Single-cell phenotyping using mass cytometry

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Single-cell resolution phenotyping

- Today’s technologies are capable of deeply characterizing and quantifying protein expression patterns on live cells.

- (re)Defining resolution
  1. Number of cells (e.g. throughput)
  2. Number of parameters measured (e.g. dimensionality)
  3. Capacity to detect the metric (e.g. sensitivity)
  4. Optical measurements (e.g. “pixilation”)
Overview

1. Flow Cytometry and Mass Cytometry

2. Introduction to CyTOF2 Mass Cytometer

3. CyTOF workflow and data
Immunofluorescent flow cytometry

**Strengths**

- Very fast acquisition rate
- Cost-effective for small, simple panels
- Robust systems with support from competing companies
- *Cells can be recovered (sorted)*

**Limitations**

- Panels with >12 markers become very difficult to design, require compensation
- Significant signal overlap concerns at high dimensions
- Intracellular phospho-staining introduces additional issues

Raychaudhuri et al., 2015
Spectral overlap and the limitations of immunofluorescent cytometry

- Emission spectral profile of FITC
- Percentage of FITC emission spectral profile detected in the 585/40 channel
- Emission spectral profile of PE
- Percentage of PE emission spectral profile detected in the 525/50 channel
- Overlapping emission spectral profile of FITC and PE not detected in either the 525/50 or 585/40 channel
- Band pass filter
Mass cytometry allows for the simultaneous detection of up to 50 markers

**Strengths**
- No autofluorescence, no need for compensation
- Up to 50 parameters with little increase in design complexity
- Cost effective for large panels
- Easily barcode up to 20 samples prior to staining
- Intracellular phospho-staining

**Limitations**
- Slower acquisition
- Cells destroyed during acquisition
- Upfront Cost
- Single instrument upkeep requires dedicated operator
- Fluidigm’s historical control over technology and reagents

Raychaudhuri et al., 2015
Considering aspects of resolution to answer systems-level questions

- Fluorescence-based flow cytometry
  - Resolution favors *throughput* over *dimensionality*. *Sensitivity* is dependent on abundance of target and Ab affinity, and instrument

- Mass cytometry
  - Great balance of resolution in terms of *throughput* and *dimensionality*. *Sensitivity* is comparable to flow

- Excellent approach for the monitoring the immune system as well as complex solid tissues, especially when interested in targeting heterogenous cell populations
The CyTOF2 mass cytometer

- Time-of-flight
- Quadrupole
- ICP
- Nebulizer

Integrate per cell

Heavy (>100 Da)
Reporter atomic ions

Light (<100 Da)
Overly abundant ions

Cell 1

Cell 2

Cell 3

Mass

Element

Cell 1: A, B, C, D...
Cell 2: 1, 2, 3...
Cell 3: 4, 5, 6...

.FCS file

Analysis

Element A vs Element B

Staining for CyTOF

- Overall staining protocol similar to flow cytometry

- Intracellular staining requires fix/perm

- DNA intercalators used to identify singlets vs doublets and discriminate intact/live cells

- Inclusion of EQ beads for normalization of mass signal across samples and experiments
Typical mass cytometry workflow

1. Experimental and panel design

2. Panel validation on tissue of interest

3. Full experimental run / data collection

4. Data analysis
Experiment and Panel design

1. Consistent sample processing and preparation is key!

2. Validated “off-the shelf” panel vs. custom panel design
   - *Clones confirmed to be working in clinical flow will save time*

3. Include common “indexing” proteins
   - e.g. CD3, CD19, CD45, etc.

4. Put less highly expressed markers in most sensitive mass channels

5. Avoid conjugating metals with risk of oxidation or isotopic impurity to highly expressed markers
Panel Validation

- Cells tested: pooled
  1. PBMCs (donated by Farshad)
  2. KG-1 (human bone marrow myelogenous leukemia)
  3. Cord Blood (purchased from Stemcell)
  4. K562 (human myelogenous leukemia line)
  5. HMC (human mast cell line)
Data collection and analysis: cleaning

Data collected in .fcs format, must be “cleaned” prior to analysis

Normalization and Debarcoding
Data collection and analysis: The "problem" of dimensionality

- 2 parameters: 1 plot
- 3 parameters: 3 plots
- 9 parameters: 36 plots
- 32 parameters: 496 plots

Data Collection and analysis: MATLAB, R, and Cytobank

**MATLAB**
- Very stable
- Faster processing
- Great for custom data visualization
- Must learn syntax
- Amazing documentation

**R**
- Free
- Constant evolution
- Many packages available
- Potentially unstable

**Cytobank**
- Individually licensed
- Internet connection required (one user at a time)
- Very user friendly
The R package “cytofkit” and phenograph

Interactive Visualization of cytofkit Results

cytokit RData:
- Choose Plot: CD11, cytotox, lyric /Data
- Upload complex
- Submit

Plot Method:
- Scatter Plot
- Heat Map
- Subset Progression

Plot Annotation:
- Add Cluster Labels
- Repel Cluster Labels
- Label Samples or Shapes
- Separate Plot by Samples

Sample Filter:
- 1320-13, G2, STEM, G2M

Data Summary:
- Expression Data: 1564 metrics x 31034 genes
- Cluster Method: cytofkit (Phenograph) /DensVM
- Visualize Method: t-SNE, PCA, IsoMAP
- Progression Method: t-SNE

* Recently made available as a standalone web app!
CytoBank: web-based SPADE analysis

"Spanning-tree Progression Analysis of Density-normalized Events."

"SPADE clusters phenotypically-similar cells into a hierarchy that allows high-throughput, multidimensional analysis of heterogeneous samples"

*Available in MatLab and CytoBank and Flowjo*
Additional ongoing projects

- ALS and MS blood samples
- Cord blood from allergic vs non-allergic
- Case studies with genetic mutations affecting immune function
- Cancer cell lines
- Kidney
- Skeletal muscle
- Spleen
- Lung
- Heart
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Question and Answer Session

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Advantages of mass cytometry

- No significant signal overlap → no compensation
- No background signal "autofluorescence" → lower sensitivity to noise
- *Less* complex for high-parameter panels
- Simultaneous detection of both intracellular and intercellular targets
- Barcoding multiple samples
Neodymium

- Overstaining
- Oxidation
- Isotopic impurity

Abundance sensitivity (M +/- 1)
<1%

Oxide formation (M +16)
~2-3%
High oxidizers: La, Ce, Pr, Nd
Low oxidizers: Eu

Isotopic purity Nd142
Metal dependent
Into: 141 (0.5%), 143 (0.3%), 144, 146