Innovative Time-Resolved Approaches to Flow Cytometry

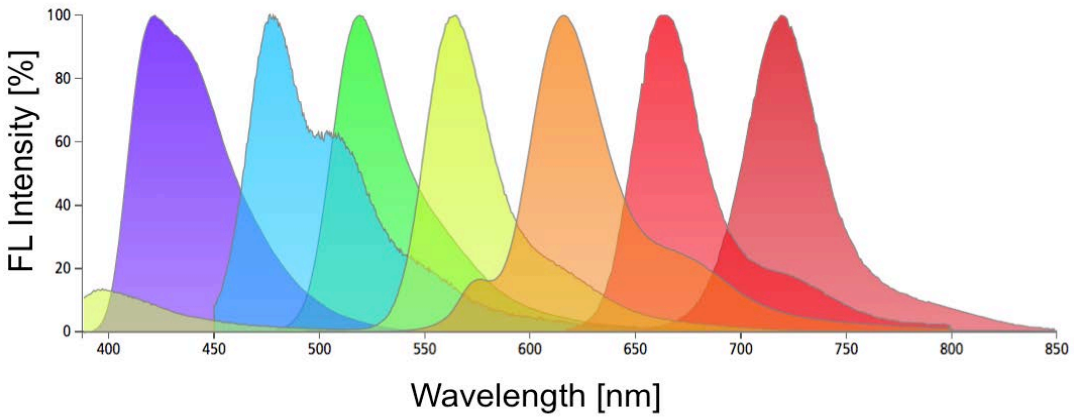
December 12, 2019

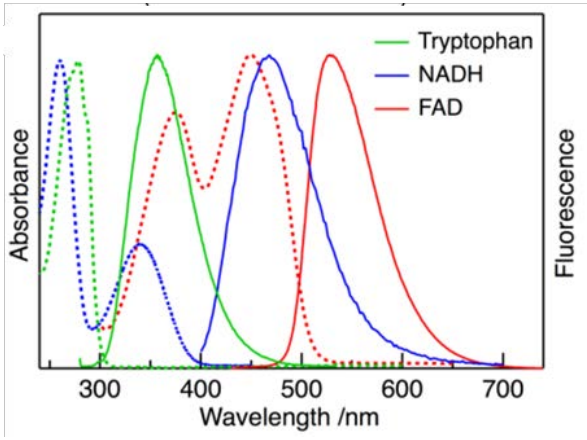
NIH Flow Cytometry Interest User Group Winter 2019 Meeting

Giacomo Vacca, *President*

Kinetic River Corp.

Unmet Needs in Fluorescence Flow Cytometry





UNMET NEEDS:

1. Spectral spillover → limited # channels
   - PAIN: long, complex runs
   - PAIN: incomplete information
2. Spectral spillover → compensation
   - PAIN: population spreading
   - PAIN: wasted time
3. Autofluorescence → high background
   - PAIN: reduced sensitivity
   - PAIN: reduced dynamic range

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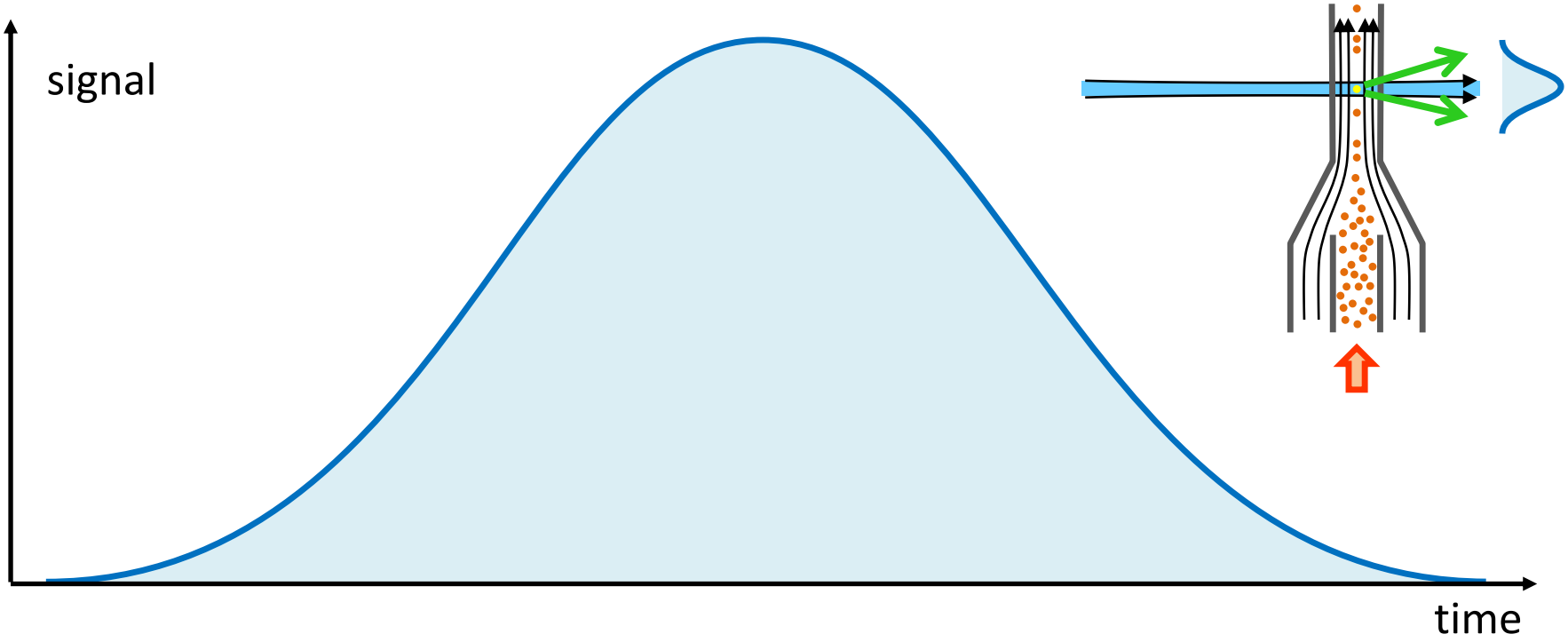
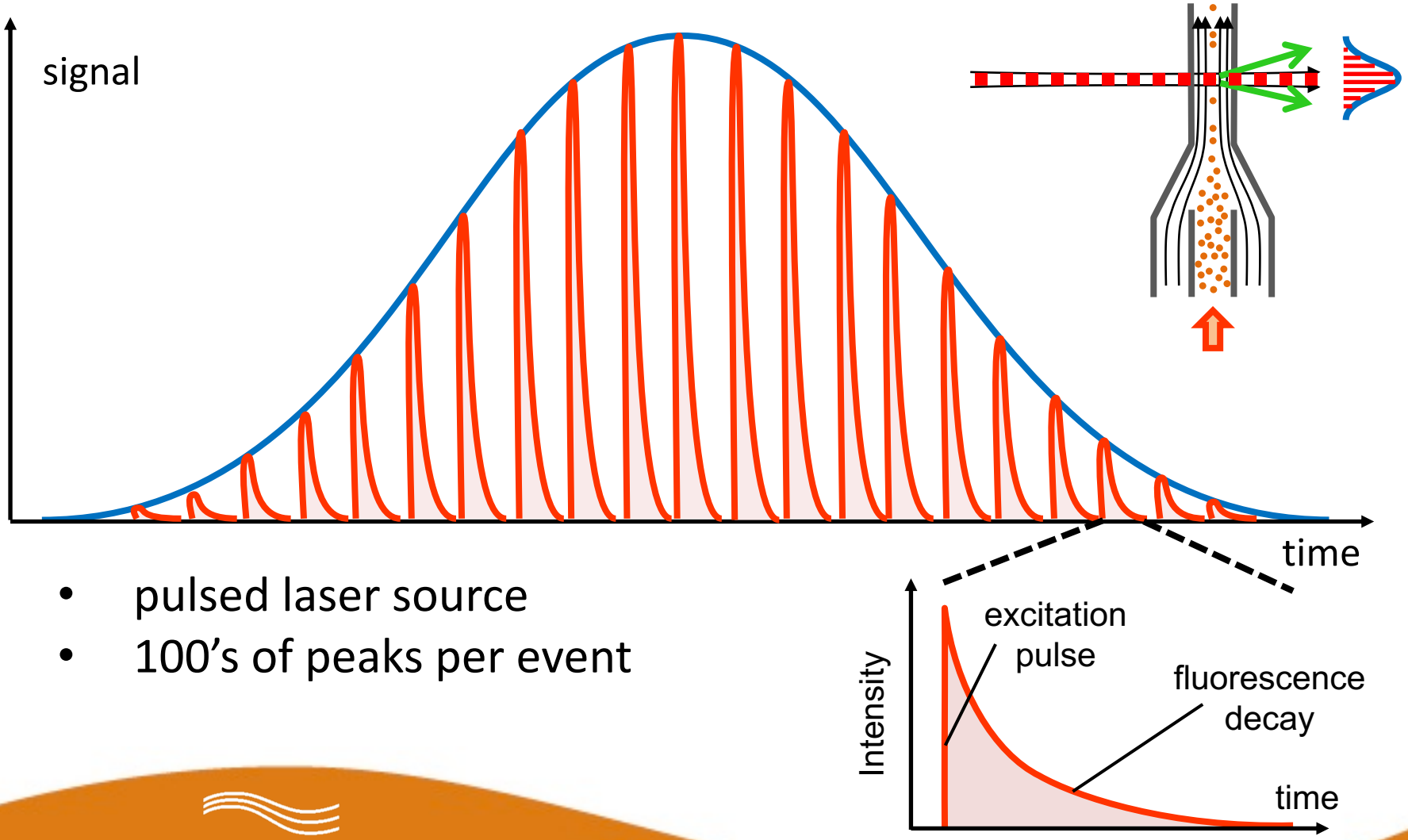
Traditional Flow Cytometry



- continuous light source
- one peak per event

Time-Resolved Flow Cytometry



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

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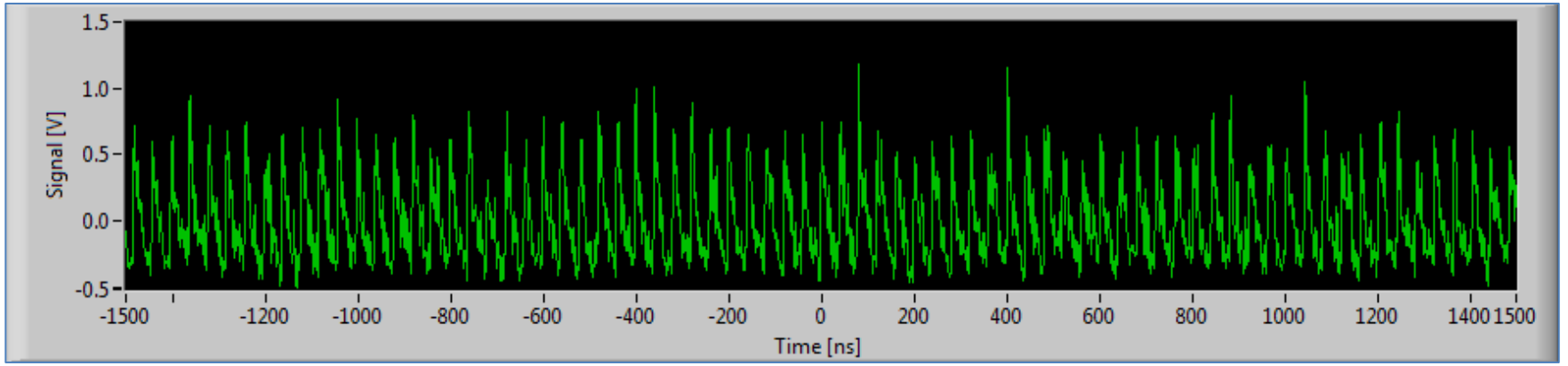
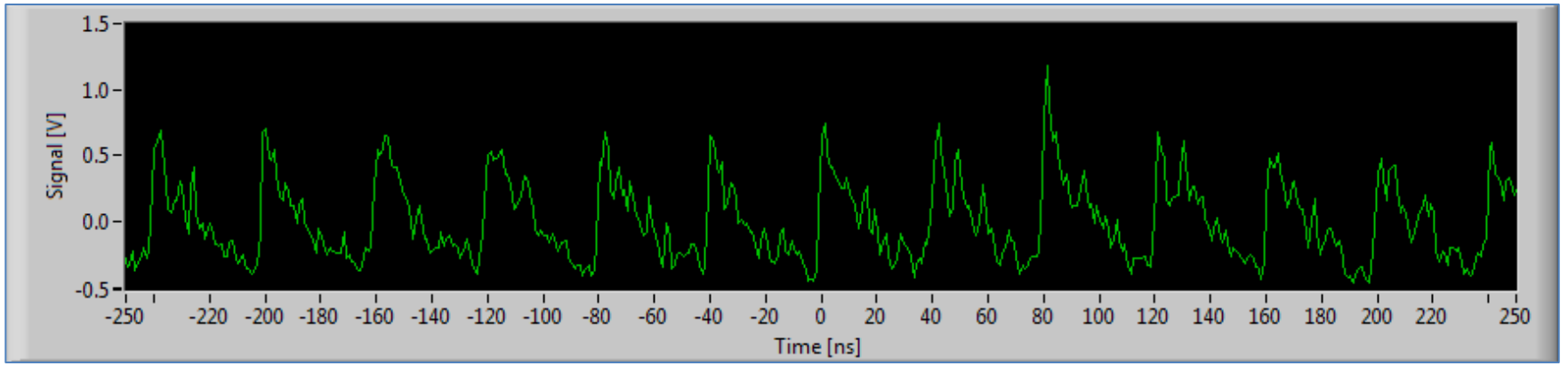
Time-Resolved Flow Cytometry





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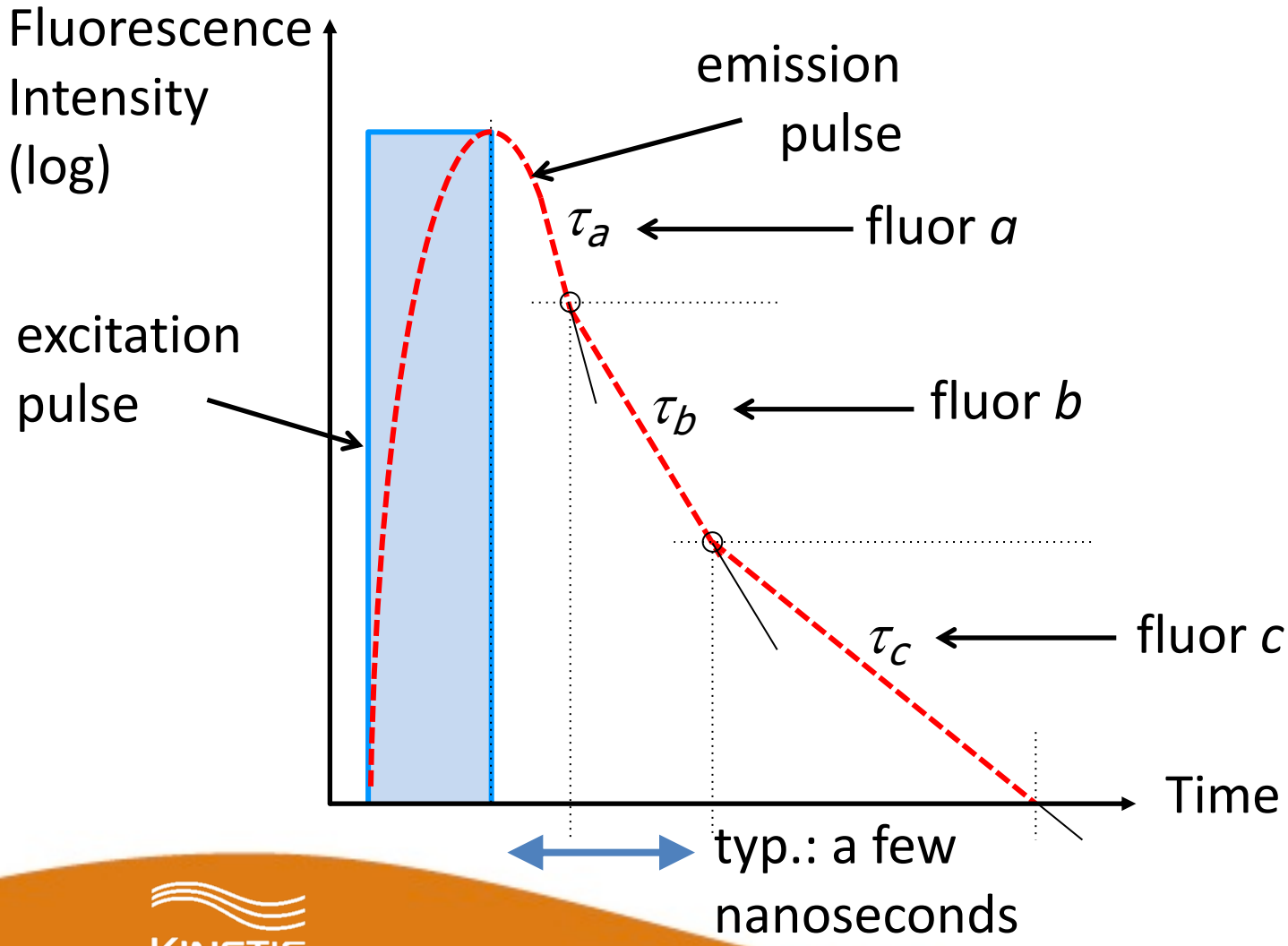
Multiple Fluors Recorded on the Same Detector at the Same Time



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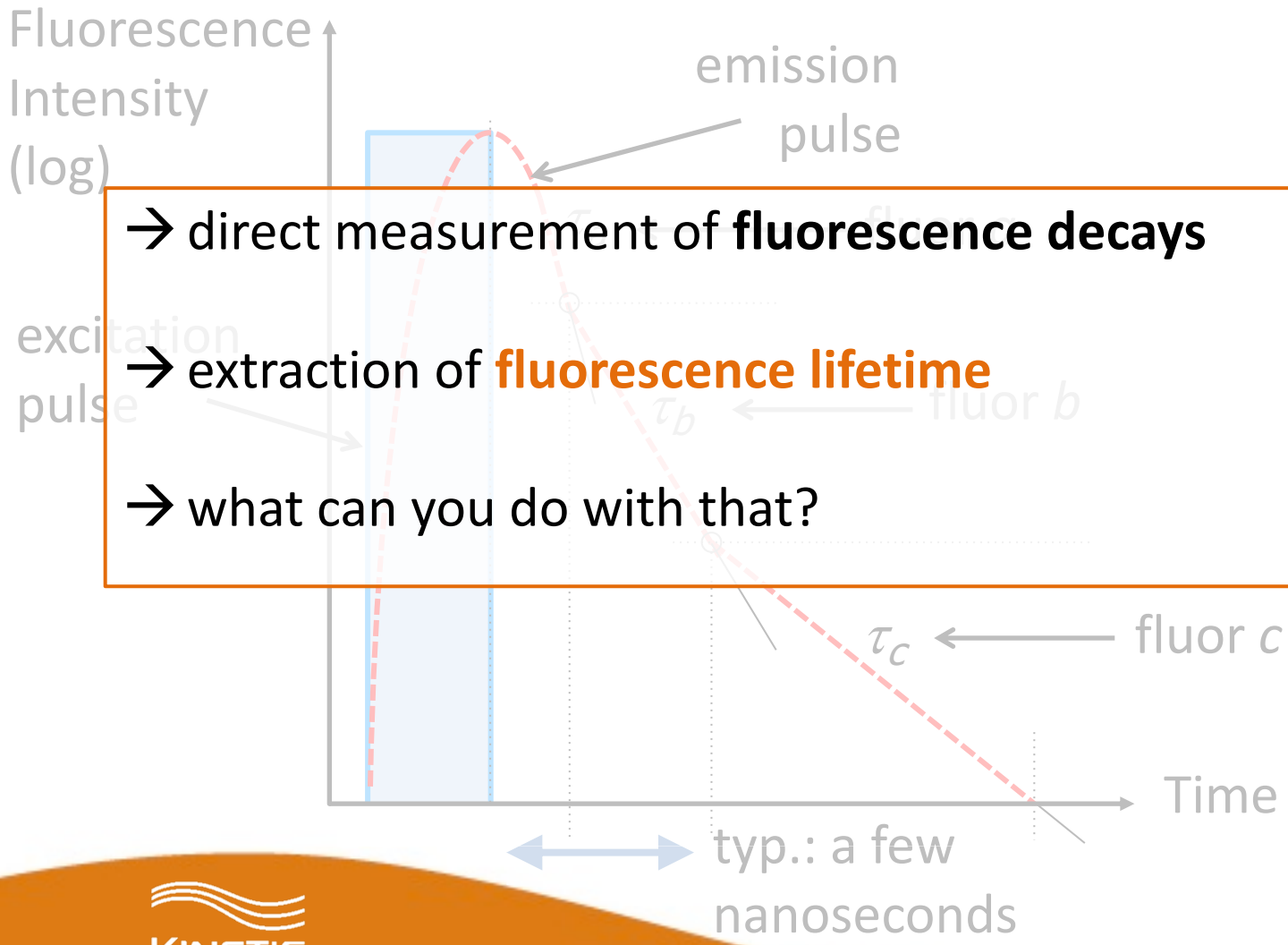

→ direct measurement of **fluorescence decays**

→ extraction of **fluorescence lifetime**

→ what can you do with that?

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LIFETIME AS A PARAMETER:

DANUBE

FLUORESCENCE LIFETIME FLOW CYTOMETER

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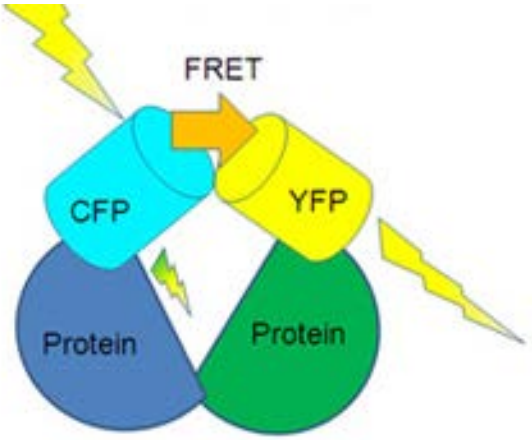
Fluorescence Lifetime As a Parameter

- can be sensitive local probe
- quantitate FRET (Förster Resonance Energy Transfer)

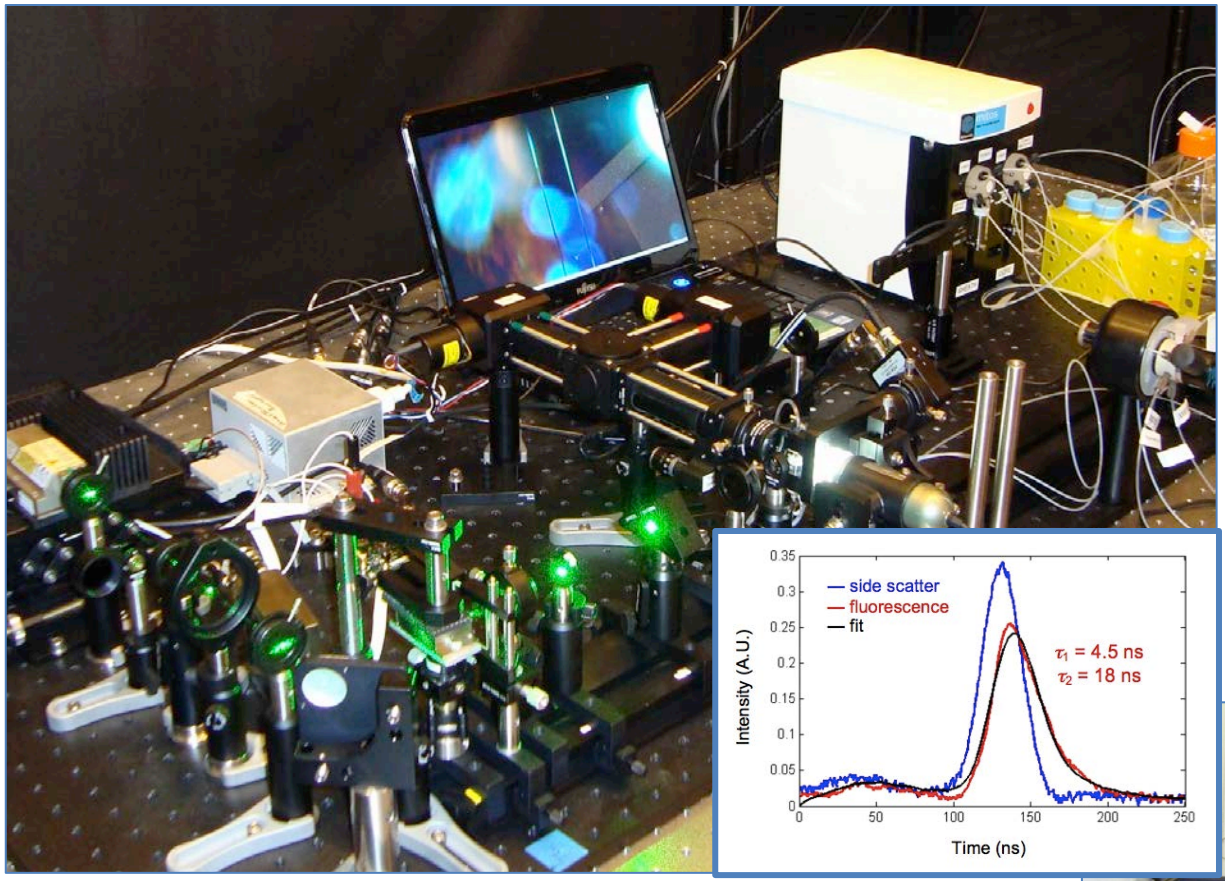


- probe molecular environment
  - Ca^{2+}, Cl^{-}, O_2, pH, temperature
- probe protein-protein interactions

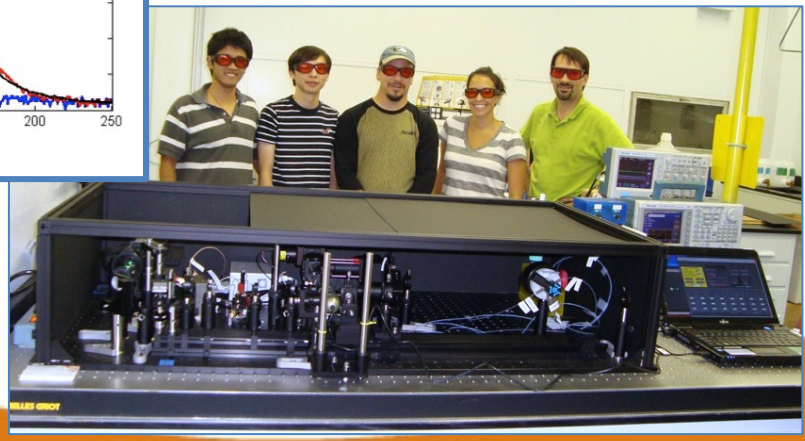
APPLICATIONS

- cell signaling
- cancer cell analysis
- label-free identification of cancer cells

Danube: Fluorescence Lifetime FC



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



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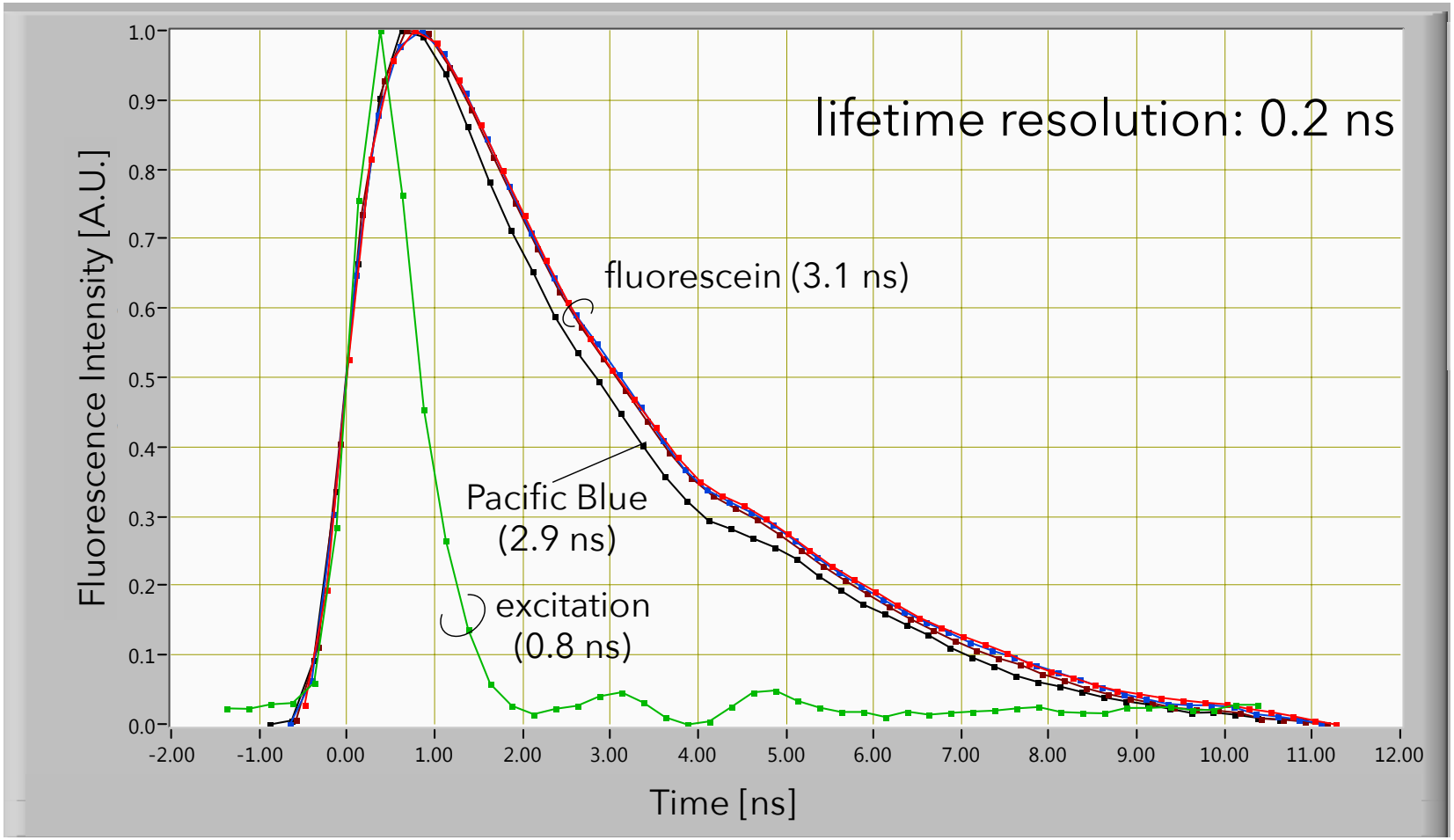
Danube: 200-ps Lifetime Resolution



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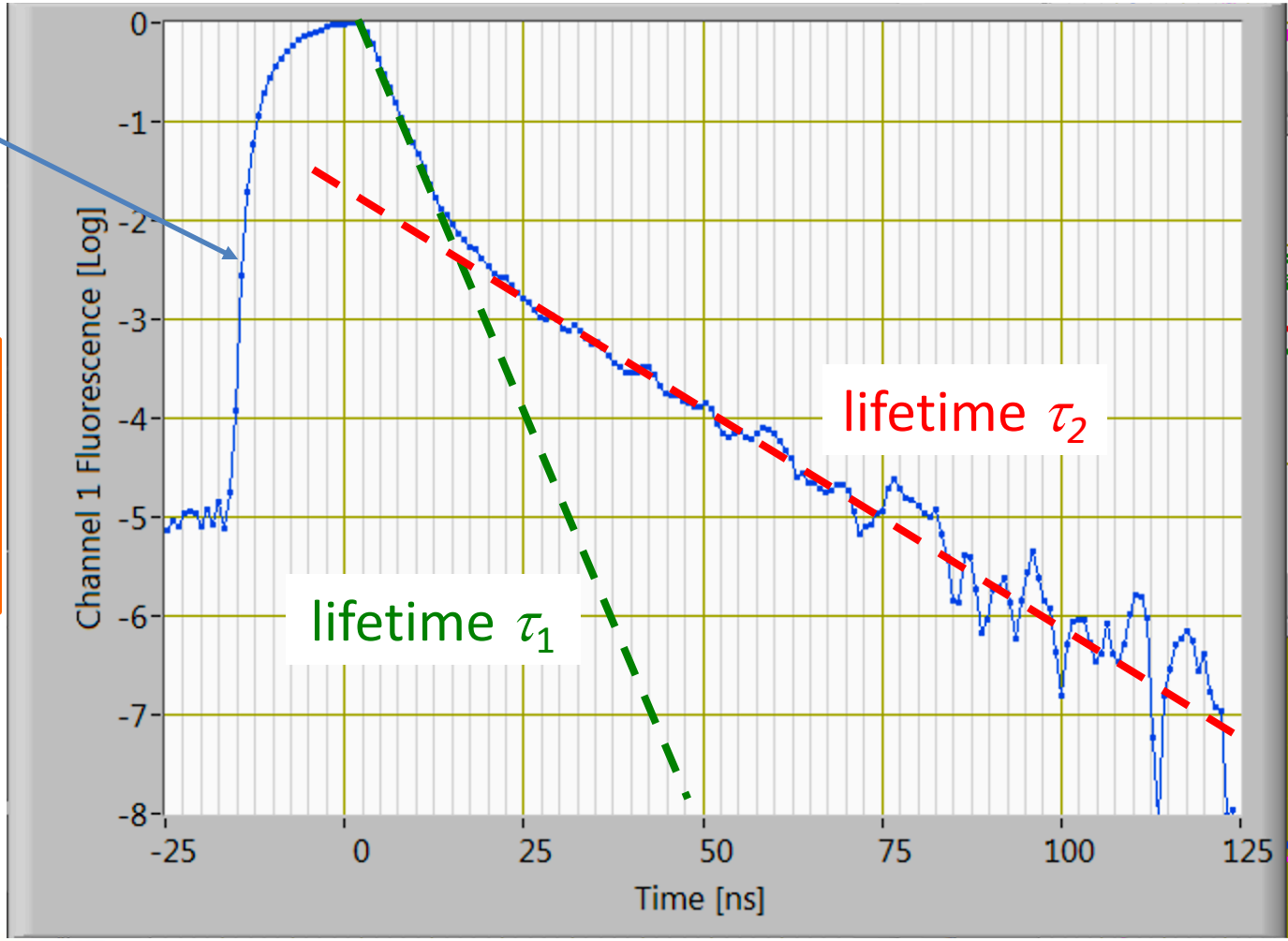
Danube: Multicomponent Decays

excitation pulse

Quantitative time-resolved flow cytometry



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LIFETIME AS A MEANS TO AN END:

TIBER

LABEL-FREE METABOLIC PROFILE FLOW CYTOMETER

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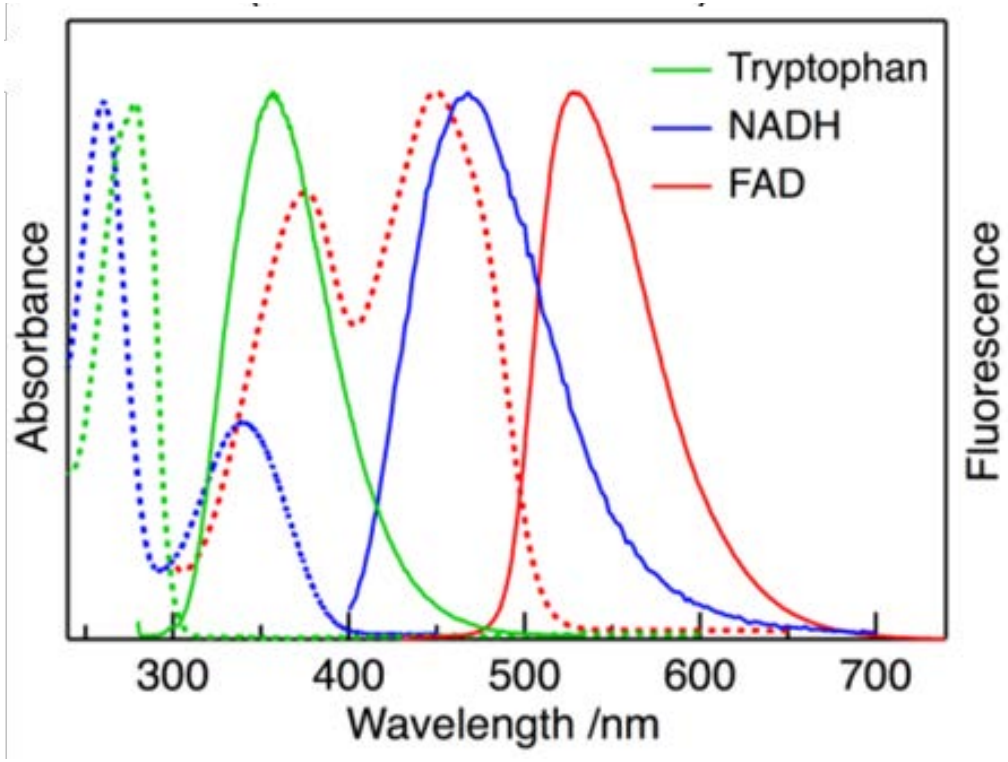
Some Sources of Cellular AF



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

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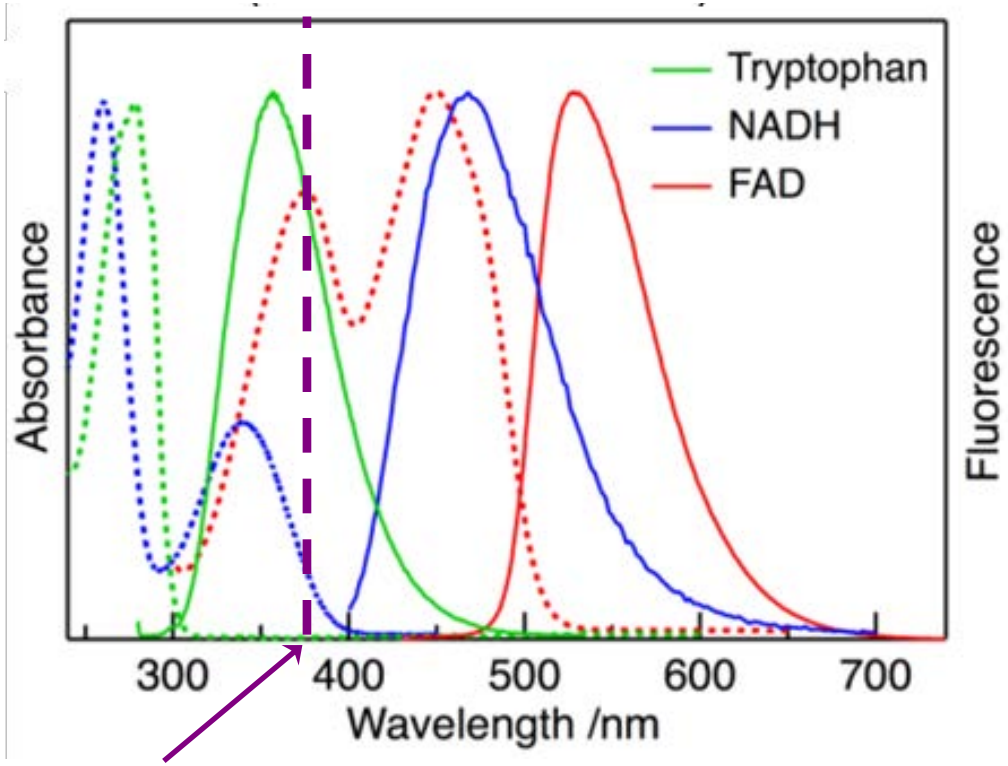
Some Sources of Cellular AF



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

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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)	aerobic glycolysis (cytosolic)
Free: Bound NADH Ratio	$\alpha_{free}/\alpha_{bound}$ is **LOW**	$\alpha_{free}/\alpha_{bound}$ is **HIGH**
Free NADH Lifetime, τ_{free}, is **SHORT**, <0.5 ns		
Protein-bound NADH Lifetime, τ_{bound} , is **LONG**, >1.5 ns		

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Metabolic Profiling by FLIM

- wtHEK293 cells
- lifetime of NADH autofluorescence

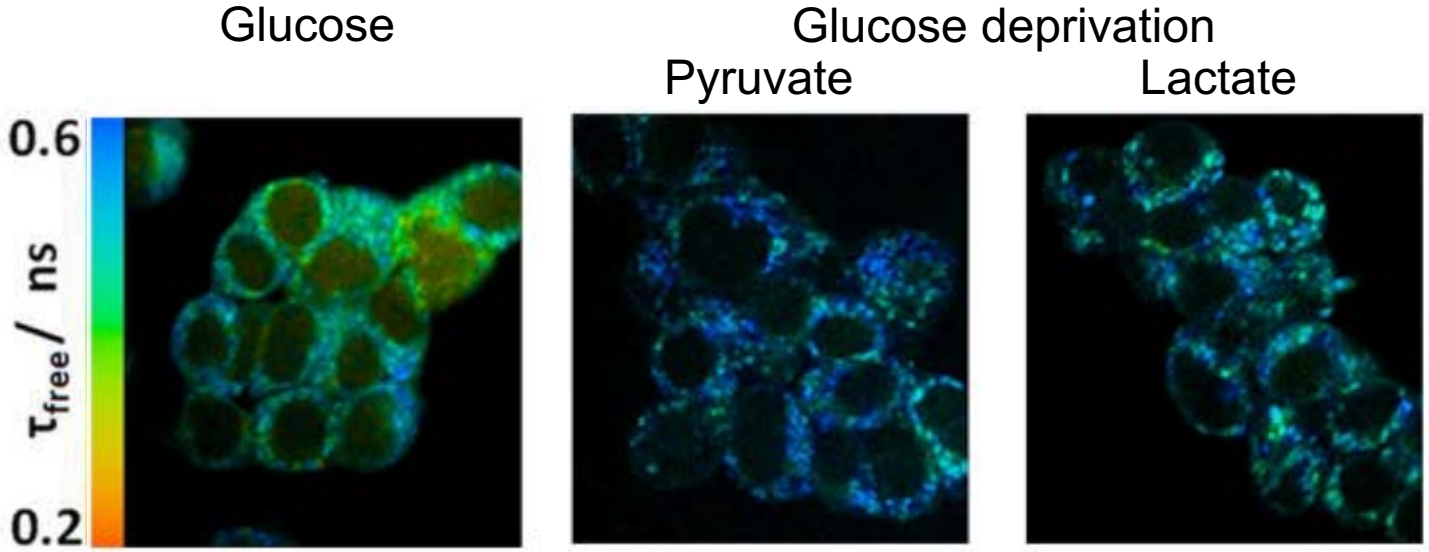

Fig. 2f, Blacker et al., Nat. Comm. 5:3936 (2014) | DOI: 10.1038/ncomms4936

NADH Free vs. Bound by FLIM

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melanoma mouse model

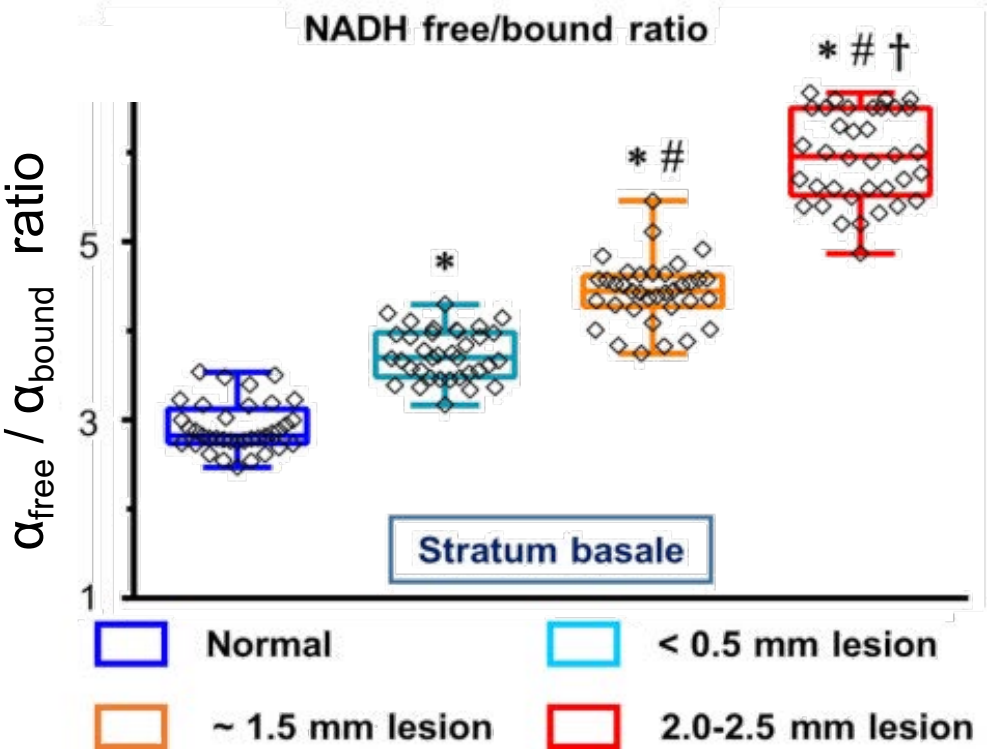

Fig. 3, Pastore, M. N., et al. (2017) Exp. Derm. 26, 607-614

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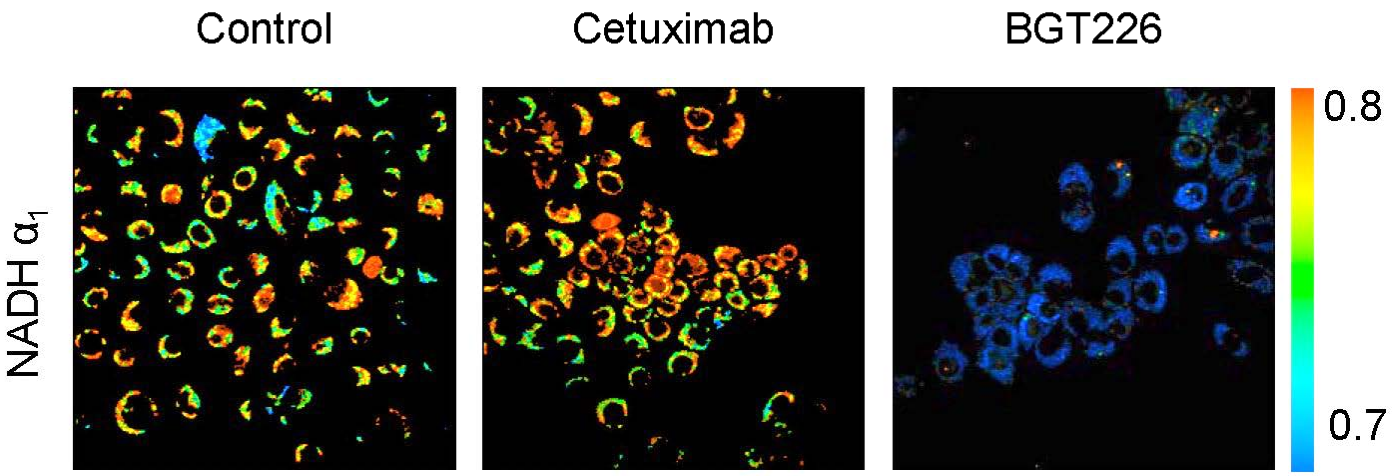
Tiber: Label-Free Cancer Cell ID

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

cancer cell: NADH lifetime ↑

normal cell: NADH lifetime ↓



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

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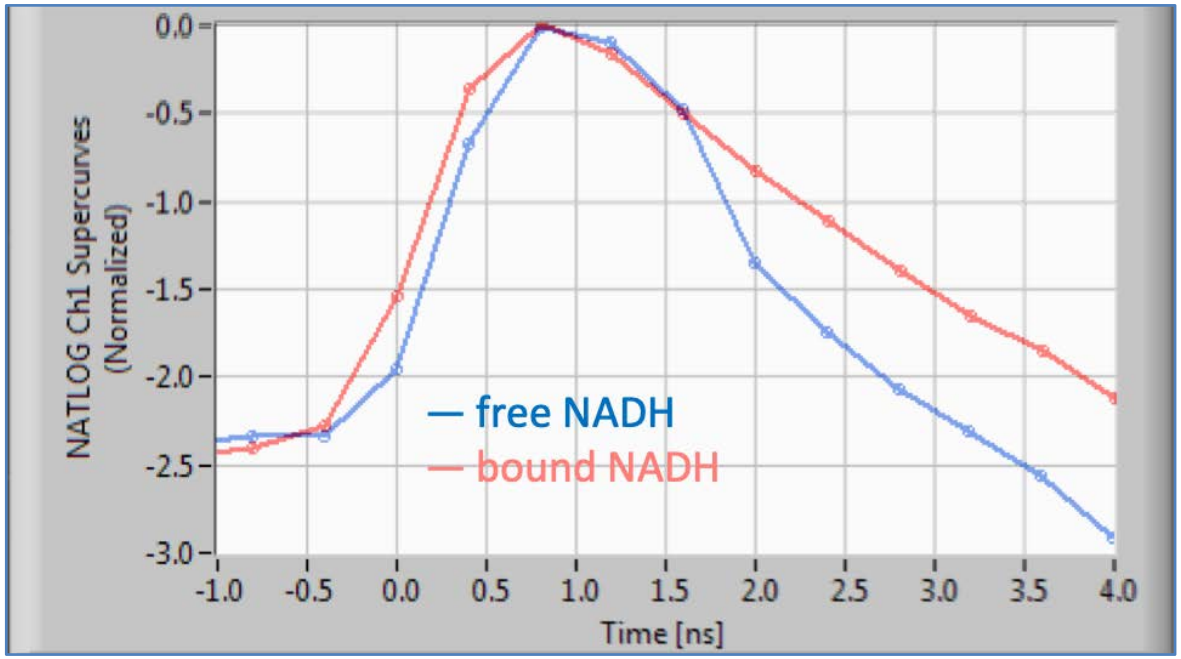
Tiber: Free vs. Bound NADH in Flow

- 375-nm excitation
- label-free
- blue FL channel
- NADH in solution, free & bound to lactate dehydrogenase
- direct time-resolved measurements
- **FC results mirror FLIM**
- next: NADH in cells in flow



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LIFETIME AS A MEANS TO AN END:

ARNO

COMPENSATION-FREE FLOW CYTOMETER

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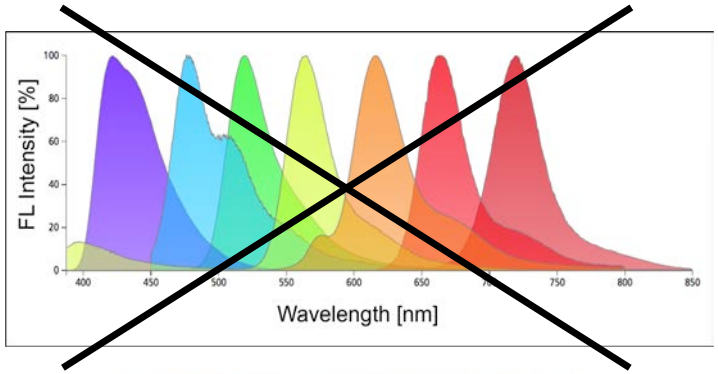
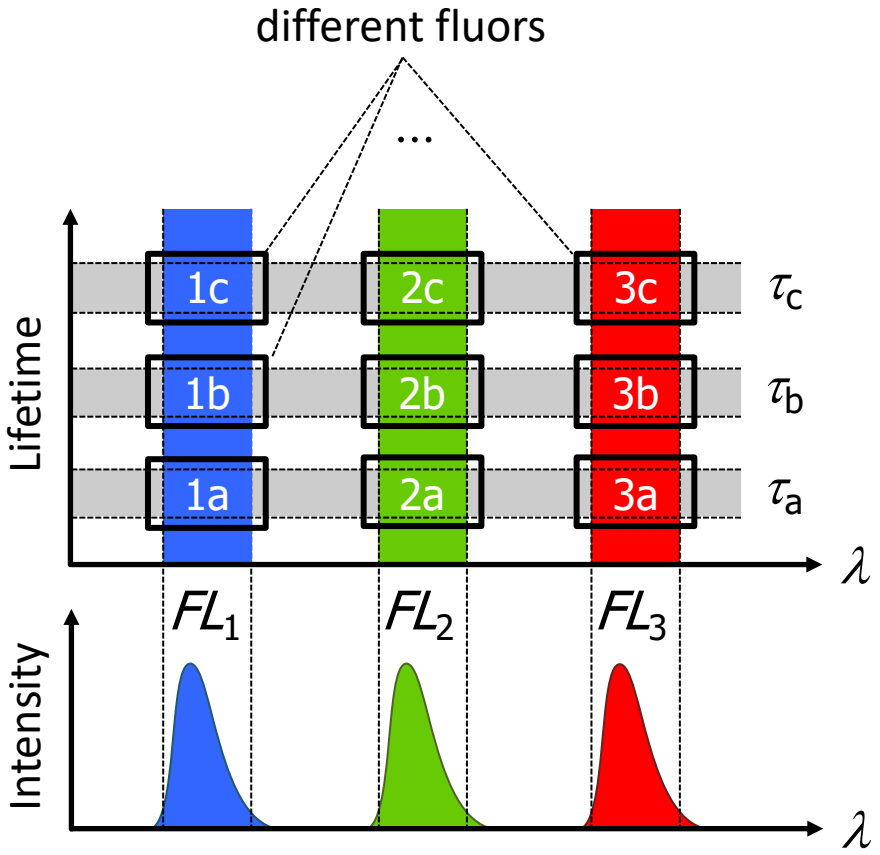
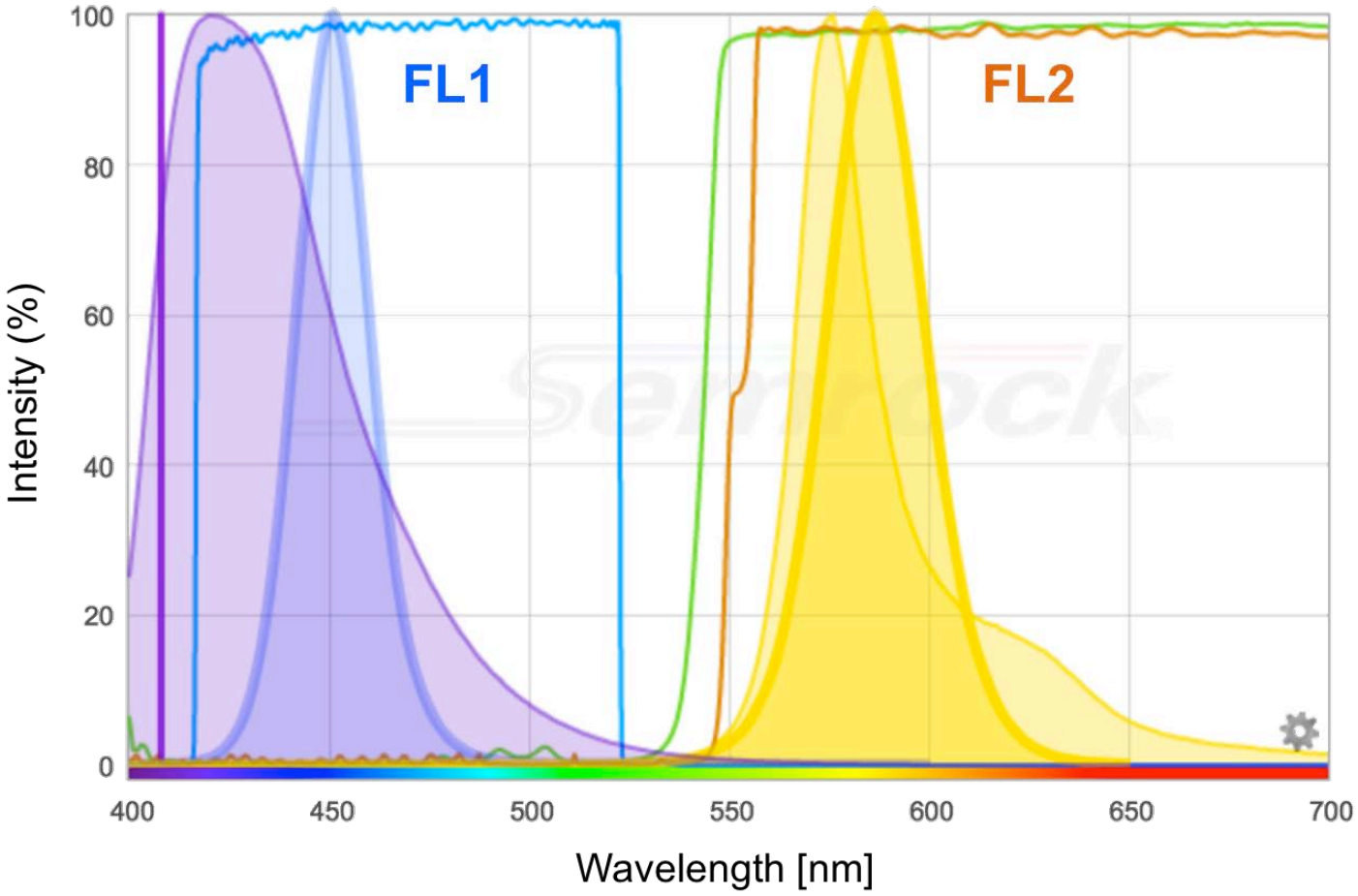
Lifetime As Means To an End

- distinguish fluors
  based on color
  AND lifetime
  - → reduce spectral spillover
  - → **compensation-free**
  - → expand # parameters





A Four-Color Example



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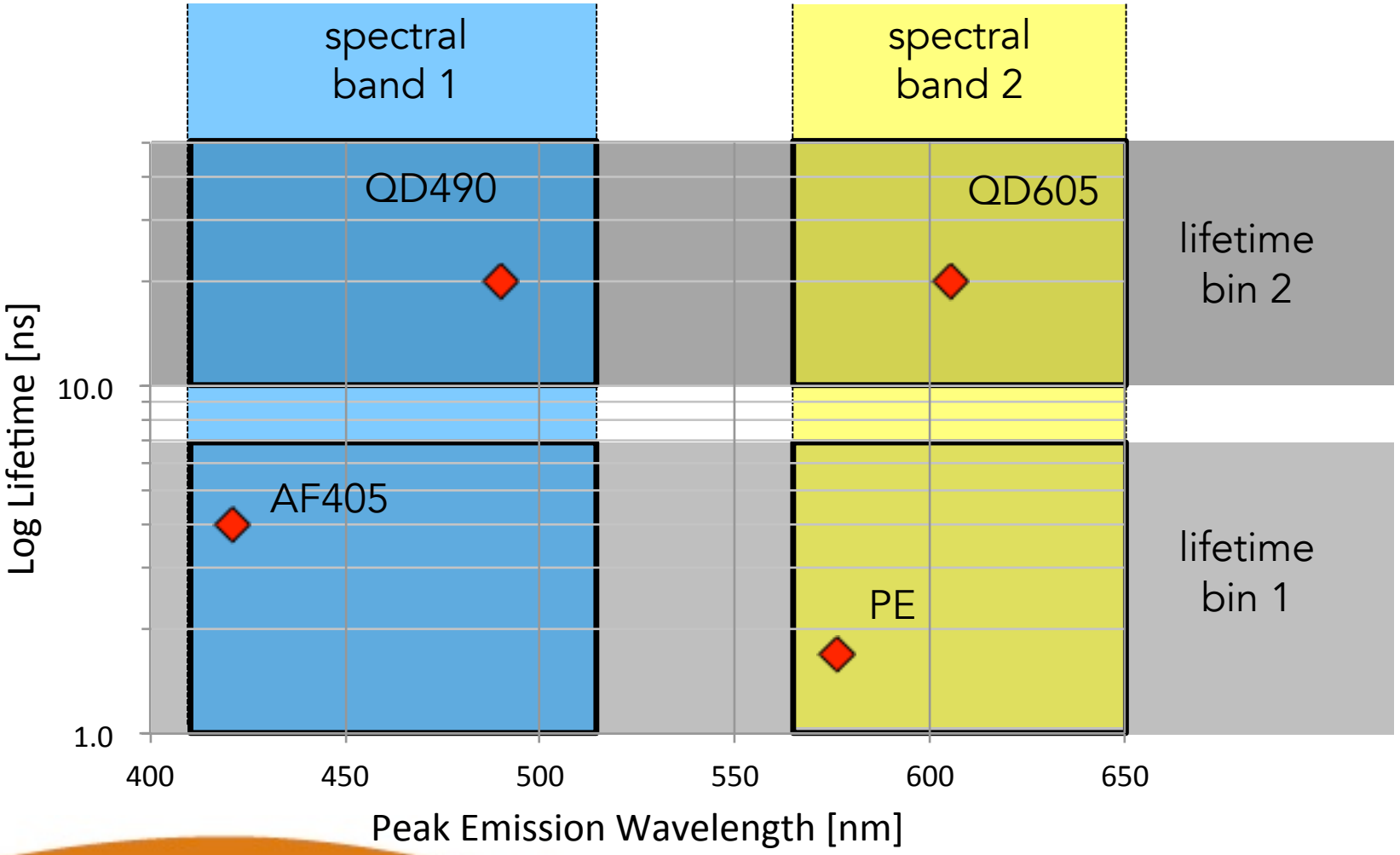
4 Colors: 2 Spectral Bands, 2 Lifetimes



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Arno Development Platform

- 3rd generation platform
- 6 parameters (4x FL, FSC, SSC)
- bead SSC singlet CVs ≈ 2%
- bead FL CVs ≈ 6%

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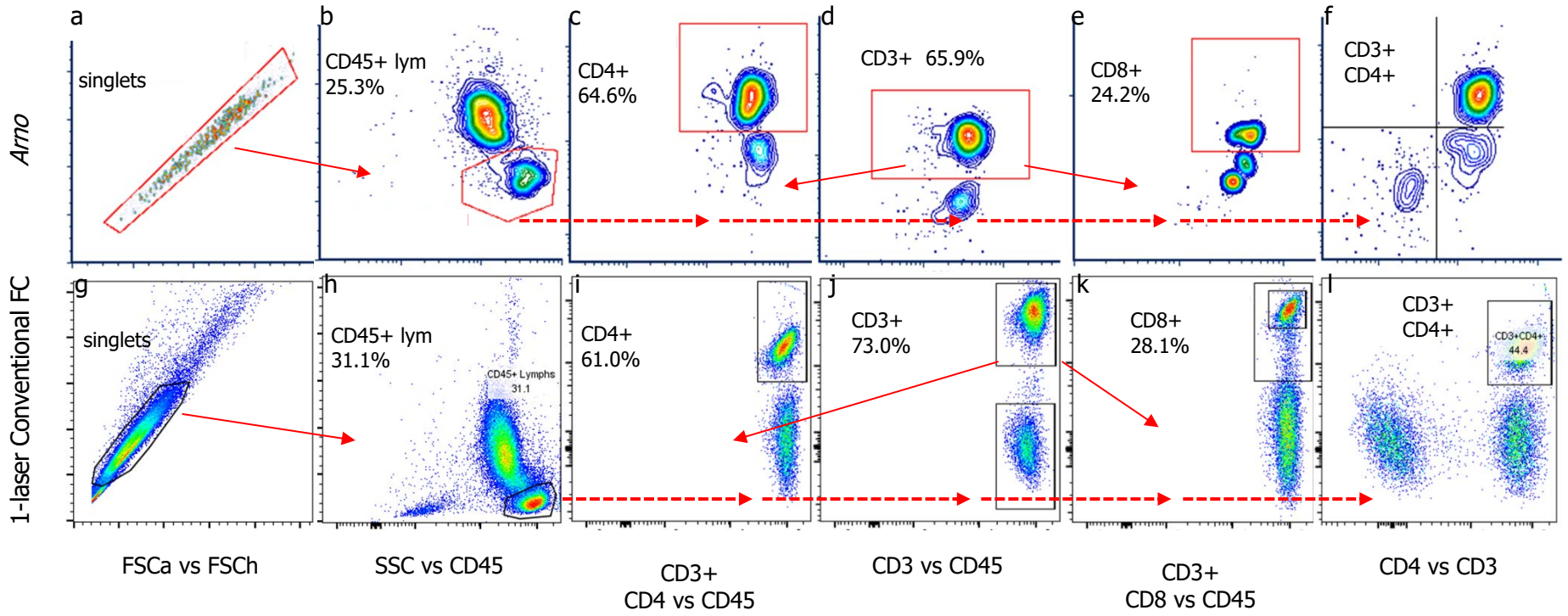
Demo *Arno* Cell Assay: 4 Colors, 2 PMTs, 1 Laser



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

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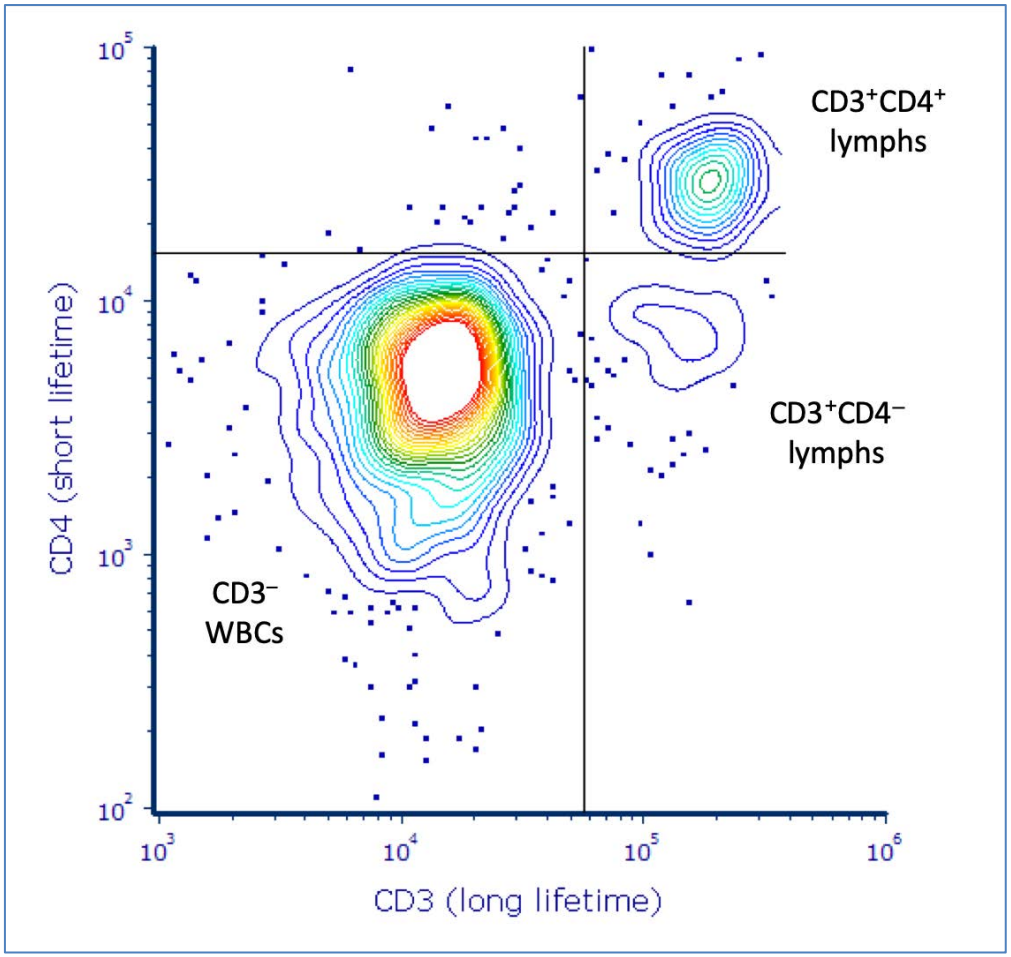
Demo *Arno* CD45/3/4/8 Assay

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



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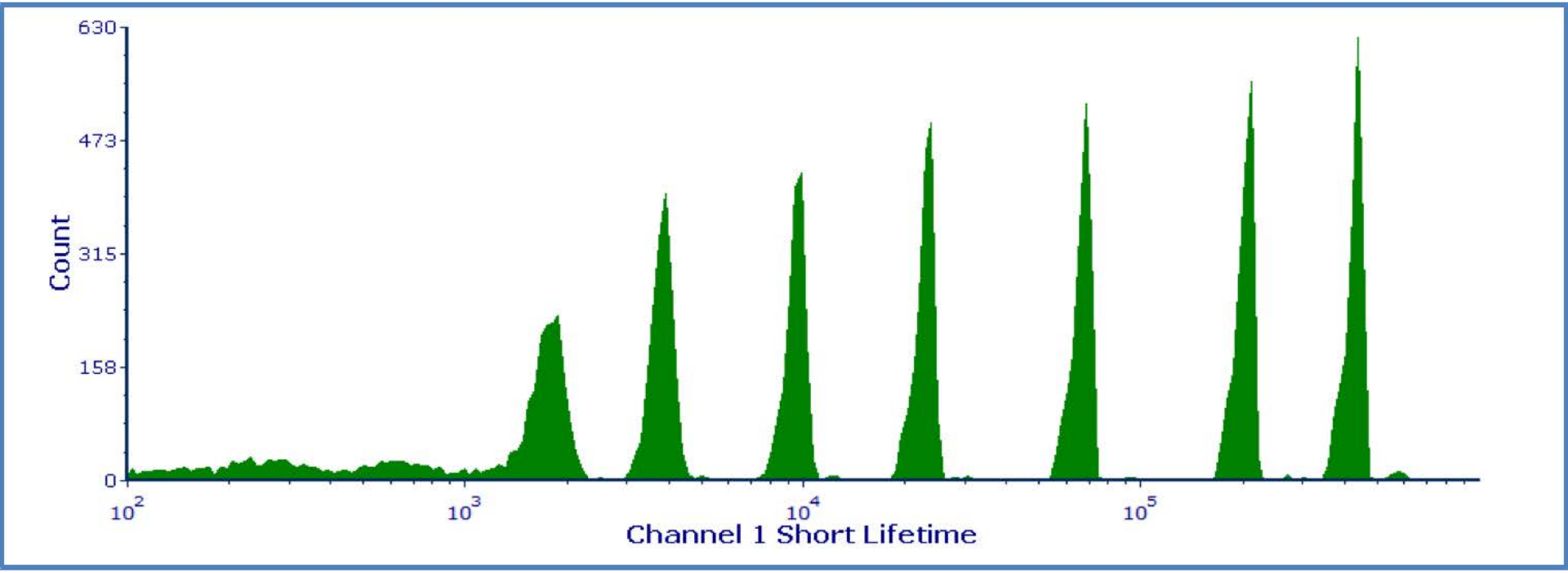
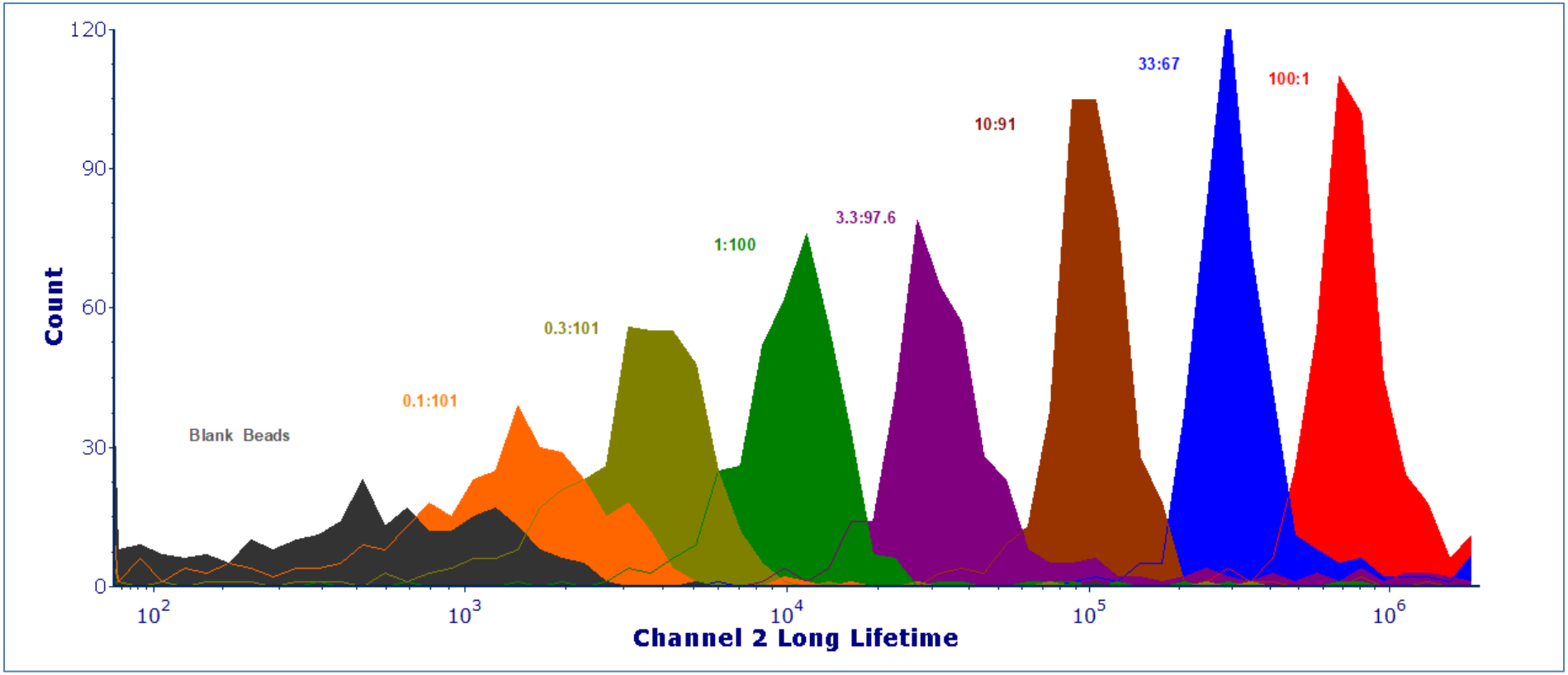
Arno 8-Peak Rainbow Beads



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

Arno Titrations on Comp Beads



- titrated CD8 / QD605 on universal capture beads
- **3 decade dynamic range (for now)**

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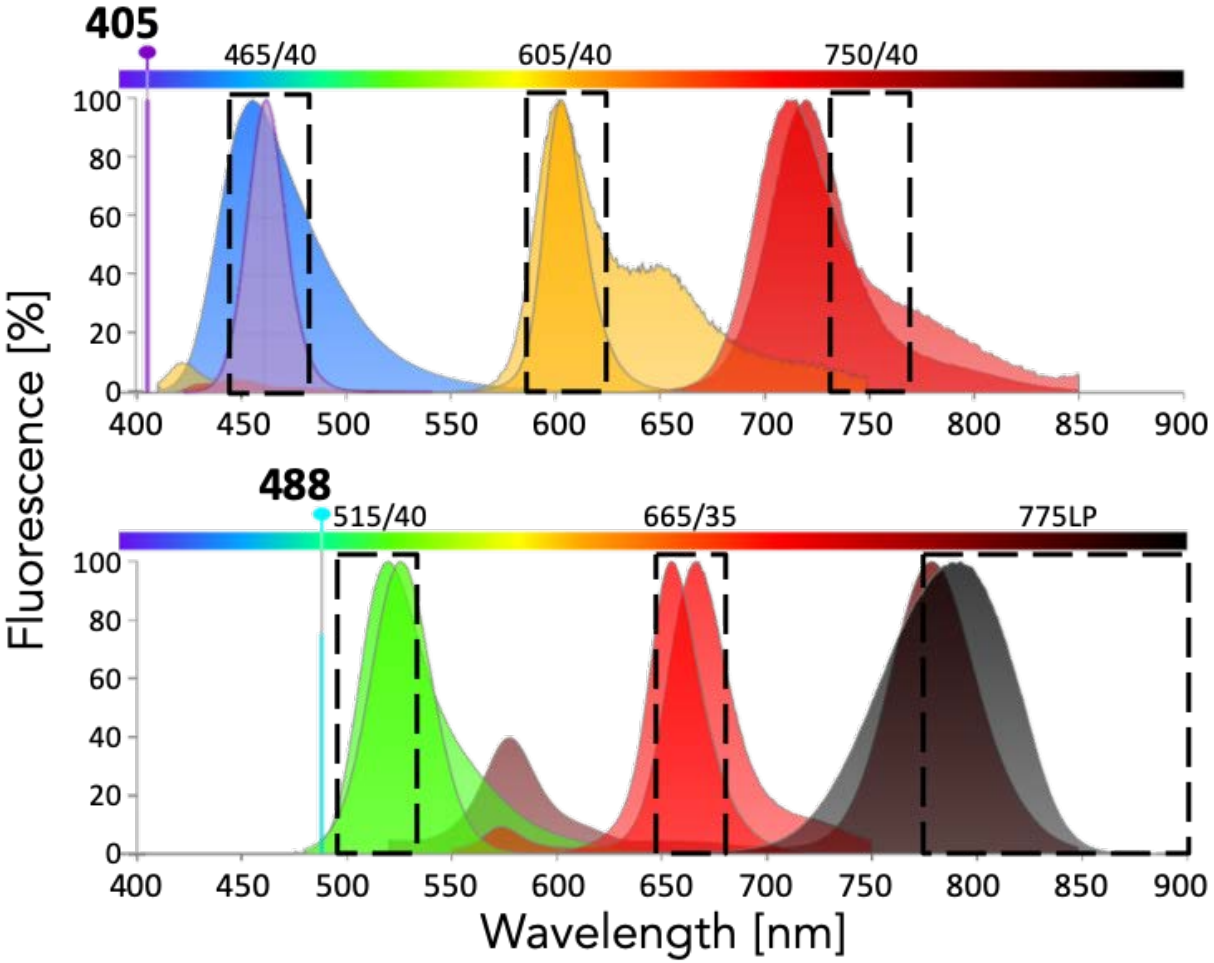
14-Parameter, No-Comp *Arno*



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

Compensation-free multicolor flow cytometry

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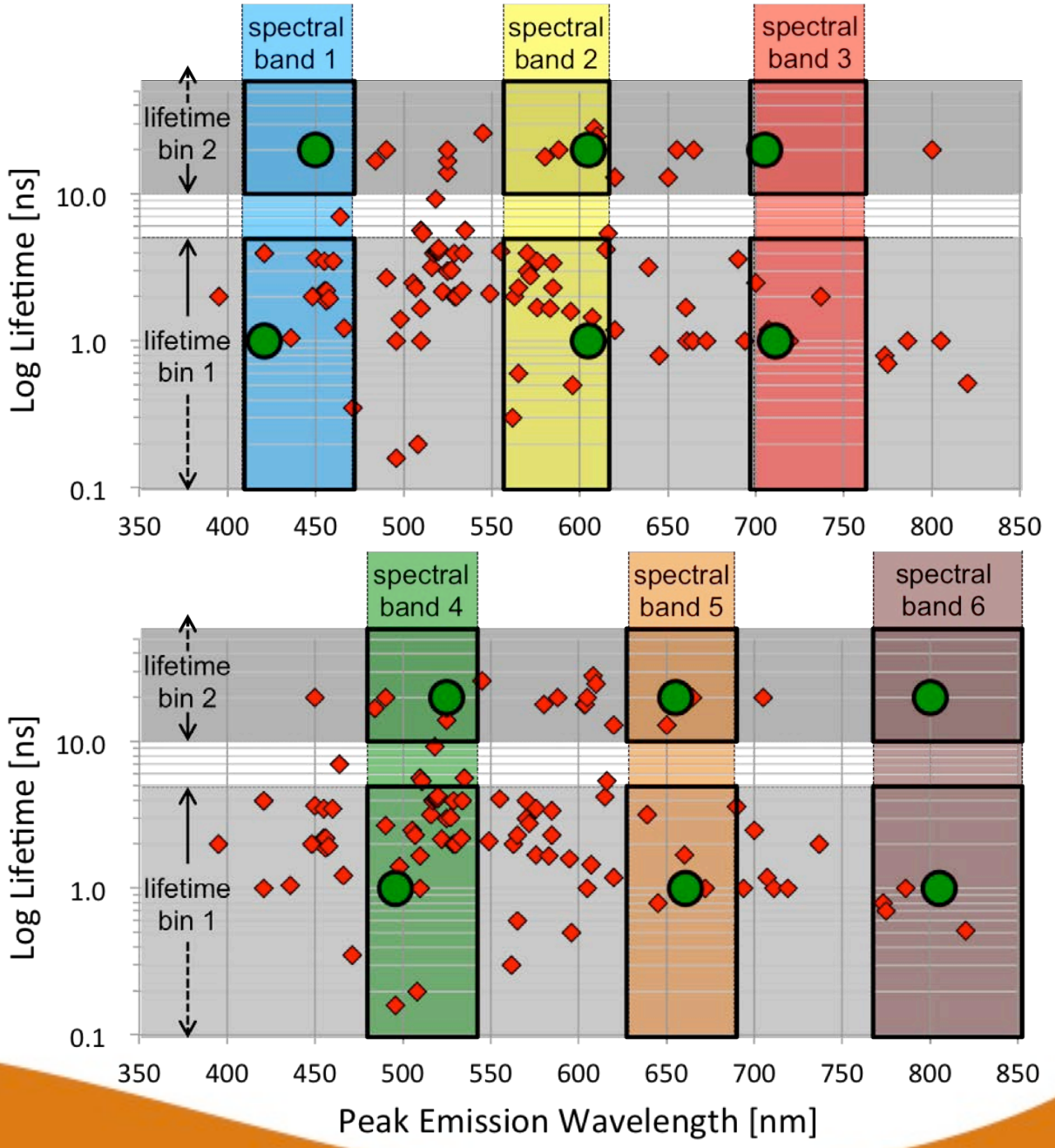
12 Antibodies with 6 Colors

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



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A FEW THINGS YOU MAY NOT KNOW ABOUT US

Kinetic River: A Growing Team

Giacomo Vacca, PhD
Founder & President

KP Shevgaonkar, MS
Biomedical Scientist

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

Eli Kashi, MS
Mechanical Engineer

Haely Shah, MS
Algorithm Engineer

Jinman Huang, PhD
Sr. Staff Scientist

Tim Gray, MS
Mfrg. Engineer

Ashley Sloat, PhD
Patent Agent IP Advisor

Richard McKay, PhD
Technical Sales Advisory Board

Alastair Hood, PhD
Advisory Board

Rosemary Coates, MBA
Advisory Board

Sean Murphy, MS
Advisory Board

Linda Vahdat, MD, MBA
Advisory Board

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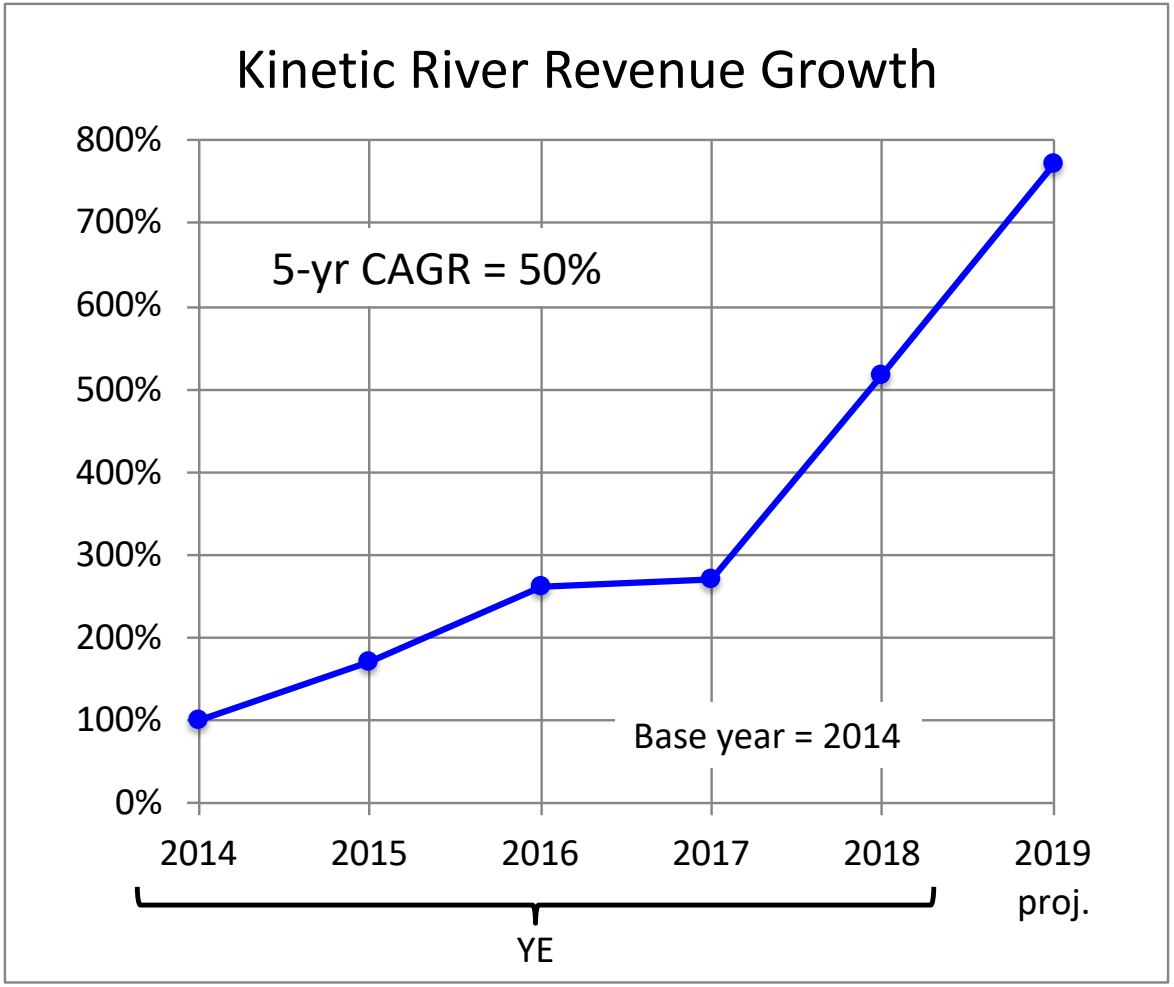
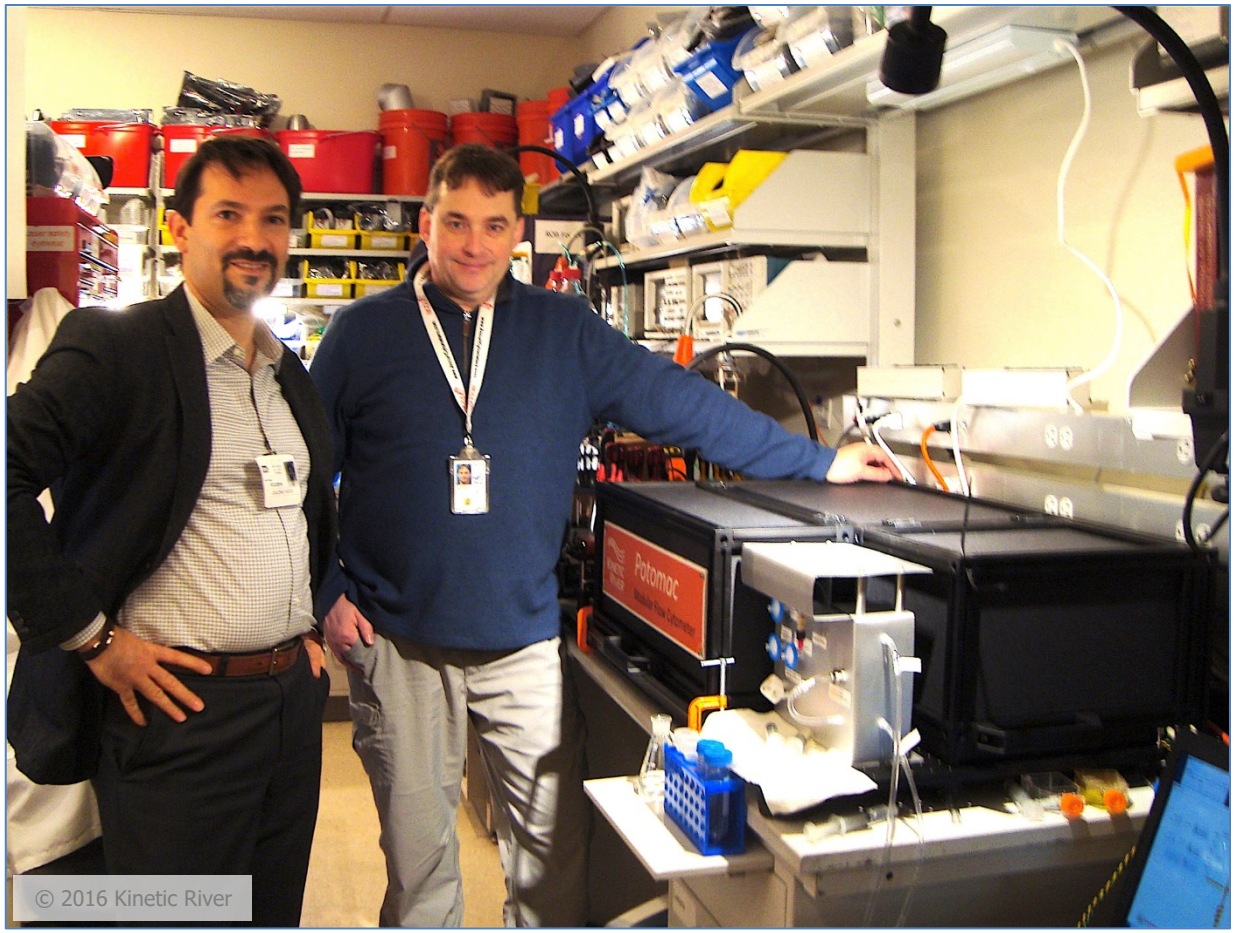
Kinetic River: A Growing Business

- **$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 Analyzer at NCI



- customized design
- Dr. Bill Telford (Core Lab Mgr.)
- 488 + external lasers (stacked beams)
- 7 detectors (5 FL + FSC, SSC)
- customized for external 266-nm excitation

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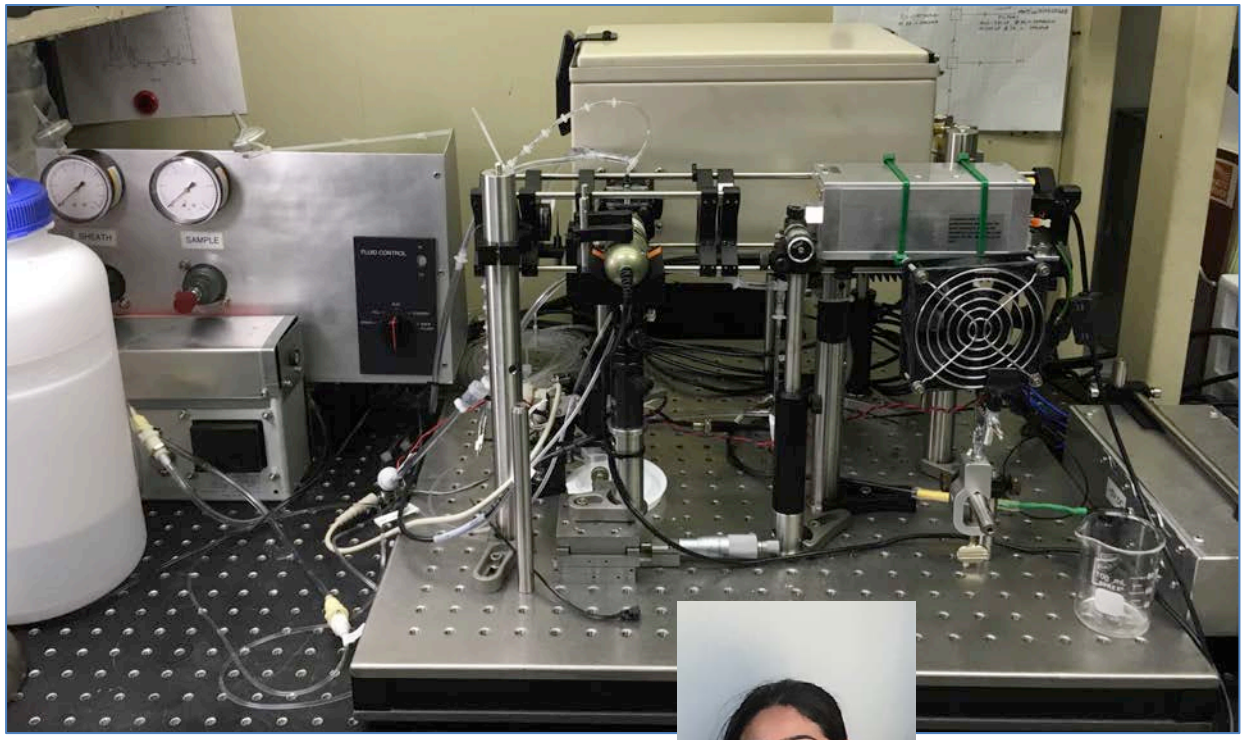
We Can Run Samples For You!



- scientific collaboration
- sample analysis as a service
- or “try before you buy”
- e.g.:
  - Danube (lifetime)
  - Tiber (metabolism)
  - Colorado (elimin. autofluorescence)

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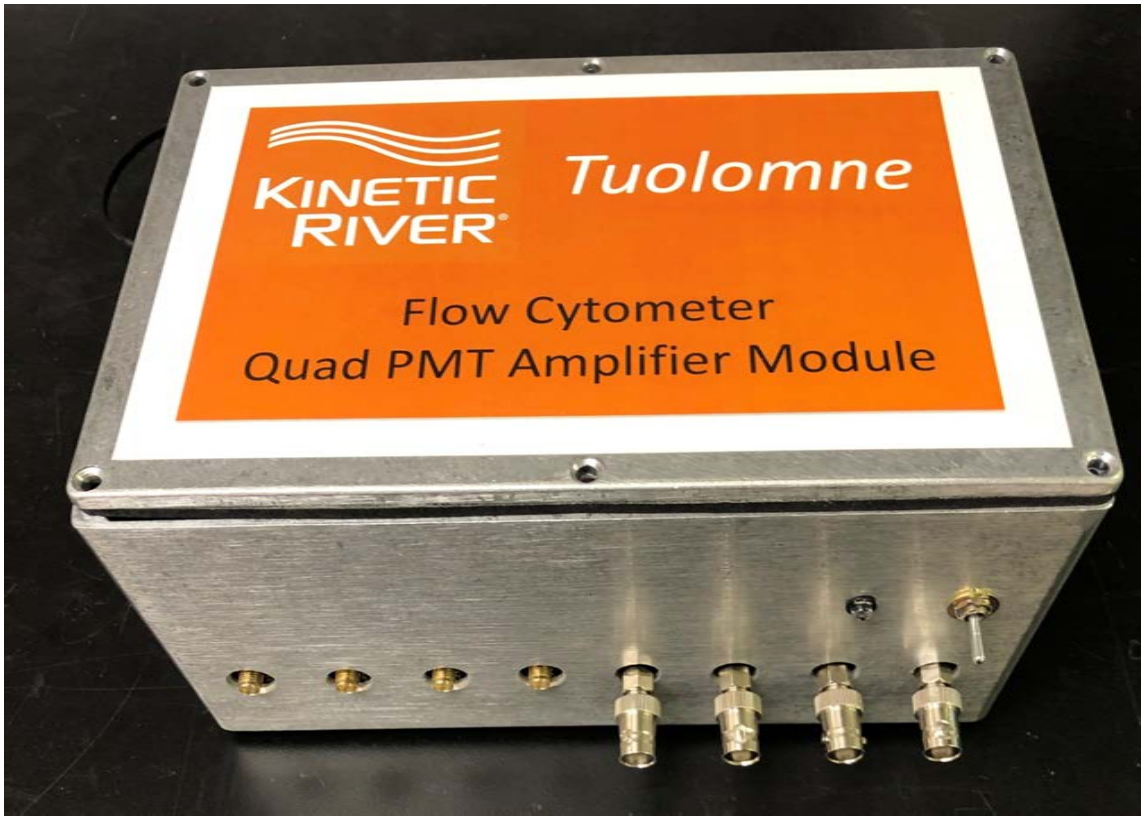
Tuolomne: Standalone PMT Amplifiers

- 4x detector amplifier
- handles PMTs, SiPMS
- regular or inverted output
- replaces DarklingX (RIP)



Shasta: Standalone Fluidic Control

- dual hydrostatic control
- extremely stable flow
- can be used to replace built-in fluidics
- Shasta: uses house air
- Shasta+: built-in pressure sources

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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, E. Kashi, H. Shah (Kinetic River)
- D. Vacca, A. Singhal (summer interns)
- R. Jimenez, E. Shain, C. Heyes, S. Gunupudi (consultants)

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