Presenter Disclosure Information

Holden Maecker

The following relationships exist related to this presentation:

BD Biosciences, Salary, Consultant

Prospects for New Clinical Flow Cytometry Assays

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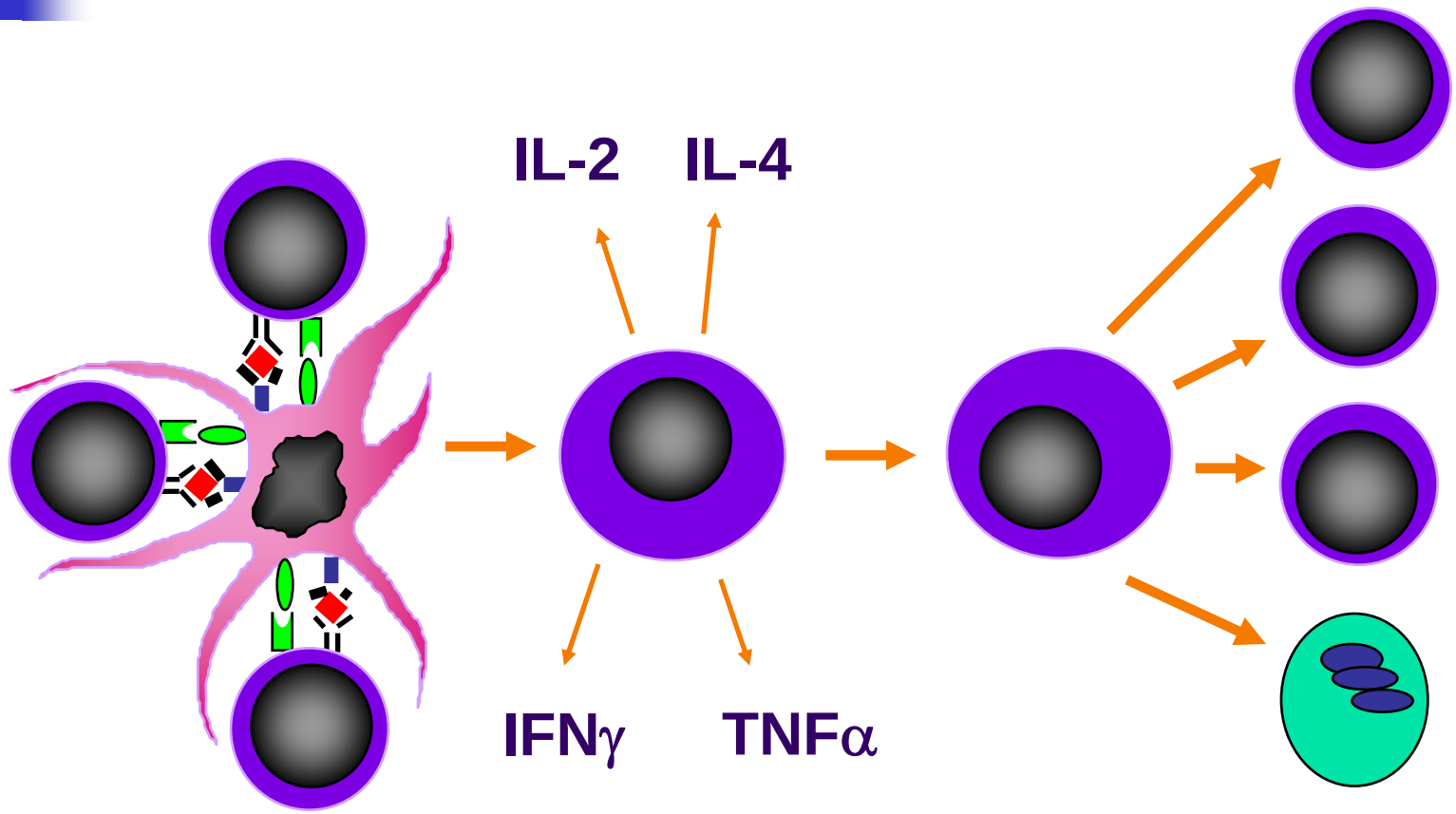
Early & Late Cellular Immunity Functions



Protein phosphorylation

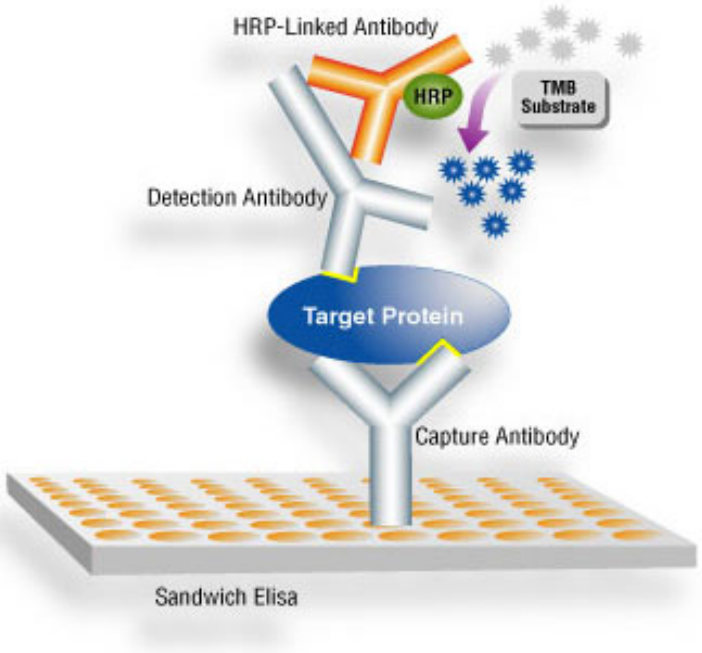
Cytokine expression

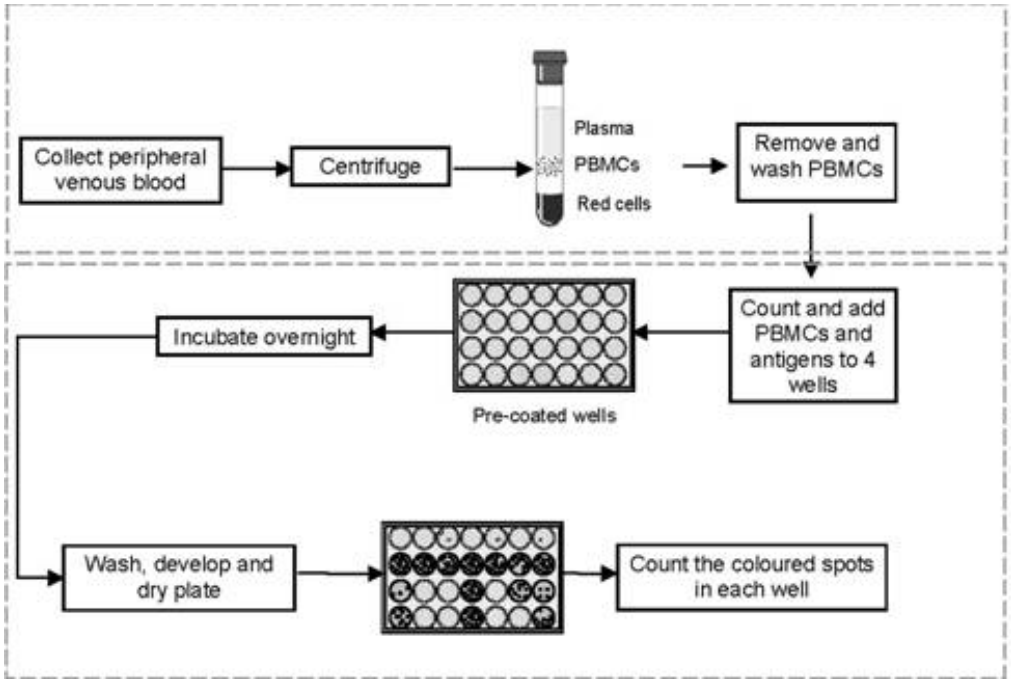
Cyto-toxicity

Proliferation/ Death

FDA-approved cellular immunity tests

- Quantiferon TB - IFNg release assay for T cell response to TB
- T-Spot TB ELISPOT test





What these tests do and don’t tell us

- Do give evidence of a cellular response to the antigen, i.e., previous exposure

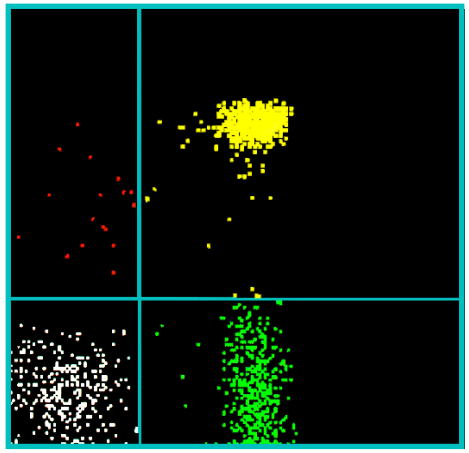
- Don’t demonstrate protection from disease
- Don’t distinguish active from latent disease

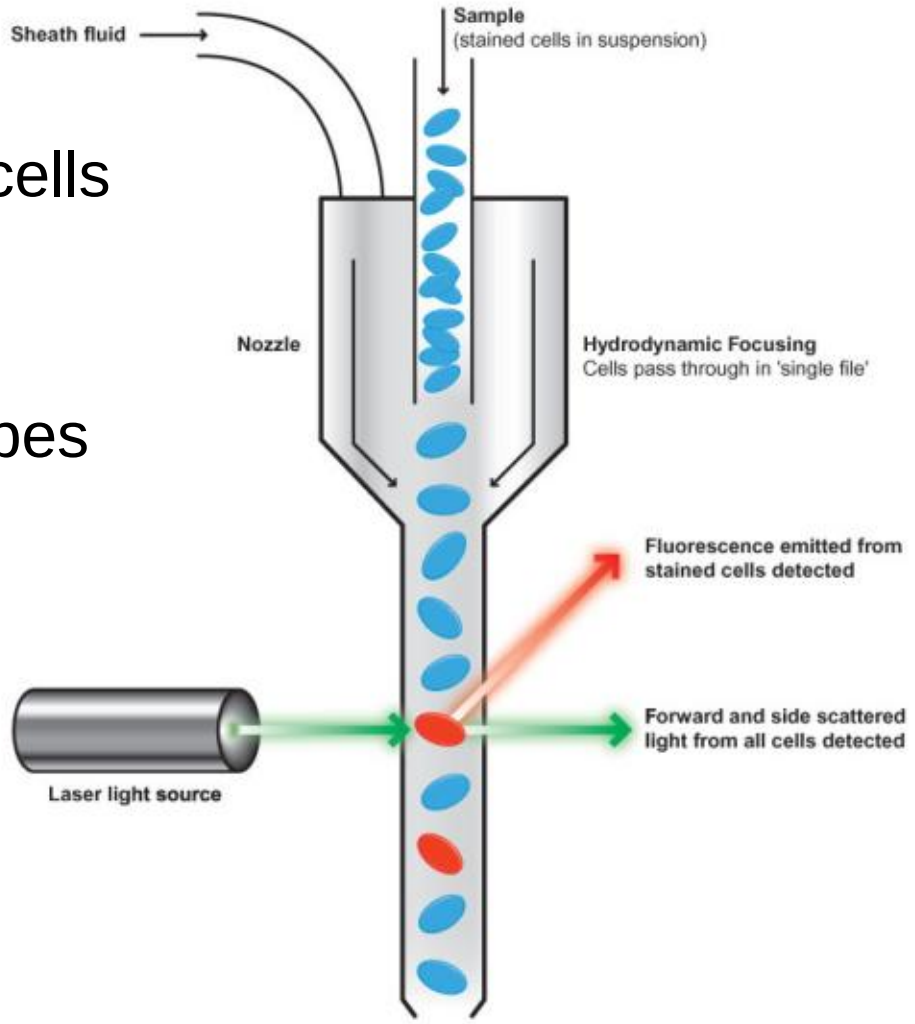
What else we would like to know

- QUANTITY of T cells specific for antigen:
  - Functional and non-functional
- QUALITY of T cells specific for antigen:
  - Breadth of epitopes recognized
  - Types of cytokines that can be produced
  - Degranulation or lytic capacity of T cells
  - Phenotypic markers on the T cells:
    - Memory/effector markers (CCR7, CD45RA, etc.)
    - Markers of exhaustion (e.g., PD-1)
    - Perforin, granzymes, etc.

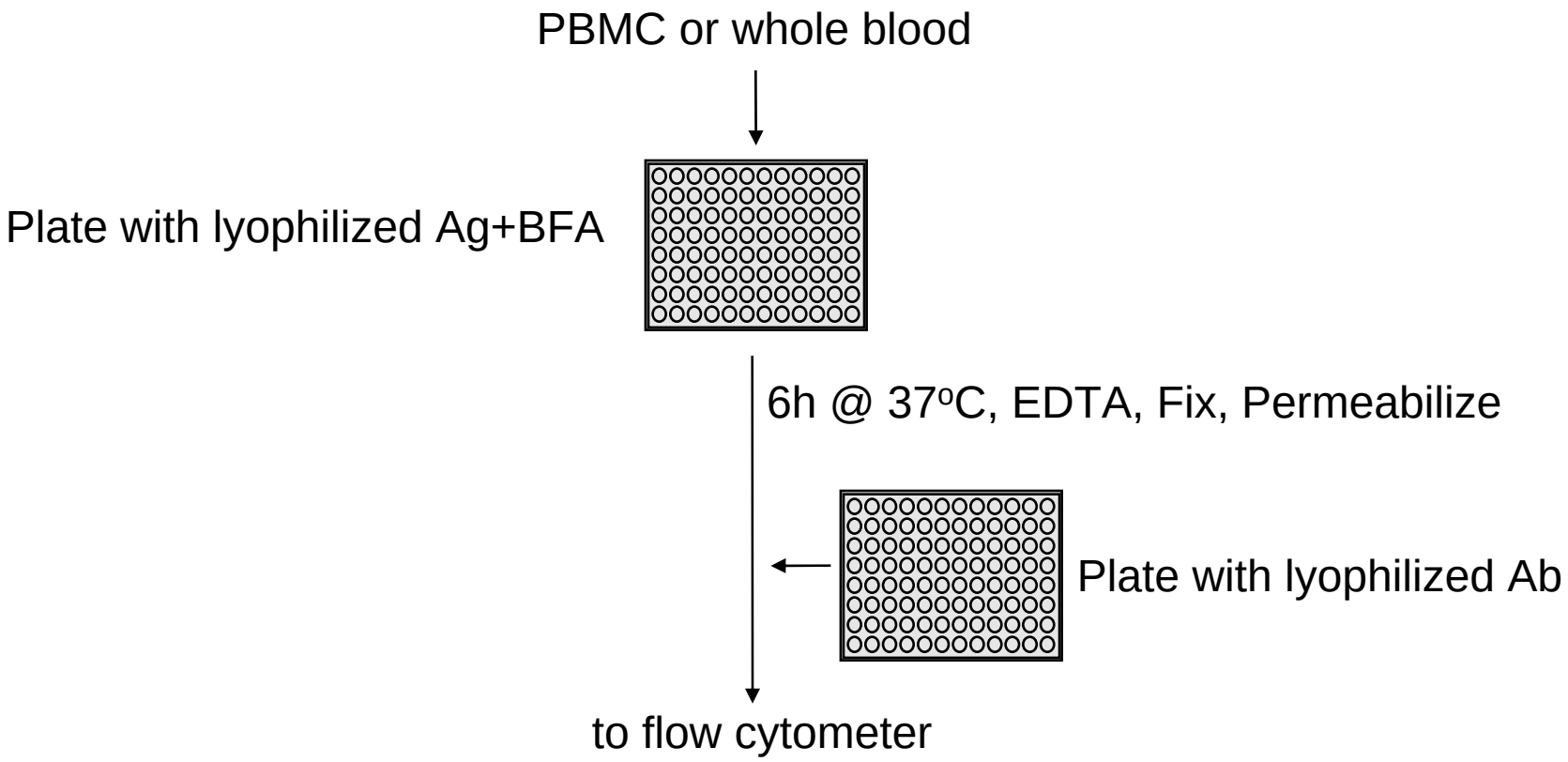
Why flow cytometry?

- Measures multiple markers on individual cells
- Can detect rare cell populations
- Can see both phenotypes and functions



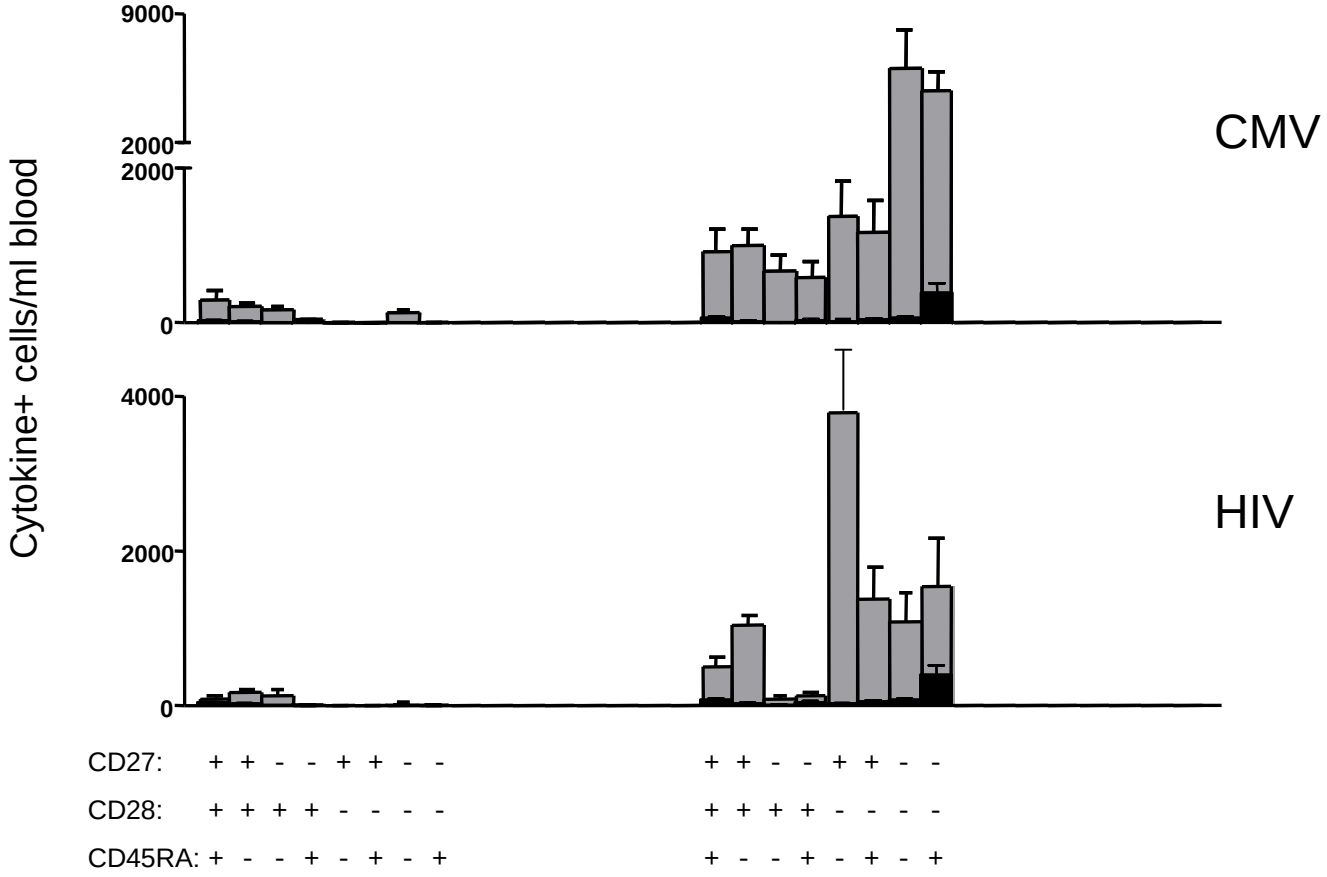


Intracellular Cytokine Staining (ICS): Simplified and standardized with Lyoplates



Maecker et al., 2005, BMC Immunol 6: 13

ICS: HIV response monitoring

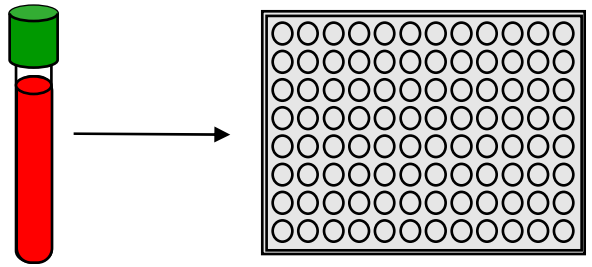


Nomura et al., 2006, AIDS Research & Therapy 3: 18

Phospho-Flow Assays

- Measure phosphorylation events in very short-term stimulated whole blood, PBMC, etc.
- Can measure multiple cell-surface and intracellular markers in combination, using multiparameter flow cytometry
- Can detect signaling events through T cell receptors, surface Ig, cytokines, etc.
- May detect signaling defects in aging or immune-mediated diseases

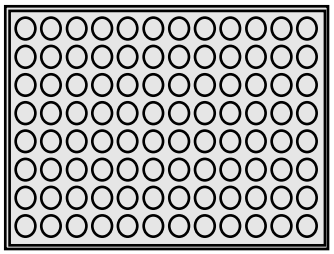
Principle of Phospho-Flow



PBMC or whole blood

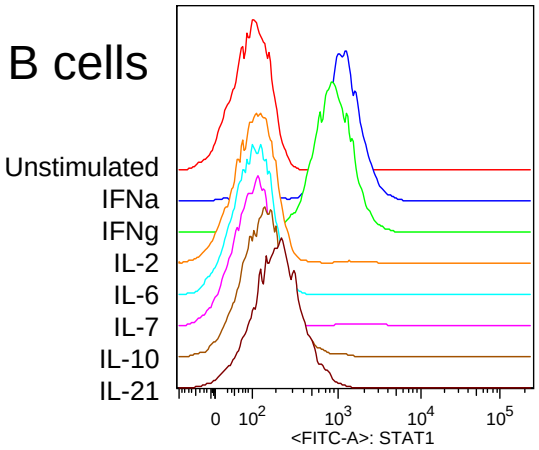
Antigenic stimulus

Incubate 2-30 min

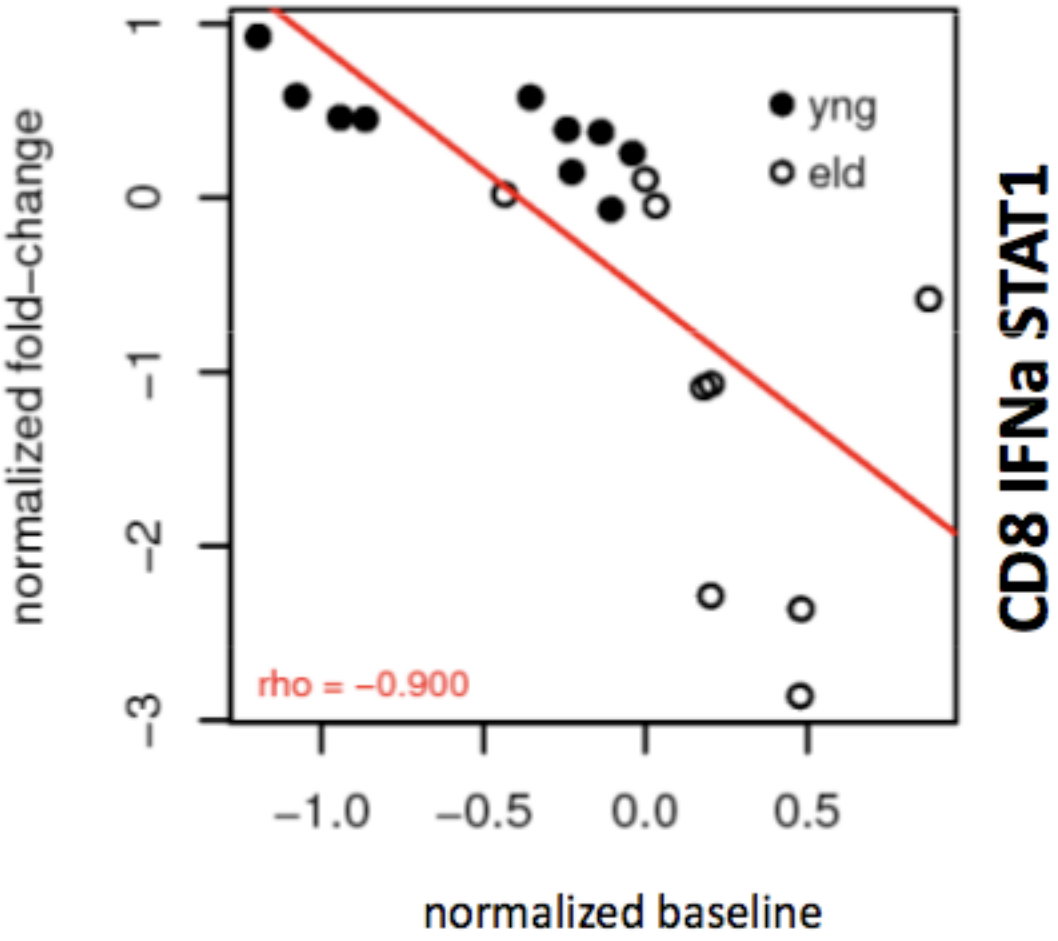


- Fix cells
- Permeabilize
- Stain

Gate on cells of interest

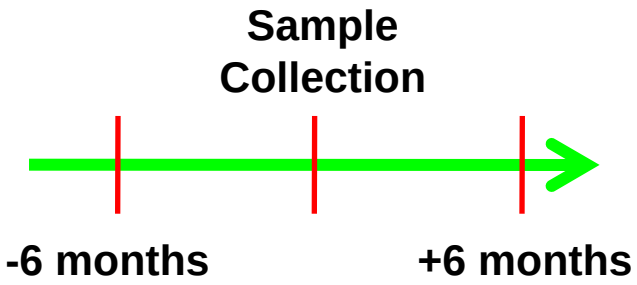


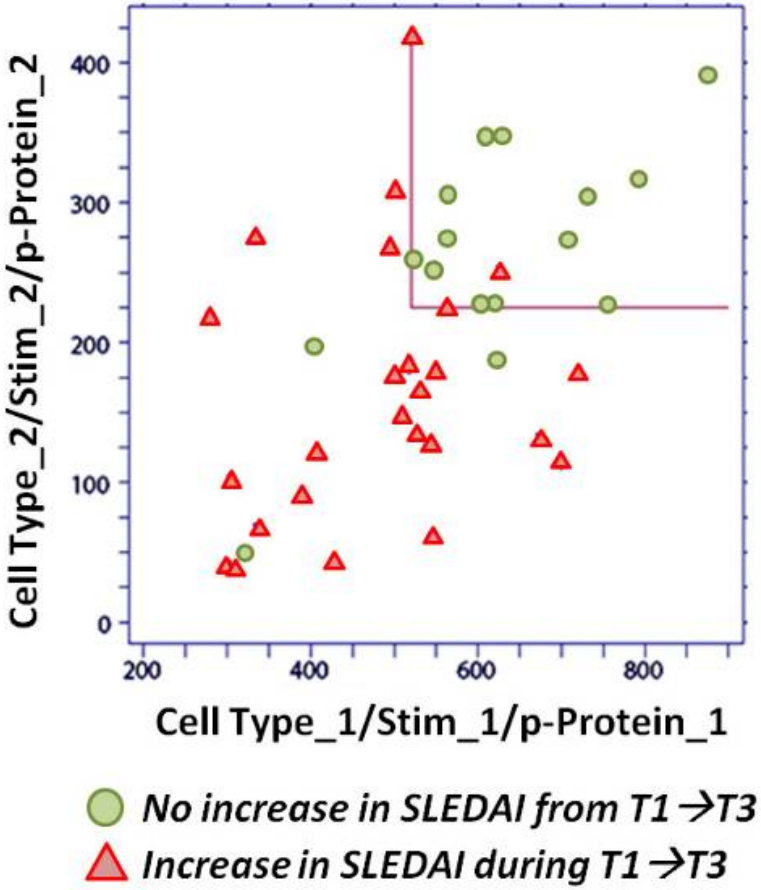
Phospho-flow defects in elderly



Shen-Orr et al., manuscript in preparation

SLE: predicting clinical activity



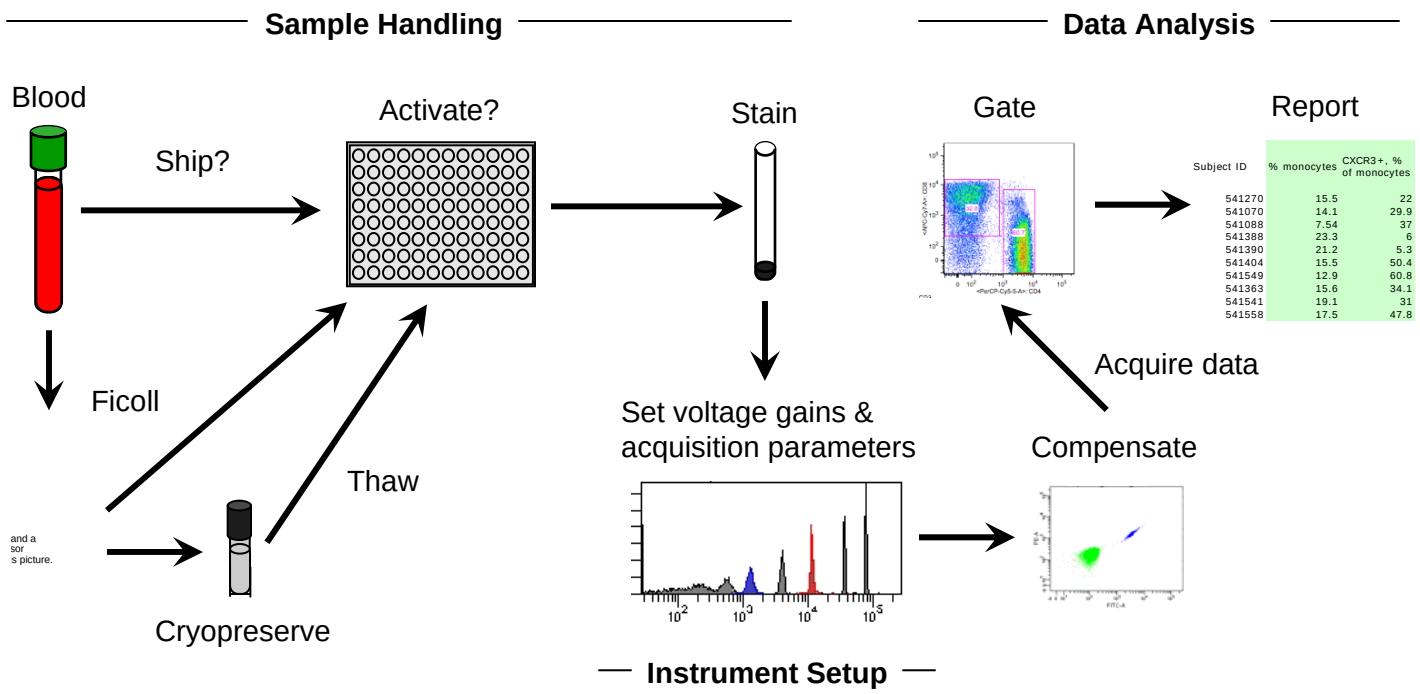


Data courtesy of Garry Nolan, Stanford University

So why isn't everyone doing it?

- Assays are technically demanding
  - Require specialized equipment and training
  - Need for standardization for meaningful results
  - Sample handling logistics can be difficult
- Financial constraints

Choices and sources of variability



Conclusions

- Clinical tests for cellular immunity are largely lacking
- Flow cytometry is a powerful technology for dissecting cellular immune responses
- There is promise that flow cytometric assays could be useful in infectious diseases, autoimmunity, and other areas
- Simplification and standardization of methodology will be necessary to create clinically useable tests