Past and Future of Flow Cytometry
. . . and some Secrets

FACS Users Meeting
Stockholm
February 4, 2009

BD

The Short Story

1974: The first sorter by BD-FACS

1978: Start of Monoclonal Center

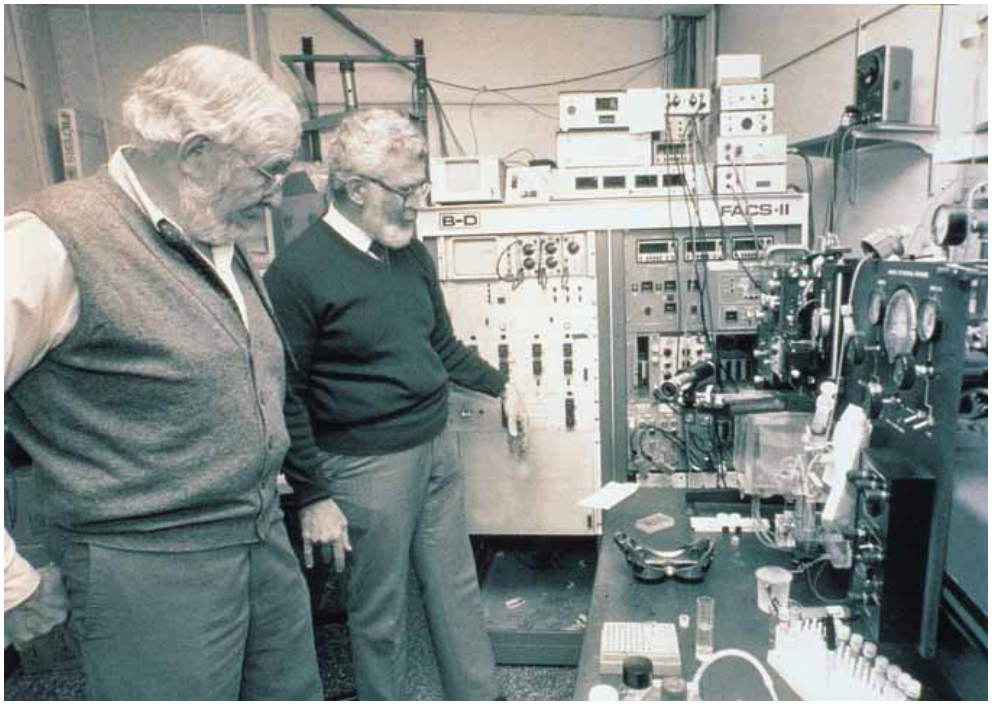
2008: + $ 1 billion in revenues

So, how did we get to this reward?

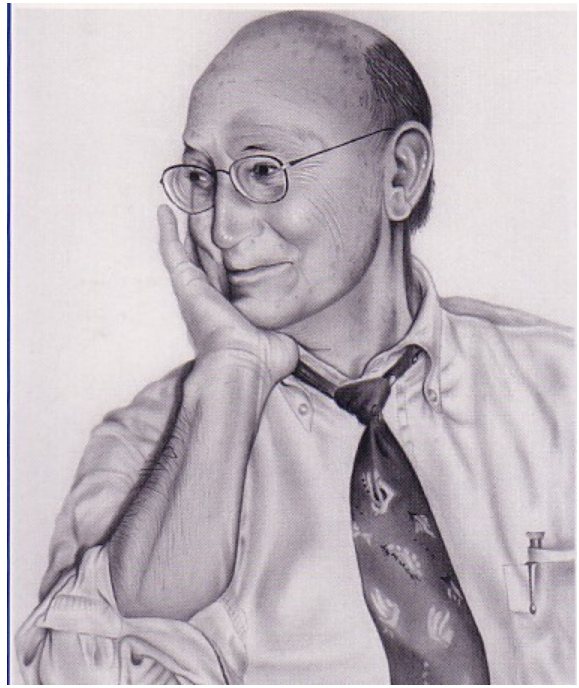
The Start: BD & FACS Systems

- Dick Sweet:
  - Applied ink jet concept to fluid stream
- Mack Fulwyler:
  - Integrated cytometry and sorting
- Len Herzenberg:
  - Expanded cytometry to antibodies
- Bernie Shoor:
  - Convinced BD to make investment

The pioneers on the BD side:



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."*

Others had their start too:

- Wolfgang Goehde (Biophysics/Partec)
- Lou Kaminsky (ODS)
- Wallace Coulter (Coulter Electr/BCI):

. . . With the common denominator: DNA

. . . In contrast, ‘FACS Systems’ was focused on Immuno-fluorescence. . .

Basics (1): The Cells and Views

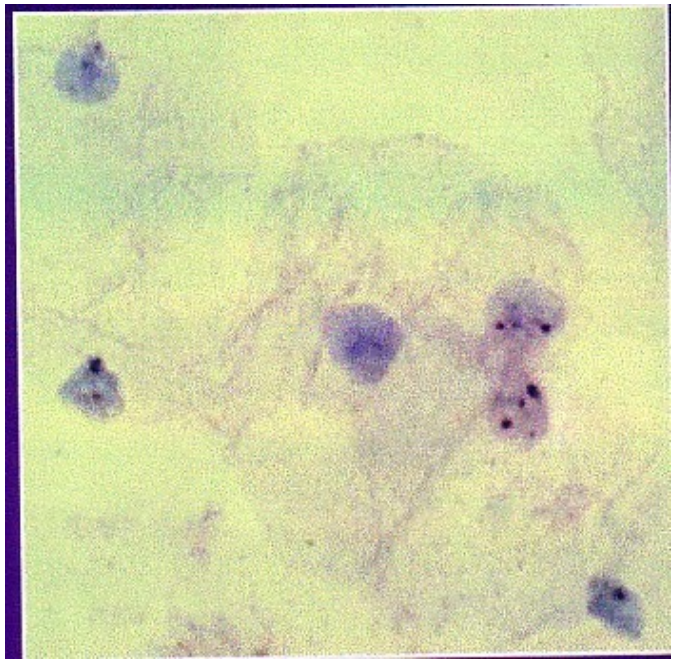


Figure 1: Cells can be stained for light transmission viewing

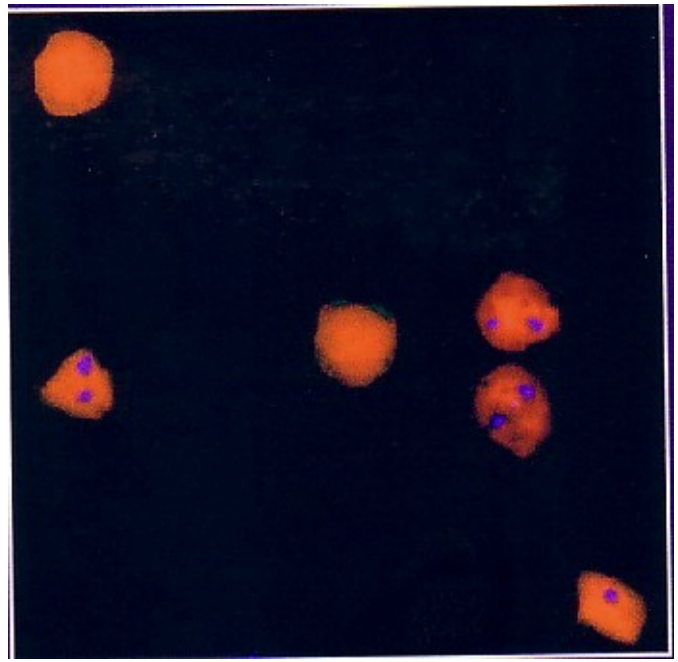
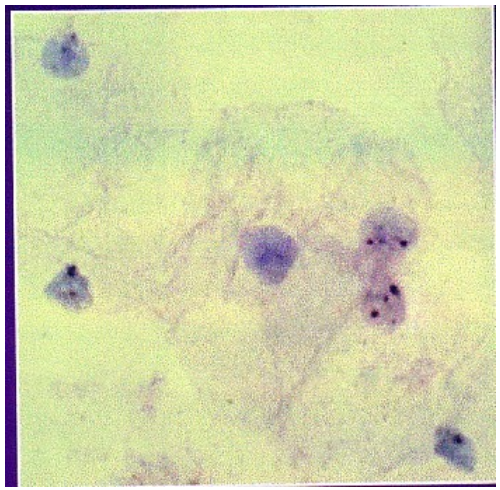
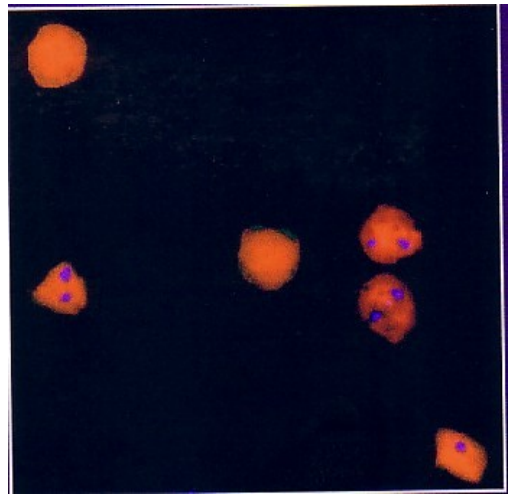


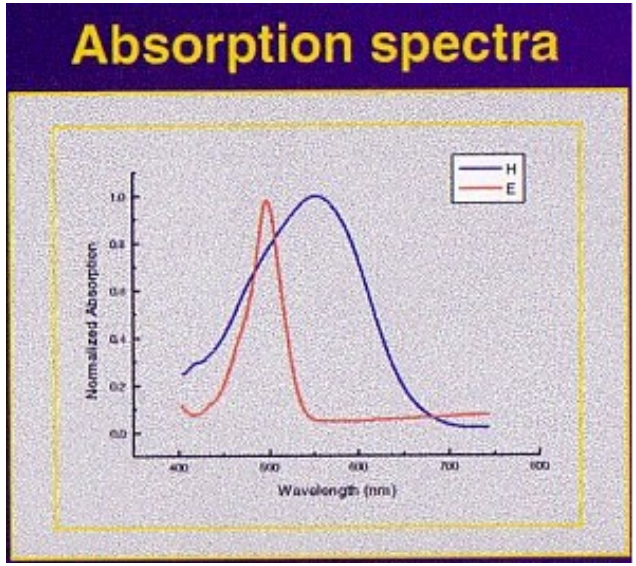
Figure 2: Cells can be stained for fluorescence viewing

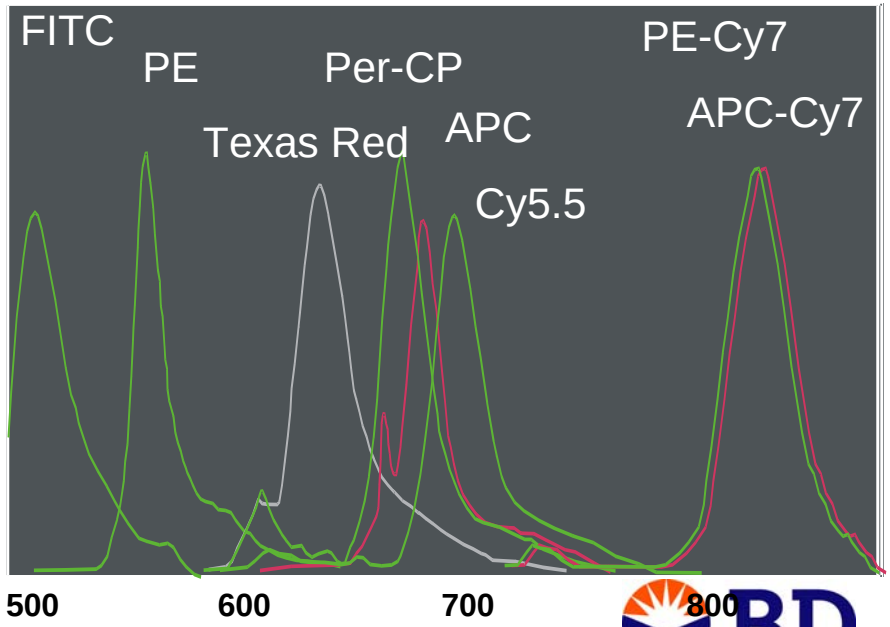
Basics (1): The Cells and Views



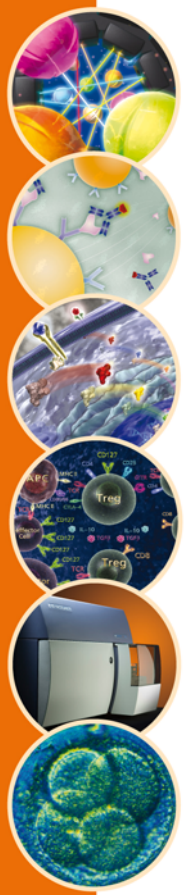
Absorption
&
Fluorescence







Basics (2): Tissue and Suspensions



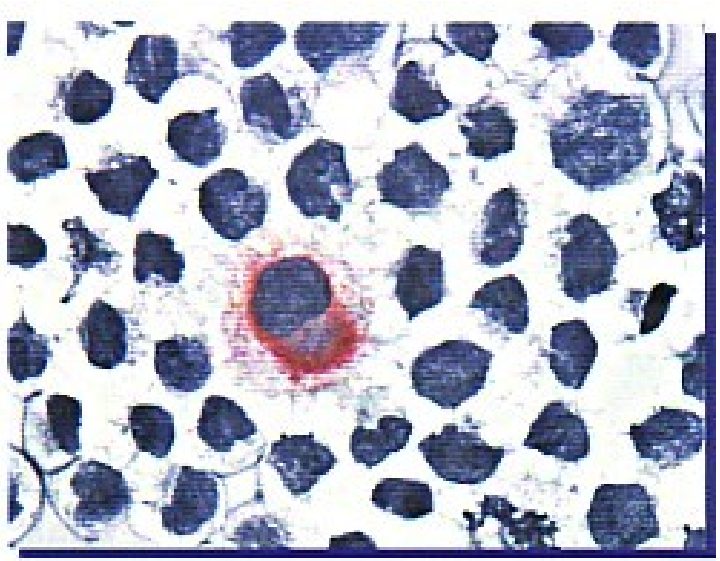


Figure 3: Cells can be studied in an organized context: a tissue

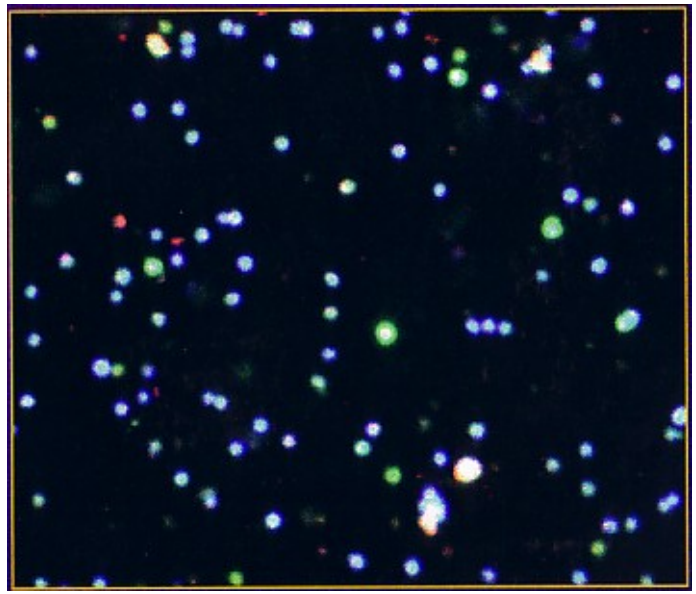


Figure 4: Cells can be studied in a suspension context: a blood sample

Basics (3): The Number of Cells

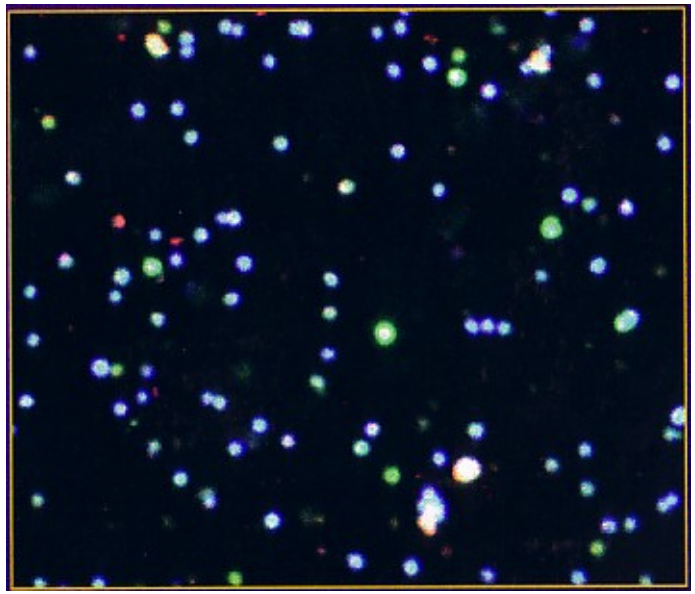
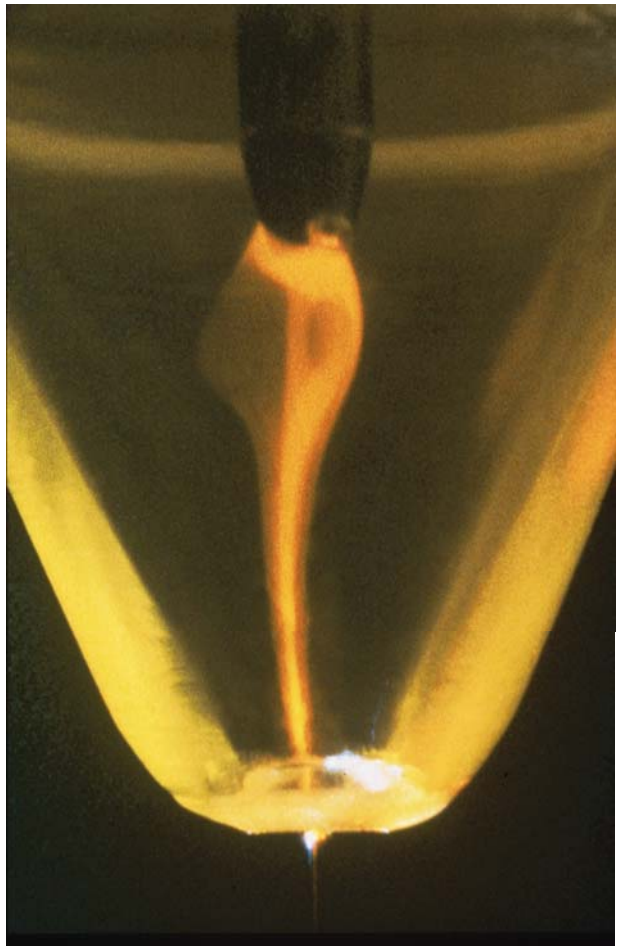


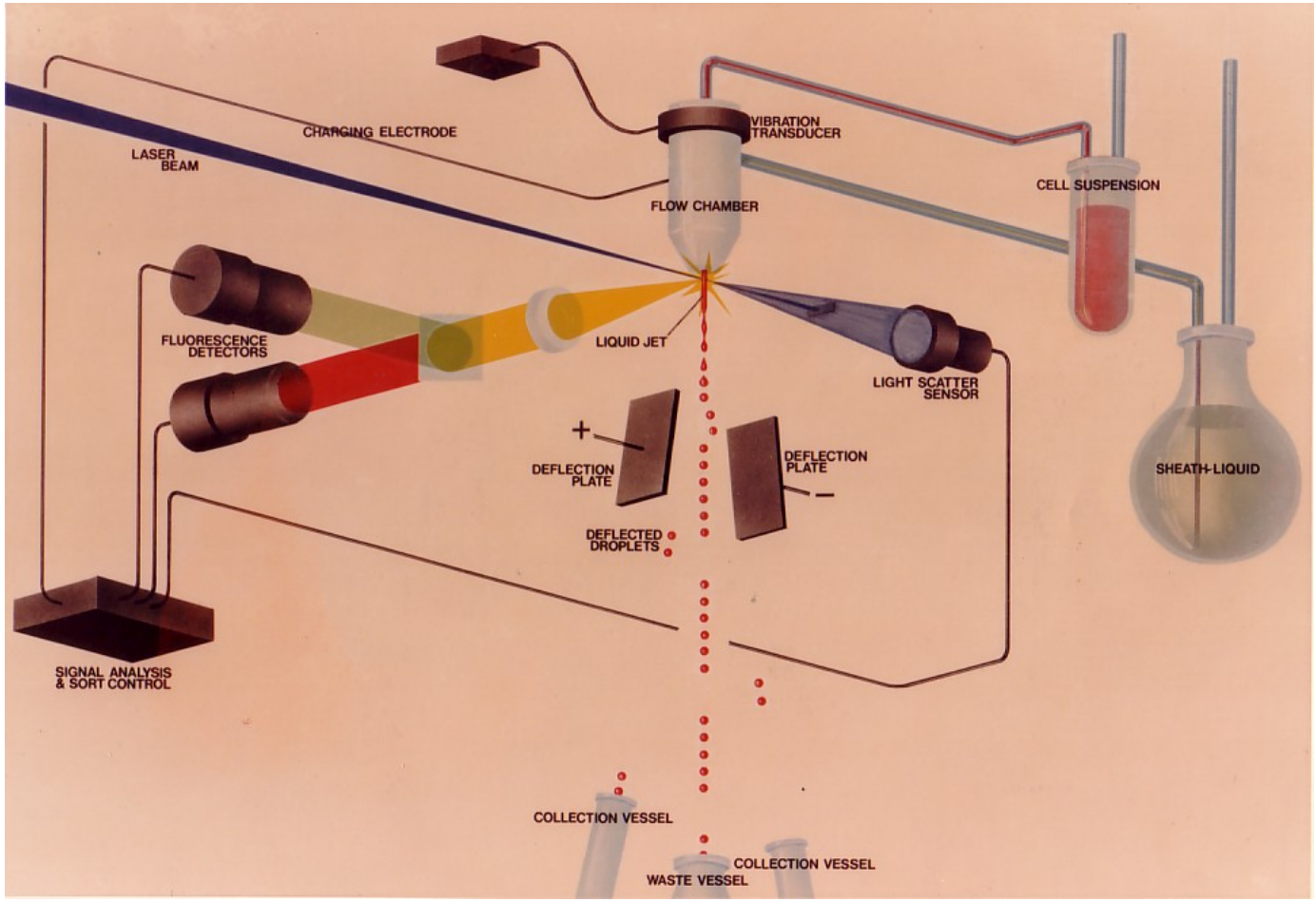
Figure 5: Cells can be looked at within a limited field of view

Figure 6: Cells can be mechanically organized in a string and could be made to run in a continuous mode

Basics: Make an endless cell stream



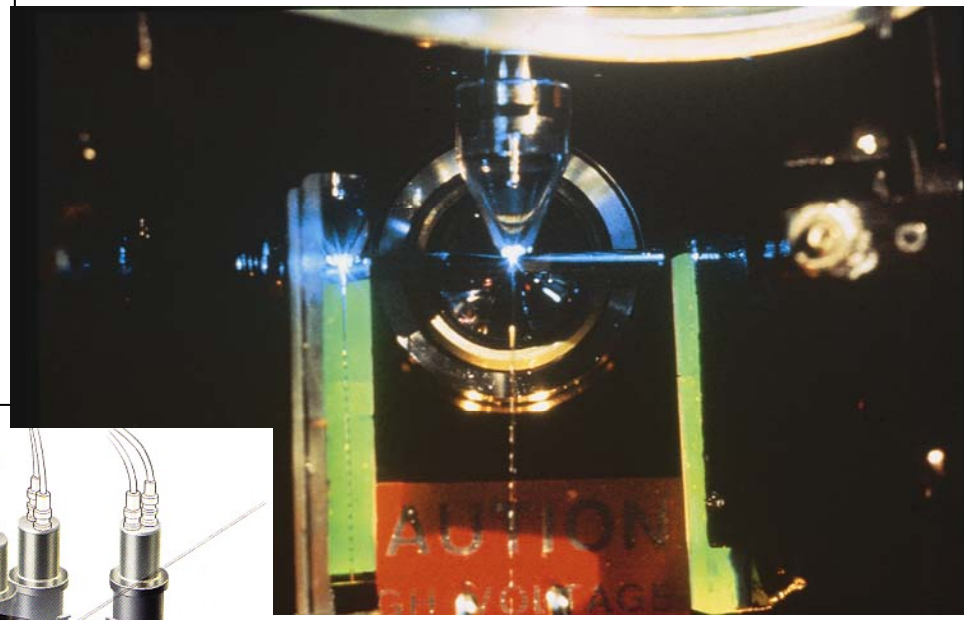
Basics: With cells aligned, detection is next

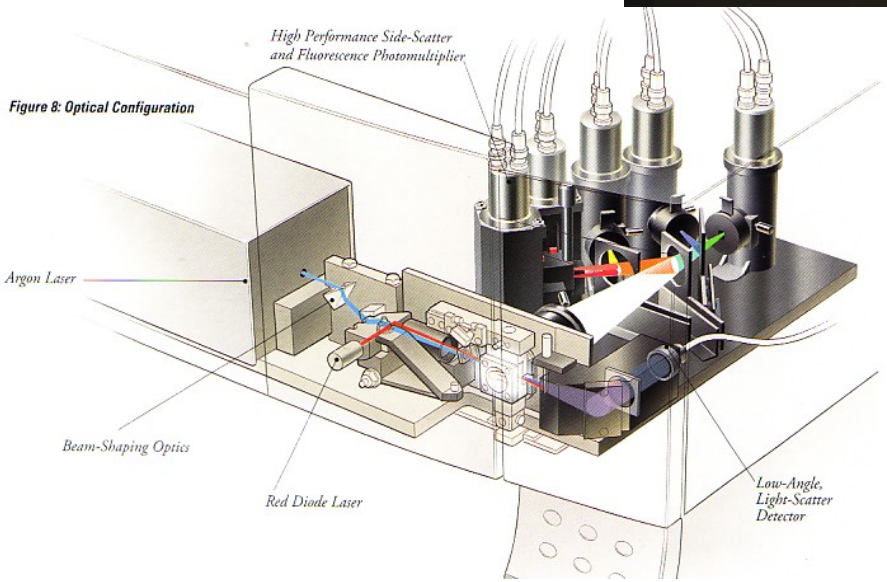


Basics: Detection of cells requires essential components

-Light Sources:
- for light scatter
- for excitation of dyes

- Light Detectors:
- for scattered light
- for fluorescent light

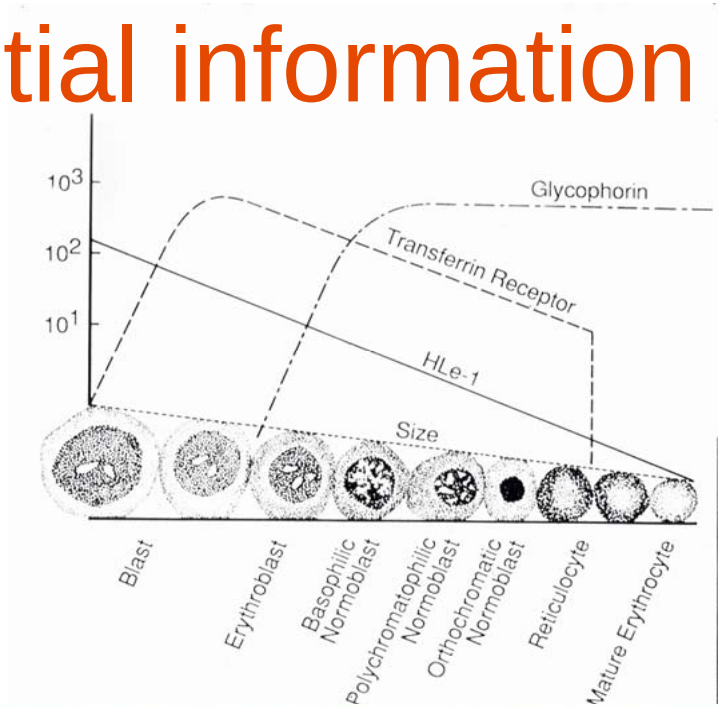


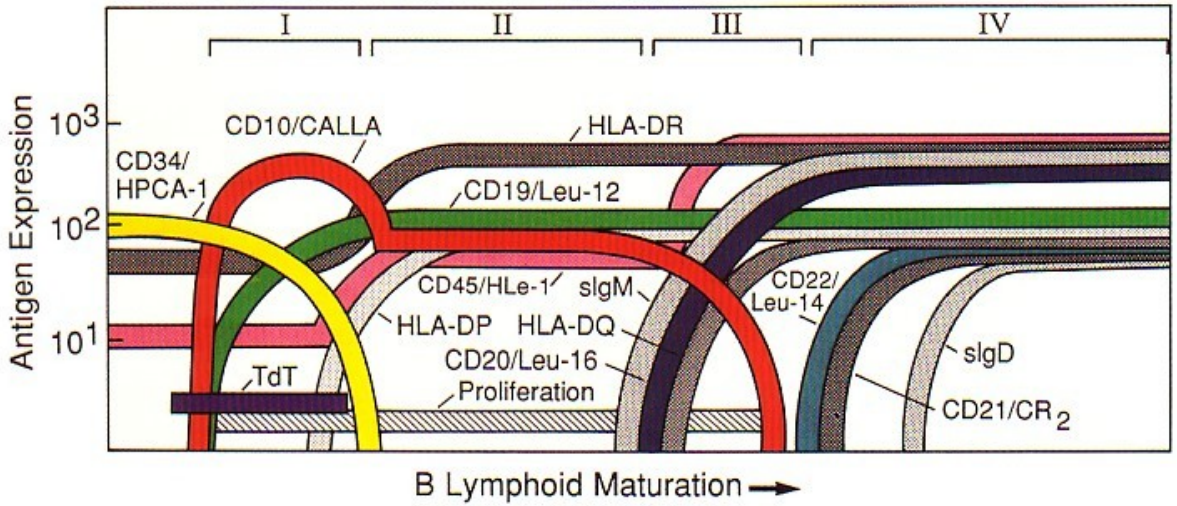


- Collector Lens (Integrated)
- Optical Filters

Basics: Detection of cells delivers essential information

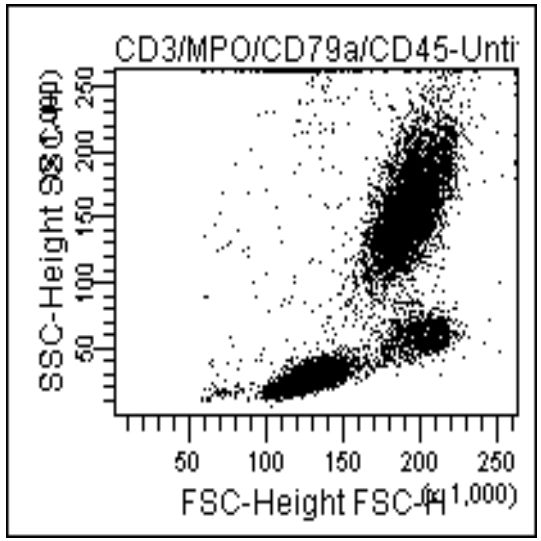
For each cell:
- Size
- Internal Structure
- Specific proteins (outer & inner)
  - ***Monoclonal** Antibodies*

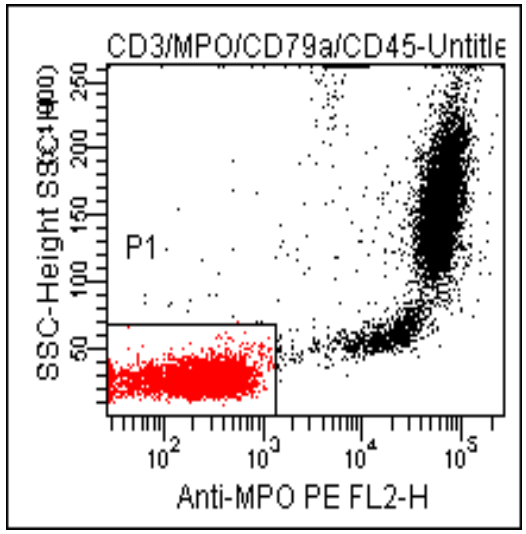


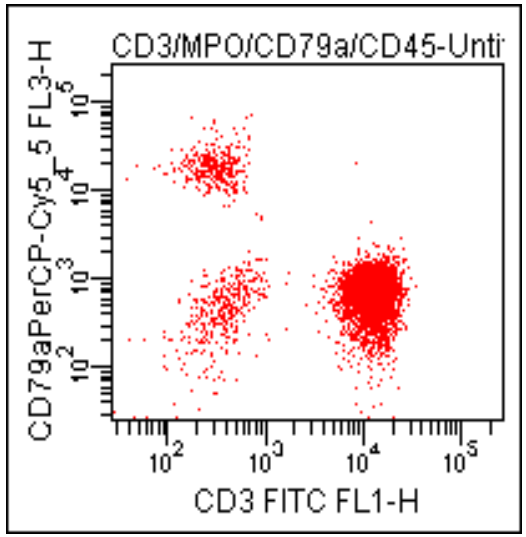


BD

Data Presentation:

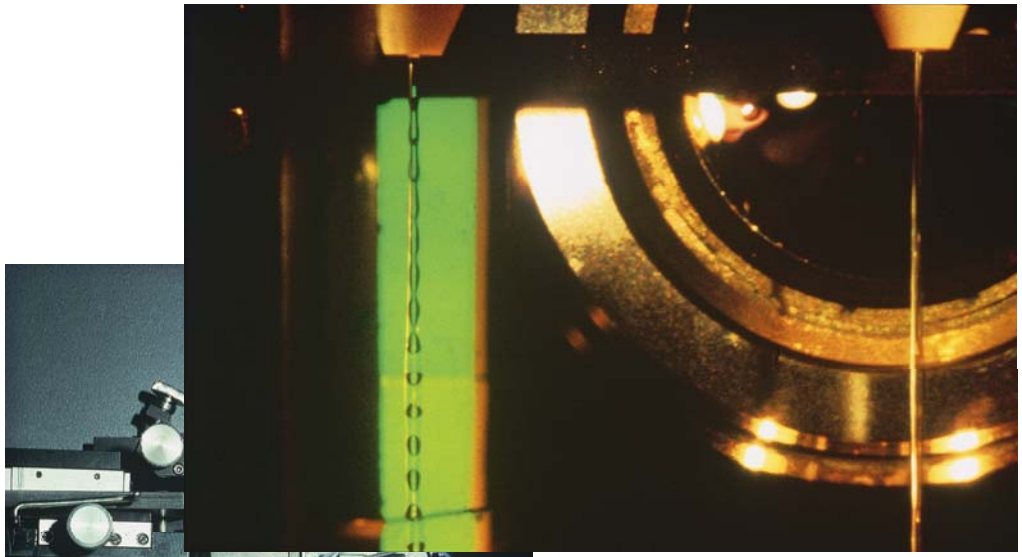


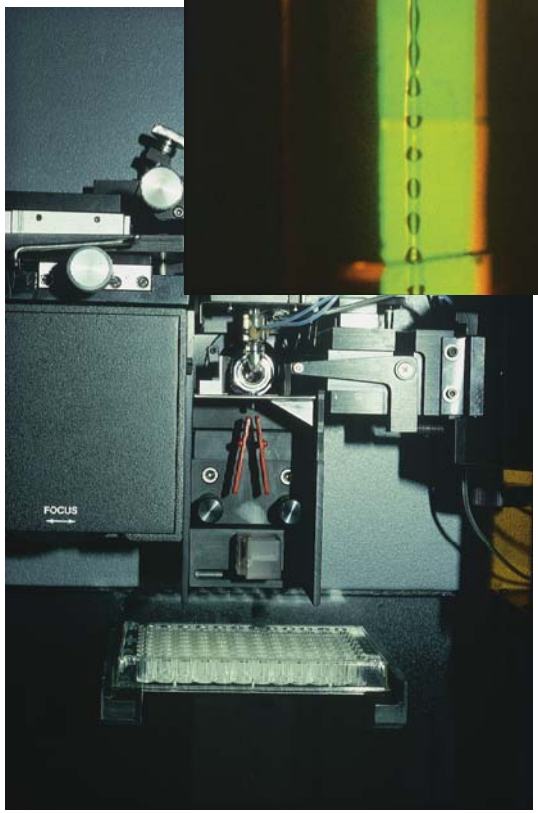


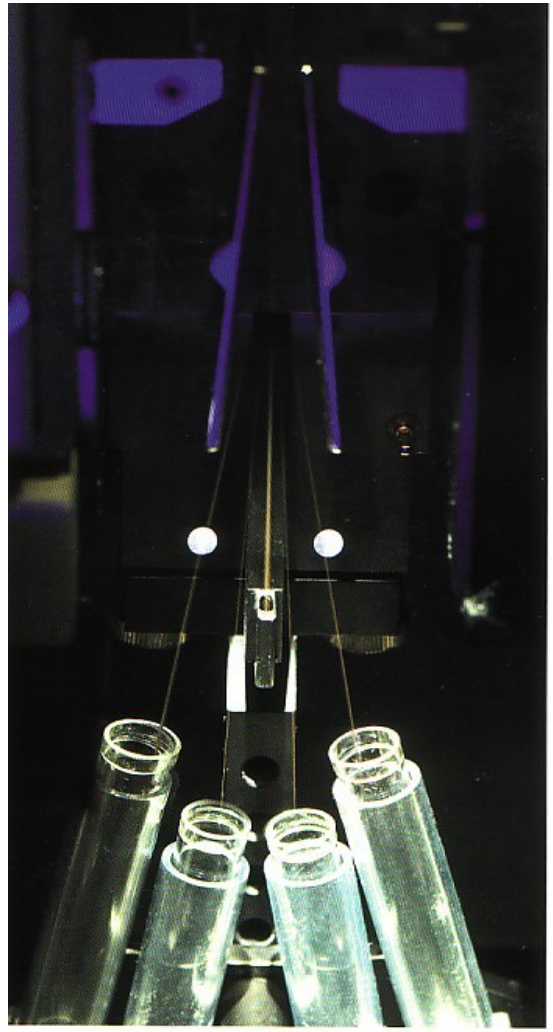


Data obtained with the BD IntraSure reagent kit

Basics: Individual cell sorting, “the ultimate claim to fame”

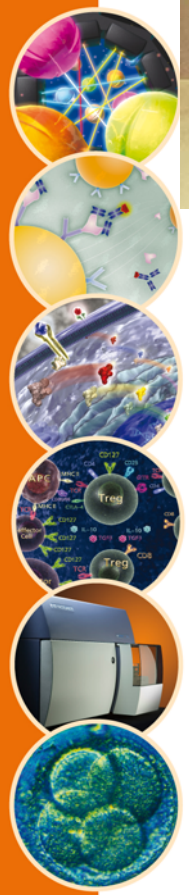






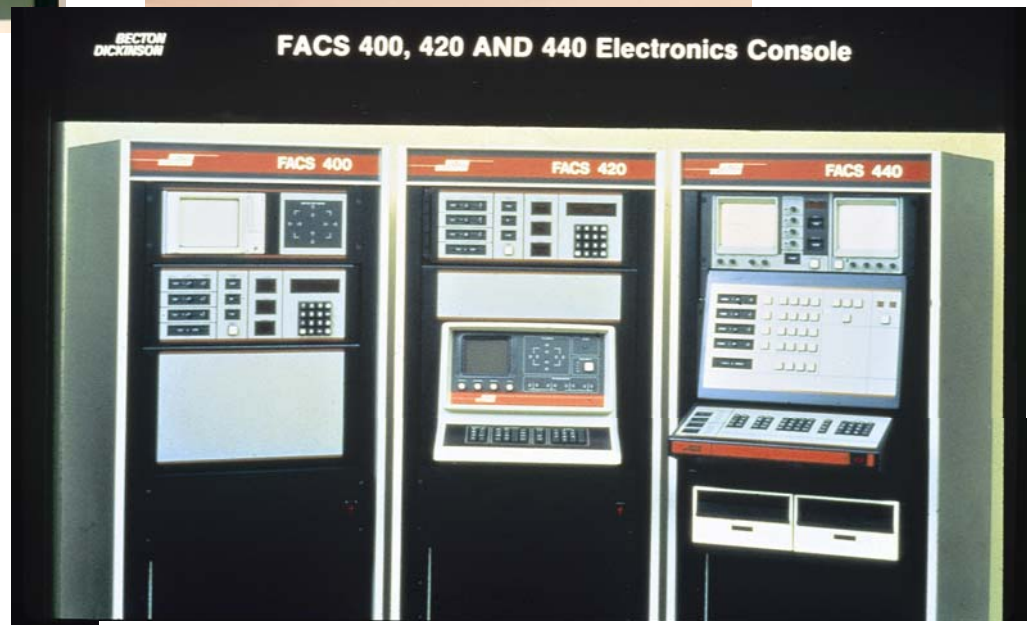
‘FACS’

How the ‘FACS systems’ have changed over time:



The ‘consoles’

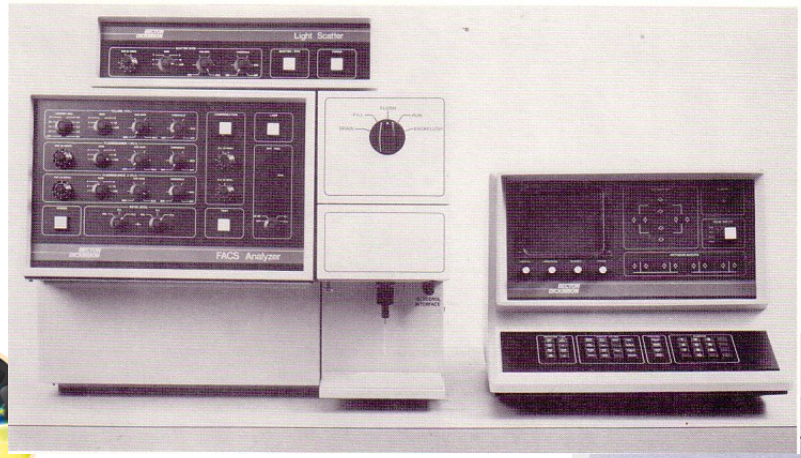
1976 - 1982



Lesson: Reduced capabilities can lead to poor sales.

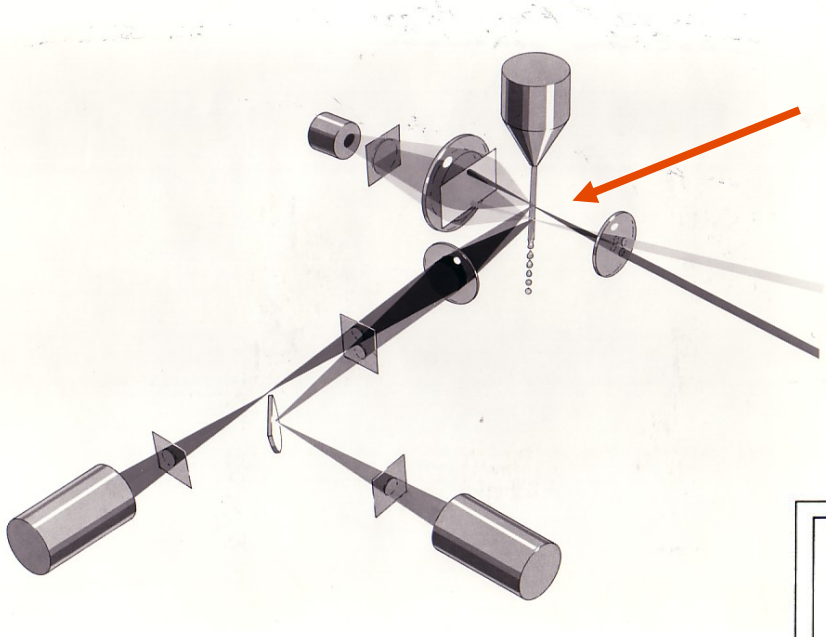
The workstations (1985 – 2007):

The Analyzer Generations:



Lesson: Filling a gap sometimes is a trap !

Notes on Optics (1):

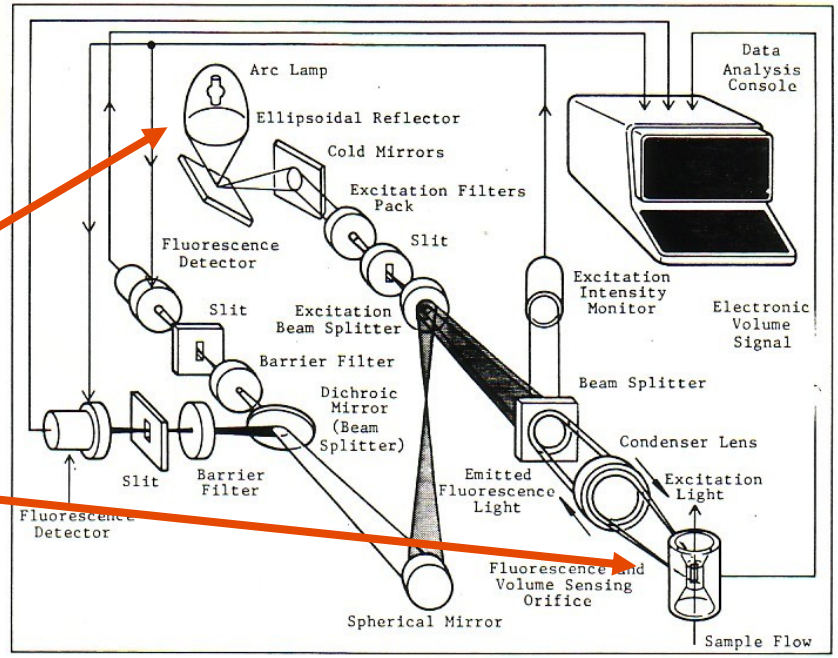


Key decision 1:

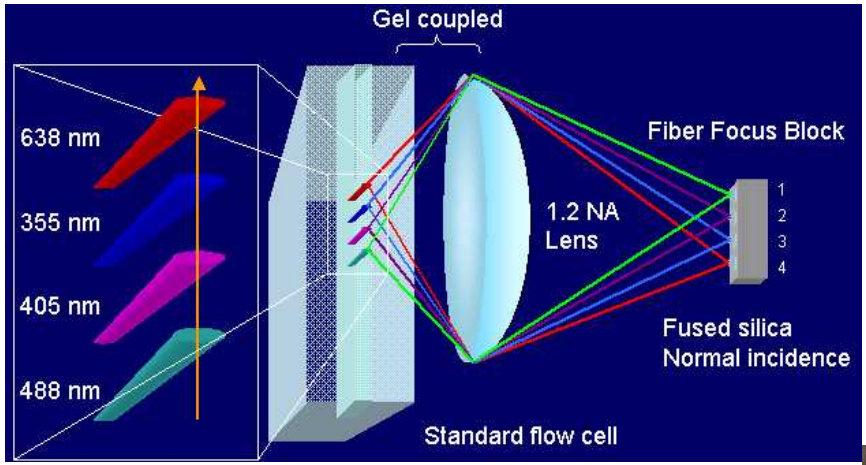
"the space – and time – separated beams" – at the basis of the sensitivity issue.

Key decision 2:

How a poor decision leads to a series of very important developments in this technology.



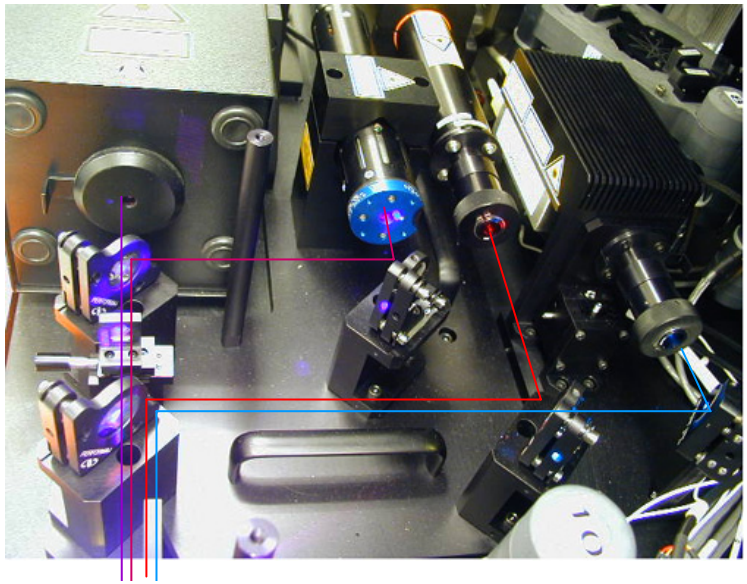
Notes on Optics (2):

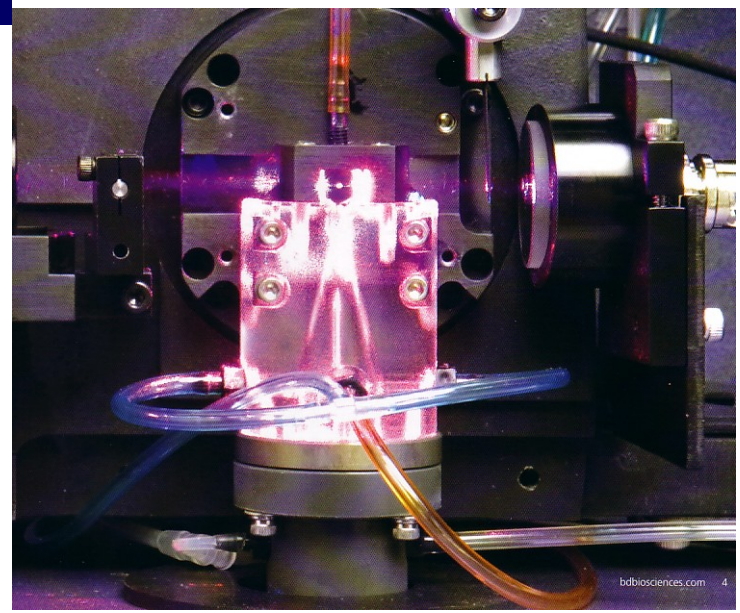


Time-space separation:

- Reduced background noise
- Electronic support
- Integrated cuvette / lens
- Frame stability

➢*Maximized sensitivity*

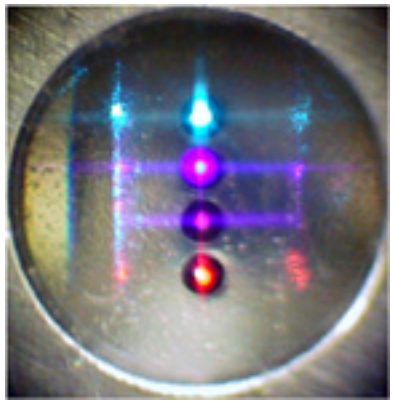


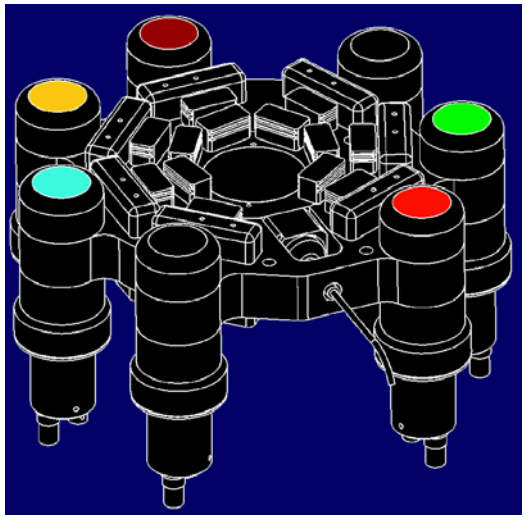


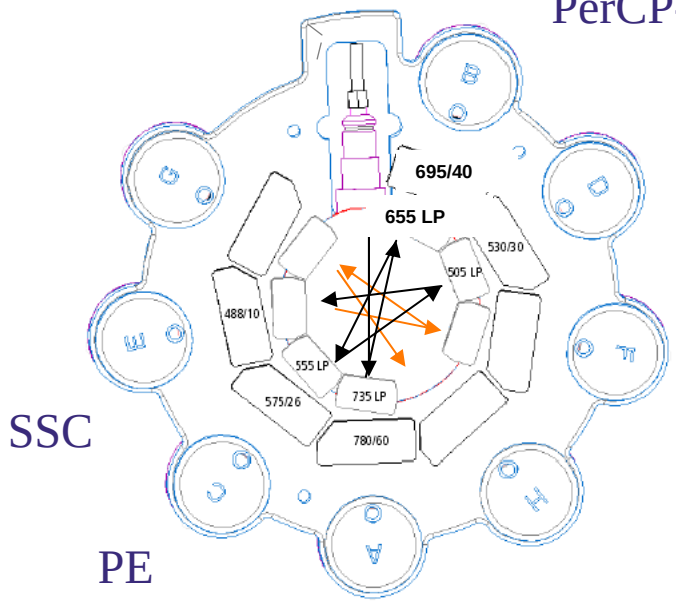
Notes on Optics (3):

Photon Collection:
- Integrated collector
- Pinholes reduce noise
- New photon travel

➢*Maximized Sensitivity*





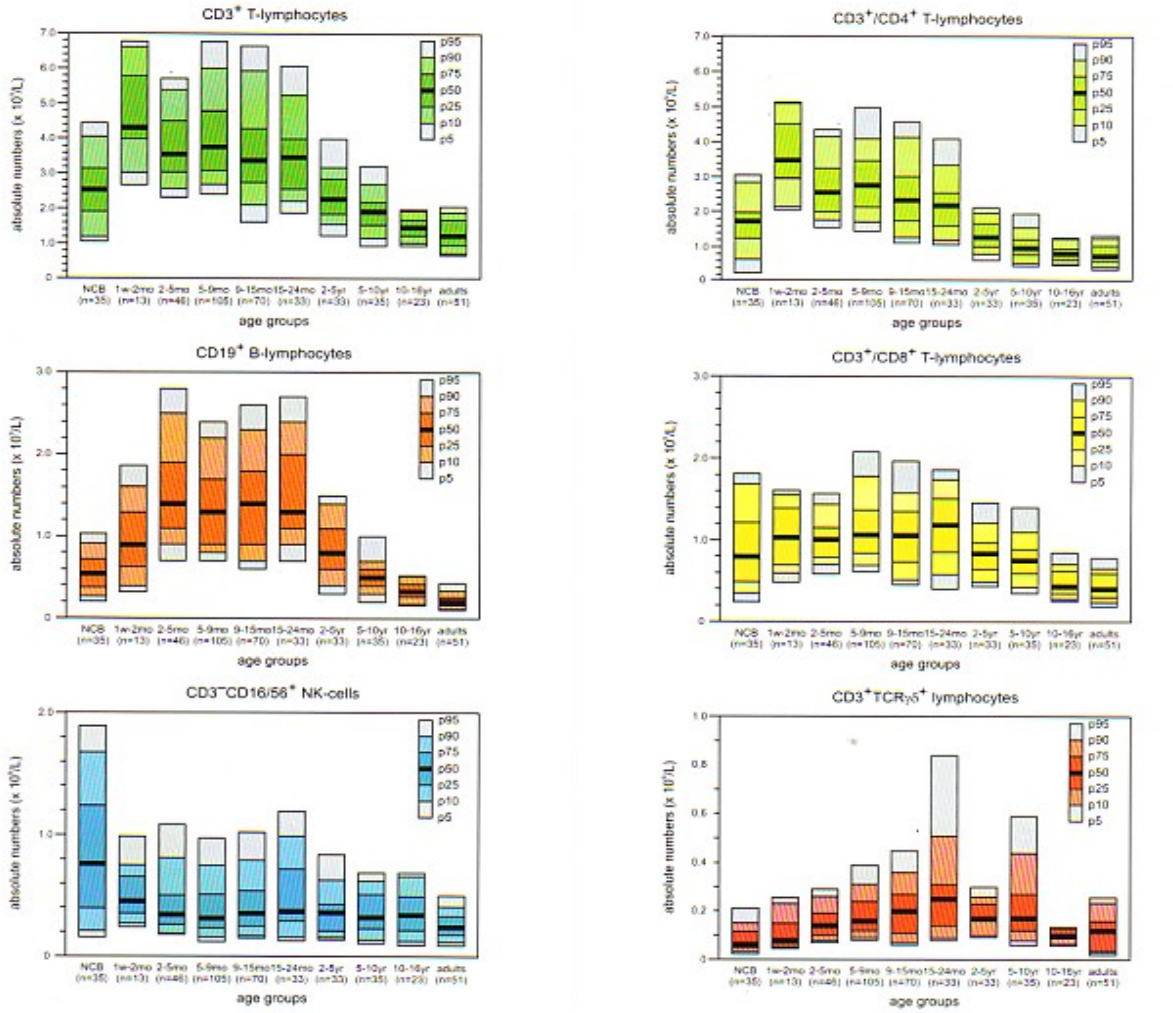


Performance Conclusions:

- Recognized reputation for quality
- Lab-to-lab comparison
- Attracts pioneers in new fields
- Reputation upheld by support (Technical & Applications)

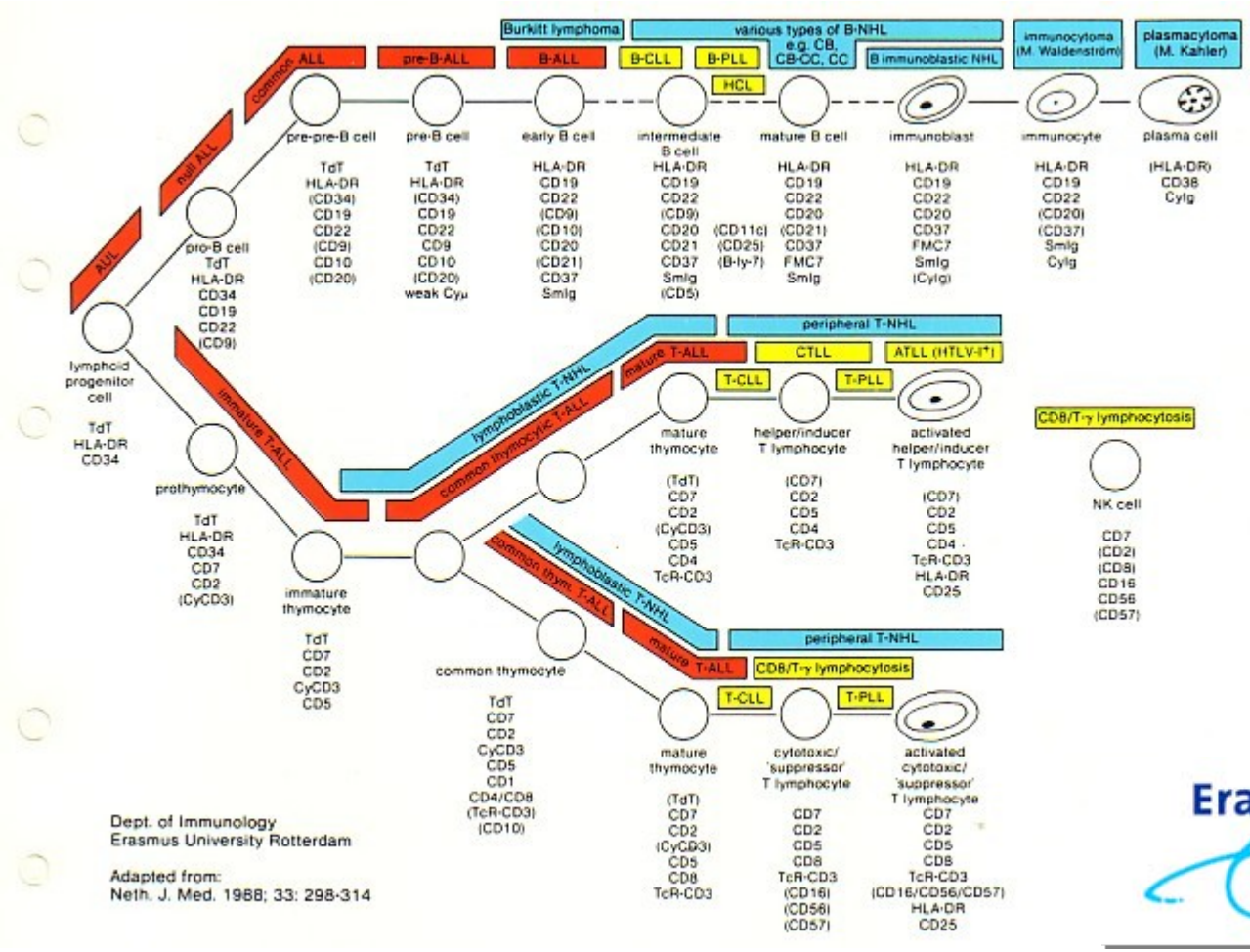
- **Comparable performance** all systems
- Maximized sensitivity across systems
- Supported by **reagent quality levels**

Normal Values Determination:



. . . standardization allowed to assemble Normal Value charts that are generally used in clinical laboratories.

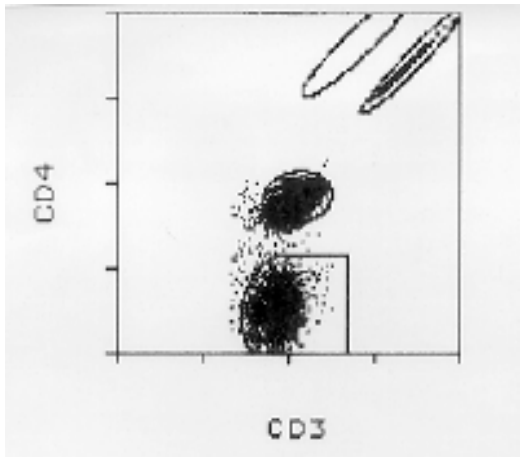
Construction of development paths:



Development charts created the basis that allowed better descriptions of disease states through phenotyping . . .

Area 1 of significant benefit:

- Diagnosis of AIDS took a direct benefit of the technology development. BD was able to set the standard via automated analysis . . .



FACSCount SW Version 1.5 4/05

Date : 9/20/07 10:28

Control output
Lab Normal ID : 20092007001809
Absolute Counts - cells/ul

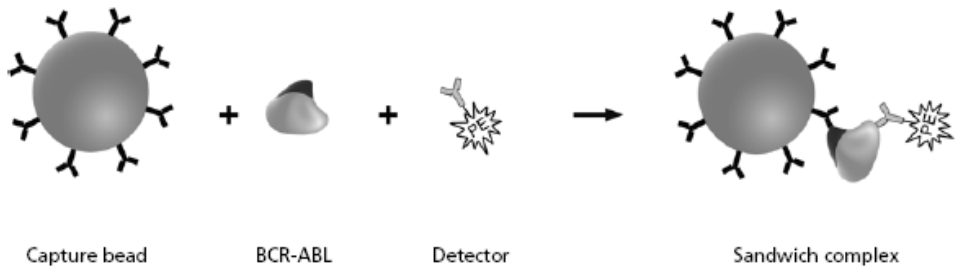
CD4	:	1166
CD8	:	706
Total CD3 (CD4)	:	1899
Total CD3 (CD8)	:	1859
Total CD3 Avg	:	1879
CD4/CD8	:	1.65
CD4/CD3	:	0.62
CD8/CD3	:	0.38

  - Presently correcting setup to implement the pediatric %CD4 signal.

Lesson: Science evolves, diagnostic capabilities need to follow.

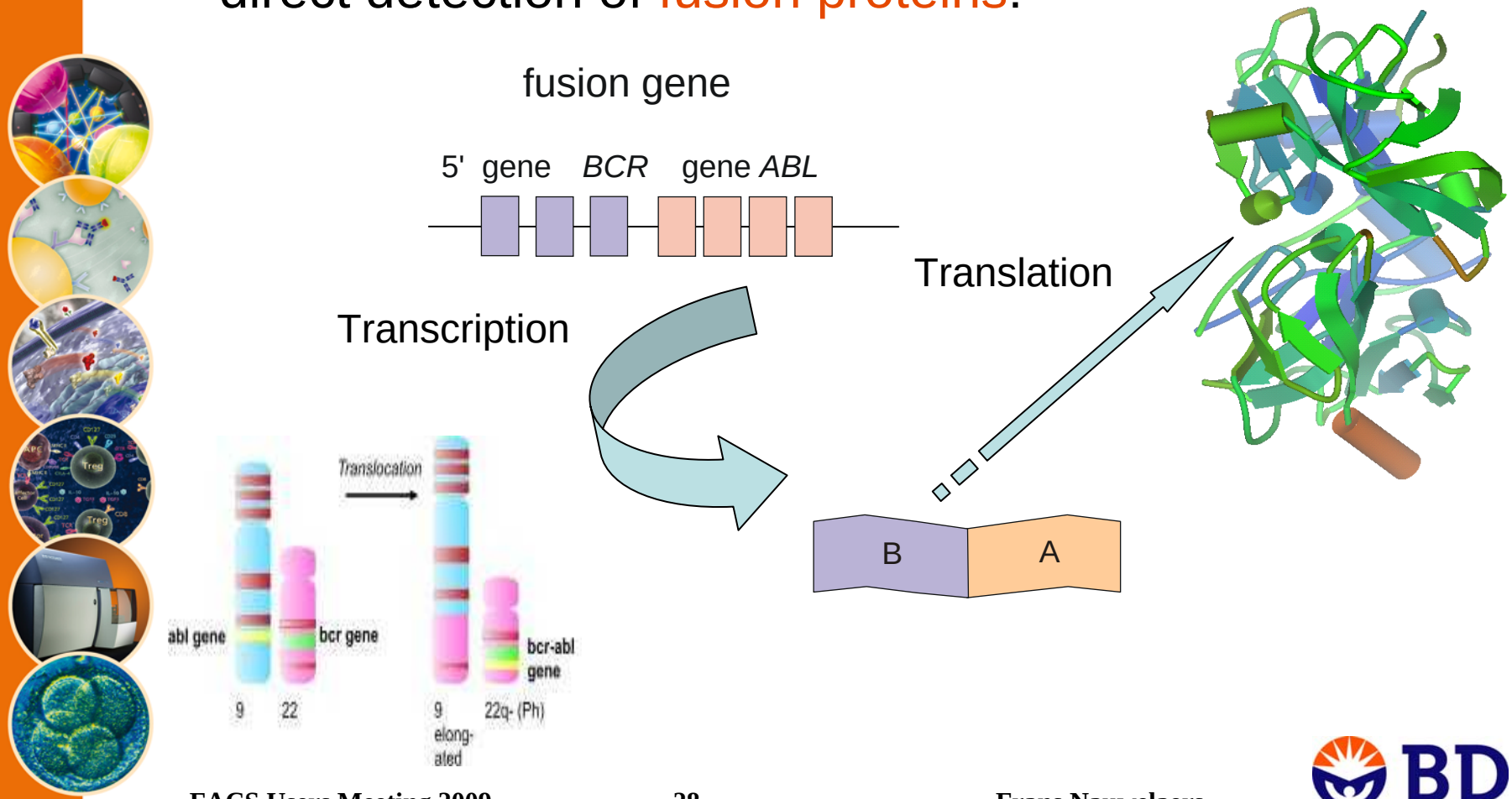
Area 2 of significant benefit:

Diagnosis of leukemia received a major benefit through a systematic improvement of immuno-phenotyping.
The current 6- and 8-color panels can lead to an easier definition in the therapy/MRD phase.
This can be complemented with the development of the fusion protein detection:

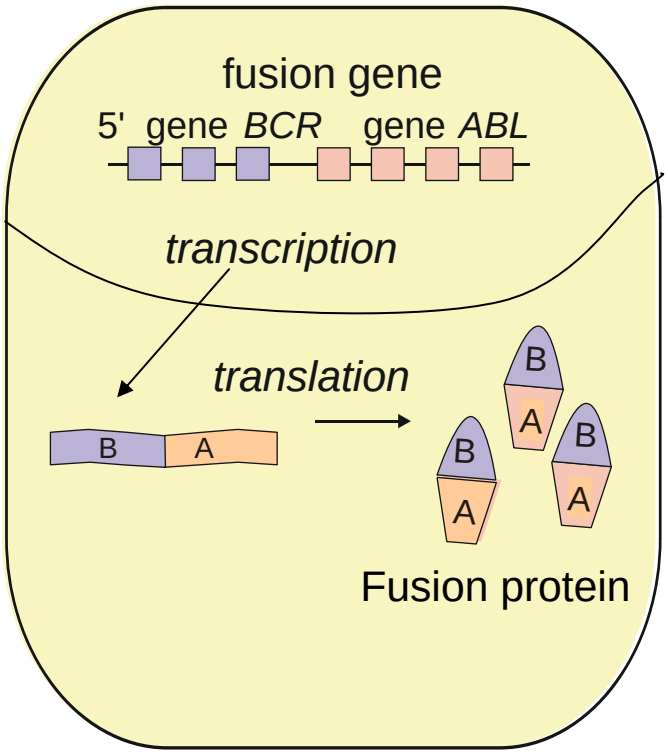


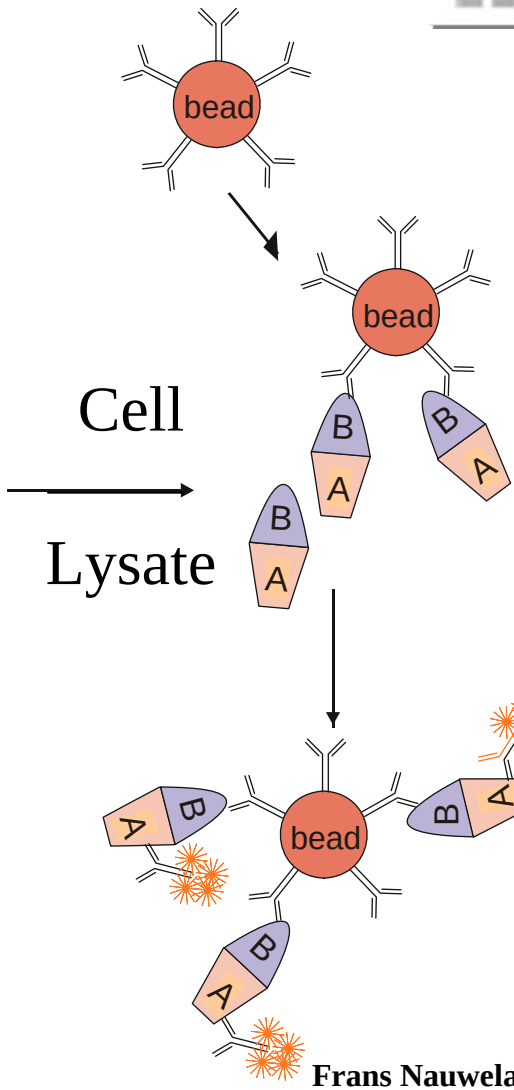
Area 2 of significant benefit:

Improving the diagnosis of leukemia through the direct detection of fusion proteins:



Development of Fusion Protein Assays in Partnership with dynomics





BCR ANTIBODY CONJUGATED CBA BEAD

CAPTURE OF FUSION PROTEIN

DETECTION WITH PE-ABL ANTIBODY

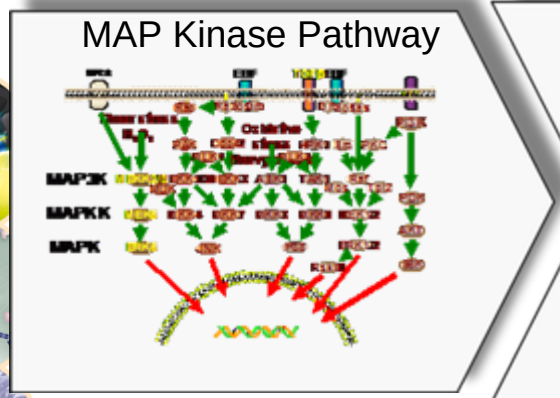
Patents:

US 6,610,498 B1 (26 August 2003)
US 6,686,165 B2 (3 February 2004)

BD

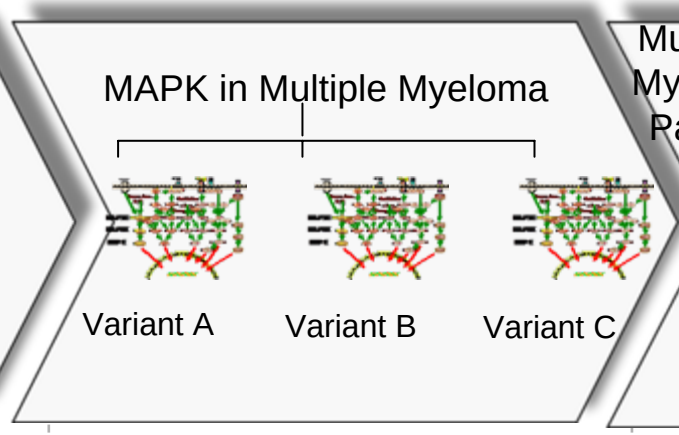
In testing of new drugs, Phospho-proteins can help enabling “personalized medicine”.

Today



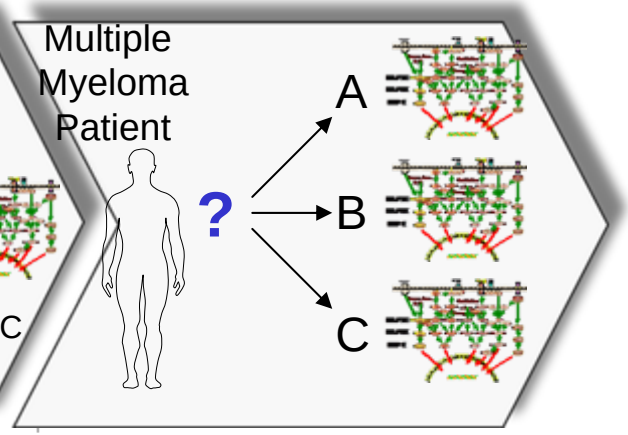
Mapping complex signaling pathways drive cellular response to stimuli (e.g. uncontrolled growth & proliferation)

In 3-5 Years

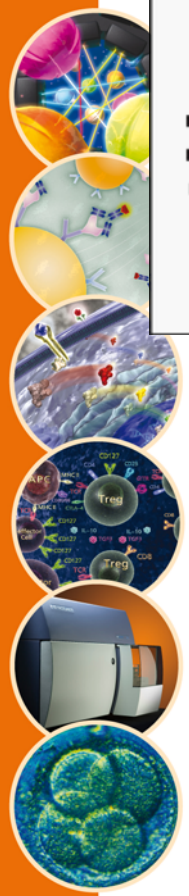


Understanding signal pathway variations by disease state (e.g. types of cancer) and among patient subpopulations within a given disease

In 4-8 Years



Detecting the signaling pathway variants responsible for propagating each patient’s disease and optimizing a therapeutic response based on this determination

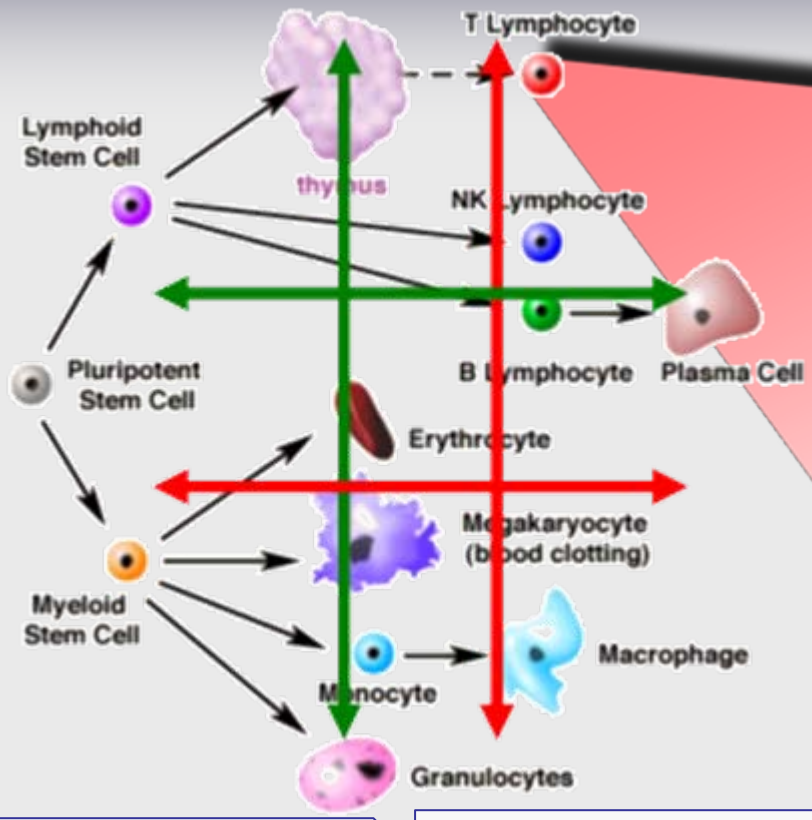


BD

Phospho-proteins open a window on the internal workings of cells.

Intercellular communication

Cytokines, Chemokines, Growth Factors



Antigen Detection

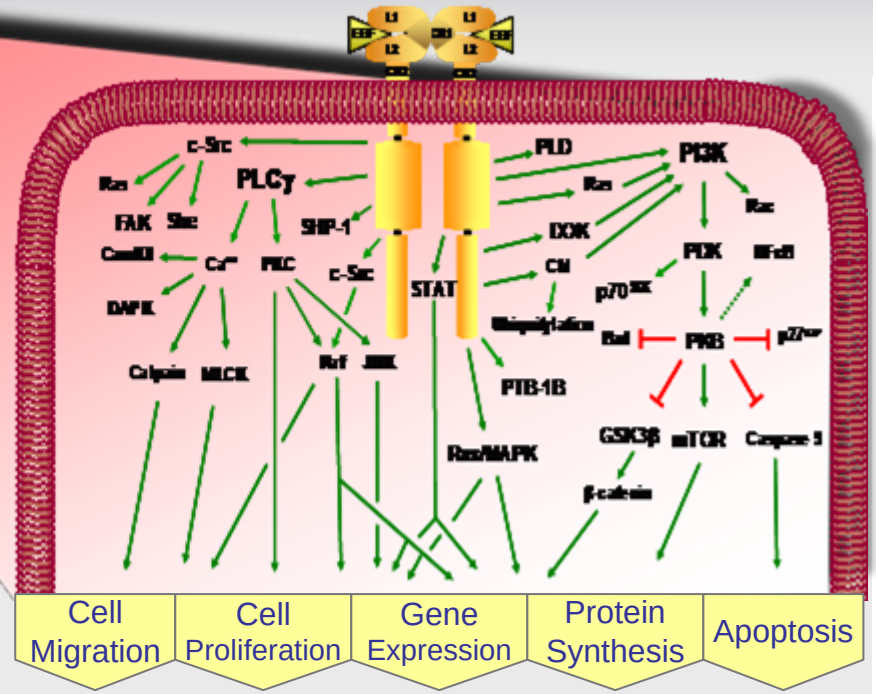
Immune Function

Quantitation

Immunophenotyping

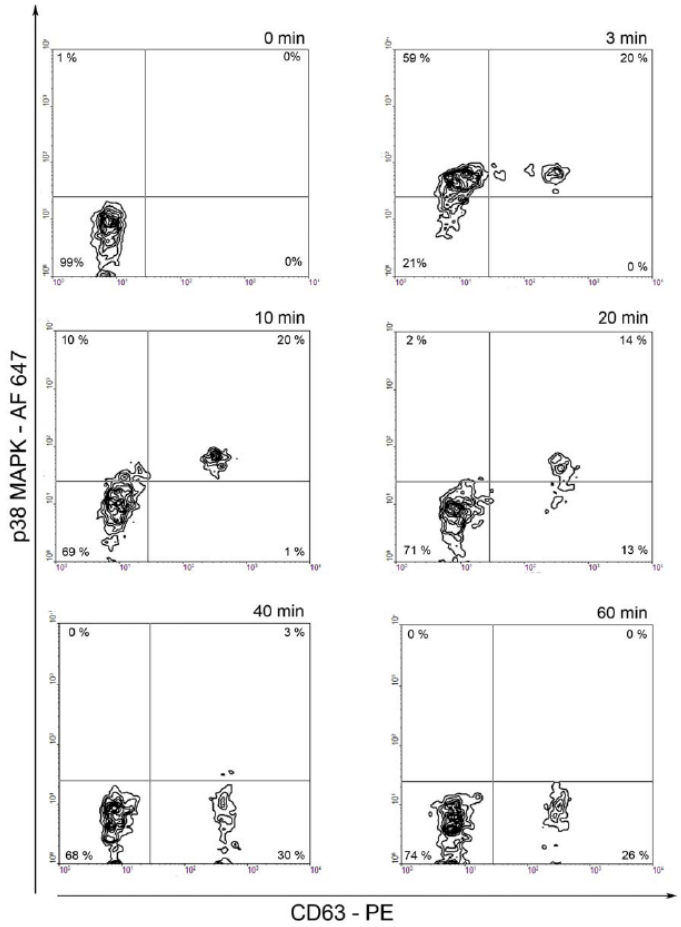
Intracellular communication

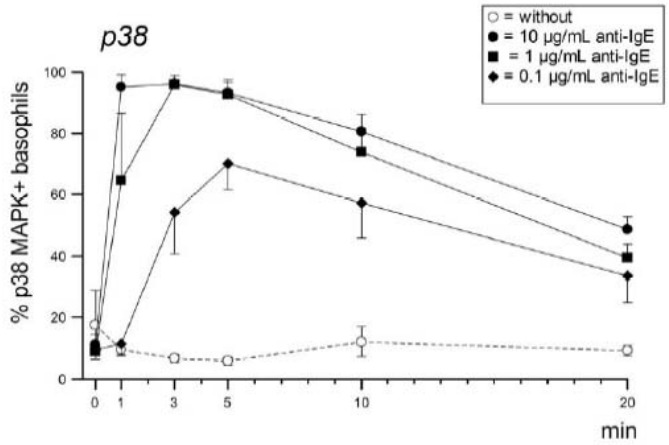
Kinases & Phosphatases

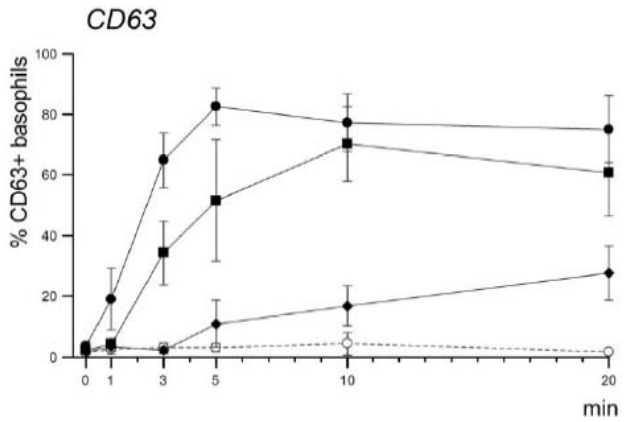


Cell Signal Transduction

A new barrier broken in Allergy?







Revealing of intracellular phospho-proteins showing difference in kinetics between ‘cell activation’ and ‘cellular response’ (Univ. Antwerp)

How about a non-leukemia cancer option?

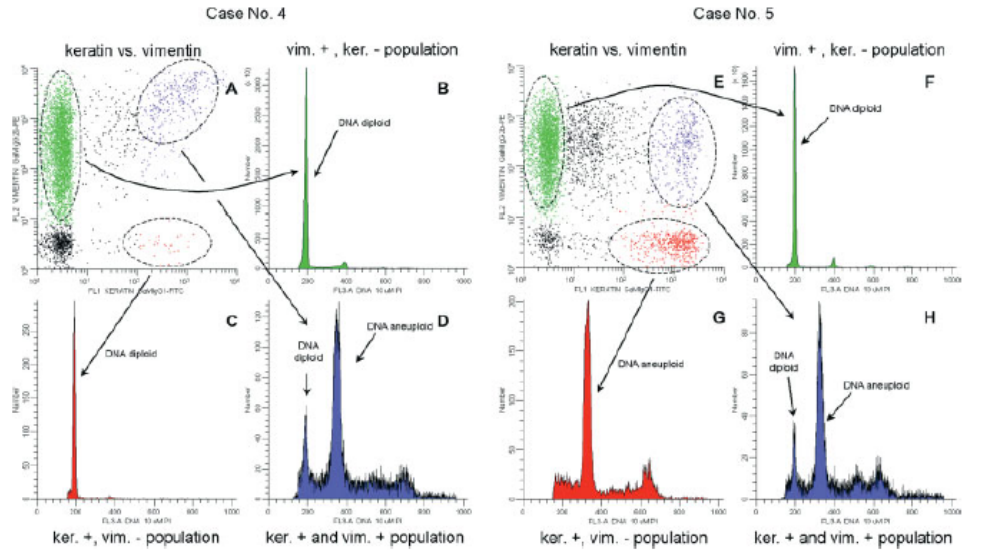


Figure 3. Keratin/vimentin co-expression in two cervical carcinomas. (A, E) Keratin expression (abscissa) versus vimentin expression (ordinate). Note: K + V+ double-positive cells (purple). (B, F) Gating on the V + K− cell populations (green) shows unimodal DNA histograms (as in Figures 2B, 2F and 2J). (C, G) The K + V− cells (red) represent a DNA diploid (C) or a DNA aneuploid population (G). (D, H) K + V+ cell populations comprise both a DNA diploid and a DNA aneuploid population

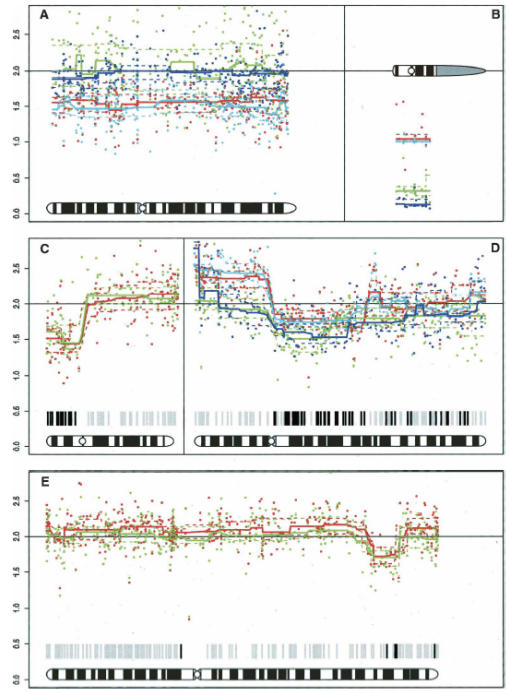


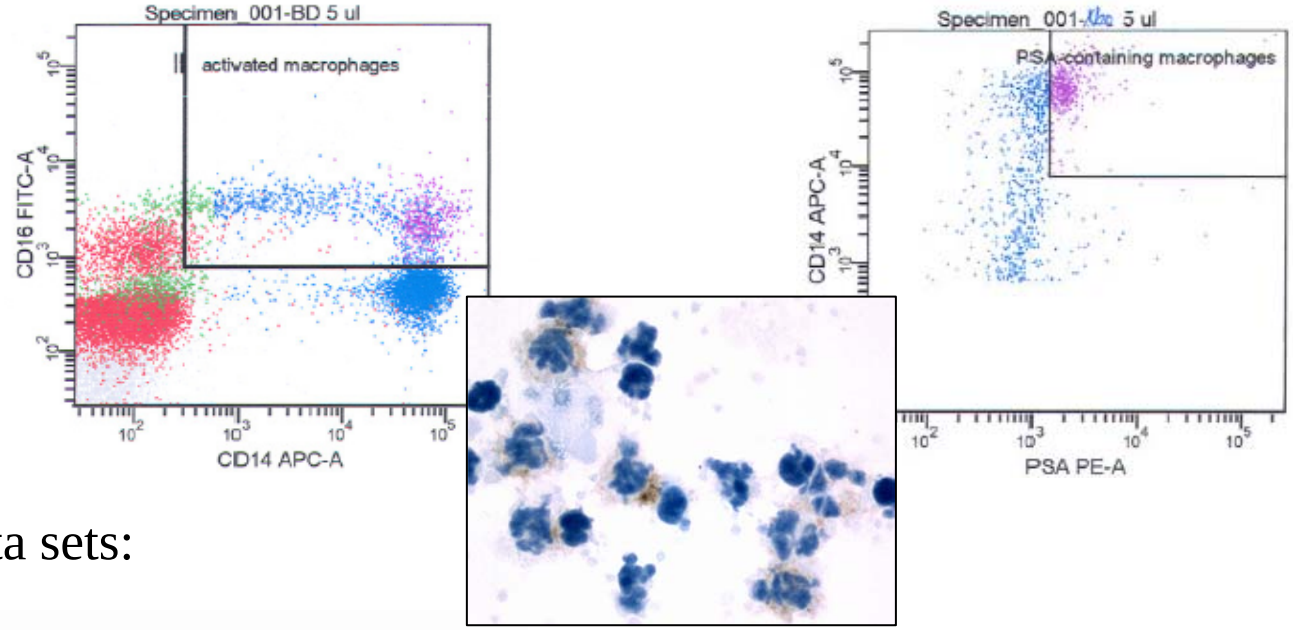
Figure 4. Chromosomal plots from BeadArrays. Each panel depicts the smoothed copy number as a continuous line and the 10th and 90th percentiles as dashed lines. The unsmoothed copy number values are shown as dots. (*A,B*) X- and Y-chromosome from leukocyte DNA. Red, 44 male; blue, 106 female; cyan, 108 male; green, 514 female. (*C–E*) Comparisons of copy numbers. The green lines depict FFPE tumor tissue, and the red lines depict fresh frozen tumor tissue. Bars below the plot indicate heterozygous SNPs in the corresponding normal sample. At black bars, the SNP has switched to homozygosity in the tumor. Physical loss is called when the upper percentile line drops below 2; gain is called when the lower percentile line exceeds 2. (*C*) Chromosome 17 in tumor 106. (*D*) Chromosome 5 in tumor 44. Blue line, FFPE BAC array; cyan line, fresh frozen BAC array. (*E*) Chromosome 2 in tumor 514.

With cell sorting as unique support:

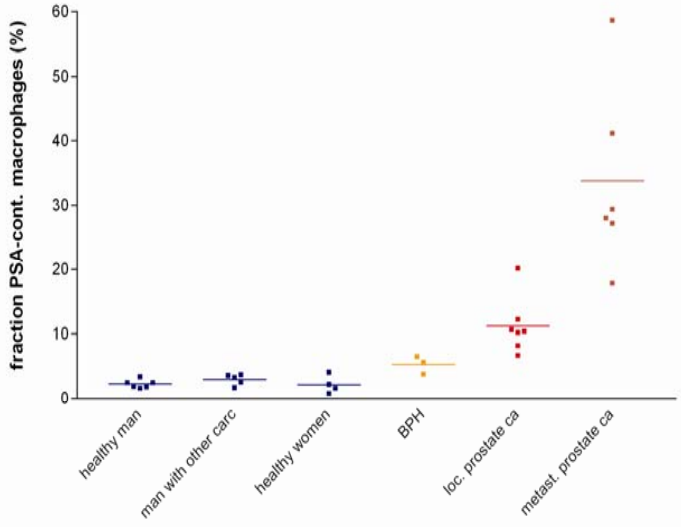
- solid tumors can be treated and cells recovered at 90% efficiency.
- cancer and normal (internal control) cells can be sorted from same patient sample
- highly purified populations can be used for molecular work and thus much better gene analysis (Univ. Leiden)

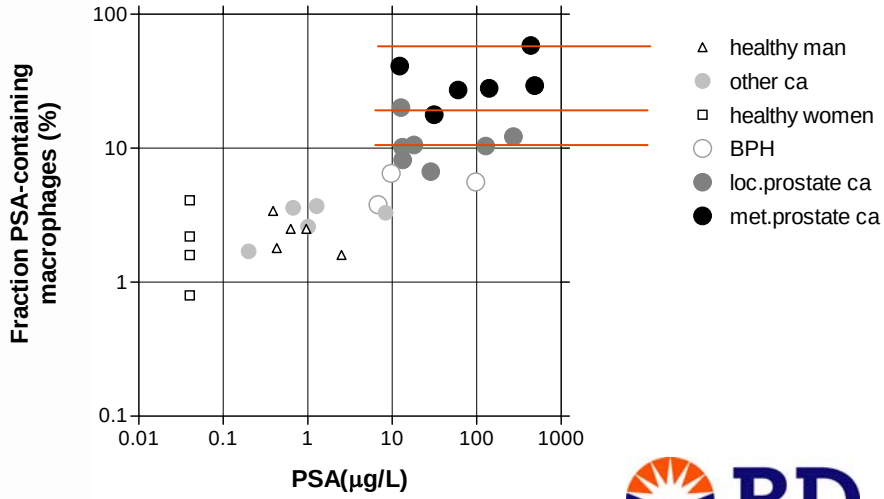
A Prostate Cancer sPSA-reflex Assay?

Flow cytometry data:
(Atrium MC, The Netherlands)

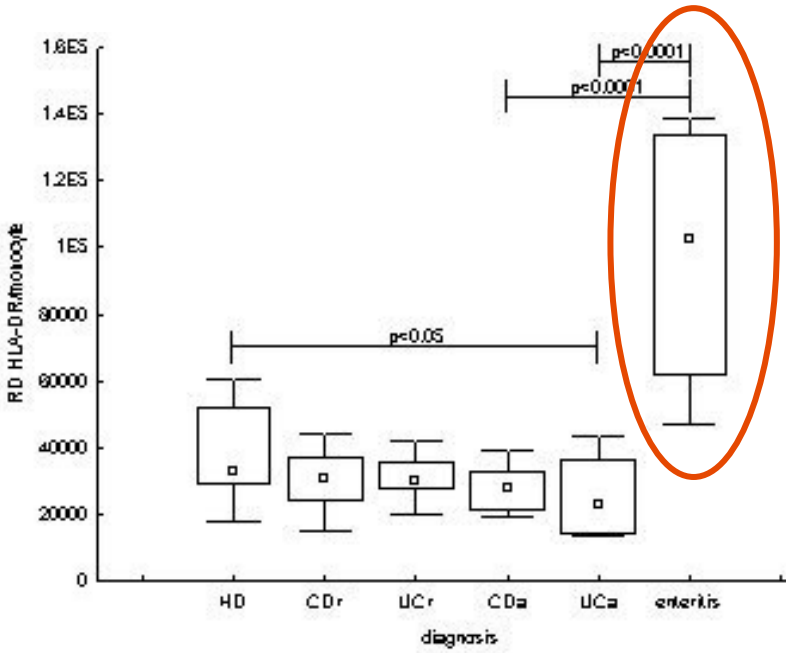


Clinical data sets:

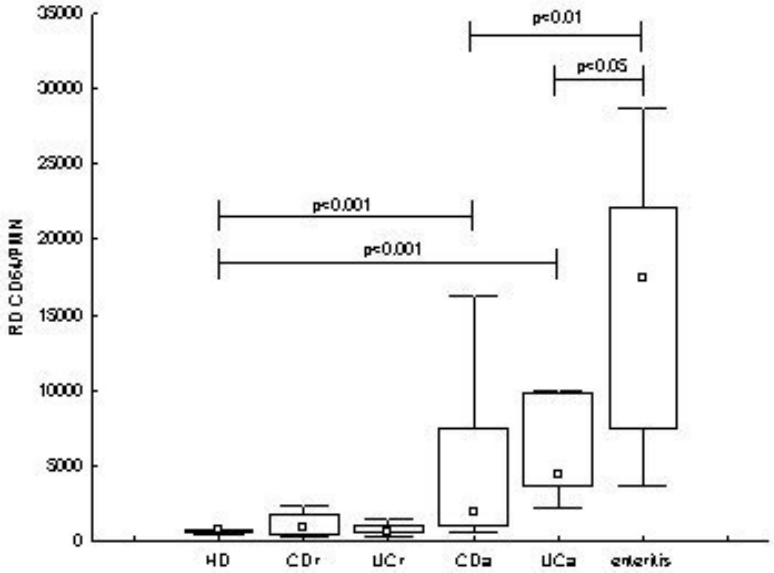




Help with a Crohn's Disease Assay (IBD)?



Monocyte selection and HLA-Dr expression



Granulocytes and CD64 expression
(quantitative determination / BD QuantiBrite-PE)

Figure: Clinical Data: Bacterial Enteritis vs. Crohn's Disease and Ulceritive Colitis (active) vs. IBD (in remission)

(Hietzing Hospital, Vienna)

How about tomorrow?

- There is a vast benefit of a cooperation between academia and industry. The EU promotes this, and we have seen the benefit in our multicolor application in the Leukemia field. As a great example:

EuroFlow

ERIC

- There is a definite need to further simplify the intracellular staining procedure to better work with the phospho-proteins under a multicolor setup, so as to diagnose the pathway.

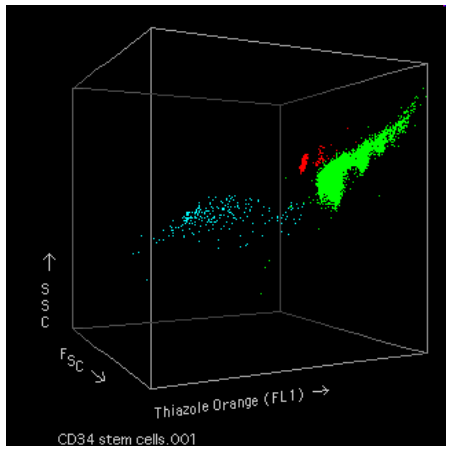
- At the same time, there's a need for the analysis of activation status of the cell combined with the quantification of the release factors (cytokines, chemokines).

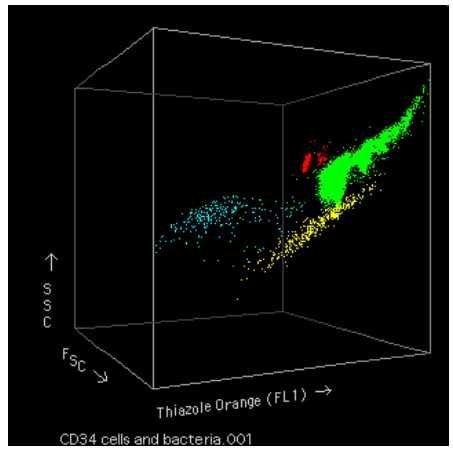
- The most flexible approach for this combined measurement is the utilization of the CBA assay with the Cellular Analysis:

BD

How about tomorrow?

- There is a definite need to sort larger particles: e.g. plant cells, egg cells, megakaryocytes.
- At the same time, there's a need for the analysis and sorting of the ever smaller: e.g. microsomes in blood





Bacteria contaminating cellular preparations

- The most flexible answer today is:

the 'Influx' system – partially looping back to the original design concept: let's make what researchers need and observe what can be done with the possibilities that are offered.

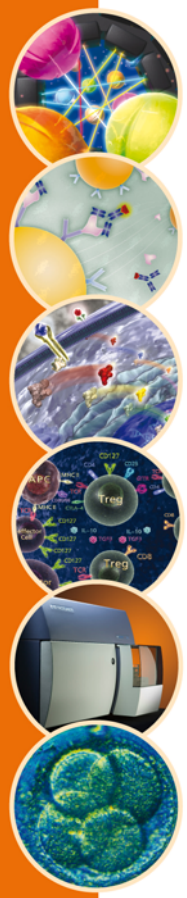
The conclusion for this field:

- Way back in 1987 in a profiling session, Ed Ludwig asked the question: ‘How big is this flow market?’ – We had to say we didn’t know. He said he’d be satisfied with an order of magnitude : -)

- In 2007, he asked the question again: ‘Have we figured out how big the flow market is?’.

- The most reasonable answer we came up with still is:

‘Most likely as big as our imagination.’



Thank you

and

a safe journey home