Understanding Biological Heterogeniety through Mass Cytometry: Present and Future Directions

University of Virginia CyTOF Interest Group Meeting
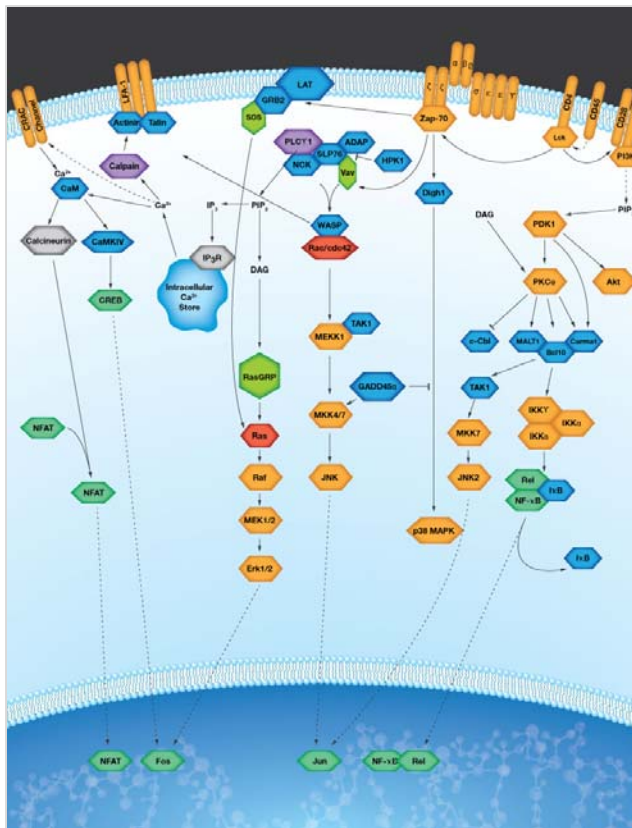December 5, 2014

Michelle Poulin
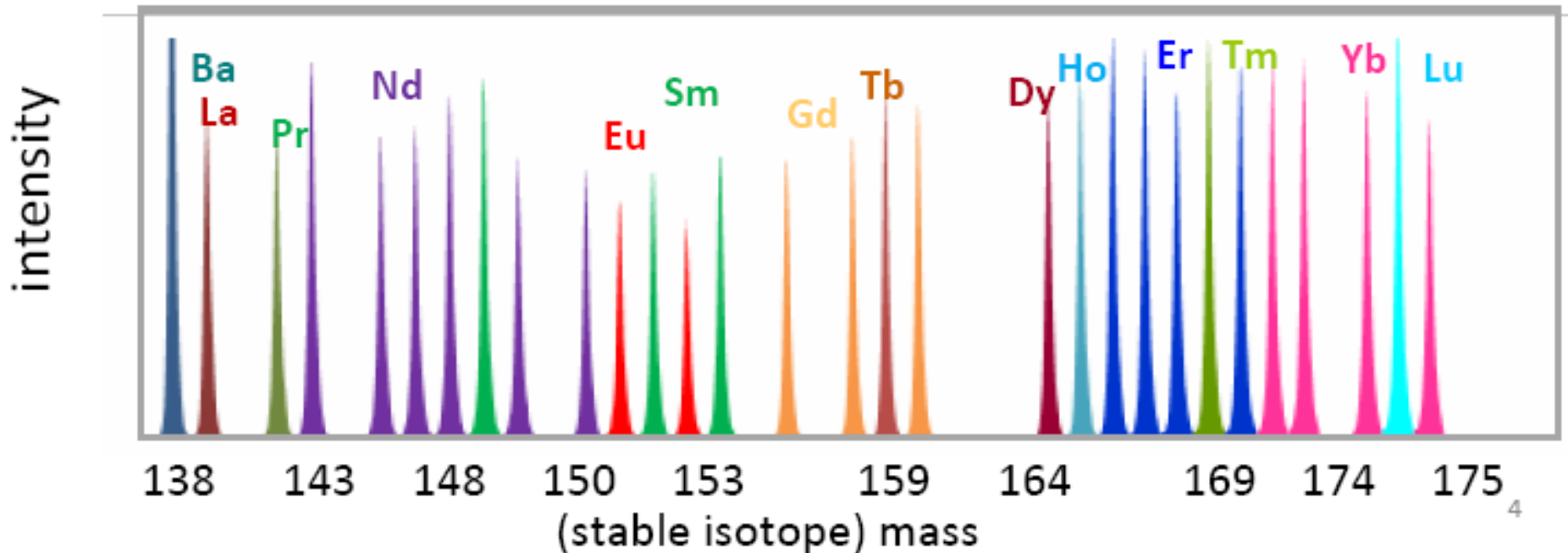
Field Applications Scientist

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.

FLUIDIGM

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

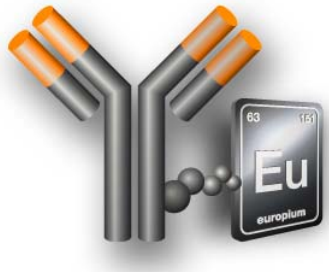FLUIDIGM®

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**

F FLUIDIGM®

CyTOF® Mass Cytometry Research

Single-Cell Signaling Signatures Correlate with Surgical Recovery

RESEARCH ARTICLE

SURGERY

Clinical recovery from surgery correlates with single-cell immune signatures

Brice Gaudillière,[1,2]* Gabriela K. Fragiadakis,[2,3]* Robert V. Bruggner,[2,4] Monica Nicolau,[2,5,6] Rachel Finck,[2,3] Martha Tingle,[1] Julian Silva,[1] Edward A. Ganio,[1] Christine G. Yeh,[1] William J. Maloney,[7] James I. Huddleston,[7] Stuart B. Goodman,[7] Mark M. Davis,[3] Sean C. Bendall,[2,3] Wendy J. Fantl,[2,3,8] Martin S. Angst,[1†‡] Garry P. Nolan[2,3†‡]

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?

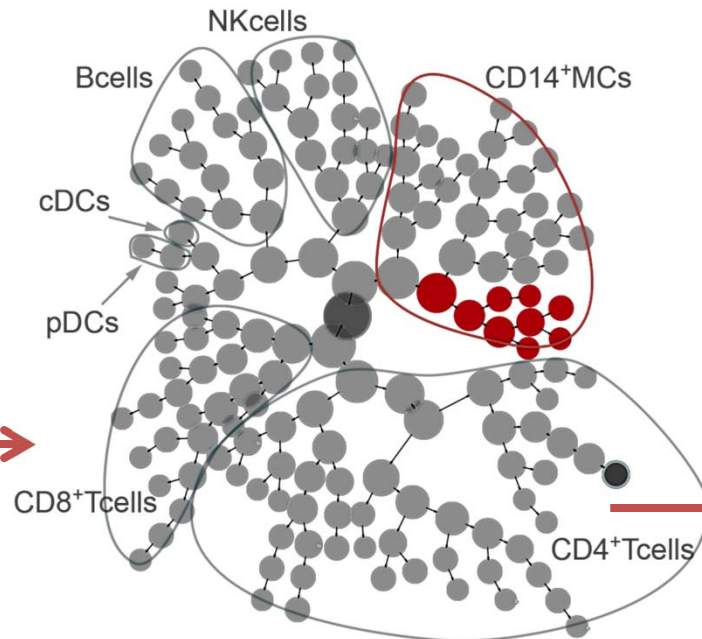# Correlation of Signaling Pathways with Clinical Recovery

Blood from 26 Hip Replacement Patients: baseline to 6 weeks

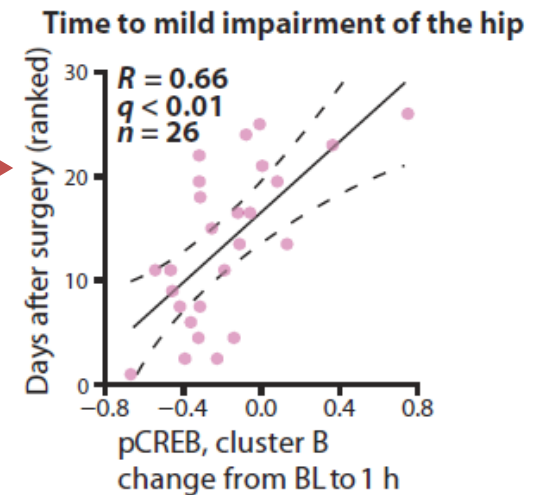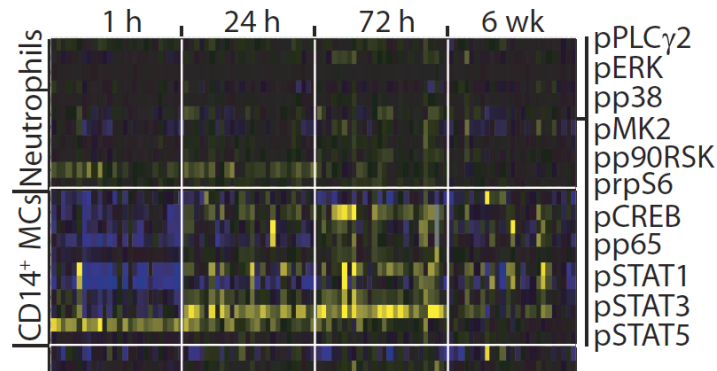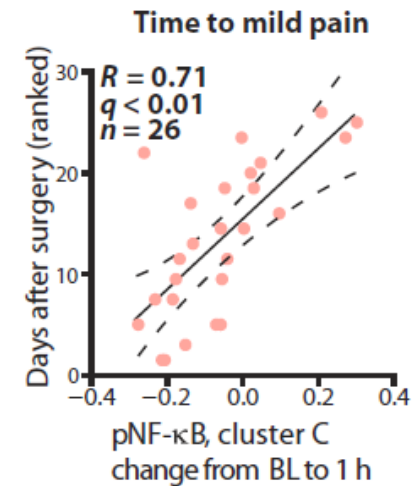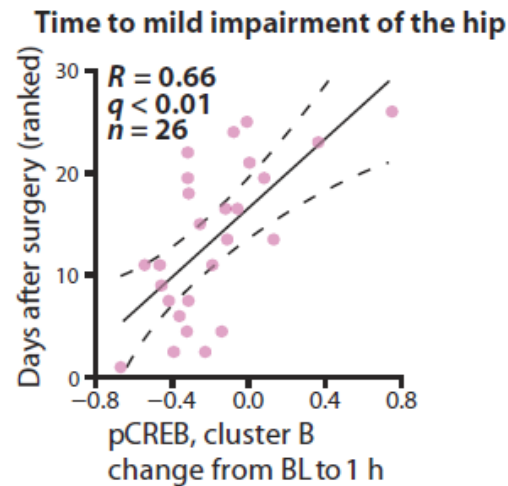Correlate cell types and signaling status to clinical recovery

Clustering (21)

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

Signaling (11)

pLCγ2	pCREB
pERK	pp65
pp38	pSTAT1
pMK2	pSTAT3
pp90RS	pSTAT5
prpS6	

NKcells
Bcells
CD14$^+$MCs
cDCs
pDCs
CD8$^+$Tcells
CD4$^+$Tcells

Time to mild impairment of the hip

$R = 0.66$
$q < 0.01$
$n = 26$

Days after surgery (ranked)

pCREB, cluster B change from BL to 1 h

1 h   24 h   72 h   6 wk

Neutrophils | CD14$^+$ MCs

pPLCγ2
pERK
pp38
pMK2
pp90RSK
prpS6
pCREB
pp65
pSTAT1
pSTAT3
pSTAT5

Gaudilliere *et al.*, *Sci. Transl. Med.* **6**, 131 (2014)

Correlation of Signaling Pathways with Clinical Recovery

Gaudilliere *et al.*, *Sci. Transl. Med.* **6**, 131 (2014)

Uniquely CYTOF®

- **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**
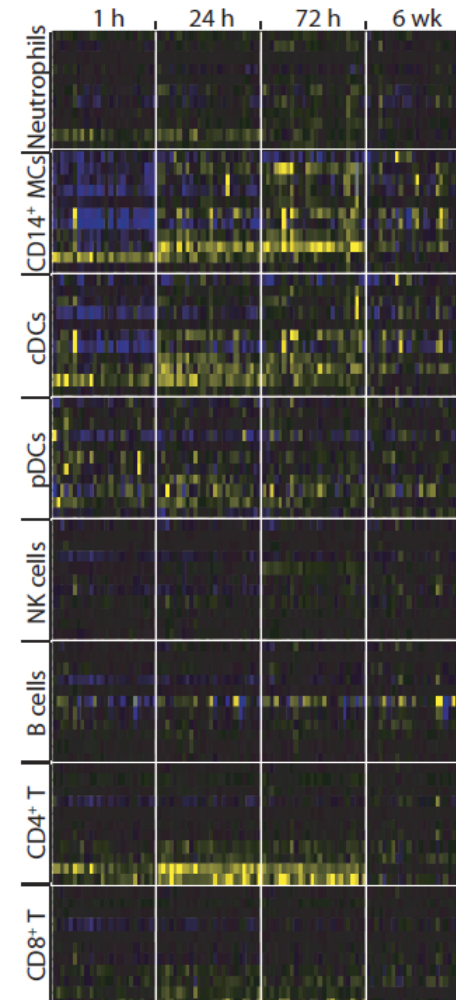
Gaudilliere *et al., Sci. Transl. Med.* **6**, 131 (2014)

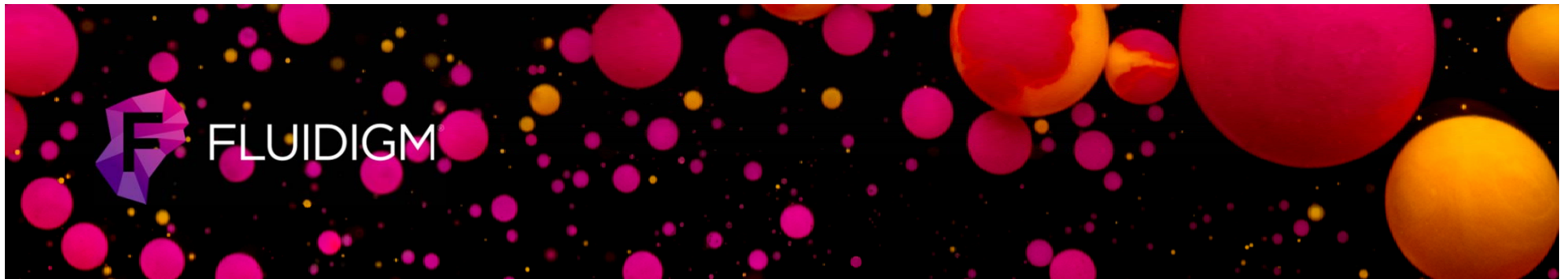# Roadmap

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

FLUIDIGM®

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

FLUIDIGM®

New Metals

Maxpar labeling kits and pre-conjugated antibodies

- 161Dy

- 163Dy

- 173Yb

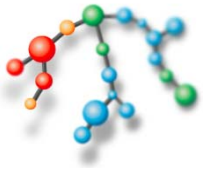Pre-conjugated antibodies

- 155Gd

- 89Y

Now 36 metals to build your panel

Panel Kits

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

FLUIDIGM®

13 Panel kits Now Available

Catalog No.	Name	Reactivity	Markers	Category
201304	Peripheral Blood	Human	17	CD and Surface Markers
201302	Peripheral Blood Basic I	Human	7	CD and Surface Markers
201315	Peripheral Blood Basic II	Human	7	CD and Surface Markers
201314	B Cells	Human	12	CD and Surface Markers
201305	T Cells	Human	16	CD and Surface Markers
201307	T Cells, Expansion	Human	10	CD and Surface Markers
201311	HSPC, Expansion	Human	7	CD and Surface Markers
201308	Intracellular Cytokine I	Human	11	Cytokines
201306	Spleen / Lymph Node	Mouse	16	CD and Surface Markers
201303	Spleen / Lymph Node Basic	Mouse	6	CD and Surface Markers
201310	Intracellular Cytokine I	Mouse	8	Cytokines
201309	Signaling I	Cross	7	Signaling and Transcription
201313	Cell Cycle and Proliferation	Cross	5	Cell Cycle and Proliferation

F FLUIDIGM®

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

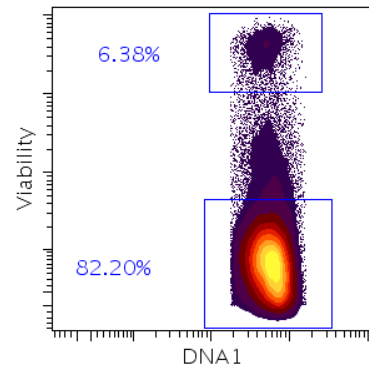Also available in 194Pt and 198Pt monoisotopic form

Fienberg *et al., Cytometry A* **81**, 467 (2012)
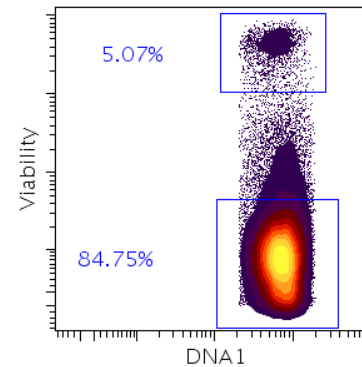
Cisplatin works with all staining protocols
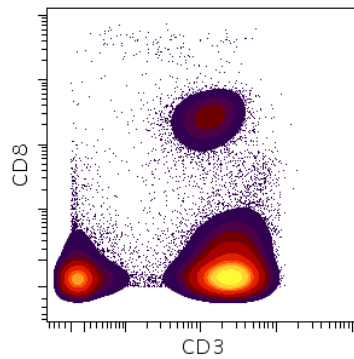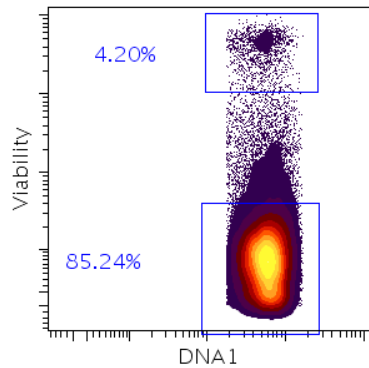
Surface Stain   **Cytoplasmic stain**   **Nuclear stain**
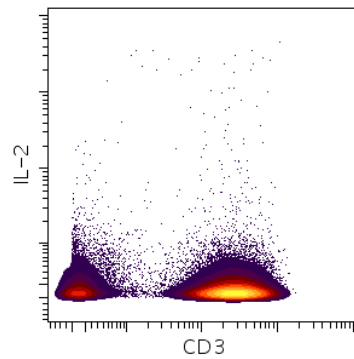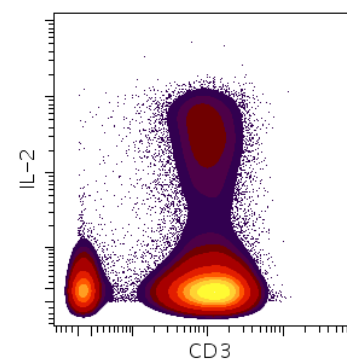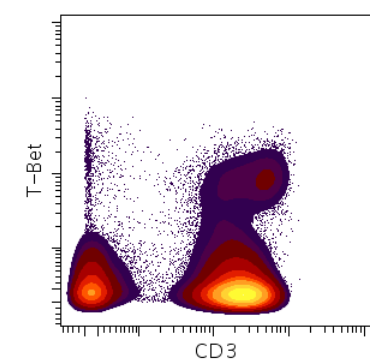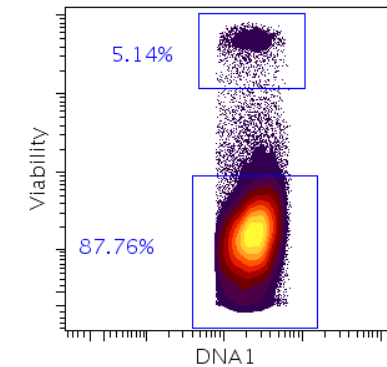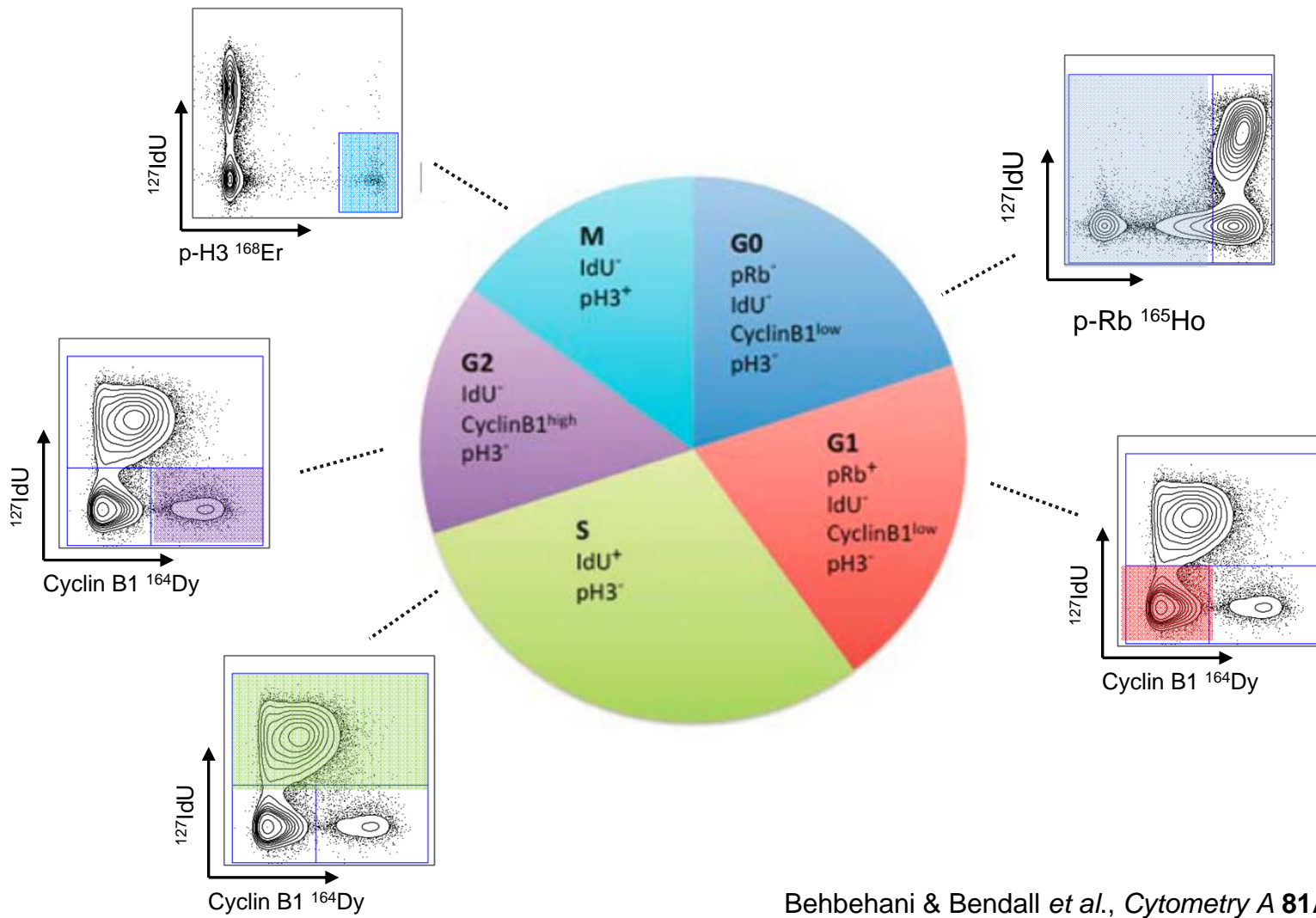
Cell-ID™ 127IdU

IdU = iododeoxyuridine

127I-containing pyrimadine 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
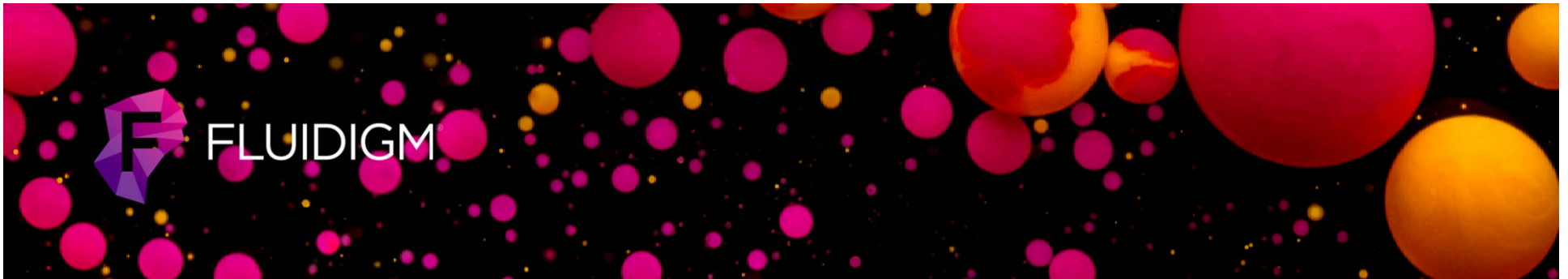
Easy to use compared to fluorescent assays

127IdU for Cell Cycle Analysis

Behbehani & Bendall *et al.*, *Cytometry A* **81A**, 552-566 (2012)

Maxpar® Panel Designer

Panel Designer Benefits

Optimal panel design

Simplifies choice of reagents

Improved data quality

FLUIDIGM®

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
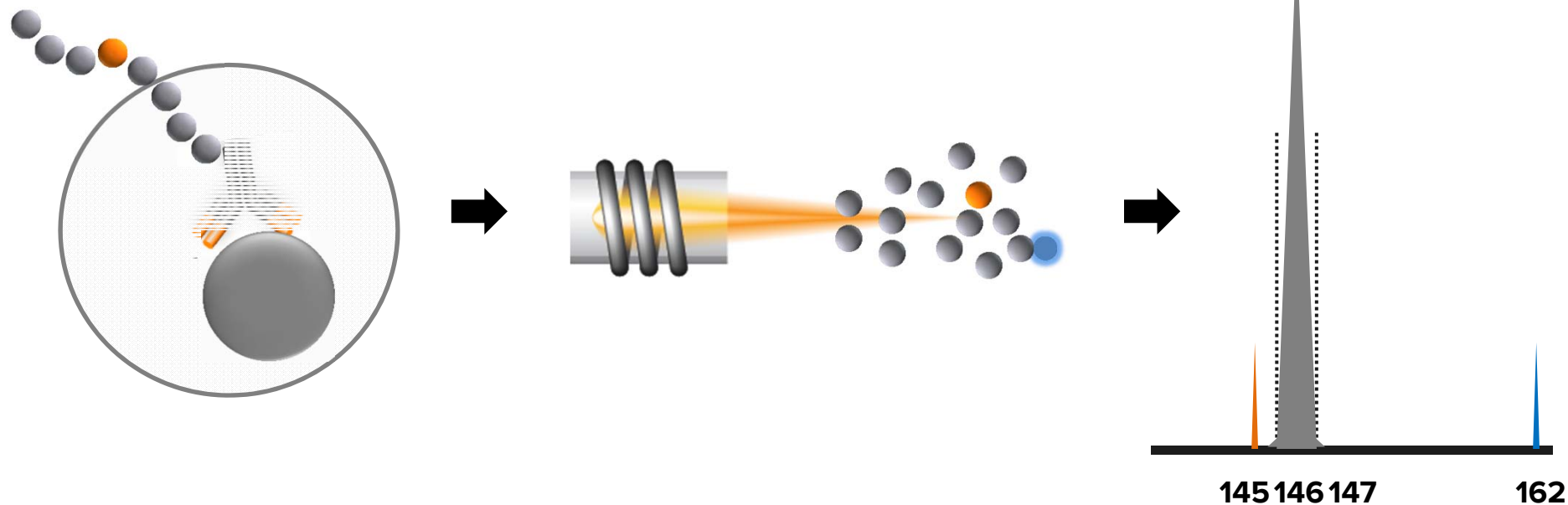
Sources of Signal Overlap

Impurity: 0-4% in impurity channels

Oxides: 0-3% in M+16 channel

Abundance Sensitivity: 0-1% in M+/-1 channels

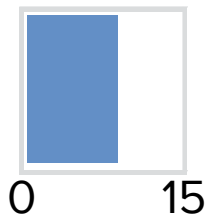145 146 147　　　162
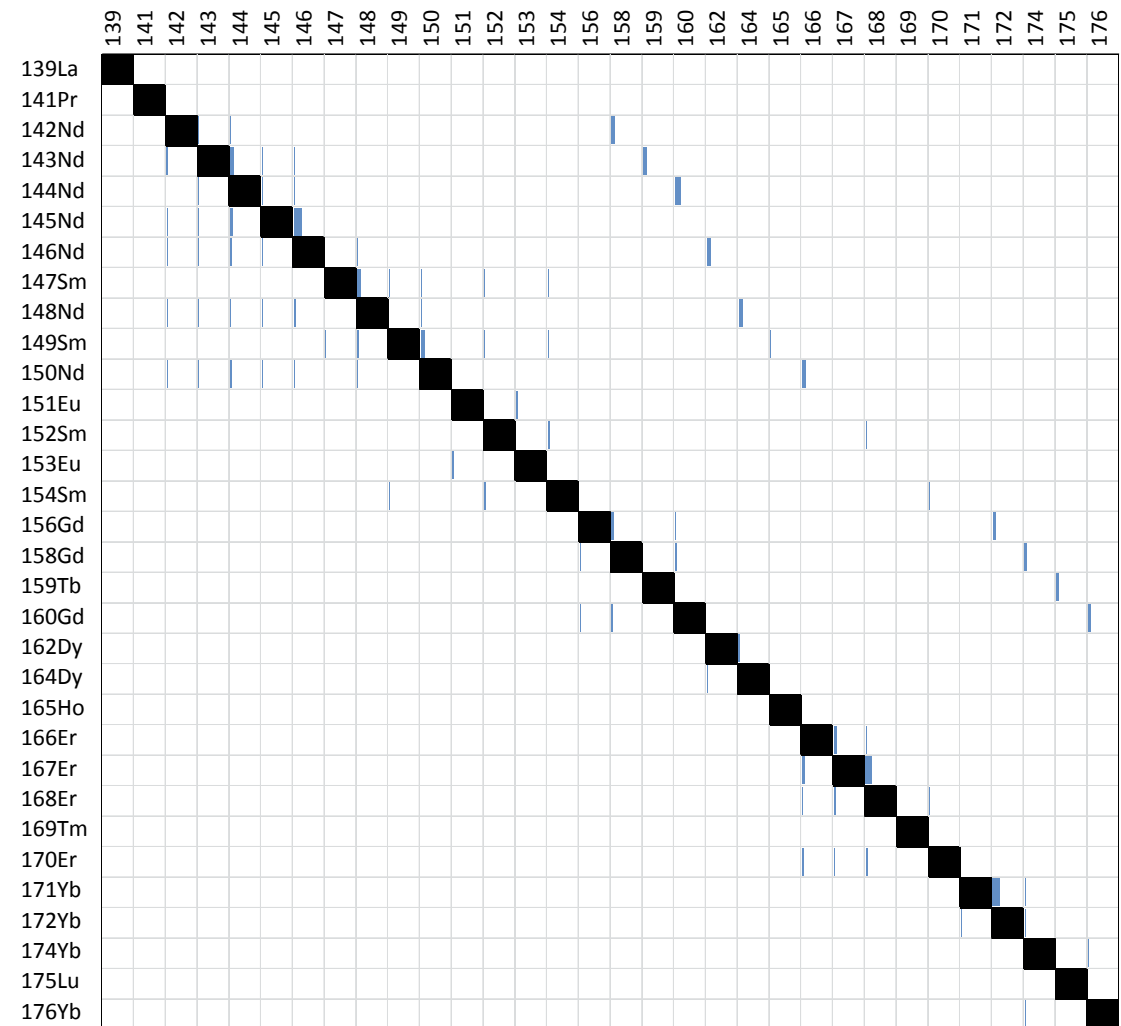

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Signal Overlap

Flow Cytometry - 12 marker
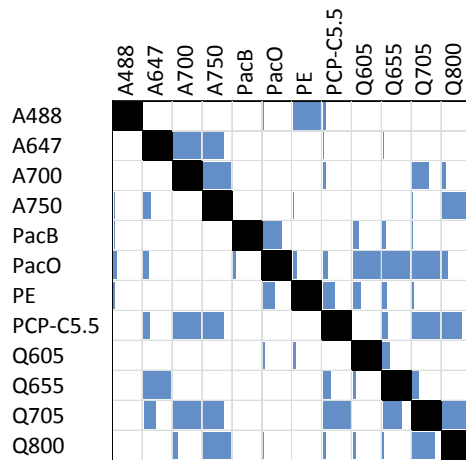
Mass Cytometry - 32 marker

% Overlap

0   15

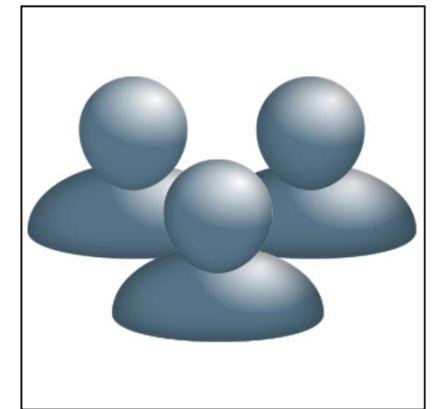# Maxpar® Panel Designer for Mass Cytometry

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
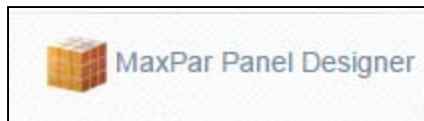
Create account here:

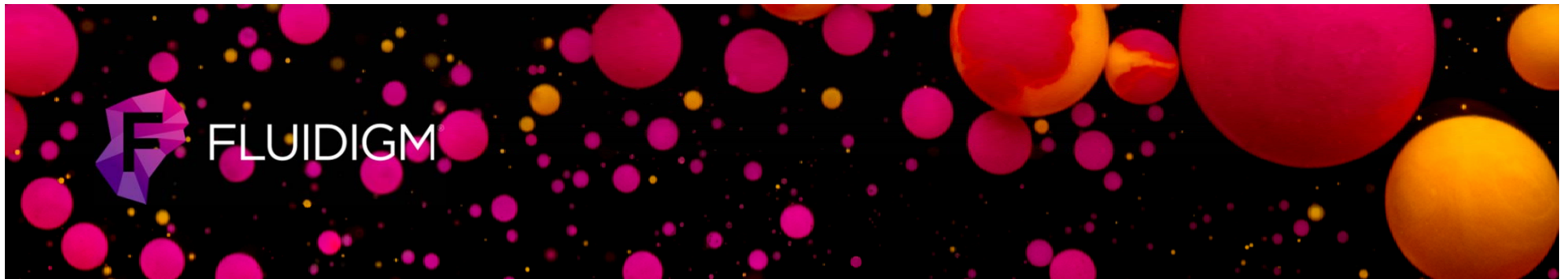   http://www.dvssciences.com/login.php

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
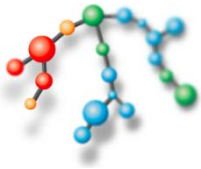
Barcoding kit

Metal Conjugated Neutravidin

More new metals, antibodies, and panel kits

FLUIDIGM®

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.

Target	Clone	Metal	Target	Clone	Metal
CD19	HIB19	142Nd	CD15	W6D3	164Dy
CD117	104D2	143Nd	CD34	581	166Er
CD11b	ICRF44	144Nd	CD3	UCHT1	170Er
CD64	10.1	146Nd	CD44	IM7	171Yb
CD7	CD7-6B7	147Sm	CD38	HIT2	172Yb
CD123	6H6	151Eu	HLA-DR	L243	174Yb
CD45	HI30	154Sm	CXCR4	12G5	175Lu
CD33	WM53	158Gd			

FLUIDIGM®
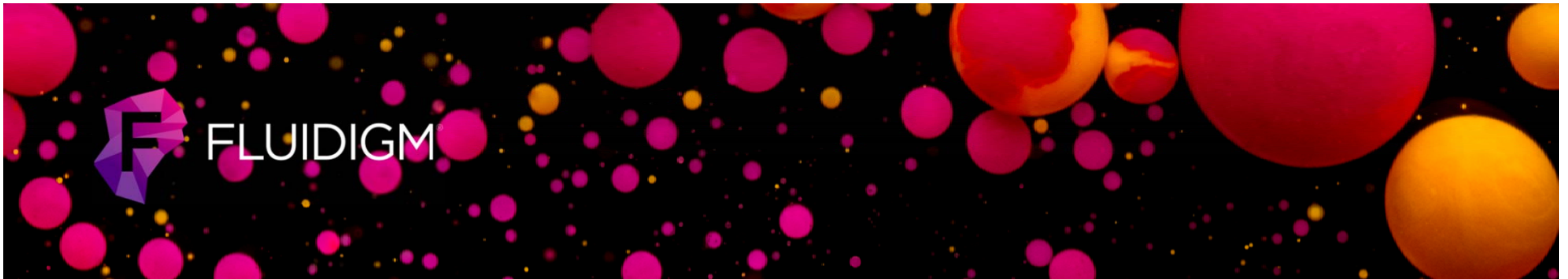
Barcoding

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

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Barcoding: Workflow

Stimulate cells

Fix and perm

Barcode

Combine up to 20 samples in 1 tube
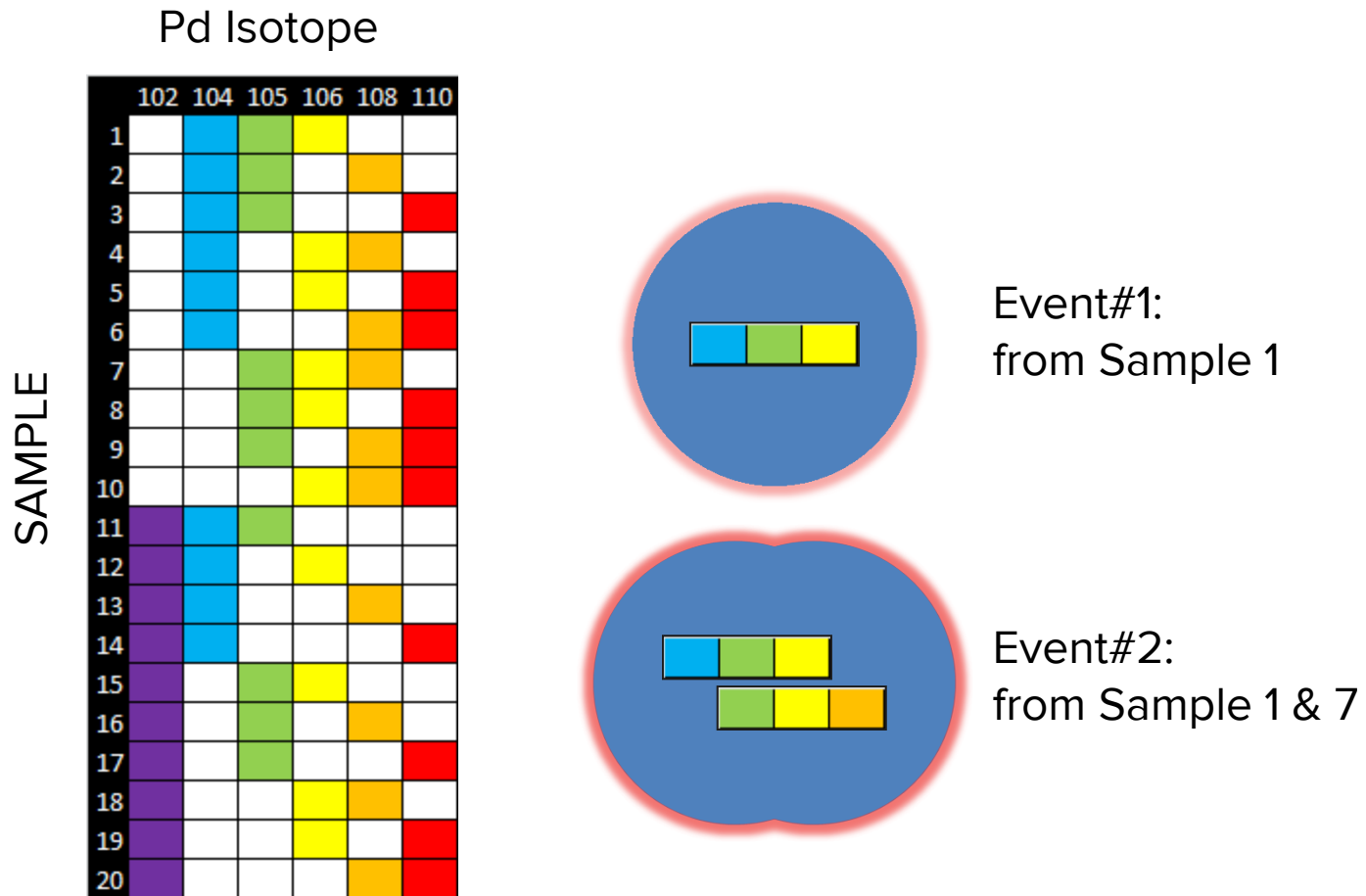
Stain with Panel and Ir

Collect data on CyTOF

De-barcode

Analyze data

FLUIDIGM®

'Doublet-free' Barcoding

3-digit barcoding enables elimination of cross-sample doublets

Pd Isotope

SAMPLE

Event#1:
from Sample 1

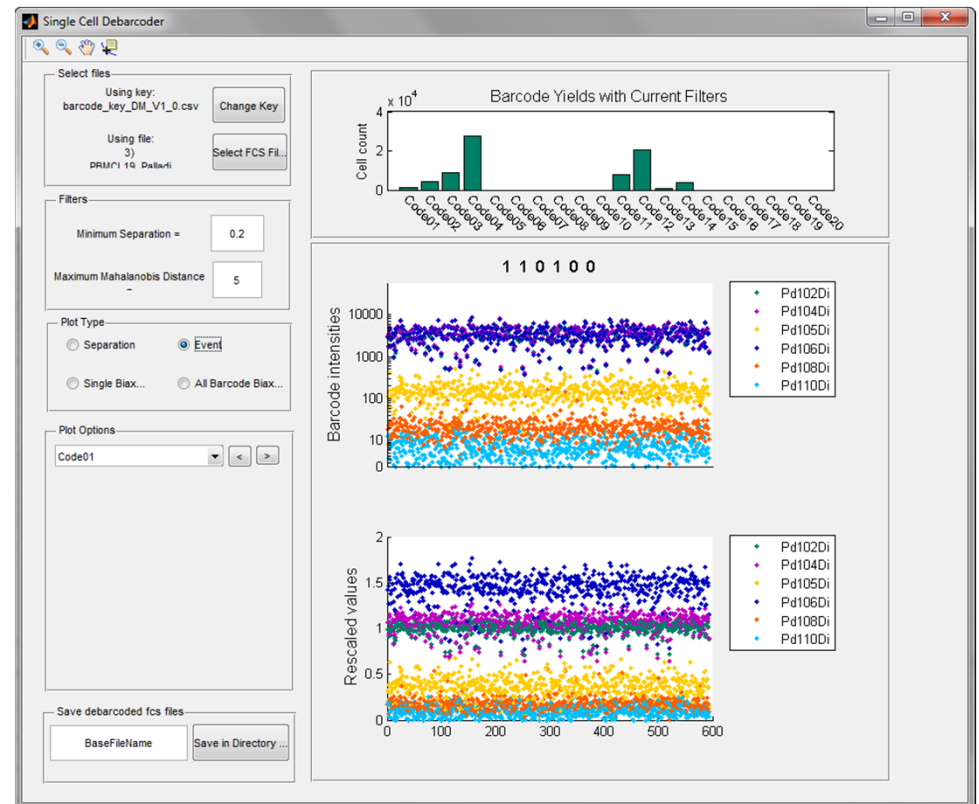Event#2:
from Sample 1 & 7

FLUIDIGM®

Single cell de-barcoder

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

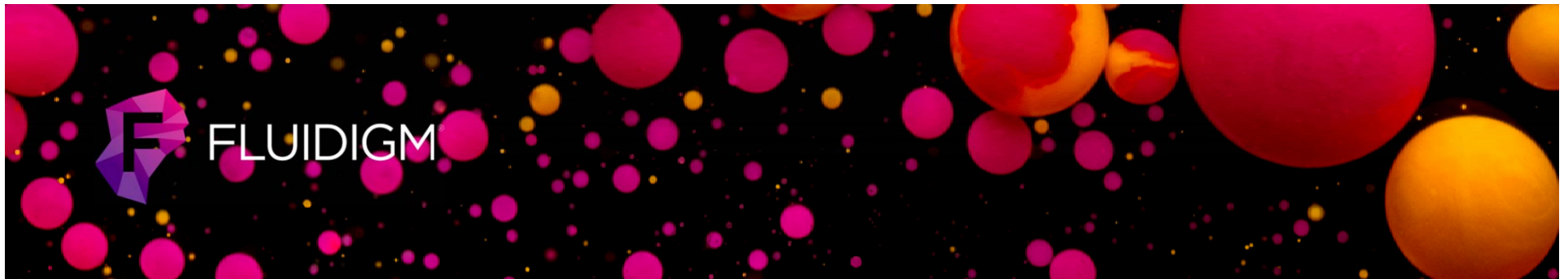Beta sites engaged

Expect feedback in Q1 on

- Kit

- Protocol

- De-barcoder

Launch expected in Q2

FLUIDIGM®

Neutravidin

Neutravidin

Tetrameric protein with strong affinity for biotin ($K_d = 10^{-15}$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 tetramers that can bind to antigen specific T cells (unique application)**

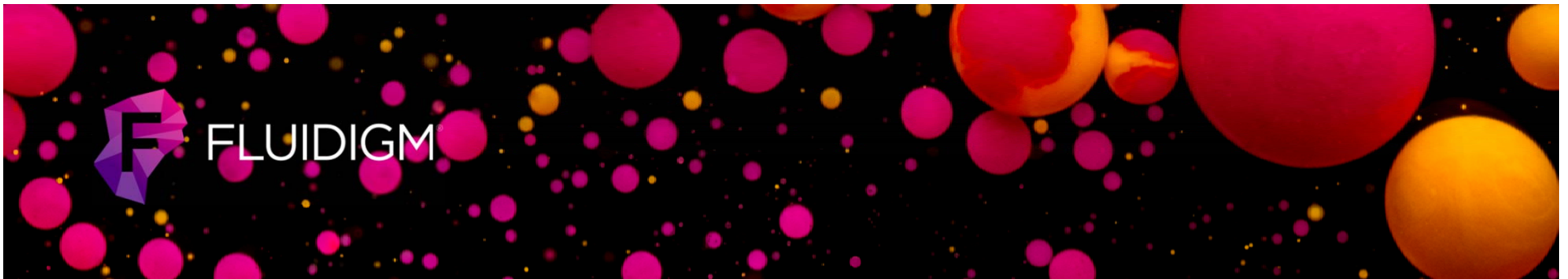FLUIDIGM

Tetramers
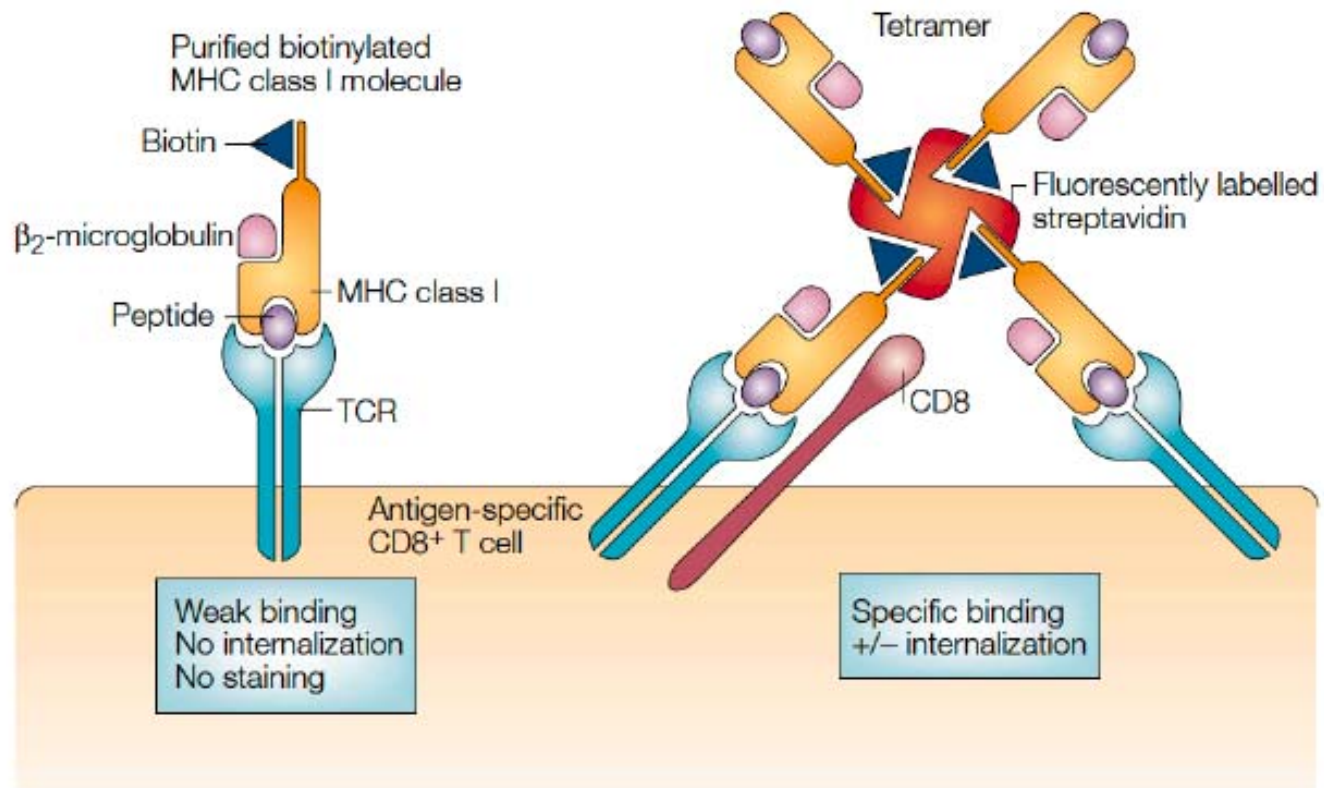
Neutravidin: status

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.
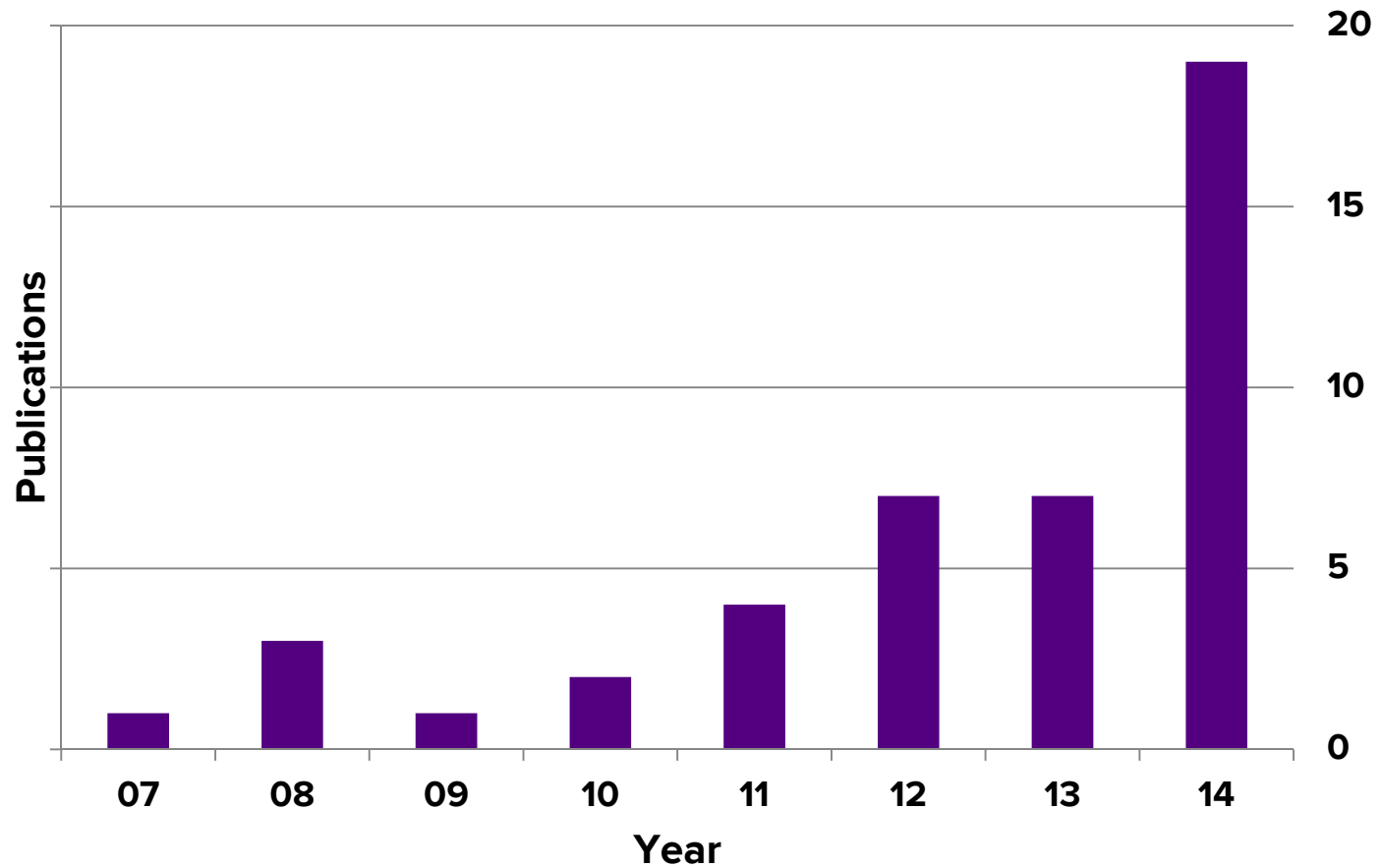
Launch when product is ready

New Publications

Publications

New Publications Q3/4 2014

Becher, B., et al. **High-dimensional analysis of the murine myeloid cell system**. Nat Immunol 2014.

Behbehani, G.K., et al. **Transient partial permeabilization with saponin enables cellular barcoding prior to surface marker staining**. Cytometry A 2014.

Chang, Q., et al. **Single-cell measurement of the uptake, intratumoral distribution and cell cycle effects of cisplatin using mass cytometry**. Int J Cancer 2014.

Edgar, L.J., et al. **Identification of hypoxic cells using an organotellurium tag compatible with mass cytometry**. Angew Chem Int Ed Engl 53 (43): 11473-11477, 2014.

Fergusson, J.R., et al. **CD161 Defines a Transcriptional and Functional Phenotype across Distinct Human T Cell Lineages**. Cell Rep 2014.

Gaudilliere, B., et al. **Clinical recovery from surgery correlates with single-cell immune signatures**. Sci Transl Med 6 (255): 255ra131, 2014.

Krishnaswamy, S., et al. **Conditional density-based analysis of T cell signaling in single-cell data**. Science 2014.

Mingueneau, M., et al. **Single-cell mass cytometry of TCR signaling: Amplification of small initial differences results in low ERK activation in NOD mice**. Proc Natl Acad Sci U S A 2014.

O'Gorman, W.E., et al. **The Split Virus Influenza Vaccine rapidly activates immune cells through Fcgamma receptors**. Vaccine 32 (45): 5989-5997, 2014.

Sen, N., et al. **Single-cell mass cytometry analysis of human tonsil T cell remodeling by varicella zoster virus**. Cell Rep 8 (2): 633-645, 2014.

Sachs, Z., et al. **NRASG12V oncogene facilitates self-renewal in a murine model of acute myelogenous leukemia**. Blood 2014.

Strauss-Albee, D.M., et al. **Coordinated Regulation of NK Receptor Expression in the Maturing Human Immune System**. J Immunol 2014.

Swadling, L., et al. **A human vaccine strategy based on chimpanzee adenoviral and MVA vectors that primes, boosts, and sustains functional HCV-specific T cell memory**. Sci Transl Med 6 (261): 261ra153, 2014.

Yao, Y., et al. **CyTOF supports efficient detection of immune cell subsets from small samples**. J Immunol Methods 2014.

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Single-Cell Workflow Example 1

Single-cell genomics
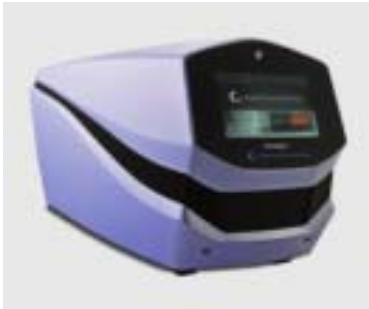
mRNA seq. (miRNA, gene expression)

RNAs or genes expressed in target cells
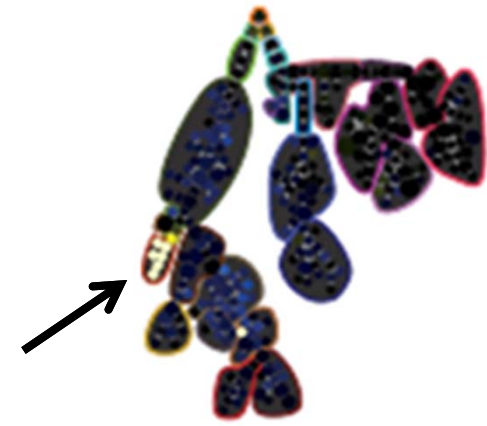
Metal label antibodies to coded proteins

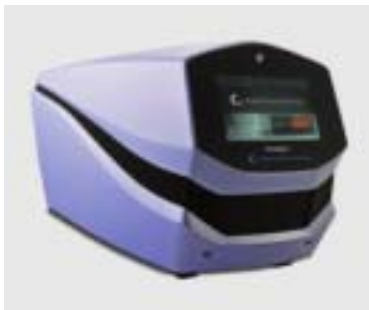**Single-cell proteomics assay on CyTOF 2**

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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.

Michelle.Poulin@fluidigm.com

Jeannie.Gaylor@fluidigm.com

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