Quick Start Guide

First steps with ZEN 2011 (blue edition)
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1 Concept

ZEN is a modular image-processing and analysis software for modern microscopy from Carl Zeiss. In addition to basic functionality for image acquisition and microscope definitions, elementary image processing and annotations, image analysis and documentation optional modules for specific tasks are available.

1.1 Image Acquisition

A range of different camera types can be used with ZEN, from simple TV cameras through to high-resolution and high-sensitivity cameras. The cameras of the Carl Zeiss AxioCam family guarantee optimum integration.

1.2 Image Processing

The acquired image is immediately displayed on the monitor. It can then be optimized using a wide range of techniques:

- Contrast, brightness and color adjustment
- Noise suppression, smoothing and contour enhancement
- Sharpness enhancement/emphasizing of details
- Correction of illumination influences and white balance

ZEN can also be used to add any annotations that you may require to the images. All elements, from scale bars and colored markings through to text and graphics, have been integrated into the program.

1.3 Image Analysis

You are able to perform simple interactive measurements in the basic program. The measured values (e.g. lengths, areas and perimeters) are made available in a data table, and can be processed further using spreadsheet programs. The interactive measurement can be executed via the Graphics menu from the menu bar, or via the Graphics tab in the view controls of the Measurement View.

1.4 Documentation

Besides the image itself, the ZEN image format CZI also saves additional data, such as the image number, date of acquisition, microscope settings, exposure values, size and scale details, contrast procedures used etc. Annotations and measured values are also saved with the image.
2 Start ZEN blue software

➢ Your Microsoft operating system is ready for operation.
➢ You have successfully installed ZEN blue on your computer.

1. Double click on ZEN blue program icon on your desktop.

   ⇦ The software starts. After a while you see the ZEN blue login screen.

3. Activate checkbox Don’t show this dialog next time if you don’t want to see the login dialog with the next start of the software. If you want to see the dialog again, go to menu bar and click on File | Login.

4. Click on Start System button to start ZEN blue with full software functionality.

5. Click on Image Processing button to start ZEN blue with image processing functions only.
   ⇦ You successfully started ZEN blue software.
3 Program interface

The ZEN (blue edition) program interface is divided into three main areas. Via the tabs in the **Left Tool Area (5)** you can access all the main tools for microscope control (Locate tab), acquisition (Acquisition tab), image processing (Processing tab), image analysis (Analysis tab) and report generation (Reporting tab). The **Center Screen Area (6)** is used to display your images, while the **Right Tool Area (7)** provides you with an overview of all open documents and is used for advanced file management.

Fig. 1: Program interface (ZEN lite)

1 Title bar
2 Workspace Configuration
3 Menu bar
4 Tool bar
5 Left Tool Area
6 Center Screen Area
7 Right Tool Area
8 Status bar

3.1 Title bar

Fig. 2: Title bar

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>?</td>
<td>Activates the &quot;drag &amp; drop&quot; help function. A question mark appears beside the mouse pointer. Move the mouse pointer to a place in the software where you need help. Left-click on the desired location. The online help opens.</td>
</tr>
<tr>
<td></td>
<td>Minimizes the program window.</td>
</tr>
</tbody>
</table>
Symbol | Description
---|---
| Maximizes the program window across 2 screens. This option is only possible if you are working with 2 screens with the same resolution.
| Maximizes the program window to the main screen.
| Reduces the program window to any selected size.
| Closes the program window.

3.2 Menu bar

The menu bar contains all the menus you need to manage, edit and view your projects.

3.3 Workspace configuration

Here you find settings to adjust your workspace. Select light/dark Design of the user interface or enlarge the screen with Workspace Zoom. Save and reload all your personal settings as a Workspace configuration. With the Dock all tool windows button in the top right corner you can dock all undocked tools to left tool area with one click.

See also
- Adjust workspace appearance [➙ p.12]

3.4 Tool bar

Here you gain quick access to important functions, e.g. saving or opening files. Further right you find settings for Workspace Configuration, i.e. Design settings.
Information

You can adapt the tool bar to your personal requirements in the Extras menu | Customize Tool Bar.

3.5 Left Tool Area

In the Left Tool Area you find the Main Tabs for microscope and camera settings (Locate tab), image acquisition (Acquisition tab), image processing (Processing tab), image analysis (Analysis tab) and reporting (Reporting tab). The Main Tabs are organized in an order which follows the typical workflow of experiments in bioscience or material science.

![Left Tool Area (ZEN pro, desk, system)](image)

3.6 Center Screen Area

The Center Screen Area is structured in 4 areas. The Document bar (1) is on top. On the left side you find the tabs for the general and specific Image Views (2). In the middle of Center Screen Area is the Image Area (3), images, reports and tables were shown here. Under the image area you find the General- and View Specific Controls (4) organized in tabs. View specific control tabs are flagged blue.
3.7 **Document bar**

*Fig. 8: Document bar*

Here you see tabs of all opened documents. Click on a tab to view the image/document. On the right end of the document bar, you find buttons to switch view mode (*Expose* and *Splitter* mode) and further view options (*View* menu).

3.8 **Status Bar**

Here you will see important information on the system status:
Scaling options

Displays which lateral scaling is currently being used. The automatic checkbox is activated by default. The scaling will be calculated automatically based on your hardware settings (i.e. objective, adapters, etc.). If the automatic checkbox is deactivated, you can also load/import scalings or start the scaling wizard in the Options menu.

System Information

Always shows the latest, currently active process that the system is performing.

Progress Bar

Displays the progress of the currently active process. Each new process added supersedes older still active processes. If you click on the icon, a window opens all currently running chronologically listed processes. You can stop a process that is running using the Stop button.

Performance Indicators

In this group you will see an overview of the performance of individual computer components:

- Free RAM indicates how much physical memory is still available;
- Free HD indicates how much space is still available on the hard drive onto which the next image is to be acquired (see Extras/Options/Save);
- CPU indicates the usage of the Central Processing Unit.
- The status bar provides an overall assessment of the system usage.

Frame Rate

Indicates the current frame rate in frames per second (fps) used by the active camera for producing new images. Please note that above in most cases a speed of > 100 frames per second this value can not always be accurately determined.

Pixel Value and Position

Pixel value displays the gray value in the image at the current position of the mouse pointer. In the case of multichannel images the gray value/channel is displayed for up to 4 channels.

Position displays the X/Y position (in pixel coordinates) of the mouse pointer in the image.

Information (i)

If you click on the icon, a window opens with a List of System Messages.
**Storage Folder**
Displays the location where new images are automatically saved. This path can be changed in the menu **Tools | Options | Saving**.

**User**
Shows the Windows user name of the logged in user.

**Time**
Shows the current Windows system time.
4 Adjust workspace appearance

4.1 Set user language

➢ You have successfully started ZEN (blue edition)
1. Click on menu Tools | Options.
   ⇨ The Options dialog opens. The entry General in the Software group is selected.
2. Select user language from the Fixed Language dropdown list.

Information

If the Select Automatically checkbox is activated the software uses the language which is set in the system settings of your computer.

➢ The message appears to restart the application. Confirm message by clicking on OK.
3. Click on OK.
   ⇨ The Options dialog closes.
4. Exit and restart software.
   ➢ You have successfully set user language.

4.2 Select design
1. Select light/dark design from design dropdown list in workspace configuration area.

4.3 Zoom in/out workspace
1. To zoom in or out of the workspace move the slider left or right.

2. To reset workspace zoom to default click on Reset button.
4.4 Show/hide areas

1. Click on show/hide buttons to show or hide areas.

4.5 Undock/dock tool window

This function allows you to undock/dock a tool window. An undocked tool window can be positioned anywhere on the screen.

1. Click undock button to undock a tool window.

2. Click dock button to dock a tool window back to its place in left tool area.

Information

With dock all tools function in Workspace Configuration [➔ p.7] you can globally attach all undocked tool windows back to the left tool area.
4.6 Show all mode of tool windows

1. Click Show all button (deactivated) to show advanced settings or function of a tool window.

   ![Show all button (deactivated)](image)

2. Click Show all button (activated) to show only the basic functions of a tool window.

   ![Show all button (activated)](image)
5 Step by step to the first image

5.1 Configure Microscope Components

This chapter refers to the manual configuration of the microscope components in ZEN lite. All microscope components definitions will be stored in the meta data of the acquired image.

➢ You have selected the Camera tab.

1. Click to the blue header of the Microscope Components tool.

➢ The tool will open. Consider that the button Show all is activated.

2. Under Objective select that objective you will use for your acquisitions.

3. Select all other microscope components you eventually will use (i.e. Optovar, Reflector, etc.).

Information

If you have activated the automatic button in the Statusbar under Scaling (standard settings), the scaling will be calculated on the basis of your definitions. If you want to perform a manual scaling, read the chapter Create Manual Scaling.

➢ You have successfully configured your microscope components.

See also

➢ Status Bar [⇒ p.9]

➢ Create manual scaling [⇒ p.16]
5.2 Create manual scaling

➢ You oriented the object micrometer horizontally on the microscope stage.
➢ You selected correctly all definitions for your microscope in the **Microscope Components** tool. In our example we use an objective with a 10x magnification.

1. Acquire an image (see Acquire a first image with ZEN blue [➙ p.19]) of the scale in your object micrometer using the objective to be scaled manually.

2. Switch off the automatic scaling in **Status** bar by deactivating the **automatic** button.

3. Open the **Options** menu and click on the entry **Create New Scaling**.
4. Click on single line button as reference line in the calibration wizard and activate the automatic Line Detection button.

Information

The function automatic Line Detection calculates the theoretical maximum of the reference line’s both end points to the closest scale lines in the image. Thus the distance will be calculated with sub-pixel accuracy.
5. Draw in the reference line along the scale.

6. Enter the true distance between both scale lines in the calibration wizard. In our example this is 500 micrometer.

7. Enter a name for the scaling (i.e. Obj 10x) and click the Save scaling button.
You performed a manual scaling for your objective. Repeat this sequence for all objectives you will need a manual scaling for. Always ensure that you did select the correct objective in the tool Microscope Components and for this performed and selected the matching scaling in the status bar.

**Information**

If you defined manual scalings for your available objectives, and if you activate in the Status bar under Scaling the checkbox Automatic again, the system will use the measured scalings instead of the theoretic ones. You will recognize this via the label “measured” instead of “theoretic” beside the pixel size.

### 5.3 Acquire a first image with ZEN blue

This section guides you to your first image with ZEN blue software.

➢ You have connected and configured a microscope camera (i.e. AxioCam MR) to your system.

➢ You configured the microscope components (e.g. objective, camera adapter) und use the automatic or a manual scaling.

➢ You have started the ZEN blue software and selected Camera tab (ZEN light only) or Locate tab.

1. Position your specimen on the microscope and adjust the microscope to see a focused image through the oculars.

2. Adjust the tube slider of the microscope to the TV adapter, e.g. 50% camera and 50% ocular.

3. Click on Live button.

   ➞ The live mode will be activated. You will recognize the live mode by the green signal and by the hatched tab in the document bar. In the Center Screen Area you will see the camera live image. By default the live image shows a cross hair helping to navigate on the specimen. In the chapter Live Image Adjustments you will learn how to optimize live image display.

4. Click on Set Exposure button.
The exposure time will be automatically determined and set.

**Information**

If you do not see a focused image please refocus the specimen on the microscope. You may activate the focus bar as an additional aid. Open the context menu in the Center Screen Area via the right mouse key. There select the entry **Focus Bar**. The focus bar will be shown.

5. Click **Snap** button.

   ⇦ You successfully acquired your first image with **ZEN blue**. Save the image in the file system via the menu **File | Save as**.

**See also**

- Optimize Live Image Settings [➙ p.20]
- Document bar [➙ p.9]

### 5.4 Optimize Live Image Settings

➢ You started the live display via the **Live** button and see the camera’s live image in the Center Screen Area.

1. Activate the **RangeIndicator** controlbox in the **Dimensions** tab. This will mark overexposed (too bright) areas in the live image in red and underexposed (too dark) areas in blue.

1. On the **Display** tab click the **0.45** button. The display curve will be adapted to a gamma value of 0.45. This will set the optimum color presentation. If you do not see this button, activate the **Show all** mode.
2. Move the controls under the display curve left and right in order to directly adjust the values for **Brightness (White)**, **Gamma**, and **Contrast (Black)** in the live image.

1 Control Contrast (Black)
2 Control Gamma
3 Control Brightness (White)

**Information**

With the settings above the display of the live image will be adapted. These settings will also be transferred to your acquired image. This will not change the camera settings.

### 5.5 Add Annotations

➢ You configured all microscope components you are going to use.
➢ You activated the automatic scaling.
➢ You acquired an image with **ZEN (blue edition)**.

1. Select the **Graphics** tab.

2. Click the button **Insert Scale Bar**.

→ The scale bar will appear directly in the image.
Information

Click with the right mouse key to any requested annotation in the image to edit this annotation (e.g. color, line width). This will open the context menu. Select the entry Format Graphical Elements... In this dialog you have numerous formatting possibilities.

3. Click the button Draw Arrow.
   
   - The button will turn into blue to indicate its activation. Now you may draw an arrow into your image.
   
   - You added the annotations ScaleBar and Arrow from the toolbar to your image. Below the toolbar you will find an additional configurable toolbar. Using the button Customize you may adapt this toolbar to your needs.
6 Close ZEN blue software

➢ You have acquired or processed an image, created a table or a report with ZEN blue.

1. Click on File | Exit to end ZEN blue software. Alternatively you can press ALT+F4 on your keyboard or click on Close icon in program bar.

Information

If you haven’t saved your files the save/keep documents dialog will open before the program closes. Select files you want to save or unselect files you don’t want to save.
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