

User Guide

ZEN 2 core SP1

Imaging Software





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1 About this Help

1.1 Welcome

Welcome to the ZEN 2 core SP1 Online Help.

ZEN 2 core SP1 is the new image acquisition and analysis software from ZEISS. Its design and intuitive user interface help you examine samples quickly, easily, and reliably, especially in quality assurance environments. Furthermore, in ZEN 2 core SP1, you can choose between a free examination environment or a workflow-based design.

1.2 Conventions in this Document

The following text formats are used in this document:

Example	Description
Save icon	Clickable user interface elements, e.g. buttons and icons
On/Off button	Hardware buttons on the microscope
Image panel	Non-clickable user interface elements, e.g. name of a dialog
<i>Ctrl</i> key	Keyboard shortcut
Input text	Text to be entered by the user
<i>Link</i> [▶ 12]	Link to further information

Additional information is indicated as follows:

i INFO
Helpful additional information, e.g. about necessary additional actions.

1.3 Display of Safety Notes and Safety Labels

The display of safety notes in the documentation and software follows a system of risk levels, that are defined as follows:

 **WARNING**

Risk of personal injury

WARNING indicates a potentially hazardous situation which, if not avoided, could result in major personal injury or death.

 **CAUTION**

Risk of personal injury

CAUTION indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate personal injury.

NOTICE

Risk of property damage

NOTICE indicates a property damage message. In addition, NOTICE is used for data loss or corrupt data as well.

The safety icons / labels on the device or in the documentation refer to potential dangers or information that are defined as follows:

Icon / Label	Name	Description
	<p>Crushing Fingers</p>	<p>This icon warns you of a potential risk of crushing fingers.</p>

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Safety

Refer to the safety notes and instructions in the documentation of all necessary devices (e.g. microscope peripherals, cameras, computers, computer accessories, etc.) before installing and using the software.

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Notice of the Producer

This software product was designed, realized, verified, validated and released in a certificated process environment. The quality management system is certified following the rule of DIN EN ISO 9001.

The fields of application of the Software are common tasks and applications in microscopy respectively imaging (so called "Off-The-Shelf Software"). Though the user acknowledges that in any kind of use the end user of the software is responsible for the validation of the Software for the end user's dedicated intend of use considering all requirements of law and standards (e. g. FDA/21 CFR part 11, IvDD, etc.). If necessary the end user has to establish, to document, to implement and to maintain a special process to fulfill all the requirements to be conform with the validate rules of law and standards. It is pointed out that displayed measure values (eg length measurement) may not be used directly as analytical values for diagnostic results.

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2 First Steps

2.1 Starting the Software and Logging In

Prerequisites ■ The software and all required licenses have been installed.

- Procedure**
- 1 On your Windows Desktop, double-click the ZEN 2 core SP1 program icon. The **Login** screen is displayed if user management is enabled. Otherwise you are logged in automatically.
 - 2 Click your name in the list of users.
 - 3 Enter your password and click **Login**. The **Home Screen** is displayed.
 - 4 Click the icon of the operating mode you wish to use.

INFO

When you start the software for the first time, user management is disabled.

2.2 Closing Down the Software

- Procedure**
- 1 Click the **Home** icon. The **Home Screen** is displayed. If you have unsaved templates, analysis results, or reports, choose whether to save or discard them.
 - 2 Click .
 - 3 In the **Shut Down** dialog, confirm that you wish to close the software. Any unsaved data is discarded.
 - 4 If desired, turn off the microscope hardware. For more information, see your microscope instruction manual.

2.3 Working with ZEN starter

You can use the software even if no dongle is attached. The software is then run as the free version **ZEN starter**, where not all features are available (e.g. image acquisition) and no modules or extensions are available.

Nevertheless, you can still perform many typical actions, for example:

- Loading and viewing existing microscope images
- Performing analyses
- Creating reports

3 Basic Concepts

3.1 Introduction

This section describes the concepts that you should understand before using ZEN 2 core such as user roles, operating modes, workbenches, and tools. It also provides an overview of the user interface.

3.2 Jobs and Free Examinations

ZEN 2 core SP1 is designed to support two fundamental ways of using your microscope:

- Working with jobs (creating, running, editing and managing jobs)
- Performing free examinations

Jobs In ZEN 2 core SP1, the term *job* refers to a collection of examination tasks. Jobs can be created to ensure that the same examination tasks are carried out each time the job is run, in the same manner, and with the same settings. Jobs are used mainly in routine quality control examinations where it is essential that identical examinations are performed for each sample.

Free examinations Free examinations can be used to inspect a sample quickly, easily, and flexibly without defining examination tasks. A typical use is to examine a faulty sample where the cause of the fault is unknown or for one-off examinations that will not be repeated. In such cases only the examination results, reports and images need to be saved rather than the examination tasks.

Operating modes The above ways of using your microscope are reflected in the operating modes of the software.

3.3 User Roles

User management is an optional component. It is disabled by default. When user management is enabled, three types of user role are defined initially in the software:

- Administrator
- Supervisor
- Operator

The available modes and tasks you can perform in the software depend on your user role. User roles can be added and modified in **Maintenance** mode.

If user management is disabled, the user has all user rights at the same time.

Administrators Administrators install and configure the software. This includes:

- Managing system settings
- Managing users
- Specifying the connected hardware in the Microscope Tool Box application (MTB)
- Configuring and managing the archives.

Supervisors Supervisors perform the following main tasks:

- Creating job templates for the operators to run
- Performing free examinations

They are also able to perform the following tasks:

- Running jobs
- Managing jobs in the archive (running, editing, deleting)
- Defining and evaluating job reports

Operators Operators can only perform a limited number of tasks:

- Searching for a job
- Running a job
- Browsing the job results

3.4 Overview of Supervisor Tasks

As a supervisor, the way you perform the majority of individual tasks is independent of whether you are in **Free Examination** mode or **Create Job Template** mode. The difference between the modes is whether the tasks are joined together to create a workflow for the operator to perform.

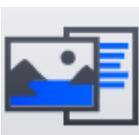
For a detailed overview of the tasks in each mode see:

- *Operating Modes* [▶ 20]
- *Workflow Create Job Template* [▶ 36]
- *Workflow Free Examination* [▶ 33]

The tasks that are independent of a mode are described in a corresponding chapter in the order they are typically performed.

3.5 Operating Modes

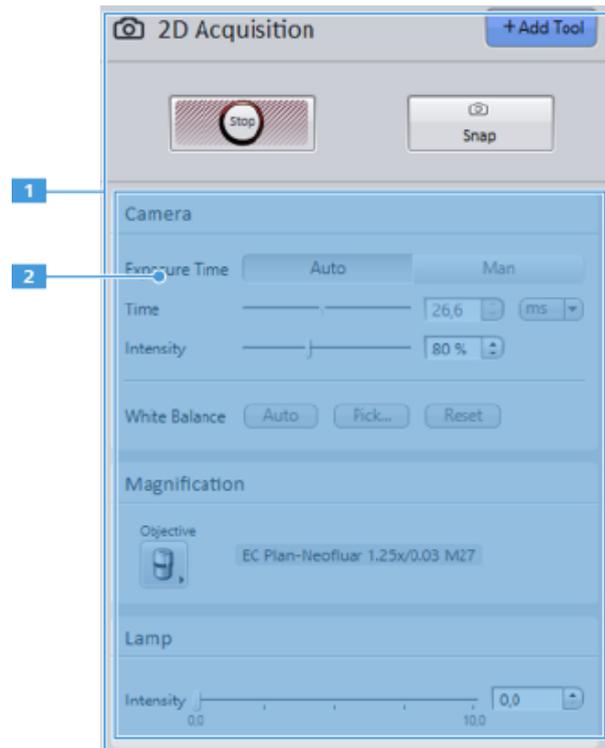
ZEN 2 core contains different operating modes that correspond to the different ways of working with the microscope. The modes that are available to you depend on your user role.

Icon	Mode	Description
	<i>Free Examination</i> [▶ 33]	Inspect a sample quickly, easily, and flexibly without defining examination tasks.
	<i>Create Job Template</i> [▶ 36]	Define fixed examination steps to be performed each time a sample is examined. This mode is designed for routine examinations.
	<i>Run Job Template</i> [▶ 46]	Run an examination on a sample according to the step-by-step tasks defined in the job template.
	<i>Manage Templates</i> [▶ 160]	Edit and manage templates in the archive: <ul style="list-style-type: none"> ■ Job templates ■ Form templates ■ Report templates ■ Custom workbenches ■ Automatic measurements ■ Macros
	<i>Browse Job Results</i> [▶ 164]	View and manage job results in the archive

Icon	Mode	Description
	<i>Maintenance</i> [▶ 291]	Configure global settings, manage users, and calibrate measurements, etc.

3.6 Workbenches and Workbench Categories

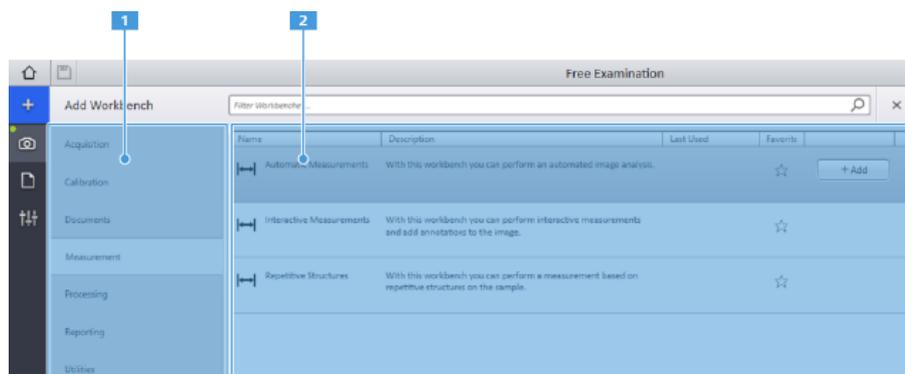
The software is based around the concept of workbenches. In ZEN 2 core, the term *Workbench* refers to a group of tools in the software. Furthermore, a workbench typically corresponds to a task in the software. Workbenches also affect the appearance of the **Center Screen Area**.



- 1 Workbench
- 2 Three tools (Camera, Magnification, Lamp)

Workbenches in turn are grouped into categories. The categories correspond to the typical microscopy tasks, e.g.:

- Acquiring an image
- Processing an image
- Measuring an image.



- 1 Workbench category
- 2 Workbenches

Typically there are multiple workbenches in each category. For example, for image acquisition the following workbenches (amongst others) are available:

- Simple acquisition, e.g. 2D Acquisition
- Advanced acquisition, e.g. Tiles

Workbenches enable you to concentrate on your microscopy tasks by providing the tools you require while keeping the user interface uncluttered.

The tools contained in a workbench may also be present in multiple other workbenches. As a supervisor you can also create new workbenches or modify existing ones by adding and removing tools according to your requirements. These customized workbenches can be saved as new workbenches and reused in other examinations or by other users.

INFO

- The appearance of workbenches and how you use them depends on your current mode.
 - In **Free Examination** mode click **Add Workbench**.
 - In **Create Job Template** mode click **Add Task**.
- The workbenches that are available depend on your hardware and licenses.

3.7 Creating and Using Custom Workbenches

You can add or remove tools from a workbench at any time. You can also save a workbench configuration as a custom workbench. This enables you to use it in other job templates or free examinations.

All custom workbenches are saved and managed in the **Archive**.

INFO

In **Create Job Template** mode, each workbench is automatically saved in the job template in its current configuration. It is also displayed in the same configuration when running the job.

Creating To create a custom workbench:

- Procedure**
- 1 Customize your workbench by adding or removing tools.
 - 2 Right-click the icon of a workbench and select **Save as custom workbench**.
 - 3 Enter a name for the custom workbench.
The name is used to identify the workbench and must be unique within the system.
 - 4 Enter a description for the workbench.
It should describe the purpose or special features of the workbench to help other users know when to select it.
 - 5 Click **Save** to save the custom workbench.

Using To use a custom workbench:

- Procedure**
- 1 Click **+ Add Task**.
 - 2 Select the **My Workbenches** category.
 - 3 Select the desired custom workbench and click **+ Add**.

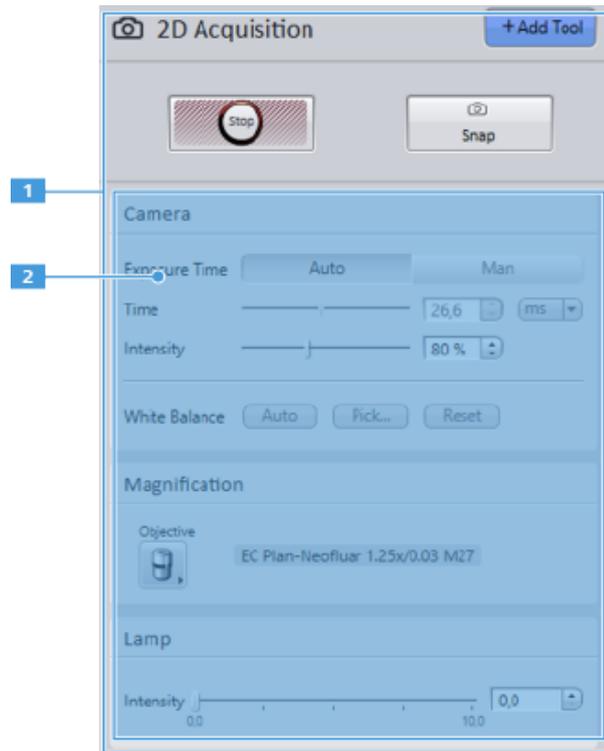
INFO

To ensure data integrity, it is not possible to overwrite existing default or custom workbenches.

3.8 Tools and Parameters

Tools enable you to perform a specific action in the software, for example:

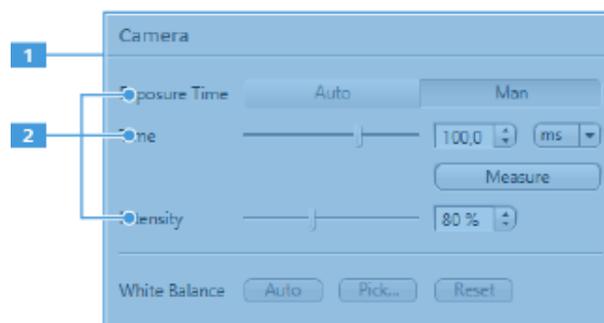
- Acquiring an image
- Selecting a different hardware magnification
- Reducing the noise
- Measuring a length



- 1 Workbench
- 2 Tools

The tools contained in a workbench may also be present in multiple other workbenches. As a supervisor you can also create new workbenches or modify existing ones by adding and removing tools according to your requirements. These customized workbenches can be saved as new workbenches and reused in other examinations or by other users.

Parameter A tool typically consists of multiple parameters. A parameter corresponds to an individual field or control within a tool.



- 1 Tool
- 2 Parameters

In **Create Job Template** mode you can specify which parameters are visible to the operator and how the operator can adjust the values.

3.9 Templates, Images, and Documents

The software supports various kinds of objects. These can be grouped into the following categories:

- Templates
- Images and documents

The categories are treated differently in the software, for example where they are managed or how you interact with them.

Templates Templates contain predefined content, for example the tasks required to run a job, or the fields required to complete a form.

Templates include the following objects:

- Job templates
- Form templates
- Report templates
- Custom workbenches
- Automatic measurements
- Macros

Templates are managed in the **Archive**. When a user's workflow requires selecting one of the above items (e.g. choosing a job template to run or which report template to use), the user is presented with the items stored in the **Archive**. In general, users only interact with items in the **Archive**; they do not need to interact with the file system.

Images and documents Images and documents refer to objects created during an examination.

Images and documents include the following objects:

- Images
- Measurement results and data tables
- Forms
- Reports

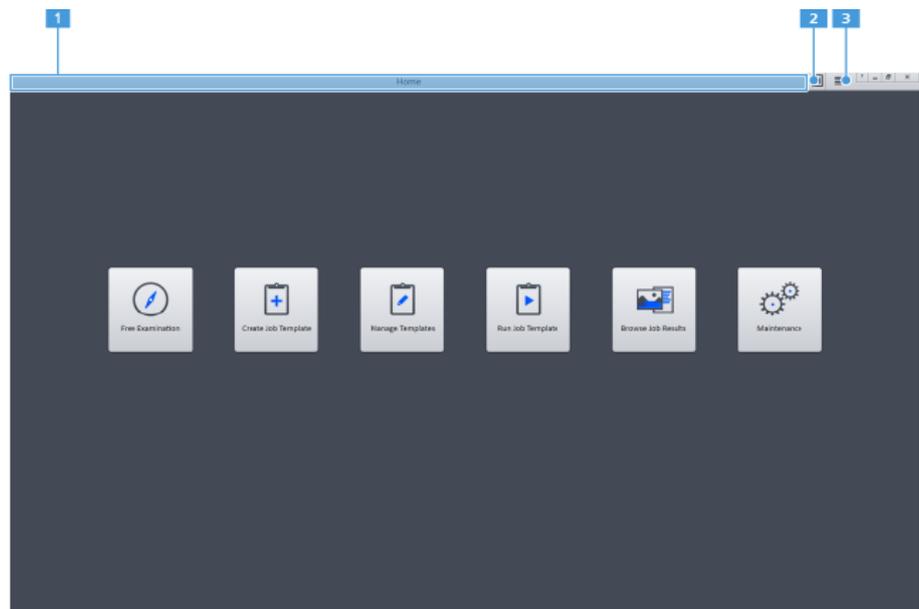
Images and documents are created by tools or tasks and are automatically stored in the **Archive** as children of the corresponding job or free examination. However, you can also import and export images and documents to/from the file system using tools in workbenches. Furthermore, in **Free Examination** mode, all images and documents are additionally displayed in the **Documents Area**.

3.10 User Interface Overview

The following topics contain an overview of the main user interface elements for the different modes you can select from the **Home Screen**.

3.10.1 User Interface - Home Screen

The **Home Screen** is displayed after you log in. The available modes depend on your user role.



1 Title Bar

2 System Messages icon

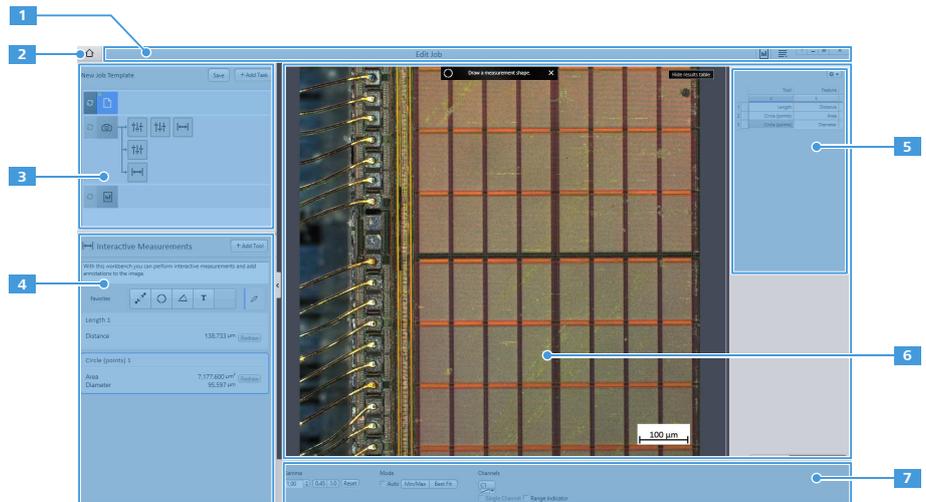
Displays system status messages such as the time required to generate an image, as well as errors and warnings

3 Options icon

Change the zoom level of the user interface

3.10.2 User Interface - Create Job Template Mode

The following figure shows the typical user interface when creating a job template.



1 Title Bar

Enables you to change the zoom of the user interface and view system messages

2 Home icon

Takes you back to the **Home Screen**

3 Task List

Enables you to define the tasks to be performed by the operator

4 Workbench Area

Contains the currently selected workbench and tools

5 Results Table

Lists the results of all the measurements in the image

6 Center Screen Area

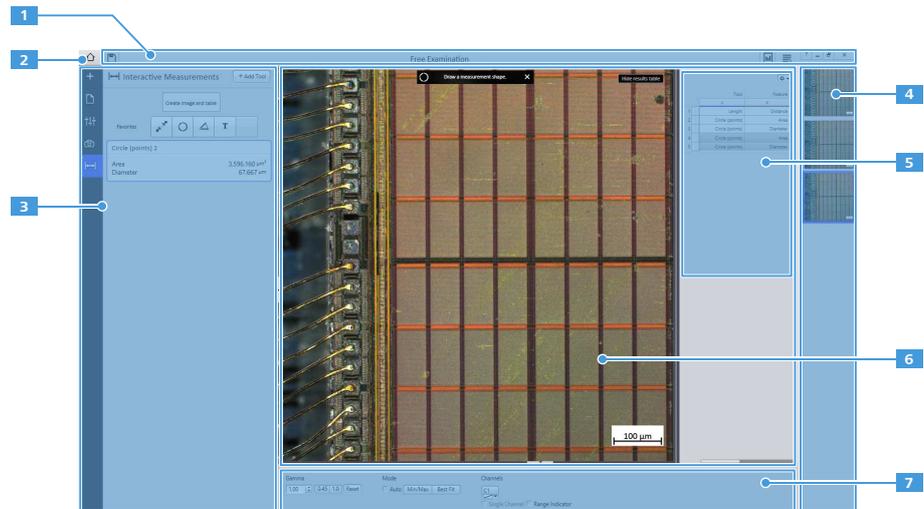
Contains the current image, measurements, measurement results, and context-specific tools

7 View Options

Enables you to adjust how an image is displayed (i.e. its appearance) without changing the image itself

3.10.3 User Interface - Free Examination Mode

The following figure shows the typical user interface when working in Free Examination mode.



1 Title Bar

Enables you to change the zoom of the user interface and view system messages

2 Home icon

Takes you to the **Home Screen**

3 Workbench Area

4 Documents Area

Contains a list of all documents (images, measurement results, and reports) within the examination

Double-click a document to display it and select it in the open task. Each time you apply a tool a new document is generated.

5 Results Table

Lists the results of all the measurements in the image

6 Center Screen Area

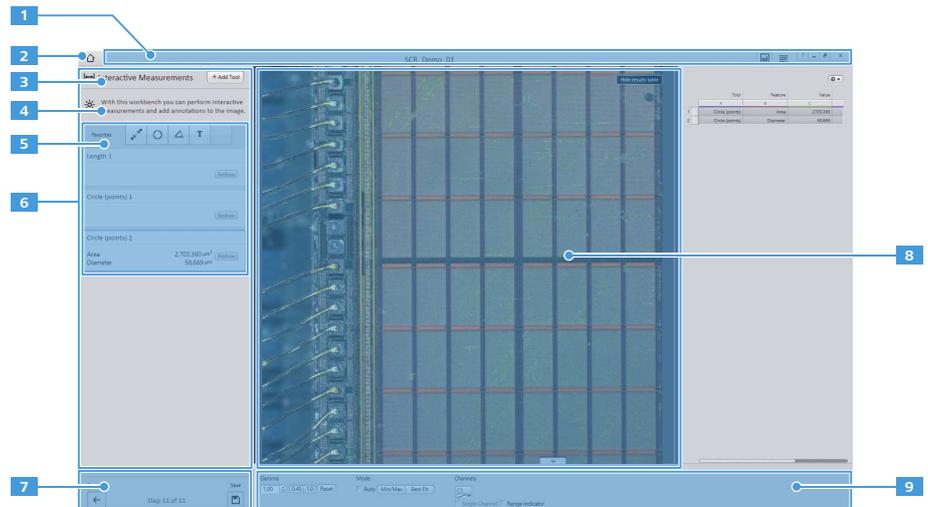
Contains the current image, measurements, measurement results, and context-specific tools

7 View Options

Enables you to adjust how an image is displayed (i.e. its appearance) without changing the image itself

3.10.4 User Interface - Run Job

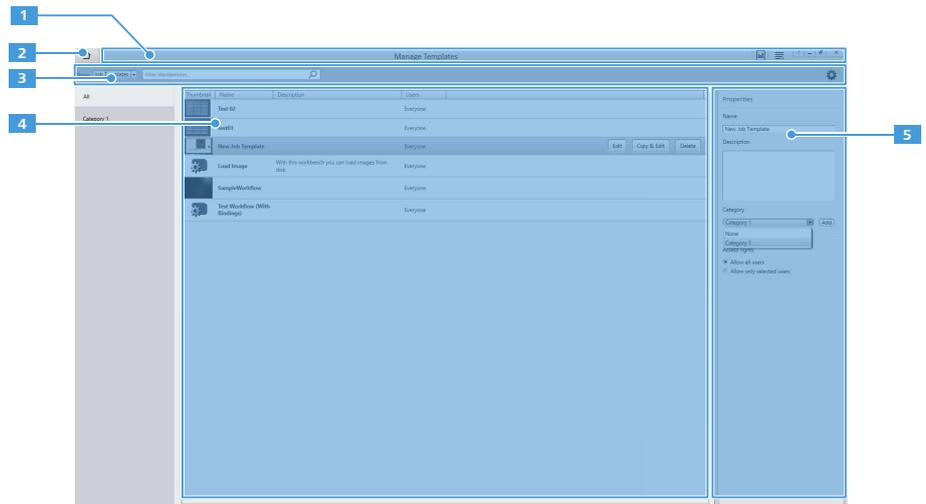
The following figure shows the typical user interface when running a job.



- 1 Title Bar**
Enables you to change the zoom of the user interface and view system messages
- 2 Home icon**
Takes you to the **Home Screen**
- 3 Task name**
- 4 Task instructions**
Additional information about how to perform the current task (for example the region of the sample to analyze)
- 5 Tools available to perform the task**
- 6 Task Panel**
- 7 Task Navigation buttons**
- 8 Center Screen Area**
Contains the current image and measurements
- 9 View Options**
Enables you to adjust how an image is displayed (i.e. its appearance) without changing the image itself

3.10.5 User Interface - Manage Templates

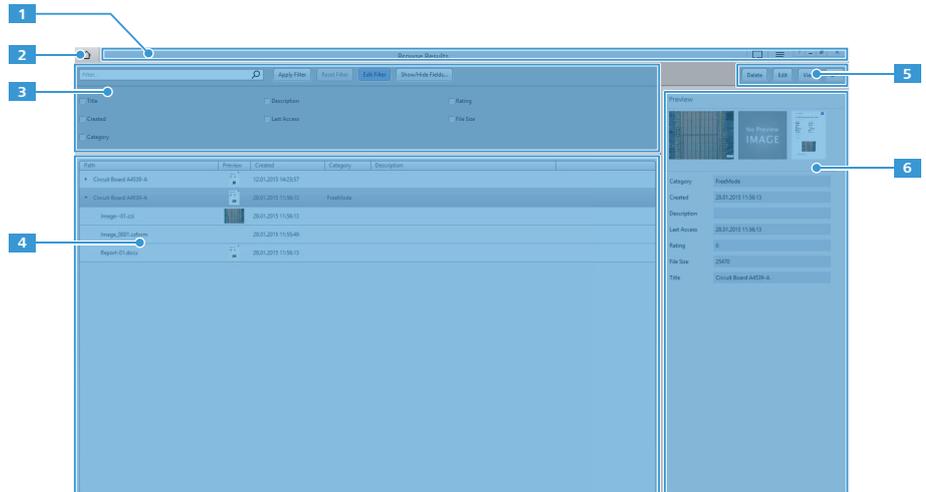
The following figure shows the typical user interface when managing templates.



- 1 Title Bar**
Enables you to change the zoom of the user interface and view system messages
- 2 Home icon**
Takes you to the **Home Screen**
- 3 Filter Area**
Enables you to filter the list of templates by type or search term
- 4 Template List**
Displays all templates specified by the filter
- 5 Properties Area**
Allows to change the properties of the selected template, e.g. the name, category, and permissions.

3.10.6 User Interface - Browse Job Results

The following figure shows the typical user interface when browsing job results.



- 1 Title Bar**
Enables you to change the zoom of the user interface and view system messages

- 2 Home icon**
Takes you to the **Home Screen**
- 3 Filter Area**
Enables you to filter the list of job results by type or search term
- 4 Results List**
Lists all job results and results from free examination sessions in a hierarchical structure
- 5 Action Area**
Enables you to perform actions with the selected job result
- 6 Preview Panel**
Shows additional information about the selected job result and a preview of the documents contained in it

4 Free Examinations

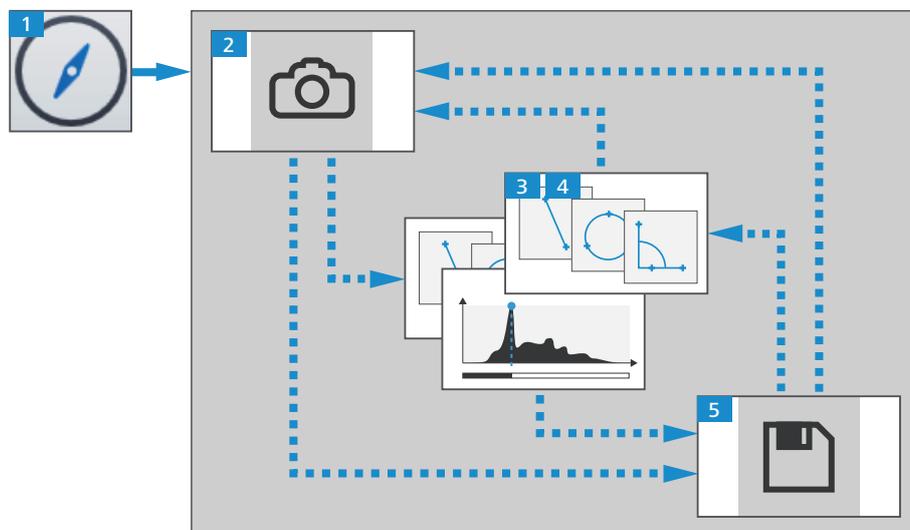
4.1 Basics of Free Examinations

Free examinations can be used to inspect a sample quickly, easily, and flexibly without defining a job consisting of multiple tasks in a fixed order. A typical use is to examine a faulty sample where the cause of the fault is unknown or for one-off examinations that will not be repeated.

This section provides an overview of typical actions in **Free Examination** mode, such as using workbenches, adding and removing tools and working with documents and images.

4.2 Workflow

In **Free Examination** mode there is no typical workflow. In this mode, workbenches can be used in any sequence.



- 1** Select **Free Examination** from the **Home Screen**.
- 2** Acquire an image.
Acquire an image using the camera or load an image from the file system.
- 3** Process the image.
Enhance the appearance of the image using various processing tools.
- 4** Analyze the sample
If desired, repeat steps 2 – 4.
- 5** Save or export the images and analysis results.

Each time you apply a tool a new image is generated in the **Documents Area**. You can apply any number of processing or analysis tools in any order.

4.3 Selecting Workbenches

In **Free Examination** mode you can change which workbenches are displayed in the **Workbench Area**. To be able to use the tools within a workbench it must be selected. However, you can add or remove a workbench from the **Workbench Area** at any time without affecting the examination results. The order of the workbenches also does not affect the examination results.

TIP

Remove any workbenches you do not require to reduce the number of workbenches displayed in the **Workbench Area**.

Adding workbenches To add a workbench to the **Workbench Area**:

Procedure 1 Click the **Add Workbench** button.



2 In the left pane, click the desired workbench category.

3 In center pane, click the desired workbench.

A description of each workbench is displayed in the right pane.

4 Click **+ Add**.

Removing workbenches To remove a workbench from the **Workbench Area**:

Procedure 1 Right-click the icon of the workbench.

2 Click **Close workbench**.

INFO

The next time you start a free examination the workspace is displayed as you left it.

4.4 Adding and Removing Tools

You can add and remove tools from a workbench so that a workbench only contains the required tools.

Adding tools To add a tool to a workbench:

Procedure 1 Select the workbench to which you wish to add a tool.

2 Click the **Add tool** button in the **Workbench Area**.

3 In the overlay select the desired tool.

The tools that are available depend on the current workbench.

Removing tools To remove a tool from a workbench:

- Procedure**
- 1 Right-click anywhere within the tool.
 - 2 Click **Delete tool** and click **OK** to confirm.

Alternatively, you can click  to remove the tool.

4.5 Documents and Images

In **Free Examination** mode, there is no **Task Area** as in **Create Job Template** mode. Instead, user-created documents such as images, tables, forms, or reports are listed in the **Documents Area**.

The **Documents Area** displays the current image and all previous images. You can navigate to a previous image by clicking on it. If you then use a tool, it is applied to the currently selected image (rather than the most recent image). The resulting image is then added to the bottom of the **Documents Area**.

4.6 Customizing Workspace

The **Free Examination** workspace always starts with the same workbenches displayed in the **Workbench Bar** as were used for the previous free examination.

- If you have a set of workbenches that you commonly use for free examinations, you can simplify your work by adding them to the **Workbench Bar**.
- Furthermore, if there are workbenches you rarely need, you can remove these from the **Workbench Bar**.
- You can also modify existing workbenches by adding and removing tools.

By combining the above methods, you can configure the free examination workspace to your requirements.

5 Create a Job Template

5.1 Basics of Create Job Template Mode

Create Job Template mode is used for designing workflows (jobs) which then can be selected and performed in the **Run Job** work mode. Create a job template containing all the necessary tasks to examine a sample. Each time the job template is run by an operator, the same tasks are performed.

This section provides an overview of the typical design steps in this mode. It describes how to create a job template, how to use workbenches to add and remove tasks, and how to modify the properties of individual tools in a workbench.

5.1.1 Job Templates

A job template contains all the examination tasks to be performed when the job is run: e.g. acquiring an image, processing it, analyzing it and creating a report.

Each task to be performed by the operator is represented by an icon in the **Task List**.

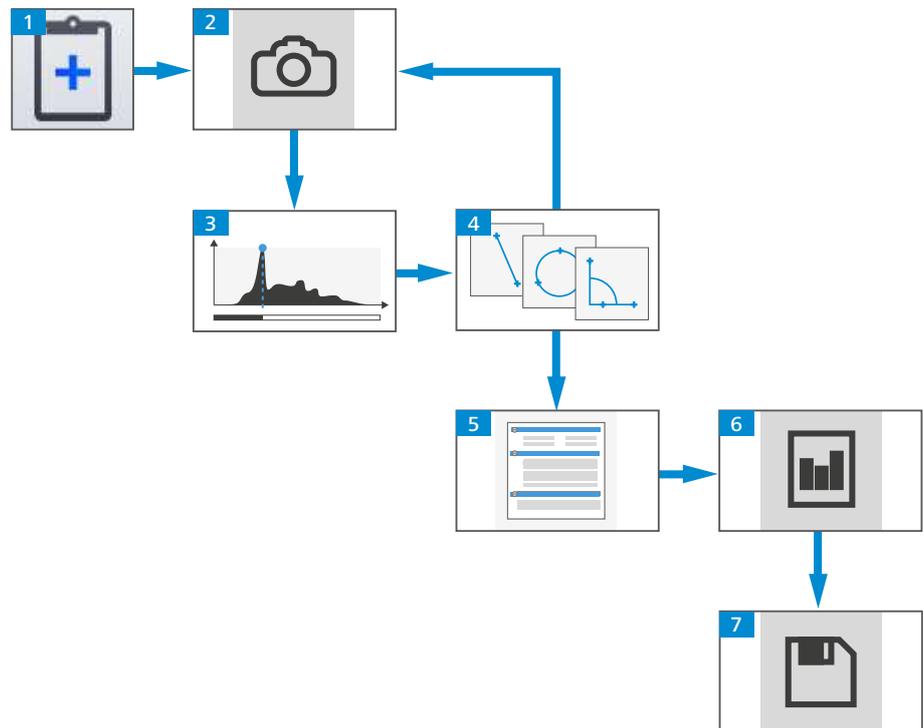
By adding tasks to the **Task List** and structuring them you can create a workflow.

For each task you can also specify the following:

- Which tools are available
- Whether tool parameters can be changed
- The default values for parameters and the range of permitted values

5.1.2 Workflow

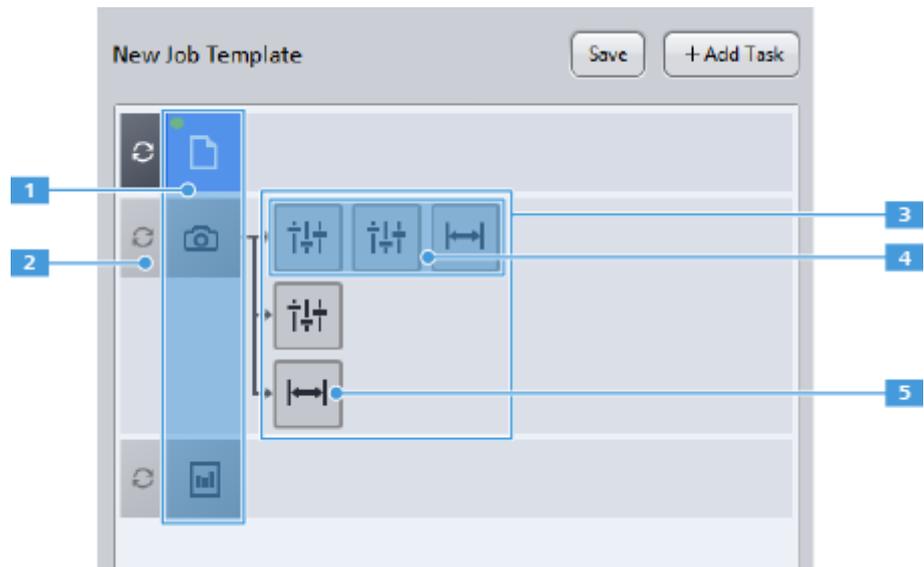
One typical workflow when creating a job template can be summarized in the following figure. Each step represents one task in the job template and is represented with an icon in the **Task List**.



- 1** Select **Create Job Template** mode from the **Home Screen**.
- 2** Acquire an image.
Use the microscope camera or load an image from the file system.
- 3** Process the image.
Enhance the image and configure which parameters can be modified, for example adjust the brightness and specify that contrast can only be altered +/- 10%.
- 4** Analyze the image.
Define the measurements to be performed and configure the accepted measurement tolerances.
If desired, repeat steps 2 – 4.
- 5** Configure the input form.
Select a form template and specify the metadata to be recorded (e.g. sample ID, current time) and how it is entered (manually, automatically).
- 6** Configure the report.
Select a report template and configure how measurement results and metadata should appear in it.
- 7** Save the job.
Specify a name for the job template and which users are allowed to run it.

5.1.3 Task List

The **Task List** indicates all the tasks to be performed when the job is run.



- 1 Image/document
- 2 Apply changes to current image/document
- 3 Task tree
- 4 Branch
- 5 Task

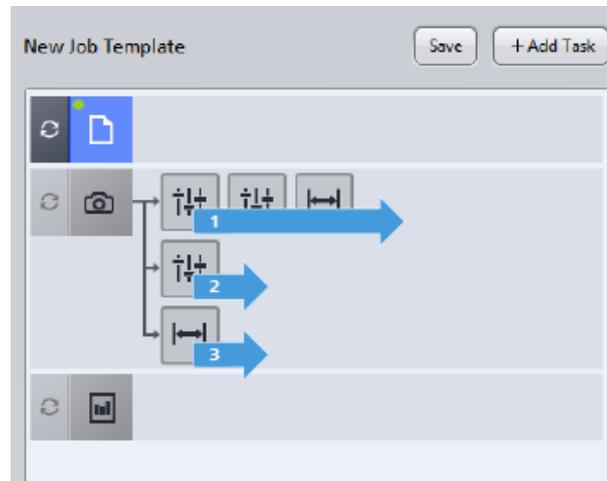
Every time you select a task, a corresponding icon is added to the task list. The result is a task tree per image or document.

The position where the task is added depends on the item currently selected in the **Task List**:

Selected item	Position new task is added
Task (default)	At the right end of the current branch Each branch can contain multiple processing or measurement tasks.
Image / document	As a new branch This enables you to perform different tasks on the same image/document without needing to reacquire it

INFO
Image and document tasks are always inserted as a new tree, regardless of the current selection.

When the job is run, the tasks are performed in the following order:



5.1.4 Task Queue

In **Create Job Template** mode, many of the image processing tools for an image are not applied immediately as they require processing time to calculate the resulting image. The refresh icon to the left of the image/document in the **Task List** indicates its current status:

-  Click the refresh icon to update the image/document

If a branch in the **Task List** contains multiple processing tools they are all applied, from left to right. The output of a processing tool provides the input for subsequent processing tools.

5.2 Creating a New Job Template

ZEN 2 core SP1 is supplied with example job templates which you can configure to your requirements.

However, you can also create a new job template from scratch.

- Prerequisites**
- You are logged in as a supervisor or you have the sufficient privileges to create a job template

- The **Home Screen** is visible

Procedure 1 Click **Create Job Template**.

An empty job template is created.

- 2 Select the category of the first task to be performed, typically **Acquisition**.

- 3 Select the workbench for the first task to be performed and click **Add**.

Alternatively, you can double-click the desired workbench.

The first task is displayed in the **Task List** and the corresponding workbench and tools are displayed in the **Workbench Panel**.

- Continue with the steps described in the typical workflow.

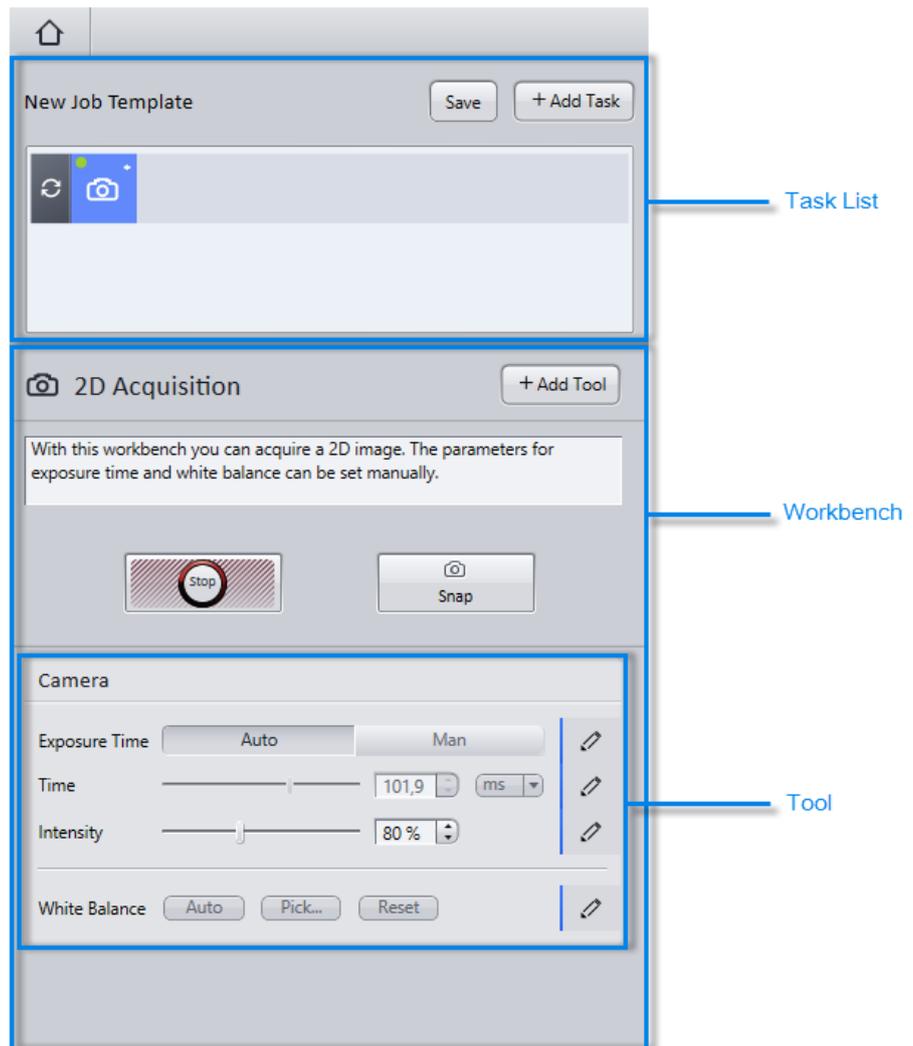


Fig. 5.1: Creating a Job Template

5.3 Customizing Job Templates

5.3.1 Adding and Removing Tasks

By adding tasks to the **Task List** and structuring them you can create the workflow for the operator when running the job.

The position where the task is added depends on the item currently selected in the **Task List**:

Selected item	Position new task is added
Task (default)	At the right end of the current branch

Selected item	Position new task is added
	Each branch can contain multiple processing or measurement tasks.
Image / document	<p>As a new branch</p> <p>This enables you to perform different tasks on the same image/document without needing to reacquire it</p>

INFO

Image and document tasks are always inserted as a new tree, regardless of the current selection.

Adding a task To add a task to the job template:

- Procedure**
- 1** Ensure that the correct item in the **Task List** is selected for the location you want to insert a new task.
 - 2** Click the **Add Task** button.
 - 3** Select the desired workbench category.
The workbenches that are available depend on the currently selected task.
 - 4** Select the desired workbench and click **Add**.
Alternatively, you can double-click the workbench.

The workbench is displayed in the **Workbench Area** and a corresponding icon is added to the **Task List**.

Removing a task When you remove a task from the **Task List**, all subordinate tasks are automatically removed.

- Procedure**
- 1** Right-click the icon of the task in **Task List**.
 - 2** Click **Delete Task**.

5.3.2 Specifying Tools for a Task

For each task to be performed when the job is run, you can specify which tools are available to complete the task. For example, for a processing task, you can specify that only the **White Balance** tool and **Gauss** tool are available.

This is done by adding and removing tools from the corresponding workbench so that only those required when running the job are included in the workbench.

INFO

If a tool is required when running a job, it must be included in the workbench. However, if you do not want the operator to be able to change the values of the tool parameters you can lock or hide individual parameters. To hide the entire task from the operator, right-click it and apply the **Run Silent** option. The task is then executed but not visible to the operator when the job is run.

Adding tools To add a tool to a workbench:

- Procedure**
- 1 Select the workbench to which you wish to add a tool.
 - 2 Click the **Add tool** button in the workbench header.
 - 3 Select the desired tool.

The tools that are available depend on the current workbench.

TIP

If you wish to add large numbers of tools to a workbench, it may be advisable to add another workbench of the same type and to distribute the tools across the two workbenches.

Removing tools To remove a tool from a workbench:

- Procedure**
- 1 Right-click anywhere within the tool.
 - 2 Click **Delete**.

Alternatively, you can click  to remove the tool.

If you remove a processing tool, the new processing result is not displayed until you click the corresponding  icon in the **Task List**.

5.3.3 Specifying Permitted and Expected Values for a Tool

When creating a job template you can specify the range of values that an operator can enter for each parameter. The range of values is defined by the following:

- Minimum permitted value
- Maximum permitted value
- Expected value

Measurement tools For measurement tools you cannot limit the range of a parameter. However, you can configure tolerances between which the measurement value must lie. For more information, see *Configuring Tolerances for a Measurement* [▶ 44].

Furthermore, you can enter the expected value of the tool. This is the value that an ideal sample has; typically it is the value from the sample specifications.

Procedure 1 Click the tools options icon.



2 Set the values as desired.

The expected value must be between the minimum and maximum permitted values.

When the job is run, the value of the parameter is set to the expected value from above. The minimum and maximum values of each parameter are also adjusted accordingly in the user interface.

TIP

If you do not want the operator to be able to change the value of a parameter, you can lock or hide the parameter.

5.3.4 Locking and Hiding Parameters in Tools

When creating a job template you can specify the operator's privileges for each parameter individually. The privilege is indicated by the following icons:

■  Parameter can be seen and changed by the operator

If the parameter can be changed by the operator, you can choose to limit the range of permitted values.

■  Parameter can be seen but not changed by the operator

■  Parameter is hidden from the operator (and thus cannot be changed)

To change the property of a parameter:

Procedure ◆ Click the icon until it has the desired state.

The icon cycles between the three states in the above order.

If all the parameters in a workbench are hidden or locked, the corresponding task is performed automatically in the background when the job is run.

If you want an entire task to be performed automatically in the background without user interaction, you can use the **Run Silent** option. This overrides the settings of individual parameters within the task.

Procedure ◆ Right-click the icon of the task in the **Task List** and select **Run Silent**.

5.3.5 Adding Information for the Operator

When the job is run, the user needs to know what to do for each task.

For each task you can enter corresponding instructions. These are then displayed to the user under the task name, when the job is run.

- Procedure**
- 1 Click in the text field at the top of the **Workbench Panel**.
 - 2 Enter instructions about how to perform the task.

The text is stored in the workbench description for the current job template. You can enter different instructions for the same workbench in different job templates.

INFO

- When running the job the operator does not see a copy of the image you acquired or the measurements you performed. Therefore, ensure that the text you enter is sufficiently descriptive and unambiguous to achieve the desired results.
- If you create a custom template, the workbench description is stored as the default instruction. However, you can modify this text individually for each job template as described above.

5.3.6 Configuring Tolerances for a Measurement

For each measurement you can configure the following parameters:

- **Expected value**
- **Lower boundary**
- **Upper boundary**

The expected value is typically the theoretical value contained in the CAD data or sample specifications.

The boundaries specify the limits between which the measurement must lie, for example to fulfil quality control criteria. However, the user can measure the value freely and is not constrained by the boundaries (i.e. the software does not limit the measurement value). It is also possible to only specify one boundary.

You can easily see whether the measurement is within the boundaries via the color code next to the value:

- Green: Within boundaries
- Red: Outside boundaries

INFO

For tools other than measurement tools you can limit the range of a parameter. In this case the user cannot select a value outside the range. For more information, see *Specifying Permitted and Expected Values for a Tool* [▶ 42].

Prerequisites ■ A measurement tool is selected

Procedure

- 1 Click the  icon.
- 2 Set the values as desired.
The expected value must be between the lower and upper thresholds.

5.3.7 Sorting and Copying Tasks

You can easily change the order of workbenches, or move them from one image node to another. For example, this is useful if you want to apply processing tools in a different order or if you want to duplicate analysis tasks.

- Procedure**
- 1 Click the desired workbench in the **Workbench Area**.
 - 2 Drag it to its new position.
The light gray bars indicate possible target positions.
 - 3 To copy a workbench, press the **CTRL** key while dragging.
A plus icon is displayed next to the icon while dragging.
All the values of the workbench are also copied.

5.4 Saving and Completing the Job Template

You can save a job template at any time during its creation. The **Save Job Template** dialog enables you to specify the following:

- Job template properties (name and description)
- Category to which the job template is assigned
- Permissions (users that can run the job)

You can also modify the above in the **Archive** later.

For each job you can specify which users are allowed to run it. Typically, a group of users is specified, but you can also specify individual users. By default, a job can be performed by any user.

Prerequisites ■ You are in **Create Job Template** mode

Procedure

- 1 Click the **Save** button.
- 2 In the **General** tab enter a meaningful **Name** and **Description**.
- 3 In the **Security** tab specify which users or user groups should be allowed to run the job.
By default all users can run the job.

6 Run a Job

6.1 Basics of Running a Job

As an operator, your main task is to run jobs that have been created by a supervisor. When you log in as an operator, you can choose between selecting a job to run and viewing the results of a job you have run previously.

The job created by the supervisor contains all the steps to be performed, from image acquisition to processing, analysis, and how the results should be reported. When running the job you are guided through the required steps and automatically presented with the necessary tools to perform it.

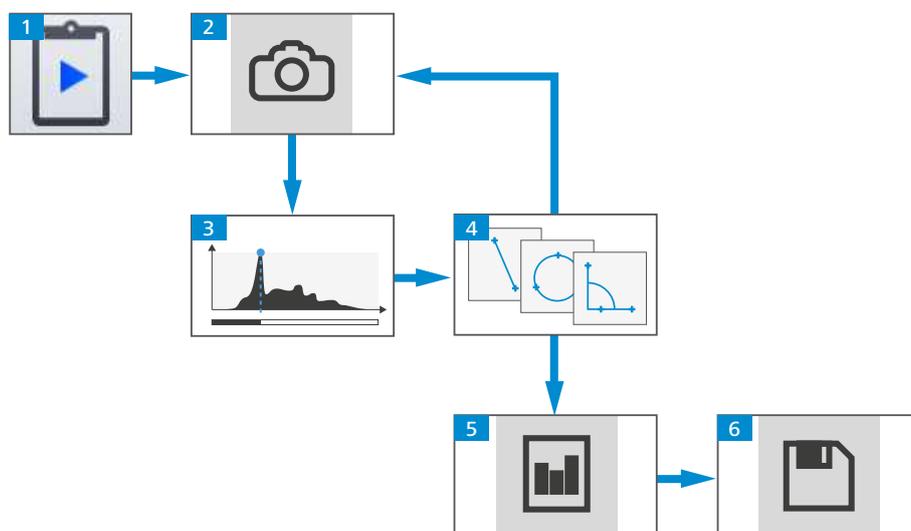
Text from the supervisor describes what to do for each task, for example where to perform an analysis on the sample. Furthermore, the supervisor can specify a default value for a tool and limit the range of values that you can apply. Values that may not be changed are locked or hidden.

For more information about an individual tool, click  and then the tool.

Once you have completed all the steps in the job, the images and the measurement results are automatically saved in a job result. You can then repeat the job with another sample or select a different job to run.

6.2 Workflow

The following diagram shows an example of the workflow when running a job. Any steps where no interaction is required are performed automatically in the background.



- 1 Select the appropriate job for the sample.

- 2 Acquire an image of the region defined by the supervisor.
- 3 Process the image as defined by the supervisor.
- 4 Perform the analyses defined by the supervisor.
You are automatically guided through the analyses and presented with the appropriate tool.
Repeat steps 2 - 4 for other areas of the sample as defined by the supervisor.
- 5 Check the information in the job report.
- 6 Save the job results.

6.3 Selecting a Job to Run

When you log in as an operator, you can choose between selecting a job to run and viewing the results of a job in the archive that you have run previously.

- Prerequisites**
- You are logged in as an operator
 - You are on the **Home Screen**

- Procedure**
- 1 Click **Run Job Template**.
 - 2 Click the desired job in the list.
 - 3 Click the **Run** button.

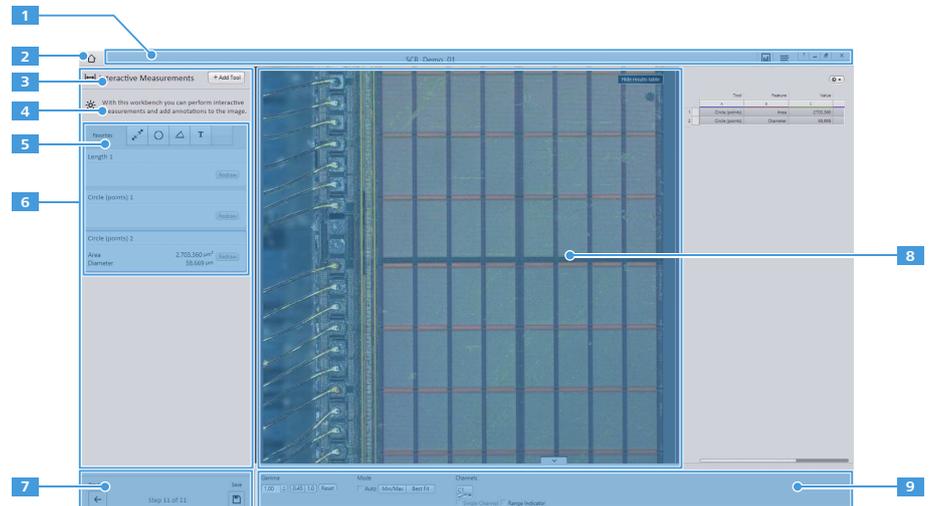
Alternatively, you can double-click the desired job.

The first step in the job is displayed (typically acquire an image).

If the job requires a different hardware configuration to your current setup, the system prompts you which components need to be changed. For more information about how to change components, see your microscope instruction manual.

6.4 Running a Job

When running a job, the user interface has the following elements:



1 Title Bar

Enables you to change the zoom of the user interface and view system messages

2 Home icon

Takes you to the **Home Screen**

3 Task name

4 Task instructions

Additional information about how to perform the current task (for example the region of the sample to analyze)

5 Tools available to perform the task

6 Task Panel

7 Task Navigation buttons

8 Center Screen Area

Contains the current image and measurements

9 View Options

Enables you to adjust how an image is displayed (i.e. its appearance) without changing the image itself

Prerequisites ■ You have selected the job to run

Procedure 1 Follow the instructions in the **Task Panel** using the tool(s) available. For more information about an individual tool, click **?** and then the tool.

2 To continue with the next step, click the **Next** icon.



3 When you have completed all the steps, click the **Save** icon.



INFO

You can click the  icon to return to a previous step, for example to change the values of a tool or the location of an analysis. If you make a change in a previous step, all measurements etc. you performed in subsequent steps are discarded.

6.5 Acquiring an Image

Acquiring an image is typically the first step when running a job. The image is acquired using the camera on your microscope. To load an image from the file system, see *Loading an Existing Image* [▶ 123].

- Procedure**
- 1** Ensure you acquire the same area of the sample as the supervisor.
Follow any instructions added by the supervisor in the workbench panel.
 - 2** If your microscope has a motorized stage, you can navigate the sample and adjust the focus using the software.
Otherwise, move the sample on the motorized stage by hand and focus manually. For more information, see your microscope instruction manual.
 - 3** If your microscope has a motorized object revolver you can select the correct zoom level using the software.
Otherwise, select the correct objective by hand. For more information, see your microscope instruction manual.
 - 4** If desired, adjust the acquisition parameters.
For more information about individual parameters, click .
 - 5** Click **Snap**.
If you are not satisfied with the image, click **Live** and repeat the above steps.
 - 6** When you are satisfied with the image, click **Next**.

6.6 Processing the Image

Image processing enables you to adjust the appearance of the image after it has been acquired, for example to compensate changes in brightness if the illumination changes between jobs.

Typical processing actions include:

- Brightness and contrast
- Reduce noise and enhance contours
- Adjust the size, rotation, and quality

The tools that you can use, and thus the properties that you can alter, depend on what the supervisor has enabled in the job template.

- Procedure**
- 1 Adjust the values of the parameters.
 - 2 All processing tools are applied in one go, in the order displayed in the workbench.
 - 3 Repeat the above steps until you are satisfied with the resulting image.
For more information about individual parameters, click .
 - 4 Click **Next**.

6.7 Analyzing the Image

6.7.1 Performing an Interactive Measurement

Interactive measurements enable you to measure e.g. distances, angles, area, and intensities of pixels. All the measurements to be performed in the current image are displayed in the **Center Screen Area** at the locations specified by the supervisor.

- Procedure**
- 1 Follow any instructions added by the supervisor in the workbench panel.
 - 2 Drag each measurement to the correct location.
 - 3 Click the measurement to move or resize it.
The corresponding handles for moving or resizing the measurement are displayed in the image.
 - 4 Click **Next**.

INFO

If the supervisor specified an expected value and upper/lower limits for a measurement value (e.g. area or diameter), a corresponding color symbol in the **Task Panel** indicates whether your measurement result lies inside the expected limits (green) or outside the limits (red).

6.7.2 Performing an Automatic Measurement

Automatic measurements enable you to analyze simple shapes based on their gray values. For example, you can automatically count and classify particles in the sample according to their size or color.

Typically the supervisor has specified all relevant measurement parameters when setting up the job template. Your actions are limited to file system interactions (loading/saving images/results) or correcting measurement results.

- Procedure**
- 1 Follow any instructions added by the supervisor in the workbench panel.
 - 2 Click the **Analysis** button.

- 3 If your interaction is required, follow the instructions from the supervisor for each step.

Once the automatic measurement has been completed the measurement results are displayed in the **Results Table**.

- 4 Click **Next**.

6.8 Creating a Report

Reports enable you to collate all the information from your examination in a single document. Typical information includes:

- Images
- Measurement data
- Metadata (e.g. examination time, hardware setup)

Each report template contains placeholders for the above information to enable you to collate the information easily. The placeholders are usually filled automatically with the correct information. Depending on the settings applied when the job was created, you might be allowed to change the content of a placeholder.

- Procedure**
- 1 Follow any instructions added by the supervisor in the workbench panel.
 - 2 Check that all the information is included in the report (for example the correct images).

A preview of the report is displayed in the **Right Tool Area**.

- 3 To change the information in a placeholder, select the desired report template in the **Add Templates** tool.

Various placeholders are listed in the **Workbench Area**.

- 4 Click the arrow icon in a placeholder and select the corresponding measurement information that you wish to add, for example image, measurement result, etc.



You can add multiple items to a single placeholder. The report preview updates accordingly.

- 5 If you require a paper copy click **Print Report**.

6.9 Saving and Completing a Job

When you have completed the last examination task, you can save the job.

- Procedure**
- 1** Check that you are satisfied with all your measurement results.
 - 2** If you are not satisfied with a result, navigate back to the corresponding task and change the parameters accordingly.
For information, see *Running a Job* [▶ 48].
 - 3** To complete the job, click the **Save** icon.

The measurement results are saved and a report is generated automatically.
 - 4** Choose what you want to do next:
 - Repeat the same job with a new sample
 - Return to the **Home Screen**

7 Image Acquisition

7.1 Overview of Image Acquisition

Acquiring an image is the first step when working with the microscope. It refers to taking a picture of the sample using the microscope camera or importing an existing image. After you have acquired an image you can process and analyze it, and, if desired, add it to a report.

7.2 Basics of Image Acquisition

7.2.1 Acquisition Methods

You can acquire an image as follows:

- Load an existing image from the **Archive** or file system

This enables you open a previously acquired image or to import an image from another system.

- Acquire a new image using the camera

The software contains various acquisition workbenches depending on whether you want to simply acquire an image quickly, or whether you want to adjust image acquisition parameters. It also contains various workbenches tailored to different types of image. The available workbenches depend on your hardware and licenses.

7.2.2 Image Types

The following types of images are supported by the software. The types that are available to you depend on your hardware and licenses.

Image type	Description
Standard image	Simple snapshot of the sample. Once you have created an image, you can process it, apply measurements, save, or export the image.
Extended depth of focus (EDF)	Takes images of various focal planes and renders the image stack together to create a single image where the entire sample surface is in focus, regardless of the height of the objects on it

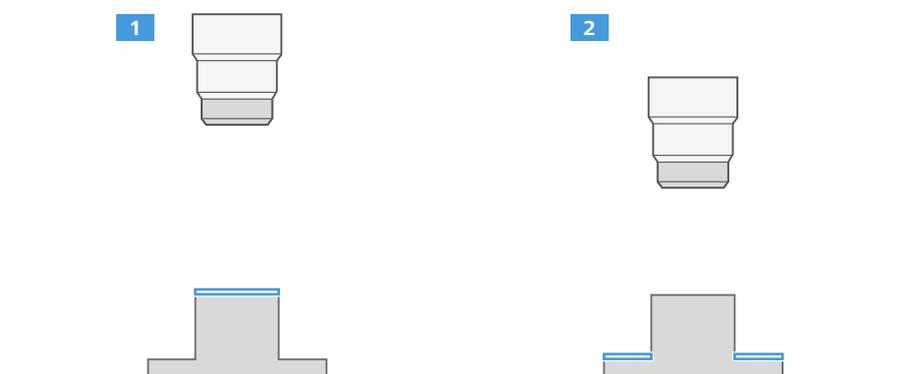
Image type	Description
Tiles	Acquires multiple images (tiles) with a <i>motorized</i> stage and stitches them together to create one large image This is only available with a motorized stage (x/y direction).
Panorama	Acquires multiple images (tiles) with a <i>manual</i> stage and stitches them together to create one large image
Linkam time series	Acquires a series of images at specific temperatures or intervals during temperature changes This is only available with a Linkam heated stage.

7.2.2.1 Extended Depth of Focus (EDF) Images

The depth of focus of the microscope optics is physically limited due to the high magnification. Therefore, objects cannot be imaged sharply over their entire physical height if their height exceeds the microscope's depth of focus.

An extended depth of focus (EDF) image helps to extend the physically limited depth of focus of the microscope:

First a Z-stack image is acquired, which consists of a sequence of images, each corresponding to a different focal plane. This is achieved by acquiring an image at various Z positions of the objective leading to a range of distances between objective and sample.



- 1 Upper objective position: upper part of sample in focus
- 2 Lower objective position: lower part of sample in focus

The in-focus regions of the individual images of the Z-stack image are then stitched together to a single EDF image. In the EDF image the entire acquired area of the sample surface is in focus, regardless of the height of the objects.

You can acquire an EDF image as follows:

- Motorized

If a motorized z-axis or focus drive is available you can define a range of Z positions and the EDF image is acquired automatically. ZEN 2 core moves the z-axis to each Z position, acquires an image at each position, and calculates the EDF image.

- Manually

If only a manual z-axis is available, you can move it to the desired positions and acquire an image at each position, thus obtaining the Z-stack image. ZEN 2 core then calculates the EDF image.

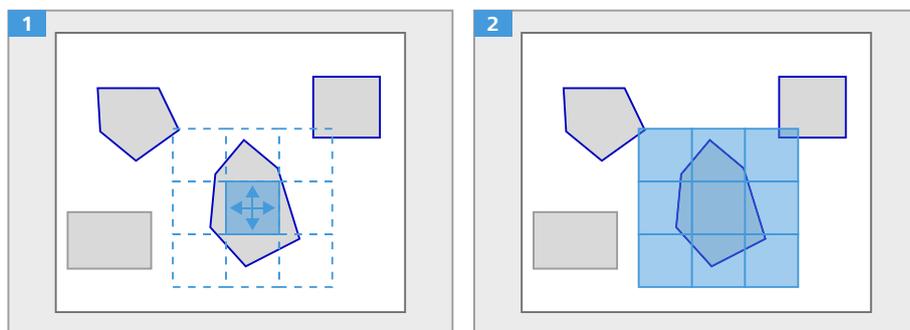
In addition to a simple EDF image, you can combine EDF with tile images.

7.2.2.2 Oversize Images (Tiles and Panorama)

Depending on your application, the field of view of your microscope might be too small for the sample area you wish to acquire. ZEN 2 core contains workbenches which enable you to acquire an oversized image exceeding the image size of a single image.

You can acquire an oversized image as follows:

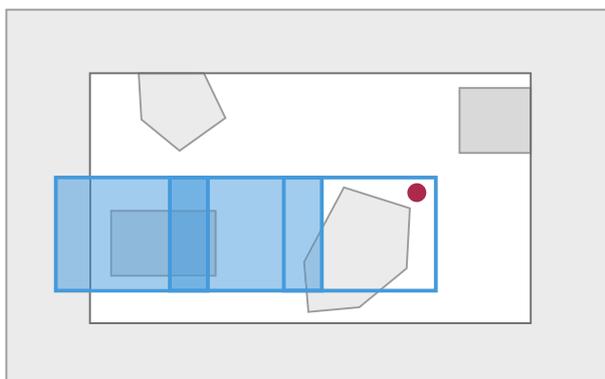
- Tiles



If a motorized stage is available, you can use one of the **Tiles** workbenches to define the area on the sample for which you wish to acquire an oversized image. The oversized image is then acquired and stitched together automatically.

For more information, see *Tiles (manual) Workbench* [▶ 178] or *Tiles (interactive) Workbench* [▶ 178]

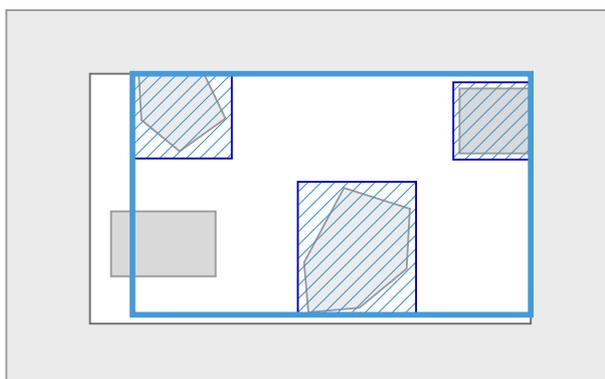
■ Panorama



If only a manual stage is available, you can acquire an oversized image with the **Panorama** workbench. In this case you acquire a set of connected, overlapping images (tiles) manually. The tiles are then stitched together to a single large image.

For more information, see *Panorama Workbench* [▶ 178].

■ Position list



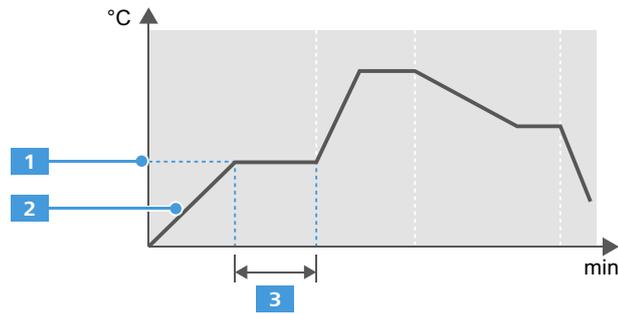
If a motorized stage is available and you wish to acquire images at different positions on the sample without acquiring the entire enclosed area, you can use the **Position List** workbench. The images are saved to a single file along with their positions. The space between the images remains empty.

For more information, see *Position List Workbench* [▶ 178].

7.2.2.3 Time Series and Temperature Series (Linkam)

If you have a temperature-controlled Linkam stage, ZEN 2 core enables you to acquire a series of images at different sample temperatures.

Within the limits of your hardware you can define an arbitrary temperature curve which is fed to the Linkam stage. A temperature curve consists of individual linear temperature ramps, which are connected one with another. Each ramp is defined by a heating or cooling rate and a target temperature, which can be sustained for a specified time interval before the next ramp starts.



- 1 Target temperature
- 2 Heating or cooling rate in °C/min
- 3 Time interval for which the temperature is sustained

For each ramp, you can acquire images as follows:

- Time series: the acquisition is triggered each time a defined time interval has elapsed.
- Temperature series: the acquisition is triggered each time the temperature has changed by a certain value.
- None: No images are acquired (for example until the heating stage has reached the start temperature)

7.2.3 Stage Movement

You can move the stage, and thus navigate the sample, as follows:

- By hand

For more information, see your microscope instruction manual.

- Using the software

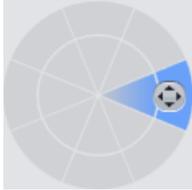
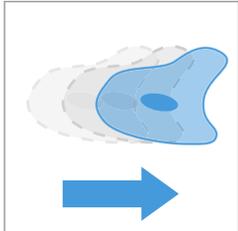
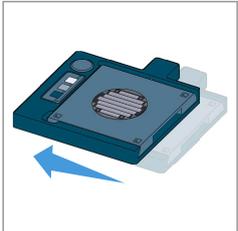
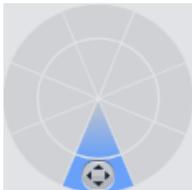
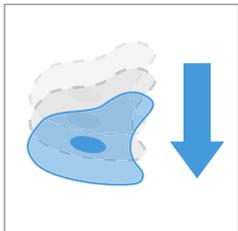
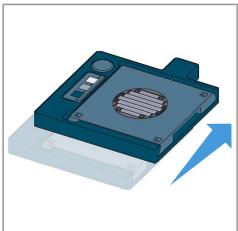
This is possible if your microscope has a motorized stage.

In the software, the **Stage Navigation** tool enables you to navigate the sample freely or jump to a specific location.

The direction of the **Navigation Circle** (software joystick) corresponds to the movement of the image, not to the actual stage movement.

For example, a movement in the positive X direction means that the area shown in the image moves to the right. As the optical components of the microscope, including the camera, are fixed, the stage actually moves to the left.

Examples:

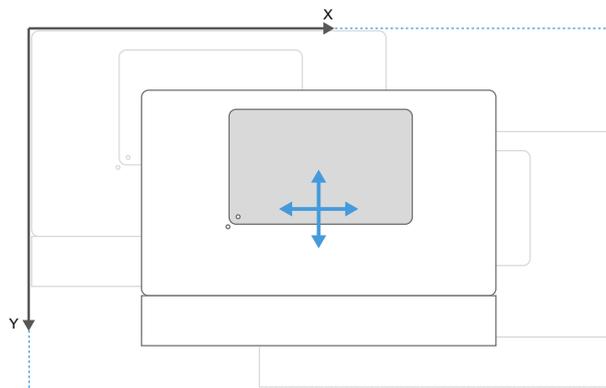
Direction	User action	Movement in camera image	Actual stage movement
+ X			
- Y			

7.2.4 Coordinate Systems

The software contains the following coordinate systems:

- Stage coordinate system
- Image coordinate system
- World coordinate system

Stage coordinates The stage coordinate system describes the position of the stage.



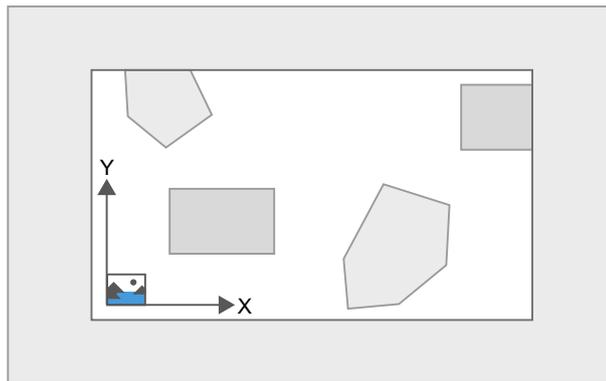
In ZEN 2 core the coordinate values are sometimes relative and sometimes absolute. Each time a stage navigation or focus tool is loaded, the software coordinate values are set to zero in X, Y, and Z direction for the current stage position, regardless of its actual physical position. When the stage is then moved the relative displacement is displayed.

To use absolute coordinates you need to reference the coordinate values first. Click the **Home/Calibrate** button of the corresponding tool. The stage is then moved to the end position and the coordinate value in the software is set to zero for this stage position. When the stage is then moved the absolute displacement is displayed.

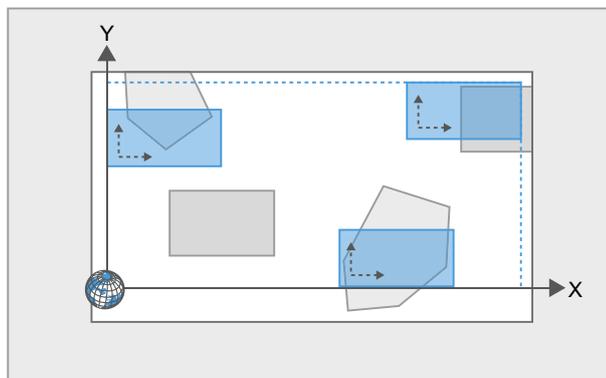
To move a motorized stage using one of the corresponding tools (e.g. **Focus** tool, **Stage Navigation** tool) you can either use the graphical elements, such as **Navigation Bar** and **Navigation Circle**, or you can enter the target coordinates directly.

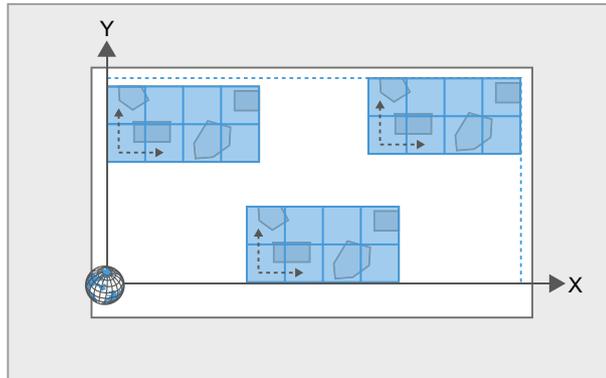
The direction in which the stage moves for positive or negative coordinate values depends on the microscope type and the microscope setup and cannot be described generally.

Image coordinates The image coordinate system describes the position of a pixel within an individual image. The coordinates are absolute, based on the top left pixel in the image. In an oversize image (e.g. a tiled image), each acquired image has its own independent coordinate system.



World coordinates The world coordinate system describes the position of a pixel within an image. In individual images, the world coordinates are identical to the image coordinates. However, for an oversize image (e.g. a tiled image), the world coordinate is based on the complete image.





7.3 Preparations

7.3.1 Navigating the Sample

The **Stage** tool enables you to move the motorized stage as follows:

- Freely using the **Navigation Circle** (software joystick)
- To a specific location by entering coordinates on the sample

CAUTION

Risk of Crushing Fingers

The drive of a microscope stage with a motorized horizontal stage axis (stage drive) is strong enough to crush fingers or objects between the stage and nearby objects (e.g. a wall).

- ◆ Remove your fingers or any objects from the danger area before moving the stage drive.
- ◆ Release the joystick immediately to stop the movement.

Adding the Stage Navigation tool To add the **Stage** tool to your acquisition workbench:

- Prerequisites**
- You are logged in as a supervisor.
 - An acquisition workbench is selected.
 - The selected acquisition workbench allows usage of the **Stage** tool.

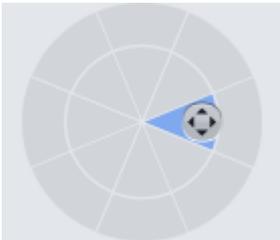
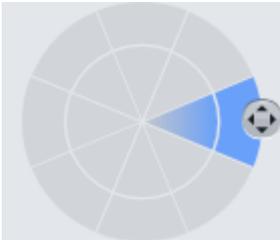
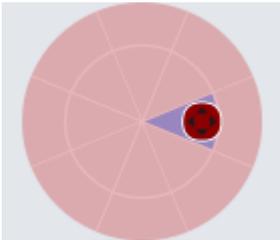
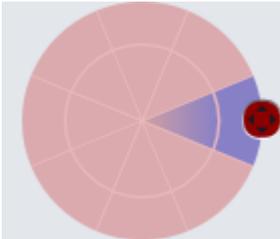
- Procedure**
- 1 Click the **Add Tool** button.
 - 2 In the tool overlay, select **Stage**.

INFO

The tile acquisition tools have an additional navigation control. For more information, see *Acquiring Tile Images* [▶ 69].

Free navigation To navigate the sample freely:

- Procedure**
- 1 Click the desired segment of the **Navigation Circle**.
You can switch between the two speed modes by right-clicking the central **Navigation Circle** button.
 - 2 Release the mouse button to stop the stage movement.

Software joystick	Movement speed
	Slow
	Medium
	Fast
	Very fast

TIP

You can also use the arrow keys to navigate the sample if the **Navigation Circle** is selected.

Specific location To jump to a specific location on the sample:

- Enter the target coordinates in the corresponding fields and press **Enter**.

INFO

You can stop the motion of the stage at any time by clicking the **Stop** button.

7.3.2 Focusing the Sample Manually

The **Focus** tool enables you to focus the sample by moving the stage up and down as follows:

- Freely using the **Navigation Bar**.
- To a specific Z coordinate.

This method is only possible, if your microscope has a motorized stage or a focus drive (inverted microscope). If no motorized stage is installed, you have to focus manually. For more information, see your microscope instruction manual.

 **CAUTION**

Risk of Crushing Fingers

The drive of a microscope stage with a motorized vertical axis (focus drive) is strong enough to crush fingers or objects between the stage and the microscope stand.

- ◆ Remove your fingers or any objects from the danger area before moving the focus drive.
- ◆ Release the joystick immediately to stop the movement.

Adding the Focus tool To add the **Focus** tool to your acquisition workbench:

- Prerequisites**
- You are logged in as a supervisor.
 - An acquisition workbench is selected.

- Procedure**
- 1 Click the **Add Tool** button.
 - 2 In the **Tool Overlay**, click **Focus tool**.

Free focusing To focus the sample freely:

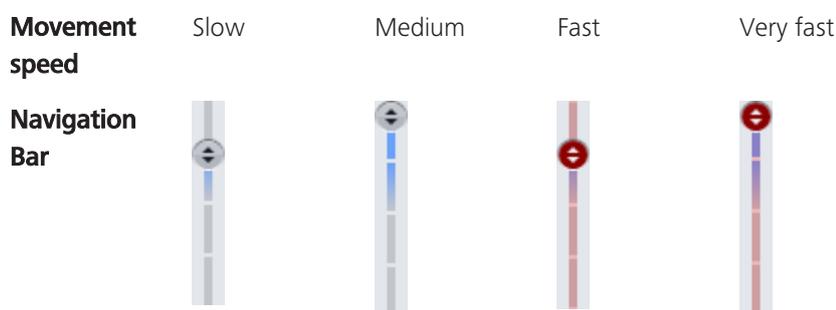
Prerequisites ■ The **Focus** tool is displayed.

Procedure 1 Click the desired **Navigation Bar** segment directly and keep the mouse button pressed.

Alternatively, you can click the **Navigation Bar** button and drag it to the desired segment.

You can switch between two speed modes by right-clicking the **Navigation Bar** button.

2 To stop the stage movement, release the mouse button or click the **Stop** button.



TIP

You can also use the arrow keys to focus the sample once you have clicked the **Navigation Bar**.

Specific stage position Initially, when you use the Focus tool, the exact position of the stage is not known. Therefore, the position indicated in **Current** is initially set to zero. If you enter a value, the stage moves by the entered amount relative to the current position.

If you want to move the focus to an absolute position, you must first click **Home** to move the stage to one of the end positions. The value of **Current** is set to this known position. You can then enter an absolute position.

INFO

You can stop the motion of the stage at any time by clicking the **Stop** button.

7.3.3 Focusing the Sample Automatically

If you have a motorized stage you can focus the sample automatically, i.e. the area of interest is kept in focus without your intervention.

The software supports the following types of automatic focus:

■ Software auto focus

The system acquires images over a range of Z heights and compares them to find the one where the area of interest is sharpest.

- Hardware auto focus

The system uses an integrated infrared camera sensor to judge the focus over a range of Z heights and to find the sharpest point.

The methods that are available to you depend on your hardware and license. It is possible to combine the methods, but the most recently selected method is used.

The auto focus methods can be enhanced by roughly focusing the sample before starting the auto focus.

**CAUTION****Risk of Crushing Fingers**

The drive of a microscope stage with a motorized vertical axis (focus drive) is strong enough to crush fingers or objects between the stage and the microscope stand.

- ◆ Remove your fingers or any objects from the danger area before moving the focus drive.
- ◆ Release the joystick immediately to stop the movement.

Adding the autofocus tools To add the **Software Autofocus** or **Hardware Autofocus** tool to your acquisition workbench:

- Prerequisites**
- You are logged in as a supervisor.
 - An acquisition workbench is selected.

- Procedure**
- 1 Click the **+ Add Tool** button.
 - 2 In the **Tool Overlay**, double-click **Hardware Autofocus** or **Software Autofocus**.

Software autofocus To focus the sample automatically using software auto focus:

- Prerequisites**
- The **Software Autofocus** tool is displayed.

- Procedure**
- 1 Navigate to the region of interest of the sample.
 - 2 Set the following parameters as desired:
 - 1 **Quality**
 - 2 **Range Coverage**
 - 3 **Sampling**
 - 4 **Sharpness Measure**
 - 3 If you wish to detect the focus in the range around the current focus position, click **Relative Range**.
Deactivate **Automatic Range** and use **Range** to specify the height above/below within which the system finds the best focus point

- 4 If you wish to detect the focus in a specific range, click **Fixed Range**.
Move to the start of the range and click **Set First** and the end of the range and click **Set Last**.
- 5 If only a section of the acquired image should be compared to find the focus, activate **Spot Meter / Focus ROI**.
Drag in the image to define the region of interest.
- 6 To set the focus, click **Find Focus**.
The software autofocus is a one-off procedure. If you move the sample, repeat the above steps.

Hardware autofocus To focus the sample automatically using hardware auto focus:

Prerequisites ■ The **Hardware Autofocus** tool is displayed.

Procedure 1 Navigate to the region of interest of the sample.

2 Set the desired **Resolution and Speed** and **Sample Texture**.

3 To set the focus, click **Once**.

4 If you require a continuous auto focus, click **On** and set the auto focus frequency using **Period**.

5 If the hardware consistently focuses at an incorrect height, activate **Handwheel on**, focus the sample manually, and click **Z-Pos -> AF-Pos**.

The system notes this offset and then keeps the correct height in focus even if you move the sample. This is useful if for example, the system focuses on the top surface of a transparent sample and you wish to focus on the bottom surface.

7.3.4 Selecting the Objective

You can change the magnification by changing the objective. The **Magnification** tool enables you to switch between objectives installed in your microscope.

Adding the Magnification tool To add the **Magnification** tool to your acquisition workbench:

Prerequisites ■ You are logged in as a supervisor.

■ An acquisition workbench is selected.

Procedure 1 Click the **+ Add Tool** button.

2 From the **Tool Overlay**, select **Magnification**.

Changing the objective To change the objective:

Prerequisites ■ The **Magnification** tool is selected.

Procedure 1 Click the objective icon.

A list of available objectives with their main properties is displayed.

2 Select the desired objective.

- Motorized objective revolver: The revolver is turned automatically to the corresponding position.
- Manual objective revolver: The revolver cannot be turned automatically. ZEN 2 core prompts you to turn it manually.
For more information, see your microscope instruction manual.
- Coded objective revolver: Do not select the objective in ZEN 2 core. Change the objective by turning the objective revolver manually. ZEN 2 core recognizes the selected objective automatically.

TIP

If you need to be sure the magnification used during acquisition agrees with the magnification information stored in the image metadata, use a motorized or a coded revolver.

7.3.5 Setting the Temperature and Vacuum

You can use the **Linkam Heating Stage** workbench to control the temperature and vacuum system of the Linkam heating stage, without acquiring a time series.

You can control the two systems independently of each other.

A typical use is to set a temperature or pressure that is used throughout an examination and which does not need to change over time.

Prerequisites ■ The **Linkam Heating Stage** workbench is selected.

- Procedure**
- 1 To use the temperature control: activate **Temperature control on** and set the target temperature in **Limit**.
 - 2 To use the pressure control: activate **Vacuum control on** and set the target pressure in **Setpoint Pressure**.
 - 3 Wait until the **Status** of the desired systems is **Holding**.

7.4 Acquiring Images

7.4.1 Acquiring a New Image

You can acquire an image using your microscope's camera. Depending on your microscope, you may have to perform focus and positioning operations manually or using the software.

Prerequisites ■ The sample is sufficiently illuminated

- Procedure**
- 1 Click the **Add workbench** button.
 - 2 In the left panel, click **Acquisition**.

- 3 In the center panel, select the desired acquisition workbench and click **Add**. The selected workbench with its default tools is displayed in the **Workbench Area**. A live image of the sample is displayed in the right tool area.
- 4 Focus the sample using one of the focus tools or focus manually.
- 5 If desired, add or remove tools from the workbench.
- 6 Adjust the parameters in the tools until you are satisfied with the result in the live image.
- 7 Click the **Snap** icon to acquire the image.
Free Examination mode: The image is added to the list of documents in the right panel.

Live mode You can switch back to a live image at any time by clicking the **Live** icon.

7.4.2 Acquiring an Extended Focus Image

You can acquire an EDF (Extended Depth of Focus) image using one of the EDF acquisition workbenches. Depending on your microscope you perform focus operations manually or using the software.

Acquiring an EDF image manually To acquire an EDF image manually:

- Prerequisites**
- The **EDF (manual focus)** workbench is selected.
 - The sample is sufficiently illuminated and in focus.

- Procedure**
- 1 Set up the camera using the **Camera** tool.
 - 2 Select the desired **Mode** in the **Manual Extended Depth of Focus** tool:
 - **Timer**: Enables you to specify the acquisition interval in seconds. Choose an interval that leaves you enough time to move the stage to the next position between two acquisitions.
 - **F12 Key**: Enables you to trigger the acquisition manually by pressing the *F12* key on your computer keyboard. This leaves you as much time as you need to move the stage between two acquisitions.
 - 3 Stereomicroscope: Choose the desired **Z-Stack Alignment** method.
 - 4 Start the acquisition by clicking the **Start** button at the top of the **Workbench Area**.
 - 5 Acquire your first image.
 - **Timer**: The image is acquired automatically after the specified **Interval**.
 - **F12 Key**: Press the *F12* key to acquire the image.
 - 6 Move the stage to the next Z-position.
For more information see your microscope instruction manual.
 - 7 Acquire the next image.

- 8 Continue moving the stage another step and acquiring an image over the desired focus range.
- 9 Finish the acquisition by clicking **Stop** in the **Workbench Area**.

For more information on how to move the stage manually, see your microscope instruction manual.

Acquiring an EDF image automatically To acquire an EDF image automatically:

- Prerequisites**
- The **EDF (motorized focus)** workbench is selected.
 - The sample is sufficiently illuminated and in focus.

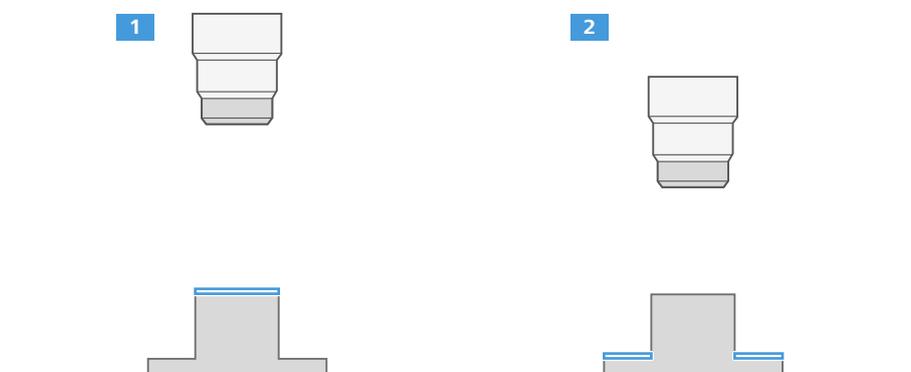
- Procedure**
- 1 Set up the camera using the **Camera** tool.
 - 2 Set up the focus range over which to acquire the EDF image in the **Motorized Extended Depth of Focus** tool.
 - Move the stage away from the sample until the top of the sample is no longer in focus. Define this focal plane position as the upper end of the range by clicking **Top > Assign**.
 - Move the stage towards the sample until the bottom of the sample is no longer in focus. Define this focal plane position as the lower end of the range by clicking **Bottom > Assign**.
 - 3 The step size is calculated automatically. If you want another step size, set the value for the **Step Size** to specify the distance the stage travels between two acquisitions.

The number of images to be acquired is displayed automatically as **Slices**.

Alternatively you can use **Optimal**: The optimal **Step Size** is determined automatically depending on your microscope setup.

- 4 Start the acquisition by clicking the **Start** button in the **Workbench Area**.

ZEN 2 core acquires the slices and calculates the EDF image automatically.



- 1 This position or slightly higher: **Top > Assign**
- 2 This position or slightly lower: **Bottom > Assign**

TIP

The algorithm used to calculate the EDF image works best if the acquired images differ significantly. Try to acquire as few images as possible. Make sure an acquired image contains focused areas which are not focused in the other images, i.e. each new image contains new information relevant for the EDF calculation.

7.4.3 Acquiring Tile Images

The tiles acquisition workbenches enable you to acquire an image of a large sample area. You define the area to be acquired and the system then acquires the corresponding tiles (images of neighboring sample areas) automatically and assemble them to a large image.

7.4.3.1 Acquiring a Tile Image

- Prerequisites**
- The **Tiles (manual)** or **Tiles (interactive)** workbench is selected.
 - The sample is sufficiently illuminated and in focus.
 - The microscope is equipped with a motorized stage.

- Procedure**
- 1 Set up the camera using the **Camera** tool.
 - 2 Select the objective in the **Magnification** tool.
 - 3 Define the region of which you wish to acquire the tile image using the **Tiles Setup (manual)** or **Tiles Setup (interactive)** tool and select a **Focus Correction**.

The **Center Screen Area** displays the area you wish to acquire and a preview of the tiles to be acquired, including overlap.

- 4 Set up the stitching method to be applied after acquisition using the **Tile Stitching** tool.
- 5 In the **Workbench Area**, click the **Start** button.

ZEN 2 core acquires the tiles and assembles the tile image automatically.

7.4.3.2 Acquiring a Tile Image with Extended Depth of Focus

The **Tiles with EDF (interactive)** workbench enables you to combine a tile image with extended depth of focus (EDF) acquisition. A Z-stack image is acquired for each tile.

- Prerequisites**
- The **Tiles with EDF (interactive)** workbench is selected.
 - The sample is sufficiently illuminated and in focus.
 - The microscope is equipped with a motorized stage.

- Procedure**
- 1 Set up the camera using the **Camera** tool.
 - 2 Select the desired objective in the **Magnification** tool.

- 3 Define the region of which you wish to acquire the tile image using the **Tiles Setup (interactive)** tool.
- 4 Set up the focus range and number of slices to be acquired for each tile in the **Motorized Extended Depth of Focus** tool.
- 5 Set up the stitching method to be applied after acquisition of using the **Tile Stitching** tool.
- 6 In the **Workbench Area**, click the **Start** button.

ZEN 2 core acquires the tiles with extended depth of focus and assembles the tile image automatically.

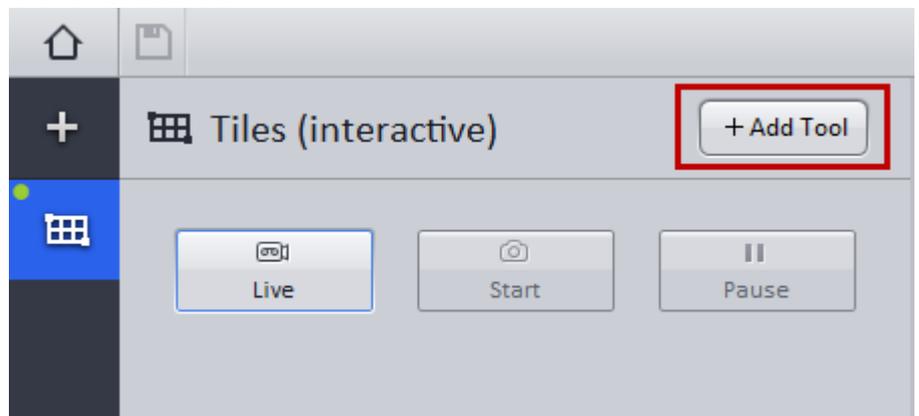
INFO

Each tile is acquired multiple times at different focus positions. Thus, combining tile acquisition with extended depth of focus increases the acquisition time considerably.

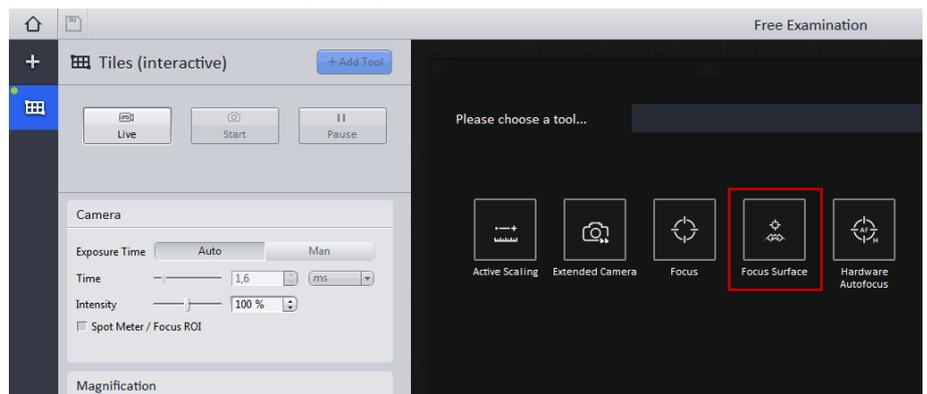
7.4.3.3 Using Focus Support Points for Tile Images

- Prerequisites**
- You have selected a tiles workbench e.g. **Tiles (interactive)**.
 - You have set up a tiles acquisition (e.g. 3x3 tiles).

- Procedure**
- 1 Click on **+ Add Tool**.

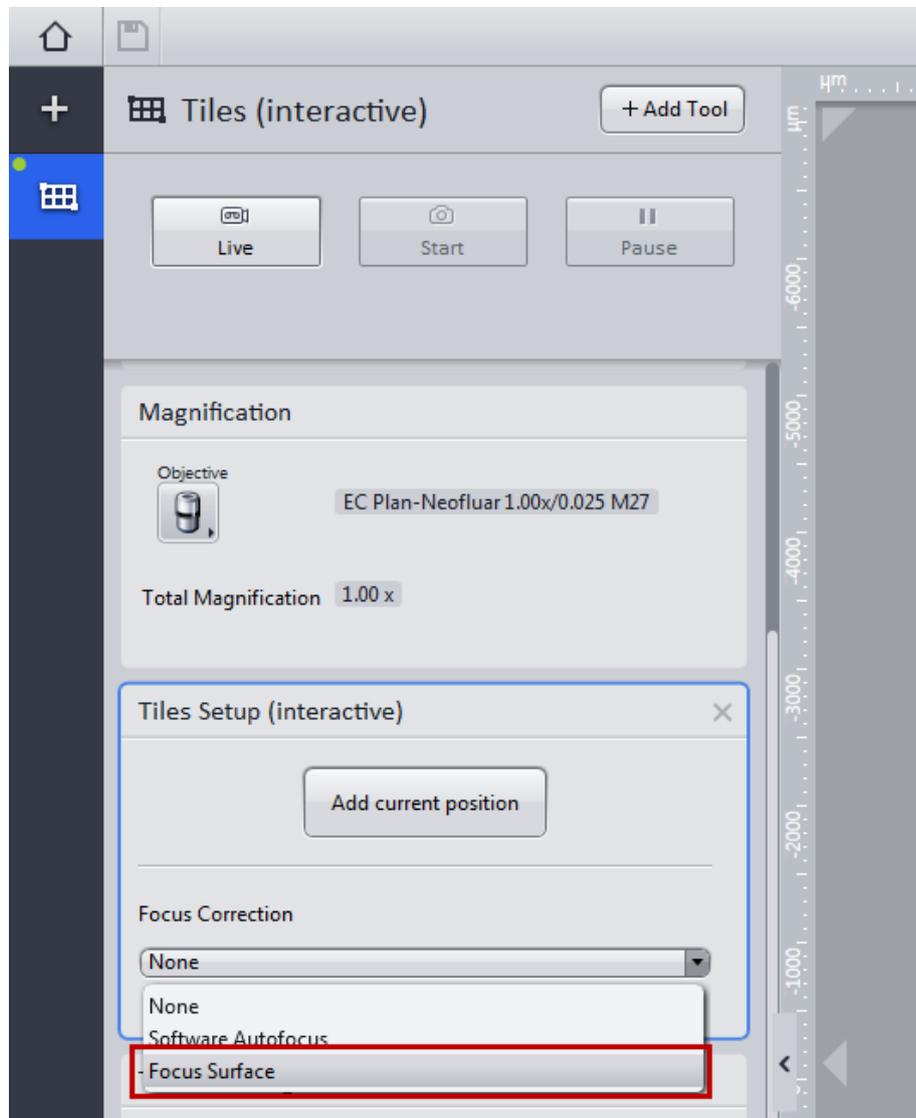


- 2 Double click on the **Focus Surface** tool.

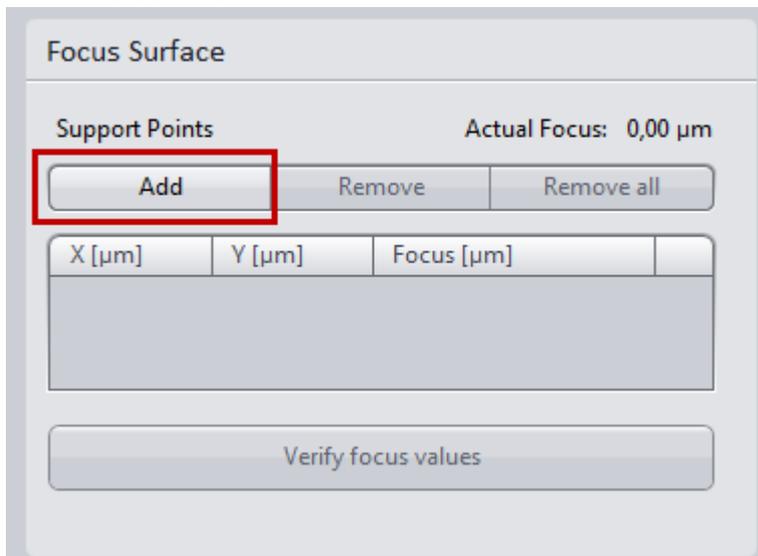


The tool will be added to the workbench.

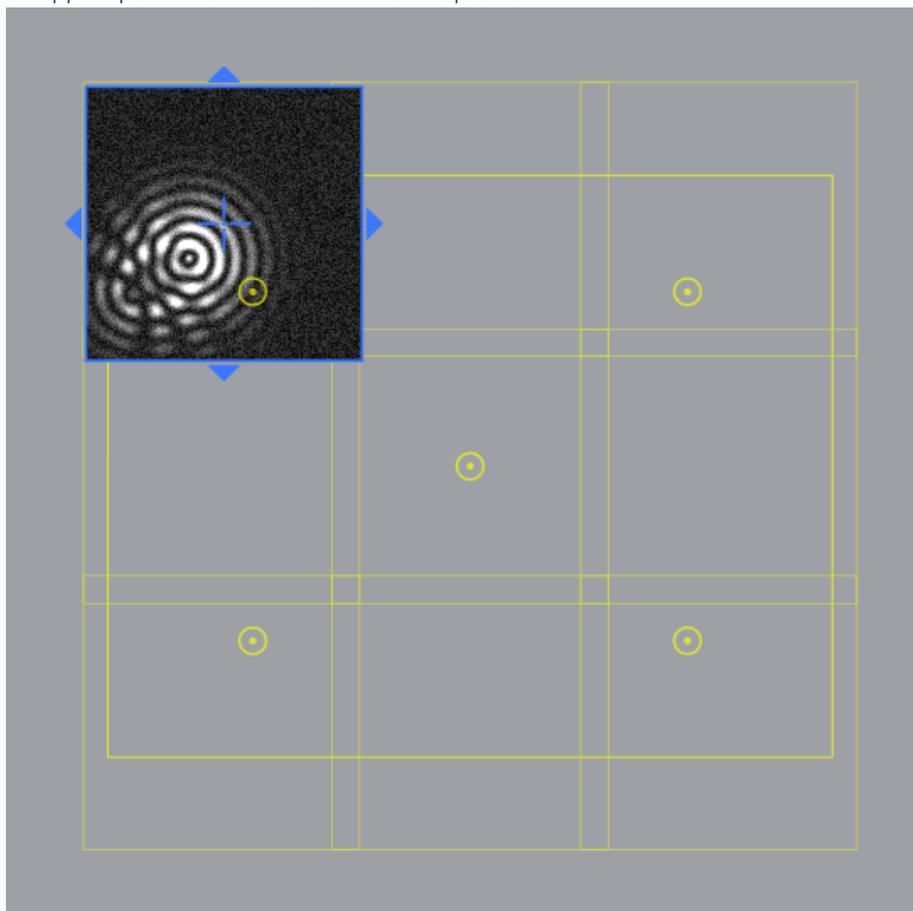
- 3 In the **Tiles Setup (interactive)** tool under **Focus Correction** select **Focus Surface**.



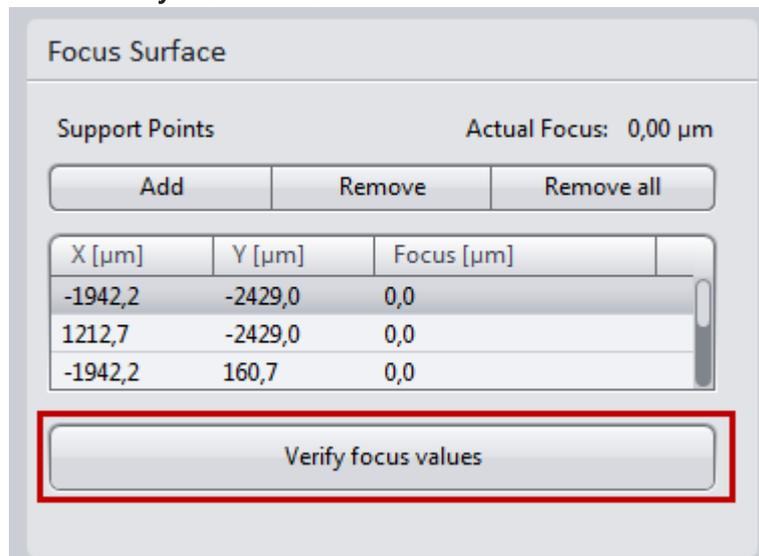
4 In the **Focus Surface** tool click on **Add**.



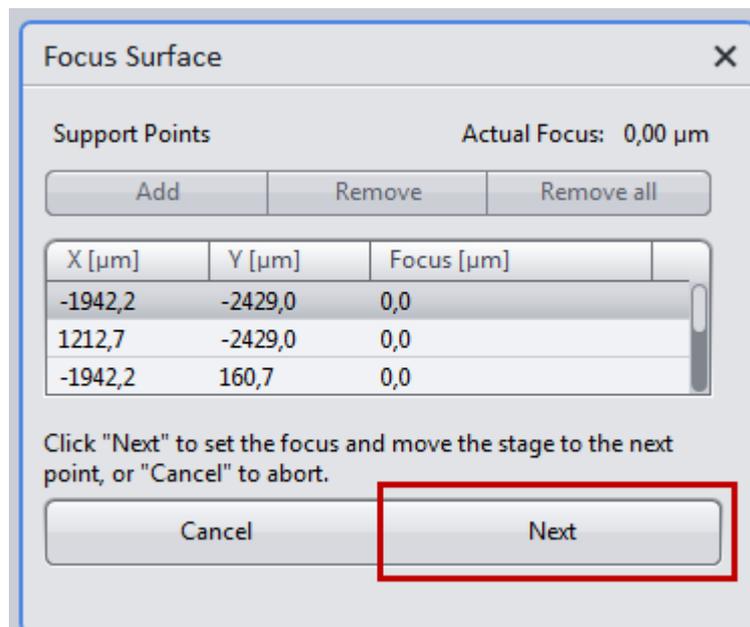
5 support point are added to the tiles acquisition.



- 5 Click on **Verify focus values**.



- 6 The stage moves to the first support point. Check if the image is in focus. If not, refocus the image.
- 7 Click on **Next**.



- 8 Repeat the last two steps until you have verified all support points.

The message **Verified successfully** appears in the Focus Surface tool. You can now continue and acquire your tiles image.

7.4.4 Acquiring a Panorama Image

The **Panorama** workbench enables you to acquire an image of a large sample area with a manual stage.

You acquire tiles (images of neighboring sample areas) and move the stage between two tile acquisitions manually.

Prerequisites ■ The **Panorama** workbench is selected.

■ The sample is sufficiently illuminated and in focus.

Procedure **1** Set up the camera using the **Camera** tool .

2 Select the desired objective in the **Magnification** tool.

3 Set up the stitching method to be applied after acquisition using the **Tile Stitching** tool.

4 Click the **Start** button in the **Workbench Area**.

The following elements appear in the **Center Screen Area**:

- the **Live Navigator**, a live preview image with tile overlay
- an acquisition tool to acquire tiles

5 Move the stage until the sample area of interest is visible in the **Live Navigator** image.

6 Click **Acquire Tile Image** in the acquisition tool in the **Center Screen Area**.

7 Double click one of the eight neighbor fields next to the acquired tile.

The **Live Navigator** is placed accordingly. The preview image displayed inside the **Live Navigator** is identical to the last acquired tile.

i INFO

If you click on the Live image, it gets semi transparent.

8 Match the **Live Navigator** image to the edge of the last acquired tile by moving the stage accordingly.

9 Acquire the tile by clicking **Acquire Tile Image** button.

Repeat the previous steps until you have acquired an image of the entire sample area of interest.

10 Click the **Stop** button in the **Workbench Area**.

The acquired image is complete. If you have selected stitching, ZEN 2 core finishes the acquisition by aligning the tile images along their edges.

7.4.5 Acquiring a Temperature Series Image

The **Linkam Acquisition** workbench enables you to conduct a temperature-dependent experiment and to acquire images at pre-defined temperatures or time intervals.

- Prerequisites**
- A temperature-controlled Linkam stage is installed in your microscope (for more details, read the Linkam product information).
 - The **Linkam Acquisition** workbench is selected.
 - The sample is sufficiently illuminated and in focus.
- Procedure**
- 1 Click  to add a temperature ramp.
 - 2 Specify the target temperature (**Limit**) and the change in temperature (**Rate**).
 - 3 If desired, specify a **Hold Time**.
This is the duration the temperature is maintained before the next ramp starts.
 - 4 In **Acquisition Type** specify how images are acquired during the ramp:
 - 1 **None**: No settings
 - 2 **Time**: Specify the time interval and the corresponding unit
 - 3 **Temperature**: Specify the temperature step in °C.
 - 5 Set up the camera for the selected temperature ramp using the **Camera** tool.
 - 6 Click  to add more temperature ramps in the table and modify them as required.
 - 7 Click **Start Linkam** in the **Workbench Area**.

For more information on editing the temperature curve, see *Linkam Heating Stage Acquisition Setup* [▶ 202].

The temperature curve is applied to the Linkam stage and the images are acquired as defined for the individual temperature ramps. In the **Center Screen Area** a **Linkam View** is displayed. The **Linkam View** shows the temperature curve diagram, which is extended for each image acquired.

After acquisition of the temperature series you can move the blue vertical line along the time axis of the temperature curve. The image acquired at the corresponding temperature is shown on the right.

7.4.6 Acquiring an Image Using Best Image

The **Best Image** workbench enables you to acquire 10 versions of the same image. Different predefined display options are applied to the individual images. After the acquisition you can select the image which suits you most.

- Prerequisites**
- The **Best Image** workbench is selected.
- Procedure**
- 1 Acquire the set of images by clicking the **Start** button in the **Workbench Area**.
 - 2 At the bottom of the **Center Screen Area**, select the most suitable image by left-clicking it.
 - 3 Click the **Select** button in the **Best Image** tool.

7.5 Optimizing the Acquisition

7.5.1 Defining a ROI

In the **Extended Camera** tool, you can define a subset of the camera sensor to be acquired, the region of interest (ROI). The rest of the camera sensor area is omitted, thus speeding up the acquisition.

Procedure 1 Enter the size of the ROI in pixels into the input fields **Size**.

The size of a pixel ($\mu\text{m} \times \mu\text{m}$) is displayed below the **Acquisition ROI** overview window and depends on the physical dimensions of the camera sensor and your **Binning** setting.

2 To position the ROI, enter the **Offset** in X and Y directions.

The **Offset** has its origin in the upper left corner and increases to the right and downwards.

Alternatively, you can resize and move the ROI (the blue frame in the overview window) using the mouse.

7.5.2 Specifying the Microscope Setup

The **Light Path** tool serves the following purposes:

- Indicates the current microscope setup at a glance
- Enables you to change hardware components if the corresponding component is motorized

The light path setup may affect the behavior of the workbenches and tools and is stored in the image metadata.

Prerequisites ■ The **Light Path** tool is selected.

- The component can be changed.
This is indicated by the small arrow symbol in the lower right corner of the icon.

Procedure 1 Click the component you wish to change.

2 Select the settings of the new component from the list.

- If the component is motorized, it is automatically moved into position on the microscope.
- If the component is not motorized, the component cannot be changed automatically. This is indicated by a hand symbol in the icon. ZEN 2 core prompts you to change it manually.

For more information on manually changing the component, see your microscope instruction manual.

If the component is coded, e.g. a coded objective revolver, you can change it manually without clicking in the **Light Path** tool. The **Light Path** tool is updated automatically. Using motorized microscopes same thing happens when using the TFT.

7.5.3 Working with Camera Presets

The **Extended Camera** tool contains a **Settings** section. This allows you to select a set of preset values. You can also import or export these presets to use them on another system.

You can perform the following actions with presets:

Action	Description	Procedure
Apply preset	The current parameter values are overwritten with those stored in the preset	■ Select the desired preset from the list
Restore <i>preset</i> values	The current parameter values are reset to those stored in the preset	■ Click the Reload button.
Restore <i>initial</i> value	The current parameter values are overwritten with the ZEISS default values	■ Click the Default button.
Save changes to the current preset	The parameter values in the preset are overwritten with those of the current tool	■  > Save
Save changes as a new preset	A new preset is created with the current parameter values	Procedure 1  > Save As 2 Enter the new name for the preset
Export a preset	The parameter values stored in the preset are saved in a file	Procedure 1  > Export 2 Specify the location in the file system.
Import a preset	A preset from the file system is added to the list of presets and the current parameter values are overwritten with those stored in the preset.	Procedure 1  > Import 2 Select the desired preset file from the file system.

Action	Description	Procedure
Delete a preset	<p>The currently selected preset is deleted.</p> <p>The next preset in the list is selected and the values from the preset applied. If the list is empty, the default values for the tool are applied.</p>	 > Delete

INFO

Modified presets are indicated by a * next to the name.

8 Image Processing

8.1 Overview of Image Processing

Once you have acquired an image you can optimize its appearance by applying various image processing functions. These enable you to enhance the regions of an image that you are interested in analyzing.

In **Create Job Template** mode you can specify which optimizations are available when the job is run. If you specify multiple image processing functions, they are applied in order from top to bottom in the **Task List**. For more information, see *Task Queue* [▶ 39].

INFO

- Some acquisition workbenches enable you to optimize the image as part of the acquisition. For more information, see Overview.
- ZEN 2 core also contains an interface with the ImageJ software. This standalone software contains many advanced processing possibilities.

8.2 Basics of Image Processing

8.2.1 Types of Image Processing Tools

The image processing tools enable you to enhance the image details you are most interested in by modifying the image after acquisition. The tools can be classified as follows:

■ Adjust

These tools enable you to adjust basic image settings like brightness, contrast or color settings.

■ Geometric

These tools enable you to apply basic image transformations such as flipping or rotating the image.

■ Sharpen

These tools enable you to make the image look sharper and to enhance details, e.g. contours or edges.

■ Smooth

These tools enable you to remove noise from the image by smoothing it. Most smoothing filters work in a similar way - possibly you have to try several filters to find the most suitable.

■ Utilities

These tools enable you to perform miscellaneous image operations, such as changing the color depth, splitting and combining color channels, or creating various test images.

8.2.2 Pixel Type

The pixel type specifies the amount of information stored in a grayscale or color image. This in turn is specified by the following:

- Number of channels
- Range of pixel values per channel
- Number format

Number of channels The number of channels specifies the number of colors supported by the image.

- 1 channel
 - Grayscale image
 - No color information
 - The pixel values of the channel specify relative shades of gray.
- 3 channels
 - Color image
 - One channel each for red, green and blue
 - The pixel values of each channel specify the relative shades of the corresponding color.

Range of pixel values per channel The range of pixel values per channel corresponds to the number of shades that can be distinguished in the channel. The larger the range, the more shades are distinguished, leading to a higher image quality.

This is also referred to as the bit depth or color depth.

Number format Specifies how pixel values are stored digitally. Typically integer values are used.

For processed images, where processing tools may result in non-integer values, real and complex number formats are available. However, when an image is displayed, the pixel values are always mapped to integer values. Therefore, real and complex values are only used internally and relevant if it is important to maintain the entire image information, for example if you wish to apply further processing tools at a later point in time.

- Real pixel values (Float) increase the precision of image calculations such as the ratio of two images.
- Complex pixel values (Complex) usually result from a transformation of the image into the Fourier space.
- Complex pixel values are stored with their real and imaginary part.

Supported pixel types ZEN 2 core supports the following pixel types.

Pixel Type	Number of channels	Range of pixel values per channel	Number format
8 Bit B/W	1 (gray value)	0 ... 255	Integer
16 Bit B/W		0 ... 65,535	Integer
32 Bit B/W Float		0 ... 2×10^{23}	Real values
2x32 Bit Complex		<ul style="list-style-type: none"> ■ Real: 0 ... 2×10^{23} ■ Complex: 0 ... 2×10^{23} 	Real and complex values
24 Bit RGB	3 (color)	0 ... 255	Integer
48 Bit RGB		0 ... 65,535	Integer
3x32 Bit RGB Float		0 ... 2×10^{23}	Real values
3x64Bit RGB Complex		<ul style="list-style-type: none"> ■ Real: 0 ... 2×10^{23} ■ Complex: 0 ... 2×10^{23} 	Real and complex values

8.3 Applying an Image Optimization

ZEN 2 core SP1 contains various image processing tools you can apply to your images. To apply an image processing tool, you must select the **Image Processing** workbench and add the desired tool (if not already available).

Adding an image processing tool To add an image processing tool:

- Prerequisites**
- You are logged in as a supervisor.
 - The **Image Processing** workbench is selected.

Procedure 1 Open the **Tool Overlay** by clicking the **+ Add Tool** button in the **Workbench Area**.

2 Double-click the desired tool in the **Tool Overlay**.

The categories help you find the tool you need for your job.

The tool is added to the **Image Processing** workbench. You can now adjust it as required for the job, add more tools, or remove tools you do not need.

If you are working in **Create Job Template** mode and a branch in the **Task List** contains multiple processing workbenches, they are all applied, from left to right. The output of a processing workbench provides the input for subsequent processing workbenches.

The same holds for the tools within one processing workbench: the processing tools are applied from top to bottom and the output of a processing tool provides the input for the subsequent processing tool.

8.4 ImageJ

8.4.1 Basics of the ImageJ Extension

The ImageJ Extension integrates the ImageJ software into ZEN 2 core. This enables you to make use of ImageJ's image processing capabilities from within your workflows.

You can send images to ImageJ and retrieve them back into ZEN 2 core with a single click each.

INFO

The **Single Instance Listener**, a feature of ImageJ, is crucial for the ImageJ Extension to work. The **Single Instance Listener** does not work for the latest ImageJ 2 versions. We recommend to use one of the Fiji Life-Line versions you can find on the Fiji homepage for which the **Single Instance Listener** does work.

8.4.2 Activating the ImageJ Extension

You activate the ImageJ extension via the **Extensions Manager** and then configure it in the **Options** dialog.

Configuring ZEN 2 core

Prerequisites

- ImageJ is installed on your system.
The Life-Line version of the Fiji distribution is recommended, which contains all necessary ImageJ plugins and works with ZEN 2 core.
- You are logged in as an administrator.
- The **Home Screen** is displayed.

Procedure

- 1 Activate the **ImageJ Extension** in the **Extensions Manager**:
Maintenance > Extensions Manager
- 2 Confirm the configuration by clicking the **Apply** button.
If you activate ImageJ for the first time, a "missing path" warning is displayed. Confirm it by clicking **OK**.
- 3 Open the **Options** window:
Maintenance > Options
- 4 Select the **ImageJ** tab.
- 5 Select the path to the ImageJ executable from the drop-down list:

- Select one of the paths suggested by ZEN 2 core or:
- Click on  to specify a different path.

Configuring ImageJ To configure how ImageJ interacts with ZEN 2 core:

Prerequisites ■ ImageJ is installed on your system.
The Life-Line version of the Fiji distribution is recommended, which contains all necessary ImageJ plugins and works with ZEN 2 core.

- Procedure** 1 Open the ImageJ installation specified in the ZEN 2 core options.
- 2 Activate the **Single Instance Listener**:
You find the **Single Instance Listener** under **Edit > Options > Misc...**

8.4.3 Installing and Preparing ImageJ

The ImageJ extension of ZEN 2 core requires ImageJ to be installed and additionally to be configured correctly.

To prepare ImageJ for the use in ZEN 2 core, perform the following steps:

- Procedure** 1 Create a folder which can be fully accessed without Windows administrator rights.
- 2 Install ImageJ in the created folder. Check for ImageJ updates after installation. We recommend to use the Fiji distribution, which includes all required plugins. If you use Fiji, you can skip the next two steps.
- 3 Download the OME Bio-Formats library (LOCI Plugins for ImageJ).
- 4 Copy the downloaded file loci_tools.jar to the ImageJ\plugins folder.
- 5 Make sure that **Run single instance listener** is activated in ImageJ.
You find it under the **Edit > Options > Misc...**

ImageJ is now correctly installed and prepared to work with ZEN 2 core. If you have several ImageJ installations on your computer, make sure to direct ZEN 2 core to the above installation using the **General Options** dialog.

8.4.4 Adding the ImageJ Workbench

The ImageJ workbench enables you to connect ZEN 2 core with ImageJ. The communication between the two programs is bidirectional. You can use ImageJ to complement the image processing tools available in ZEN 2 core.

Prerequisites ■ ImageJ is installed on your system and configured for ZEN 2 core.
■ The **ImageJ Extension** is activated in ZEN 2 core and configured.
■ You are logged in as a supervisor.

- Procedure** 1 Add the **ImageJ Connection** workbench:
+ Add Task > Utilities > ImageJ Connection > + Add

- 2 Use the **Send Image** and **Retrieve Image** buttons to send the image to ImageJ and to retrieve it back into ZEN 2 core after processing it with ImageJ.

8.4.5 Exchanging Images between ZEN 2 core and ImageJ

The **ImageJ** tool enables you to send images to ImageJ or to retrieve images from ImageJ, e.g. after processing them with ImageJ.

Sending or Retrieving Images

Sending an image to ImageJ To send an image to ImageJ:

- Prerequisites**
- The **ImageJ** tool is selected.
 - An image is displayed.

- Procedure**
- 1 Send the image to ImageJ by clicking the **Send Image** button.
The image is sent to ImageJ. Any modifications (e.g. processing or measurements) are applied before it is sent.
 - 2 Follow the instructions in ImageJ.
Depending on the image data, ImageJ shows an import dialog. For more information, see the ImageJ instruction manual.

Retrieving an image from ImageJ To retrieve an image from ImageJ:

- Prerequisites**
- The **ImageJ** tool is selected.

- Procedure**
- 1 Open ImageJ and prepare the image you wish to retrieve.
 - 2 In ZEN 2 core, click the **Retrieve Image** button in the **ImageJ** tool.

Image Type Conventions

ImageJ does not support all file types and pixel types that can be used in ZEN 2 core.

In these cases the image must be converted in ZEN 2 core before sending it to ImageJ.

See the tables below for further details.

ZEN 2 core to ImageJ

ZEN 2 core	ImageJ	Comments
.tif, .jpg, .bmp, .png, .gif	Original	The image is imported unchanged.
.ome.tif	Original	The image is imported unchanged.
.czi 2D 8/16 Bit B/W	32 Bit RGB	You can change the pixel type back to B/W in ImageJ using the Image > Type command.

ZEN 2 core	ImageJ	Comments
.czi 2D 12 Bit B/W	Not supported	Convert the image to 16 bit B/W using the Change Pixel Type tool before sending it to ImageJ.
.czi 24/48 Bit RGB	32 Bit RGB	
.czi 36/42 Bit RGB	Not supported	Convert the image to 24/48 bit RGB using the Change Pixel Type tool before sending it to ImageJ.
<ul style="list-style-type: none"> ■ Multi-channel ■ Z stack ■ Temperature /Time series 	MD image	If necessary, reassign the dimensions in ImageJ using Image > Hyperstacks for example.
Tiled image	Partially supported	Only the first tile is imported.

ImageJ to ZEN 2 core

ImageJ	ZEN 2 core	Comments
.tif, .jpg, .bmp, .png, .gif	Original	The image is imported unchanged.
.ome.tif	Original	The image is imported unchanged.
2D images, B/W or RGB	.tif, B/W or RGB	Any 2D image other than the types listed above is received as a .tif-image.
<ul style="list-style-type: none"> ■ Multi-channel ■ Z stack ■ Temperature /Time series 	MD image	All channels or layers are imported into a corresponding multidimensional image.
Tiled image	Partially supported	Only the first tile is imported.

9 Image Analysis

9.1 Overview of Image Analysis

Once you have acquired an image you can analyze its properties by performing various types of measurements. The measurement results can then be added to a report.

In **Create Job Template** mode you can specify which measurement tools are available when the job is run as well as tolerances (upper and lower limits for the measurement value).

9.2 Basics of Image Analysis

9.2.1 Types of Analysis Tools

ZEN 2 core contains the following types of analysis tools:

- Interactive Measurements

These enable you to measure distances, angles, area, and intensities of pixels.

- Automatic Measurements

These enable you to analyze simple shapes based on their gray values.

For example, you can automatically count and classify particles in the sample according to their size or color.

9.3 Interactive Measurements

9.3.1 Types of Interactive Measurements

Interactive measurements enable you to measure the properties of a sample, for example, angles, area, and intensities of pixels. The tools can be classified as follows (the available tools depend on your hardware setup and licenses):

- Annotations

Enable you to add text labels to an image, mark objects of interest, or determine the coordinates of a point in the standard or relative coordinate system.

- Area Tools

The majority of Area Tools enable you to calculate the area (in pixels) enclosed by various shapes. Furthermore, the mean intensity of the enclosed pixels is also calculated. Some Area Tools are used to calculate the length of curves, such as the Spline Curve tool.

- Measurements

Enable you to measure angles and distances between lines and points.

For more information on an individual tool, see the tool reference.

9.3.2 Using Interactive Measurement Tools

Interactive measurement tools enable you to measure distances, angles, areas, and intensities of pixels. In the **Favorites** section you can arrange your favorite tools by simply dragging them from the tools selection to the favorites bar.

Prerequisites ■ An image is displayed.

- The **Interactive Measurement** workbench is selected.

Procedure 1 Select the desired measurement tool from the tool selection.

2 Alternatively select a tool from the **Favorites** bar.

If it is not visible, click **+ Add Tool** and double click on the desired tool.

Note that only parameters of the active tool are displayed in the workbench area.

3 Click to place the measurement tool in the image.

For more information about how to use each tool, see *Interactive Measurements Workbench* [▶ 180].

4 To add more measurements, repeat the above steps.

The selected tool remains active until you press *Esc* or close the window **Apply selected tool (Esc to abort)**.

If desired you can modify the measurement as follows:

- Sorting interactive measurements

- Editing interactive measurements

- Adding an annotation to interactive measurements

- Repeating / correcting an interactive measurement

9.3.3 Layering Interactive Measurements

Each measurement is stored in an imaginary layer. By default, the first measurement is the bottom layer and the most recent measurement is the top layer. You can change the order of the layers, for example if two measurements overlap and one is obscuring the other.

To move a measurement up or down a layer:

Procedure 1 Right-click the measurement.

2 Select how you want to move the measurement:

- **Bring Forwards:** up one layer

- **Send Backwards:** down one layer

- **Bring to Front:** to top
- **Send to Back:** to bottom

9.3.4 Adding an Annotation to Interactive Measurements

You can add an annotation to an interactive measurement, for example to label an area of the sample.

Adding an annotation To add an annotation:

Prerequisites ■ At least one measurement is visible in the **Center Screen Area**.

- Procedure**
- 1** Double-click the measurement in the **Center Screen Area**.
 - 2** Enter the desired text.
To add an extra line of text press the *ENTER* key.
 - 3** Click outside the measurement.

INFO

You can change the formatting of the annotation in the same way as you change the appearance of the measurement result.

- You cannot apply different text formatting to the annotation and the measurement result.
- You cannot apply different formatting to individual words or characters of the annotation.

Removing an annotation To remove an annotation:

Prerequisites ■ At least one measurement is visible in the **Center Screen Area**.

- Procedure**
- 1** Double click the desired measurement.
 - 2** Delete all the text in the annotation.
 - 3** Click outside the measurement.

9.3.5 Editing Interactive Measurements

You can change the following properties of an interactive measurement:

- Size and position of the entire measurement (measurement result and individual nodes)
- Position of measurement result
- Number and position of individual nodes

INFO

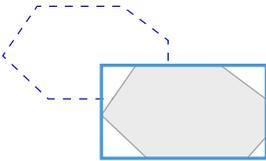
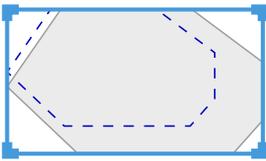
For some tools you can change their orientation (rotation or placement of the measurement arcs).

For more information, refer to the help topic for the corresponding tool.

Entire measurement To change the properties of the entire measurement:

Prerequisites ■ At least one measurement is visible in the **Center Screen Area**.

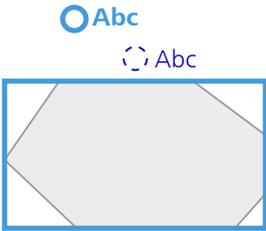
Procedure ◆ Click the desired measurement and perform one of the following actions:

Graphic	Aim	Action
	Move measurement	Click within the bounding box and drag
	Resize freely	Drag the sides of the bounding box
	Resize (proportional)	Press <i>CTRL</i> and drag the corners of the bounding box

Measurement result To change the properties of the measurement result:

Prerequisites ■ At least one measurement is visible in the **Center Screen Area**.

Procedure ◆ Click the desired measurement and perform the following action:

Graphic	Aim	Action
	Move measurement result	Drag the node of the measurement result

Annotation position To alter the position of an annotation:

Prerequisites ■ At least one measurement is visible in the **Center Screen Area**.

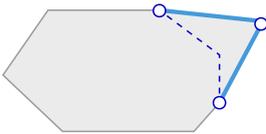
- Procedure**
- 1 Click the desired measurement.
 - 2 Place the cursor over the annotation node. The cursor changes to a hand icon.
 - 3 Drag the node to the new location.

Individual nodes To change the properties of individual measurement nodes:

Prerequisites ■ At least one measurement is visible in the **Center Screen Area**.

■ The measurement was created by defining multiple nodes.

- Procedure**
- 1 Right-click the desired measurement.
 - 2 Click **Edit Points**.
 - 3 Perform one of the following actions:

Graphic	Aim	Action
	Move node	Drag node
	Add node	Click between two nodes
	Remove node	Press <i>CTRL</i> and click node

9.3.6 Hiding an Interactive Measurement

Normally, when a job is run, interactive measurements are automatically placed on the image at the location specified in **Create Job Template** mode. However, it is also possible to create an interactive measurement task where the image has no measurements pre-placed. In this case, ensure that the operator knows where to perform the measurement (e.g. using a suitable workbench description).

- Procedure**
- 1 Click the desired measurement.
 - 2 Delete the measurement using the *DEL* key on your keyboard.

The tool remains in the **Workbench Area** but the measurement is no longer displayed in the image. The operator running the job later can create the measurement freely instead of modifying a pre-placed measurement.

TIP

You can also use this feature to correct a faulty measurement while working in **Create Job Template** mode:

- Delete the measurement from the image and click the **Redraw** button in the tool to repeat the measurement correctly.

9.4 Automatic Measurements

9.4.1 Basics of Automatic Measurements

Automatic measurements enable you to analyze simple shapes based on their gray values. For example, you can automatically count and classify particles in the sample according to their size or color.

Setting up an automatic measurement consists of the following steps:

- 1 Classifying measurements
- 2 Specifying the region of an image to be analyzed
- 3 Configuring object detection
- 4 Automatically correcting object detection
- 5 Manually correcting object detection
- 6 Defining values to be measured
- 7 Previewing measurements

You are guided through these steps using the **Image Analysis Wizard**.

Automatic measurements can be run as follows:

- Interactively
- Automatically

When run interactively, the user can adjust the settings in each of steps 2-5 if enabled in the measurement definition. Since this mode requires a good knowledge of the sample and the software, it is typically used by supervisors in **Free Examination** mode.

When run automatically, the user cannot adjust any settings. The interaction is limited to acquiring or selecting the image and saving the measurement results. This is typical for inclusion in a job template. In this case it is common to assign the automatic measurement to a macro and insert the macro into the template rather than the automatic measurement itself.

9.4.1.1 Analysis Tab

The **Analysis** tab is displayed at the bottom of each step of the **Image Analysis Wizard**.

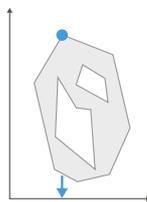
It enables you to adjust the following global settings:

Parameter	Description
Show Objects	Shows/hides the detected objects in the image.
Show All Classes	<ul style="list-style-type: none"> ■ Activated: Displays the detected objects from all classes ■ Deactivated: Displays the detected objects from the selected class and subclasses.
Fill	<ul style="list-style-type: none"> ■ Activated: Displays the detected objects as a filled shape ■ Deactivated: Displays the contour of detected objects
Opacity	Adjusts the transparency of detected objects This only has an affect if Show Objects is activated.
	Deletes all detected objects from the image
Create Tables	Displays the measurement values for the individual objects and the summation classes.

9.4.1.2 Measurement Features

The software can automatically detect and measure the following properties of objects:

ACP X
Unscaled

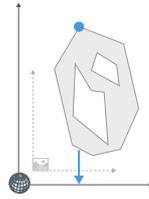


The x coordinate of the first pixel in the first line of a region

To identify measurement objects, the image is scanned from top left to bottom right. The so-called ACP (anti-coincidence point) is the first point that has been identified for a new object. The parameter **Acp X** indicates the x-coordinate of this point.

- Unit: pixels
- Value range: 1 ... image size in x-direction

ACP X
Unscaled
WCS

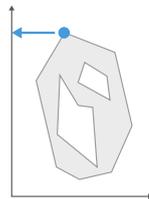


The x coordinate of the first pixel in the first line of a region

To identify measurement objects, the image is scanned from top left to bottom right. The so-called ACP (anti-coincidence point) is the first point that has been identified for a new object. The parameter **Acp X** indicates the x-coordinate of this point in the world coordinate system (WCS).

- Unit: pixels
- Value range: 1 ... image size in x-direction

ACP Y
Unscaled

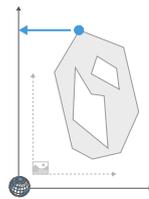


The y coordinate of the first pixel in the first line of a region

To identify measurement objects, the image is scanned from top left to bottom right. The so-called ACP (anti-coincidence point) is the first point that has been identified for a new object. The parameter **Acp Y** indicates the y-coordinate of this point.

- Unit: pixels
- Value range: 1 ... image size in y-direction

ACP Y
Unscaled
WCS

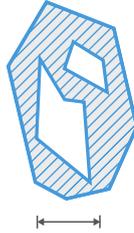


The y coordinate of the first pixel in the first line of a region

To identify measurement objects, the image is scanned from top left to bottom right. The so-called ACP (anti-coincidence point) is the first point that has been identified for a new object. The parameter **Acp Y** indicates the y-coordinate of this point in the world coordinate system (WCS).

- Unit: pixels
- Value range: 1 ... world coordinate size in y-direction

Area



Area of a region

Area of a region excluding any holes it may contain. The areas of the holes are not included in the measurement. If you want to include them, use the **Area filled** parameter.

■ Unit: Unit of area of the scaling assigned to the image (e.g. μm^2)

Area
Convex

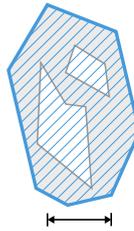


Area of convex hull of a region

The current region is surrounded by a convex polyline. The (filled!) area of the resulting region is then measured.

■ Unit: Unit of area of the scaling assigned to the image (e.g. μm^2)

Area
Filled

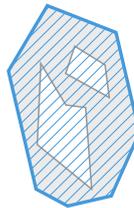


Area of filled region

Area of a region including any holes it contains. The holes are interpreted as belonging to the region or are filled prior to the measurement. If you do not want the holes to be measured, use the **Area** parameter.

■ Unit: Unit of area of the scaling assigned to the image (e.g. μm^2)

Area
Filled
Unscaled

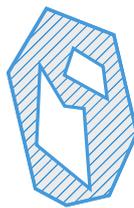


Area of filled region

Area of a region including any holes it contains. The holes are interpreted as belonging to the region or are filled prior to the measurement. If you do not want the holes to be measured, use the **Area** parameter.

■ Unit: pixels²

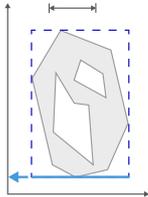
Area
Unscaled



Area of a region unscaled

The **Area unscaled** parameter corresponds to the **Area** parameter. However, the scaling of the image is not taken into account for the measurement. The (unfilled!) area of a region is displayed in pixels in each case.

Bound Bottom

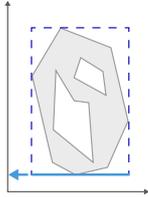


- Unit: pixels²

Minimum y-coordinate of the bounding box of a region

Indicates the y-coordinate of the bottom edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.

Bound Bottom Unscaled

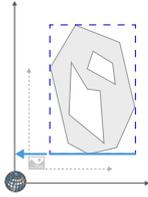


- Unit: Unit of the scaling assigned to the image (e.g. μm)

Minimum y-coordinate of the bounding box of a region

Indicates the y-coordinate of the bottom edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.

Bound Bottom Unscaled WCS

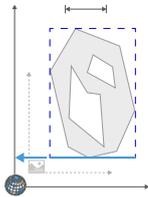


- Unit: pixels

Minimum y-coordinate of the bounding box of a region

Indicates the y-coordinate in the world coordinate system (WCS) of the bottom edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.

Bound Bottom WCS

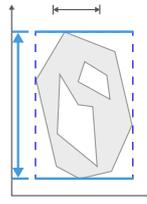


- Unit: Unit of the scaling assigned to the image (e.g. μm)

Minimum y-coordinate of the bounding box of a region

Indicates the y-coordinate in the world coordinate system (WCS) of the bottom edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.

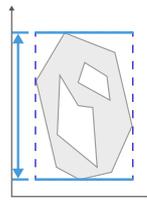
Bound Height



Indicates the height (size in y-direction) of a bounding box for a region. The box is drawn in parallel to the x and y axis.

- Unit: Unit of the scaling assigned to the image (e.g. μm)
- Formula: Bound top - Bound bottom

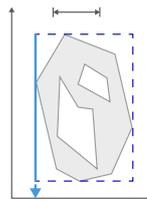
Bound Height Unscaled



Indicates the height (size in y-direction) of a bounding box for a region. The box is drawn in parallel to the x and y axis.

- Unit: pixels
- Formula: Bound top - Bound bottom

Bound Left

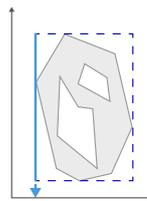


Minimum x-coordinate of the bounding box of a region

Indicates the x coordinate of the left-hand edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.

- Unit: Unit of the scaling assigned to the image (e.g. μm)

Bound Left Unscaled

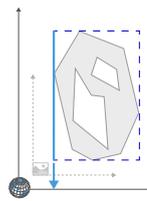


Minimum x-coordinate of the bounding box of a region

Indicates the x coordinate of the left-hand edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.

- Unit: pixels

Bound Left Unscaled WCS

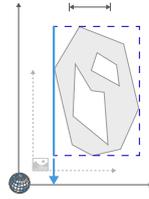


Minimum x-coordinate of the bounding box of a region

Indicates the x coordinate in the world coordinate system (WCS) of the left-hand edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.

- Unit: pixels

Bound
Left
WCS

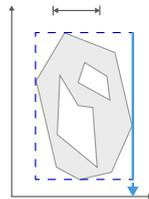


Minimum x-coordinate of the bounding box of a region

Indicates the x coordinate in the world coordinate system (WCS) of the left-hand edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.

■ Unit: Unit of the scaling assigned to the image (e.g. μm)

Bound
Right

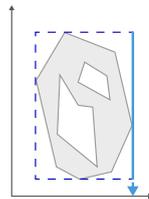


Maximum x-coordinate of the bounding box of a region

Indicates the x coordinate of the right-hand edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.

■ Unit: Unit of the scaling assigned to the image (e.g. μm)

Bound
Right
Unscaled

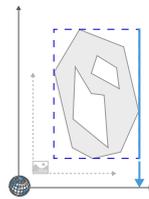


Maximum x-coordinate of the bounding box of a region

Indicates the x coordinate of the right-hand edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.

■ Unit: pixels

Bound
Right
Unscaled
WCS

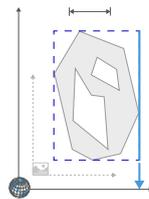


Maximum x-coordinate of the bounding box of a region

Indicates the x coordinate in the world coordinate system (WCS) of the right-hand edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.

■ Unit: pixels

Bound
Right
WCS

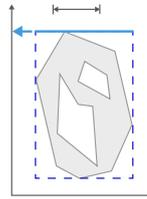


Maximum x-coordinate of the bounding box of a region

Indicates the x coordinate in the world coordinate system (WCS) of the right-hand edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.

- Unit: Unit of the scaling assigned to the image (e.g. μm)

Bound
Top

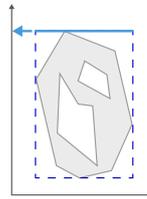


Maximum y-coordinate of the bounding box of a region

Indicates the y coordinate of the top edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.

- Unit: Unit of the scaling assigned to the image (e.g. μm)

Bound
Top
Unscaled

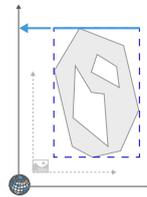


Maximum y-coordinate of the bounding box of a region

Indicates the y coordinate of the top edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.

- Unit: pixels

Bound
Top
Unscaled
WCS

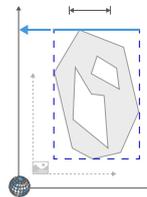


Maximum y-coordinate of the bounding box of a region

Indicates the y coordinate in the world coordinate system (WCS) of the top edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.

- Unit: pixels

Bound
Top
WCS

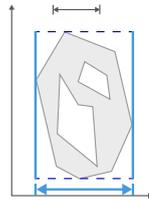


Maximum y-coordinate of the bounding box of a region

Indicates the y coordinate in the world coordinate system (WCS) of the top edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.

- Unit: Unit of the scaling assigned to the image (e.g. μm)

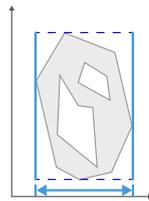
Bound Width



Indicates the width (size in x-direction) of a bounding box for a region. The box is drawn in parallel to the x and y axis.

- Formula: Bound right - Bound left
- Unit: Unit of the scaling assigned to the image (e.g. μm)

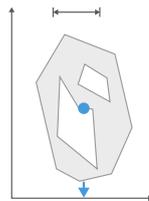
Bound Width Unscaled



Indicates the width (size in x-direction) of a bounding box for a region. The box is drawn in parallel to the x and y axis.

- Formula: Bound right - Bound left
- Unit: pixels

Center X

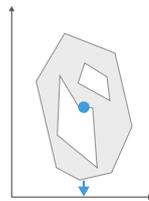


The x coordinate of the geometric center of gravity of a region

Depending on the shape of the object, this point may also lie outside a region. The associated y-coordinate is determined via the **Center Y** parameter.

- Unit: Unit of the scaling assigned to the image (e.g. μm)

Center X Unscaled

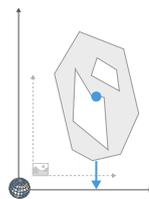


The x coordinate of the geometric center of gravity of a region

Depending on the shape of the object, this point may also lie outside a region. The associated y-coordinate is determined via the **Center Y** parameter.

- Unit: pixels

Center X Unscaled WCS

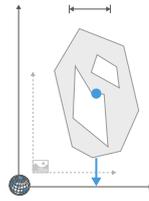


The x coordinate in the world coordinate system (WCS) of the geometric center of gravity of a region

Depending on the shape of the object, this point may also lie outside a region. The associated y-coordinate is determined via the **Center Y** parameter.

- Unit: pixels

Center X
WCS

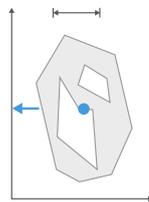


The x coordinate in the world coordinate system (WCS) of the geometric center of gravity of a region

Depending on the shape of the object, this point may also lie outside a region. The associated y-coordinate is determined via the **Center Y** parameter.

- Unit: Unit of the scaling assigned to the image (e.g. μm)

Center Y

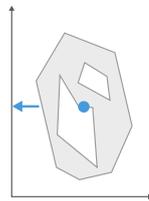


The y coordinate of the geometric center of gravity of a region

Depending on the shape of the object, this point may also lie outside a region. The associated y-coordinate is determined via the **Center X** parameter.

- Unit: Unit of the scaling assigned to the image (e.g. μm)

Center Y
Unscaled

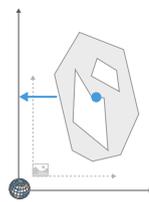


The y coordinate of the geometric center of gravity of a region

Depending on the shape of the object, this point may also lie outside a region. The associated y-coordinate is determined via the **Center X** parameter.

- Unit: pixels

Center Y
Unscaled
WCS

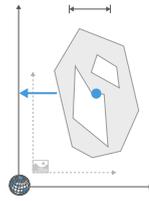


The y coordinate in the world coordinate system (WCS) of the geometric center of gravity of a region

Depending on the shape of the object, this point may also lie outside a region. The associated y-coordinate is determined via the **Center X** parameter.

- Unit: pixels

Center Y
WCS



The y coordinate in the world coordinate system (WCS) of the geometric center of gravity of a region

Depending on the shape of the object, this point may also lie outside a region. The associated y-coordinate is determined via the **Center X** parameter.

- Unit: Unit of the scaling assigned to the image (e.g. μm)

Circularity

$\text{Sqrt}(\text{Roundness})$

$\text{Roundness} = 4 \times \text{Area} / \pi \times \text{FeretMax}^2$

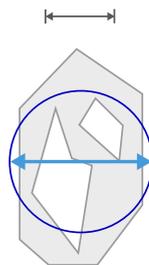
Compactness

$4 \times \pi \times \text{Area} / \text{PerimeterConvex}^2$

Convexity

$\text{PerimeterConvex} / \text{PerimeterCrofton}$

Diameter

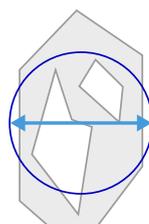


Diameter of a circle with an area equal to that of the object

The object is measured using the **Area** parameter. A circle with the same area as the object is created. The diameter of this circle is returned.

- Formula: $\text{Sqrt}((4 / \pi) \times \text{Area})$
- Unit: Unit of the scaling assigned to the image (e.g. μm)

Diameter
Unscaled

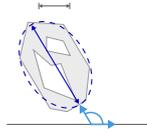


Diameter of a circle with an area equal to that of the object

The object is measured using the **Area** parameter. A circle with the same area as the object is created. The diameter of this circle is returned.

- Formula: $\text{Sqrt}((4 / \pi) \times \text{Area1})$
- Unit: pixels

Ellipse
Angle

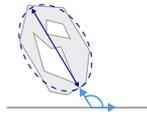


Angle of the major axis of the ellipse

The major axis of an ellipse with the same geometric moment of inertia as the current region is determined in accordance with the **Ellipse major** parameter. The angle to the x-axis is then determined. The indication of the angle always relates to a counterclockwise direction.

- Unit: degrees
- Value range: 0 ... 180°

Ellipse
Angle
Unscaled



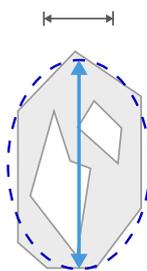
Angle of the major axis of the ellipse

The major axis of an ellipse with the same geometric moment of inertia as the current region is determined in accordance with the **Ellipse major** parameter. The angle to the x-axis is then determined. The indication of the angle always relates to a counterclockwise direction.

- Unit: degrees
- Value range: 0 ... 180°

This tool uses unscaled pixels for calculating the angle. The results may differ from the results of Ellipse Angle.

Ellipse
Major

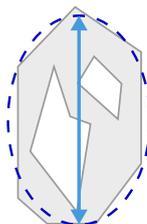


Length of the major axis of the ellipse

Length of the major axis of an ellipse with the same geometric moment of inertia as the region. The moment of inertia is calculated about the center of gravity of the region.

- Unit: Unit of the scaling assigned to the image (e.g. μm)

Ellipse
Major
Unscaled

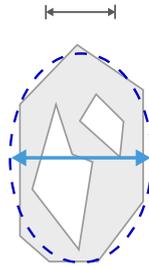


Length of the major axis of the ellipse

Length of the major axis of an ellipse with the same geometric moment of inertia as the region. The moment of inertia is calculated about the center of gravity of the region.

- Unit: pixels

Ellipse
Minor

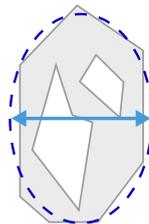


Length of the minor axis of the ellipse

Length of the minor axis of an ellipse with the same geometric moment of inertia as the region. The moment of inertia is calculated about the center of gravity of the region.

■ Unit: Unit of the scaling assigned to the image (e.g. μm)

Ellipse
Minor
Unscaled

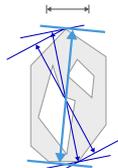


Length of the minor axis of the ellipse

Length of the minor axis of an ellipse with the same geometric moment of inertia as the region. The moment of inertia is calculated about the center of gravity of the region.

■ Unit: pixels

Feret
Maximum

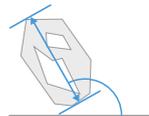


Maximum feret of a region

The maximum feret of a region is determined on the basis of distance measurements. Two straight lines are positioned on opposite sides of the object, like a sliding caliper, at 32 angle positions. The corresponding distance is measured for each angle position. The maximum value determined is the maximum feret.

■ Unit: Unit of the scaling assigned to the image (e.g. μm)

Feret
Maximum
Angle



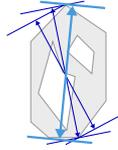
Angle of the maximum feret of a region in relation to the x-axis

The maximum feret is determined as described in **Feret Maximum**. The angle of the maximum feret in relation to the x-axis is then determined. The indication of the angle always relates to a counterclockwise direction.

■ Unit: degrees

■ Value range: 0 ... 180°

Feret
Maximum
Unscaled

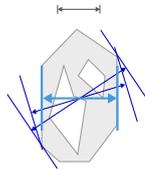


Maximum feret of a region

The maximum feret of a region is determined on the basis of distance measurements. Two straight lines are positioned on opposite sides of the object, like a sliding caliper, at 32 angle positions. The corresponding distance is measured for each angle position. The maximum value determined is the maximum feret.

■ Unit: pixels

Feret
Minimum

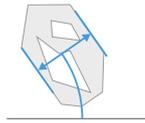


Minimum feret of a region

The minimum feret of a region is determined on the basis of distance measurements. Two straight lines are positioned on opposite sides of the object, like a sliding caliper, at 32 angle positions. The corresponding distance is measured for each angle position. The minimum value determined is the minimum feret.

■ Unit: Unit of the scaling assigned to the image (e.g. μm)

Feret
Minimum
Angle



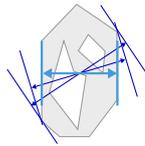
Angle of the minimum feret of a region in relation to the x-axis

The minimum feret is determined as described in **Feret Minimum**. The angle of the minimum feret in relation to the x-axis is then determined. The indication of the angle always relates to a counterclockwise direction.

■ Unit: degrees

■ Value range: 0 ... 180°

Feret
Minimum
Unscaled

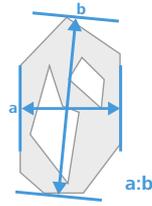


Minimum feret of a region

The minimum feret of a region is determined on the basis of distance measurements. Two straight lines are positioned on opposite sides of the object, like a sliding caliper, at 32 angle positions. The corresponding distance is measured for each angle position. The minimum value determined is the minimum feret.

■ Unit: pixels

Feret Ratio

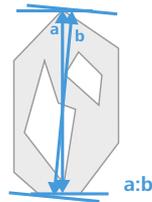


Feret ratio

The ratio of **Feret Minimum** to **Feret Maximum** is calculated. This ratio makes it possible to make statements on the form of the measured objects. If the feret ratio has a low value, long, elongated objects are present. Values approaching 1 indicate the presence of compact or circular objects, as in this case **Feret Minimum** and **Feret Maximum** have very similar values. The **Form circle** is also suitable for making statements on the circularity of an object.

- Formula: $\text{FeretMin} / \text{FeretMax} (a / b)$
- Unit: none
- Value range: 0 ... 1

Feret Vertical to Maximum



The ratio between the feret distance at 0° and the **Feret Maximum**

- Unit: none

Fiber Length

Length of a fiber-like region

To calculate the fiber length, a structure that is actually similar to a fiber is required. Here it is not the distance between a start and end point that is determined. The check can be done using the **Form circle**, among other things.

- Formula: $\frac{1}{4} \times (\text{Perimeter} + (\text{Sqrt}(\text{Perimeter}^2 - 16 \times \text{Area})))$
- Unit: Unit of the scaling assigned to the image (e.g. μm)

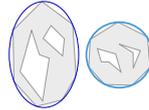
Fiber Length Unscaled

Length of a fiber-like region

To calculate the fiber length, a structure that is actually similar to a fiber is required. Here it is not the distance between a start and end point that is determined. The check can be done using the **Form circle**, among other things.

- Formula: $\frac{1}{4} \times (\text{Perimeter1} + (\text{Sqrt}(\text{Perimeter1}^2 - 16 \times \text{Area}))$
- Unit: pixels

Form
Circle



Form factor of a region

Describes the form of a region on the basis of its circularity. A perfect circle is given the value 1. The more elongated the region is, the smaller the form factor. The calculation is based on the **Area filled** and **Perimeter Crofton** parameters.

- Formula: $4 \times \pi \times \text{Area} / \text{PerimeterCrofton}^2$
- Unit: none
- Value range: 0...1

ID

Sequential ID of the object

ID of the
Parent

Sequential ID of the object's parent

Image
Index
Block

Index for the individual image slices of a multi-block image.

Multi-block images are composed of blocks of different dimensions.

Image
Index
Position

Index for the individual image slices of a **Position List** image.

If the image was acquired using the **Position List** tool, the **Image Index Position** returns the number of the image that contains the object.

In contrast, the **ID** parameter is a global counter, i.e. the number of the object.

The **Index Position** can be used to guarantee unambiguous assignment between measured regions in the image and the individual lines of a data table, especially in cases where several images are measured, as automatic assignment is then no longer possible. To achieve this, the **Index Region** parameter must also be selected as a

region feature and also be inserted into the graphics plane as an annotation(Draw Features). It is also advisable to activate the **Image name** parameter as a region feature so that the correct original image can be reloaded.

- Restriction: This value is only available for images that have previously been acquired with AxioVision or saved in AxioVision ZVI format.

Image
Index
Scene

Index for the individual image slices of a scene image

Indicates the unambiguous number of the scene in an image. The word **Scene** refers to a coherent object on a slide that contains several objects for examination. The **Index Scene** can be used to guarantee unambiguous assignment between measured regions in the image and the individual lines of a data table, especially in cases where several images are measured, as automatic assignment is then no longer possible. To achieve this, the **Index Region** parameter must also be selected as a region parameter and must also be inserted into the graphics plane as an annotation (Draw Features). It is also advisable to activate the **Image name** parameter as a region parameter so that the correct original image can be reloaded.

Image
Index
Time

Index for the individual image slices of a time series image.

Image
Index Z

Index for the individual image slices of a Z stack image.

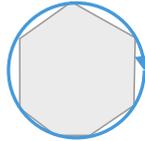
Index

Intensity Maximum of channel 'C1'	The pixel value of the brightest pixel in the object.
Intensity Mean Value of channel 'C1'	The average brightness (pixel value) of the pixels in the object.
Intensity Minimum of channel 'C1'	The pixel value of the darkest pixel in the object.
Intensity Range of channel 'C1'	The difference between the pixel value of the brightest and darkest pixels in the object, i.e. Intensity Maximum of channel 'C1' - Intensity Minimum of channel 'C1'
Intensity Standard Deviation of channel 'C1'	The standard deviation of the brightness (pixel value) of the pixels in the object.
Number of the region holes	Calculates the number of holes enclosed by the object
Perimeter	Perimeter of a region This parameter is specially optimized for measuring the perimeters of circles. If the measured region contains holes, the total perimeter including the perimeters of the hole structures is determined. If

you only want the perimeter of the outside contour to be determined, use the **Perimeter filled** parameter.

- Unit: Unit of the scaling assigned to the image (e.g. μm)

Perimeter
r
Unscaled



Perimeter of a region

This parameter is specially optimized for measuring the perimeters of circles. If the measured region contains holes, the total perimeter including the perimeters of the hole structures is determined. If you only want the perimeter of the outside contour to be determined, use the **Perimeter filled** parameter.

- Unit: pixels

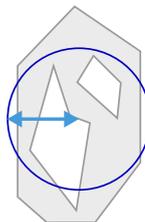
Radius

Radius of a circle with an area equal to that of the object

The object is measured using the **Area** parameter. A circle with the same area as the object is created. The radius of this circle is returned.

- Formula: $\text{Sqrt}((1 / \pi) \times \text{Area})$
- Unit: Unit of the scaling assigned to the image (e.g. μm)

Radius
Unscaled



Radius of a circle with an area equal to that of the object

The object is measured using the **Area** parameter. A circle with the same area as the object is created. The radius of this circle is returned.

- Formula: $\text{Sqrt}((1 / \pi) \times \text{Area1})$
- Unit: pixels

Region
Class
Color

Returns the color of the class to which the object is assigned

Region Class ID	Returns the ID of the class to which the object is assigned
Region Class Name	Returns the name of the class to which the object is assigned
Roundne ss	Formula: $4 \times \text{Area}$

9.4.2 Creating an Automatic Measurement

To be able to automatically analyze a sample you first have to create a new automatic measurement or modify an existing one.

- Prerequisites**
- An image is displayed
 - The **Automatic Measurement** workbench is selected

- Procedure**
- 1 In the **Image Analysis** tool, click on  and select **New**.
 - 2 Enter a meaningful name for the automatic measurement.
 - 3 Click **Setup Image Analysis**.
 - 4 Complete all the steps of the wizard.
These are described in detail in the following topics.
 - 5 Click **Finish**.

You can then run the automatic measurements as follows:

- Interactively
- Automatically

Alternatively you can include it in a job template or assign it to a macro.

9.4.3 Automatic Measurement Wizard

9.4.3.1 Classifying Measurements (Classes)

This step in the **Image Analysis Wizard** enables you to create measurement classes for the sample. A class corresponds to a type of object to be detected, for example objects with a specific brightness or shape.

This step enables you to define how many measurement classes are required. The properties of a class (i.e. how an object is detected) are defined in a later step.

When you create a class it is added to the list of classes, together with a summation class (indicated by a red plus icon). The class itself contains the measurement values for each individual object detected. The summation class contains the sum of all measurement values for all objects.

Classes can be nested. In this case, the summation class contains the sum of all subordinate summation classes.

Example The following example shows how classes could be grouped:

Structure	Measurement feature	Measurement results
Root		
Light particles (summation)	Count Area	Total number of light particles Total area of all light particles
Light particles	Intensity Area	Intensity of each light particle Area of each light particle
Square particles (summation)		Total area of all square particles
Square particles	Area	Area of each square particle
Round particles (summation)		Total diameter of all round particles
Round particles	Diameter	Diameter of each round particle
Dark particles (summation)	Count	Number of dark particles
Dark particles	Area	Area of each dark particle

You can also adjust the appearance of each class:

- Name
- Channel used

In a multichannel image you can select which channel should be evaluated

- Color

The objects detected by the automatic measurement are displayed in this color on the image

To define the classes for the automatic measurement:

Procedure 1 Click **Add Class**.

The new class and the summation class are always inserted below the currently selected item.

- 2 Enter a meaningful name for the class and the summation class.
- 3 In a multichannel image, select which channel should be evaluated.
- 4 Select the color in which the detected objects are displayed.
- 5 Click **Next**.

For detailed information about each parameter, see *Classes* [▶ 228].

9.4.3.2 Specifying Region of Image to be Analyzed (Frame)

This step in the **Image Analysis Wizard** enables you to specify the following:

- The areas of the image to be measured (measurement frames)
- How objects at the edge of the image or frame are treated

Procedure 1 If you wish a user to be able to adjust the settings in this step when the job is run, activate **Interactive**.

- 2 Specify the measurement area:
 - Use the drawing tools to create one or more new areas.
 - You can modify measurement areas by dragging them in the image or entering coordinates.
 - If you wish to measure the entire image, click the trash can icon to delete all measurement areas.



- 3 In **Mode**, specify how objects at the edge of the image or the measurement area are treated.
- 4 Click **Next**.

For detailed information about each parameter, see *Frame* [▶ 228].

9.4.3.3 Configuring Object Detection (Automatic Segmentation)

This step in the **Image Analysis Wizard** enables you to specify how objects are detected automatically based on their brightness. For this a black and white image is generated (the original image stays untouched) and the algorithm detects groups of pixels based on their brightness compared to neighboring pixels.

All the objects detected with the current settings are highlighted in the image.

TIP

- If not all objects are detected automatically it is better to adjust the parameters so that too many objects are detected. You can then use the following steps to exclude objects, for example based on their size or roundness.
- You can manually add or remove difficult to detect objects in a later step.

- Procedure**
- 1** If you wish this step to be included when the job is run, activate **Execute**. Otherwise the step is skipped.
 - 2** If you wish a user to be able to adjust the settings in this step when the job is run, activate **Interactive**.
 - 3** Select the class for which you wish to modify the settings.
You can specify different settings for each class.
 - 4** Use the **Smooth** and **Sharpen** settings to optimize the image, for example by reducing noise and enhancing contours.
 - 5** Specify the minimum number of pixels to be considered an object in **Minimum Area**.
 - 6** Select a color channel and the color model to use.
You can specify different settings for each channel.
 - 7** Specify the upper and lower brightness levels that correspond to an object for the selected channel:
 - Enter gray values in **Low** and **High**.
 - Adjust the sliders in the histogram.
 - In **Threshold Definition** select **Click** and click representative areas in the image.
 - + adds further objects by increasing the threshold boundaries to include the brightness values of the selected object
 - removes objects with the selected brightness values and reduces the threshold boundaries
 Use **Tolerance** and **Neighborhood** to adjust how pixels are treated that are close to the selected pixels in terms of brightness or proximity.
 - In **Threshold Definition** select **Automatic** and specify the desired algorithm to automatically detect the threshold boundaries
 - 8** Use **Fill** and **Separate** to specify how holes in objects and overlapping objects are treated.
 - 9** Click **Next**.

For detailed information about each parameter, see *Automatic Segmentation* [▶ 230].

9.4.3.4 Automatically Correcting Object Detection (Condition)

This step in the **Image Analysis Wizard** enables you to specify which of the detected objects are measured, based on various properties such as area, intensity, or roundness.

All the objects detected with the current settings are highlighted in the image.

TIP

- You can manually add or remove difficult to detect objects in the next step.

- Procedure**
- 1** If you wish this step to be included when the job is run, activate **Execute**. Otherwise the step is skipped.
 - 2** If you wish a user to be able to adjust the settings in this step when the job is run, activate **Interactive**.
 - 3** Select the class for which you wish to modify the settings.
You can specify different settings for each class.
 - 4** Click **Edit**.
 - 5** Double click the properties to use to select detected objects.
 - The selected properties are added to a so-called block. The properties are joined by "And" conditions: only detected objects which fulfill all the properties in a block are measured; all other detected objects are excluded.
 - You can filter the list by entering the first few letters of the name of a property in **Search Feature** or by selecting the corresponding category of property in the drop-down.
 - For more information about the properties and their values, see *Feature Selection Dialog* [▶ 241].
 - 6** To create an alternative block, click **Add Block**.
The blocks are joined by an "Or" condition. Objects that fulfill all the conditions in at least one block are measured.
 - 7** Double click the properties to add to the new block.
 - 8** Repeat steps 5-7 as desired.
 - 9** When you have selected all the desired properties, click **OK**.
 - 10** For each property, click a representative object in the image.
The threshold values for the property are set based on the values of the selected object.

- 11** If desired, correct the lower and upper threshold values in **Minimum** and **Maximum** respectively.
- If the object's property is not between the thresholds it is excluded.
 - If you do not want to include a threshold for a property, deactivate the corresponding checkbox. In this case, the threshold is set to the minimum or maximum possible value respectively.

- 12** Click **Next**.

For detailed information about each parameter, see *Condition* [▶ 236].

9.4.3.5 Manually Correcting Object Detection (Interactive Segmentation)

This step in the **Image Analysis Wizard** enables you to add or remove individual objects to be measured. The step can be considered a manual "fine-tuning" of the automatic detection results of the previous steps. After this step, all the objects you wish to measure should be selected and any you do not wish to be measured should be removed.

All the objects detected with the current settings are highlighted in the image.

TIP

If you need to make large numbers of manual corrections, it might be quicker to try adjusting the parameters in the previous steps first.

- Procedure**
- 1** If you wish this step to be included when the job is run, activate **Interactive**. Otherwise the step is skipped.
 - 2** Select the class for which you wish to modify the settings.
You can specify different settings for each class.
 - 3** To add a new object or remove part an object, click **Draw** or **Erase** and use a drawing tool in the image.
 - **Draw**: Adds the area drawn as a new object
 - **Erase**: Removes the area drawn from any existing objects; the remaining parts of the objects are measured

For more information about how to use the drawing tools, see *Interactive Segmentation* [▶ 237].
 - 4** To split an existing object into multiple objects click **Cut** and draw a line in the image where the object should be split.
The resulting objects are measured separately.
 - 5** To extend the size of an object click **Merge** and use a drawing tool in the image.
If the area drawn overlaps an existing object, the area and object are joined into a single object.

- 6 To fill an object containing a hole, click **Fill** then click an object in the image. The hole is filled and the complete object is measured.
- 7 To delete objects, click the desired action and then click an object in the image:
 - **Remove**: Deletes the selected object; the object is no longer measured
 - : Removes all objects; no objects are measured
- 8 To expand or reduce the size of an object based on the brightness of surrounding pixels, click **+** or **-** and click a representative area in the image. The amount by which the object expands or reduces depends on the brightness of the selected pixel, its proximity to other objects, and the values of the **Intensity** and **Color** parameters.
 - **+**: Expands the closest object
 - **-**: Reduces the closest object
- 9 Click **Next**.

For detailed information about each parameter, see *Interactive Segmentation* [▶ 237].

9.4.3.6 Defining Values to be Measured (Features)

This step in the **Image Analysis Wizard** enables you to define the properties of the detected objects to be measured, such as location, diameter, or intensity.

All the objects detected with the current settings are highlighted in the image.

Prerequisites ■ The objects you wish to measure are selected. If not, change the values in the previous steps first.

- Procedure**
- 1 Select the class for which you wish to modify the settings. You can specify different settings for each class.
 - 2 Click **Edit**.
 - 3 Double click the properties to be measured for the detected objects.
 - You can change the order of the measurements using the up and down arrow icons.



 - You can remove a property from the list using the trash can icon.


 - 4 If you want a property to be measured but not displayed in the image, deactivate the corresponding **Display** checkbox. This prevents the image becoming cluttered.

- 5 When you have selected all the desired properties, click **OK**.
- 6 If you wish the same measurements to be applied to all classes, click **Copy to All**.
- 7 Click **Next**.

For detailed information about each parameter, see *Features* [▶ 240].

9.4.3.7 Previewing Measurement (Measure)

This step in the **Image Analysis Wizard** displays a preview of the measurement results of the selected class. These results are calculated roughly and may differ from the actual results when the automatic measurement is performed.

- Procedure**
- 1 Check the measurement results for each class.
If you are not satisfied with the results, change the settings in the previous steps.
 - 2 Click **Finish** to save the automatic measurement.

For detailed information about each parameter, see *Measure* [▶ 241].

9.4.4 Running an Automatic Measurement

Automatic measurements can be run as follows:

- You can change the parameters of any step for which the **Interactive** checkbox is activated.
- The rest of the automatic measurement is performed automatically, except for where essential

- Prerequisites**
- An image is displayed
 - The **Automatic Measurement** workbench is selected

- Procedure**
- 1 In the **Program** list, select the desired automatic measurement definition.
 - 2 Select **Analyze Interactive**.

The results of the automatic measurement are displayed in the **Results Table**. If you select a row in the **Results Table** the corresponding object is highlighted in the image and vice versa.

9.5 Analysis Results

9.5.1 Displaying Analysis Values

You can view a table of the analysis results for all analyses performed for the current image.

- Prerequisites**
- A measurement workbench is selected.
 - You have performed at least one measurement

Procedure ◆ Click **Show results table** in the **Center Screen Area**.

The **Results Table** is displayed on right of the center tool area.

9.5.2 Sorting Analysis Values

You can sort the analysis results so that the values of interest are displayed at the top of the list.

Sorting single column To sort analysis results according to one criterion:

Prerequisites ■ The **Results Table** is displayed

- Procedure**
- 1 Click the header of the column you wish to sort.
 - 2 Click the header again to sort the column in the reverse order.

Sorting multiple columns To sort analysis results according to multiple criteria:

Prerequisites ■ The **Results Table** is displayed

Procedure 1 Click on **Options**.



- 2 Click **Sort Data**.
- 3 Select the first column to sort and the sort order (ascending, descending).
- 4 Click the plus icon and specify the next column to sort.



You can change the order of columns using the up and down arrow icons.



- 5 Click **Apply**.

9.5.3 Filtering Analysis Values

You can filter the analysis results so that only relevant values are displayed.

Prerequisites ■ The **Results Table** panel is displayed.

Procedure 1 Click the **Options** icon.



- 2 Click **Filter Data**.
- 3 Select the column to be filtered and the filter criteria.
 - **Equal**: the value of the cell must be identical to that in the filter string
 - **Not equal**: the value of the cell is any value apart from the filter string
 - **Contains**: the filter string is present in the value of the cell, e.g. "xel" matches "pixels"

- 4 Enter the filter string to be compared to the cell value.
- 5 If desired, add further criteria using the **AND** and **OR** buttons.
And: The criteria in both the current and subsequent row must be fulfilled
Or: The criteria in either the current row, the subsequent row, or both must be fulfilled
- 6 Activate the **Filter** checkbox.
Only rows where the selected columns fulfil the criteria are displayed.

9.5.4 Saving and Exporting Analysis Results

You can export the results of analysis tools, for example to copy them to another system or to analyze them in a statistics program.

- Prerequisites**
- You have performed at least one measurement
 - The **Save File** workbench is selected

- Procedure**
- 1 Select the **Save Table** tool.
 - 2 Click  and select the desired file type and location in the file system.

9.6 Configuring Analyses

9.6.1 Working with Image Analysis Presets

Many tools contain a **Settings** section. This allows you to save and load typical parameter values, saving the need to enter the values each time you use the tool.

The presets are available for all users of the system. You can also import or export these image analysis programs to use them on another system.

You can perform the following actions with programs:

Action	Description	Procedure
Apply Image Analysis program preset	The current program values are overwritten with those stored in the preset	◆ Select the desired preset program from the list.
Save changes to the current preset	The parameter values in the preset are overwritten with those of the current program preset	◆  > Save

Action	Description	Procedure
Save changes as a new preset	A new preset is created with the current parameter values	<ol style="list-style-type: none">  > Save As Enter the new name for the Image Analysis program.
Import a preset	A preset from the file system is added to the list of presets and the current parameter values are overwritten with those stored in the preset	<ol style="list-style-type: none">  > Import Select the desired preset program file from the file system.
Delete a preset	<p>The currently selected preset is deleted.</p> <p>The next program preset in the list is selected and the values from the preset applied. If the list is empty, the default settings for the image analysis are applied.</p>	  > Delete

INFO

Modified presets are indicated by a * next to the name.

9.6.2 Configuring Interactive Measurements

You can configure various properties of interactive measurements, for example:

- Line color, strength, and style
- Text font, color, and size
- Opacity
- Measurement results displayed in the in the **Center Screen Area** next to the interactive measurement and in the corresponding tool

- Prerequisites**
- An interactive measurement workbench is selected.
 - You have performed at least one interactive measurement.

- Procedure**
- 1 Select the desired interactive measurement tool.
 - 2 Click the "gear wheel" icon.



- 3 Adjust the parameters as desired.
The measurements are updated instantaneously.

10 Image and Document Management

10.1 Basics of Image and Document Management

You can import and export the following images and documents to/from the file system using tools in workbenches:

- Image
- Measurement results and data tables
- Tool presets

All other items, such as job templates, custom workbenches, automatic measurements, report templates and macros are managed in the **Archive**.

10.2 Supported Image and Document Formats

Images and documents refer to objects created during an examination.

Images and documents include the following objects:

- Images
- Measurement results and data tables
- Reports (which can be exported to an external storage device)

Image formats The following file formats are supported for images:

- **.czi**: Carl Zeiss Image
Contains the image and all associated measurements, settings, and metadata in a proprietary format
- **.bmp**: Windows Bitmap
- **.gif**: Graphics Interchange Format
- **.jpg**: Joint Photographic Engineering Group
- **.png**: Portable Network Graphic
- **.zvi**: AxioVision format for Carl Zeiss Image

This file format is supported to enable older Zeiss images to be loaded.

The following table lists the advantages and disadvantages of each file format:

Property	czi	bm	gif	jpg	png	zvi
Access		p				

Formats

Property	dzi	bmp	gif	jpg	png	zvi
– Read	x	x		x		x
– Write	x	x		x	x	
Advantages						
– All raw image acquisition data retained	x					
– Raw processed image data retained	x	x				
– Measurements usw. saved as extra layer	x				x	
– All metadata retained	x					
– Small file size			x	x	x	
– High compatibility across devices (open format)		x	x	x	x	
– Lossless compression			x		x	
					x	
– Transparency supported			x		x	
Characteristics						
– Can only be opened by Zeiss ZEN software	x					
– Large file size	x	x				
– No layers		x		x		
– No transparency		x		x		
– Loss of image quality due to compression				x		
– Measurements "burnt into" images and cannot be removed		x	x	x		
– No metadata		x	x	x	x	
– Only 256 colors			x			

Table formats Measurement results and data tables can be imported and exported in the following file formats.

File extension	Description
.czt	Zeiss Table Document
.csv	Comma Separated values
.xml	eXtended Markup Language Document

Report formats Reports can be exported in the following file formats.

File extension	Description
.docx	Microsoft Word 2010 format
.pdf	Adobe PDF format
.xps	Microsoft XML paper specification format

10.3 Loading an Existing Image

You can load any supported image type from the file system.

- Prerequisites**
- The file you wish to import is supported by ZEN 2 core
 - The **Load File** workbench is selected

- Procedure**
- 1 Click on  to open the file browser and select the desired image.
 - 2 To display the image in the **Center Screen Area**:
 - **Create Job Template** mode: click **Refresh**

 - **Free Examination** mode: click **Apply**

Create Job Template mode This step is performed automatically in the background when the operator runs the job. Ensure that the operator has sufficient privileges to read the file.

10.4 Exporting Images

The **Save File** workbench enables you to export an image, e.g. to use it for publishing or to archive it on an external storage device.

The workbench contains several tools to export images. The basic **Save Image** tool is visible by default and enables you to export an image in the most common image formats such as .czi, .tif, .jpg, or png. You can select the image format in the dialog window that opens when you apply the tool.

For detailed control of the export settings such as resolution or compression, or if you wish to export a movie, you can add one of the more advanced export tools:

Prerequisites ■ The **Save File** workbench is selected.

- Procedure**
- 1 In the **Workbench Area**, click **+ Add Tool**.
 - 2 Select the desired export tool.

For more information on the individual export tools, see *Save File Workbench* [▶ 179].

TIP

If you are working in **Free Examination** mode, you can right-click the image you wish to export in the **Document Area** and select **Save As...** In the dialog you can adjust parameters for every image format like **Burn in Annotations**. You can save several images simultaneously by selecting them with the *Ctrl* key.

11 Forms and Reports

11.1 Basics of Forms and Reports

In ZEN 2 core the concepts of forms and reports are closely related.

Forms provide a simple way to add user-defined information to an examination. Reports enable you to collate all the documents and information from your examination (including that entered in forms) in a single document.

As a supervisor you can use templates to configure the properties of a form or report and specify what the user has to add when the job is run.

- Forms templates are created and managed within the software.
- Report templates are managed within the software, but created using the Microsoft Word Add-In.

Typically, the information entered in a form should also be displayed in the job report. ZEN 2 core enables you to import a form template into the report template. This saves you having to define the same data twice (in the form template and the report template).

11.2 Forms

11.2.1 Basics of Forms

Forms provide a simple way to add user-defined information to an examination. Information can be entered into a form automatically (e.g. current time and date) or manually by the operator (e.g. current sample number).

The software comes with several pre-defined form templates. As a supervisor you can create new form templates or modify existing ones. You can specify the following form template properties:

- The fields the form contains, and thus the information to be entered
- Whether the information is entered manually or automatically
- Source for automatic data
- Layout of the form

Typically, the information entered in the form should also be displayed in the job report. ZEN 2 core enables you to export a form template in order to import it into a report template later. This saves you having to define the same data twice (in the form template and the report template).

You can also copy exported form templates to a different system to share them, for example with colleagues. All form templates are stored in the Archive where they can be accessed by other users.

11.2.2 Selecting a Form Template

Forms provide a simple way to add information to a job when it is run by an operator. The software comes with several predefined form templates. These are stored in the **Archive**.

- Prerequisites**
- The desired form template is in the **Archive**.
 - The **Input Forms** workbench is selected.

- Procedure** 1 Click the folder icon.



A list of the form templates in the **Archive** is displayed.

- 2 Select the desired form template and click **Apply**.

If the available form templates in the **Archive** are not suitable for your purposes, you can create a new form template or modify an existing one. In the **Archive** you can also import form templates from the file system, e.g. to copy a template from another system.

11.2.3 Creating or Modifying a Form Template

Forms provide a simple way to add information to a job when it is run by an operator. If the available form templates in the **Archive** are not suitable for your purposes, you can create a new form template or modify an existing one.

The **Form** workbench is selected.

- Procedure** 1 To create a new form template, click the plus icon.



- 2 To modify an existing form template, click the folder icon and select the desired form template.



- 3 Modify the form template as desired:

- Customize the layout (e.g. add or remove fields)
- Customize the properties of the fields
- Translate the form

- 4 When you are satisfied with the form template, click the **Apply** button to use the form template in your job template.

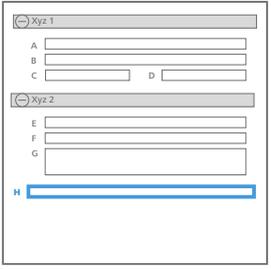
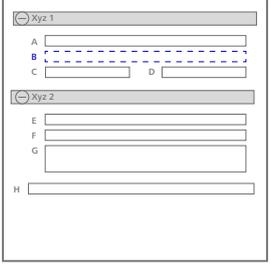
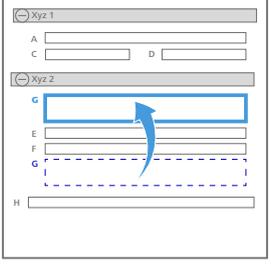
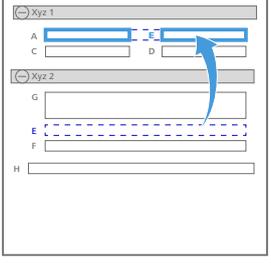
11.2.4 Customizing the Layout of Form Templates

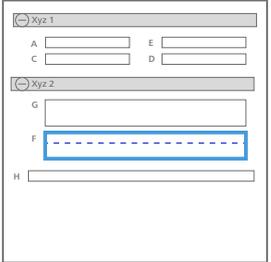
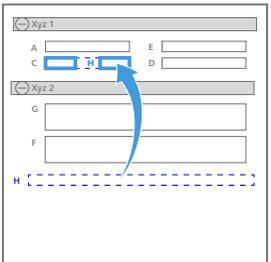
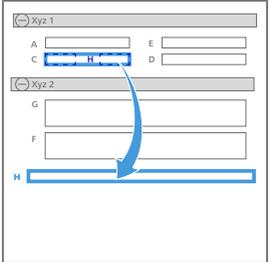
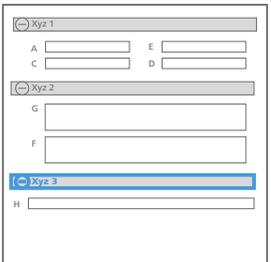
For each field in a form template you can modify its size and position within the template. You can also combine multiple fields into a group.

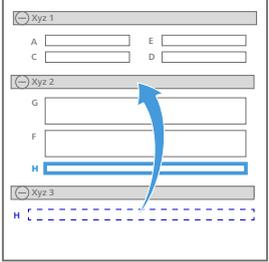
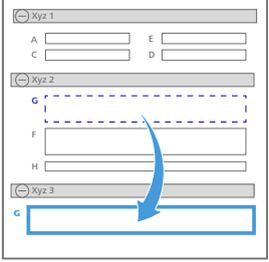
Prerequisites ■ The **Input Forms** workbench is selected.

- Procedure**
- 1** In the **Selected form template** tool, click the **Edit** button.
 - 2** Customize the layout of form template using the methods listed below.
 - 3** When you are satisfied with the form template, click the **Apply** button to use the form template in your job template.

Layout methods

Action	Procedure	Example
Add field	Click the  icon. The new field is added at the bottom of the form template.	
Remove field	Select the field and click the  icon.	
Sorting fields and groups	Click the field and drag it to its new location. If two or three fields are placed in the same row, their widths adjust automatically.	
Change field width	You cannot change the width of a field manually. The width depends on the number of fields in the row. If two or three fields are placed in the same row, their widths adjust automatically.	

Action	Procedure	Example
Change field height	Select the field and drag the handles up or down.	
Add column	<p>Select a field label or group label and drag it onto an existing field or group.</p> <p>The two items are placed next to each other. The left hand column can be split further, and a total of three columns are possible.</p> <p>If you drag a field onto a group (or vice versa), the width of the entire group is resized.</p>	
Remove column	Select the field or group and drag it to the bottom of the form template.	
Add group	<p>Click the  icon.</p> <p>The new group label is added at the bottom of the form template.</p>	

Action	Procedure	Example
<p>Group fields</p>	<p>Drag the fields to be added to the group onto the group label.</p> <p>Grouped fields can be hidden or shown by clicking the group label.</p> <p>Groups cannot be nested (i.e. you cannot create a group within a group).</p>	
<p>Remove field from group</p>	<p>To remove a field from a group, select the field and drag it to the bottom of the form template.</p>	

11.2.5 Customizing the Properties of Form Template Fields

For each field in a form template you can customize the type and format of information that can be entered into it and whether the information is entered manually or automatically.

Prerequisites ■ The **Input Forms** workbench is selected.

Procedure **1** In the **Selected form template** tool, click the **Edit** button.

2 Select the field you wish to modify.

3 Select the desired properties in the **Field settings** tool.

4 If you want to create a drop-down list, select **Metadata** or **Enumeration** in the **Type** dropdown.

You can define and sort the list entries in the **Predefined Values** section.

5 When you are satisfied with the form template, click the **Apply** button to use the form template in your job template.

For details on individual parameters, see the reference section.

11.2.6 Translating Form Templates

You can translate the labels of fields in a form template. When running a job, the field labels in the form are displayed in the language of the operator's user interface if the form template has been translated into this language. Otherwise the field labels are displayed in the default language (English).

Prerequisites ■ The **Input Forms** workbench is selected.

■ The form to be translated is loaded and selected.

Procedure **1** In the **Selected form template** tool, click the **Edit** button.

2 Activate the **Form is translated** checkbox.

3 Select the input language from the dropdown list.

4 Select a field in the form template.

5 Click the **Label** field of the **Field Settings** tool.

A new tool is displayed in the **Workbench Area** with the name of the currently selected field.

6 In the new tool, enter the translated name in the corresponding language field.

7 Repeat steps 3 to 5 for all other target languages.

You can see a preview of the form in a specific language at any time by clicking the **Language** dropdown in the **Form designer** workbench.

The list of available languages is the same as the available user interface languages.

8 When you are satisfied with the form template, click the **Apply** button to use the form template in your job template.

11.3 Reports

11.3.1 Basics of Reports

Reports enable you to collate all the information from your examination in a single document. Typical information includes:

■ Images

■ Measurement data tables

■ Metadata (e.g. examination time, hardware setup)

This document serves as a protocol of your examination.

ZEN 2 core SP1 comes with several pre-defined report templates. Each report template contains placeholders for the above information to enable you to collate the information easily. The placeholders also ensure that each time the job is run, the same information is added to the report.

Creating a new job report or modifying the layout and placeholders of an existing report template is performed in an external add-in for Microsoft Word 2010 or higher. Install the add-in on a computer where Microsoft Word is already installed. Using the templates in ZEN 2 core SP1 does not require an installation of Word, you can also create templates in the Microsoft Word format in ZEN 2 core SP1.

You can also export report templates to copy them to a different system, for example to share them with colleagues. All report templates are stored in the Archive where they can be accessed by other users

11.3.2 Selecting a Report Template

The software comes with several pre-defined report templates. Each report template contains placeholders to enable you to collate examination information easily into a single document.

- Prerequisites**
- The desired report template is in the **Archive**.
 - The **Reports** workbench is selected.

- Procedure** 1 Click the plus icon.



A list of form templates from the **Archive** is displayed in the **Select Report Templates** dialog.

- 2 Activate the checkbox of the desired report template and click **Select**.

If you activate multiple checkboxes, the selected report templates are joined together into one document.

If the available report templates in the **Archive** are not suitable for your purposes, you can create a new report template or modify an existing one using the ZEN Word Add-In. In the **Archive** you can import report templates from the file system, e.g. to copy a report template from another system.

11.3.3 Assigning Examination Information to Report Placeholders

Reports enable you to collate all the information from your examination in a single document. Typical information includes:

- Images
- Measurement data tables
- Metadata (e.g. examination time, hardware setup)

Each report template contains placeholders for the above information to enable you to collate the information easily. The placeholders also ensure that each time the job is run, the same information is added to the report.

- Prerequisites** ■ At least one report template is listed in the **Add Templates** tool.

- Procedure** 1 Select the desired report template in the **Add Templates** tool.

Various placeholders are listed in the **Workbench Area** and a preview of the report is displayed in the **Center Screen Area**.

- 2 Click the arrow icon in a placeholder and select the corresponding measurement information that you wish to add, for example image, table containing measurement results, etc.



As a rule you can add a single item to each placeholder. The image placeholder is an exception: you can add multiple images to a single image placeholder.

The report preview updates accordingly.

- 3 Repeat the above step until you have added information to all the placeholders.
- 4 Click **Create Report** to create the document in the **Documents Area**.
You can export the report from the **Documents Area** by using the context-menu.
- 5 If you require a paper copy of the template click **Print Report**.

11.3.4 Types of Placeholders

Each report template contains placeholders to enable you to collate examination information easily. The placeholders also ensure that each time the job is run, the same information is added to the report.

The following types of placeholders are supported:

Name	Description
Image	<p>Enables the user to add any image from the current examination</p> <p>For either type of image you can specify the metadata to be displayed, for example acquisition time, username, resolution, etc.</p>
Table	<p>Enables the user to add any measurement result from the current examination</p> <p>Tables can be connected with an image to show the measurement data.</p>
Properties	<p>Enables you to create a table of metadata, and specify the source (e.g. an image or a form)</p> <p>Metadata can include, for example, acquisition time, username, resolution, etc.</p>
Label	<p>Adds a text label that can have a different value in each of the languages supported by ZEN 2 core</p>

Name	Description
	This is useful if your report template is used in different languages.
File	Enables you to specify files (txt, rtf, doc, docx, htm, html, czxml) created during an examination that should be added to the report (currently not supported in ZEN 2 core)
Group	Groups placeholders If, for example, you create a group containing an image followed by a table, the user can add multiple images and multiple tables; each table is displayed under the corresponding image.

INFO

If you have any fixed element that should be included in each report in the same manner, such as a company logo, use the common Microsoft Word functions instead of a placeholder.

11.3.5 Creating a New Report Template

The software comes with several pre-defined report templates. If none of the available templates are suitable, you can create a new report template using the ZEN Word Add-In for Microsoft Word.

INFO

To create a new report template, Microsoft Word 2010 or higher and the ZEN Word Add-In must be installed.

- 1 Start Microsoft Word.
- 2 Create a new blank document.
- 3 Add the desired placeholders to the document.
- 4 Customize the appearance of the document according to your company or institution policies.
- 5 Save the document to your file system.
- 6 To use the template in ZEN 2 core SP1 you must import it into the **Archive**.

11.3.6 Modifying a Report Template

The software comes with several pre-defined report templates. If none of the available templates are suitable, you can modify an existing report template using the ZEN Word Add-In for Microsoft Word.

INFO

To modify a report template, Microsoft Word 2010 or higher and the ZEN Word Add-In must be installed.

- 1 Export the template to be modified from the **Archive** to your file system.
- 2 Start Microsoft Word.
- 3 Open the report template document from your file system.
- 4 Modify the placeholders or appearance of the document as desired.
You can use any standard features of Word, e.g. page layouts, text formatting, tables, etc.
- 5 Save the document to your file system.
- 6 To use the template in ZEN 2 core you must import it into the **Archive**.
If you keep the filename of the report, you will be asked whether you want to replace the existing report in the **Archive**.

11.3.6.1 Adding a Form Placeholder

Typically, the information entered in a form should also be displayed in the job report. ZEN 2 core enables you to import a form template into the report template. You can specify which data of a form will be displayed in the report using the form placeholder.

- Procedure**
- 1 Export the desired form template from the **Archive** to the file system.
 - 2 Open the report template and place the cursor where the form data should be included.
 - 3 In the toolbar ribbon, click **ZEN Report Templates**.
 - 4 Click **Properties**.
A blank metadata table is added to the document.
 - 5 In **Data Source Types**, select **Form Source**.
 - 6 Select the form to be imported.
 - 7 Specify the fields to be imported from the form.
 - 8 Save the report.

INFO

If you only wish certain fields to be used in the report, export the entire form and select the desired fields when importing into the report.

11.3.6.2 Adding an Image Placeholder

Image placeholders are filled interactively or automatically with the images generated during an examination..

You can also specify the metadata to be displayed directly underneath the image, for example the acquisition time, username, resolution, etc.

TIP

- If you want to include an image in the report that was not acquired during the examination, e.g. an image of an ideal sample or the company logo, use the standard Microsoft Word functions.
- If you want to display the metadata at a position other than directly beneath the image, use the **Properties** placeholder to create a metadata table and link it to the selected image. For more information, see *Adding Image Metadata* [▶ 136].

Prerequisites ■ ZEN Word Add-In is installed

- The report template to be modified is loaded

Procedure **1** In the document click the location where you wish to insert the placeholder.

2 In the toolbar ribbon, click **ZEN Report Templates**.

3 Click **Image**.

A blank image placeholder is added to the document. You can check that the size of the placeholder is sufficient by loading a test image: click **Save** and select the desired file.



This image is overwritten with the examination image when the report is generated.

4 In **List of Tags**, double-click the metadata to be displayed in the report.

- Use the up and down arrow icons to sort the order of the metadata.



- If you want to hide the **Property Name** column, activate the **Hide property name** checkbox.

5 Save your changes.

11.3.6.3 Adding a Table Placeholder

Table placeholders enables the user to add any measurement result from the current examination, e.g. of measurement results.

Prerequisites ■ ZEN Word Add-In is installed

■ The report template to be modified is loaded

Procedure **1** In the document click the location where you wish to insert the placeholder.

2 In the toolbar ribbon, click **ZEN Report Templates**.

3 Click **Table**.

A blank table placeholder is added to the document. You can check that the size of the placeholder is sufficient by loading a test table (for example a representative table exported from the software): click **Save** and select the desired file.



This table is replaced by the table that is assigned to the placeholder during report generation.

4 Save your changes.

11.3.6.4 Adding a Chart Placeholder

Chart placeholders enable the user to add any graph or chart from the current examination, e.g. a temperature curve.

Prerequisites ■ ZEN Word Add-In is installed

■ The report template to be modified is loaded

Procedure **1** In the document click the location where you wish to insert the placeholder.

2 In the toolbar ribbon, click **ZEN Report Templates**.

3 Click **Chart**.

A blank chart placeholder is added to the document. You can check that the size of the placeholder is sufficient by loading a test chart: click **Save** and select the desired file.



The chart is replaced by the chart assigned to the placeholder during report generation.

4 Save your changes.

11.3.6.5 Adding a Properties Placeholder (Metadata)

11.3.6.5.1 Adding Image Metadata

Image metadata placeholders enable you to create a table of metadata from an image acquired during an examination. Metadata can include, for example, the acquisition time or resolution, etc.

Metadata placeholders also enable you to place image metadata at an arbitrary location in the report (by default, image metadata is displayed directly beneath the image). The metadata is extracted when the placeholder is replaced with an image during report generation.

TIP

If you want to display an acquired image with its metadata below, it is usually simpler to use an **Image** placeholder. For more information, see *Adding an Image Placeholder* [▶ 135].

- Prerequisites**
- ZEN Word Add-In is installed
 - The report template to be modified is loaded
 - The report template contains at least one image placeholder
- Procedure**
- 1 In the document click the location where you wish to insert the metadata table.
 - 2 In the toolbar ribbon, click **ZEN Report Templates**.
 - 3 Click **Properties**.
A blank metadata table is added to the document.
 - 4 Click **Image source**.
 - 5 Select the metadata values to be displayed and click **Add**.
 - To add multiple values, press the *CTRL* key.
 - Use the up and down arrow icons to sort the order of the metadata.
- 
- 6 To allow the user to specify the image from which the metadata is extracted, leave the placeholder blank.
Alternatively, to specify that the metadata is extracted from a specific image placeholder, click **Link to Object** and in the drop-down select the desired placeholder.
 - 7 If you want to hide the **Property Name** column, activate the **Hide property name** checkbox.
 - 8 Save your changes.

11.3.6.5.2 Extracting Data from a Table

Table metadata placeholders enable you to extract and manipulate information from tables created during an examination, e.g. from measurement results.

For each column in the table you can specify which of the following to include:

- The value from a specific row
- A calculation based on all the values in the column (e.g. average, maximum value)

TIP

Before inserting the placeholder it is recommended to create a table in ZEN 2 core with an identical structure to that required in the report. Export the table to the file system.

- Prerequisites**
- ZEN Word Add-In is installed
 - The report template to be modified is loaded
 - An example table is available in the file system

- Procedure**
- 1 In the document click the location where you wish to insert the table.
 - 2 In the toolbar ribbon, click **ZEN Report Templates**.
 - 3 Click **Properties**.
A blank metadata table is added to the document.
 - 4 Click **Table source**.
 - 5 Load the example table: click the folder icon and select the desired file.

 - 6 Select the desired **Column name**.
 - 7 To display the value from a specific row, select **Row** in the **Display type** column and the row number in the **Selected** column.
 - 8 To calculate a value based on all the values in the column, select **Calculated** in the **Display type** column and the type of calculation in the **Selected** column.
 - 9 Save your changes.

11.3.6.5.3 Adding Form Data

Form placeholders enable you to quickly add the information entered in a form to a report.

You can import a form from ZEN 2 core to ensure you have the identical fields.

- Prerequisites**
- ZEN Word Add-In is installed
 - The report template to be modified is loaded
 - The form template has been exported from ZEN 2 core

- Procedure**
- 1 In the document click the location where you wish to insert the form.
 - 2 In the toolbar ribbon, click **ZEN Report Templates**.
 - 3 Click **Properties**.
A blank metadata table is added to the document.
 - 4 Click **Form source**.
 - 5 Click the folder icon and select the form template in the file system.



- 6 Select the metadata values to be displayed and click **Add**.
 - To add multiple values, press the *CTRL* key.
 - Use the up and down arrow icons to sort the order of the metadata.



- 7 Save your changes.

11.3.6.5.4 Adding System Information

System metadata placeholders enable you to create a table containing data such as company information, of metadata from an item acquired during an examination, e.g. from an acquired image or a results form.

Metadata can include, for example, measurement values, acquisition time, username, resolution, etc.

- Prerequisites**
- ZEN Word Add-In is installed
 - The report template to be modified is loaded

- Procedure**
- 1 In the document click the location where you wish to insert the placeholder.
 - 2 In the toolbar ribbon, click **ZEN Report Templates**.
 - 3 Click **Properties**.
A blank metadata table is added to the document.
 - 4 Click **System Data source**.
 - 5 Select the metadata values to be displayed and click **Add**.
 - To add multiple values, press the *Ctrl* key.
 - Use the up and down arrow icons to sort the order of the metadata.



- 6 Save your changes.

11.3.6.6 Adding a Multi-Language Label

Label placeholders enable you to add text to the report template that adapts to the language of the user interface of ZEN 2 core. This enables you to create localized version for different languages without needing to create and manage a separate template per language. If you do not need a report in multiple languages, you do not need to use this placeholder.

Prerequisites ■ ZEN Word Add-In is installed

■ The report template to be modified is loaded

Procedure **1** In the document click the location where you wish to insert the placeholder.

2 In the toolbar ribbon, click **ZEN Report Templates**.

3 Click **Label**.

A blank label placeholder is added to the document.

4 Enter the text for the label in each language in the corresponding field.

If you do not enter text for a language, the default language (English) is used.

5 Save your changes.

11.3.6.7 Adding a File Placeholder

File placeholders are replaced with files (txt, rtf, doc, docx, htm, html, czxml) created during an examination.

Prerequisites ■ ZEN Word Add-In is installed

■ The report template to be modified is loaded

Procedure **1** In the document click the location where you wish to insert the placeholder.

2 In the toolbar ribbon, click **ZEN Report Templates**.

3 Click **File**.

A file placeholder is added to the document.

4 Click folder icon and select a sample that has been created with ZEN 2 core.



A preview of the file is displayed in the document.

5 Save your changes.

11.3.6.8 Grouping Objects

Group placeholders help you to structure the report template. If, for example, you create a group containing an image followed by a table, the user can add multiple images and multiple tables; each table is displayed together with the corresponding image.

Prerequisites ■ ZEN Word Add-In is installed

■ The report template to be modified is loaded

Procedure **1** In the document click the location where you wish to insert the placeholder.

2 In the toolbar ribbon, click **ZEN Report Templates**.

3 Click **Group**.

A blank group placeholder is added to the document.

4 Add new placeholders to the group or drag existing placeholders into the group.

5 Save your changes.

12 Scaling and Units

12.1 Basics

Scalings specify the number of pixels in an image that correspond to a certain actual length of an object in the image, for example that 100 pixels correspond to 1 μm .

Units specify how lengths are displayed, e.g. in millimeters or inches.

As a supervisor you can perform the following actions:

- Create a new scaling by measuring an object of known length
- Add a scaling to existing images retrospectively
- Select whether a custom or a theoretical scaling is used for an image

The theoretical scaling is calculated automatically based on the properties of the hardware components (e.g. zoom of the objectives, number and separation of pixels on camera chip, etc.)

INFO

The units used to display scalings and measurements are a global setting for all users and can only be changed by an administrator.

12.2 Creating a Custom Scaling

You can create a custom scaling for the current hardware setup by measuring an object of known length. If enabled by the administrator, you can select that this custom scaling is used as the basis for current measurements with this hardware setup.

INFO

This tool should typically only be used in **Free Examination** mode. If you add it to a job template a custom scaling will be created each time the job is run. Furthermore, only one scaling can be stored per hardware configuration, i.e. when a new custom scaling is created, the existing one is overwritten.

- Prerequisites**
- You are in **Free Examination** mode
 - You have permission to overwrite custom scalings

- Procedure**
- 1 Place an object of known length on the microscope stage.
 - 2 Acquire an image of the object using any acquisition method.

- 3 Add the **Create Measured Scaling** workbench from the **Calibration** category.
- 4 Select the desired measurement tool:
 - **Line** tool
 - **Parallel Line** toolFor more information about using the tools, see the Workbench and Tool Reference.
- 5 Measure the object on the screen.
If you want the measurement tool to automatically snap to edges activate **Automatic Line Detection**.
- 6 In **Correspond to [Value]** enter the actual length of the object and select the correct units.
The system calculates the scaling.
- 7 Enter a meaningful name for the scaling and click **Save Scaling**.

INFO

The current hardware configuration, including the selected objective, is automatically stored as part of the scaling. You can only apply the scaling in the future when the identical hardware configuration is used. Therefore, if you have multiple hardware configurations, you need to create a scaling for each one.

12.3 Assigning a Scaling to an Acquired Image

If an image does not contain a scaling recognized by the software, for example because it was created on another device, you can assign a scaling retrospectively. Any measurements subsequently performed on the image use the assigned scaling.

- Prerequisites**
- You know the actual scaling of the image
You can calculate this by acquiring an image of an object of known length on the same external device and noting the number of pixels.

- Procedure**
- 1 Load the image which you want to assign a scaling.
 - 2 Add the **Assign Measured Scaling** workbench from the **Processing** category.
 - 3 Enter the known values for horizontal (**X**) and vertical (**Y**) scaling and select the corresponding units.
A pixel can correspond to different lengths in each direction.
 - 4 Click **Assign Scaling to Image**.

INFO

- Measurements and scale bars that are burnt into the images are not updated.

12.4 Selecting a Scaling Method

As a supervisor you can specify how the scalings for images are calculated:

- **Theoretic**

Based on the properties of the hardware components (e.g. zoom of the objectives, number and size of pixels on camera chip, etc.)

- **Custom scaling**

Based on a manual (user-defined) measurement created using the **Create Scaling** tool.

This feature can be disabled by the administrator.

Prerequisites ■ A custom scaling for the current hardware setup exists
If not, only the theoretical scale is available

- Procedure**
- 1 Acquire an image of the object using any acquisition method.
 - 2 Add the **Assign Measured Scaling** workbench from the **Processing** category.
 - 3 In **Scaling** select the desired scaling method:
 - **Theoretic**

- [Custom_Name]

The settings of the hardware components used in the scaling are displayed below.

- 4 Click **Assign Scaling to Image**.

The selected scaling method is applied to all images in the current job with the current hardware setup.

12.5 Managing Custom Scalings

You can import/export custom scale presets, for example to copy them to another system.

- Procedure**
- 1 Open **Manage Scalings**:
Home Screen > Maintenance > Manage Scalings
 - 2 Perform the actions listed below as required.

Action	Description	Procedure
Export a preset scaling	The scaling values are saved in a file	<ol style="list-style-type: none"> 1  > Export 2 Specify the location in the file system.

Action	Description	Procedure
Import a preset scaling	A preset from the file system is added to the list of scalings and the current parameter values are overwritten with those stored in the preset.	<ol style="list-style-type: none">  > Import Select the desired scaling file from the file system.
Delete a preset scaling	<p>The currently selected scaling is deleted.</p> <p>The next scaling in the list is selected and the values from the scaling applied. If the list is empty, the default values are applied.</p>	◆  > Delete

12.6 Assing a Scaling (Manual Hardware)

If an image does not contain a scaling, for example because it was acquired with manual hardware (i.e. a microscope where the individual hardware components cannot be detected), you can assign a scaling retrospectively. Any measurements subsequently performed on the image use the assigned scaling.

The total magnification of the microscope, and thus the scaling, is calculated based on the magnification of individual components.

Prerequisites ■ You know the magnifications of the hardware components

- Procedure**
- 1 Acquire an image of the object using any acquisition method.
 - 2 Add the **Assign Theoretical Scaling** workbench from the **Processing** category.
 - 3 Select the magnification of each component.
 - 4 Click **Assign Scaling to Image**.

INFO

- Measurements and scale bars that are burnt into the images are not updated.

13 Shuttle & Find Module

13.1 Introduction

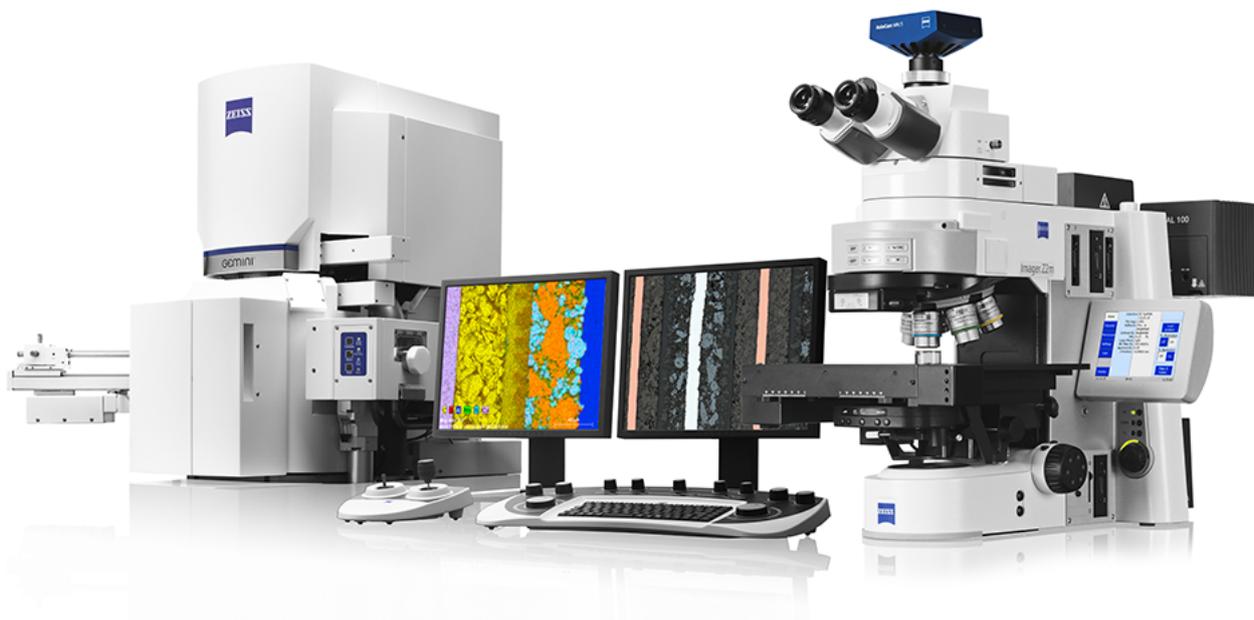


Fig. 13.1: SEM / LM system for correlative microscopy

The **Shuttle & Find** module in ZEN 2 core allows the relocation of sample positions in two different microscopes, e.g. a light microscope and a scanning electron microscope (SEM), and the correlation of two images to one merged image. This technique is called correlative microscopy. It is used to combine the two worlds of scanning electron microscopy and light microscopy and brings them together in one image. Note that in the documentation and in the software GUI the abbreviation 'S&F' is often used for 'Shuttle & Find'.

The samples can be mounted in especially designed correlative holder systems (with three correlative calibration markers) from ZEISS. Also user-defined holder systems with three calibration markers can be used. As the shape and size of materials samples vary strongly, a range of flexible correlative holders were designed to fulfill customers' needs.



Fig. 13.2: Example of a correlative ZEISS sample holder

13.2 Shuttle & Find Workflow

The Shuttle & Find (S&F) workflow in ZEN 2 core can be described in the following steps:

Image Acquisition on the Light Microscope (LM)

Before acquiring an image with the light microscope and using it for correlative microscopy, it is necessary to set up the system correctly e.g. stage calibration, camera orientation, calibrating objectives and setting the correct scaling. Note, that we do not describe these topics within this guide as we focus on the Shuttle & Find workflow only.

■ Step 1 : Calibrating the Holder

After starting the software you first need to calibrate the correlative holder for the LM system to setup the correlative coordinate system. Note that the holder calibration must be done twice on both systems the LM and the SEM. For the calibration you have to use the **S&F Marker Calibration** workbench, see *S&F Holder Calibration Workbench* [▶ 182].

■ Step 2: Acquiring the LM image

Now you can perform the image acquisition on the LM. To learn more about image acquisition in ZEN 2 core, read the corresponding topics of the Online Help.

■ Step 3: Drawing ROIs / POIs

In this step you can draw in regions or points of interests onto your sample images. These are usually the positions you want to investigate further on the other (SEM) system. For drawing in the ROIs /POIs use the **S&F ROI / POI Drawing** workbench, see *S&F ROI/POI Drawing Workbench* [▶ 182].

■ Step 4: "Shuttling" the Sample to the SEM

Now you can bring your sample to the SEM system. At this point do not remove the sample from the sample holder.

For transferring the image data we recommend to use the **Archive** functionality of the software. If both system PCs have access to a network, image data can be easily exchanged in that way. If there is no network connection you must transfer the image data via a storage device (USB stick or external hard disc) and open the file via the workbench **Documents | Load File**.

Image Acquisition on the Scanning Electron Microscope (SEM)**■ Step 1 : Calibrating the Holder**

After bringing the image data and the sample holder including the sample to the SEM, again you first need to calibrate the correlative holder for the SEM system. For the calibration you have to again use the **S&F Marker Calibration** workbench, see *S&F Holder Calibration Workbench* [▶ 182].

■ Step 2: "Finding" the Sample Positions on the SEM

After calibration you can now start and relocate the sample positions.

Therefore use the **S&F Find** tool (tool in the **Acquisition** workbenches), see *S&F Find Tool* [▶ 218]. Of course you should also bring the image data from the LM acquisition to recognize your drawn in ROIs/POIs. If you have loaded the LM image, you will see the ROI/POIs in a list and can move the scanning stage to these positions by one click with the mouse.

■ Step 3: Acquiring the SEM image

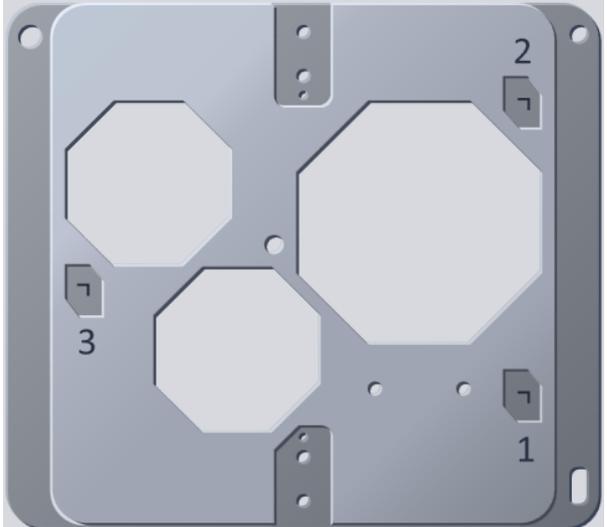
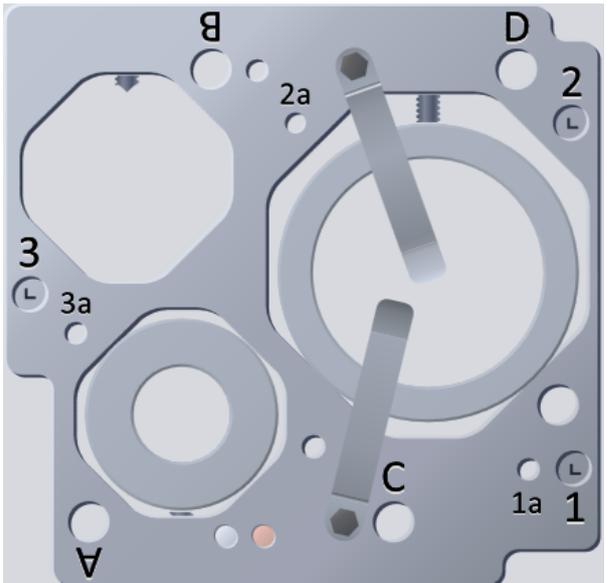
Now you can perform the image acquisition on the SEM.

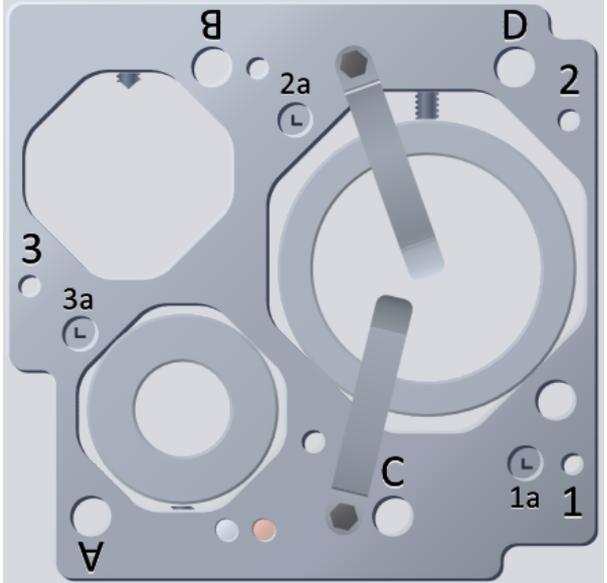
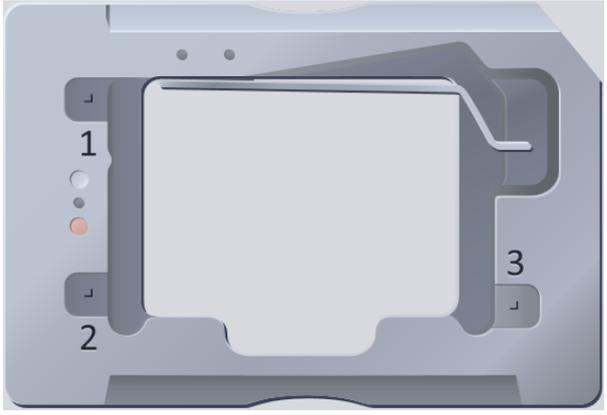
■ Step 4: Generating the Overlay Image

After having acquired the SEM image from the same ROIs/POIs like on the LM system, you now can combine both (or more) images together and generate an overlay image. Therefore use the **S&F Overlay** workbench, see *S&F Overlay Workbench* [▶ 182].

13.3 Correlative Sample Holders

Name	Image
MAT Flat Stubs	
MAT Particle	

Name	Image
MAT Universal A	 <p>The diagram shows a square sample holder with three octagonal sample positions. Callout 1 is at the bottom right, callout 2 is at the top right, and callout 3 is on the left side.</p>
MAT Universal B	 <p>The diagram shows a square sample holder with a central circular sample position. Two slanted bars are positioned across the center. Callouts include 1 and 1a at the bottom right, 2 and 2a at the top right, 3 and 3a on the left side, and A, B, C, and D at various corners and points.</p>

Name	Image
MAT Universal B v2	 <p>The diagram shows a top-down view of the MAT Universal B v2 sample holder. It features a central circular area with two rectangular strips (labeled C) positioned across it. Surrounding this central area are several circular features and ports. Labels include: 'A' at the bottom left corner; 'B' at the top left corner; 'D' at the top right corner; '1' and '1a' at the bottom right corner; '2' at the top right corner; '2a' and 'L' near the top center; '3' and '3a' on the left side; and 'C' near the bottom center. There are also several small circular features and a red dot near the bottom center.</p>
Geoslides	 <p>The diagram shows a top-down view of the Geoslides sample holder. It features a central rectangular area with a white slide. The holder has several ports and features. Labels include: '1' on the left side; '2' at the bottom left corner; and '3' on the right side. There are also several small circular features and a red dot near the bottom left corner.</p>

14 GxP Module

14.1 Introduction

GxP is a general term for **Good Practice** quality guidelines and regulations. These guidelines are used in many fields, including the pharmaceutical and food industries. The titles of these good practice guidelines usually begin with "Good" and end in "Practice", with the specific practice descriptor (x) in between.

GxP represents the abbreviations of these titles, where **x** (a common symbol for a variable) represents the specific descriptor. A "c" or "C" is sometimes added to the front of the initialism. The preceding "c" stands for "current." For example, cGMP is an acronym for "current Good Manufacturing Practices". The term GxP is frequently used to refer in a general way to a collection of quality guidelines.

The **GxP Module** provides different functionality, which provide the pre-condition to make the system compliant to 21 CFR part 11. The module can also be used to make the production process more reproducible and traceable. Beside the standard functionality, the module offers following functionality:

- Audit Trail, see *Audit Trail Concept* [▶ 152]
- Encrypting of job templates and job results
- Release procedure for job templates
- Electronic signature

i INFO

The validation and the qualification procedures which are needed for a 21 CFR Part 11 system are not part of this module and need to be performed separately.

14.2 Audit Trail Concept

By licensing the **GxP** module the **Audit Trail** functionality becomes available in the software. It is accessed via the **Maintenance** tab.

The Audit Trail implementation in ZEN 2 core allows to record/log all software specific actions performed by the user. Note that the functionality applies for the **Run Job** mode. In **Free Examination** mode the functionality is not effective. The Supervisor creates a Job Template and after the release procedure, the template e is activated and ready to use for production. Changes in the released job template will be recorded in the Audit Trail.

In the **Audit Trail** window you see the log entries in a list as soon as the module is active. You can export these log files in **.PDF** or **.html** format. It is also possible to show the results of a particular date. Therefore under **Time Range** you can adjust the desired dates by the help of a calendar. Of course a **Search** by keywords is implemented as well.

All audit relevant actions performed with the software are recorded and logged. The following list shows the most important actions which are logged:

Actions to be logged	Text / Log (exemplary)
Starting up and shutting down the software	"Starting ZEN 2 core Version 2.0, MTB 2011 2.4.0.8"
Loggin in / Loggin off (Category: Logon/Logoff)	"User "Z" has logged on."
Entering and exiting different modes (Category Enter / Exit Mode):	"User "Admin" has entered Maintenance Mode."
Executing job templates (Category: Execute)	"Execute template "XYZ" with Status "Active"."
Changing of parameters (Category: Execute)	In task 2 "image Processing" the following parameters have changed: Brightness from "0" to "50".
Changing status of job templates (Category: SetStatus)	"Status of template "XYZ" changed from "Active" to "Inactive"."
Changing of Templates in the Archive (Manage Templates)	"Job template "Template 2" was created."
User Management	"User "XY" was created."
Messages and Errors (Category: Warning)	"Scaling is invalid."
Using the Electronic Signature	"Status of template 'XYZ' changed form 'Draft' to 'Active' (Electronic Signature)."

14.3 Status Handling of Job Templates

You must be logged in with dedicated user rights (e.g. as **Supervisor**) to manage the status of job templates.

The status handling of job templates will be recorded in the Audit Trail. It ensures that an operator can only work with job templates which are released (or "activated") by a supervisor including an electronic signature. To activate a job template and assign an electronic signature the supervisor must enter his password.

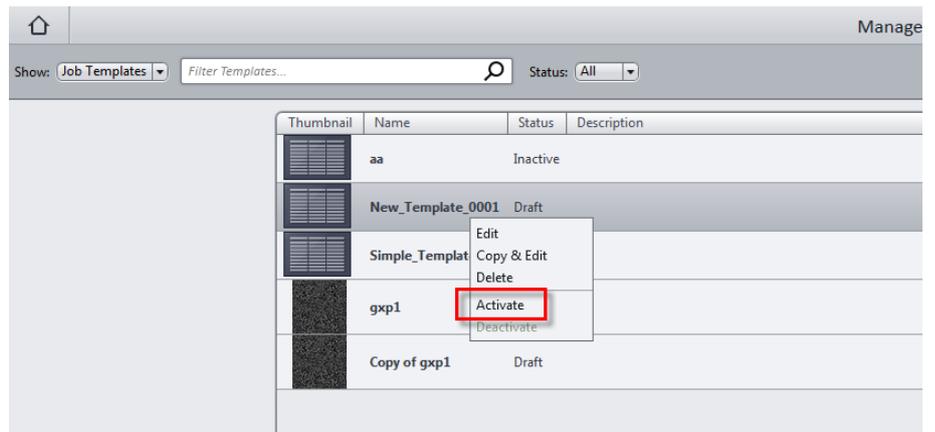
If you have licensed the **GxP** module, all job templates which are created new get a particular status. After you have created a new job template (see *Basics of Create Job Template Mode* [▶ 36]) the template has the status "**Draft**". To make the template available for operators the status has to be changed to "**Active**". If the template status was set to active you cannot delete the template anymore. You can set the template status to "**Inactive**", if you don't want the operators to work with the template.

Changing status of job templates

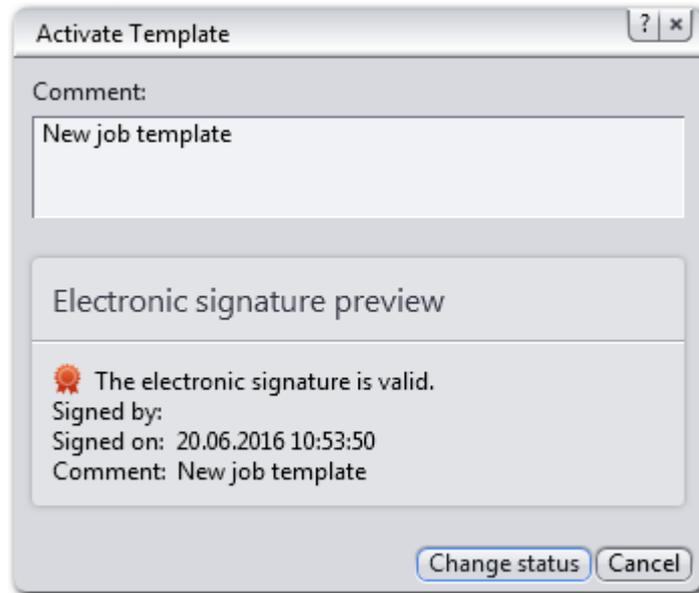
Prerequisites ■ Under **Home | Manage Templates** you can see all your templates and their status.

Procedure 1 Right-click on a template with the status **Draft**.

2 Click on **Activate**.

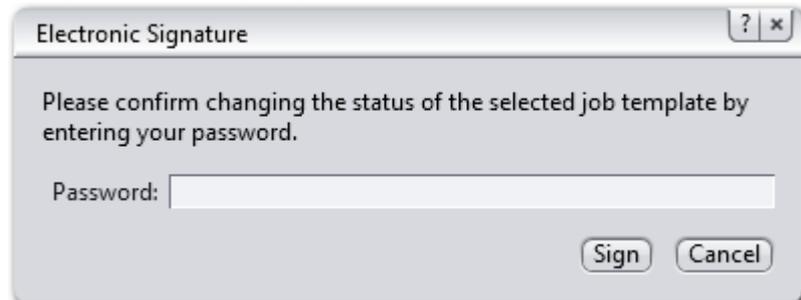


The dialog for the electronic signature opens.



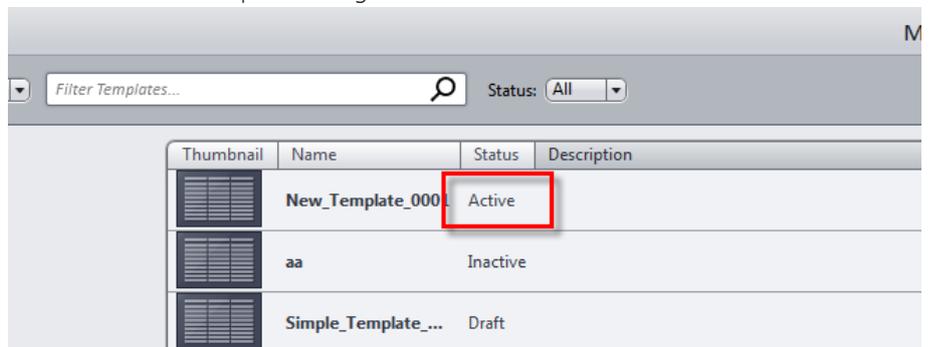
- 3 Click on **Change status**.

Now you must enter your password to confirm the action.



- 4 Click on **Sign**.

The status of the template changed to **Active**.



You have successfully changed the status of the job template. The template is now visible for operators and can be executed. When the job template is executed, all actions and changes will be logged in the Audit Trail.

15 Macros and Automation

15.1 Basics of Macros

Macros are part of the Open Application Development (OAD) framework and enhance the ZEN 2 core software.

Macros can be used for the following tasks:

- Control every aspect of the user interface, such as automating repetitive tasks or routine tasks composed of several steps
- Interact with external software, such as ImageJ/Fiji

The **OAD Macro** tool enables you load, preview, and run macros which are available on your system. ZEN 2 core contains the **Macro Editor**, which enables you to edit, run, or debug macros according to your needs or to write macros from scratch. The modular software interface of ZEN 2 core means that you can write macros but not record them automatically.

Python is used as the programming language for macros. The programming interface is implemented in the **Macro Object Model**. To open the **Macro Object Model** documentation, start the **Macro Editor** and navigate to **Help > Macro Object Model...**

15.2 Macro Editor

The **Macro Editor** is an integrated development environment that enables you to create, edit, run, and debug macros.

Edit and create macros The available macros are managed in the **User Documents** area on the right of the **Macro Editor**.

If desired, you can create new macros, which are then added to the **User Documents** and made available on the system.

The central editor component supports syntax highlighting for Python, which is the language used for macros in ZEN 2 core.

Run and debug macros The **Macro Editor** contains debugging functionality such as debugging line-by-line or using breakpoints.

The **Watch** tab in the bottom area of the **Macro Editor** enables you to type the macro functions or variables you wish to observe while debugging a macro.

15.3 Creating a Macro

Prerequisites ■ The **OAD Macros** workbench is selected.

Procedure 1 Create a new macro using the toolbar below the list of macros (**User Documents**).

A newly created macro is given a standard name composed of the string "Macro-" plus an ascending number.

2 Rename the macro and give it a meaningful name

3 To edit the macro open it in the **Macro Editor**.

The first lines of a macro are displayed in the **Preview** section of the **OAD Macro** tool. Therefore, it is recommended to begin the macro with a comment of the macro's main features.

15.4 Managing Macros

To manage macros on your system:

Procedure 1 Expand the **Selection** section of the **OAD Macro** tool.

2 Perform the desired function from the toolbar.

Function	Description
	Creates a new macro A newly created macro is given a standard name composed of the string "Macro-" plus an ascending number. It is recommended to rename the macro and give it a meaningful name.
	Deletes the selected macro from the system
	Contains options to modify macros
– New	Creates a new macro A newly created macro is given a standard name composed of the string "Macro-" plus an ascending number. It is recommended to rename the macro and give it a meaningful name.
– Duplicate	Creates a copy of the macro. The copied macro is named automatically.
– Save	Saves changes to a macro in the archive If there are any unsaved changes, a macro is marked with an asterisk after its name and cannot be renamed.

Function	Description
– Save As...	Enables you to save a copy of the macro and to give the copy a different name.
– Rename	Use Rename after creating or duplicating a macro to give the new macro a meaningful name.
– Delete	Deletes the selected macro from the system

INFO

Right-clicking anywhere inside a tool, including the list of macros (**User Documents**), allows you to close the tool. To modify macros, use the options icon from the toolbar below **User Documents**.

15.5 Running a Macro

Prerequisites ■ The **OAD Macros** workbench is selected.

Procedure 1 Select the desired macro in the **Selection** section of the **OAD Macro** tool.
If you wish to load a macro you have available in a file, you need to add it to ZEN 2 core first. For more information, see *Importing and Exporting a Template* [▶ 163].

2 Open the **Macro Editor**

3 Click the **Run** button.

If the macro does not end automatically or does not work properly, you can enforce termination by clicking the **Stop** button.

15.6 Debugging a Macro

Macros are debugged from within the **Macro Editor**.

Prerequisites ■ The macro to be debugged is opened in the **Macro Editor**.

Procedure ◆ Right-click a variable name in the macro source code and select **Add Watch**.
The variable is added to the **Watch** tab.

After entering all variables and functions to be monitored, you can debug the macro using one of following actions:

Action	Description
Run	Runs the macro without debugging
Debug	Runs the entire macro in debug mode

Action	Description
	Execution of the macro halts at previously defined breakpoints.
Step Over	Runs the macro line by line If the current line contains a function call, the function is executed. The debugger continues in the next line of the macro.
Step Into	Runs the macro line by line If the current line contains a function call, the debugger jumps into the function. The function is then executed line by line. After the function has finished, the debugger jumps back into the macro to the next line after the function call.
Stop	Halts the debugger at the current line.
Reset	Deletes any macro or debugger memory entries Use reset to ensure a clean debug run after having debugged before, e.g. if you made corrections to the code and wish to debug anew.
Breakpoint	Marks the current line as a breakpoint If you wish to use simple debugging instead of the line-by-line debugging methods, execution halts at a breakpoint. This enables you to monitor your previously defined variables and functions at predefined positions. Debugging this way is usually faster than using the line-by-line debugging methods, but requires knowledge about where to look for possible bugs.
Set Line	Runs and debugs the current line, i.e. the line of the current cursor position.

16 Archive and Template Management

16.1 Archive

The **Archive** is the central location for storing and managing result data and templates.

Access to the items in the **Archive** depends on the current user's privileges, which can be set up in the software for each user of the system.

ZEN 2 core supports the following archives:

- **Local Archive:** suitable for a single system (free)
- **IMS:** client/server archive solution

16.2 Manage Templates

16.2.1 Basics of Template Management

The **Archive** is the central location for managing the following items:

- Job templates
- Form templates
- Report templates
- Custom workbenches
- Automatic measurements
- Macros

When a user's workflow requires selecting one of the above items (e.g. choosing a job template to run or which report template to use), the user is presented with the items stored in the **Archive**. In general, users only interact with items in the **Archive**; they do not need to interact with the file system.

The items in the **Archive** can be managed as follows:

- Edit
Modify an existing item
- Copy
Duplicates an item so that
- Add to a category
Categories enable users to filter the items

- Import / export to the file system

Enables you to add an item from the file system to the **Archive** or vice versa, for example to exchange items with other systems
- Modify access rights

Specify which users / groups can use the item
- Modify further properties (name, description)

Enable users to find the desired item easily

16.2.2 Editing and Copying Templates

You can edit or copy an existing template as follows:

Prerequisites ■ The **Manage Templates** mode is selected.

- Procedure**
- 1 Select the type of template to be edited using the **Show** drop-down.
 - 2 Select the template to be edited.

If you know the name of the template you can enter the first few letters in the **Filter** to reduce the number of entries.
 - 3 Right-click and select the desired function:
 - **Edit**: Opens the selected template so you can change its values or settings. When you are finished editing the existing template is overwritten with the new values.
 - **Copy & Edit**: Copies the template and then opens the copy for editing.
 - **Delete**: Deletes the selected template.

16.2.3 Supported Template Formats

Templates contain predefined content, for example the tasks required to run a job, or the fields required to complete a form. You can import and export the templates.

The following file formats are supported for each type of template:

Template type	File extension
Job templates	.czjob
Form template	.formconfig
Report templates	.docx
Custom workbenches	.czjob
Automatic measurements	.czias
OAD Macros	.czmac

16.2.4 Modifying Template Properties and Category

Each template has the following properties:

- Name

The name must be unique within the **Archive**

- Description

This should be a meaningful explanation of what the template is used for. It should enable users to select the correct template.

Furthermore, each template can be assigned to a category. Categories enable you to group similar templates together. You can create any number of categories, e.g. for types of sample or institutions in your company. However, each template can only be assigned to a single category. You cannot modify or delete existing categories.

Prerequisites ■ The **Manage Templates** mode is selected.

Procedure 1 Select the type of template to be edited using the **Show** list.

2 Select the template to be edited.

If you know the name of the template you can enter the first few letters in the **Filter** to reduce the number of entries.

3 Modify the **Name** and **Description** as desired.

4 Select the category to which the template should be assigned.

To create a new category, click **Add** and enter the name.

16.2.5 Modifying Template Permissions

For each template you can specify which users can access it. Typically, a group of users is specified, but you can also specify individual users. By default, each template can be performed by any user.

Permissions can be specified using the following methods:

- ZEN 2 core users and user groups

- Windows Active Directory or IMS users

Prerequisites ■ The **Manage Templates** mode is selected.

Procedure 1 Select the type of template to be edited using the **Show** list.

2 Select the template to be edited.

If you know the name of the template you can enter the first few letters in the **Filter** to reduce the number of entries.

3 Click the **Save** button.

4 Click **Allow only selected users**.

Initially the list is empty.

- 5 Specify users or roles using one of the following methods.
The methods cannot be combined.

Zeiss users and user groups To specify permissions based on ZEN 2 core users and user groups:

- Procedure**
- 1 Click **++**.
 - 2 Select the user(s) and/or group(s) that can access the template.
 - : Users
 - : User roles
 - 3 To select multiple entries, press the *CTRL* key.

Windows Active Directory To specify permissions based on Windows Active Directory settings:

- Procedure**
- 1 Click **AD++**.
The standard Windows Active Directory dialog box is displayed.
 - 2 Specify the categories to be used to define permissions (e.g. users, user groups).
 - 3 Enter the name(s) of the Windows user(s) or user group(s) that can access the template.
For more information, see the Windows instruction manual.

16.2.6 Importing and Exporting a Template

Templates stored in the **Archive** can be accessed by any user of the system or, if the microscope is connected to a network, by any user in the network. If you want to transfer a template between non-connected systems, you can export it from one **Archive** and import it the other **Archive**.

In contrast to all other templates, report templates cannot be edited within ZEN 2 core. If you want to modify a report template, you have to export it so that you can open it in Word and use the Add-In to modify the report. Once you have modified the report template you can then import it back into the **Archive**.

Prerequisites ■ The **Manage Templates** mode is selected.

- Procedure**
- 1 Select the type of template to be edited using the **Show** list.
 - 2 Select the template to be edited.
If you know the name of the template you can enter the first few letters in the **Filter** to reduce the number of entries.
 - 3 Click  and select the desired function:
 - **Export**
 - **Import**

- 4 Select the desired file format and location on the file system.

If you import a template with the same name as an existing template you can choose to overwrite it or import the template with a new name.

16.3 Browse Job Results

16.3.1 Basics of Job Results

Each time a job template is run and completed, a job result is created and stored in the **Archive**. The job result contains all the images and measurement results that the user acquired or performed.

In **Browse Job Results** mode you can view and manage the job results, for example to check the quality of measurements performed by a user or to transfer job results to another system. You can also load a job result, change data, and store it under a new name, for example to correct a measurement.

16.3.2 Filtering and Selecting Job Results

Typically the **Archive** contains a large number of job results. You can filter the list to help you find the desired result quickly.

Prerequisites ■ The **Browse Job Results** mode is selected.

- Procedure**
- 1 If you wish to search for a text in all fields, enter it in the **Filter** text box. The search starts automatically once you begin typing.
 - 2 If the list of job results is too long you can use advanced filter options: click **Edit Filter**.
 - 3 Use **Show/Hide Fields...** to activate the desired filtering fields.
 - 4 Activate the checkboxes of the properties to be filtered.
 - 5 For each property, select a condition and value:
 - **=**: The property value must be identical to the value
The value is not case-sensitive
 - **< / >** The property must be smaller (<) or greater (>) than the value
 - **<= / >=** The property must be smaller/equal (<) or greater/equal (>) than the value
 - **Contains** The value must be contained somewhere in the property (e.g. "mag" matches "image")
The value is not case-sensitive
 - **Between** The property must be between the lower and upper values
 - **Before / After**
The date of the property must be before/after that of the value

- 6 Click **Apply Filter**.
- 7 Select the desired job result in the list.

16.3.3 Viewing, Editing, and Deleting Job Results

Each time a job template or a free mode session is run and completed, a job result is created and stored in the **Archive**. You can view these job results, for example to check their accuracy. Furthermore, you can also change data in a job result and store it under a new name, for example to correct a measurement. Lastly you can delete a job result if it is no longer needed (for example to remove duplicates).

Prerequisites ■ The **Browse Job Results** mode is selected.

- Procedure**
- 1 Select the desired job result.
You can filter the job results if the list is too long.
 - 2 To check the job result, click **View**.
You can view all the documents in the job and the corresponding results. However, you cannot make any changes.
 - 3 To create and modify a copy of the job result, click **Edit**.
You can view the results in free mode, continue your work, or change results and save the job result under a new name.
 - 4 To permanently remove the job result, click **Delete**.
This action cannot be undone.

16.3.4 Importing and Exporting Job Results

Job results stored in the **Archive** can be accessed by any user with corresponding rights or, if the system is connected to an IMS server, by any user of the IMS using a ZEN 2 core client. If you want to transfer a job result between non-connected systems, you can export it from one **Archive** and import it the other **Archive**.

Prerequisites ■ The **Browse Job Results** mode is selected.

- Procedure**
- 1 Select the desired job result.
If you know the name of the template you can enter the first few letters in the **Filter** to reduce the number of entries.
 - 2 Click on **Options** and select the desired function:

 - **Export**
 - **Import**
 - 3 Select the desired file format and location on the file system.
If you import a job result with the same name as an existing job result you can choose to overwrite it or import the job result with a new name.

16.3.5 Adding documents to a job result

It is also possible to add further images / documents to existing job results. To do this, proceed as follows:

- Prerequisites**
- You are in **Free Examination** mode.
 - You have acquired an image and created other documents, e.g. a report.

- Procedure**
- 1** Click on the **Save** icon right to the **Home** button.
The **Save results** dialog opens.
 - 2** Skip to the **Add to results** tab.
 - 3** Select the job result you want to add the new image to.
 - 4** Click on **Save**.

The image and documents is saved to the existing job results.

17 Display Settings

17.1 Configuring the Center Screen Area

You can configure the appearance of the **Center Screen Area** according to your preferences:

Property	Function
Zoom	<p>Adjusts the digital zoom factor of the image.</p> <ul style="list-style-type: none"> ■ In / out enlarges the image in steps of 125% of the current size or reduces the image in steps of 80% of the current size ■ 100 % zooms to a 1:1 depiction of the image ■ Fit to view scales the image to completely fill the Center Screen Area.
Rulers	<p>Shows vertical and horizontal rulers next to the image.</p> <p>The zero point is the top left corner of the Center Screen Area.</p>
Viewport scale bar	<p>Shows the current scale of the image.</p> <p>This is derived from the hardware setup (e.g. the magnification of the objective). You can also configure the scaling manually.</p>
Navigator	<p>Shows a miniature overview of the entire image.</p> <p>A frame indicates the section currently displayed. Drag the frame to navigate the image.</p>
Graphics	<p>Shows all measurements or annotations in the image.</p>

To adjust the appearance:

- Procedure**
- 1 Right-click in the right tool area outside the image.
 - 2 Select the desired option.

17.2 View Options

To change the appearance of the image in the **Center Screen Area** you can apply a number of image settings in the **View Options**. The **View Options** are located at the bottom of the **Center Screen Area**.

You can adjust the following:

Parameter	Description
Gamma	<p>Defines whether details in brighter or darker image regions are enhanced. Setting the gamma value causes the value of each pixel to be multiplied by an individual factor. This factor depends on the pixel value (brightness) itself.</p> <ul style="list-style-type: none"> ■ Value < 1: Details in dark image regions are enhanced. ■ Value > 1: Details in bright image regions are enhanced.
Mode	<p>This setting specifies the range of visible gray values. Gray values below this range are displayed black, gray values above the range are displayed white. Can help solve display issues, e.g. if an image appears completely black.</p>
– Auto	Automatically adjusts the range of displayed pixel values using the selected method: Min/Max or Best Fit .
– Min/Max	Sets the contrast to normal: the range of displayed pixel values matches the minimum and maximum pixel values occurring in the image.
– Best Fit	Increases the contrast: the range of displayed pixel values is limited by the pixel values resulting from the Best fit: Black % and Best fit: White % thresholds defined in the Histogram tool.
Channels	<p>Allows you to set the currently displayed channel and enables you to assign a single color to it or to display the selected channel in false colors using a look-up table.</p> <p>Color images are temporarily converted to a grayscale image before a color or look-up table is assigned to the resulting single grayscale image. The red, green, and blue channels of a color image can not be modified individually. However, if you have a multi-channel image, you can apply different colors to each channel.</p>
– Color	Displays the image in a predefined color. Each pixel is displayed in a shade of the selected color, corresponding to the pixel's brightness.
– LUT	Displays the image in false colors of your choice. The false colors are stored in predefined look-up tables (LUT). Each pixel is displayed in one of the colors stored in the look-up table, depending on the pixel's brightness.

Parameter	Description
– Cust...	Displays the image in a user-defined color selected from the RGB color palette. Each pixel is displayed in a shade of the selected color, corresponding to the pixel's brightness.
– None	The image is displayed as is.
Single Channel	The channels are displayed separately.
Range Indicator	Activated: Bright pixels that appear to be overexposed are displayed red and dark pixels that appear to be underexposed are displayed blue. The Channels settings are deactivated.

INFO

The **View Options** settings are only applied to the preview image to support you during acquisition, e.g. to optimize the acquisition parameters or to find regions of interest. If you wish to apply the settings to the image, use the appropriate image processing tool.

18 Workbench and Tool Reference

18.1 Overview

The software contains pre-configured workbenches for the typical tasks in the software. Each workbench contains one or more tools.

This reference describes the features of each workbench, the tools it contains, and how to use each tool.

The workbenches and tools are grouped into categories to help you find the required item more easily.

18.2 Categories

18.2.1 Acquisition Workbenches

This workbench category enables you to acquire images using various methods or to load existing microscope images from the file system.

INFO

Due to the high number of tools and for better readability, the information is spread across two tables. Hence, you need to read both tables to find out which tools are available in each workbench.

The following table shows which tools are available for each workbench in the **Acquisition** category.

Workbench	Tools
Camera	Extended Camera
Focus	Focus
Hardware	Hardware
Histogram	Histogram
Lamp	Lamp
Light Path	Light Path
Magnification	Magnification
Software	Software
Stage	Stage

Workbench	Tools									
2D Acquisition	x	o	o	o	o	o	o	o	o	o
2D Acquisition (automatic)	o	o	o	o	o	o	o	o	o	o
Best Image	o	o	o	o	o	o	o	o	o	o
EDF (manual focus)	x	o			o	o	o	o		
EDF (motorized focus)	x	o	o	o	o	o	o	o	o	o
Link Acquisition	x	o	o	o	o	o	o	o	o	o

Workbench	Tools										
Load Image											
Panorama	x	o				o	o	o	o		
Position List	x	o	o	o	o	o	o	o	x	o	o
Tiles (interactive)	x	o	o	o	o	o	o	o	x	o	o
Tiles (manual)	x	o	o	o	o	o	o	o	x	o	o
Tiles with EDF (interactive)	x	o	o	o	o	o	o	o	x	o	o
x: available by default											
o: optional											

Workbench	Tools								
	MotORIZED EDF	Manual EDF	Tiles setup (interactive)	Tiles setup (manual)	Stitching	Link am	Loaded Image	Best Image	Position List
2D Acquisition									
2D Acquisition (automatic)									
Best Image								X	
EDF (manual focus)		X							
EDF (motorized focus)	X								
Link am Acquisition						X			

Workbench	Tools			
Load Image				X
Panorama			X	
Position List				
Tiles (interactive)		X	X	X
Tiles (manual)			X	X
Tiles with EDF (interactive)	X	X	O	X
x: available by default				
o: optional				

18.2.2 Calibration Workbenches

This workbench category enables you to create a new scaling by measuring an object of known length.

18.2.3 Documents Workbenches

This workbench category enables you to import and export images, documents, and measurement results.

The following table shows which tools are available for each workbench in the **Documents** category.

Workbench	Tools									
	Load Image	Load Table	Save Image	Save Table	Save Report	Image Export	Movie Export	OME TIFF Export	ZVI Export	
Load File	x	x								
Save File			x	x	x	o	o	o	o	

x: available by default
o: optional

18.2.4 Measurement Workbenches

This workbench category enables you to measure images as follows:

- **Automatic Measurements**

Enable you to analyze simple shapes based on their gray values

For example, you can automatically identify, count, and classify particles in the sample according to their size or color.

- **Interactive Measurements**

Enable you to measure distances, angles, area, and intensities of pixels

18.2.5 Processing Workbenches

This workbench category enables you to optimize the appearance of an image, manipulate tables of measurement results, add a scaling retrospectively or select whether a custom scaling or a theoretical scale is used for an image.

18.2.6 Reporting Workbenches

This workbench category enables you to perform the following tasks:

- Create and manage form templates
- Open and manage report templates

The following table shows which tools are available for each workbench in the **Reporting** category.

Workbench	Tools			
	Add Templates	Form Templates Management	Form Designer	Form Fields Settings
Forms	x			
Reports		x	¹⁾	¹⁾

x: available by default

¹⁾ Only available after selecting a form template and clicking the **Edit** button

18.2.7 Utilities Workbenches

This workbench category contains workbenches to manage extensions and database connections.

The following table shows which tools are available for each workbench in the **Utilities** category.

Workbench	Tools		
	Image Export IMS	Linkam Heating Stage Control	OAD Macro
Image Export to IMS	x		
ImageJ Connection			
Linkam Heating Stage		x	
OAD Macros			x

x: available by default

18.2.8 Custom Workbenches

The software contains pre-configured workbenches for the typical tasks in the software. However, you can also create a custom workbench that contains the exact tools you require for your tasks.

This workbench category contains a list of all available custom workbenches.

18.3 Workbenches

18.3.1 Acquisition

18.3.1.1 2D Acquisition (automatic) Workbench

This workbench enables you to acquire an image quickly with the most common settings and automatic camera exposure.

If you wish to change the settings, you can add all tools required for controlling the hardware such as camera tools, focus tools, stage control tools, or tools to change other microscope components settings.

18.3.1.2 2D Acquisition Workbench

This workbench enables you to acquire an image using advanced settings according to your requirements. The camera tool, which enables you to control camera settings such as exposure or white balance, is visible by default.

If you wish to change more settings, you can add all tools required for controlling the hardware such as focus tools, stage control tools, or tools to change parts of the microscope.

18.3.1.3 Best Image Workbench

The **Best Image Acquisition** workbench enables you to acquire several versions of the same image. Different predefined display options are applied to the individual images. You can then select the most suitable one using the **Best Image** tool.

18.3.1.4 EDF (manual focus) Workbench

This workbench enables you to acquire an extended depth of focus (EDF) image if a manual focus drive or stage is installed on your microscope.

The software creates a single image from individual images acquired at different Z positions of the stage. The depth of focus of the resulting EDF image corresponds to the focus range of the individual images covered during acquisition. Stage movement and acquisition of the individual images is controlled manually.

18.3.1.5 EDF (motorized focus) Workbench

This workbench enables you to acquire an extended depth of focus (EDF) image if a motorized z drive is installed on your microscope.

The software creates a single image from individual images acquired at different Z positions of the stage. The depth of focus of the single image corresponds to the focus range of the individual images covered during acquisition. The stage movement and acquisition of the individual images is controlled automatically.

18.3.1.6 Linkam Acquisition Workbench

This workbench enables you to acquire a series of images at different temperatures. You can combine time-dependent or temperature-dependent acquisitions into one experiment.

The result is a series of images at each temperature/time, as well as a temperature profile of the experiment. You can display the corresponding image for each data point of the temperature profile.

18.3.1.7 Load Image Workbench

This workbench enables you to open an image from a storage device, e.g. a previously acquired and saved image.

18.3.1.8 Panorama Workbench

This workbench enables you to acquire an oversize image with a manual stage, for example if the total sample area to be acquired exceeds the area that can be acquired with a single acquisition. You can manually acquire multiple images (tiles) of neighboring areas on the sample, which then are assembled to an oversize image.

You can save the oversize image as tiles or you can stitch the tiles together into a single oversize image.

18.3.1.9 Position List Workbench

This workbench enables you to define a set of images to be acquired at different positions of the sample. This is useful if you process a set of almost identical samples and know the positions of the sample areas from which you wish to acquire images.

The software moves the stage automatically to the defined positions and acquires an image at each position. The acquired images are stored into a single file along with their position information.

18.3.1.10 Tiles (interactive) Workbench

This workbench enables you to acquire an image of a large sample area composed of tiles: a tile corresponds to the area the camera is able to acquire with a single acquisition.

You can specify multiple areas of interest on your sample. The software defines the total area which needs to be acquired and thus the required number of tiles. The tiles are then acquired automatically (the stage movement is controlled by the software) and merged into a single image.

18.3.1.11 Tiles (manual) Workbench

This workbench enables you to acquire an image of a large sample area composed of tiles: a tile corresponds to the area the camera is able to acquire with a single acquisition.

You specify the area of interest on your sample you wish to acquire by defining a corresponding region in the software. You can define the region by the number of tiles to be acquired or by the size in micrometers. The tiles are then acquired automatically (the stage movement is controlled by the software) and merged into a single image.

18.3.1.12 Tiles with EDF (interactive) Workbench

This workbench enables you to acquire an image of a large sample area by specifying different positions on your sample which you wish to combine into a large image. The software shows a preview of the area to be acquired and splits it into tiles: a tile corresponds to the image area the camera is able to acquire with a single acquisition.

The stage movement and acquisition of the individual tiles is controlled automatically.

In addition, each tile is acquired at different focus depths, i.e. Z positions of the stage. As a result, a stack of tiles is acquired for each position, which is stitched together into one tile with an extended depth of focus (EDF).

18.3.2 Calibration

18.3.2.1 Create Measured Scaling Workbench

This workbench enables you to create a new scaling definition by measuring an object of known length.

18.3.3 Documents

18.3.3.1 Load File Workbench

This workbench enables you to load an image or table from the file system. You can then use the imported image or table in the current experiment.

18.3.3.2 Save File Workbench

This workbench enables you to save the following objects to the file system in various supported formats.

- Images
- Measurement results
- Reports

Images and tables can also be exported in other file formats. In addition to specifying a file format, exporting enables you to adjust various other parameters such as scaling, compression, how annotations (graphics) are treated.

18.3.3.3 Load Multiple Images Tool

This tool enables you to load multiple images all at once from the file system. You can load any supported image file.

Parameter	Description
File Names	Shows the path of the images to be loaded. Click on  to open the file browser and select the desired images. To select a range of images hold down the <i>Shift</i> key and click on the first and the last image of the range. Alternatively you can hold down the <i>Alt</i> key to select multiple single images.

18.3.4 Measurement

18.3.4.1 Automatic Measurement Workbench

This workbench enables you to analyze simple shapes based on their gray values. For example, you can automatically count and classify particles in the sample according to their size or color.

18.3.4.2 Interactive Measurements Workbench

This workbench enables you to measure distances, angles, area, and intensities of pixels in images. You can also save the measurements results tables and images.

Under **Favorites** you can save your favorite measurement tools for fast access. Simply click on **+ Add Tool** to see all available tools. Then move the desired tool per Drag & Drop on the favorites bar.

18.3.5 Processing

18.3.5.1 Image Processing Workbench

This workbench enables you to optimize the appearance of an image by applying various image processing tools, for example to reduce noise or enhance a region of interest.

18.3.5.2 Table Processing Workbench

This workbench enables you to merge results tables or create a histogram based on measurement results.

18.3.5.3 Assign Measured Scaling Workbench

This workbench enables you to calculate the scale for images acquired with microscopes where the individual hardware components can be detected automatically.

18.3.5.4 Assign Pixel Size Workbench

This workbench enables you to assign a scale to an image retrospectively, for example if the image does not contain a scale recognized by the software because it was created on another device.

18.3.5.5 Assign Theoretical Scaling Scaling Workbench

This workbench enables you to calculate the scale for images acquired with manual hardware (i.e. a microscope where the individual hardware components cannot be detected automatically).

18.3.6 Reporting

18.3.6.1 Forms Workbench

The **Forms** workbench enables you to create and manage form templates.

Forms provide a simple way to add information to a job when it is run. Information can be entered into a form automatically (e.g. current time and date) or manually by the operator (e.g. current sample number).

18.3.6.2 Reports Workbench

The **Reports** workbench enables you to open and use report templates and generate reports.

Reports enable you to collate all the information from your examination in a single document. This document serves as a protocol of your examination.

18.3.7 Utilities

18.3.7.1 Linkam Heating Stage Workbench

This workbench enables you to control the temperature and vacuum of the Linkam heating stage.

18.3.7.2 OAD Macros Workbench

This workbench enables you to load, preview, and edit macros.

18.3.7.3 ImageJ Connection Workbench

This workbench enables you to send images to ImageJ or to retrieve (processed) images from ImageJ.

Parameter	Description
Send Image	Sends the current image to ImageJ and enables you to apply image processing in ImageJ.

Parameter	Description
	ImageJ might display a comprehensive import options window, depending of the type of the sent image. For more information, see the ImageJ online help.
Retrieve Image	Retrieves the image of the current ImageJ window.

18.3.8 Shuttle & Find

18.3.8.1 S&F Holder Calibration Workbench

This workbench enables you to calibrate your correlative holders before starting the image acquisition. On an LM system, the **Camera** tool, which enables you to control camera settings such as exposure or white balance, is visible by default. Other tools to control the microscope can be added to the workbench.

18.3.8.2 S&F Overlay Workbench

This workbench enables you to create overlay images from different microscopes e.g. LM and SEM image files.

18.3.8.3 S&F ROI/POI Drawing Workbench

This workbench enables you to draw in ROIs or POIs to the image.

18.4 Tools

18.4.1 Acquisition

18.4.1.1 Best Image Tool

This tool enables you to select an image from the set of images acquired with the **Best Image** workbench.

Parameter	Description
Select Image	Selects the image which is used throughout the job. If this button is grayed out, select an image of the set by left-clicking it. If no images are visible at the bottom of the Center Screen Area , acquire a set of images using the Start button from the Best Image workbench.

18.4.1.2 Camera Tool

This tool is the basic tool to control the camera. It contains options for exposure time and white balance of the acquired images.

Parameter	Description
Exposure Time	Enables you to control the exposure settings of your camera
– Auto / Man	<ul style="list-style-type: none"> ■ Auto: The exposure time is calculated automatically every time an image is acquired. ■ Man: If you are not satisfied with the automatic result, you can adjust the measured exposure time manually. The exposure time specified this way can be changed and adjusted manually at any time.
– Time	<p>Duration of the acquisition</p> <p>The weaker the illumination of the sample, the longer the required exposure time.</p>
– Measure	Measures the exposure time manually once, which is used for all subsequent images. The exposure time determined this way can be changed and adjusted manually at any time.
– Intensity	<p>Enables you to compensate for underexposure or overexposure if you are not content with the auto exposure result</p> <ul style="list-style-type: none"> ■ 5% - 100%: Darkens the image (compensates for overexposure) ■ 100% - 200%: Brightens the image (compensates for underexposure)
White Balance	<p>Only visible, if you are using a color camera.</p> <p>Enables you to adjust the colors of an image.</p> <p>White Balance enables you to remove a color cast (e.g. a red or green tint) from the live image that may result from non-neutral lighting. As a result the colors appear neutral.</p>
– Auto	Selects the reference point for white balance correction automatically and adjusts the hue of all other pixels accordingly. To use this function properly, a color neutral position must be visible on the sample. If the camera's field of view is full of colored structures, navigate to a color neutral spot or insert a color neutral surface under the objective.
– Pick...	Enables you to specify the reference point for white balance correction manually. The hue of all other pixels is adjusted accordingly.

Parameter	Description
	To achieve an optimum result, pick a color neutral spot (white or gray) on the sample.
– Reset	Resets any color changes and sets the white balance value to 6500 K.
Spot Meter / Focus ROI	Activated: The exposure time and focus measurements use the intensity values within a specified area instead of the entire camera sensor area. This improves the results for the area to be acquired.

18.4.1.3 Extended Camera Tool

This tool enables you to apply advanced settings to the camera. Besides the usual options for exposure time, you can set different image properties or select a region of interest of the total camera sensor area. In addition, the tool contains a number of post processing options and camera-specific settings.

Exposure Time

If you use automatic exposure, you can select an area on the camera sensor which is used to calculate the exposure time.

Parameter	Description
Time	Specifies the duration of the image acquisition. Select the unit of time (min, ms, s, μ s) from the drop-down list on the right and enter the desired value.
Auto Exposure	<ul style="list-style-type: none"> <input checked="" type="checkbox"/> Activated: The exposure time is calculated automatically every time an image is acquired. The exposure time in the corresponding input field fluctuates accordingly. <input type="checkbox"/> Deactivated: You can set the exposure time manually.
Set Exposure	Starts a one-off measurement of the exposure time, which is then used for all subsequent images. Deactivates Auto Exposure . If you are not satisfied with the result, you can adjust the measured exposure time manually.
Auto Exposure Intensity	Enables you to compensate for underexposure or overexposure if you are not content with the auto exposure result

Parameter	Description
	<ul style="list-style-type: none"> ■ 5% - 100%: Darkens the image (compensates for overexposure) ■ 100% - 200%: Brightens the image (compensates for underexposure)
Spot Meter / Focus ROI	<p>Activated: The exposure time and focus measurements use the intensity values within a specified area instead of the entire camera sensor area. This improves the results for the area to be acquired.</p> <p>If the red Spot Meter / Focus ROI frame is not visible in the live image, right-click in the live image and select Spot Meter / Focus ROI from the context menu.</p>

Binning

Parameter	Description
Binning	<p>Binning combines the information of neighboring camera pixels into a single larger pixel. The camera sensitivity is increased by improving the signal-to-noise ratio, but the resolution is decreased by the same factor.</p> <p>For example, if the binning is set to 2×2, the analog signal (charge) of four pixels are combined before digitization. The camera sensitivity is increased by a factor of four but the resolution is reduced by a factor of four.</p> <p>Increasing the binning means weaker signals can be detected for a given exposure time.</p>

18.4.1.3.1 White Balance Section

This section is only visible if you are using a color camera. The section enables you to adjust the color balance to a neutral hue independent of the light source used.

Save suitable white balance settings using the **Settings** section to ensure color reproducibility of images acquired in the future.

Parameter	Description
Auto	Compensates for the color temperature of the light source automatically to yield a neutral hue

Parameter	Description
	<p>The entire camera sensor area is measured. If there are no pure white areas on the sample and Auto does not yield the desired results, measure and compensate for the color temperature of the light source as follows:</p> <ul style="list-style-type: none"> <li data-bbox="778 539 1442 680">■ Transmitted light: Move the sample such that a clear and transparent region is illuminated or remove the sample from the microscope. Click the Auto button to perform the auto white balance. <li data-bbox="778 707 1442 813">■ Reflected light: Use a neutral surface (e.g. a piece of white paper) as a sample. Click the Auto button to perform the auto white balance. <p>You can now acquire white balanced images of your sample with the above settings.</p>
Pick...	<p>Enables you to select a reference pixel for white balance from the live image</p> <p>The selected pixel should be neutral white.</p>
3200 K	<p>Applies a predefined color balance setting to compensate for the color temperature of a halogen light source at approximately 3200 K</p>
5500 K	<p>Applies a predefined color balance setting to compensate for the color temperature of an LED light source at approximately 5500 K</p>
Show Channels	<p>Enables you to set the color balance of each color channel (red/cyan, green/magenta and blue/yellow) individually to make the image appear neutral</p>
Color Temperature	<p>Changes the overall color temperature of the image from cool (blue cast) to warm (red cast)</p> <p>The color channels (red/cyan, green/magenta and blue/yellow) are adjusted automatically. The Color Temperature setting can work against the settings applied using Show Channels.</p> <p>Use Color Temperature for fine tuning in combination with Pick... if Pick... does not give perfect results.</p>
Saturation	<p>Changes the colorfulness of the image</p>
Reset	<p>Resets any color changes and sets the white balance value to 6500 K.</p>

18.4.1.3.2 Acquisition ROI Section

This section enables you to define a region of interest (ROI) on the camera sensor which is used for acquisition. A smaller ROI can increase the acquisition speed.

The region of interest is indicated by a blue frame in the preview window and can be moved and resized freely. The preview window always shows the entire camera sensor area which can be acquired.

The **Pixel Size** shown below the preview window indicates the size in μm to which a pixel corresponds. This depends on the camera sensor properties and on the binning.

Parameter	Description
Maximize	Selects the entire available image sensor area as the region of interest
Center	Positions the region of interest precisely at the center of the image
Size	Sets the width and height of the region of interest in pixels
Offset	Specifies the position of the top left corner of the Acquisition ROI (blue frame) with respect to the top left corner of the preview window.
Refresh Overview	An image is acquired and displayed in the preview window with the current ROI settings. This has no effect on the image in the Center Screen Area .

18.4.1.3.3 Post Processing Section

This section allows you to apply basic image processing functions (e.g. for image enhancement) while acquiring the image. This can be helpful if certain image processing steps are necessary for any acquired image and saves image processing work later in a job. It also enables you to compensate for constant offsets impeding the image quality.

Depending on the camera model, different settings are available. The following image processing functions are the most common:

- Black Reference
- Shading Correction
- Noise filter
- Unsharp mask

Parameter	Description
Black Reference	<p>A camera-specific correction that compensates for camera sensor faults such as individual bright pixels that can occur at long exposure times (approximately 5 seconds and longer)</p> <p>This correction is recommended for applications that involve low-light conditions and thus long exposure times (e.g. live cell imaging, fluorescence imaging).</p> <p>Activated: The acquired image correction data is applied to each acquired image.</p>
– Define	<p>Acquires the reference image that is applied to subsequent images if Black Reference is activated. The reference image should be updated at certain intervals since the camera sensor properties could change over time.</p> <p>In order to measure the reference image make sure that no light can hit the camera sensor. Ideally, remove the camera from the microscope and seal it with its cap before performing the measurement.</p>
Shading Correction	<p>Compensates for uneven exposure of an image. The uneven exposure (shading) might be caused by non-uniform illumination (e.g. vignetting) or dirt and dust on glass (lens) surfaces.</p> <p>Activated: The last acquired reference image is applied to each acquired image, depending on the Global/Specific setting.</p>
– Global	<p>Shading correction is applied only to images that are acquired with the same objective as the reference image. The following components are taken into account:</p> <ul style="list-style-type: none"> ■ Objective and Optovar ■ Camera bit depth and RGB/BW mode ■ Camera model and port position <p>This corresponds to objective-specific shading correction and is the standard setting. Fluorescence-specific components are not taken into account.</p> <p>To have a full set of reference images, you have to acquire a separate reference image for each objective available in the microscope. If you are using a motorized or coded objective revolver and no reference image is available for the current objective, Shading Correction is deactivated automatically.</p>

Parameter	Description
<ul style="list-style-type: none"> – Specific 	<p>Shading correction is applied only to images that are acquired under the same fluorescence settings as the reference image. The following components are taken into account:</p> <ul style="list-style-type: none"> ■ Contrasting method and condenser ■ Fluorescence reflector and beam splitter ■ Spinning disc fluorescence filter
<ul style="list-style-type: none"> – Define 	<p>Acquires a reference image that is applied to subsequent images if Shading Correction is activated.</p> <p>The reference image should contain information about the illumination only and no specific information, e.g. the structure of a sample. To achieve this, set the illumination intensity to medium, defocus, and acquire an image of the empty light path (transmission), or defocus and acquire an image of a uniform surface such as a piece of paper (reflection).</p> <p>To work properly, the reference image must not contain any overexposed areas.</p>
<p>Enable Noise Filter</p>	<p>Activated: Noise in the acquired image is filtered according to the adjusted threshold. Affects acquired images only; the live image does not change.</p>
<ul style="list-style-type: none"> – Threshold 	<p>The noise filter reduces the extent to which individual pixels deviate from the average value of their nearest neighbors. The Threshold corresponds to a tolerance value. If the deviation of the middle pixel value from the average value of the pixels immediately surrounding it exceeds the tolerance value (i.e. it is interpreted as noise), it is replaced by the average value.</p> <p>The higher the value, the greater the tolerance for noise. The lower the value, the stronger the noise reduction.</p> <p>This technique reduces the noise of individual pixels, in particular with EMCCD cameras and CMOS cameras. The applied method reduces the noise of individual pixels without destroying fine structure in the image, as in most cases these are larger than individual pixels.</p> <p>This filter is also suitable for the dynamic removing of individual "hot pixels" from an image without having to acquire a reference image in advance.</p>

Parameter	Description
Enable Unsharp Mask	Enhances contrasts at fine structures and edges. Thus, the resulting image appears clearer and sharper.
– Strength	Controls the amount of contrast enhancement applied to fine structures and edges. The higher the strength, the darker or lighter the resulting edges, compared to the original image.
– Radius	Determines the size of detail to be enhanced. A small radius enhances smaller details. The radius also affects the appearance of enhanced edges. A large radius leads to a visible halo along enhanced edges. The larger the radius, the broader the halo.
– Color Mode	Determines the calculation method, which affects the appearance of the output image. <ul style="list-style-type: none"> ■ RGB: <ul style="list-style-type: none"> – The Unsharp Mask filter calculates the sharpness for each color channel individually. – The color saturation and the color of structures may be changed and color noise may occur. ■ Luminance: <ul style="list-style-type: none"> – The Unsharp Mask filter calculates the sharpness based on the luminance signal computed from the RGB channels. – This mode avoids possible color noise or shift in color saturation, which could be induced by certain image textures.
– Auto Contrast	Activated: You can adjust the Contrast Tolerance (0-20). Auto Contrast only works in RGB color mode.
– Contrast Tolerance	Increasing the contrast during unsharp masking is achieved by broadening the distribution of intensities. This corresponds to a spread of the image histogram. Contrast Tolerance controls how much the intensity distribution is spread and thus how strong the contrast is increased. <ul style="list-style-type: none"> ■ Contrast Tolerance = 0: No spread of intensities, no increase of contrast ■ Contrast Tolerance = 20: Maximum spread of intensities, maximum increase of contrast

Parameter	Description
– Clip To Valid Bits	<ul style="list-style-type: none"> ■ Activated: The processed image is composed of the same colors as the original image (i.e. the value range of the output image is adjusted to the color range of the input image). ■ Deactivated: Colors not present in the original image may appear in the processed image.

TIP

For a comprehensive set of image processing tools, which can be applied after image acquisition, use the **Image Processing** workbench.

18.4.1.3.4 Settings Section

The **Settings** section manages user-defined camera settings.

You can perform the following actions with presets:

Action	Description	Procedure
Apply preset	The current parameter values are overwritten with those stored in the preset	■ Select the desired preset from the list
Restore <i>preset</i> values	The current parameter values are reset to those stored in the preset	■ Click the Reload button.
Restore <i>initial</i> value	The current parameter values are overwritten with the ZEISS default values	■ Click the Default button.
Save changes to the current preset	The parameter values in the preset are overwritten with those of the current tool	■  > Save
Save changes as a new preset	A new preset is created with the current parameter values	<ol style="list-style-type: none"> 1  > Save As 2 Enter the new name for the preset
Export a preset	The parameter values stored in the preset are saved in a file	<ol style="list-style-type: none"> 1  > Export 2 Specify the location in the file system.

Action	Description	Procedure
Import a preset	A preset from the file system is added to the list of presets and the current parameter values are overwritten with those stored in the preset.	<ol style="list-style-type: none">  > Import Select the desired preset file from the file system.
Delete a preset	<p>The currently selected preset is deleted.</p> <p>The next preset in the list is selected and the values from the preset applied. If the list is empty, the default values for the tool are applied.</p>	  > Delete

INFO

Modified presets are indicated by a * next to the name.

18.4.1.3.5 Mode Section

The **Mode Section** determines how the software retrieves the camera sensor data.

Parameter	Description
Color Mode	This parameter is available for color cameras only.
– RGB	The image data of a color camera is transmitted unchanged. This corresponds to the standard operating mode of a color camera.
– B/W	<p>The color information of a color camera are discarded and converted into a grayscale image. The data of related color channels are averaged. The saturation of the camera appears reduced as a result.</p> <p>This process does not change the spectral properties of a color camera. The information of the colored pixel on the camera sensor are color interpolated for technical reasons prior to being changed in a monochrome image. The image information of the color sensor still undergoes color interpolation. An infrared filter also restricts the spectral sensitivity of the color camera compared to the spectral curve of the sensitivity of a black/white camera.</p>
Live Speed	Specifies the live image update speed.

Parameter	Description
	<p>Enables you to focus or to find regions of interest on a sample quickly. A high live image update speed reduces the exposure time of the live image, even at longer exposure times used for image acquisition.</p> <p>To achieve a similar impression of image brightness, however, the image data supplied must be adjusted digitally, which may generate a certain amount of noise or reduce the resolution of the live image.</p>

18.4.1.3.6 Model Specific Section

This section of the **Extended Camera** tool contains additional, model-specific camera settings depending on which camera model you use on your system.

Parameter	Description
Reset	Resets all parameters to factory setting.
Camera Identifier	Displays a unique camera identifier for the active camera. The camera identifier consists of the product name and part of the serial number. It helps you to identify the image source if you use different cameras in one system.
Orientation	<p>Modifies the orientation of the camera image. Depending on the camera port properties, the acquired image may be displayed in an undesired orientation. Use this parameter to correct the orientation during the acquisition process.</p> <p>The orientation is performed by the camera driver. This enables you to correct the live image without using image processing operations from ZEN 2 core.</p> <p>The available orientation options vary with the camera model.</p>
– Original	No change
– Flip Horizontally	Mirrors the camera image about a vertical axis.
– Flip Vertically	Mirrors the camera image about a horizontal axis.
– Rotate 90 CW	Rotates the image 90 degrees clockwise.
– Rotate 90 CCW	Rotates the image 90 degrees counter-clockwise.

Parameter	Description
– Rotate 180	Rotates the image 180 degrees.
– Mirror at +45 Diagonal	Mirrors the camera image about the axis running from the lower left corner to the upper right corner.
– Mirror at -45 Diagonal	Mirrors the camera image about the axis running from the upper left corner to the lower right corner.

18.4.1.4 Focus Tool

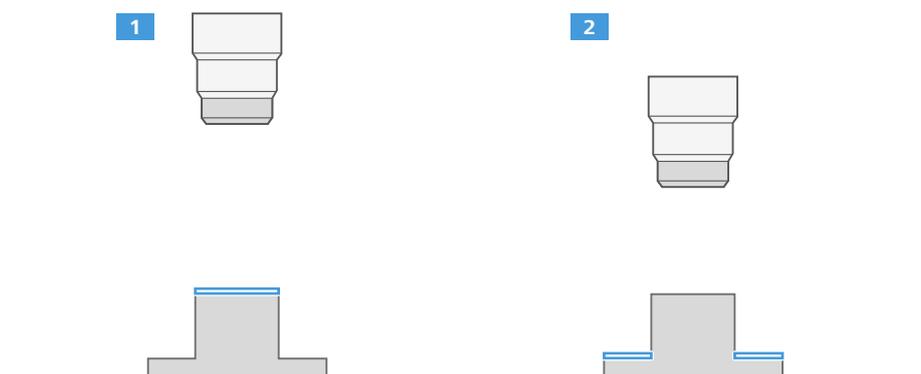
 **CAUTION**

Risk of Crushing Fingers

The drive of a microscope stage with a motorized vertical axis (focus drive) is strong enough to crush fingers or objects between the stage and the microscope stand.

- ◆ Remove your fingers or any objects from the danger area before moving the focus drive.
- ◆ Release the joystick immediately to stop the movement.

This tool changes the vertical distance (i.e. Z direction) between stage and objective. This enables you to focus the sample, or, for a sample with an uneven surface, to focus the area of interest.



- 1** Higher objective position, higher sample features in focus
- 2** Lower objective position, lower sample features in focus

Parameter	Description
Current	Displays the stage position in μm

Parameter	Description
	<p>Initially, when you use the Focus tool for the first time after switching on the microscope, the exact position of the stage is not known. Therefore, the position indicated by Current is initially set to zero. If you enter a value, the stage moves by the entered amount relative to the current position. If you want to move the focus to an absolute position, you must first click Home to move the focus to one of the end positions. The value of Current is set to this known position. You can then enter an absolute position.</p> <p>The Current input field defines the target position of the stage in μm. The stage starts moving immediately after the coordinates have been entered and confirmed by pressing the <i>Enter</i> key or by clicking anywhere outside the Current input field.</p>
<p>Navigation Bar</p>	<p>Enables you to move the stage freely in Z direction</p> <p>To move the stage, drag the Navigation Bar button in the desired direction. If released, the Navigation Bar button snaps back to the center and the stage stops.</p> <p>The Navigation Bar allows four speeds.</p> <div style="border: 1px solid #ccc; padding: 5px; margin-bottom: 10px;"> <p>Normal modes:</p> <ul style="list-style-type: none"> ◆ Inner segments: Slow ◆ Outer segments: Medium </div> <div style="border: 1px solid #ccc; padding: 5px;"> <p>High-speed modes:</p> <ul style="list-style-type: none"> ◆ Inner segments: Fast ◆ Outer segments: Very Fast </div>
<p>Stop</p>	<p>Stops any stage movement immediately.</p>
<p>Backlash Correction</p>	<p>Activated: Enhances the positional accuracy by performing an extra movement. When activated the focusing takes slightly longer</p>

Parameter	Description
Handwheel on	<ul style="list-style-type: none"> ■ Activated: Turning the handwheel also adjusts the focus ■ Deactivated: The handwheel is deactivated: turning it does not affect the focus
Step Size	<p>Defines the difference in μm by which the stage moves at each step. Indirectly this defines the speed of the stage movement.</p> <p>The Step Size also determines the accuracy of the focus position.</p>
Home	<p>Moves the focus to one of the end positions. The value of Current is set to this known position.</p> <p>This ensures that the position shown as Current corresponds to the actual stage position.</p>
Work	<p>Moves the stage back to the position it was in before using the Load button (i.e. the work position)</p> <p>If you have moved the stage (e.g. using the Navigation Bar) after moving it into the load position, the work position is lost and the Work button will not work.</p>
Load	<p>Increases the distance between objective and stage by $8,000 \mu\text{m}$</p> <p>This aids you in exchanging the sample. After exchanging the sample, you can move the stage back into its work position by using the Work button.</p> <p>Make sure not to move the stage (e.g. using the Navigation Bar) after moving it into the load position. Otherwise, the previous position is lost and the Work button will not work.</p>
Z-Position	<p>Specifies which position of the motorized z drive is used as the origin (zero value)</p>
– Set Zero	Sets the current focus position as the origin (zero value)
– Calibrate	Performs an automatic calibration

18.4.1.5 Hardware Autofocus Tool

This tool enables you to control the Auto Focus device, which constantly keeps the sample in focus. The Auto Focus device finds and regulates the focus position automatically, for example when you move the sample.

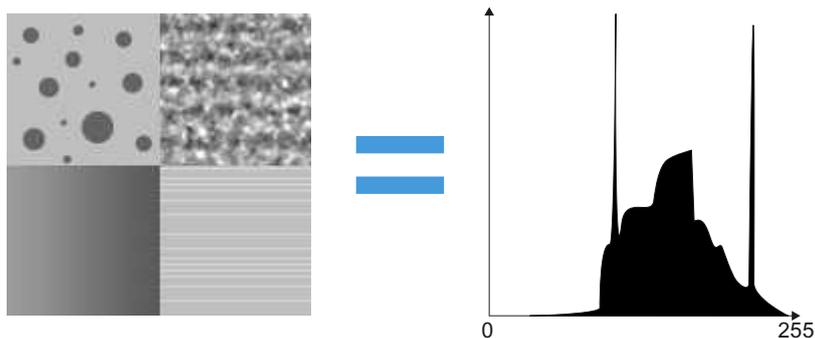
Parameter	Description
On	Continuous or periodic activation of the Auto Focus device The execution period is specified by Period .
Standby	Deactivates the Auto Focus device and switches it to standby.
Once	Performs a one-off autofocus
Stop	Switches the Auto Focus device off The focus is not monitored. You can use the Software Autofocus tool to find the focus position for a desired area on the sample.
Period	Specifies the interval between two subsequent Auto Focus device measurements
Focus Position	Indicates the focus distance determined by the Auto Focus device
Handwheel on	<ul style="list-style-type: none"> ■ Activated: The focus position can be overridden manually. ■ Deactivated: The handwheel on the microscope is deactivated. The focus position is only controlled by the Auto Focus device/the software.
Offset	Displays the difference between the measured Focus Position and the current Z position of the microscope's stage/objective
Z-Pos->AF-Pos	<p>Defines the current Z position as the new distance to be monitored and regulated by the Auto Focus device</p> <p>This setting is useful if the sample is sandwiched between object slide and cover glass: You can use one of the surfaces as a reference point for the Auto Focus device. This way, the distance between objective and the reference point plus a defined offset can be maintained.</p>
Reset	Cancels the Z-Pos->AF-Pos setting and resets the Auto Focus device to default settings
Resolution and Speed	Specifies how accurate the Focus Position is measured and how fast this can be done:
– Exact	Accuracy high, speed low
– Balance	Accuracy medium, speed medium
– Fast	Accuracy low, speed high

Parameter	Description
Sample Texture	Improves the autofocus measurement by taking the structure of the sample into account
– Reflective	Use Reflective if the sample is composed of a smooth material which mostly reflects light, such as semiconductor materials or metals.
– Semi refl.	Use Semi refl. if your sample cannot be classified as either reflective or non-reflective. If you are not sure about the sample's reflectivity you can use this setting as a starting point.
– Diffuse	Use Diffuse if your sample is composed of a rough material which mostly scatters light, such as paper.

18.4.1.6 Histogram Tool

This tool enables you to control how the image data is displayed. It adjusts the displayed intensity range of each channel. You can use the Histogram tool to make an image appear lighter or darker, to change the contrast, or, for a color image, to correct the color balance by modifying the color channels individually.

The image histogram is a graphical representation of the intensity (i.e. brightness) distribution of an image. The intensity is represented by the pixel value. For each pixel intensity value, the number of pixels in an image is counted. In the image histogram, the horizontal axis represents the pixel value and the vertical axis represents the number of pixels with that pixel value.



Working with the histogram

Action	Description
Change brightness	Move the range of displayed pixel values by dragging the central handle in the histogram.
Change contrast	Crop or extend the range of displayed pixel values.

Action	Description
	Alternatively, you can redefine the range directly by entering the Black and White pixel values. Any pixel value lower than Black is displayed black, any pixel value higher than White is displayed white.
Emphasize details in a range of pixel values	<p>Adjust the display curve by changing the gamma value.</p> <ul style="list-style-type: none"> ■ Gamma < 1: Details in darker image regions (low pixel values) are increased, details in brighter image regions (high pixel values) are decreased. ■ Gamma > 1: Details in brighter image regions (high pixel values) are increased, details in darker image regions (low pixel values) are decreased. <p>Alternatively, you can increase or decrease details of arbitrary ranges: enter Spline Mode and reshape the display curve by adding or removing anchor points to the spline (see below).</p>
Adjust the histogram for each color channel	If you are using a color camera and a multichannel setup, you can adjust the range and curve for each color channel separately. Select the desired channel from the drop-down list (default value: Current).

Histogram parameters

Parameter	Description
Spline Mode	<p>Enables you to freely edit the display curve and thus to increase or decrease the degree of displayed detail for arbitrary pixel value ranges</p> <ul style="list-style-type: none"> ■ Activated: Click to add anchor points to the display curve or drag existing anchor points to adjust the display curve. ■ Deactivated: Adjust the display curve using the Gamma value.
Auto	Automatically adjusts the range of displayed pixel values for each color channel using the selected method: Min/Max or Best Fit .
Min/Max	Sets the contrast to normal: the range of displayed pixel values matches the minimum and maximum pixel values occurring in the image.
Best fit	Increases the contrast: the range of displayed pixel values is limited by the pixel values resulting from the Best fit: Black % and Best fit: White % thresholds.

Parameter	Description
– Best fit: Black %	<p>Defines the lower threshold of the range of displayed pixel values</p> <p>For example, if the value is set to ten, the ten percent of all pixels with the lowest values are displayed black. The lower threshold of the range of displayed pixel values is calculated accordingly.</p>
– Best fit: White %	<p>Defines the upper threshold of the range of displayed pixel values</p> <p>For example, if the value is set to ten, the ten percent of all pixels with the highest values are to be displayed white. The upper threshold of the range of displayed pixel values is calculated accordingly.</p>
Options	Enables you to manage display settings, e.g. to save or to load them.
Black	Defines the black point. All pixels with this value or lower appear black.
White	Defines the white point. All pixels with this value or higher appear white.
Gamma	The Gamma value defines the ratio between the color values of the original image and the displayed image. Define here, whether details in brighter or darker image regions are enhanced.

18.4.1.7 Lamp Tool

This tool enables you to control the illumination of your sample.

Parameter	Description
Intensity	<p>Sets the intensity of the sample illumination</p> <ul style="list-style-type: none"> ■ 0: Illumination off ■ 10: Maximum illumination

18.4.1.8 Light Path Tool

This tool indicates the hardware components currently incorporated into the microscope setup. The tool serves the following purposes:

- Indicates the current microscope setup at a glance
- Enables you to change hardware components if the corresponding component is motorized

This may affect the behavior of the workbenches and tools.

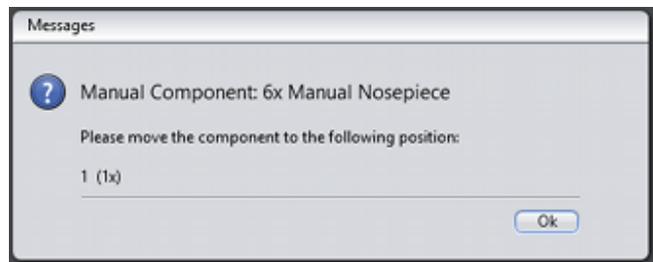
An icon representing a microscope component may have additional icons:

Symbol	Description
--------	-------------

Hand icon in the lower left corner

Indicates that the components are controlled manually. If you change the setting you must physically change the hardware component accordingly.

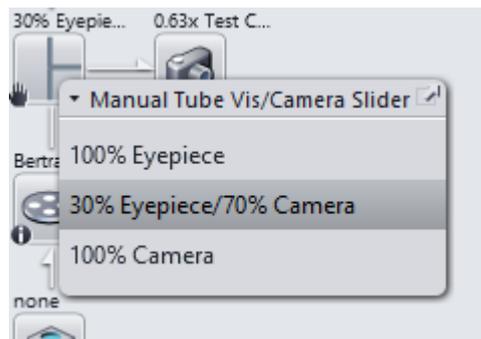
For example, if you change the software setting for the nosepiece, the software asks you to adjust the nosepiece at the microscope.



Arrow icon in the lower right corner

Indicates that the component represented by the component icon can be changed. The change can be automatic or manual (requires user action).

To change the component, left-click the icon representing the component, and select the desired option from the list.



18.4.1.9 Light Path Editing Tool

This tool is only available if you work with motorized components, e.g. motorized reflector changer or objective revolver.

With this tool you can adjust different hardware settings and assign it to an action button. If you click on the corresponding button, the hardware setting will be executed. 4 buttons are available meaning you can adjust and execute up to 4 different hardware settings.

Parameter	Description
Action buttons (Setting1-4)	<p>These 4 buttons can be assigned with hardware settings. To assign a hardware setting simply click on the button. The button then appears in blue color. Now you can edit the settings under Edit Settings.</p> <p>If a hardware settings is assigned to a button, the setting will be executed immediately when you click on the corresponding button.</p>
Edit Settings	Here you can specify the name, tooltip and hardware settings for the selected button.
- Button Name	Here you can enter a name for the selected button. The text will then appear on the button.
- Tooltip	Here you can enter a tooltip for the selected button. The tooltip will appear when you move the mouse pointer over the button.
Lightpath representation	<p>Here you see the graphical representation of the light path of your microscope system (if supported/available). Each icon represents a component of the light path (e.g. filter wheel or reflector changer).</p> <p>If you click on the component, a settings dialog opens. There you can adjust if the setting should be included in the hardware setting (checkbox activated) and of course the the specific settings for the component (e.g. which reflector should be used). If a component is included in a hardware setting, it is also highlighted in blue color.</p>

18.4.1.10 Linkam Heating Stage Acquisition Setup

This tool enables you to set up a temperature-dependent experiment with a Linkam stage and to acquire images at set intervals.

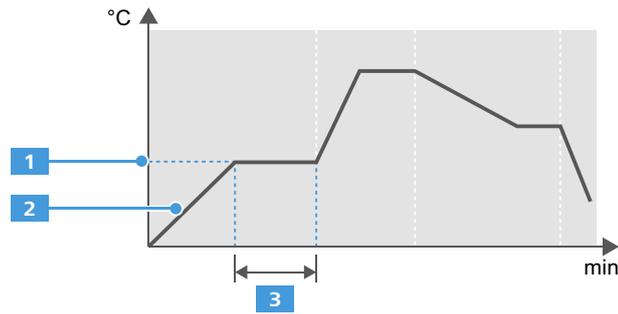
You can set up a temperature curve for the Linkam stage which is composed of individually defined temperature ramps connected to each other. For each temperature ramp you can define a condition when to acquire an image:

- Each time a defined time interval has elapsed
- Each time the temperature has changed by a defined value

As a result you obtain a time series or a temperature series of images per temperature ramp.

The temperature curve is represented by a table in the **Linkam Heating Stage Acquisition Setup** tool. Each row corresponds to one temperature ramp.

Temperature Profile Designer



- 1** Limit
- 2** Rate
- 3** Hold Time

Parameter	Description
Rate	<p>Specifies the heating or cooling rate of your Linkam stage in °C/min. For active cooling your stage must be equipped accordingly.</p> <p>You only need to define the Rate and the Limit (i.e. target temperature). The tool automatically determines whether to cool or to heat.</p>
Limit	<p>Specifies the target temperature of the Linkam stage. If this temperature is reached, it is sustained for the duration given by Hold Time.</p>
Hold Time	<p>Specifies the duration for which the temperature specified by Limit is sustained.</p> <p>After this, the next temperature ramp defined in the next table row starts.</p>
Icon Bar	Manages the temperature curve
– 	Moves the selected temperature ramp (table row) up
– 	Moves the selected temperature ramp (table row) down
– 	Duplicates the selected table row
– 	Removes the selected row

Acquisition Type Specifies whether a temperature or a time series is acquired for the currently selected ramp (i.e. table row).

Parameter	Description
None	No images are acquired during the selected temperature ramp.
Time	A time series is performed.
– Interval	An image is acquired whenever the time defined by Interval has elapsed.
Temperature	A temperature series is performed.
– Temperature Step	An image is acquired each time the temperature increases or decreases by this value.
Focus Correction	
– None	If selected, no focus correction is performed during the acquisition.
– Software Autofocus	If selected, during the experiment an focus correction is performed via the software autofocus. Under Repeat every you can set how often the correction is performed (e.g. after each image or after 2 images).
Annotations for temperature, pressure and time	Click on Show annotations in image to show the annotations in the image.

18.4.1.11 Magnification Tool

The **Magnification** tool enables you to set the objective, and thus the magnification, currently used on your microscope.

Parameter	Description
Objective	The behavior of the Objective icon depends on your type of objective revolver:
– Manual objective revolver	<ol style="list-style-type: none"> 1 Click the Objective icon and select the desired objective. 2 Turn the objective revolver manually to the corresponding position.

Parameter	Description
– Motorized objective revolver	<ul style="list-style-type: none"> Click the Objective icon and select the desired objective. <p>The objective revolver is turned to the corresponding position automatically.</p>
– Coded objective revolver	<ul style="list-style-type: none"> Turn the objective revolver manually to the desired position. <p>The objective is detected automatically by the software and displayed in the Magnification tool correspondingly.</p>
Zoom Level	<p>Only available for stereo or zoom microscopes.</p> <p>Use the slider to adjust the desired zoom level of your microscope.</p>
Total Magnification	<p>Only available for stereo or zoom microscopes.</p> <p>Shows the value of the total magnification of your microscope system. The value is calculated as follows:</p> $\text{Total Magnification} = \text{Magnification Objective} * \text{Magnification Camera Adapter (optional)} * \text{Zoom Level}$

18.4.1.12 Manual Extended Depth of Focus Tool

This tool increases the depth of focus by acquiring a sequence of images and combining them into a Z-stack image.

The tool combines the sharp regions of the individual images of the Z-stack into a single image. The depth of focus of this image is considerably larger than that of a single image.

In order to acquire images at different focus positions, you have to move the stage manually in Z direction. Whenever you change the stage position, a different plane of the sample is focused by the objective. You can trigger the image acquisition of the individual images manually or automatically at certain time intervals.

Parameter	Description
Mode	Determines whether the acquisition is triggered by a countdown timer or manually.
– Timer	Acquires an EDF (Extended Depth of Focus) image automatically after a countdown timer interval.
– F12 Key	Acquires an EDF image when you press the <i>F12</i> key.

Parameter	Description
Interval	Sets the countdown timer interval (in seconds), after which an image is acquired. Only active in Timer mode.
Z-Stack Alignment	Selects whether the individual images of the Z-stack image are aligned before being combined and at which quality. Use this method with a stereo microscope, where the images Z-stack images are displaced compared to each other.
– No Alignment	The Z-stack image is not aligned before being combined. You should select this setting for any microscope other than a stereo microscope, e.g. a compound microscope.
– Normal	High speed with normal image quality
– High	Low speed with high image quality
– Highest	Lowest speed with best image quality

18.4.1.13 Motorized Extended Depth of Focus Tool



Risk of Crushing Fingers

The drive of a microscope stage with a motorized vertical axis (focus drive) is strong enough to crush fingers or objects between the stage and the microscope stand.

- ◆ Remove your fingers or any objects from the danger area before moving the focus drive.
- ◆ Release the joystick immediately to stop the movement.

This tool creates an extended depth of focus (EDF) image by acquiring a sequence of images at different focus positions and combining the in-focus areas of each image.

The tool accepts a range of Z-positions of the stage and an interval/step size at which to acquire the individual images. The motorized stage is then automatically moved to the defined positions, at each position an image is acquired, and the in-focus areas of the acquired images are combined to an EDF image.

Parameter	Description
Focal plane	Defines the upper and lower end of the range of focus positions used to acquire the individual images
– Top > Assign	The current stage position is defined as the upper end of the range Move the stage until the top of the sample is no longer in focus and then click Top > Assign .
– Bottom > Assign	The current stage position is defined as the lower end of the range Move the stage until the bottom of the sample is no longer in focus and then click Bottom > Assign .
Step Size	Enables you to define the distance the stage travels between two image acquisitions
Slices	Displays the number of images to be acquired that results from the range (Focal Plane) and the Step Size
Optimal	Determines the optimal Step Size and number of Slices automatically depending on your microscope setup.
Z-Stack Alignment	Selects whether the individual images of the Z-stack image are aligned before being combined and at which quality. Use this method with a stereo microscope, where the images Z-stack images are displaced compared to each other.
– No Alignment	The Z-stack image is not aligned before being combined. You should select this setting for any microscope other than a stereo microscope, e.g. a compound microscope.
– Normal	High speed with normal image quality
– High	Low speed with high image quality
– Highest	Lowest speed with best image quality
High Resolution	Increases the resolution of the EDF calculation

18.4.1.14 Panorama Tool

This tool enables you to select a focus correction method that is applied each time a tile of the panorama image is acquired. This tool is only visible if both hardware and software autofocus are available.

Parameter	Description
Software Autofocus	Performs a Software Autofocus each time a tile of the panorama image is acquired.
Hardware Autofocus	Uses the Hardware Autofocus device to focus the sample continuously.

18.4.1.15 Position List Tool

This tool enables you to acquire tiles at arbitrary positions of your sample. In contrast to the tile acquisition tools, the tiles in the position list are not necessarily positioned next to each other.

Use the **Live Navigator** to define the positions. Drag the small blue-framed preview window to the areas of your sample where you wish to acquire a tile.

Parameter	Description
X Position, Y Position	Displays the current position of the Live Navigator
Add current position to list	Adds the current position to the position list The X and Y values are defined by the Live Navigator position. To change the Z value you need to load the Focus tool.
Position List	Shows the current list of positions and their X, Y, and Z values Using the options you can modify the list and its entries, e.g. sort the positions or change their X, Y, or Z values retrospectively.
Focus Correction	Allows you to choose which Z position to use for acquisition of the tiles: <ul style="list-style-type: none"> <input type="checkbox"/> Use the value defined in the list <input type="checkbox"/> Use software/hardware auto focus

18.4.1.16 Software Autofocus Tool

**Risk of Crushing Fingers**

The drive of a microscope stage with a motorized vertical axis (focus drive) is strong enough to crush fingers or objects between the stage and the microscope stand.

- ◆ Remove your fingers or any objects from the danger area before moving the focus drive.
- ◆ Release the joystick immediately to stop the movement.

This tool automatically focuses the sample using software algorithms. The tool scans a defined Z range and acquires an image at defined heights. The resulting images are compared in order to find the focus, i.e. the Z position where the acquired image is as sharp as possible.

Use this tool after you have found the desired area on your sample and have prepared your sample for acquisition.

You can accelerate the autofocusing process by roughly pre-focusing the sample manually.

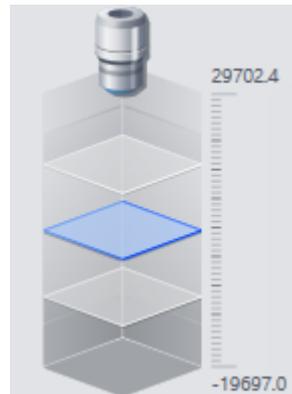
Parameter	Description
Find Focus	Triggers the software autofocus to find the focus.
Quality	Specifies the method to estimate the image sharpness and thus the precision of the calculated autofocus
– Basic	Applies a simple and fast method to estimate the image sharpness at each autofocusing step Significantly faster, but not as precise as Best .
– Best	Applies a more complex, optimized method to estimate the image sharpness at each autofocusing step More precise, but significantly slower than Basic .
Range Coverage	Specifies how the range is scanned:
– Smart	Acquires images at different positions until a first sharpness maximum is measured This position is assumed to be the in-focus position.
– Full	Acquires images over the entire range of positions

Parameter	Description
	<p>The in-focus position is defined by the position where the maximum sharpness was measured.</p> <p>The Full method requires more time than the Smart method but yields better results.</p>
Sampling	<p>Defines the distance the stage/objective is moved between two autofocus measurement positions</p> <p>An image is acquired at each position and its sharpness is compared to the previously acquired image to determine the focus.</p> <p>The accuracy of the calculated focus increases with a smaller displacement per step, but the required total measurement time also increases.</p> <p>The Step Size that results from the Sampling setting depends on the microscope setup (e.g. on the objective used) and is calculated automatically.</p>
Sharpness Measure	<p>Specifies how the focus position is calculated:</p>
– Contrast	<p>Calculates the best focus position by maximizing the local contrast in the selected image area</p> <p>Use this method if the sample has structures with clearly visible edges leading to sharp intensity changes in the image.</p>
– Intensity	<p>Calculates the best focus position by maximizing the total intensity in the selected image area</p> <p>Use this method if the sample has uniform structures leading to slowly varying intensities in the image.</p>
– Auto	<p>Decides automatically whether to use the contrast method or the intensity method, based on the image properties</p>
Autofocus Search Range	<p>Defines the Z range, which is used to find the focus</p>
– Relative Range	<p>The autofocus search range is defined by positions relative to the current objective position.</p> <p>This mode is recommended if you have already focused the sample roughly by hand.</p> <p>Automatic Range:</p>

Parameter**Description**

- Activated: The range for the autofocus search is calculated automatically, depending on the current objective.
- Deactivated: You set the range manually.
 - **Range:** the total distance in μm
 - **Step Size:** the distance between subsequent focus positions in μm

The **Step Size** depends on the microscope setup and on the selected **Sampling**. It is calculated automatically.

Overview image

The overview image indicates the objective and the stage. The blue plane indicates the stage, the light gray box indicates the current range.

The scale adapts automatically.

You can drag the blue plane to move the stage up or down.

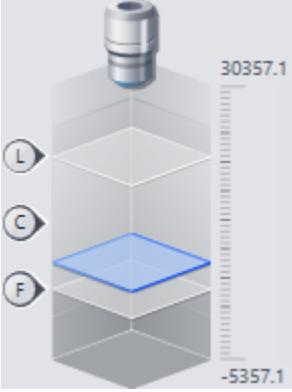
– Fixed Range

The autofocus search range is defined by absolute positions.

This mode is useful if you have a rough estimate where to look for the in-focus position.

- **Set First:** Defines the Z position from where the **Range** starts.
- **Set Last:** Defines the Z position where the **Range** ends.

Overview image

Parameter	Description
	 <p>The overview image indicates the objective and the stage. The blue plane indicates the stage, the light gray box indicates the current range.</p> <p>You can drag the blue plane to move the stage up or down. Alternatively you can click the flags to quickly move the stage into the first position (F), the center position (C), or the last position (L) of the range.</p> <p>You can also use the overview image to set the first or last position: Move the blue plane outside the light gray box into the desired position and then click Set First or Set Last.</p>
Autofocus ROI	Specifies which region of the camera sensor is used to find the in-focus position:
<ul style="list-style-type: none"> <li data-bbox="531 1350 762 1429">– Spot Meter / Focus ROI 	<ul style="list-style-type: none"> <li data-bbox="770 1350 1442 1462">■ Activated: Only the image region within the Spot Meter / Focus ROI region is used to calculate the autofocus. <li data-bbox="770 1485 1442 1630">You can set the Spot Meter / Focus ROI region by right-clicking on the live image and activating the Navigator. You can resize the red rectangle and move it to the region to be used for focusing. <li data-bbox="770 1653 1442 1724">■ Deactivated: The entire camera sensor area is used to calculate the autofocus.

18.4.1.17 Stage Tool

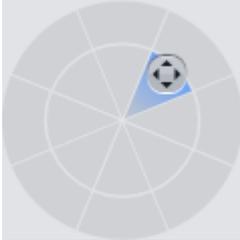
CAUTION

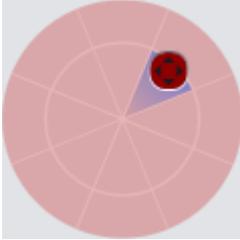
Risk of Crushing Fingers

The drive of a microscope stage with a motorized horizontal stage axis (stage drive) is strong enough to crush fingers or objects between the stage and nearby objects (e.g. a wall).

- ◆ Remove your fingers or any objects from the danger area before moving the stage drive.
- ◆ Release the joystick immediately to stop the movement.

This tool enables you to navigate the sample in a microscope equipped with a motorized stage. You can use the **Navigation Circle** (software joystick) to move the stage or enter the coordinates directly.

Parameter	Description
Navigation Circle	<p>Enables you to move the stage freely in the X and Y direction and in both diagonal directions.</p> <p>To move the stage, drag the Navigation Circle icon in the desired direction. If released, the icon snaps back to the Navigation Circle center and the stage stops.</p> <p>The Navigation Circle allows four speeds:</p> <ul style="list-style-type: none"> ■ Normal modes: <ul style="list-style-type: none"> – Inner segments: Slow – Outer segments: Medium  <ul style="list-style-type: none"> ■ High-speed modes:

Parameter	Description
	<ul style="list-style-type: none"> – Inner segments: Fast – Outer segments: Very Fast
	
	<p>To enter the high-speed mode, right-click the Navigation Circle icon. The Navigation Circle turns red. To return to normal speed, right-click the Navigation Circle icon again.</p>
Stop	<p>Stops any stage movement immediately.</p> <p>Use this button if you entered X-Position and/or Y-Position and wish to interrupt the stage movement immediately (e.g. to prevent a collision).</p>
X-Position, Y-Position	<p>Specifies the target coordinates for the stage movement.</p> <p>The stage starts moving immediately after the coordinates have been entered and confirmed; either by pressing the Return key or by clicking anywhere outside the current input field.</p>

TIP

You can also control the **Navigation Circle** and thus the motorized stage with the keyboard. To activate keyboard control left-click anywhere inside the segmented **Navigation Circle**. To change between the two speed modes, right-click the central **Navigation Circle** icon.

- To move the stage at the lower speed, use the arrow keys (diagonal movements are also possible).
- To move the stage at the higher speed, use **Shift** + arrow keys.

18.4.1.18 Tiles Setup (interactive) Tool

This tool enables you to acquire an oversize image that cannot be acquired with a single camera shot due to the limited size of the camera sensor.

The tool enables you to move the stage freely and to add regions of your sample you wish to include in the oversize image. A rectangle that encloses all the selected regions is displayed. The tool then acquires several images (tiles) such that the entire sample area corresponding to the rectangle is acquired.

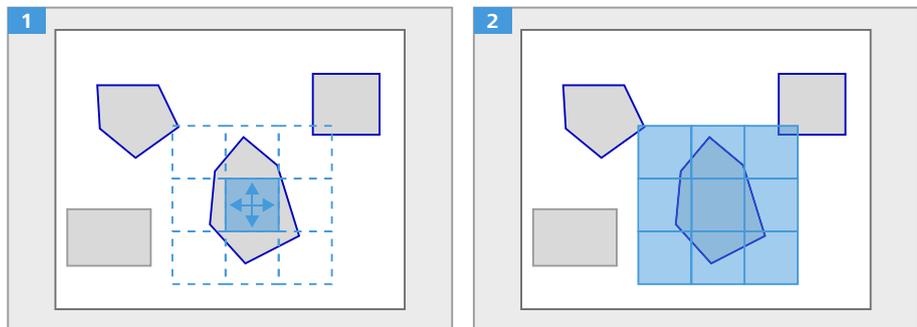
Parameter	Description
Add current position	Adds the position of the current preview image (Navigator View) to the list of regions to be included in the final oversize image.
Focus Correction	If the sample height varies by a large amount, you can select an autofocus correction method (Software Autofocus, Hardware Autofocus or Focus level) which is applied during the acquisition of each tile.

18.4.1.19 Tiles Setup (manual) Tool

This tool enables you to acquire an oversize image that cannot be acquired with a single camera shot due to the limited size of the camera sensor.

Several images (tiles) are acquired and joined together and create a single oversize image.

You can either define the total area to be acquired by a number of tiles or by its size directly.



Parameter	Description
Create tile region	Creates a preview of the area to be acquired The tile region is created around the current preview image (Navigator View) position. The red grid shows the tiles to be acquired, including an overlap. The yellow frame shows the total (sample) area of interest.
Tiles	Defines the total area to be acquired by a number of tiles
Size	Defines the total area to be acquired in μm times μm .

Parameter	Description
X, Y	<ul style="list-style-type: none"> ■ Tiles: Define the number of tiles to be acquired in both directions. The Size is calculated automatically. ■ Size: Define the size of the area to be acquired. The required number of tiles is calculated automatically.
Focus Correction	If the sample height varies by a large amount, you can select an focus correction method which is applied during the acquisition of each tile.

18.4.1.20 Tiles Stitching Tool

This tool enables you to combine a set of tiles into one large image.

This tool takes a tile image with the individual tiles placed next to each other as input and returns a single large image. The tiles are shifted and rotated against each other to make the transitions between them as seamless as possible. In addition, the tool enables you to correct uneven exposure (shading), either automatically or by means of a reference image.

Parameter	Description
Perform Stitching	Activated: Stitching is performed after acquisition of the individual tiles
Edge Detector	<p>When acquiring tiles to create a single large image, the stage movement is not precise down to the pixel level of the camera sensor. To bypass this technical limitation and to have a margin to compensate for this inaccuracy, tiles are usually overlapped by a few percent.</p> <p>To align the tiles, the overlaps between neighboring tiles are analyzed. An edge detector may improve analysis results.</p>
– Yes	Applies an edge detection algorithm to the tiles internally to improve analysis of the overlaps between neighboring tiles. This may improve the alignment of the tiles and thus the stitching result.
– No	Omits edge detection. The quality of alignment of the tiles may be reduced.
Minimal Overlap	The amount of overlap between neighboring tiles (in % of the area of a single tile) expected by the stitching tool. The tool evaluates this amount of overlap or more as required.

Parameter	Description
	The value to the overlap that was used for acquisition of the tiles is set. Larger values may improve the result but increase calculation time.
Max Shift	Specifies the maximal extent of shift (in % of the area of a single tile) which can be applied to a tile during stitching.
Comparer	Specifies how the conformance of the tiles in the overlapping regions is evaluated.
– Basic	Basic comparison (faster)
– Best	Complex comparison (slower)
– Optimized	Optimized comparison
Global Optimizer	Specifies the number of overlaps evaluated during stitching. Evaluating more overlaps per tile yields a better stitched image, but requires more calculation time.
– Basic	Only one overlap per tile is evaluated.
– Best	All overlaps of a tile are evaluated.
Defaults	Resets all tool settings to the default values.
Reset	Enables you to return the output image back to its original form (input) after applying the stitching.
Redo	Enables you to return to the output form by reapplying the desired stitch settings.

18.4.1.21 Focus Surface Tool

With this tool you can add and edit support points for a tiles acquisition. You can set different focus positions for each support point, this will help to keep the sample in focus on every position.

Parameter	Description
Add	<p>Initially adds five new support points to a tiles acquisition, if no support points have been added before. To add more support points click on the button again. A new support point will be added at the current stage position.</p> <p>Note that you must have set up the tiles acquisition (e.g. 3x3 tiles) before you can add support points.</p> <p>You can edit these point by their position by clicking on the corresponding point and moving it via Drag&Drop.</p>

Parameter	Description
	Note that before you start an acquisition, each support point must be verified. This means you must check and confirm each support point if it is in focus.
Remove	Removes a selected support point from the image. Note that at least 4 support point must remain to use the support points functionality for a tiles acquisition. If there are only 4 support points left, this button will be grayed out.
Remove All	Removes all support points at once.
List of support points	Here you see the positions (X/Y) and focus position of each support point that you have added. If you click on a entry the corresponding support point in the image will be selected.
Verify Focus Values	Starts the verifying process for the support points. This process is necessary before you start your tiles acquisition. The software starts always with the first support point. You have to check if it is in focus and at the desired position. If you click on the Next button, the point will be verified and the software moves to the next support point. Note that the verification of the focus points should be done before every new acquisition, as the focus drive might move during acquisition. If all support points are verified the message "Verified successfully" appears in the tool. That means you can now start your tiles acquisition.

18.4.1.22 S&F Find Tool

With this tool you can relocate (find) positions on your sample. First you have to acquire an image on the LM and usually you draw in certain ROIs / POIs. You can then bring the sample and the holder to the SEM. There you can relocate the sample positions by one click on the corresponding ROI / POI.

Note that the **S&F Find** tool can be added to each acquisition workbench. Therefore first add an acquisition workbench (e.g. 2D Acquisition) and click on **+ Add Tool**. Then double click on the **S&F Find** tool in the list of tools. When running the software on a SEM system the **S&F Find** tool is available in one of the **Acquisition** workbenches.

Parameter	Description
Reference Image	Here you can load a reference image including ROI/POIs.
ROI / POI list	In this list you see all the ROIs (regions of interest) and POIs (points of interest) which are drawn in the loaded reference image.
Move stage to load position before xy movement	If activated, the stage moves first to the load position before moving in x or y position (e.g. before moving to the next ROI / POI). Activate this option if you work with uneven samples to avoid collision of the objective and the sample.
Move to center	Moves the stage to the center position of the reference image.
Move to selection	Moves the stage to the selected region or position (the position must be selected in the list). In case a region was selected, the magnification will be adjusted to the extension of the ROI (on the SEM only).

18.4.2 Calibration

18.4.2.1 Create Measured Scaling Tool

This tool enables you to create a new scaling definition by measuring an object of known length. You can then use the scaling to perform the following:

- Scale images retrospectively
- Apply it as the standard scale for all future images and measurements acquired with an identical hardware setup

Parameter	Description
 Select	Enables you to select and modify existing measurements.
 Draw Reference Line	Enables you to specify a length by drawing a line. Use the Line tool for diagonal lines or to measure the distance between two points
 Draw Parallel Reference Line	Enables you to specify a length by drawing parallel lines. The length is interpolated by calculating the "mid" line between the two parallel lines. Use the Parallel Reference Line tool when you, for example, wish to ensure that the measured length is in the middle of the ruler lines of an object micrometer.

Parameter	Description
Automatic Line Detection	Activated: The measurement tools automatically snap to edges/contours in the image.
[Measured value] correspond to [Value]	The measured number of pixels on the screen and the actual length of an object of known length.
Units	The units of the object of known length This setting does not override the global units setting specified by the administrator.
Scaling	Displays the current scaling.
Name	Type in a custom name for the measured scaling.
Save Scaling	Saves the current scaling under the selected name It can then be used by all other users.

18.4.3 Documents

18.4.3.1 Load File

18.4.3.1.1 Load Image Tool

This tool enables you to load an image from the file system. You can load any supported image file.

Parameter	Description
File Name	The path and filename of the image to be loaded Click on  to open the file browser and select the desired image.

18.4.3.1.2 Load Table Tool

This tool enables you to load a data table from the file system. You can load any supported file, regardless of whether it is a result table generated by the software or external software. For example, you could load a table containing theoretical measurement results to check whether the actual measurements are correct.

Parameter	Description
File Name	The path and filename of the table to be loaded Click on  to open the file browser and select the desired file.

18.4.3.1.3 Load Multiple Images Tool

This tool enables you to load multiple images all at once from the file system. You can load any supported image file.

Parameter	Description
File Names	Shows the path of the images to be loaded. Click on  to open the file browser and select the desired images. To select a range of images hold down the <i>Shift</i> key and click on the first and the last image of the range. Alternatively you can hold down the <i>Alt</i> key to select multiple single images.

18.4.3.2 Save File

18.4.3.2.1 Save Image Tool

This tool enables you to save the current image to the file system in any supported file format. It can then be shared with users of other systems.

Parameter	Description
Path	The path and filename where the image should be saved Click on  to open the file browser and select the desired location and file format.

18.4.3.2.2 Save Report Tool

This tool enables you to save the current report to the file system. It can then be shared with users of other systems.

INFO
Report templates are imported and exported using the Archive .

Parameter	Description
Path	The path and filename where the report should be saved Click on  to open the file browser and select the desired location and file format.

18.4.3.2.3 Save Table Tool

This tool enables you to save the current measurement results table or data table to the file system in any supported file format.

Parameter	Description
Path	The path and filename where the table should be saved Click on  to open the file browser and select the desired location and file format.

18.4.3.2.4 Export

18.4.3.2.4.1 Image Export Tool

This tool exports an image in various formats.

Parameter	Description
File type	Selects the file type of the exported image. Depending on the selected file type, additional settings for image quality and compression are available.
Resize	Sets the resolution of the exported image in per cent of the original image.
Original Data	Activated: A copy of the original image is saved, along with the modified image.
Apply Display Curve and Channel Color	Activated: The current channel color and display curve are applied to the image before exporting, i.e. a modified image is exported. You can specify the channel color and display curve in the View Options . To save both the original and the modified image, activate Original Data ; for more information see above.
Burn-in Graphics	Activated: Graphics and annotations, e.g. from measurements, will replace the underlying image pixels. The underlying image pixels will be lost in the process.
Use Full Set of Dimensions	Exports the entire image region.
Define Subset	Enables you to export one of the following image subsets:

Parameter	Description
	<ul style="list-style-type: none"> ■ Region ■ Tiles, optionally with overlap
Region	Enables you to define an image region to be exported.
- Full	Exports the entire image area.
- Rectangle	Enables you to specify a rectangular subsection of the image to be exported.
Keep Tiles	<p>Only effective if a region of interest (ROI) is defined.</p> <p>Activated: Extracts all tiles that are inside or partly inside the defined ROI. The tiles are extracted completely and not cut-off.</p> <p>This setting is recommended when you wish to apply DCV processing functions to the resulting image.</p>
Tiles	<p>Enables you to export tiles where each tile is saved as a single image.</p> <p>You can use the tiles as represented in the software or you can specify a number of rows and columns to re-tile the image before export.</p>
- Existing Tiles	<p>If the current image is tiled, each tile is exported as a single image.</p> <p>Otherwise a single image is exported.</p>
- Re-Tile	<p>Splits the current image into a specific number of tiles.</p> <ul style="list-style-type: none"> ■ Columns, Rows: Number of equally spaced columns and rows the image is split into ■ Overlap: Percentage by which neighboring tiles will overlap.
Export to	Specifies the export path of the image. You can select any local or network path available on your machine.
Create Folder	<p>Creates a folder at the path location specified above and saves all image data to that folder.</p> <p>The Prefix is used as the folder name.</p>
Generate xml file	Creates an XML file containing the metadata of the image.
Generate zip file	Compresses all exported files. The result of Export Image is one single ZIP file.

Parameter	Description
Prefix	Enables you to specify a prefix that is added to all exported files

18.4.3.2.4.2 Movie Export Tool

This tool compiles a movie from a series of input images.

Prerequisite You have acquired or opened an image from a Time Series.

Parameter	Description
Mode	Sets the file type and codec of the exported movie. The available codecs depend on the file type.
Format	Sets the width and height, the frame rate, and the quality of the movie. The following formats are available: <ul style="list-style-type: none"> – Predefined A list of preset formats corresponding to well-established TV or HD video formats. The number of available formats varies with the selected Mode. – User Defined Allows you to set width, height, and frame rate freely. – Original Size Sets the width and height of the movie to the input image dimensions.
Quality	Refers to the compression of the video data. This setting is independent of Mode and Format .
Burn-in Graphics	Activated: Graphics and annotations, e.g. from measurements, will replace underlying pixels in the exported movie frames. Underlying pixels will be lost in the process.
Fitting	Defines how the image data is scaled, zoomed, or cropped according to the movie format.
Mapping	Specifies whether the movie into which the images are combined has a fixed frame rate (1 Frame per Image) or a fixed duration (Fixed Duration). <ul style="list-style-type: none"> – 1 Frame per Image <ul style="list-style-type: none"> ■ One image will be mapped to one movie frame. ■ The movie duration adapts to the number of images and the selected frame rate. ■ Choose this mapping for optimum movie quality results.

Parameter	Description
– Fixed Duration	<ul style="list-style-type: none"> ■ One image may be mapped to several frames ■ The frame rate adapts to the number of images and the desired movie duration.
Image Count	Displays the number of frames contained in the movie to be exported
Final Movie Length	Displays the duration of the movie to be exported
Use Full Set of Dimensions	Exports the entire image region into the movie
Define Subset	Exports a region of the original images, or tiles of the images, optionally with overlap. A separate movie file is created for each tile.
Region	If you select a region, only that region of each original image will be exported to the movie.
Tiles	If you define tiles, each tile of the original images will be exported to a single movie.
– Existing Tiles	Activated: All tiles will be exported as represented in ZEN 2 core.
– Re-Tile	Activated: You can re-tile the original images into a desired number of columns and rows. An additional overlap of the tiles is optional.
Export to	Specifies the export path of the movie. You can choose any local or network path available on your machine.
Prefix	Enables you to specify a prefix that is added to all exported files

18.4.3.2.4.3 OME TIFF Export Tool

This tool exports an image in the OME (Open Microscopy Environment) TIFF format. You can use this image in other programs supporting this open format. The images are then available as a multi-page TIFF file. An image exported as a group of tiles is stored as a multi-page TIFF file.

Parameter	Description
Resize	Sets the resolution of the exported image (in % of the original image size).

Parameter	Description
BigTIFF	Activated: The image is stored in BigTIFF format. The BigTIFF format allows file sizes larger than 4 GiB.
Compress	Activated: The image data is compressed.
Use Tiles	If the exported image is a tile image, each tile is exported to a separate file.
Merge all Scenes	If the exported image is a scene image, all scenes are exported as one large image.
Shift Pixel	
Original Data	Activated: A copy of the original image is saved, along with the modified image.
Apply Display Curve and Channel Color	<p>Activated: The current channel color and display curve are applied to the image before exporting, i.e. a modified image is exported.</p> <p>You can specify the channel color and display curve in the View Options Area.</p> <p>To save both the original and the modified image, activate Original Data; for more information see above.</p>
– Burn-in Graphics	Activated: Graphics and annotations, e.g. from measurements, will replace the underlying pixel in the exported image. Underlying pixels will be lost in the process.
Use Full Set of Dimensions	Exports the entire image region.
Export to	Defines the export path of the image. You can select any local or network path available on your machine.
Prefix	Enables you to specify a prefix that is added to all exported files

18.4.3.2.4.4 ZVI Export Tool

This tool exports an image in the Zeiss AxioVision format (ZVI).

Parameter	Description
Export to	Defines the export path of the image. You can select any local or network path available on your machine.
Prefix	Enables you to specify a prefix that is added to the exported file

18.4.4 Measurement

18.4.4.1 Automatic Measurement

18.4.4.1.1 Image Analysis: Setup Image Analysis Tool

This tool enables you to create, perform, or modify an automatic image analysis.

Parameter	Description
Program	Lists all the predefined automatic measurement programs.
 Options	Enables you to manage automatic measurement as follows:
– New	Create a new empty automatic measurement program.
– Rename	Enables you to change the name of the selected automatic measurement program. Enter a meaningful name that describes the aim of the automatic measurement program. The name applies to all users.
– Edit	Enables you to specify whether the following steps in the automatic measurement are performed when the job is run without having to click through the entire wizard.
– Save	Overwrites the selected automatic measurement with the current settings in the archives. The changes then apply to all users.
– Save As	Saves the current automatic measurement under a different name
– Import	Imports an automatic measurement from the file system, if, for example, it was exported from another system
– Export	Exports the automatic measurement to the file system, for example so that you can copy it to another system
– Delete	Deletes the selected automatic measurement It is then no longer available to any users. Any job templates or macros containing the automatic measurement must be updated before they can be run.
Class tree	Preview of the measurement classes
Setup Image Analysis	Starts the Image Analysis Wizard to configure the automatic measurement

Parameter	Description
Analyze	Runs the automatic measurement
Interactive	All steps for which Interactive is activated can be modified.

18.4.4.1.2 Image Analysis Wizard

18.4.4.1.2.1 Classes

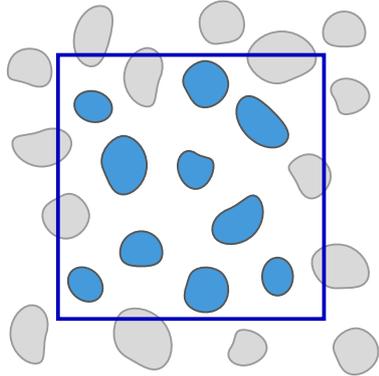
This step in the **Image Analysis Wizard** enables you to create measurement classes for the sample. A class corresponds to a type of object to be detected, for example objects with a specific brightness or shape.

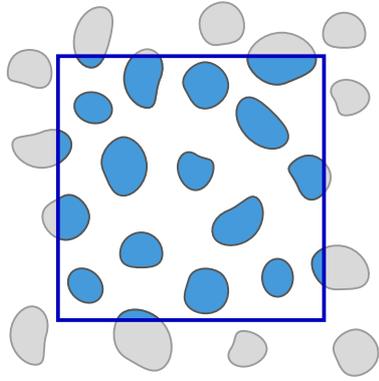
Parameter	Description
Class tree	Lists all the classes of measurement. Each class also contains a summation class. The class itself contains the measurement values for each individual object detected. The summation class contains the sum of all measurement values for all objects. Classes can be nested. In this case, the summation class contains the sum of all subordinate summation classes.
Add Class	Adds a new class and summation class under the selected item.
Remove Class	Removes the selected class and any subclasses.
Name	The name of the selected class or summation class. Enter a meaningful name to help identify classes quickly.
Channel	Specifies which channel should be evaluated (multichannel image only).
Color	The color in which objects detected by the automatic measurement are displayed in the image.
Back	Returns to the previous step in the wizard.
Next	Proceeds to the next step in the wizard.

18.4.4.1.2.2 Frame

This step in the **Image Analysis Wizard** enables you to specify the following:

- The areas of the image to be measured (measure frames)
- How objects at the edge of the image or frame are treated

Parameter	Description
Interactive	Activated: The user can change the settings in this step when the automatic measurement is run
Mouse Pointer icon	Enables you to select and modify existing measurement frames Press <i>CTRL</i> to select multiple frames.
Rectangle / Circle / Contour icon	Draws a frame of the corresponding shape in the image
	Deletes all frames This means that objects in the entire image are measured.
Maximize circle	Activated: The currently selected circle is expanded to fill the entire image
Center circle	Activated: The currently selected circle is centered horizontally and vertically
Mode	Specifies how objects at the edge of the image or frame are treated Objects completely within the image/frame are always measured in their entirety.
– Inside Only	Not measured 
– Cut at Frame	Only portion of object within image/frame measured

Parameter	Description
	
Left, Right	Top left coordinates of the currently selected frame (in pixels)
Width, Height	Dimensions of the currently selected frame (in pixels)
Back	Returns to the previous step in the wizard
Next	Proceeds to the next step in the wizard

18.4.4.1.2.3 Automatic Segmentation

This step in the **Image Analysis Wizard** enables you to specify how shapes are detected automatically based on their brightness. The algorithm segments the individual RGB channels into a black and white image and detects groups of pixels based on their brightness compared to neighboring pixels.

All the objects detected with the current settings are highlighted in the image.

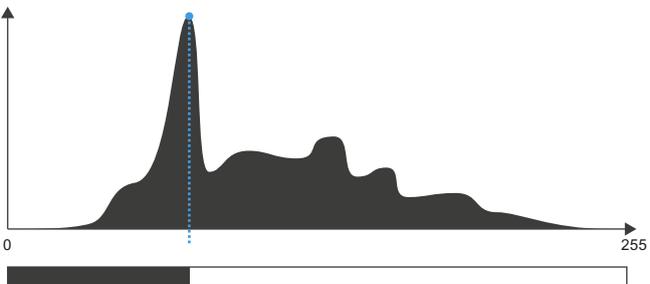
Parameter	Description
Execute	Activated: This step is included when the Image Analysis Wizard is run. Otherwise the step is skipped.
Interactive	Activated: The user can change the settings in this step when the automatic measurement is run
Class tree	Enables you to select the class to which the settings apply You can specify different settings for each class.
Smooth	Specifies the method to smooth the image and thus reduce noise
– None	No smoothing algorithm is applied

Parameter	Description
– Lowpass	<p>The lowpass filter compares the brightness of each pixel to the brightness of its neighboring pixels. If a pixel is brighter than its neighbors, the brightness of this pixel is reduced and the brightness of the neighboring pixels is increased.</p> <p>This suppresses sharp changes in brightness (i.e. contours) and leads to more gradual changes in brightness.</p> <p>Size: Determines the number of neighboring pixels taken into account. The size should correspond to the pixel size of the contours to be reduced.</p>
– Gauss	<p>Each pixel is replaced by a weighted average of its neighbors. The weighting depends on the sigma value.</p> <p>The Gaussian filter is particularly useful for contour enhancement, which is very sensitive to noise. Using a Gaussian filter before finding contours greatly improves the results</p> <p>Sigma: Determines how much neighboring pixels contribute to the weighting. Larger values broaden the applied Gaussian distribution and lead to reduced noise but also to an increasing loss of image information.</p>
– Median	<p>Each pixel is replaced by the median of its neighbors. The number of neighboring pixels taken into account depends on the size.</p> <p>In a set of values (in this case the pixel values taken into account), the median is the value for which the number of larger values is equal to the number of smaller values.</p> <p>Size: Determines the number of pixels taken into account. A higher size leads to more noise reduction but also to a large amount of blur and thus to a loss of detail in the resulting image.</p>
Sharpen	Specifies the method to enhance contrast at fine structures and edges and thus create an image that appears sharper
– None	No sharpening algorithm is applied
– Delineate	Emphasizes edges around structures in an image. It is useful for images where the gray value range of structures differs clearly from the gray value range of the pixels around them.

Parameter	Description
	<p>Threshold: The difference in gray values which specifies an edge between neighboring image regions. The Threshold value should correspond roughly to the gray value difference between foreground objects and the background.</p> <p>Size: Determines the size of image details which are enhanced – the smaller the Size value, the finer the details affected by the tool. The Size value should correspond to the size of the transition area between foreground objects and the background.</p>
– Unsharp Masking	<p>A copy of the image is created. The copy is blurred, inverted, rescaled and added to the original.</p> <p>Strength: Defines the amount of contrast enhancement applied to fine structures and edges. The higher the value, the greater the edge enhancement.</p>
Minimum Area	Specifies the minimum number of pixels that are considered to be an object
Color model	Specifies how the colors are indicated
– RGB	Red-green-blue colorspace
– HSL	Hue-saturation-lightness colorspace
Threshold	Sets the brightness boundaries between which pixels are considered
– Reset	Clears the upper and lower thresholds No pixels are considered
– Undo	Undoes the last change made to the threshold values
– Redo	Restores the last undone change to the threshold values
–  R	Activates the selected channel in the Expander Histogram.
 G	
 B	
–  H Hue	Activates the selected channel in the Expander Histogram.
 L Lightness	

Parameter	Description
	
Saturation	
– Low	The lower threshold Only pixel values above this value are considered. The range of possible values depends on the bit depth of the image.
– High	The upper threshold Only pixel values below this value are considered. The range of possible values depends on the bit depth of the image.
– Invert	Only pixels outside the threshold boundaries are considered, i.e. those pixels <i>below</i> the lower threshold or <i>above</i> the higher threshold
– Full Range	Sets the lower threshold to 0 and the upper threshold to 256 The entire range of pixel values is considered
Histogram	Shows/hides the histogram Use the sliders under the histogram to adjust the Low and High threshold values.
Threshold definition	Enables you to select pixels to be considered using the image rather than the histogram
– Click	Select representative areas in the image manually The threshold boundaries are adjusted depending on the values of Pick Behavior , Tolerance , and Neighborhood .
– Automatic	The threshold values are calculated automatically depending on the algorithm selected in Method .
Pick Behavior	Specifies whether manually selected pixels are added to the selection or removed from it
– +	Adds further objects by increasing the threshold boundaries to include the brightness values of the selected object
– -	Removes objects with the selected brightness values and reduces the threshold boundaries
Tolerance	Specifies how many additional pixel values are included in the selection based on their brightness

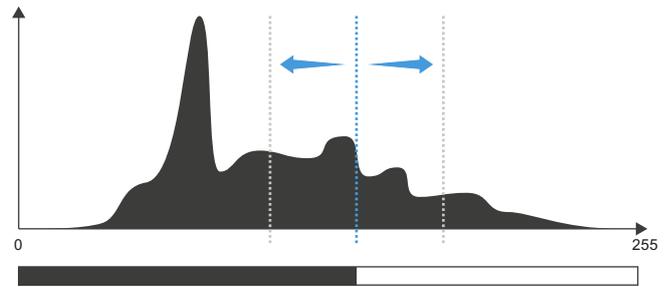
Parameter	Description
	A higher value means that more pixel values similar to the selected one are included. A lower value means that only the exact pixel value selected is included.
Neighborhood	<p>Specifies how many additional pixel values are included in the selection based on their physical proximity to the selected pixel</p> <p>A higher value means that more pixels surrounding the selected pixel are included. The threshold boundaries are adapted so that all the pixel values of these neighboring pixels are included. A lower value means that the boundaries are adapted based on only the pixels directly next to the selected pixel.</p>

Parameter	Description
Method	<p>Specifies the algorithm used to automatically detect the threshold boundaries. The most suitable algorithm depends on your precise requirements.</p> <p>The value also depends on the bit depth of the image.</p>
<ul style="list-style-type: none"> - Otsu 	<p>The pixel values below the threshold are designated as background and those above the threshold as foreground. It iterates through all possible threshold values and for each value calculates the spread of the pixel intensities of the background and foreground pixels. The threshold is set at the value that minimizes both spreads.</p> <p>This method is particularly suited to light objects on a dark background.</p>
<ul style="list-style-type: none"> - Maximum Peak 	<p>The threshold is set to the pixel value that occurs most frequently.</p> 

<ul style="list-style-type: none"> - Iso Data 	<p>The pixel values below the threshold are designated as background and those above the threshold as foreground. An initial threshold value is chosen, and the mean pixel intensity of the foreground and background pixels is</p>
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Parameter	Description
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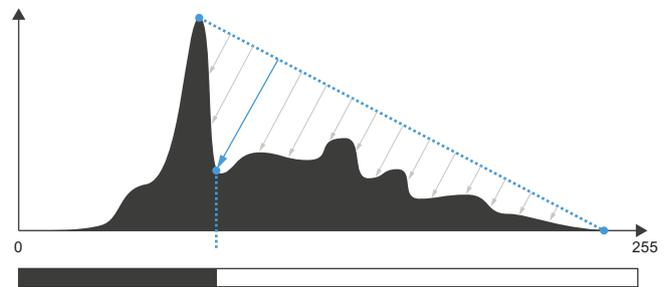
calculated. These two mean values are averaged and the result serves as the input threshold for the next calculation. The process is repeated until the threshold value no longer changes.



– **Triangle Threshold**

The algorithm constructs a line between the peak of the highest frequency pixel intensity and the lowest pixel intensity. The distance between the line and the histogram is computed for all values along the line. The pixel intensity where the line is longest is used as the threshold.

This method is particularly suited when the foreground pixels only have a weak peak in the histogram.



– **Three Sigma Threshold**

The pixel value that occurs most frequently is calculated. The standard deviation of the values in the peak is calculated. The threshold is set to the pixel intensity that is the sum of the peak value and three times the standard deviation.

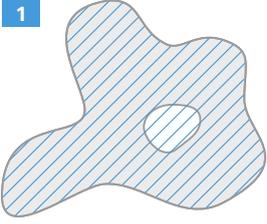
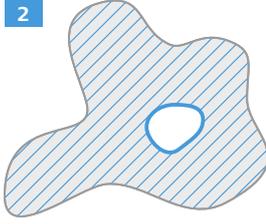
Parameter	Description
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Fill

Specifies how holes in detected objects are treated

Activated (1): If a detected object completely surrounds another potential area, then the enclosed area is also included

Deactivated (2): Only the detected object is included

Parameter	Description
	<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> <p>1</p>  </div> <div style="text-align: center;"> <p>2</p>  </div> </div>
Separate	Specifies how overlapping objects are treated
– Morphology	<p>Pixels are "eroded" from the edge of the shape until it splits into two shapes. The result is two rounded objects, potentially with a large gap between them.</p> <p>Count: Specifies the number of pixels to be eroded from each side</p> <div style="text-align: center;">  </div>
– Watersheds	<p>The effect of this algorithm is best understood with an analogy:</p> <p>The shape is considered to contain two "valleys" with the two brightest pixels corresponding to the bottom of one valley each. If water were poured onto the shape, there would be a boundary ("watershed") that defines where water flows into one valley or the other.</p> <p>The shape is split along this boundary. The result is two shapes separated by a thin 1-pixel boundary. The rest of the shape perimeter remains unchanged.</p> <div style="text-align: center;">  </div>
Back	Returns to the previous step in the wizard
Next	Proceeds to the next step in the wizard

18.4.4.1.2.4 Condition

This step in the **Image Analysis Wizard** enables you to exclude detected objects based on various properties such as area, intensity, or roundness.

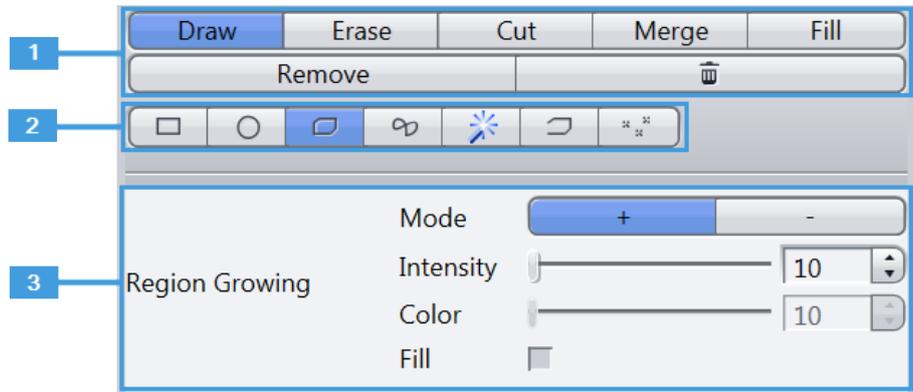
Parameter	Description
Execute	Activated: This step is included when the job is run

Parameter	Description
	Otherwise the step is skipped.
Interactive	Activated: The user can change the settings in this step when the automatic measurement is run
Edit	Enables you to specify the properties that should be considered for the selected class
Undo	Undoes the last change made to the threshold values
Redo	Restores the last undone change to the threshold values
Name	Lists the properties that are considered Only detected objects which fulfill all the properties are considered; all others are excluded.
Minimum	Specifies the lower threshold of the property If an object's property is above this value the object is measured. If you do not want to use this threshold, deactivate the checkbox. The threshold is set to the minimum possible value (typically 0).
Maximum	Specifies the upper threshold of the property If an object's property is below this value the object is measured. If you do not want to use this threshold, deactivate the checkbox. The threshold is set to the maximum possible value.
Back	Returns to the previous step in the wizard
Next	Proceeds to the next step in the wizard

18.4.4.1.2.5 Interactive Segmentation

This step in the **Image Analysis Wizard** enables you to add or remove individual objects or areas to be measured.

The tools in this step are split into two categories:

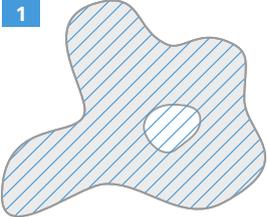
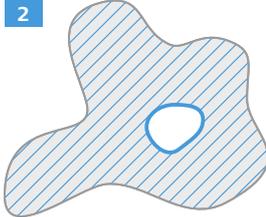


- 1** Freehand tools
Enable you to add or remove individual areas or objects by drawing shapes in the image
- 2** Drawing tools
Enable you to add or remove a corresponding shape from an existing object. The action depends on which freehand tool is selected.
- 3** Region Growing tools
Enable you to expand or reduce the size of an individual object based on the brightness of surrounding pixels

The tool category that is selected is indicated by a white background. The tools in separate categories cannot be combined.

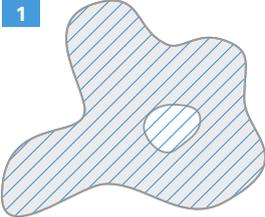
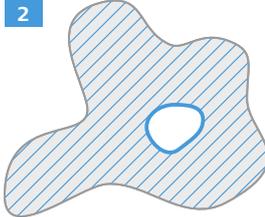
Freehand tools

Parameter	Description
Interactive	Activated: The user can change the settings in this step when the automatic measurement is run
Class tree	Enables you to select the class to which the settings apply You can specify different settings for each class.
Draw	Adds the area drawn as a new object / extends an existing object
Erase	Removes the area drawn from an existing object; the remaining area is measured
Cut	Splits an object into multiple objects along the line drawn; each object is measured separately
Merge	If the area drawn overlaps an existing object, the area and object are joined into a single object
Fill	If a selected object completely surrounds another potential area, then the enclosed area is also included (1).

Parameter	Description
	<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> 1  </div> <div style="text-align: center;"> 2  </div> </div>
Remove	The selected object is not measured
	Removes all objects; no objects are measured

Region Growing tools

Parameter	Description
Mode	Expand or reduce the size of an object based on the brightness of surrounding pixels
- +	Expands the closest object
- -	Reduces the closest object
Intensity	<p>Specifies how many additional pixel values are included in the selection based on their brightness</p> <p>A higher value means that more pixel values similar to the selected one are included. A lower value means that only the exact pixel value selected is included.</p>
Color	<p>Specifies how many additional pixel values are included in the selection based on their color (only available for multi-channel images)</p> <p>A higher value means that more colors similar to the selected one are displayed. A lower value means that only the exact color selected is displayed. The comparison is based on the RGB (red green blue) colorspace.</p>
Fill	<p>Specifies how holes in detected objects are treated</p> <p>Activated (1): If a detected object completely surrounds another potential area, then the enclosed area is also included</p> <p>Deactivated (2): Only the detected object is included</p>

Parameter	Description
	<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;"> 1  </div> <div style="text-align: center;"> 2  </div> </div>
Undo	Undoes the last change made to the threshold values
Redo	Restores the last undone change to the threshold values
Back	Returns to the previous step in the wizard
Next	Proceeds to the next step in the wizard

18.4.4.1.2.6 Features

This step in the **Image Analysis Wizard** enables you to define the properties of the detected objects to be measured, such as location, diameter, or intensity.

Parameter	Description
Class tree	Enables you to select the class to which the settings apply You can specify different settings for each class.
Region Features	Lists the object properties to be measured
– Edit	Enables you to select the properties to be measured
– Copy to All	Copies the measurements for the selected class to all other classes
– Name	Sorts the measurements alphabetically
– Display	Deactivated: The measurement is performed but not displayed in the image. This prevents the image becoming cluttered.
Region Annotations	Allows you to add annotations to the image, for example to indicate areas of particular significance
– Edit	Enables you to edit the image annotations
– Copy to All	Copies the annotations for the selected class to all other classes
– Name	Sorts the annotations alphabetically
Back	Returns to the previous step in the wizard
Next	Proceeds to the next step in the wizard

18.4.4.1.2.7 Measure

This step in the **Image Analysis Wizard** displays a preview of the measurement results of the selected class. These results are calculated roughly and may differ from the actual results when the automatic measurement is performed.

Parameter	Description
Class tree	Enables you to select the class to which the settings apply You can specify different settings for each class.
Results table	Displays the measurement results per object Click a row of the table to highlight the corresponding object in the image or vice versa
Back	Returns to the previous step in the wizard
Finish	Proceeds to the next step in the wizard

18.4.4.1.2.8 Region Condition Editor Dialog

Specify the properties that must be fulfilled for an object to be measured. All the properties within at least one block must be fulfilled. You can create complex rules by joining blocks using "or" conditions.

Parameter	Description
Selected Features for Condition	Lists the blocks of properties that must be fulfilled for an object to be measured
Search Features	You can filter the list by entering the first few letters of the name of a property in Search Feature or by selecting the corresponding category of property in the drop-down.
Add Block	Creates a new "Or" block of conditions If several Or blocks are defined, an object is measured if it fulfills all the conditions in at least one block.
Clear Block	Removes all properties from the selected block
Remove Block	Deletes the selected Or block and the properties it contains
Remove All	Deletes all Or blocks and the properties within them

18.4.4.1.2.9 Feature Selection Dialog

Specifies the properties of the detected objects to be measured, such as location, diameter, or intensity

Parameter	Description
Selected Features for Condition	Lists the object properties to be measured
– Name	Name of the property to be measured
– Display	Deactivated: The measurement is performed but the result is not included in the Results Table
Search Features	You can filter the list by entering the first few letters of the name of a property in Search Feature or by selecting the corresponding category of property in the drop-down.
– Name	Name of the available properties for the current class
	Sorts the order in which the measurement results are displayed
	Removes a property from the list

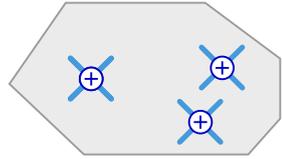
18.4.4.2 Interactive Measurement

18.4.4.2.1 Annotations

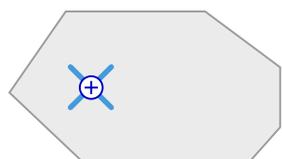
18.4.4.2.1.1 Arrow Tool

Icon	Description	Use
	Adds an arrow to the image to indicate a feature.	<ul style="list-style-type: none"> Click to specify the location of the tail and head of the arrow.

18.4.4.2.1.2 Events Tool

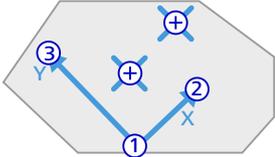
Icon	Description	Use
	<p>Adds multiple crosses to the image to indicate the location of features.</p>	<ul style="list-style-type: none"> ■ Click to specify the location of the crosses. <p>The coordinates and intensity (pixel value) are displayed next to each cross.</p>
		

18.4.4.2.1.3 Marker Tool

Icon	Description	Use
	<p>Adds a cross to the image to indicate the location of a feature and measures the intensity of the selected pixel.</p>	<ul style="list-style-type: none"> ■ Click to specify the location of the cross. <p>The coordinates and intensity (pixel value) are displayed next to the cross.</p>
		

18.4.4.2.1.4 Points Relative Tool

Icon	Description	Use
	<p>Enables you to draw perpendicular axes to define a relative coordinate system in the image. You can then mark points in the image and display the locations of the points in the relative coordinate system.</p>	<ol style="list-style-type: none"> 1 Click to set the origin of the relative coordinate system. 2 Click to set the direction of the positive x and y axes.

Icon	Description	Use
		<p>3 Click to define points in the new coordinate system.</p> 

18.4.4.2.1.5 Text Tool

Icon	Description	Use
	<p>Adds a text box to the image.</p>	<p>■ Click to specify the location of the top left corner of the text box.</p> 

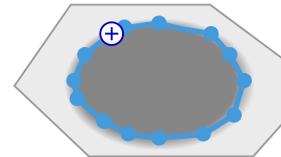
TIP

To change the rotation of the text box, click and drag the node above the text box (**rotate** icon)

18.4.4.2.2 Area Tools

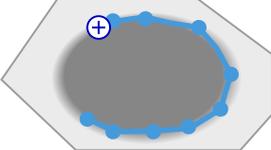
18.4.4.2.2.1 Active Contour Tool

Icon	Description	Use
	Measures the area enclosed by a line (in pixels) along a contour of constant brightness. It also measures the mean intensity of the enclosed pixels. The software tries to place the points on pixels with the closest intensity to the first point.	<ol style="list-style-type: none"> 1 Hold down the left mouse button and move the cursor along the points through which the line should pass. 2 Right click to complete the line. The line is "closed", i.e. the last node is joined to the first node by a straight line to create an enclosed shape.

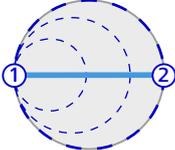


18.4.4.2.2.2 Active Curve Tool

Icon	Description	Use
	Measures the distance (in pixel) along a contour of constant brightness. The software tries to place the points on pixels with the closest intensity to the first point.	<ol style="list-style-type: none"> 1 Hold down the left mouse button and move the cursor along the points through which the line should pass. If the software detects a contour nearby, it automatically places the point on the contour. 2 Right click to complete the line. The line remains open, i.e. the last node is not joined to the first node.

Icon	Description	Use
		

18.4.4.2.3 Circle (Diameter) Tool

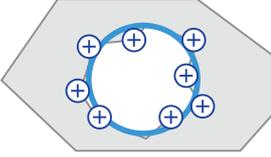
Icon	Description	Use
	Measures the diameter of a circle.	<ul style="list-style-type: none"> ■ Click and drag to specify the location and size of the circle, starting at a point on the circumference. <p>By default, the diameter is measured horizontally in the image, regardless of how you draw the circle.</p> 

TIP

To change the orientation of the diameter, click and drag the center node (**hand** icon).

18.4.4.2.4 Circle (Points) Tool

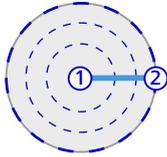
Icon	Description	Use
	Measures the area and diameter of a best fit circle defined by at least three points.	Use this tool if, for example, only an arc of the circle (i.e. not the entire circle) is visible in the image.

Icon	Description	Use
		<ol style="list-style-type: none"> 1 Click several (at least three) positions at the circumference to specify the points describing the circle. 2 Finish editing by right-clicking. Note: This final click creates the last circle point. 

TIP

To change the orientation of the radius, click and drag the center node.

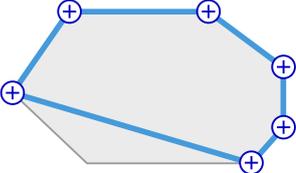
18.4.4.2.2.5 Circle (Radius) Tool

Icon	Description	Use
	<p>Defines a circle measurement via the radius.</p>	<ol style="list-style-type: none"> 1 Click to specify the center of the circle. 2 Drag to specify the size and orientation of the radius. 

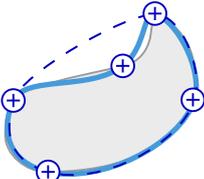
TIP

To change the orientation of the radius, click and drag the center node (**hand** icon).

18.4.4.2.2.6 Contour (Polygon) Tool

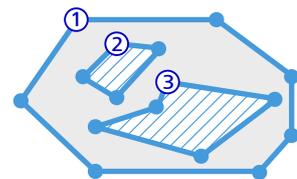
Icon	Description	Use
	<p>Measures the area enclosed by a line that passes through all the selected points.</p>	<ol style="list-style-type: none"> Click multiple times to select the points through which the line should pass. Right click to complete the line. <p>The line is closed, i.e. the last node is joined to the first node by a straight line to create an enclosed shape.</p> 

18.4.4.2.2.7 Contour (Spline) Tool

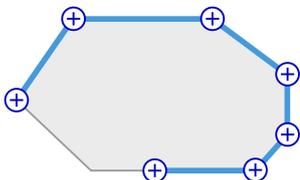
Icon	Description	Use
	<p>Measures the area enclosed by a polynomial curve that passes through all the selected points.</p>	<ol style="list-style-type: none"> Click multiple times to select the points through which the polynomial curve should pass. Right click to complete the curve. <p>The curve is closed, i.e. the last node is joined to the first node by a curve to create an enclosed shape.</p> 

18.4.4.2.2.8 Contour with Holes Tool

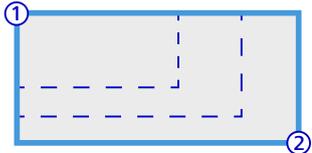
Icon	Description	Use
	Measures the area enclosed by a polygon less the area enclosed by multiple holes within the polygon.	<ol style="list-style-type: none"><li data-bbox="1090 461 1442 573">1 Click multiple times to define the edge of the outer polygon.<li data-bbox="1090 584 1442 864">2 Right-click to complete the line. The line is closed, i.e. the last node is joined to the first node by a straight line to create an enclosed shape.<li data-bbox="1090 875 1442 1032">3 Click within the shape to define a hole to be subtracted from the polygon.<li data-bbox="1090 1043 1442 1122">4 Right-click to complete the hole.<li data-bbox="1090 1133 1442 1211">5 Repeat steps 3 and 4 as desired.<li data-bbox="1090 1223 1442 1377">6 Right-click outside the outer polygon to complete the measurement.



18.4.4.2.9 Curve (Polygon) Tool

Icon	Description	Use
	<p>Measures the distance along a line that passes through all the selected points.</p>	<ol style="list-style-type: none"> 1 Click to select the points through which the line should pass. 2 Right click to complete the line. <p>The line remains open, i.e. the last node is not joined to the first node.</p> 

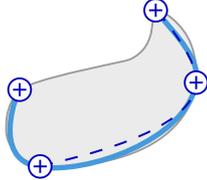
18.4.4.2.10 Rectangle Tool

Icon	Description	Use
	<p>Measures the area enclosed by a rectangle.</p>	<p>Click and drag to specify size and location of the rectangle.</p> 

TIP

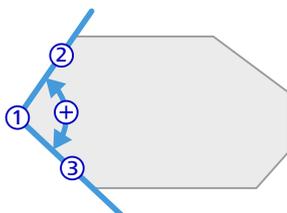
To change the rotation of the rectangle, click and drag the node above it (**rotate** icon).

18.4.4.2.2.11 Spline Curve Tool

Icon	Description	Use
	<p>Measures the distance along a polynomial curve that passes through all the selected points.</p>	<ol style="list-style-type: none"> 1 Click to select the points through which the polynomial curve should pass. 2 Right click to complete the curve. <p>The curve remains "open", i.e. the last node is not joined to the first node.</p> 

18.4.4.2.3 Measurements

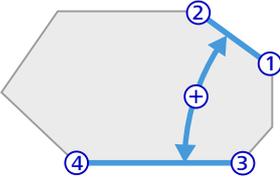
18.4.4.2.3.1 Angle (Connected) Tool

Icon	Description	Use
	<p>Measures the angle between two connected lines.</p>	<ol style="list-style-type: none"> 1 Click to set the position where the two lines should meet. 2 Click to specify the end point of each line. 

TIP

To change the location of the angle arc, click and drag the arc node (**hand** icon).

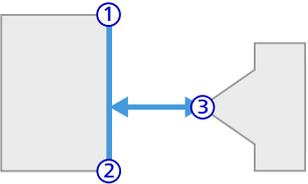
18.4.4.2.3.2 Angle (Disconnected) Tool

Icon	Description	Use
	<p>Measures the angle (<math><180^\circ</math>) between two lines that do not join.</p>	<ol style="list-style-type: none"> 1 Click to set the start and end points of the first line. 2 Click to define the start and end points of the second line. <p>The angle measured depends on the order of the points as shown below. You cannot measure an angle >180°.</p> 

TIP

To change the location of the angle arc, click and drag the arc node (**hand** icon).

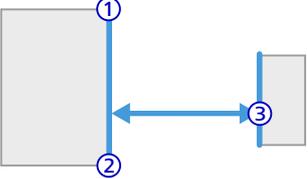
18.4.4.2.3.3 Caliper Tool

Icon	Description	Use
	<p>Measures the perpendicular distance between a line and a point.</p>	<ol style="list-style-type: none"> 1 Click to define the start and end point of the line. 2 Click to define the location of the point. <p>The perpendicular distance between the line and the point is displayed.</p> 

TIP

To change the location of the distance indicator, click and drag the center node (**hand** icon).

18.4.4.2.3.4 Distance Tool

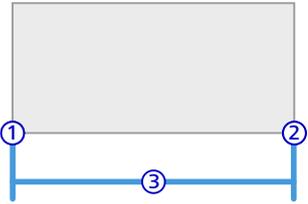
Icon	Description	Use
	Measures the distance between two parallel lines.	<ol style="list-style-type: none"> 1 Click to define the start and end point of the first line. 2 Click to define the location of second parallel line. <p>The perpendicular distance between the lines is displayed at the location of the third click.</p> 

TIP

To change the location of the distance indicator, click and drag the center node (**hand** icon).

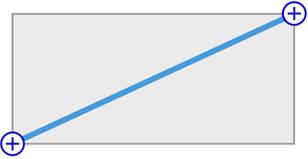
18.4.4.2.3.5 Length Tool

Icon	Description	Use
	Measures the distance between two points. The distance indicator is displayed with an offset.	

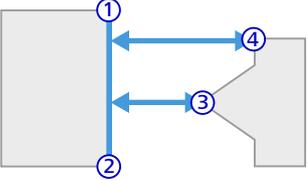
Icon	Description	Use
		<ol style="list-style-type: none"> 1 Click to specify the points to measure between. 2 Click to specify the location of the measurement line (i.e. the size of the offset). The measurement is placed parallel to the distance to be measured. 

TIP
To change the location (offset) of the distance indicator, click and drag the center node (**hand** icon).

18.4.4.2.3.6 Line Tool

Icon	Description	Use
	<p>Measures the distance between two points directly.</p>	<p> Click to specify the start and end points of the line</p> 

18.4.4.2.3.7 Multi Calipers Tool

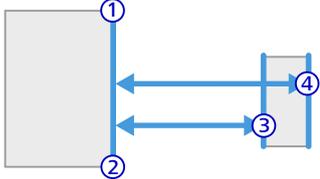
Icon	Description	Use
	Measures the perpendicular distances between a line and multiple points.	<ol style="list-style-type: none"> 1 Click to define the start and end point of the line. 2 Click to define the location of the first point. 3 Click to define the locations of the other points. <p>The perpendicular distances between the line and the points are displayed.</p> 

TIP

To change the location of the distance indicator, click and drag the center node (**hand** icon).

18.4.4.2.3.8 Multi Distance Tool

Icon	Description	Use
	Measures the distances between multiple parallel lines.	<ol style="list-style-type: none"> 1 Click to define the start and end point of the first line. 2 Click to define the location of second parallel line. 3 Click to define the location of further parallel lines.

Icon	Description	Use
		<p>The perpendicular distances between the lines are displayed.</p> 

TIP

To change the location of the distance indicator, click and drag the center node (**hand** icon).

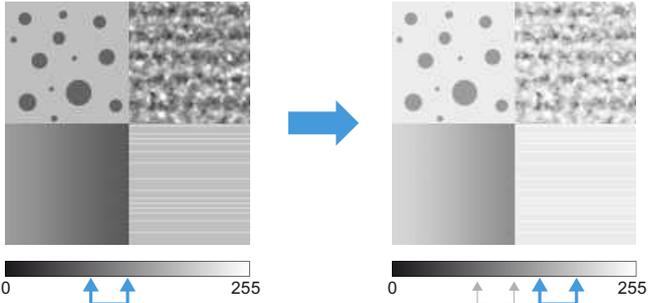
18.4.5 Processing

18.4.5.1 Image Processing

18.4.5.1.1 Adjust

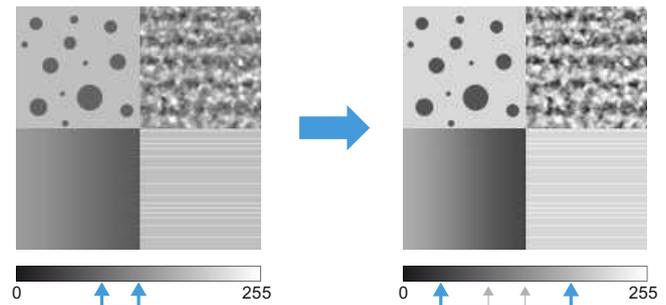
18.4.5.1.1.1 Brightness/Contrast/Gamma Tool

This tool provides various methods to change the brightness and contrast of an image, depending on the pixels you want to adjust, e.g. all pixels, just darker pixels, etc.

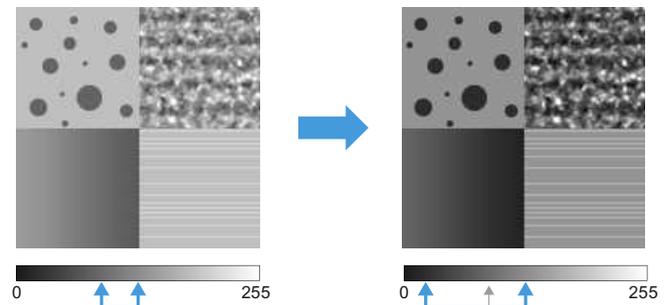
Parameter	Description
<p>Brightness</p>	<p>Increases or decreases the value of each pixel by a constant value up to the limits (given by the pixel type).</p> <p>The range of brightness values (i.e. the relative brightness of light and dark areas of the image) remains constant.</p> 

Parameter**Description****Contrast**

Broadens or narrows the range of brightness values (i.e. the relative brightness of light and dark areas of the image). A high contrast helps distinguish the light and dark areas of the image. Increasing the contrast can cause subtle graduated tones to be lost, whilst decreasing the contrast can cause the image to look soft.

**Gamma**

Enhances details in brighter or darker image regions. Setting the gamma value causes the value of each pixel to be multiplied by an individual factor. This factor depends on the pixel value (brightness) itself.



- Gamma < 1:
 - Details in bright image regions reduced
 - Details in dark image regions enhanced
- Gamma = 1: No change
- Gamma > 1:
 - Details in bright image regions enhanced
 - Details in dark image regions reduced

18.4.5.1.1.2 Color Balance Tool

This tool adjusts the hue of a color image. You can set the hue of each color channel for three predefined brightness ranges independently.

Parameter	Description
Range	Specifies the brightness range to which the hue settings apply
– Shadows	Dark pixels only
– Midtones	Intermediate pixels only
– Lights	Light pixels only
Hue	Sets the hue of each color channel for the selected brightness range

18.4.5.1.1.3 Color Temperature Tool

This tool adjusts the color temperature of a color image by changing the hue.

Color temperature defines how a color appears dependent on the ambient lighting. If for example, an image was acquired under light weighted towards the blue end of the spectrum, you can decrease the color temperature to make the image appear more like the sample would have been acquired in neutral lighting conditions.

Parameter	Description
Temperature Delta	<p>Specifies the hue by which the pixels are changed. Changing the value by 1 corresponds to a color temperature change of 10 Kelvin.</p> <ul style="list-style-type: none"> ■ Negative values reduce the color temperature, resulting in a warmer redder hue. ■ Positive values increase the color temperature, resulting in a colder bluer hue. <p>You can increase or decrease the color temperature by up to 3,000 Kelvin.</p>

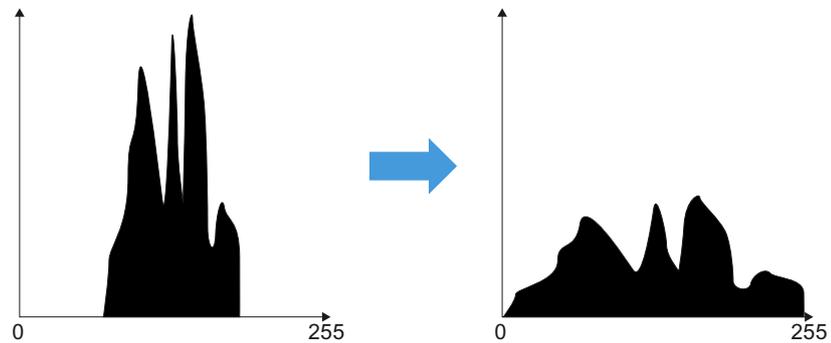
18.4.5.1.1.4 Histogram Equalization Tool

This tool increases the global contrast of an image.

Histogram equalization is particularly useful if the regions of interest and the background have similar pixel values, i.e. all the pixel values in the image lie close together.

Histogram equalization maps the highest occurring pixel value to the maximum pixel value given by **Upper Threshold** and the lowest occurring pixel value to the minimum pixel value given by **Lower Threshold**. The pixel values in between are mapped accordingly.

As a result, the pixel values are drawn apart and the contrast is increased.

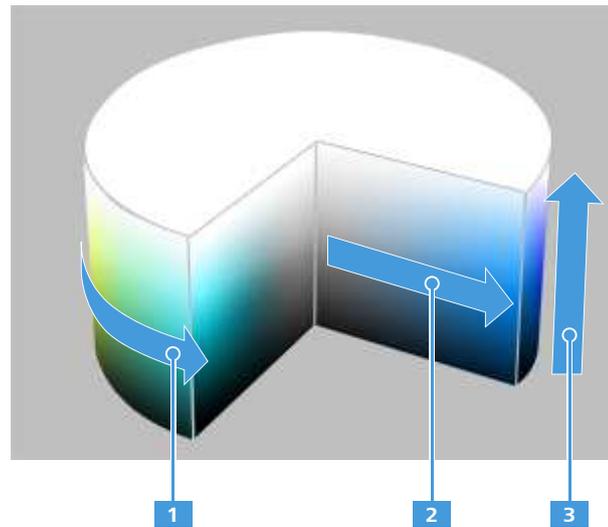


Parameter	Description
All z-layers, All time points	<p>Only visible, if the image is a Z stack/time series</p> <ul style="list-style-type: none"> ■ Activated: The tool is applied to each image of the Z stack/time series. ■ Deactivated: The tool is applied to the currently selected image only.
Upper Threshold	<p>Defines the maximum value to which the highest occurring pixel value is mapped. Upper Threshold can be set to between 90 % and 100 % of the maximum pixel value.</p>
Lower Threshold	<p>Defines the minimum value to which the lowest occurring pixel value is mapped. Lower Threshold can be set to between 0 % and 10 % of the maximum pixel value.</p>

18.4.5.1.1.5 Hue/Saturation/Lightness Tool

This tool adjusts the color impression of an image by modifying the parameters in the HSL color space.

The HSL color space is an alternative color system. All colors available in the RGB color space are mapped to a cylinder.



- 1 Hue
- 2 Saturation
- 3 Lightness

In the HSL color space, a color is defined by the following (cylindrical) coordinates:

- Angle about the cylinder axis: Hue, which goes from red over green to blue.
- Radial distance from the cylinder axis: Saturation, which is a measure for the colorfulness.
- Height along the cylinder axis: Lightness, which expresses the brightness of a color relative to an equivalently illuminated white.

Parameter	Description
Hue	<p>Shifts the color impression of the image towards red or blue.</p> <ul style="list-style-type: none"> ■ Positive angles shift the hue towards red. ■ Negative angles shift the hue towards blue. <p>As shown above, the hue is defined by the angle about the HSL cylinder axis. The values -180 and +180 therefore have an identical effect.</p>
Saturation	<p>Sets the colorfulness of the image between completely colorless (i.e. grayscale) at 0 and maximum colorful at 200.</p>
Lightness	<p>Describes how bright or dark a color pixel appears.</p> <p>The lightness of a color pixel corresponds to the brightness of an equally bright gray pixel. It is the average of the red, green, and blue value of this pixel.</p>

Parameter	Description
	The lightness slider is normalized such that -100 yields a uniform black image and +100 yields a uniform white image.

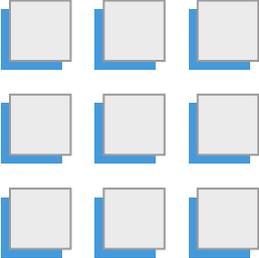
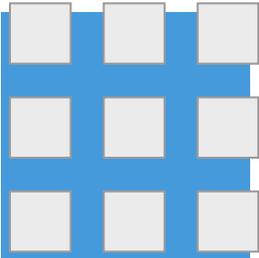
18.4.5.1.1.6 Shading Correction Tool

This tool compensates for uneven exposure of an image, such as vignetting. The uneven exposure (shading) might be caused by non-uniform illumination, non-uniform camera sensitivity, or dirt and dust on glass (lens) surfaces.

The tool loads a reference image and applies it to the unevenly exposed image. The reference image should contain information about the illumination only and no specific information, e.g. the structure of a sample. You can record the reference image as follows:

- Transmission microscope**
- 1 Remove the sample and sample holder from the light path.
 - 2 Move the objective until the light source is out of focus.
A brightness distribution without any structural information is visible.
 - 3 Acquire the image.
This image can be used as a reference image for the current light path. If you change any component of the light path, such as objective or beam splitter, you have to acquire a new reference image for the new light path settings.
- Reflection microscope**
- 1 Use a white piece of paper as the sample.
 - 2 Move the objective until the piece of paper is out of focus.
A brightness distribution without any structural information is visible.
 - 3 Acquire the image.
This image can be used as a reference image for the current light path. If you change any component of the light path, such as objective or beam splitter, you have to acquire a new reference image for the new light path settings.

Parameter	Description
in1	Loads the input image which is exposed unevenly and needs to be corrected.
in2	loads the reference image containing the illumination pattern to be applied to the image from in1
Shading Mode	The Shading Mode defines how ZEN 2 core processes tile images used as in1 input images. Tile images result from certain acquisition methods, such as tiles or panorama. If you apply Shading Correction to a simple image, you can ignore this setting.

Parameter	Description
– Camera Shading	 <ul style="list-style-type: none"> ■ Applies the shading correction to each tile of a tile image separately. ■ The size of the reference image (in2) should correspond to the size of a single tile. <p>Automatic</p> <p>Activated: Calculates a corrected image from the input image itself. The correction image from in2 is ignored.</p> <p>If no reference image is available, such as a background image acquired without a sample, you can try this method.</p>
– Global Shading	 <ul style="list-style-type: none"> ■ Applies the shading correction globally to the whole input image composed of all tiles. ■ The size of the correction image (in2) should correspond to the size of the whole input image composed of all tiles.
Display Mode	Specifies how the reference image is applied
– Additive	<p>The reference image (in2) is normalized and then subtracted from the input image (in1).</p> <p>Use this if your reference image contains the (in-focus) background you wish to remove.</p>
– Multiplicative	<p>The input image (in1) is divided by the normalized reference image (in2). This is the default setting.</p> <p>Use this if your reference image contains the illumination information as described above and you wish to correct for incorrect shading.</p>

Parameter	Description
Offset	Adjusts the brightness of the final image by adding a constant value to each pixel value.

NOTE

If the input image and the reference image do not match in size, ZEN 2 core acts in the following manner:

- If the reference image is smaller than the input image, the reference image is applied to the upper left corner of the input image.
- If the reference image is larger than the input image, the upper left corner of the reference image is applied to the input image.

18.4.5.1.1.7 White Balance Tool

This tool enables you to adjust the colors of an image.

White balance enables you to remove a color cast (e.g. a red or green tint) from an image and to make the colors appear neutral. Poor white areas are changed to pure white.

The color temperature adjusts the overall hue of the image.

You can use white balance and color temperature to remove detrimental effects of ambient lighting. If for example, an image was acquired under light weighted towards the blue end of the spectrum, you can use white balance and/or decrease the color temperature to make the image appear more like the sample would have been acquired in neutral lighting conditions.

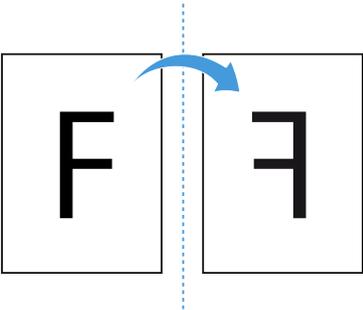
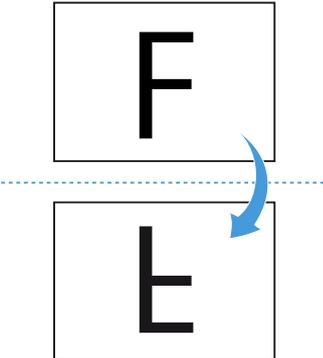
Parameter	Description
Automatic	Selects the white balance point automatically and adjusts the hue of all other pixels accordingly.
 Pick	Enables you to specify the white balance point manually. The hue of all other pixels is adjusted accordingly. To achieve an optimum result, pick a neutral white pixel.
Temperature Delta	Specifies the hue by which the pixels are changed. Changing the value by 1 corresponds to a color temperature change of 10 Kelvin. <ul style="list-style-type: none"> ■ Negative values reduce the color temperature, resulting in a warmer redder hue. ■ Positive values increase the color temperature, resulting in a colder bluer hue.

Parameter	Description
	You can increase or decrease the color temperature by up to 3,000 Kelvin.

18.4.5.1.2 Geometric

18.4.5.1.2.1 Mirror Tool

This tool mirrors the image about a vertical or horizontal axis.

Parameter	Description
Display Mode	
- Horizontal	Mirrors the image about a vertical axis.
	
- Vertical	Mirrors the image about a horizontal axis.
	

18.4.5.1.2.2 Resample Tool

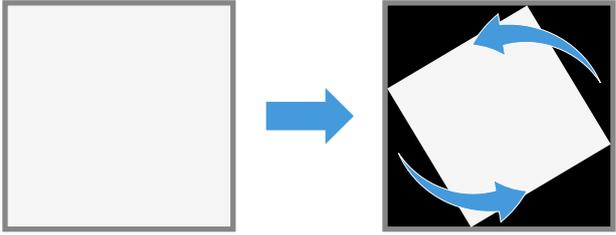
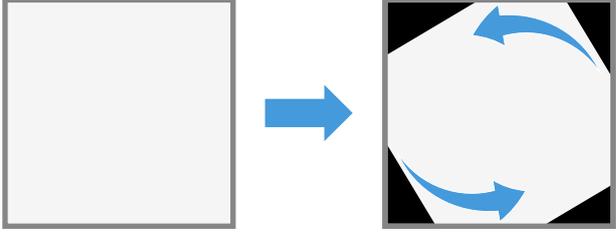
This tool resizes an image in each direction separately.

Parameter	Description
Adapt Sizes	<ul style="list-style-type: none"> ■ Activated: The image pane adjusts to the resized image. The entire resized image is visible. ■ Deactivated: The size of the image pane remains constant. If the image is enlarged, the parts of the image outside the image pane are cropped. <p>Use Shift in X and Shift in Y to specify the section of the resized image that should be retained in the image pane.</p>
Interpolation	<p>If the image is resized, the number of pixels changes. Interpolation defines the method used to calculate new pixels.</p>
– Nearest Neighbor	<ul style="list-style-type: none"> ■ Lowest quality ■ Shortest calculation time
– Linear	<ul style="list-style-type: none"> ■ Medium quality ■ Average calculation time
– Cubic	<ul style="list-style-type: none"> ■ Highest quality ■ Longest calculation time
Shift X, Shift Y	<p>Use Shift X and Shift Y to display the desired section of the resized image.</p> <p>Only available if Adapt Sizes is deactivated.</p>
Scaling X, Scaling Y	<p>Defines the scaling factor by which the image is resized. You can select the scaling factor for each direction separately.</p>

18.4.5.1.2.3 Rotation Tool

This tool rotates the image counter-clockwise.

Parameter	Description
Horizontal Alignment	<p>If, for example, the sample was not aligned correctly on the motorized stage, it will appear rotated in the image. To correct this, you can draw a line in the image to indicate which part of the sample should be parallel to the base of the image. The image is then rotated accordingly.</p> 

Parameter	Description
	<ol style="list-style-type: none"> 1 Click the Horizontal Alignment button. 2 In the image, click to set the start and end points of the correction line. <p>The tool sets the rotation angle accordingly.</p>
Angle	Sets the rotation angle by which the image rotates counter-clockwise.
Center X, Center Y	<p>Sets the pivot point about which the image rotates.</p> <p>Center X = 0 and Center Y = 0 corresponds to the upper left corner of the image. The Center X and Center Y values determine the number of pixels by which the pivot point moves in each direction.</p> <ul style="list-style-type: none"> ■ Positive Center X values move the pivot point to the right. ■ Positive Center Y values move the pivot point down.
Change Size	<ul style="list-style-type: none"> ■ Activated: <div style="display: flex; align-items: center; margin: 10px 0;">  </div> <ul style="list-style-type: none"> – The image pane is variable and adjusts to the rotated image. – Corners are preserved. – The image size varies. – Empty areas are filled black. ■ Deactivated: <div style="display: flex; align-items: center; margin: 10px 0;">  </div>

Parameter	Description
	<ul style="list-style-type: none"> – The image pane is fixed and corresponds to the original image dimensions. – Corners rotated outside the fixed image pane are cropped. – The image size is preserved. – Empty areas are filled black.

18.4.5.1.2.4 Shift Tool

This tool shifts the content of an image horizontally or vertically. The size of the image pane (the area filled by the original image) does not change. Pixels shifted outside the image plane are deleted.

Parameter	Description
Shift X	<p>Sets the number of pixels by which the image is shifted horizontally.</p> <ul style="list-style-type: none"> ■ Positive values: The image is shifted to the right. ■ Negative values: The image is shifted to the left.
Shift Y	<p>Sets the number of pixels by which the image is shifted vertically.</p> <ul style="list-style-type: none"> ■ Positive values: The image is shifted down. ■ Negative values: The image is shifted up.

18.4.5.1.3 Sharpen

18.4.5.1.3.1 Delineate Tool

This tool emphasizes edges around structures in an image. It is useful for images where the gray value range of structures differs clearly from the gray value range of the pixels around them. In contrast to other sharpening tools, the halo effect around the emphasized edges is reduced.

Parameter	Description
Threshold	The difference in gray values which specifies an edge between neighboring image regions

Parameter	Description
	The Threshold value should correspond roughly to the gray value difference between foreground objects and the background.
Size	Determines the size of image details which are enhanced – the smaller the Size value, the finer the details affected by the tool. The Size value should correspond to the size of the transition area between foreground objects and the background.

18.4.5.1.3.2 Enhance Contours Tool

This tool emphasizes fine structures in an image. It finds image regions where pixel values change rapidly, identifies these regions as contours and enhances them.

Parameter	Description
Strength	Determines the size of image details which are enhanced - the higher the strength value, the finer the details affected by the tool. If you set the strength value too high, too many image details are enhanced. You obtain a grainy image and lose image information.

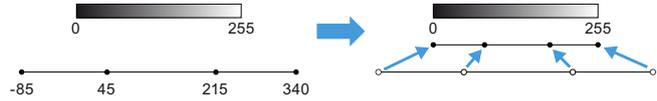
Parameter	Description
Normalization	Defines how out-of-range pixel values are mapped The calculated pixel values of the output image may be out-of-range and are mapped into the available range.

– Clip

Values exceeding the pixel value range are set to the highest value available (white), values falling short of the pixel value range are set to the lowest value available (black).



- Automatic** Normalizes the pixel values automatically to the available pixel value range. The highest resulting value is mapped to the maximum pixel value, the lowest resulting value to 0. As a result, the whole range of resulting pixel values is compressed evenly.



- Wrap** If a resulting value is larger than the maximum pixel value of the image, the difference exceeding the maximum pixel value is added to 0. Similarly, if a resulting value is below 0, the resulting pixel value is the maximum pixel value minus the difference falling below 0.



- Shift** Normalizes the output to the value "pixel value + maximum pixel value/2". As a result, all resulting values are mapped to the available value range.

The middle value of the pixel value range remains constant. Values left and right of the middle value are changed progressively, so that values inside the pixel value range are changed only slightly. Values outside the pixel value range are changed strongly and mapped to the fringes of the pixel value range.



- Absolute** Converts negative pixel values into positive values. Positive pixel values exceeding the maximum pixel value are set to the maximum pixel value.



18.4.5.1.3.3 Unsharp Masking Tool

This tool enhances contrasts at fine structures and edges. Thus, the resulting image appears sharper.

Parameter	Description
Strength	Defines the amount of contrast enhancement applied to fine structures and edges. The higher the Strength , the greater the edge enhancement.
Radius	Defines the size of detail to be enhanced and the appearance of enhanced edges <ul style="list-style-type: none"> ■ Small radius: enhances small details ■ Large radius: enhances large details <p>A large radius leads to a halo along enhanced edges. The larger the radius, the broader this halo.</p>
Color Mode	Defines the calculation method, which affects the appearance of the output image
– RGB	<ul style="list-style-type: none"> ■ The sharpness is calculated for each color channel separately. ■ The color saturation and the color of structures may be changed and color noise may occur.
– Luminance	<ul style="list-style-type: none"> ■ The sharpness is calculated based on an average brightness signal of all color channels. ■ This mode does not show any color noise or change of color saturation.
Threshold Mode	Specifies how the boundaries between sharpened image regions are calculated <p>It is only effective if the Threshold Low value is not equal to 0 or the Threshold High value is not equal to 100.</p>
– None	No adjustment
– Binary	The boundaries follow the threshold values
– Linear	The boundaries follow a linear course calculated from the threshold values
Threshold Low, Threshold High	Defines the minimum and maximum contrast along edges and structures which are to be affected by the Unsharp Masking filter

Parameter	Description
	<p>Areas with a contrast in the range between Threshold Low and Threshold High are considered, areas showing a contrast outside the range are ignored.</p> <p>Use the threshold limits to avoid the following:</p> <ul style="list-style-type: none"> ■ Threshold High: Setting it too high overemphasizes edges which already show a high contrast. ■ Threshold Low: Setting it too low leads to sharpening of false edges in relatively uniform areas. This can result in undesired speckles in low contrast areas. <p>If you set Threshold High too low or Threshold Low too high, the Unsharp Mask filter will be reduced in its effect.</p>
Clip To Valid Bits	<ul style="list-style-type: none"> ■ Activated: The output image is composed of the same colors as the input image (i.e. the value range of the output image is adjusted to the color range of the input image). ■ Deactivated: Colors not present in the original image may appear in the output image.

18.4.5.1.4 Smooth

18.4.5.1.4.1 Binomial Filter Tool

This tool reduces noise in an image. Each pixel is replaced by a weighted average of its neighbors. The weighting depends on the kernel size.

The binomial filter is very similar to a Gaussian filter in its effect. It is faster in terms of calculation time but offers fewer options.

Parameter	Description
Kernel Size	Sets the number of neighboring pixels taken into account. A higher kernel size leads to more noise reduction but also to a larger amount of blur in the resulting image.

18.4.5.1.4.2 Denoise Tool

This tool removes noise from an image using a real or a complex wavelet transformation. While common filter-based noise reduction tools always smooth the entire image and reduce details such as thin lines, the wavelet method preserves details as far as possible.

This tool uses the method of bivariate shrinkage with local variance estimation (thresholding) is used. [Bivariate Shrinkage with Local Variance Estimator, Levent Sendur and Ivan W. Selesnick, IEEE Signal Processing Letters, Vol. 9, No. 12, December 2012]

Parameter	Description
Complex Wavelets	<p>Uses the Dual-Tree Complex Wavelet Transform which provides extra coefficients (information) for analysis and yields better results</p> <ul style="list-style-type: none"> ■ Lower probability of producing block artefacts ■ Computationally intense, slower
Real Wavelets	<p>Uses the discrete wavelet transform which has fewer coefficients (less information) for analysis</p> <ul style="list-style-type: none"> ■ Higher probability to produce block artefacts ■ Computationally easier, faster

18.4.5.1.4.3 Gauss Tool

This tool reduces noise in an image. Each pixel is replaced by a weighted average of its neighbors. The weighting depends on the sigma values.

The Gaussian filter is very similar to a binomial filter in its effect. It is slightly slower in terms of calculation time but allows you to define the strength by its sigma values instead of defining the filter size directly.

Parameter	Description
Sigma X, Sigma Y	<p>Determines how much neighboring pixels in horizontal and vertical direction contribute to the weighting.</p> <p>Larger values in sigma broaden the applied Gaussian distribution and lead to reduced noise in the corresponding direction, but also to an increasing loss of image information (blur).</p>

TIP

The Gaussian filter is particularly useful for edge detection, which is very sensitive to noise. Using a Gaussian filter before detecting edges greatly improves the results.

18.4.5.1.4.4 Lowpass Tool

This tool reduces noise in an image. Each pixel is replaced by the average of its neighbors. The area which contributes to the average value is determined by the kernel size.

Parameter	Description
Count	Specifies the number of times the tool is applied More repetitions leads to stronger noise reduction but also to more loss of image detail.
Kernel Size X, Kernel Size Y	Determines the number of neighboring pixels taken into account

18.4.5.1.4.5 Median Tool

This tool reduces noise in an image. Each pixel is replaced by the median of its neighbors. The number of neighboring pixels taken into account depends on the kernel size.

Parameter	Description
Kernel Size X, Kernel Size Y	Determines the number of pixels taken into account in horizontal and vertical direction. Larger kernel sizes lead to reduced noise in the corresponding direction, but also to an increasing loss of image information (blur).

i INFO

In a set of values (in this case the pixel values taken into account), the median is the middle value for which the number of larger values is equal to the number of smaller values.

18.4.5.1.4.6 Sigma Tool

This tool reduces noise in an image. The noise is removed selectively in image areas that have a relatively uniform brightness. As a result, fine object structures are not modified.

Each pixel is replaced by an average of its neighbors. In order to calculate the average, only the brightness values that lie within a defined range (\pm sigma) around the brightness value of the central pixel are taken into account.

Parameter	Description
Sigma	<p>Defines which neighboring pixels are taken into account.</p> <p>For example, if Sigma equals 50, only neighboring pixels deviating by less than ± 50 from the brightness value of the central pixel are used to calculate the new value of this pixel.</p>
Kernel Size X, Kernel Size Y	Specifies the number of neighboring pixels taken into account for each pixel.

18.4.5.1.4.7 Single-Pixel Filter Tool

This tool removes single pixel phenomena.

Single pixel phenomena can occur due to a faulty exposure of single pixels or lines of pixels, resulting from one of the following:

- Adverse ambient conditions
- Adverse timing of successive image acquisitions
- The properties of the camera sensor itself

The incorrectly exposed pixels are typically recognizable as particularly light or dark points or lines.

Parameter	Description
Threshold	<p>Specifies if a single bright pixel is removed (i.e. adapted to its neighboring pixels). The effect of Threshold depends on the neighboring pixels.</p> <p>The higher the Threshold, the brighter a pixel has to be compared to its neighboring pixels to be removed by the filter.</p>

18.4.5.1.5 Utilities

18.4.5.1.5.1 Add Channels Tool

This tool combines the channels of two images into a multi-channel image. The number of resulting channels equals the sum of the channels of each image.

The color channels of an RGB color image are not considered separate channels, i.e. if you combine two RGB images the result is a multichannel image with two channels (and not six).

Parameter	Description
in1, in2	Specifies the two images to be combined into a multi-channel image

18.4.5.1.5.2 Change Pixel Type Tool

This tool changes the amount of information stored in a grayscale or color image. For example, you can use this tool to change a color image into a gray scale image or a 24 Bit RGB image into a 48 Bit RGB image. This can be useful if you want to compare or combine images with different pixel types.

Parameter	Description
Pixel Type	<p>Specifies the following properties of the image:</p> <ul style="list-style-type: none"> ■ Number of channels ■ Range of pixel values per channel ■ Number format <p>For supported values, see <i>Pixel Type</i> [▶ 80].</p>

NOTE

Consider the following before changing the pixel type:

- If you select a pixel type smaller than the current pixel type, you might reduce the image quality and lose image information. This information cannot be restored later.
- If you select a pixel type larger than the current pixel type (e.g. 48 Bit RGB for an 24 Bit RGB image), the image quality does not improve. However, the range available for certain image processing operations is extended.

18.4.5.1.5.3 Combine RGB Tool

This tool generates a color image from three grayscale input images. Each grayscale image contains the pixel values of one of the color channels; red, green, and blue.

You can use this tool to create a color image as follows:

- Combine the individual channels of a color image

If a color image is split into individual images for each channel (for example to modify one of them), you can recombine the images (channels) into a single color image.

- Combine images acquired using a B/W camera in combination with red, green, and blue color filters

Acquire three grayscale images applying one of the filters for each image and combine them into a color image.

If you use a color image as an input for **Combine RGB**, the tool first converts it into a grayscale image. It does not extract the data from the corresponding color channel.

Example: If you use a color image as the input of the blue channel, the tool calculates a mean grayscale image and does not extract the data from its blue channel. This grayscale image for the blue channel is then combined with the input images for the red and green channels.

Parameter	Description
in1, in2, in3	Specifies the gray scale images representing the red, green, and blue channel of the output image. Avoid using color images as input.
Output Pixel Type	Sets the pixel type (i.e. color depth) of the output image. <i>Pixel Type</i> ▶ 80]

18.4.5.1.5.4 Copy Annotations Tool

This tool copies the annotations of one image into another image.

Parameter	Description
Preserve Scaled Size	Has an effect if the size of the target image is different from the size of the source image: <ul style="list-style-type: none"> ■ Activated: The copied annotations remain unchanged. ■ Deactivated: The copied annotations are scaled according to the possible image size difference.

18.4.5.1.5.5 Copy Image Tool

This tool generates a copy of the current image. It includes the image contents only; any annotations, measurements, tables et cetera will be omitted.

18.4.5.1.5.6 Create Gray Scale Image Tool

This tool can generate various types of grayscale images. You can use this tool for creating test images, e.g. to test the effects of the different image processing tools.

Parameter	Description
Pattern	Specifies whether a uniform grayscale image or a grayscale gradient image is created
– Uniform	Creates a uniform image from a single grayscale value
– 2D Gray Scale Horizontal	Creates a grayscale gradient in horizontal direction.
– 2D Gray Scale Vertical	Creates a grayscale gradient in vertical direction.
Width, Height	Sets the width and height of the output image in pixels.
Min. Gray Value, Max. Gray Value	Sets the upper and lower value of the grayscale gradient. A very small difference between the two values results in a visibly coarse gradient.
Pixel Type	Sets the pixel type (i.e. color depth) of the image.

18.4.5.1.5.7 Generate Image Pyramid Tool

This tool generates a set of images at different resolutions. This enables you to zoom in, out, and navigate efficiently even in very large tile images.

Parameter	Description
Background	Defines the background color used in the image pane when scaling down the image.

18.4.5.1.5.8 Image Generator Tool

This tool creates test images from scratch, in a variety of patterns.

NOTE

If you use this tool in a job after loading or acquiring and processing an image, any image data from previous steps is lost and cannot be exported.

Parameter	Description
Width, Height	Specifies the width and height of the generated image in pixels.
Z-Slices	Specifies the number of layers if you wish to create a Z-stack test image.

Parameter	Description
Channels	Specifies the number of channels if you wish to create a multi-channel test image.
Time Slices	Specifies the number of successive images if you wish to create a time series test image.
Min. Gray, Max. Gray	Sets the upper and lower pixel values of the selected pattern.
Pixel Type	Specifies the pixel type of the generated image, e.g. 8 Bit B/W or 24 Bit RGB. For more information, see <i>Pixel Type</i> [▶ 80].
Pattern	Specifies the pattern of the output image. For more information, see <i>Pattern</i> .

18.4.5.2 Table Processing

18.4.5.2.1 Append Table Tool

This tool enables you to merge tables of measurement results. You can select any results or data table in your current experiment. If you want to merge a table from your file system, you must first import it.

The resulting merged table can be included in a report, exported to the file system, or used to create a histogram.

INFO

If you merge tables with different columns, the resulting table contains the columns of both tables, which might result in a large table with a large number of empty cells.

E.g. table A contains columns for angles and areas and table B contains columns for diameters and circumferences. The merged table consists of four columns, with empty cells where either of the tables A or B does not provide values.

Parameter	Description
Placeholders	<p>The placeholders enable you to select the tables to be merged. You can only merge two tables. The tables are merged in the order displayed (i.e. the table added to the right placeholder is appended below the table in the left placeholder.</p> <p>Click the  arrow in a placeholder and select the corresponding table.</p>

Parameter	Description
Apply	Merges the two tables and creates a new combined table

18.4.5.2.2 Calculate Histogram Tool

This tool calculates a frequency distribution from a table of measurement results.

Parameter	Description
Columns	The column of the table of measurement results for which the frequency distribution is calculated
Class Boundaries	Specifies how to establish the interval for the classification:
– >=, ..., <	
– >, ..., <=	
Automatic Classification	Activated:
Logarithmic	Activated: Classes with a low number of elements are emphasized against classes with a high number of elements.
Class Count	
Display Mode	Specifies how the count of each class is displayed:
– Count	The number of elements in each class is displayed.
– Count Cumulative	For each class, the added up count from zero to this class is displayed.
– Percentage	The number of elements in each class is displayed as a percentage of the total count of elements.
– Percentage Cumulative	For each class, the added up percentage from zero to this class is displayed.
Sum	The sum of the values of the elements in each class is displayed
Sum Cumulative	For each class, the added up sum from zero to this class is displayed.
Percentage Sum	The sum of values of the elements in each class is displayed as a percentage of the total sum of all values.
Percentage Sum Cumulative	For each class, the added up sum from zero to this class is displayed as a percentage of the total sum of all values.

18.4.5.3 Assign Measured Scaling Tool

This tool enables you to calculate the scale for images acquired with microscopes where the individual hardware components can be detected automatically. The scale can be calculated using the following methods:

- Theoretic

Based on the actual properties of the hardware components (e.g. zoom of the objectives, number and separation of pixels on camera chip, etc.)

- Custom scale

Based on a manual (user-defined) measurement created using the **Create Measured Scaling** tool.

Parameter	Description
Scaling	Enables you to specify whether the theoretical or custom scale is used.
Scaling info	The name and properties of the hardware components of the current or selected hardware setup.
Assign scaling to image	Applies the selected scale to the image and all subsequent images in the job.

18.4.5.4 Assign Pixel Size Tool

This tool enables you to assign a scale to an image retrospectively, for example if the image does not contain a scale recognized by the software because it was created on another device.

Parameter	Description
Scale Factor	Enables you to enter the known horizontal (X) and vertical (Y) scale.
Scale Unit	Select the unit of the known scale (e.g. millimeters or inches).
	Locked: Keeps the Scale Factor of X and Y synchronizes. Open: Allows to adjust the Scale Factor of X and Y different.
Assign scaling to image	Applies the selected scale to the image and all subsequent images in the job.

18.4.5.5 Assign Theoretical Scaling Tool

This workbench enables you to calculate the scale for images acquired with manual hardware (i.e. a microscope where the individual hardware components cannot be detected automatically).

The total magnification of the microscope, and thus the scale, is calculated based on the magnification of individual components.

Parameter	Description
Objective	Select the magnification of the objective. Click  or  to add or remove an objective magnification.
Zoom	Select the current zoom of the objective. Click  or  to add or remove a zoom level.
Camera Adapter	Select the magnification of the camera adapter. Click  or  to add or remove a camera adapter magnification.
Total Magnification	The theoretical magnification resulting from the individual magnifications of the above components.
Camera pixel distance	Select the corresponding pixel distance for your camera. Click  or  to add or remove a cameras pixel distance.
Assign scaling to image	Applies the selected scale to the image and all subsequent images in the job.

18.4.6 Reporting

18.4.6.1 Form

18.4.6.1.1 Form Templates Management Tool

This tool enables you to select form templates and add them to the job template.

Parameter	Description
Form template preview	Preview of the selected form template and its name The appearance depends on the view settings (see below)

Parameter	Description
	Allows you to select a different form template
	Creates a new blank form template
	Deletes the current form template
	Specifies what is shown in the preview window: <ul style="list-style-type: none"> <input checked="" type="checkbox"/> Only filename <input checked="" type="checkbox"/> Filename and small preview <input checked="" type="checkbox"/> Filename and large preview
Edit	Enables you to configure the layout of the form template and the properties of each field
Apply	Adds the form template to the job template

18.4.6.1.2 Form Designer Tool

This tool enables you to specify the layout of the fields in a form template and to enter translations of the field names for other languages.

Parameter	Description
Filename	The name of the form template in the software and also the filename of the form template (.formconfig). The name must be unique within the system.
	Save the current form template (overwrite existing template or save with a new filename)
	Deletes the current form template
	Adds a new field at the bottom of the form template You can then drag it to the desired location or configure its properties.
	Adds a new group at the bottom of the form template. You can drag individual fields into the group or drag the group to the desired location.
	Deletes the currently selected field or group
	Undo / redo your last action

Parameter	Description
Form is translated	<p>Specifies which language the field names are displayed in when the job is run:</p> <ul style="list-style-type: none"> ■ Activated: User interface language ■ Deactivated: Default language (English) <p>Note: the field names are not translated automatically, but must be entered manually for each field in the selected target language</p>
[Language]	Displays a preview of the form template in the selected language
Apply	Adds the form template to the job template

18.4.6.1.3 Form Field Settings Tool

This tool enables you to specify the properties of fields in a form template.

Parameter	Description
Label	<p>The name of the field.</p> <p>This value can be translated.</p>
Data link	Specifies whether the value in the field is entered manually (value = This form) or automatically (all other values).
– This form	Field is filled manually with value entered by user
– User name	The name of the user running the job in which the form template is used
– Current date	The date when the form is displayed to the user
– Current time	The time when the form is displayed to the user
– Current date and time	The date and time when the form is displayed to the user
Type	<p>Specifies the format in which the data must be entered in the field, for example that only numbers are allowed.</p> <p>The user is prevented from proceeding if the data is in the incorrect format.</p>
– String	Data is treated as text

Parameter	Description
	Letters, numbers, and punctuation are allowed
– Double	Numbers (including decimals)
– Integer	Only whole numbers (no decimals)
– Date Time	Date (day, month, year) and time (hour, minute, second) The date and time formatting depends on the system settings.
– Date	Specifies a date (day, month, year) The date formatting depends on the system settings.
– Time	Specifies a time (hour, minute, second) The time formatting depends on the system settings.
– Enumeration	An entry from a user-defined drop-down list (see Predefined Values)
– Metadata	Data about the job template, e.g. the department to which it is assigned
Alignment	Alignment of the data relative to the field
Access	How the user running the job can interact with the field
– Read only	User can see value but not change it
– Write	User can enter any value that conforms with the constraints specified under Type
– Select	User can select any value from a pre-defined list
– Select and add	User can select any value from a pre-defined list or add a new entry to the list
Dimension	Specifies the expected input type if Type is set to Integer or Double A drop-down with Units is displayed next to the corresponding field.
Unit	Specifies the default units for the corresponding Dimension
Predefined Values	Allows you to create and manage the list of values that can be entered into the field
– 	Add a new entry

Parameter	Description
– 	Remove the selected entry
– 	Move selected entry higher in the list
– 	Move selected entry lower in the list
Default Value	Specifies the value from the above list that is pre-selected when the form is displayed.

18.4.6.1.4 Form Localization Tool

This tool enables you to enter the translations for field names.

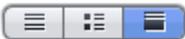
Parameter	Description
locName	The name of the field in the source language. If you want to change this value, use the Label field in the Field settings tool.
Language	Enter the translation of the field name in the corresponding target language

18.4.6.2 Reports

18.4.6.2.1 Add Templates Tool

This tool enables you to select report templates and add them to the job template.

Parameter	Description
Print Report	Prints the report to any local or network printer
Create Report	<ul style="list-style-type: none"> ■ Free Examination mode: Adds the report to the Document and Images area and saves the report template in the Archive ■ Create Job Template mode : Saves the report template in the Archive
Report template preview	Preview of the selected report template and its name The appearance depends on the view settings (see below)
	Allows you to select the report template to be used

Parameter	Description
	If you select multiple report templates, they are joined together into one document.
	Removes the current report template The report template is not deleted and can be selected again by clicking the  icon.
	Specifies what is shown in the preview window: <ul style="list-style-type: none"> ■ Only filename ■ Filename and small preview ■ Filename and large preview
Placeholders	Each report template contains placeholders to enable you to collate the information easily. The placeholders also ensure that each time the job is run, the same information is added to the report. <ol style="list-style-type: none"> 1 Click  and select the desired report template. A preview of the form template is displayed in the Center Screen Area and in the Add Templates tool. 2 Select the template preview in the Add Templates tool. The placeholders available for this template are displayed. 3 Click the  arrow in a placeholder and select the corresponding measurement information that you wish to add, for example image, measurement result, etc.
Show / Hide Annotations	If your image has drawn in graphical elements or annotations (measurements, etc) you can show or hide these annotations in the report. To hide annotations simply deactivate the checkbox on top of the corresponding image.
	

18.4.7 Utilities

18.4.7.1 Linkam Heating Stage Setup Tool

This tool enables you to control temperature and vacuum of the Linkam heating stage. You can control the two properties independently of each other.

Temperature

Parameter	Description
Temperature control on	Activated: Turns the heating stage on
Temperature	Indicates the current temperature
Status	
– Heating	Temperature increasing at the specified Rate
– Cooling	Temperature decreasing at the specified Rate
– Holding	Temperature is being maintained at the Limit value
– Standby	None of the above: ready for a command
Rate (°C/min)	Speed the temperature should change
Limit (°C)	Target temperature When it is reached the stage is maintained at this temperature

Vacuum

Parameter	Description
Vacuum control on	Activated: Turns the vacuum pump on
Measured Pressure	Indicates the current pressure
State	
– Compressing	Pressure increasing until Setpoint Pressure reached
– Expanding	Pressure decreasing until Setpoint Pressure reached
– Holding	Pressure is being maintained at the Setpoint Pressure value
– Standby	None of the above: ready for a command
Setpoint Pressure	Target pressure When it is reached the stage is maintained at this pressure

18.4.7.2 OAD Macro Tool

This tool enables you use macros to automate tasks such as applying a series of processing tools to an image or batch converting images from one file type to another. You can use macros to connect ZEN 2 core with external software, e.g. ImageJ.

Parameter	Description
Selection	Contains the User Documents : A list of all macro files available in the configured macro folder. To configure the macro folder, use Manage Templates from the Home Screen .
Preview	Displays the first lines of the selected macro. It is advisable to begin any macro with a comment describing the main functionality of the macro.
Properties	Displays metadata specified for the selected macro.
– Name	Enter a meaningful name for the macro.
– Keywords	Keywords can be used to further specify the selected macro.
– Description	A description of the macro's functionality.

18.4.8 Shuttle & Find

18.4.8.1 Calibration Settings Tool

Using this tool you can calibrate the correlative holder you will use in your experiment. Note that you have to calibrate the holder on both systems, the SEM and the LM. When working with the **Shuttle & Find** module the calibration is always the first step. To perform the calibration adjust the settings in the **Holder Calibration Settings** tool and click on the **Start** button above the tool. The software will then guide you through the calibration process.

Parameter	Description
Sample Holder	Here you need to select the correlative holder which shall be calibrated and used for your experiment.
Move stage to load position before xy movement	If activated, the stage moves first to the load position before moving to the next marker. Activate this option if you work with uneven samples to avoid collision of the objective and the sample.
Automatic movement to next marker	If activated, the stage automatically moves to the next marker position after the last marker position was confirmed.
Stage movement direction	Here you can set the direction of the stage movement. Depending on the stage settings in the MTB 2011 configuration software it can be necessary to invert the stage axes. If you click on Invert X , the X axis is inverted. If you click on Invert Y , the Y axis is inverted.

18.4.8.2 ROI / POI Definition Tool

With this tool you can draw in ROIs or POIs to the acquired image. Select whether you want to draw in a ROI (rectangular shape) or POI (point) and mark the interesting areas of your sample.

Parameter	Description
Arrow / selection mode	Activates the selection mode. If you click on a drawn in ROI/POI you can move or change the size.
Rectangle mode	Using the rectangle mode you can draw in rectangular regions of interest. Click on the rectangle button and draw in the ROI in the image area.
Points mode	Using the points mode you can draw in single points of interest. Click on the points button and draw in the POI in the image area.
ROI / POI list	Here you see all added ROIs / POIs.

18.4.8.3 Image Overlay Tool

With this tool you create an overlay image of SEM (Scanning Electron Microscope) and LM (Light Microscope) images. Simply choose an reference image (e.g. the LM image) and move the desired SEM image from the image gallery (below the image area) per drag and drop in to the image area. After you have adapted the SEM image to fit the LM image click on **Create Correlation**. A new output image will be generated and appears in the image gallery. Note that all images you want to use for the overlay image have to be loaded / opened in the software before.

Parameter	Description
Reference Image	Here you select the reference image for the overlay image. The reference image is usually the LM image with a lower magnification. The reference image is always the image in the background and cannot be adapted.
Image List	In the list you see the images which were added to the image area. We recommend to start with only one image. The added image has a green frame and can be adapted in its size and position. Therefore click on the image and use the buttons in the edges and on top of the image. Note that you can also set so called "Pins" to fix the added image at a certain position. To set a pin right click within the added image (inside the green frame) and click on Set Pin in the context menu. Now you can move the added image around the "pinned" position.

Parameter	Description
Interpolation Mode	Here you can select the desired interpolation mode for the image generation. While the interpolation method and results depend on the images used, in most cases we recommend to use the linearic interpolation mode.
- Nearest Neighbor	This is the simplest and fastest method. Calculation method: The output pixel is given the gray value of the input pixel that is closest to it.
- Linear	This is our recommended method as it delivers good and fast results in most cases. Calculation method: The output pixel is given the gray value resulting from the linear combination of the input pixels closest to it.
- Cubic	As the calculation method here is more complex this mode will take slightly longer. The results are not forcedly better than with the linear mode. Calculation method: The output pixel is given the gray value resulting from a polynomial function of the input pixels closest to it.

19 Maintenance

19.1 Basics of Maintenance

Maintenance mode enables administrators to specify global settings for the software. You should only adjust these settings if you understand the consequences for users of the system.

19.2 General Options

19.2.1 General Options

The following options enable you to specify the language of the user interface.

Parameter	Description
Language	Sets the language of the user interface The language settings are set globally for all users.
– Select Automatically	<ul style="list-style-type: none"> ■ Activated: The user interface is displayed in the language of the user's operating system. If the language is not available, the default language (English) is used. ■ Deactivated: Value of Fixed Language will be used. You must restart the software for the language settings to take effect.
– Fixed Language	<p>The specified language is used for all users, regardless of their user interface settings.</p> <p>This setting only applies if Select Automatically is deactivated.</p> <p>You must restart the software for the language settings to take effect.</p>
Confirmations	<ul style="list-style-type: none"> ■ Activated: A prompt is displayed after clicking the  icon. ■ Deactivated: The application closes when a user clicks the  icon. Nevertheless the user is prompted to save any unsaved work.

19.2.2 Startup Options

The following options specify how the software starts.

Parameter	Description
Show Splash Screen	Activated: A splash screen is displayed while the software is loading.
Stage/Focus Calibration	Activated: Each time the software starts a dialog box is displayed which enables you to calibrate the motorized stage and/or focus.

19.2.3 Naming Options

The following options enable you to specify how items are named automatically.

Parameter	Description
Category	Select the item for which you wish to modify the automatic naming options. The other fields update accordingly. You can specify different settings for each type of item.
Prefix	Specifies text to be displayed at the start of the automatic name A typical prefix is the name of the item, e.g. Image .
Digits	Specifies the number of digits for the counter value. If the current counter value has fewer digits than this setting, the counter value is filled with leading zeroes. Example: Digits = 4 , current counter value = 17 , automatic name contains 0017 .
Format	Specifies the main part of the automatic name You can configure any name by entering text or using the placeholders. To add a placeholder, double-click it in the ID list or enter % and the desired placeholder ID. The results are displayed in the Preview field. Example: Enter %h-%m-%s if you want the exact creation time to be displayed in the automatic name. Example result: 14-53-07 .

Parameter	Description
Initial Counter Value	Specifies the lowest value of the counter The counter increments in integer steps. Leading zeroes are added according to the Digits setting.
Suffix	Specifies text to be displayed at the end of the automatic name
Preview	Shows how the automatic name will be displayed with the current settings.
Save/Restore Counter Value	<ul style="list-style-type: none"> <input checked="" type="checkbox"/> Activated: The counter value will be increased across sessions and users. <input type="checkbox"/> Deactivated: The counter value resets each time you log in. A separate counter value is used for each user. Nevertheless, existing images are not overwritten.
Format-IDs	Enables you to create an automatic name using the placeholders Double click a placeholder to add it to the Format field.

The changes to the names apply to all items created subsequently (i.e. existing items are not renamed).

19.2.4 Documents Options

The following options specify how images are displayed initially after being acquired as well as whether the DataZone is displayed and the information it contains.

Parameter	Description
Default Settings for New Images	Specifies how images are displayed initially after being acquired
– Show Rulers	Activated: Rulers are displayed on the top and left of the Center Screen Area . The scale and units of the ruler depend on the settings in Manage Scalings .
– Auto Fit	<ul style="list-style-type: none"> <input checked="" type="checkbox"/> Activated: Image will be adjusted to fill the available Center Screen Area. <input type="checkbox"/> Deactivated: Images are displayed unscaled.
– Use Interpolation for Image Display	Activated: Image will be smoothed when zooming in.

Parameter	Description
– Set Logarithmic Scale in Histogram	<ul style="list-style-type: none"> ■ Activated: The logarithmic scale emphasizes pixels in the histogram which occur with a lower frequency. ■ Deactivated: The linear scale emphasizes all pixels equally.
– Show Viewport Scalebar in 2D View	<p>Activated: An overlay window containing a viewport scale bar is displayed when the user zooms into an acquired or loaded image.</p> <p>The overlay window is closed automatically when the zoom is reset.</p>
– Show Viewport Scalebar in Live Window	<p>Activated: An overlay window containing a viewport scale bar is displayed when the user zooms into the live image.</p> <p>The overlay window is closed automatically when the zoom is reset.</p>
– Show Navigator in 2D View	<p>Activated: Displays the navigator in the Center Screen Area</p> <p>The Navigator indicates the section of the image that is currently displayed in the Center Screen Area</p>
– Use Pan Mode in 2D View for Tile Images	<p>Specifies how to pan a tiled image in 2D mode:</p> <ul style="list-style-type: none"> ■ Activated: Drag using left mouse button ■ Deactivated: Press the middle mouse button and then drag using left mouse button
Image Data Zone	
– Show Image Data Zone	<p>Activated: Additional image information is displayed in the Center Screen Area</p>
Image Data Zone Optional Data	<p>Enables you to specify which hardware and acquisition metadata is displayed in the Image Data Zone</p>
Image Data Zone Contact Data	<p>Enables you to specify which user information metadata is displayed in the Image Data Zone</p>

19.2.5 Acquisition Options

19.2.5.1 General Section

This section specifies how manual hardware is treated.

Parameter	Description
Show a Request to Move Manual or Coded Hardware Components	<ul style="list-style-type: none"> ■ Activated: If a user changes the hardware setup in the software (for example using the Light Path tool) the software prompts the user to change the physical hardware setup. ■ Deactivated: No prompt is displayed <p>This may result in a discrepancy between the physical hardware setup and that indicated in the software.</p>

19.2.5.2 Camera/Live Section

This section specifies global acquisition options

Parameter	Description
Enable Stage/Focus Control in Live View	Activated: Enables the position of the motorized stage to be set from within the Center Tool Area (instead of only using the Navigation tool)
Automatically Add Scalebar Annotation at Snap	Activated: A scale bar annotation is displayed in the lower right corner of the image.
Show Camera Export Options	Activated: a Save dialog is displayed after each acquisition.

19.2.5.3 Tiles Section

This section specifies global tile settings.

Parameter	Description
Enable Stage Moving with Live Navigator Handle	No effect in the current software version. A motorized stage follows the Live Navigator , independent of this setting.
Delimiter for CSV Export/Import	Allows you to choose the character used for separating the entries when a list of tiles is exported into or imported from a text file
Activate Stitching During Acquisition for New Experiments	Activated: Acquired tiles are stitched in the background while the remaining tiles are being acquired

19.2.5.4 Panorama Section

This section specifies global panorama settings.

Parameter	Description
Automatically Start Live Mode in the Panorama View	Activated: a live image is shown immediately after clicking Start Panorama .
Show Information Title in the Panorama View	No effect in the current software version.
Show Acquisition Animation	Activated: An animation is displayed during each tile acquisition.
Automatically Move Stage/Live after an Acquisition	<p>Activated: The Live Navigator is moved after acquisition of a tile. This indicates that the acquisition of a tile is finished and that the stage is ready to be moved to the next position.</p> <p>Deactivated: The Live Navigator will stay at the position where the last tile has been acquired. You have to move it manually to one of the neighboring positions manually before moving the stage.</p>
Enable Transparency Effect on Selected Tile Image	No effect in the current software version.

19.2.6 User Options

The following options enable you to specify personal and company information.

Parameter	Description
User Information	<p>Allows you to enter user information which will be displayed with your job templates.</p> <p>This is useful for single-user licenses (user management is deactivated).</p>
Company Information	Allows you to enter company information and upload the company logo.

19.2.7 ImageJ Options

The following options enable you to configure ImageJ.

Parameter	Description
ImageJ Folder	Shows the program folder of the currently selected ImageJ executable
ImageJ executable	<p>Specifies the path to the main executable file of your ImageJ installation.</p> <p>ZEN 2 core automatically searches the standard windows program folders for ImageJ and shows the paths to all found ImageJ executables in the drop-down list.</p> <ul style="list-style-type: none"> ■ Your installation is found: Select the path from the drop-down list. ■ Your installation is not found or you wish to use a different installation: Select ... from the drop-down list and specify the path of the ImageJ executable.
– Shift Pixels to 16bit	Each color or gray channel is converted to 16 bit before export to ImageJ.
Default preferred conversion	Specifies the preferred conversion for images received from ImageJ
Default preferred file format	Specifies the file format in which the image is saved before being opened in ImageJ
– Automatic	Selects the format in which the file is saved automatically, depending on the image properties.
– Czi	Saves the file in the CZI format. This image is then imported in ImageJ.
– Ome Tiff	Saves the file in the OME TIFF format. This image is then imported in ImageJ.
– Tiff	Saves the file in the TIFF format. This image is then imported in ImageJ.
– Tiff With Display Mapping	Saves the file in the TIFF format including a display curve. This image is then imported in ImageJ.

19.3 User and Group Management

19.3.1 Basics of User and Group Management

The software can be used with or without user management.

Without user management User management is disabled by default. This means that every user has the same rights. No username or password is required and there are no user roles within the software (i.e. the user can perform any action).

With user management If user management is enabled, each user has an account which is used to log into the software. Each user account is assigned to one or more user groups.

User groups define the privileges (actions the user can perform in the software) for the users assigned to the group. Groups typically correspond to the roles in the software (e.g. supervisor or operator). However, you can also create new user groups if required.

Typically, one user is assigned to one group, but can be assigned to multiple user groups if required. Users have the sum of all permissions of the groups to which they are assigned.

19.3.2 Creating and Managing User Accounts

Each user of the system must have a user account to be able to log in to the system. You can add new user accounts or modify existing ones as follows.

- Prerequisites**
- You are logged in as an administrator
 - **Enable User Management** is activated

- Procedure**
- 1 Select **Home Screen > Maintenance > User Management**.
 - 2 Click **Users**.
 - 3 Perform one of the following actions.

Action	Parameter	Description
 Create new user account A user account has no privileges until it is assigned to at least one user group	Name	Specifies the user name used within the software
	Description	Enables you to add user details
	Password, Confirm Password	Specifies the initial password. Otherwise the initial password is blank. There are no restrictions on the password length or characters.

Action	Parameter	Description
	User can change password	Deactivated: Only Administrators can change the user's password
	User has to change password on next login	After the next successful login, the user has to change their password Ensure User can change password is activated.
 Manage user account settings	User Information	Enables you to change the user name and the user details used within the software.
	User may log in	Deactivated: user cannot access the system This enables you to quickly disable an account without deleting it
	User can change password	Deactivated: Only Administrators can change the user's password
	User has to change password on next login	After the next successful login, the user has to change their password Ensure User can change password is activated.
	Reset password	Sets the password to a blank string.
 Delete user account from the system	-	This cannot be undone. However, data and objects created or modified by the user are not deleted.

19.3.3 Creating and Managing User Groups

User groups define the privileges (actions the user can perform in the software) for the users assigned to the group. Groups typically correspond to the roles in the software (e.g. supervisor or operator). However, you can also create new user groups if required. The groups have specific rights and users are assigned to a user group.

- Prerequisites**
- You are logged in as an administrator
 - **Enable User Management** is activated

- Procedure**
- 1 Select **Home Screen > Maintenance > User Management**.
 - 2 Click **Groups**.
 - 3 Perform one of the following actions.

Action	Parameter	Description
 Create new user group Initially a user group has no privileges associated with it. You need to set the appropriate group privileges after creating the group.	Name	Specifies the group name used within the software
	Description	Enables you to add user group details, such as information about which privileges users have after being added to the group
	Type	Specifies where the user group is stored and managed and also the method used to verify that a user is a member of the group. <ul style="list-style-type: none"> ■ Local Within the software ■ Active Directory Using Windows Active Directory ■ LDAP Using Lightweight Directory Access Protocol ■ IMS Using Imagic user groups
 Copy an existing user group	-	All properties of the user group are copied Users of the existing user group are not automatically assigned to the new group.
 Manage group account settings	Data > Name	Enables you to change the group name used within the software

Action	Parameter	Description
	Data > Description	Enables you to change user group details, such as information about which rights users have after being added to the group
	Privileges	Defines which privileges are assigned to the group's members. Several pre-defined sets of privileges are available. It is also possible to select privileges manually from the list.
		Procedure
		<ol style="list-style-type: none"> 1 Activate the desired privileges from the list. Or: Select a pre-defined set of privileges. 2 Click OK.
	Delete user group from the system	- This cannot be undone. User accounts assigned to the user group are not deleted. However, the users do lose the privileges conferred by the group.

19.3.4 Assigning a User to a Group

Initially, a user is not assigned to any user groups. To be able to use the software, the user must be assigned to at least one user group. The user then inherits the privileges assigned to the user group.

A user can be a member of multiple user groups. Users have the sum of all permissions of the groups to which they are assigned.

- Prerequisites**
- You are logged in as an administrator
 - **Enable User Management** is activated
 - The group to which the user should be assigned exists and has the correct privileges

- Procedure**
- 1 Select **Home Screen > Maintenance > User Management**.
 - 2 Click **Users**.
 - 3 Select the user from the list and click .
 - 4 Click **Group**.

- 5 Click  and select the user groups to which the user should be assigned.
- 6 Click **OK**.

19.3.5 Modifying Group Privileges

Privileges are assigned to user groups. They specify what actions members of the group can perform in the software.

The software contains various pre-defined roles, each with different sets of privileges. Typically, the software contains one user group for each role. However, you can create any number of user groups with arbitrary privileges.

Privilege/ Privilege sets	Account Manager	Supervisor	Novice	Operator
Manage users and groups	x			
Create jobs		x		
Manage archive	x	x		
Manage application settings	x			
Free examination		x	x	
Edit jobs		x		
Run jobs		x	x	x
See all templates		x	x	x
See all job results		x		

- Prerequisites**
- You are logged in as an administrator.
 - **Enable User Management** is activated.

- Procedure**
- 1 Select **Home Screen > Maintenance > User Management**.
 - 2 Click **Groups**.
 - 3 Select the desired user group and click .
 - 4 Click **Privileges**.

- 5 Select the privileges of the user group.

You can click a pre-defined role or activate individual checkboxes to create a custom set of privileges.

- 6 Click **OK**.

If users are already assigned to the group, they inherit the modified privileges next time they log into the software.

19.3.6 Global Login Options

Global login options apply to all users, regardless of the user groups to which the user is assigned. Open the global options via **Home | Maintenance | User Management | Options**.

Parameter	Description
Check the following rules for a password	<p>Here you can specify certain rules or criteria for a password that is created. If the checkbox is activated, the rules must be fulfilled when a new password is created.</p> <p>The following rules can be adjusted:</p> <ul style="list-style-type: none"> ■ Minimal number of lower case characters (e.g. when you set "2", the password must contain at least two lower case characters e.g. "e" and "f") ■ Minimal number of upper case characters (e.g. when you set "2", the password must contain at least two upper case characters e.g. "C" and "G") ■ Minimal number of digit characters (e.g. when you set "3", the password must contain at least 3 digits (from 0 - 9), e.g. "5", "6", "7") ■ Minimal number of special characters (e.g. when you set "1", the password must contain at least one special character, e.g. "&") ■ Minimum length (e.g. when you enter "9", the password must consist of at least 9 characters (any from above).
Force users to change password after period of time	<p>Activated: The user must change his password after the specified period of time elapses.</p> <p>Deactivated: The password never expires.</p>
– Days before expiry	Specifies the period of time after which the password expires.

Parameter	Description
Lock screen after certain time span	<p>Activated: After a period of inactivity the screen is locked and the user must enter his/her password to continue working.</p> <p>Deactivated: The password never expires.</p>
– Minutes until screen lock	Specifies the time span after which the screen is locked.
Enable Auto-Login	<p>■ Activated: No password is required</p> <p>The user is logged in automatically based on the Windows username.</p> <p>Create a user group in the software that is based on Windows Active Directory (Type = AD) and ensure that all relevant Windows users are present in the group and that the group has sufficient privileges in the software.</p> <p>■ Deactivated: Each user has to log in with their own password.</p>
Export/Import user database	Enables you to export or import the user database, including all user groups and privilege sets, for example to exchange it with another system.
– Export...	Specify the location on the file system where the database should be exported
– Import...	Select the database location on the file system

19.4 Archive Management

19.4.1 Configuring an IMS Archive

The archive options enable you to specify how and where the **Archive** is stored, as well as the hierarchy of attributes that can be assigned to templates.

Prerequisites ■ You are logged in as an administrator.

Procedure 1 Open the **Archive Options**:

Home Screen > Maintenance > Archive Options

2 As **Archive Type**, select **IMS Archive** from the drop-down list.

3 Refer to the table below to configure the **Settings**. The information is provided by the admin of your IMS archives.

Parameter	Description
IMS host	URL and port of the IMS server
Default username	Username for accessing the IMS server during setup
Default password	Password for accessing the IMS server during setup
Results archive name	Name of the data table (IMS archive) in the IMS database where results should be stored
Results archive uri field name	Name of the field in the above data table, where the job hierarchy is stored
Configuration archive name	Name of the data table (IMS archive) in the IMS database where the templates are stored
Configuration archive uri field name	Name of the field in the above data table, where the template hierarchy is stored

19.4.2 Configuring a Local Archive

The archive options enable you to specify how and where the **Archive** is stored, as well as the hierarchy of attributes that can be assigned to templates.

Prerequisites ■ You are logged in as an administrator.

- Procedure**
- 1 Open the **Archive Options**:
Home Screen > Maintenance > Archive Options
 - 2 As **Archive Type**, select **Local Archive** from the drop-down list.
 - 3 Refer to the table below to configure the **Settings**.

Parameter	Description
Archive Path	The location of the Local Archive on the file system
Archive Hierarchy	Enables you to create a hierarchical structure of properties that can be assigned to templates
+ icon	Adds a new property below the currently selected one To add a new property parallel to the top property, ensure that none are currently selected
- icon	Removes the currently selected property
Expand all	Expands the hierarchical list so that all properties are visible

19.5 Scale Management

19.5.1 Specifying Units and Scaling Method

As an administrator you can specify which units are used to display scales and measurements in images.

Furthermore, you can specify whether other users can select a scaling method.

- Selection disabled

If a custom scale has been created for the current hardware configuration it is used. Otherwise the theoretical scale is used.

- Selection enabled

Other users can choose to use the custom scale (if available) or the theoretical scale.

The theoretical scaling is calculated automatically based on the properties of the hardware components (e.g. zoom of the objectives, number and separation of pixels on camera chip, etc.)

Prerequisites ■ You are logged in as an administrator

Procedure 1 Open **Manage Scalings**:

Home Screen > Maintenance > Manage Scalings

2 In **Units for Scaling** specify the metric or imperial units for all scales and measurements in images.

3 Specify whether other users can select a scaling method:

1 Selection *disabled* (**Automatic Scaling** activated)

2 Selection *enabled* (**Automatic Scaling** deactivated)

INFO

- After changing these settings, the units are applied to all measurements and images that are subsequently performed or acquired.
- Measurements in job templates, job results, and images that have already been acquired are not subsequently displayed in the new units.
- Images in which the measurements or scale are "burnt in" cannot be rescaled.
- Custom scales are hardware-specific. Such a scale is only available when the identical hardware is in use as when the scale was created.

19.5.2 Managing Custom Scalings

You can import/export custom scale presets, for example to copy them to another system.

- Procedure**
- 1 Open **Manage Scalings**:
Home Screen > Maintenance > Manage Scalings
 - 2 Perform the actions listed below as required.

Action	Description	Procedure
Export a preset scaling	The scaling values are saved in a file	<ol style="list-style-type: none"> 1  > Export 2 Specify the location in the file system.
Import a preset scaling	A preset from the file system is added to the list of scalings and the current parameter values are overwritten with those stored in the preset.	<ol style="list-style-type: none"> 1  > Import 2 Select the desired scaling file from the file system.
Delete a preset scaling	<p>The currently selected scaling is deleted.</p> <p>The next scaling in the list is selected and the values from the scaling applied. If the list is empty, the default values are applied.</p>	  > Delete

19.6 Module Management

19.6.1 Managing Modules

Modules contain additional software functions. The modules that are available to you are controlled by licences. You can purchase additional licenses to enable additional modules. For more information, including how to install licenses, contact your ZEISS representative.

The available modules are managed in the **Module Manager**. There you can enable or disable modules. If a module is disabled, the corresponding features are hidden in the software.

- Prerequisites**
- You are logged in as an administrator.
 - The **Home Screen** is displayed.

- Procedure**
- 1 Open the **Module Manager**:
Maintenance > Module Manager

- 2 You can activate or deactivate any module via the corresponding checkbox.
- 3 Some activated modules require further configuration:
 - Check for new options under **Maintenance > Options**.
 - Configure the newly activated modules accordingly.

19.7 Extension Management

19.7.1 Managing Extensions

Extensions are optional software add-ons. For example, ZEN 2 core is shipped with the ImageJ extension, an interface which enables you to integrate the ImageJ software into your workflow and thus make use of ImageJ's image processing capabilities.

You can activate the available extensions in the **Extensions Manager**.

Prerequisites ■ You are logged in as an administrator.

■ The **Home Screen** is displayed.

Procedure 1 Open the **Extensions Manager**:

Maintenance > Extensions Manager

- 2 You can activate or deactivate any extension via the corresponding checkbox.
- 3 Confirm the configuration by clicking the **Apply** button.
- 4 Some activated extensions require further configuration:
 - Find more options under **Maintenance > Options**.
 - Configure the newly activated extensions accordingly.

19.7.2 Activating the ImageJ Extension

You activate the ImageJ extension via the **Extensions Manager** and then configure it in the **Options** dialog.

Configuring ZEN 2 core ■ ImageJ is installed on your system.

Prerequisites

The Life-Line version of the Fiji distribution is recommended, which contains all necessary ImageJ plugins and works with ZEN 2 core.

■ You are logged in as an administrator.

■ The **Home Screen** is displayed.

Procedure 1 Activate the **ImageJ Extension** in the **Extensions Manager**:

Maintenance > Extensions Manager

- 2 Confirm the configuration by clicking the **Apply** button.

If you activate ImageJ for the first time, a "missing path" warning is displayed. Confirm it by clicking **OK**.

- 3 Open the **Options** window:
Maintenance > Options
- 4 Select the **ImageJ** tab.
- 5 Select the path to the ImageJ executable from the drop-down list:
 - Select one of the paths suggested by ZEN 2 core
 - or:
 - Click on  to specify a different path.

Configuring ImageJ To configure how ImageJ interacts with ZEN 2 core:

Prerequisites ■ ImageJ is installed on your system.
The Life-Line version of the Fiji distribution is recommended, which contains all necessary ImageJ plugins and works with ZEN 2 core.

- Procedure**
- 1 Open the ImageJ installation specified in the ZEN 2 core options.
 - 2 Activate the **Single Instance Listener**:
You find the **Single Instance Listener** under **Edit > Options > Misc...**

19.8 Graphical Elements

19.8.1 Managing Default Measurement Features

For each type of measurement, you can configure which measurement results (features) are displayed by default in the **Center Screen Area** next to the measurement.

When performing a measurement you can override this setting and select different features to be displayed.

Prerequisites ■ You are logged in as an administrator.
■ The **Home Screen** is displayed.

- Procedure**
- 1 Open **Manage Features of Graphical Elements**:
Maintenance > Manage Features of Graphical Elements
 - 2 Select the desired type of measurement in the left panel.
 - 3 Specify which features can be selected or displayed by users using the checkboxes in the **Available** column.
 - 4 Specify which features are displayed by default using the checkboxes in the **Label** column.

19.9 Hardware Configuration

19.9.1 Selecting the Hardware Configuration

- Prerequisites**
- You are logged in as an administrator.
 - The **Home Screen** is displayed.

Procedure 1 Open **Change Microscope Configuration**:

Maintenance > Change Microscope Configuration

After connecting to the MTB, all available hardware setups configured in the MTB are listed.

- 2 Select the desired hardware setup.
Ensure that the microscope supports the new configuration.
- 3 Click **Activate Selected Configuration**.

19.10 S&F Settings

The settings are accessed via **Home | Maintenance | S&F Settings**.

If you use the software for correlative microscopy, here is where you need to set if you work with the **LM** (light microscope) or **SEM** (scanning electron microscope) system. The adjustment has to be done only once when you start working with the software. Additionally you see the list of all available correlative holder templates. Of course you can create new holder files or export/load existing holder files here. Additionally, not-used holders can be deactivated.

19.11 Import/Export Settings

Here you can backup and restore software specific settings. To generate a backup of all software specific settings (e.g. user settings, templates, etc.) simply click on **Backup**. The backup file (*.zip) will be generated in the specified folder.

To restore settings you need to have at least generated one backup file before. If you click on **Restore** you have to specify the backup file (*.zip) location and click **Open**. All settings which are stored in the backup file will be restored.

We recommend to perform backups of the software settings on a regular base (e.g. each week / month) depending on your personal needs.

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