

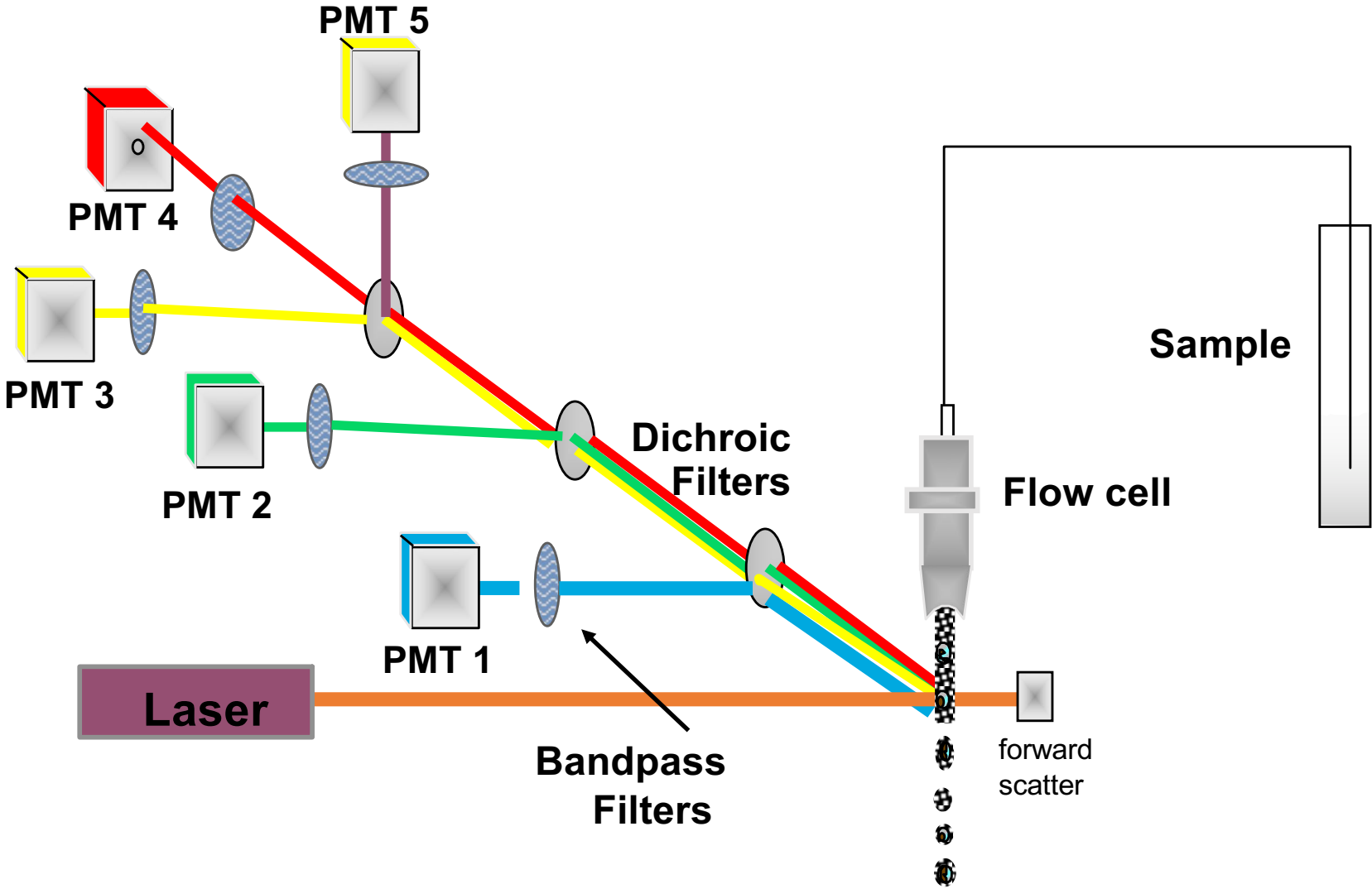
# Refresher: Conventional Flow, Spectral Flow, CyTOF



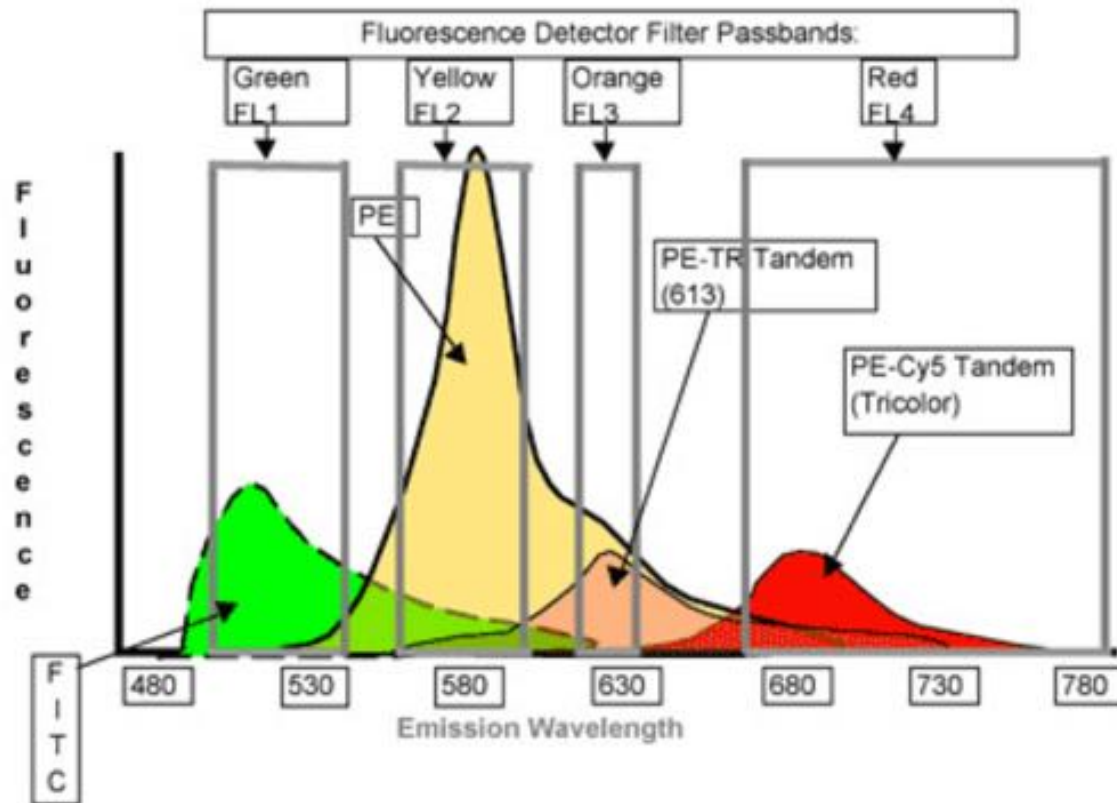
Lisa Borghesi  
Professor of Immunology  
Director, Unified Flow Core

# Conventional flow cytometry

“one detector, one color”



# Spectral Overlap



From Practical Flow Cytometry Third Edition, Howard M. Shapiro p.164.

# Our high speed analyzers

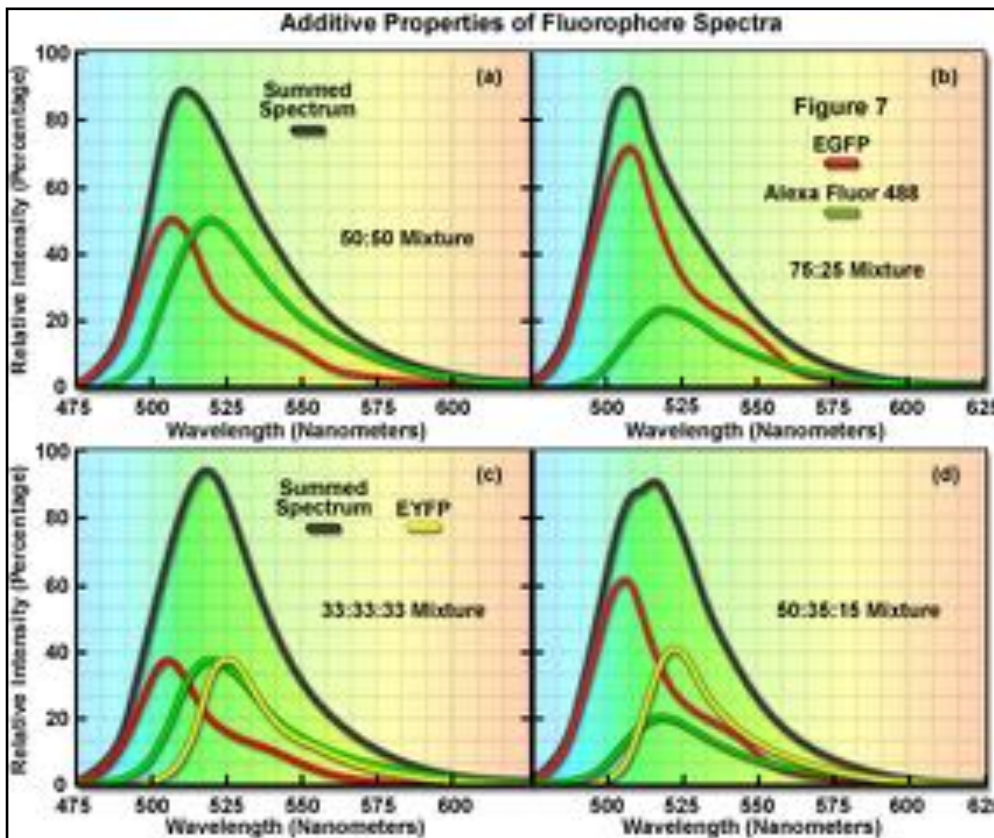
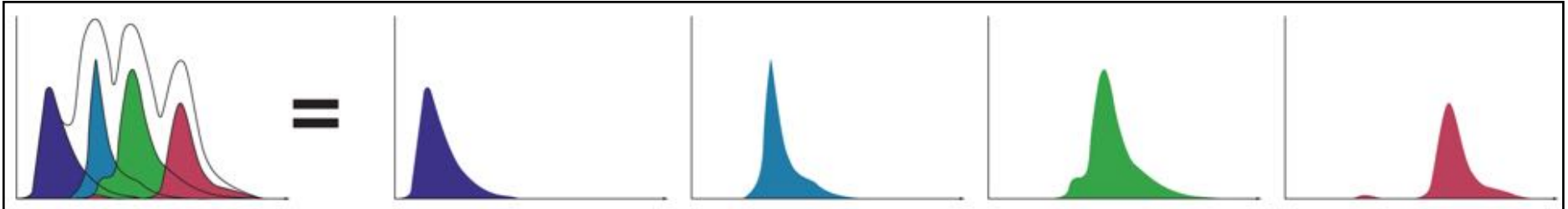


3 BD LSRFortessa, 2 BD LSRII, 1 NxT Attune  
= conventional



3 Cytek Auroras (2 in Unified Core, 1 at HCC)  
= spectral

# Spectral flow cytometry: Cytek Aurora



collect ALL of the light →  
increased sensitivity

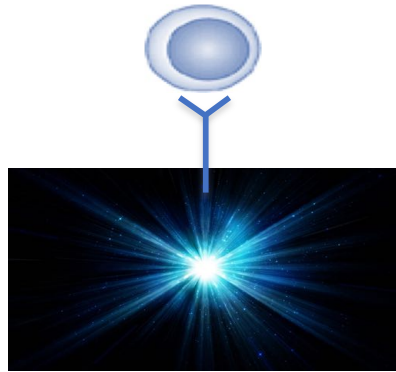
discriminate fluors with  
overlapping spectra → not  
possible with conventional flow  
cytometry

64 channels

# Spectral cytometry rivals CyTOF capabilities

## FACS

Antibodies labelled with **fluorochromes**

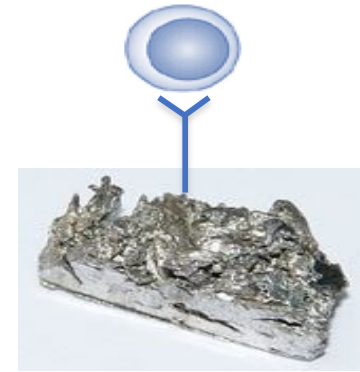


Conventional cytometry, 10-15 colors; beyond that gets tough

Spectral technologies = game changer, 64 channels

## CyTOF

Antibodies labelled with **elemental isotopes**



40-50 parameters in a single tube

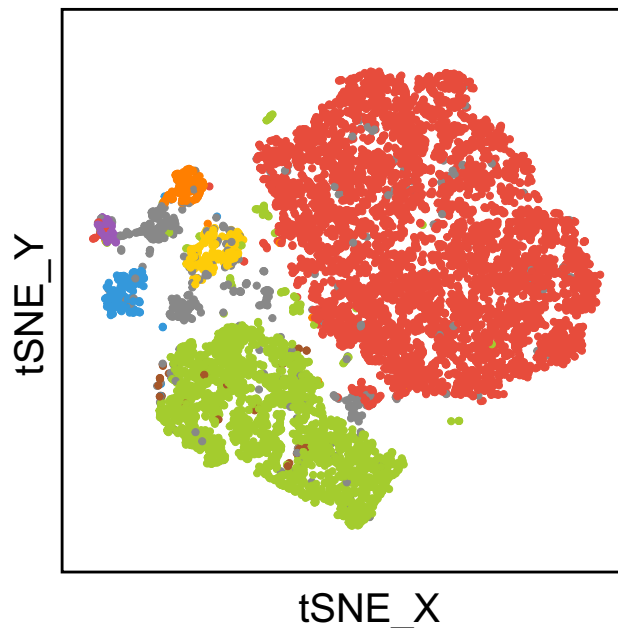
Ex. gadolinium (Gd), samarium (Sm), terbium (Tb), thorium (Th), ytterbium (Yb)

# Pitt Aurora Flow Panels Repository

- Panels for each mouse and man
- Designed/validated by our Pitt colleagues
- Excel spreadsheet located in same PittBox that contains this ppt

Thanks to our users for sharing their panel designs!

# Next Tutorial: Harness the Full Information Content of Your Data with Algorithmic Analysis



- **High-dimensional analysis** – Human brain is not so hot at >3 dimensions.
- **Highlight patterns in the data** – Manual analysis with iterative bivariate plots is highly inefficient.
- **Facilitates population discovery** – Populations can be overlooked b/c biases and a priori knowledge dictate analysis.
- **Fun!**



# Acknowledgements



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