

Manual Zeiss Axio Zoom.V16 microscope and ZEN pro software

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Fred Hartjes



Starting up

- **Power on**
 - Actuate the knob on the EMS 3 control unit
 - Switch on the coaxial illumination
 - Switch on the ring illumination at the VisiLED MC100 unit
- **Login** on the PC under your own account. Login under timepix does not work
- **Open ZEN**
 - All programs => Carl Zeiss Microscopy => ZEN2 => ZEN (blue edition)
 - In case of a selection window for various versions: select <ZEN2 pro>
 - If requested select <Calibrate Now> on the popup window
 - Check the Light Path (main tab Locate) for any failures
 - In case of a broken camera indication => reboot the PC
 - If you use ZEN for the first time the displayed image will be upside down. To correct check the blue camera tab, press show all in the tab header, and press Model Specific at the bottom of the menu. At Orientation change from original to Rotate 180. This only has to be done for the first login.
- **Check the path for storing pictures**
 - Should be: Tools => Options => Saving => Auto Save Path => D:\Zeiss pictures
- **Select channel if requested**
 - Acquisition => Channels => Add channel: select any channel from the list.



Standard operation

- **Use the Sycop 3 unit for zooming and focussing**
 - Zoom: joy stick left/right
 - Coarse focus: joy stick forward/backward
 - Fine focus: wheel left or right from the joy stick
- **Viewing**
 - Camera image and image through eye pieces cannot be viewed simultaneously. Pull the slide to the right for camera viewing. The focus value of the camera is about 20 um bigger than the focus in the eye piece mode. For rapid focussing and image selection viewing through the lens is recommended.
 - Note: the magnification (max 180x) refers to the image on the eye pieces, not to the camera image.
- **Illumination**

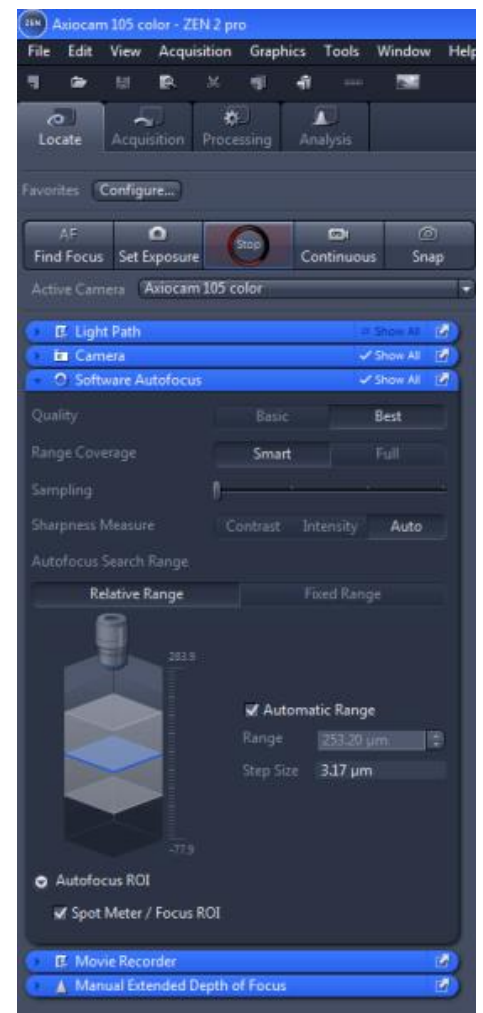
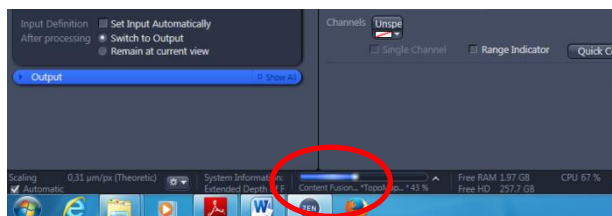
- There are two illumination sources that can both be tuned in intensity. In most cases the balance between these two sources has big effect on the image quality. Take your time to find the best setting.
 - Through the lens by the unit on the left. Could be blocked by the slide COAX on the microscope head.
 - Ring illumination by the VisiLED MC 1000 unit. Could either a full ring or any ring sector.
- **Viewing an image:** press the Live button
- **Toggle full screen => F11**
- **Creating image files and storing them on disk**
 - Press Snap => picture will be stored in RAM in the Zeiss czi format and appears in the right hand column under Images and Documents. Pressing the floppy symbol saves the picture as czi on the default path.
 - To save it on disk in a general format like jpg or png, click the right mouse button.
- **Adjusting image brightness and contrast => use the image analysis display**

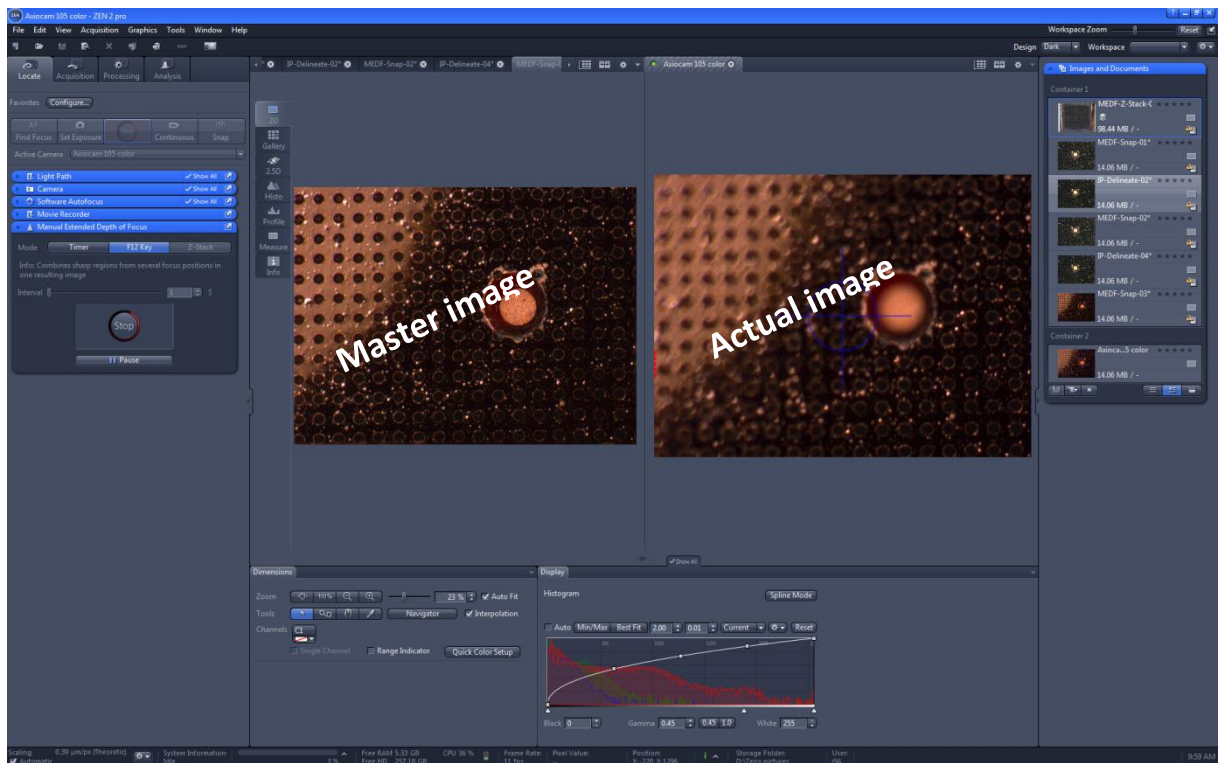
Advanced functions

Both methods (Autofocusing and Manual Extended Focus) require a surface having certain visible structure like scratches, grains or tiny dust particles. Therefore the methods do not work on shiny surfaces and flat, transparent objects.

● Autofocusing

- Main tab Locate.
- Sub tab: Live
- Roughly focus the microscope on the desired point.
- Set focussing parameters on the Software Autofocus tab
 - Recommended settings for accurate Z measurement are shown on the right picture
 - To execute the autofocus procedure, press on sub tab "Find Focus". After completion (see the progress bar at the bottom of the ZEN window) the focus value is read out as the Z-Position on the Sycop 3.





- **Creating an extended focus image**

With this method one creates an image where items on different height are still all seen in focus. To make this, a number of images is acquired at different focal heights where each new image is used to update a master image. For the software licence that is built in (Manual Extended Focus), the images have to be created by hand.

- Main tab: Locate, set Live on and focus the microscope to about the Z-centre of the object
- Determine your focussing strategy:
 - What is the range of focus of all items you want to see in the final image?
 - What is a suited step height you want to apply (e.g. one or two clicks of the fine focus wheel)?
- Set “Life” off and open the blue tab Manual Extended Depth of Focus
 - Set Mode: F12 Key
 - Press Start. Two images will appear: the right one is the actual image direct from the camera, the left one is the resulting master image (for the first step this is an older image).
 - Press the F12 key to acquire a new image. Every time this is done the master image disappears for a moment and returns when it is updated with newly acquired image.
 - Change the focus with the fine focus wheel and press F12 again to collect a new image at a different focus height. You will see that the master image gradually improves when the information of more and more different focal heights is added to it. Continue with this until you are satisfied with the master image.
 - Terminate the process by pressing on Stop. The master image is stored in the Images and Documents tab on the right side of the ZEN window.