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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.
- 2. Alternatively click on Start | All Programs | Carl Zeiss | ZEN 2011 | ZEN 2011 (blue edition) entry (blue icon).
 - \Rightarrow The software starts. After a while you see the **ZEN blue** login screen.

Hardware Settings and Login	; ×
ZEN	
Start System	Image Processing
Don't show this dialog next time	

- 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, got 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	5 Left Tool Area
2 Workspace Configuration	6 Center Screen Area
3 Menu bar	7 Right Tool Area
4 Tool bar	8 Status bar

3.1 Title bar

ZEN system 2011

Fig.	2:	Title	bar
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Symbol	Description
?	Activates the "drag & drop" 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.
-	Minimizes the program window.

? = 💷 🗖

Symbol	Description
00	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.
8	Reduces the program window to any selected size.
×	Closes the program window.

3.2 Menu bar

File Edit View Acquisition Graphics Tools Window Help

Fig. 3: Menu bar

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

3.3 Workspace configuration

Workspace Zoom — Reset 🗹
Design Light Vorkspace Standard

Fig. 4: Workspace Configuration

Here you find settings to adjust your workspace. Select light/dark **Design** of the user interface or enlarge the screen with **Worspace 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 | **CustomizeTool 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.



Fig. 6: Left Tool Area (ZEN pro, desk, system)

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.

CFP-YFP-05.zvi*	YFP-cntrl-05.zvi ⁽⁸⁾ CHO-3-channe	I_fluornce ®	(<u>111</u> 00 o -
2D Split Galery Ortho Cut Cut 2,5D SD SD Histo Colocal. Profile Mesure			
Dimensions Player Graphics		♥ ✓Show all	
Z-Position 1	1) , , , , , , , , , , , , , , , , , , ,	Texasred	Spline Mode

Fig. 7: Overview Center Screen Area

Document bar
 Image area
 Image view tabs
 View specific and general controls organized in tabs

3.7 Document bar

		(+)	\$ •
CFP-YFP-05.zvi* ^(X) YFP-cntrl-05.zvi ^(X) CHO-3-channelce.zvi* ^(X)	11 CD ¢	*	



Here you see tabs of all opend documents. Click on a tab to view the image/document. On the right end of 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

Scaling	4,0 µm/px (theoretic)	
✓ automatic		₩

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 a** menu.

System Information

System Information: Experiment

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

Progress Bar

Experiment Completion... 50 %

Displays the progress of the currently active process. Each new process added supersedes old-

er still active processes. If you click on the A 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: Position: R:1076 G:115 B:29 X: 420 Y: 229

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.

Options		
Software General Startup	Language	
Naming	Fixed Language English	
Saving	日本語	- 11
Documents	中文(中华人)	民共和国)
Acquisition	Deutsch	
User	English	
DataTables	\ \	
Hardware		



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.

		? _ m # ×
V	Vorkspace Zoom 🥚	Reset 🖌
	Design Light 💌	Workspace Standard 💌 🐲

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

- me Grafik Extras Fenster Hilfe · Arbe a D 23 3 9 9 USa1_Darm_5x_3.jpg Amera Versibeitung Analyse Bericht . 0 00 111 0 84 14,06 ME H 8 × 19 10 A 1.0x Acridine Orange dind Profi Reflektor 1.0x mt-Vergrößerung 10x 問 \$ xxx Q Q -26 % 2 Auto-Fit 1 .0 to Min/Max BestFit C1
- 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.

• Time Series			Show all	Ľ
Duration)	1	•	Cycles	J
As Long as Possible				
Interval	0.0	-	ms	•
🔲 Use Camera Streaming if Possib	le			
		Measu	ure Speed	



Information

With **dock all tools** function in Workspace Configuration [\rightarrow 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.

Time Series		D Show all	Ľ
Duration]	1	Cycles	D
As Long as Possible			
Interval	0.0	*) (ms	-
🔲 Use Camera Streaming if Possib	le		
	<u>А</u> (Ме	asure Speed	

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

• Time Series		🗸 Show all 📓
Duration	1	Cycles 🔻
🗌 As Long as Possible		
Interval	0.0	🔹 (ms 🔻
🔲 Use Camera Streaming if Possib	le	
		Measure Speed
Start Manual 🔻		
Stop Manual 💌		
Pause Begin Manual		
Pause End Manual 💌		
Switches		

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.

Camera	Process	ing Repo	P rting		
0		@]		0	
Set Expos	ure	Live	Continuous	s Sna	p
		orononante	2000 ApJ 2000 A		20
	oscope c	omponents	8	LI SHOW AN	~
Cam	iera	M 9 0		C Show All	
T Mov	ie Record	er			TPAL 1

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

0	Microscope Components	□ Show All	
0	💿 Camera	□ Show All	2)
0	E Movie Recorder		2

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

🗾 🔳 Microscope 🤇	Components	🖌 Show All 🛛 📝
Objective	(10x	• + m
Optovar	(1.0x	•
Reflector	BF	•
CameraAdapter	(1.6x	•
Total Magnification	16x	

- 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**.

 $\,\Rightarrow\,$ You have successfully configured your microscope components.

See also

- Batus Bar [→ p.9]
- B 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.

scale_obj10x.czi - ZEN lite 2011		
Datei Bearbeiten Ansicht Aufnahme Grafik Extras Fenster Hilfe		Arbeitsbereichs-Zoom
		Design Hell Arbeitsbereich V
Kamera Verarbeitung Bericht scale_obj10x.cz	8	💷 🌢 👻
	Indite Indite	Centainer 1
Skalierung	Freies RAM 1,17 GB CPU 9 % Bildrate: Pixelwert: Position: Orc	Iner: Anwender: 16:08
Automatisch	0% Freie HD 146,25 G8 - fps - X:-116 Y: 615 C:\	Users\M1CRU\Pictures m1cku 10.00

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



3. Open the **Options** menu **I and** click on the entry **Create New Scaling**.

	Create New Scaling
	Assign Active Scaling to Image
	Import
	Export
	Delete
0 lt	More
-theoretic-	idle

 $\Rightarrow\,$ The calibration wizard will appear in the image area.



4. Click on **single line** button as reference line in the calibration wizard and activate the **automatic Line Detection** button.

Please Draw a Reference Line	₹ 🔽	ine Detection
	(Save scaling



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 "**theo-retic**" 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.

Camera	Processin	g Repo) rting			
0	1	@1	6	3	0	
Set Exposu	ire	Live	Cont	inuous	Sna	р
Link Came	eras era Axio	Cam Emul	ation (dvn	amic)		
Link Came	eras era (Axio)	Cam Emul	ation (dyn	amic)	G Show All	2
Link Came	eras era (Axio) oscope Cor	Cam Emuli	ation (dyn	amic)	Show All	
Link Came Active Came E Micro To Came	eras era (Axiot oscope Cor era	Cam Emuli	ation (dyn	amic)	 Show All Show All 	2 2

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

 $\Rightarrow\,$ 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

- B Optimize Live Image Settings [→ p.20]
- Document bar [\rightarrow 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.

Dimensions G	raphics		
Zoom 💬	+ 100% Q		53 % 🕄 🔽 Auto Fit
Tools	•	Navigator	Interpolation
Channels 1			
I √ s	ingle Channel	✓ Range Indicator	Quick Color Setup

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.
 - \Rightarrow 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.

Imprint

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