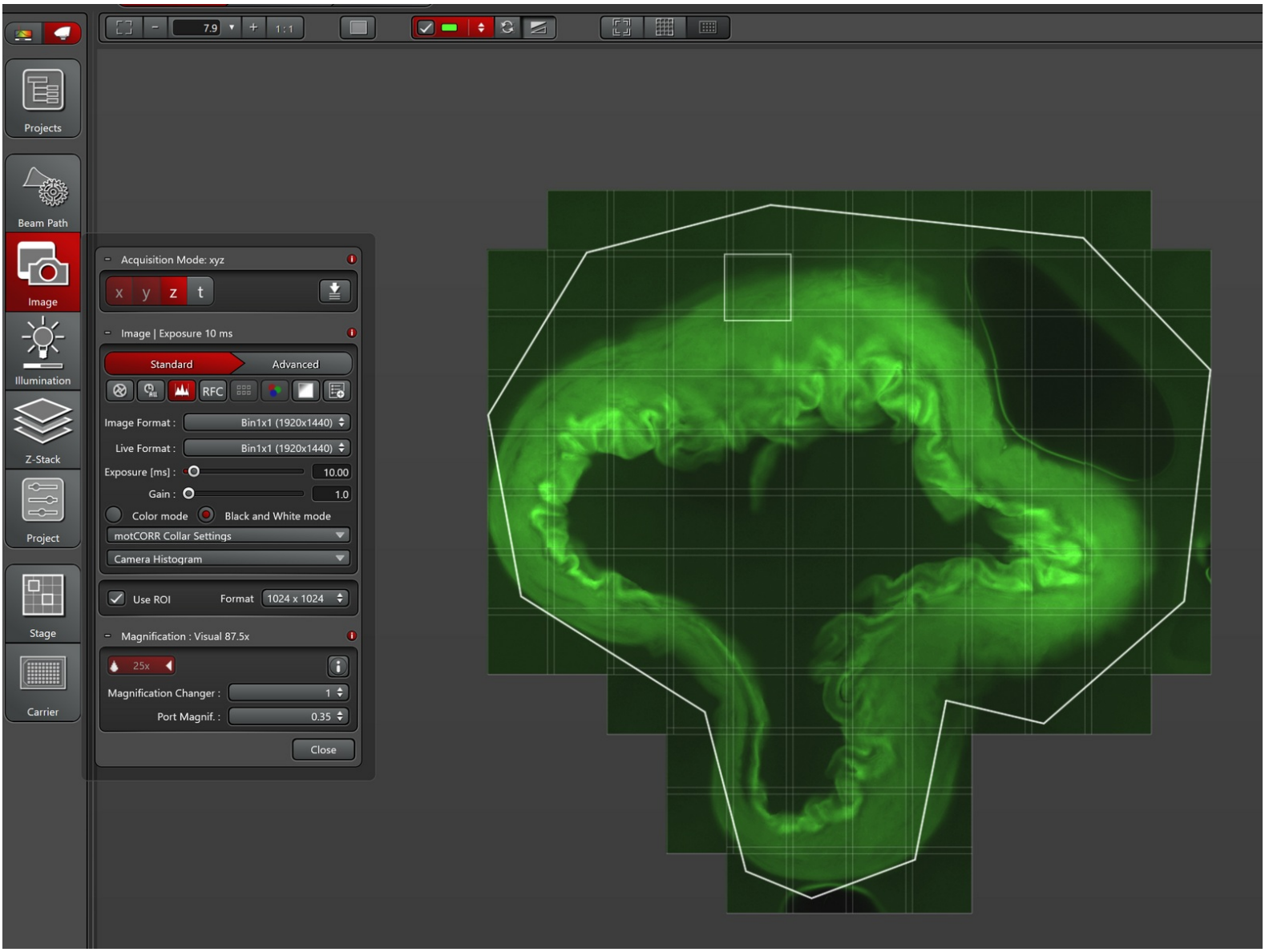
Leica SP8 upright (MP DIVE): create overviews using the camera

On the SP8 upright MP, you can use the built-in camera to create fast widefield overviews of your sample both in fluorescence and brightfield mode.

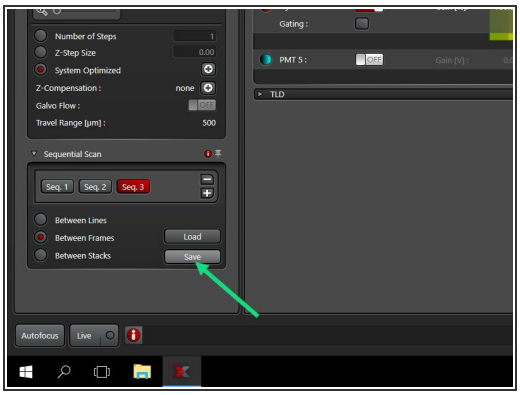
Written By: Karin Seubert



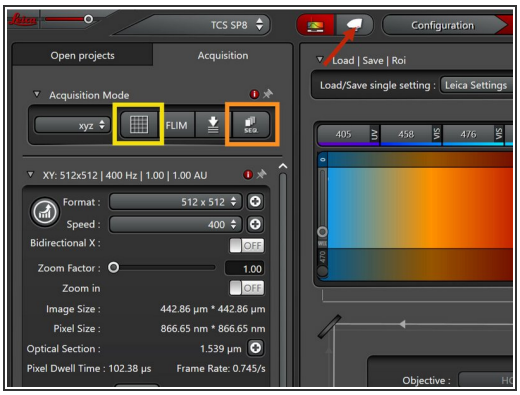
INTRODUCTION

If you have a large sample and you want to have a fast overview, the built-in Leica DFC7000 T Color CCD Camera is the ideal partner for this job. It can be operated in both fluorescence and brightfield mode and will capture a widefield image.
Please be aware that thanks to the widefield characteristics, up to your whole sample will be roughly in focus without any further focus definition. However, if you have a very thick sample, it might also lead to seamingly blury samples due to the out-of-focus light.

Step 1 — Activate Widefield Mode and Navigator





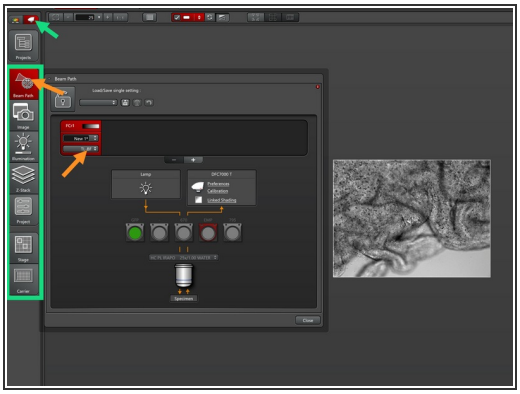
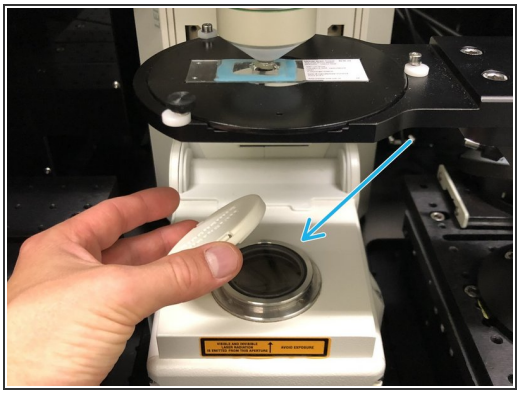
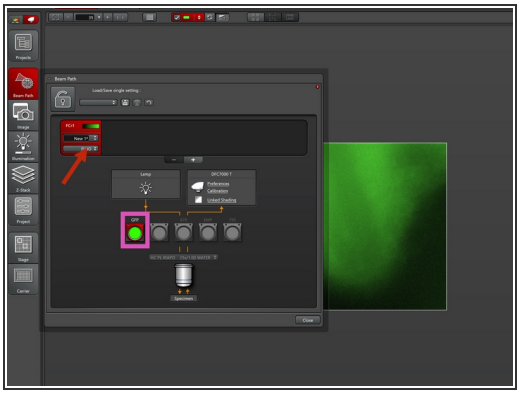


⚠ **Switching off the RLD detectors** for MP imaging is crucial in order not to harm them!!

- If needed, **save your sequential settings**.
- **Deactivate the sequential settings** (must be gray).
- **Deactivate all HyD-RLD detectors**.

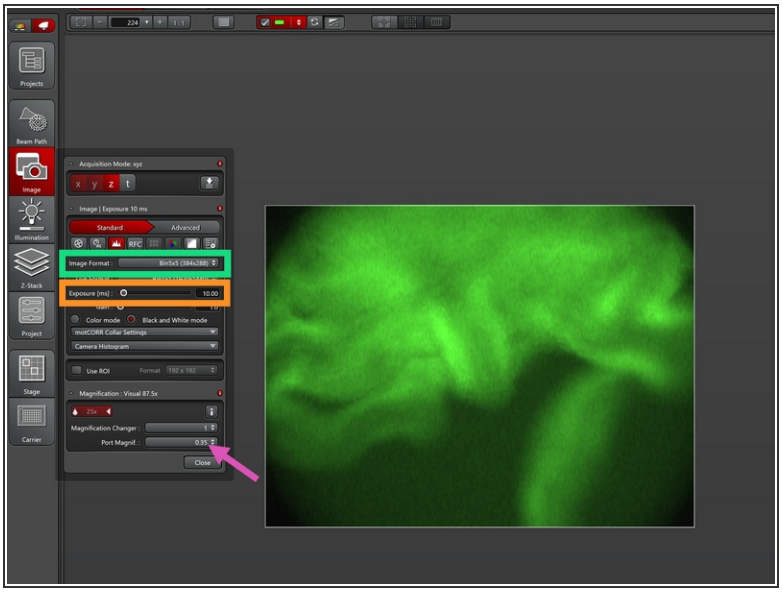
- **Activate the camera** (widefield mode).
- Go to the **"Navigator"**.

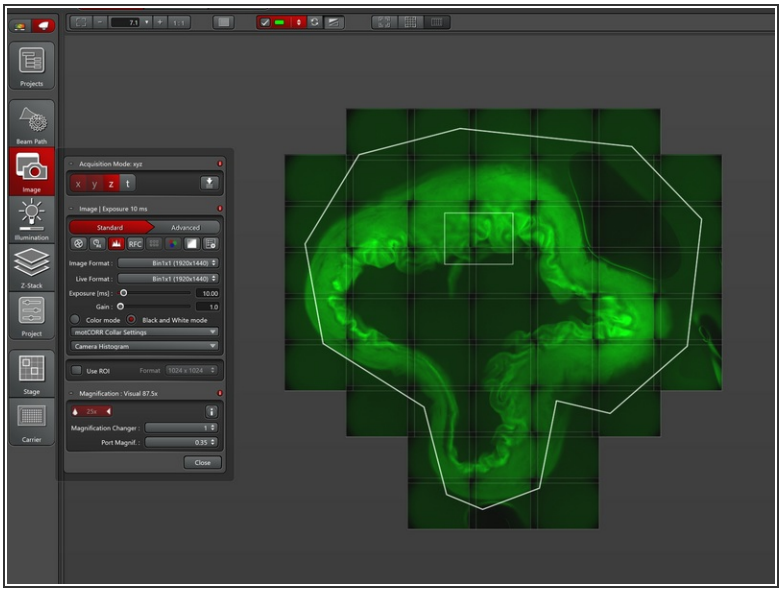
Step 2 — Fluorescence vs. Brightfield

- If you have the camera (widefield) option activated, you will see some widefield-specific tabs on the left.
- In the **"Beam Pass"** tab, select **"TL-BF"** if you want to image with **brightfield**.
  - Before using brightfield, please remember to remove the protection cap.
- If you want to use **fluorescence** instead, select **"FLUO"**.
  - At the SP8 MP DIVE, only one filter cube for **GFP** is installed.

Step 3 — Image Acquisition Settings

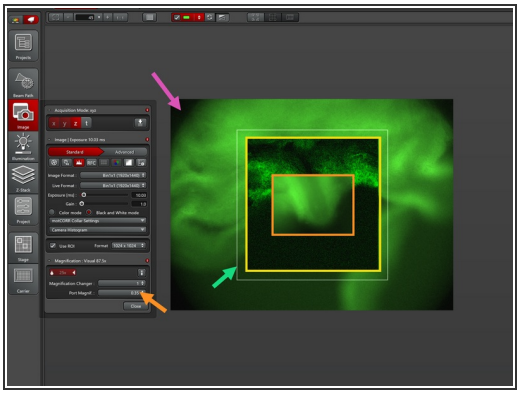


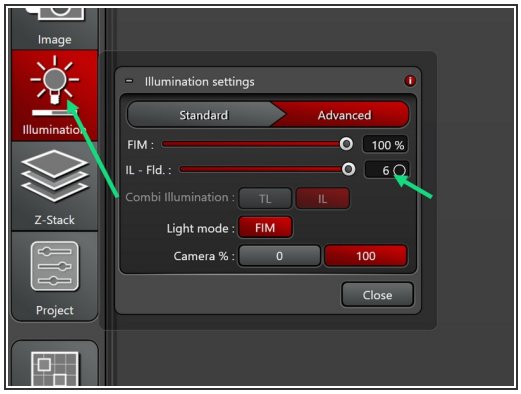


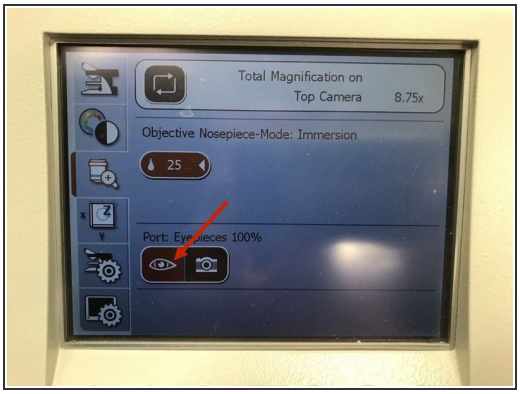
- Go **"Live"**.
  - ⓘ The camera has a rectangular format, which is different from the standard square format in confocal.
- Adjust the **Exposure Time**. Longer exposure times will lead to better signal.
- You can use binning (up to 5x5) in the **"Image Format"**, which will sacrifice some resolution in order to get better signal with unchanged exposure time.
  - ⓘ This is a good strategy if you have a light-sensitive sample.
- For the fastest overview, leave the **"Port Magnification"** at 0.35.
- You can now use all the tools available in the Navigator (i.e. Spiral, Polygon, etc.) in order to define your region to be imaged.
- Press "Start" to acquire the overview

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Step 4 — Magnification and FOV







- You may notice some dark shadows at the edges.
- Make sure that in the **"Illumination"** tab the field aperture **"IL-Fld"** is at 6. If smaller, the dark shadows grow bigger. However, with a Port Magnification of 0.35, there is always a little shadow.
- A workaround (even though slower) is to **increase the Port Magnification**. As a result, the field of view (FOW) will be smaller. This will also decrease pixel size and therefore **increase resolution**.
- For comparison, the confocal FOV with zoom=1 is shown.
- Another option is to use only part of the camera chip via **"ROI": the FOV gets smaller**, however without a change in pixel size (indicated by the white line).
- If you want to use the eyepieces again (e.g. for focusing), you will need to switch manually to the correct beam path on the external controller.