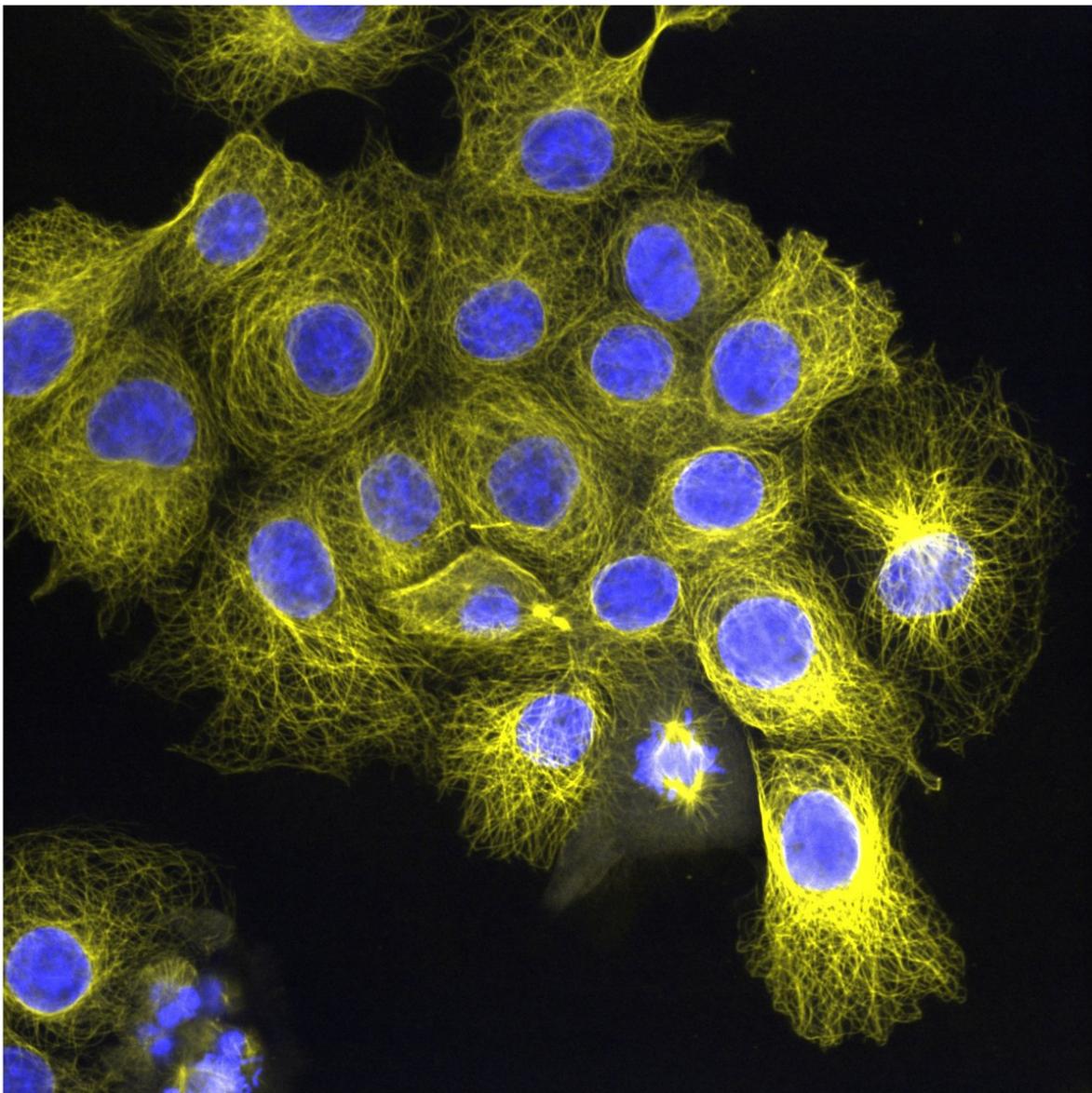


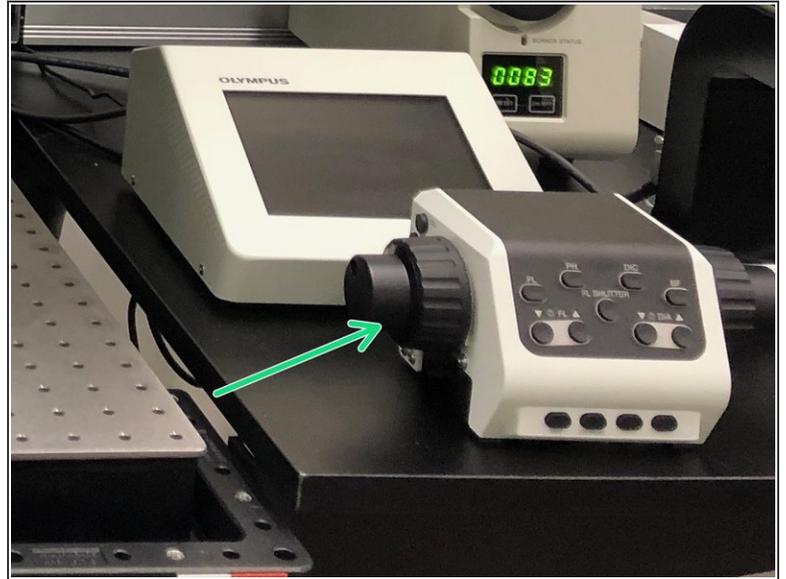
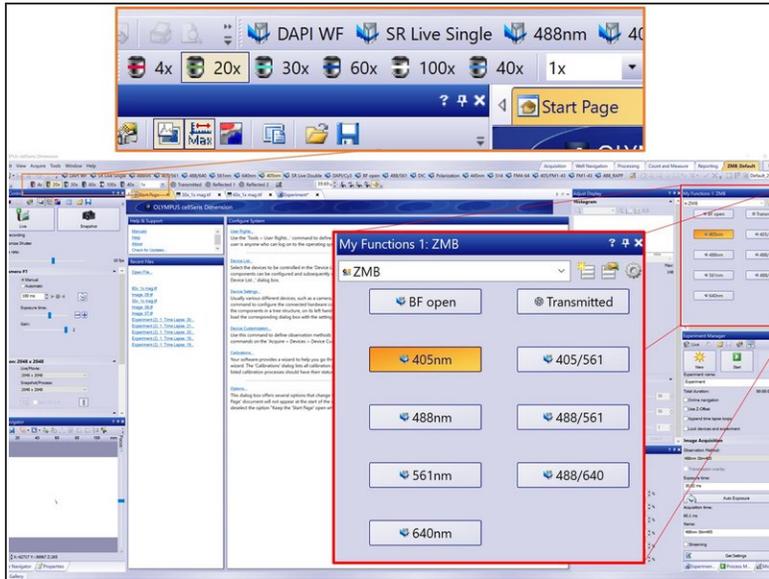
Olympus Spinning Disk - 2: Multichannel + Z-stack acquisition

How to acquire a multicolor image + z-stack with the Olympus Spinning Disk located at the Center for Microscopy and Image analysis, UZH, Room Y42-H-83.

Written By: Joana Raquel Delgado Martins

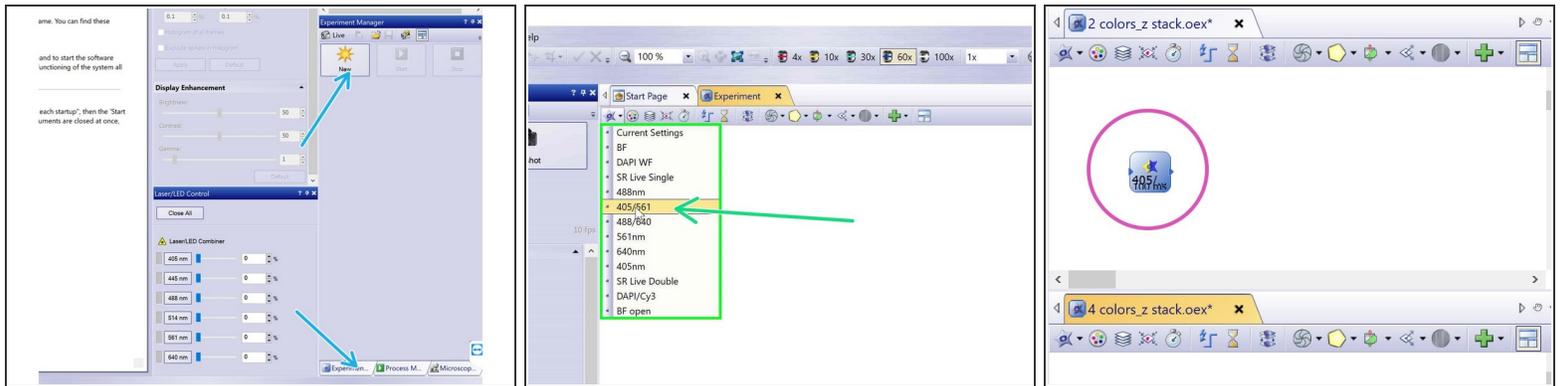


Step 1 — Place and focus your sample



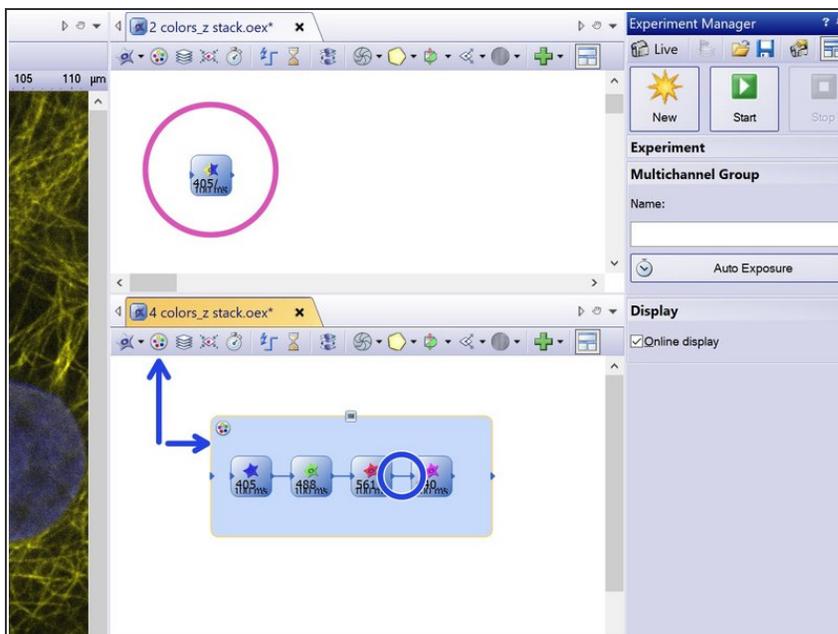
- Choose a suitable objective and place your sample on the microscope.
- ① The 30x, 60x and 100x objectives use **silicon oil** that you will find on the table next to the microscope.
- Choose one of the available observation methods on "My functions" to find and focus your sample through the microscope (e.g. "BF open").
- Additionally to the microscope wheels you can also use the keyboard to navigate and focus your sample:
 - ① Keep "Ctrl" key pressed and click on the Live image to move the stage and center your image.
 - ① Keep "Ctrl" key pressed and use the mouse wheel on the live image to focus.

Step 2 — Create a new experiment



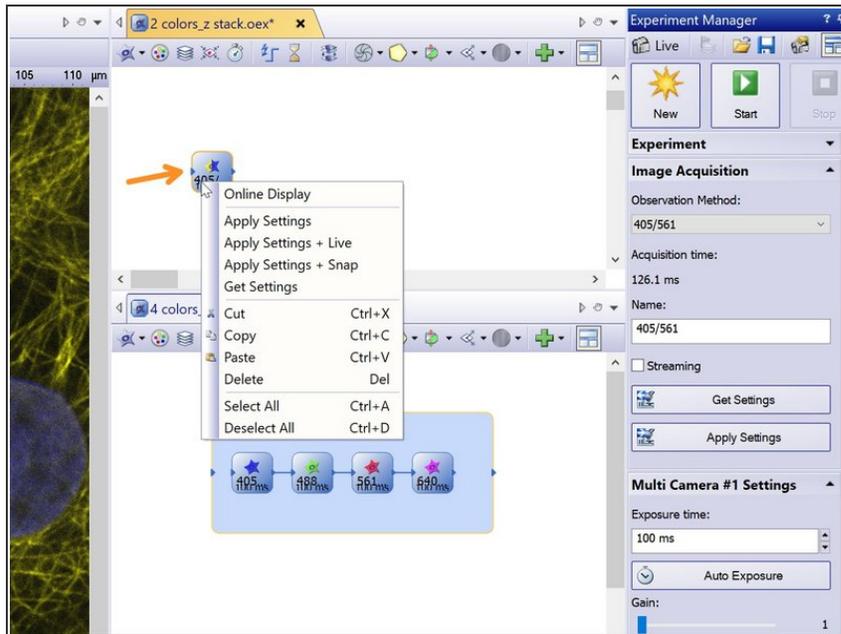
- Start a new experiment by clicking on the "New" button in the "Experiment Manager" (tab on the bottom right).
- Add a new "Image Acquisition" object by clicking on the respective icon.
- Chose your acquisition settings from the drop down menu.
- Add it to your experiment by clicking on the white area.

Step 3 — Multichannel acquisition - Examples



- Example 1 - dual camera simultaneous acquisition - 405/561
- Example 2 - sequential acquisition - 405/488/561/640
- When using more than two "Image Acquisition" objects, connect them and add a multichannel group around them.

Step 4 — Adjust imaging settings



- Right click on your acquisition object (e.g. 405/561) and choose "Apply settings + Live".
- ⓘ Alternatively you go to "Live" and then double click on the observation method.

Step 5 — Set Camera Parameters

The screenshot shows the Olympus software interface with several panels. A red box highlights the 'Pixel Clock' dropdown menu, which is currently set to '480 MHz'. Below it, the 'Multi Camera' dropdown menu is also visible, showing '480 MHz' and '129 MHz'. The interface includes various tabs and settings for camera configuration.

Typical frame rates (frames/s)

READOUT MODE		AREA READOUT MODE			LIGHTSHEET READOUT MODE	
Scan mode		Fast scan	Standard scan	Ultra-quiet scan	Fast scan	
X (pixels)	Y (pixels)	CoaxPress (16 bit)	USB 3.0 ^{**} (16 bit)	CoaxPress and USB 3.0 (16 bit)	CoaxPress (16 bit)	USB 3.0 ^{**} (16 bit)
2304	2304	88.1	31.6	23.2	88.9	31.6
2304	2048	100	35.5	26.1	100	35.5
2304	1024	200	71.1	52.3	199	71.1
2304	512	400	142	104	397	142
2304	256	799	284	208	787	284
2304	128	1590	568	415	1540	568
2304	6	22 800	9330	5950	15 800	9330
2304	4	41 000	18 600	10 700	22 800	18 600

** The faster frame rates are available at 8 bit and 12 bit.

i The Camera can be run at 8 bit, 12 bit as well as 16 bit (recommended).

● Toggle here the bit depth ON = 16 bit, OFF = 8bit.

● Change here the pixel clock (*determining the read out speed of the camera sensor*):

i 23 MHz: Ultra-quiet scan (recommended).

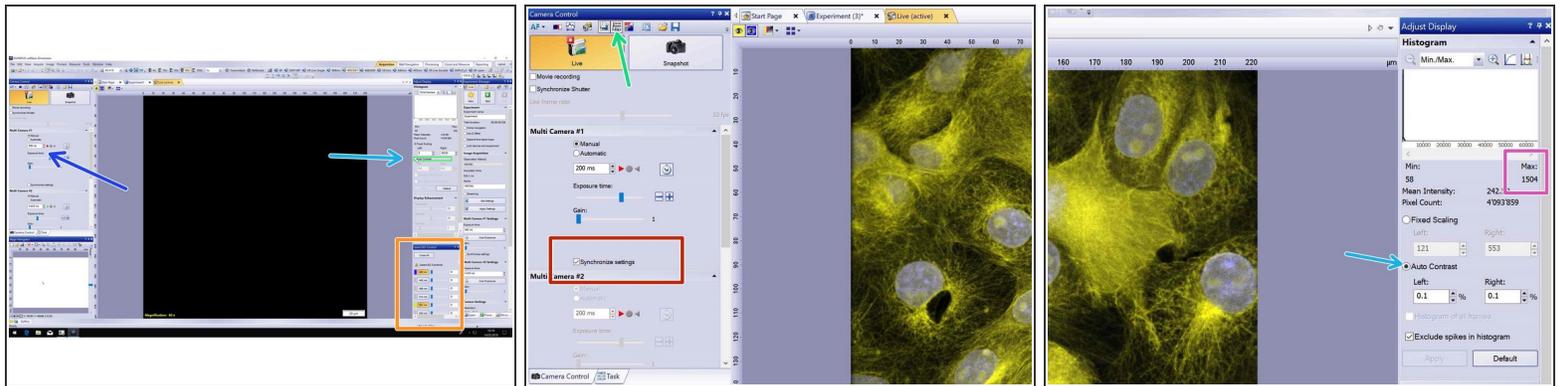
i 129 MHz: Standard scan .

i 480 MHz: Fast scan.

● Find here in addition the typical (corresponding) frame rates (frames/s) for each modi.

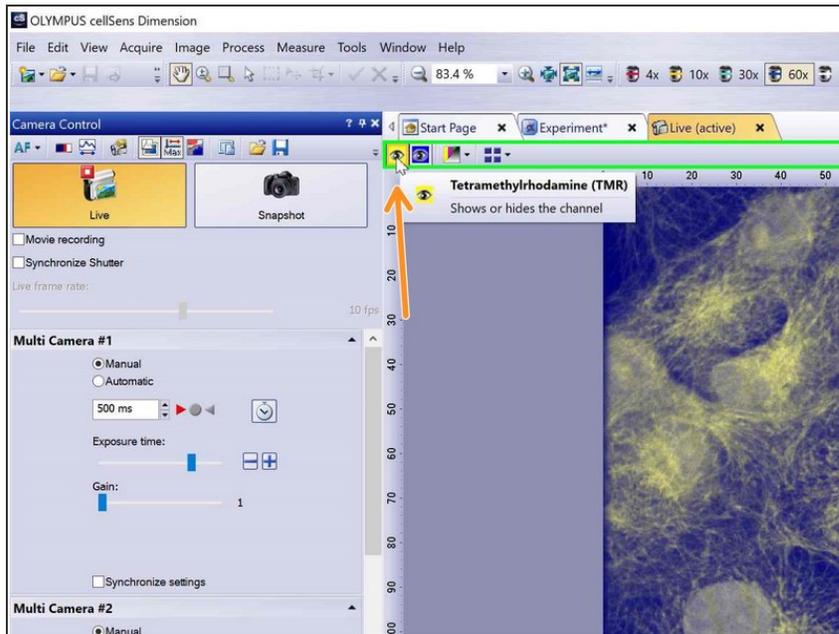
! If you are acquiring at 8 bit the histogram max should be lower than 255, at 12 bit lower than 4095, while if you are using 16 bit the max should be lower than 65535.

Step 6 — Adjust imaging settings



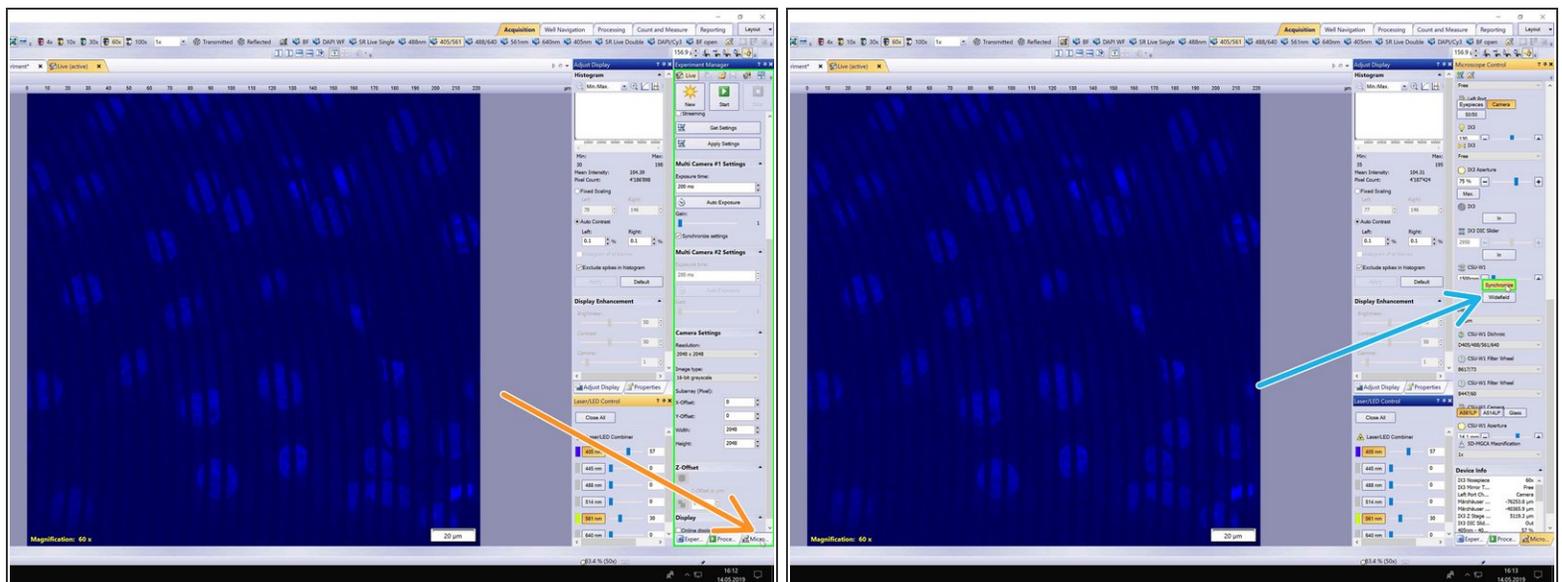
- Adjust contrast on the Adjust Display tab by choosing "Auto contrast".
- Adjust camera exposure times ("Camera control" tab) .
 - ① Type in values (multiples of 10, 20, 50, 100, 200, etc) rather than using the scroll.
- ☛ If you are using double camera settings also define the exposure time for the second camera.
 - If you want to have the same acquisition time for both cameras select "Synchronize settings".
- Here you can toggle the camera bit depth between 16 bit and 8 bit. Make sure you use the appropriate camera settings.
- Adjust laser intensities on the "Laser/LED Control" tab.
- Check the histogram and avoid saturating your signal.

Step 7 — Show/Hide channels



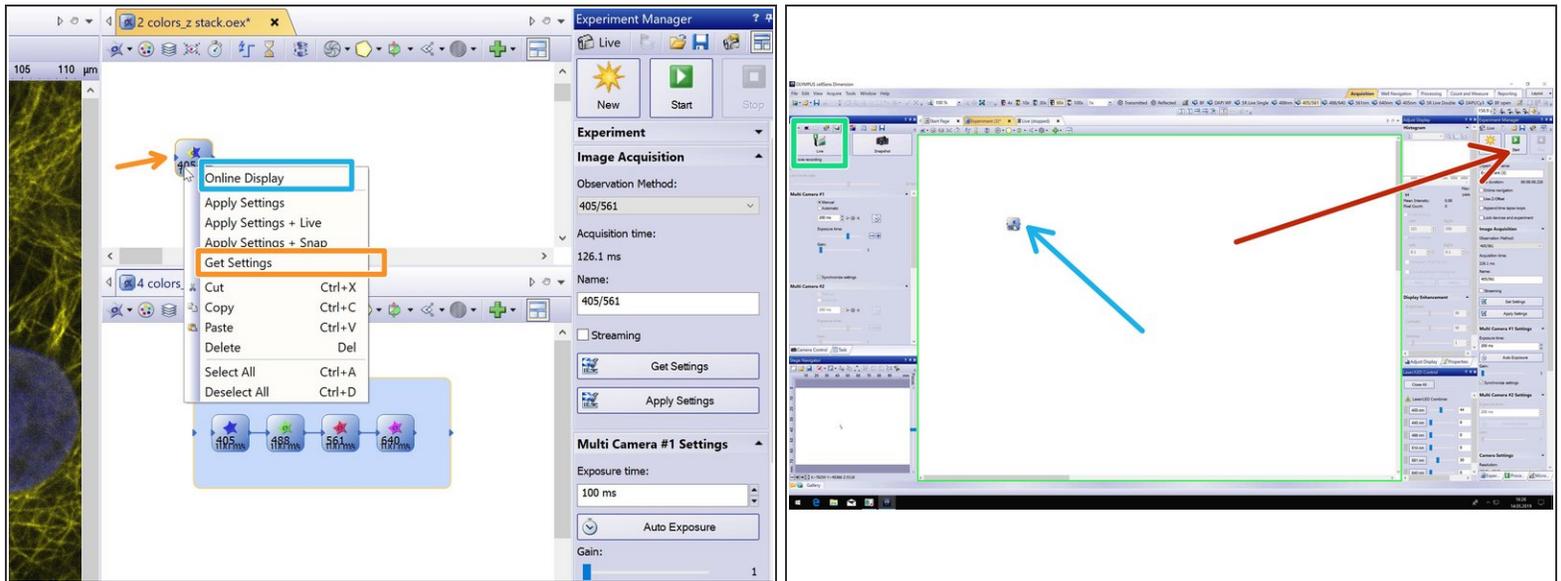
- If you are using the dual camera by toggling this button you can show or hide channels.

Step 8 — Synchronize disk



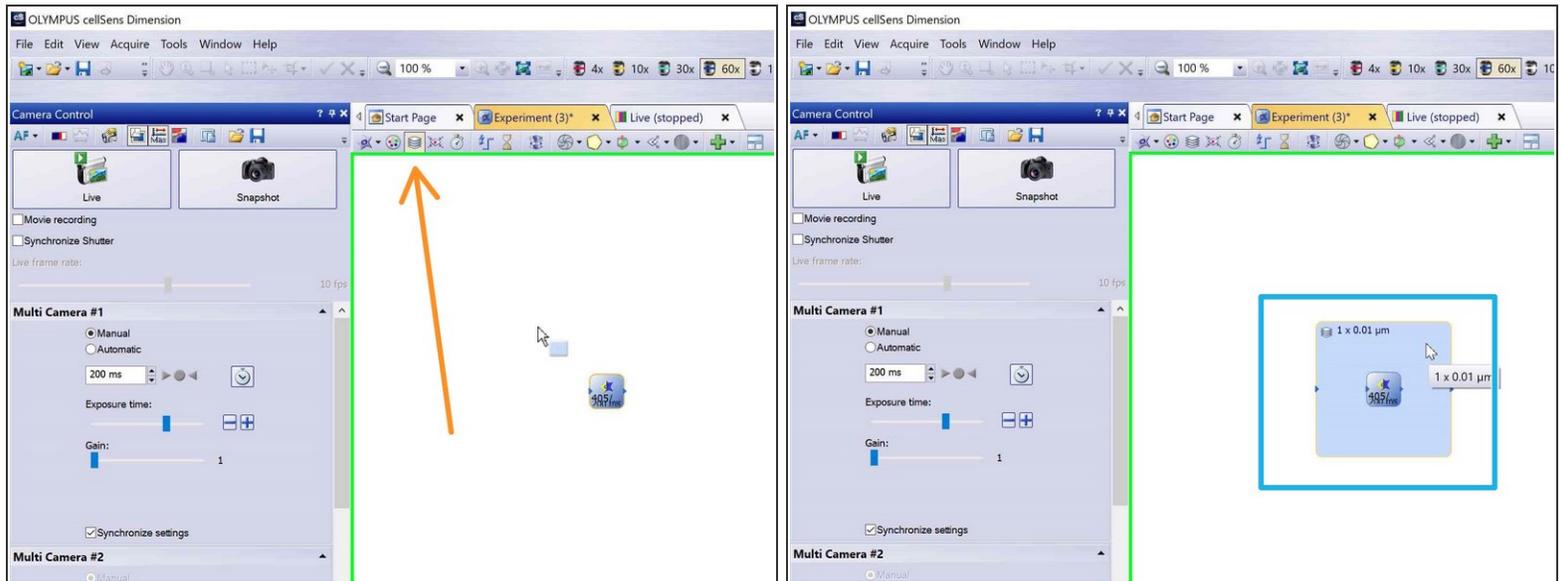
- To avoid image artifacts from the spinning disc you need to synchronize the disk with the camera.
- Select the "Microscope" tab.
- Scroll down and press the "Synchronize" button.

Step 9 — Get settings



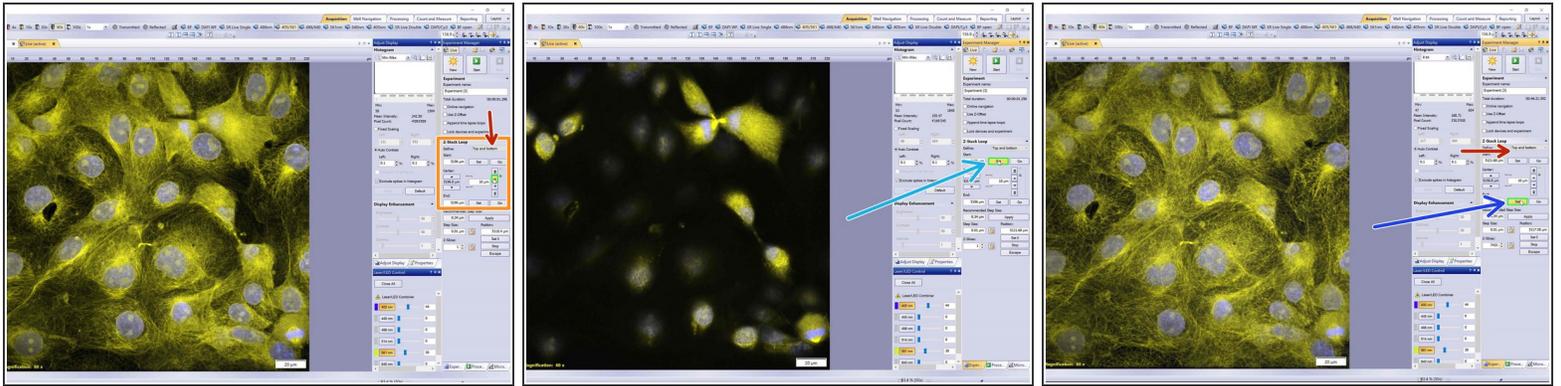
- Once you are satisfied with your settings, stop Live mode by clicking on the "Live" button.
- Right click on the observation methods and choose "Get Settings".
- ⓘ This will get the settings from the microscope and update your experiment accordingly.
 - The option "Online display" will allow you to visualize your image during acquisition.
 - ⓘ For a multicolor image you can add it to the multicolor box.
 - You can now start your experiment by pressing "Start".
- ⓘ If you want to continue adding objects to your experiment (e.g. Z- Stack or time series), follow the next steps.

Step 10 — Insert z-stack



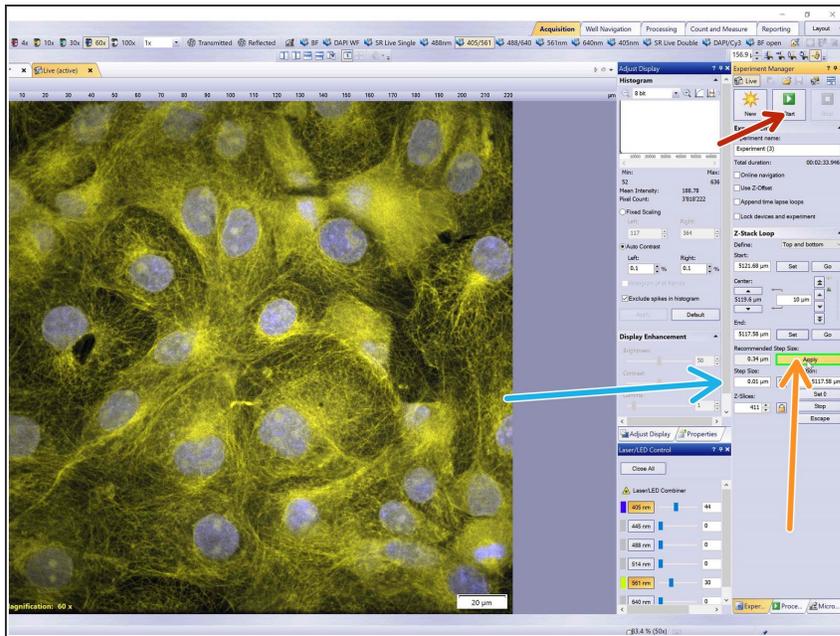
- Click the z-stack icon.
- Draw a rectangle around your "Image Acquisition" object (e.g. 405/561).
- If you want to use the faster Piezo stage follow these [Steps](#).

Step 11 — Define top and bottom



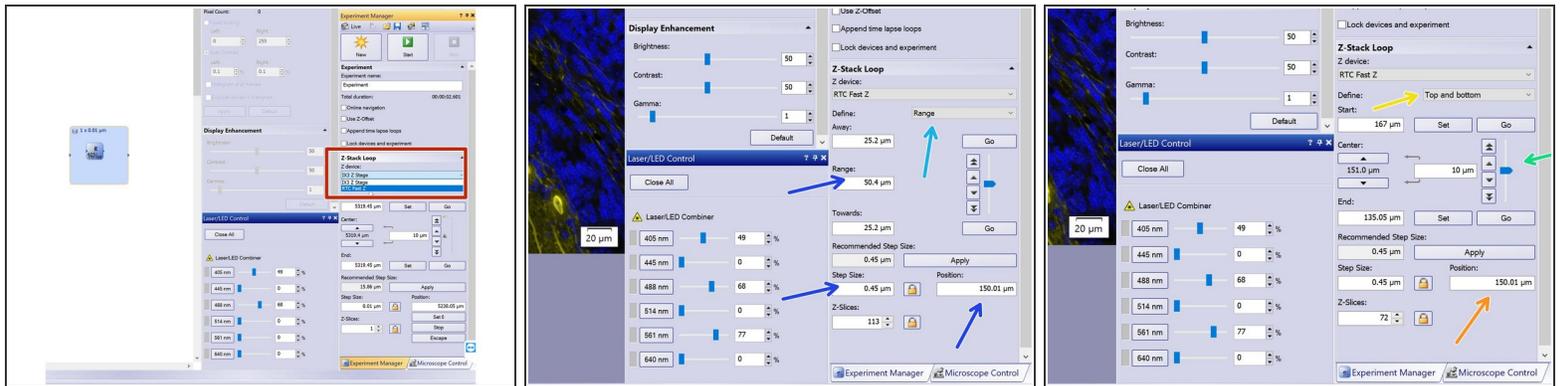
- Go to the "Z-Stack Loop" section in the "Experiment Manager".
- Define stack "Top and bottom".
- You can use the arrows or Ctrl+mouse wheel to move to the top of your stack.
- Click "Set".
- Move to the bottom of your stack and press again "Set".
- Stop Live mode.
- Alternatively you can also choose to define a z range from the drop down menu.

Step 12 — Define step size



- If you want to use the recommended Z-step size click "Apply".
- Otherwise define your step size in the appropriate field.
- Start your experiment.

Step 13 — Piezo stage - RTC Fast Z



- For faster acquisition of z-stacks you can also use the option RTC Fast (Piezo stage) from the drop down menu.
- ⓘ The travel range of this stage is 300µm and you can define your z-stack according to "Range" or "Top Bottom".
 - To use "Range":
 - Set center "Position" at 150µm, the desired range and step size accordingly.
 - To use "Top Bottom":
 - Make sure you set "Position" at 150µm.
 - Use the slider or arrows on the side to define your top and bottom limits.
- ⚠ Make sure the Piezo is in the middle of the travel range (150) before you start your experiment.

Step 14 — Available guides



- Check the next guide for more info on - [Olympus Spinning Disk - 3: Overviews + multi-positions + xyz images](#),
- [Olympus Spinning Disk - 1: Start up](#), [Olympus Spinning Disk - 2: Multichannel + Z-stack acquisition](#), [Olympus Spinning Disk - 3: Overviews + multi-positions + xyz images](#),
- [Olympus Spinning Disk and ScanR - Datamover](#), [Olympus Spinning disk - Load new/different device settings](#).
- [Olympus Spinning Disk - Photomanipulation unit](#).
- [Olympus Spinning Disk - Widefield mode](#).
- [Olympus Spinning Disk - Shut Down](#).