Introduction to Spectral Cytometry using Cytek® Aurora and Northern Lights
Day 1
Objectives

By the end of Day 1, you should be able to:

• Analyze full spectrum signatures
  ✓ Understand how full spectrum signatures are generated
  ✓ Identify unique signatures
  ✓ Normalize spectrum signatures
  ✓ QC and troubleshoot signatures
Spectral Cytometry
A little background
Trends in Cytometry

Trends in Cytometry

1980  1 Laser, 2 Colors
1990  2 Lasers, 4 Colors
2000  3 Lasers, 8 Colors
2010  3 Lasers, 13 Colors
2017  Aurora: 3 Lasers, 20 Colors
2019  Aurora: 5 Lasers, 40 Colors
Flow Cytometry Review

**Fluorescence**
- Laser Beam
- Cuvette
- Flow Cell

**Light Collection**
- Dichroic mirrors
- Filters
- Detectors
- Photomultiplier Tubes (PMT's)

**Electronic measurement**
- Time (microseconds)
- Volts

**Data**
- Flow cytometry graph

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Flow Cytometry is a laboratory technique that uses a stream of cells to passively through a small aperture, where they are illuminated by a laser and their light scattering and/or fluorescent properties are measured.
Quick Poll #1

How many fluors have been successfully run together on a 5 Laser Aurora?
What are we looking at?

Is this the whole picture?
Conventional Detection of FITC

FITC = Green! ... or emission from 515-545 (530/30 BP)
Spectral Flow Cytometry

Conventional flow cytometry uses mirrors and filters to select specific wavelength ranges for detection of signal from different fluorophores on individual PMTs. Spectral flow cytometry uses dispersive optics, such as prisms or gratings, to disperse the collected light across a detector array, allowing the full spectra from each particle to be measured.

Spectral Detection of FITC

FITC = Full emission from 490-650
Cytek’s Approach

**Unique Optical Design**
- High Sensitivity Collection Optics
- Lasers are spatially separated
- Dedicated detector array

**Full Spectrum Analysis**
- Spectral signature created via capture of the entire emission spectrum

**Spectral Unmixing**
- Calculates the contribution of each known fluorophore’s spectra to the total
What Kind of Detectors Are in the Aurora?

### Avalanche Photodiodes (APDs)
- 3 APD detector modules – up to 50 Channels

### Photomultiplier Tubes (PMTs)
- 1 PMT detector module – 3 Channels
High Sensitivity APDs

• Quantum Efficiency is the ability of a detector to convert photons into electrons

• APDs have higher QE than PMTs across a broader range of emission wavelengths

Data from Hamamatsu Photonics
## 5 Laser Aurora: Detector Arrays

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### Red
The superior sensitivity of Cytek Spectral Cytometers is from light detectors known as:
5 Laser Aurora Design
Building a Spectral Signature
Full Spectrum Signatures

- Fluorescent dyes excited by the onboard lasers are measured.

- Measurement is from the laser line to the infrared region.
Full Spectrum Enables Use of Highly Overlapping Dyes

Markers that are co-expressed CAN effectively be used in combination

Plot gated on singlet lymphocytes
Validating Data Obtained Using Highly Overlapping Dyes

BV711

Qdot 705

CD3- Lymphocytes

CD3+ Lymphocytes

Control

Test
Overlay spectra to determine degree of similarity

- 1 = spectra match perfectly
- 0 = spectra are completely different
Complexity Index: Assess Overall Panel Dye Choices

• Overall measure of uniqueness of all dyes used in the panel
  • Lower complexity index = easier combination of dyes to work with
  • Useful for assessing fluorochrome selection in panel design

• Example Complexity Index Values:
  • 10 Dyes at Left: 2.5
  • Cytek 35 Color Panel Dyes: 46.4
Quick Poll #3

How many fluorescent light detectors are in a 5 laser Aurora?
Exercise 1 - Handout

Aims:
• Identify Peak emission channels for fluors
• Identify secondary emission channels for fluors
• Identify exciting laser(s)
• Distinguish Unique signatures
Part 1 – Understanding Signature Characteristics

Exciting Laser(s): ALL
Peak channel: YG9
Secondary Peak Channel(s) UV15, V15, B13, R7
Part 2 – Can these fluors be used together?

YES
Fluorochrome Selection Guide Review

Unique signatures
Fluorochrome Selection Guidelines

https://cytekbio.com/blogs/resources/tagged/data-sheets
By the end, you should be able to:

- Understand the basics of flow cytometry
- Understand spectral flow cytometry
- Recognize unique spectral signatures
  - Recognize raw signature vs normalized signatures
  - Be able to troubleshoot from the signatures
- Use Spectral Unmixing
  - Workflow
  - Reference controls
- Apply the tools of Panel design
  - Concept and tools
  - Validation
  - Control optimization
- Apply Instrument setup concepts to sample optimization
  - CAS
- Generate high quality data
  - Data analysis and troubleshooting
Questions?

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