Analyzer Training Guidelines

- Follow the New User Instructions on our web page for how to access our services. A fully completed new user form must be on file before we accept any training appointments.

- 24 hour advanced notice for a cancellation is required to avoid the full charge for training.

- Training is instrument, fluorophore and application specific. Example: You will not be able to set up the flow cytometer by yourself to do cell cycle if you were trained to run GFP expressing cells.

- You must bring samples for training. The samples you bring should resemble the cells you will be using for future experiments. If at some point you change cell type you may need staff help to make adjustments to your instrument settings and acquisition template.

- Our training rate can be found on our web page. The first training session usually takes 2 hrs. The rest of your samples may take longer to run.

Test Tube Analyzer Training Guidelines

- Cells must be in a **Falcon 12x75mm polystyrene** test tube. No substitutions. No polypropelene tubes. No VWR or other manufacturer tubes.

- Samples must be in a very good single cell suspension. If you vortex and can see clumps, use Falcon 12 x 75 mm polystyrene test tubes to filter (Falcon #352235, VWR # 21008-948) cells to remove the clumps. A filter is built into blue cap. Gently put your pipet tip on filter and push cells through strainer into tube.

- Bring controls as outlined below. Controls should be brought to verify compensation settings and positive and negative cell boundaries each time you use the flow cytometer.
  - Unlabeled cells
  - Single color controls for each fluorophore
    - ⇒ GFP only
    - ⇒ PE only
    - ⇒ PI only
  - Secondary control only (if you are using primary and secondary antibodies).
  - FMO controls. Fluorescence minus one controls are samples labeled with all fluorphores except one. These controls help you set boundaries for positive and negative cells when analyzing samples.

- Minimum cell volume is 500ul. Maximum cell volume is 1 ml.

- Cell concentration should be about 1 million/ml.

- Resuspend cells in media or PBS with protein such as 5% FBS or 1%BSA. This will limit cell lose due to cells sticking to the test tube.

HTS (96 well robot) Analyzer Training Guidelines

- Use BD plates. Other plates may work but the well depth may be different. The result can be that you leave a significant amount of sample behind.
- Bring all controls outlined above in a **Falcon 12x75mm polystyrene** TEST TUBES. Follow the tube guidelines as outlined above.
- Fill 4 wells with 250ul 10% bleach and 4 wells with 250ul H_2O. If you do not have 8 wells to spare, bring an extra 96 well plate to clean the HTS.
- Add 250ul/well. You will lose 20ul/well due to the dead volume of the tubing. To keep the percentage of cell loss to a minimum, resuspend in a large volume (250ul).