Keyence BZ-X800 Fluorescence Microscope Instructions

- Fix cover slips to the slide; you can use nail polish
- Imperative to use oil with 100x
- Incubator (live cells). Regulate temp, CO_2, humidity
  → Prolonged studies
- Lift condenser before loading samples
- Load slides outside of the microscope
- Have cover slip face down; bottom right corner of slide less than 90°
- Writing on bottom and you should see the text (place on top)
- BZ-X800- viewer pics
  Analyzer- counts and image processing

★ Probably won't use other 2 software's

- Resolution sensitivity- binning- combine in single pixel
  → High resolution: 100x very fine structures
- Longer exposure- risk to photobleaching
- High sensitivity- higher
- Standard- 90% of the time sufficient
- Manual Focus
  → Scroll mouse- change Z (move lens up or down) (fine focus)
  → Hold control and scroll (coarse focus)

★ 100x manual and <u>NOT</u> autofocusing

- Navigation- find sample and creates map
- Correction collar- adjust lens (only for 20 and 40x)
  → Adjusts beam to compensate for thickness of the vessel
  → Ring with numbers on the lens
  → Will have to change for multi well plates
- Low photo bleach- excitation light not continuously on to preserve samples
- Multiple markers- multi color box on the top left of the screen
  → Channel cube
  → Camera setting auto
  → Layers different color images
- Image processing- per individual channel, then merge

★ 3 Main Options

1. Black Balance
   → Set square over background
   → Eliminates background, low level fluorescence
   → Keep sample parameters consistent- save conditions
   → Load conditions and pull up next file
   → Important for quantitative analysis

2. Lookup Table
   → Shadow similar to black balance
   → Highlight- brighter
   → Leave gamma
3. Haze Reduction
   → Looks for localized peaks
   → More crisp and clear images
   → Usually preview 2-3 gets the job done
   → Works well on fine structures; fluorescence
   → Won't work well with brightfield

- Overlay images
- Insert scale bar, numeric display, calibration setting micron per pixel
- Click "line image" after capturing photo to unfreeze microscope/screen
- X40-x100 (best frame of reference)
  → Drop immersion oil (gets through around 2 cover slips)
  → Lens paper to clean it
  → Pipette dropper to dab oil
  → Do not suck up air bubbles
  → White through catches extra oil
  → Only need small amount (about as much as a ball point pen)
  → Click yes after adding oil
  → Scroll down and don't do autofocus (it lowers lens and creates an air gap which presses back to the slide and creates an air bubble)
- Hold control, coarse focus and then go to fine focus
- Z stacking- scroll up until out of focus (top of sample)
- Scroll down to find bottom of the sample
  – Images in different planes
  – Z stack puts them together
  – Set upper and lower limit
  – Pitch= step size **0.3 microns**
  – Start capture takes multiple images for Z-stack
  – Analyze images and scroll with yellow bar
  – Load (L) bottom right corner
- Now we want to squish into one image (full focus, standard image)
- Z- stacking typically for thick samples, but could be used for cultures too (anywhere betweem cover slip and slide can be cells within water)
- Image tab- can do settings before capturing but then that will be the raw image. It also depends on your project requirements
- Menu- capture setting- save (reproducibility between samples)
  → Can also load settings from image file
- 100x-4x
  – Click wipe off oil off slide
  – 70% ethanol or sparkle lens cleaner

  - NEVER use elm wipe on lens (scratches lens)
  - Use optical lens paper. Fold over a couple of time
  - Add small bit of lens solution
  - Air out so it is not soaking wet
  - Clean only in circular motions gently
  - Move finger to the spots without oil
  - Another sheet of dry lens paper and wipe one more time
  - Clean white trough/ring
  - Dried oil can scratch lens
  - Best practice to just clean in between lenses. 40x highest risk of getting damaged
- Counting software- still cells
- Algae preservation?
- XY measure- count; click cells (> 100 cells)
- 2D measurements, area measurement
- 6mo. To test out software
  - Different pics/areas in slide
  - Number of slides
  - Extrapolations density to whole slide/sample
  - Take standard deviations between samples number of trials to find optimal sweet spot
- Algae- high sensitivity
- Colony counter
  - Food/beverage geared
  - Doesn't do sample prep
  - Separates on morphology
  - Check sheet
  - Count
  - Document
  - Transfer to excel