New England Cytometry
Fall Meeting
October 27, 2022

The history of flow cytometry

from early pioneers

to modern high performance systems

Diether Recktenwald
Desatoya LLC
Reno NV 89507, USA

Diether@desatoya.com

Phone: +1-408-658-6074

Slides are taken from many different sources to reflect the technologies at their time.

Sources are shown, when they were available.

The selection of the material is influenced by what was available to me, and by how I experienced the developments during my professional activities in the field from 1980 to the present.

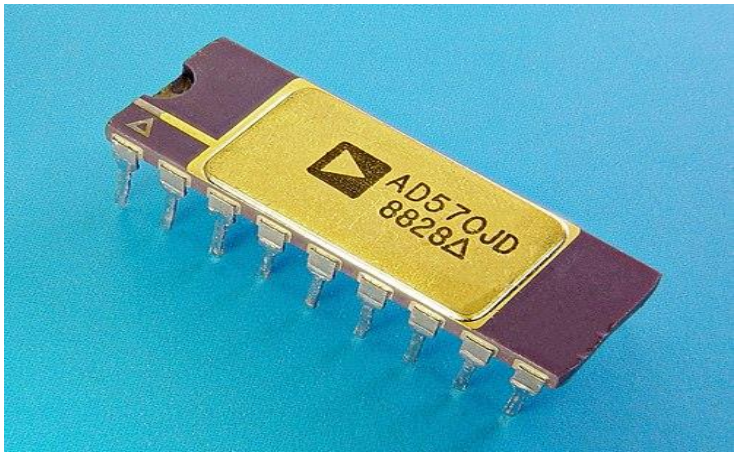
A detailed history of cytometry has been published by Howard Shapiro in 2004 in Cytometry 58A: 13-20 titled “The Evolution of Cytometers”

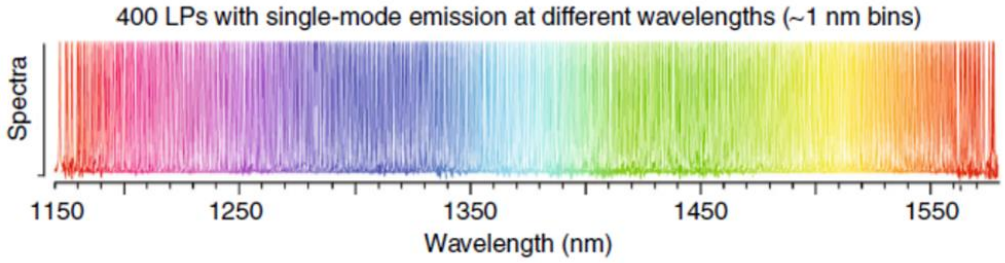
I have been spending most of my career with BD Biosciences, and at present I perform limited professional advice services for several companies outside of BD Biosciences.

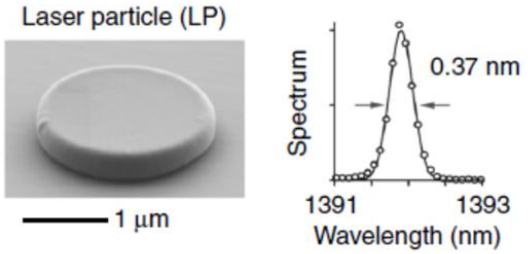
Diether
Recktenwald
October 2022

Essential Technologies

- Optics (Microscopy)
- Light sources detectors
- Analog and digital electronics
- Software & algorithms
- Affinity reagents
- Labels



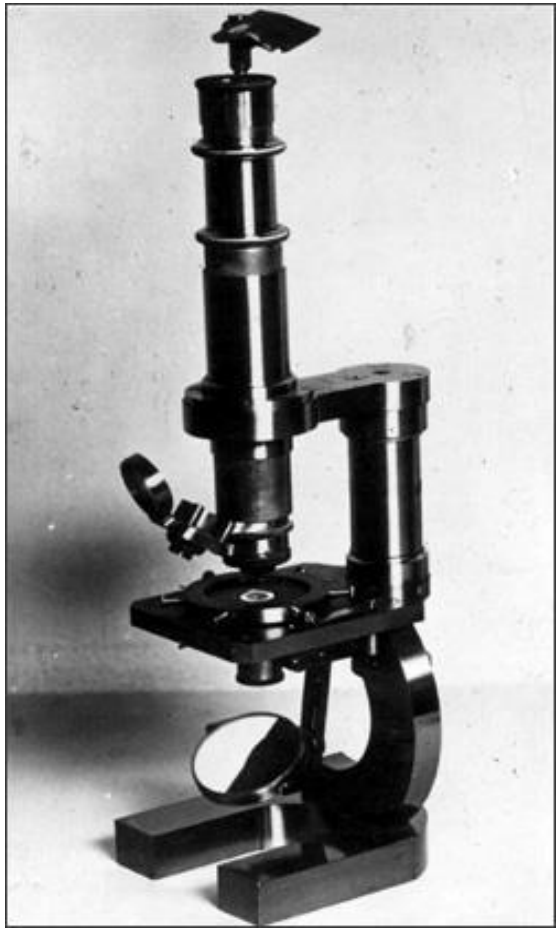




Laser Particles as Labels for Cell Analysis
Kwok S.J.J. et al (2019)

https://doi.org/10.1038/s41377-019-0183-5

Early History



- 1665 – English physicist, Robert Hooke used a microscope lens to observe “pores” in cork
- 1674 – Anton van Leeuwenhoek built a simple microscope with only one lens to examine blood cells
- 1872 – Ernst Abbe calculated the maximum resolution in microscopes
- 1930 – L.A. Kubetsky invented the PMT in Russia
- 1932 – Frits Zernike invented the phase-contrast microscope (label-free observations)
- 1969 – Willard Boyle and George E. Smith at Bell laboratories invented the CCD
- 1960 – Theodore Maiman developed the first working laser at Hughes Research Lab
- 1971 – Intel launches 4-bit 4004 microprocessor

Persistent Supporters from Two Large Companies

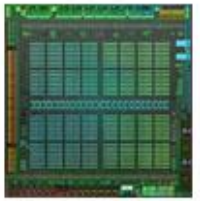
Wallace H. Coulter
1913-1998

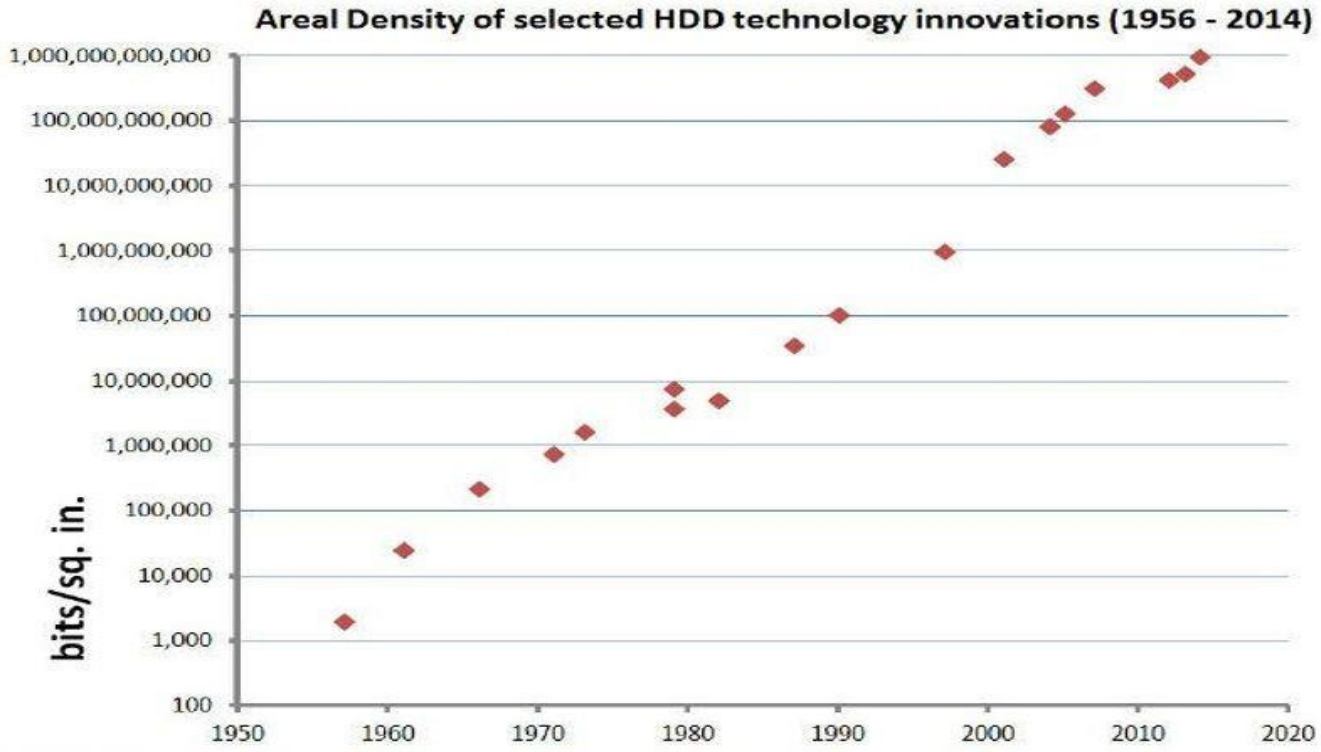
Joseph R. Coulter, Jr.
1924-1995

BEC now Danaher

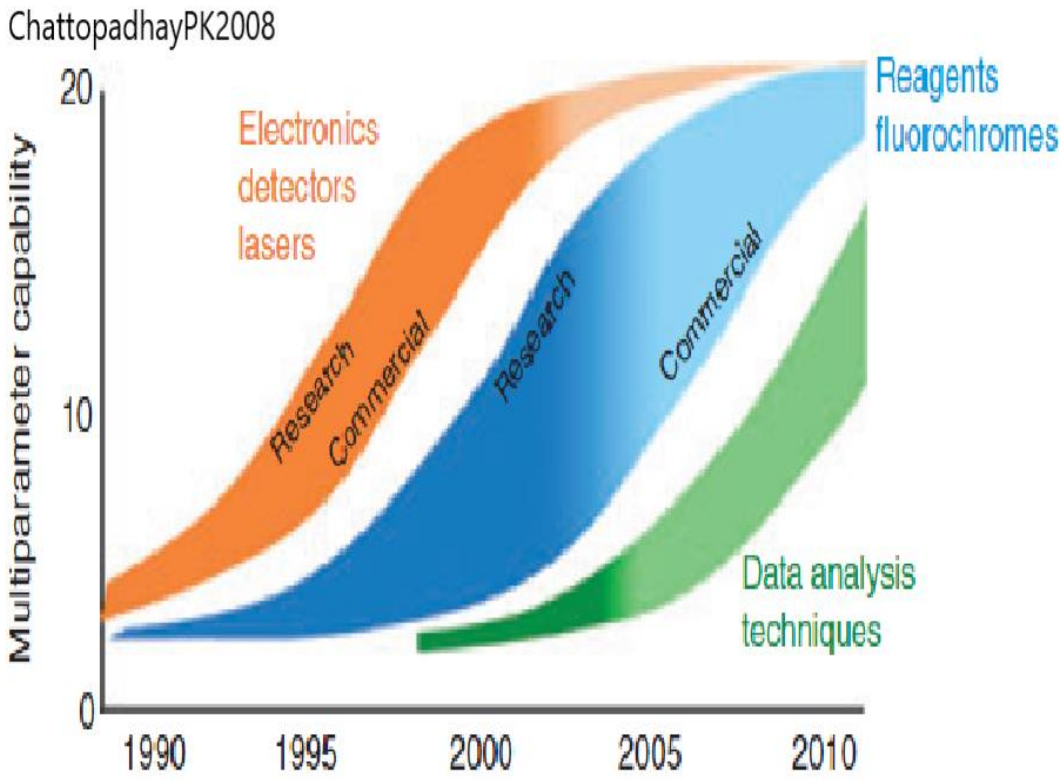
BDX

Computing Performance

1950s	1960s	1970s	1980s	1990s	2000s	2010s
Silicon Transistor	TTL Quad Gate	8-bit Microprocessor	32-bit Microprocessor	32-bit Microprocessor	64-bit Microprocessor	3072-Core GPU
						
1 Transistor	**16 Transistors**	**4500 Transistors**	**275,000 Transistors**	**3,100,000 Transistors**	**592,000,000 Transistors**	**8,000,000,000 Transistors**



Technology Development History

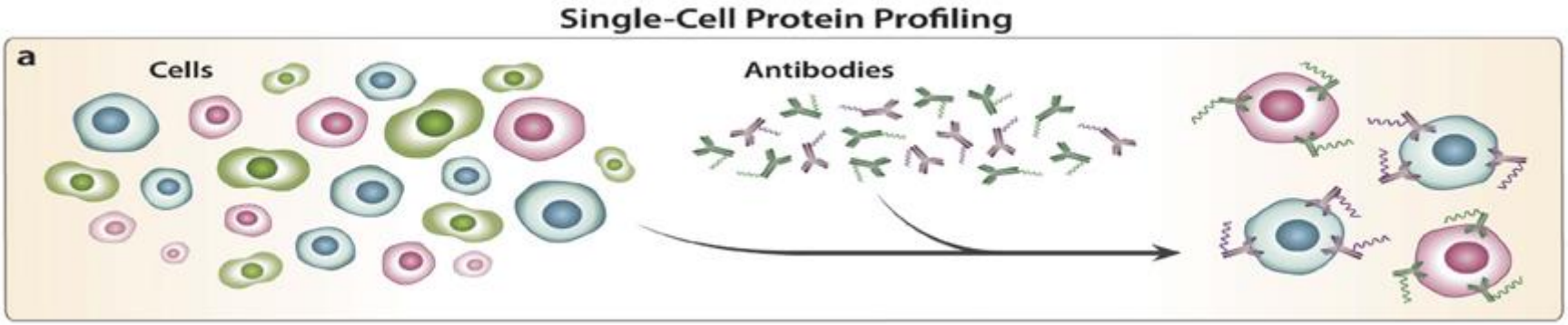


Today, October 2022:

Instrumentation 100

Fluorochromes 70
NA barcoding 100s

Data analysis 100s



Shahi P et al. (2017) Abseq; DOI: 10.1038/srep44447

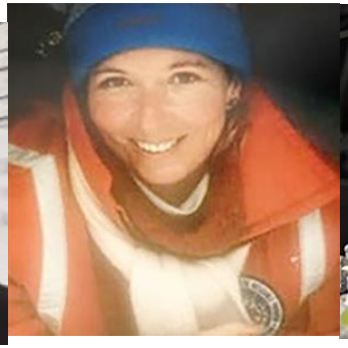
Academic Innovators

Wolfgang Goehde, ICP22
Len Herzenberg, Wayne Moore, Dave Parks, FACS™
Klaus Rajewski, Andreas Radbruch, 1st European FACS™, MACS™
Joel Gray, Brian Mayall, Phil Dean, chromosome sorting
Gary Salzmann, Jim Jett, John Nolan, early software, single molecule detection
Steven W. Graves,Gregory Kaduchak, “acoustic cytometer”
Joe Trotter, WinMDI
Alexander Glazer & Lubert Stryer, Barbara Prezelin, algae pigments & tandems
Roger Tsien, fluorescent proteins
Paul Alivisatos, qDots
Paul Robinson, full spectrum flow cytometry
Alan J Heeger, PI-coupled fluorescent polymers
Bahram Jalali, Eric Diebold, high speed imaging flow cytometer/sorter

Flow cytometry group leaders, system improvements & voice of the customer








Industrial Innovators

L.A. Kamentsky, M.R. Melamed, H. Derman, spectrometer for ultra-rapid cell analysis
Howard Shapiro, multibeam flow cytometer, low cost system, the BOOK
Mack Fulwyler, Dick Sweet, Bernie Shoor, industrial FACS™ cell sorter
Bob Auer, Yong Chen, Coulter flow cytometry product line
Chia Huei Chen, John Kimura, commercial algae pigment conjugates
Linda Lee, small molecule DNA/RNA dyes
Rosaria & Dick Haugland, many new fluorescent dyes
Ger van den Engh, William Stokdjik, innovative electronics
Bob Hoffman, Joe Trotter, BD cytometry product innovation
Bruce Bagwell, innovative algorithms for cytometry
Morgan Conrad, Pierre Bierre, algorithms for cytometry
Collin Rich, Nathan Bair, simplified portable cytometer
Masataka Shinoda,Motohiro Furuki,Masanobu Yamamoto, SONY cytometers
Ming Yan, Wenbin Jiang, Eric Chase, advanced full spectrum cytometry

1964 Kamentsky Sorter

BEC web-site 2013

LA Kamentsky, MR Melamed & H. Derman, Spectrophotometer: New instrument for ultrarapid cell analysis, Science 150, 1965

Particle Technology

Particle Technology Inc. - COULTER -1971

Fulwyler began consulting for Coulter in the late 1960's. Spinning out LASL FCM and Particle manufacturing technologies.

In 1971, Mack Fulwyler resigned from LASL and established PTI as a Coulter subsidiary company

1976 PTI dissolved, technology transferred to Florida

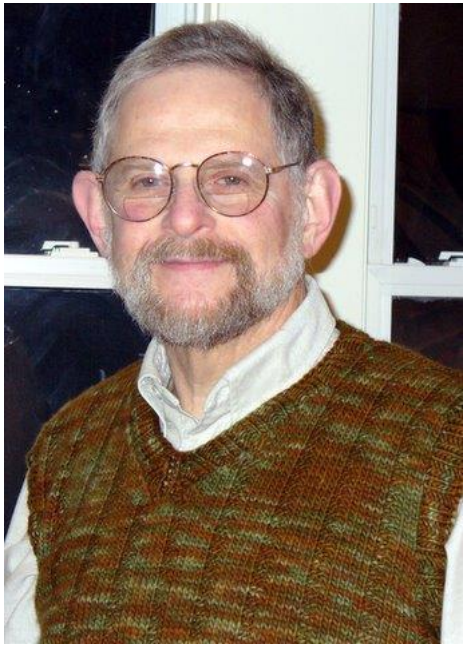
Epics II 1975, Designed by Mack Fulwyler and Jim Corell
Delivered to NCI/NIH

TPS 1974 - 1979, Designed by Bob Auer

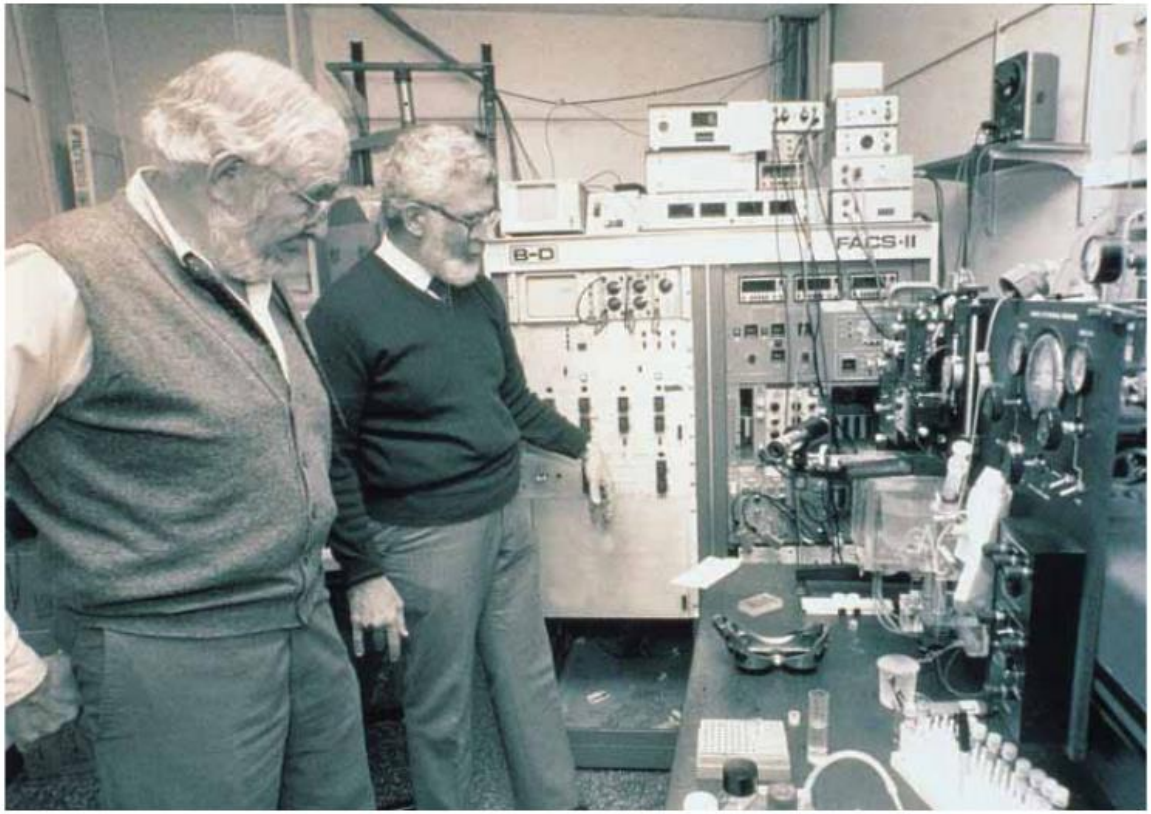
From Beckman-Coulter website 2013

Multi-beam Flow Cytometers

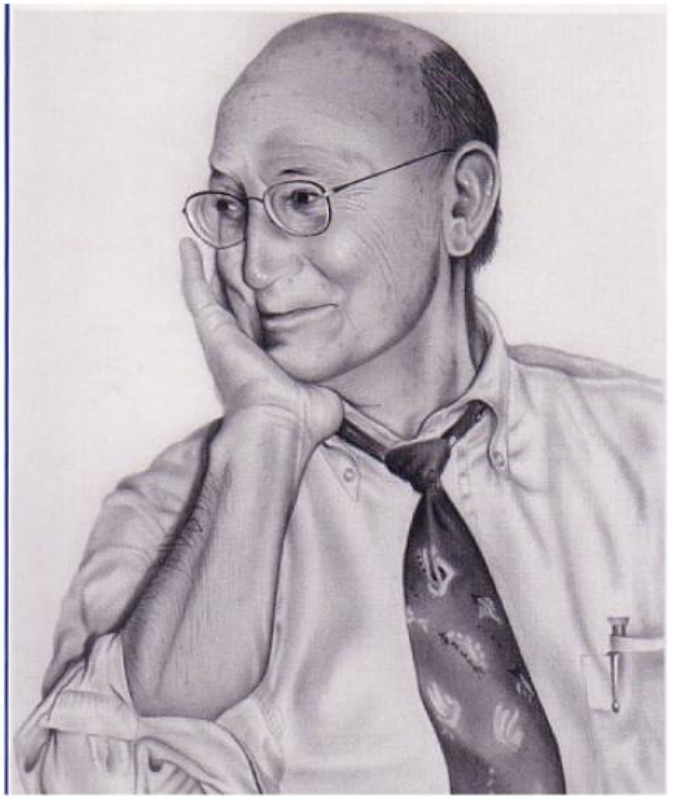
Howard M. Shapiro - 1973-76
Shapiro and the Block instruments designed a series of multibeam flow cytometers that did differentials and multiple fluorescence excitation and emission



From Beckman-Coulter website 2013



Bernie Shoor and Len Herzenberg



Mack Fulwyler

From the original business plan (1976): *"We are convinced that the FACS cell sorter can be sold to at least 50 leading research laboratories in the world."*

- FACS history from the Smithsonian archives
  http://siarchives.si.edu/research/videohistory_catalog9554.html

Flow cytometer breadboard at BD Biosciences

History of Early Commercial Cytometry Instruments

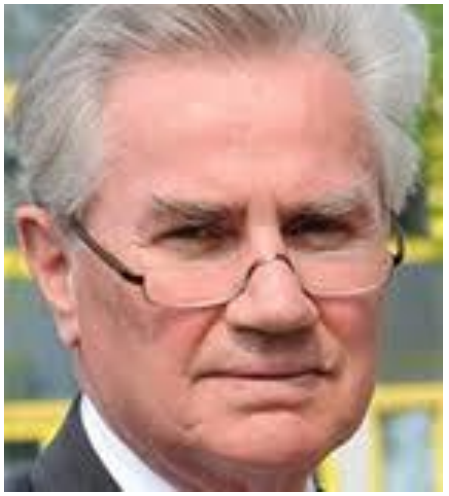
1968 1st fluorescence-based device (ICP 11) by Wolfgang Göhde, University Münster, Germany, commercialized in1968/69 by German developer and manufacturer Partec through Phywe AG.

1971 Cytofluorograph, Ortho

1973 PAS 8000, Partec

1974 1st FACS instrument, BD

1977 Epics Instrument,Coulter



Manufacturers of Flow Cytometers

Past and Present

SONY make.believe

iCyt

EMD MILLIPORE

Guava

OWL Bio

MACS Miltenyi Biotec

DVS Sciences

Fluidigm

Cytomation

BECKMAN COULTER

PARTEC

PHYWE excellence in science

Sysmex

Xitogen

Stratedigm Cytometry Without Compromise

BD

Cytopia Influx

accuri CYTOMETERS

BIO-RAD

ZE5 Se3 from Propel

handyem Democratize cytometry™

ThermoFisher SCIENTIFIC

Acousticyte Attune

BigFoot, Propel

CYTEK TRANSCEND THE CONVENTIONAL

Bay bioscience

Bay bioscience

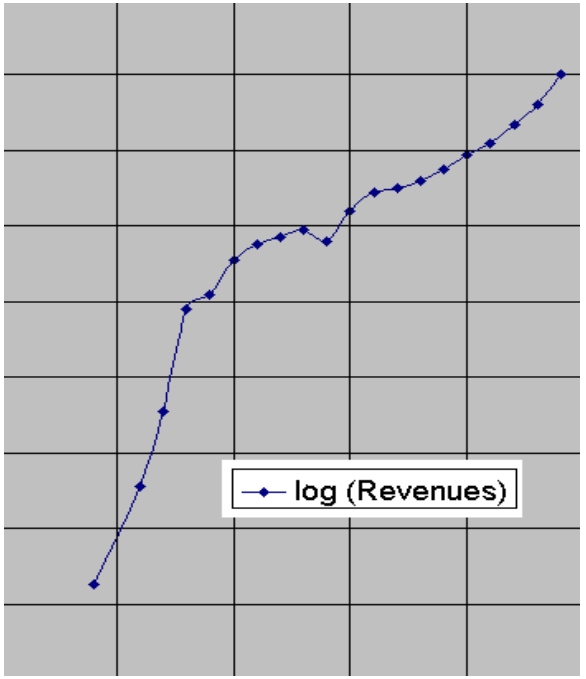
Ortho-Clinical Diagnostics a Johnson & Johnson company

nanocellect Biomedical, Inc.

...

An intrapreneurial founder

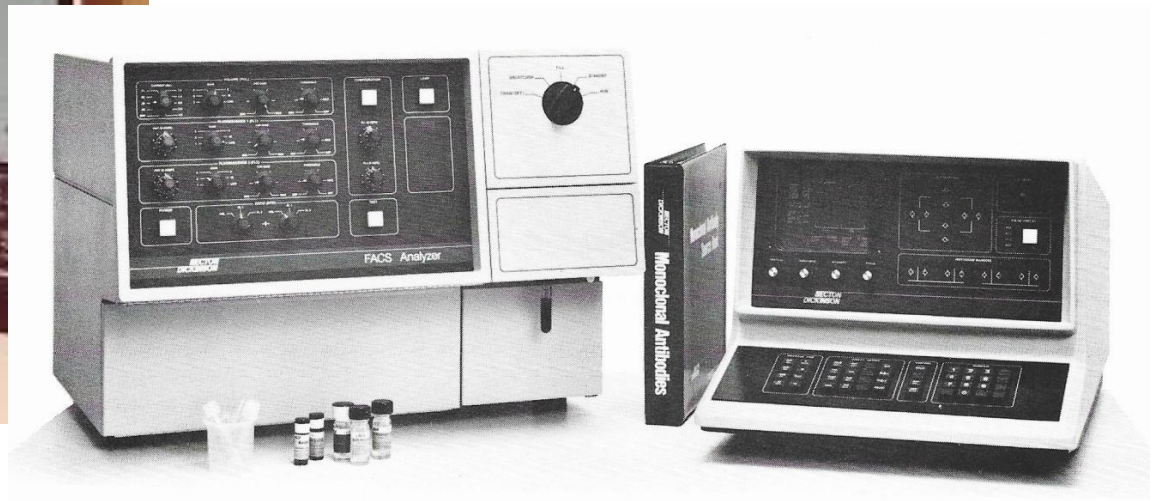
Bernie Shoor completed his B.A. in physics from NYU in 1946. After receiving his degree, he worked for the Army Signal Corps and subsequently the Sperry Gyroscope Company. In 1966, Shoor began working for Endevco Corporation, a small scientific instrument company which was eventually bought by BD. In 1970, Shoor became manager of BD's Mountain View, California laboratory. (from the Smithsonian Videohistory Collection THE HISTORY OF THE CELL SORTER RU 9554)



Rapid growth from a small beginning to a large business

Early Flow Cytometry Practice

Many system control knobs and switches with oscilloscope display for pulses and histograms.



Requiring the early users to perform complex adjustments and to service the instrument.

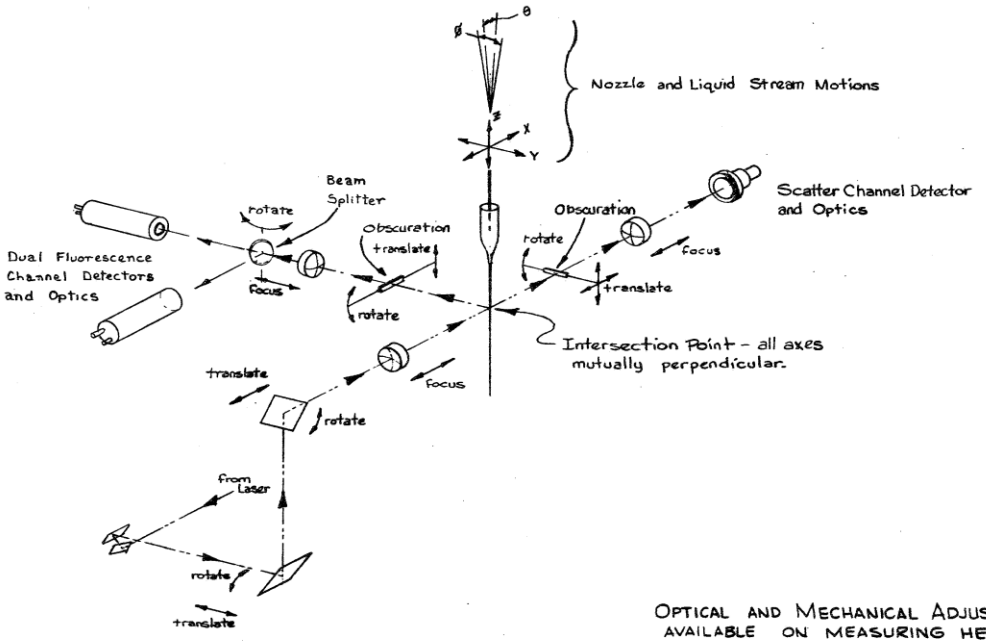


FIGURE 1-8

OPTICAL AND MECHANICAL ADJUSTMENTS AVAILABLE ON MEASURING HEAD

PULSE HEIGHT ANALYZER (CONTINUED):

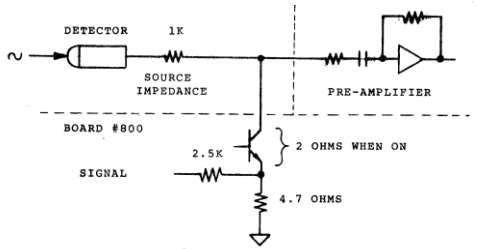
In the standard version, a single parameter PHA is provided which is capable of analyzing one parameter at a time, as selected on the control panel. The analyzer is capable of dividing the input signal into 1024 channels (on the basis of amplitude) and accumulating up to 10^6 events (cells) in each channel. Typically, the memory bank is sub-divided (by front panel controls) into four quadrants, each containing 256 channels. This permits storing and displaying four sequential experiments.

OPTIONAL DUAL PARAMETER STORAGE AND DISPLAY:

In addition to providing normal single parameter analysis and display, the dual parameter system is capable of storing and displaying correlated data from two parameters at the same time. This information is stored in a 64 x 64 matrix which may be sub-divided into four 32 x 32 matrices or two 32 x 64 matrices. Display of correlated data is on the x-y CRT oscilloscope and may be selected in either contour or isometric display. Regions of interest may be intensified and data accumulated for these regions.

OTHER AUXILIARY SYSTEMS:

The PHA, with or without the optional dual-parameter storage and display, can be interfaced with x-y plotters, mini-computers, teletype keyboards, etc., using direct plug-in interface boards.



SET-UP PROCEDURE FOR TIMING AND CONTROL LC

A. SYSTEM TO CALIBRATE MODE

1. All cards installed, power ON

2. Scope Switching Panel

a. push CALIBRATE SIGNALS switch to lit position

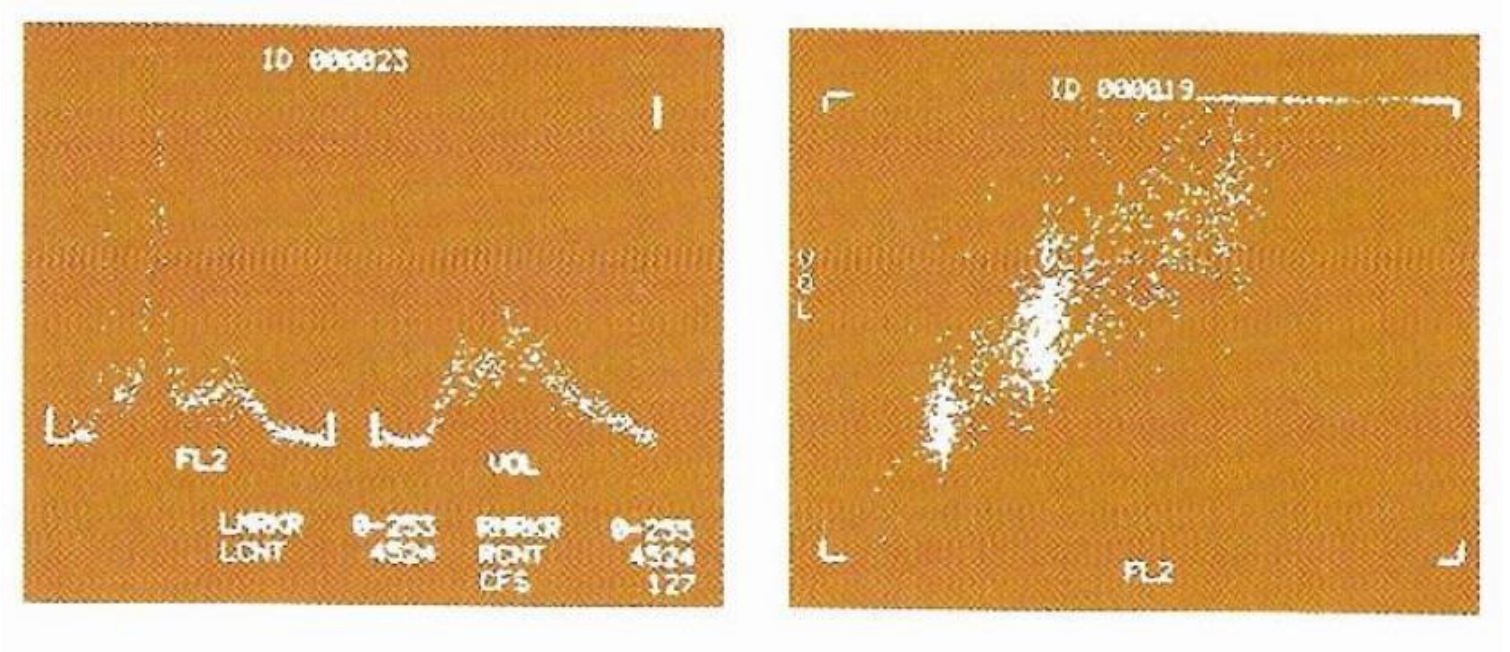
b. TRIGGER switch to CALIBRATE

Sir Gustav Nossal shows a FACS II in 1977, the 1st FACS in the Southern hemisphere.

ADVANCED FEATURES OF THE FACS ANALYZER

- Mercury-arc lamp illumination as standard with fluorescence filter sets for use with a wide variety of fluorochromes
- Microprocessor-controlled measurements of cell volume and two fluorescence parameters, including ratios of any two parameters
- Two-parameter analysis with independent two-parameter gating
- Display of data as side-by-side profile histograms, each with 256 channels of resolution, or two-parameter dot plots
- Paired histogram comparisons by overlap
- Logarithmic and linear amplification of fluorescence and volume signals with linear gain controls
- Single-knob fluid control providing orifice and sample backflush
- Interchangeable fluid orifices from 50 to 150 microns in diameter and 75 to 225 microns in length for operation with a wide variety of samples
- Operates from a standard electrical outlet (115 VAC, 15 Amp) requiring no compressed gas or external fluid source
- Optional CONSORT 20 data processing package for mass storage and fully automated analysis of histogram data
- Typical sample analysis with data output: one to three minutes per sample

FACS Analyzer Console Data Output



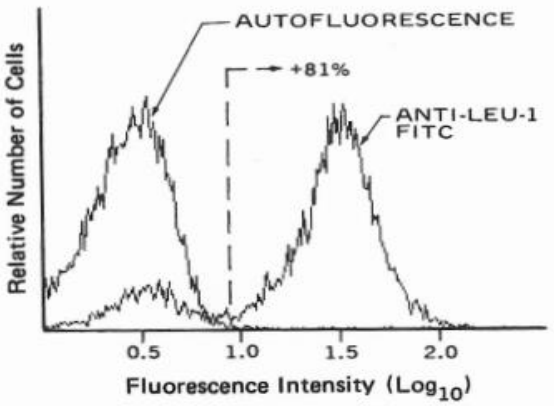
1D side by side histograms and 2D dotplot (top)

Volume gated histogram of leukocytes stained with CD4-FITC (left)

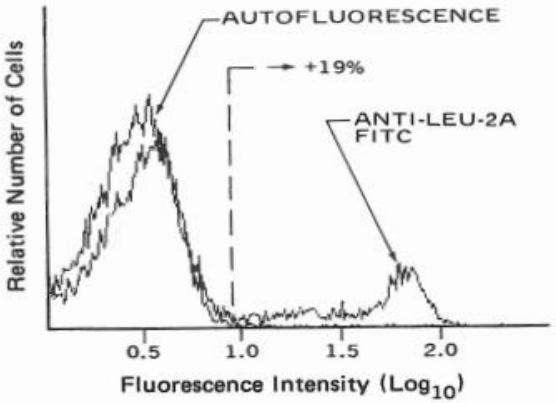
FACS ANALYZER DATA

Shown below are volume-gated fluorescence profiles of normal human peripheral blood lymphocytes (Ficoll-Hypaque gradient preparation). Reagents are **Becton Dickinson Monoclonal Antibodies†** to human lymphocyte antigens: Leu-1, Leu-2a, Leu 3a + 3b, Leu-4, Leu-5 and HLA-DR. These antibodies were directly conjugated with fluorescein isothiocyanate (FITC), except Anti-Leu-5, which was analyzed with indirect immunofluorescence. Total number of cells analyzed for each run was approximately 2 x 10^4 at count rates of up to 1000 cells per second. Data were processed using CONSORT 20 software.

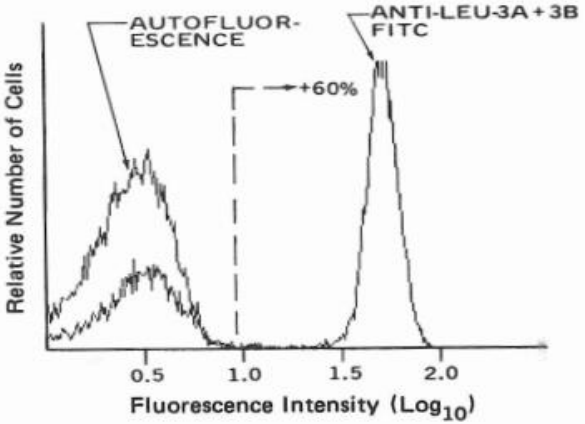
Anti-Leu-1
(Catalog No. 5303)†
Pan T



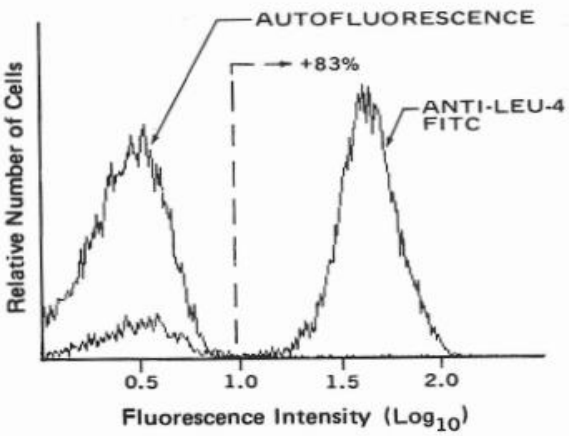
Anti-Leu-2a
(Catalog No. 5313)†
T Cytotoxic/Suppressor



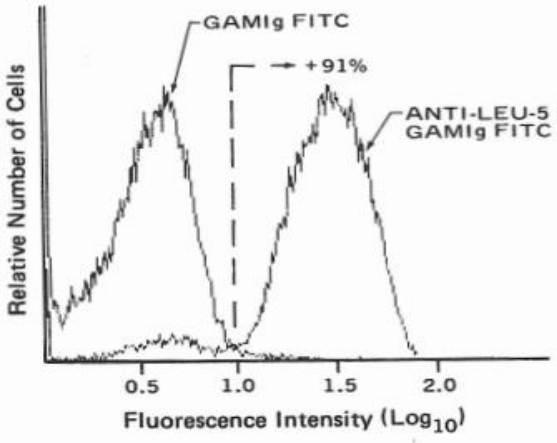
Anti-Leu-3a + 3b
(Catalog No. 5413)†
Helper/Inducer



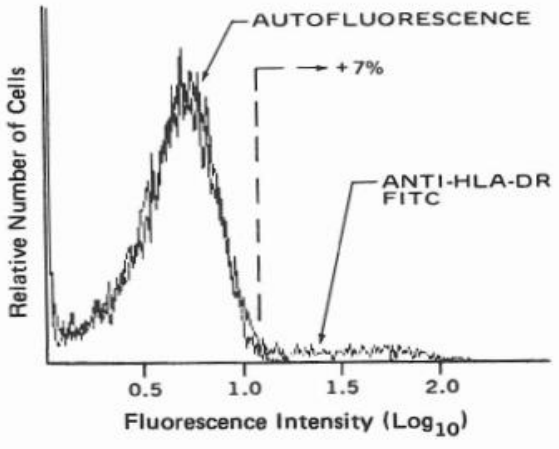
Anti-Leu-4
(Catalog No. 5343)†
Pan T



Anti-Leu-5
(Catalog No. 5380)†
Pan T/E-Rosette



Anti-HLA-DR
(Catalog No. 5363)†
B Cell and Monocyte



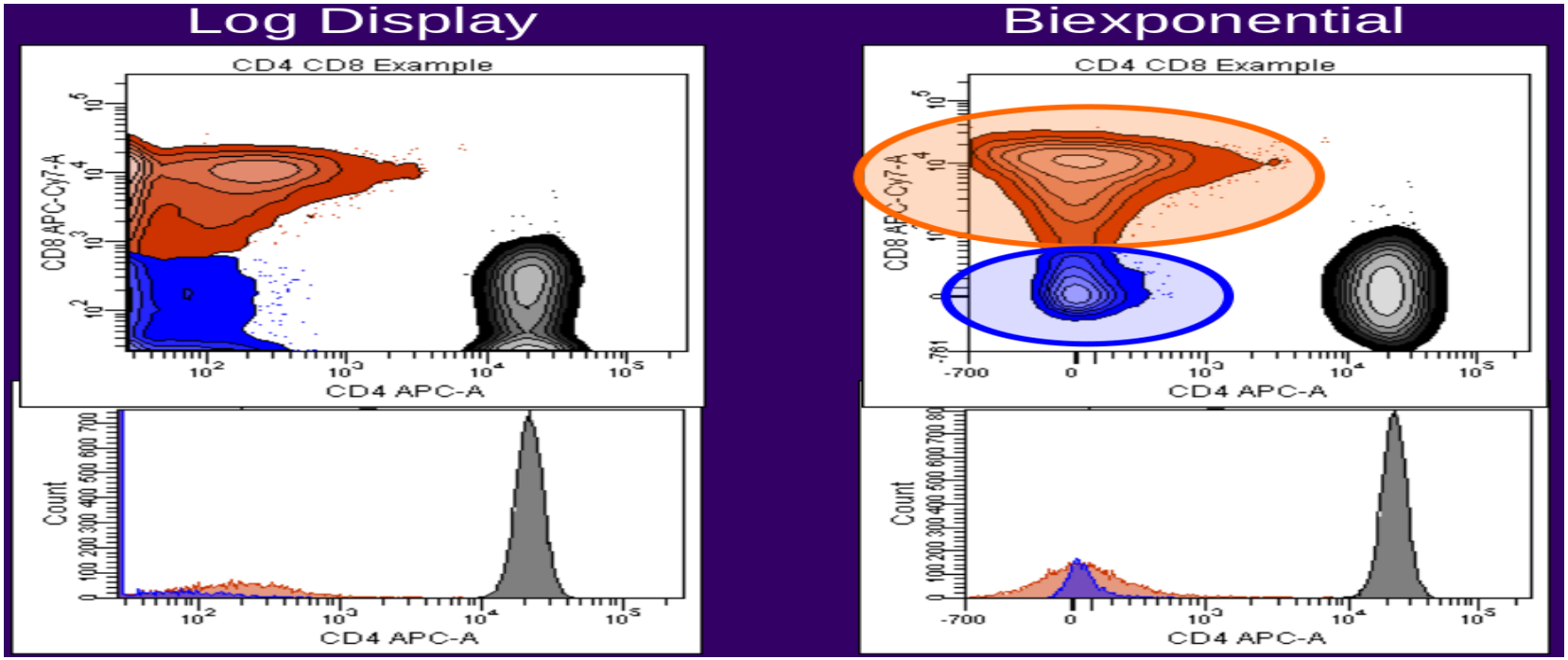
Computers changing the instrument appearance

- Digitize in 14-bits
  - 16,384 levels
- Sample at 10Mhz
  - 10 million times/sec
- 16 channels

FACS™ digital Vantage cell sorter

Recognizing Issues with log Data Display

- Since log(0) is undefined we cannot visualize numbers <= 0 using a log scale (on-axis events)
- At the low end the CV tends to increase unlike the high end where it is usually constant
- The inherent character of immunofluorescence data is
  - Log-like at the high end
  - Linear near zero
  - Contains negative corrected numbers (after baseline and compensation) which are valid scores
- Asilomar 2002: Dave Parks – suggested log transformation is not the best visualization approach for flow Immunofluorescence data



Joe Trotter, BD, retired

Spectra from Single Particles using Diffraction Grating

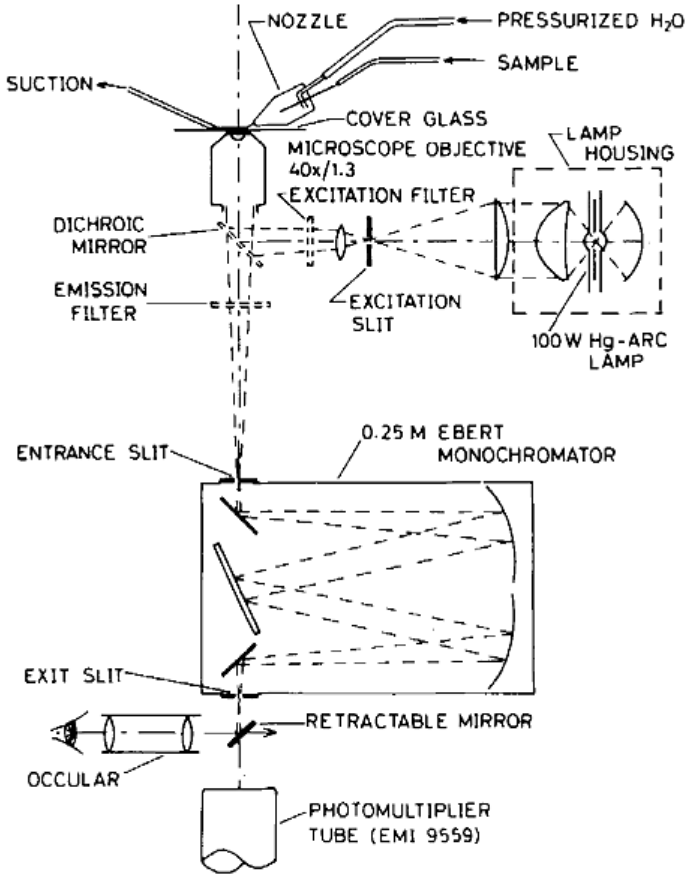


FIG. 1. Optical outline of the flow cytometer including a grating monochromator for monochromatic fluorescence detection.

SteenHB1986Cytometry7_104

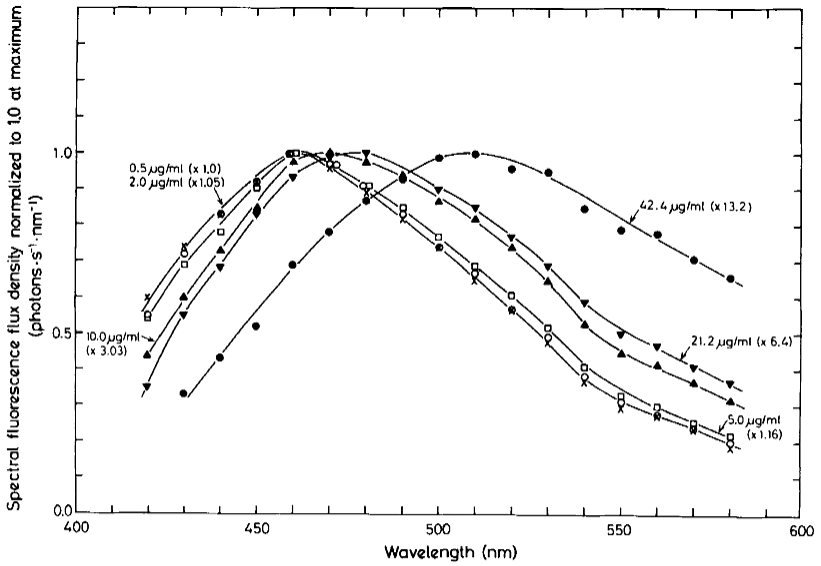


FIG. 2. Corrected fluorescence spectra of rat thymocytes fixed in ethanol and stained with Hoechst 33258 in the concentrations indicated on the curves. Each spectrum has been multiplied by the normalization factor given in parentheses. The total fluorescence intensity is thus approximately inversely proportional to this factor. The similarity between the spectra obtained with the two lowest dye concentrations is an indication of the reproducibility of the measurements.

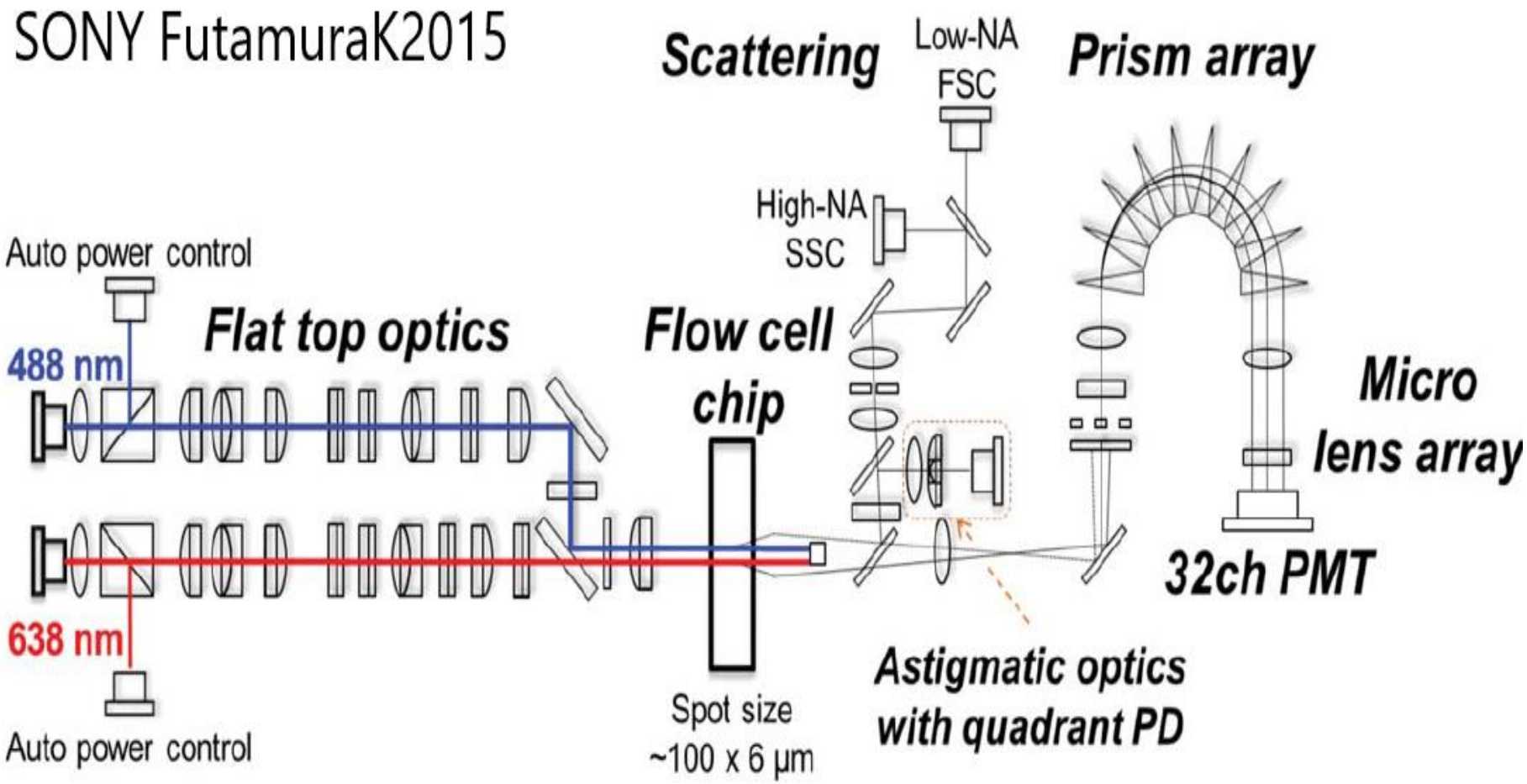
SteenHB1986Cytometry7 104

Others:

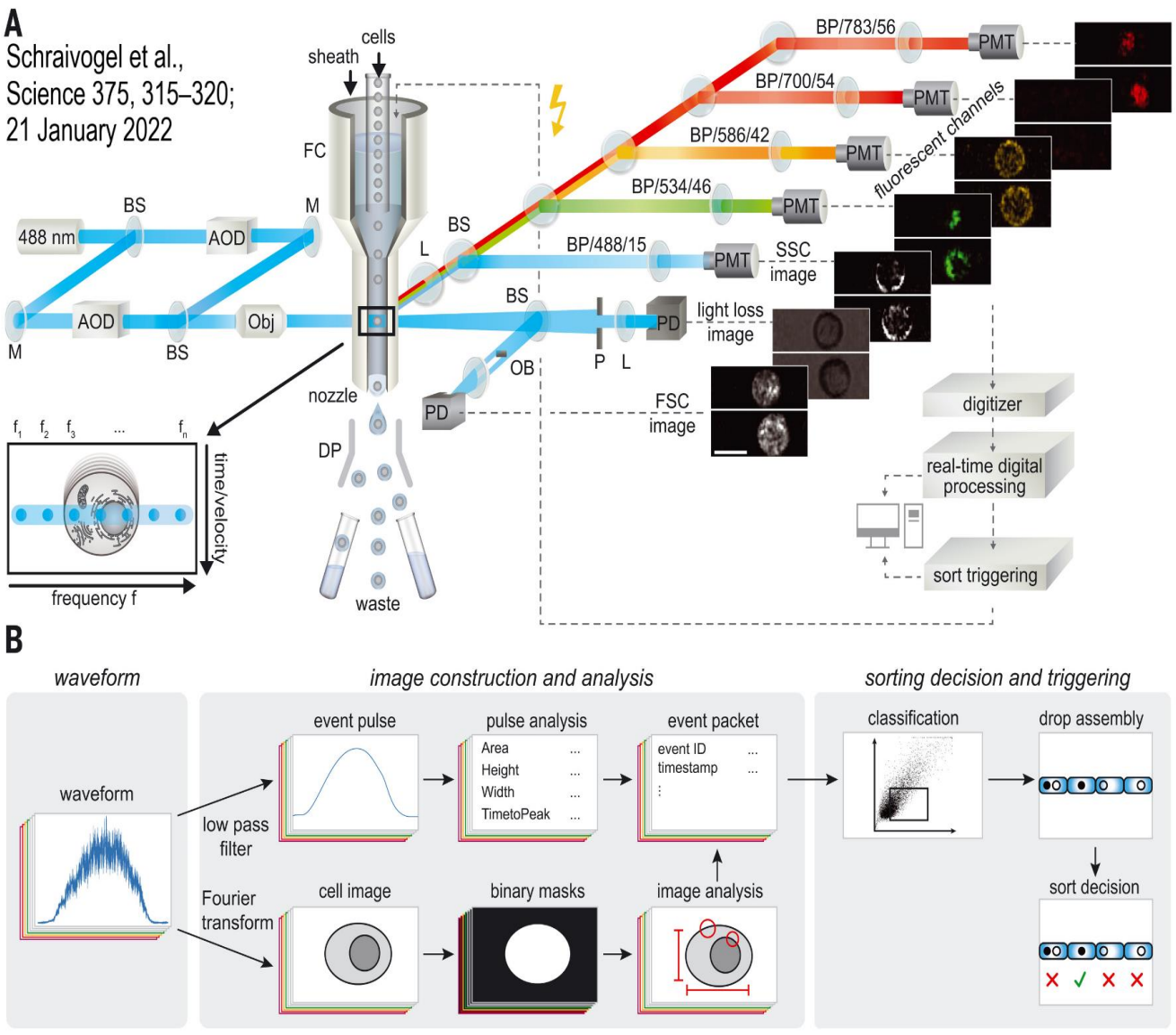
Gauci MR 1996, prism based flow cytometry

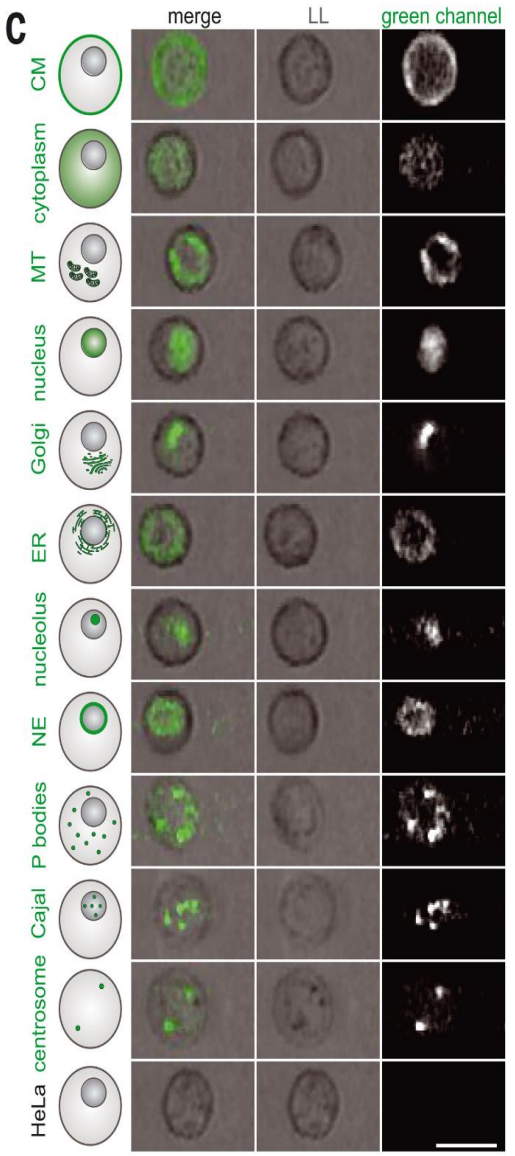
Buican T 2004, FT interferometry

Modern Day Flow Cytometry

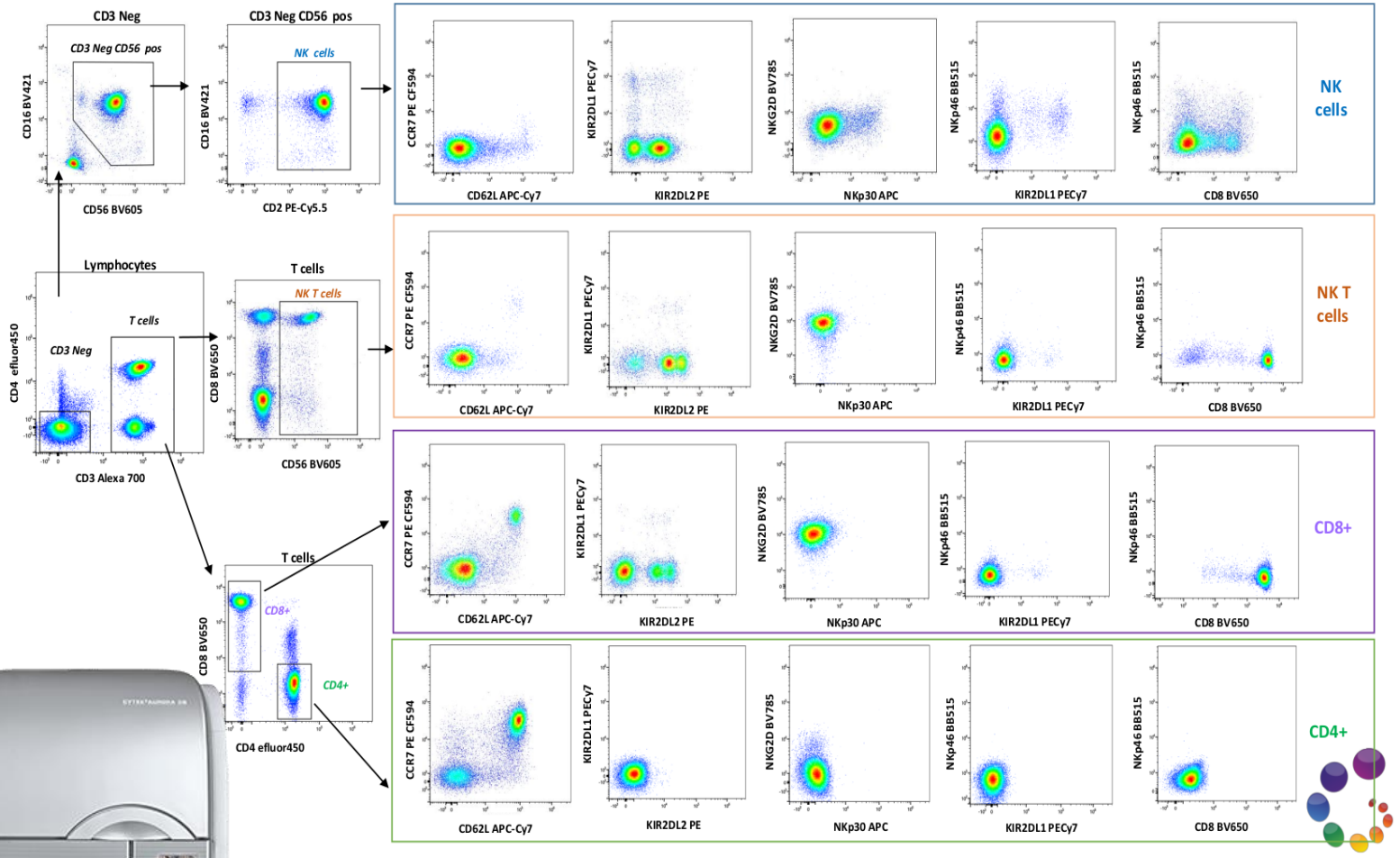


Flow Sorting based on Morphology





Multi-laser Full Spectrum Cell Sorter



Immune Cell Profile with 35 fluorophors

I thank Fred Preffer for making the contact and the organizers for the invitation to give this talk to the New England Flow Cytometry Group.

I thank Allen Poirson for providing me input about the SONY cytometry team, and Bob Auer for input about the Beckman Coulter team.

If you are interested in further detail about the history of flow cytometry I suggest reading Howard's text at
Howard Shapiro (2004) Cytometry 58A: 13-20
"The Evolution of Cytometers"

Diether Recktenwald, diether@desatoya.com, +1-408-658-6074
http://www.desatoya.com