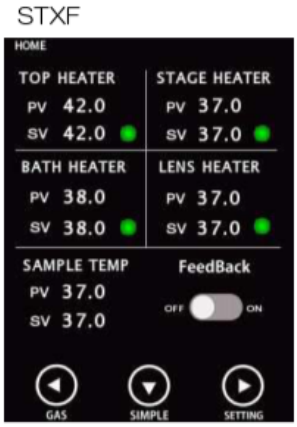
Live Cell Imaging

- **With $C0_2$ and water reservoir:**

1. Turn on all components according to sticky notes
   - The camera and computer are usually always on so start at number 2
2. Add 26 mL of distilled H_20 into the water reservoir using the syringe provided
3. Turn on Tokai Hit STX Stage Top Incubator
4. Turn on the gas:

   (a) Open cylinder valve 1
      * Make sure this valve turned several times to ensure cylinder is completely open
   (b) Increase the pressure of gauge B should be set to 7-10 psi
5. Use a combination of the gas flow nozzle on the Tokai Hit STX Stage Top Incubator, knob 2 on the gas tank adapter, and the knob 3 to control the outgoing pressure (gauge B in picture above) to get the gas flow nozzle at 100 mL/min.
6. Ensure that if the sample sensor device is not being used, the "Feedback" switch is turned off on the Tokai Hit STX Stage Incubator. If this is not correct, then the top heater will get to its correct temperature, creating condensation on the cover to the live-cell imaging module on the microscope.
7. Check to make sure all components of the live-cell module are the correct temperature using the Tokai Hit STX Stage Top Incubator screen.



8. Choose objective by using the rotatable switch on the front of the microscope
   - If you are using the 60X objective, make sure to place a drop of the Nikon immersion oil on the objective

9. Remove cover to microscope stage
10. Remove the stage the entire stage adapter
11. Wash magnetic plate holder and cover with ethanol (be careful with cover glass, it is very fragile)
12. Take lid off cells and place plate on the stage adapter
13. Secure plate with magnetic holder
14. Place stage adapter back into its original position
15. Place the microscope stage over back into its original position, covering the stage adapter
16. Make sure the light source is turned on
17. **Adjust parameters for these channels as such to these values (These are just general guideline):**
    - DIC:
        * Bit Depth: 16-bit (Prime BSI Settings)
        * Format: Binning 2x2 (Prime BSI Settings)
        * Around 10% laser power (Ti2 Thinkpad Tab)
        * 50-100 ms of exposure (Prime BSI Settings) (this can have a higher exposure time because it is less phototoxic)
    - TRITC, DAPI, FITC, Cy5 (in Spectra Pad Tab):
        * Bit Depth: 16-bit (Prime BSI Settings)
        * Format: Binning 2x2 (Prime BSI Settings)
        * 5-10% laser power (Spectra Pad Tab)
        * 30-50 ms of exposure (Prime BSI Settings)
18. **Check the Kohler illumination**
    (a) Find best focus for cells with eyepiece
    (b) Turn FS disk at the top of the microscope all the way to the left
    (c) Turn lights off in the room
    (d) Look into microscope and adjust condenser with big knobs located on the left and right sides of the microscope until octagon in field of view (FOV) is as sharp as possible (be careful to make sure the condenser does not touch the stage)
        - If the octagon is not visible, adjust the condenser
    (e) Adjust the position of the octagon using the two red hex tools on the side of the microscope. There should be a hole on both sides of the microscope near the polarizer
    (f) Turn the FS disk all the way to the right
19. **Apply shading correction only for all channels used in your experiment:**
    (a) Lower objective until no cells or textures from cells can be seen
    (b) Open shading correction panel using the acquire tab at the top of the Nikon desktop application
    (c) Select per optical configuration
    (d) For each channel in your experiment:
        - Change to the appropriate channel in the Camera Tab
        - Click use current image as shading image
20. Readjust focus on cells
21. Change the region of interest (ROI) to 1952x1952
    - This is optional. There can still be shading issues after the fact around the edges so this can cut them out
22. Turn PFS on using the Ti2 Thinkpad
23. **Set Job Parameters in the ND Acquisition Tab:**
    - Ensure the only the Tabs that are needed for your experiment are checked
    - Check the Use PFS check box
    - Pick out x XY positions, ensuring that they do not overlap by using the XYZ overview tab

- Click the optimize button in the XY Pos. Tab (this reorders points to minimize the movement of the stage when taking pictures)
- Select the correct channels for your experiment in the lambda tab
- Set parameters for your experiment in the Time Tab
- Change settings in other tabs as needed for your experiment
- Name file appropriately for your experiment

24. Run experiment
25. Turn gas off:

(a) Loosen knob 3 until it there is little resistance in turning it forward and backward
- Gauge B should be at 0 psi

(b) Close valve 1

26. Turn off Tokai Hit STX Stage Top Incubator
27. Remove top heater carefully and wipe the dew off the inside surface
28. Remove sample from microscope and place all magnetic pieces back
29. Remove remaining water from water bath using the syringe
30. Using a cloth with alcohol, clean the chamber. If any water drops are left, it could cause mold.
31. Turn off all instruments in reverse order starting at 5 and ending at 2

- **Without $C0_2$ or water reservoir:**
  1. Turn on all components according to sticky notes
     - The camera and computer are usually always on so start at number 2
  2. Choose objective by using the rotatable switch on the front of the microscope
     - If you are using the 60X objective, make sure to place a drop of the Nikon immersion oil on the objective
  3. Remove cover to microscope stage
  4. Remove the stage the entire stage adapter
  5. Wash magnetic plate holder and cover with ethanol (be careful with cover glass, it is very fragile)
  6. Take lid off cells and place plate on the stage adapter
  7. Secure plate with magnetic holder
  8. Place stage adapter back into its original position
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  10. Make sure the light source is turned on
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      (d) For each channel in your experiment:
          - Change to the appropriate channel in the Camera Tab
          - Click use current image as shading image
  14. Readjust focus on cells
  15. Change the region of interest (ROI) to 1952x1952

- This is optional. There can still be shading issues after the fact around the edges so this can cut them out

16. Turn PFS on using the Ti2 Thinkpad
17. **Set Job Parameters in the ND Acquisition Tab:**
    - Ensure the only the Tabs that are needed for your experiment are checked
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    - Select the correct channels for your experiment in the lambda tab
    - Set parameters for your experiment in the Time Tab
    - Change settings in other tabs as needed for your experiment
    - Name file appropriately for your experiment
18. Run experiment
19. Remove sample from microscope and place all magnetic pieces back