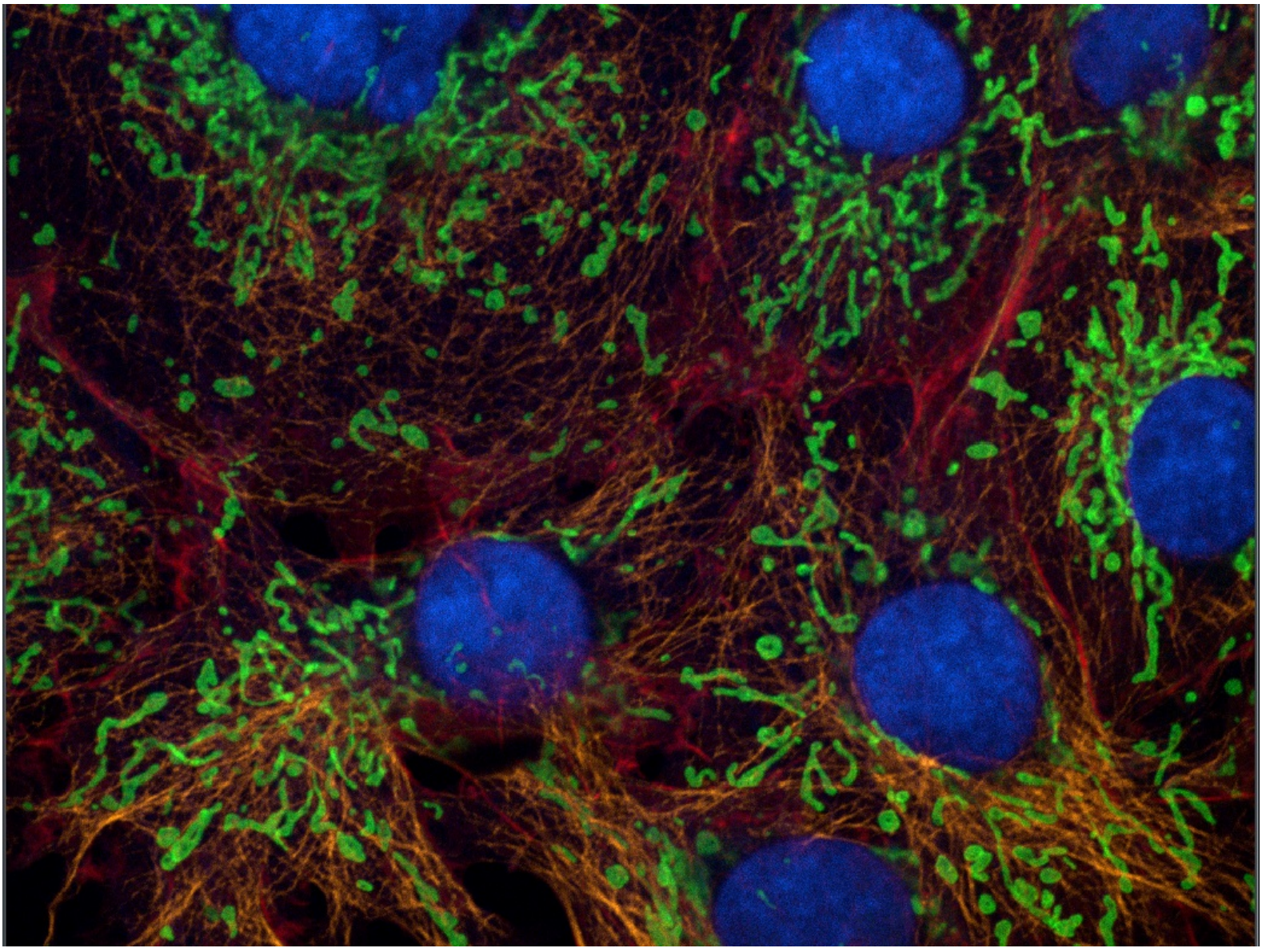
Visitron Spinning Disk - Acquire an Image in XY and Z

Steps for image acquisition on the Visitron Spinning disk microscope from the Center for Microscopy and Image Analysis, University of Zurich.

Written By: Jana Döhner



INTRODUCTION

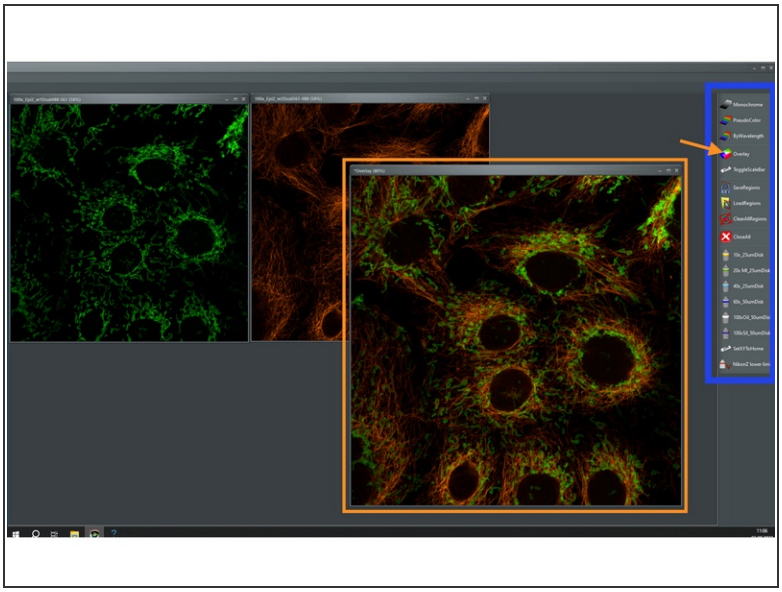
In this guide of the Center for Microscopy and Image Analysis we describe the major steps/aspects required for image acquisition on the Visitron Spinning Disk Microscope.

It introduces you to the "VisiView" software for acquiring a (multichannel) image in 2D as well as 3D. For start-up of the system, mounting and focusing a sample, environmental control, multiposition, xyzt acquisition as well as finishing your session please refer to corresponding guides.

Please find detailed information about the available system here.

Step 1 — VisiView Software Interface

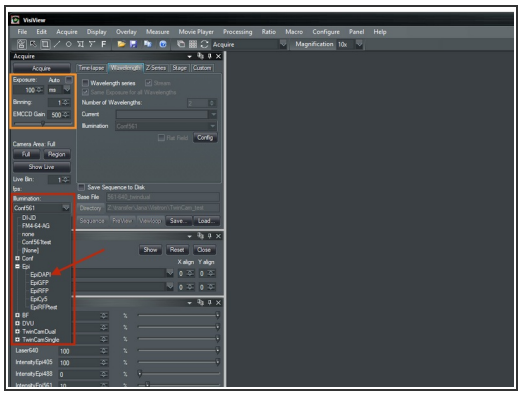
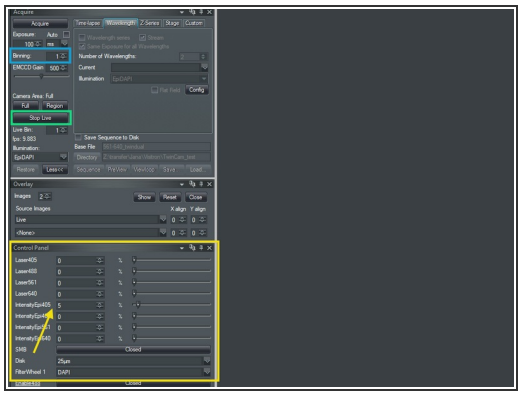
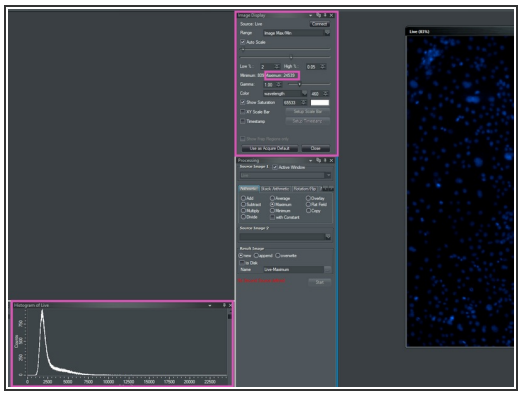




- **Acquire**: control acquisition parameters as illuminations, exposure, gain, binning e.g., adjustments of z-stacks, timelapse or multi area detection
- **Control Panel**: controlling LED and/or laser intensities.
- **Overlay**: set which acquired images should be overlayed for display
- **Histogram**: display of the intensity distribution of the active image
- **Image Display**: option for auto scaling, intensity values, adding annotations to image e.g.
- **Processing**: there are different processing options available within VisiView software
- **Macros**: most used functions and toggling between objectives.

ⓘ *Please note, the interface can slightly differ between users (where each window is located, or if active e.g.). Windows can be activated/opened via the menu bar on top, and can be rearranged via drag and drop.*

Step 2 — Set Imaging parameters

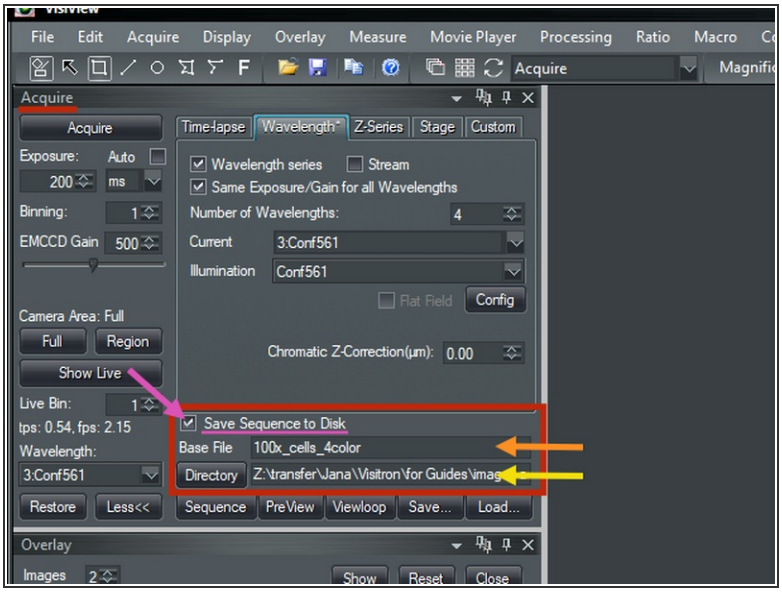
- Have your sample mounted and focused as described in Start-up guide.
- Select a **"Wavelength/Illumination"** (here e.g. EpiDAPI) within the "Acquire" window.
- Adjust Camera **"Exposure"** time and **"EMCCD gain"**.
- Adjust **LED/Laser intensity** within the "Control Panel".
- Click **"Show Live"**.
- Check "Histogram" and "Image Display" to optimize imaging parameters (as exposure, gain and intensity). **Avoid any saturation.**

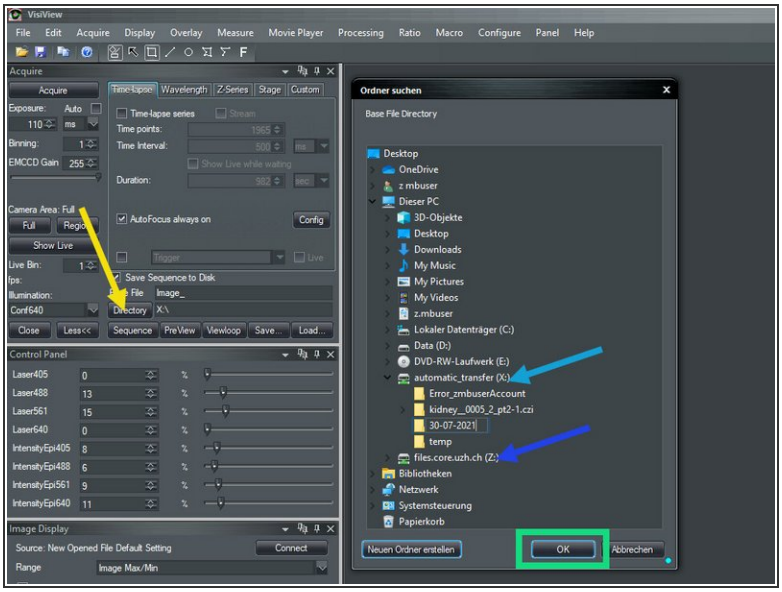
  ⓘ *Camera(s) run in 16 bit mode, so your max should be lower than 65535.*

- **"Binning" and "Live Bin"** - *Binning can be used to increase sensitivity in low-light conditions, read-out times get shortened, but resolution will also decrease.* "Binning" - any adaptions here will be applied to the final image acquisition. "Live Bin" - only applied during live mode.

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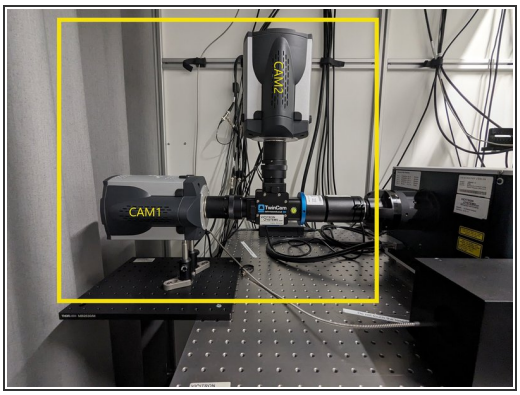
Step 3 — Saving your data

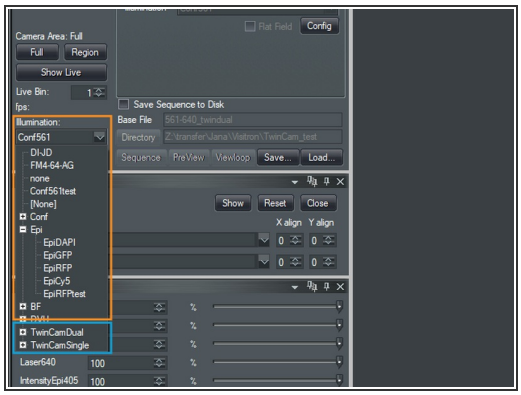


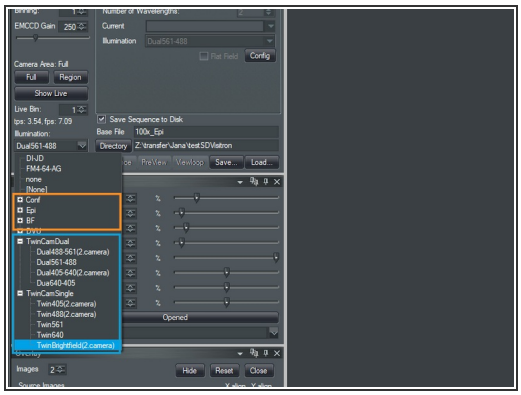


- Depending on which tabs are active you can decide if you wanna save your data automatically or manually. For some options data must be automatically saved.
- Within the "VisiView" software in "Acquire" window define where your data should be saved:
  - Define "Base File" name.
  - Define Storage location via "Directory".
- There are two options to save your data:
  - Choose **"automatic_transfer (X:)"** and generate a new folder. Details can be found in the following guide "Visitron spinning Disk - Datamover".
  - Save directly to network on **"files.core.uzh.ch (Z:)"**. Details can be found here.
- Finally press "Ok".

Step 4 — Multichannel Acquisition - Options







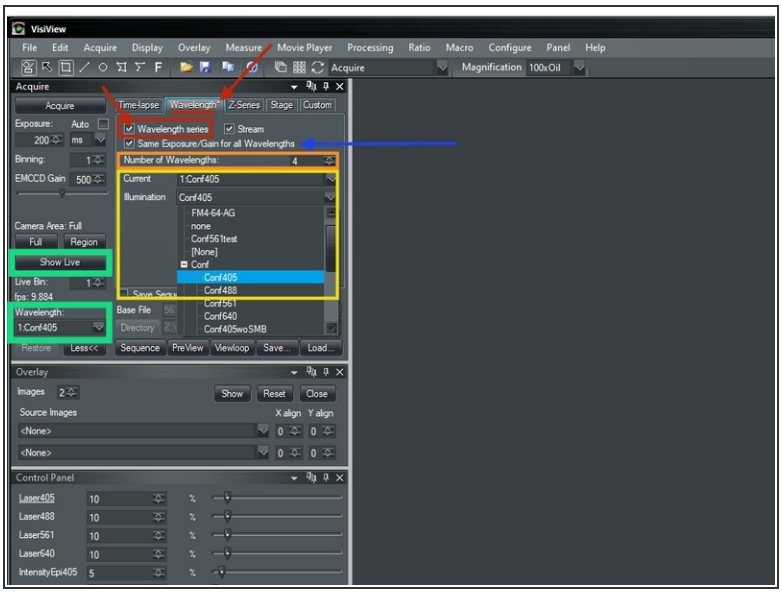
ⓘ *The system is equipped with 2 EMCCD cameras.*

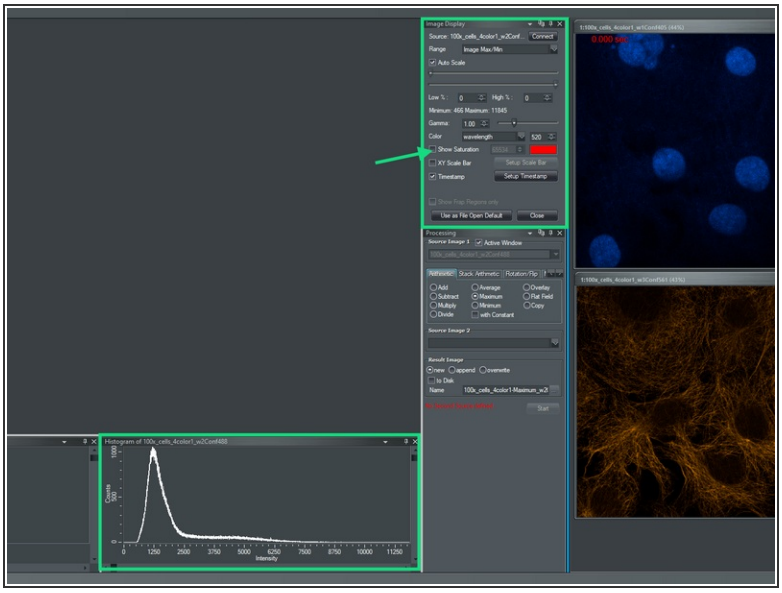
- Imaging can be performed either with one camera (CAM1) or with both (CAM1 and CAM2) using the TwinCam module, depending which wavelength setting is chosen.

ⓘ *The TwinCam module allows for fast and sensitive simultaneous acquisition of two-color (live) samples.*

- Preset illumination selections for one camera approach - Conf~, Epi~, and BF drop down.
- Preset illumination selections for dual camera (TwinCam) approach - TwinCamDual~ and TwinCamSingle drop downs.
- For **one camera approach** please follow the **next steps**.
- For **dual camera approach** using the TwinCam module please refer to the following guide **"Visitron Spinning Disk - Dual camera TwinCam"** (*guide is still in preparation*).

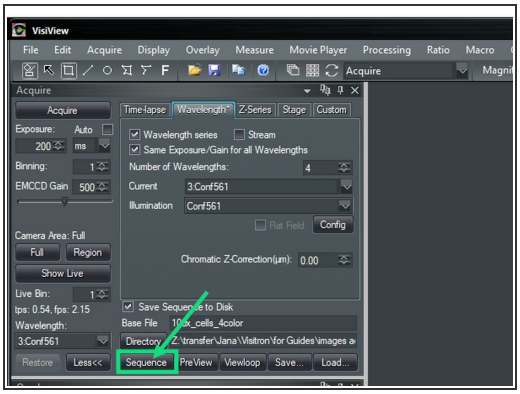
Step 5 — Multichannel acquisition

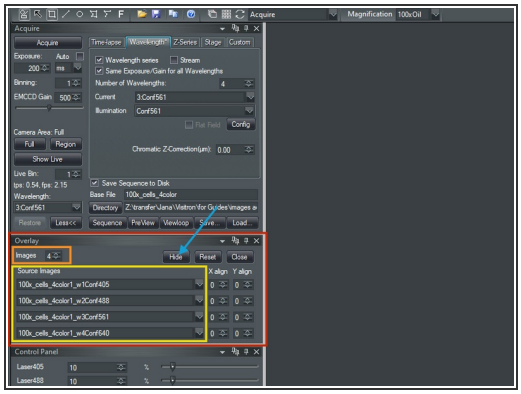


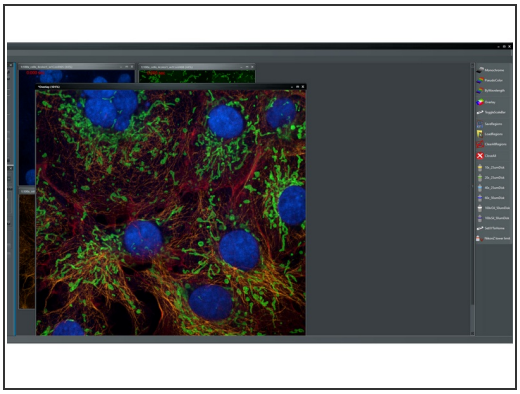


- Within the "Acquire" window select "Wavelength" tab and activate **"Wavelength Series"**. The **"*"** represents if this series is activated.
- Define how many channels you would like to image (here 4 e.g.).
- For each channel ("Current") select an **"Illumination" wavelength**.
- **Adjust** your **imaging settings** (exposure, gain, LED/laser power) for each channel by selecting it first under "Wavelength" and then click **"Show Live".**
  - Saturation can be displayed in any wished Pseudocolor by checking the box "Show Saturation".
- Once satisfied with your settings for each channel click "Stop Live".
  - You can define if "Exposure/Gain" should be same for each wavelength by marking "Same Exposure/Gain ..." if applicable. Further re-adjustments might be required.

Step 6 — Multichannel acquisition - continued

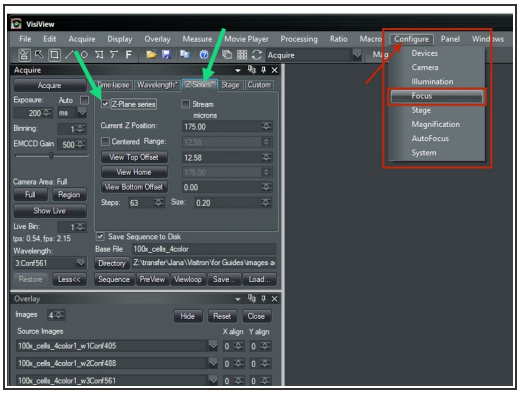


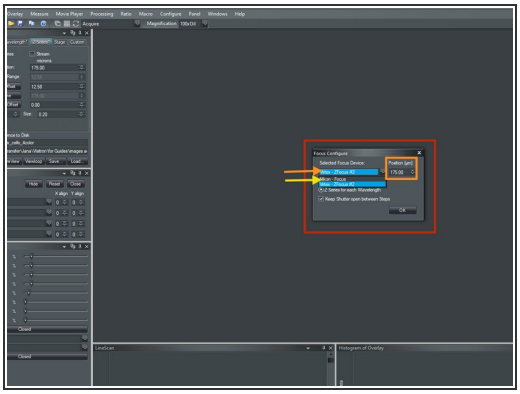
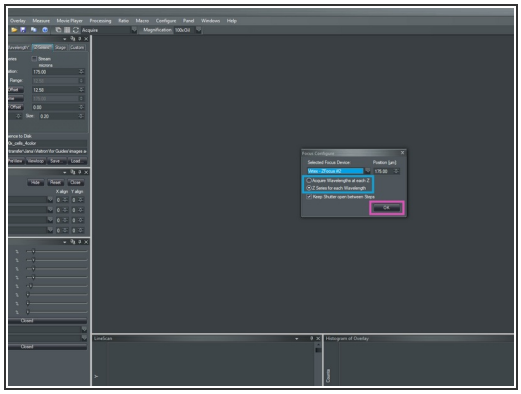




- Once you are satisfied with your settings, click on "Sequence" to **start acquisition**.
- Depending whether you selected "Save Sequence to Disk", the data will be already saved or not.
- To observe an **overlay** of the acquired channels:
  - Select how many channels you wanna overlay.
  - Select which ones in the drop down menus.
  - Click "Show".
    - *Here it shows "Hide" instead of "show", as the overlay is already open.*

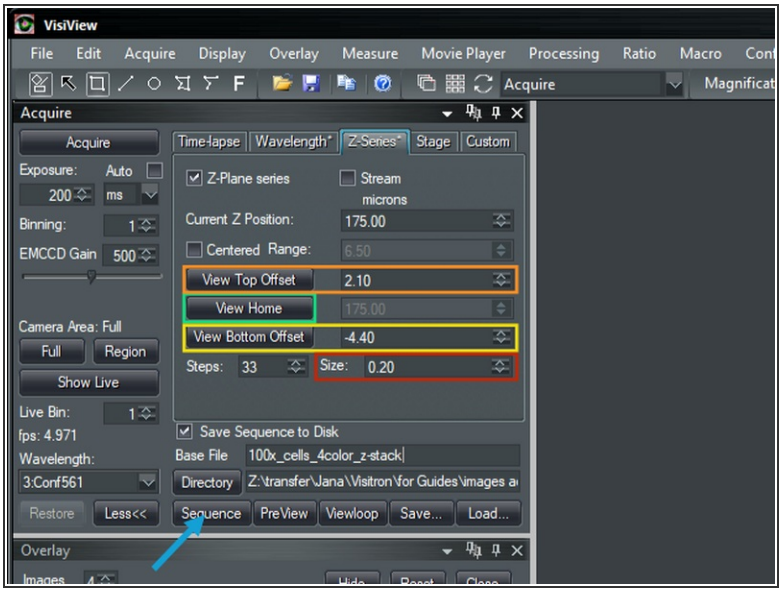
Step 7 — Define a Z-stack

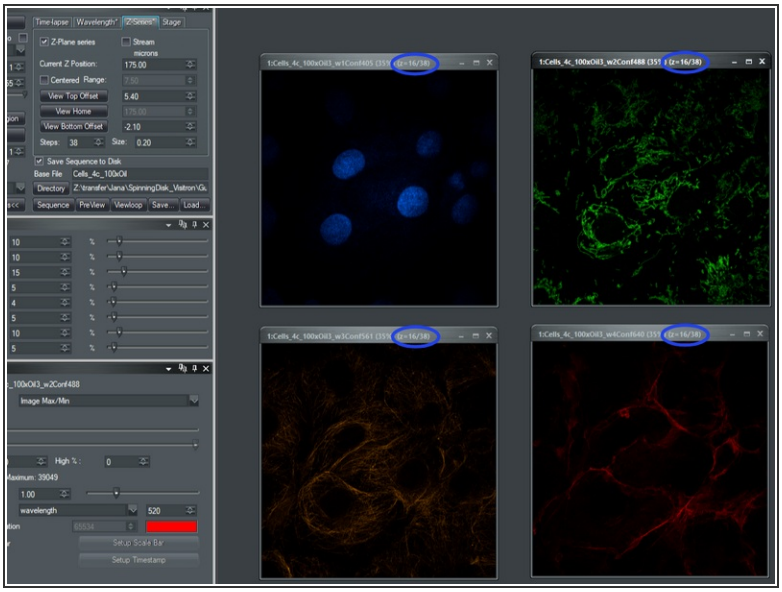


- Select **"Z-Series" tab** and activate "Z-Plane series".
- To configure which device be used go to "Configuration" and select "Focus".
- Within the opened window "Focus Configure" select your "Focus Device" from the drop down menu.
  - **Virtex - ZFocus#2**: Fast Piezo stage - the travel range of this device is 350um. Make sure the Piezo is in the middle of the travel range (175) before you start your experiment.
  - **Nikon - Focus** - full travel range of the stage, so used for thicker samples (more than 350 um).
  - Select if you wanna "Acquire Wavelengths at each Z" or acquire "Z Series for each Wavelength".
- Click "Ok".

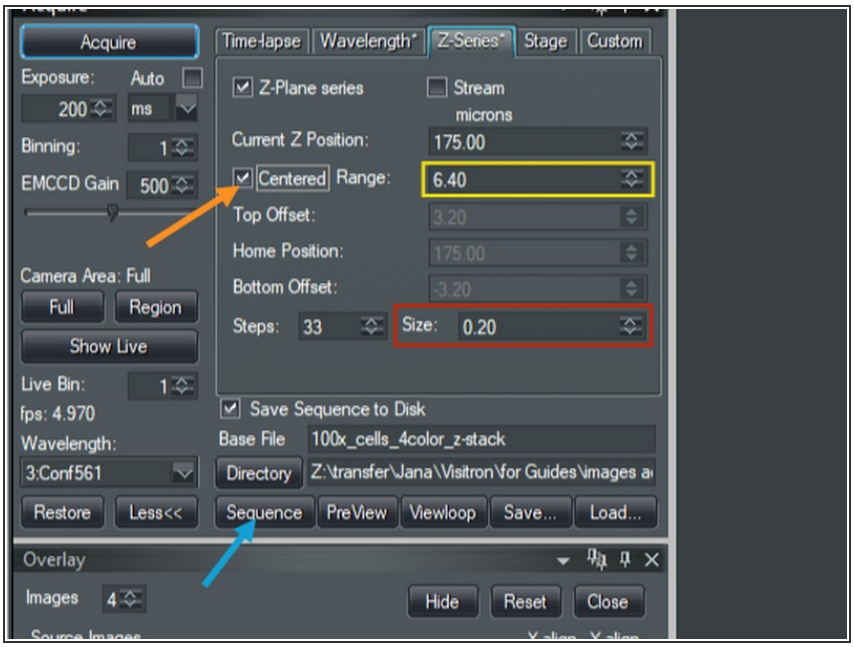
Step 8 — Define a Z-Stack - Top and bottom





- Click **"View Home"** and focus.
- Define now your top and bottom limits of your sample:
  - Click **"View Top Offset"** and adjust the value that you reach the top of your sample.
  - Click **"View Bottom Offset"** and adjust the value accordingly for the other side of your sample.
  - Stop Live View when satisfied.
- Define your **step "Size"** (unit = um). Z-stack direction can be alternated by adding a "-" in front of your defined Step-Size.
- Click **"Sequence"** to acquire a z-stack.
- One can toggle through the different z-layers using the keyboard arrow keys up/down.

Step 9 — Define a Z-Stack - Centered Range



- Alternatively you can also choose to define a **centered range.**
  - Check "Centered Range".
  - Define your volume size.
  - Make sure that your current focus is in the middle of your sample.
- Define your **step "Size"** (unit = um). Z-stack direction can be alternated by adding a "-" in front of your defined Step-Size.
- Click **"Sequence"** to acquire a z-stack.