# Gallios Training

**Beckman Coulter Company Guide**
**https://www.beckmancoulter.com/ucm/idc/groups/public/documents/webasset/glb_bci_150264.pdf**

**Calendar for time sign up:**
**http://bidflow.calendarhost.com/**

**Sheath & Waste Tanks**

- Please check before starting
- External Tanks
  - Will beep/ notify if empty or full
- Reservoirs
  - Blue reservoir is cleansing solution

**Power Supply**

- Circuit breaker → on and off switch (hard re-set)

**Configuration box**

- Can change filters/ configurations
- Can take out filters/ swap around
- If you have to change check with someone in lab

**Drip Chamber**

- If running for 10+ minutes / sample then drip chamber must empty before next sample

**Carousel**

- Can set up tubes & leave
- Samples 400-500 ml volume per sample recommended for running (minimum 200 mL)

## Kaluza – Acquisition (software)

- Turning machine on/ off done via software
- Blue Orb- Acquisition (use to turn on)
- Red Orb- Analysis portion
  - Can analyze while samples are running

**Fluorescence Chanels**
**https://www.bsf.a-star.edu.sg/Ads%20Pictures/Gallios%20Filters%20Config.pdf**

**Main Menu**

1. FL1- FITC
2. FL2 – PE
3. FL3 -ECD/ PE Texas red/ PI
4. FL4 -PE CY5
5. FL5- PE CY7
6. FL6 –APC
7. FL7 -A700
8. FL8 – APC CY7
9. FL9 – Pacific Blue
10. FL10 – Pacific Orange

- Instrument Control →Initialize→ Cycles up instrument
- Clean → cleans lines (blue solution)
- Open
    - New protocol
    - Import old protocol
- Can choose to enable all detectors OR pick corresponding
- EDIT work list → edits carousel tubes
    - Duplicate (links to protocol)
    - Name tubes/ link to specific protocol → Done editing
    - Clear work list → resets/ deletes
- Choose output location → Users file → Make folder

**Instrument Controls**

- Acquire single (acquires one)
- Acquire → runs through entire sample
- Prime 3x → clears lines/ clogs
- Set up mode (blue) → acquires *without* saving
    - Use for setting up voltages, compensation
    - Dictate # events

**Acquisition Controls**

- Choose number of events to accumulate OR time
- Compensation – sliders
- Voltage – sliders
- Speeds → low, med high

**Plots & Gates**

- Histograms, Plots, Gates
- Add all plots (option)
    - Can set up to preference
    - Additional plots

**Running samples**

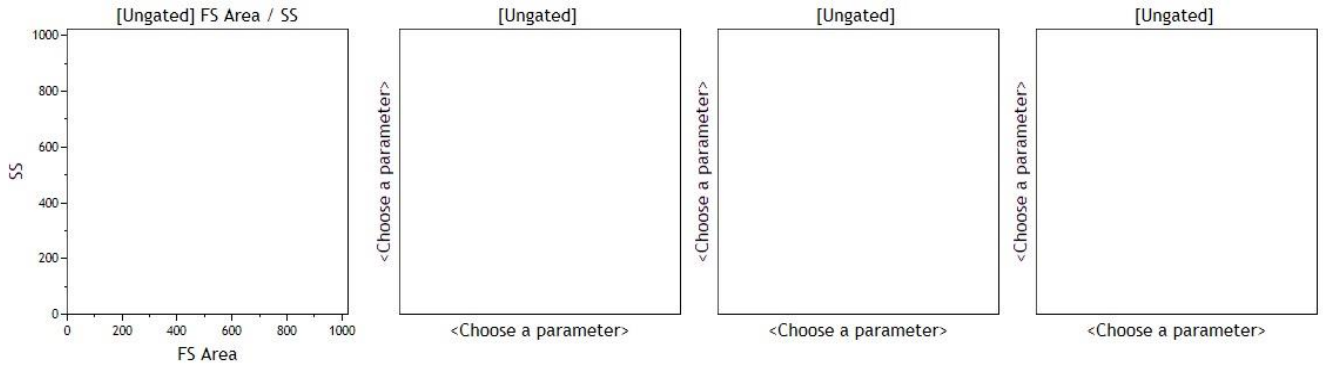
- Right click → Radial Menu → brings up options
- Features → Undo/ Redo

**Hardware**

- Discriminator → "Threshold"
    - Set to 100 by default→ can adjust based on sample
- Particle Size
    - Submicron: microparticles
    - Small: Basic whole blood components (3micron-20micron)
    - Large: < 20 micron
- Voltage → fine adjustment
- Gain → course adjustment

**Starting Experiment**

- Access Kaluza (blue orb) and load tubes accordingly
- Open
    - New protocol
    - Import old protocol
- Choose Proper Band Passes:
    1. FL1- FITC
    2. FL2 – PE
    3. FL3 -ECD/ PE Texas red/ PI
    4. FL4 -PE CY5
    5. FL5- PE CY7
    6. FL6 –APC
    7. FL7 -A700
    8. FL8 – APC CY7
    9. FL9 – Pacific Blue
    10. FL10 – Pacific Orange
- Instrument Control →Initialize → Cycles up instrument
- Prime 3x
- Enter Set up mode
    - # of events, amount of time, and speed of acquiring
    - Determine Threshold and particle size wanted
    - Create graphs and plots wanted for recording data
        - Basic Example:




- Acquire Single on unstained sample: KEEP SET UP MODE ON
    - Adjust Gain → then adjust voltages for FSC & SSC
    - Adjust Fluorescence channels
    - Rename graphs
    - Turn off set up to record data and run remaining sample
- Acquire Single on compensation controls: SET UP MODE
    - Adjust Gain → then adjust voltages for FSC & SSC
    - Adjust Fluorescence channels
    - Rename graphs
    - Can also type in via compensation matrix
    - Turn off set up to record data and run remaining sample
- Repeat above step for each control
- Acquire for rest of mixed samples
- Use set up modes to run through compensation controls
- Can save worklist & save compensation
- Hit Red Orb to go to Kaluza Analyzation software

**Kaluza Analyzis** (Red Orb)

- New Composite: can select all files → new composite → merges data sets
- Plots & Gates: new options from acquisition
    - Overlay plots
    - Comparisons