

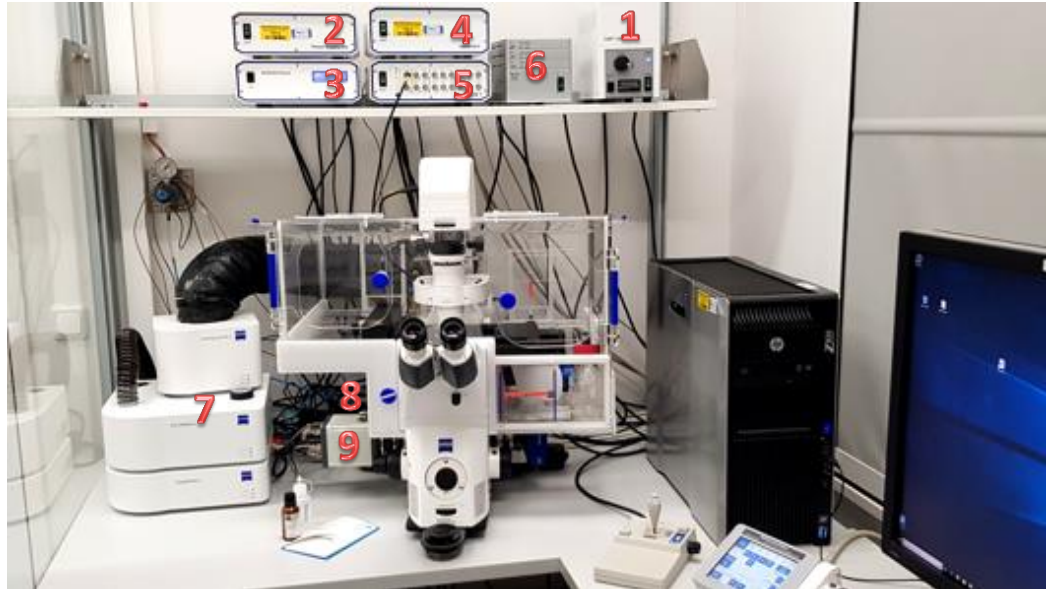
**Standard Operating Procedure
Workflow Zeiss AxioObserver Z1
ApoTome**

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Please note:

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Last change: 12.02.2020



Starting the System

- Start the computer behind the monitor
- Activate the HBX lamp if needed <1>
- Turn on all the switches <2> ...<6>
- If you need the heating turn on the tower > 2h before your experiment <7>
- Turn on the microscope <8> and the Camera <9>

Starting the Software

- Start ZEN blue Software
- Use the software mode: „ZEN pro“
- Please initialize the stage. Make sure the Objectives are in the load position and no obstacles are in the range of the stage

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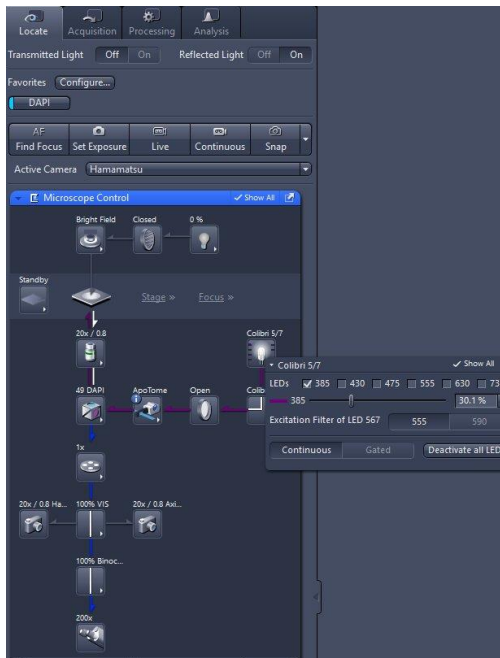
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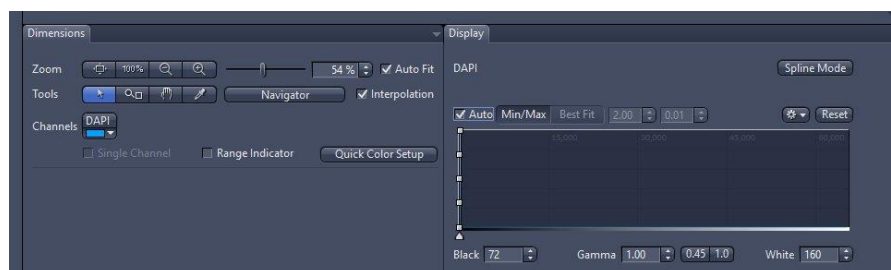
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Load specimen

- Get started in the locate tab
 - Choose the proper Objective (be careful changing between different immersion types)
 - Bring the Objectives in the load position
 - Insert your specimen
 - Place your sample with the joystick (push the button on top to increase the speed of the stage) directly over the objective
 - Have a look if the correct filter is chosen (e.g. DAPI filter or trans. Brightfield)
 - If you want to use fluorescence to focus on your sample you have to activate the LED line fitting to your filter configuration, or choose the Lamp. Apply some power and open the shutter
 - Choose the lightpath to be directed on the ocular
 - Look through the oculars and focus your sample. You might want to start with a positive control to adjust your setup



If you want to focus in the acquisition tab please make sure you activated the "Auto" contrasting method in the –Display- Adjustments



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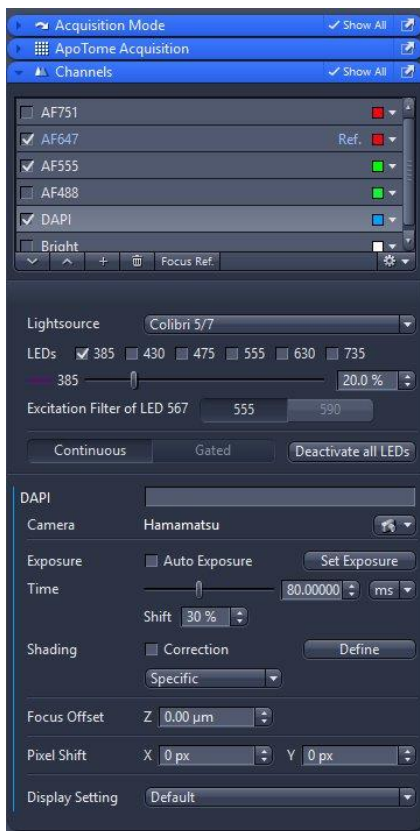
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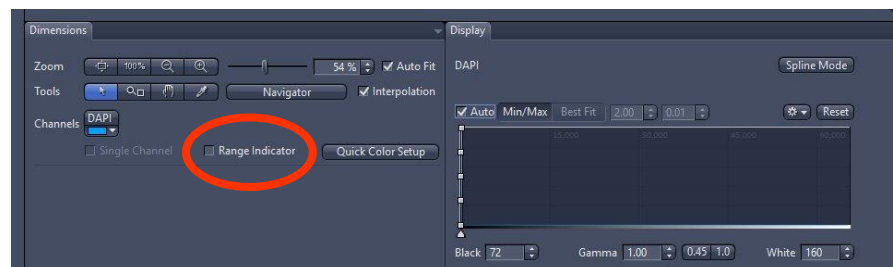
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Acquisition setup



- Change to the acquisition tab and open the channels toolbox
- You might need to add fluorophores with the + button. Be aware there are only 6 different LED lines and 5 different filter cubes available (The “All LED Template”-experiment setup offers you all possible options and fits to all possible staining-conjugates suitable to this instrument)
- Highlight one of the channels to start your setup and press the “live” button.
- Change LED power in % and exposure time in “ms” till you get a good signal intensity. Activate the range indicator and watch out for red signal in the gray scale image to make sure you’re not acquiring saturated images
- Set up all channels of interest for your specimen properly
- Tick all the channels you want to acquire and use the “Snap” button to create an overlay multichannel image



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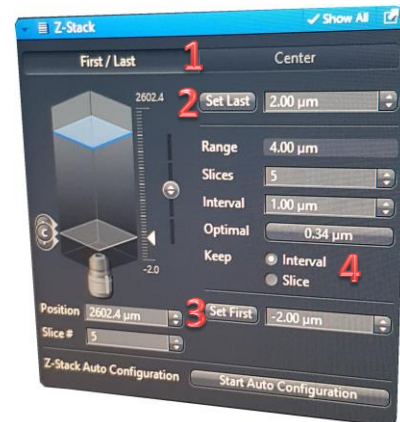
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Z-Stack settings

- First activate the Z-Stack option 
- Decide if you want to define only your center position to equally build a stack around this point, or if you want to define the first and last stack position **<1>**
- If you choose the first/last option move with the z drive to your lowest position of interest in the “Live” mode and push the “Set Last” button **<2>**
- Move to the highest position and press “Set First” **<3>**
- Take care to choose a proper stepping size **<4>** therefore change the interval, or the amount of slices in the given range. You can also choose the “Optimal” option related to the selected objective
- Activate as many preadjusted channels as needed and press “Start Experiment”



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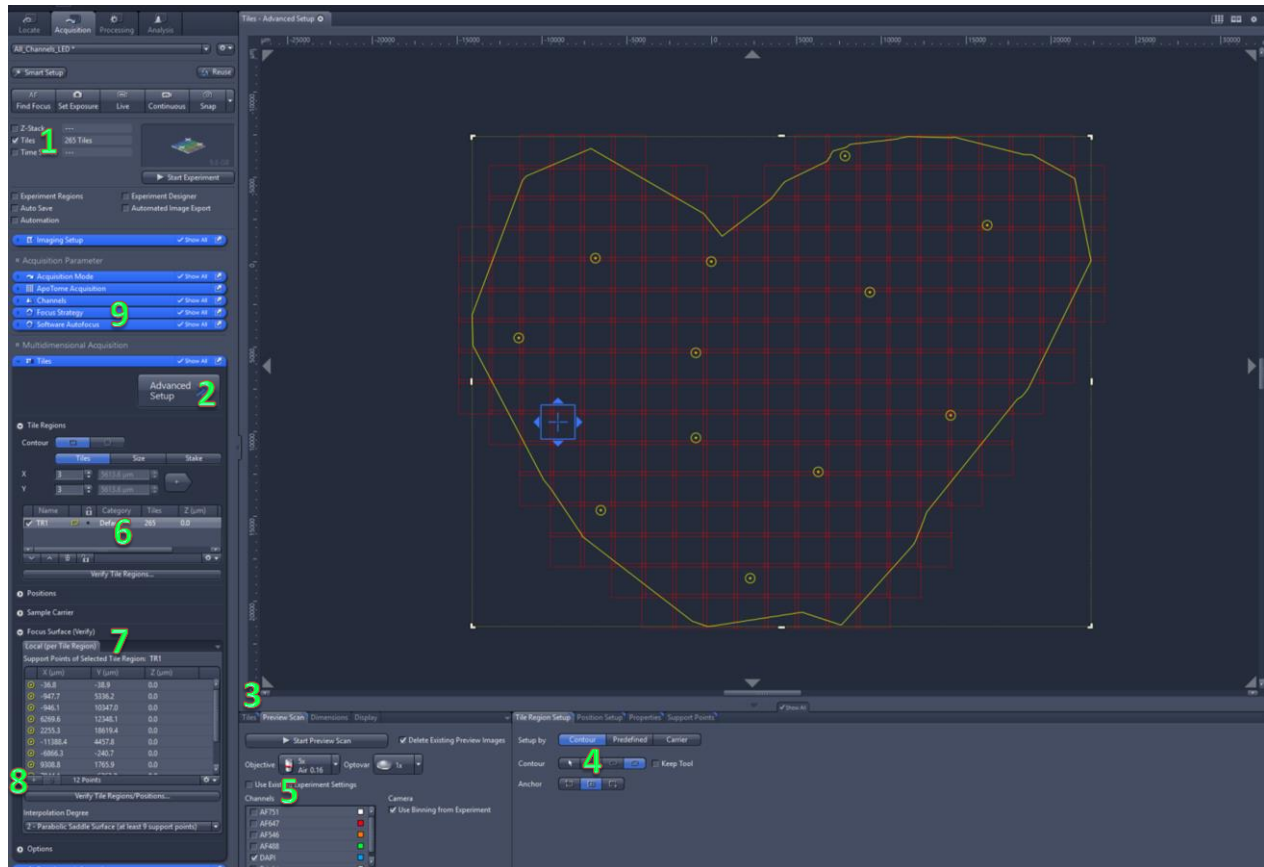
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Tiling setup



- Activate the „Tiles“ option <1> and select the „Advanced setup“ <2> in the Tiles drop-down menu
- Activate the “show Live/continuous in” “separate container” option in the Tiles setup tab <3>
- Choose a huge area with the squared contour tool <4> from the tile region option for the preview scan option
- If a preview scan is opted <5> choose the smallest objective available in the objective drop down menu and untick the option called “use existing experiment setup” only choose one channel for a fast overview and press “Start Preview Scan” (be aware the acquired signal might be extremely low; adjust the “Display” settings if needed)
- Use the region tool <4> to draw the new region of interest. Deactivate/delete the old overview region if existing in the “Tile Regions” drop down menu <6>
- Choose the “Focus Surface” <7> drop down menu to add local focus points with the “+” button <8> for larger specimen to obtain a flat focal plane
- Make sure the “Local Focus Surface” option is selected in the “Focus Strategies” <9> drop down

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Shutdown Procedure

- Clean the objective you used
- Change to the smallest magnification and bring the stage into the load position
- Save the images you want to save on the server if possible. Clean up used disk space if possible
- Shutdown the software
- Check for the next user in the scheduler (if the next person is registered within the next hour you can leave the system switched on. Only perform a Windows sign out)
- Or shutdown instrument and computer if the next scheduled appointment is more than one hour in the future, or if you are the last user
- If you cancel, or cannot come to your appointment you still have to make sure the system will be switched off

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Shutdown policy

- You **MUST** look in the scheduler at the **END** of your imaging session, to confirm the arrival time of the next user, since the next user could have changed or cancelled their booking while you were at the instrument.
- If the next user arrives more than an hour after you, or if you are the last person in the scheduler then you **MUST** switch off the system.
- If you have a scheduler reservation and cannot make it then you **MUST** cancel your reservation in the scheduler, so that the user before you can decide upon the appropriate switch down procedure.
- If your reservation cancellation occurs more than an hour after the previous reservation then it becomes your responsibility to **ENSURE** that the instrument is switch down.
- If you are unable to cancel, because you are within the 2 hours cancellation time limit or do not have internet access, then it is **STILL** your responsibility to **ENSURE** that the instrument is switched down.
- If you cannot switch down the instrument yourself (because you are somewhere else indisposed) then arrange for a colleague or an IMCES staff member to switch down the system for you.

Inform the person **DIRECTLY** in person or by phone - do **NOT** assume they will read their emails in time!