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PEARLS OF LABORATORY MEDICINE

Cell Sorting using Flow Cytometry

Michael Timm, BA

Development Coordinator, Department of Laboratory Medicine and Pathology, Mayo Clinic

10.15428/CCTC.2019.307215

01/02/2019

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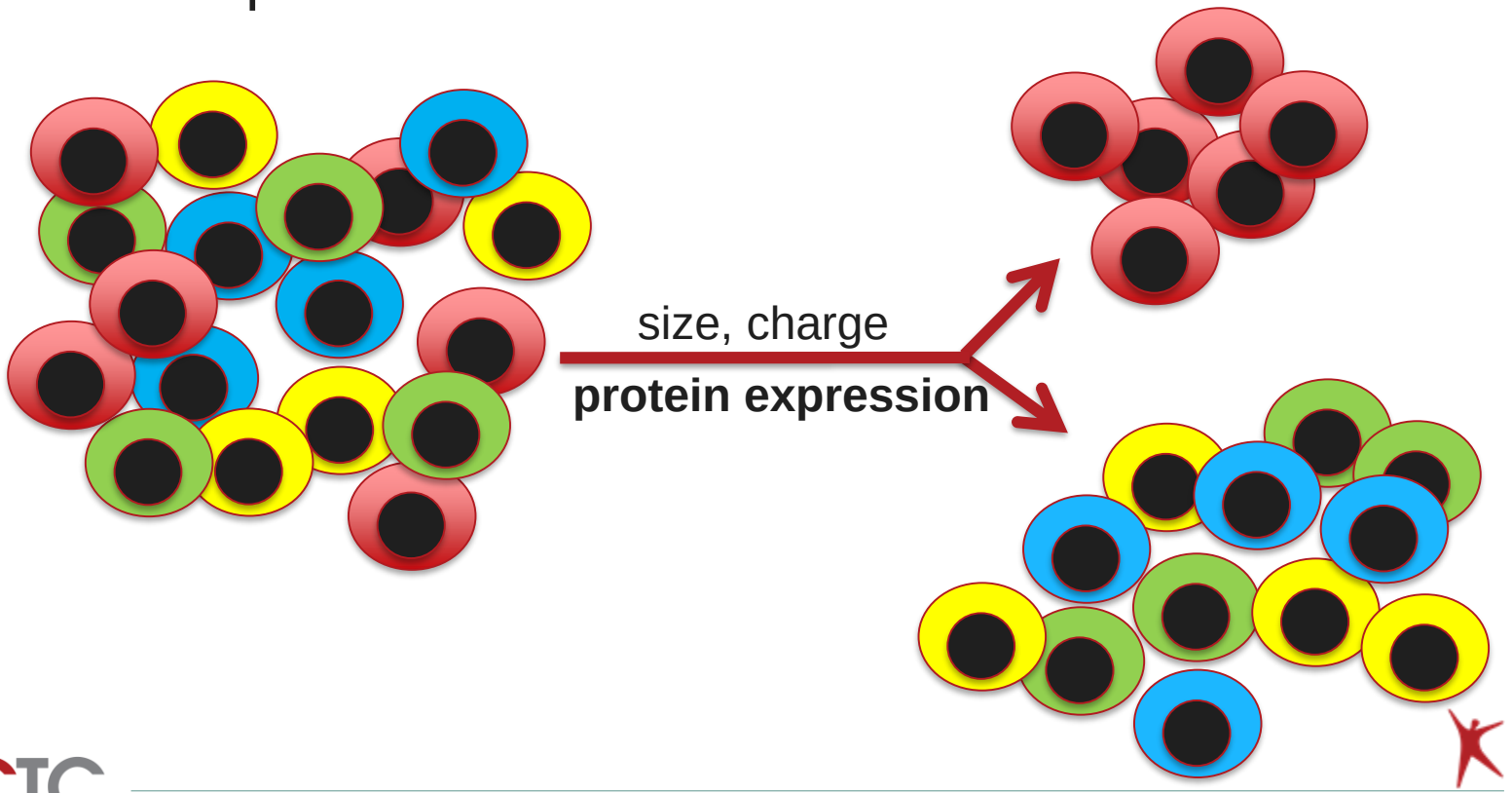
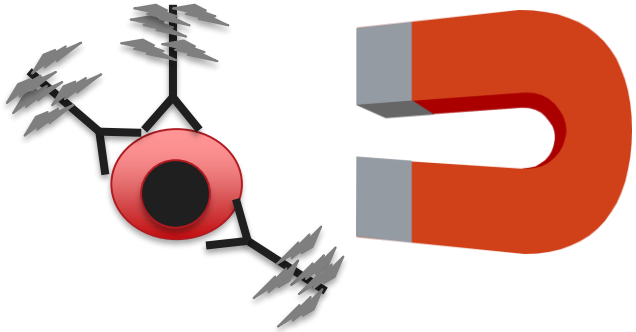
What is Cell Sorting?

- A process of physically separating a cell population in a suspension from the rest

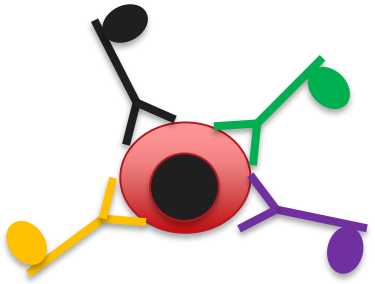


Types of Cell Sorting in the Routine Use Today

- **Magnetic Activated Cells Sorting (MACS)**
  - Magnetic nanoparticles bound to the antibody
  - BULK = all cells collected at once (fast and great for large quantities)
  - Only one cell characteristic used



- **Fluorescence Activated Cell Sorting (FACS)**
  - Fluorescent dyes bound to different antibodies
  - SINGLE CELL = each cell separately analyzed
  - Multiple characteristics used
  - High specificity and purity



History of Cell Sorting

Late 1800s

- Lord Rayleighs principle of droplet formation from a stream of liquid

Mid-1960's

- Sweet develops first INK JET at Stanford University
- Fulwyler at Los Alamos National Laboratory first successful sort of cells based cell volume

Late 1960's

- Len Herzenberg coined the commonly used term FACS – Fluorescence Activated Cell Sorter

Early 1970s

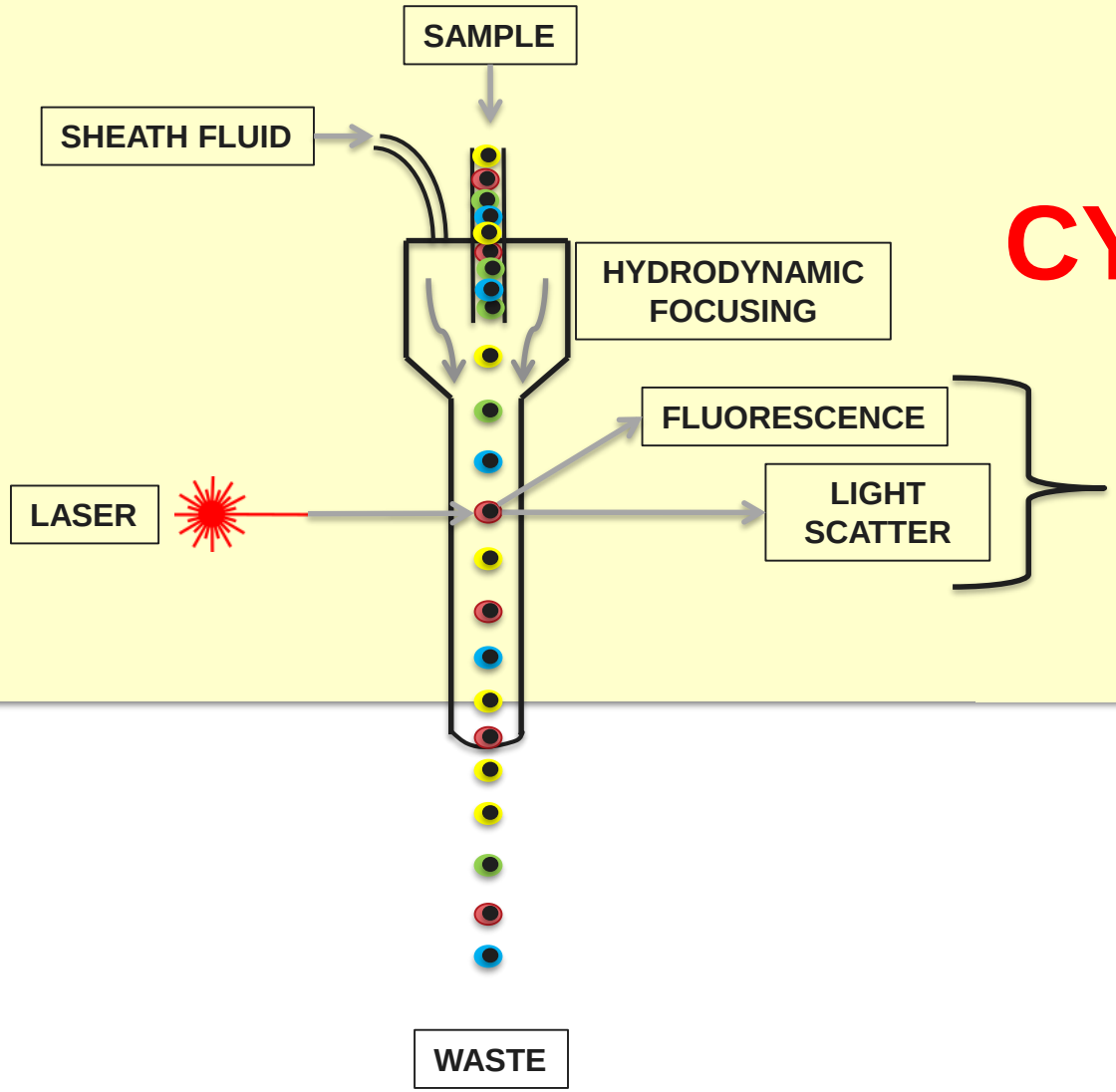
- Becton Dickinson launched first commercially available cell sorter the FACS-1. BD still owns the trademark for FACS to this day

FACS vs. Flow Cytometry

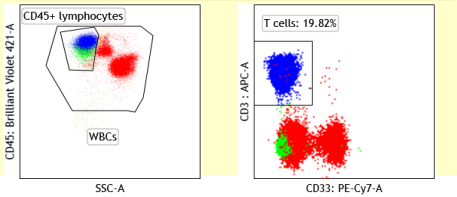
- All the principles of flow cytometry immunophenotyping apply to FACS
  - Fluidics
  - Optics
  - Electronics

- FACS has an additional component of cell collection, defined by gating

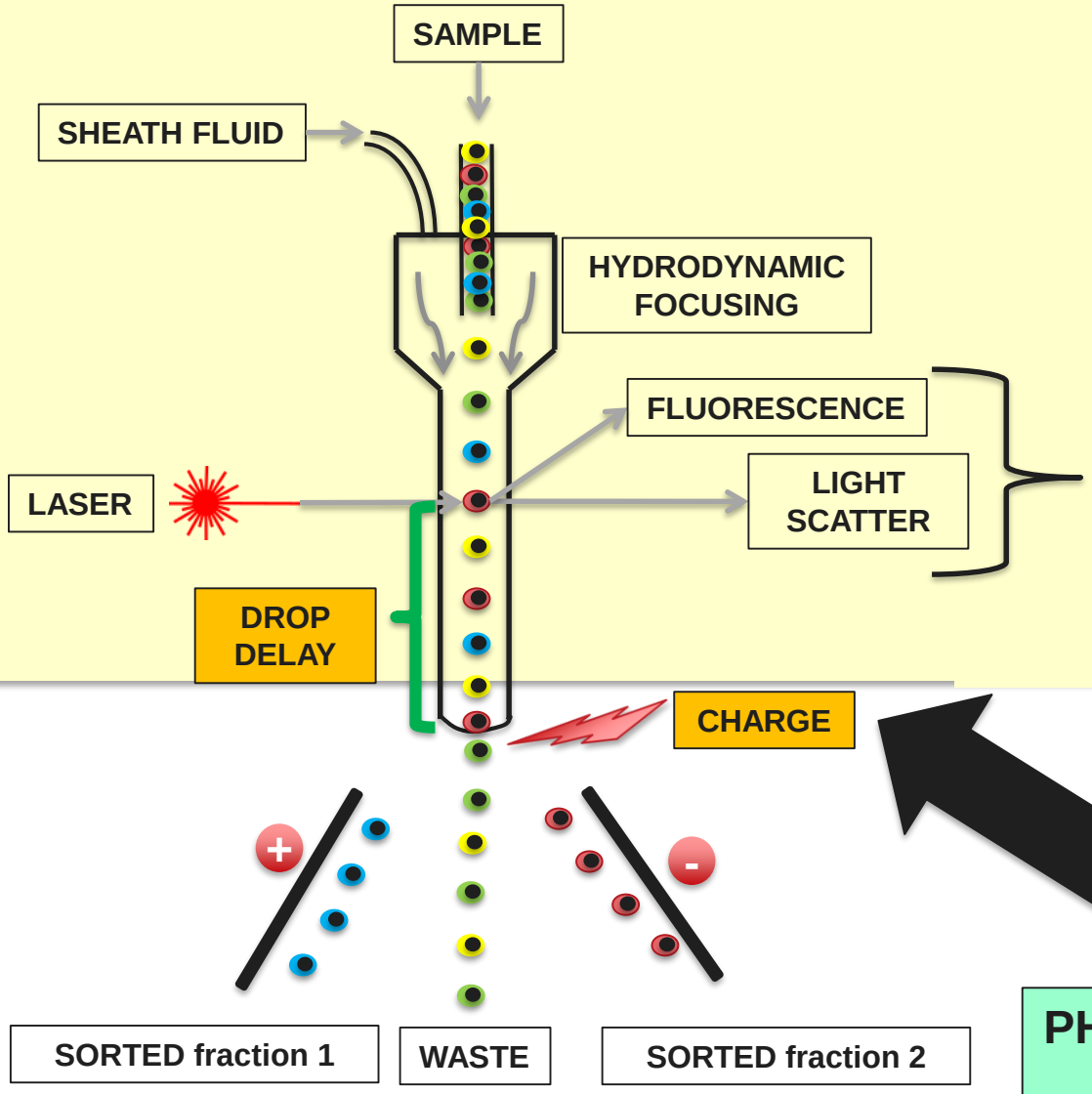
FLOW CYTOMETRY



DIGITAL OUTPUT (DOT PLOTS)



FACS



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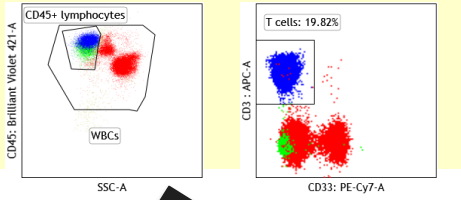


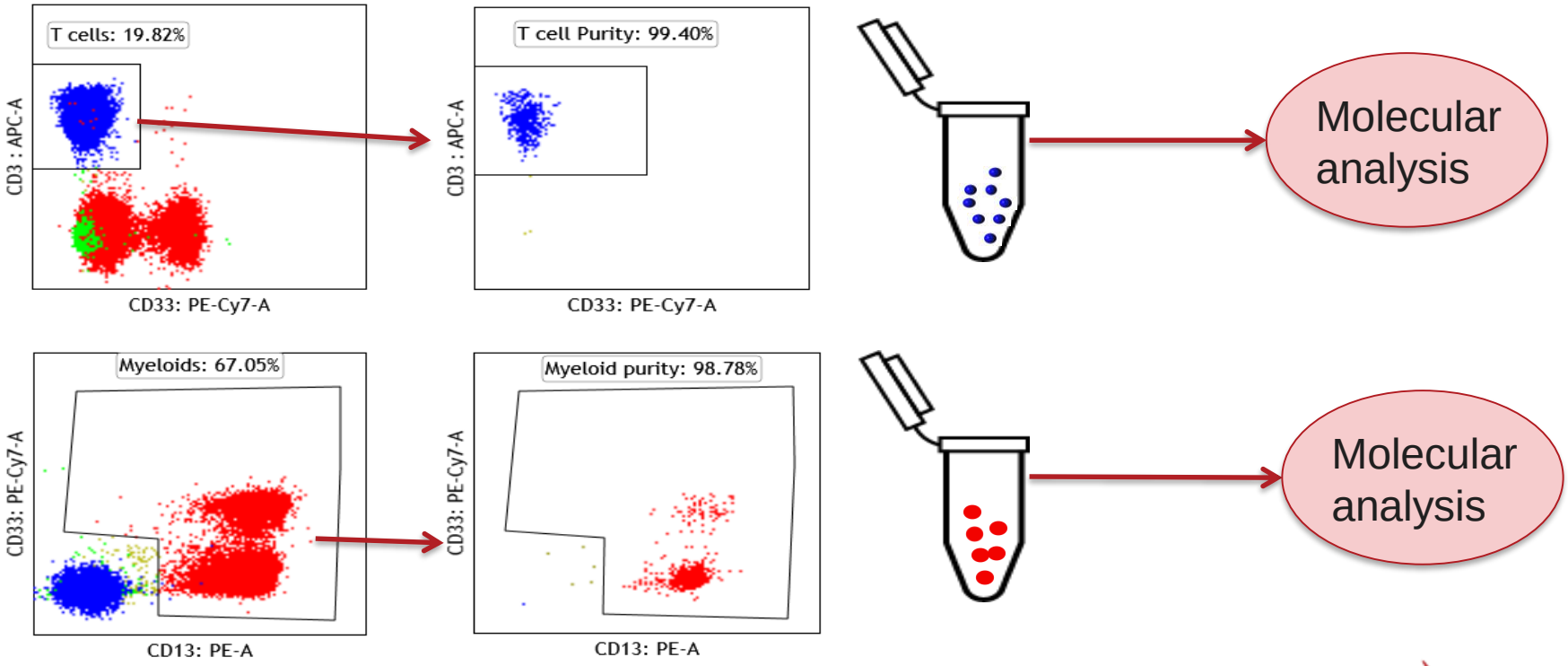
PHYSICAL OUTPUT (CELLS)

FACS Experiment

Sorting criteria

Purity assessment

Downstream assay



Applications of Cell Sorting

- Subset analysis of any lineage
- Increased sensitivity and specificity of genetic testing (PCR, FISH, NGS, microarrays…etc)
- Isolation of stem cells
- Isolation of engineered cells

- Cloning (single cell sorting)
- Protein engineering
- Drug discovery

Evolution of Cell Sorting

	EARLY FACS	MODERN FACS
INSTRUMENT SIZE	LARGE	SMALL
EASE OF USE	DIFFICULT	EASY
NUMBER OF FLUOROCHROMES	2	UP TO 9
NUMBER OF STREAMS (COLLECTION OUTPUTS)	1	UP TO 4
SPEED	SLOW	FAST
COLLECTING TUBES	LIMITED OPTIONS	MANY OPTIONS

Choosing the Right Instrument – Workflow Considerations

- COMPLEXITY - number of sort streams for recovery
  - 1 or 2 – small benchtop models
  - More than 2 – large floor models
- NEED FOR LIVE CELLS (culturing, cloning)
  - Sterile sorts
- SPACE AND COST
  - Floor models are larger and much more expensive
- AEROSOLIZATION
  - Need a hood or integrated aerosol management system

Optimizing and Troubleshooting a Sorting Assay

- Depends on the DOWNSTREAM ASSAY
  - Cell number, purity, viability, fixation
- Cell aggregation can be a problem
  - Filtering, diluting, adding EDTA or DNAse
- Instrument set up
  - PMTs and compensation
- Collection
  - May need a specific buffer, or specific collection tubes
- Flow rate is inversely proportional to the purity of the target population
- Purity vs. recovery mode

References

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4. Hrezenberg LA, Parks D, Sahaf B, Perez O et al. The history and future of the fluorescence activated cell sorter and flow cytometry: A view from Stanford. Clinical Chemistry 2002;48:1819-27
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Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

- **Employment or Leadership**: None declared
- **Consultant or Advisory Role:** None declared
- **Stock Ownership:** None declared
- **Honoraria:** None declared
- **Research Funding:** None declared
- **Expert Testimony:** None declared
- **Patents:** None declared

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