

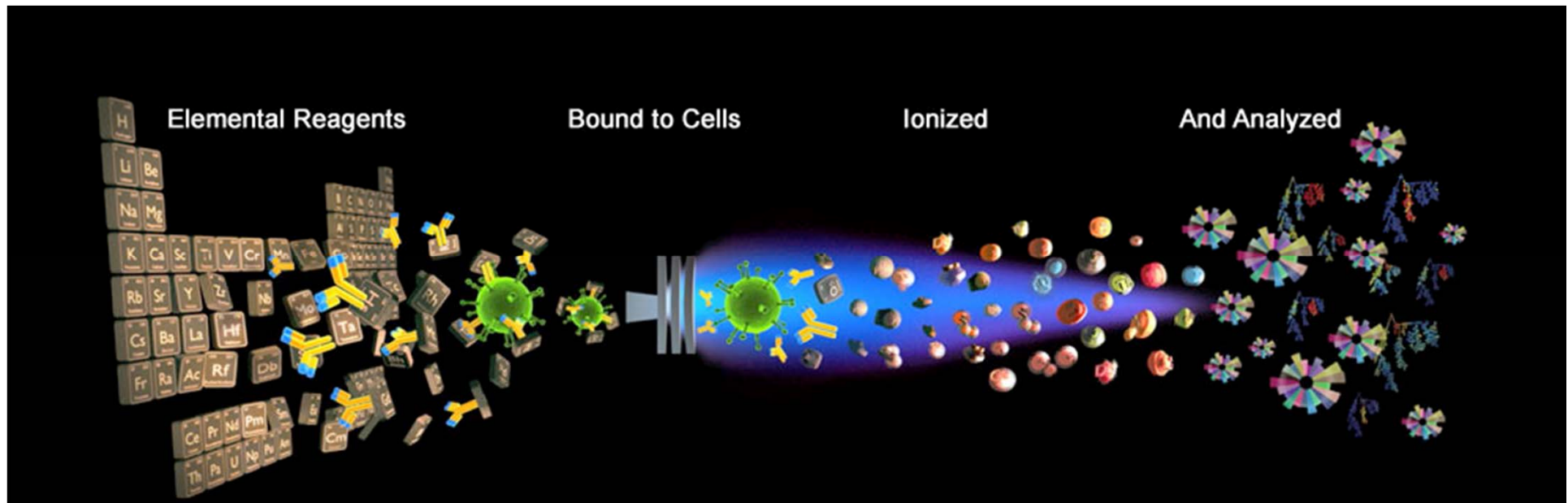
U N I V E R S I T Y O F B E R G E N

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 ($\mu\text{L}/\text{min}$)
Replicate sample CV (normalized)	<3%



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CyTOF Research

Applications

Phenotyping

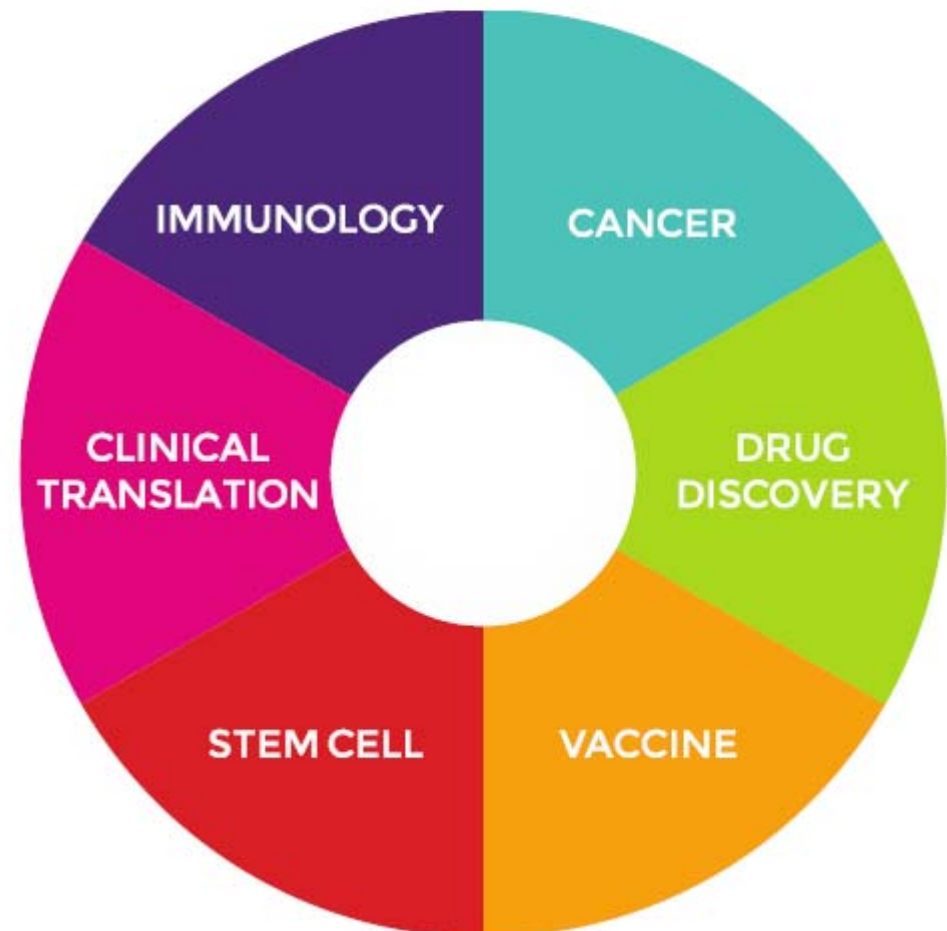
Signaling and transcription

Cytokines and growth factors

Cell death and apoptosis

Cell cycle and proliferation

Research areas

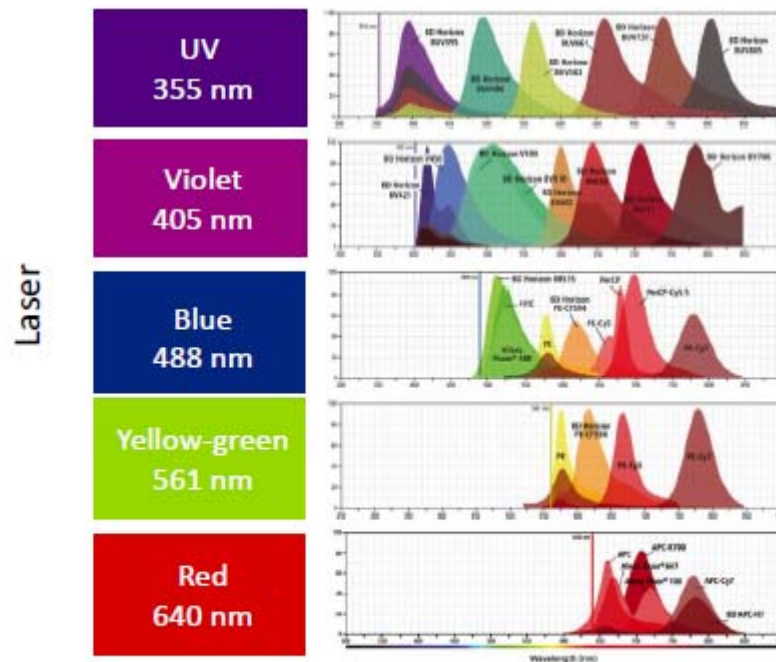


Antibody-mediated multiparameter protein detection

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



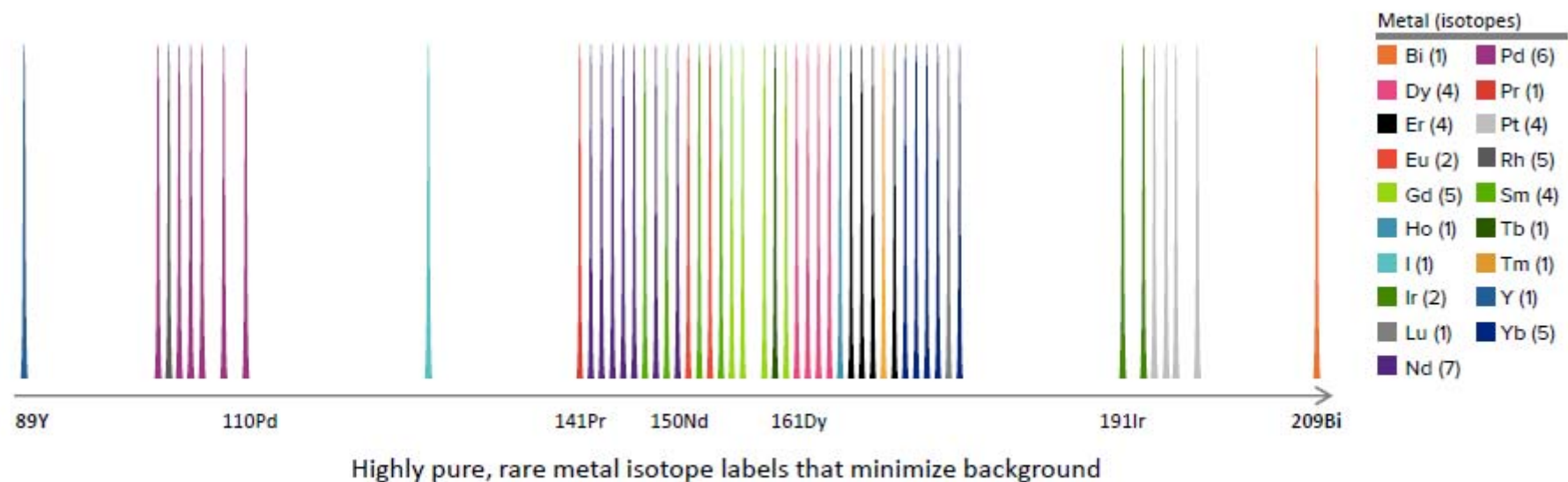
Staining intensities and background

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



Helios™ and Hyperion™ Imaging System

Powered by CyTOF technology



Helios™
Mass Cytometry



Hyperion™
Imaging Mass
Cytometry

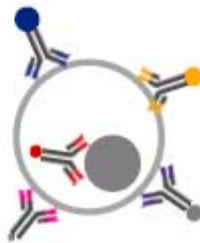
CyTOF Workflow



1

Select

Maxpar[®] panel and prepare mixture from individual antibodies.



2

Stain

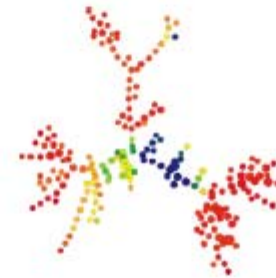
cells using protocols and buffers validated by Fluidigm.



3

Acquire

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

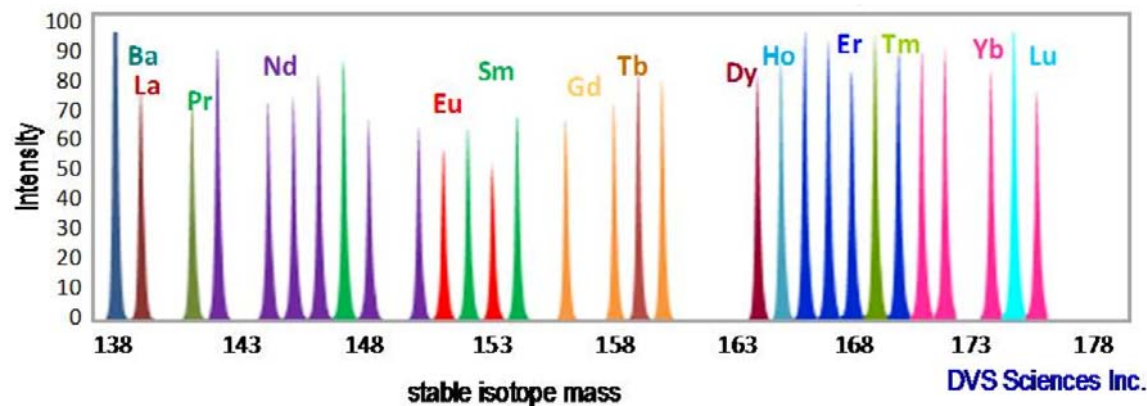


4

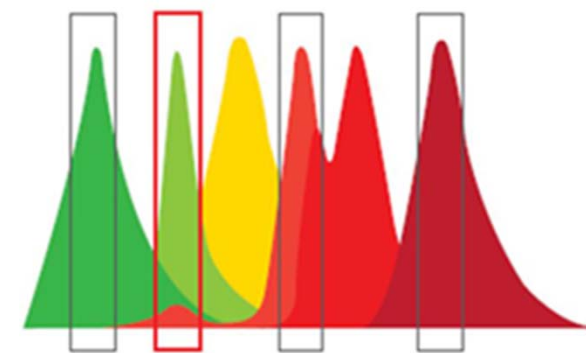
Analyze

data using proven analytical tools.

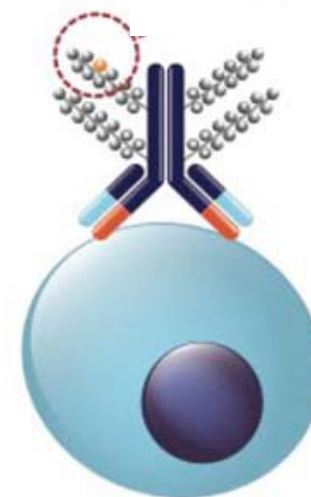
Mass Cytometry – Method



Conventional Flow Cytometry



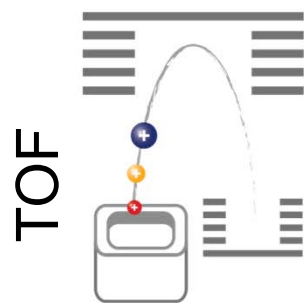
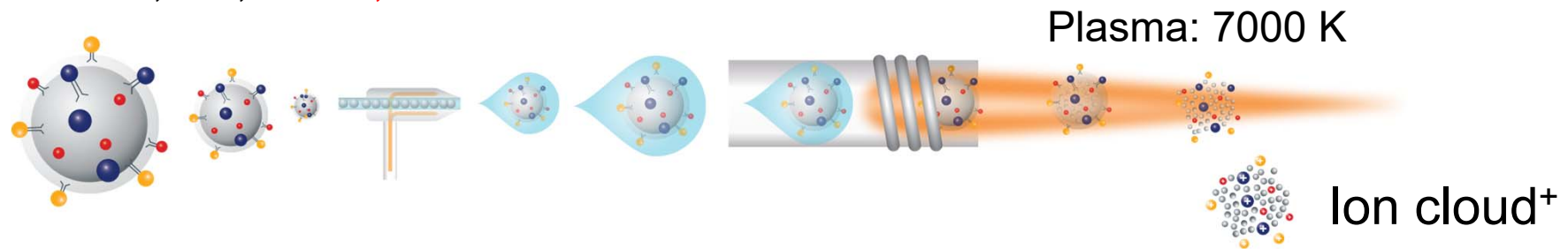
1 H Hydrogen																	2 He Helium				
3 Li Lithium	4 Be Beryllium															5 B Boron	6 C Carbon	7 N Nitrogen	8 O Oxygen	9 F Fluorine	10 Ne Neon
11 Na Sodium	12 Mg Magnesium															13 Al Aluminum	14 Si Silicon	15 P Phosphorus	16 S Sulfur	17 Cl Chlorine	18 Ar Argon
19 K Potassium	20 Ca Calcium	21 Sc Scandium	22 Ti Titanium	23 V Vanadium	24 Cr Chromium	25 Mn Manganese	26 Fe Iron	27 Co Cobalt	28 Ni Nickel	29 Cu Copper	30 Zn Zinc	31 Ga Gallium	32 Ge Germanium	33 As Arsenic	34 Se Selenium	35 Br Bromine	36 Kr Krypton				
37 Rb Rubidium	38 Sr Strontium	39 Y Yttrium	40 Zr Zirconium	41 Nb Niobium	42 Mo Molybdenum	43 Tc Technetium	44 Ru Ruthenium	45 Rh Rhodium	46 Pd Palladium	47 Ag Silver	48 Cd Cadmium	49 In Indium	50 Sn Tin	51 Sb Antimony	52 Te Tellurium	53 I Iodine	54 Xe Xenon				
55 Cs Cesium	56 Ba Barium	*	72 Hf Hafnium	73 Ta Tantalum	74 W Tungsten	75 Re Rhenium	76 Os Osmium	77 Ir Iridium	78 Pt Platinum	79 Au Gold	80 Hg Mercury	81 Tl Thallium	82 Pb Lead	83 Bi Bismuth	84 Po Polonium	85 At Astatine	86 Rn Radon				
87 Fr Francium	88 Ra Radium	**	104 Rf Rutherfordium	105 Db Dubnium	106 Sg Seaborgium	107 Bh Bohrium	108 Hs Hassium	109 Mt Meitnerium	110 Uun Ununnilium	111 Uuu Unununium	112 Uub Unbibium			114 Uuq Ununquadium			116 Uuh Ununhexium				
			* Lanthanides	57 La Lanthanum	58 Ce Cerium	59 Pr Praseodymium	60 Nd Neodymium	61 Pm Promethium	62 Sm Samarium	63 Eu Europium	64 Gd Gadolinium	65 Tb Terbium	66 Dy Dysprosium	67 Ho Holmium	68 Er Erbium	69 Tm Thulium	70 Yb Ytterbium	71 Lu Lutetium			
			** Actinides	89 Ac Actinium	90 Th Thorium	91 Pa Protactinium	92 U Uranium	93 Np Neptunium	94 Pu Plutonium	95 Am Americium	96 Cm Curium	97 Bk Berkelium	98 Cf Californium	99 Es Einsteinium	100 Fm Fermium	101 Md Mendelevium	102 No Nobelium	103 Lr Lawrencium			



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Introduction – Mass Cytometry

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

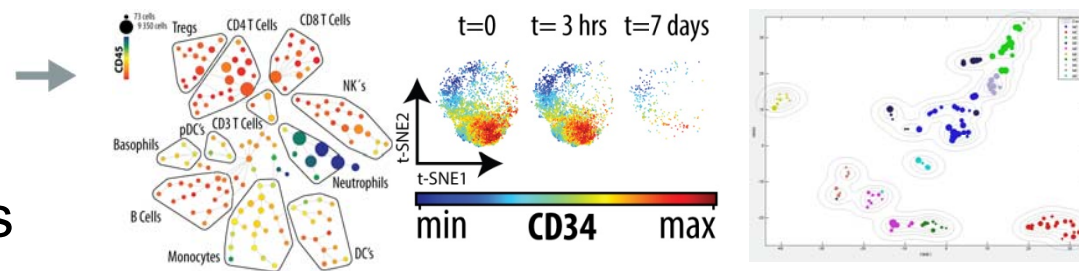


Normal FCS Files

Quadropole

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

SPADE, t-SNE and PhenoGraph



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



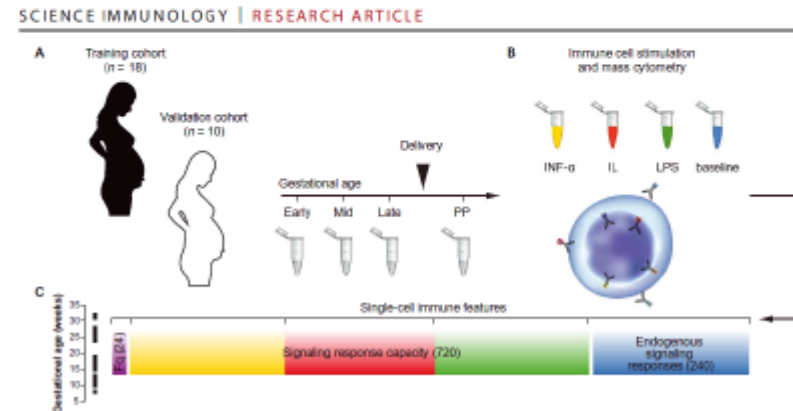
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Many high-impact papers with breakthrough findings

“An immune clock of human pregnancy.”

Science Immunology (2017)

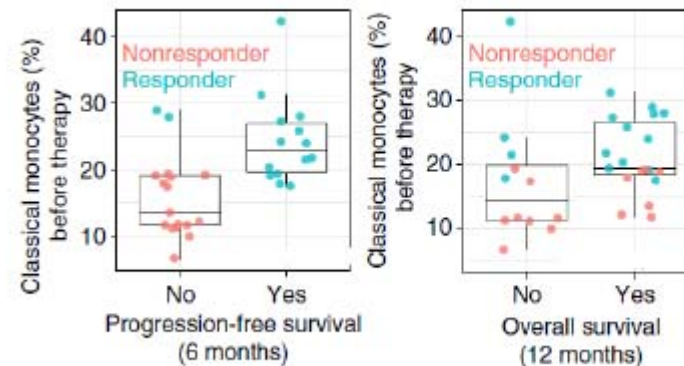
Aghaeepour, N. et al.



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

Nature Medicine (2018)

Krieg, C. et al.



Summary - Suspension

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

Hyperion™
Tissue Imager

Detection

CyTOF technology

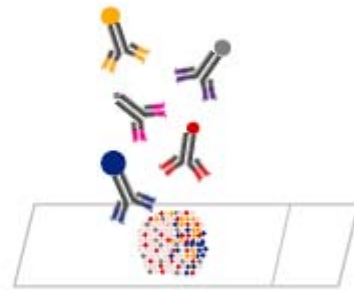
Hyperion Imaging System Workflow



1

Design

panels using IHC-validated antibodies conjugated to metal tags.



2

Stain

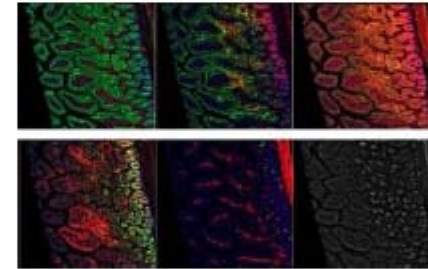
tissues (FFPE and frozen) or fixed cells with metal-conjugated antibodies.



3

Image

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



4

Analyze

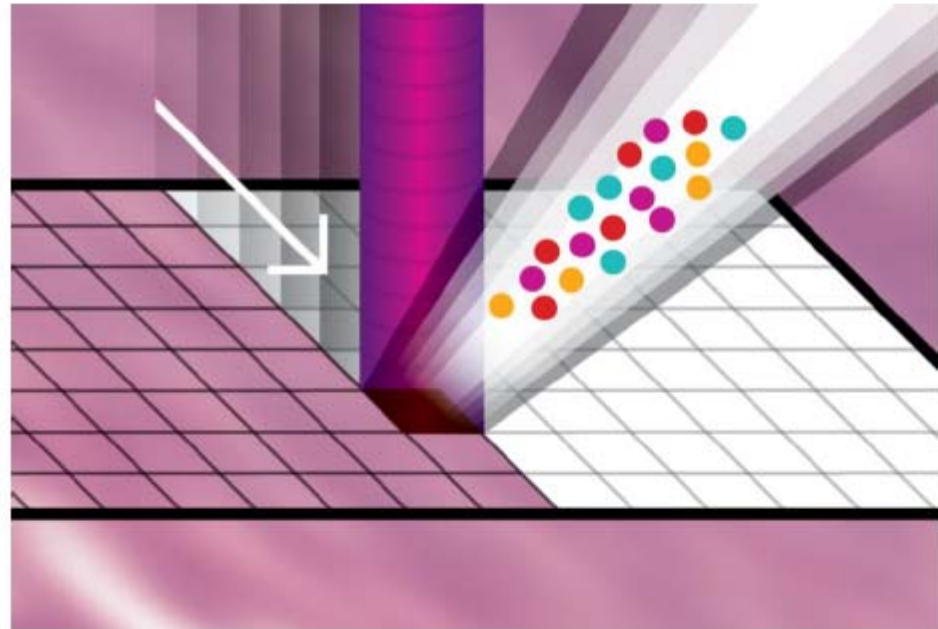
using post-analytical imaging and secondary analysis software tools.

How Imaging with the Hyperion Imaging System works

Load the sample into the Hyperion Imaging System

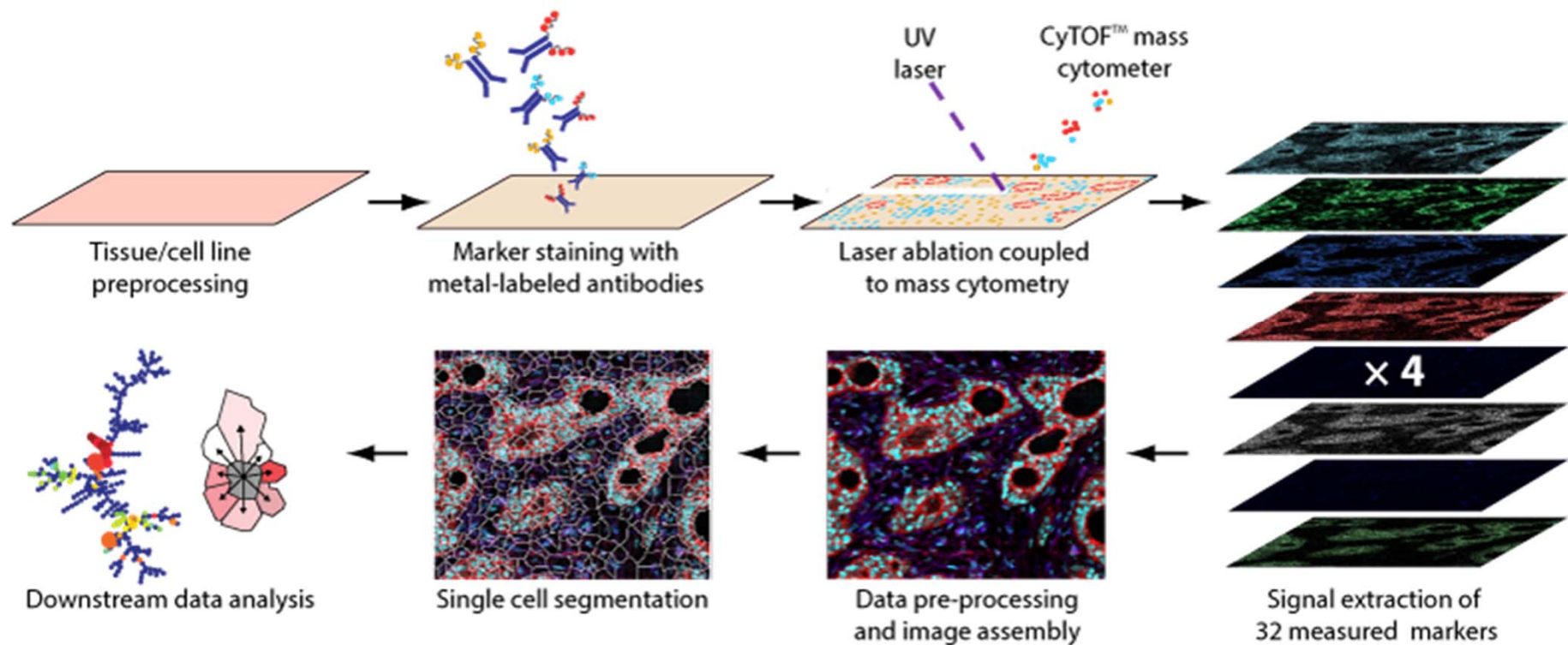


Precise laser imaging of the region of interest



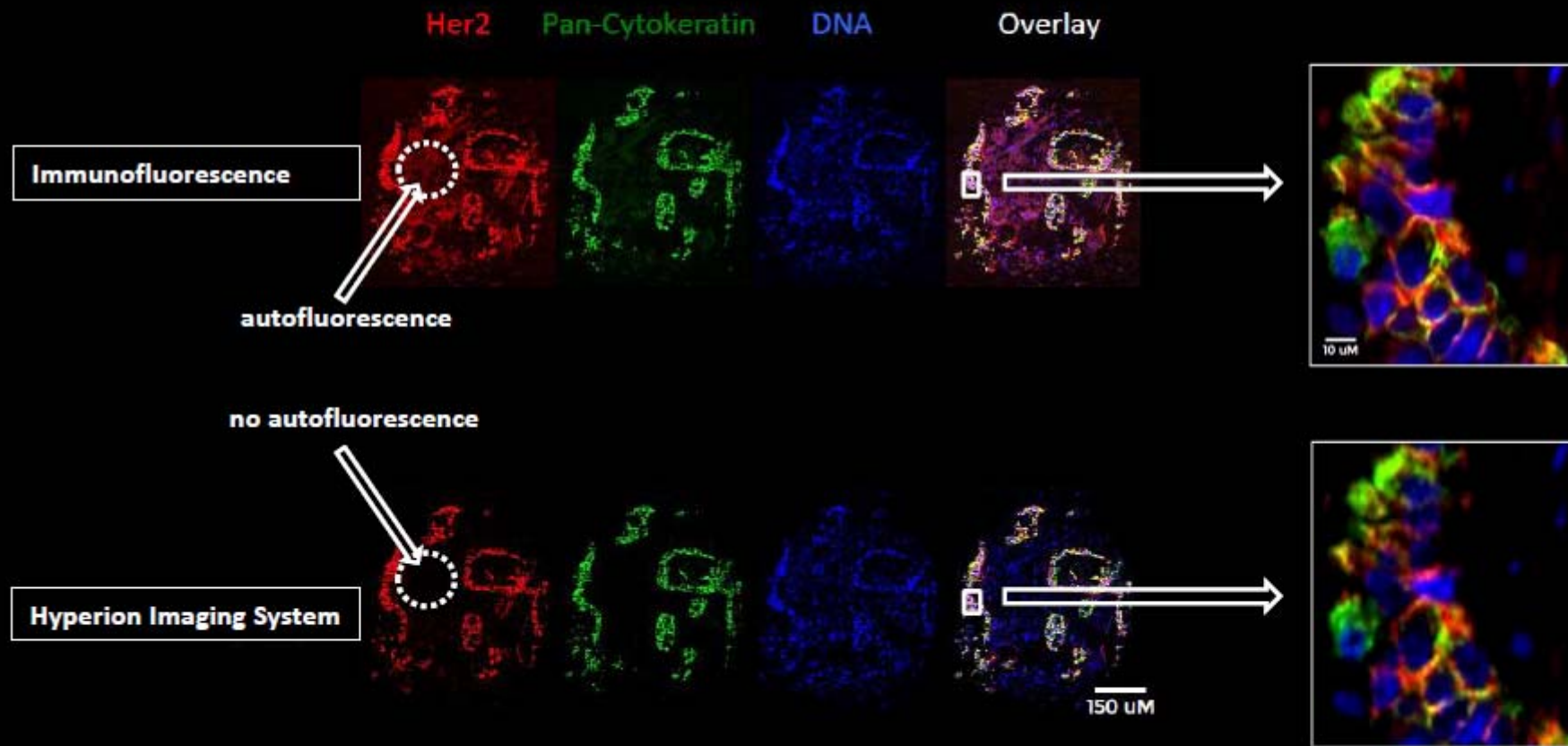
Laser beam focuses on $1\text{ }\mu\text{m}^2$ spots in the selected region
Collects metal-tagged proteins
Sends for CyTOF analysis

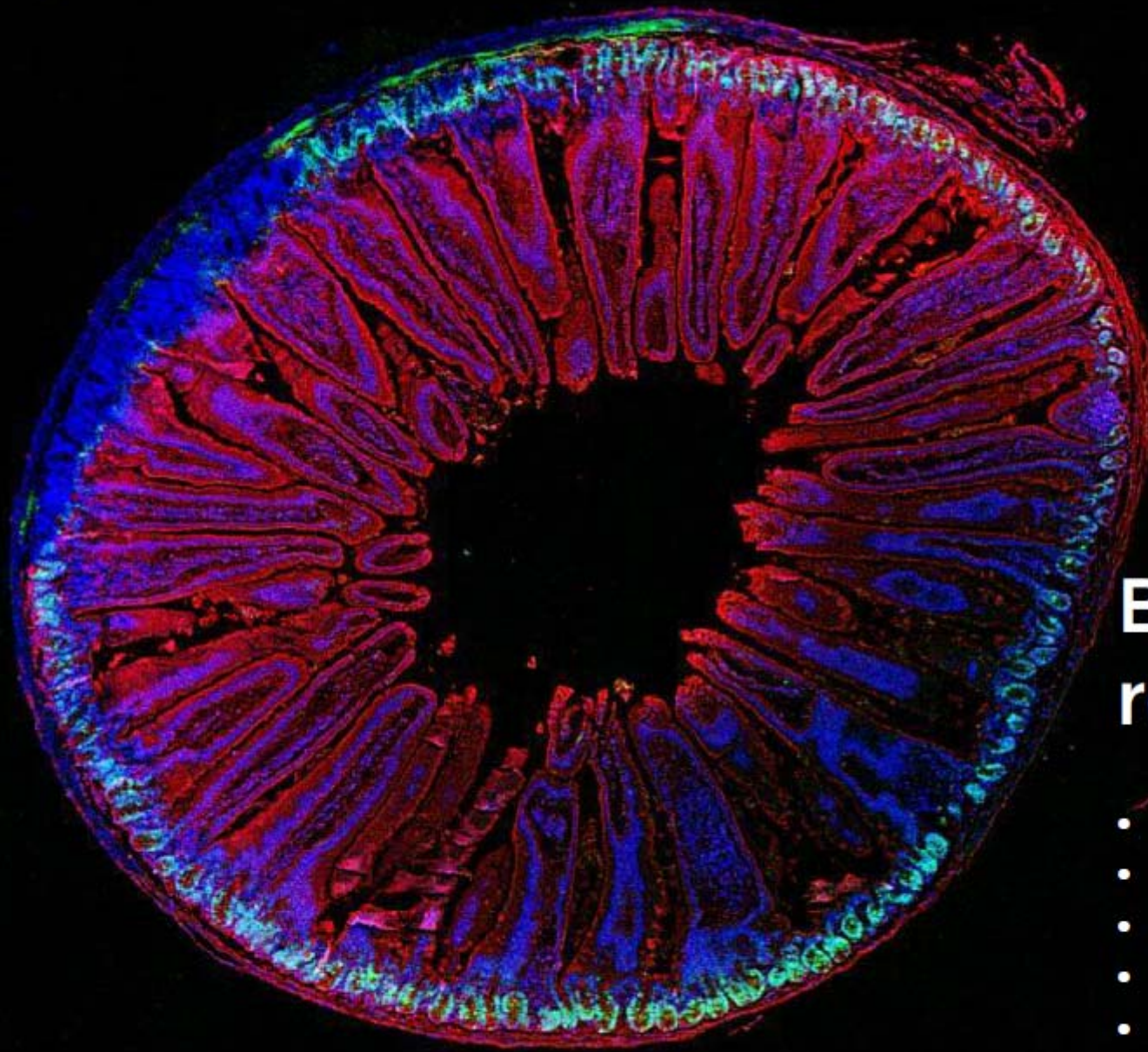
Image and single cell segmentation



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

– Courtesy of Dr. Bernd Bodenmiller, University of Zurich





Basic research

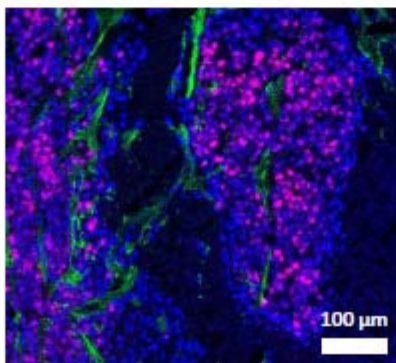
- Stem cell differentiation
- Embryonic development
- Hematopoiesis
- Liver regeneration
- Wound healing

AvantiLipid Ki67 DAPI

FFPE Mouse gut

Hyperion Imaging analysis pipeline

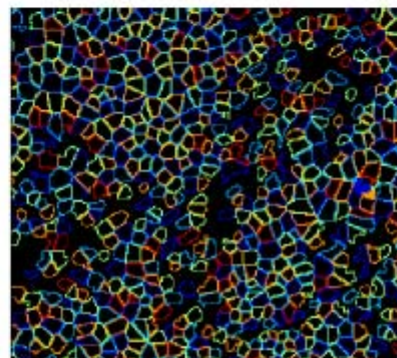
A three step workflow using MCD Viewer and histoCAT™



1

View and Validate

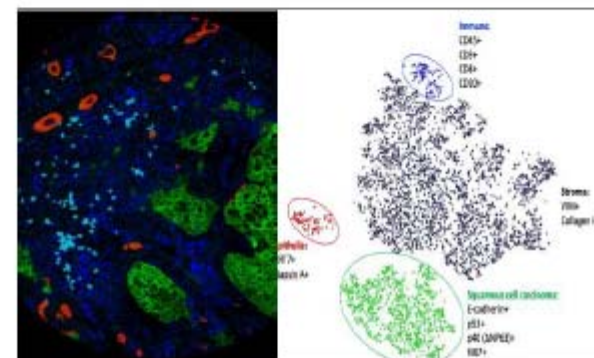
Detect presence of protein(s) of interest, produce high quality plots with chosen combinations of markers and confirm experimental success. Export files in various formats.



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.