



THE UNIVERSITY OF
CHICAGO
BIOLOGICAL
SCIENCES

Aurora User Training Hands-on

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Startup Procedure

1. Check sheath and waste tanks. Fill/empty as needed
2. If the Aurora is not on, turn it on
3. Log into windows with your CNET ID
4. Open SpectroFlo
5. Run daily QC (optional if it has already been run today)
6. Switch to the acquisition tab to begin experiment

Running Daily QC



Cytek Assay Settings

- One of the easiest things about the aurora is that you don't usually need to adjust the gains (voltages) for every detector
- The the Cytek Assay Settings and daily QC automatically set the gains for you!
- When to run daily QC? Once a day.
 - **Always:** if you turned on the Aurora or the QC hasn't been run today
 - **Optional:** if someone else ran the QC today, you have the option to run it again or proceed to acquiring samples



What are the cytek assay settings?

- The term “cytek assay settings” has been used to refer to two things:

The gains set on the

Instrument Control  

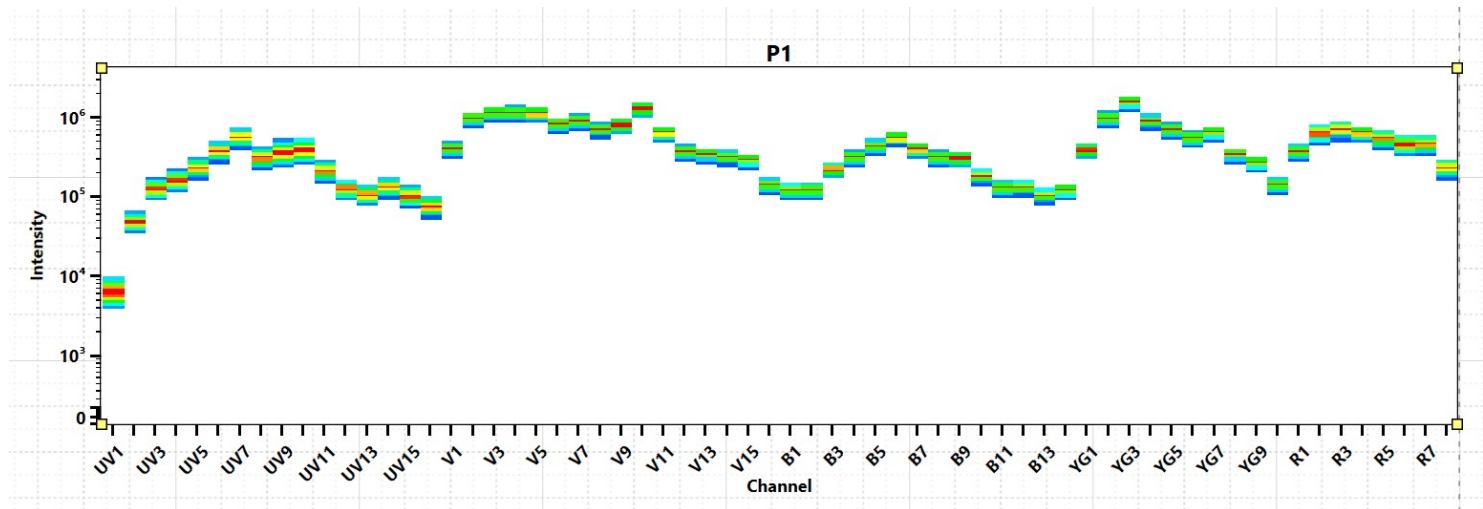
User Settings: CytekAssaySettings NEW 0719 (A ▾

 Save  Save As

GAIN	THRESHOLD	SIGNAL	LASERS
FSC	SSC	SSC-B	
176 ▾	422 ▾	180 ▾	
Violet	Blue	YellowGreen	Red
V1	V2	V3	V4
564 ▾	893 ▾	745 ▾	583 ▾
V5	V6	V7	V8
925 ▾	427 ▾	542 ▾	1,094 ▾
V9	V10	V11	V12
826 ▾	569 ▾	451 ▾	436 ▾
V13	V14	V15	V16
360 ▾	428 ▾	585 ▾	435 ▾

All Channels %: ▾

MFIs of the QC beads:



What are the cytek assay settings?

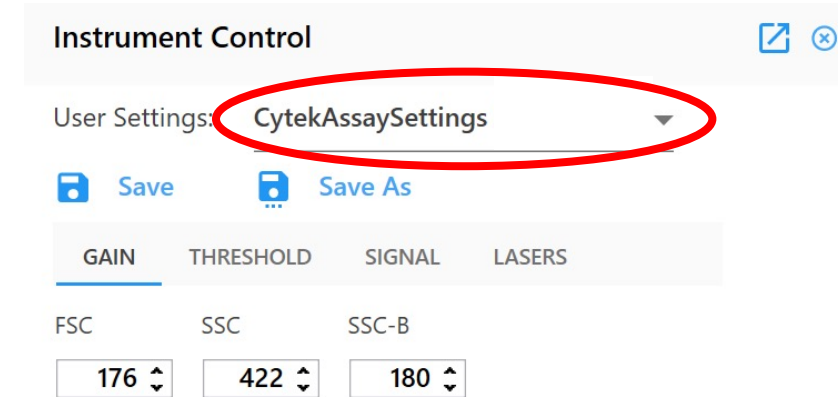
- At cytek headquarters, cells were stained with individual fluorophores and they determined the best gains for each detector:
 - each fluorophore has a distinct signature
 - minimize spreading errors after unmixing
- QC beads were run at those optimized gains to determine target MFIs for each detector
- The cytek assay settings on each machine are essentially the target MFIs determined at cytek HQ on their one specific aurora

What does daily QC do?

- Every day when daily QC is run, gains are automatically adjusted so that the positive peak of the QC beads match the target MFIs (the cytek assay settings)
- Therefore, every time QC is run, the gains will change slightly
- This works because the gains and the MFI are directly proportional:
 - If the MFI is 500, the gain is 1000 and you want to decrease the MFI to 250, then the gain should be set at 500.

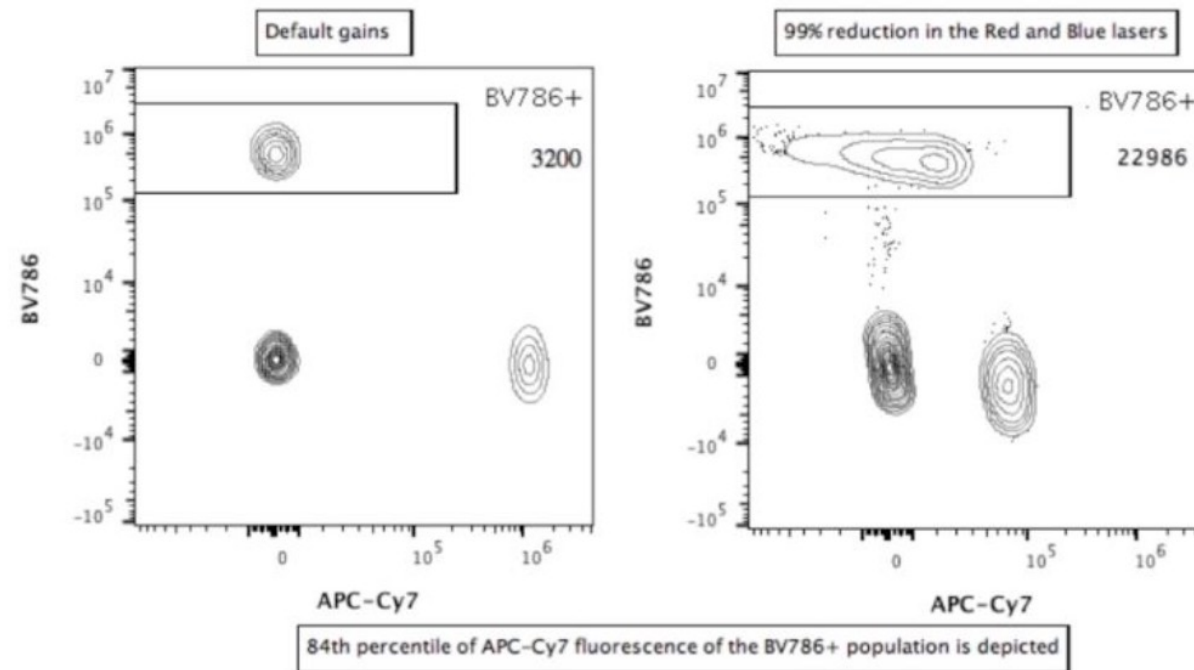
So what do I need to do when I start my experiment?

1. Run daily QC – always if you are first of the day
 - Dilute beads in water
 - Only reuse diluted beads if stored in the fridge
2. Open your experiment and check that the assay settings are set to “CytekAssaySettings”
3. Change your FSC and SSC as needed for your specific cells, but do not change any gains on the lasers without first talking to CAT Facility staff



What happens if you don't use the default Cytek Assay Settings?

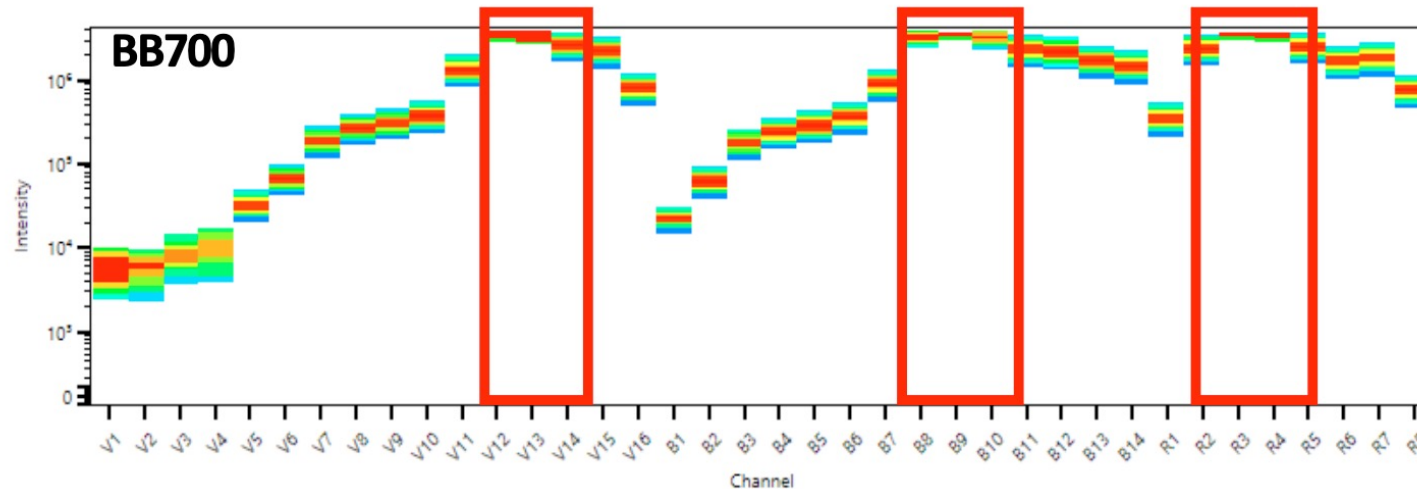
- Remember that the CAS are optimized for minimizing spreading error



Ferrer-Font, et al. <https://www.biorxiv.org/content/10.1101/784884v1>

When should the gains be lowered?

- If a fluorophore is off scale, you should lower the gains
 - Talk to David or Laura about how to do this
 - For your next experiment, use less reagent
- If you have a fluorescent protein that cannot be lowered (i.e. a reporter mouse)



Using the Instrument

Workflow for using the SpectroFlo software on the aurora

1. Run unstained cells
2. Preview a fully stained sample to check if any gains need to be lowered
3. Record reference controls
4. Spectral unmixing (recommended but optional at this step)
5. Run full stained samples
6. Analyze samples in FlowJo or FCS Express
7. (Spectral unmixing can be done again if needed)

What should you do at the end of your experiment?

- Please help us keep the aurora clean by running the **flow cell clean** when you have completed your experiment
- If you are the **last person of the day**, run a **fluidics shutdown** instead of the flow cell clean

There is a handy sign on the front of the aurora to help you fill the tubes easily!!!

