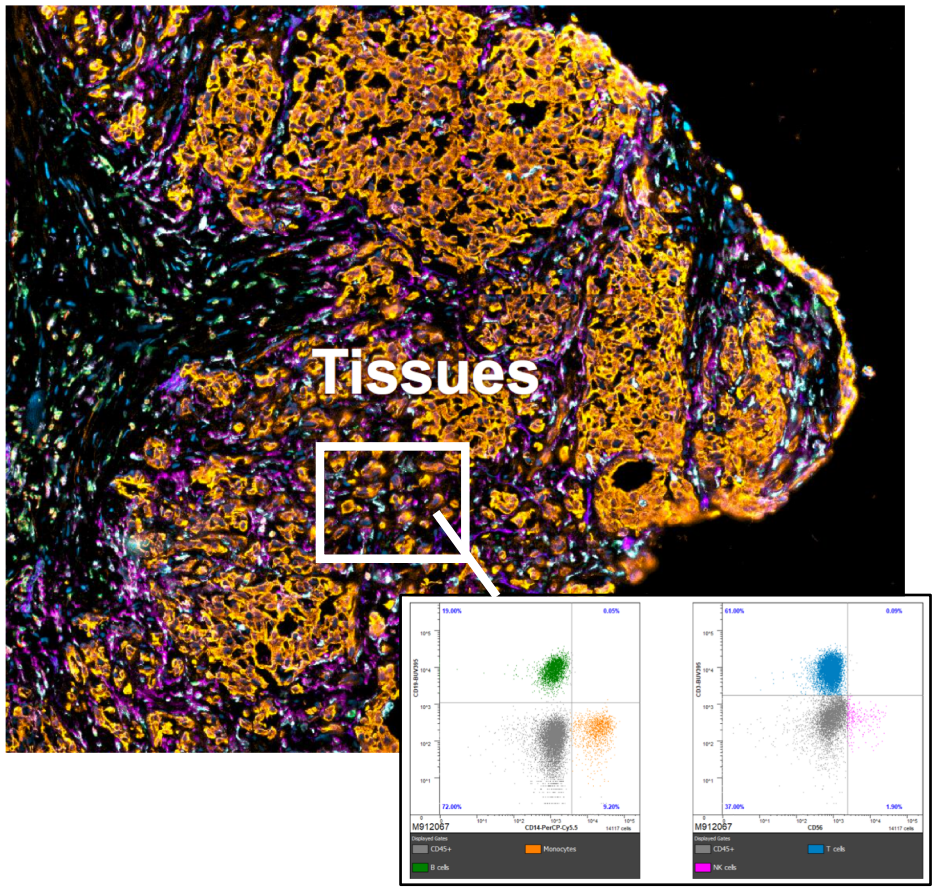
Intro to Chip Cytometry



Tissue Positionality

+

FACS-style Deep Phenotyping

(30-50-plex)

Lisa Borghesi
Professor of Immunology
Director, Unified Flow Core

ZellScanner ONE
Canopy Biosciences chip cytometer

- Spatial localization
- Deep phenotype (30-50+ plex)
- FACS-style gating, *extract per-cell fluorescence of co-expressed markers*

- Your trusted, validated Ab clones
- Non-destructive, re-interrogate same specimen later

Outline

Overview of chip cytometry

ZellScanner ONE unique capabilities

Chip cytometry method

Validated Ab panel info, core user fees

Reference: resources, images, publications, detailed Ab panels, etc.

What is chip cytometry?

Imaging method that reports positional and quantitative information on highly defined immune phenotypes within tissue sections.

ZellExplorer software is unique:

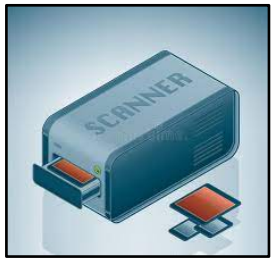
- FACS-like gating, visualize co-expression of multiple markers at single cell level
- Export fcs for cross-analysis in FlowJo

New Capabilities

ZellScanner ONE chip cytometer

- easy-to-use
- high plex, 30-50+ markers
- leverage your validated Ab panels
- FACS-type gating, per-cell marker co-expression, quantitative
- non-destructive, reanalyze sample w/ newly published biomarkers

Existing Capabilities



Slide scanners

- easy-to-use
- low plex, 9-12 color
- fast, quantitative

Confocal

- high-res, subcellular info
- low plex, 5-6 colors
- laborious, quantitative analysis challenging

GeoMx® DSP

CODEX

Digital spatial profilers

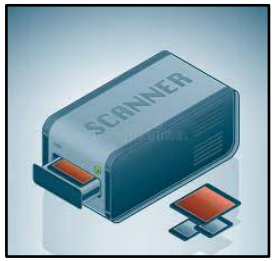
- high plex, 50-100+ markers
- entirely different method – development/validation
- customized reagents

New Capabilities

Chip cytometry (limitations)

- serial rounds of staining
  5 Ab/round, medium-throughput
- software challenges of:
  - resolving tightly aggregated cellular clusters
  - accurately resolving overlapping cells in different layers, “spatial spillover” or “stereology”

Existing Capabilities



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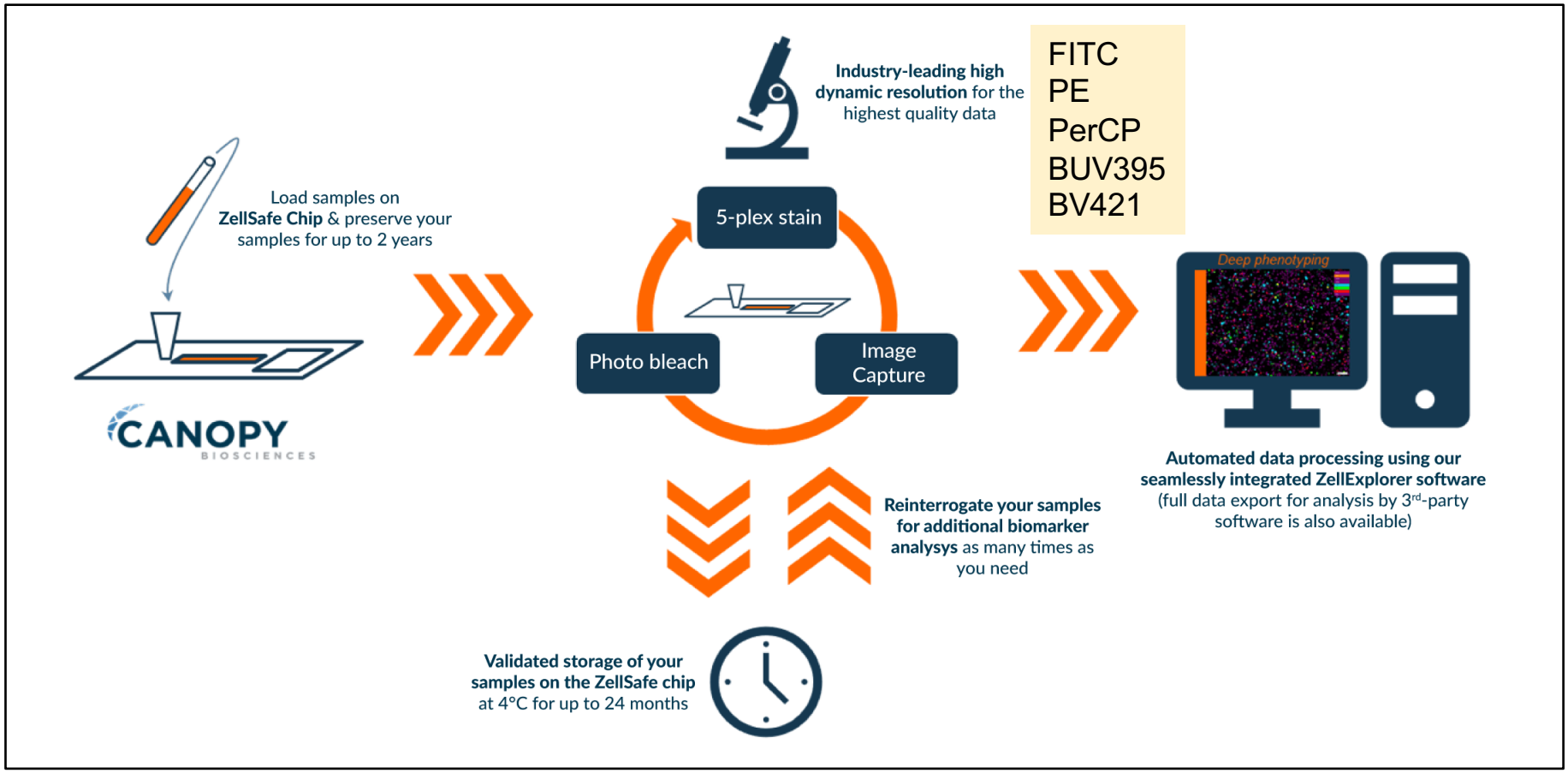
ZellScanner ONE Workflow



Process: Iterative rounds of staining w/ 5-plex cocktails, image, photobleach, repeat.

Timing: Roughly 0.5-1 day. Scanning all 99 positions takes 2 hours. Then focus on region of interest. A 1 mm structure = 9 fields of view. Photobleaching is the most time-consuming step, 20s/position.

ZellScanner ONE, Kane Lab murine splenic T cells

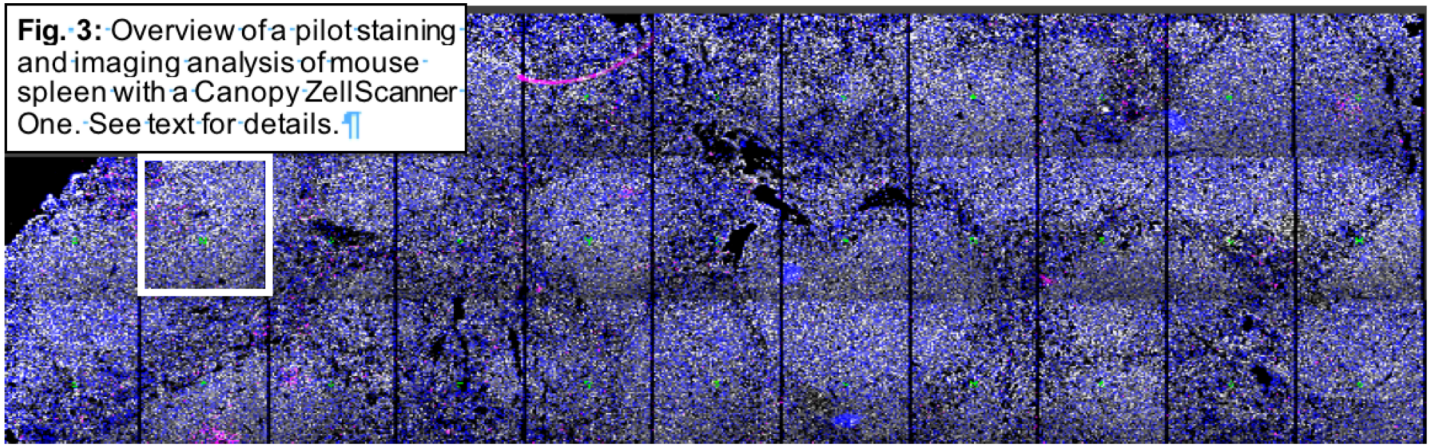


Fig. 3: Overview of a pilot staining and imaging analysis of mouse spleen with a Canopy ZellScanner One. See text for details.¶

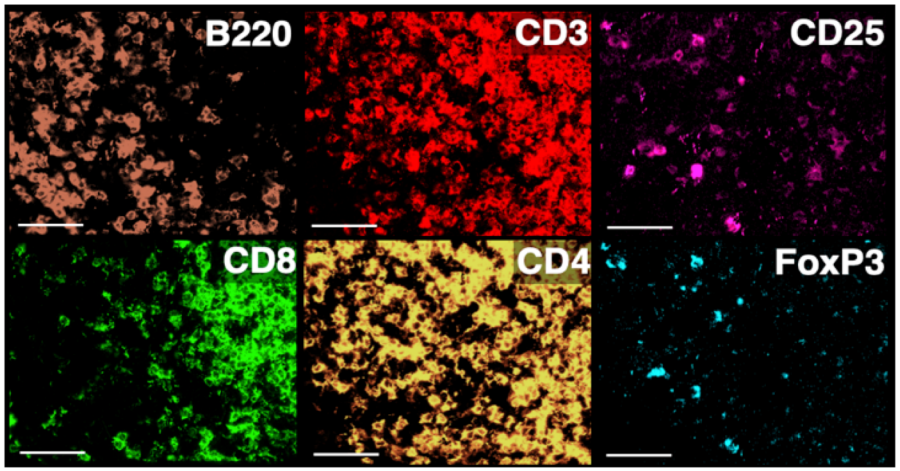


Fig. 4: Representative markers analyzed in pilot data for mouse spleen using Canopy ZellScanner One. Selected segments represent a sub-section of the area within the white box in Fig. 3. Note that FoxP3 is a nuclear transcription factor, leading to tight punctate staining, while other markers are cell-surface and cytoplasmic (ER, Golgi). Scale bar = 50 μM.

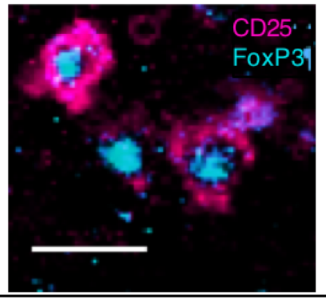
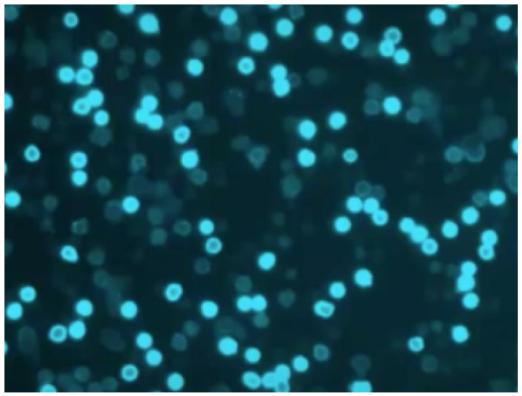
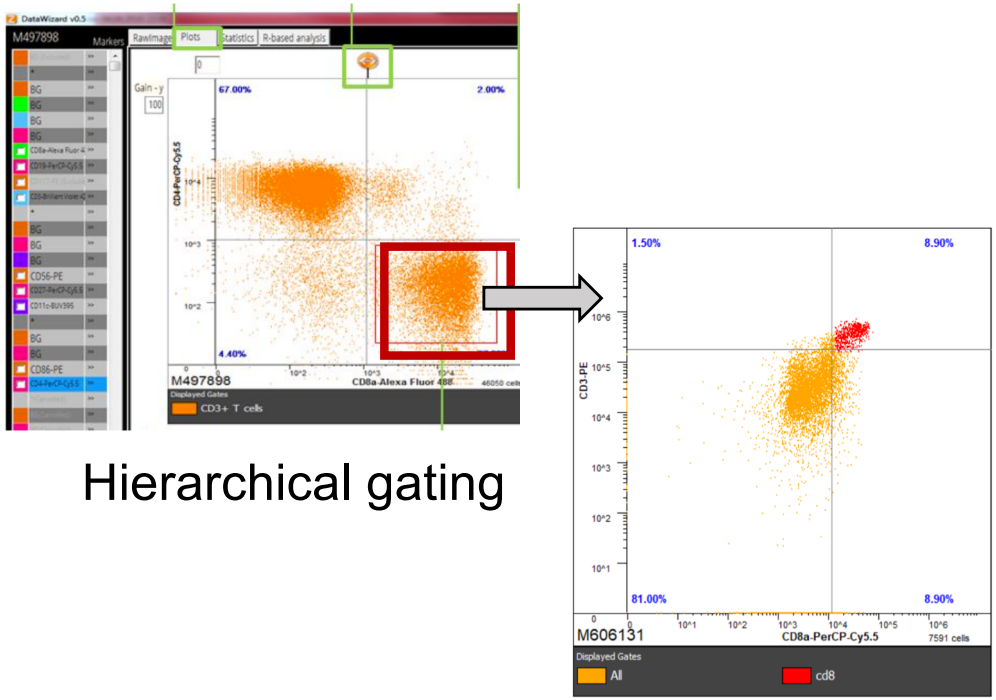


Fig. 5: Overlay of CD25 and FoxP3 staining for Treg. A sub-section from Fig. 4 was isolated and digitally enlarged. CD25 and FoxP3 were then overlaid. Scale bar = 10 μM.¶

ZellExplorer Software



Cells recognized by software



Hierarchical gating

- Also, export as fcs for analysis via FlowJo or computational algorithms

Canopy Biosciences Validated Abs & Panels

Species	Fresh Frozen	FFPE	PBMC
Human	60-plex	35-plex	120-plex
NHP	12-plex		35-plex
Mouse	40-plex		48-plex

See detailed validated Ab panels here:

https://2botn2272e4g2c0whuz2v3eb-wpengine.netdna-ssl.com/wp-content/uploads/2021/07/chipcytometry-marker-lists.pdf

(located on main page: https://canopybiosciences.com/chipcytometry-2/)

Costs

FACS Fee: $36/hr (unassisted core member)

ZellSafe chips + buffer: Kits of 100 are ~$6K = ~$60/chip

→ Flow core is looking into best way of stocking chips, allowing you to purchase individually

contact:
Dewayne Falkner (falkner@pitt.edu)
Unified Flow Core, Director of Operations

Reference Slides At End

Publications

Vendor Contact, Wendy Laird

Our other demo images
The Rays lab, lung
Oberbarnscheidt Lab, kidney

Detailed comparison of imaging instruments at Pitt

Canopy validated antibody panels
(Hu, NHP, murine // fresh-frozen or FFPE)

Acknowledgements

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Fadi Lakkis
Mark Gladwin
Larry Moreland
John McDyer
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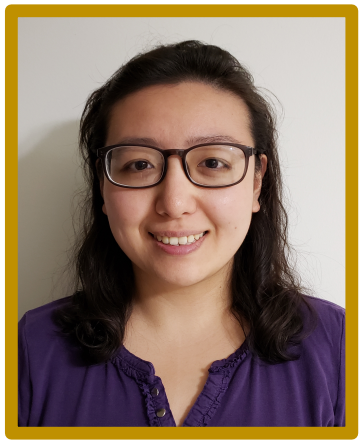

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Cytometry Specialist

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Publications

- Human gut issue-resident memory T cells subsets, FitzPatrick 2021 *Cell Reports*, 34:108661
- Unconventional MAIT T cells in human colon tissue, Leng 2019 *Cell Reports* 28:3077-91
- CAR T cells in murine intracerebral lymphoma, Mullazanni 2019 *PNAS* 116: 2475-84)
- T cells from atopic dermatitis patients in which blood samples were too limiting for analysis by flow cytometry, Roesner 2015 *J Inv Dermatol* 135:2324-27; Roesner 2016 *J Immunol* 196:3245-52
- Mouse lung macrophages, Happle 2014 *Sci Transl Med* 6:250ra113

Vendor Contact

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ZellScanner ONE, The Rays murine lung

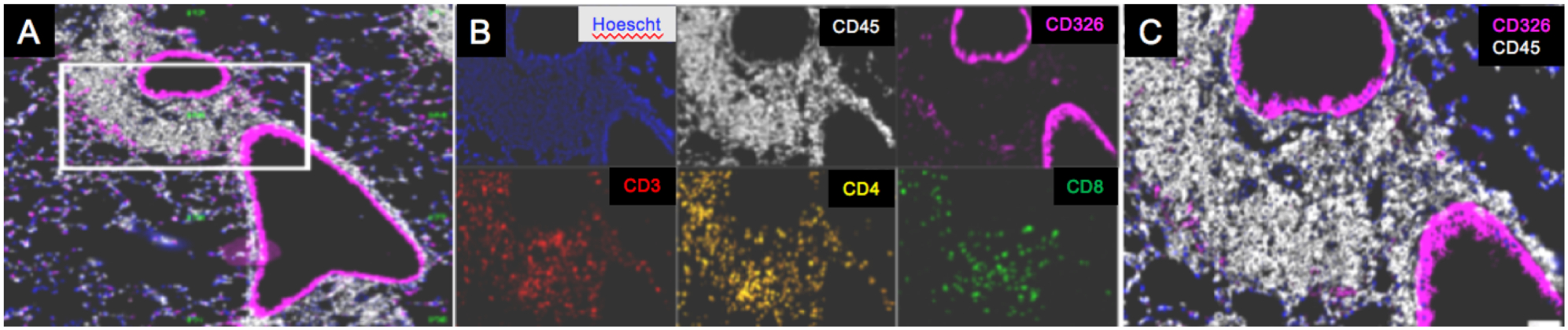

Fig 2. Zellscanner one pilot. **(A)** Overview of lung section from mouse model of severe asthma, stained for the indicated twelve markers and imaged on ZellScanner. **(B)** Representative markers analyzed in lung tissue section (white box in A) **(C)** Overlay showing epithelial cells (CD326, pink) and leukocytes (CD45, white).¶

ZellScanner ONE, Oberbarnscheidt murine kidney

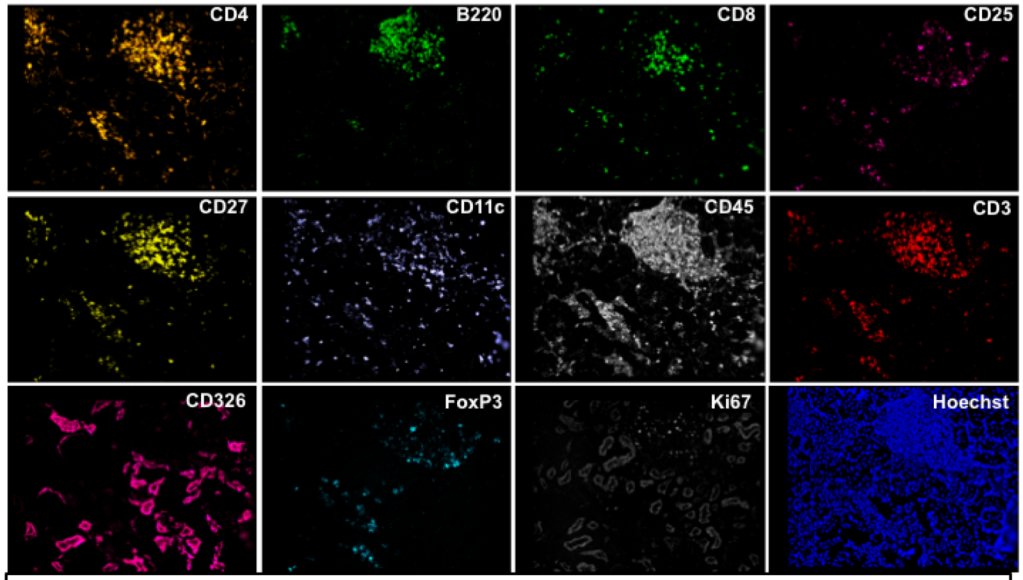

Fig. 2: Canopy ZellScanner 12-plex kidney tissue staining.

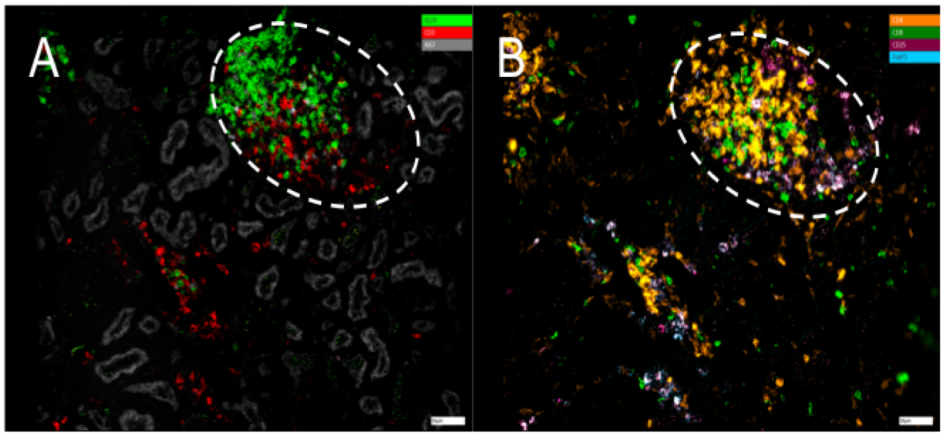

Fig. 3: TLO like structure in KTx (12-plex staining). (A) displays B cells and T cells. (B) Overlay of CD4, CD8, CD25 and FoxP3. Acquired with ZellScanner instrument.

Detailed Comparison of UPitt Instruments

	ZellScanner ONE	Perkin Elmer Vectra Polaris	NanoString GeoMx	CODEX	Confocal microscopy
Platform	Chip-based multiplexing, Ab-fluor	Slide scanner, Ab-fluor	Slide scanner, Oligo-antibody conjugates, barcoding	Slide scanner, Oligo-antibody conjugates, fluorescent dNTPs	Microscopy, Ab-fluor
Multiplexing theoretical limit	unlimited	9-plex	unlimited	unlimited	unlimited
Multiplexing practical limit	120-plex (hu)	9-plex	~90-plex (hu)	50	5-6 colors
Leverage existing flow expertise & antibody panels?	yes	yes	no	no	somewhat
Easy cross-validation by flow cytometry using same Ab clones?	yes	yes	no	no	yes
Robust in a multi-user setting w/ users of varying experience?	yes	yes	Learning curve	Learning curve	Learning curve
Hierarchical gating allows single cell resolution using complex markers?	yes	no	no	no	no
Validated immunology panels commercially available?	Tissue: Hu, Ms Suspension: Hu, Ms, NHP	no	Tissue: Hu, Ms	Tissue: Hu, Ms	no
Non-proprietary antibodies?	yes	yes	no, supplied by AbCam only	no, supplied by Akoya only	yes
Reanalyze exact same specimen w/ new biomarkers weeks/months later?	yes	no	no	no	no

Canopy Biosciences Validated Antibody Panels, next 4 slides

- **Human, fresh-frozen**
- **Human, FFPE**
- **Mouse, fresh-frozen**
- **NHP, fresh-frozen**

https://canopybiosciences.com/wp-content/uploads/2021/07/chipcytometry-marker-lists.pdf

HUMAN FRESH FROZEN TISSUE

Validated Target List

A list of targets corresponding to fully validated fluorescently conjugated antibodies for use in ChipCytometry assays. Antibodies have been tested in human fresh frozen (FF) tissue for precise and consistent performance. Select from our pre-validated target list to design custom panels specific to your project needs. Validation of custom biomarkers is available upon request.

CA-IX	CD29	CD86	CTLA-4	N-cadherin
CD3e	CD31	CD90	Cytokeratin 18	OX40
CD4	CD38	CD95	E-cadherin	Pan-cytokeratin
CD8a	CD39	CD123 (IL3RA)	EGFR	PD-1
CD10	CD40	CD141	Endoglin	PD-L1
CD11c	CD44	CD155	EpCAM	SMA
CD14	CD45	CD161	FoxP3	SMAD1/2/3
CD16	CD45RA	CD193 (CCR3)	GATA3	TIGIT
CD19	CD45RO	CD223	Granzyme B	TIM-3
CD20	CD56	CD278 (ICOS)	HER2	VEGFR-2
CD21	CD68	CD299	HLA-A	Vimentin
CD25	CD69	CD335	HLA-DR	
CD27	CD73	Collagen IV alpha	Ki-67	

HUMAN FFPE TISSUE

Validated Target List

A list of targets corresponding to fully validated fluorescently conjugated antibodies for use in ChipCytometry assays. Antibodies have been tested in human formalin-fixed paraffin-embedded (FFPE) tissue for precise and consistent performance. Select from our pre-validated target list to design custom panels specific to your project needs. Validation of custom biomarkers is available upon request.

CD3	CD25	CD123	HLA-A	S1PR1 (EDG-1)
CD4	CD27	CD138	HLA-DR	SMA
CD8	CD38	CD223	Ki-67	Sox-10
CD11c	CD45	CD357	Pan-cytokeratin	TCF1
CD14	CD45RA	CD366	PD-1	TIGIT
CD16	CD45RO	EGFR	PD-L1	Vimentin
CD19	CD56	FoxP3	p-AMPK alpha-1,2 (Thr183, Thr172)	
CD20	CD68	HER2		

MOUSE FRESH FROZEN TISSUE

Validated Target List

A list of targets corresponding to fully validated fluorescently conjugated antibodies for use in ChipCytometry assays. Antibodies have been tested in mouse fresh frozen tissue (FF) for precise and consistent performance. Select from our pre-validated target list to design custom panels specific to your project needs. Validation of custom biomarkers is available upon request.

CD3e	CD25	CD68	F4/80	MHC II
CD4	CD27	CD69	FAP	NK1.1
CD5	CD31	CD103	FoxP3	Pan-cytokeratin
CD8a	CD44	CD140b	GFAP	PD-1
CD11b	CD45	CD206	Ki-67	PD-L1
CD11c	CD45R (B220)	Desmin	Ly6C	SMA
CD19	CD64	EpCAM	Ly6G (GR1)	VEGFR-2

NON-HUMAN PRIMATE FRESH FROZEN TISSUE

Validated Target List

A list of targets corresponding to fully validated fluorescently conjugated antibodies for use in ChipCytometry assays. Antibodies have been tested in non-human primate fresh frozen (FF) tissue for precise and consistent performance. Select from our pre-validated target list to design custom panels specific to your project needs. Validation of custom biomarkers is available upon request.

Biotin	CD16	CD56	IgG
CD3	CD20	CD159a	IgM
CD14	CD45	IgA	

Have fun!