



Latest Innovations in Flow Cytometry from Kinetic River

June 10, 2020

Webinar

Giacomo Vacca, *President*

Kinetic River Corp.



Innovations in Flow Cytometry

- *Potomac*: Kinetic River's flagship customizable, modular FC – **a tool for unique problems**
- Time-Resolved Flow Cytometry
- *Arno 1.0*: compensation-free FC – **simplify your assays**
- *Arno 2.0*: high-parameter-number FC – **immunology for less**
- *Tiber*: label-free FC – **cancer diagnostics**
- *Danube*: fluorescence lifetime FC – **protein analysis in cells**
- Q&A (but also feel free to ask as we go)



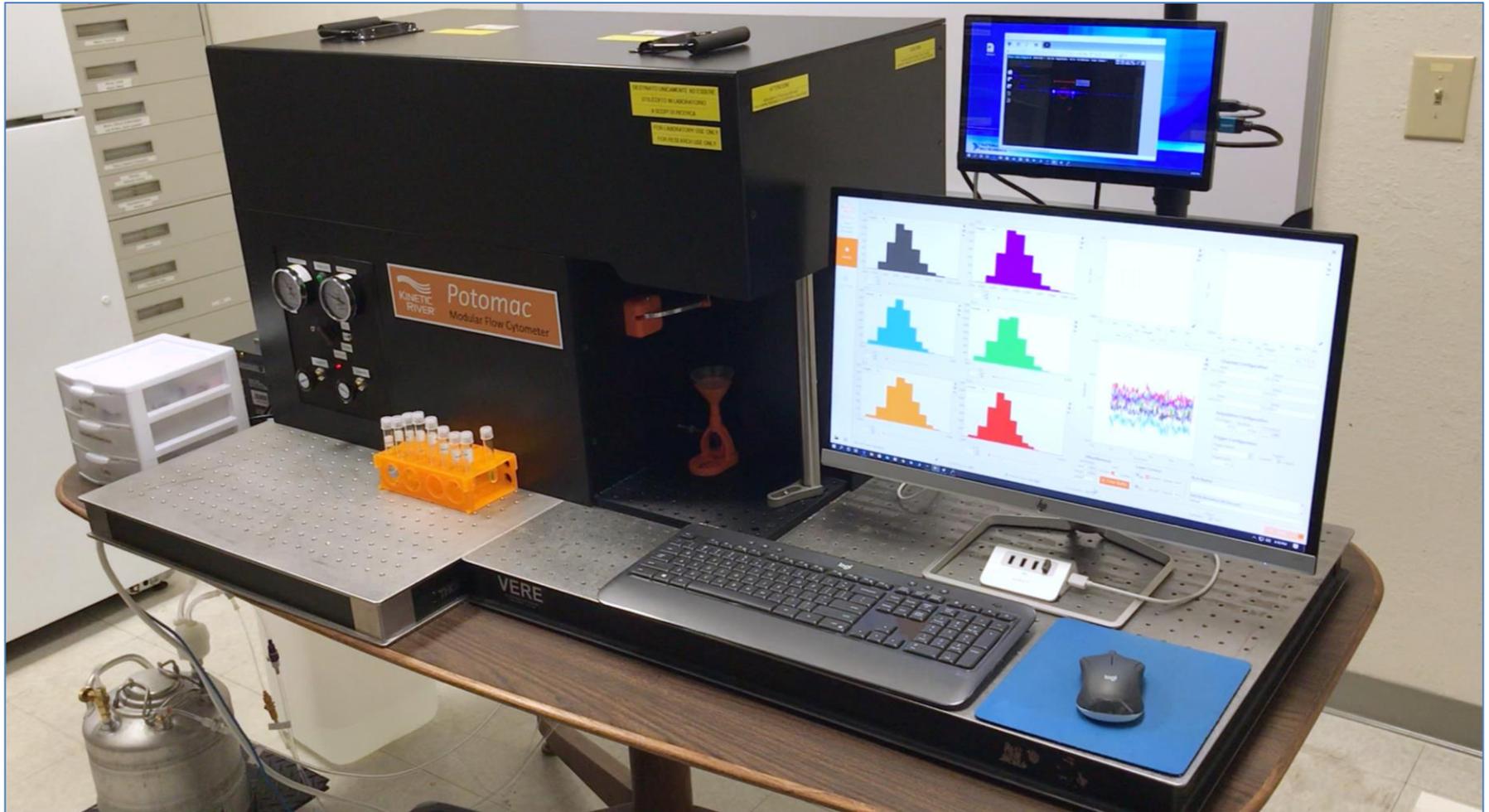
OUR CELL ANALYZER PLATFORM

POTOMAC

**CUSTOMIZABLE, MODULAR FLOW CYTOMETER
A TOOL FOR UNIQUE PROBLEMS**



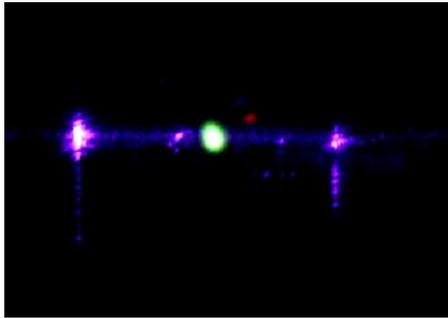
Potomac: Customizable, Modular FC



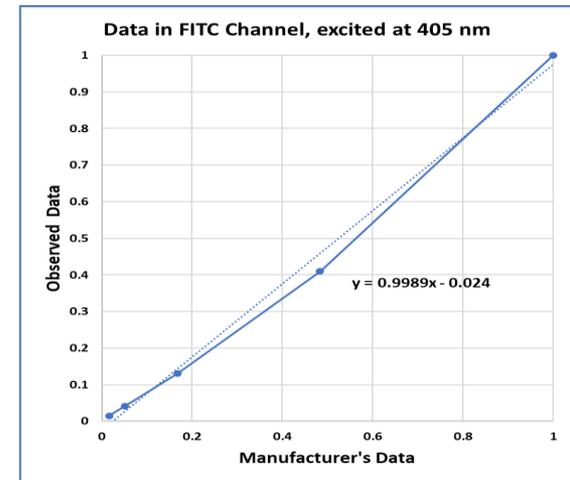
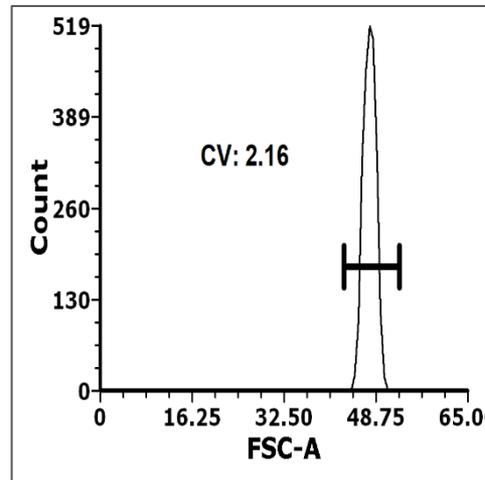
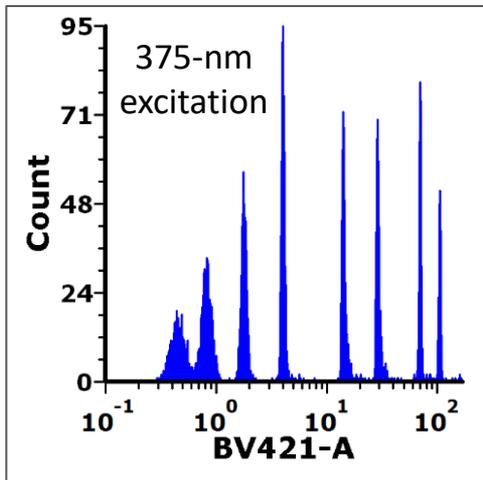
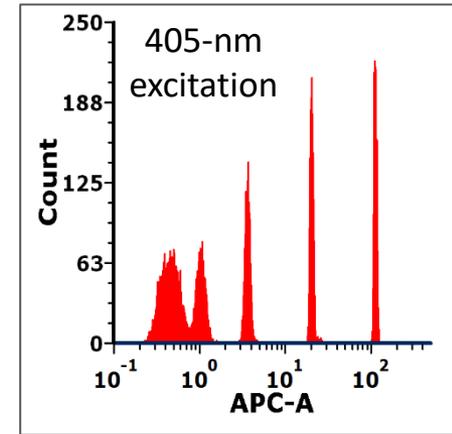
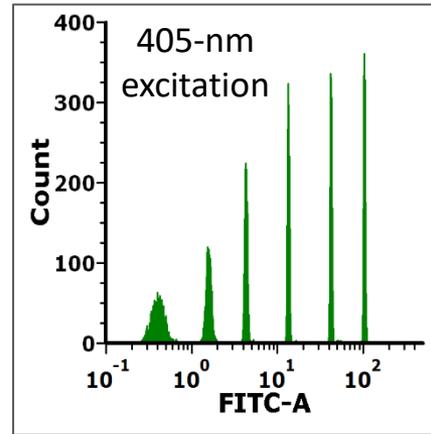
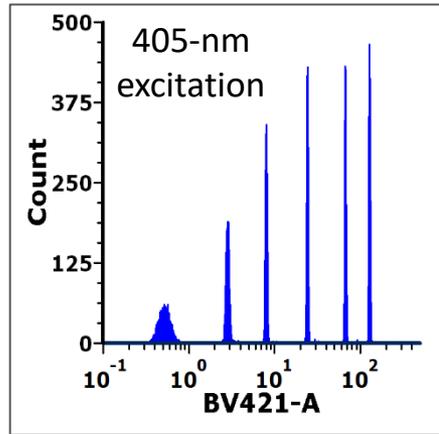
Potomac: Customizable, Modular FC



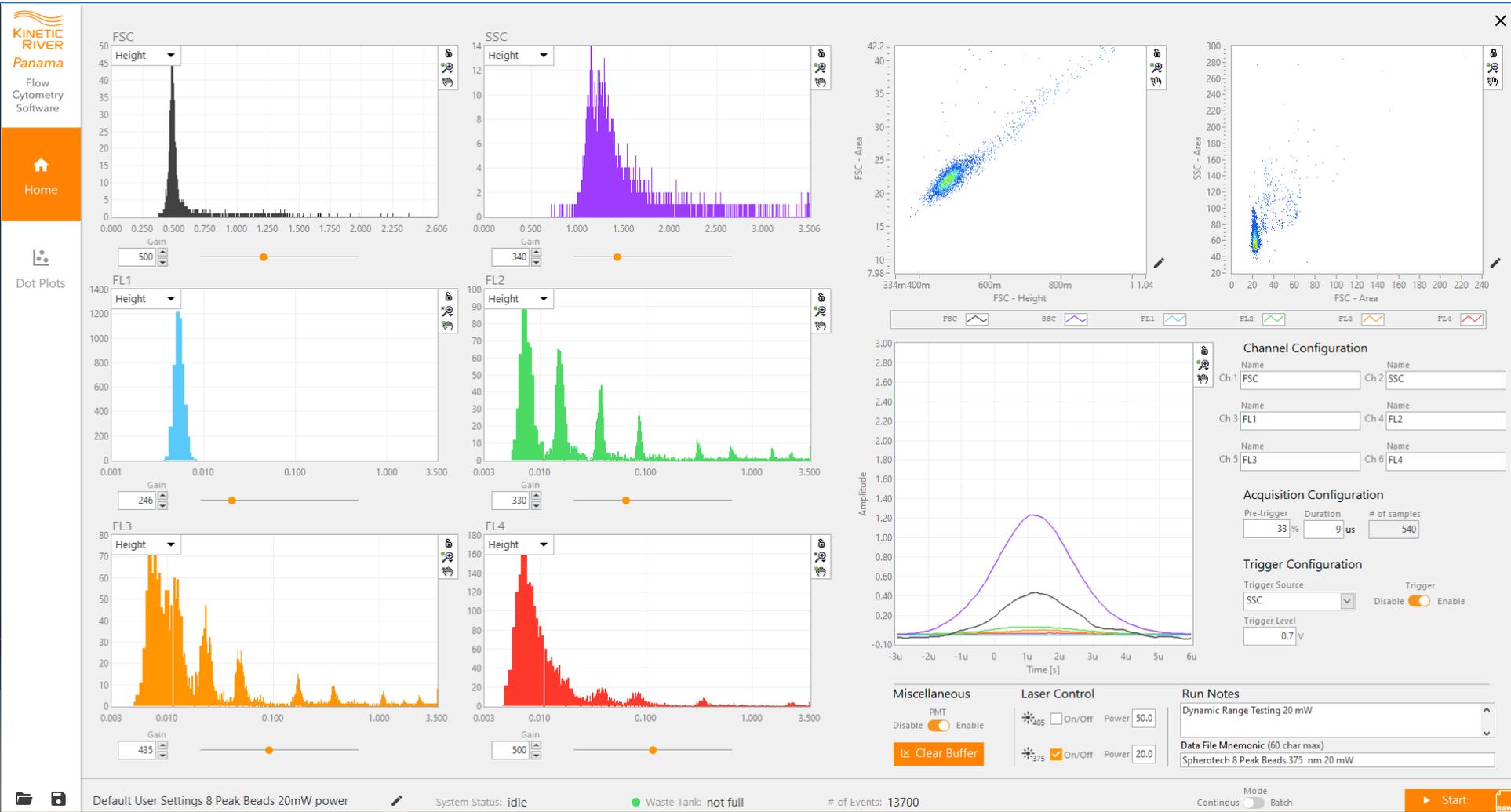
Potomac: Performance



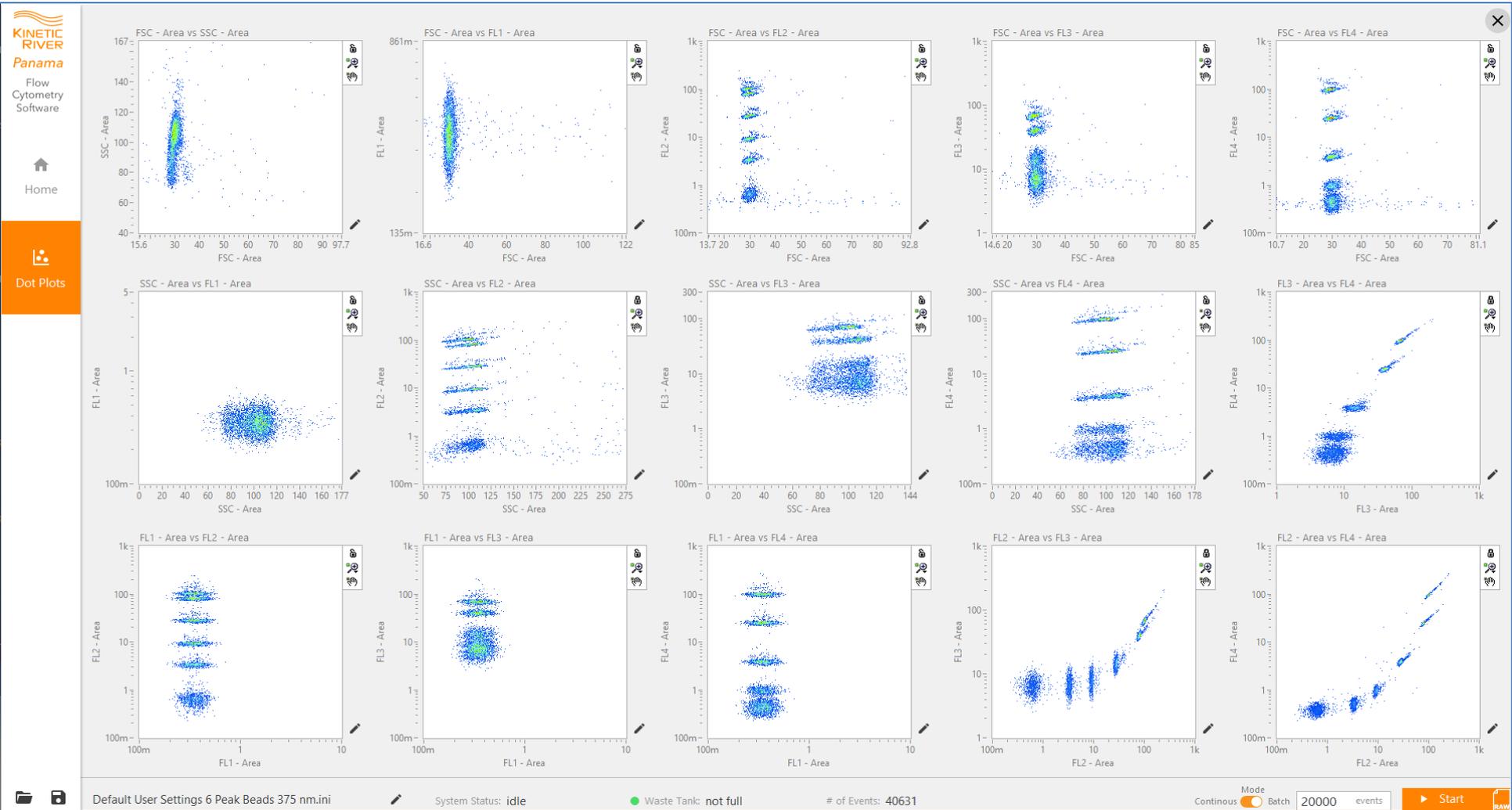
sample flow rate:
18 uL/min →
130+ uL/min



Potomac: Panama Software



Potomac: Panama Software



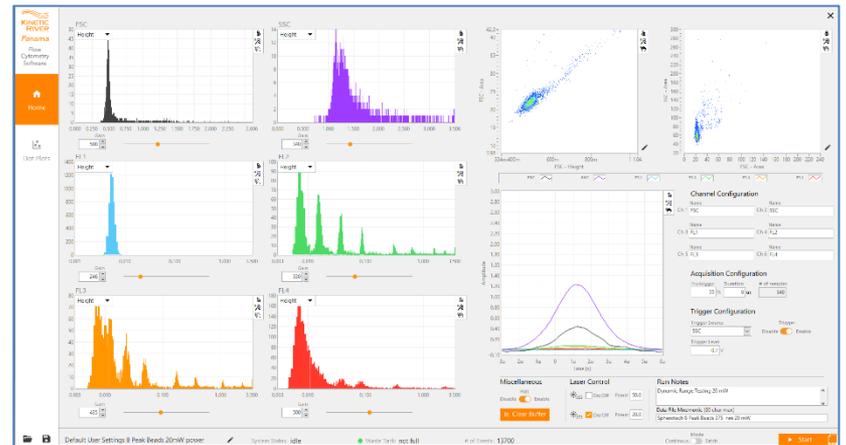
Potomac: Built to Order

- Unit for NCI
 - excitation at 488, 266 (external)
 - FSC, SSC, 5x FL channels
 - swappable excitation arm
- Unit for CNR (Naples, Italy)
 - excitation at 405, 375, 266 (external)
 - FSC, SSC, 4x FL channels; all PMTs for max sensitivity
 - flexible integrated *Shasta* fluidics
- Unit for Excelitas
 - excitation at 488, 405
 - FSC, SSC, 6x FL channels (SiPMs)
 - syringe-drive fluidics



Potomac

- modular
- customizable
- **any** laser you want (even **deep UV**)
- **any** detector you want
- **any** flow rate you want

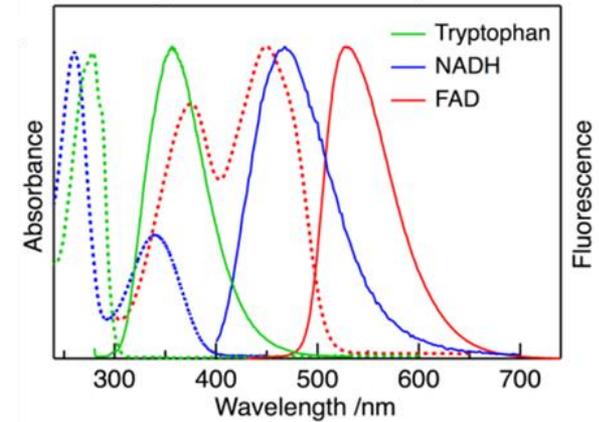
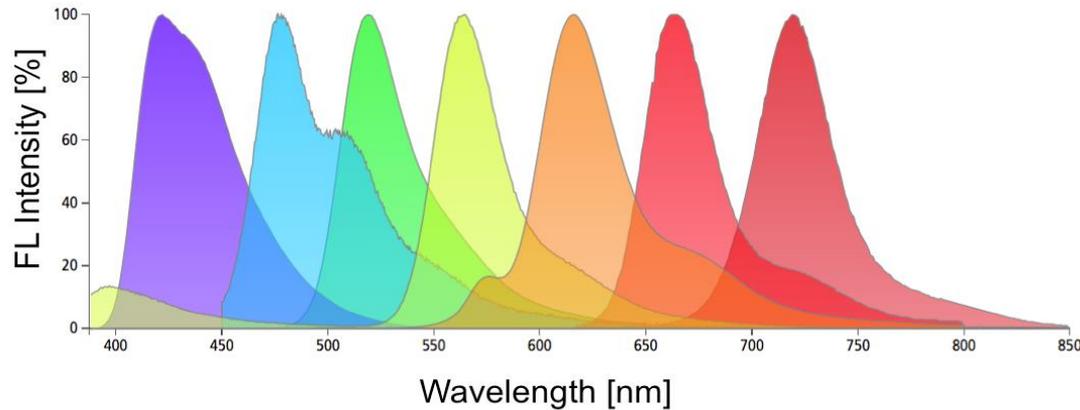


A BRIEF INTRO:

TIME-RESOLVED FLOW CYTOMETRY



Unmet Needs in Fluorescence Flow Cytometry



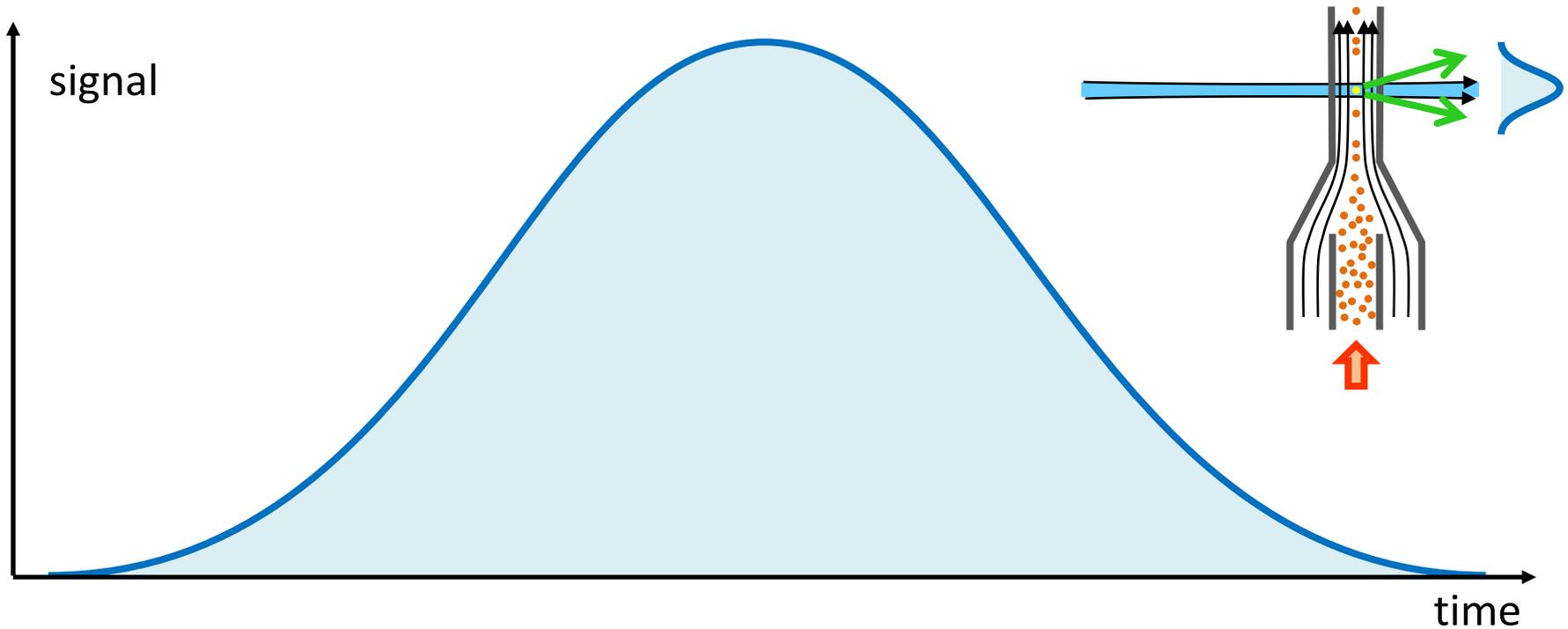
UNMET NEEDS:

1. Spectral spillover
→ compensation
2. Spectral spillover
→ limited # channels
3. Autofluorescence
→ high background

Measurement of Cellular Autofluorescence

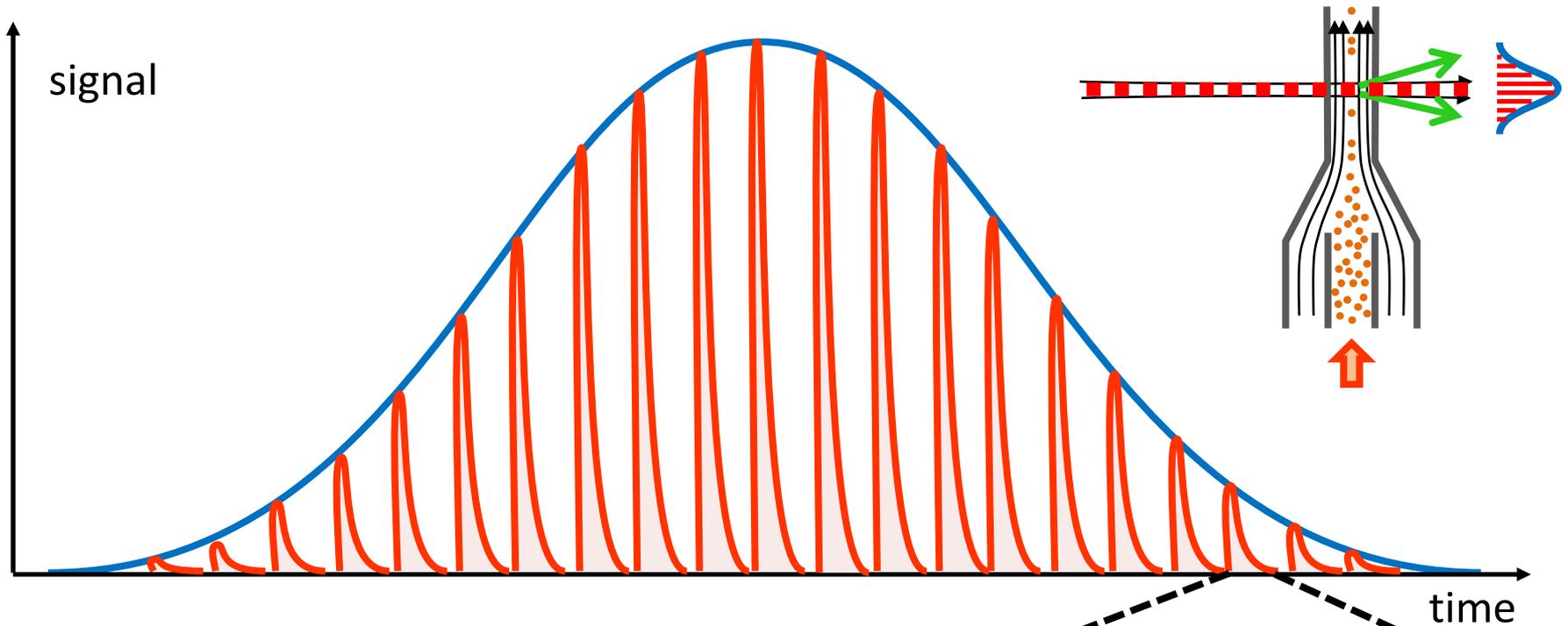


Traditional Flow Cytometry

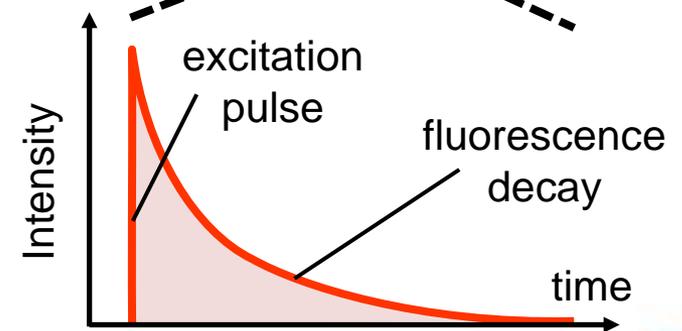


- continuous light source
- one peak per event

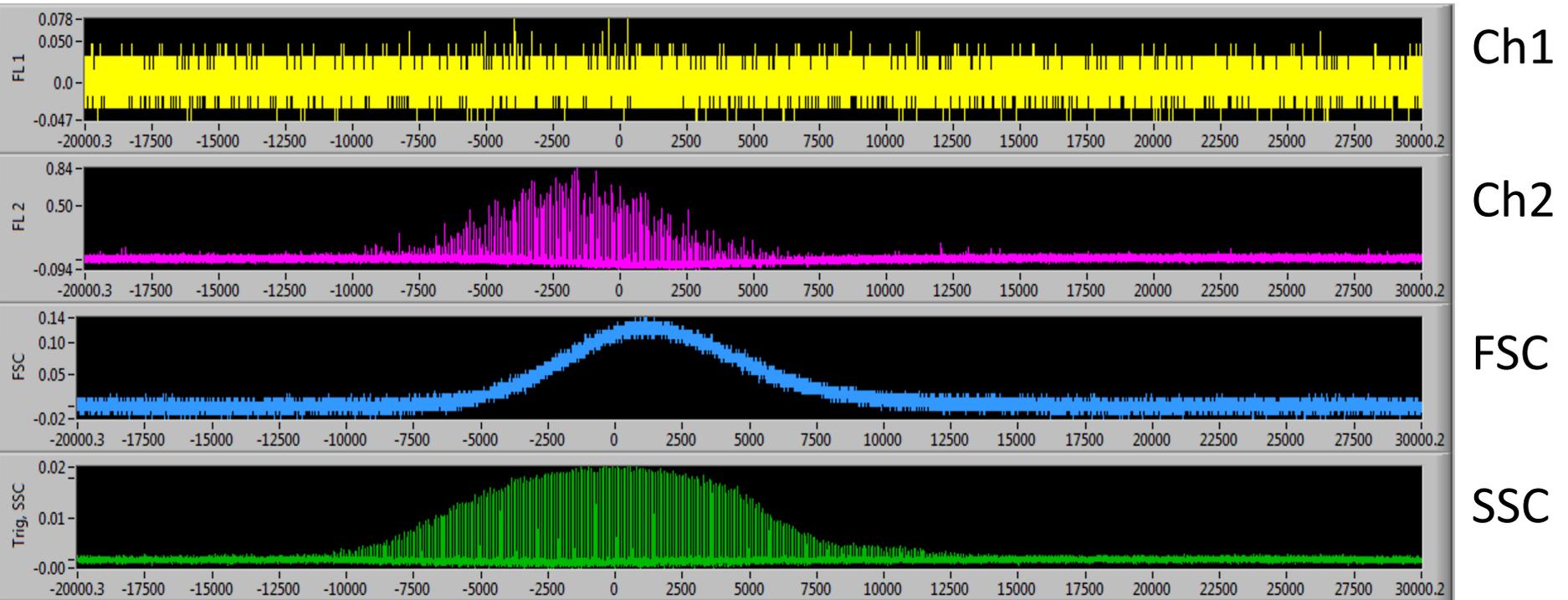
Time-Resolved Flow Cytometry



- pulsed laser source
- 100's of peaks per event

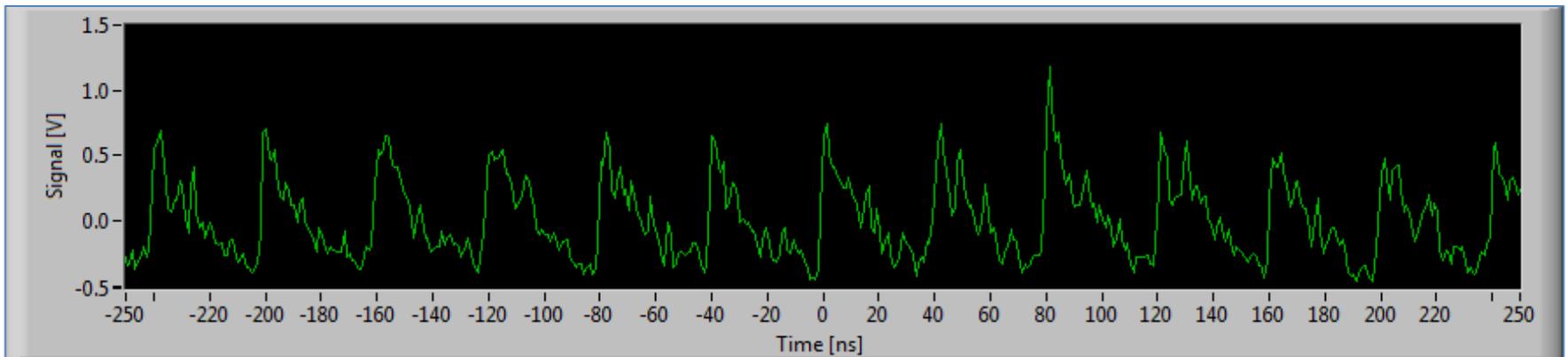
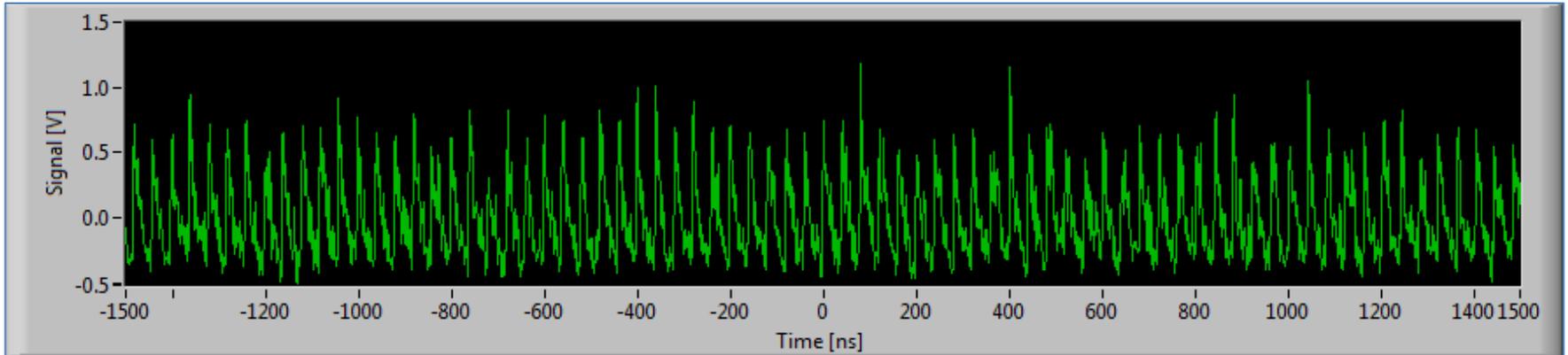


Example of Raw Time-Resolved Data

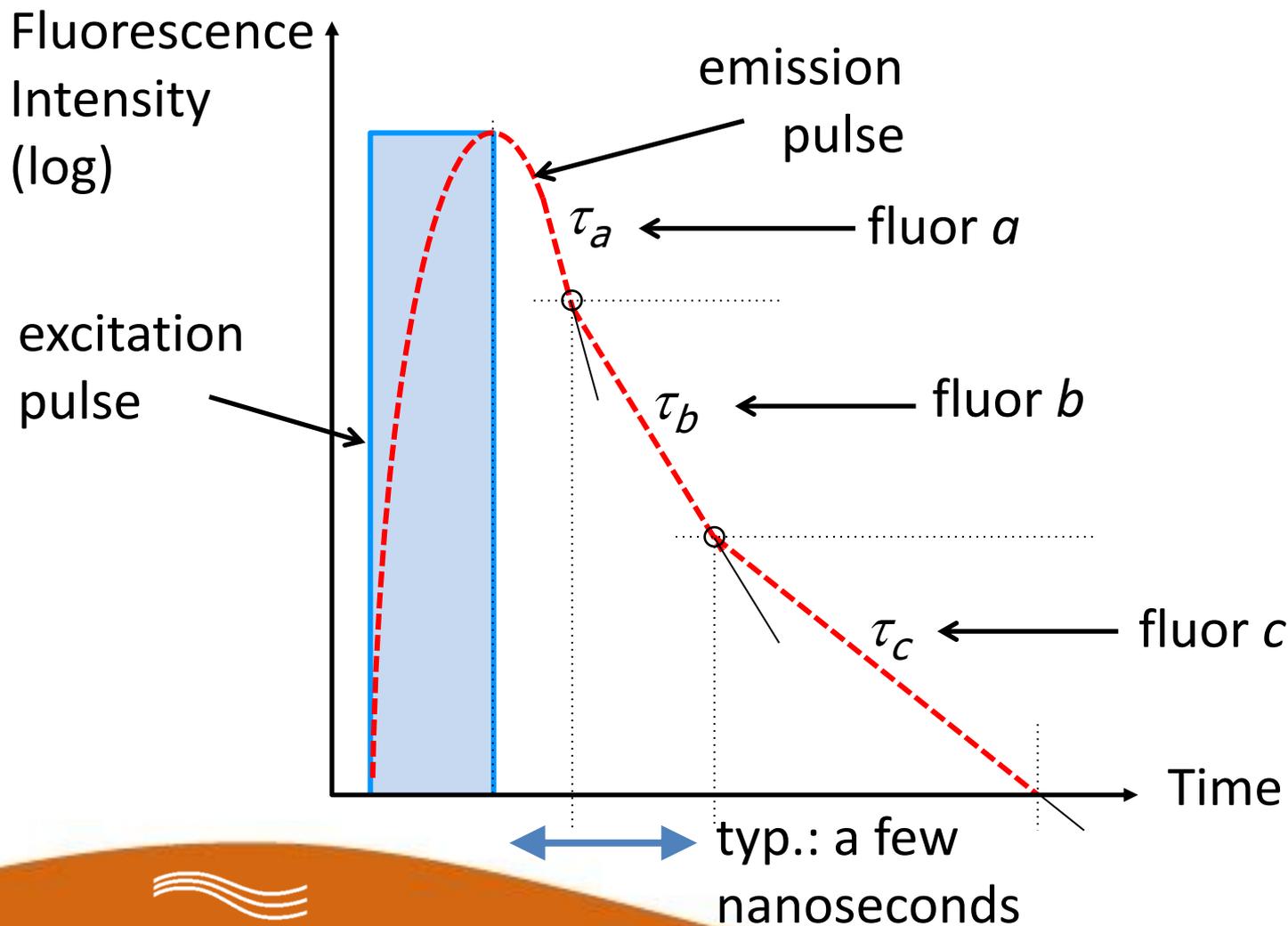


- signal follows traditional transit envelope
- but made up of 100's of individual pulses

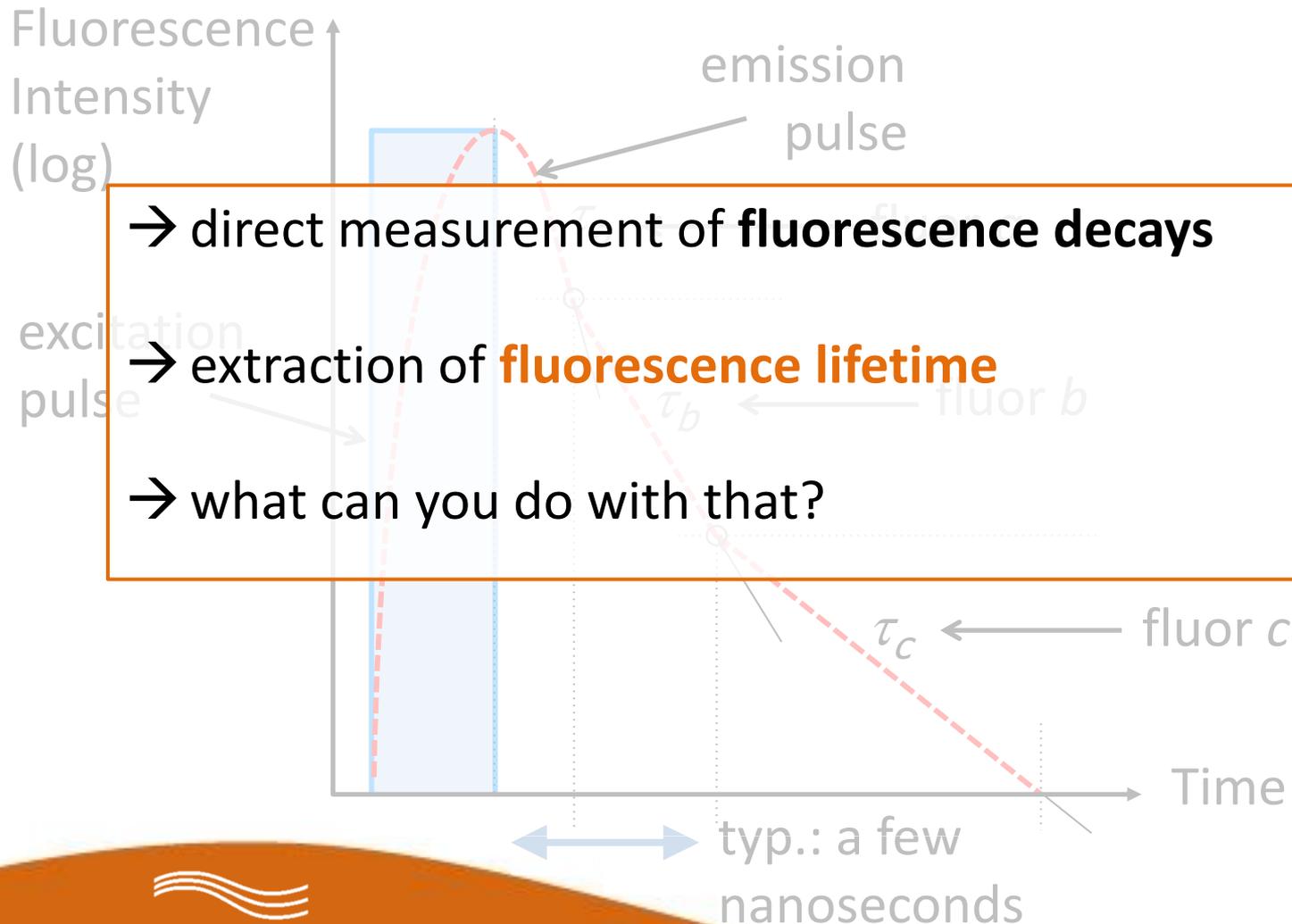
Time-Resolved Data Detail



Can Resolve Multiple Lifetimes in Same Decay



Can Resolve **Multiple Lifetimes** in Same Decay



LIFETIME AS A MEANS TO AN END:

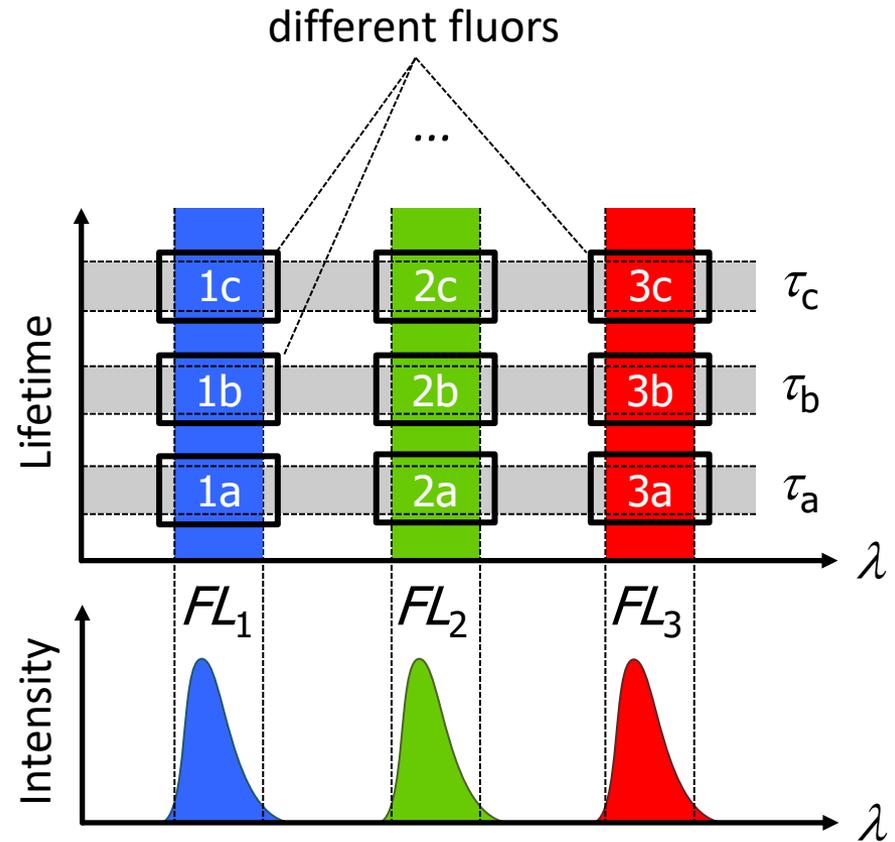
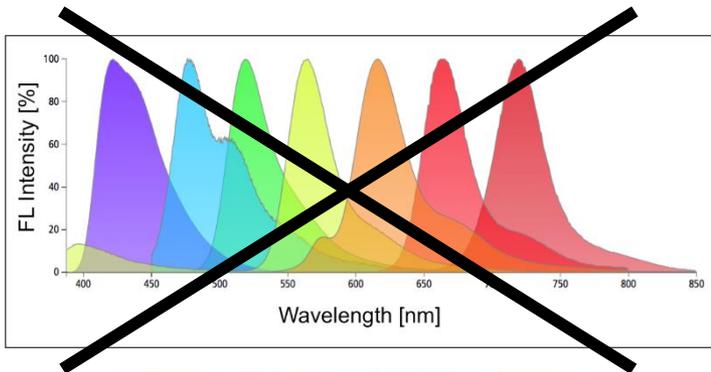
ARNO 1.0

**COMPENSATION-FREE FLOW CYTOMETER
SIMPLIFY YOUR ASSAYS**

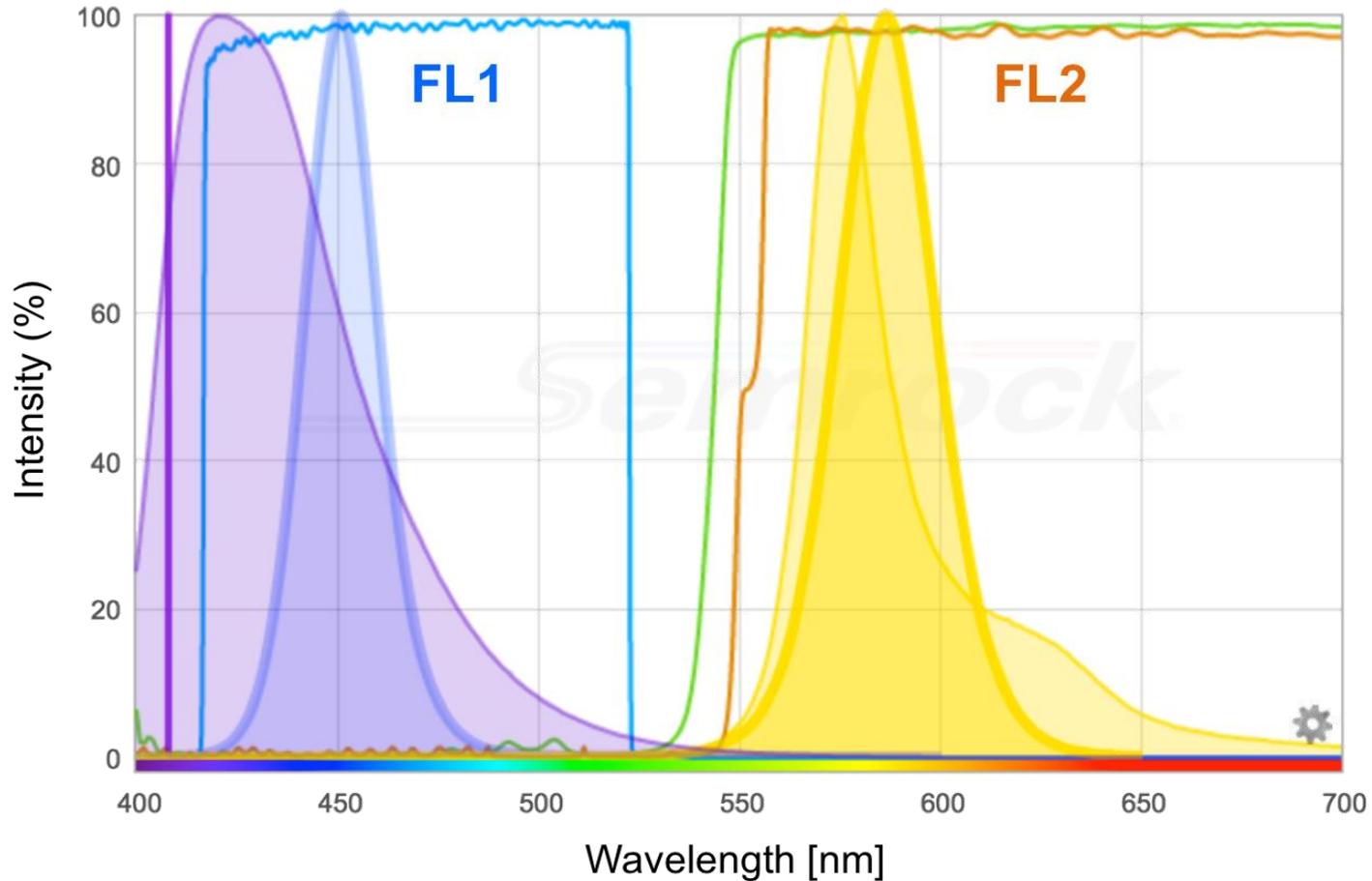


Lifetime As Means To an End

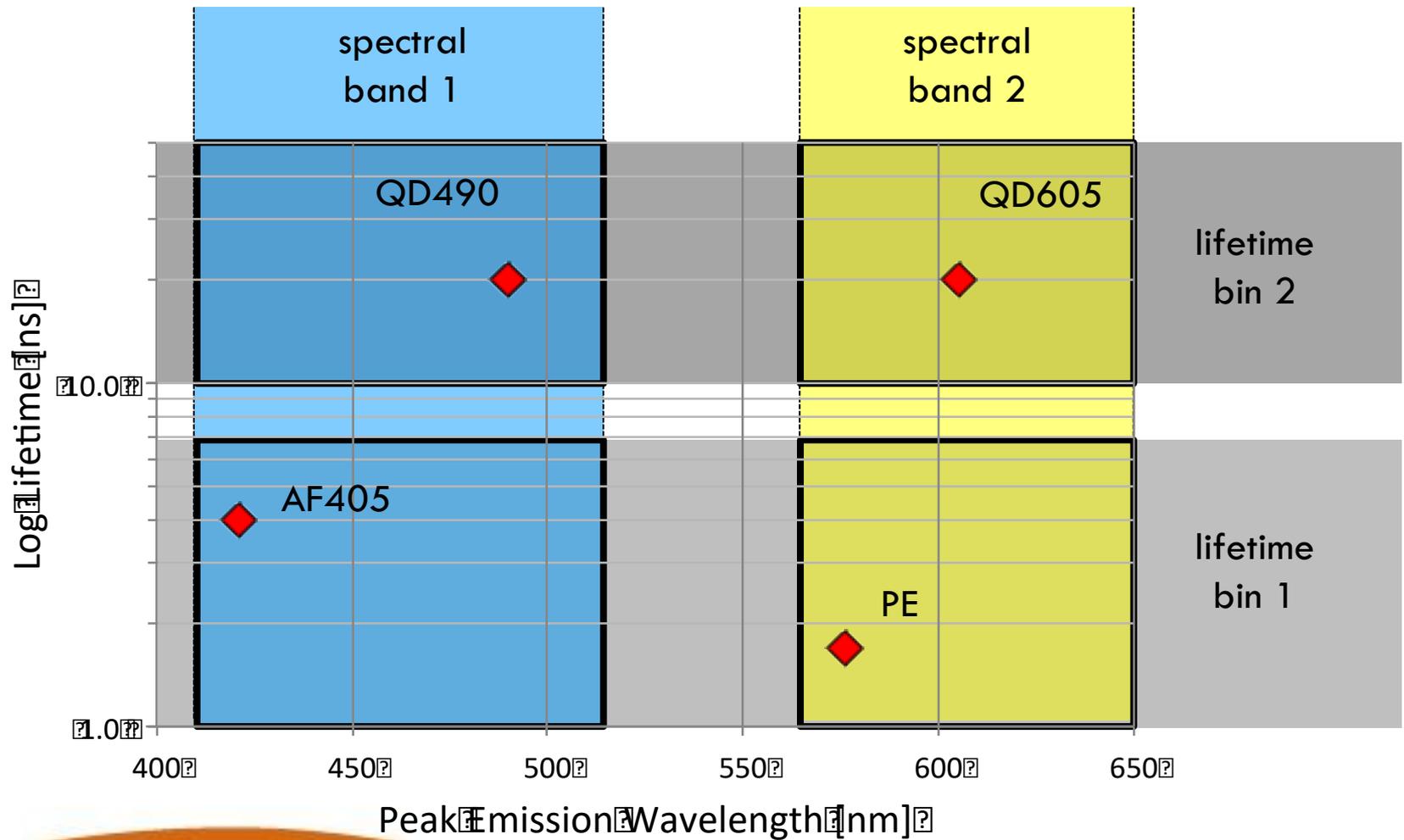
- distinguish fluors based on color AND lifetime
 - **reduce spectral spillover**
 - **compensation-free**



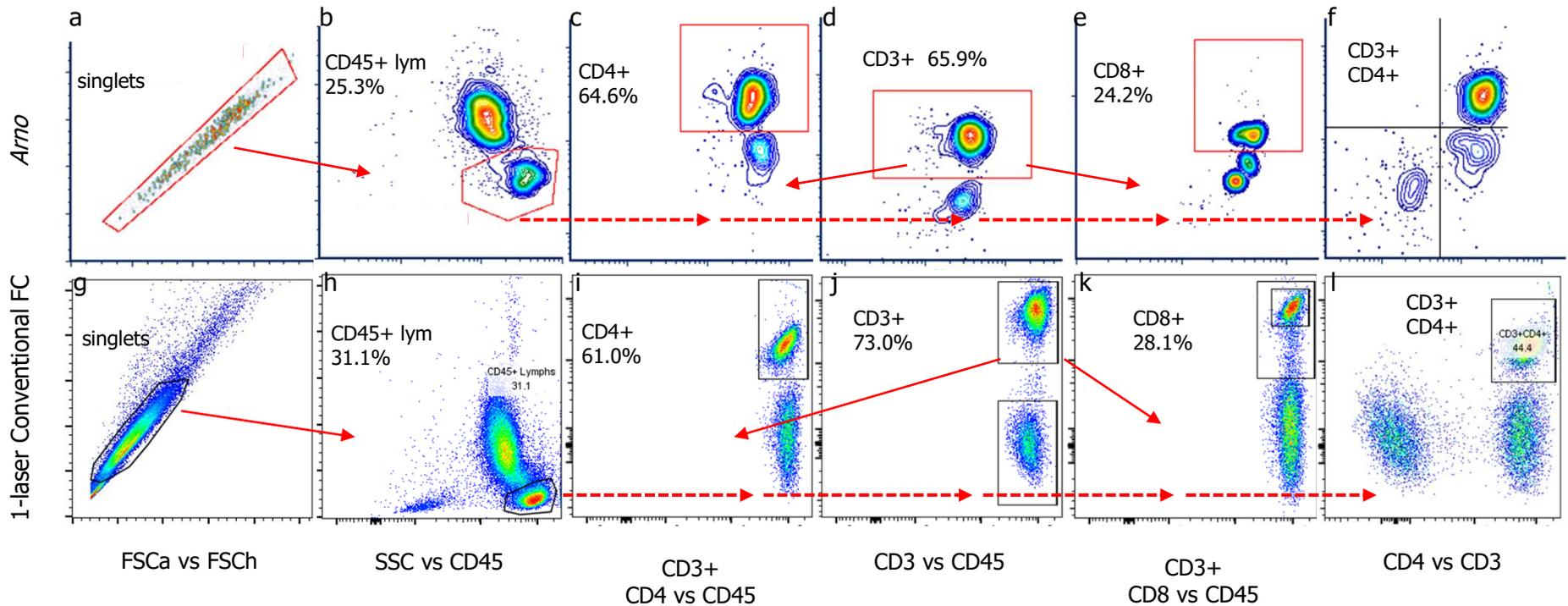
A Four-Color Demo



4 Colors: 2 Spectral Bands, 2 Lifetimes



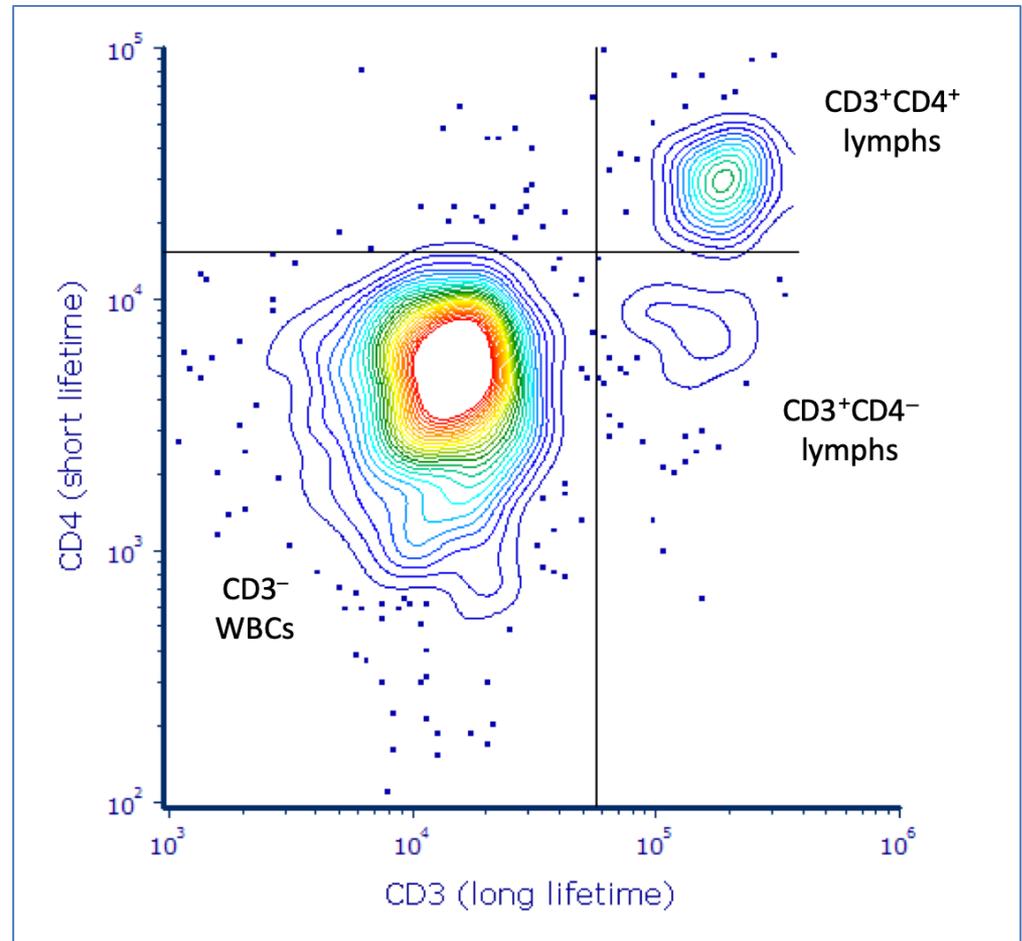
Demo *Arno* Cell Assay: 4 Colors, 2 PMTs, 1 Laser



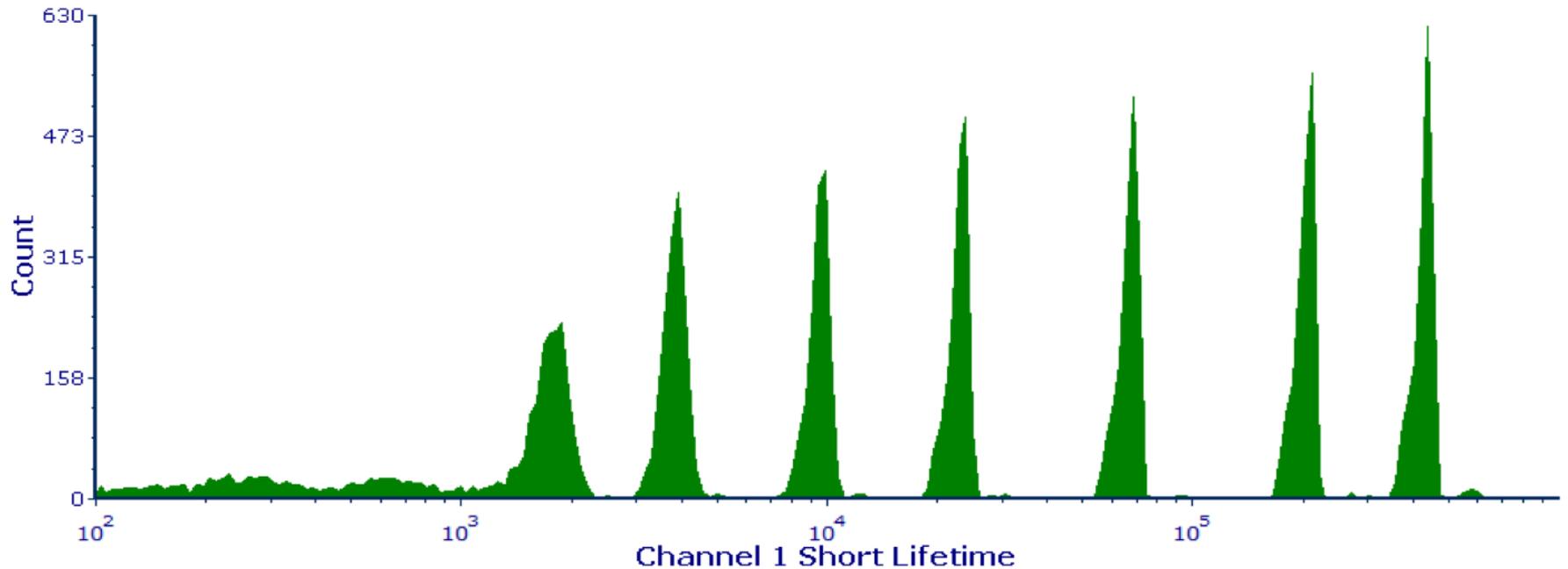
- each detector measures 2 fluors
- Dri Leukocytes
- CD45 / CD3 / CD4 / CD8

Demo *Arno* CD45/3/4/8 Assay

- Dri Leukocytes
- different markers measured simultaneously, on the same detector
- CD4: short lifetime
- CD3: long lifetime
- signals discriminated **based on color and lifetime**

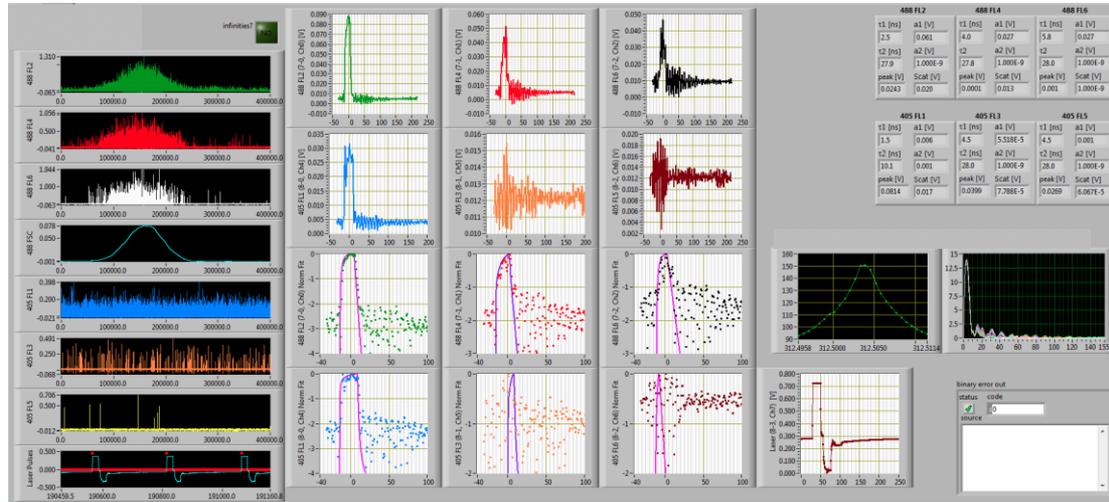
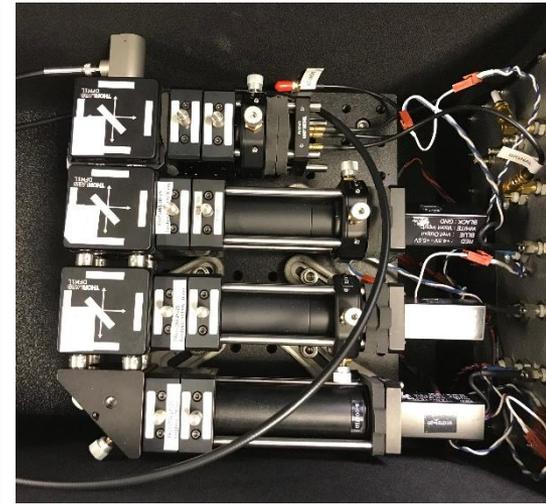
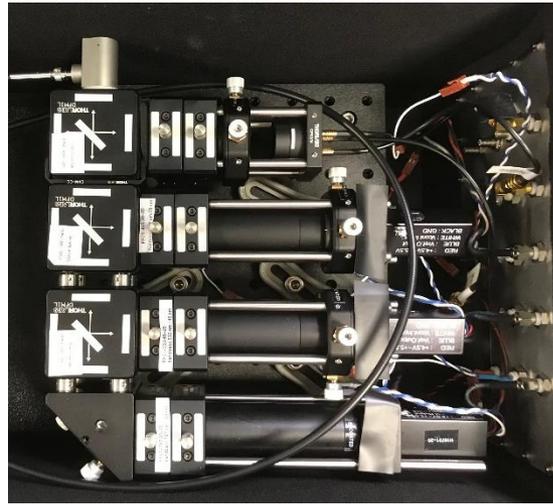
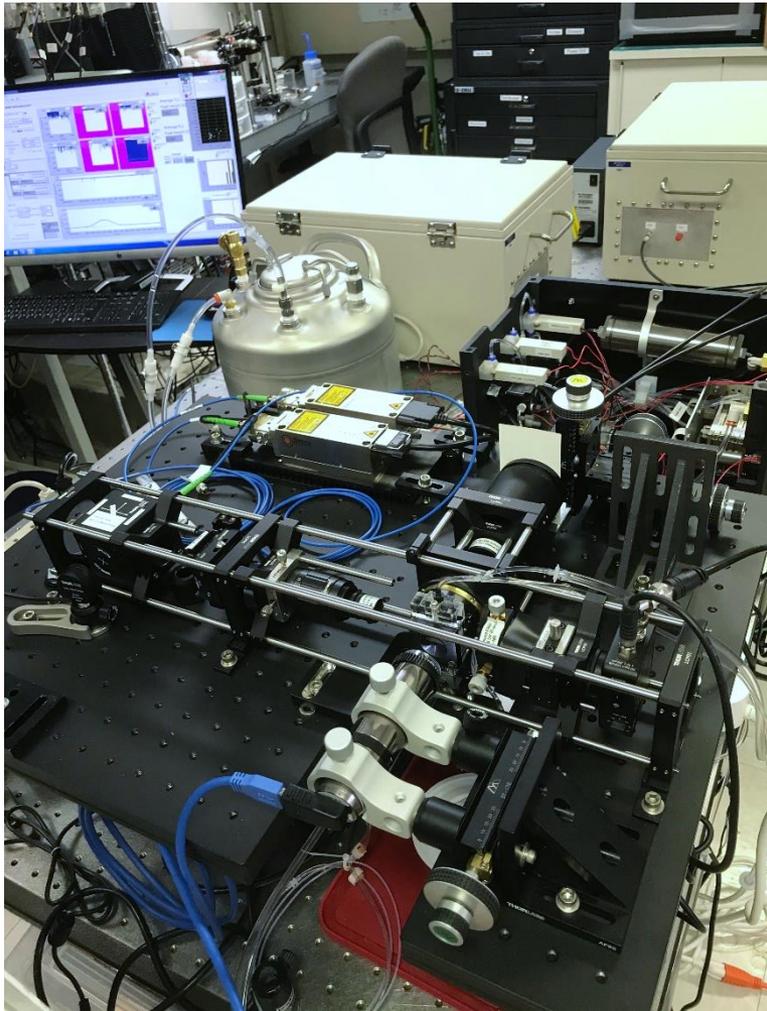


Arno 8-Peak Rainbow Beads

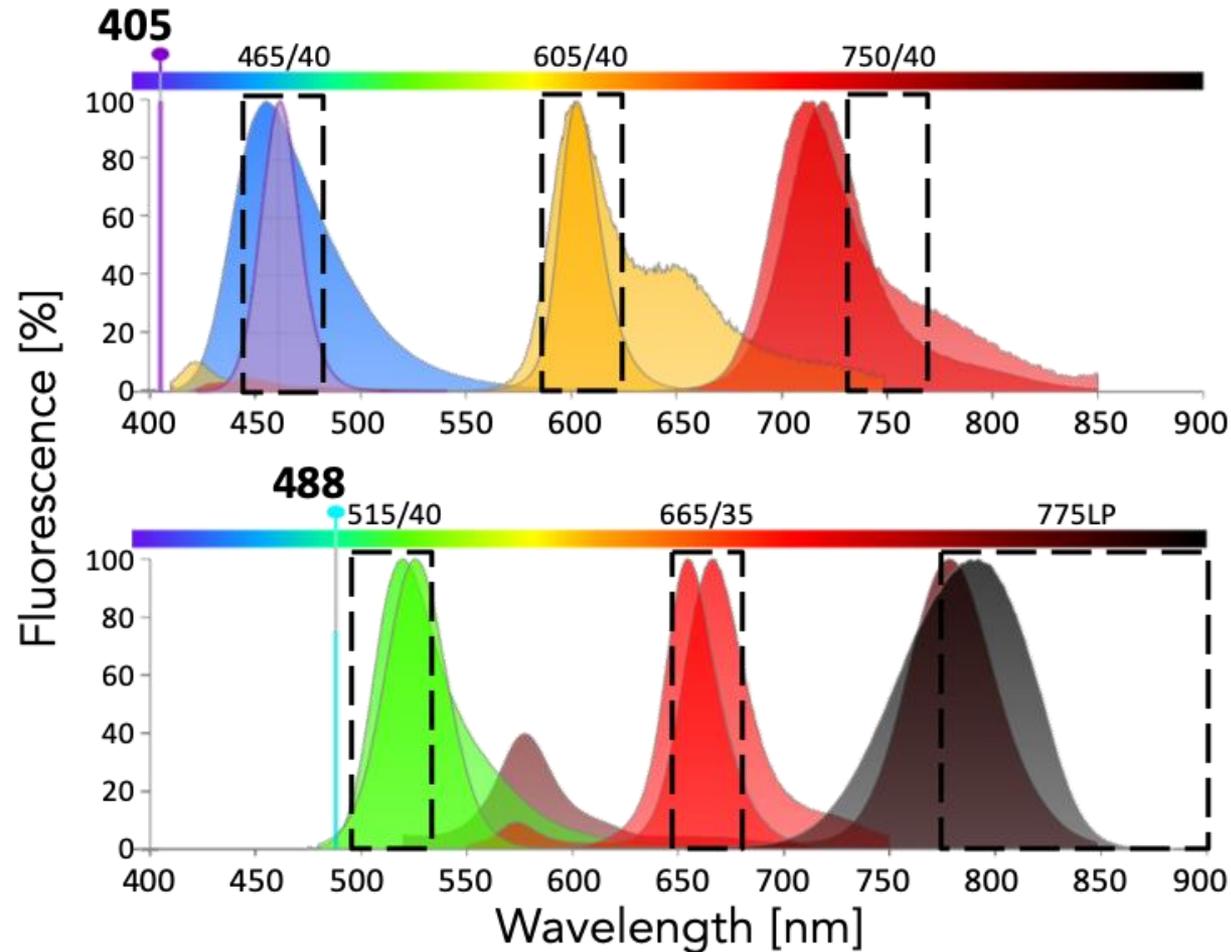


- Spherotech Rainbow beads
- data from blue channel, short τ
- 7/8 peaks resolved (MESF = 90)

Arno Current Platform



15-Parameter, No-Comp *Arno*

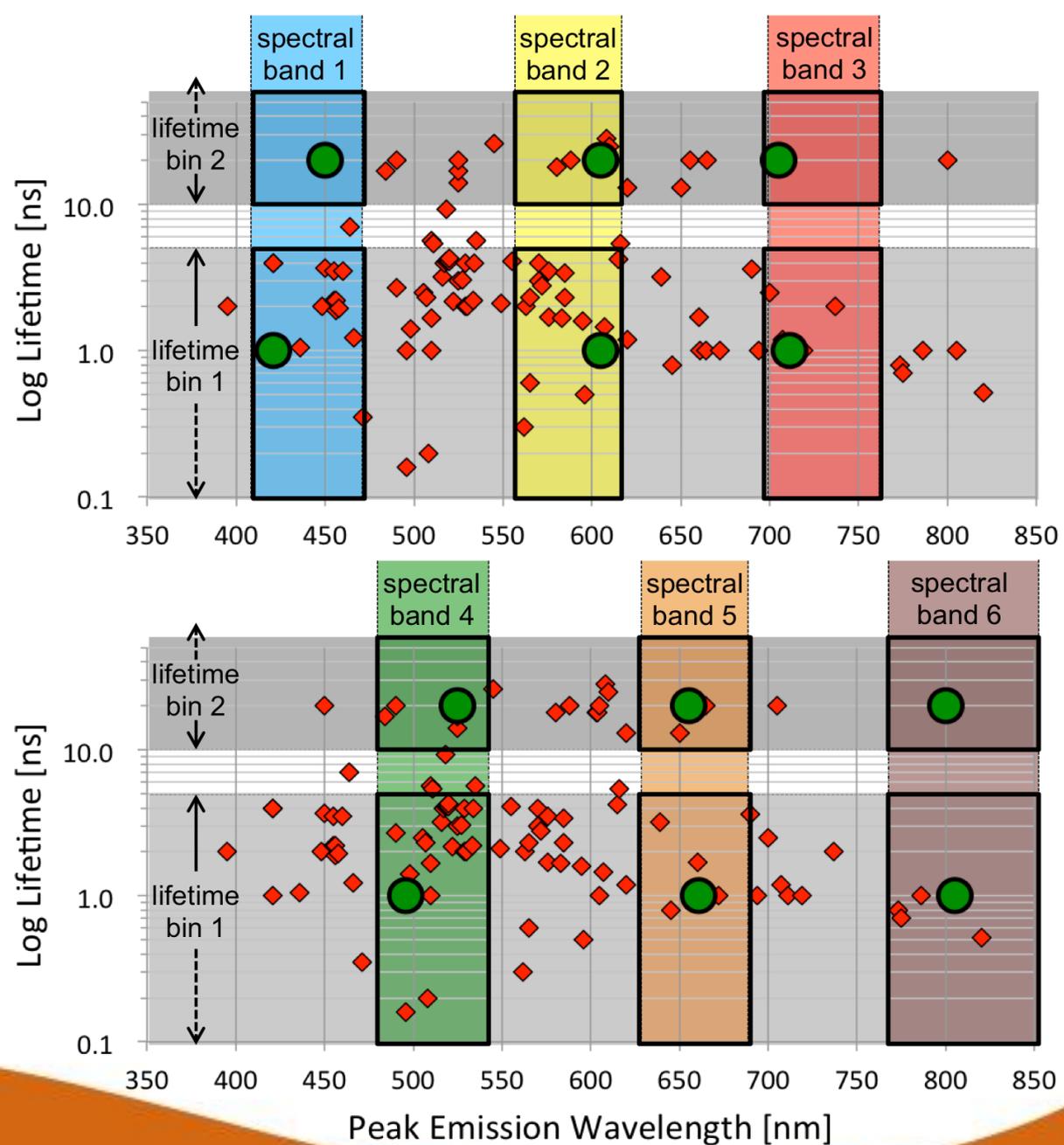


- 405, 488 nm excitation
- 12 FL channels
- < 2% spillover in any channel

**Compensation-free
multicolor
flow cytometry**

12 Antibodies with 6 Colors

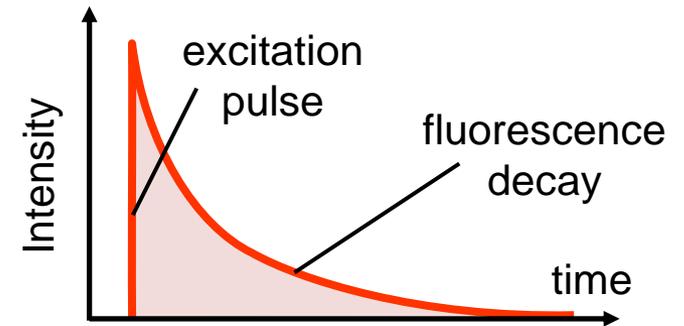
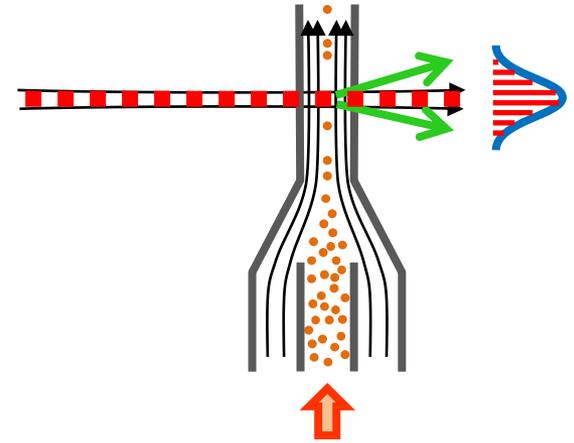
- 2 lasers:
405, 488 nm
- 6 detectors
- 2 lifetimes in each channel



Arno 1.0

- **2** lasers
- **6** FL detectors
- **12** FL channels
- + FSC, SSC₁, SSC₂
- **no** compensation

15 parameters



LIFETIME AS A MEANS TO AN END:

ARNO 2.0

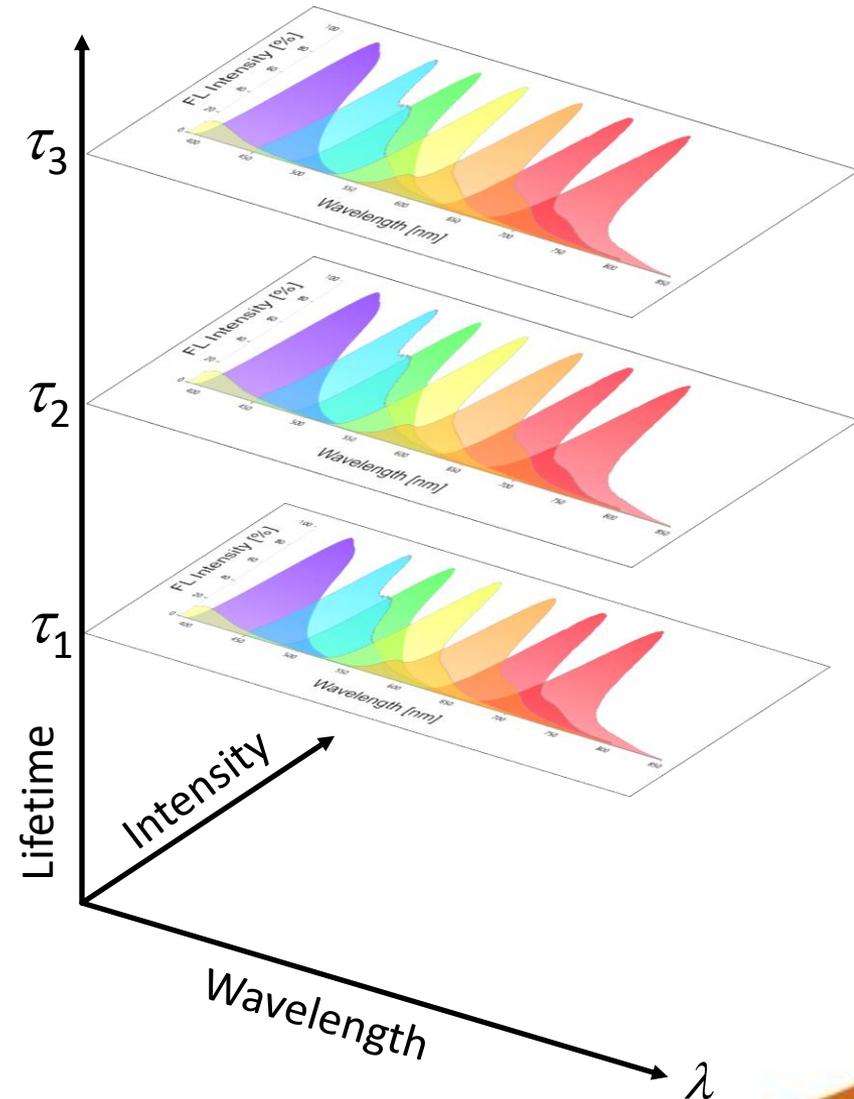
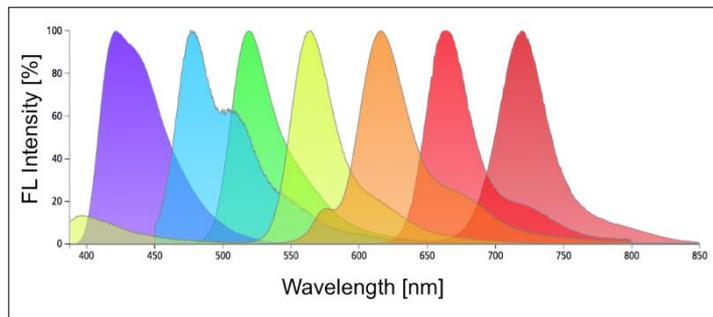
**HIGH-PARAMETER-# FLOW CYTOMETER
IMMUNOLOGY FOR LESS**



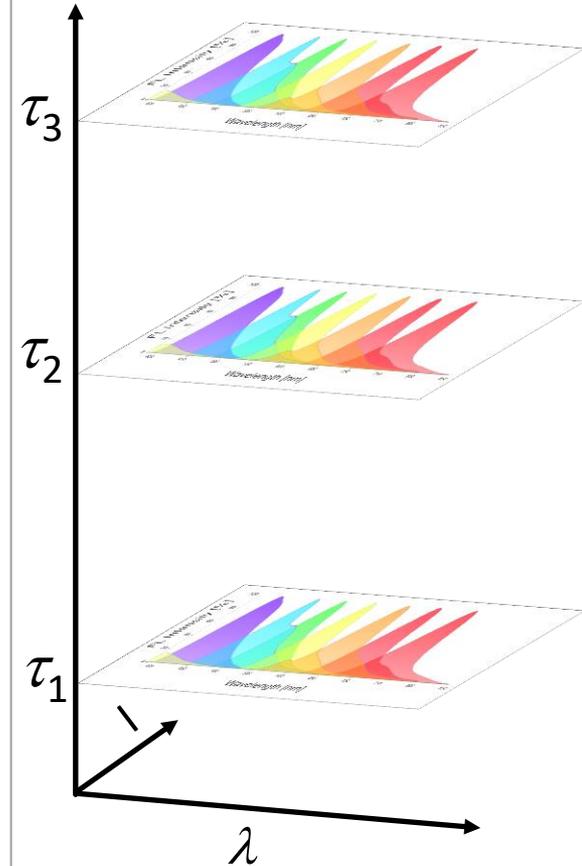
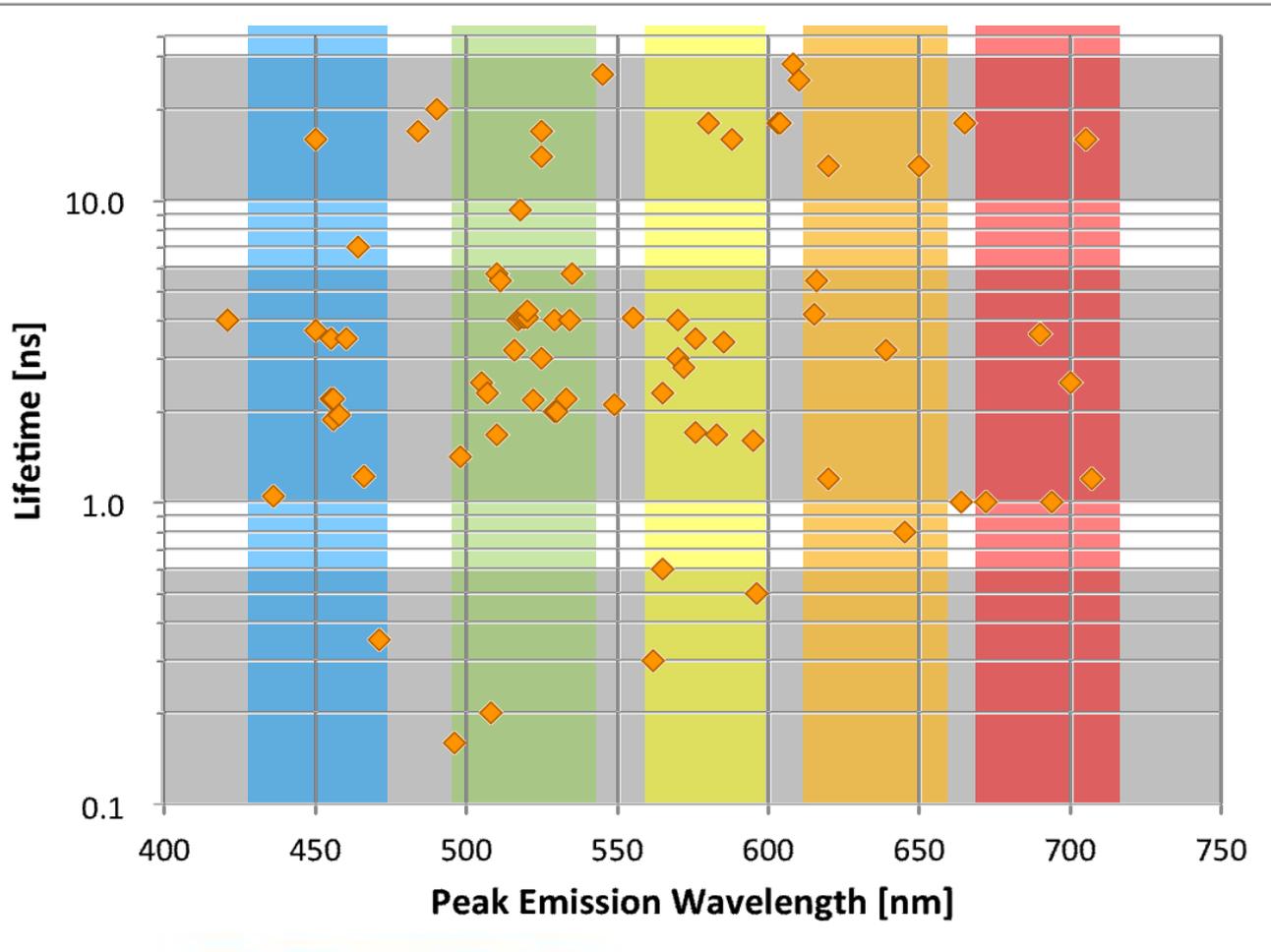
Lifetime As Means To an End

- distinguish fluors based on color AND lifetime

→ expand # parameters

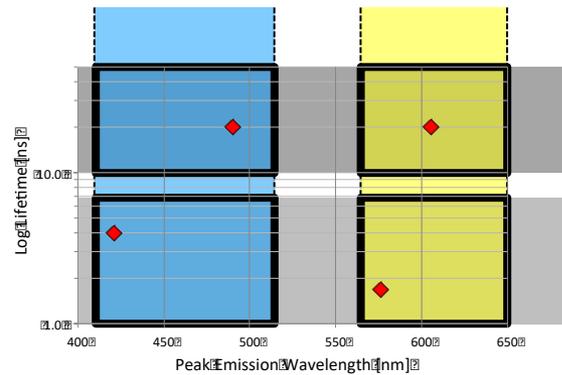


Example of *Arno 2.0* Single-Laser 3x5 Panel



From 4 ... to 15+ Parameters (Per Laser)

→ 30- to 45-Color FC



3–6
spectral
bands

x

3
lifetimes

x

3
lasers

≈

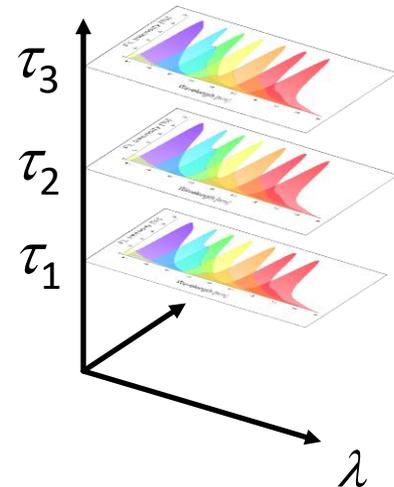
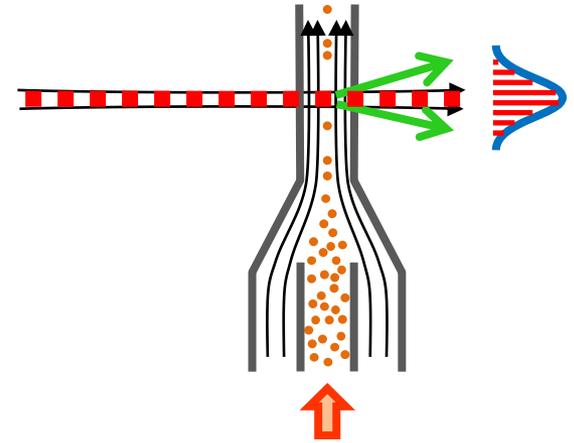
30 – 45
colors

only 3 lasers,
15 detectors

Arno 2.0

- **3** lasers
- **15** FL detectors
- **45** FL channels
- + FSC, SSC₁, SSC₂

} **30–48** parameters



LIFETIME AS A MEANS TO AN END:

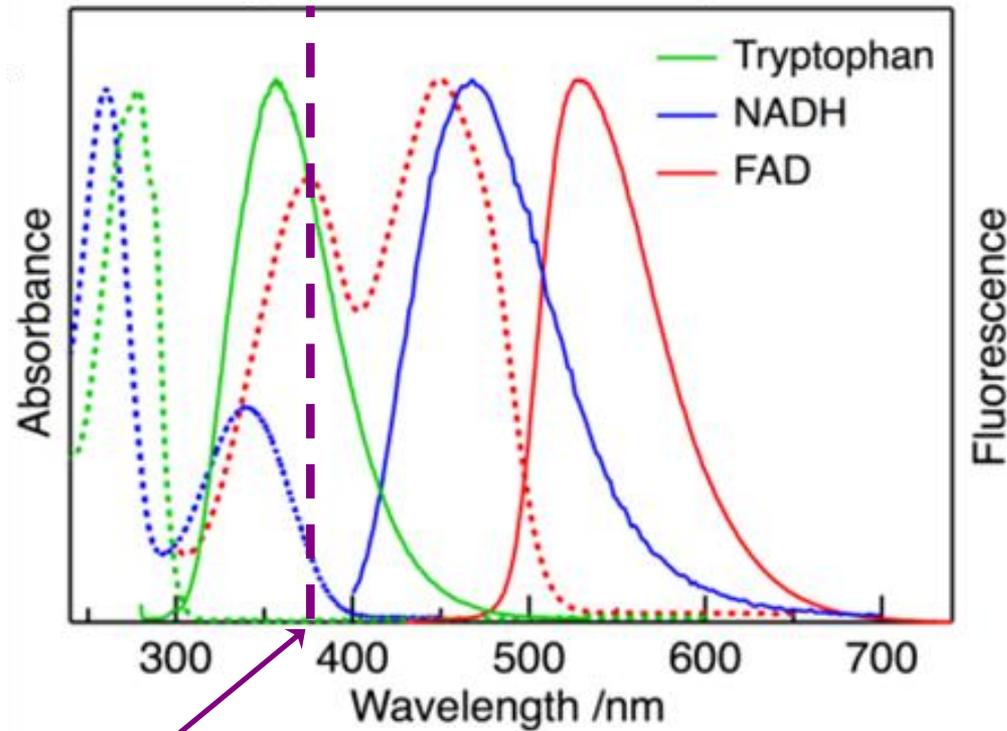
TIBER

LABEL-FREE FLOW CYTOMETER

CANCER DIAGNOSTICS



Some Sources of Cellular Autofluorescence



excitation

Islam, M. et al. (2013), *Int'l J Mol Sci* **14**, 1952-1963

Protein Binding Affects NADH Lifetime

free NADH: short lifetime

α_{free} : free fraction



protein-bound NADH: long lifetime

α_{bound} : bound fraction



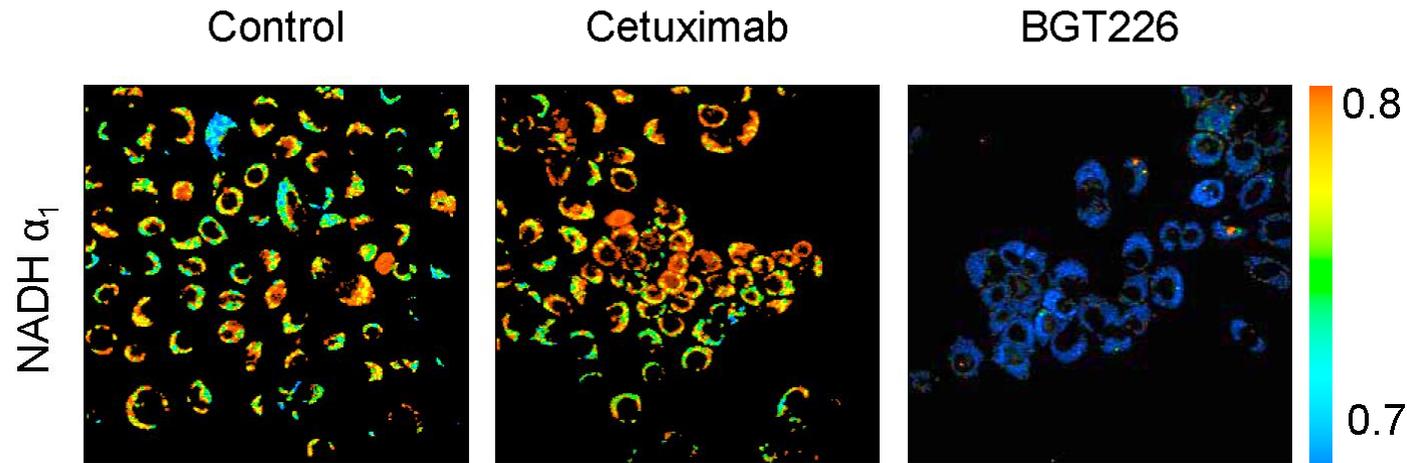
TABLE 1.	Normal Cells	Cancer Cells
Metabolic Pathway	OxPhos (mitochondria)	glycolysis (cytosolic)
Free: Bound NADH Ratio	$\alpha_{\text{free}}/\alpha_{\text{bound}}$ is LOW	$\alpha_{\text{free}}/\alpha_{\text{bound}}$ is HIGH
Free NADH Lifetime, τ_{free} , is SHORT , <0.5 ns		
Protein-bound NADH Lifetime, τ_{bound} , is LONG , >1.5 ns		

Metabolic Profiling by FLIM

- squamous cell carcinoma (SCC61 line)
- response to different cancer drug treatments

cancer cell: free NADH 

normal cell: free NADH 



From Fig. 4B, Shah, A. T., et al. (2014) *PLOS ONE* 9, e90746

NADH Free vs. Bound by FLIM

melanoma mouse model

TABLE 1.	Normal Cells	Cancer Cells
Metabolic Pathway	OxPhos (mitochondria)	aerobic glycolysis (cytosolic)
Free: Bound NADH Ratio	$\alpha_{\text{free}}/\alpha_{\text{bound}}$ is LOW	$\alpha_{\text{free}}/\alpha_{\text{bound}}$ is HIGH
Free NADH Lifetime, τ_{free} , is SHORT , <0.5 ns		
Protein-bound NADH Lifetime, τ_{bound} , is LONG , >1.5 ns		

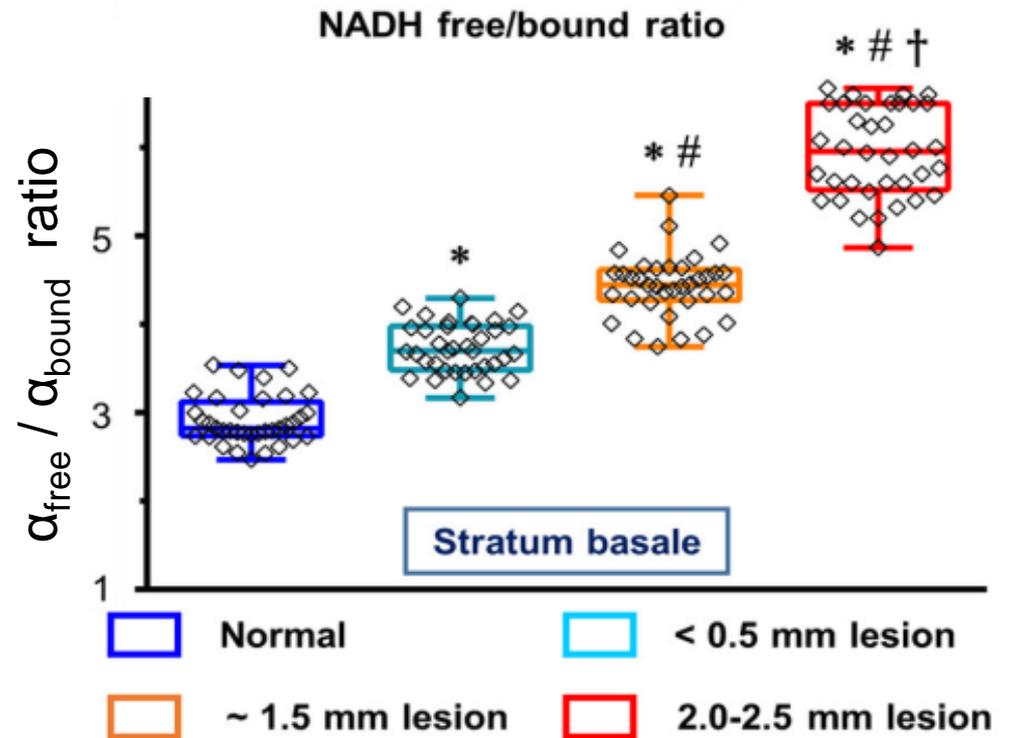
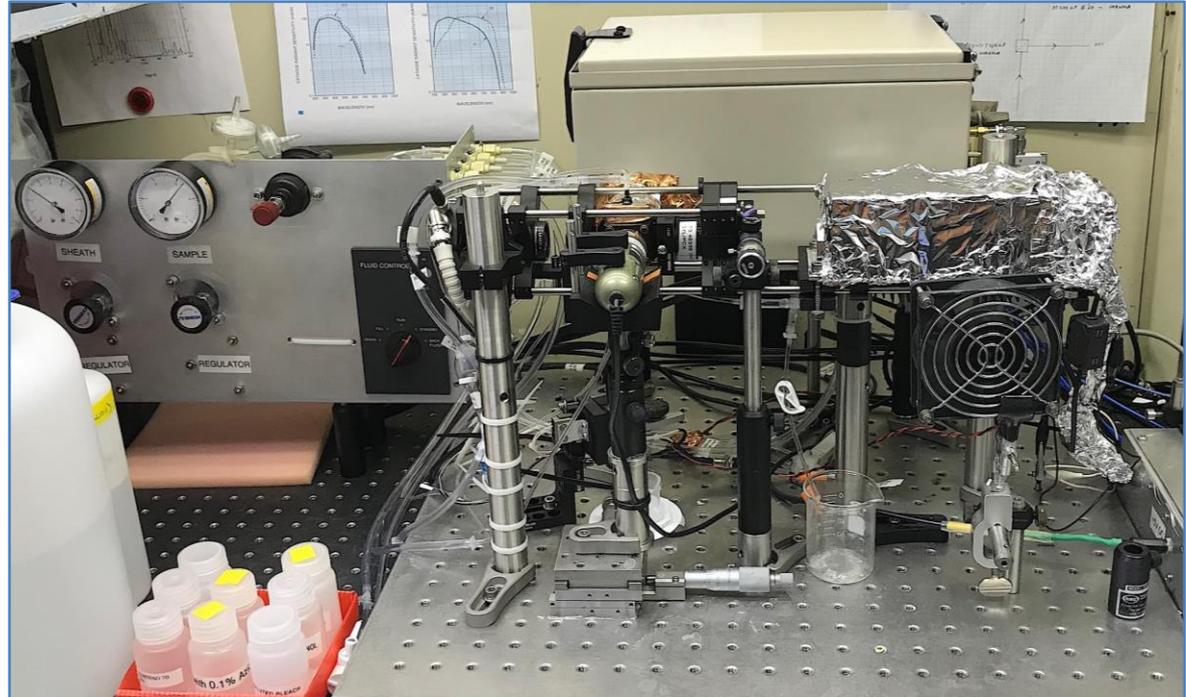


Fig. 3, Pastore, M. N., et al. (2017) Exp. Derm. 26, 607-614

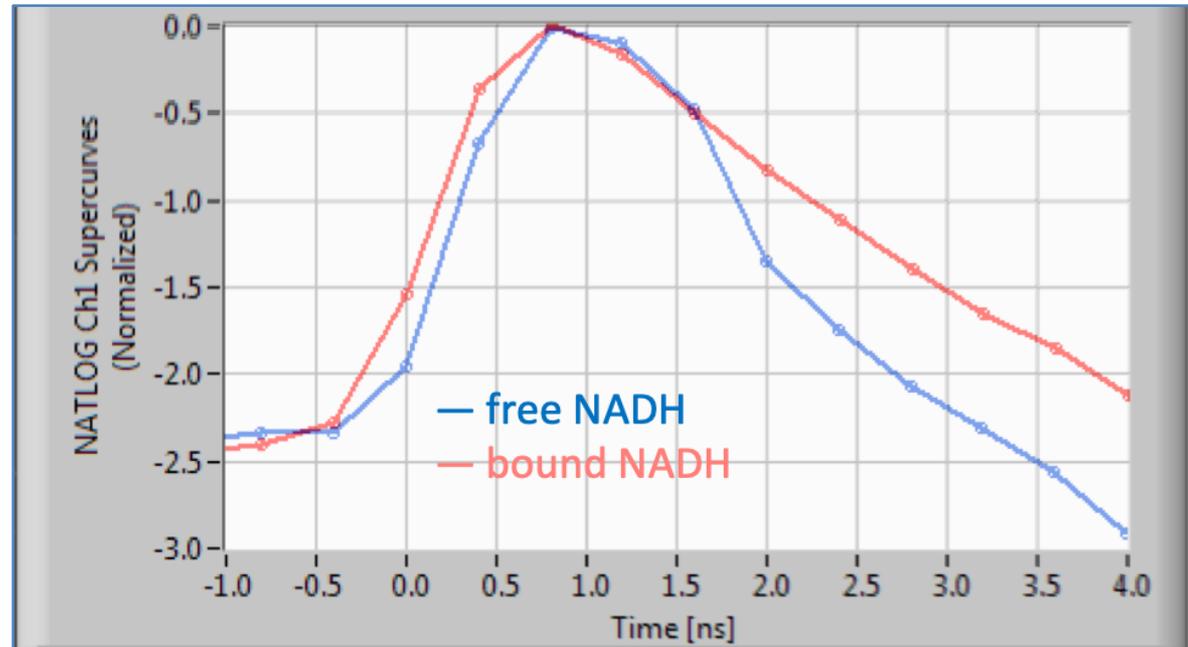
Tiber: Free vs. Bound NADH in Flow

- 375-nm excitation
- label-free
- blue FL channel
- direct time-resolved measurements



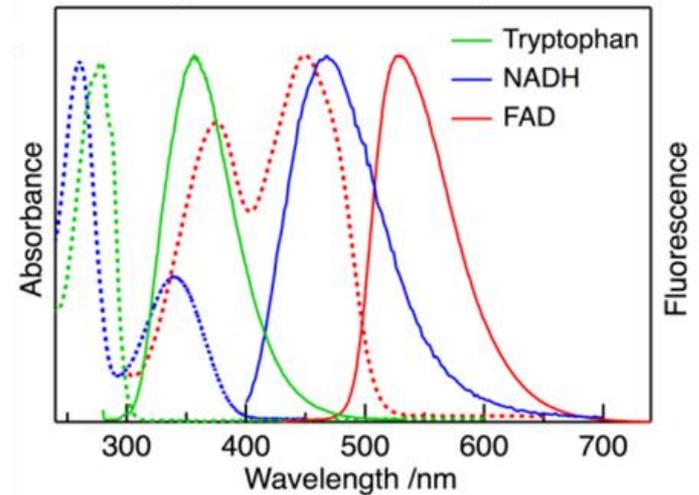
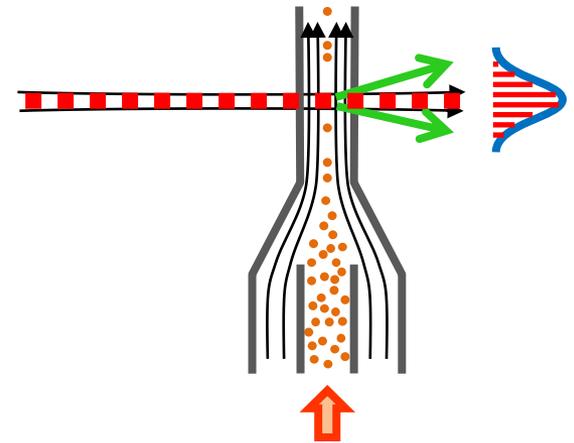
Tiber: Free vs. Bound NADH in Flow

- NADH in solution, free & bound to lactate dehydrogenase
- **FC results mirror FLIM**
- next: NADH in cells in flow



Tiber

- label-free
- autofluorescence
- metabolic profiler
- cancer-cell detector



LIFETIME AS A PARAMETER:

DANUBE

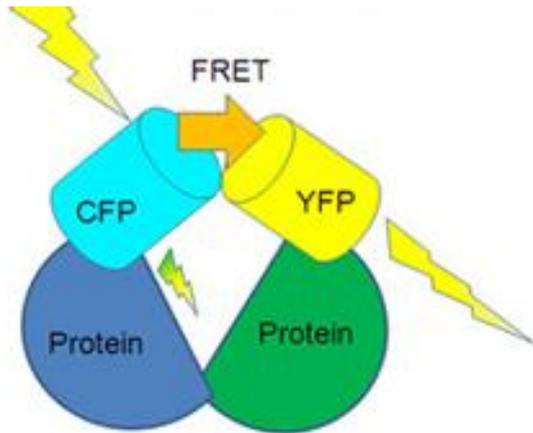
FLUORESCENCE LIFETIME FLOW CYTOMETER

PROTEIN ANALYSIS IN CELLS



Fluorescence Lifetime As a Parameter

- can be sensitive **local** probe
- quantitate **FRET** (Förster Resonance Energy Transfer)
- probe **molecular environment**
 - Ca^{2+} , Cl^- , O_2 , pH, temperature
- probe **protein-protein interactions**



APPLICATIONS

- cell signaling
- cancer cell analysis

© 2013 PE

Fluorescence Lifetime As a Parameter

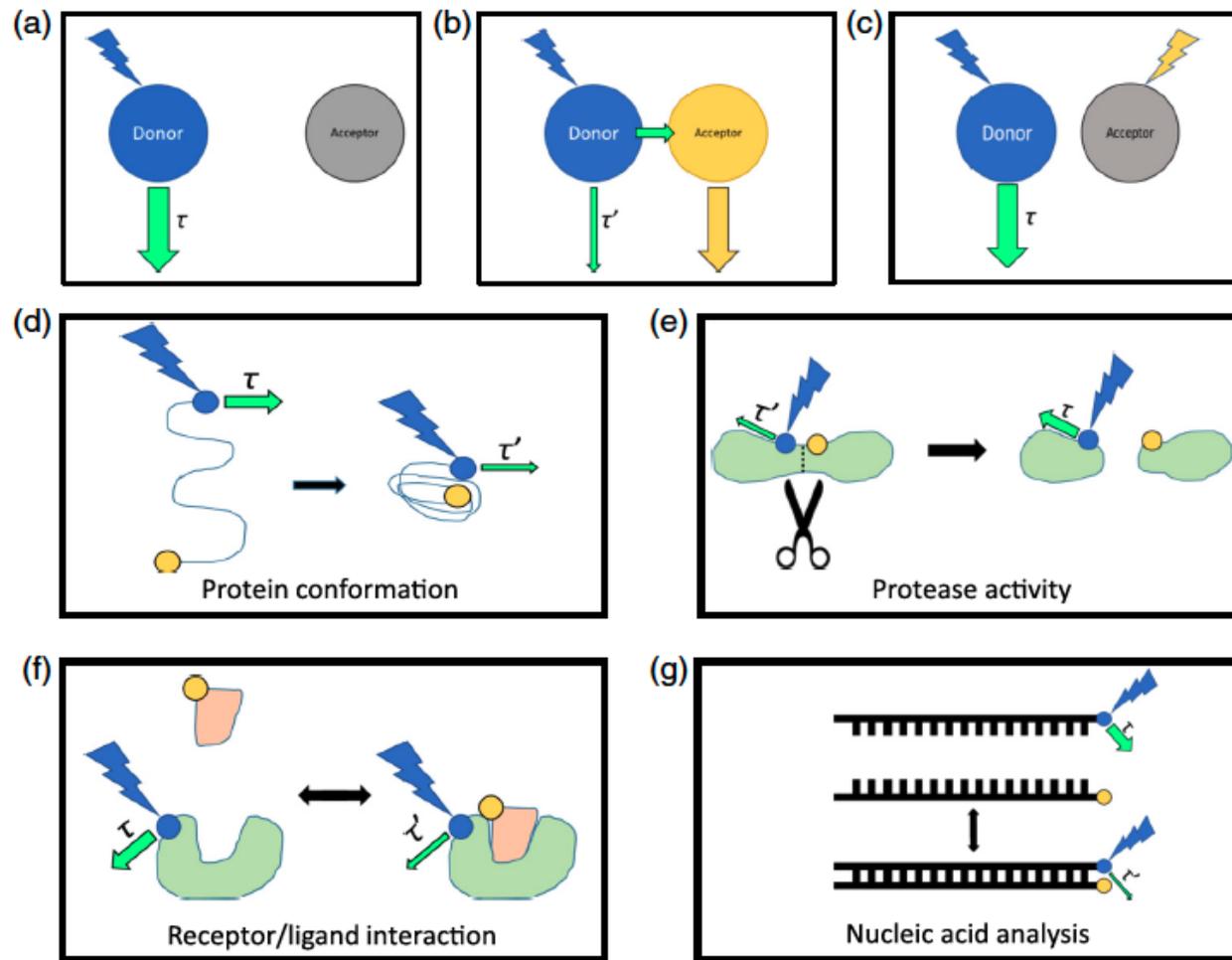
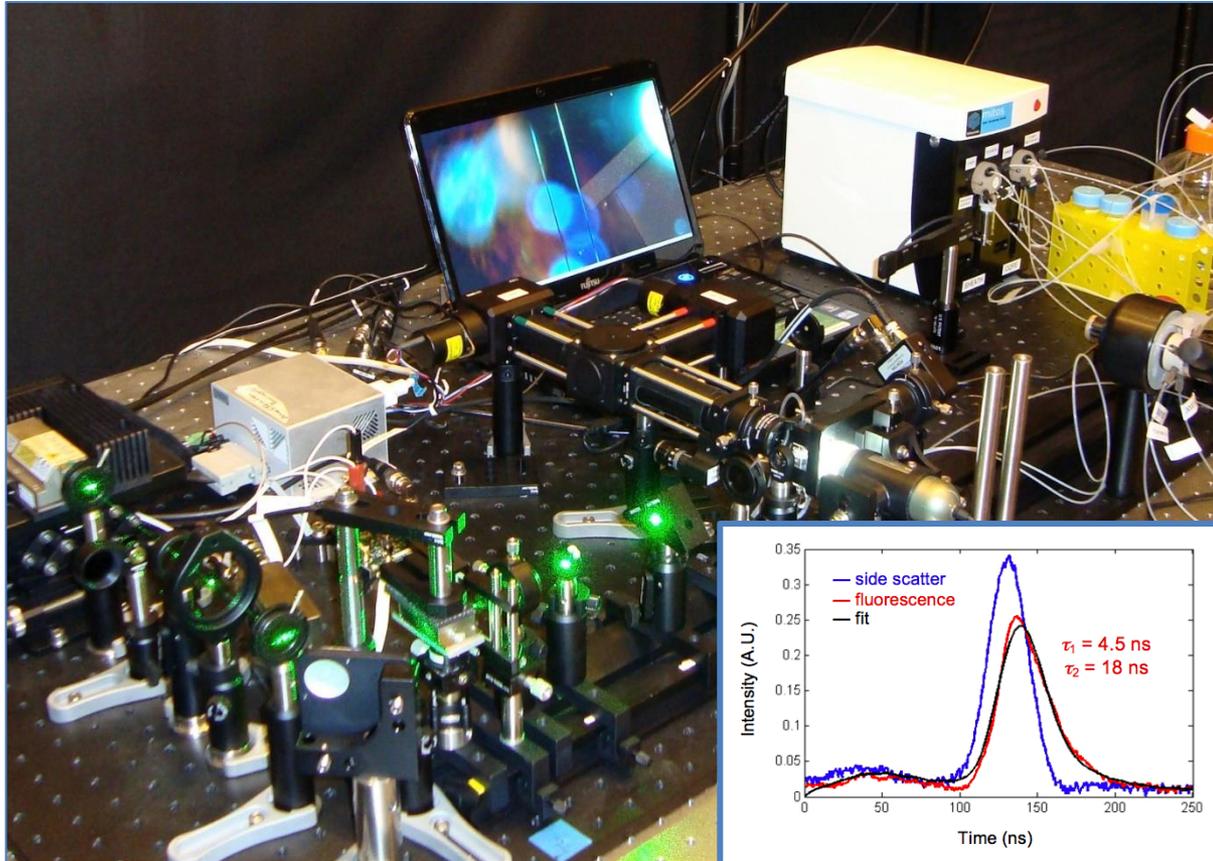
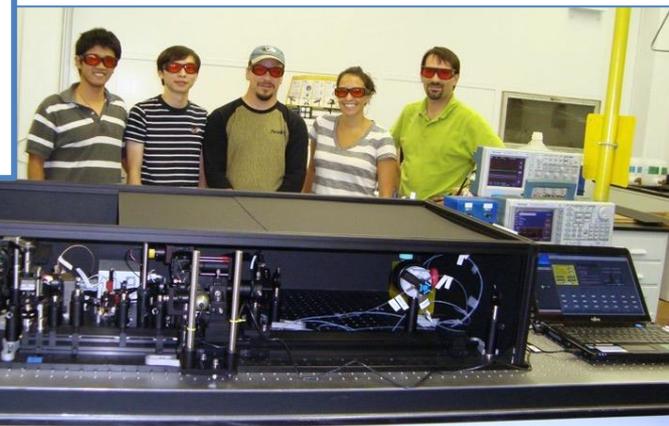
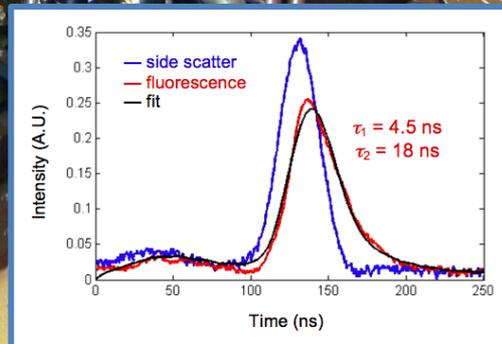


Fig. 2, R. Datta et al., *J. Biomed. Opt.* **25**(7), 071203 (2020)

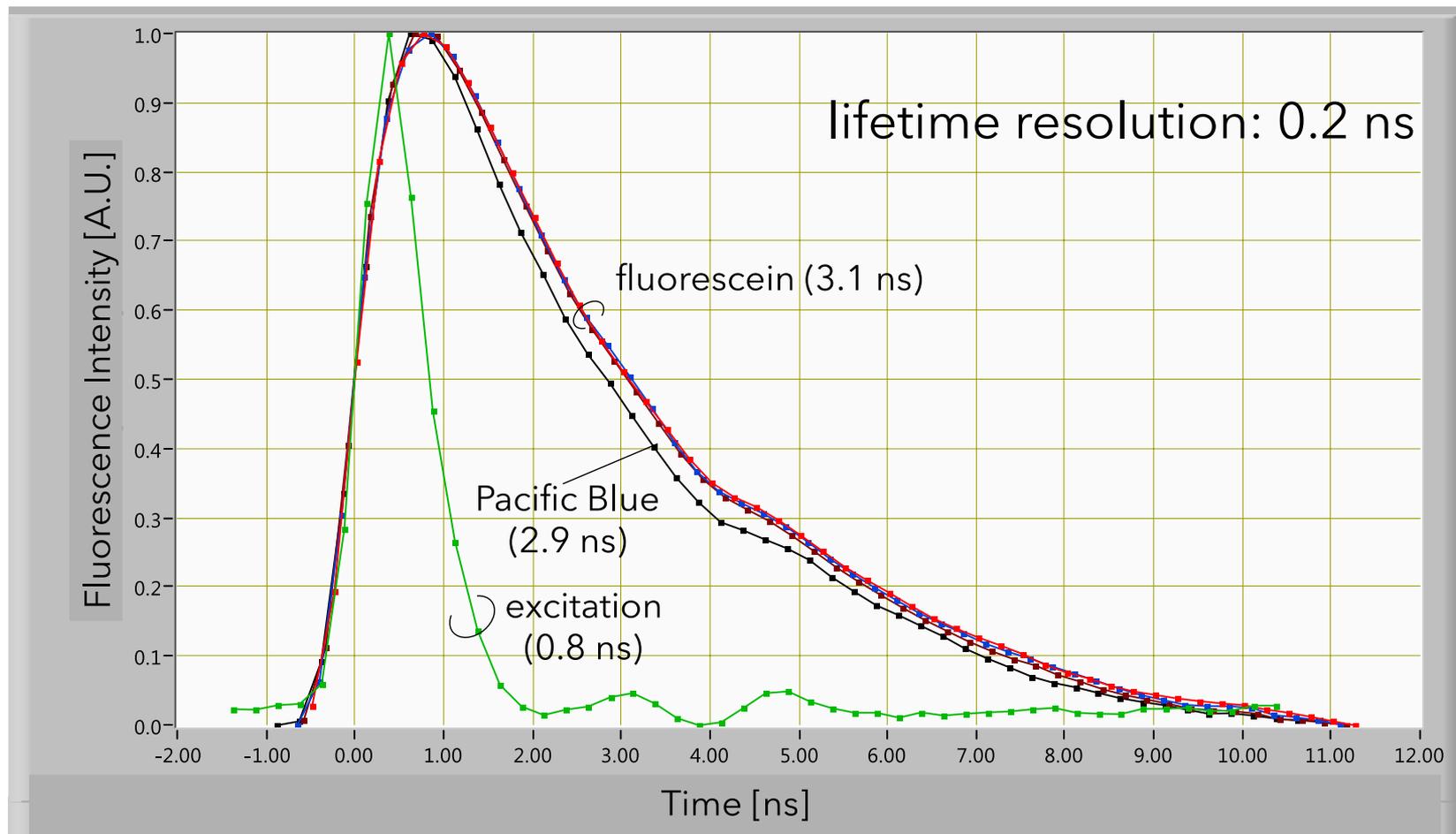
Danube: Fluorescence Lifetime FC



- reports fluorescence lifetime
- multiexponentials
- FRET, cell signaling, cell metabolism
- Prof. J. Houston's lab (NMSU)



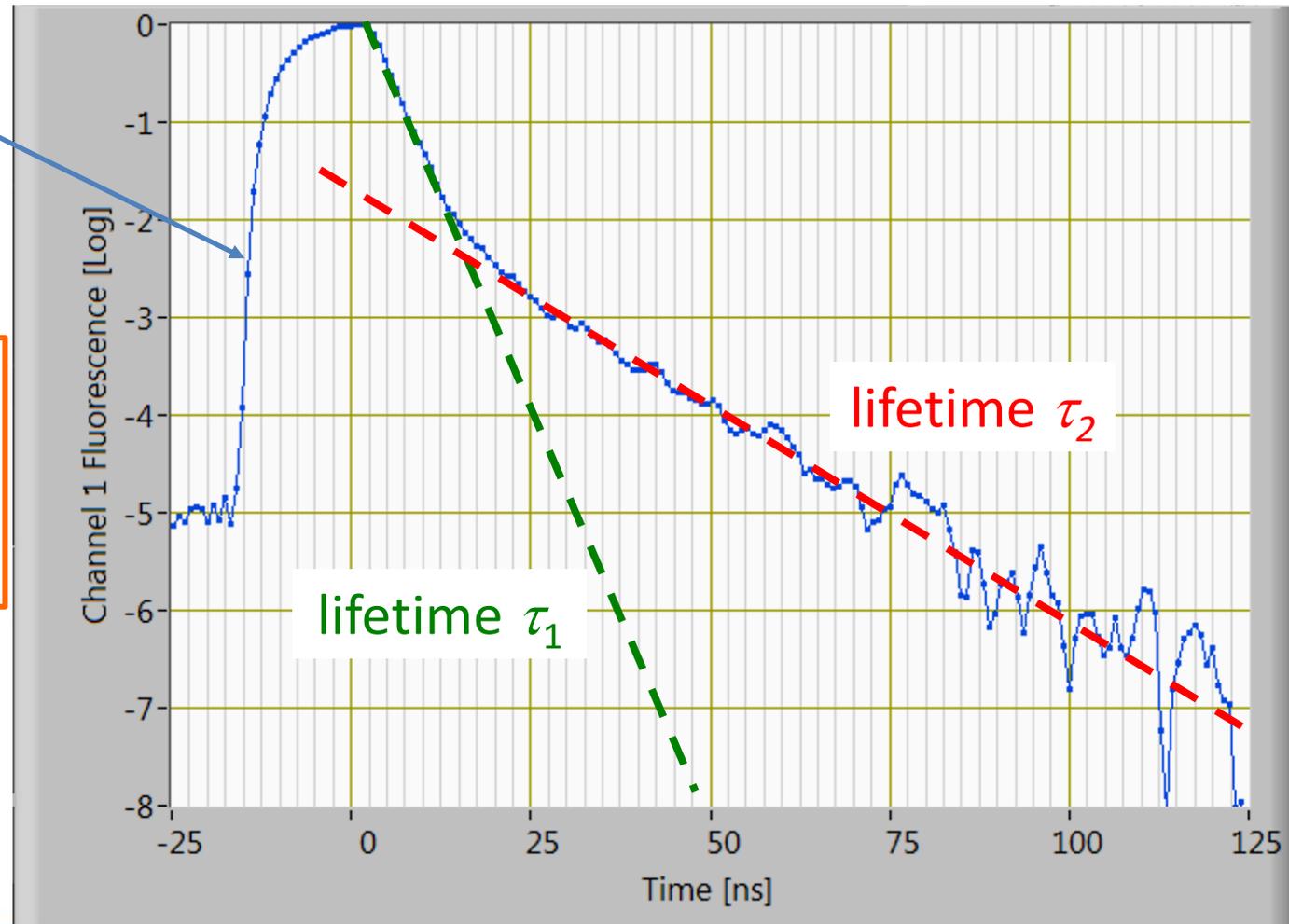
Danube: 200-ps Lifetime Resolution



Danube: Multicomponent Decays

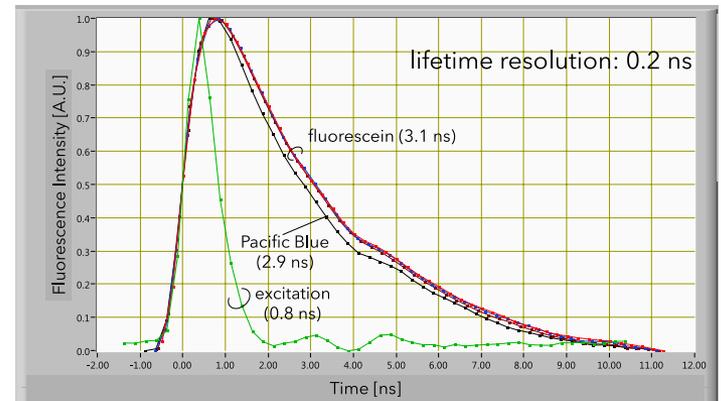
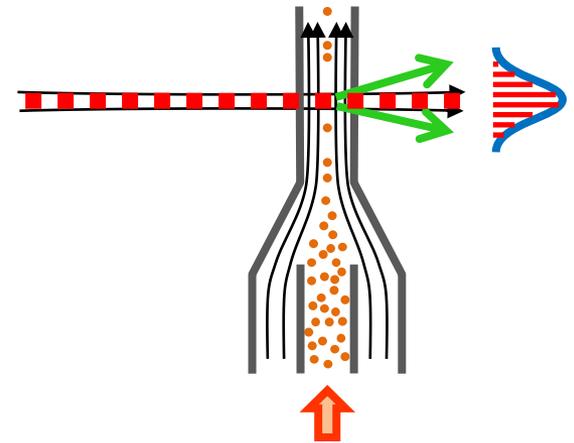
excitation pulse

Quantitative
time-resolved
flow cytometry



Danube

- fluorescence **lifetime**
- **200-ps** resolution
- protein-protein interactions
- subcellular environment



KINETIC RIVER

A FEW THINGS YOU MAY NOT KNOW ABOUT US



Kinetic River: 10th Anniversary



Kinetic River: 3x From 2019 to 2020



Giacomo Vacca, PhD
Founder & President



KP Shevgaonkar, MS
Biomedical Scientist



Alan Chin, PhD
Sr. Staff Scientist, Project Mgr.



Eli Kashi, MS
Mechanical Engineer



Jinman Huang, PhD
Sr. Staff Scientist



Tim Gray, MS
Mfg. Engineer



Ashley Sloat, PhD
*Patent Agent
IP Advisor*



Richard McKay, PhD
*Technical Sales
Advisory Board*



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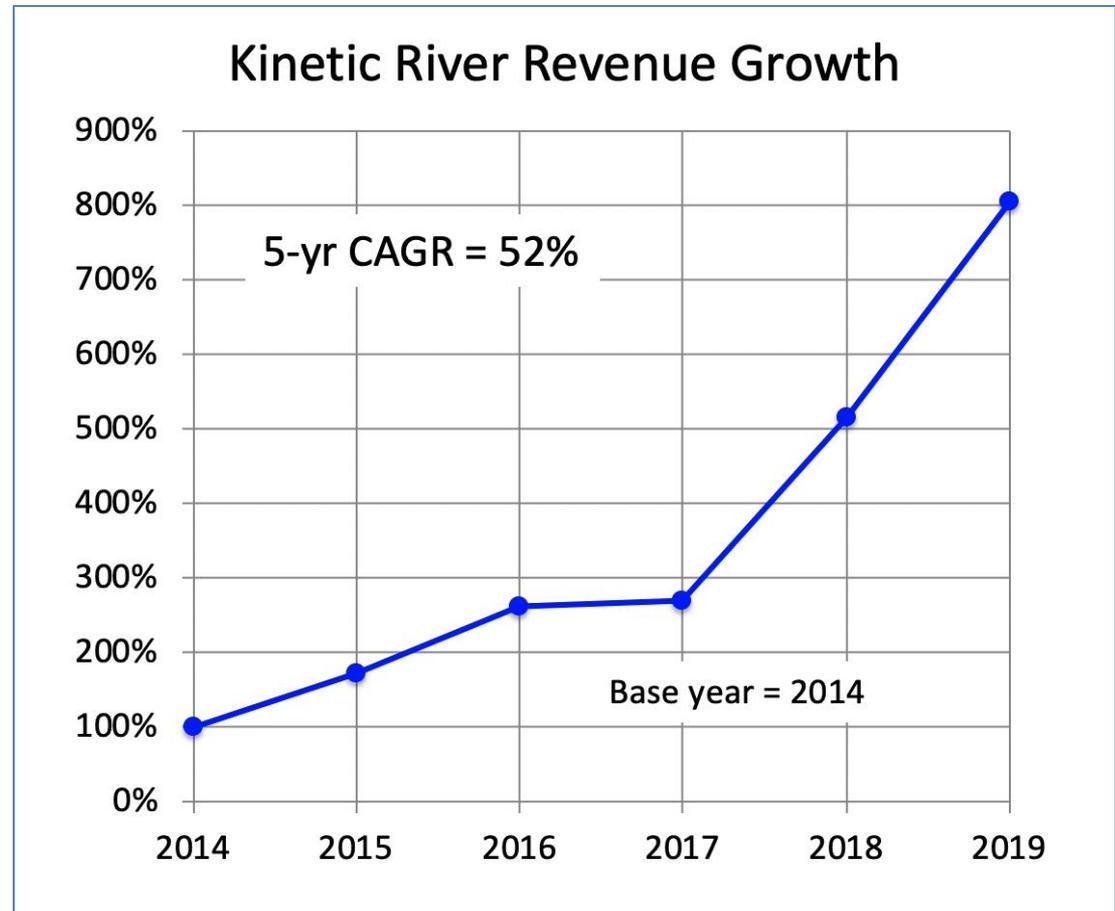


Linda Vahdat, MD, MBA
Advisory Board



Kinetic River: A Growing Business

- **\$2.2M in NIH-NIGMS grants**
- 2-year SBIR Phase II *Arno* development funding (current)
- new sales / engineering offices in US East Coast, Italy / Southern EU
- expanding consulting activities (expert witness)



Kinetic River: 10 Patents and Counting

- **4** patent families
- **10** patents issued
- **9** patents pending
- applications in US and internationally



Acknowledgements

- Research supported in part by the **National Institute of General Medical Sciences** of the U.S. **National Institutes of Health** under grant numbers, 1R43GM131619-01, 1R43GM128546-01, 1R43GM123906-01, and 2R44GM123906-02A1.
- K.P. Shevgaonkar, R. McKay, A. Chin, J. Huang, E. Kashi, H. Shah (Kinetic River)
- D. Vacca, A. Singhal (summer interns)
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