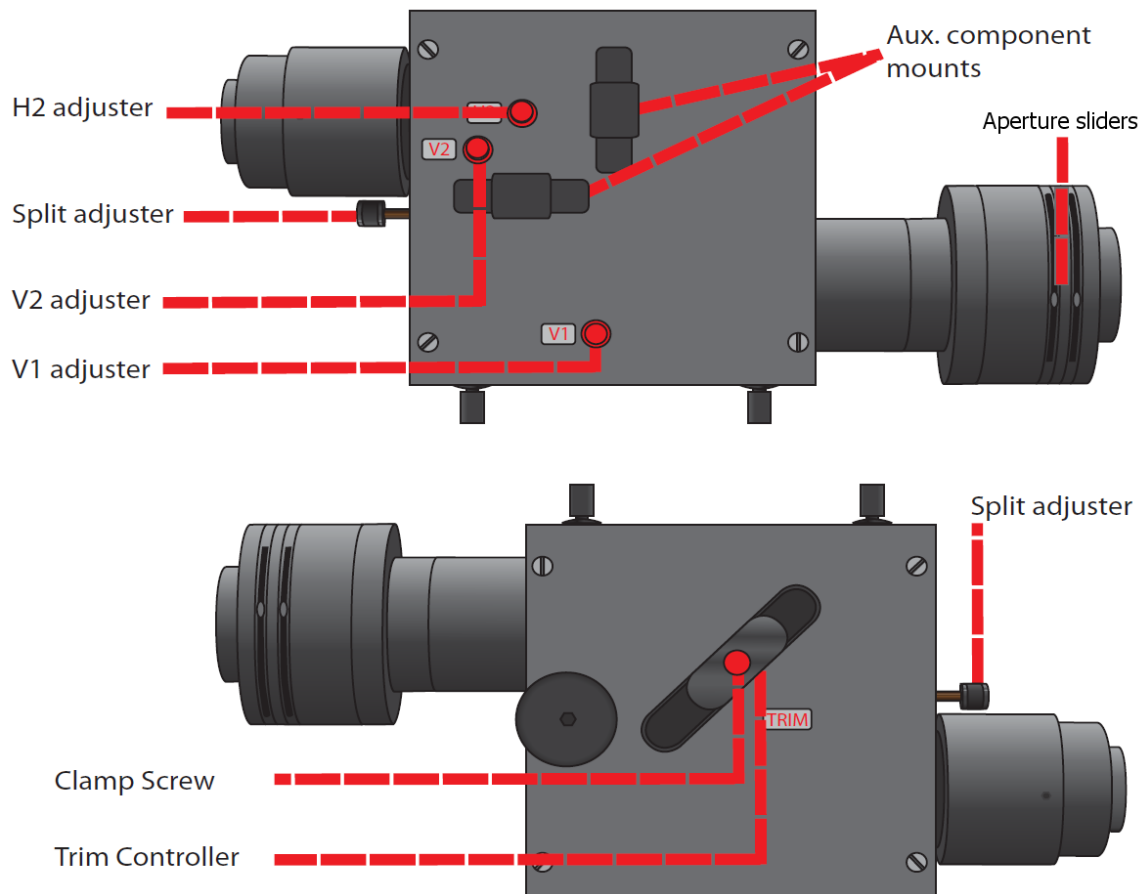
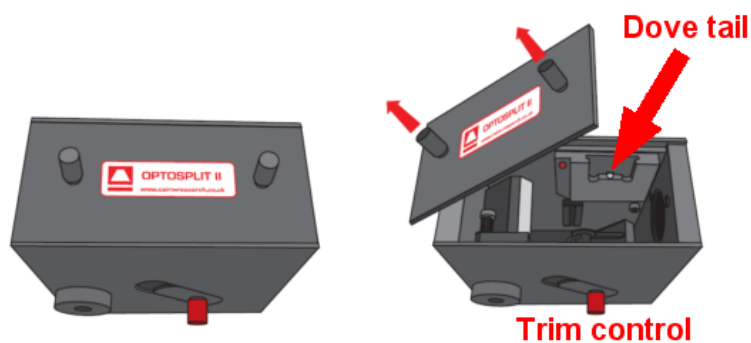


## OptoSplit II



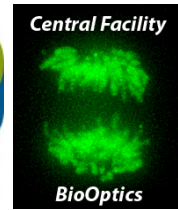
## Setup and alignment - GFP/RFP cube

- Insert the filter cube, mount the FRAP calibration slide and focus in the fluorescent layer

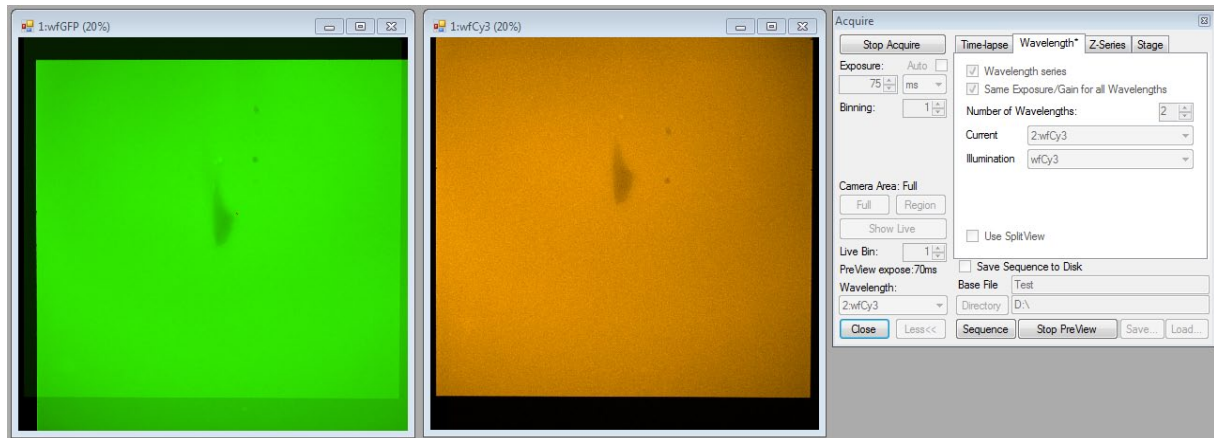


- Set the software for standard 2-channel, sequential imaging (wfGFP/wfCy3)
- Click "View loop" for continued acquisition

# LIVE Spinning Disk/ Nanodissection Unit



- Close the aperture sliders until the horizontal and vertical apertures are visible as black borders in the images. They should be sharp and parallel to the camera/image borders. If there is some slant or if the borders appear blurred, loosen the hex screws of the focussing and rotating rings, and rotate either the focussing ring and/or the camera

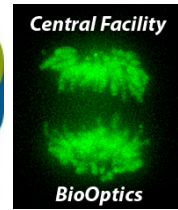


*Note:* the green image is actually an overlay of 2 images, with the second image being weaker

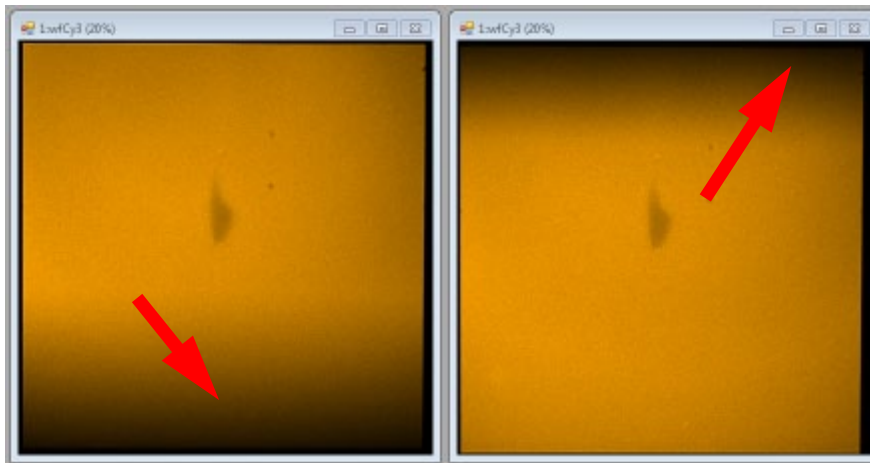
- Center the strong green signal with the knobs SPLIT and V1
- Center the red signal and/or the weak green image with knobs H2 and V2



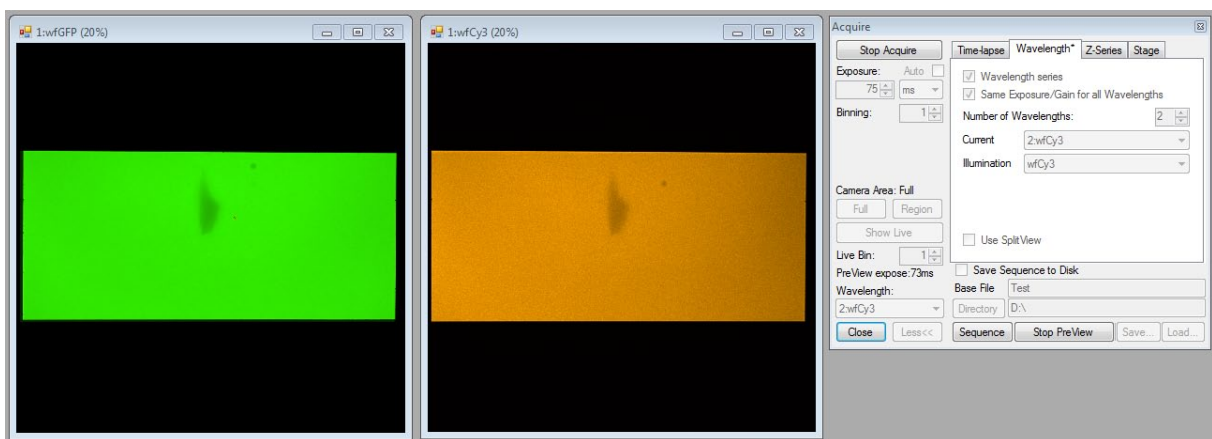
# LIVE Spinning Disk/ Nanodissection Unit



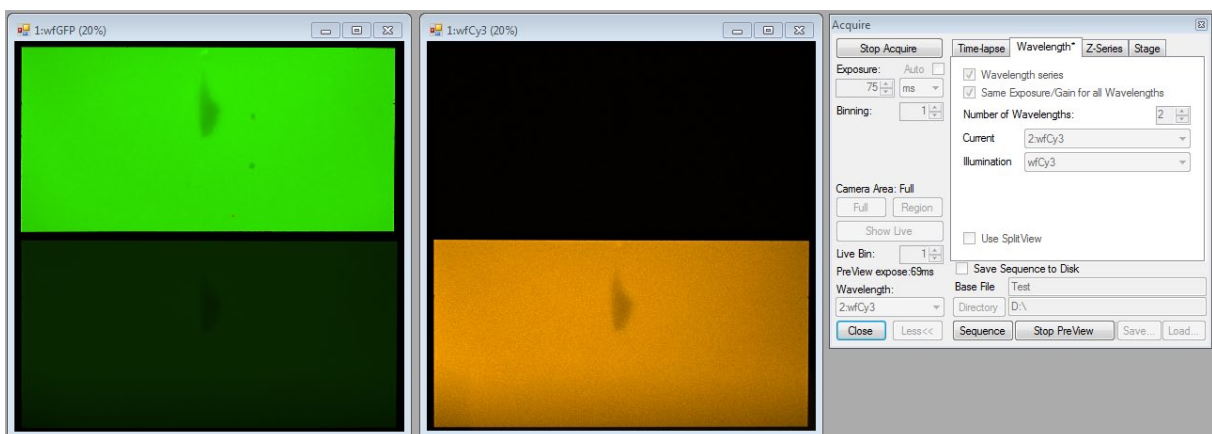
- Check for vignetting and correct with the TRIM control, if necessary (may require re-alignment of the images)



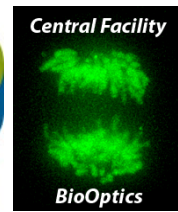
- Close the horizontal apertures to about half the field of view



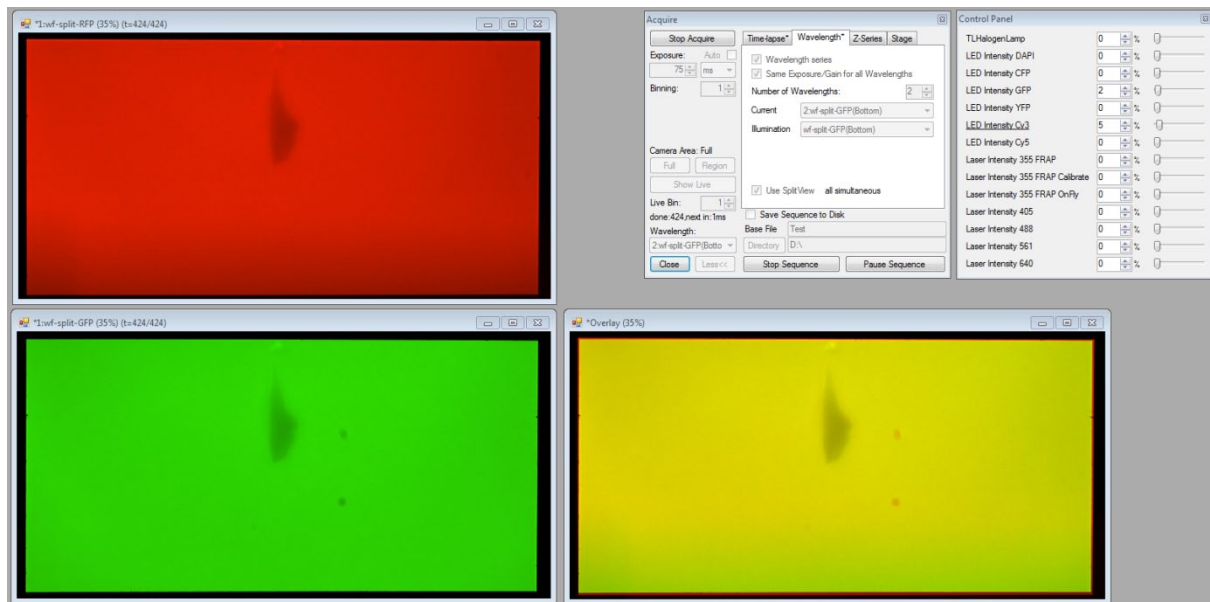
- Use the SPLIT knob to move the red channel to the bottom, the bright green to the top



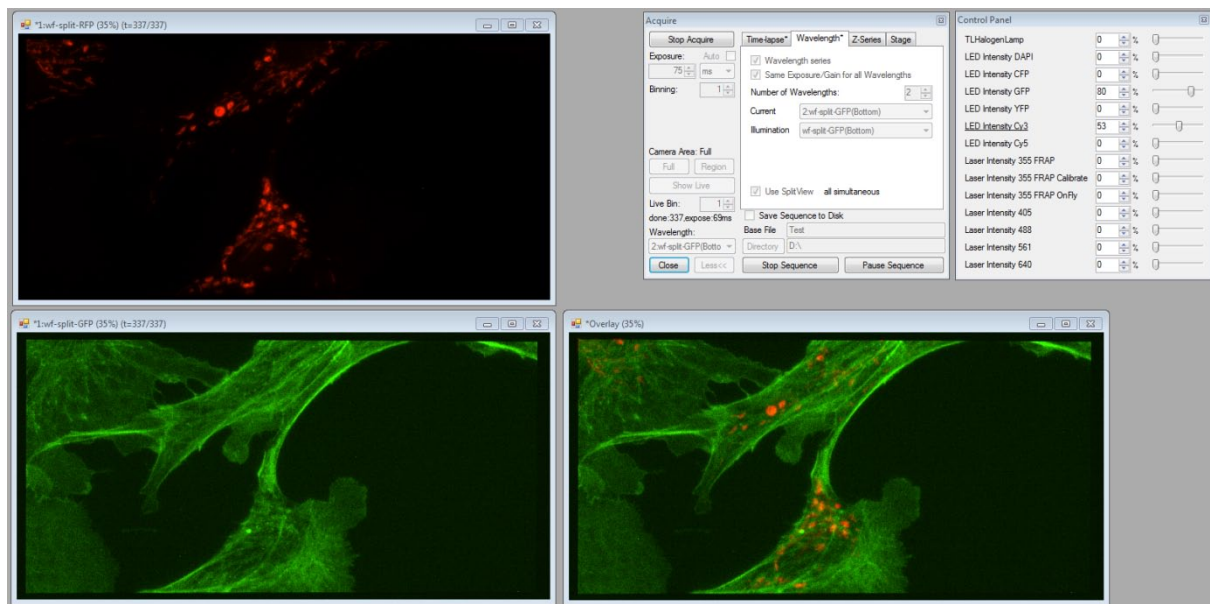
# LIVE Spinning Disk/ Nanodissection Unit



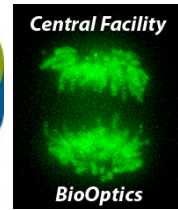
- In the "Wavelength" tab, set the camera to Split View with simultaneous acquisition. Activate the overlay. Use the knobs SPLIT/H2 and/or V1/V2 for minor adjustments



- Mount the sample

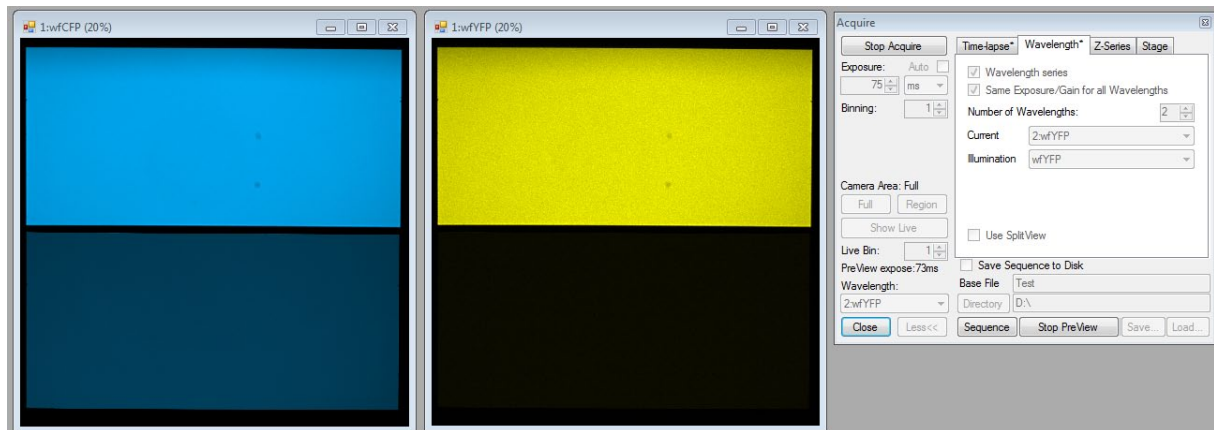


# LIVE Spinning Disk/ Nanodissection Unit



## CFP/YFP

- Align as described above. When splitting the images, move the *yellow* image to the top (the true cyan channel will move to the bottom. The top will show a lot bleed through. Its intensity may be even stronger than the actual cyan channel, depending on the light intensities used



## Removal of the cube

- Remove the cube from the light path
- Set to 2-channel sequential illumination (wfGFP/wfCy3 or wfCFP/wfYFP illumination) and click "View Loop"
- Open the apertures to leave a small black border
- Use the knobs SPLIT and V1 to center both images on the full camera chip
- Check for vignetting and correct with TRIM
- Open the apertures all the way