Human Immunology & Immunotherapy Initiative (HI^3)

SCHOOL OF MEDICINE

UNIVERSITY OF COLORADO **ANSCHUTZ MEDICAL CAMPUS**

HI3 Member Meeting
February 14, 2017
2:00-3:00pm
Shore Family Auditorium
Nighthorse Campbell Building

SCHOOL OF MEDICINE
Human Immunology & Immunotherapy Initiative (HI³)
UNIVERSITY OF COLORADO **ANSCHUTZ MEDICAL CAMPUS**

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Medicine

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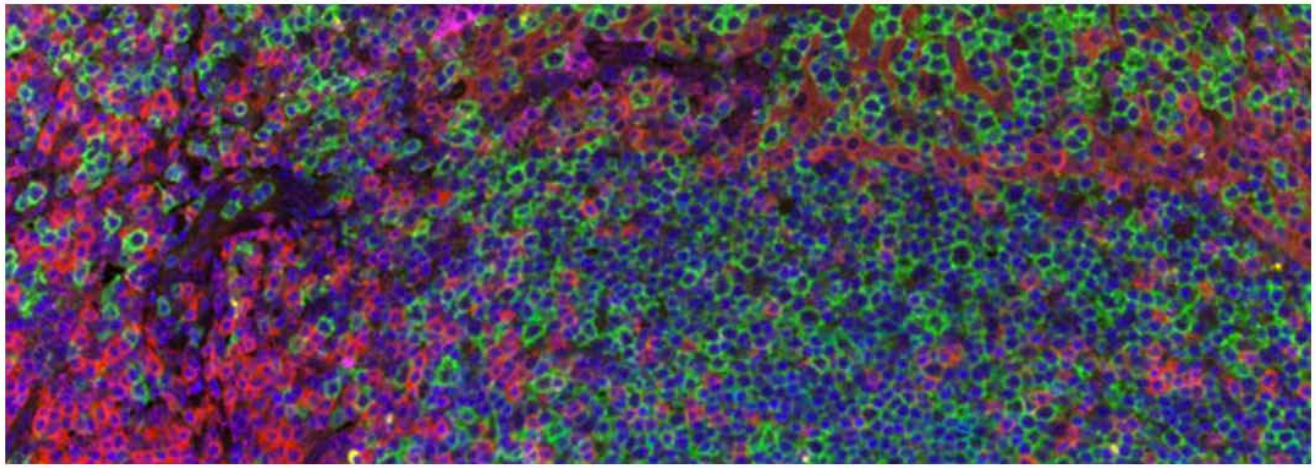
ucdenver.edu/HumanImmunology ← **Visit the HI3 website**



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  - HI3 Internal Advisory Committee
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- Training
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Human Immunology & Immunotherapy Initiative (HI³)

After decades of investigation establishing principles in animal models, it has recently become possible to treat and cure some diseases in humans by interventions that target immunological functions. Immunotherapy, described by Science magazine as the Breakthrough of the Year in 2013, has led to major changes in the standard of care for some diseases and is particularly useful in infectious disease, autoimmunity, allergy/asthma, and especially cancer.

The **mission** of the **Human Immunology and Immunotherapy Initiative (HI^3)** is to develop the infrastructure to create an all-inclusive facility that provides experimental models for the preclinical testing of new candidate therapeutics, reliable immunotherapeutic production, consistent clinical trials research support, and organized immune monitoring capabilities. In addition, the HI3 will train future scientific leaders within the realm of immunotherapy and recruit exemplary faculty to the CU Anschutz medical campus that will complement and enhance existing strengths.

Human Immunology & Immunotherapy Initiative (HI^3)

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Activities

- Faculty Recruitment
- GMP Immunotherapeutic Production
- Training Program
- Clinical Research Program
- Translational Research Networking & Preclinical Models (TRNPM)
- Human Immune Monitoring Shared Resource (HIMSR)

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HI3 Faculty Recruitment UPDATE

- **Basic Human Immunology**
  - ✓ Committee formed, position posted on CU Careers
  - ✓ 4 candidates will be interviewed by the end of April
- **Autoimmunity**
  - ✓ Committee formed, position posted on CU Careers
  - ✓ HI3 Autoimmunity Program Director
  - ✓ Interviews to begin in late April
- **Cancer**
  - ✓ Working with several cancer recruits ongoing at CU and CHCO
  - ✓ Terry Fry, MD (NCI) – verbal acceptance January 2017, informed NCI of upcoming departure, proposed start January 2018
- **Data Scientist/Bioinformatician**
  - ✓ Committee formed, position posted on CU Careers
  - ✓ 3 candidates will be interviewed by the end of March

Space?

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HI3 GMP Immunotherapeutic Production UPDATE

- **Collaborate with GMP facilities toward the production of clinical grade biological reagents and cell-based immunotherapeutic products**
  - ✓ Clinimmune
  - ✓ The Gates Center for Regenerative Medicine

- **Facilitate the use of campus CLIA labs for monitoring patient responses used for clinical decision-making**
  - ✓ Exsera BioLabs - complement and autoimmune diagnostics
  - ✓ Colorado Molecular Correlates Laboratory (CMOCO) – anatomic pathology
  - ✓ Clinimmune – flow cytometry, cell sorting, histocompatibility

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HI3 Training Program UPDATE

- **Develop and establish training programs across the training continuum at the pre-doctoral, post-doctoral, and junior faculty level**
  - ✓ Establish fellowships to support training and research for future leaders in immunotherapy – 2 PhD candidates, 2 post-doctoral fellows, 1 junior faculty
  - ✓ Work with selected faculty and established campus services to provide educational resources

- **Form a Training Program Subcommittee to play a role in the design and structure of the program** *1

Are you interested?

Please see me – Aimee Bernard

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HI3 Clinical Research Program UPDATE

- **HI3 Clinical Research Program Director**
  - ✓ Physician scientist (MD) to develop and oversee program
- **Clinical Research Program Manager *2**
  - ✓ Successfully guide HI3 investigators through the complicated web (CRSC/CCTSI, CTRC, CCRO/RI, UCCC, CHCO CC) of established CU clinical research support services
  - ✓ Provide expertise and coordination with campus clinical research services to HI3 research teams to effectively operationalize clinical research
  - ✓ Establishes and maintains bi-directional communication and collaboration between HI3 research teams and campus clinical research services from initiation to completion of clinical studies

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Translational Research Networking & Preclinical Models Facility UPDATE

- **Provide a nexus for multiple aspects of translational immunology research**
  - Director: Roberta Pelanda, PhD
  - Manager Humanized Mouse Core HI3: Julie Lang, PhD

Services include:

✓ **Enabling and promoting collaboration among investigators** along the continuum of basic-clinical-translational research by networking and/or matchmaking clinicians, clinician/scientists, and basic scientists with shared interests

✓ Establishing mechanisms that ensure **availability of human tissue** for research

✓ Developing and maintaining **preclinical mouse models** for testing of candidate therapeutics (e.g. human immune system mice or **hu-mice**) *3

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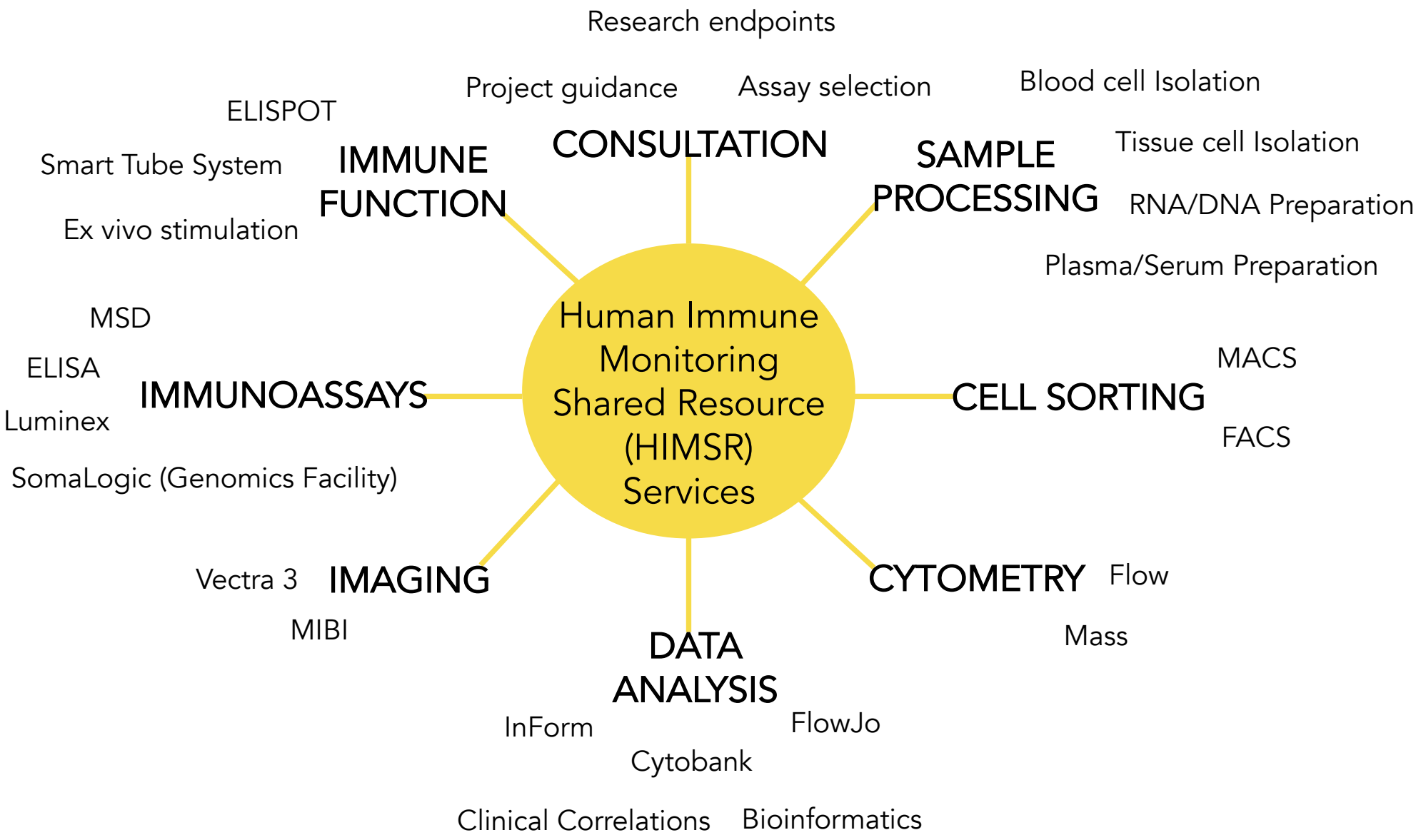
Human Immune Monitoring Shared Resource (HIMSR) UPDATE

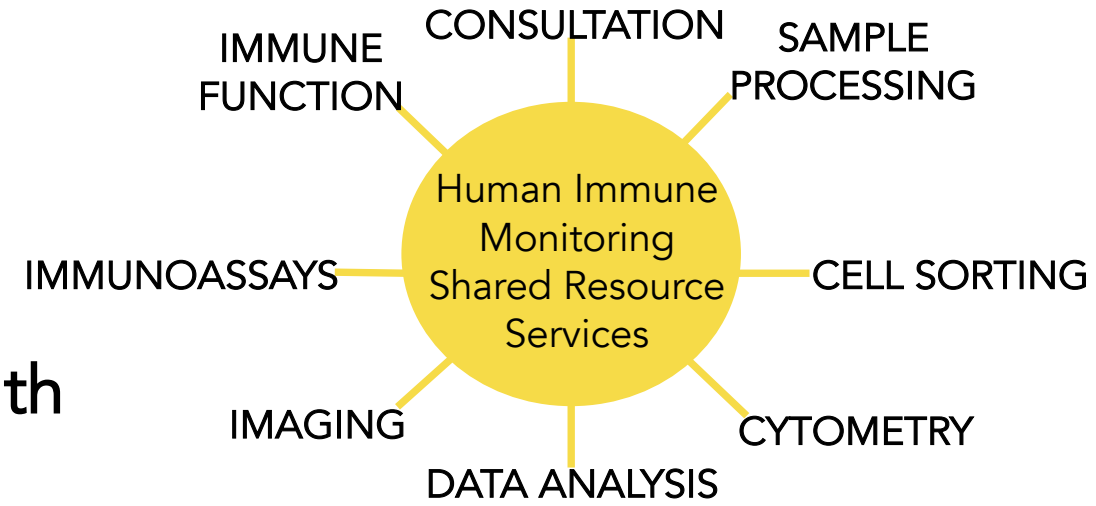
- **HIMSR Team Members**
  - Director: Jill Slansky, PhD
  - Assistant Director: Kim Jordan, PhD
  - Experimental Design Consultant: Elena Hsieh, MD
  - PRA/Histology Specialist: TBD
  - Flow cytometry and protein purification specialist: Jennifer McWilliams, PhD
  - Immuno&Micro Flow Facility Manager: Erin Kitten, BS

Human Immune Monitoring Shared Resource (HIMSR) Services



HIMSR Consultation

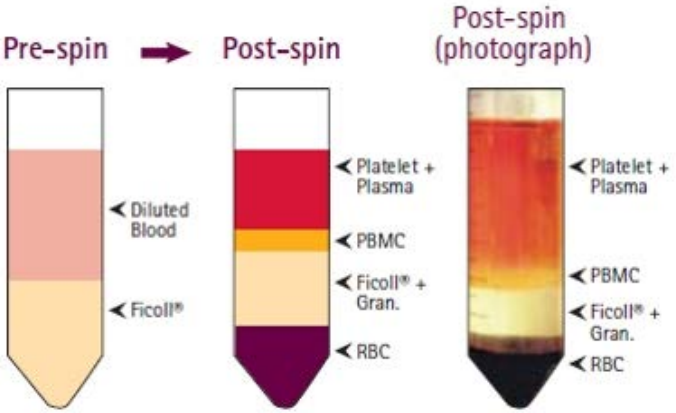
- **Intentions**
  - Provide immunology expertise
  - Discuss project goals and HIMSR assistance
  - Identify and design feasible immune monitoring experiments
  - Discuss funding sources and budgets
- **What we've been working on**
  - Study design
  - Experimental descriptions for methods sections
  - Letters of support and collaboration
  - Grant budgets
  - Grant facilities pages



- **Currently, we are working with 20+ interested groups at various funding stages and across different services**

HIMSR Sample Processing

Ficoll Gradient PBMC isolation



CPT tube PBMC isolation

Plasma and PBMC aliquots

Tissue cell isolation- Miltenyi gentleMACs

RNA and DNA isolation

Temporary cryopreservation, immune monitoring assays, genomics, proteomics

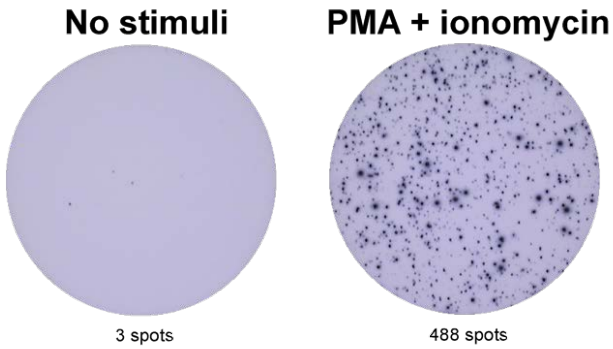
HIMSR Immune Function

In Development

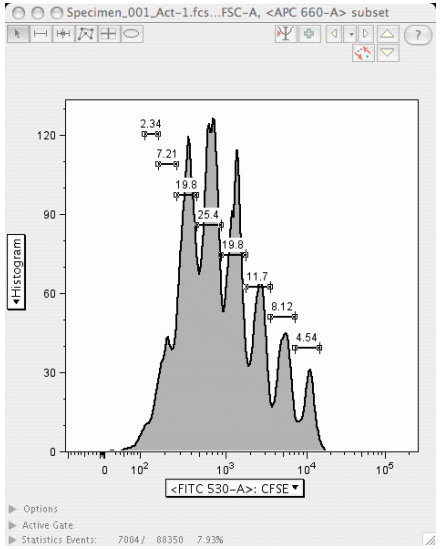
Ex vivo Stimulation: SMART Tube

- Whole blood or PBMC stimulation
- Preservative added after incubation, ready for freezing.
- Study samples thawed together and stained (surface markers, signaling molecules, or cytokines)

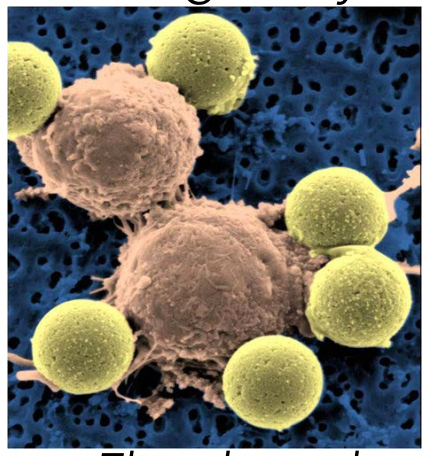
ELISPOT



Proliferation



Killing Assays



- *Flow-based*
- *Incucyte (UCCC Tissue Culture core)*

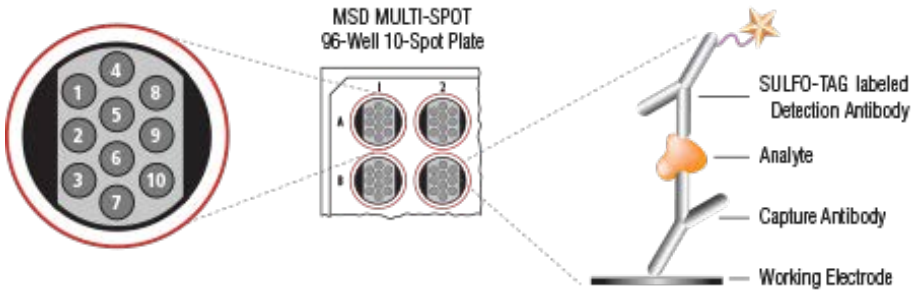
Flow cytometry

Mass cytometry

HIMSR Immunoassays

Meso Scale Discovery

A division of Meso Scale Diagnostics, LLC.



- **Parsec** automated instrument
- Up to 10 analytes per 25 ul of plasma
- ~40-plex validated assays

- **$75,000 in free reagents once the Parsec arrives and we have space for it!** *4

Luminex®

- Manual preparation of plates to read at the Cancer Center Flow Core
- Up to 500 analytes per sample

SomaLogic

- Upstream sample preparation for SOMAscan assays performed in the Cancer Center Genomics Facility
- Up to 1,300 analytes per sample

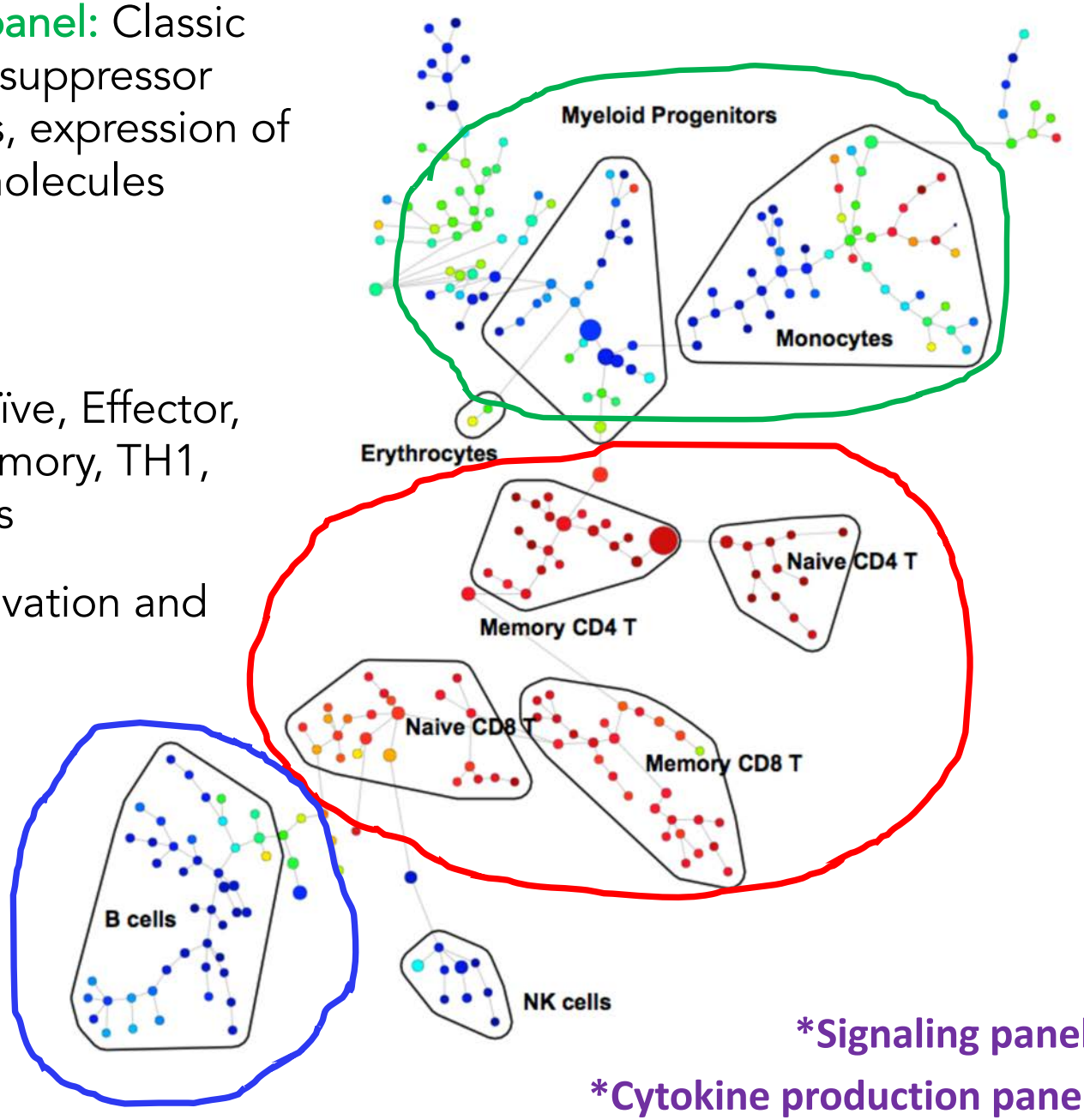
HIMSR Flow and Mass Cytometry

Monocyte/DC Phenotyping panel: Classic and activated monocytes, suppressor cells, dendritic cell subsets, expression of activation and inhibitory molecules

T cell Phenotyping panel: Naïve, Effector, Effector Memory, Central Memory, TH1, TH2, TH17, Regulatory T cells

T cell activation markers: Activation and Inhibitory receptors

B cell Phenotyping panel: Immature, naïve, memory B cells, plasmablasts, isotype, activation and inhibitory molecules



*Signaling panel

*Cytokine production panel

HIMSR Flow and Mass Cytometry

T cell Phenotyping panel

CD3, CD4, CD8, FOXP3, CD25, CD45RA, CD45RO, CD62L, CCR6, CXCR3, CXCR4, CCR7, CD66

B cell Phenotyping panel

IgM, IgG, IgD, IgA, CD19, CD20, CD10, CD27, CD138, CD38, CD81, CD5, CD22, CD66

Monocyte/DC Phenotyping panel

Lineage, HLA-DR, CD14, CD16, CD15, CD33, CD66, CD11b, CD11c, CD1c, CD141, CD123, CD80, PDL1

T cell activation markers

CD3, CD4, CD8, CD66, CD45RO, Tim-3, CTLA4, CD25, LAG-3, PD-1

Signaling panel

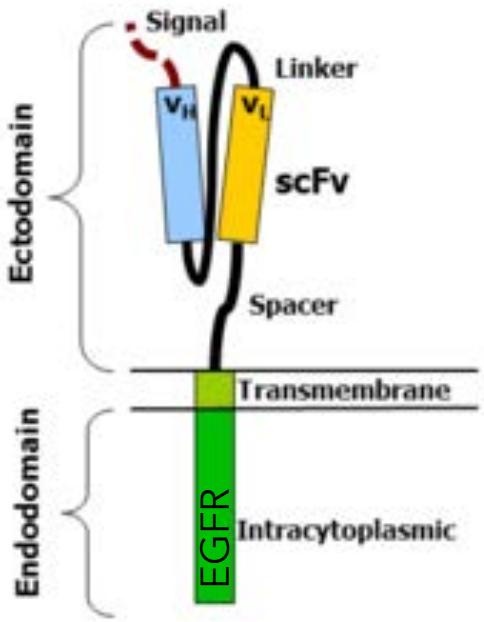
CD3, CD4, CD8, CD19, CD56, CD11b/CD66, phospho-S6, IkBa, phospho-AKT

Cytokine Production panel

CD3, CD4, CD8, CD19, CD56, CD11b/CD66, TNFa, IFNg, IL-4, IL-6, Granzyme B

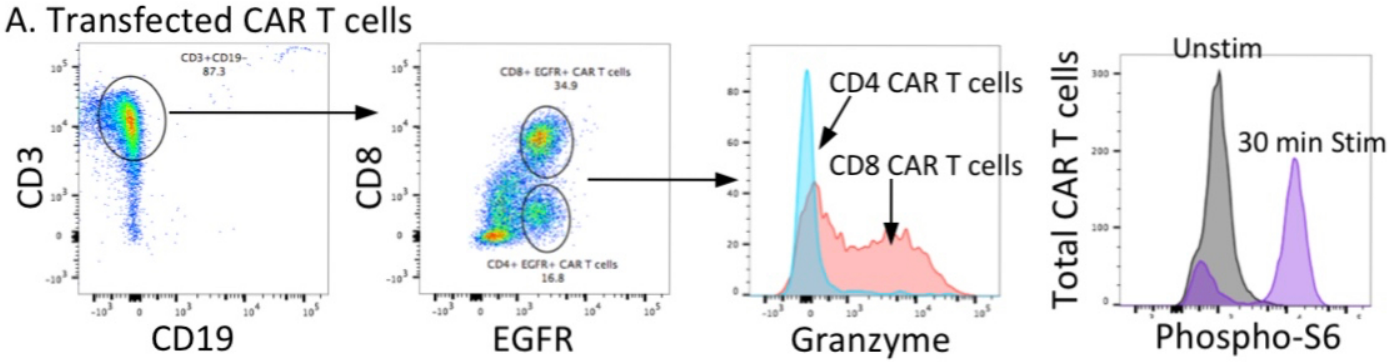
- **Are you interested in adding your favorite cells markers? Contact us!** *5
- We will also provide these antibody clone names and staining protocols for investigators wishing to perform the staining in their own laboratories.

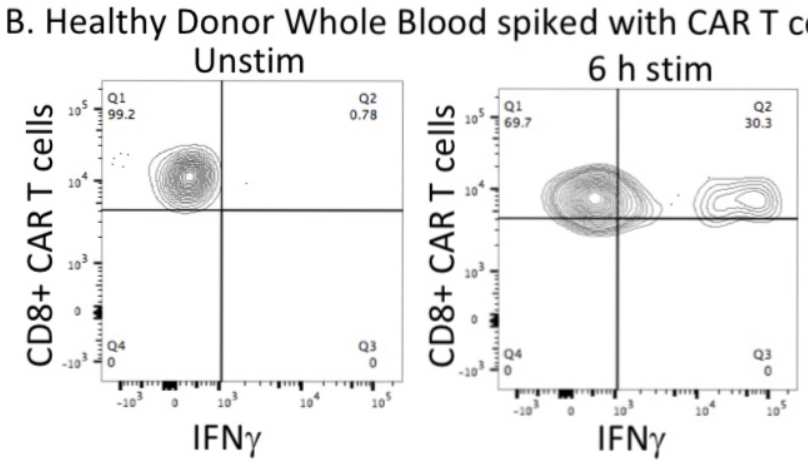
Monitoring CAR-T cell frequency and function in ALL patients



HIMSR will be:

- Processing peripheral blood at various time points post-infusion
- Stimulating cells (SMART tubes) and collecting plasma
- Analyzing frequency, subsets, and effector function of CAR T cells
- Measuring inflammatory cytokines in plasma

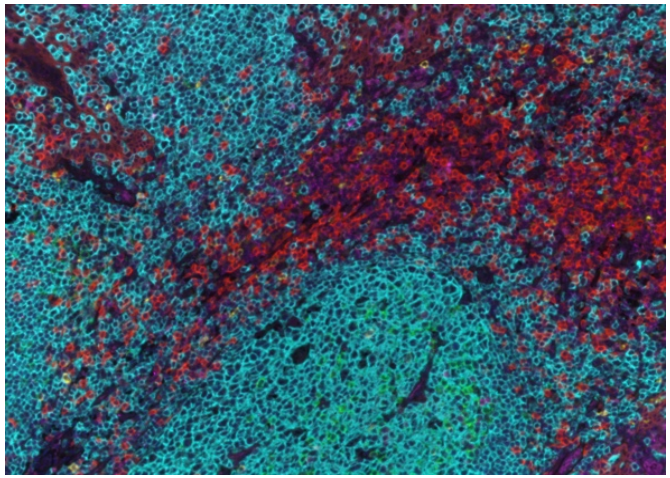




Cancer Center Immunotherapy RFA, Enkhee Purev, M.D., PI

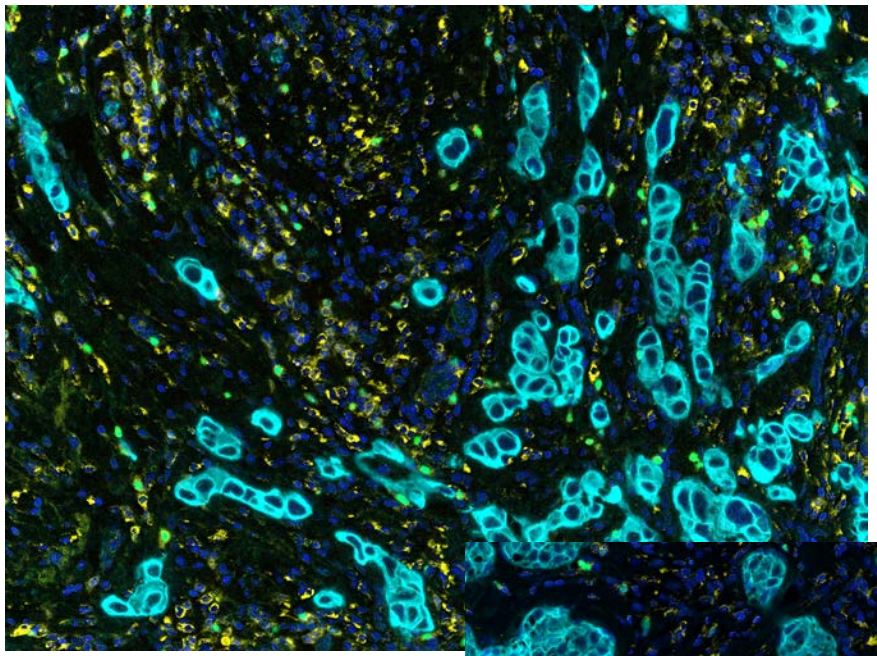
HIMSR Imaging - *in situ* visualization: Vectra 3.0 (Perkin Elmer)

Automated brightfield and fluorescence imaging platform

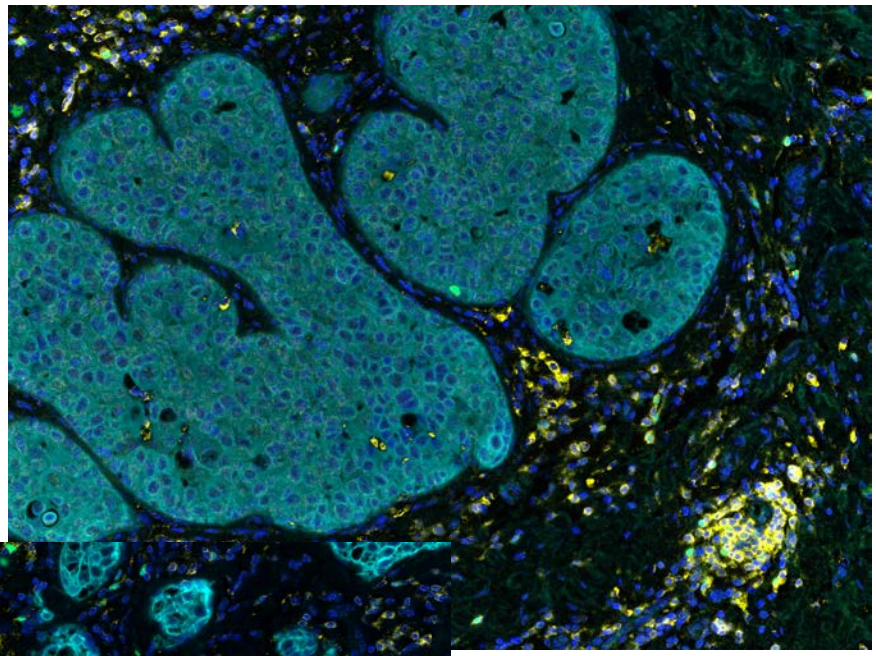


- Serial staining protocol allows for DAPI plus 6 fluorescent tissue or cell markers
- Brightfield images (brown, red, green, H&E)
- Intended for formalin—fixed paraffin-imbedded tissue (paraffin blocks)
- Whole slide scans (10x) with selected regions of interest for analyses (20x)
- Tissue Microarrays
- IRB exempt protocol submitted to obtain human tissue for staining protocol optimization (UCCC Tissue Biobanking & Processing Shared Resource)
- Working on a series of immune cell panels… **coming soon!**

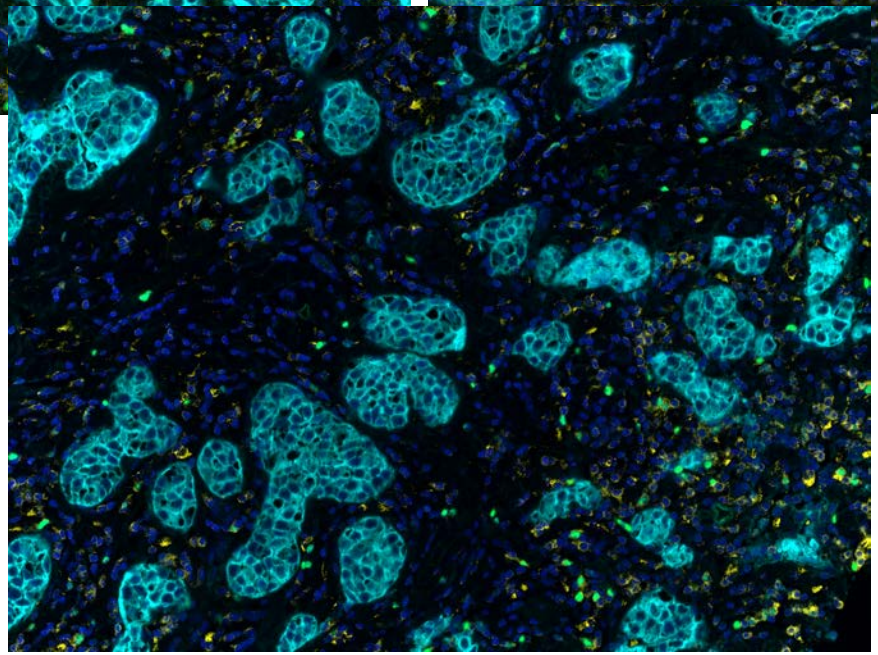
Imaging –
Quantification of immune infiltrate in Young Women's Breast Cancer



Case 1



Case 2

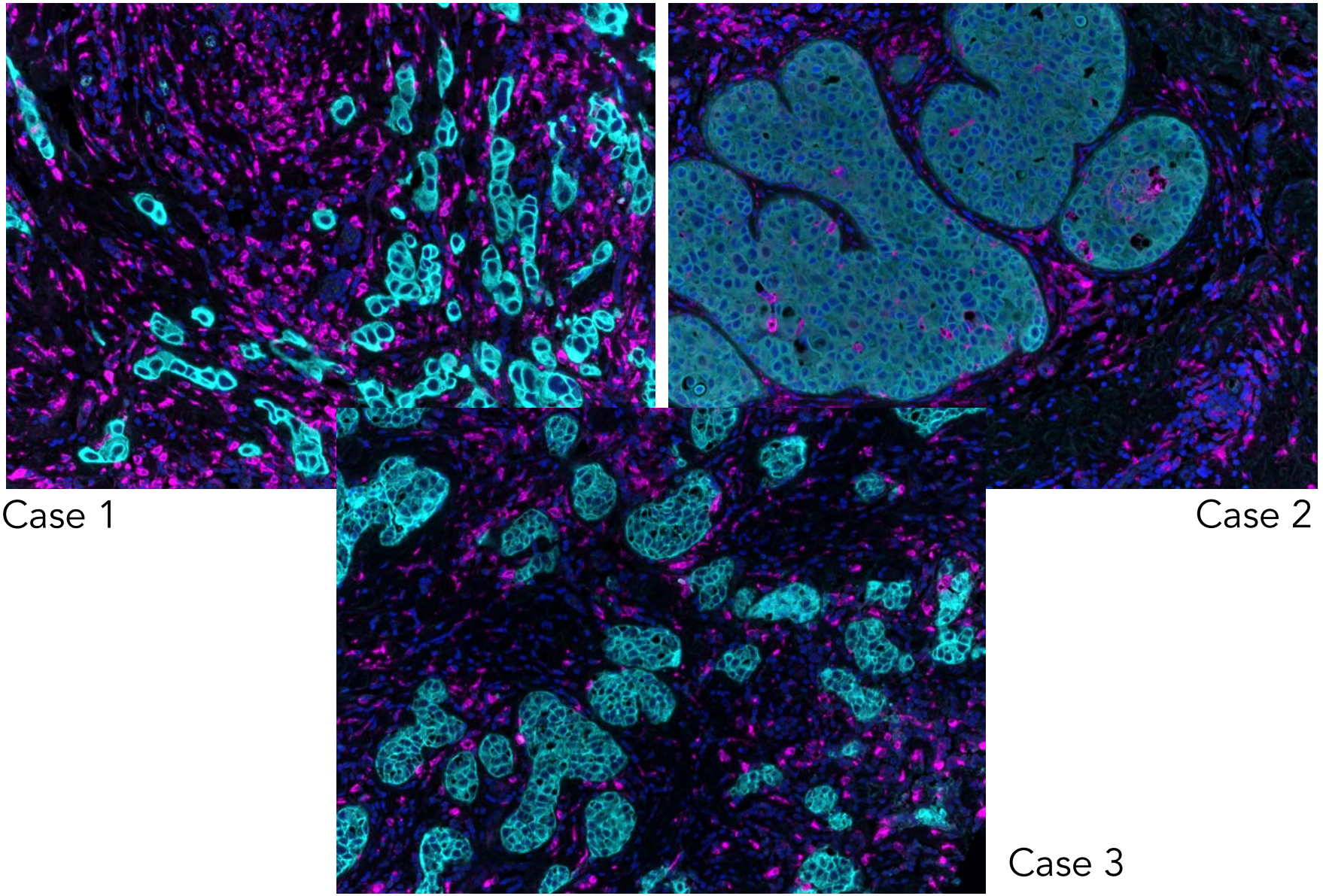


Case 3

CD4 FOXP3 Cytokeratin DAPI

Young Women's Breast Cancer Translational Program, Dr. Virginia Borges

Imaging –
Quantification of immune infiltrate in Young Women's Breast Cancer



Case 1

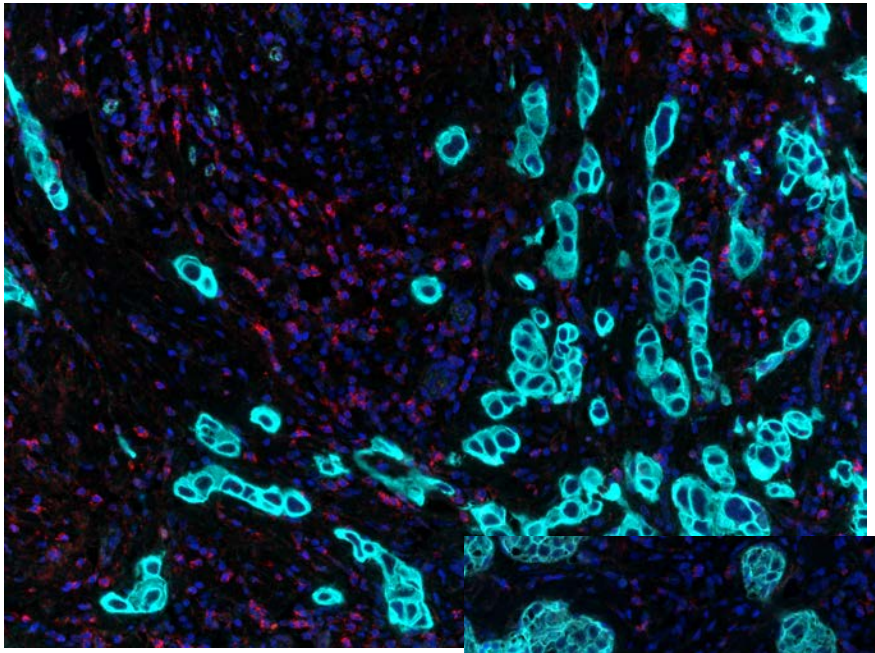
Case 2

Case 3

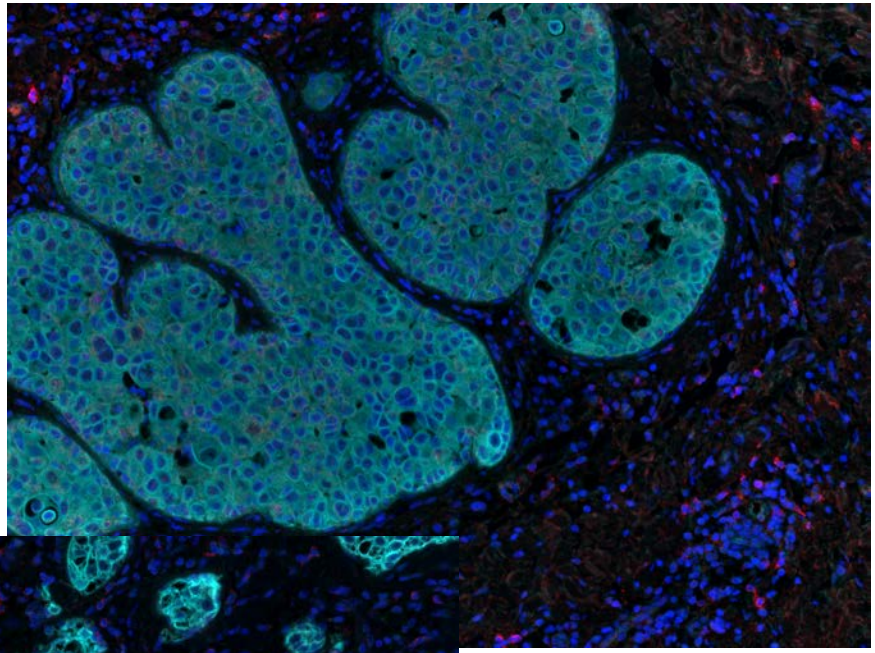
CD68 Cytokeratin DAPI

Young Women's Breast Cancer Translational Program, Dr. Virginia Borges

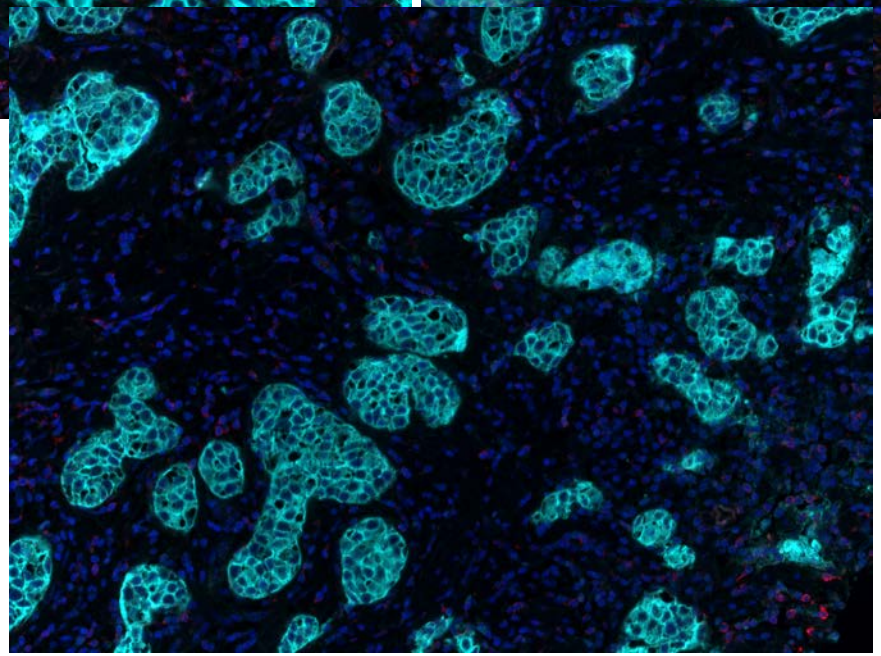
Imaging –
Quantification of immune infiltrate in Young Women's Breast Cancer



Case 1



Case 2



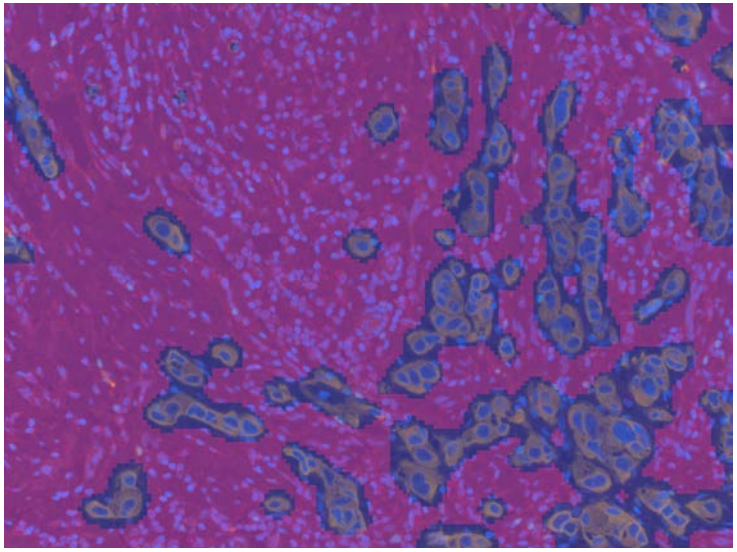
Case 3

CD8 Cytokeratin DAPI

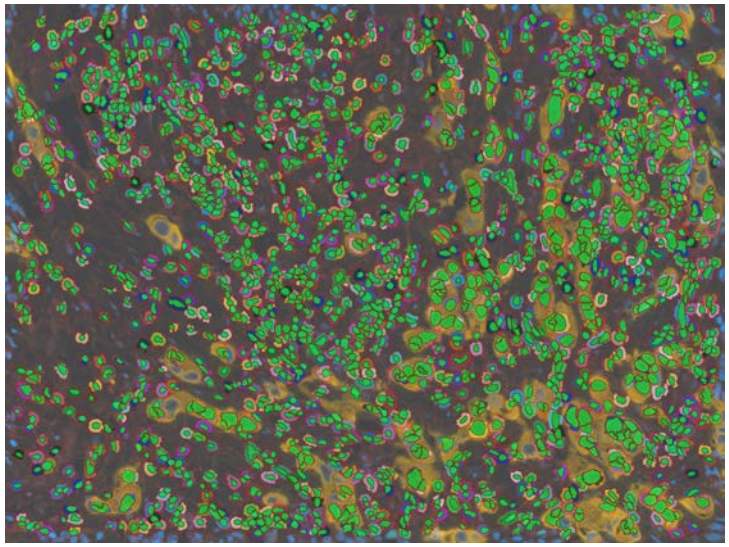
Young Women's Breast Cancer Translational Program, Dr. Virginia Borges

Features of inForm software analyses

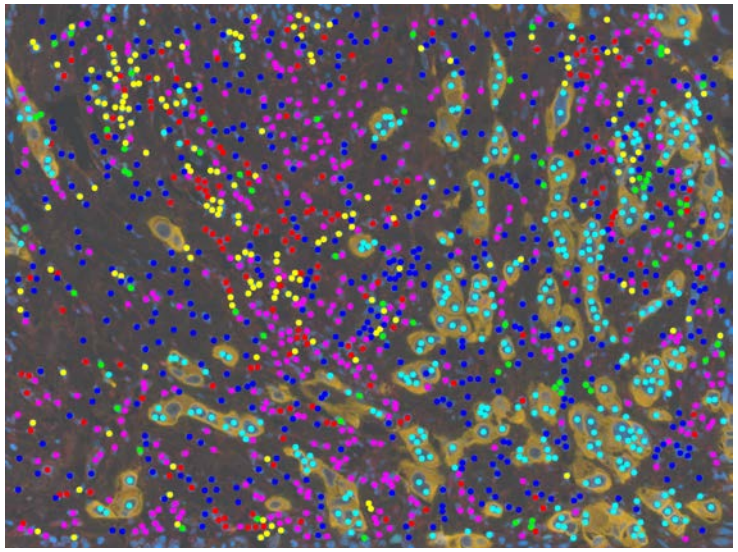
Tissue Segmentation: tumor v stroma



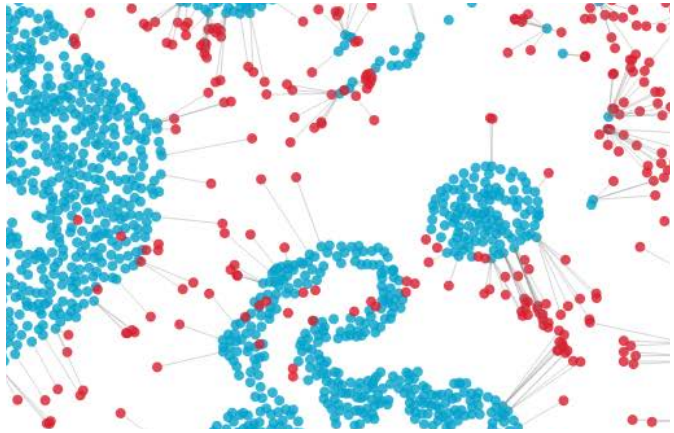
Cell Segmentation: staining intensities



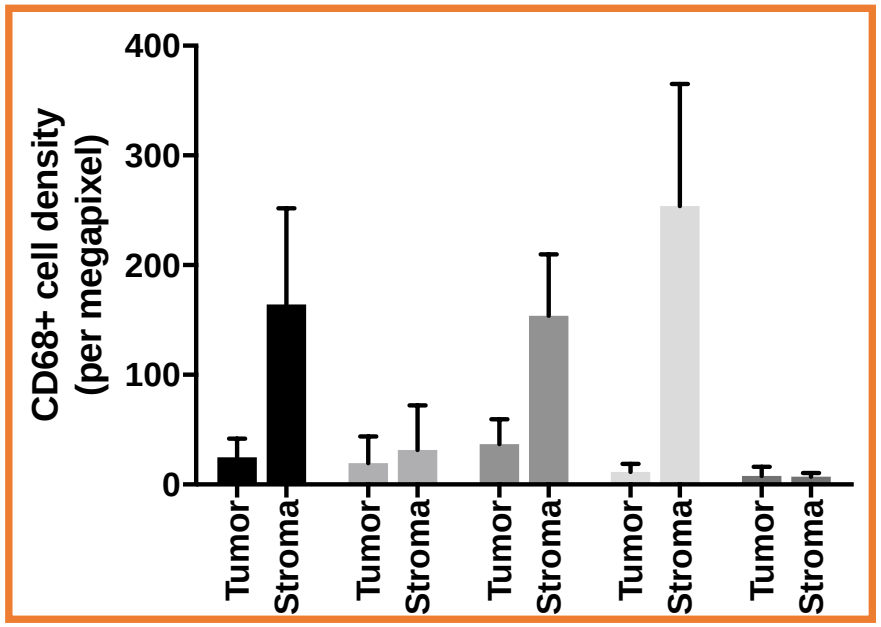
Cellular Phenotyping: frequencies

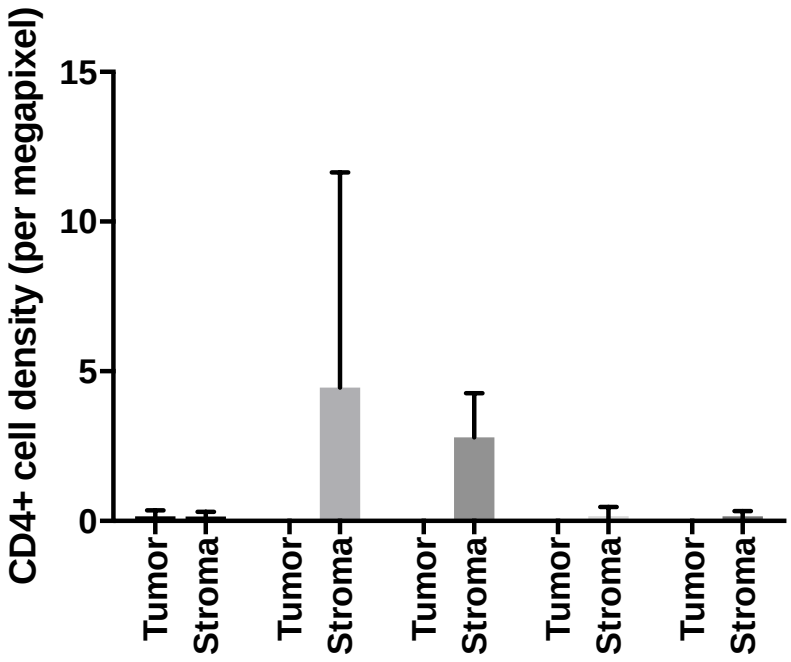


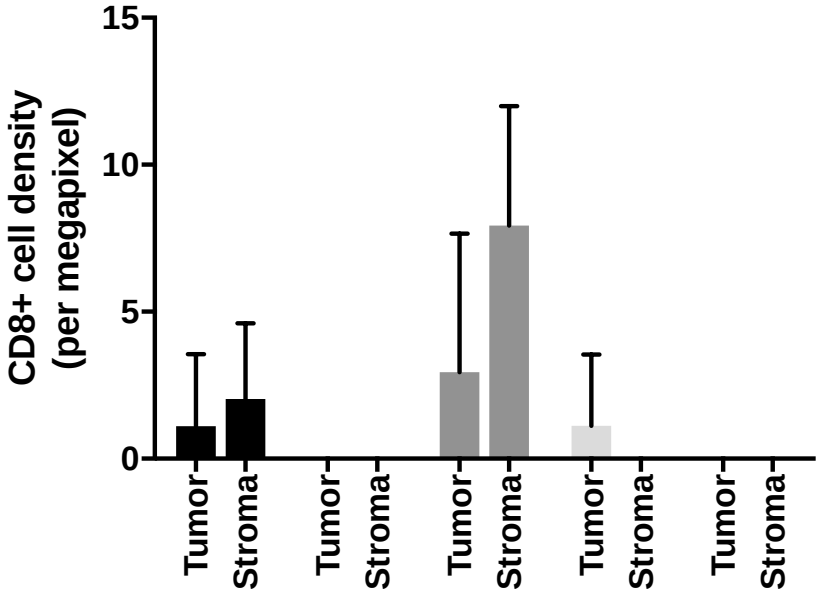
- Traditional scoring function with user-set thresholds
- Spatial information (x, y coordinates) allowing for nearest-neighbor analysis

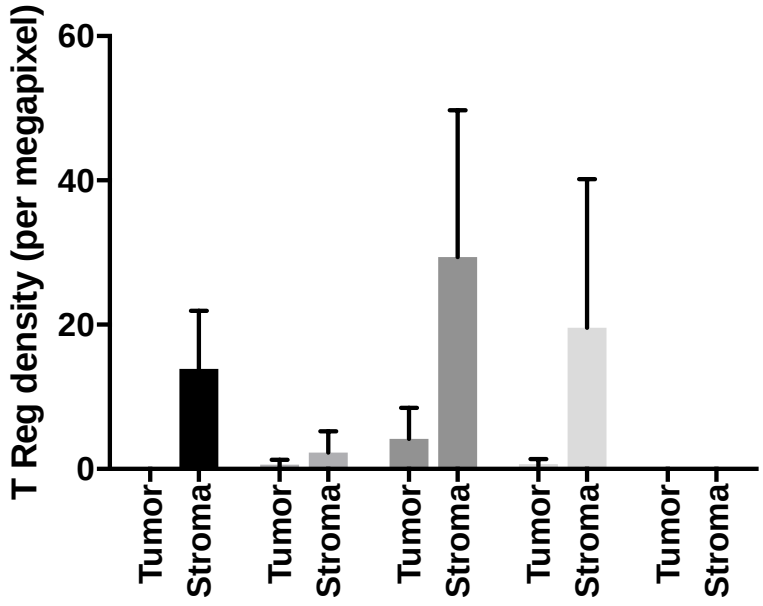


Imaging and Data Analysis –
Quantification of immune infiltrate in Young Women's Breast Cancer









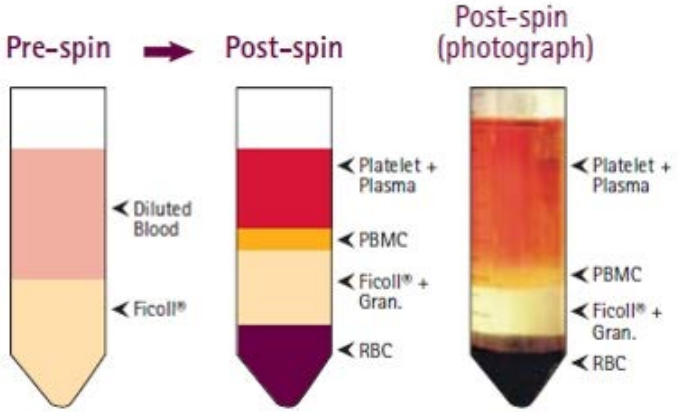
Case 1
Case 2
Case 3
Case 4
Case 5

Young Women's Breast Cancer Translational Program, Dr. Virginia Borges

Example HIMSR project

Immunomonitoring of metastatic colorectal cancer patients treated with anti-PD-1 therapy

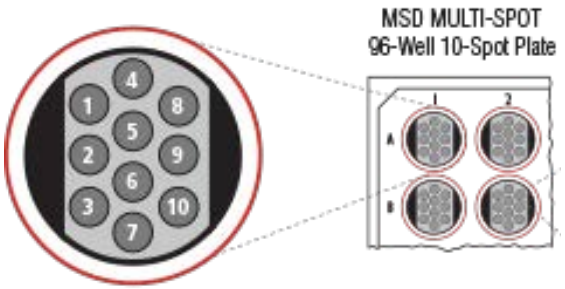
1. Ficoll Gradient PBMC isolation



2. Temporary storage of plasma and PBMC

3. Flow cytometry

4. Cytokine Array



5. *In situ* immune infiltrate in biopsies

Cancer Center Immunotherapy RFA, Karyn Goodman, M.D. and Stephen Leong, M.D., PI's

HIMSR Data Analyses and Bioinformatics

Self-use analysis stations:

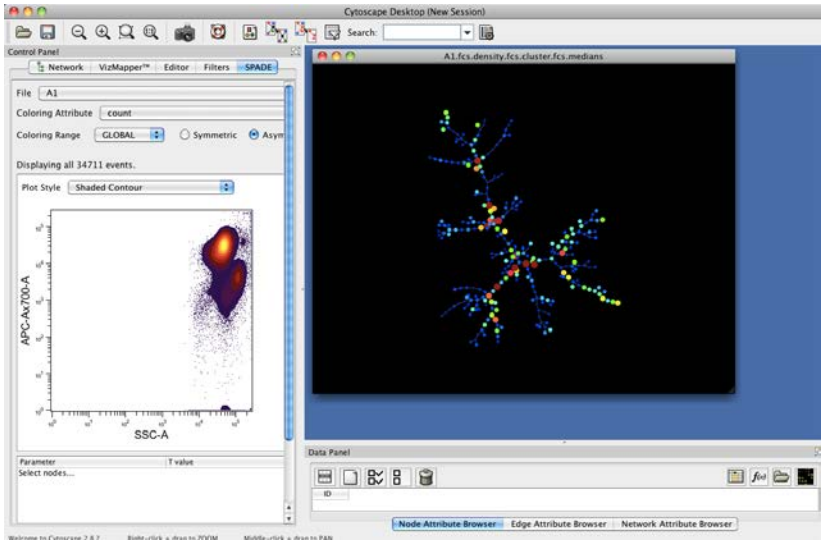
1. Image analysis

inForm software

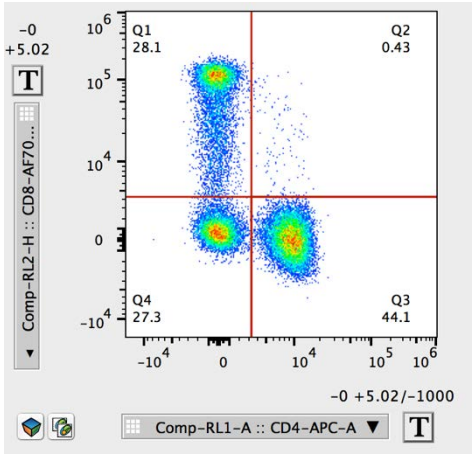
P18-8402a (Slansky lab)

2. Flow and Mass Cytometry analysis

Cytobank CU Premium account



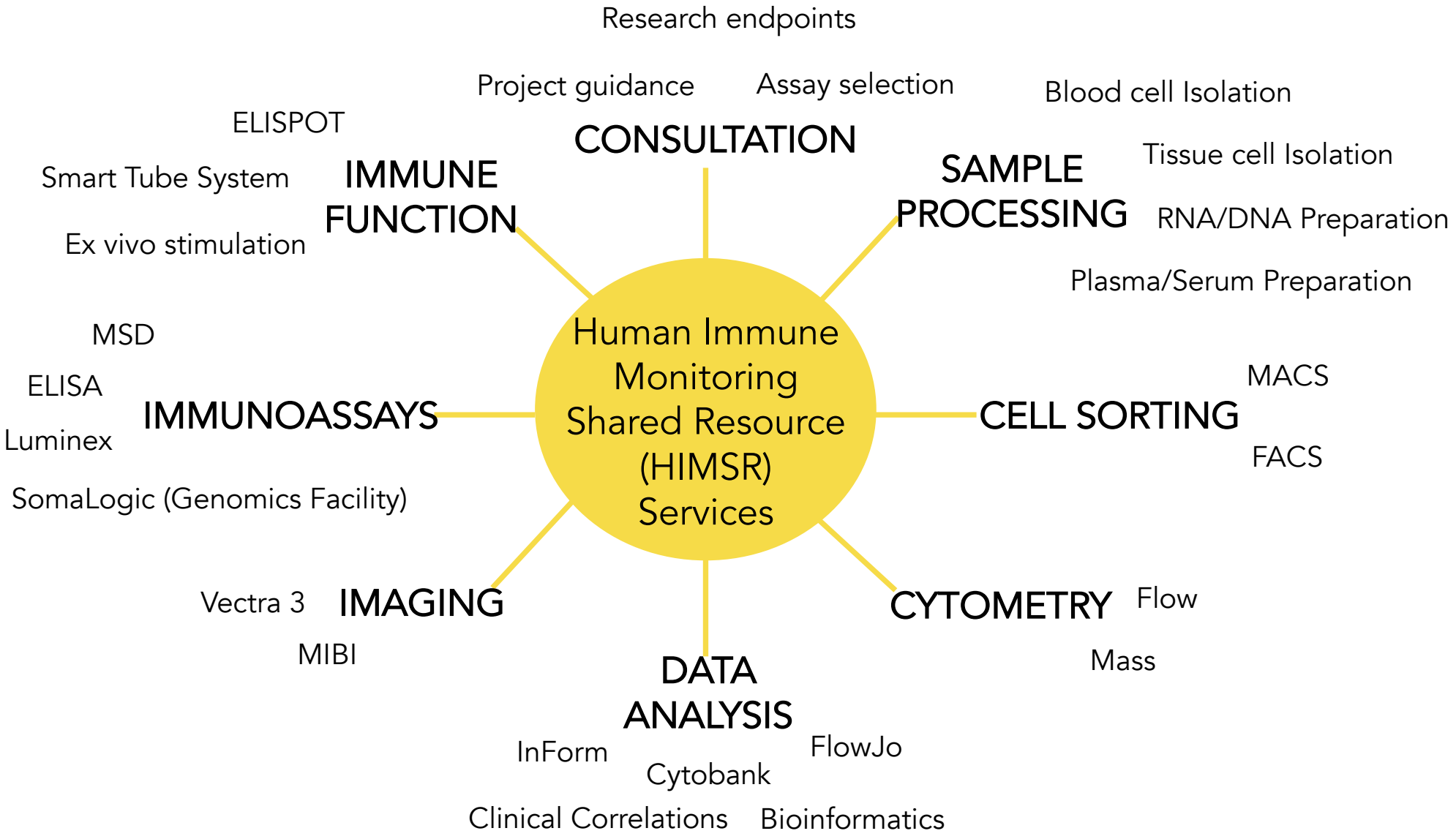
FLOWJO



Data analysis services:

- Flow: basic marker analysis and expression level
- Mass Cytometry: data normalization, barcode unmixing, marker analysis and expression level
- Vectra: images only, tissue and cell segmentation, phenotyping, scoring
- Depth of analyses dependent on investigator's need
- **Bioinformatician**

Human Immune Monitoring Shared Resource (HIMSR) Services



Interested in working with HIMSR?

Contact Kim Jordan and/or visit iLabs