Flow Cytometry: the power of single cell analysis

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FOMD FLOW CORE MANAGER

Cytometry is …

The measurement of the physical or chemical characteristics of cells or other biological particles at a single cell level

Not limited to flow cytometry!

As a cytometrist…

You are a cellular detective!

Your job is establishing cellular identity

Power in resolution

- Determining cell type a from b and distinguishing identity
- Determining what your cells are doing

Interrogation 101

What to they contain, express, or produce?

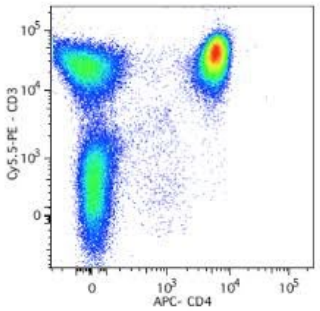
What do they do?

What do they look like?

Who do they associate with?

Standard cytometry techniques

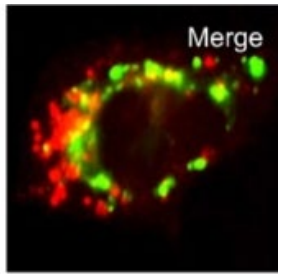
Flow Cytometry



- Zero spatial resolution
- 20* measured parameters
- Highly quantitative
- 10^6+ cells analyzed

- *Fast, sensitive and quantitative multispectral analysis on large population of cells*

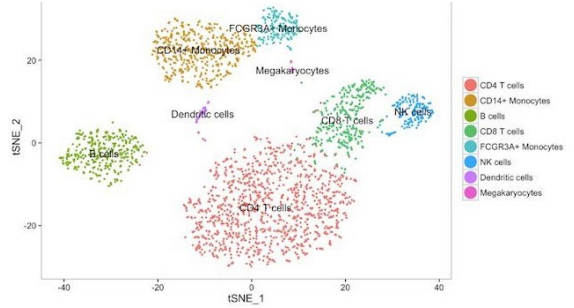
Microscopy



- Good spatial resolution
- 1-6 parameters measured
- Semi- to highly quantitative
- ~10^2 cells analyzed

- *Small cell populations, low throughput*

Genomic cytometry

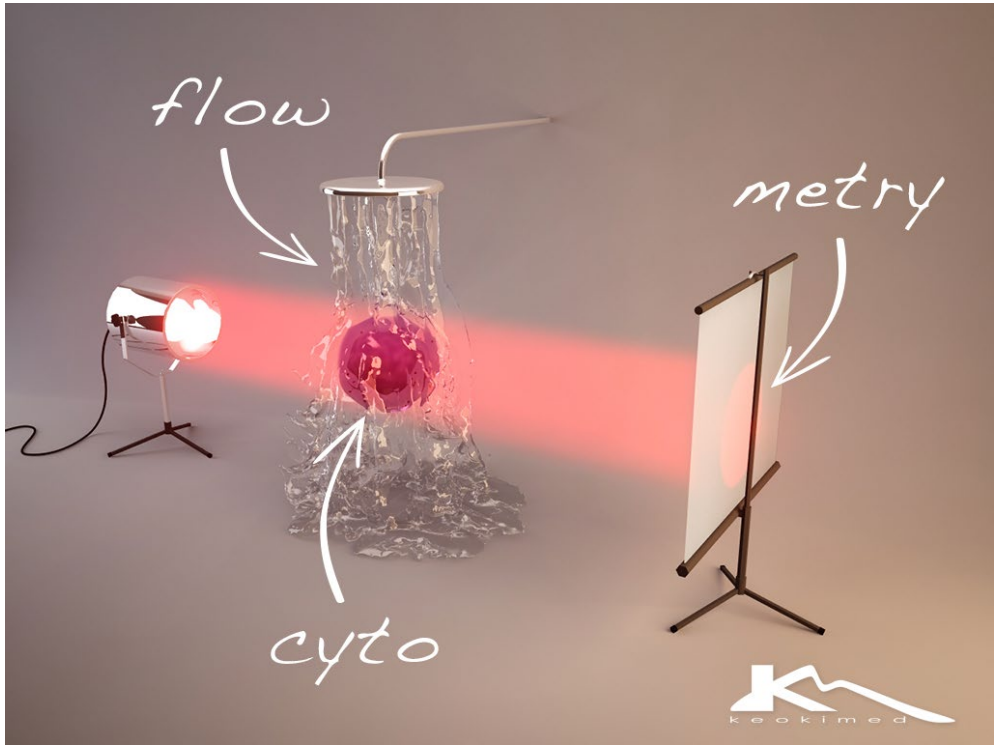


- Super high dimensional single cell profiling
- 100-1000s of genes per cell
- ~10^4 cells analyzed

- *Potential issues with doublets and high levels of gene drop-out*

What is this flow cytometry?

…is a technology that allows analysis of multiple characteristics of particles (cells) as they flow through a beam of light



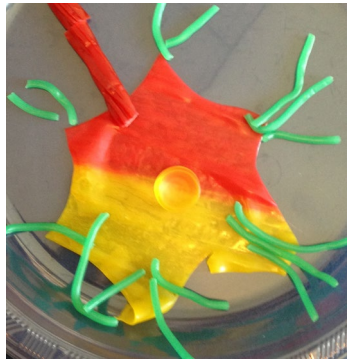
What are we measuring?

Single cells

Light

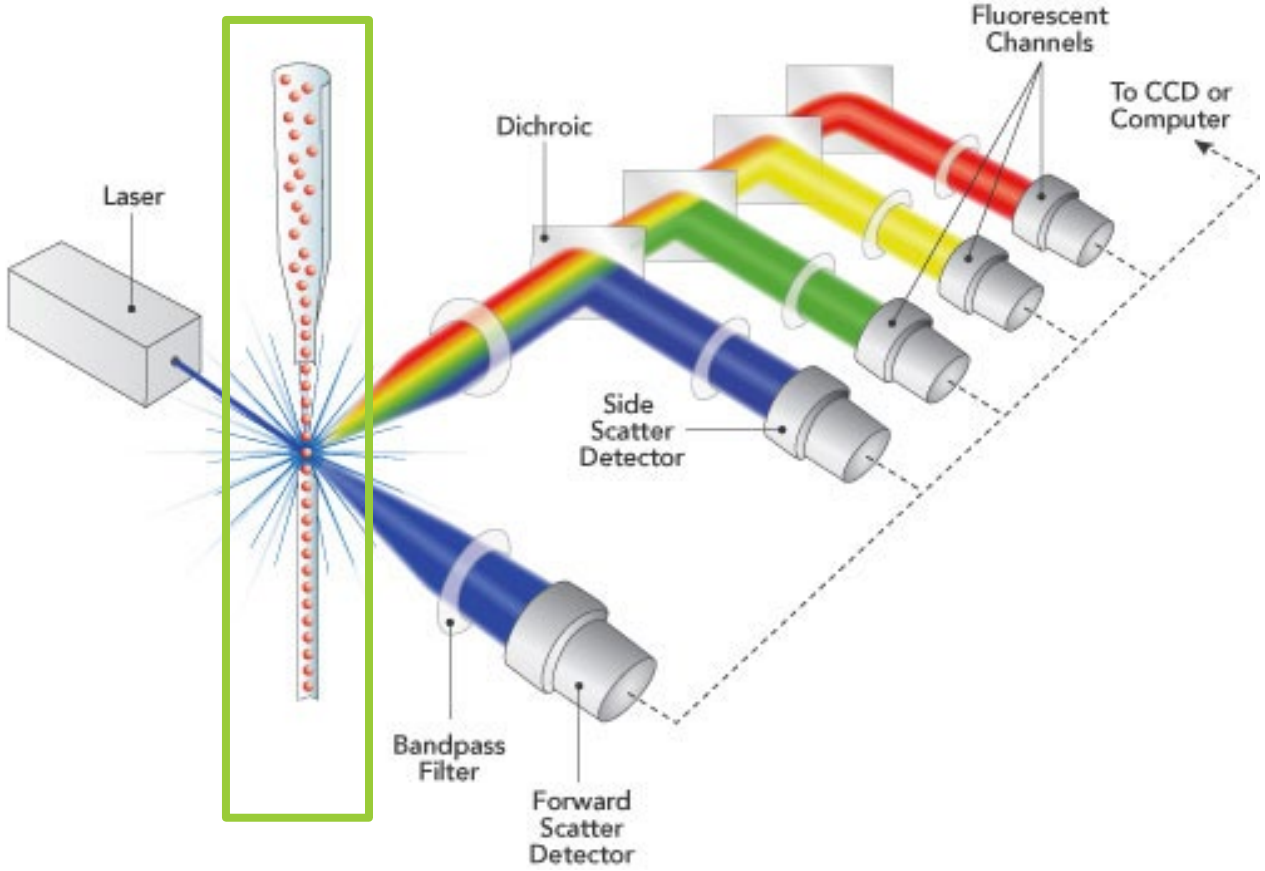
- Scatter
- Fluorescence

Flow Cytometry



Microscopy

Same basics in all machines

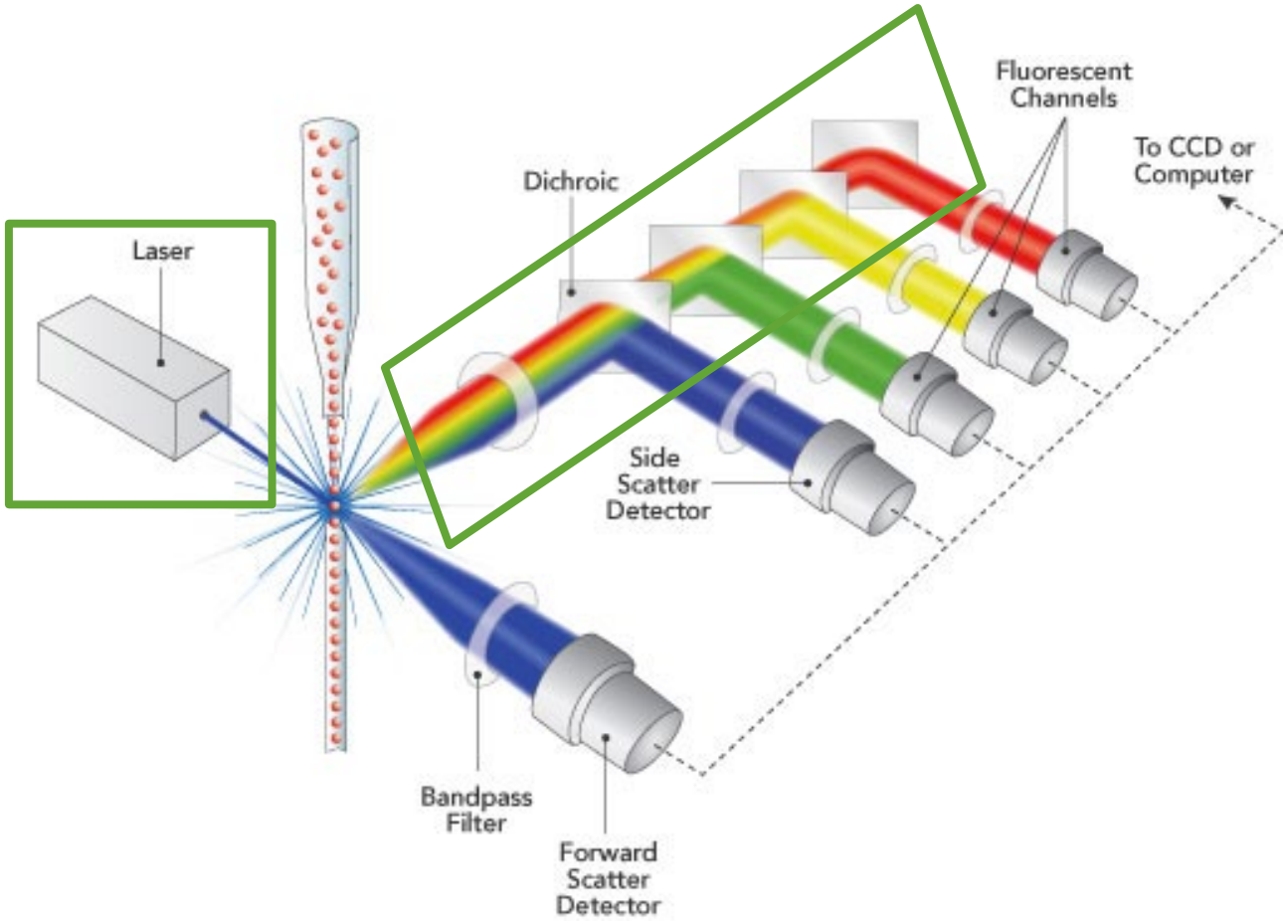


Fluidics:

- Stream of fluid that transports particles

Image courtesy of BD Biosciences

Same basics in all machines



Optics:

- Lasers that illuminate particles (intersect at the **flow cell**)
- Optical filters that direct light signals

Image courtesy of BD Biosciences

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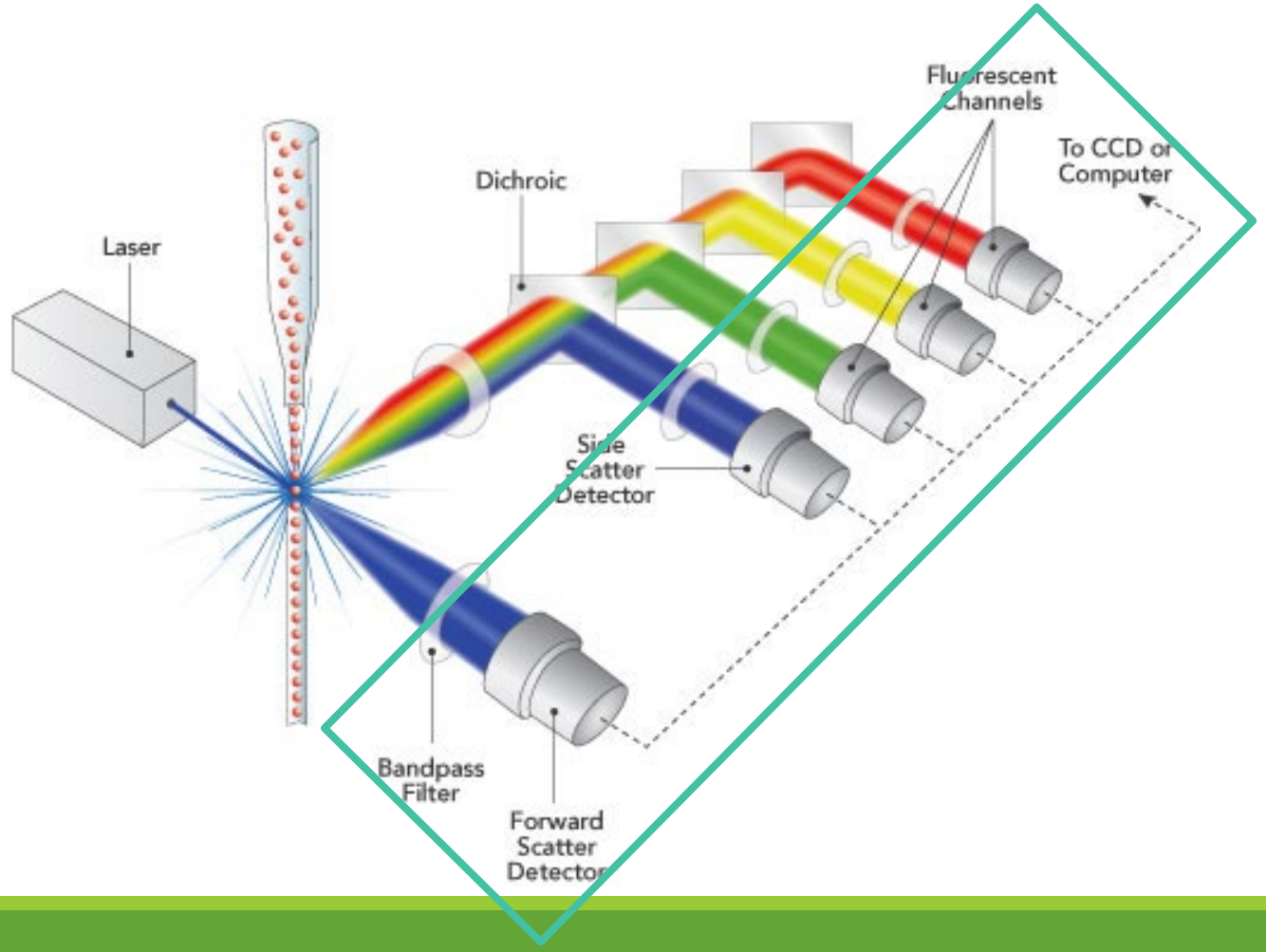

Detectors and electronics:

- Convert light signals to electronic information that can be processed by a computer

Image courtesy of BD Biosciences

Three main kinds of flow cytometry

Analyzers

- "Standard" units; will give you FSC, SSC, and fluorescence
- Parameters depend on laser setup

Sorters

Imaging cytometers

Image courtesy of BD Biosciences

Three main kinds of flow cytometry

Analyzers

- "Standard" units; will give you FSC, SSC, and fluorescence

Sorters

- Can remove specified cells from total population into new tubes= sort
- This is FACS= Fluorescence Activated Cell **Sorting**

Imaging cytometers

Image courtesy of BD Biosciences

Three main kinds of flow cytometry

Analyzers

- "Standard" units; will give you FSC, SSC, and fluorescence

Sorters

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Imaging cytometers

- Similar to an analyzer but get fluorescent images of each particle

Image courtesy of Amnis

Why use flow cytometry?

Analyze distribution of single cells

- Not an average

WB

FC

Many thousands of cells analyzed

- Quickly

Statistical information very quickly

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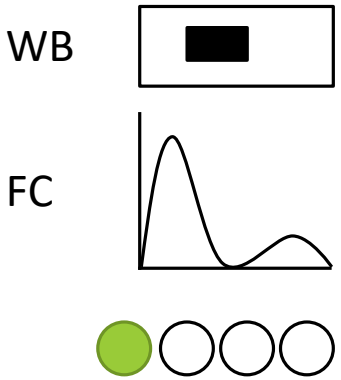

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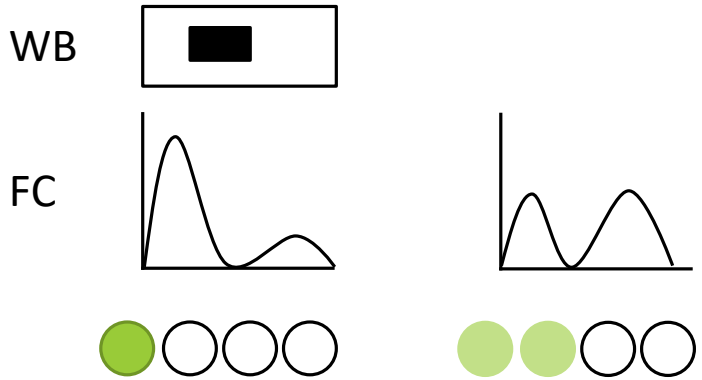

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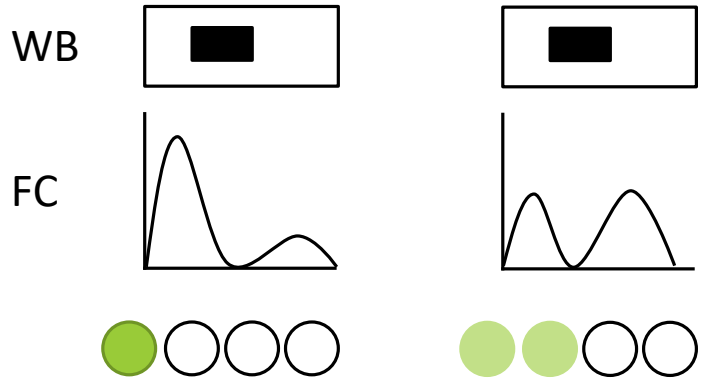

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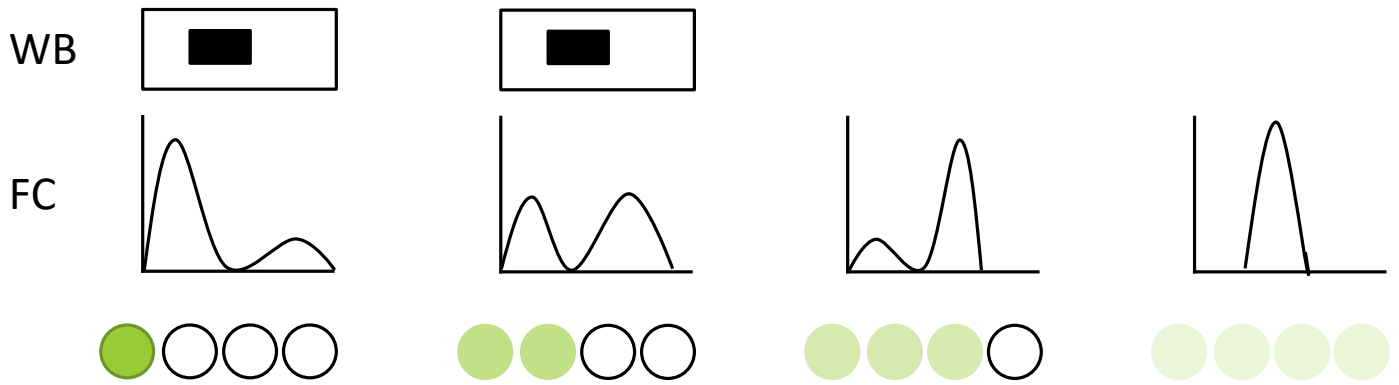

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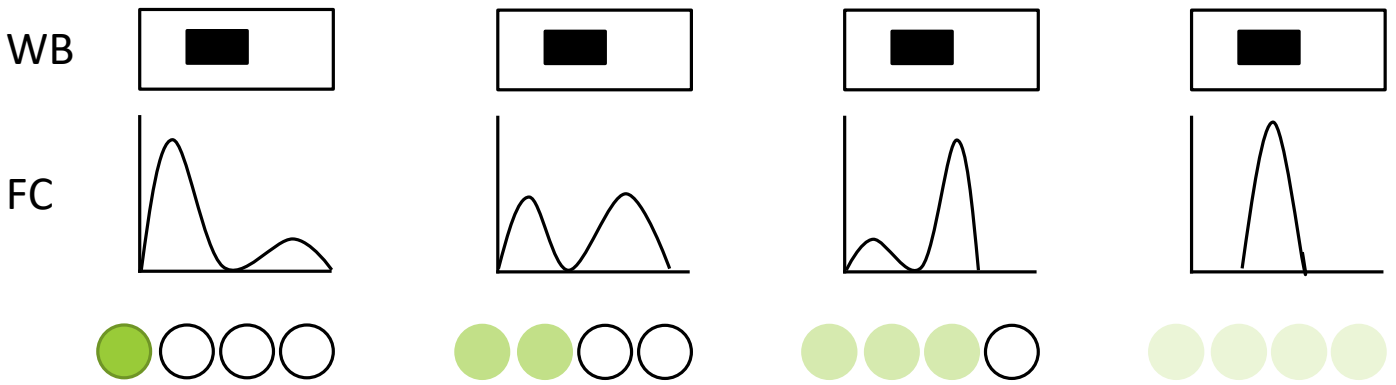

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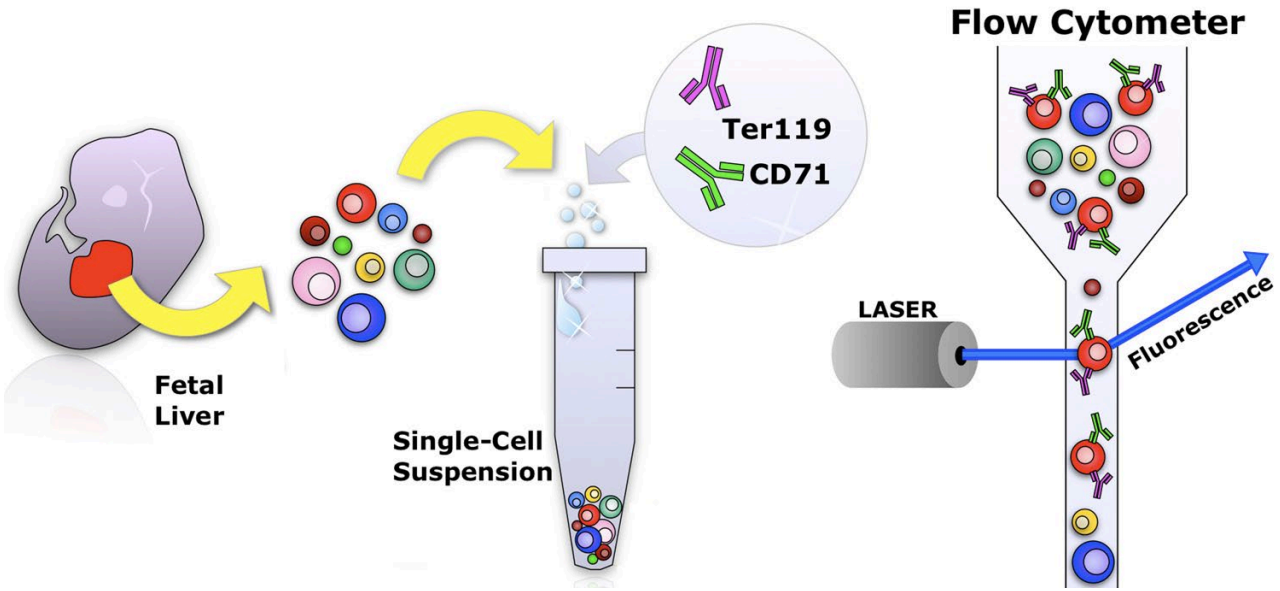
Common flow cytometry assays

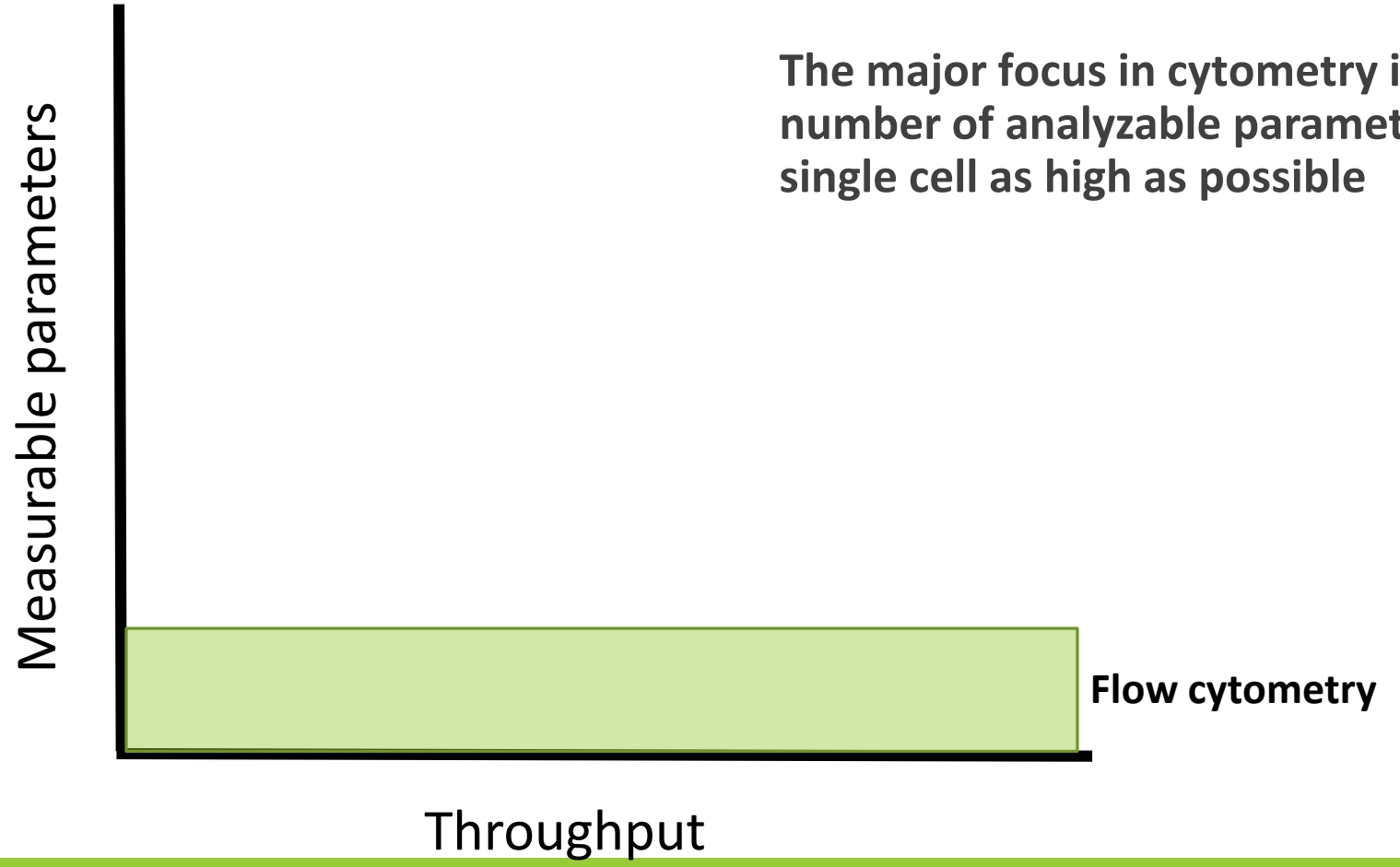
Broad applicability to many fields!

Some of the more common applications:

- Cell cycle
- Viability
- Activation of signaling pathways
- Cell phenotyping and identification
- Drug delivery
- Cell activation
- Cellular differentiation
- And the list goes on and on……



Parameter space race



The major focus in cytometry is to push the number of analyzable parameters from a single cell as high as possible

Super multi-plexed flow cytometry

Cutting edge flow cytometers are equipped with 50 possible channels and 6-10 lasers

Current max panel size ~25 parameters

$$$

Customized solutions for high parameter cell analysis

The BD FACSymphony™ system is a novel cell analyzer that leverages the inherent benefits of flow cytometry and enables the simultaneous measurement of up to 50 different characteristics of a single cell. This high parameter flow cytometer is a powerful analytical tool that enables scientists to identify and analyze distinctive phenotypes in heterogeneous populations.

ZE5 Cell Analyzer (formerly YETI)

State-of-the-art, integrated high-throughput sample loader can easily handle your samples in any type of microtiter plate up to 384 wells, including standard or deep 96 well, 5mL tube racks, and single 5mL tubes. Sample integrity is maintained with on-board agitation and temperature control.

With the smallest benchtop footprint in its class and high speed system design enabling event rates of >100,000/second, ZE5 provides unmatched performance in limited lab space.

ZE5 can be configured with up to five spatially separated lasers and 30 detectors providing the flexibility you need for multi-laser fluorescence detection without compromise. Its dual Forward Scatter design allows either simultaneous standard and small particle detection or multi-laser scatter detection. The innovative EYE profiles your instrument with 10 distinct wavelengths of LEDs to verify the optical filter configuration and track detection performance over time.

Propel Labs' intuitive EVO software provides unattended start-up and quality control, automated fluorescence compensation, a fluorochrome selector panel, and a runlist design wizard. Integrated training modules, remote access capability, and the ability to analyze files while acquiring saves time and streamlines your workflow.

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– Back to Top

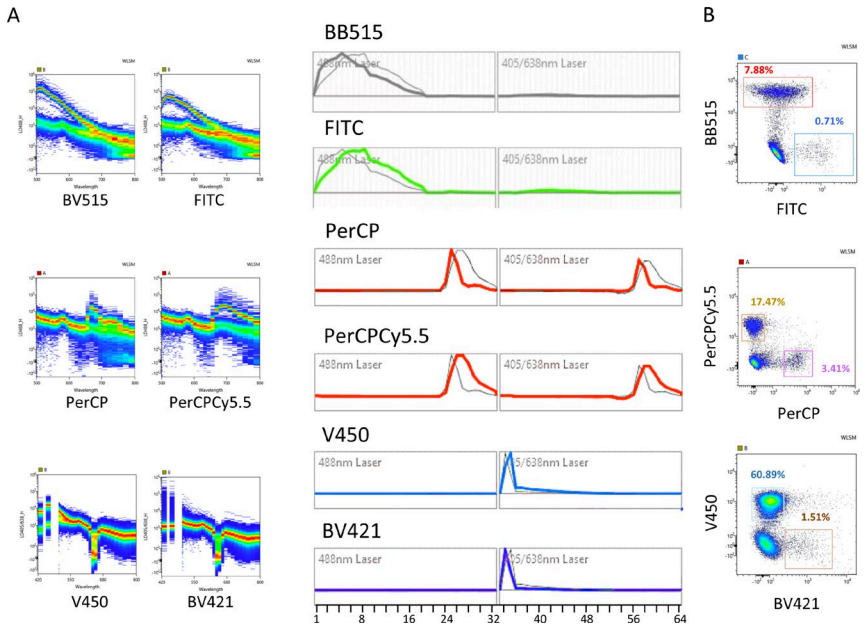
Spectral cytometry

In standard flow cytometry, 1 PMT= 1 colour

- So to add more colours, we add more lasers and more PMTs

In spectral cytometry, there are 32 or 48 detectors set up to measure the entire spectra of each fluorochrome

- In the software, each spectra is identified due to it's unique signature and "unmixed" from the other colours



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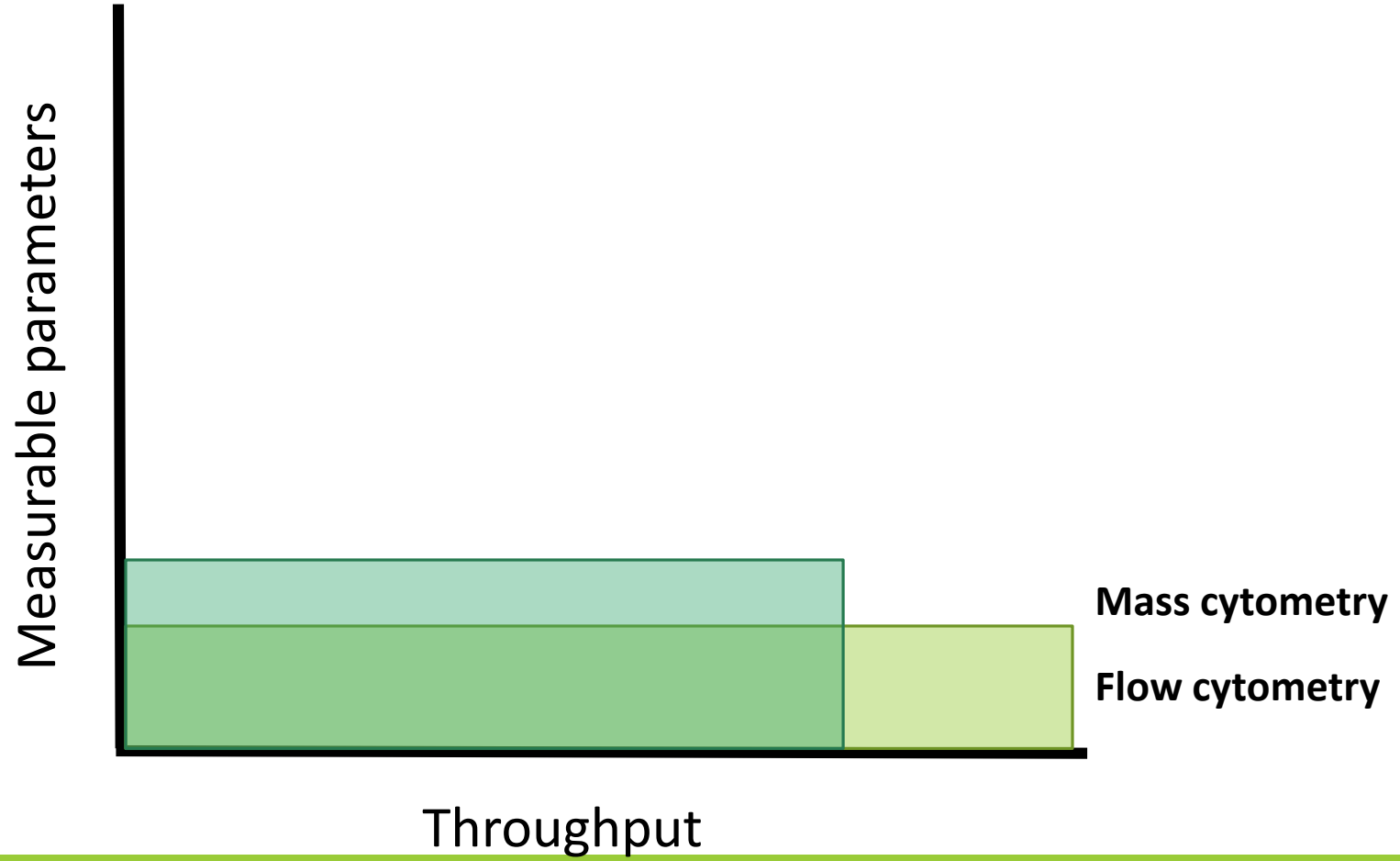
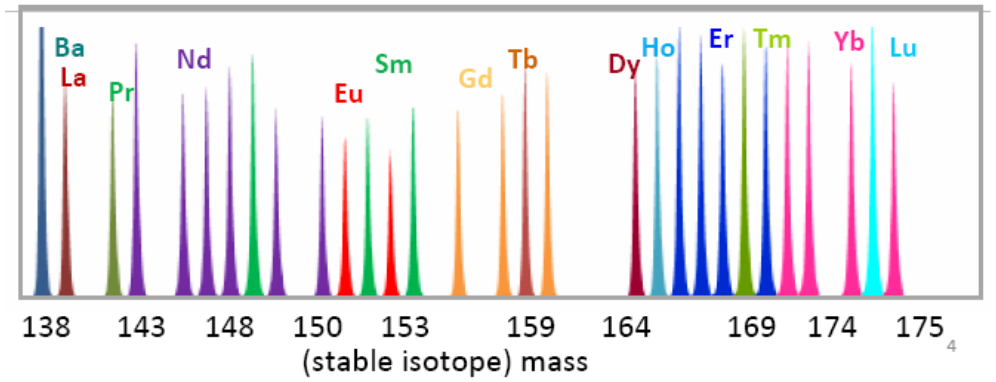
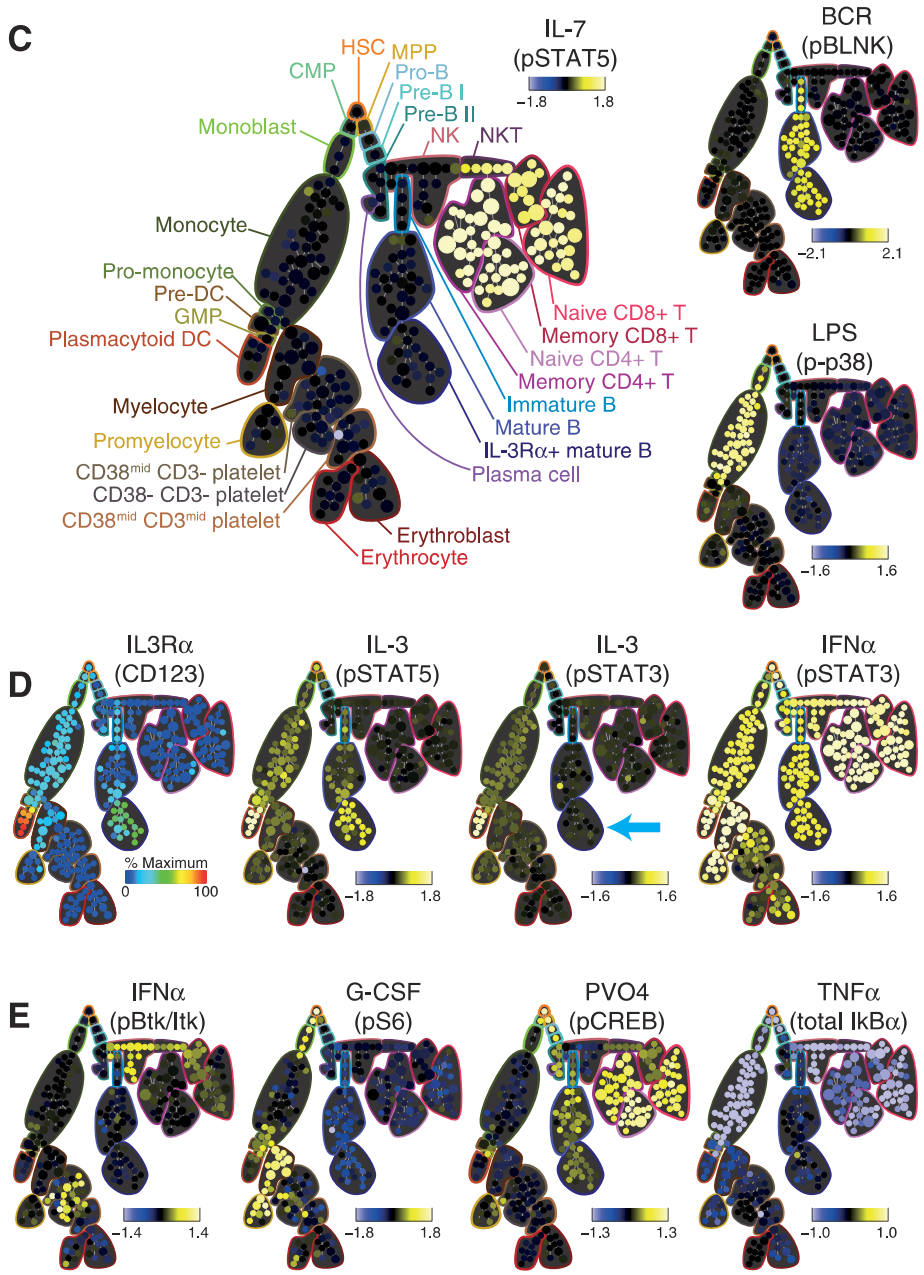


MASS CYTOMETRY



Bendall SC et al. (2011)



Identify all PBMC subsets using 21 phenotypic markers

And look at signaling responses following activation- **all in 1 sample!!**

In each of the identified subsets, cell activation signatures can be studied following various stimuli from a single sample!

18 Functional Markers

pSTAT3	pSrcFK
pSTAT5	pCrkL
pSHP2	pCREB
pZAP70/Syk	pERK1/2
pBtk/Itk	pP38
pSLP-76	pMAPKAPK2
pPLCγ2	pS6
IkBα	pH3
pNFkB	Ki67

Bendall SC et al. (2011)

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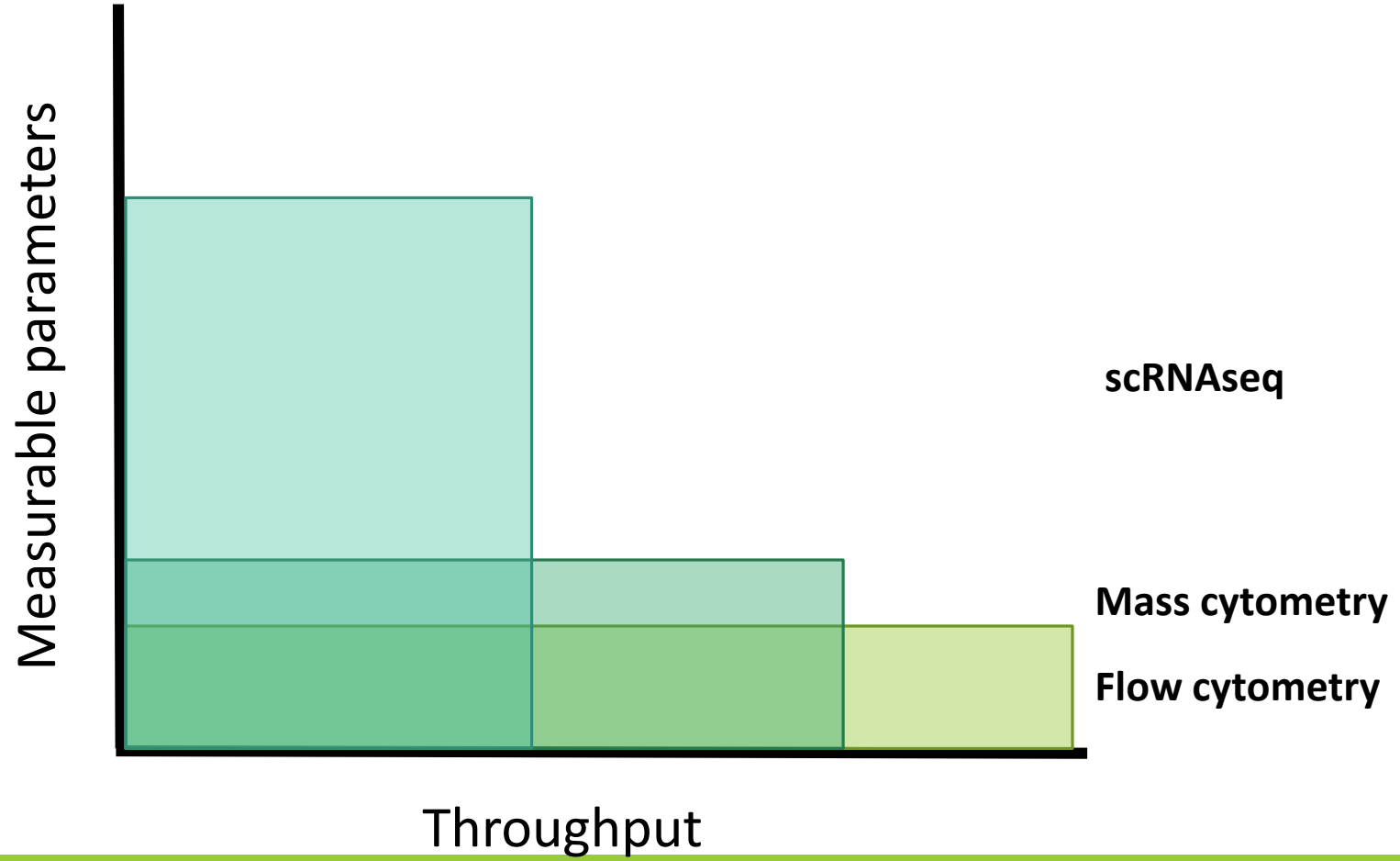
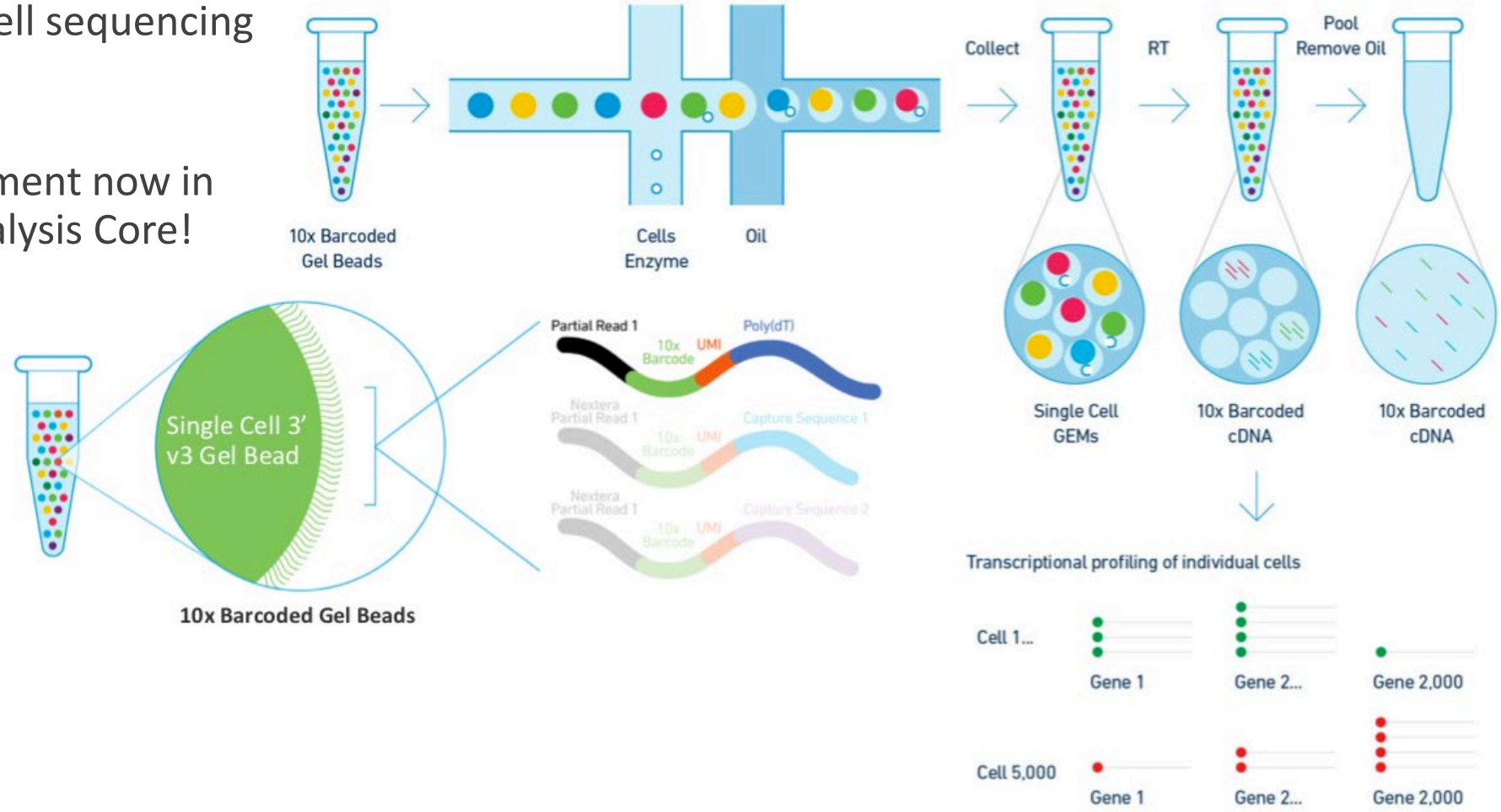


Genomic cytometry

The world of single cell sequencing

10x Genomics instrument now in the High Content Analysis Core!



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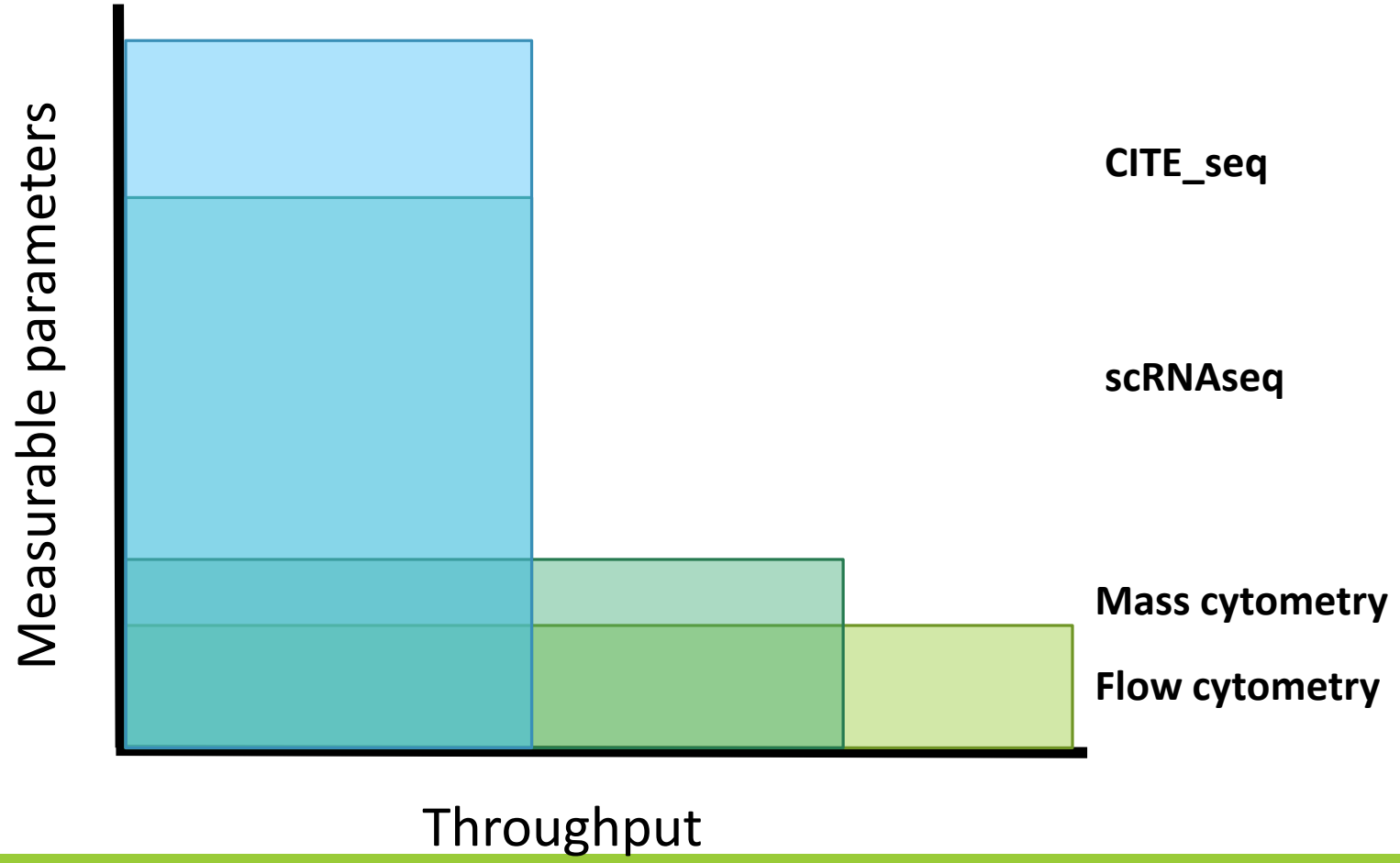
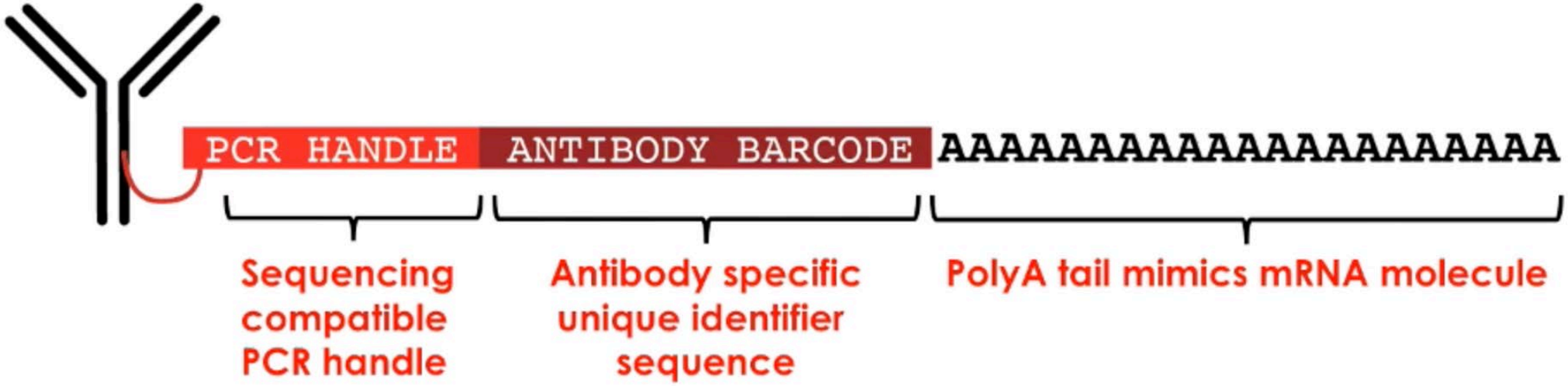


Combining single cell gene expression with protein detection…?

Protein abundance readout using a DNA-barcoded poly-adenylated oligo

- = An oligo tag that mimics a transcript!



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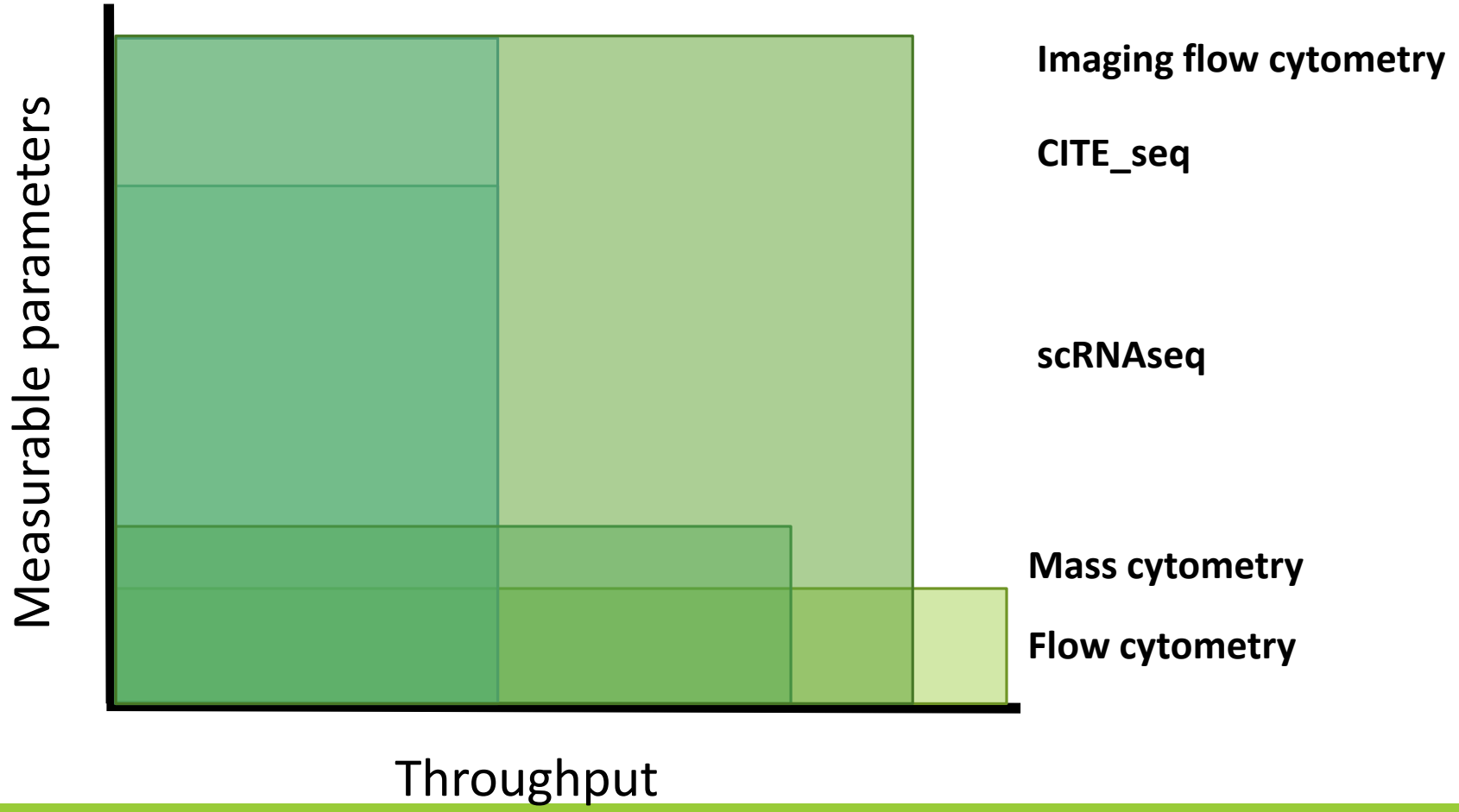

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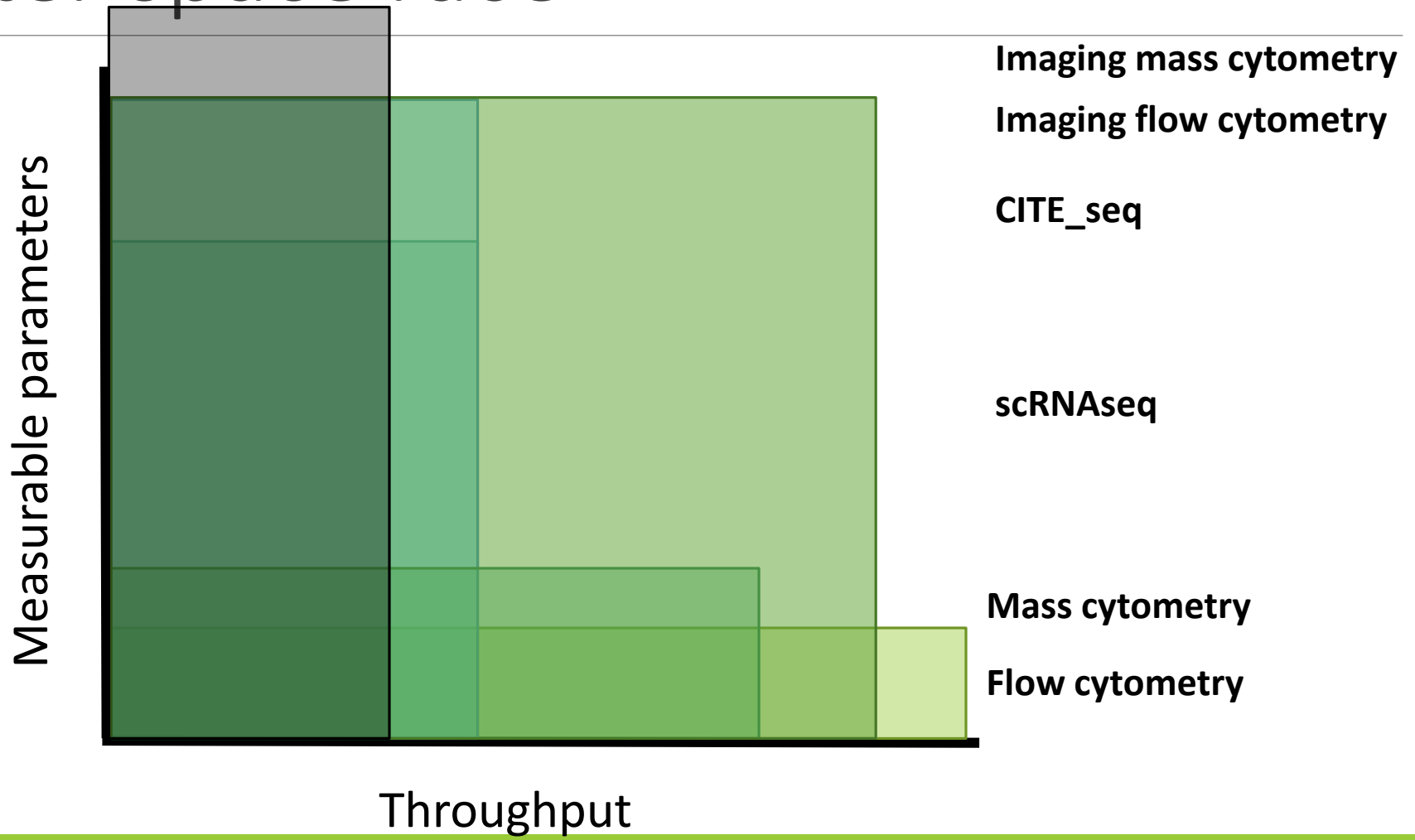
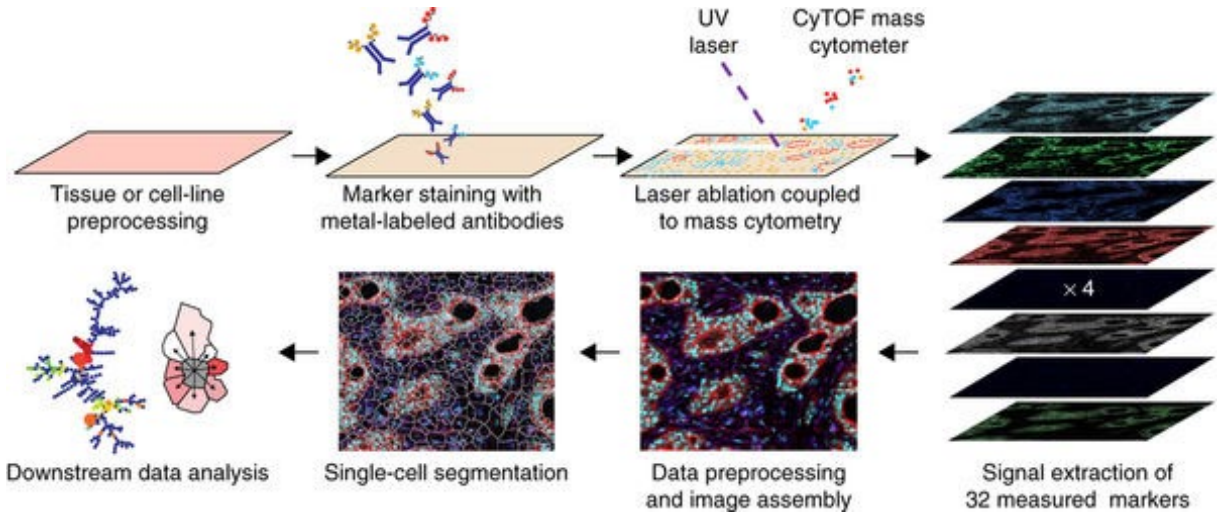


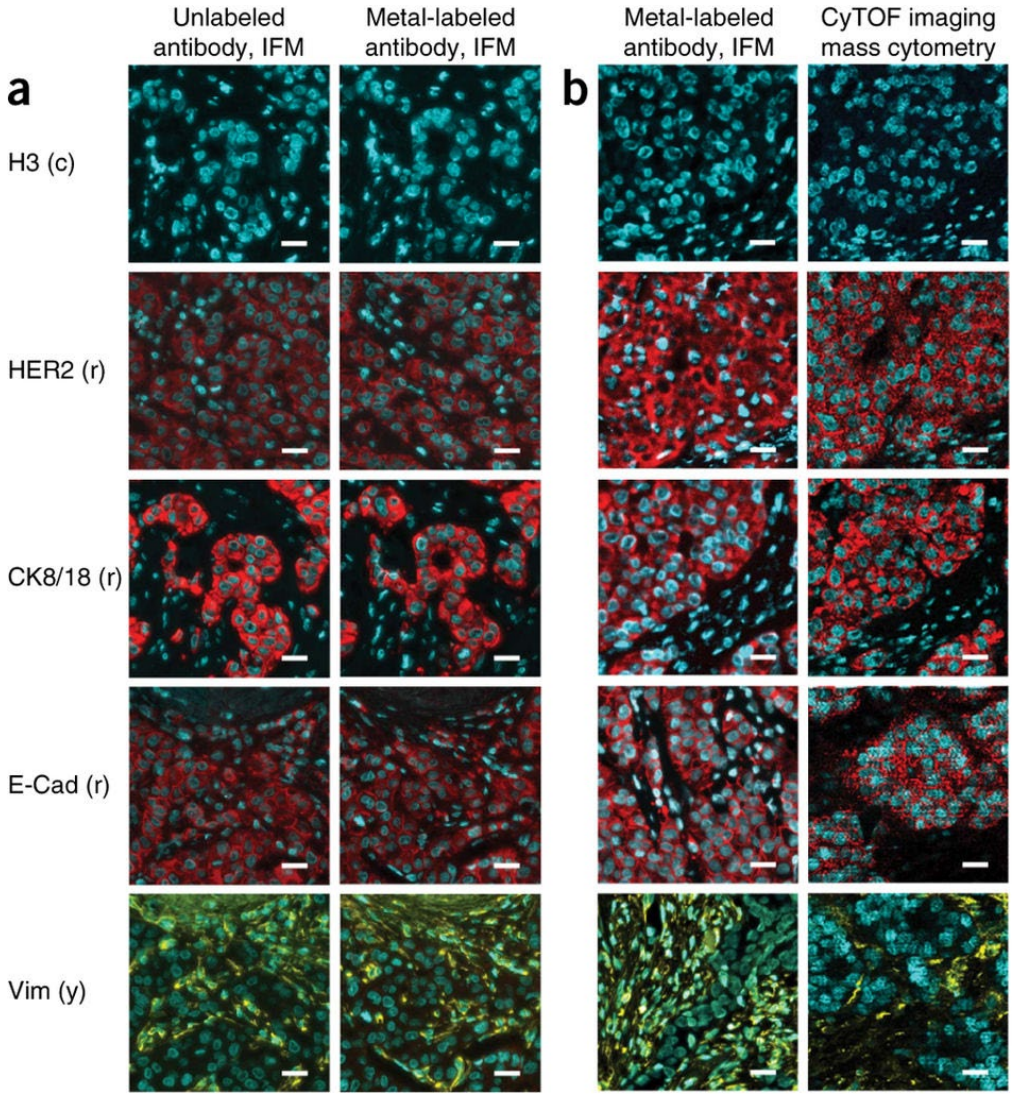
Imaging mass cytometry

Each previous technique relies on dissociating tissues into single cells, removing any potential to study cell interactions/ tissue architecture

Enter: IMAGING MASS CYTOMETRY and the ability to visualize up to 37 protein markers in the spatial context of the tissue microenvironment

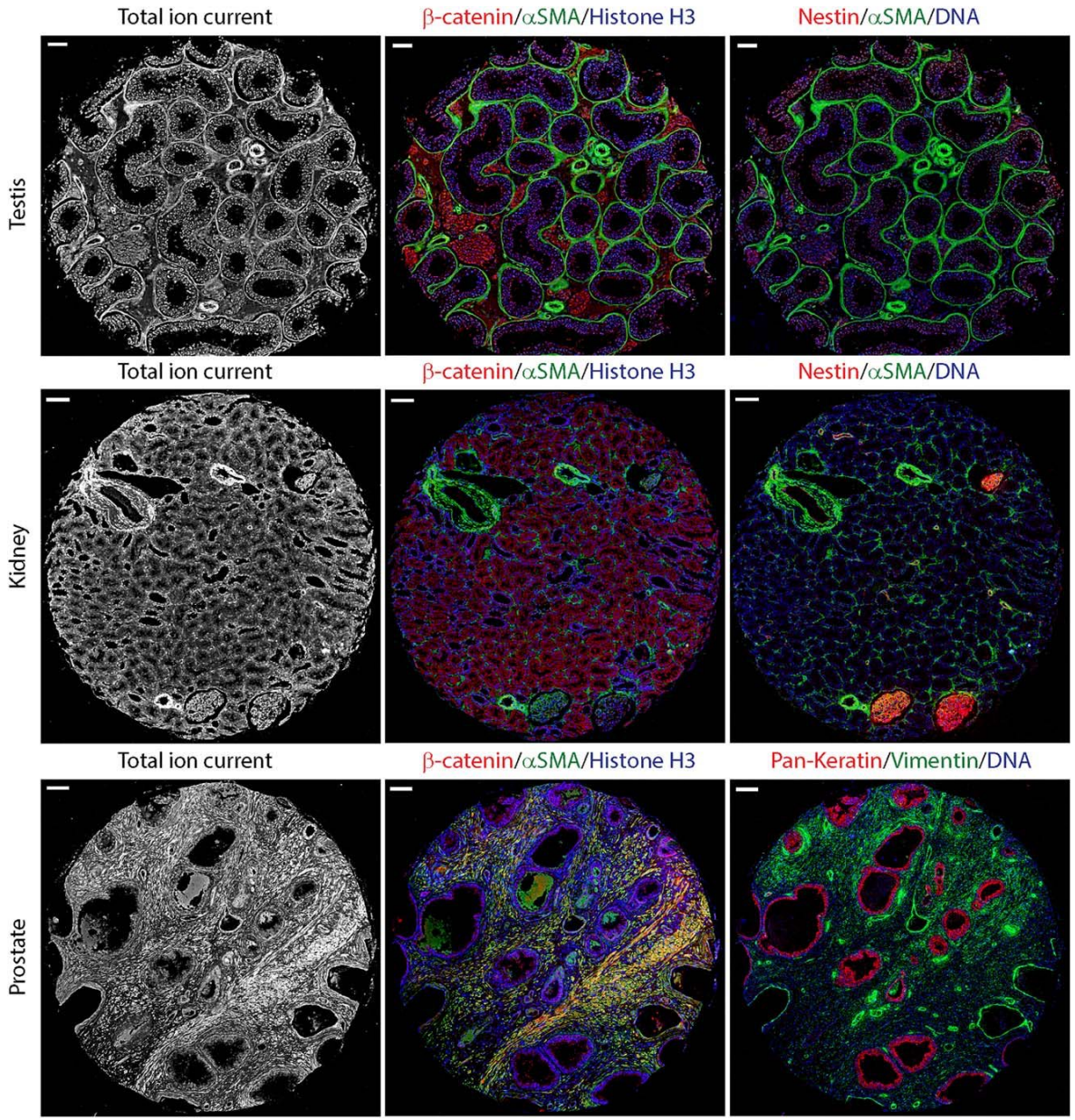


Geisen C et al. (2014)



(a) IFM on serial breast cancer tissue sections of the luminal HER2+ subtype (case no. 37) using unlabeled and metal-labeled antibodies recognizing the indicated markers. (b) IFM and CyTOF imaging mass cytometry on breast cancer tissue sections of the luminal HER2+ subtype (case nos. 210, 23 and 37) using metal-labeled antibodies recognizing the indicated markers. E-cadherin (E-Cad) and vimentin (Vim) were not analyzed on serial sections. Both Hoechst 33258 in IFM images and H3 in all images are shown in cyan (c). r, red; y, yellow; CK8/18, cytokeratin 8/18. Scale bars, 25 µm.

Geisen C et al. (2014)



Chang Q et al. (2017)

So what does this all mean?

There are an ever growing number of techniques to gain great insight into your cells

Many of these techniques can be applied on existing Core infrastructure

AND

It’s a really fun time to be a cell detective!