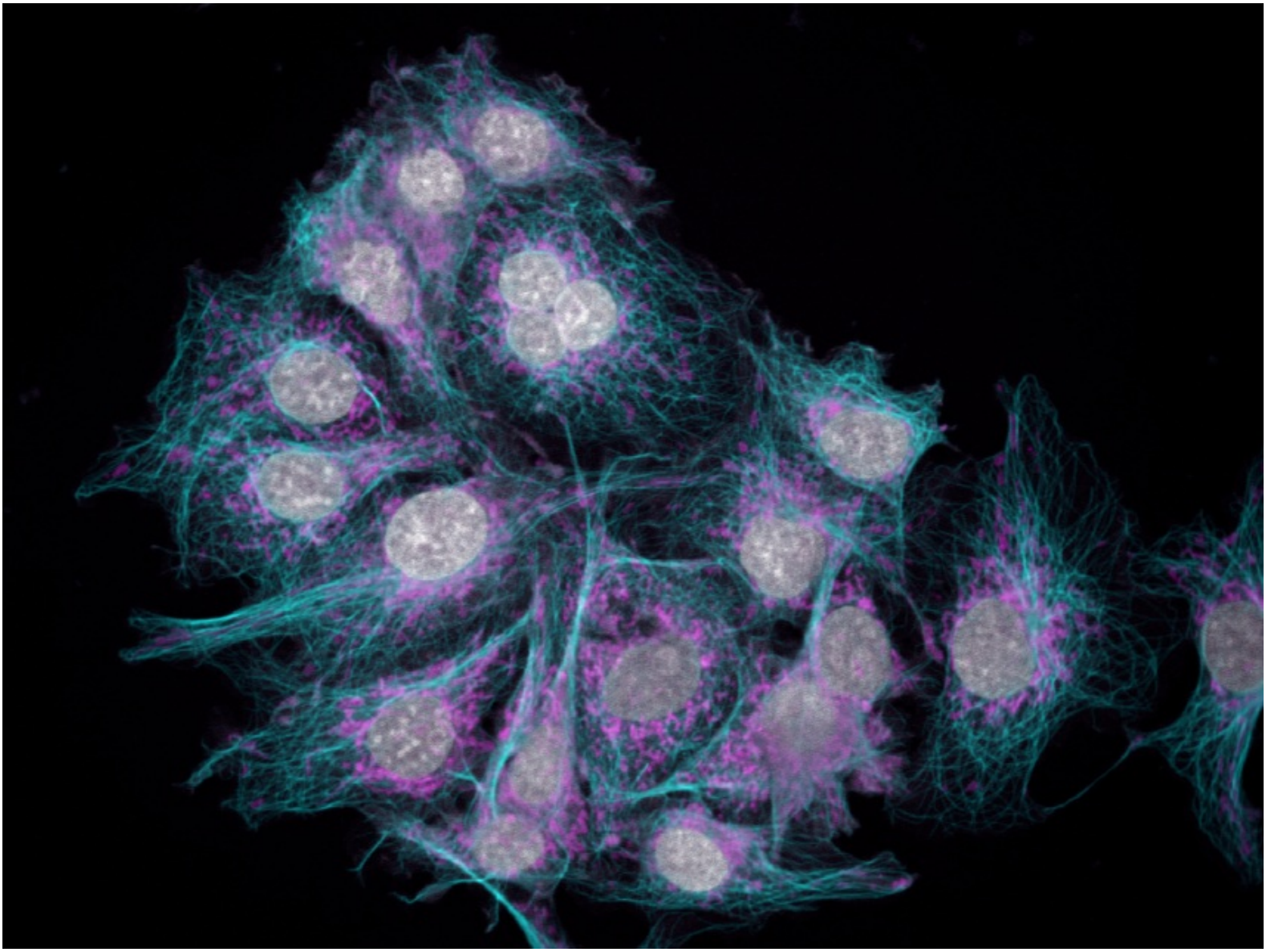
Nikon SD 3: Acquisition in XY and Z

Written By: z mbstaff



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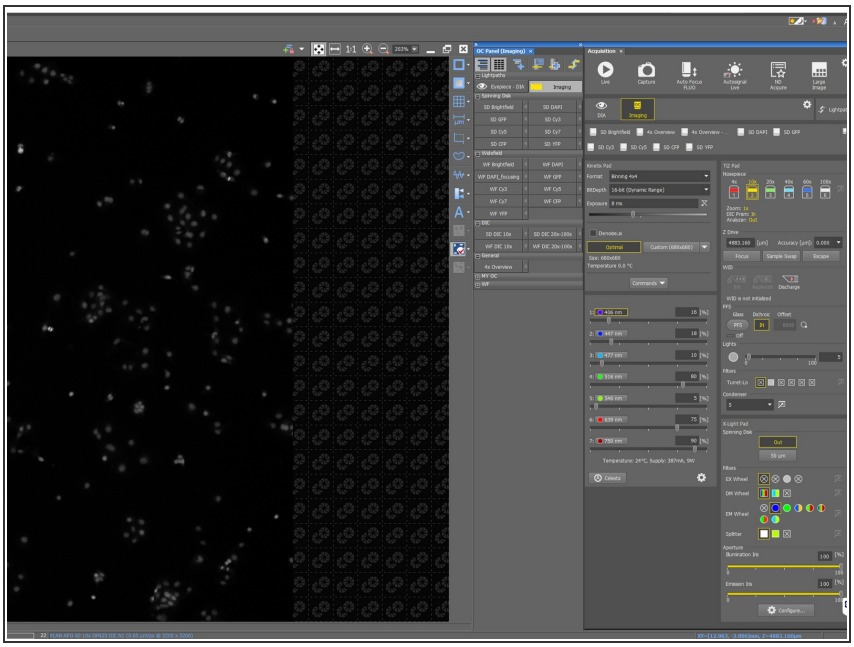
INTRODUCTION

In this guide we describe the major steps/aspects required for image acquisition on Nikon spinning disk confocal microscope.

It introduces you to the "NIS-elements" software for acquiring an image in 2D as well as 3D. For start-up of the system, mounting and focusing a sample, as well as finishing your session please check corresponding guides.

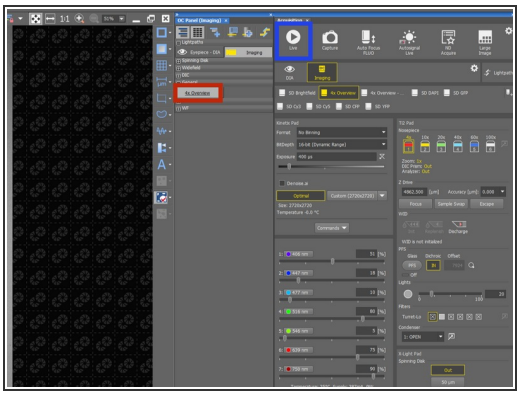
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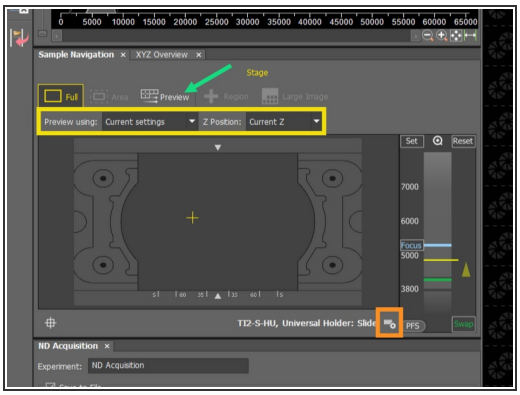
Step 1 — Check that you have private OCs to use

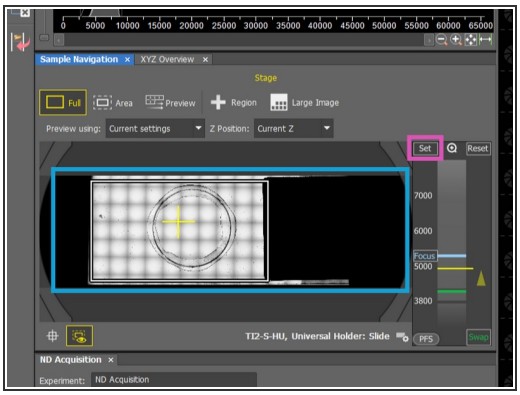


- Check this guide, how to create a set of OCs for your experiment

Step 2 — Create an overview map



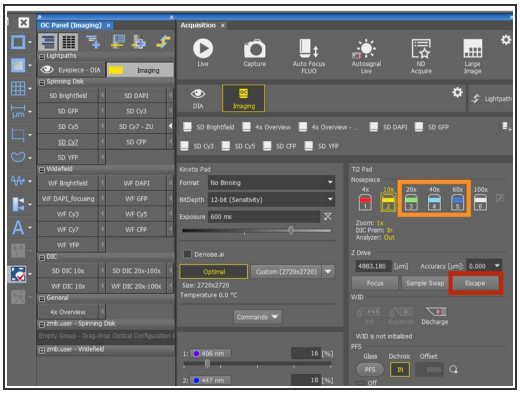
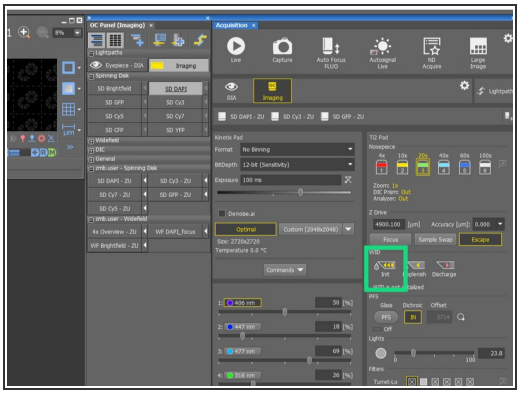
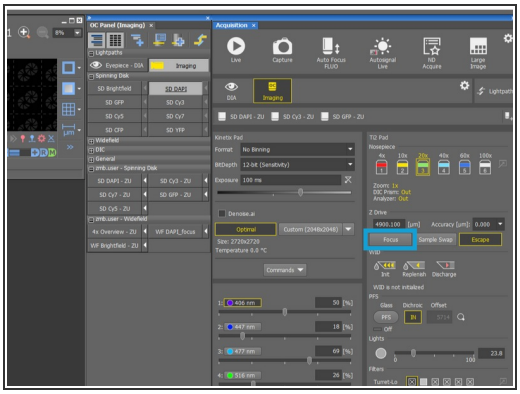




- Choose OC for Overview and low mag objective
- Go live and focus on the sample
- Choose from the sample holder - if you cannot find the exact same one, choose from the most similar one.
- Choose Current settings and Current Z position - this will use the last used OC, which you used for focusing
- Click on Preview
- Microscope will acquire a tilescan, which you can use later for the navigation in the sample
- By clicking on Set you can update your focus position.

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Step 3 — IF: using WATER imm. objective

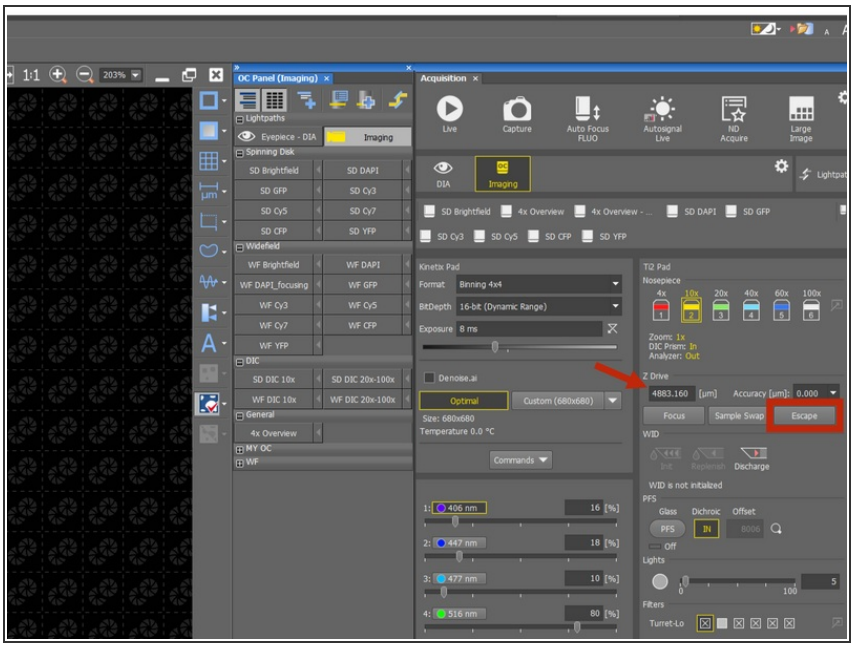




- Click on the Escape, this will lower objective to the load position. You can check that the Z-drive value should decrease to 499/500.
- Change to water immersion objective
- Initialize water dispenser - this will take few minutes, as the tubes has to be filled with water
- Now you can release the objective by clicking on Focus. Continue to manually focus on your sample in Live mode.

  ⓘ **It is good practice to visually check that there is a drop of water on the objective.**
- If, for some reason, you don't need to use automatic dispensing system you can put manually a drop of water on the objective (bottle on the table).

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Step 4 — IF: using SILICON imm. objective



- Lower the objective with the escape button. You can check that the Z-drive value should decrease to 499/500.
- Take the frame with your sample out from the microscope.

Step 5 — IF: using SILICONE imm. objective

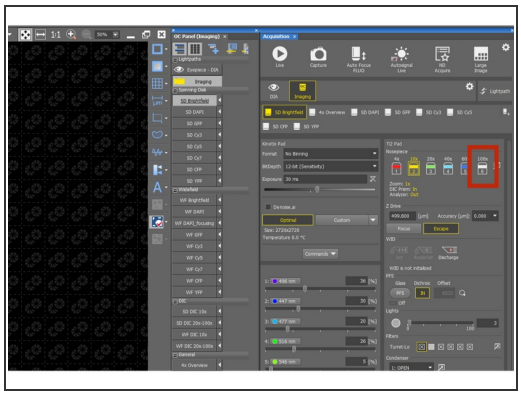
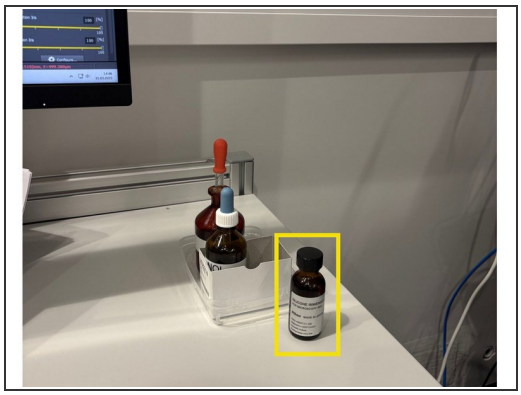
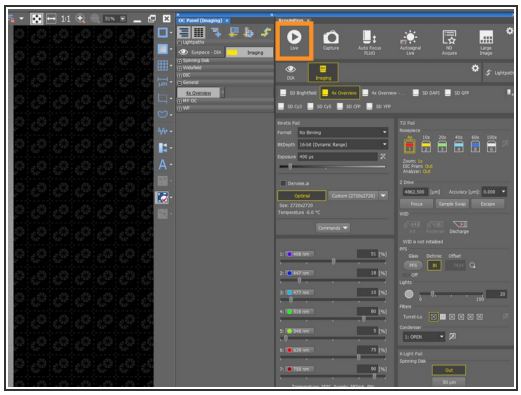


- Take out the water dispensing system (it holds there with magnet) and carefully go below the stage to place it onto the magnet next to the stage.

  ⚠ **This is important step, so the needle wont be contaminated by silicon oil. BE CAREFUL WHEN HANDLING THE NEEDLE. ALWAYS GO WITH THE NEEDLE POINTING *BACKWARDS*. So, in the case of crash you won't bend the needle**

  ⚠ Bending of the needle, will result in damaging the water dispensing system

Step 6 — IF: using SILICONE imm.

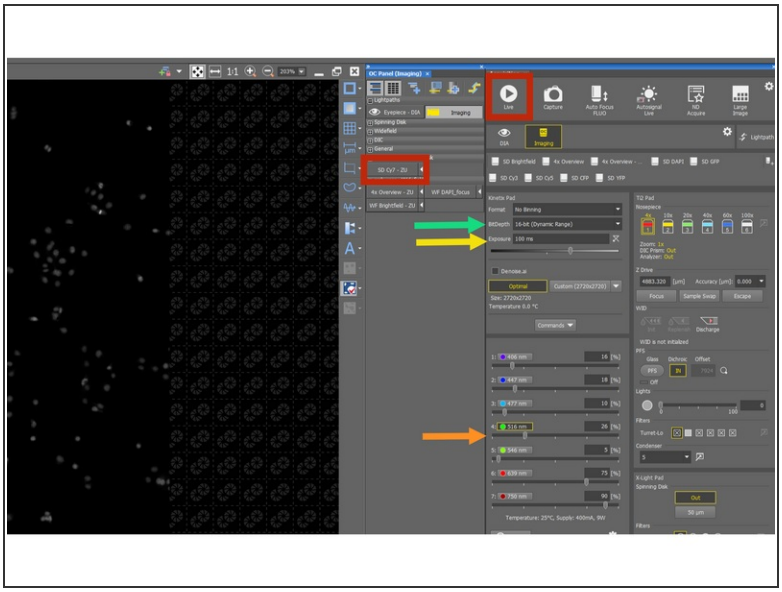




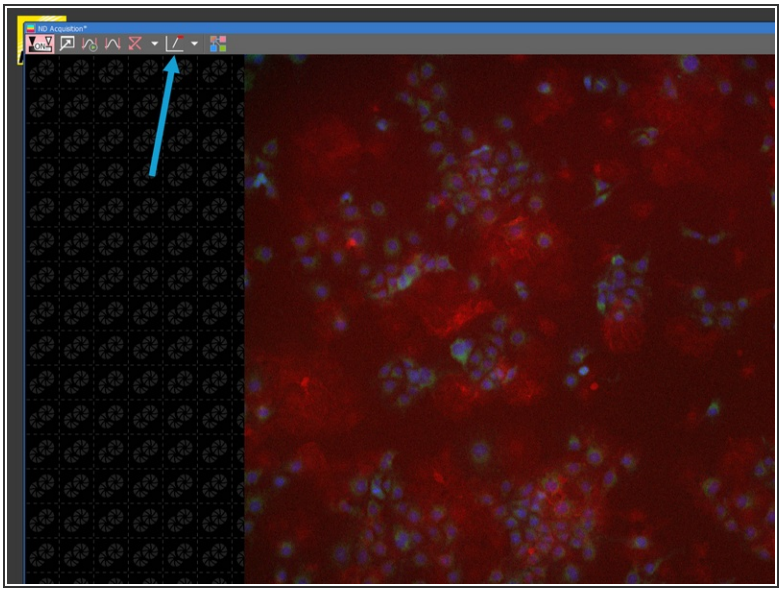
- Change the objective for 100x Silicon oil
- Put a drop of the immersion on the objective/sample (bottle on the table).

  ⚠ **The sillicon oil is very liquid!!! Please handle the oil carefully and go with the bottle closer to the objective or put a small drop on the sample handling it on the table**
- Put the sample/frame back on the stage and Release the objective from escaped position
- Fine focus in live mode with mouse wheel or on the joystick (change the speed to 1 arrow!)

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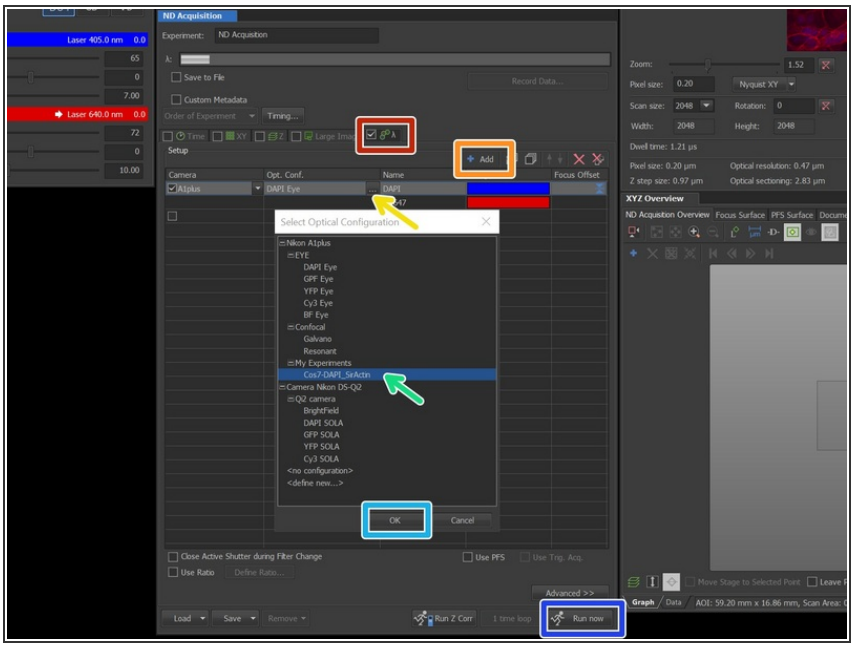
Step 7 — Adjust imaging settings





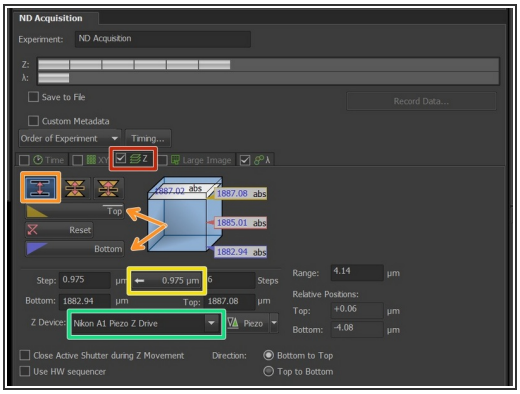
- You can choose the OC and go Live
- Adjust the laser power
- Adjust the exposure time
- **Make sure that the bit dept and ROI is the same for all the OCs (channels) that needs to be imaged**
- You can turn on the saturation control to monitor image saturation
- Continue setting up the other channels

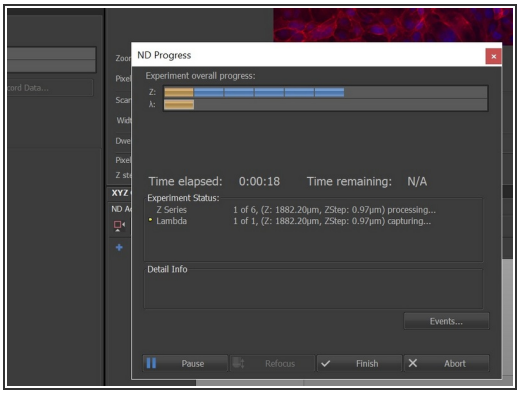
Step 8 — ND acquisition panel: 2D image

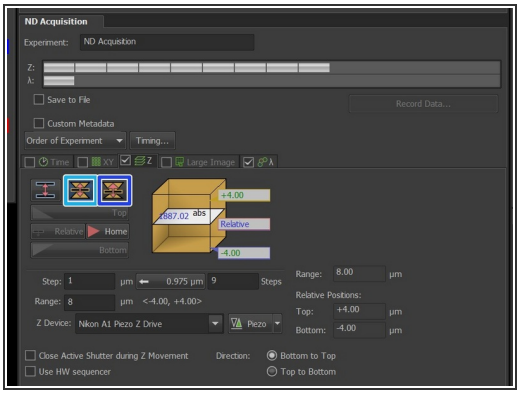


- Tick the **λ** for multichannel image
- Add a new line.
- Click on **...** to select the OC
- Choose the OC
- Confirm with **OK**
- Click on **Run now**
- If you want to save the image automatically, click on the **Save to file** and specify the path and file name

Step 9 — ND acquisition panel: 3D image

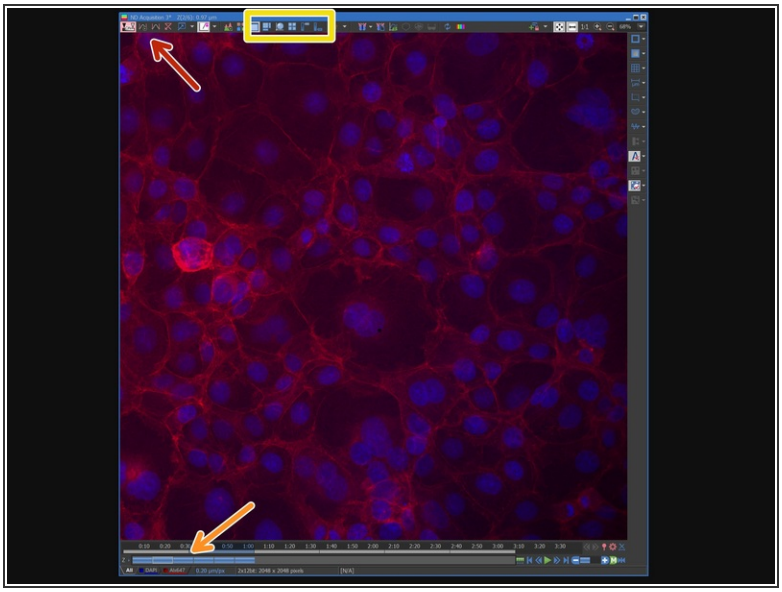


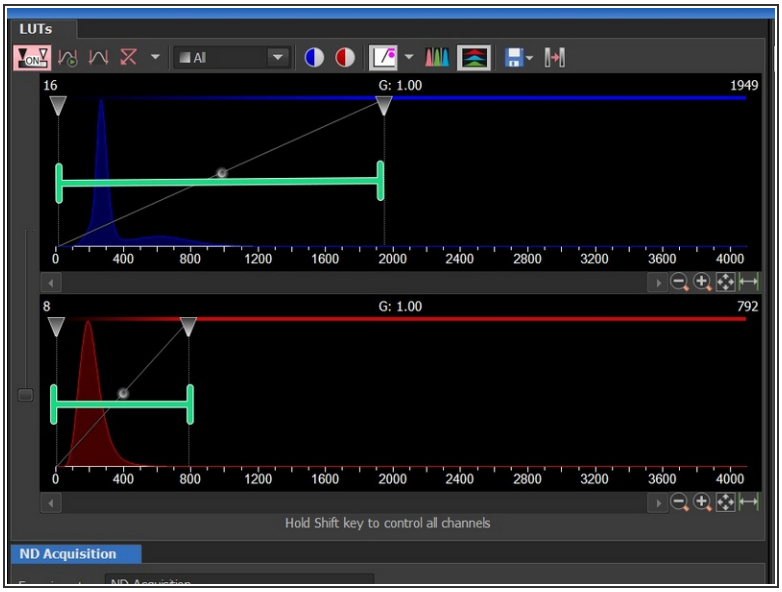




- To acquire Z-stack tick the **Z**. Make sure that **λ** is pre-selected from previous step.
- You can go **Live** and focus through your sample to set/adjust the z-stack boundaries (**"Bottom"** and **"Top"**).
- Click on the value in the box for automatically optimizing the z sampling.
  - ⓘ Depending on your experiment and acquisition needs, you might increase the step to speed up the acquisition or decrease the step if you will deconvolve the data later. Nyquist calculator
- Make sure that **Nikon A1 Piezo Z drive** is selected, as this allow move faster and more precise movement in Z.
- Click on **Run now** in the *ND acquisition* tab and see the progress of the acquisition in new pop-up window
- In this **Symmetric mode**, you can set the Z-stack relative to the current focus position (Home position). You define the range above and below the current focus point (e.g., +4 µm above and -4 µm below).
- In this **Asymmetric mode** you define the range above and below the current focus point (e.g., +8 µm above and -3 µm below).

Step 10 — Viewing





- Click on the **Autoscale** button to enhance the contrast
- You can toggle between the Z-steps
- You can preview the Z-stack also in a **3D viewer** or as **MIP** (=Maximum Intensity Projection)
- If the autoscale button does not help to see the structure of interest (e.g., due to some much brighter debris) you can also adjust the contrast in the *LUTs tab*.