Understanding Biological Heterogeniety through Mass Cytometry: Present and Future Directions

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Field Applications Scientist
Biology is heterogeneous

Biological systems consist of heterogeneous cell types, each with diverse functions and functional states.

Such complexity demands high dimensional proteomic panels that simultaneously measure breadth and depth of the system.
Uniquely CyTOF®: Atomic Mass Spectrum

- **Large panels, simplified design:** 120 mass channels, >34 mass tags with minimal overlap and similar intensity
- **Fewer samples:** no single-metal controls, more information per sample, conserving cells and reagent
Mass Cytometry

CyTOF® 2
Mass Cytometer

MaxPar®
Metal-Conjugated Reagents

Fluidigm.Cytobank™
Data analysis

= 

• Discovery of new biology
• Comprehensive Functional profiling

--- For ---
• Basic Science
• Drug Discovery
• Clinical Research
CyTOF® Mass Cytometry Research
Single-Cell Signaling Signatures Correlate with Surgical Recovery

Clinical recovery from surgery correlates with single-cell immune signatures

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Does Biological Response to Surgery Correlate with Surgical Recovery?

- Surgery significantly perturbs biological function.
- Surgical recovery varies significantly from patient to patient.
- Post-operative pain, fatigue, and initial loss of function are common.

Can biological response to surgery be correlated to recovery from surgery?
Correlation of Signaling Pathways with Clinical Recovery

Blood from 26 Hip Replacement Patients: baseline to 6 weeks

Clustering (21)
- CCR7
- CD3
- CD4
- CD7
- CD8
- CD11b
- CD11c
- CD14
- CD16
- CD19
- CD25
- CD33
- CD45
- CD45RA
- CD56
- CD66
- CD123
- CD127
- CD235
- HLA-DR
- FoxP3

Signaling (11)
- pLCγ2
- pERK
- pp38
- ppMK2
- pp90RSK
- prpS6
- pCREB
- pp65
- pSTAT1
- pSTAT3
- pSTAT5

Correlate cell types and signaling status to clinical recovery

Correlation of Signaling Pathways with Clinical Recovery

• 32 marker phenotypic and signaling panel reveals immune response to hip replacement surgery.

• Deep immune profiling enabled correlation of signaling responses to clinical recovery metrics (regain function, reduction in fatigue and pain).

• Clinical recovery correlated:
  • with signaling responses but not with cell frequency
  • most strongly with CD14+ monocyte functional state

Roadmap

Expand number of metals to increase panel size
Expand number of metal conjugated antibodies and Panel Kits for simplified panel design
Maxpar® Panel Designer for simplified panel design
Reagents for new applications
Mass Cytometry publications update
New Products
New Products - 2014

69 metal conjugated antibodies
  • Total = 315 (2/3 human, 1/3 mouse)

5 Panel Kits for simplified panel design
  • Total = 13

5 new metals
  • Panel size = 36

Reagents for new applications – Cell ID™ line
  • IdU for cell cycle; Cisplatin for dead cell discrimination

Panel Designer
  • 190 customers with access
New Metals

Maxpar labeling kits and pre-conjugated antibodies

• 161Dy
• 163Dy
• 173Yb

Pre-conjugated antibodies

• 155Gd
• 89Y

Now 36 metals to build your panel
Maxpar Panel kits provide all the necessary reagents for profiling human and mouse systems.

Kits include:

- Panel of up to 17 Metal Conjugated Antibodies
- Nucleic Acid Intercalator
- Staining buffers

Applications include:

- Phenotyping
- Cytokines
- Signaling
- Cell Cycle
### 13 Panel kits Now Available

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Cell-ID™ Reagents

Line of reagents that bind cells ‘generally’ as opposed to targeting specific proteins

Members include nucleic acid intercalators, cisplatin, IdU

Coming products: Barcoding kit
Cell-ID™ Cisplatin

Cell staining agent that:

- Contains natural abundance (194/195/196/198) Pt
- Accesses interior of dead or permeabilized cells
- Forms covalent bonds to protein nucleophiles like R-SH and R-S-CH3 groups
- Remains tightly bound through all subsequent incubations and wash steps

Superior alternative to 103Rh intercalator for dead cell identification

Also available in 194Pt and 198Pt monoisotopic form

Fienberg et al., CytometryA 81, 467 (2012)
Cisplatin works with all staining protocols
Cell-ID™ 127IdU

IdU = iododeoxyuridine

127I-containing pyrimidine nucleoside recognized as a thymidine substitute in DNA synthesis

Incorporates into DNA of proliferating cells and thus is a marker of S-phase of the cell cycle

Easy to use compared to fluorescent assays
127I\textit{dU} for Cell Cycle Analysis

Behbehani & Bendall \textit{et al.}, \textit{Cytometry A} 81A, 552-566 (2012)
Maxpar® Panel Designer
Panel Designer Benefits

- Optimal panel design
- Simplifies choice of reagents
- Improved data quality
Mass Cytometry Panel Design

Mass cytometry uniquely isolates signal from over 30 probes into single channels with minimal signal overlap, thereby enabling system-wide single-cell proteomic studies.

Sources of signal overlap are very small in Mass Cytometry.

Optimal panel design utilizes a strategy that:

• Maximizes signal and minimizes signal overlap into channels assigned to low abundance targets.

• Minimizes signal overlap to and from channels for variable expression targets.
Sources of Signal Overlap

Impurity: 0-4% in impurity channels
Oxides: 0-3% in M+16 channel
Abundance Sensitivity: 0-1% in M+/−1 channels
Signal Overlap

Flow Cytometry - 12 marker

Mass Cytometry - 32 marker

% Overlap

0 15

A488 A647 A700 A750 PacB PacO PE PCP-C5.5 Q605 Q655 Q705 Q800

A488 A647 A700 A750 PacB PacO PE PCP-C5.5 Q605 Q655 Q705 Q800

139La 141Pr 142Nd 143Nd 144Nd 145Nd 146Nd 147Sm 148Nd 149Sm 150Nd 151Eu 152Sm 153Eu 156Gd 158Gd 159Tb 160Gd 161Ho 162Dy 164Dy 165Ho 166Er 167Er 168Er 169Tm 170Er 171Yb 172Yb 174Yb 175Lu 176Yb
Maxpar® Panel Designer for Mass Cytometry

Input probes from:
- Fluidigm catalog
- Personal catalog

Generate Panel that minimizes signal overlap into low signal targets

Save and share panels/catalogs with collaborators
Logging into Panel Designer

Create account here:

http://www.dvssciences.com/login.php

Fluidigm promotes your account
Log in with username and password
Click Panel Designer icon
Begin Designing!
Coming Products
Coming Products

Human AML Phenotyping Panel Kit
Barcoding kit
Metal Conjugated Neutravidin
More new metals, antibodies, and panel kits
AML Phenotyping Panel Kit
Hu AML

MaxPar® Acute Myeloid Leukemia (AML) Panel Kit

Catalog #: XXXXX
Package Size: 25 tests

Contents:
MaxPar® Metal-Conjugated Antibodies (see table for panel)
MaxPar® Cell Staining Buffer (500 mL)
MaxPar® Fix and Perm Buffer (25 mL)
Cell-ID™ Intercalator – Ir (125 mM; 25 μL)
MaxPar® Water (500 mL)

Storage:
- Antibodies, Buffers, and Water: 4°C. Do not freeze.
- Intercalator-Ir: -20°C.

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Barcoding
Barcoding for multiplexing samples

Kit that allows multiplexing (ie combining) 20 samples into one tube prior to sample processing

Each sample is stained with a unique 3-digit Pd barcode

Benefits

• Improved data consistency as all 20 samples processed as one
• Increases throughput by reducing staining and acquisition time
• Use of Pd does not interfere with existing panel designs
• 3-digit barcoding enables gating out of cross-sample doublets
Barcoding: Workflow

- Stimulate cells
- Fix and perm
- Barcode
- Combine up to 20 samples in 1 tube
- Stain with Panel and Ir
- Collect data on CyTOF
- De-barcode
- Analyze data
‘Doublet-free’ Barcoding

3-digit barcoding enables elimination of cross-sample doublets

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<td>19</td>
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<td>20</td>
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Event#1: from Sample 1
Event#2: from Sample 1 & 7
Assigns each event in multiplexed file to its barcoded population
User filters out uncertain events
Results in separate fcs file for each barcoded sample
Barcoding: status

Beta sites engaged

Expect feedback in Q1 on

- Kit
- Protocol
- De-barcoder

Launch expected in Q2
Neutravidin
Neutravidin

Tetrameric protein with strong affinity for biotin ($K_d = 10^{-15} \text{M}$)

Contains lysine residue available for labeling with metal using amine-labeling chemistry

Uses for metal-conjugated form

- Secondary to bind biotinylated antibodies (note we already have anti-biotin that works really well)
- **Build tetrarmers that can bind to antigen specific T cells (unique application)**
Tetramers

Kleenerman et al., Nat Rev Immunol 2002)
Neutravidin: status

Preliminary feasibility data collected at beta sites show excellent results, but issue with background staining.

MBL has launched 166 biotinylated monomers for sale – this will enable customers to build a large array of tetramers once neutravidin is launched.

Launch when product is ready.
New Publications
New Publications Q3/4 2014


Single-Cell Workflow Example 1

Single-cell genomics

mRNA seq. (miRNA, gene expression)

Single-cell proteomics assay on CyTOF 2

RNAs or genes expressed in target cells

Metal label antibodies to coded proteins
Single-Cell Workflow Example 2

Single-cell proteomics assay on CyTOF 2 to define cell phenotypes of interest

Sort population to purity

Single-cell genomics
Thank you for your attention.

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Jeannie.Gaylor@fluidigm.com