Olympus ScanR HCS (Irchel) - Part2: Image Acquisition

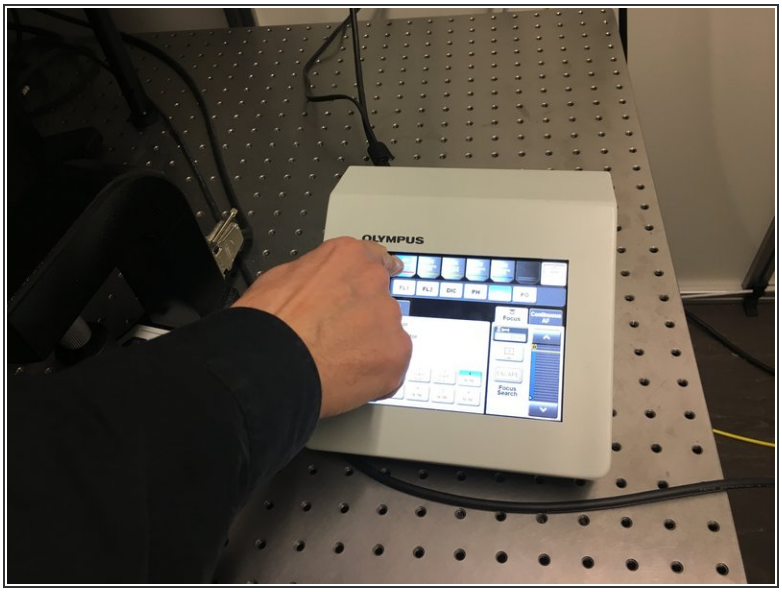
How to set up an experiment and acquire images with the Widefield - Olympus ScanR HCS (Irchel) located at the ZMB, UZH, Room Y24-F-14.

Written By: Joana Raquel Delgado Martins



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Step 1 — Place 4x objective at the lowest position

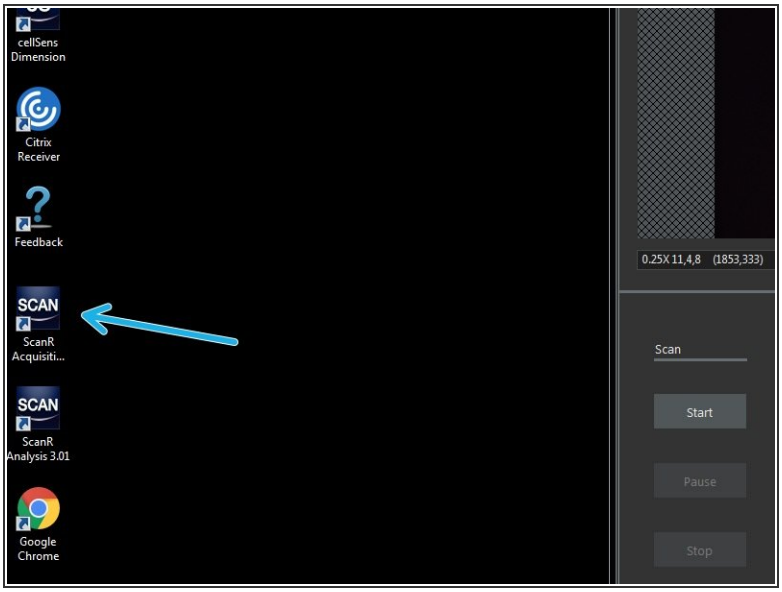




⚠ Make sure the 4x objective is selected and in the lowest position. Otherwise:

- Choose the 4x objective using the microscope touch panel.
- Move the objective to the lowest position.

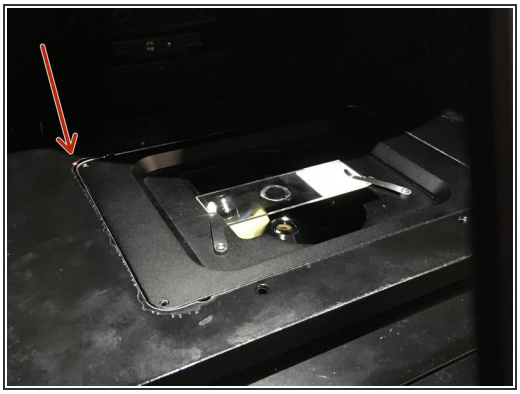
Step 2 — Initialize software

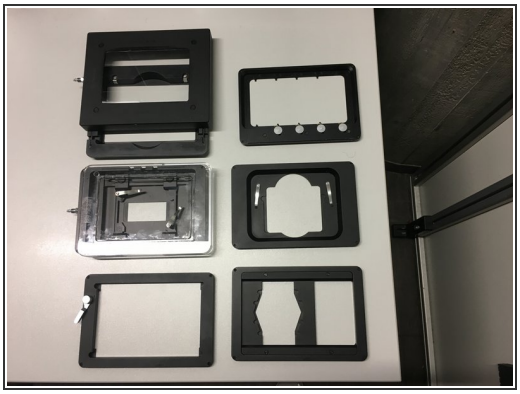




- Start ScanR Acquisition software

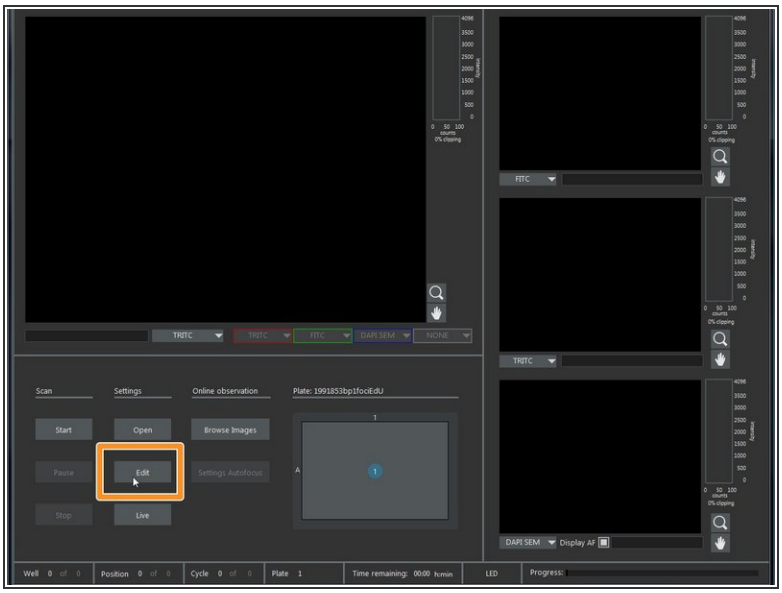
Step 3 — Place your sample

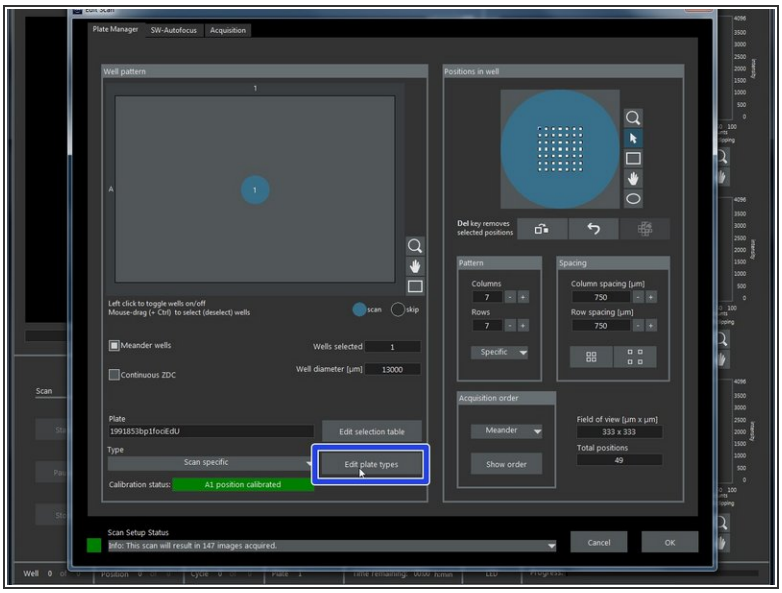




- Choose and place the appropriate holder.
- Several holders are available in the different drawers.
- Mount your sample.
- In an inverted microscope the coverslip should face the objective.

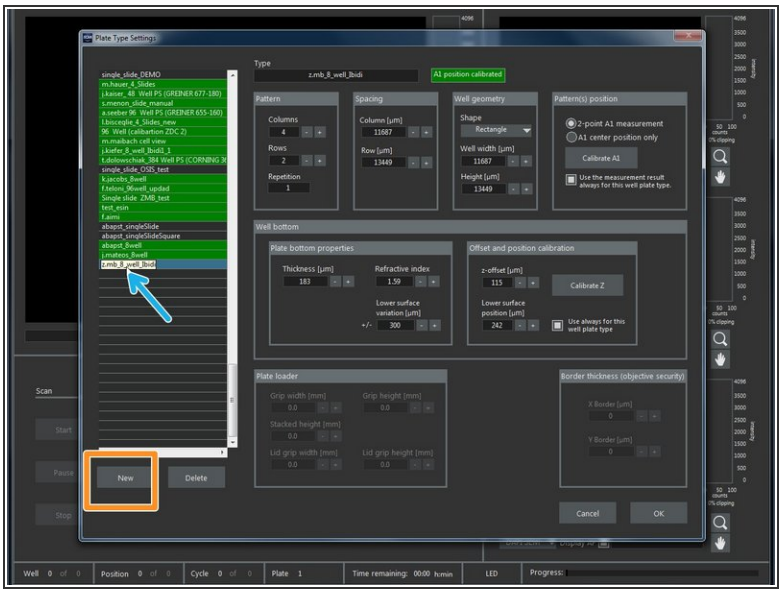
Step 4 — Define your plate/slide

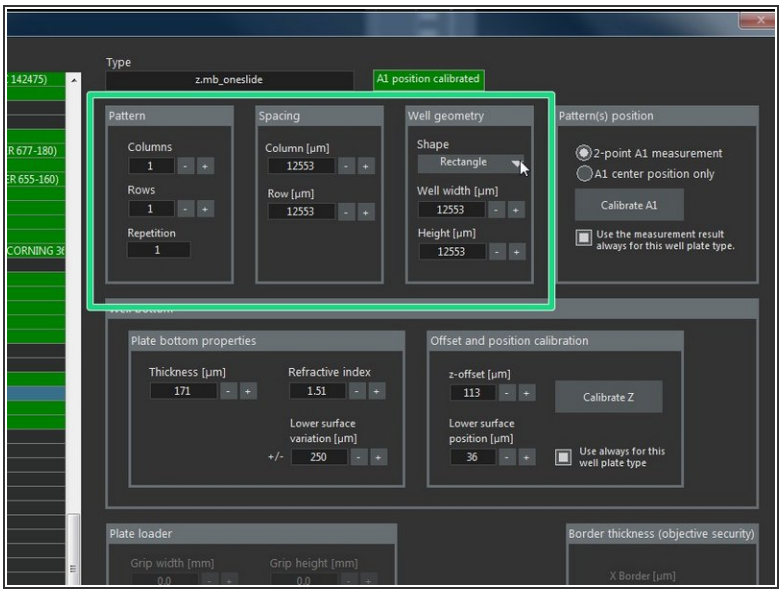




- Select "Edit" to define your plate/slide
- On the next pop up window select "Edit plate types".

Step 5 — Plate Type Settings

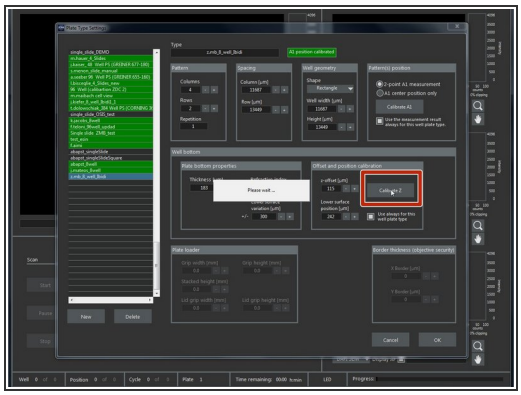


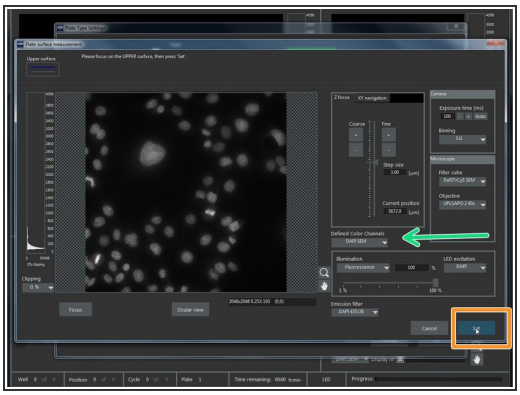


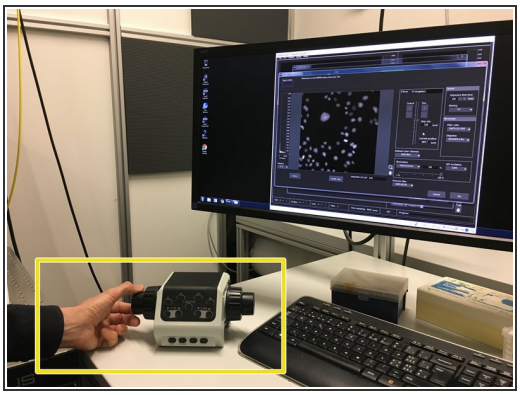
ⓘ As a starting point you can use an existing template and adapt it according to your needs.

- Chose a profile from the list that you would like to adapt, eg "z.mb_oneslide".
- Create and rename your own profile by pressing "New".
- Here you can define the Pattern / Spacing and Geometry of your Wells.

Step 6 — Calibrate Z



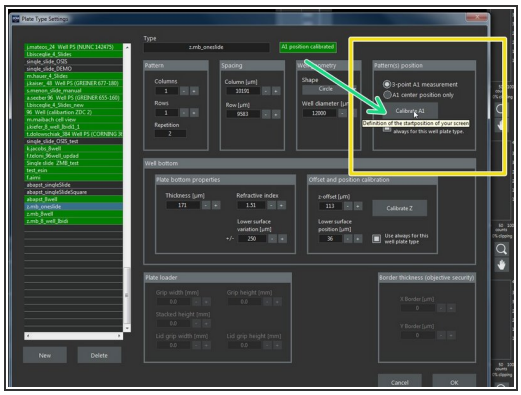


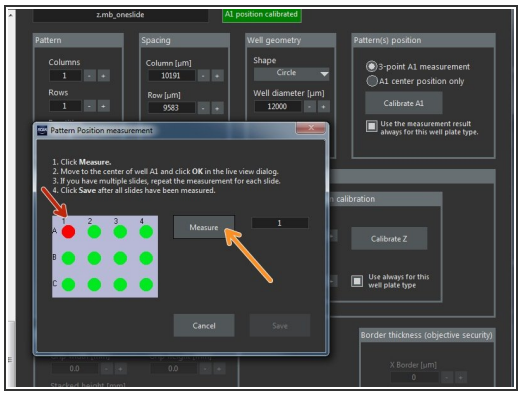


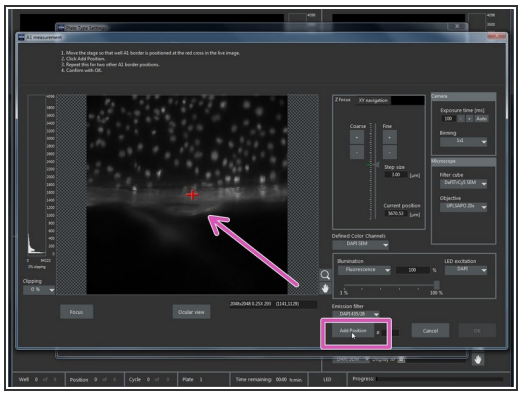
- Press "Calibrate Z" to go to Live View.
- Here you can change the "Defined Color Channels" (eg DAPI, etc).
- Focus your sample using the microscope wheels and press "Set".
- Press "Set" once you are satisfied.

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Step 7 — Pattern(s) position



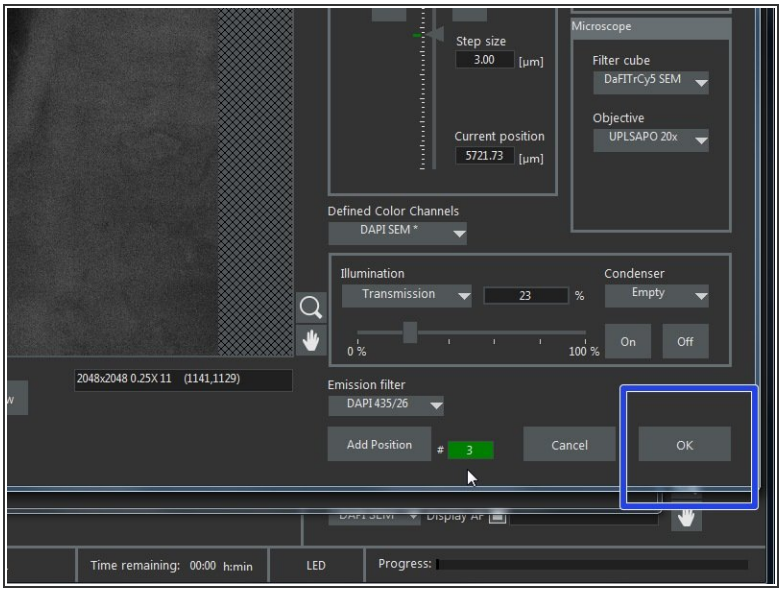


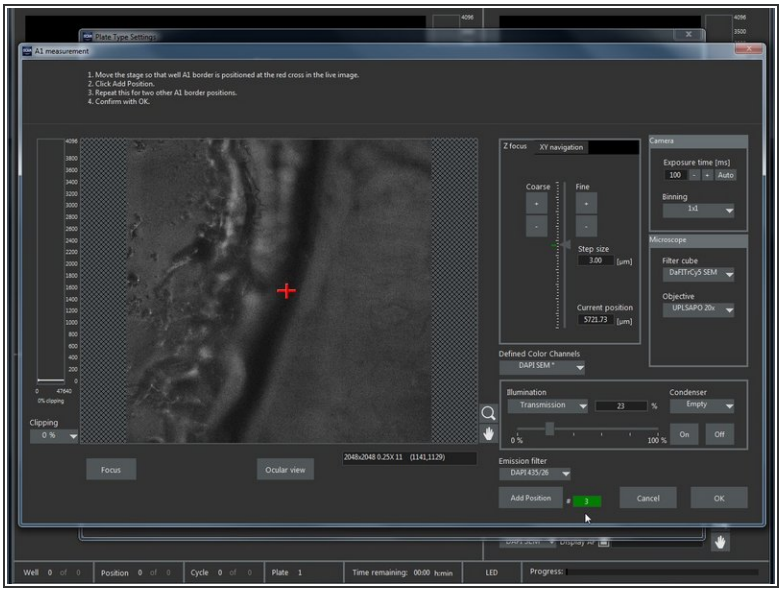


- Calibrate positions ("Plate type settings" window) using:
  - "3-point A1 measurement" - triangulation method
  - "A1 center position" - only necessary to define the center of the coverslip/well
  - Press calibrate A1
    - On the "Pattern Position measurement" window select which slide you want to measure.
    - Click "Measure" for Live view .
    - Move the stage so that the red cross is either at the center or at the edge of the slide, according to the chosen calibration strategy. Click "Add position".

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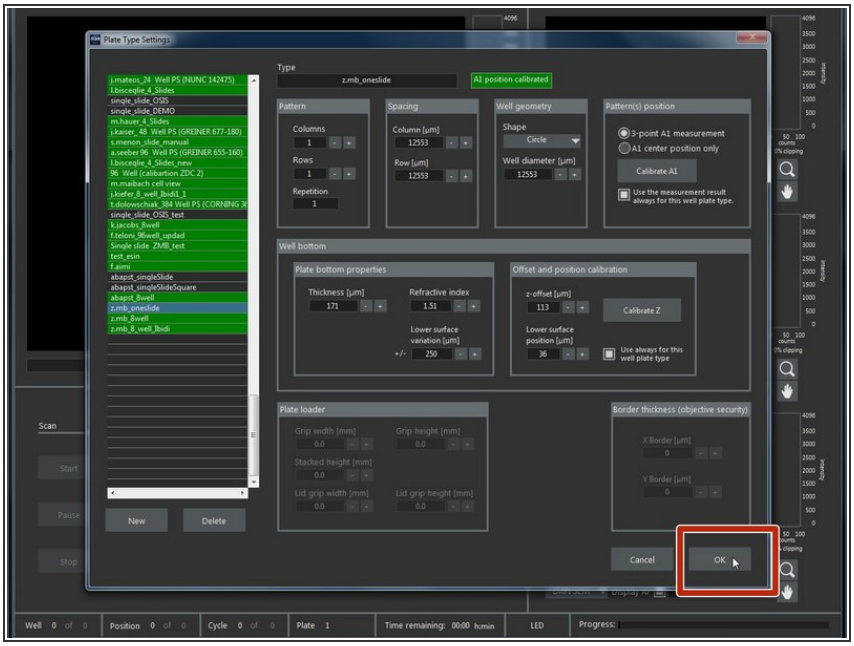
Step 8 — Pattern(s) position





- Repeat for the 2 other borders of A1 (if 3-point A1 measurement was chosen).
- Confirm with "OK" ("A1 measurement" window).

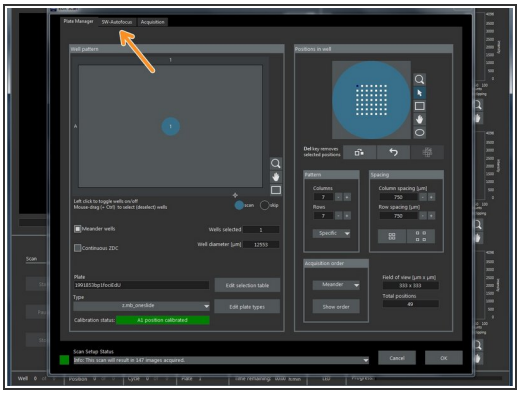
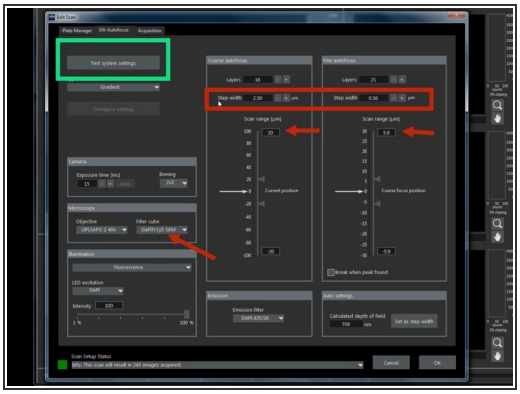
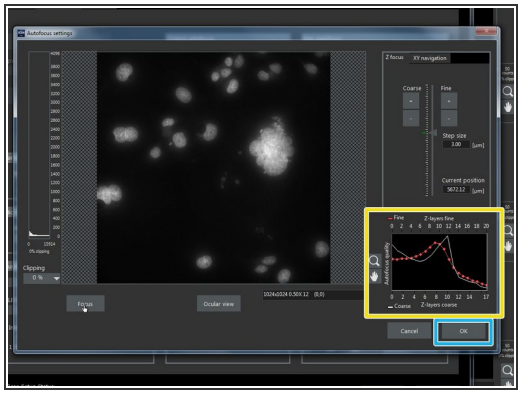
Step 9 — Plate Type Settings



- Once you are satisfied with your plate settings confirm with "OK".

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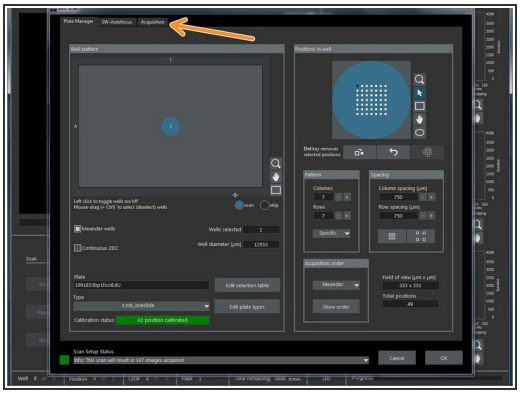
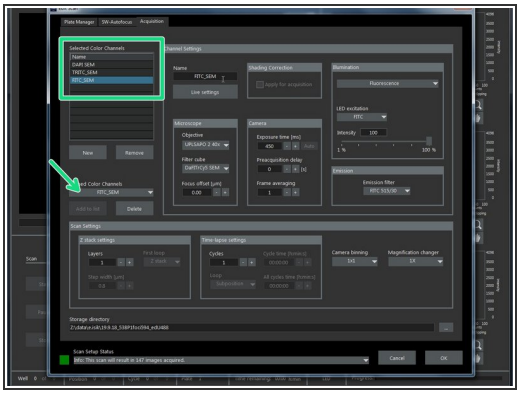
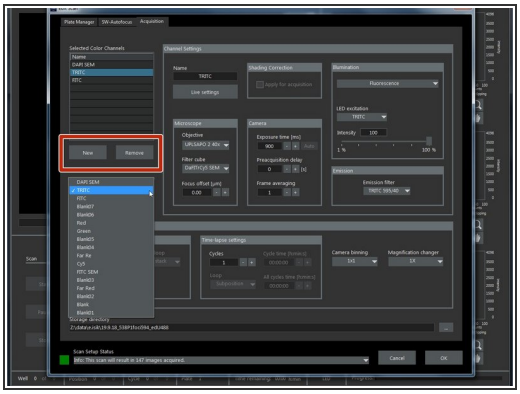
Step 10 — SW-Autofocus

- Back in the "Edit Scan" window go to the "SW-Autofocus" tab.
- Click "Test system settings".
- If the focus is not ideal adjust the step size and/or range accordingly.
- SW-Autofocus settings can be optimized with the help of the autofocus quality graph.
- Confirm your settings by clicking on "OK".

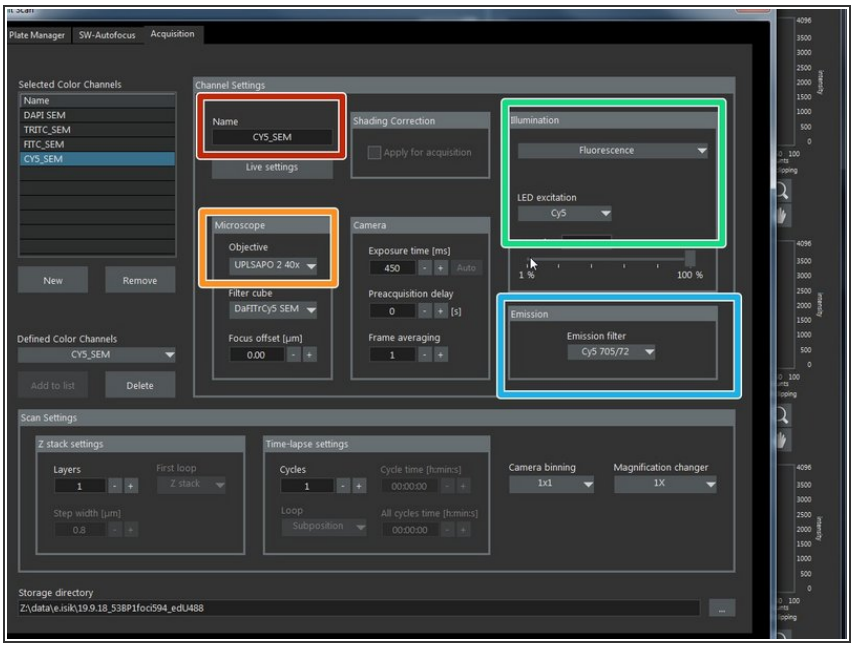
⚠ Please make sure you are using the correct filter cube. Avoid the Cy7 cube, as it blocks the laser autofocus. Use "DaFiTrCy5SEMold" instead.

Step 11 — Define Acquisition

- Back in the "Edit Scan" window go to the "Acquisition" tab.
- Here you can chose your channels for acquisition. (eg DAPI SEM, FITC SEM).
- You can also create or remove channels accordingly.

Step 12 — Defining Acquisition - Fluorescence

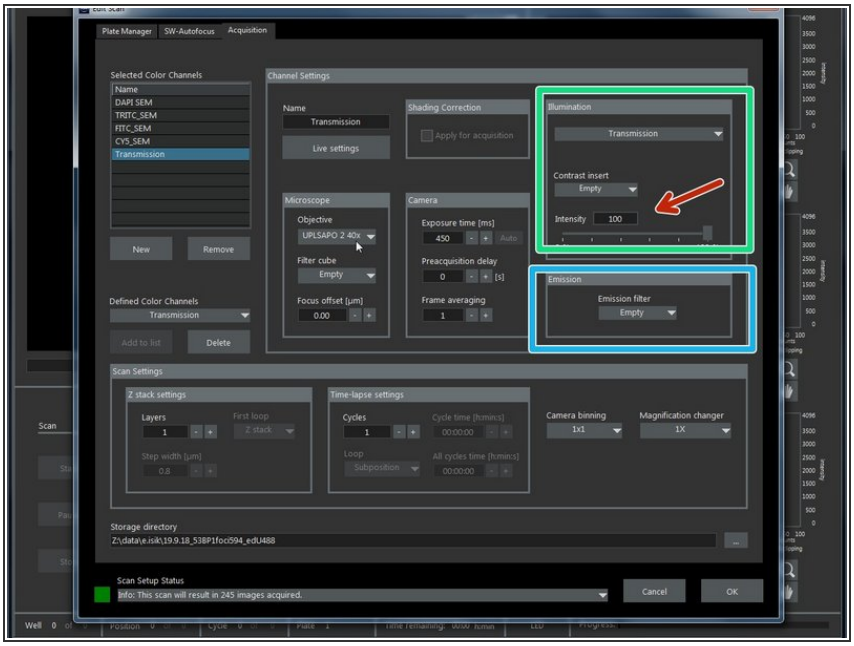


- When using an existing or defining a new channel please confirm:
  - (Re)name here.
  - Objective of choice.
  - Correct ilumination type and LED excitation (eg Fluorescence and Cy5).
  - Correct emission filters (eg. Cy5 705/72).

⚠ Please make sure you are using the correct filter cube. Avoid the Cy7 cube, as it blocks the laser autofocus. Use "DaFiTrCy5SEMold" instead.

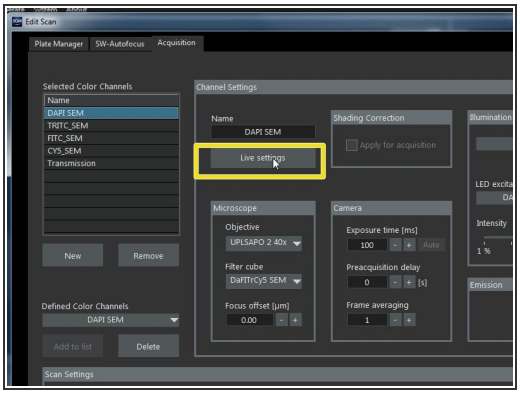
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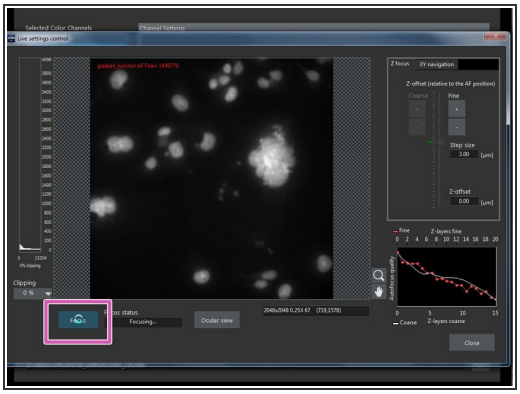
Step 13 — Defining Acquisition - Transmission

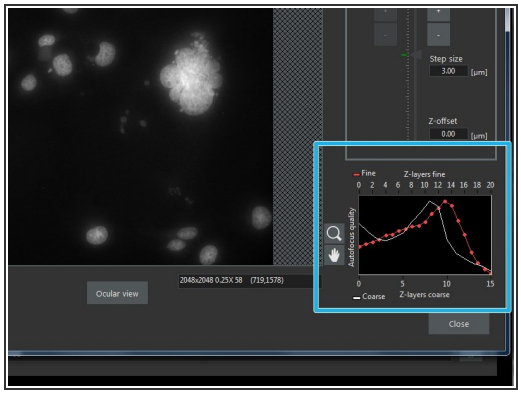


- For transmission
  - In "Illumination" chose "Transmission".
  - In "Contrast insert" choose "Empty".
  - In "Emission" choose "Empty" Emission filter.
  - ⓘ Decreasing the "Intensity" to 20 should avoid saturating the image.

Step 14 — Live view



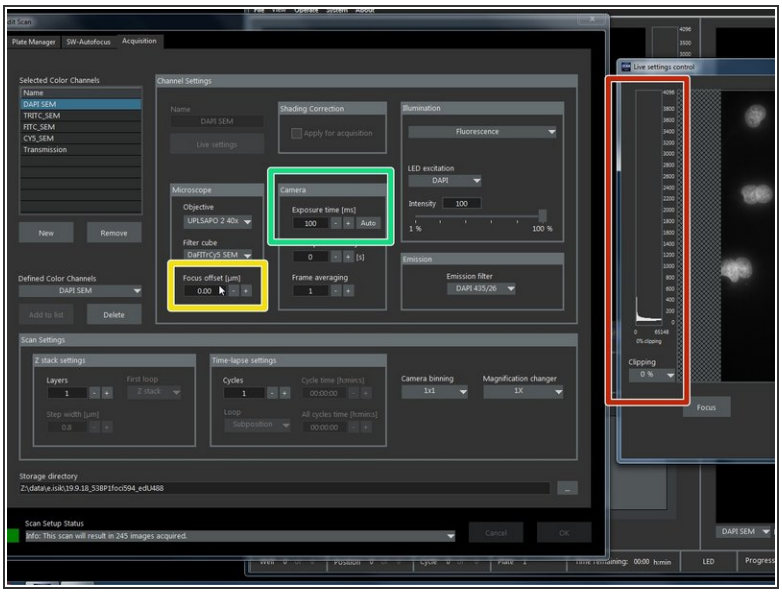




- Press"Live settings" to check settings on Live view.
- Press "Focus". The AF settings will now be applied.
- SW-autofocus settings can be optimized with the help of the autofocus quality graph.

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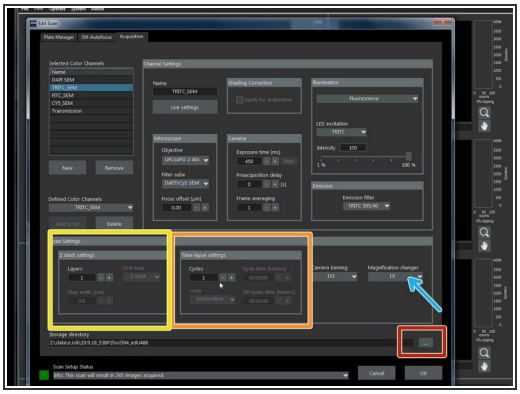
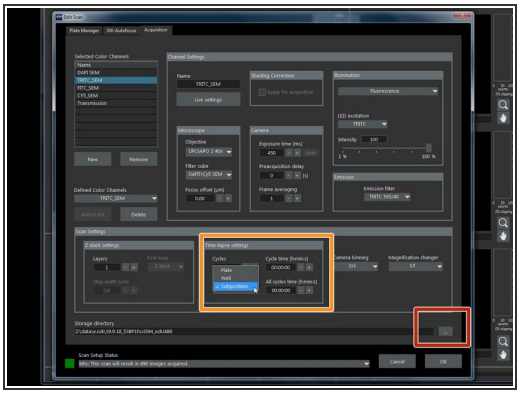
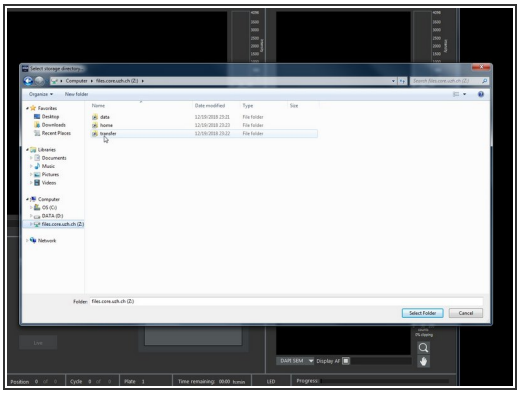
Step 15 — Live settings





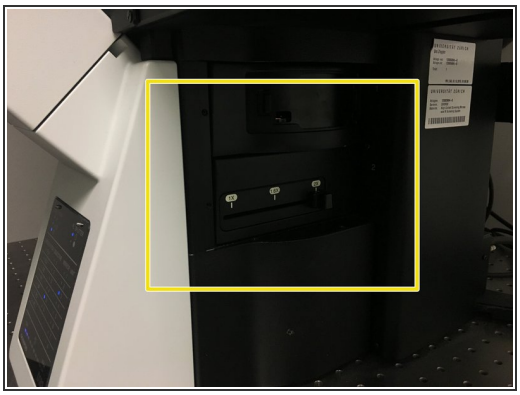
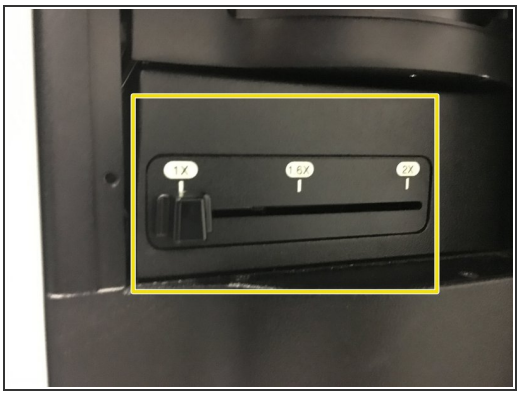
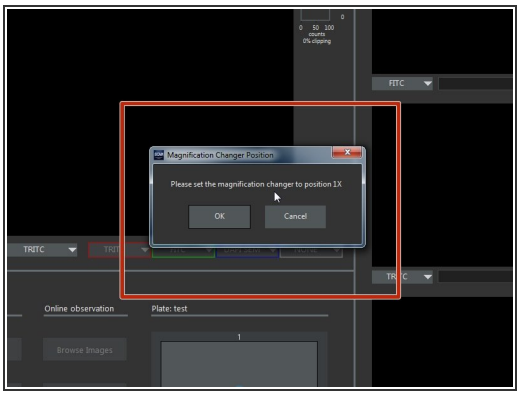
- Move the Live settings window to the side so you can change between the different channels and adjust them accordingly.
- Adjust exposure time for each channel.
- Make sure your image is not saturated - clipping should be 0%.
- Check that "Focus offset" for each channel is on "0" .

Step 16 — Acquisition - Scan settings

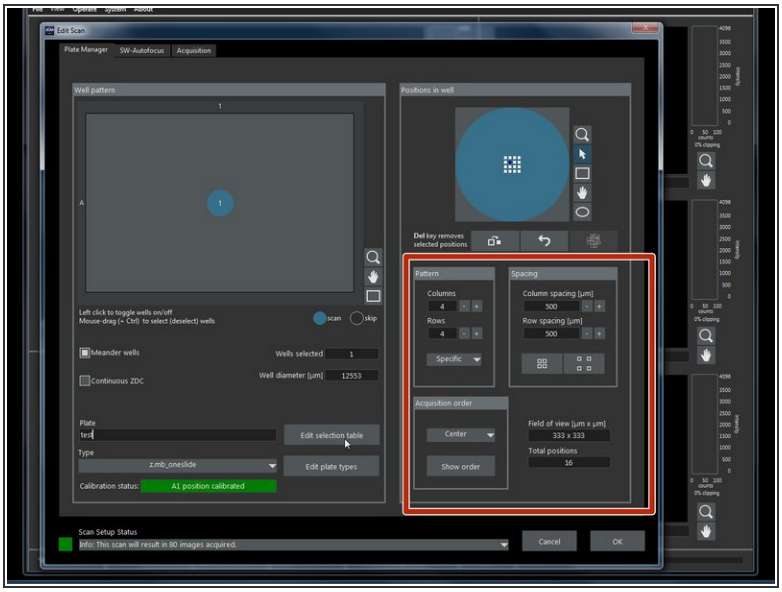
- Here you can define a time lapse.
- Here you can define z-stacks.
- Here you can define the magnification changer.
- Define your destination folder here.

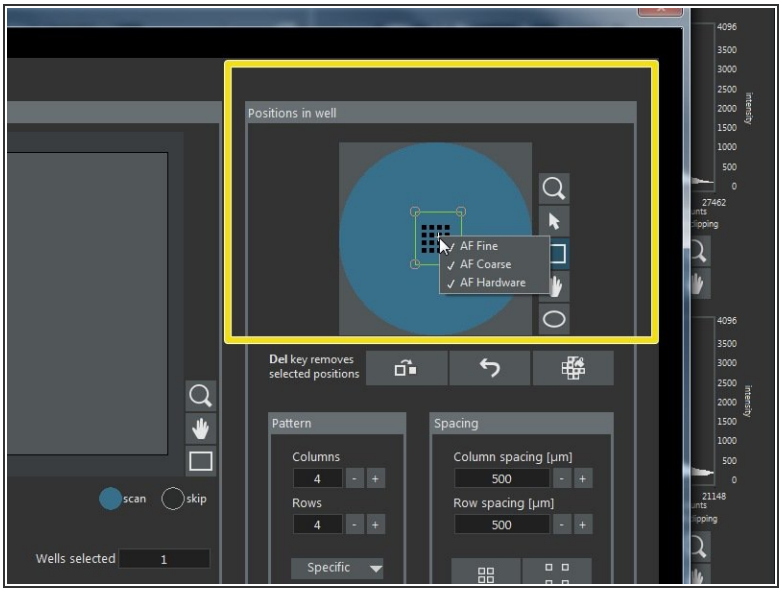
Step 17 — Magnification changer

- Make sure that the magnification changer on the right side of the microscope is correctly set. It should match the software settings.
- Otherwise you will receive a message asking you to move it into the correct position.

Step 18 — Define positions within well



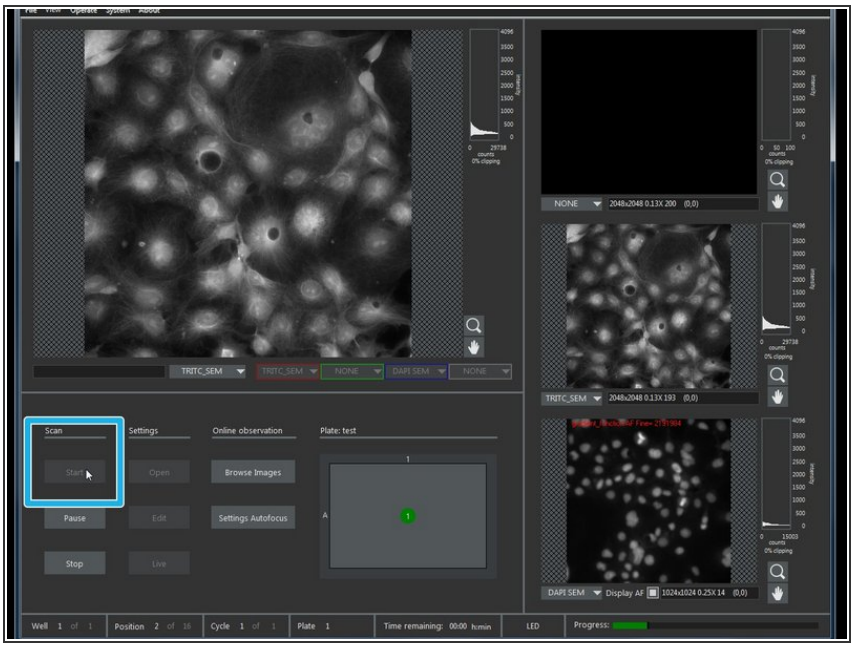


- Choose your imaging pattern here.
- Finally choose the autofocus positions/pattern you would like to apply to each well.
- Right click over defined positions.

ⓘ To optimize and speed up acquisition we recommend that you include one position per well with the 3 options "AF Coarse", "AF Fine" and "AF Hardware".

- For the remaining positions "AF Fine" might be sufficient, but this needs to be adjusted depending on the sample.
- Increase the number of positions with "AF Coarse" and "AF Hardware" if necessary.

Step 19 — Start scan



- Start your scan.