Protocol for the scope:

1. Turn on the system
   a. Shutter control (LAMBDA …)
   b. Stage controller (ProScan II)
   c. Univ. Arclamp
   d. Transmitted Lamp
   e. Microscope (back)
   f. Camera
   g. Computer.
   h. Heater (NEVTEK)
2. Buttons on the microscope
   a. The objective changer (Obj) is at the left side.
   b. Use 20x to find the cell. Phase move the polarizer to the open position (move to right). Move the phase ring to (infinity Ph1). Move the aperture (right above the phase ring) open or close position to adjust the white light.
   c. Switch between camera (L100) and eye (EYE) at the face side. Using L80 position will allow 80% of light go to the camera and 20% go to the eye.
   d. Neutral density: Located between the fluorescent lamp and the microscope body. ND4-1/4 of the light, ND8-1/8 of the light, No label -1/2 of the lights. (The position of the ½ filter is opposite to those of ND4 and ND8. There is also neutral density for the transmitted light, which should be left open. On the Zeiss microscope. 1.0 – 10%; 1.3 – 5%; 2 = 1%)
   e. Perfect focus button at the face side of the microscope body (Focus and On). Memory and Recall button help remember the position. After PFS is on, use the PFS pad to adjust focus, the regular focus knob is no longer functional. There are coarse and fine (push in the side button on the PFS pad) adjustment level control at the left side.
   f. For high magnification, switch to 100x , a drop of oil on the objective. Push the polarizer to left. Move the phase ring to DIC N2 position. Adjust Lights for DIC.

3. Metafluor: Monitor the FRET ratio in real time.
   a. Protocol: CFP YFP MCherry
   b. Configure Illumination:
   Ti Filter Block 1 – Dichoric for excitation. Number 1-4 are high-quality filters for FRET imaging. Number 5 and 6 allow seeing the cells directly using eyes.
   Ti Optical Path : Left Port (camera); Binocular (eye);
   Ti Lamp Voltage : digital control of the transmit light (can also be controlled by the button with MAX at the left side of the microscope);
   LAMBDA Wheel A: excitation filter;
   LAMBDA Wheel B: emission filters.
   LAMBDA shutter A: shutter for excitation;
   LAMBDA shutter B: shutter for transmittal light.

      c.   Use GFP-eye to look for the CFP cells.
4. Metamorph: Can be used to register several positions and then monitor multiple cells simultaneously.
    a. Camera snap – Aquire one.
    b. Live: Focus.
    c. Devices-> Configure Illumination: same panel as metafluor
    d. Keep perfect focusing on ->Apps -> Multidimension Acquisition -> Select Time lapse -> Multiple stage position -> Wave length -> Next -> Time interval 60 sec. Duration will be calculated. –> Next -> Stage position -> Open the Illumination control, select GFP-eye to look for a cell at the eye port-> switch to CFP and Open the Live windows look for the position, remember to switch the port from eye to camera-> Add position in the Multidimentions Aquire Window. For more cells, repeat this process and add more positions. (Wave length, is auto focusing needed? What is Alignment Cropping x y – Set Alignment?)
    e. After acquisition: Open Special -> Numbered names -> save as stack. Make a stack for each channel.
    f. For image resolution. Edit preference -> DPI -> 600.
5. Additional tips.
    a. The memory button at the front side does not turn off after it was turned on, but the memory can be reset by pushing the button. And to turn off the memory button, the whole microscope system needs to be turned off.
    b. Need digital control of the neutral density.
    c. A good focus at the eye port does provide a good focus at
    d. The image quality is good after saving. Be careful not to pick the thumb images with lower resolution to generate stacks.
    e. The eye is not the same focus as the camera.
    f. What we see on the screen did not 100% save to image.
    g. In metamorph, after refocus and update the multiple image acquisition positions, the images need to be saved under a different name.
    h. The PFS sometimes turns off while adding PDGF. Adding perfusion system is expected to improve on this.