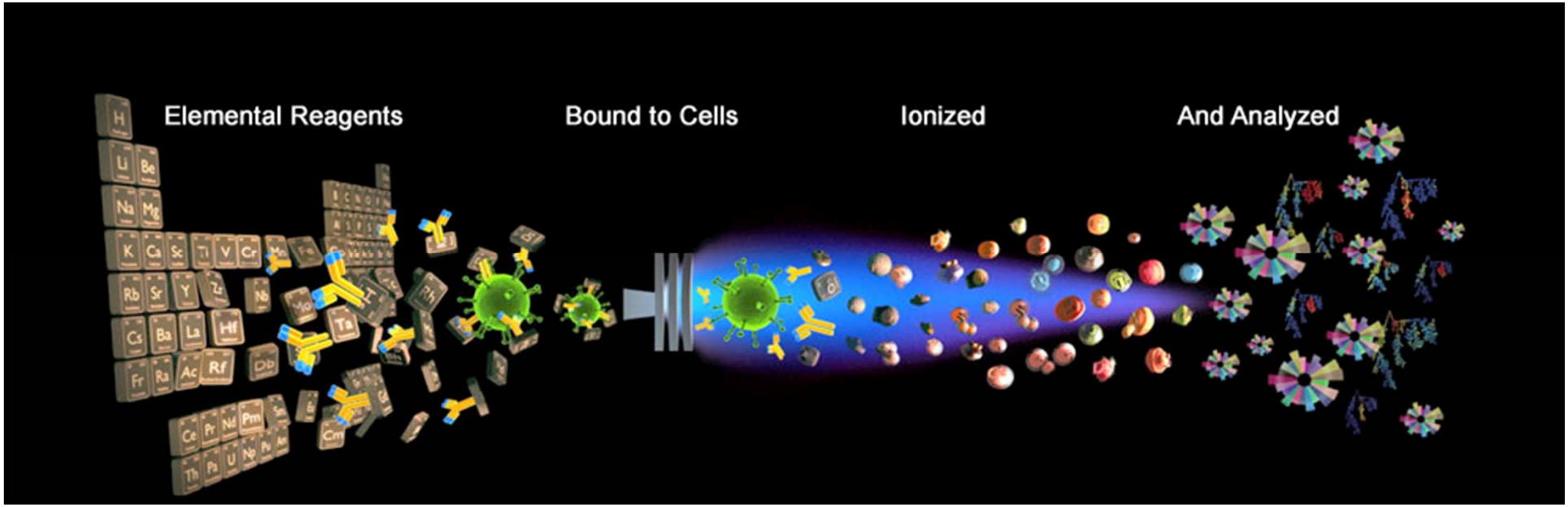
UNIVERSITY OF BERGEN

Flow Cytometry Core Facility

Discovery and Functional Profiling with Mass Cytometry

High order multiplexing of biological samples – from suspension to tissue

Jørn Skavland, PhD



Helios Mass Cytometer

Helios System

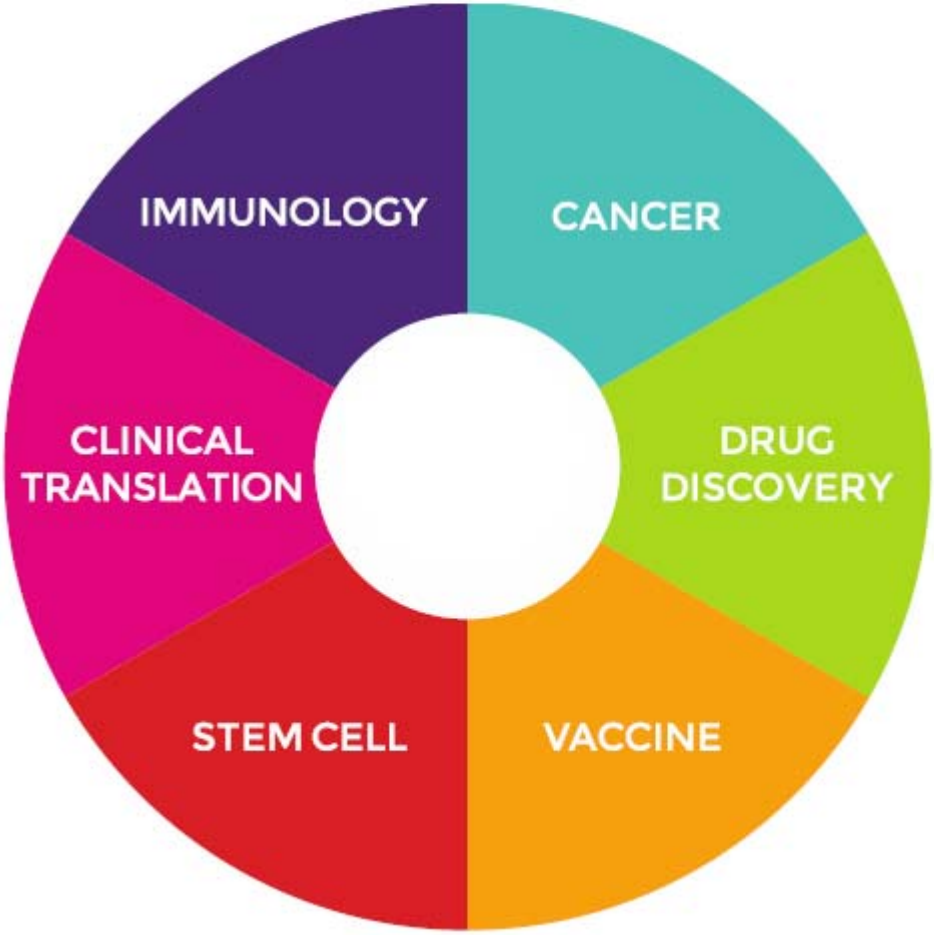
Channels	135
Mass range	75–209 amu
Abundance sensitivity	0.3% for ^{159}Tb
Instrument response	600,000 counts/pg ^{159}Tb
Detection limit	350 antibodies/cell
Dynamic range	4.5 orders of magnitude
Calibration	Automated
Operating system	Windows® 7 Pro 64-bit
Data storage	7.2TB HDD RAID (mirrored)
Sample introduction	Pneumatic single tube loader with agitation, up to 5 ml volume
Peak throughput	2,000 (events/sec)
Flow rate	30–45 (µL/min)
Replicate sample CV (normalized)	<3%

CyTOF Research

Applications

- Phenotyping
- Signaling and transcription
- Cytokines and growth factors
- Cell death and apoptosis
- Cell cycle and proliferation

Research areas

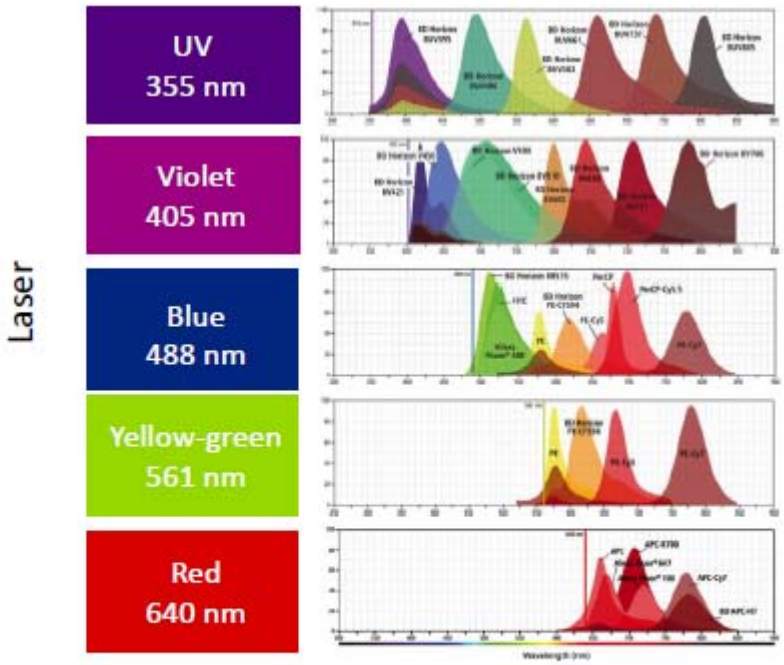


Antibody-mediated multiparameter protein detection

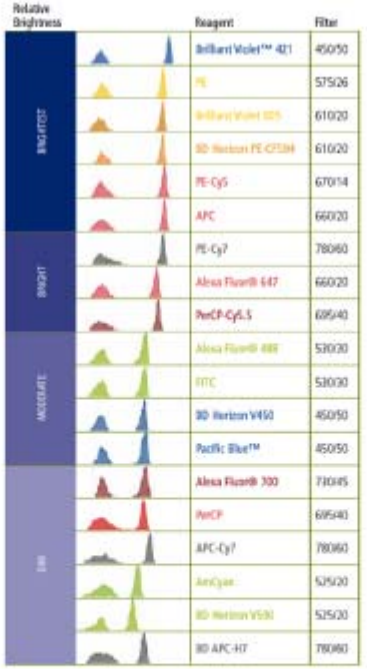
Today's gold standard

Fluorochrome-conjugated antibodies are widely used but have limited utility for high-parameter studies. These limitations impart significant complexities in experimental design and interpretation.

Fluorescence 'spillover' | Variable staining intensities | Background signal



Emission: fluorescence spillover



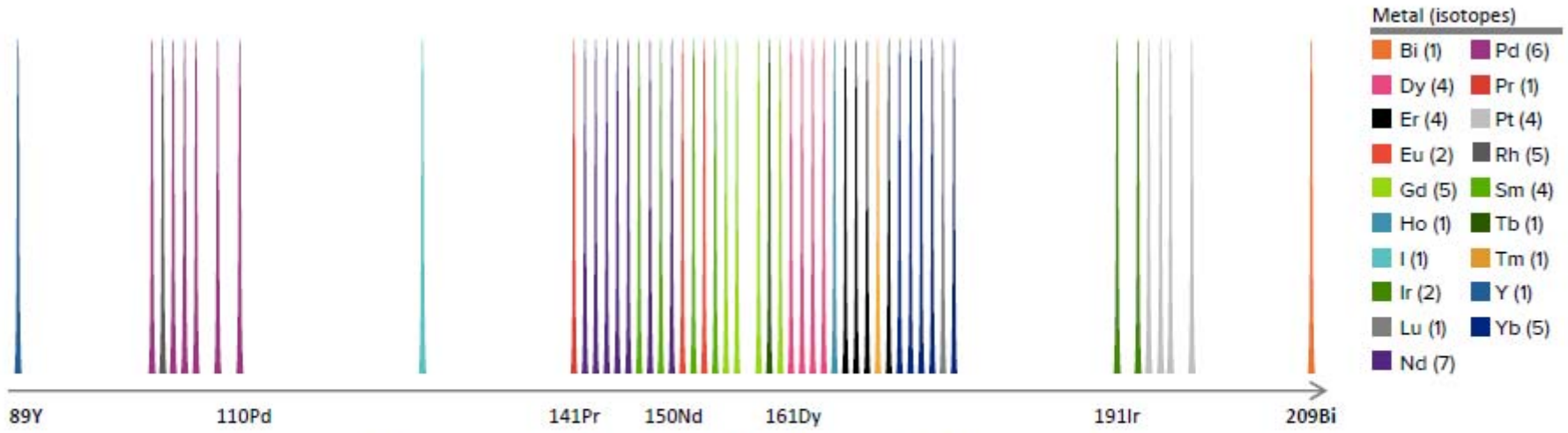
Staining intensities and background

CyTOF Technology

The new standard for high-parameter protein detection

CyTOF® technology overcomes the limitations of fluorescence-based detection modalities by separating signals based on differences in mass instead of wavelength.

Separate and distinct signals | Uniform staining | No background



Highly pure, rare metal isotope labels that minimize background

Helios™ and Hyperion™ Imaging System

Powered by CyTOF technology

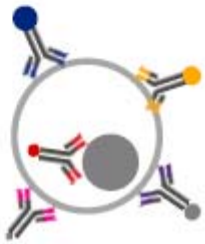
Helios™
Mass Cytometry

Hyperion™
Imaging Mass Cytometry

CyTOF Workflow

1

Select
Maxpar® panel and prepare mixture from individual antibodies.

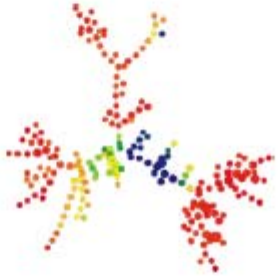


2

Stain
cells using protocols and buffers validated by Fluidigm.

3

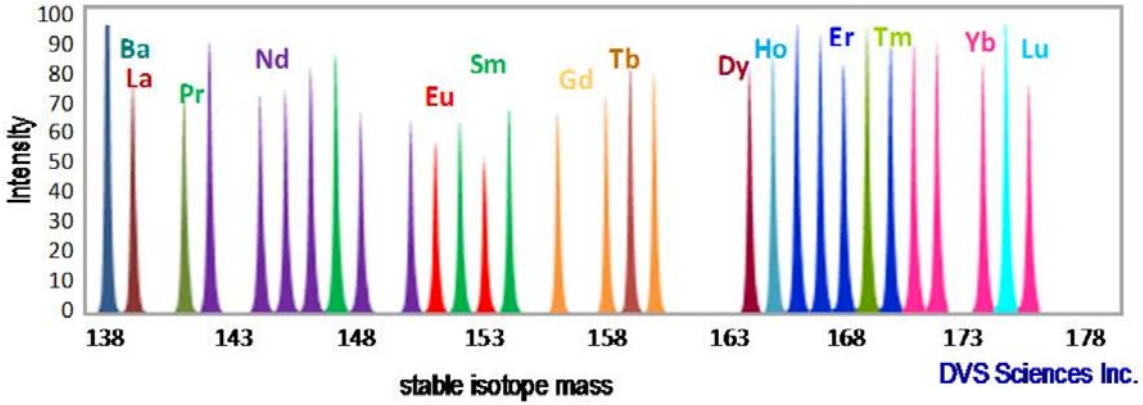
Acquire
high-parameter data for millions of cells with the Helios™ mass cytometer.

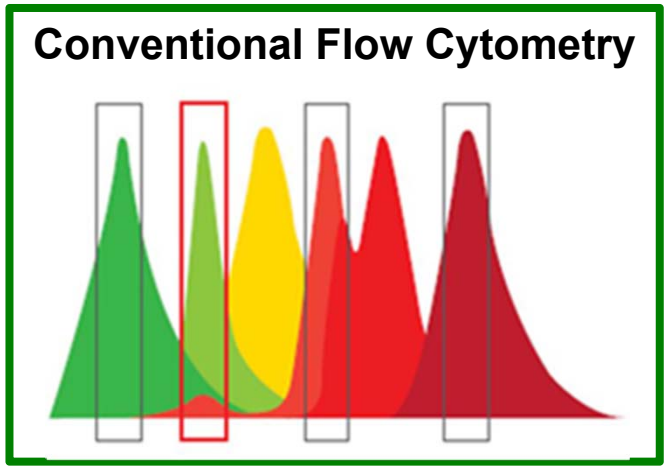


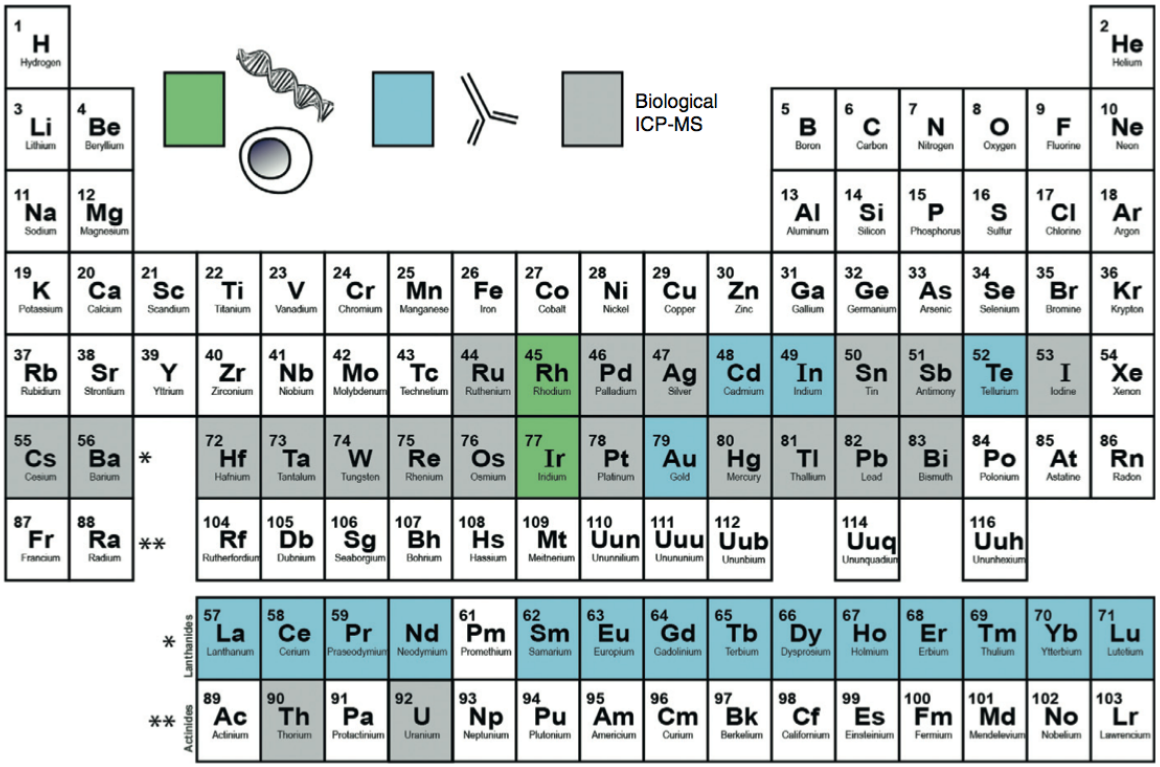
4

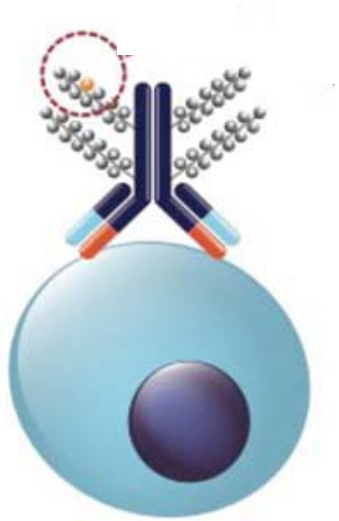
Analyze
data using proven analytical tools.

Mass Cytometry – Method









Bendall S et al. Trends in Immunology 2012

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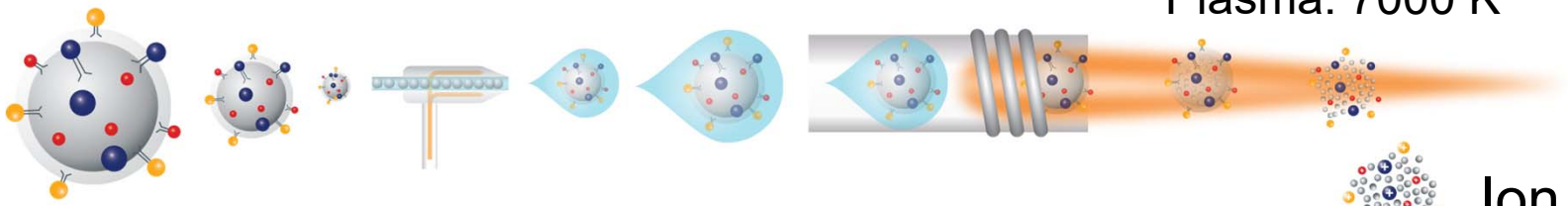
Introduction – Mass Cytometry

- Barcode, Abs, Ir-DNA, Live/Dead

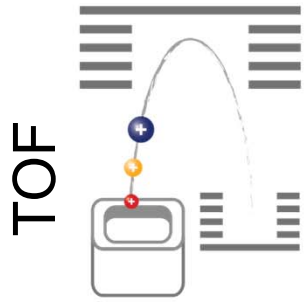


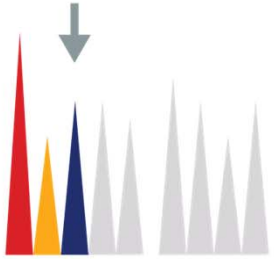
Plasma: 7000 K

Ion cloud^{+}

Quadropole

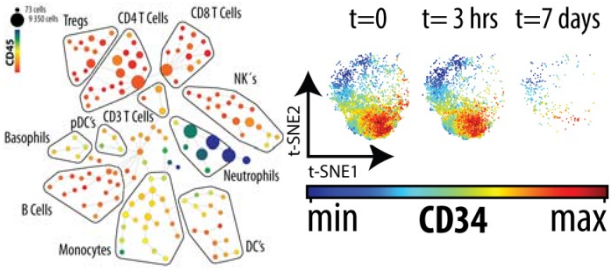
- Removes lighter atoms (C, N, O etc.)

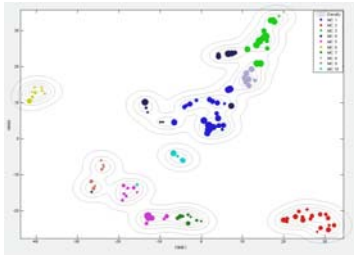




Normal FCS Files

SPADE, t-SNE and PhenoGraph





Data acquisition for Helios (pptx), Fluidigm (2015)

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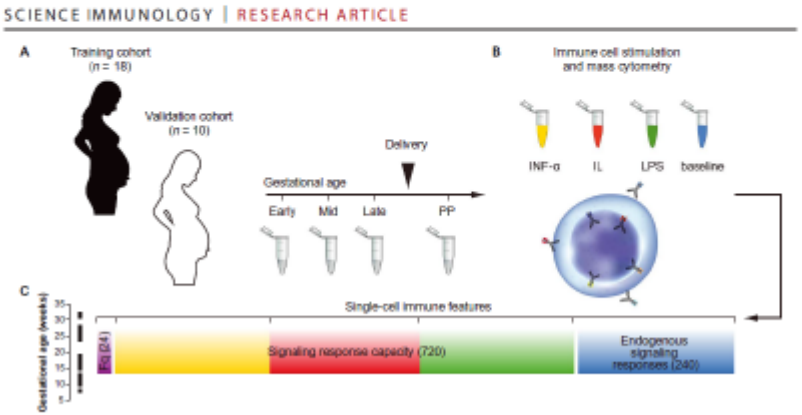
Many high-impact papers with breakthrough findings

"An immune clock of human pregnancy."

Science Immunology (2017)

Aghaeepour, N. et al.

Stanford MEDICINE

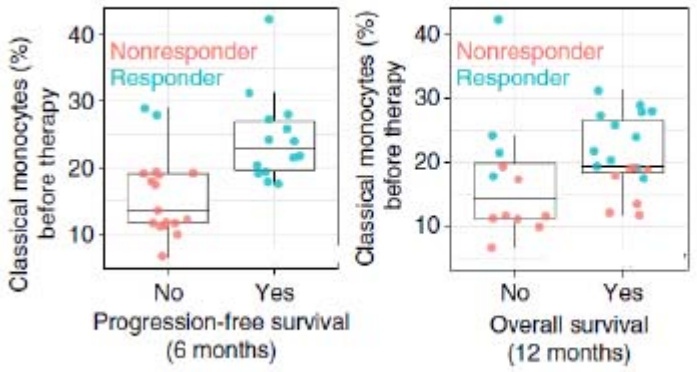


"High-dimensional single-cell analysis predicts response to anti-PD-1 immunotherapy."

Nature Medicine (2018)

Krieg, C. et al.

University of Zurich[UZH]



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Summary - Suspention

Mass cytometry:

- **Increases** the number of measurements possible on single cells (up to 54 currently)

- Possible because of **metal** conjugated **antibodies** and the resolution of the **mass spectrometer**

- Generates **complex** data: Need data **visualization** tools/algorithms, like ViSNE

Hyperion Imaging System

Highly multiplexed immunohistochemistry from FFPE, frozen tissue sections or cell smears

Comprehensive
Highly multiplexed IHC enables simultaneous detection >37 protein markers.

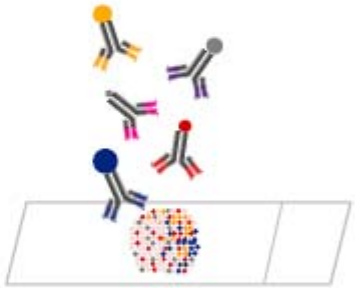
Simple
Stain samples with all antibodies simultaneously using conventional IHC protocols

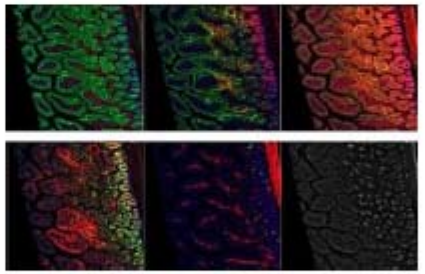
Contextual
Get subcellular resolution while preserving information about tissue architecture and cellular morphology.

Powerful
Preserve precious samples and reduce variability by eliminating dependence on serial sections.

Capture	Detection
Hyperion™ Tissue Imager	CyTOF technology

Hyperion Imaging System Workflow





1

Design
panels using IHC-validated antibodies conjugated to metal tags.

2

Stain
tissues (FFPE and frozen) or fixed cells with metal-conjugated antibodies.

3

Image
biomarkers using precise laser-directed protein capture and detection with CyTOF technology.

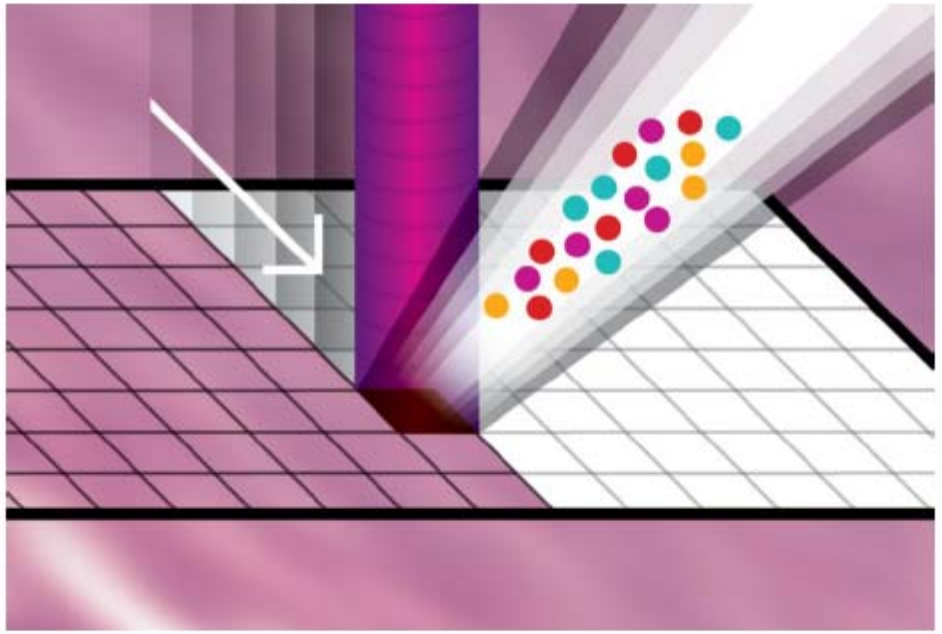
4

Analyze
using post-analytical imaging and secondary analysis software tools.

How Imaging with the Hyperion Imaging System works

Load the sample into the Hyperion Imaging System

Precise laser imaging of the region of interest

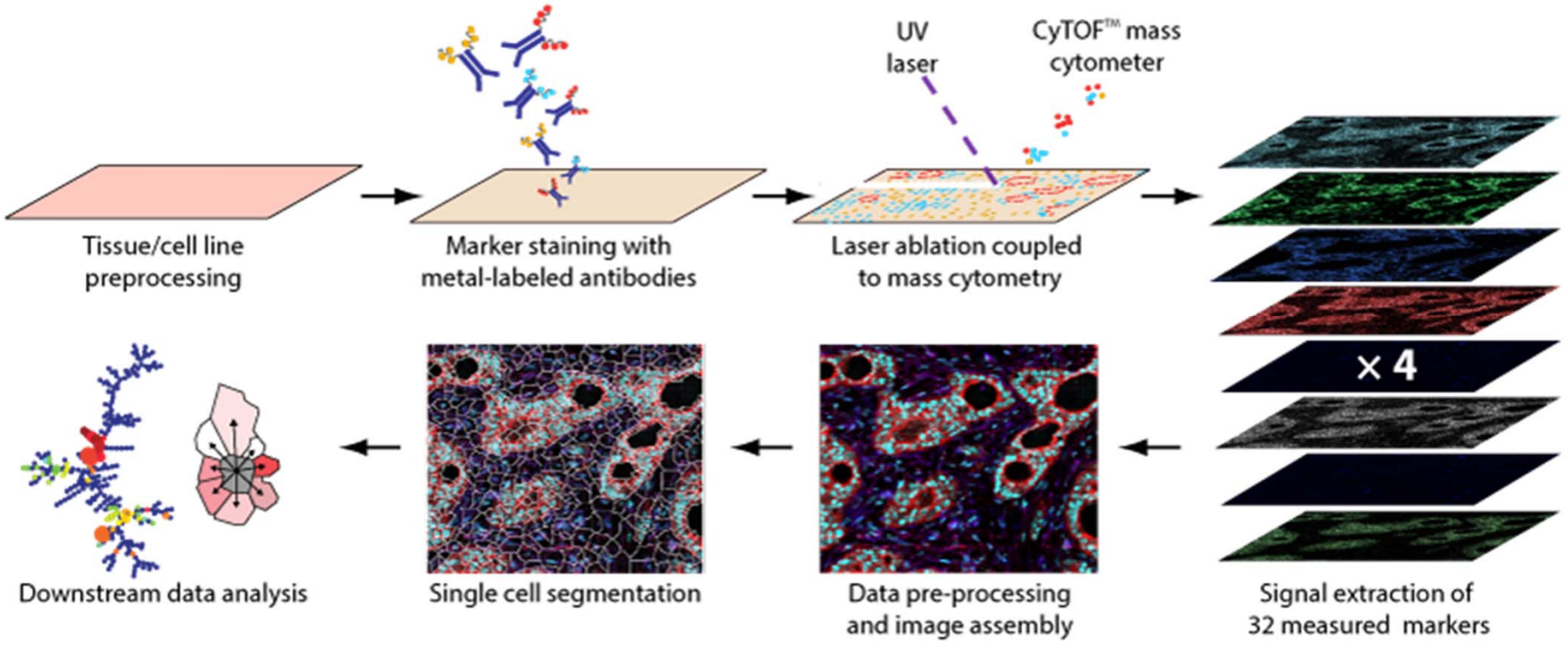


Laser beam focuses on 1 μm^2 spots in the selected region

Collects metal-tagged proteins

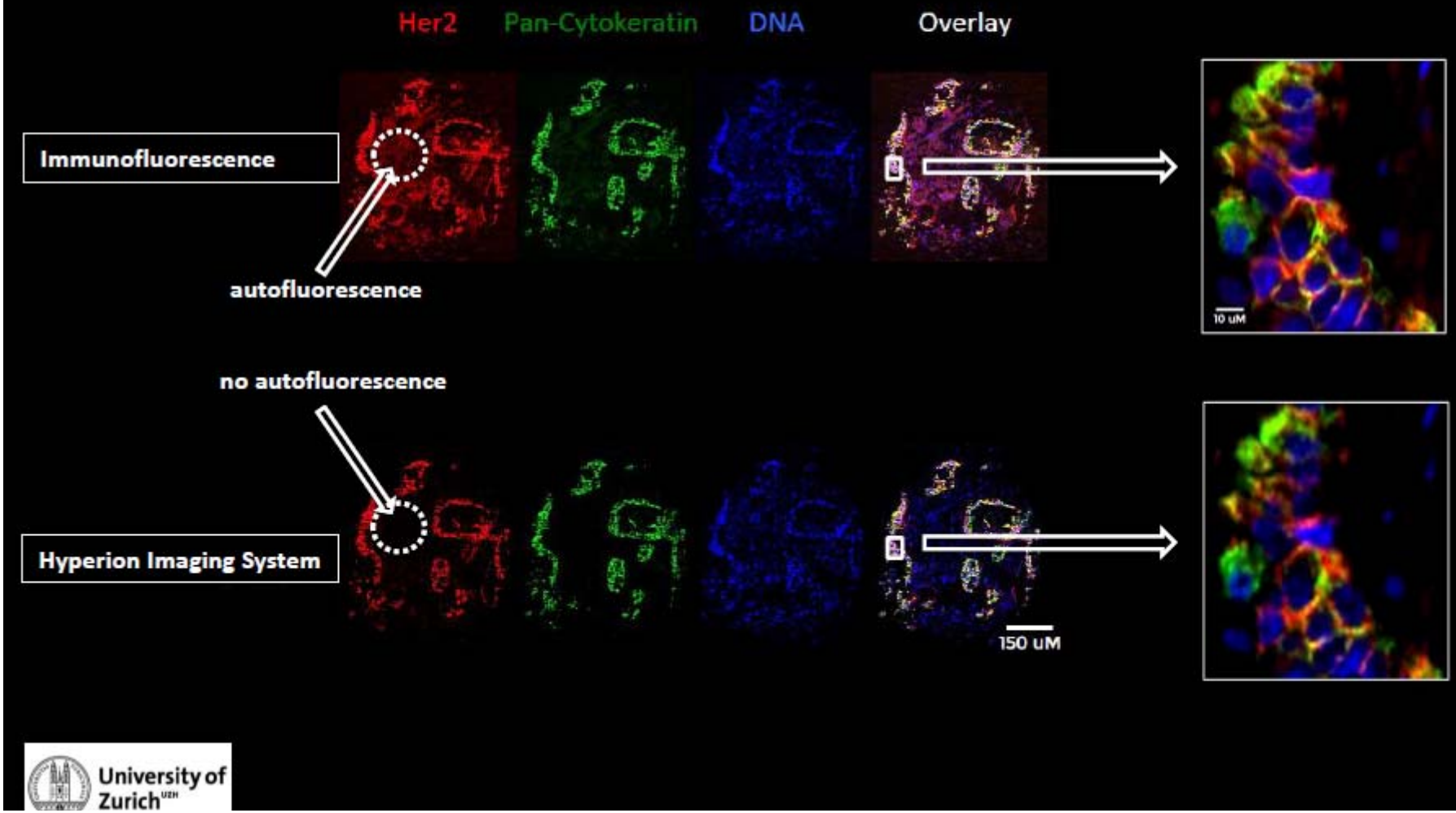
Sends for CyTOF analysis

Image and single cell segmentation

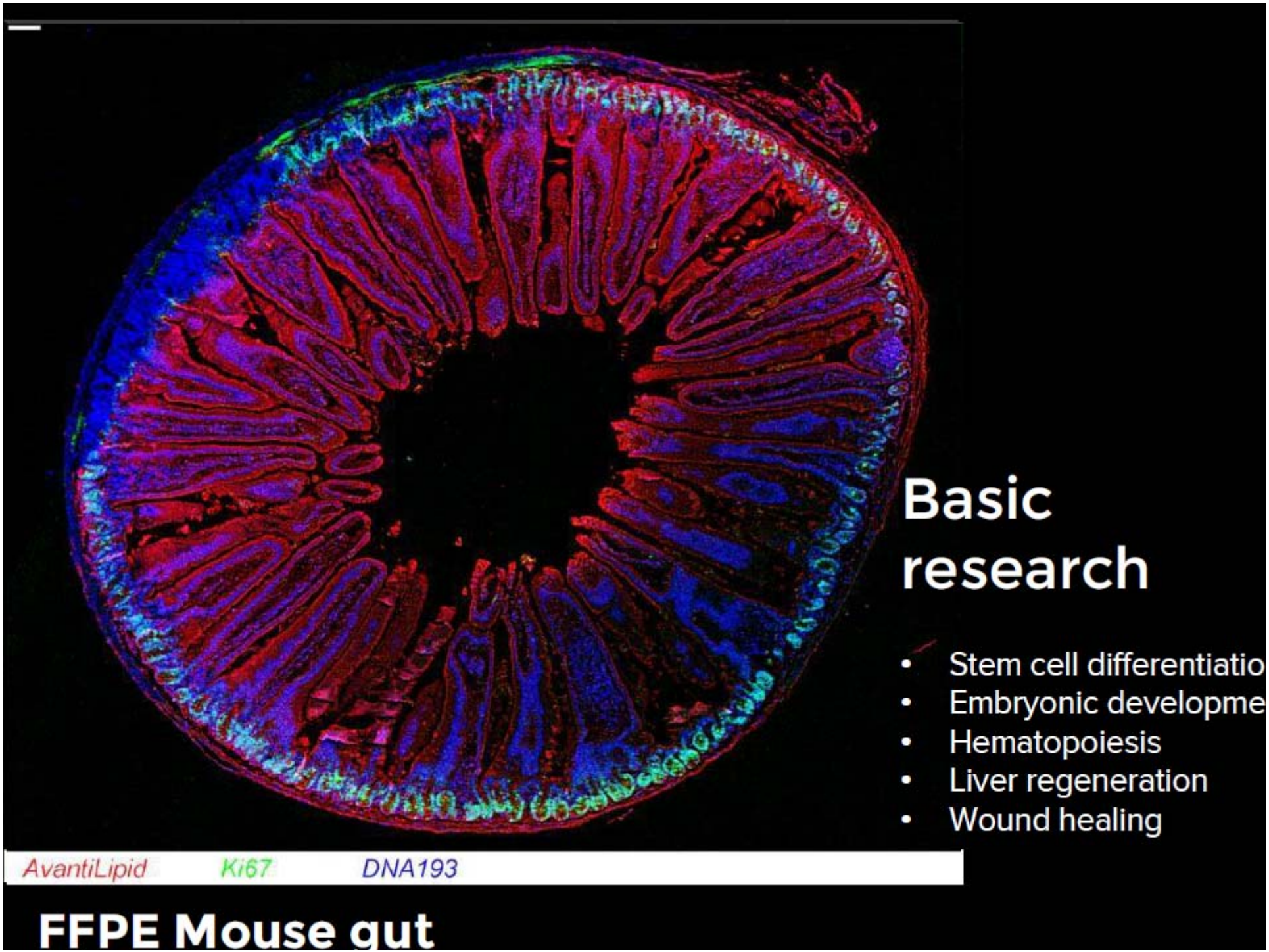


Cross-platform registration experiment: immunofluorescence vs. Hyperion in same tissue section

– Courtesy of Dr. Bernd Bodenmiller, University of Zurich

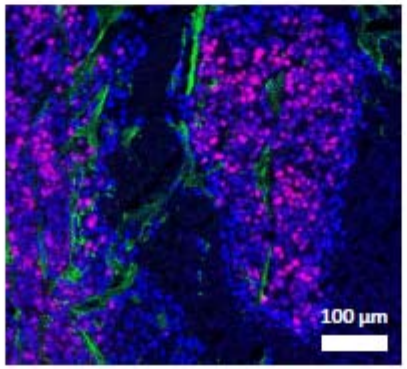


University of Zurich[UZH]


Basic research
• Stem cell differentiatio
• Embryonic developme
• Hematopoiesis
• Liver regeneration
• Wound healing
AvantiLipid
Ki67
DNA193
FFPE Mouse gut

Hyperion Imaging analysis pipeline

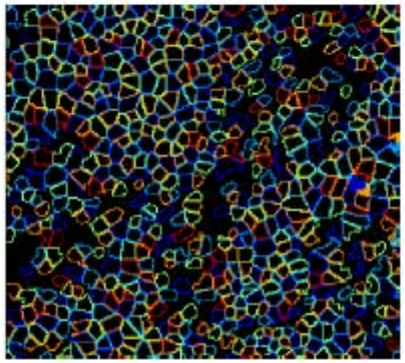
A three step workflow using MCD Viewer and histoCAT™



1

View and Validate

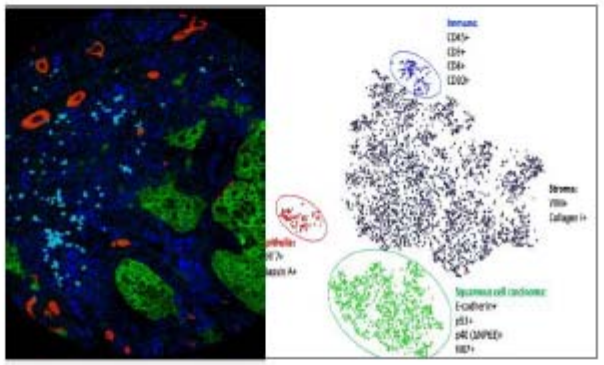
Detect presence of protein(s) of interest, produce high quality plots with chosen combinations of markers and confirm experimental success. Export files in various formats.



2

Image segment

Segment specific cell populations of interest. Quantify differential expression of the epitope within the cells and across cell population.



3

Higher order analysis

Differential expressions within a cell and between cell populations with statistical significance and in spatial context.