

Olympus Spinning Disk - DIC images

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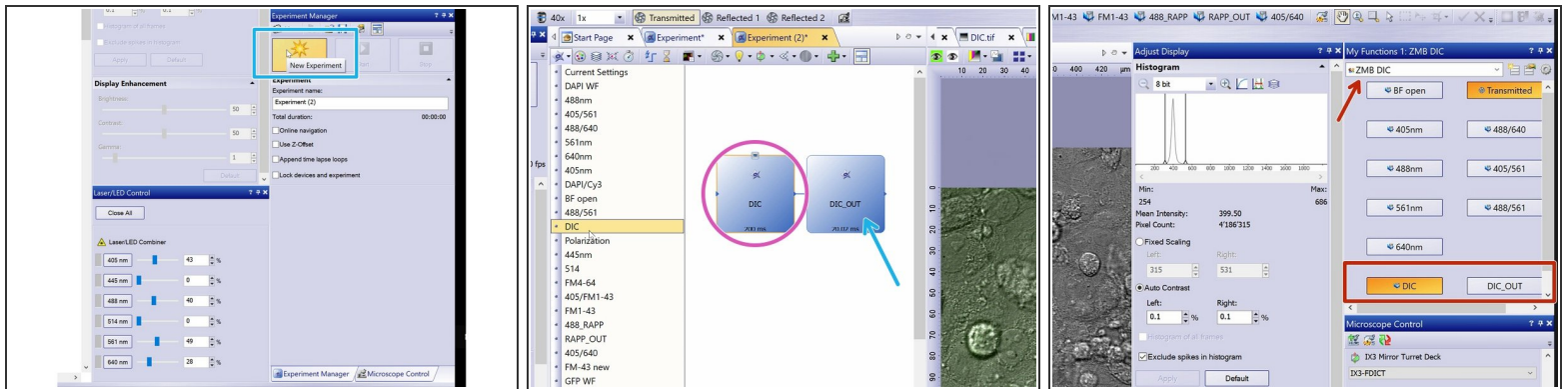
The screenshot displays the Olympus Spinning Disk software interface. The main window shows a DIC image of cells. The top menu bar includes options like '403nm', 'DAPI/Cy5', 'BF open', '405/361', 'DIC', 'Polarization', '443nm', '314', 'FW4-04', '405/FW1-45', 'FW1-45', '405/NAFF', 'NAFF_OUT', and '405/040'. The left sidebar shows a list of images, with 'Image_68.tif' selected. The top status bar displays '20.94' and a scale bar from 20 to 420 μm . The main image area shows a DIC image of cells. The bottom left corner displays the following information:

- Magnification: 30 x
- Total Magnification: 30 x
- Size (calibrated): 443.7 μm x 443.7 μm
- Calibration (X): 216.667 nm/pixel

The bottom right corner shows a 50 μm scale bar. The right sidebar contains several control panels:

- Adjust Display**: Includes a histogram and display enhancement settings (Brightness, Contrast, Gamma).
- My Functions 1: ZMB DIC**: Includes buttons for 'BF open', 'Transmitted', '405nm', '488/640', '488nm', '405/561', '561nm', '488/561', '640nm', 'DIC', and 'DIC_OUT'.
- Microscope Control**: Includes a 'Dichroic Mirror' dropdown set to 'Out' and a 'Device Units' section with various controls.
- Laser/LED Control**: Includes a 'Close All' button and a 'Laser/LED Combiner' section with various controls.

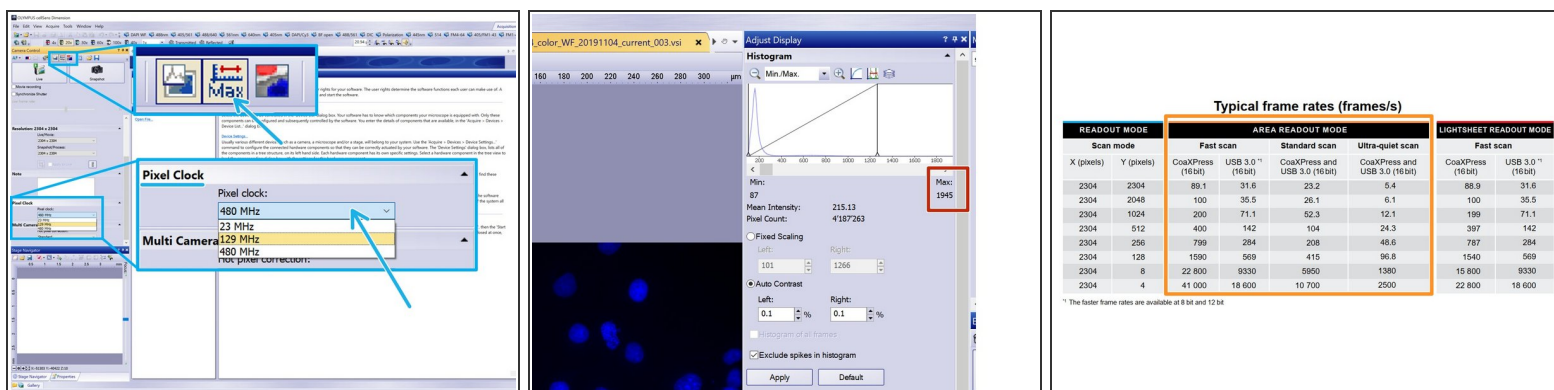
Step 1 — DIC observation method



- Start a new Experiment on the "Experiment manager" tab.
- ⚠ Make sure the microscope is properly aligned and that you have checked your Koehler illumination.
- ☑ Remove any lid you might have above your sample.
- Add your observation method DIC to the experiment.
- Do not forget to add an additional DIC_OUT observation method at the end to remove the extra components.
- 📘 An extra DIC_OUT image will be generated, you can delete or ignore it. For more info please contact the ZMB staff.
- You can find also find DIC and DIC_OUT observation methods under My functions - "ZMB DIC".

- 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.
- Under the "Microscope Control tab" you will find the two DIC Polarizers:
 - Scroll down until you find IX3 DIC slider.
- Here you can adjust the orientation of the polarizer.

Step 3 — Check image histogram and avoid clipping



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

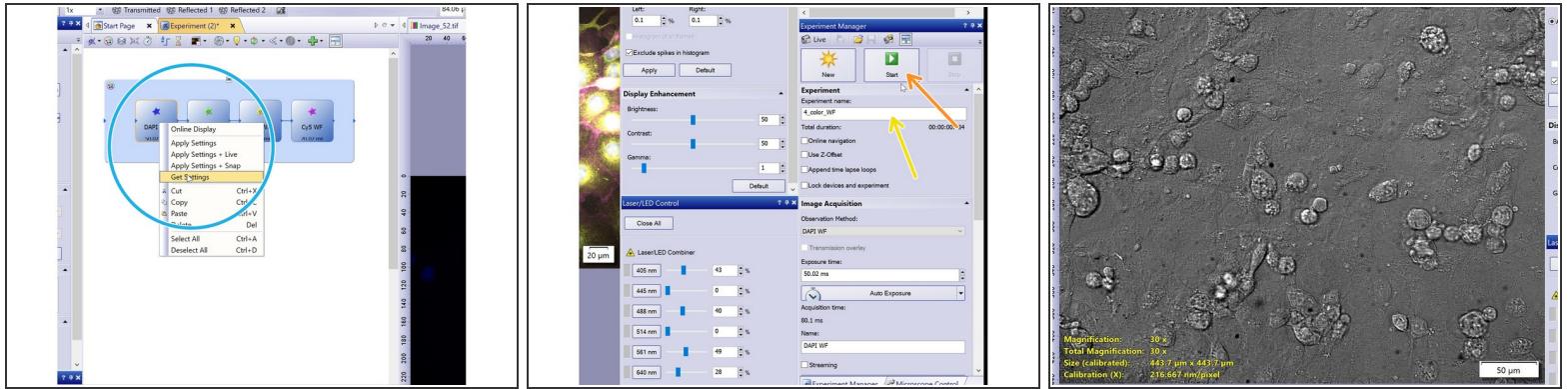
📌 The Camera can be run at 8 bit, 12 bit as well as 16 bit (recommended).

- Here you can toggle the camera bit-depth between 16, and 8-bit and adjust the pixel clock (camera read out speed).

⚠ If you are acquiring at 8 bit the histogram max should be lower than **255**, at 12 bit lower than **4095**, while if you are using 16 bit the max should be lower than **65535**.

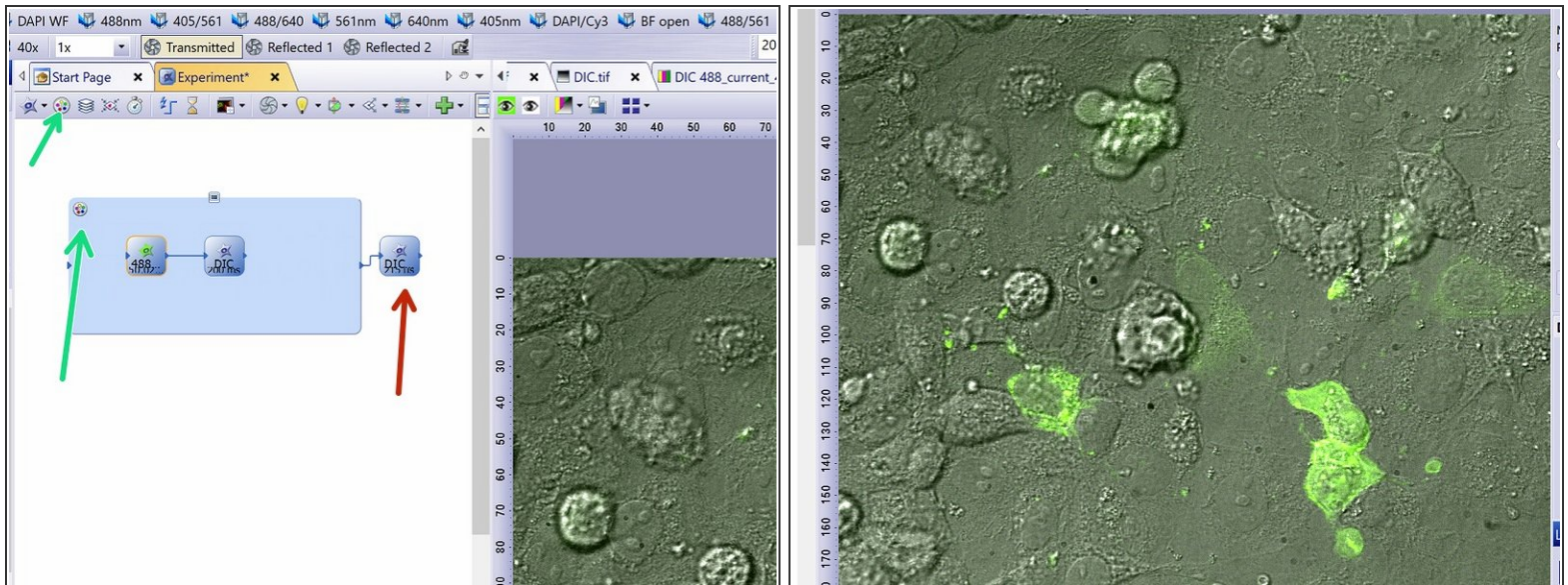
- For more details refer to the different camera modes here: [Olympus Spinning Disk - 2: Multichannel + Z-stack acquisition](#).

Step 4 — Get settings and start



- 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

Step 5 — Fluorescence + DIC



⚠ If you want to acquire fluorescence + DIC images, make sure you remove DIC components from the light path after the DIC image and before acquiring the next fluorescent image, time point, position etc.

- Remove DIC components by adding a DIC_OUT observation method.
- i** An extra DIC out image will be generated, you can delete or ignore it. For more info please contact the ZMB staff.
- Use the multicolor option to overlay the two channels.
- 📌** The 2 channels should have the same bit depth, size and format.