

GE InCell Analyzer 2500 HS - 1: Start up and perform a screen

In this guide you will learn the basics about how to operate the GE InCell Analyzer 2500 HS microscope.

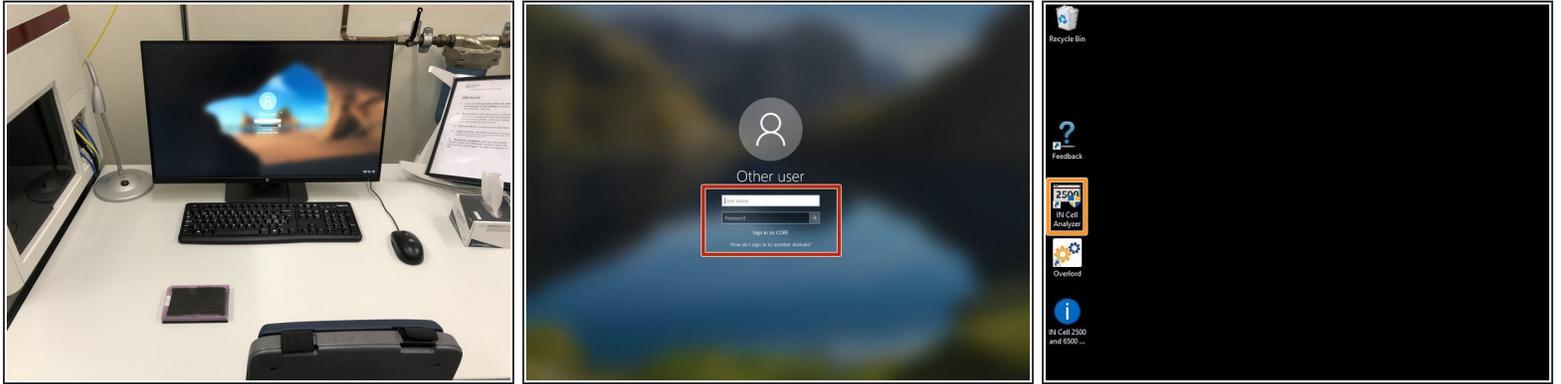
Written By: Dominik Hänni



INTRODUCTION

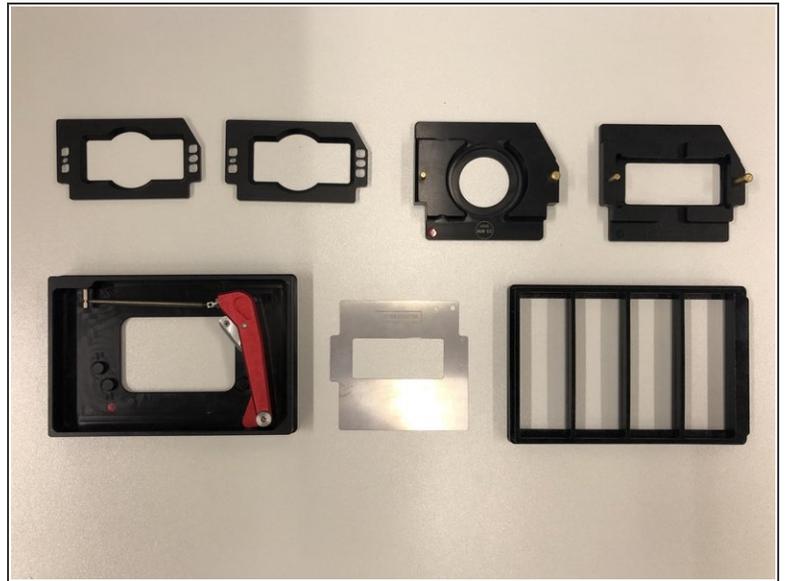
In this guide of the Center for Microscopy and Image Analysis you will learn how to set-up and perform a simple screen on the GE InCell Analyzer 2500 HS widefield fluorescence microscope.

Step 1 — Log-in and start the software



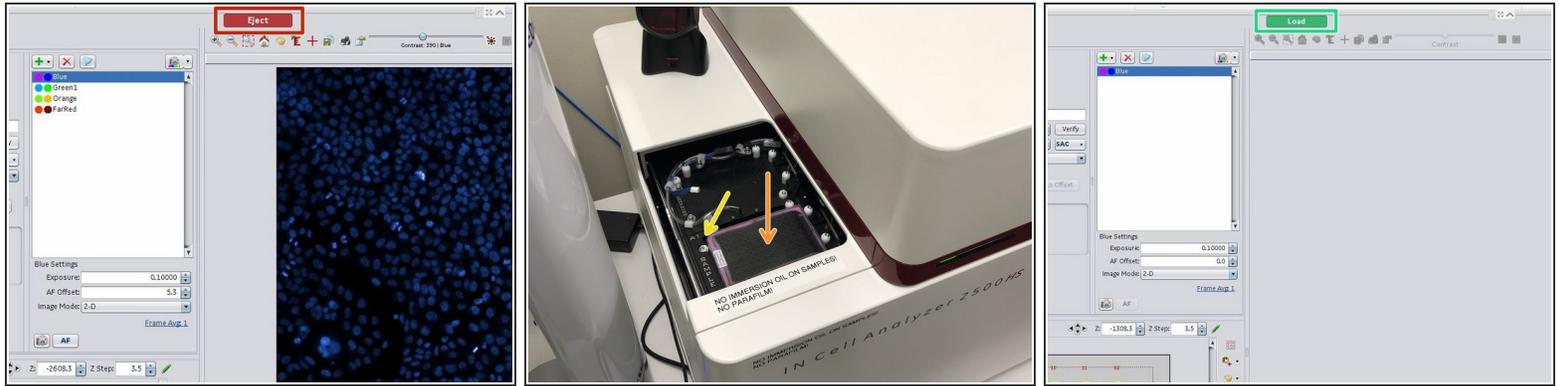
- i** The InCell microscope and the control computer are always running. Therefore, there is no need to power-on and to power off this microscope or the computer.
- Log-in with your ZMB credentials.
 - Start the "IN Cell Analyzer" software via the desktop icon.

Step 2 — Mounting samples



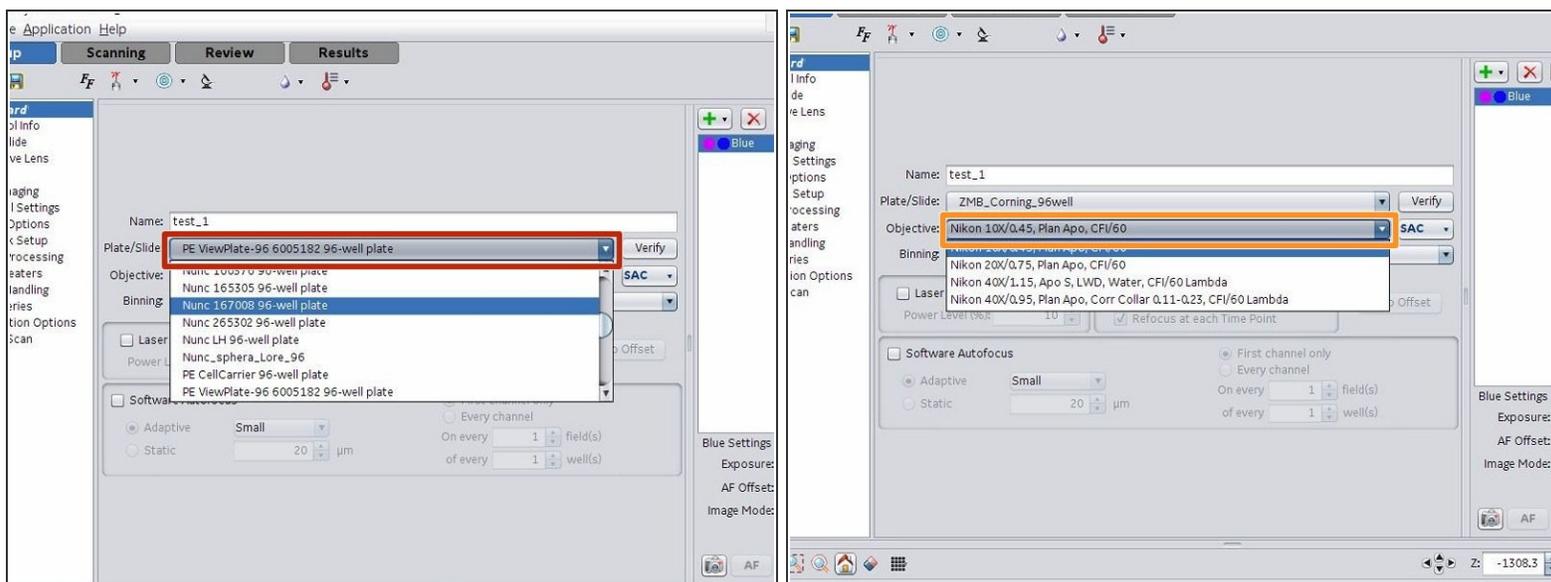
- i** The standard sample format for the InCell microscope is a 96 well plate. However there are also holders and adapters for slides, slide sized live cell chambers as well as petri dishes.
- In the cabinet close to the entrance door you can find the accessories including different sample holders.
 - Either use a well plate or choose a suitable sample holder.

Step 3 — Load a sample



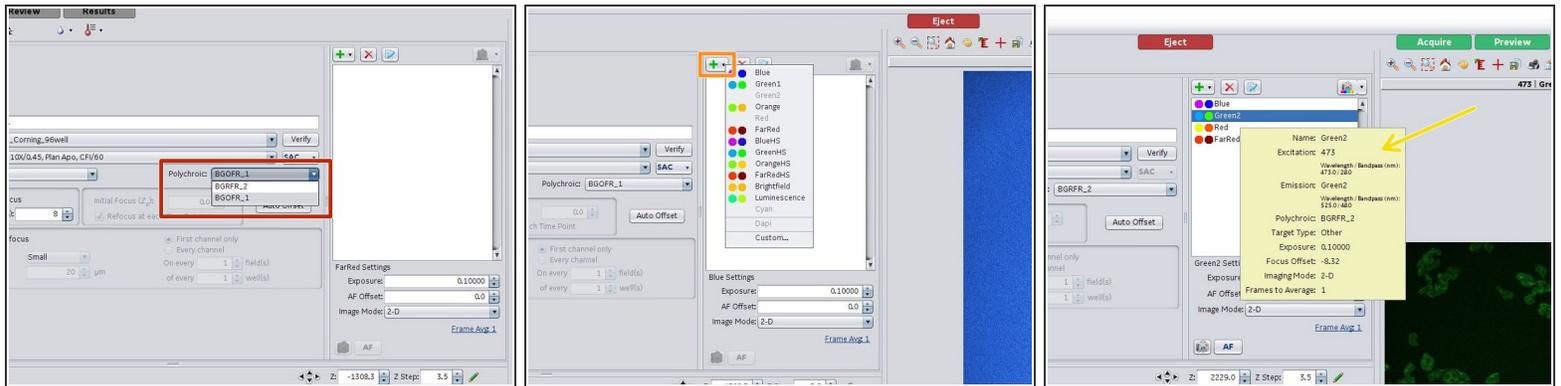
- In the software click on "Eject" to open the microscope door.
- Insert your plate or sample holder.
- Make sure that the orientation is correct (A1 should be on the top left corner).
- ⓘ Remove the lid of your sample if you are imaging in Transmission/DIC/Phase contrast mode.
- In the software click on "Load".

Step 4 — Select a plate/slide template and an objective



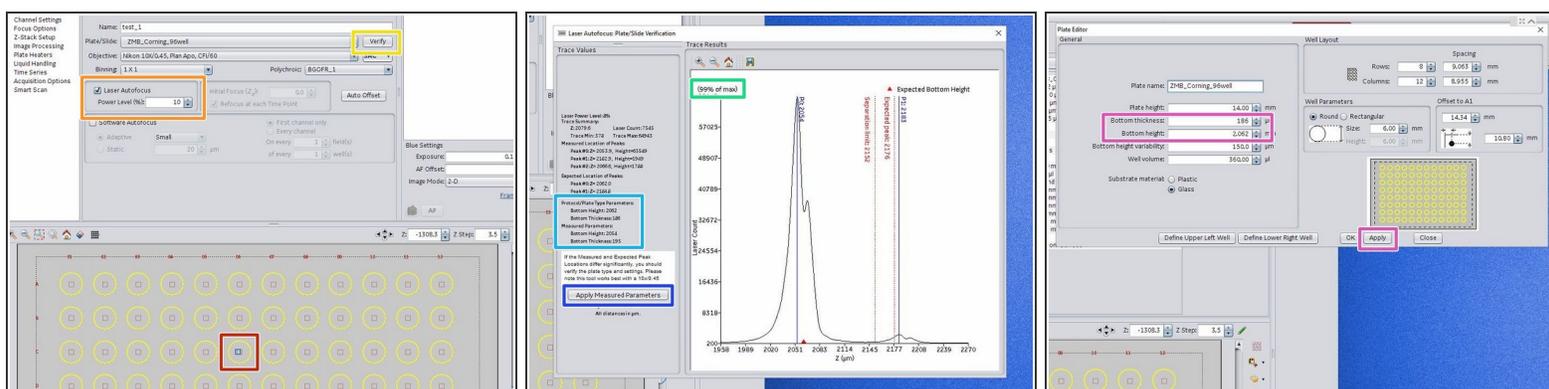
- Select a plate/slide template.
 - Select an objective.
- i** The InCell microscope is optimized for imaging regular arranged specimens such as well-plates. When producing/imaging slides we recommend to precisely mount them using the available ZMB templates. For such arranged slides we also have suitable slide definitions on the microscope.
- i** Adjusting or generating plate/slide templates and using the water immersion objective is explained in separate guides.

Step 5 — Choose a polychroic and define the imaging channels



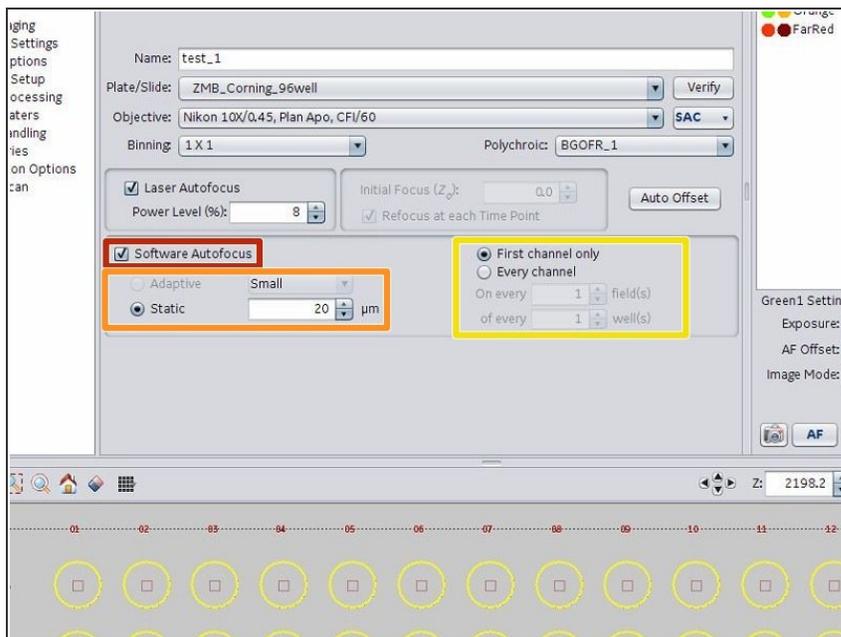
- Choose a suitable polychroic:
 - BGOFR_1: Blue (390/18), Green (475/28), **Orange (524/27)**, Far Red (632/22)
 - BGOFR_2: Blue (390/18), Green (475/28), **Red (575/25)**, Far Red (632/22)
- Add the wanted channels by using the "+" icon.
- Hoovering with the mouse over a channel opens a context information box with additional information.

Step 6 — Laser auto focus



- To move the stage, click in the center of a well or cover slip.
 - Activate the "Laser Autofocus". The needed power level depends on the objective as well as the sample.
 - Check the laser auto focus trace by clicking "Verify":
 - If the signal is saturated, reduce the auto focus laser power.
 - If the "Measured Parameters" deviate more than 10% of the "Protocol/Plate Parameters" adjust the plate parameters.
 - Click "Apply Measured Parameters" for doing that.
 - Click "Apply" in the "Plate Editor" for adjusting the plate parameters to the measured values.
- ⚠ If you execute "Verify" without being within a well/slide, it can happen that the objective presses the sample up. Since the plate is fixed within the instrument, you end up with a tilted plate and focusing is not possible anymore. To resolve such issues eject and reload your plate.

Step 7 — Optional: Software auto focus

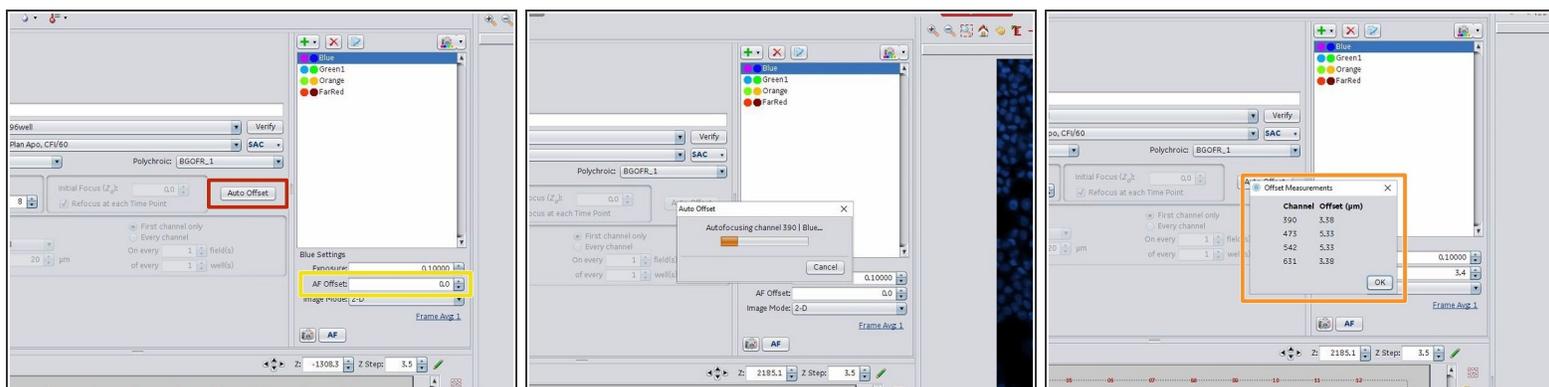


i Under most conditions the laser auto focus system of the InCell microscope is very fast and reliable. Therefore, an additional, much slower software auto focus step is usually not required.

- Check the "Software Autofocus" checkmark to engage the software autofocus.
- Specify a z axis search range.
- Define the channel as well as the frequency for performing a software auto focus.

i The InCell microscope uses the integrated image intensity as the optimization parameter for the software auto focus system. This means the software autofocus will focus on the brightest z plane.

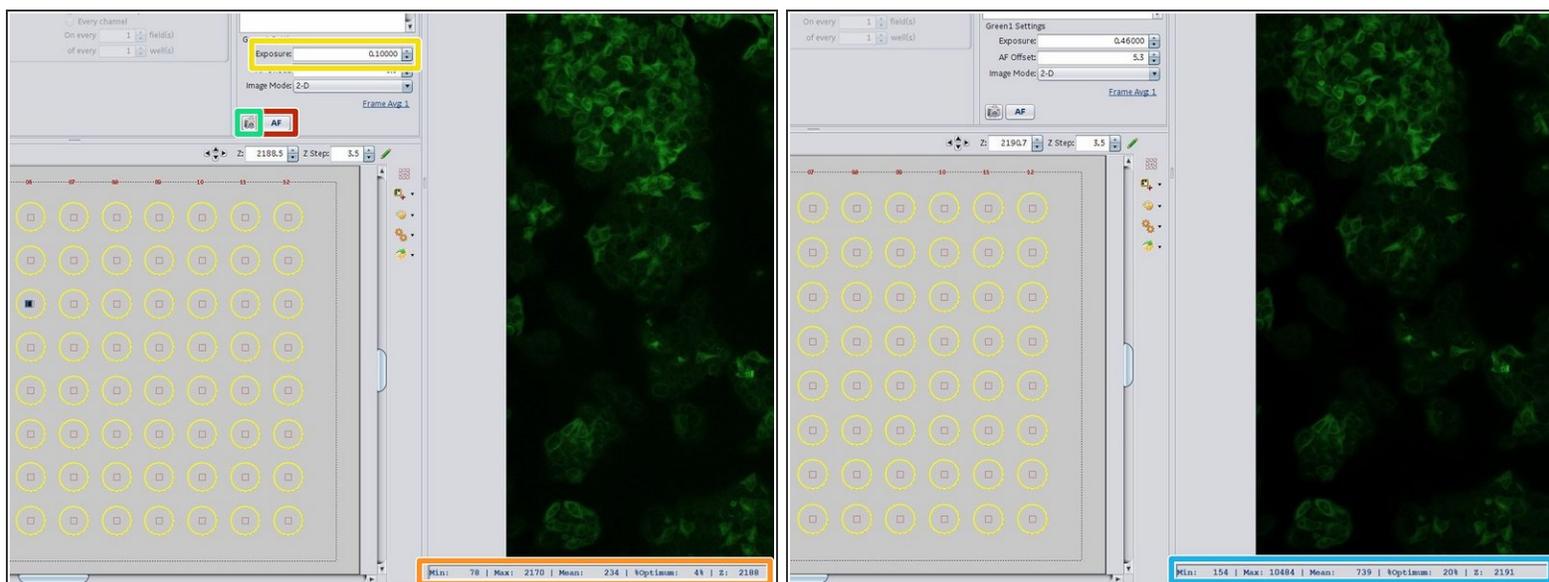
Step 8 — Auto offset



i Usually there is a z offset between the found laser auto focus position and your brightest fluorescence signal.

- Click on "Auto Offset" to automatically measure the offset for the selected channels.
- When done, the software shows you the resulting offsets. They should be in a similar range for all channel. If a channel shows a quite different value then you can:
 - Move to another position and try again.
 - Check the signal in this channel, adjust the exposure (see next step) and try again.
 - Manually adjust the offset to the same value as in another channel.
- The measured offsets will be set in the "AF Offset" window. They also can be manually adjusted there.

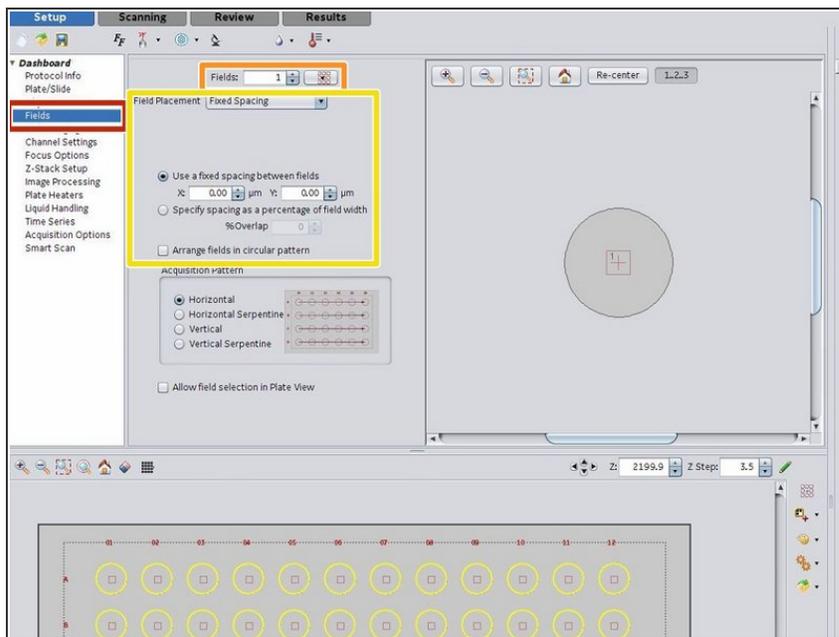
Step 9 — Adjust channel exposure



i On the InCell microscope the signal intensity can only be adjusted by the camera exposure time.

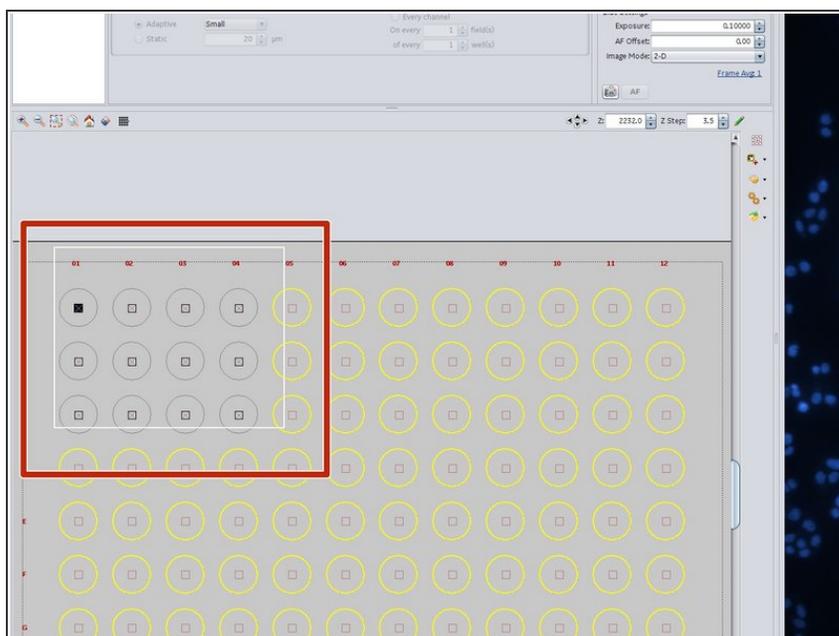
- Select the first channel and press "AF". A laser auto focus will be performed, the offset applied and an image generated.
- Check the image statistics. This microscope has a 16 Bit scmos camera so the dynamic range can be very high.
- Depending on your statistics, adjust the exposure time.
- Click on the camera symbol icon to make an image with the new exposure setting. When using the camera symbol no auto focus is performed.
- Check again your image statistics.
- Repeat this step for all channels.

Step 10 — Define scan fields



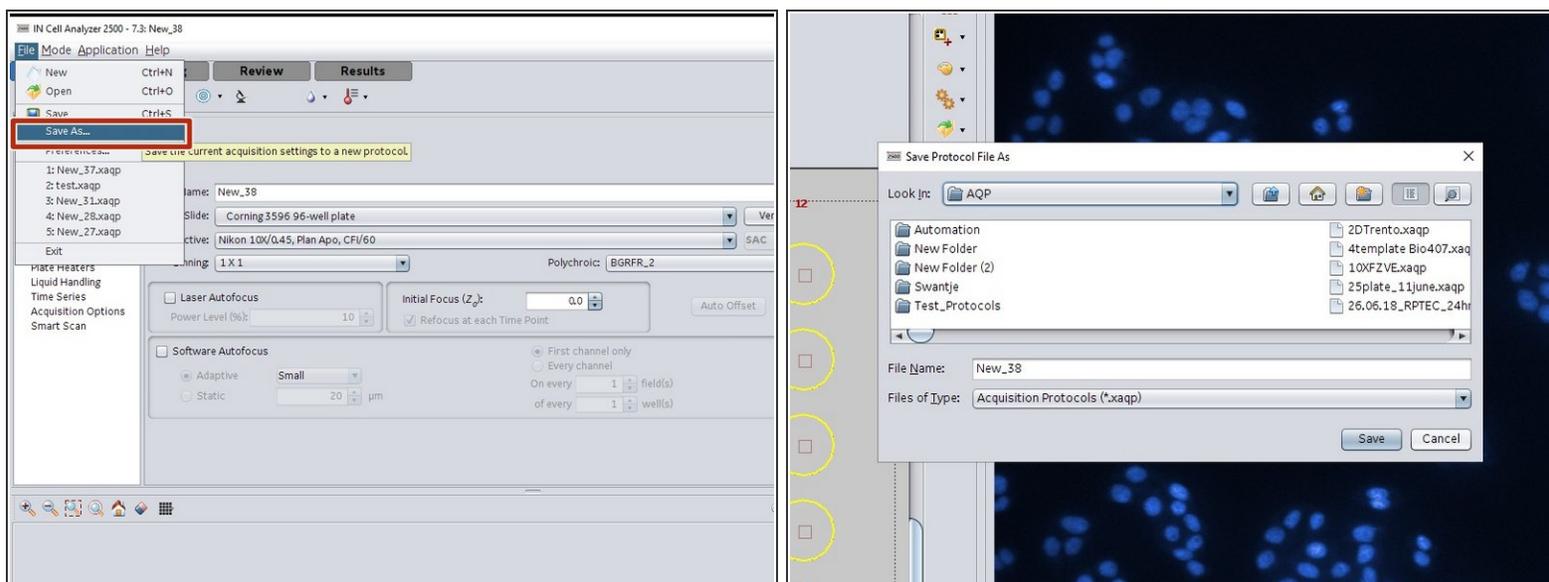
- In the left menu select the "Fields" settings.
- Here you can select the number of fields acquired in each well.
- Next you can adjust the field placement, spacing as well as the acquisition strategy for the fields in a well.

Step 11 — Select wells for acquisition



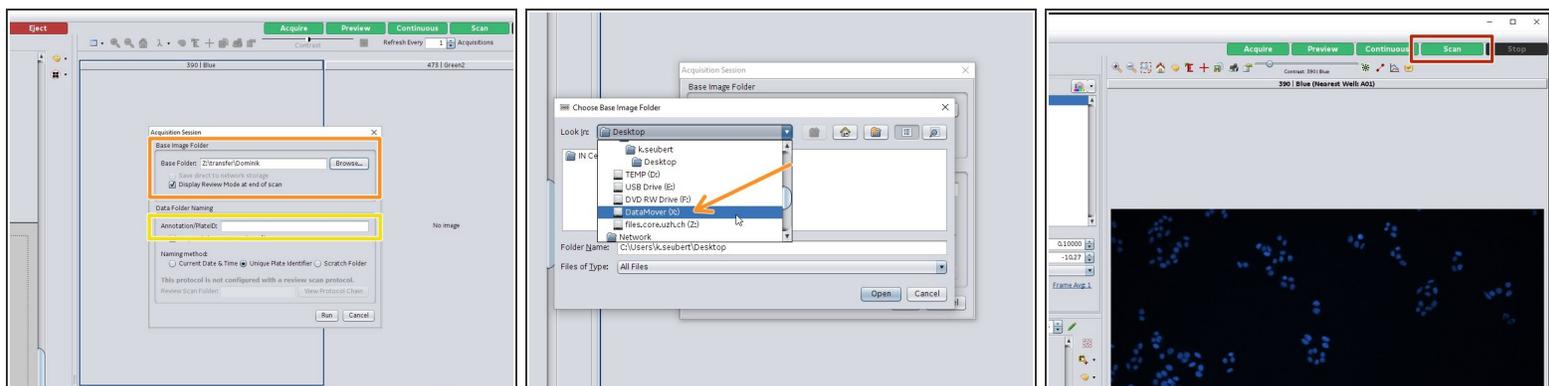
- In the plate view you can select/unselect wells for acquisition.
 - **Shift** and holding the **left mouse** button can be used to **unselect** wells.
 - **Control** and holding the **left mouse** button can be used to **select** wells.
- **i** This strategy also works for selecting/unselecting individual fields within wells.

Step 12 — Save the acquisition protocol



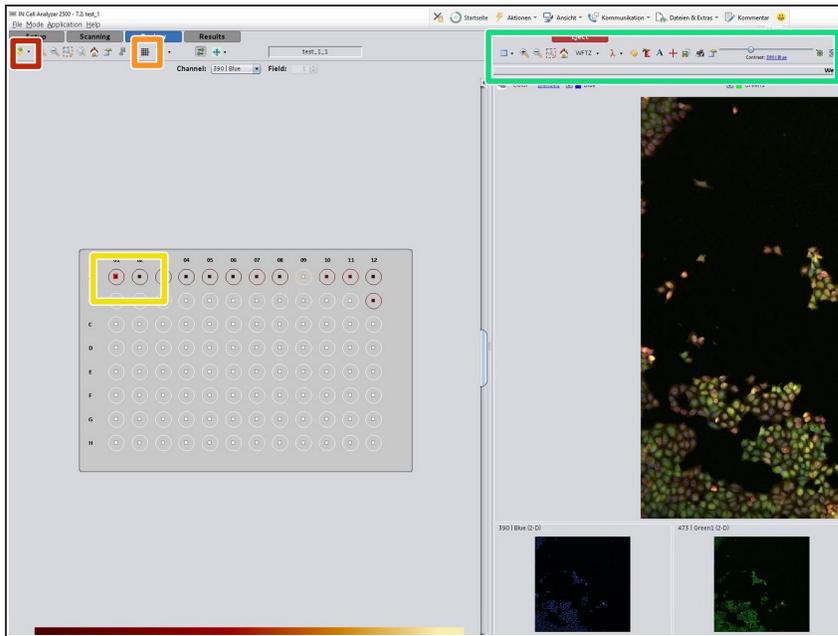
- Before running a screen, you have to save your modified acquisition protocol.

Step 13 — Run the scan



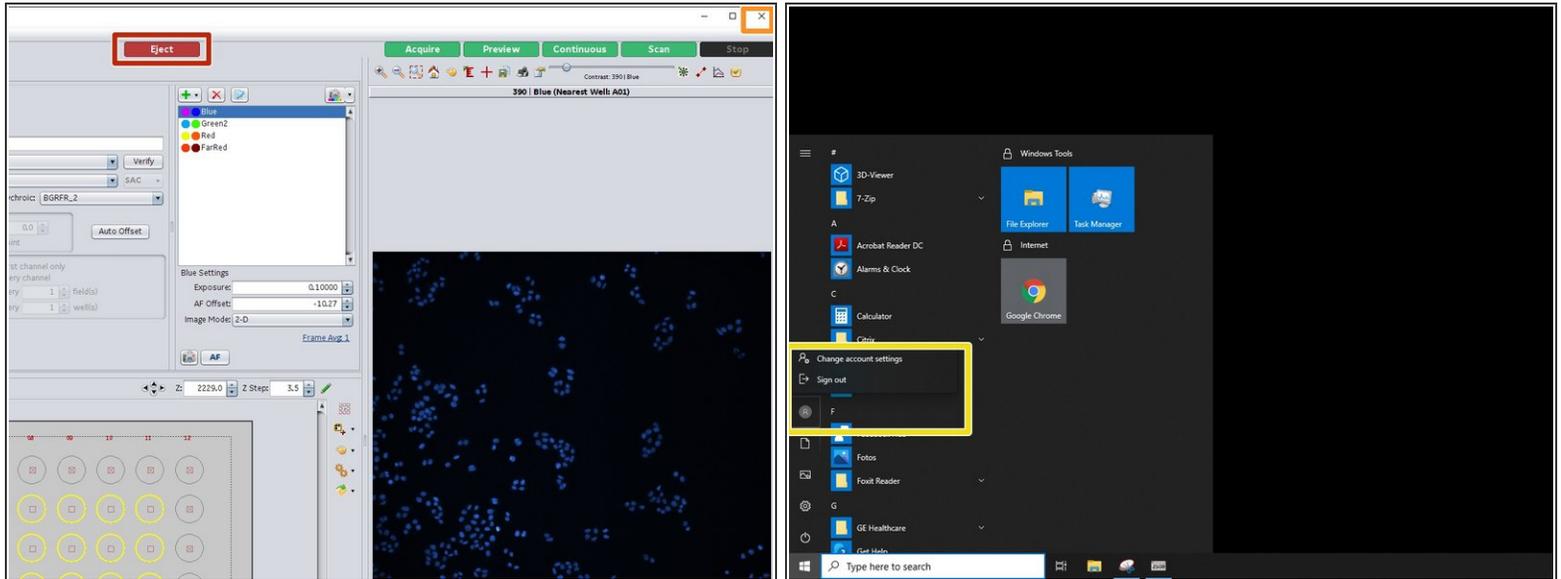
- You can give an additional experiment/plate ID which is used for naming the folders.
- Specify a storage location for your image data. We recommend saving on DataMover (X:). You can later on move your data to the ZMB data network drive via the DataMover (see also the corresponding [guide](#)).
- Click on "Scan" to start your scan.

Step 14 — Review your results



- i After a scan the review mode is automatically opened.
 - For some imaging modalities such as when using deconvolution, it is necessary to reopen the dataset after acquisition and automatic processing.
 - Here you can change the displayed parameters/preview images for the plate overview.
 - Select a field for its data being displayed.
 - Explore the channels by adjusting the image intensities.
- i We recommend to use the review mode to have a quick look at the acquired data as well as for the sometimes helpful visualization of plate parameters (e.g. z focus position).

Step 15 — Leave the instrument



- "Eject" and remove your sample. Click again on "Load" to close the microscope door.
- Close the InCell software.
- "Sign out" from the computer.

⚠ Do not shut down the computer and do not turn off the microscope.