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)

	×
CytExpert 23	
Username Password	
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- 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

CytExpert :	2.3	
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- 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.

Compensati	ion Setup	Plate Type: 66-well flat-bottom Sampling Sequence: Image: Control of the second				
Compensa	tion on:					Plate Type: 96-well flat-bottom ▼ Sampling Sequence: 📑 ▼
Area	O Height					Set As Sample Well Set the Sample Well As Empty
Use	Tube	Label	Lot No.	Sample Type	Location	Set Tube APC to Well
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v	Unstained_Bead			🔘 Cell 🔘 Bead	A1	
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✓	APC			🔘 Cell 🛛 💿 Bead		
	APC-A700			🔘 Cell 🔘 Bead		
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	Violet610			🔘 Cell 🔘 Bead		
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	ECD			🔘 Cell 🔘 Bead		
	PC5.5			🧿 Cell 🔘 Bead		
	PC7			🔘 Cell 🔘 Bead		
						✓ Mix 3 sec ♥ Backflush 4 sec
Select A	All					OK Cancel

- 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

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- 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

Tube		
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Name	Sample ID	Time

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

Plate				
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- 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



Cytoflex Setup Version: 1.01

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

aming Pules		Mix/Packfluch	Group		
	N	VIIX/Backtiush	Group		
efix: Well	6	Mix 3 sec	Name:		Apply to Sample
ample: 01-Well-A1		Ø Backflush 4 sec	Color:	· ·	
Acq. Setting Channel Compensation Matrix	Stoppin	g Rules Custom Metadata			
Gain		Threshold		Width	
FSC 31 (1~3000)	^	Primary Threshold (Trigger Level)		Channel: FSC	-
SSC 125 ↓ ▶ (1~3000)		Channel: FSC -			
FITC 206 + (1~3000)		◎ Manual 10000 (>0) ◎ Heigh	nt 🔘 Area		
PerCP 400 1 + (1~3000)	=	O Automatic			
APC 1862 🗘 🕨 (1~3000)		Logic Operator:			
APC-A700 714 ↓ (1~3000)		Secondary Threshold (Trigger Level)			
APC-A750 67 ↓ (1~3000)		Channel:			
PB450 50 ↓ (1~3000)		◯ Manual 1 (>0) ⊙ Heigh	nt 🔘 Area		
KO525 76 🗘 🕨 (1~3000)	-	O Automatic			
Default Recomm	ended		Default		Default
				Import from File	Import from Catalog
				Import Acquisition	Conditions from FCS File
					OK Cano

- 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.