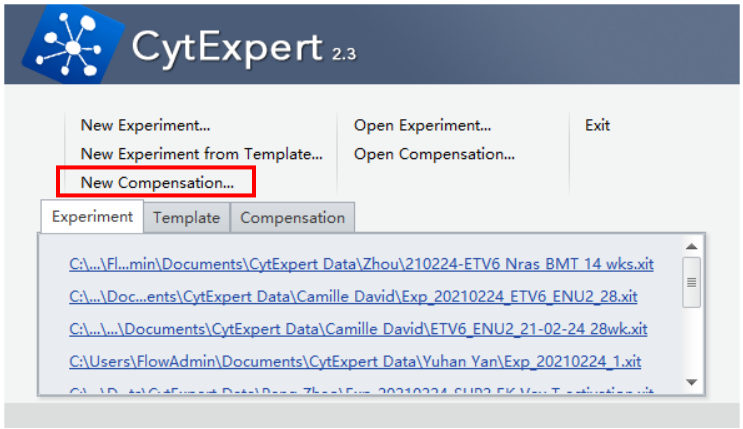
Creating Compensation File in CytExpert

1. Log into the PPMS screen using your Emory Username and Password
2. If the software isn't open, click on the CytExpert shortcut on the desktop

3. Log into the CytExpert software with your lab log in (ex. Username: Rae-Lab Password: facs123)

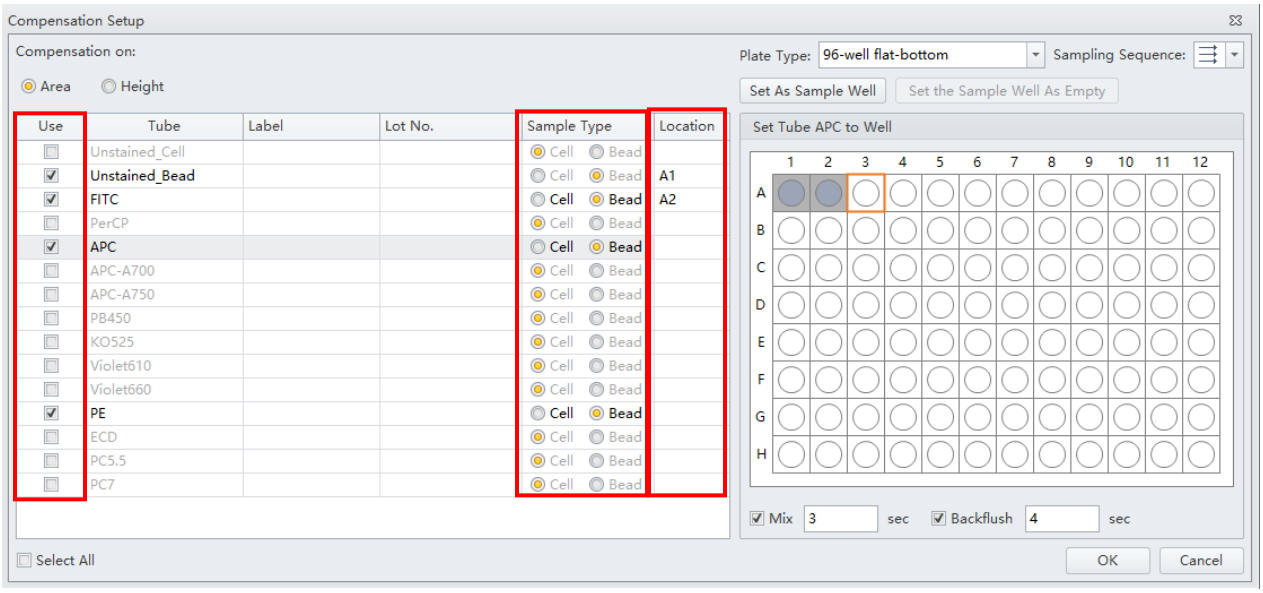
   a. Usernames are case sensitive so always capitalize your PI's last name and Lab, and don't forget the '-'
   b. The password is the same for all users. It is facs123

4. This is the screen you will see when you log in

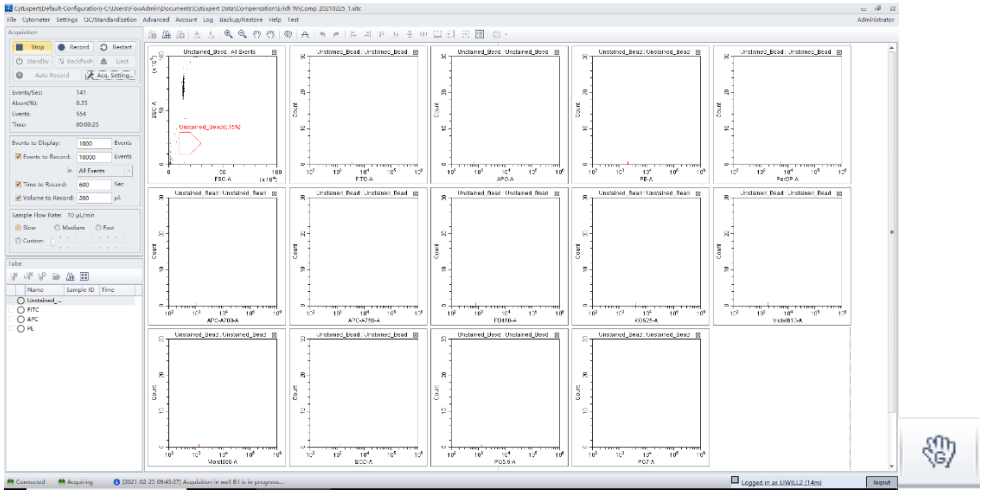


5. When starting a new experiment, you first need to create a compensation matrix to apply to it.
   a. Click on 'New Compensation…'
   b. In the file explorer that opens right click, create new folder with your name. You will store all compensation files you make on the Cytoflex in this folder.
   c. Name your compensation file and click save

6. A new window will open where you choose the channels that you are using based on the fluorochromes in your experiment.

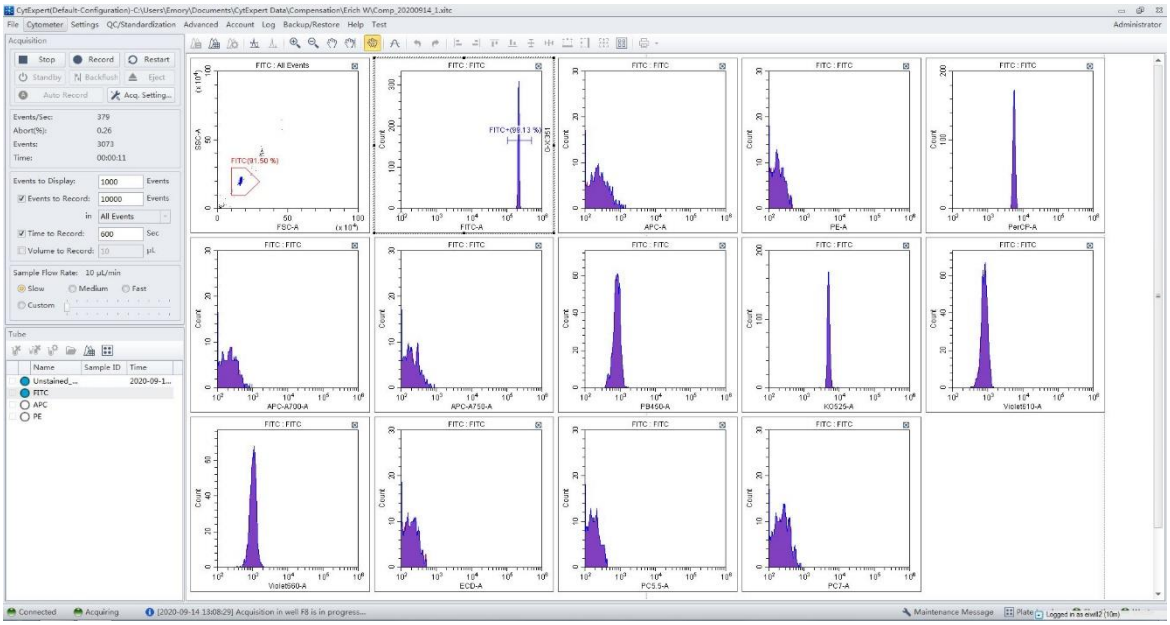


   a. If you are unsure what channel your fluorochrome is in, please refer to the filter set guide
   b. Click on the checkbox in the 'use' column to select your controls
   c. Under the 'sample type' column choose what you are using for your single stain controls
   d. Set the well location for your single stain controls
      i. Click on the row for your control in the 'Location' column so that it is highlighted in grey
      ii. Click the well location where your single stain is on the plate diagram
      iii. Click the 'Set as Sample Well' button above the plate diagram
   e. Repeat above steps for each control
   f. Once you are done click 'OK' at the bottom of the window
7. The software will open several pages of graphs (one for each control you will be using) and set up everything for you to run your controls



8. Once this is done (may take a few seconds depending on how many controls you have) you can begin to record your controls
    a. Load your plate
    b. Click on the 'Unstained' sample, you want to set your FSC and SSC settings to bring your target population onto scale
    c. DO NOT CLICK RECORD YET, only 'Start'
    d. Click on the hand tool with the letter 'G' inside of it.

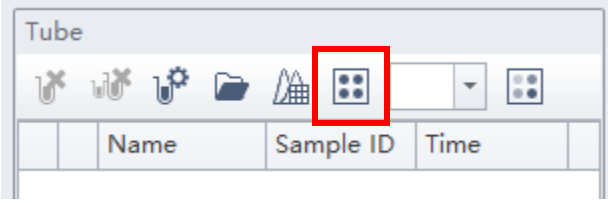
        i. This is the gain setting tool. By clicking and dragging your target population you can automatically set your gain values for those detectors.
        ii. Click and drag your target population onto scale, inside the gate displayed on the FSC v SSC graph
        iii. Click Record
    e. Repeat this process with each stained control, moving the gate as needed to enclose the peak of your fluorescence



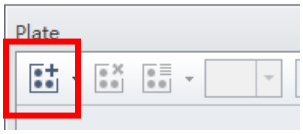
        i. You can add another decade to the display if needed
            1. Click on the small gear symbol at the top right of the plot
            2. Add another '0' at the end of
9. Once your data is recorded, click on the 'Cytometer' menu on the top right, then choose calculate compensation
    a. If there were no errors, you should get a compensation matrix displayed.
    b. The CytExpert software calculates the spillover into each channel for the compensation, even if you are not using all channels.
    c. This is OK, as you will tell the software which channels to use in your experiment.
10. Select 'Save As' on the bottom right of the Compensation Matrix window that pops up
    a. Save this matrix in the 'Compensation' > 'Your Name' folder that you made in the earlier step

Creating a New Experiment in CytExpert

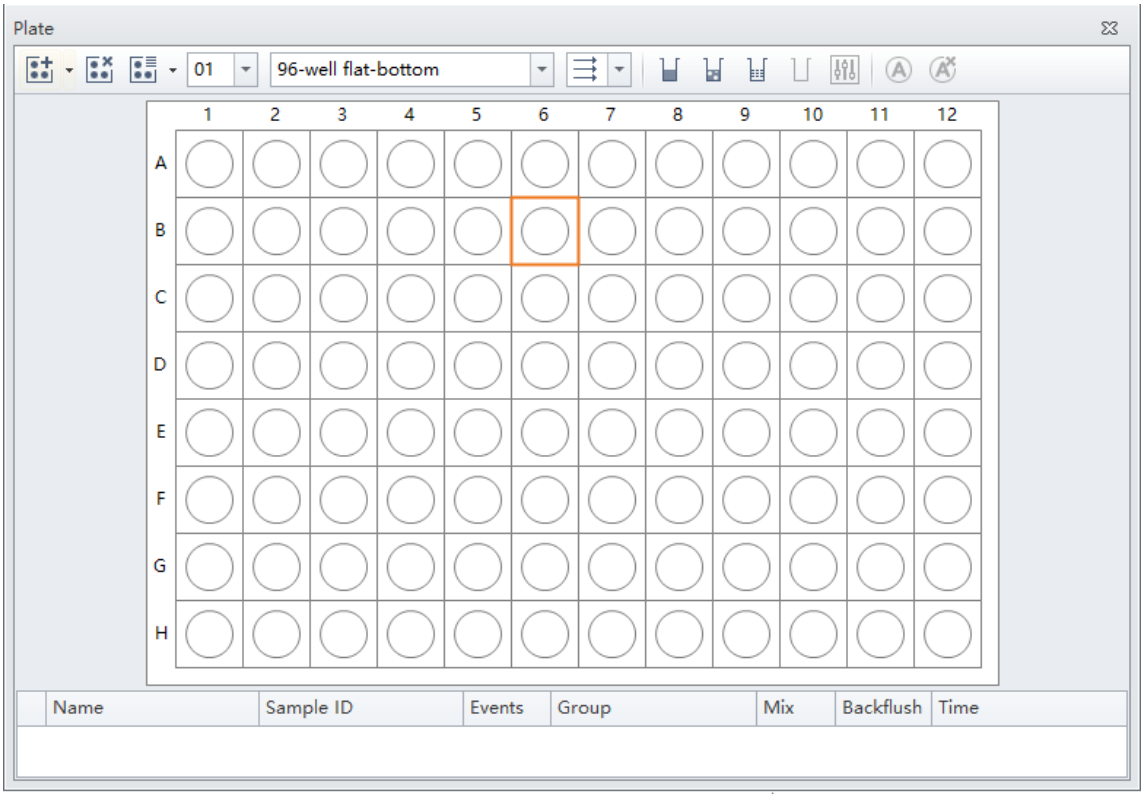
1. From the 'File' menu in the top right, select 'Create New Experiment'
2. In the file explorer that opens right click and create a folder with your name.
    a. You can store all your future experiments in this same folder
    b. Rename your experiment and click save
3. Add a plate to your experiment by clicking on the 'Plate' button under the Tube list



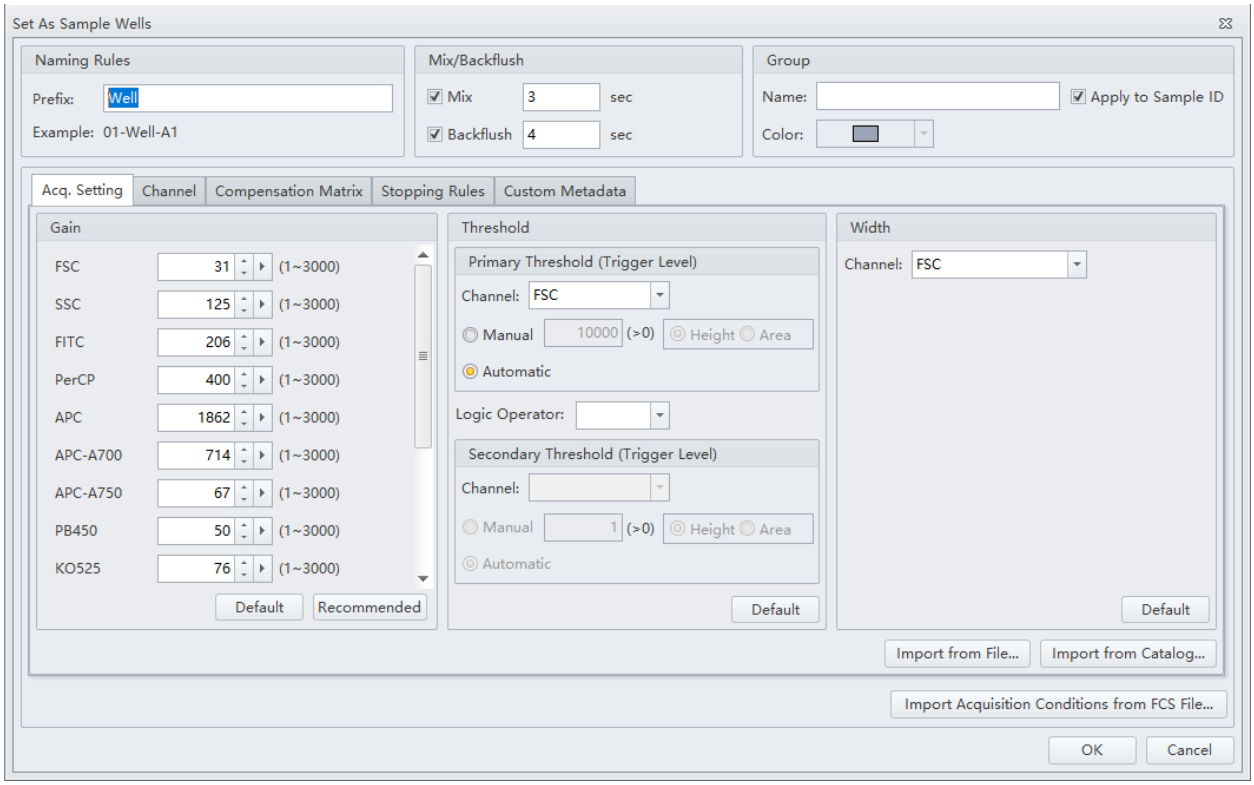
4. Click on the 'Add Plate' button in the window that opens up



    a. Select what type of plate you will be using (i.e. Flat bottom, V bottom, etc.)
5. Highlight the wells that contain your fully stained samples

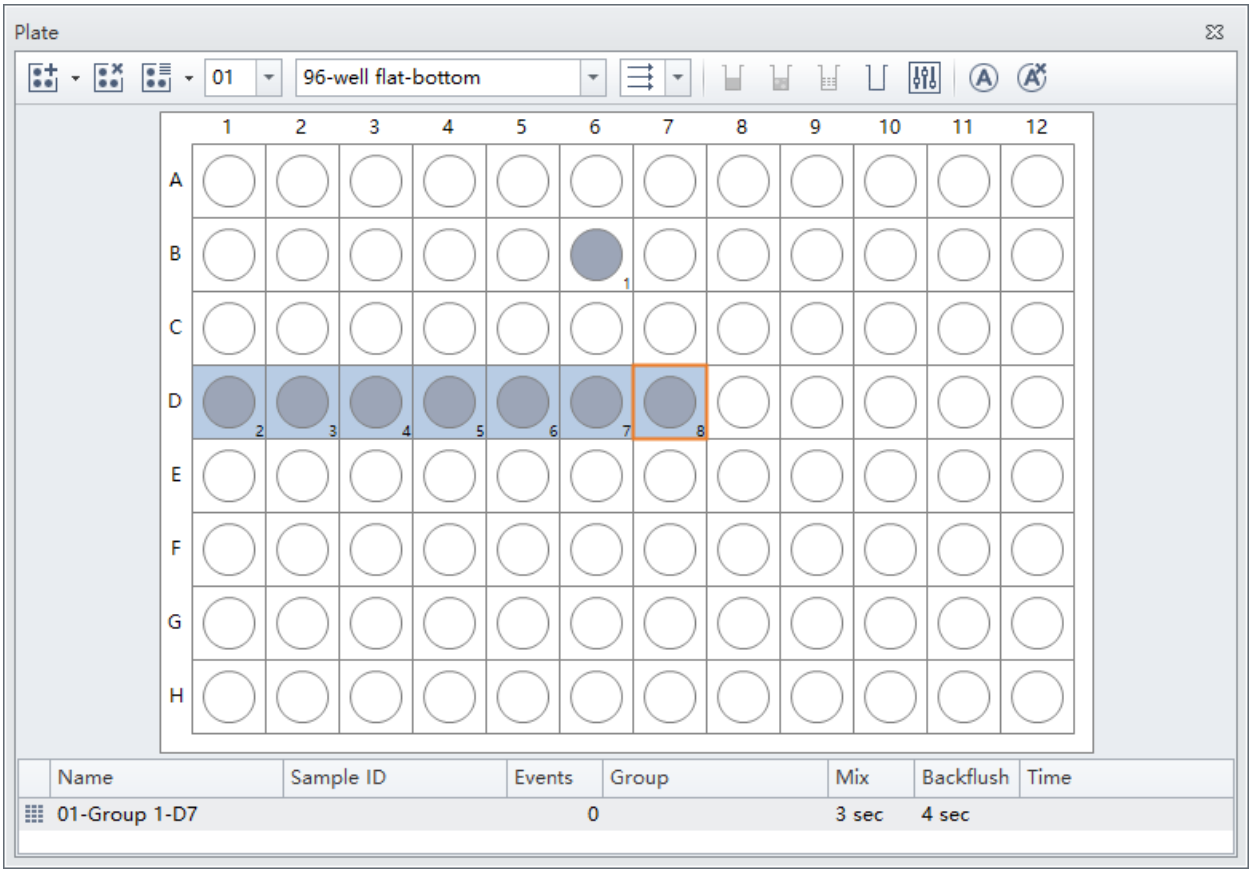


6. The following window will open for you to adjust the settings for your group



   a. First click on the Compensation Matrix Tab
   b. On the bottom right select ‘Import’
      i. Navigate to the folder you saved your compensation data from
      ii. Choose your compensation file (xxxxx.comp)
      iii. Select the option to import compensation matrix and gain
   c. Click on the Channel Tab
      i. Select only the channels you are using in your experiment
   d. Click on the Stopping Rules Tab
      i. Set your desired stopping conditions for your wells
         1. Number of events, target population, etc.
   e. When you are done with all the settings for the group click on OK on the bottom right

7. You can also set Auto Acquisition for your group.



   a. On the Plate window, highlight your group
   b. Click the button that is an A with a circle around it
      i. The Auto acquire setting allows you to select Auto Record under the controls to record your entire plate without intervention from you
8. Record your data
9. After your run, perform the Daily Clean using the cleaning plate at the machine
   a. 3 wells of the top row are filled with Clenz (blue liquid in squirt bottle)
   b. 5 wells of the second row are filled with DI water
   c. This step takes 12 minutes, so remember to book your time accordingly to leave enough time to clean before the next scheduled user
10. While the Daily Clean is running back up your FCS files to OneDrive
11. Once the Daily Clean is done close out of the software and log out of PPMS.