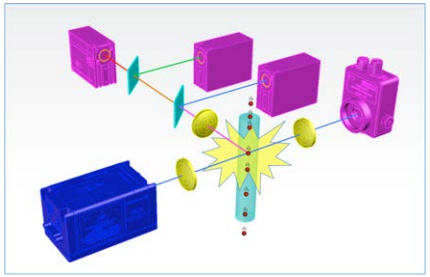
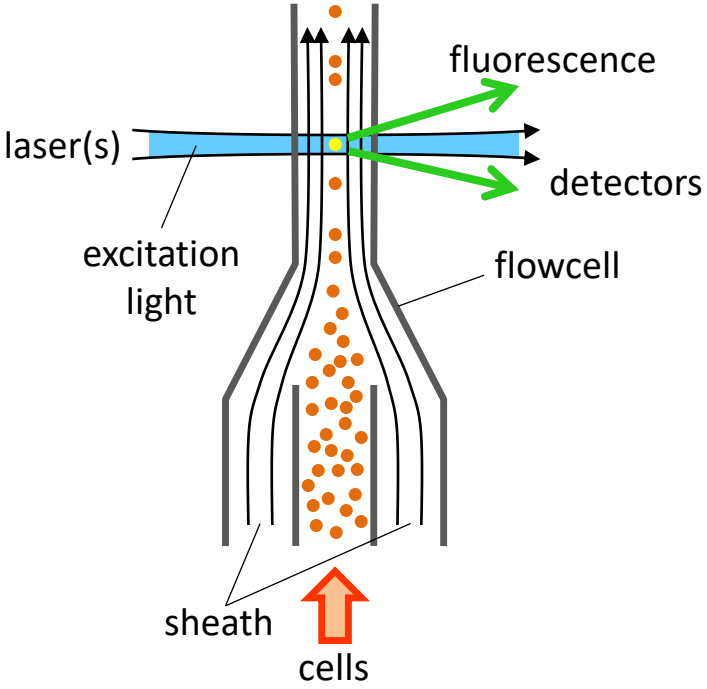
Time-Resolved Flow Cytometry: Expanding the Power of Cell Analysis

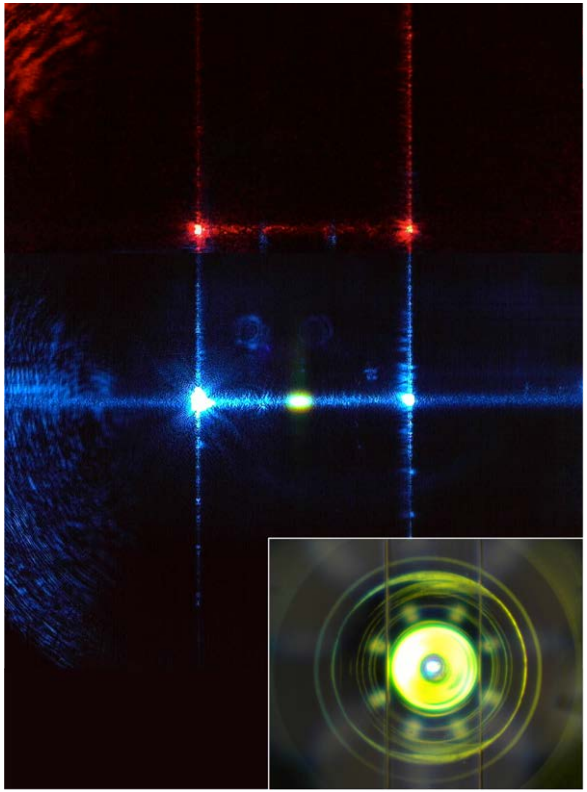
January 24, 2022

Giacomo Vacca, PhD, *President*
Kinetic River Corp.

Flow Cytometry: Cell-by-Cell Interrogation







G. Vacca / *Laser Focus World* 78 (June 2017)

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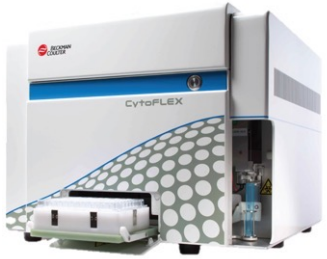
Flow Cytometry: At a Glance

Technical

- fluorescence and light scattering
- 2–10 lasers (UV–visible)
- 4–30+ detectors: PMTs, PDs, APDs, arrays
- typ. 4–12 markers/cell (but up to 30+)
- ~ 10–30k cells/second



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Market

- $5.3B/yr
- growing at 10%/yr
- hematology: add'l $4B/yr, growing at 4%/yr

Market data source: BCC Research (2019 Flow Cytometry Report)

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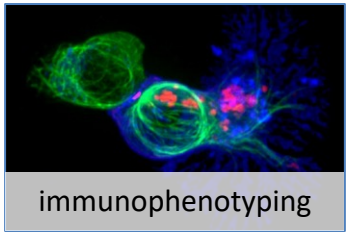
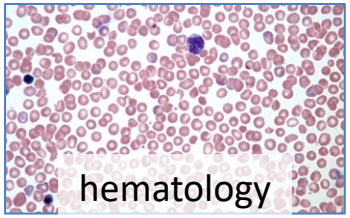
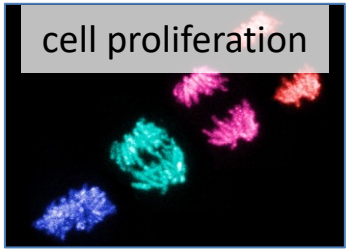
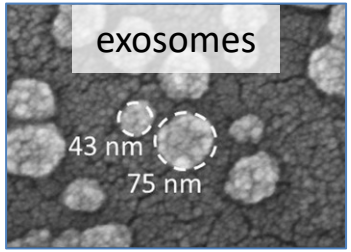
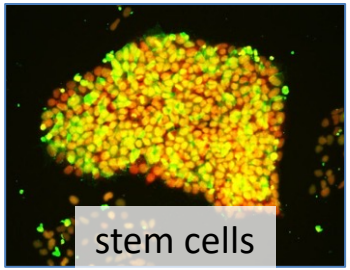
(Some) Flow Cytometry Applications

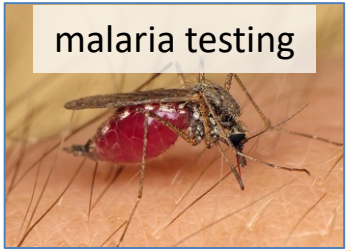












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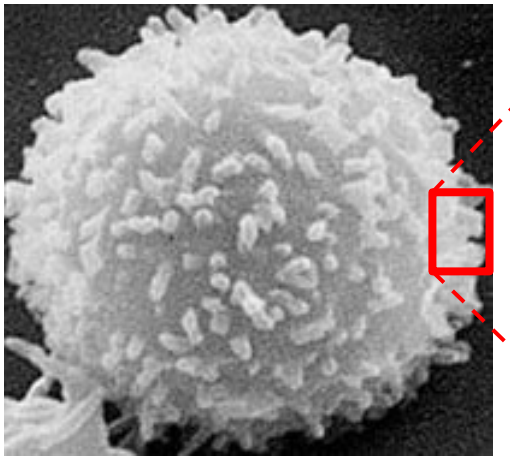
Cells Are Labeled With Fluorophores



NCI-Frederick
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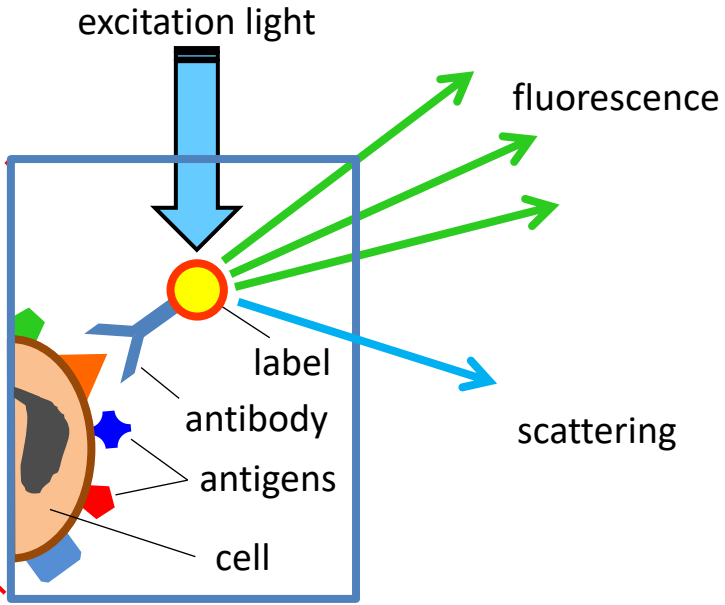

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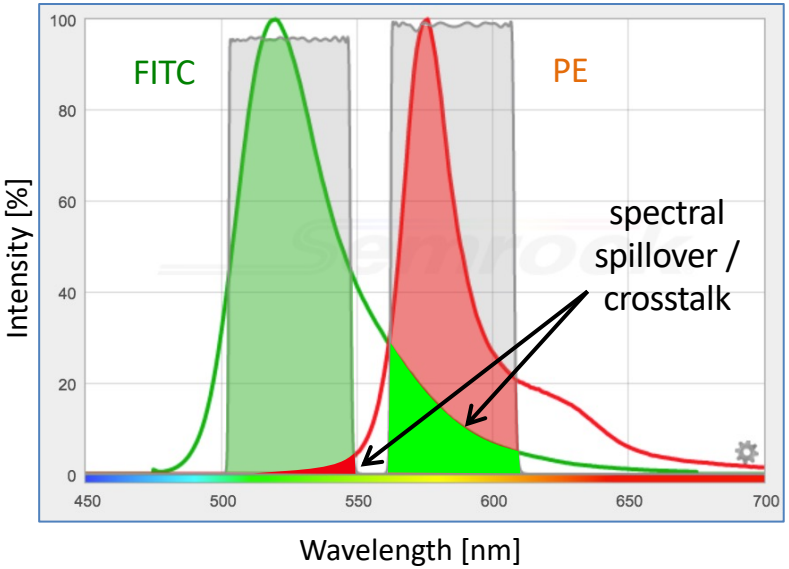
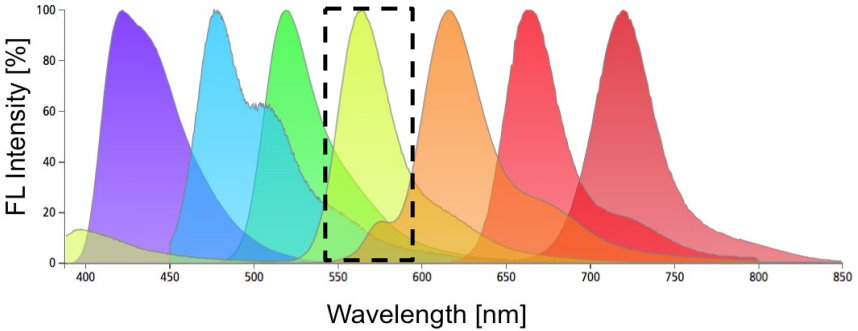
Problem: Fluor Emissions Overlap



- → spectral compensation
- → labor burden
- → operating costs
- → assay sensitivity
- → limited # of channels



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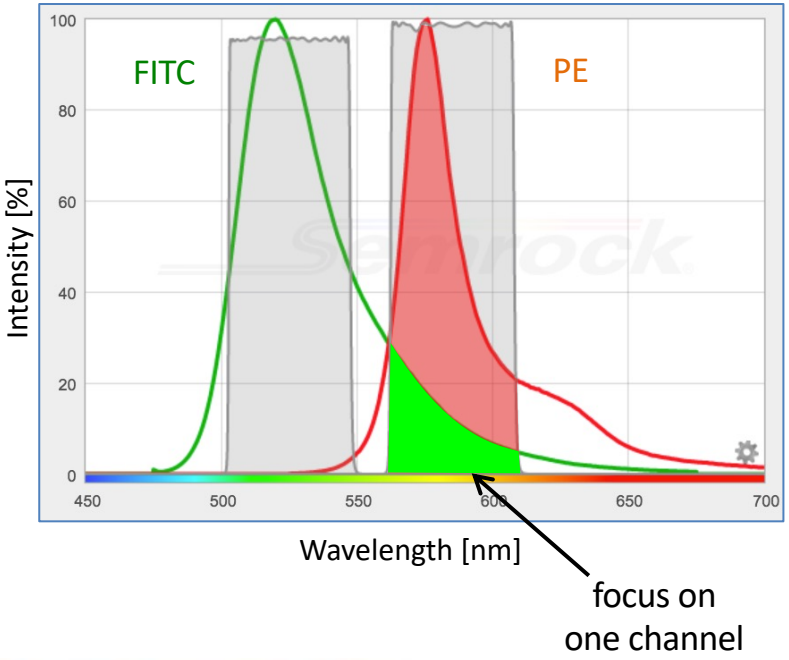
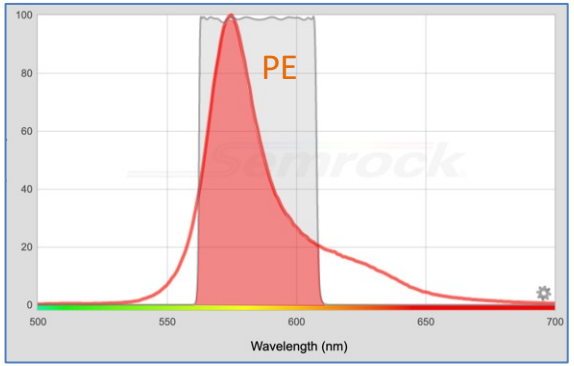
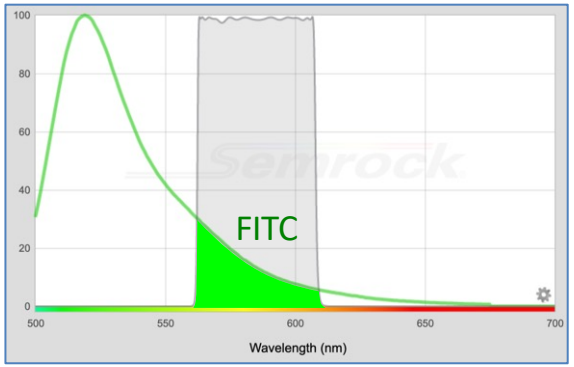
What If You Could Distinguish *Overlapping* Emissions?







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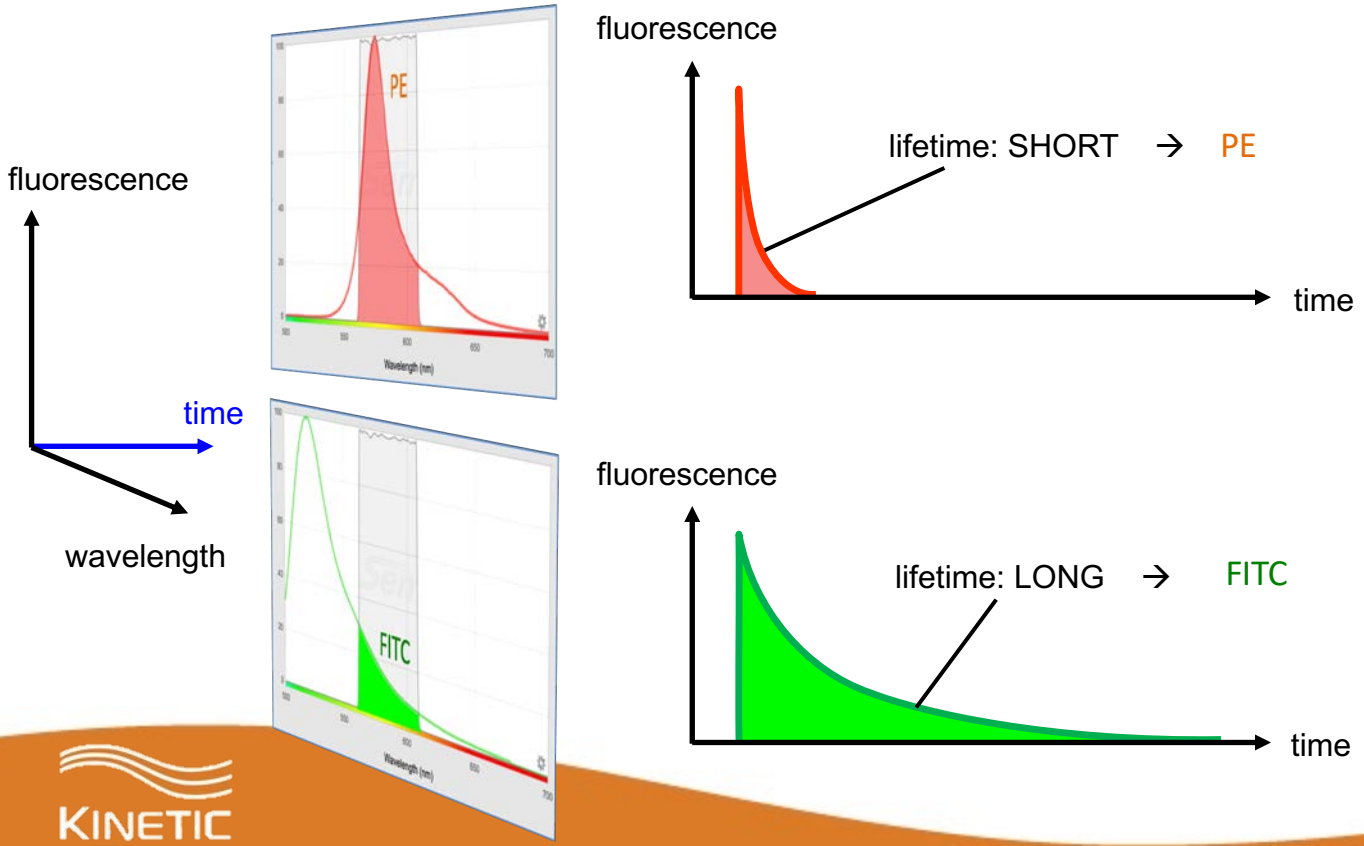

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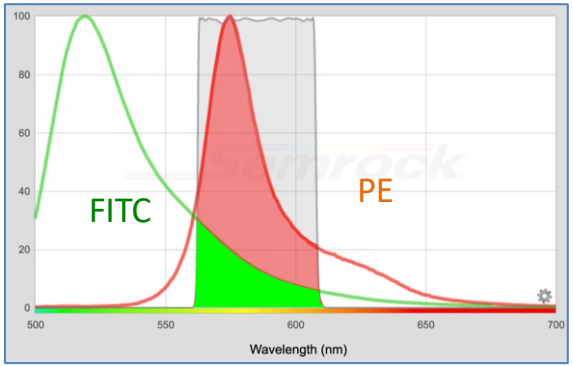
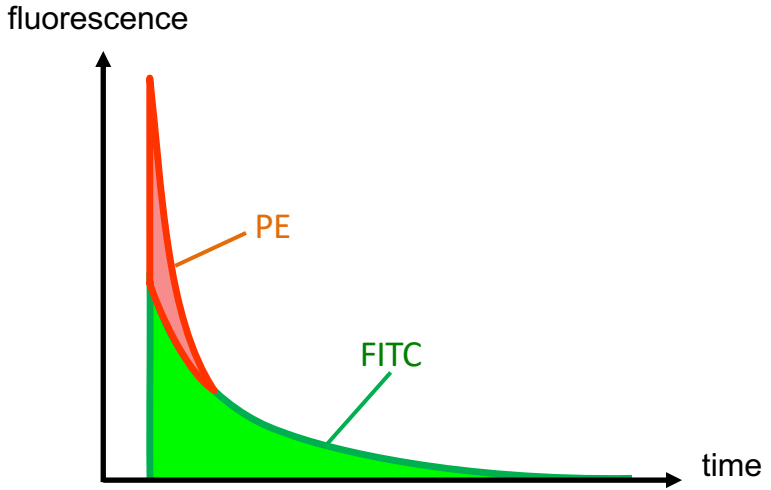
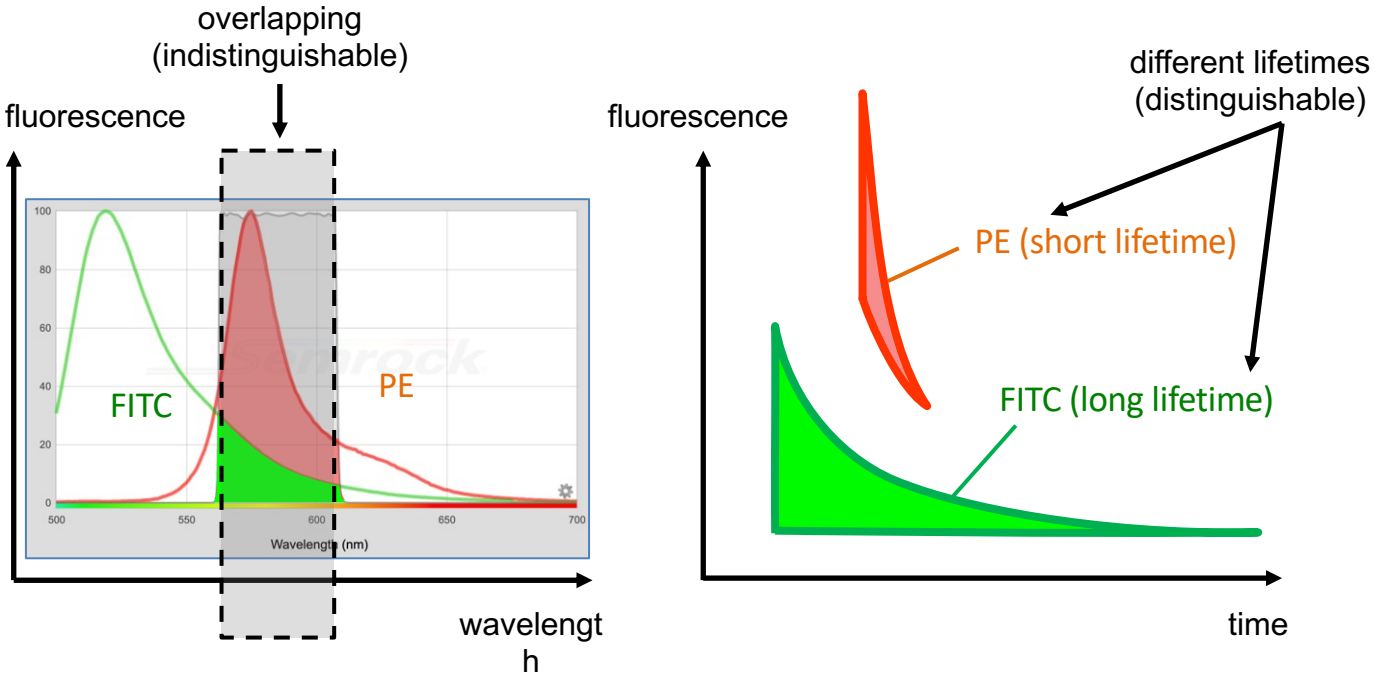
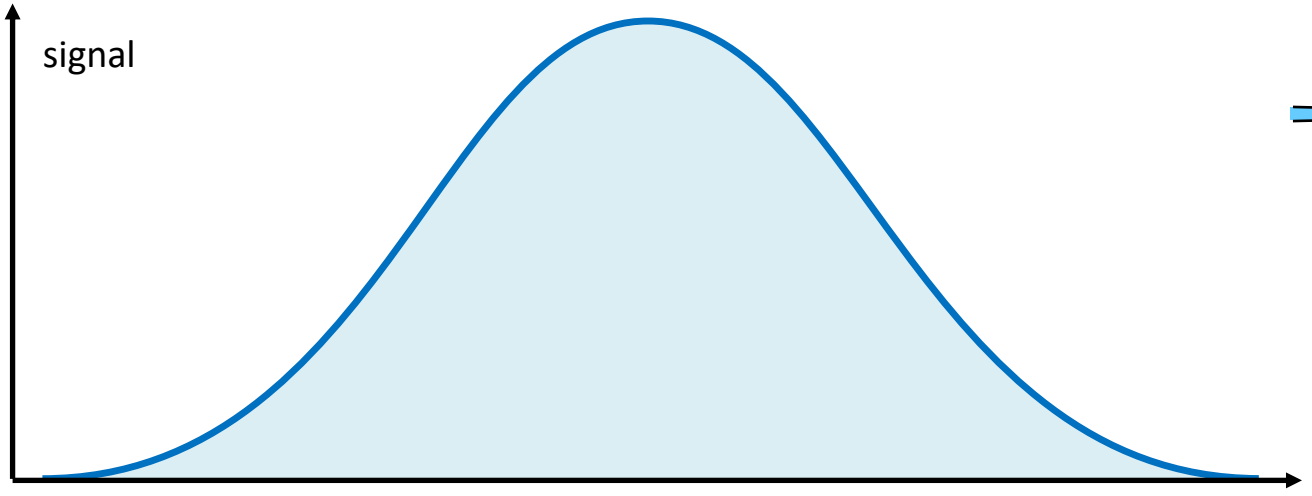


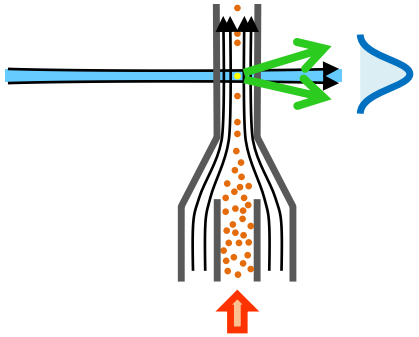


Time Enables Discrimination of Overlapping Emissions



Traditional Flow Cytometry





- continuous light source
- one peak per event

- signal rises and falls as a cell passes through the laser beam

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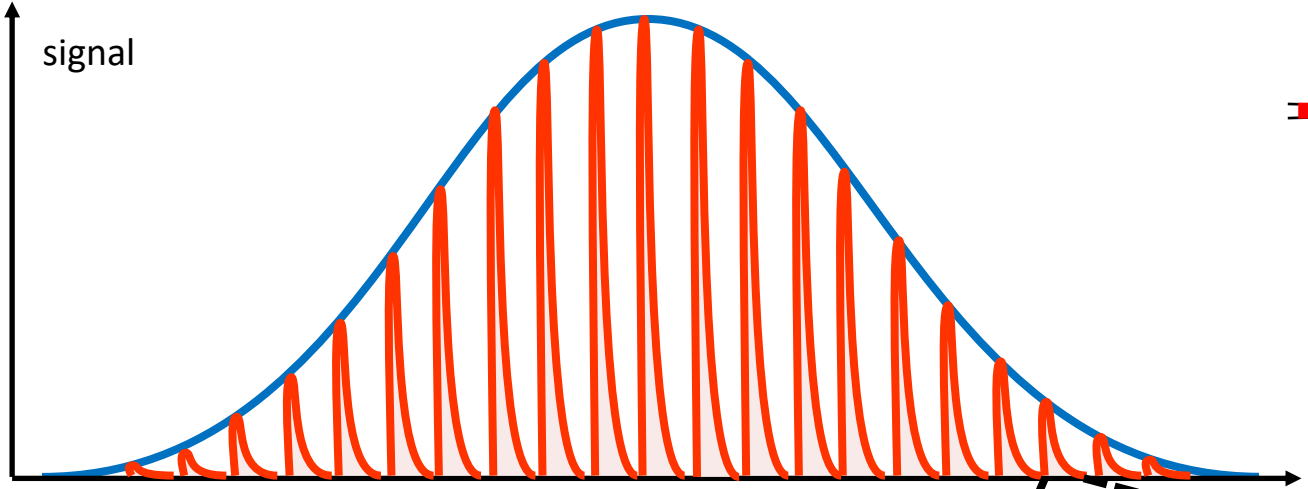
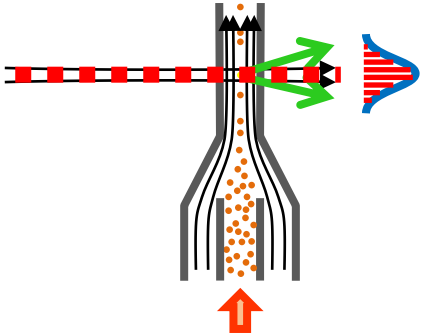
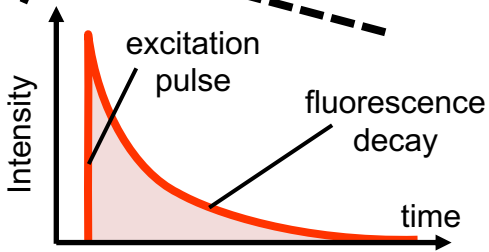
Kinetic River's Secret Sauce: Time-Resolved Flow Cytometry





- modulated laser source
- hundreds of peaks per cell
- nanosecond decays captured



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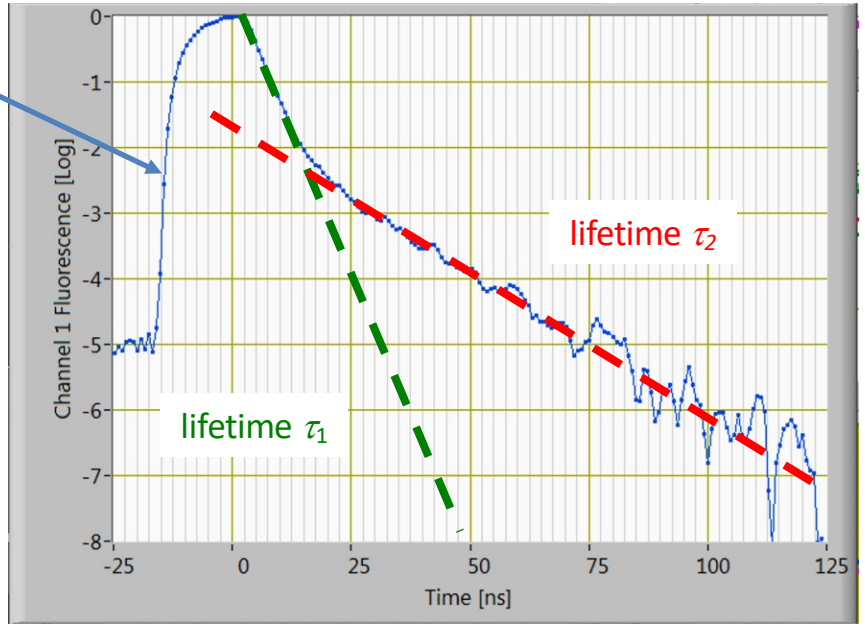
Patented Advantage: Time-Resolved Fluorescence Measurements

simultaneous measurement of multiple fluors with the same detector



Features Unlocked by Kinetic River's Time-Resolved Approach

Compensation-Free	→ **simplified** protocols, **lower costs** of controls
Lifetime Tiers	→ **2x – 3x** parameters with **same #** of lasers, detectors
40+ Fluors	→ like mass cytometry **but** cells can be sorted
Autofluorescence Removal	→ **lower** background, **higher** sensitivity
Label-Free	→ toward rapid **cancer cell** discrimination

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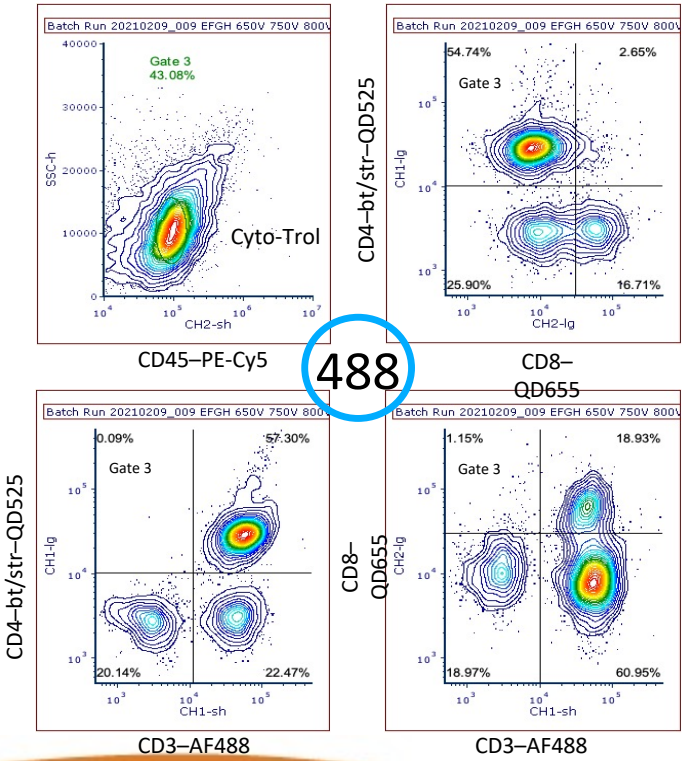
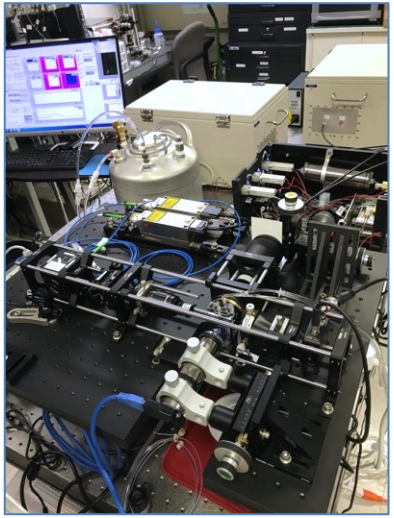
Time-Resolved FC → Compensation-Free FC



← no compensation

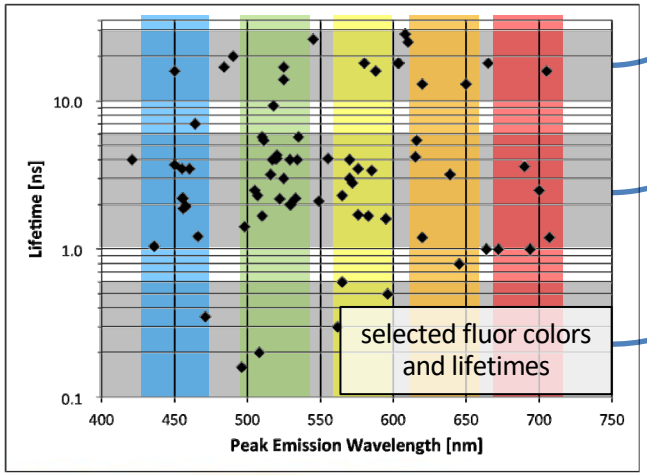
12-color platform using only

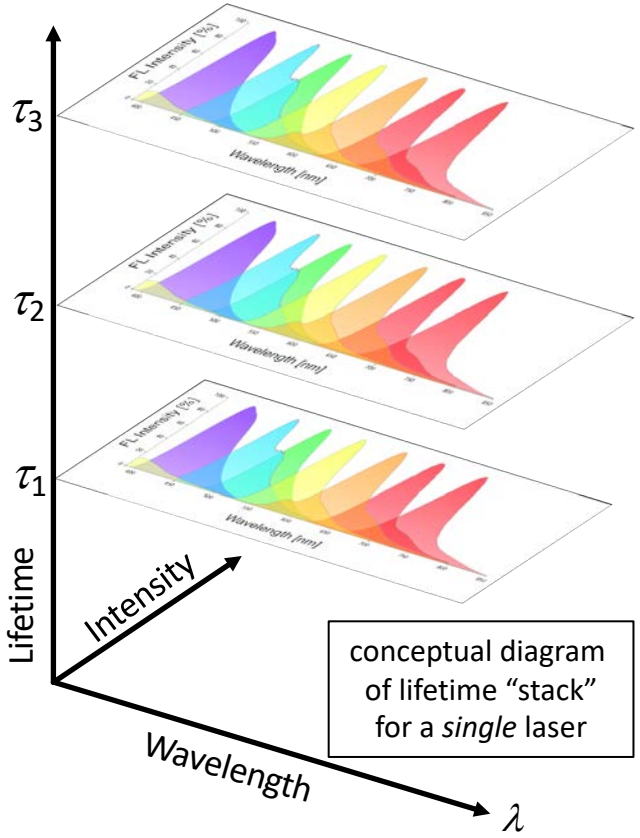
- 2 lasers (405, 488 nm)
- 6 fluorescence detectors



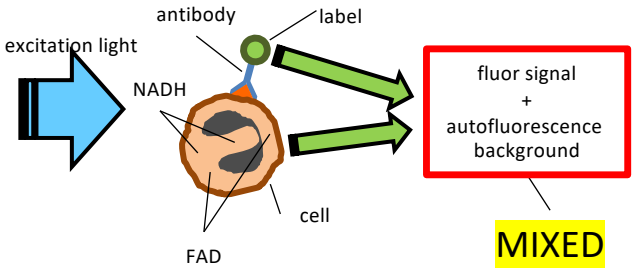
Time-Resolved FC → 2x–3x # of Parameters

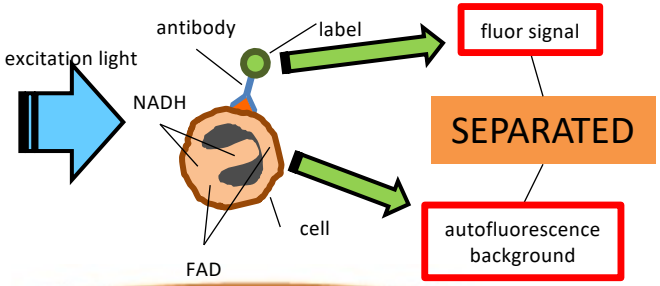
- each laser can support
  3 lifetime tiers of fluors
  - → 8–12 fluors *per laser*
  - → 40+ fluors with 5 lasers



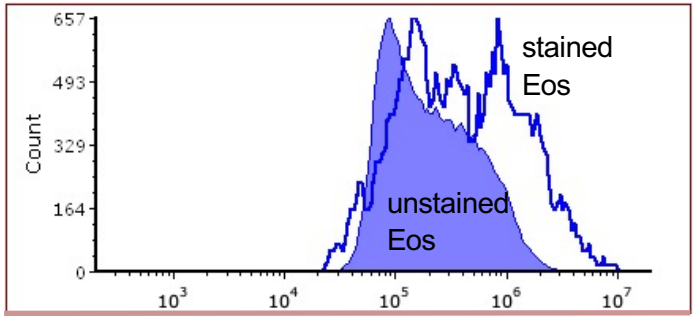


Time-Resolved FC → Autofluorescence Removal

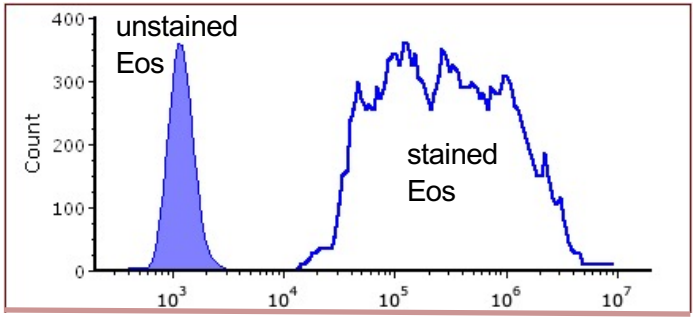




Fluorescence plus Autofluorescence



Fluorescence signal with AF removed



CD45-AlexaFluor405

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Kinetic River Team

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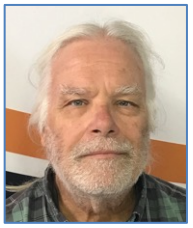
Founder & President

Alan Chin, PhD
Sr. Staff Scientist



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Rick Yarussi, MS
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Advisor

Alastair Hood, PhD
Advisory Board

Rosemary Coates, MBA
Advisory Board

Sean Murphy, MS
Advisory Board

JKI
Software Development Partner

EMC2
Distribution / Service Partner, Europe

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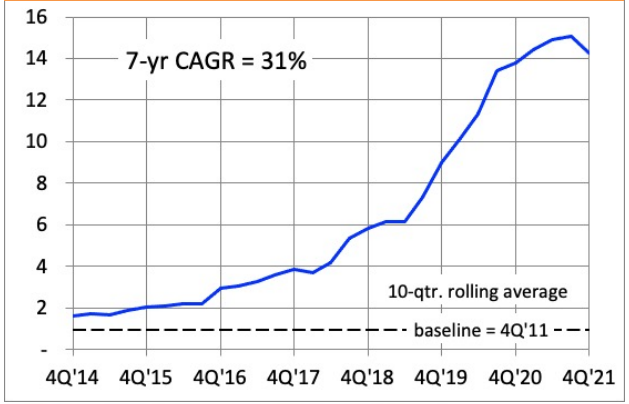
Kinetic River Key Facts

Intellectual Property

- 15 patents issued/allowed
- 13 patents pending
- 5 patent families

Revenue Growth



Key Customers

National Cancer Institute

UC Davis University of California

NM State University

National Research Council of Italy

Key Clients

Stanford School of Medicine

Sony

EMD Millipore

STgenetics

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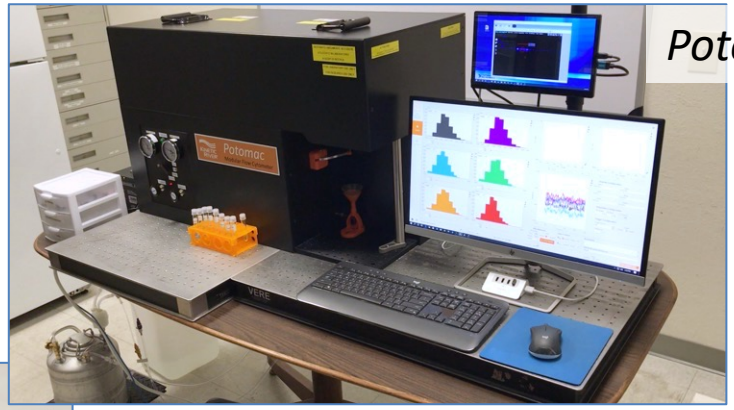
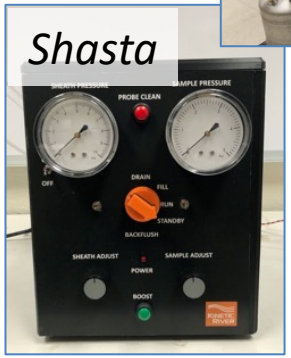
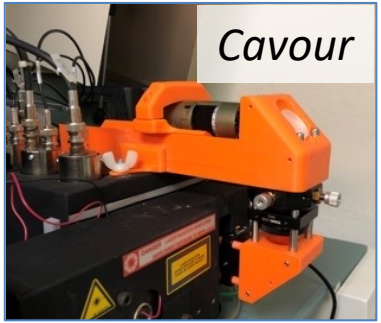
Kinetic River's On-Market Products



Potomac

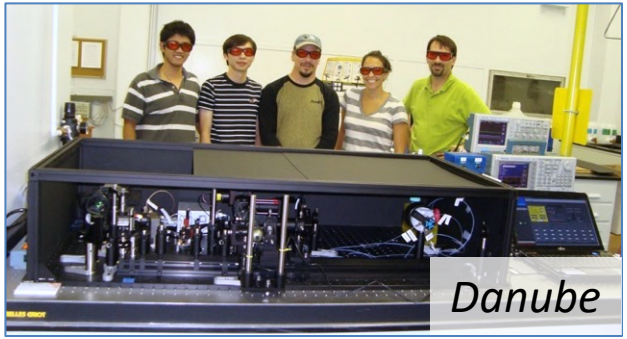
Shasta





Cavour

Tuolumne



Danube

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Acknowledgements

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- A. Chin, K.P. Shevgaonkar, E. Kashi, R. Hanson, R. McKay, J. Huang, H. Shah, K. Melampy, E. Gorina (Kinetic River)
- D. Vacca, A. Singhal, Ria Xi, Sophia Lin (summer interns/trainees)
- W. Telford, P. Chattopadhyay, B. McLaughlin, R. Jimenez, P. Cappella, G. Contini, R. Yarussi, A. Monk, A. Sloat, S. Murphy, R. Coates, A. Hood, E. Shain, T. Gray, C. Heyes, S. Gunupudi (consultants/collaborators)

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Expanding the Power of Flow Cytometry

- Time-resolved measurements eliminate bottlenecks in FC
- Reduce or eliminate spectral spillover, compensation
- Double to triple the number of detectable parameters
- Automatically remove autofluorescence background
- Label-free cancer cell discrimination

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Photonic Technology Needs

- **Sources**: pulsed OR high modulation frequency
  - pulsed: ~ 10 ns, ~ 10 MHz rep rate, ≥ 1 W peak power
  - modulation: 250+ MHz
  - from visible down to deep UV (266 nm)
- **Detectors**: compact, sensitive, high dynamic range
  - PMTs → APDs
- **Costs**: down, down, down
  - WW competition pushing down costs of analyzers

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What Kinetic River Can Do For You

- **Vehicle to Market**: design your products into state-of-the-art technology from the very beginning
- **Market Intelligence/Strategy**: we provide consulting and training courses in flow cytometry to photonics companies interested in entering the space

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