Bauer Flow Cytometry Core Aurora Description

Our custom built Aurora is a 5 laser system (355nm, 405nm, 488nm, 561nm and 640nm) that includes a built-in 96 well plate high-throughput unit. Unlike a traditional flow cytometer which collects only a narrow emission range for each fluor for each detector, the Aurora Spectral analyzer collects the emission between 400nm – 900nm across each laser for each fluorochrome (64 detectors for a 5 laser system, with all detectors being used for each fluor). This gives many fluors a unique 'fingerprint' allowing something that would look identical on a traditional flow cytometer to be distinguishable, such as GFP and FITC or APC and A647; for instance. Our 5 laser system has all spatially separated lasers and will be capable of allowing not only for the detection of any commercial fluor excited by these lasers but also potentially a 62 color multi-parametric assay (maximum fluors = # of detectors – 2). In addition, the Aurora can detect the spectral emission pertaining to cell auto-fluorescence and subtract that from all the channels, resulting in optimal data not possible on a traditional flow cytometer (a traditional flow cytometer can see excessive auto-fluorescence background in apoptosis assays or drug treated cells in most channels off the 488nm, 405nm and 561nm lasers leaving only a few fluor options off the 640nm laser to use in an experiment).

Other cool features of the Aurora include a patented flat top laser beam shape, which not only allows for a sample to be run fast (high sample pressure) without data deterioration (as commonly seen on other instruments), but when used along with the 405nm SSC, the Aurora can detect particles as small as 100nm (50nm possible when triggering off fluorescence). The Aurora is also volumetric so you can easily do cell counts along with multi-parametric analysis.

Cytek has some nice panel design tools available on their website; https://spectrum.cytekbio.com/.

Aurora Spectral Analyzer Instrument Overview

Fluidics:

The fluidics is vacuum based. The sheath fluid used is MilliQ distilled water.

Optics:

A conventional flow cytometer uses various mirrors and band pass filters to separate and collect a narrow range of each fluor emission, with one detector dedicated to each fluor.

A spectral flow cytometer uses dispersive optics, such as prisms or gratings, to disperse the emitted light across a detector array, allowing the full spectra for each fluor to be measured by all the detectors. Spectral unmixing can then be done for data analysis.

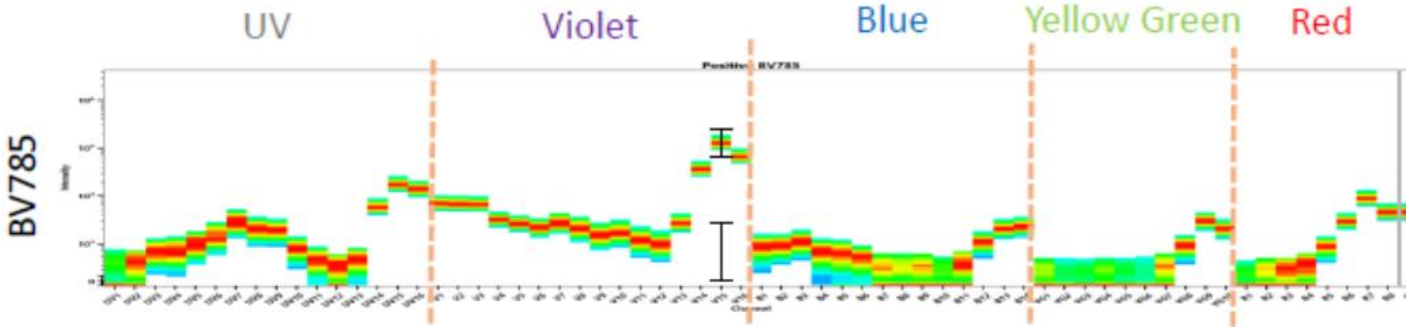
Unique Optical Design

•High Sensitivity Collection Optics

•Lasers are spatially separated. Each excitation laser has an associated solid state multi-channel semiconductor array detector module (UV=16, Violet=16, Blue=14, Yellow=10 and Red=8).

Full Spectrum Analysis

•Entire emission spectrum is captured across the different modules and then stitched together to create a spectral signature that combines emission information from all five excitation wavelengths.



Spectral Unmixing

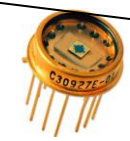
•Spectral unmixing algorithms calculate the contribution of each known fluorophore's spectra to the total collected emission signal.

Electronics:

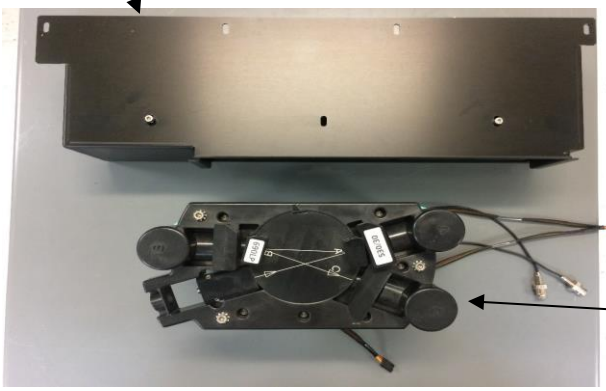
-Highly sensitive.

-80% Quantum efficiency.

Aurora Spectral Analyzer



Aurora: 64 Avalanche Photodiodes (APDs) spread across 5 spatially separated detector arrays. Very compact!



LSRII: 3 Photomultiplier tubes (PMTs) in 1 detector array. 5 detector arrays would take up an entire optical bench!

Traditional Analyzer

Software:

The Aurora uses SpectroFlo software, which is necessary for unmixing the data. Once it is unmixed, it can be analyzed on FCS Express or Flowjo software.

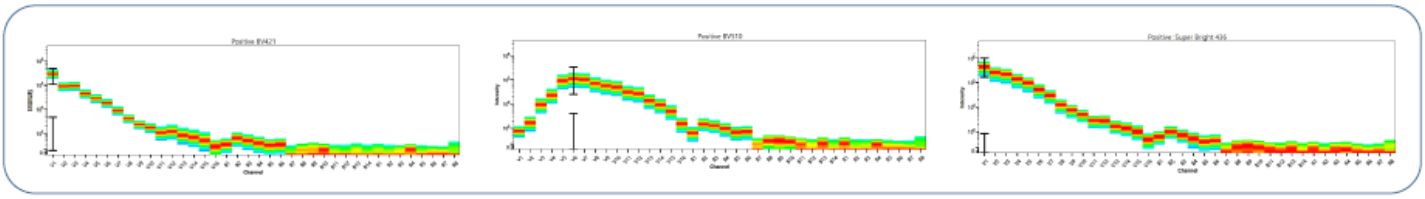
Running an Experiment on the Aurora:

- Samples are prepared and stained exactly like you would for a traditional flow cytometer.
- SpectroFlo is similar to DIVA so it shouldn't be too hard to learn.
- Setup for a large multi-color experiment is way easier on the Aurora since you generally don't need to adjust detector voltages. Also, many of the fluorochrome spectra can be saved for subsequent experiments.
- Making a new experiment on the Aurora consist of verifying that everything is on scale and if so, define and run your unstained and single stained controls (Reference Controls) in a 'Raw Worksheet', unmix and analyze in an 'Unmixed Worksheet'.
- Because the spectral unmixing is done based on the Cytek algorithm, you must have an unstained control and proper single stained controls for each fluor.
- Currently unmixing can only be done in SpectroFlo software, but once done, the unmixed data can be analyzed in FCS Express or Flowjo software.

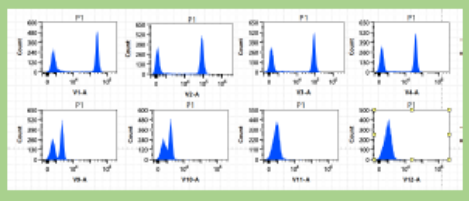
Aurora Workflow Overview

1. Run **UNSTAINED control**
2. Run **individual dye spectra controls (Reference Controls)**
3. Unmix (equivalent to Compensation step in conventional cytometer)

Reference Spectra from Single Stain Controls



Raw Worksheet



Unmixing Algorithm

Unmixed Worksheet