Sample Multiplexing for Cytometry Studies

Advances in and Advantages to Using Barcoding Methods to Scale Up Experiments and Optimize Data

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Senior Business Development Manager, EMEA
Overview

Cell barcoding is a key application to multiplex samples in large cytometry studies.

- Challenges and considerations when multiplexing
- How cell barcoding works for mass cytometry
- Selecting the right cell barcoding method

There are multiple benefits for integrating cell barcoding into clinical studies.

- Workflow efficiencies
- Improved data consistency
Sample multiplexing

- Cell samples are tagged with unique identifiers, enabling staining and analysis of a single, combined sample.

- Fluorescent cell barcoding labels samples with **dyes or fluorophore-labeled antibodies**.

- Mass cytometry barcoding labels samples with **mass-tag labeled antibodies**.

- Multiplexing is advantageous for large studies across many technology platforms (genomics, cytometry, immuno-assays).
  - Drug discovery
  - Disease profiling
  - Patient monitoring

Early barcoding method

Benefits of sample multiplexing

Improves data consistency
- Reduces sample-to-sample technical variation
- Improves cell doublet discrimination
- Eliminates carryover due to insufficient washing

Minimizes batch variations
- Run longitudinal samples obtained from each patient in the same barcoded batch to infer effect of a treatment with greatest precision (Gaudillière et al. Cytometry Part A 87 (2015): 817-829.)

Improves workflow efficiencies
- Reduces on-machine acquisition time
- Can reduce cell loss
- Can reduce use of some consumables

Enables scaled-up experiments
- Higher sample throughput due to reduced processing time in workflow

Sample multiplexing challenges with fluorescence flow cytometry

“While barcoding of samples has many benefits, it represents an additional step in the protocol, needs to be optimized on its own, and usually occupies cytometric channels which would be otherwise available to the measurement of target analytes.”


Integration of barcoding into existing panels
- Barcoding markers occupy the same channels of target analytes.

Limited number of barcodes to use at a time
- Dependent on fluorochrome selection and number of channels available for instrument

Barcoding parameter spillover
- Careful compensation of barcode parameters required, particularly for quantitative experiments such as phospho flow studies (Krutzik et al. *Current Protocols in Cytometry* 55 (2011): 6.31.1–6.31.15.)
Helios system
The new standard for high-parameter profiling, powered by CyTOF®
Cell barcoding for CyTOF

1. Barcode
2. Pool and stain
3. Acquire
4. Decode

Thaw and count cells ➔ Viability-stain ➔ Barcode-stain (according to standard surface staining protocol)

- Samples are barcoded with mass-tag labels.
- Unique 3- or 4-digit (or more) metal isotope code used as sample-specific identifier
Benefits of barcoding in mass cytometry

Scaling up experiments
- More available channels to enable large-parameter (40-plus) panel and barcoding simultaneously
- Combine more samples at once (multiplex 35-plus samples).

Improved **data consistency** and **quality**
- Less spillover into neighboring channels due to lanthanide mass-tag range

**CV% of population frequency (% of leukocytes)**

![Graphs showing CD4 T cells and B cells with and without barcoding.](image-url)

**BC = barcode**
Cell barcoding options

Palladium barcodes for universal cell multiplexing

The Cell-ID™ 20-Plex Pd Barcoding Kit (Cat. No. 201060) utilizes six stable palladium (Pd) isotopes for barcoding up to 20 samples in a 6-choose-3 format.

Live-cell CD45 based barcoding for human leukocyte multiplexing

Uses Maxpar anti-CD45 antibodies to pool 35-plus samples in one tube.

Custom barcoding

Create your own barcoding strategy for cell types or samples of interest by tagging different antibodies/reagents with metals of choice.

20-plex palladium barcoding for universal cell multiplexing

- The Cell-ID 20-Plex Pd Barcoding Kit uses 6 stable palladium (Pd) isotopes for barcoding up to 20 samples in a 6-choose-3 format.

- Palladium barcoding uses a transient partial permeabilization step to enable permanent intracellular staining.

- Fluidigm provides ready-to-use kit with reagents, 3 x 20 barcodes and debarcoding software.
A comprehensive atlas of IL-1β-induced signaling in circulating immune cells

RESEARCH ARTICLE

‘Identification of human immune cell subtypes most responsive to IL-1β-induced inflammatory signaling using mass cytometry’


Highlights

- **35-marker mass cytometry panel** including phosphoprotein markers targeted key immune cells and signaling pathways associated with IL-1β, a major mediator of COVID-19 cytokine storm.

- PBMC from three donors were stimulated with IL-1β, fixed with PFA, then **barcoded with palladium tags**, surface stained, permeabilized, then stained for intracellular phospho markers.

- Specific CD4 T cell populations exhibited the most robust response to IL-1β as measured by NF-kB and CCR6 expression.

- Systems-level study identifies potential ready-to-use assay for identifying individuals at higher risk of cytokine storm and most likely to benefit from IL-1β blockade.

McNamara Lab, University of Virginia
Live cell barcoding of human leukocytes

Live-cell barcoding uses Maxpar anti-CD45 antibodies.

- Performed prior to marker staining
- Maintains optimal detection of fixative-sensitive epitopes
- Used for leukocytes (from blood and tissue)

Utilizes **7 Cd and 4 Pt isotopes**

- 11 monoisotopic tags increase multiplexing power to more than **35 samples**.
- No interference with the lanthanide mass tag range (139–176 Da)
Catalog products for live-cell barcoding

- Seven Cd and **four new Pt isotopes** conjugated to anti-human CD45 antibodies
- Combine antibodies to design your own barcoding scheme
  - Fit current panel
  - Match scale needed

<table>
<thead>
<tr>
<th>Fluidigm Cat. No.</th>
<th>Antibody (clone)</th>
<th>Isotope</th>
</tr>
</thead>
<tbody>
<tr>
<td>3106001B</td>
<td>CD45 (HI30)</td>
<td>106Cd</td>
</tr>
<tr>
<td>3110001B</td>
<td>CD45 (HI30)</td>
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<tr>
<td>3198001B</td>
<td>CD45 (HI30)</td>
<td>198Pt</td>
</tr>
</tbody>
</table>
Beyond 35-plex live cell barcoding

- Custom and flexible barcoding
- Design your own barcoding scheme.
  - Cadmium-only
  - Mixed metal (Cd + Pt)
- Scale sample number to 50-plus.
  - $n\choose k = n!/(k!(n-k)!)$
  - 7-choose-3: **35 samples**
  - 11-choose-3: **165 samples**
Mapping tumors and tissue to guide immunotherapy

RESEARCH ARTICLE

‘Innate immune landscape in early lung adenocarcinoma by paired single-cell analyses’
Lavin, Y., Kobayashi, S., Leader, A. et al.
Cell 169 (2017): 750–765.e17

Highlights

• A comprehensive immune profiling approach points to the tumor-infiltrating myeloid cell compartment as a target for immunotherapy.
• Development of a paired-tissue analysis mass cytometry platform using an anti-CD45 barcoding strategy to simultaneously stain and analyze 3 tissue types (tumor, adjacent tissue and peripheral blood compartments)
• Second cohort of 10 patients barcoded with unique Pd identifier
• Identified a unique tumor-immune signature that is independent of TNM (tumor, nodes, metastasis) stage
# Palladium-based barcoding vs. live-cell barcoding

Choosing the right barcoding method for your research needs

<table>
<thead>
<tr>
<th>Barcode Method</th>
<th>Advantages</th>
<th>Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palladium-based barcoding</td>
<td>• Universal, cell-agnostic</td>
<td>• Adjustments to existing panels or workflows may be needed due to fix/perm step.</td>
</tr>
<tr>
<td>Cell-ID 20-Plex Pd Barcoding Kit (PN 201060)</td>
<td>• Ready-to-use kit and accompanying software</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Unique tags allow maximum antibody panel size.</td>
<td></td>
</tr>
<tr>
<td>Live-cell barcoding</td>
<td>• No fix/perm step required, fix-sensitive epitopes are preserved.</td>
<td>• Barcoding scheme designed manually</td>
</tr>
<tr>
<td>Human CD45 antibodies</td>
<td>• Easily add to existing panels.</td>
<td>• Best for blood leukocytes, CD45-negative cells excluded</td>
</tr>
<tr>
<td></td>
<td>• Flexible design allows scaling for sample number to 50-plus.</td>
<td></td>
</tr>
</tbody>
</table>
Other published cell barcoding approaches
Universal live cell barcoding for human samples

RESEARCH ARTICLE
‘A universal live cell barcoding-platform for multiplexed human single cell analysis’
Hartmann, F.J., Simonds, E.F., Bendall, S.C.
Scientific Reports 8 (2018): 10770

Universal live-cell barcoding targets ubiquitously expressed beta-2-microglobulin and CD298 cell surface markers using platinum-conjugated antibodies.

- Nonlanthanide isotopes (\(^{113}\)In, \(^{115}\)In, \(^{194}\)Pt, \(^{195}\)Pt, \(^{196}\)Pt and \(^{198}\)Pt) for barcode markers do not reduce channels currently used to label antibodies.

- Enables barcoding of cells of non-immune origin, such as tumor cells or stem cell populations
Experimental workflow

1. PBMC processing
2. Barcode samples
   **Barcoding**
3. Surface staining
4. Live/dead staining with DCED-palladium
5. Fixation with 1.6% PFA
6. Ir staining
   **Surface and Ir staining**
7. Collect data on Helios.
8. Normalize and debarcode with MATLAB®.
   **Data collection and analysis**

**Barcoding scheme**

Live cell barcoding

Consolidating signal with 2 antibodies conjugated to the same isotope

Hartmann, Simonds and Bendall (2018)
Other custom barcoding options

**In situ** barcoding

- Thiol-reactive monoisotopic mass-tagged probes enabled 20-plex barcoding of organoids in Matrigel®.
- Increased organoid sample throughput, single-cell recovery and signaling analysis

![Diagram of Thiol-reactive Organoid Barcoding in situ (TOBis)](image)


**Single-cell atlas of cancer heterogeneity**

- 9-choose-4 barcoding scheme using a mix of non-lanthanide metal isotopes for 126-plex barcoding of breast cancer cell lines

![Diagram of Single-cell atlas of cancer heterogeneity](image)


**T cell clone specificity screening**

- 14-choose-4 multiplex combinatorial tetramer staining with cellular barcoding identifies and characterizes antigen-specific T cells.

![Diagram of T cell clone specificity screening](image)

Summary

Fluidigm offers two approaches for cell barcoding:

1) Universal cell multiplexing:
   **Cell ID 20-Plex Pd Barcoding Kit**

2) Immune cell multiplexing:
   **Beyond 35-plex barcoding with CD45 antibodies**

Sample multiplexing with CyTOF enables you to:

- Integrate **customized barcoding** into high-parameter panels
- Scale up experiments and sample throughput because of **workflow efficiencies**
- **Improve data consistency** for large studies
- **Minimize batch variation** for more precise measurements of immune correlates
References

**Fluidigm application notes**

Using Monoisotopic Cisplatin-Containing Reagents for Suspension Mass Cytometry (FLDM-00446)

Seven Cadmium Labeling Kits Increase Flexibility in Panel Design (FLDM-00043)

Compatibility of Cadmium-Labeled Antibodies with Existing Protocols (FLDM-00086)

The Benefits of Palladium Barcoding on Data Quality and Workflow (FLDM-00012)

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