Flow Cytometry: the power of single cell analysis

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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 do 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
Same basics in all machines

**Optics:**
- Lasers that illuminate particles (intersect at the flow cell)
- Optical filters that direct light signals
Same basics in all machines

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
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
Three main kinds of flow cytometry

Analyzers
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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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Diagram illustrating the distribution of single cells.
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.

Flow cytometry

Throughput

Measurable parameters
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
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
Parameter space race

Throughput

Measurable parameters

Mass cytometry
Flow cytometry
MASS CYTOMETRY
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!
Parameter space race

- Throughput
  - Measurable parameters
    - scRNAseq
    - Mass cytometry
    - Flow cytometry
Genomic cytometry

The world of single cell sequencing

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

- CITE_seq
- scRNAseq
- Mass cytometry
- Flow cytometry
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!
Parameter space race

- Imaging flow cytometry
- CITE_seq
- scRNAseq
- Mass cytometry
- Flow cytometry
Parameter space race

Measurable parameters

- Imaging mass cytometry
- Imaging flow cytometry
- CITE_seq
- scRNAseq
- Mass cytometry
- Flow cytometry

Throughput
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)
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!