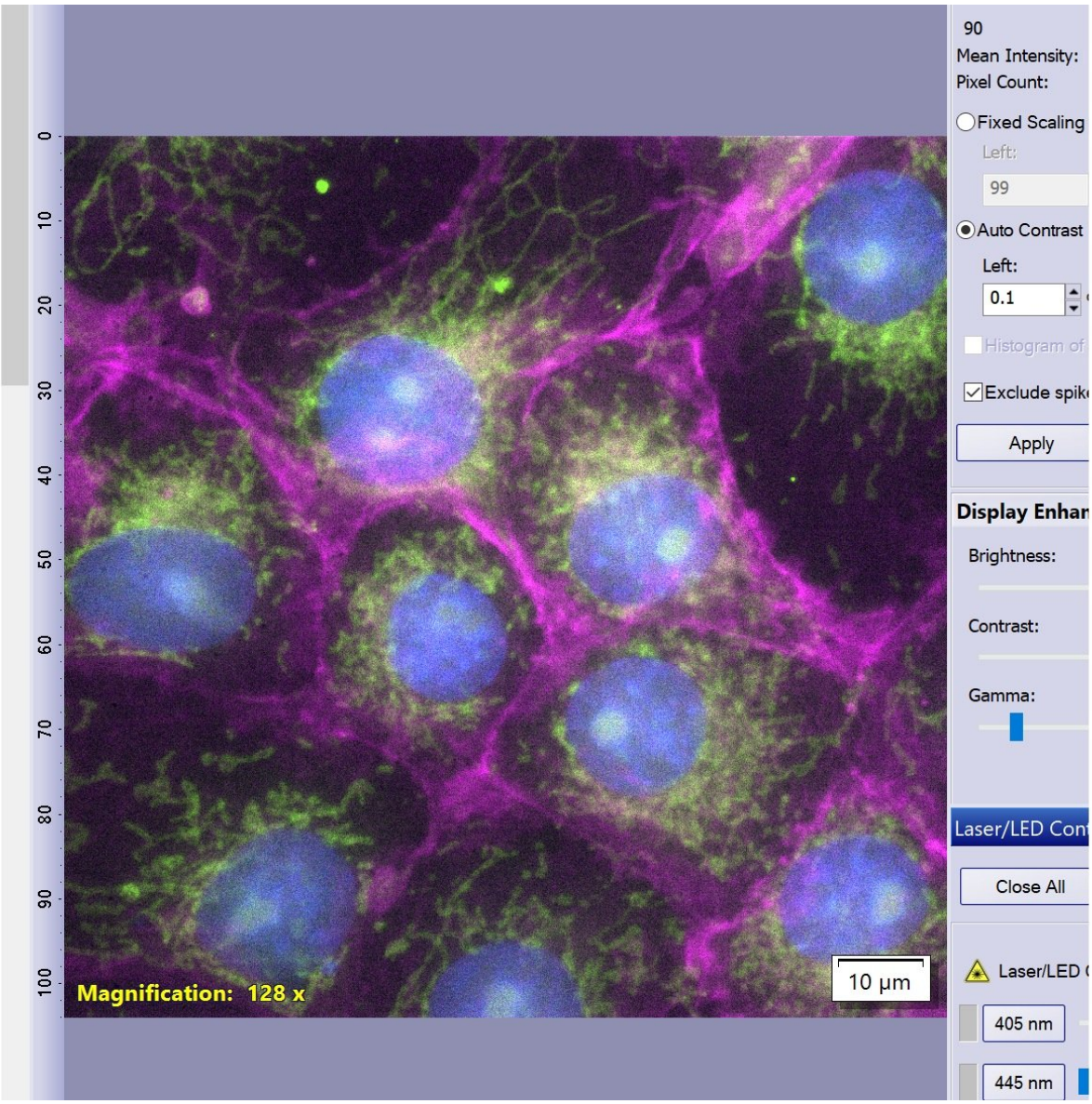
Olympus Spinning Disk - Widefield mode

Written By: Joana Raquel Delgado Martins



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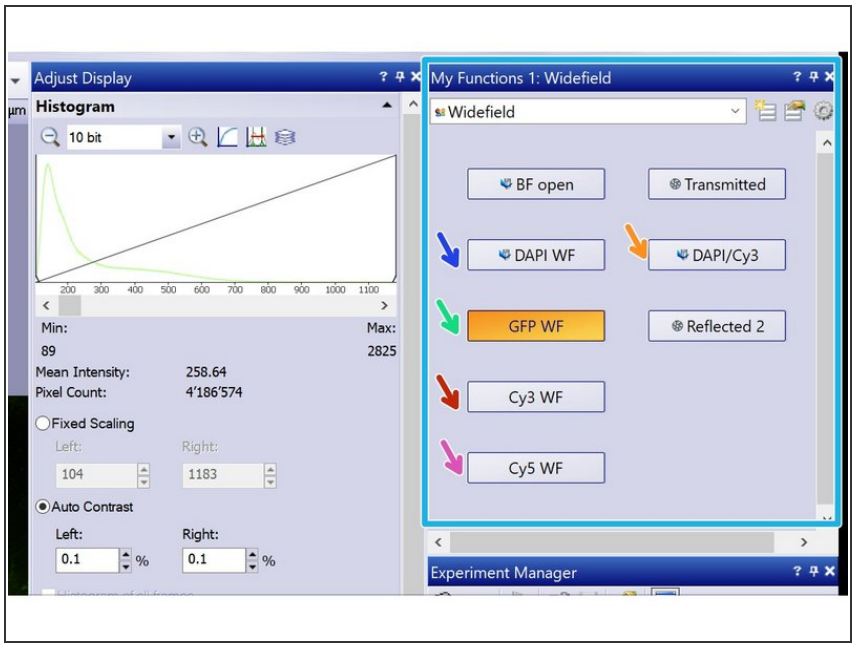
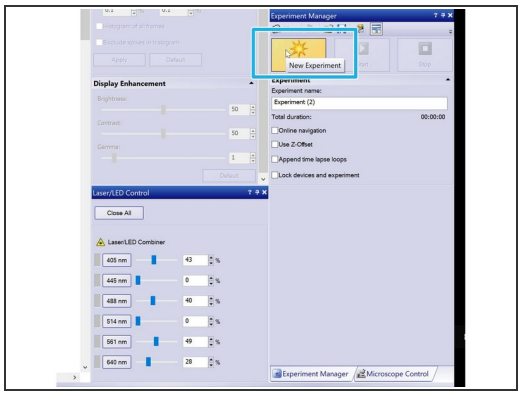
Step 1 — Widefield mode

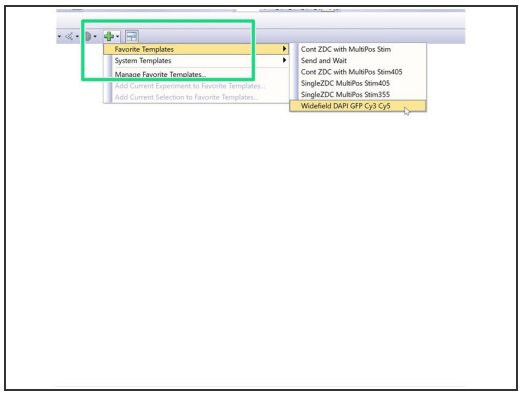


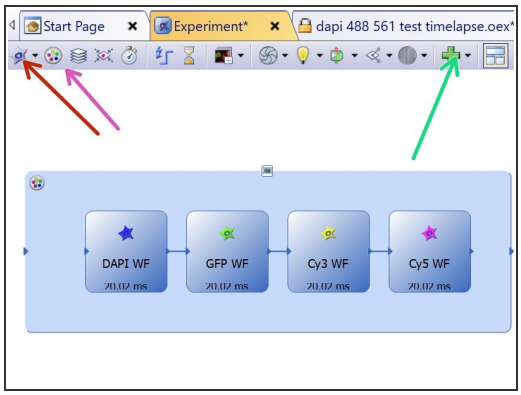
- To use the microscope on the Widefield mode different observation methods are available:
- You can find them under My functions - "Widefield" for easier access during Live mode.
  - DAPI WF
  - GFP WF
  - CY3 WF
  - Cy5 WF
  - DAPI/Cy3 (using the dual camera mode)

⚠ Be aware that the Cy5 WF observation method might also show signals is the red range as a broad light source is used (no excitation filter is present).

Step 2 — Design Experiment

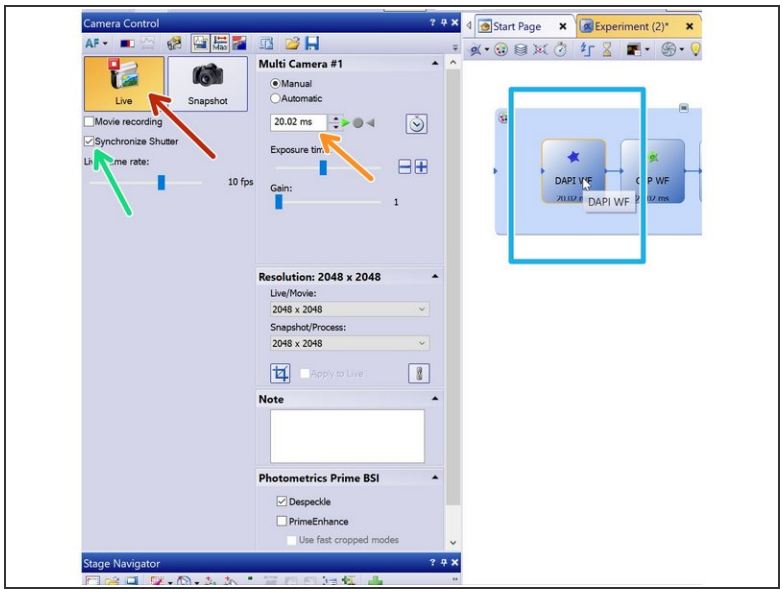


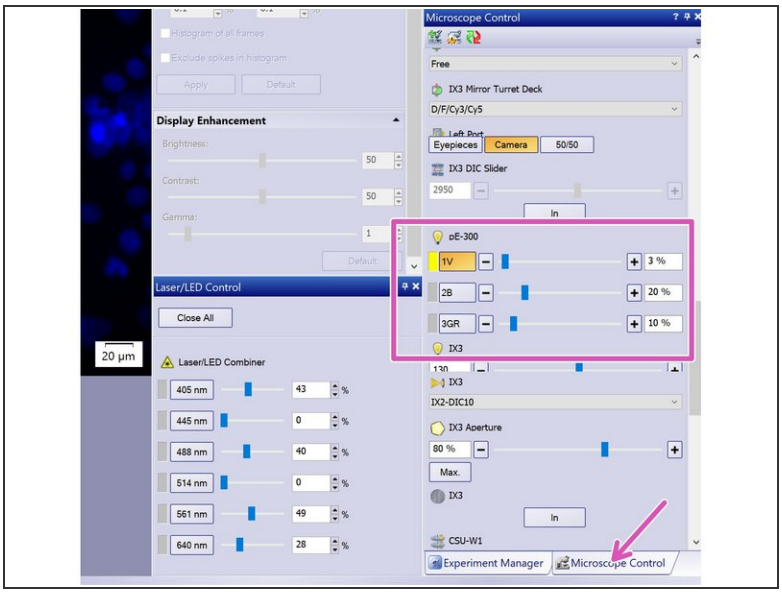




- Start a new Experiment on the "Experiment manager" tab.
- For a 4 color sequential image you can use the template - Favorite Templates - "Widefield DAPI GFP Cy3 Cy5".
  - You can also design your experiment using the available widefield observation methods from the menu.
  - For multichannel images do not forget to add the respective option.

Step 3 — Adjust LED intensity and exposure time

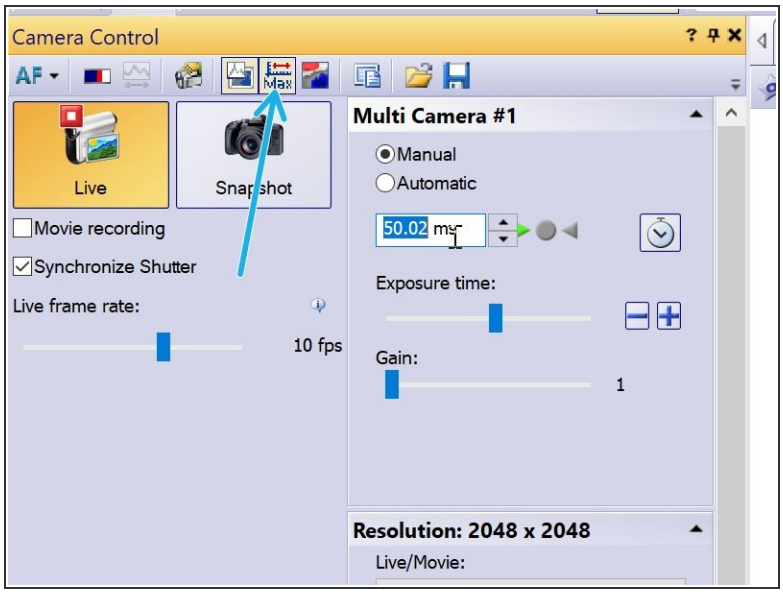


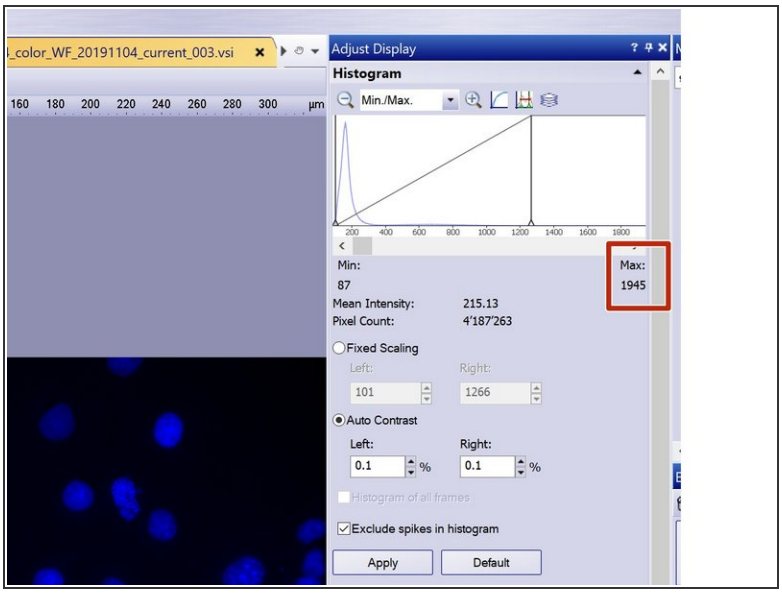


- Double click on the observation method box to activate it.
- Check the "Synchronize Shutter" box option if you would like to minimize exposure of your sample to light.
- Press Live.
- Adjust exposure time accordingly.
- You can also change LED intensity under the "Microscope Control tab":
  - Scroll down until you find pE-300

ⓘ the appropriate LED will be active and can be changed for each channel.

Step 4 — Check image histogram and avoid clipping

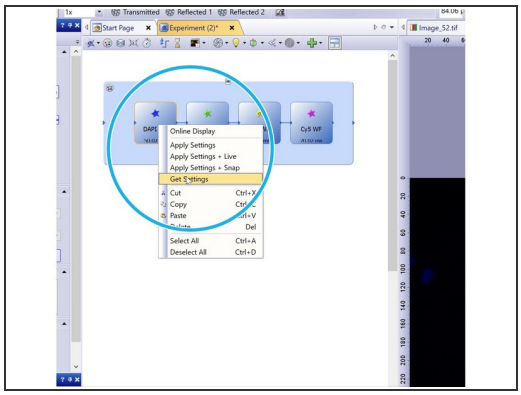


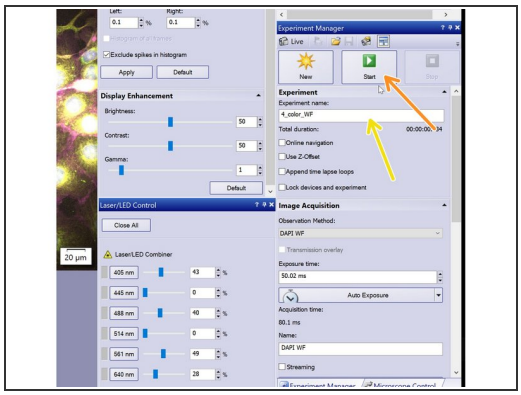


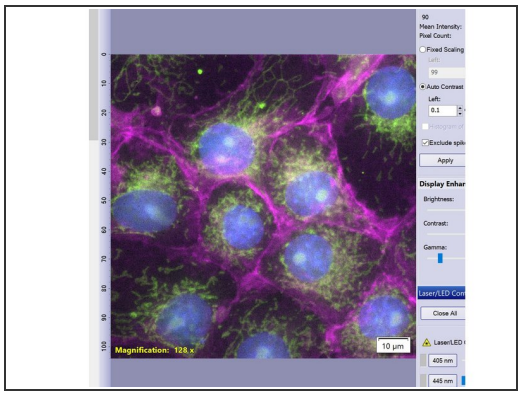
⚠ Make sure your image is not saturated with the help of the Adjust Display pannel.

- Here you can toggle the camera bit-depth between 16 and 8-bit .
- Using the camera at 16-bit (recommended), Max intensity should be less than **65 536**. At 8-bit, the Max intensity should stay bellow **255**.
- For more details refer to the different camera modes here: [Olympus Spinning Disk - 2: Multichannel + Z-stack acquisition](#).

Step 5 — Get settings







- Once you are satisfied with your settings right click on the observation method and select "Get settings".
- Repeat the procedure for the remaining observation methods.
- Name your experiment.
- Press Start.
- Visualize your image

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