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NEW FRONTIERS IN FCM APPLICATIONS

J. Paul Robinson

The SVM Professor of Cytomics

Professor of Biomedical Engineering

Director, Purdue University Cytometry Laboratories

Commissioner of the United States Patent Office from 1898 to 1901

Thanks Wikipedia

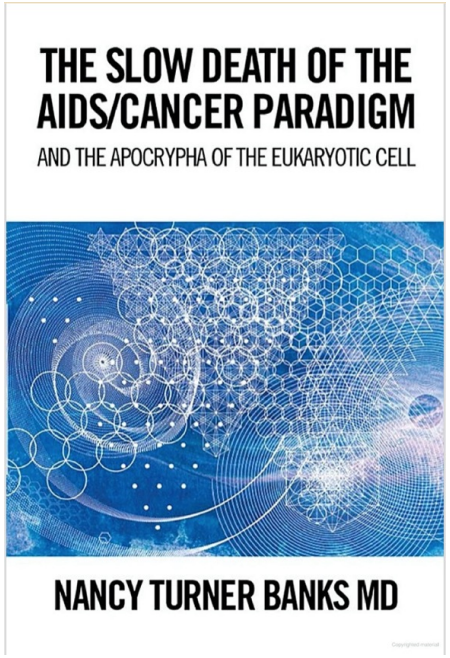
Charles Holland Duell

"***Everything that can be invented has been invented***"
(but we know this was apocryphal)!!

"Everything that can be done by flow cytometry has already been done"

Quote is attributed to a quite ignorant cytometrist…!
(if one exists)

"..flow cytometry, an unreliable cell-counting technique from the obscurity of the research lab…")



(self "published" in 2016)

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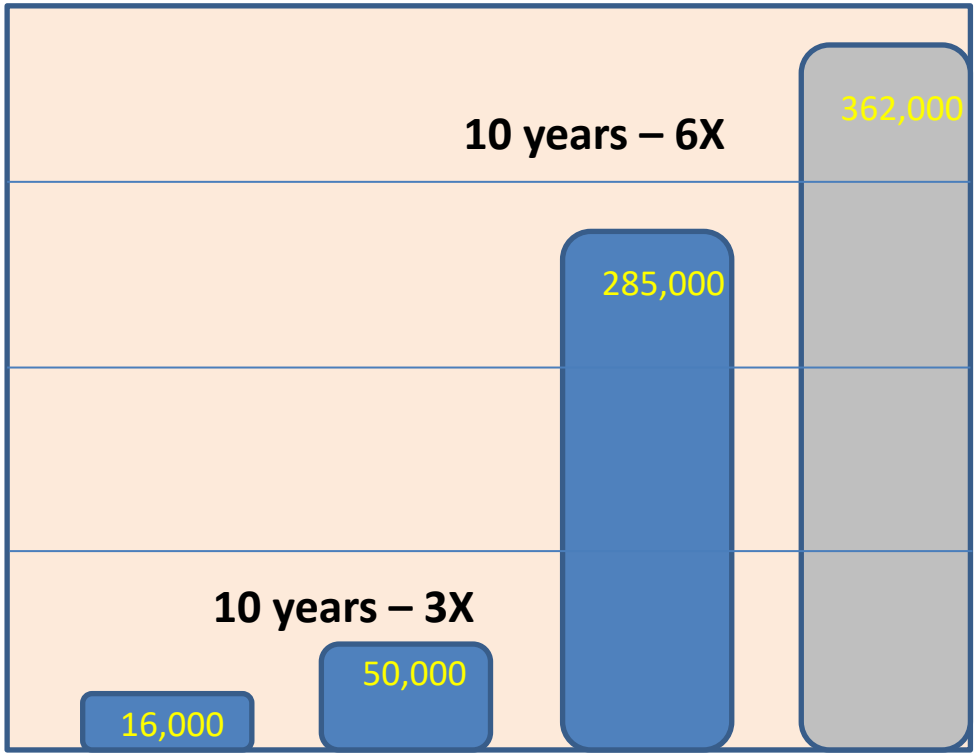
increased antibodies. The then-current immune theory claimed that in order for the B cells to produce antibodies, they needed to receive an activation signal from the thymus maturing T cells, thus the designation helper. It was not until the 1980s that the mysteries of the T cells began to be unraveled, yet twenty-first-century AIDSworld has continued to resist this knowledge and disastrously continues to employ the technique of flow cytometry to measure total CD4+T cell count, ignoring the vagaries and nondiagnostic capabilities of this measure.

over again, this time with T cell subsets. … My strongest argument is this: Measurement of T and B cells and their subsets in diseases has no clinical meaning. … Nonimmunologists have naturally assumed that any subject occupying so much space must be relevant in some way—a logical but incorrect assumption.[101] This was an early warnings against using these unreliable cell counts as a measure of morbidity. No matter. It was AIDSworld that took flow cytometry, an unreliable cell-counting technique, from the obscurity of the research laboratory[102] and introduced it to the mass market to support the notion of a cytocidal virus, all the while ignoring the known and predictable behavior of lymphocyte movements to multiple and varied external biological stressors. Ignoring the obvious, this non-specific finding of a decrease in CD4+T cells conveniently became HIV disease.

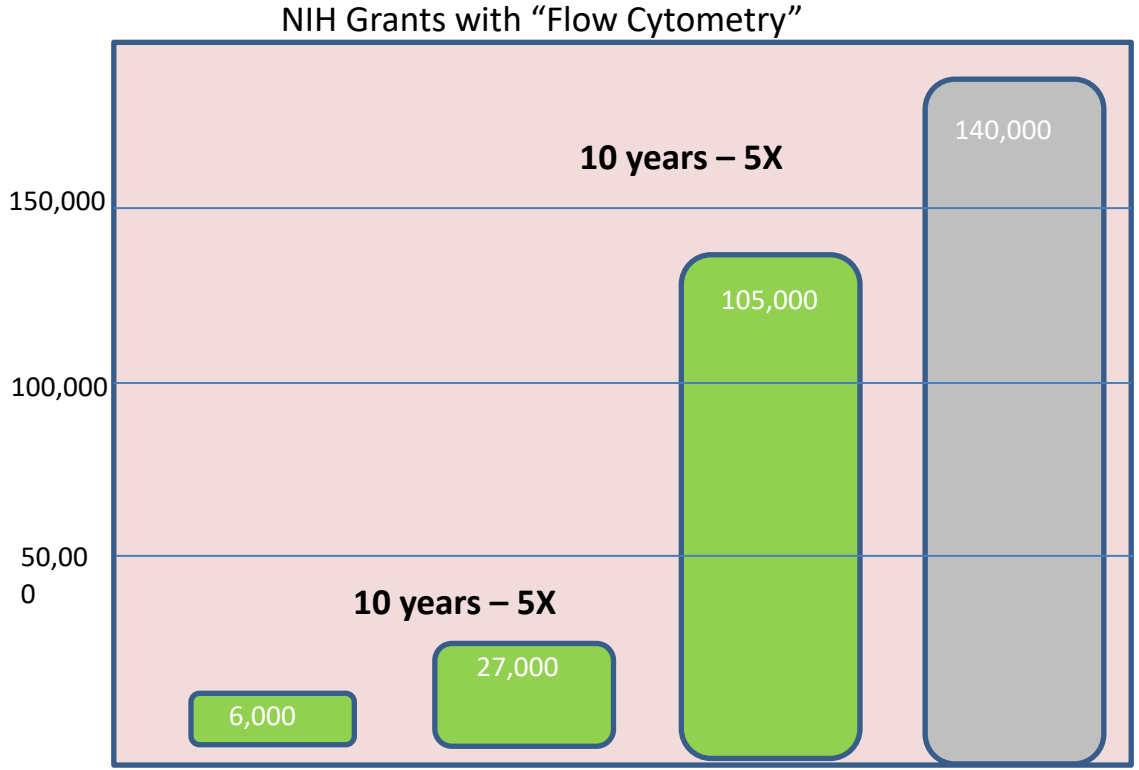
What has Flow Cytometry achieved over 50+ years?

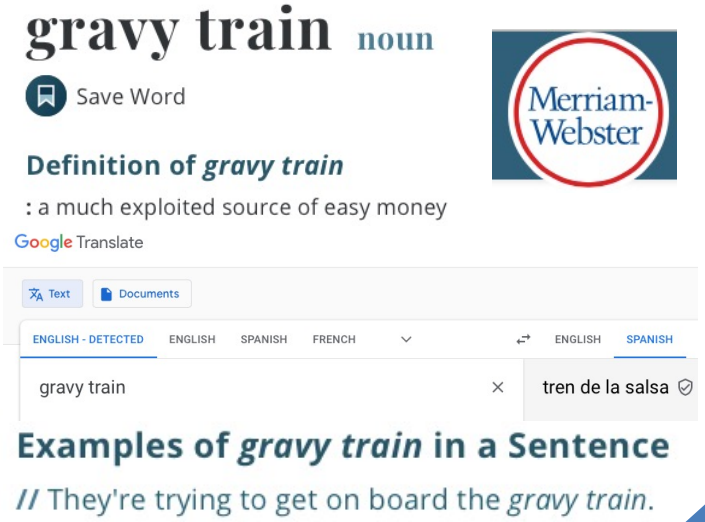
Publications with Flow Cytometry as a key Word



What has Flow Cytometry achieved over 50+ years?

National Institutes of Health





A flow cytometry “gravy train”?

40 Color - 2020

26 Color - 2019

17 Color - 2004

12 Color - 2003

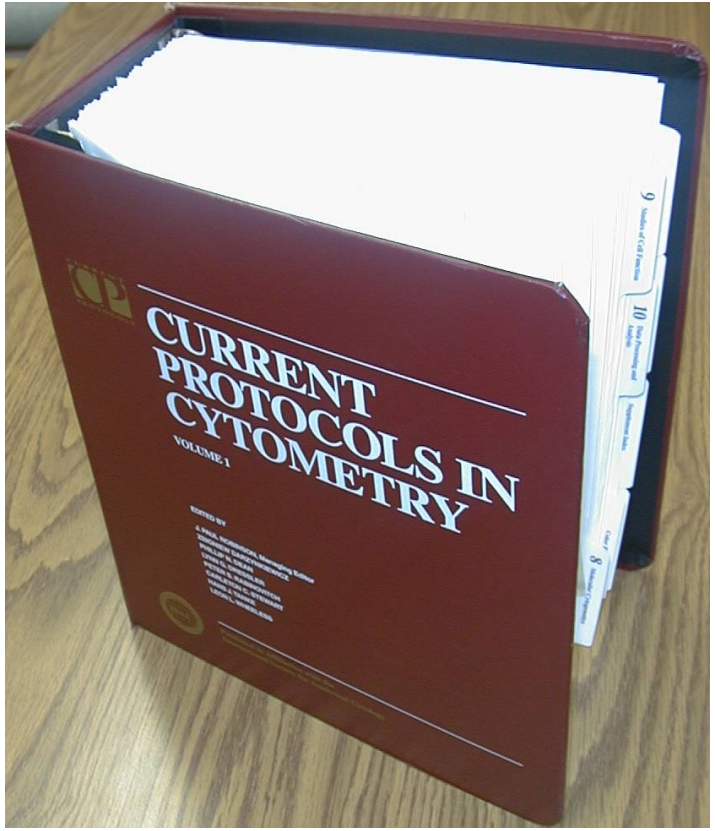
11 Color - 2001

6 Color - 1995

3 Color - 1987

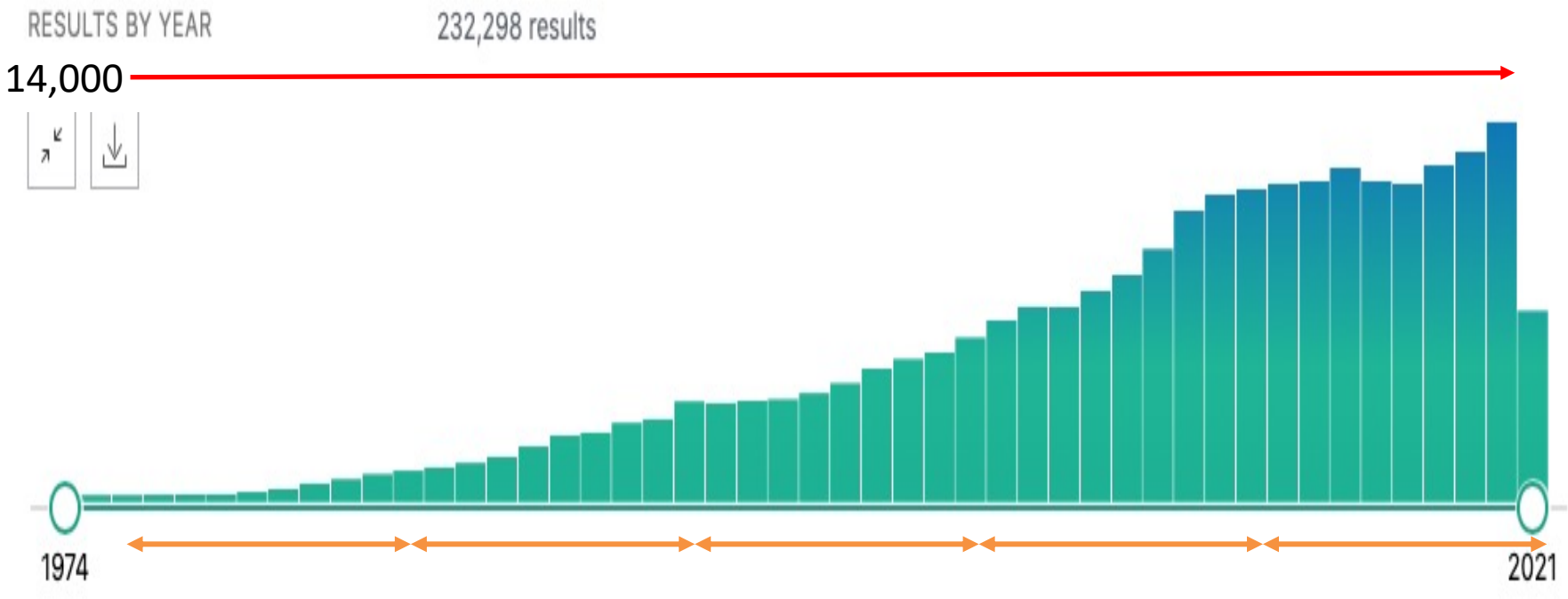
2 Color - 1977

Chapter 1 Flow Cytometry Instrumentation
Chapter 2 Image Cytometry Instrumentation
Chapter 3 Safety Procedures and Quality Control
Chapter 4 Molecular and Cellular Probes
Chapter 5 Specimen Handling, Storage, and Preparation
Chapter 6 Phenotypic Analysis
Chapter 7 Nucleic Acid Analysis
Chapter 8 Molecular Cytogenetics
Chapter 9 Studies of Cell Function
Chapter 10 Data Processing and Analysis
Chapter 11 Microbiological Applications
Chapter 12 Cellular and Molecular Imaging
Chapter 13 Multiplexed and Microparticle-Based Analyses

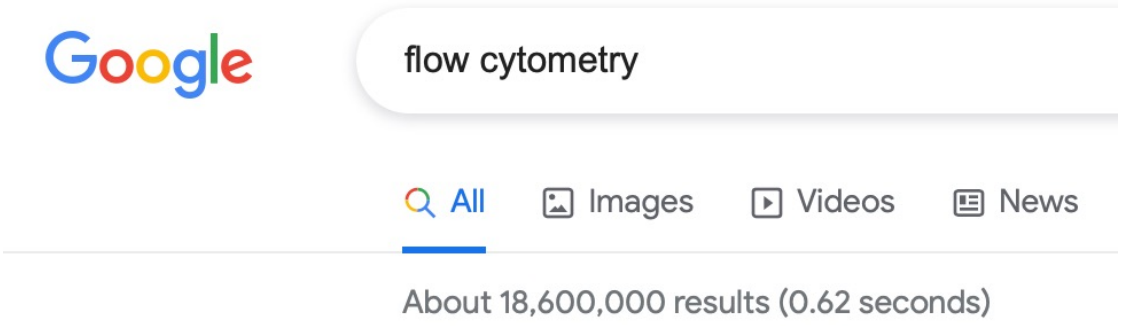


PubMed.gov

There are a lot of publications using flow cytometry….



So what is the fundamental driver for folks doing flow cytometry?



1. Using beads to calibrate an instrument
2. Immunophenotyping cells to identify subpopulations
3. Evaluating functional markers – enzymes, specific antigen targets
4. Looking for small particles
5. Cytokines, hormones, etc. using bead-based assays
6. Cell sorting to isolate a population of cells

So what can't flow cytometry do effectively?

1. It's been restricted by available fluors – and its polychromatic design
2. It's not very good at molecular targeting
3. it's poor for looking at small particles
4. it really is not quantitative at all – its at best-semi-quantitative
5. Only very specialized and rare instruments can evaluate lifetimes
6. Looking for very low fluorescence is very difficult due to high instrument noise

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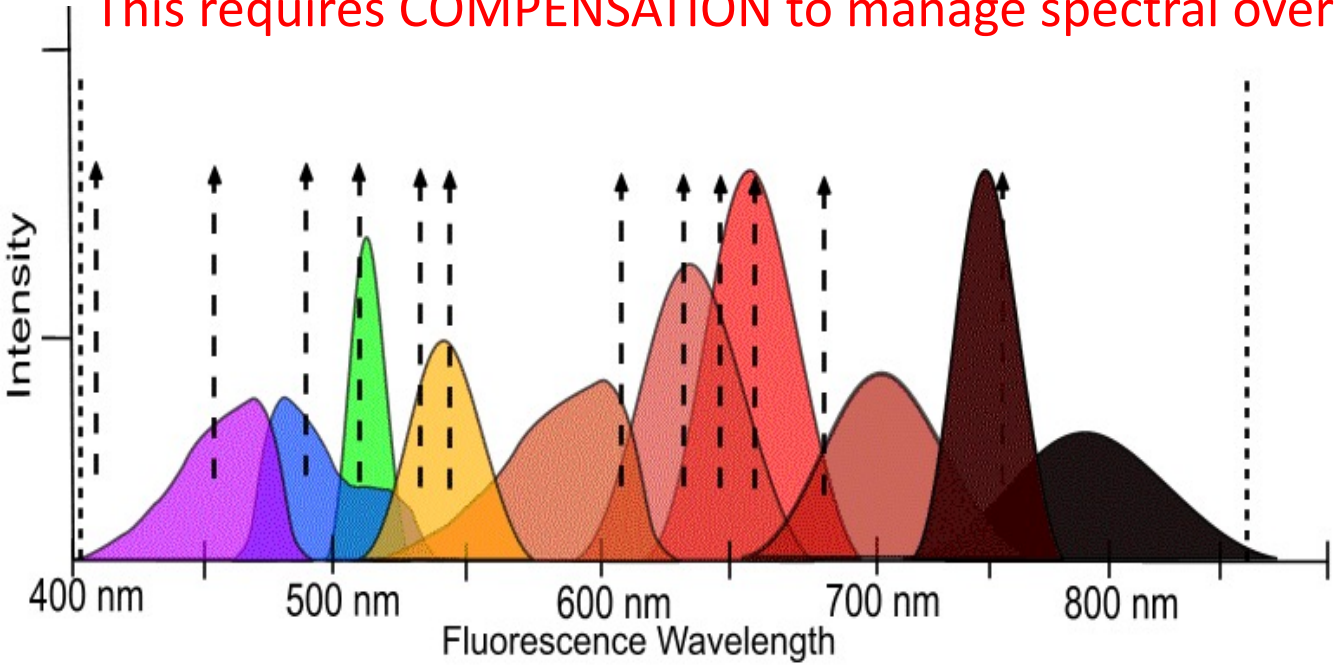
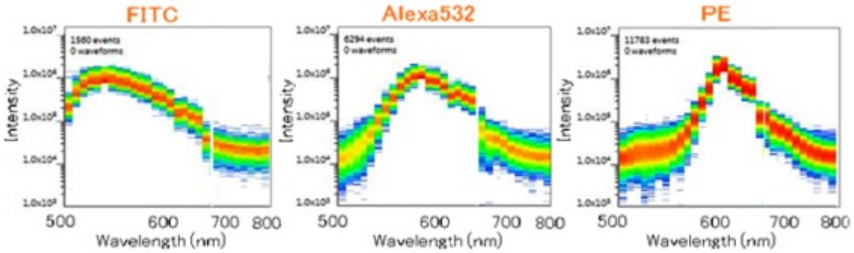
Spectral Overlap makes for very complex analysis & design

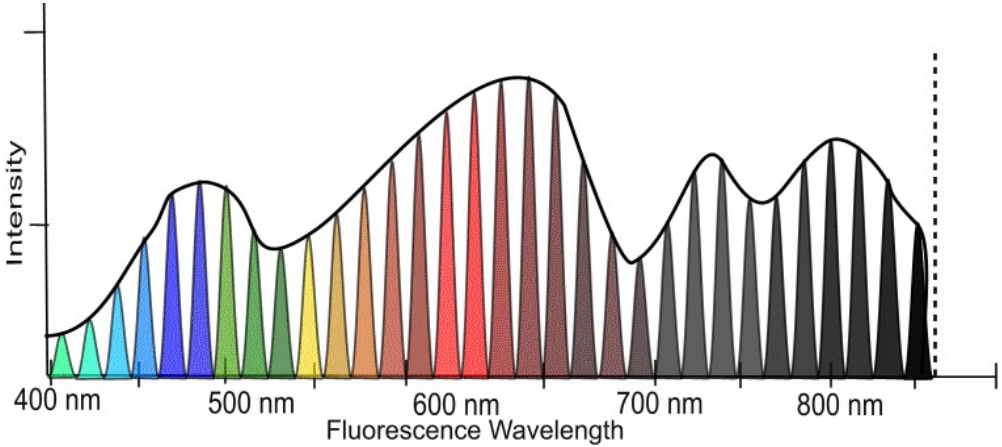
This requires COMPENSATION to manage spectral overlap



Spectral cytometry saves the day….



Cytometry Part A • 87A: 830–842, 2015



I think the future is very positive for spectral cytometry
It will open up some exciting opportunities

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How can flow cytometry enhance Molecular targeting?

1. We need better ability to target individual molecules
2. Can we better design probes that combine multiple signatures - i.e. not just fluors, but perhaps nanoparticles with physically measurable properties.
3. Broad fluors are not likely to be of great value, so this may require a new generation of fluors?

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Small particles may be very important targets, but flow cytometry is not designed to be really effective for very small particles.

We need to consider how the fundamental design of flow systems might need to be changed to better support small particle research

Current approaches – reduced laser beam profile, slower flow, very small microchannels

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MESFs have been with us for nearly 40 years.

We need to consider alternatives in terms of detectors – for example, moving to single photon technology is inherently digital – future systems might well focus on this technology.

Better and higher number array detectors are needed to accommodate a next generation instrument

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Flow lifetime analysis was first proposed by Steinkamp in 1990 but it's a highly specialty technology

Alternative approaches can be achieved making it almost routine

Imagine being able to measure any fluorescence signal but also look at drug targets for example, and being able determine whether or not a particular receptor or molecular target has been bound to another molecule – or its conformation has been altered

New single photon sensors may bring a new generation of flow technology…

1. Next generation high speed sensors are under development.
2. These sensors will deliver single photon data
3. The speed is high enough to perform life-time (around 1 ns)
4. The output is fundamentally digital –so potentially absolutely quantitative

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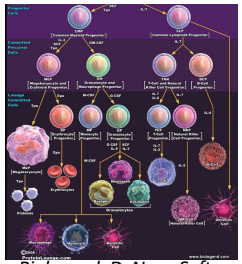
SPECTRAL & SINGLE PHOTON POTENTIAL

Spectral	**100**	**20**	**20**	50
Polychromatic	30	2-3	2	2

Phenotype

CD3
CD4
CD8
CD16
CDx



Images from: Biolegend; DeNovo Software; J.Immunol,

Conclusions

1. Flow cytometry has been highly successful over the past 55 years
2. We have learned how to take this technology and adapt it to literally hundreds of different types of assays and measurements.
3. It's a very mature, highly stable technology and very commercially viable

BUT

4. If flow cytometry is to survive into the next 20 years, it needs to move from a relatively old technology base, to next generation concepts
5. This includes how we use lasers, flow chamber design, fundamental optics changes, and next generation detectors (beyond APDs).
6. Analytical tools will always move as technology improves.

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