Multi-omics

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CANOPY BIOSCIENCES

Canopy Biosciences World-wide

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Canopy's Product range

- **Gene Editing**
  - CAS9 Protein
  - CRISPR Complete Kits
  - TUNR Flexible Gene Editing
  - miRNA CRISPR Knockout Kits
  - Custom Cell Line Engineering
- **Gene/Protein Expression**
  - Chip Cytometry
  - Nanostring
  - RNAseq Services
  - RAREseq error corrected NGS services
  - EZ Species
  - Meso Scale Discovery
  - miRNA qPCR Kits
  - miRNA Mimics
  - Multiplex PCR 20/20 Kits & Reagents
  - Cellatrix 3D Cell Culture Systems
- **Bioprocessing**
  - CHO complete
  - Host Cell Protein ELISA Kits

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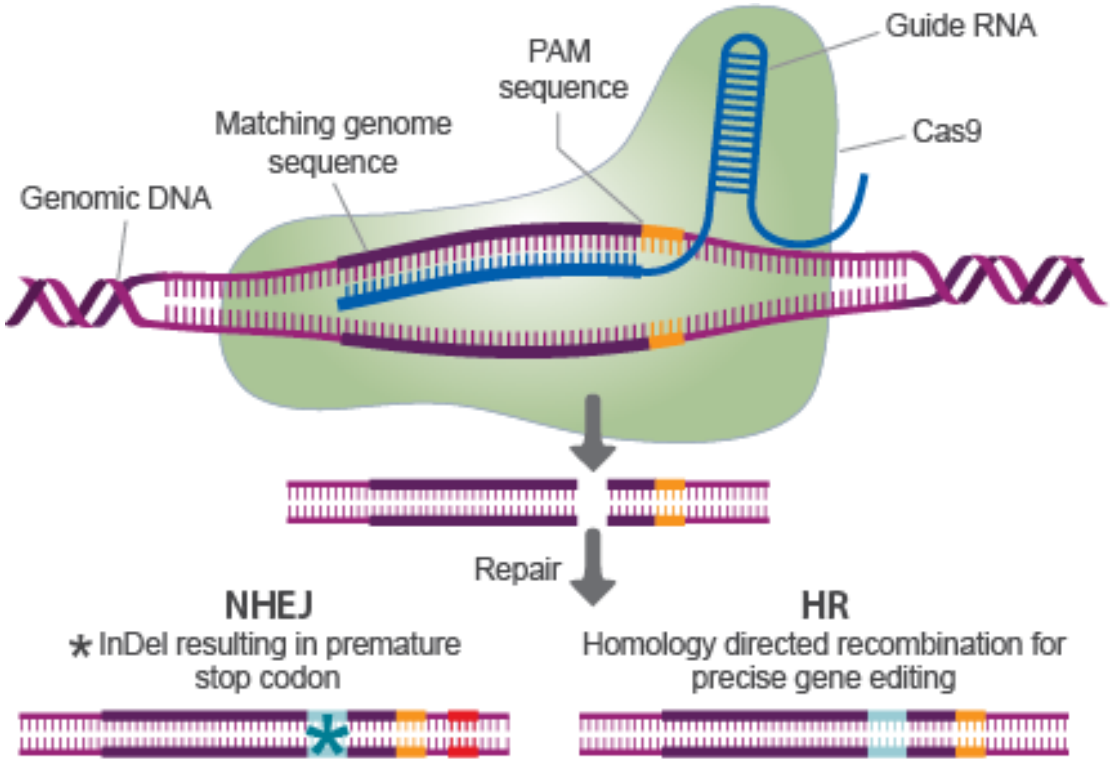
CRISPR Complete Kits

- CRISPR Complete Kit is the only CRISPR Kit for Knock-in and Point mutations
  - CRISPR kits are available for almost any project type, including knockouts, knock-ins, TUNR knockdowns, point mutations, gene replacements, humanizations, tagging, and many more.
  - Included in the CRISPR Complete Kit:
    - crRNA—custom-designed for your project
    - tracrRNA
    - Cas9 Nuclease Protein
    - Donor construct—custom-designed for your project
      (may be provided as oligonucleotide or plasmid, depending on your project needs)



Main Menu

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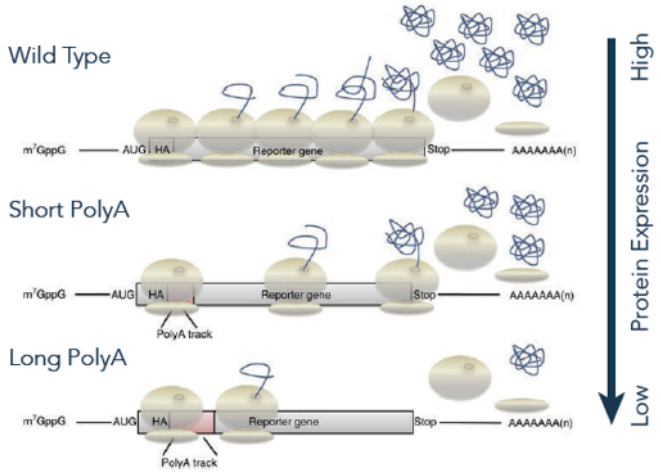
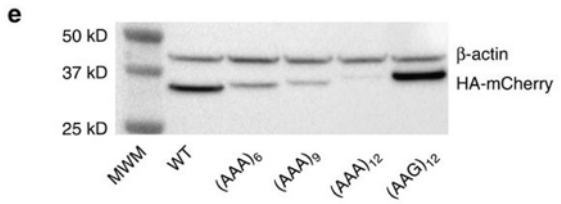
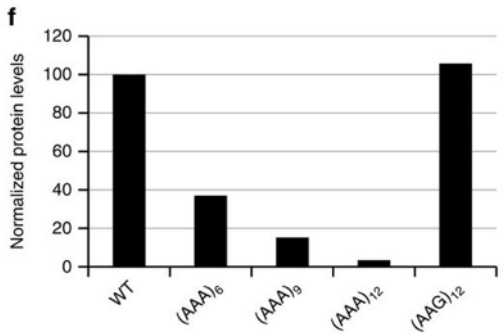
TUNR Flexible Gene Editing

- Precisely tune gene expression from 100% all the way down to complete knockout.
- PolyA Sequences Reduce Translation.
- Advantages:
  - Generate range of KD
  - Modification of endogenous expression
    - No random insertions
    - Synteny maintained
    - Intronic regions maintained
    - Endogenous promoter
  - Permanent, stable modification







Main Menu

Cell Line Engineering

Main project types

1. **Knockout:** inactivation of a gene
   a) NHEJ knockout (standard)
   b) Large deletion
2. **Knock-in:** targeted insertion of exogenous DNA
   a) Replacement of mouse gene with human gene
   b) Introduction of point mutation
   c) Correction of point mutation
   d) Tagging with reporter
   e) More
3. **Random transgenic:** random insertion of exogenous DNA
   a) Overexpression of recombinant protein
   b) Tagging with reporter
   c) more

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Successfully Edited Cell Lines

- Many cancer cell lines
- Human iPSC and ESCs
- CHO for Bioprocessing
- You can send own line or we can procure

Cell lines	Specise	Tissue
92.1	human	eye (primary uveal melanoma)
22Rv1	human	prostate carcinoma
290-Capl-CBR-Luc-cherry-3s	human	
4T1-Luc2	mouse	breast
59M	human	Ovarian tumor, epithelial
A20	mouse	B lymphocyte
A549	human	lung
Ark-2	human	endomertial serous adenocarcinoma
Ark-4	human	endomertial serous adenocarcinoma
ATCD5	mouse	chondrocyte
B16-F10	mouse	melanoma, spindle-shaped and epithelial-like
BalBC_T11	mouse	Strain specific fibroblast
BEAS-2B	human	lung
BJAB	human	blood (suspension)
BT-20	human	mammary gland/breast, epithelial
BV2	mouse	microglia
C2C12	mouse	muscle
C6	rat	neuron
Caco-2	human	Colon, epithelial
Calu-3	human	lung adenocarcinoma, epithelial
Calu-6	human	lung, epithelial
CAMA-1	human	mammary gland/breast, epithelial
CEM	human	T lymphoblast
CFPAC-1	human	pancreas, epithelial
COLO205	human	colon
COLO668	human	Small cell lung carcinoma, derived from metastatic site: brain
COV318	human	ovary
CT26	mouse	colon, fibrblast
CT-40	chicken	bursa, lymphoblast, suspension
DLD1	human	colon
Du145	human	prostate
EFM19	human	breast, epithelial
EMT6	human	breast
F244	mouse	sarcoma line
Hap1	human	near-haploid, chronic myelogenous leukemia (CML), adherent
HCC827	human	lung, adenocarcinoma, epithelial
HCC4006	human	lung, derived from metastatic site: pleural effusion, epithelial
HCT8	human	colon, ileocecal colorectal adenocarcinoma, epithelial
HCT116	human	colon, colorectal carcinoma, epithelial
HEK293	human	kidney
HeLa	human	ovarian
HepG2	human	liver
HL60	human	peripheral blood, acute promeylocytic leukemia, suspension, promyeloblast
HLEB-3	human	lens epithelial cells
HMC3	human	microglia, adherent, embryo
HME1	human	mammary epithelial
HPAC	human	pancreas
HT-29	human	Colon, epithelial
HUH7	human	liver
Ins1 832/13	rat	beta cell
iPSC (BJFF internal clone)	human	fibroblast
Jurkat, Clone E6-1	human	peripheral blood
K562	human	blood
LLC-OVA	mouse	
LN-Cap	human	prostate, metastatic derived site: left supraclavicular lymph node, epithelial
LoVo	human	colorectal adenocarcinoma
M2C		
MC38-OVA	mouse	
MCF7	human	mammary gland, derived from metastatic site: pleural effusion, epithelial
MCF10A	human	mammary gland, epithelial
MCF10DCIS.com	human	Breast cancer cells
MDA-MB-231	human	Breast cancer
MEF	mouse	embryonic fibroblast
MEG-01	human	bone marrow, patient in megakaryoblastic crisis of CML
Mel202	human	eye (primary uveal melanoma)
MONO-MAC-6	human	acute monocytic leukemia
MWCL-1	human	Waldenstrom macroglobulinemia
N2A	mouse	neuron
NCI-H1299	human	lung
NCI-H28	human	lung
NCI-H441	human	lung
NCI-H460	human	
NCI-H661	human	
NCI-H69	human	lung
NCI-H810	human	
NCI-H838	human	
NIH 3T3	mouse	fibroblast
NKM1	human	acute myeloid leukemia
NSC-34	mouse	hybrid line, fusion of motor neuron enriched, embyronic mouse spinal cord cells wi
PC3	human	prostate
PC9	human	lung adenocarcinoma, epithelial
PCL12	human	chronic B cell leukemia
PLB-985	human	myelomonoblasts
PNEC	mouse	prostate
Pymt-Bo1	mouse	?
Raji	human	B lymphoblast, suspension
Ramos1	human	B lymphocyte, suspension
RAW264.7	mouse	macrophage
Rec-1	human	lymphoblast, supension
RPCI-BMJ2	human	
RT112	human	bladder
RT4	human	bladder
SCC23	human	head and neck
SF7761	human	pediatric diffuse intrinisic pontine glioma
SF94271	human	
SHP77	human	lung, epithelial
SiHa	human	cervix, epithelial
SK-BR-3	human	Mammary gland
SNU-1077	human	uterine carcinosarcoma
SNU-539	human	uterine carcinosarcoma
SNU-685	human	uterine carcinosarcoma
SPAC1L	human	serious surface papillary carcinoma
SPAC1S	human	serious surface papillary carcinoma
SPEC2	human	serious papillary endometrial carcinoma-2
SU-DHL-6	human	peritoneal effusion, metastatic site - peritoneal cavity, B lymophocyte, suspension
SUM225	human	invasive ductal carcinoma
SW48	human	colon, epithelial
T47D	human	mammary gland, metastatic site derivied, epithelial
THP-1	human	blood, monocyte, suspension
TMD8	human	B-Cell Lymphoma
TRAMP-C2	mouse	prostate, epithelial
U251MG	human	brain, adherent
U2OS	human	bone
U373MG	human	glioblastoma
U-937	human	pleural effusion, lymphocyte, monocyte suspension
UM-UC-13	human	bladder
UM-UC-3	human	bladder
UM-UC-6	human	bladder
UM-UC-7	human	bladder
WI-38	human	fibroblast, lung
WSU-FSCCL	human	non-Hodgkin's lymphoma

Main Menu

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Timelines

- Billed in 5 milestones
- KO: from 4 months
- KI: from 5 months

Milestone	Description	Time (Weeks)	Price
Milestone 1	Test for Mycoplasm	1	10%
	Evaluation of cell line suitability for gene editing	2 - 4	
Milestone 2	CRISPR and/or TUNR design, assembly and validation in core cell line	1 - 3	5%
Milestone 3	Transfection/nucleofection and activity confirmation in transfected pool	1 - 3	50%
	Single cell dilution cloning and maintenance of single cell derived clones	3 - 7	
Milestone 4	Single cell derived clone screening, deep sequencing library preparation, deep sequencing run and analysis	2	25%
Milestone 5	Positive clone expansion, genotype confirmation, clone cryopreservation	2 - 4	10%
	Test for Mycoplasm	1	

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Cell Line Engineering Pricing Sheet

Project Type	Price
CAS9 (50µg)	$95
CAS9 (250µg)	$395
CRISPR complete kit	$990
Knock out	$14,990
Knock in	$24,990
Point Mutation	$24,990
TUNR (4xTUNRs + parental and KO)	$49,900
Transgenic	$8,990
iPSC additional fee per project	$9,990
Extra allele fee per project	$1,190
Additional clones (if identified)	$600
Screen of top 10 off target sites**	$1,390

Standard Deliverables

- Up to 2 clones*
- Biallelic modifications
- In silico off target and essentiality screens
- gRNA validation by NGS
- Sequence verified clones

* Delivery of one clones satisfies the milestone. A second clone, if recovered, is available to the researcher for no additional cost. We will screen 400 wells for KO projects and 800 wells for Kis.

** Deep sequencing of 400 bp region of the top 10 off target sites. Pricing covers screen of 2 clones.

*** If client is providing cell lines, 2 vials of 1 million cells per cell lines should be shipped to Canopy

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Canopy's miRNA Offering

Detect

qPCR Assays

Validated qPCR primers for all miRNAs (SYBR Green)

NanoString Service

Detect and quantify 800 miRNAs in a single sample

Edit

CRISPR Plasmids

All-in-one plasmids containing two sgRNAs for complete excision

Mimic

Mimics

Synthetic miRNA molecules designed for transfection efficiency and reduced OTEs

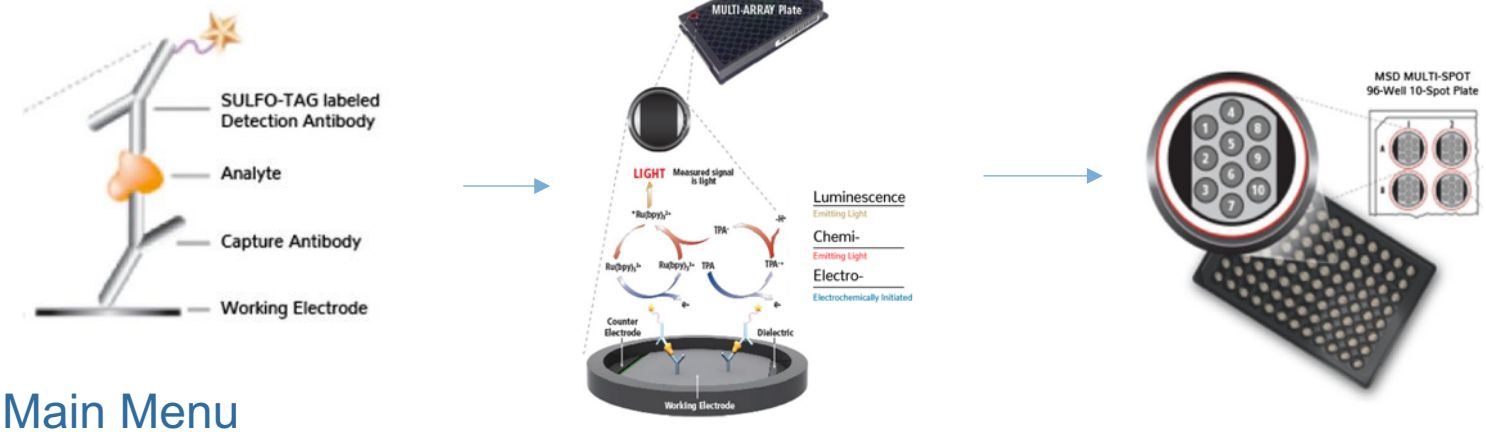
Main Menu

Meso Scale Discovery

- Multiplexed **protein** detection
- **50+** analyte panels; **10** analytes in a single sample
- Wide dynamics range
- More sensitive than Luminex

Table 1
Limits of quantification

Cytokine	LLOQ (pg/ml +/− S.D.)	
	Luminex (Biosource kit)	MSD (MSD kit)
IL-2	8.7 (+/−2.2)	2.5 (+/−3.0)
IL-4	8.6 (+/−2.9)	0.7 (+/−0.1)
IL-8	7.7 (+/−1.5)	0.7 (+/−0.1)
IL-10	11.6 (+/−0.8)	3.6 (+/−5.0)
IL-12	21.3 (+/−6.1)	2.3 (+/−2.8)
IFNg	14.4 (+/−3.0)	0.7 (+/−0.03)
TNFa	5.9 (+/−4.9)	3.2 (+/−4.3)



Main Menu

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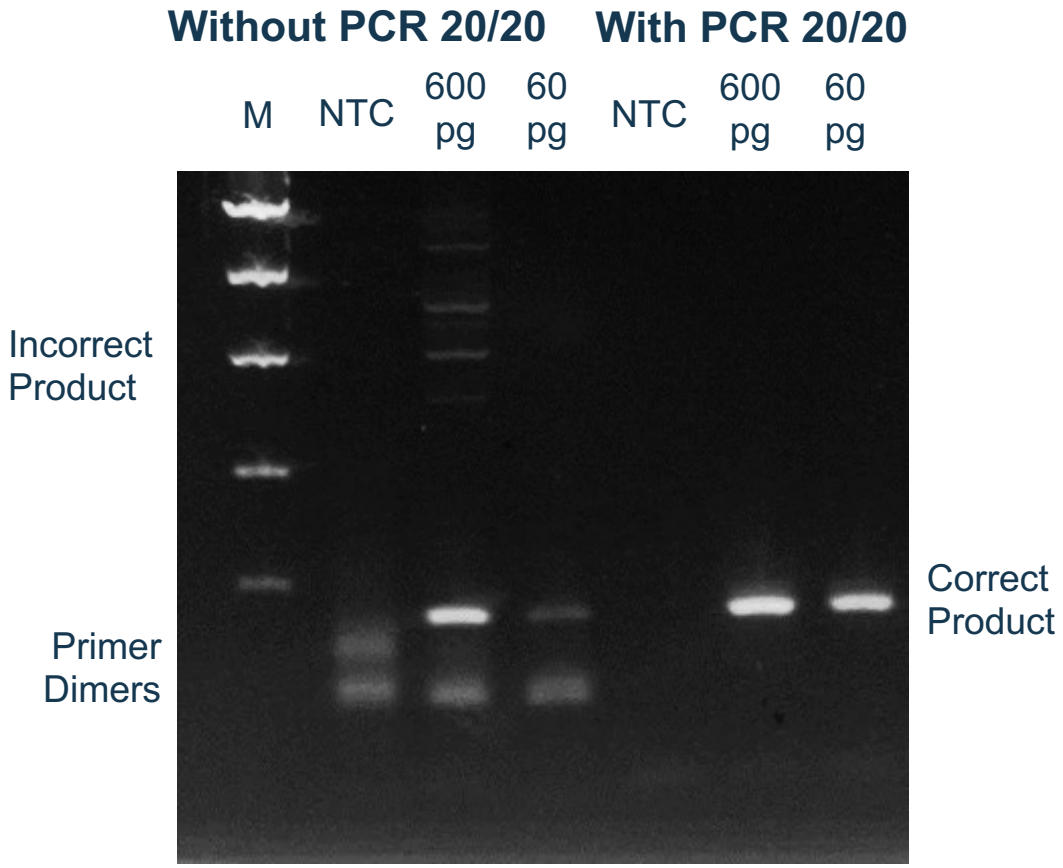
Nanostring

- Multiplex analysis of up to 800 RNA, DNA, or protein targets.
- We are a NanoString **full service provider**, from RNA isolation to comprehensive data analysis service.
- We provide a comprehensive data & pathway analysis package and reporting service.
- In as little as a two week turnaround time to receive a comprehensive data analysis report.
- We provide the majority of standard Nanostring panels off-the-shelf and custom design services as well.
- We accept a wide variety of samples including: tissue (fresh or fixed), FFPE sections, blood, serum, plasma, PBMCs, and CSF.

Main Menu

Multiplex PCR 20/20 Kits & Reagents

- 3 PCR additives to “clean up” your PCR
- Inhibit PCR enzymes from acting outside of precise temperature ranges, preventing unwanted side reactions and off target activity
- Acts directly on the polymerase to prevent non-specific enzymatic activity below 50°C; enzyme activity is restored at 60 °C
- PCR 20/20 re-engages with polymerase during cooling to prevent post-reaction amplification
  - Reduces primer dimers
  - Reduces incorrect products
  - Primers aren’t wasted = stronger signal



Main Menu

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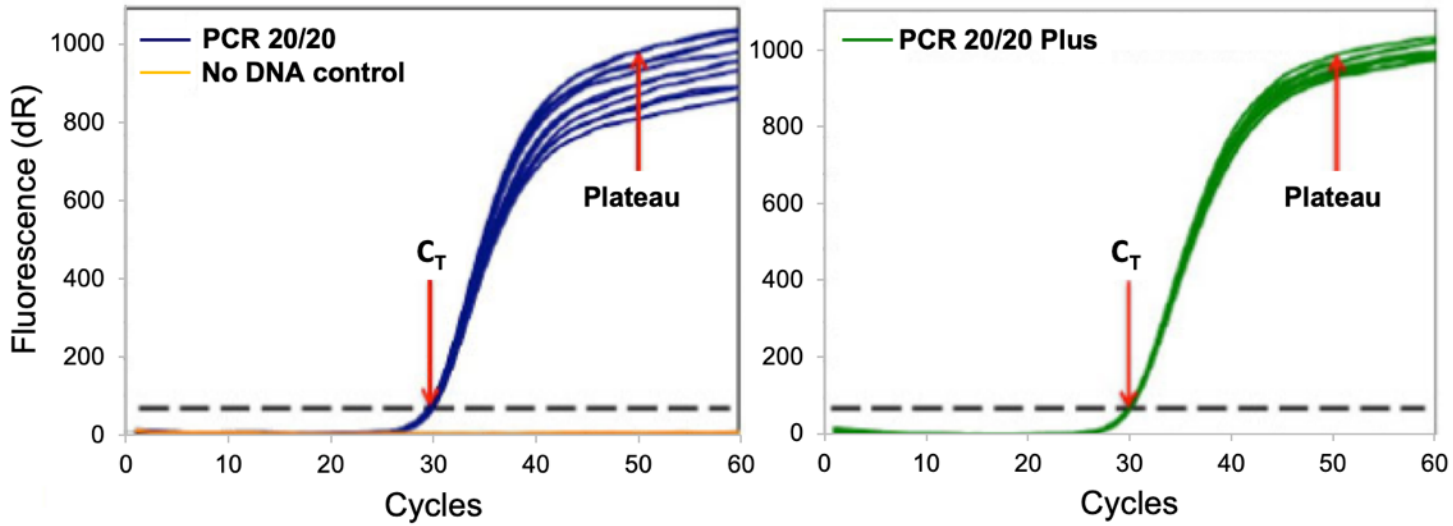
PCR 20/20 Plus

A 2-component kit

- PCR 20/20
- Focus: A double-stranded, chemically modified nucleic acid that suppresses mis-priming during the annealing and extension steps of PCR

- Improves reproducibility of data
- Increases detection of low copy number targets
- Reduces mis-priming
- Improves end-point genotyping

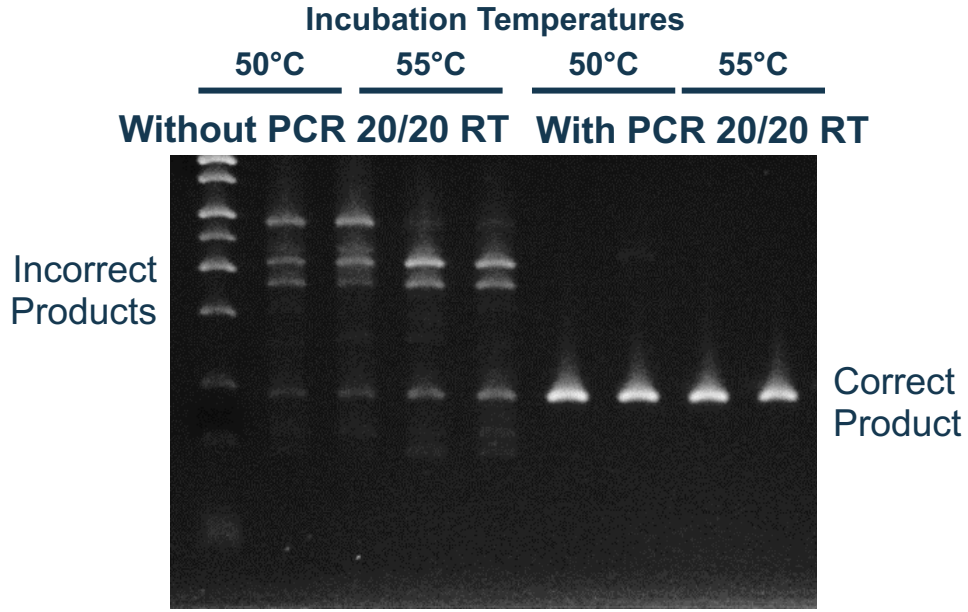


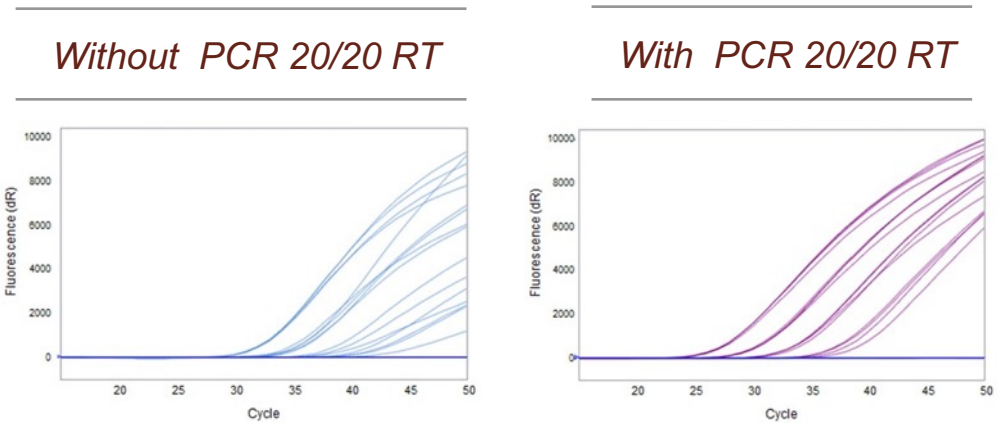
PCR 20/20 is a combination of 2 reagents that work synergistically to suppress all forms of mis-priming before, during and after amplification

Main Menu

PCR 20/20 RT

- Controls reverse transcriptases to prevent off-target amplification
- Increases specificity & yield
- Demonstrated to work with both one-step and two-step RT reactions





Main Menu

RNA Seq services

- We provide a customized service and optimized packages
- **Full service workflow:** Library prep, QC, and sequencing
- Standard sample inputs:
  - Isolated RNA: 1 µg (>50 ng/µl)
  - Tissue: 5-30 mg
  - Cell pellets: 150,000 cells
  - FFPE: 3 x 10 µm curls
  - Blood: 1 PAX gene tube
- fastq files and run summary standard. Comprehensive analysis and report available

Optimized Package Offerings		
mRNA Seq	**Total RNA Seq**	**FFPE RNA Seq**
• Poly A enrichment library prep • As little as 500 pg input • Targeting 60M paired end reads • 2x150 read length	• Ribo depletion library prep • As little as 500 ng total RNA • Targeting 60M paired end reads • 2x150 read length	• Offering both Whole Transcriptome and Targeted Exome strategies for FFPE samples • Targeting 100M paired end reads • 2x150 read length

Main Menu

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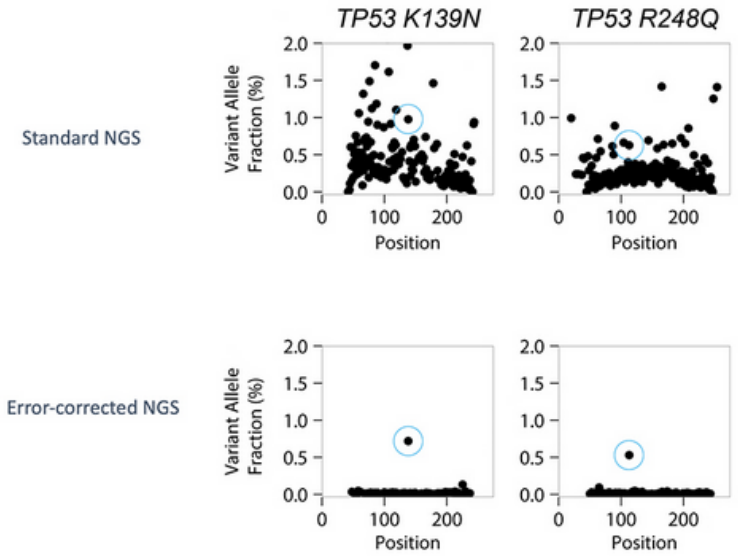
RAREseq

- Standard NGS methodology introduces errors during PCR and the sequencing workflow. These errors result in noise that makes it challenging to differentiate between errors and true variants. RareSeq™ NGS data analysis services uncovers these true variants.
- Mutations can be identified 6 years prior to onset of t-AML ... If you can find them

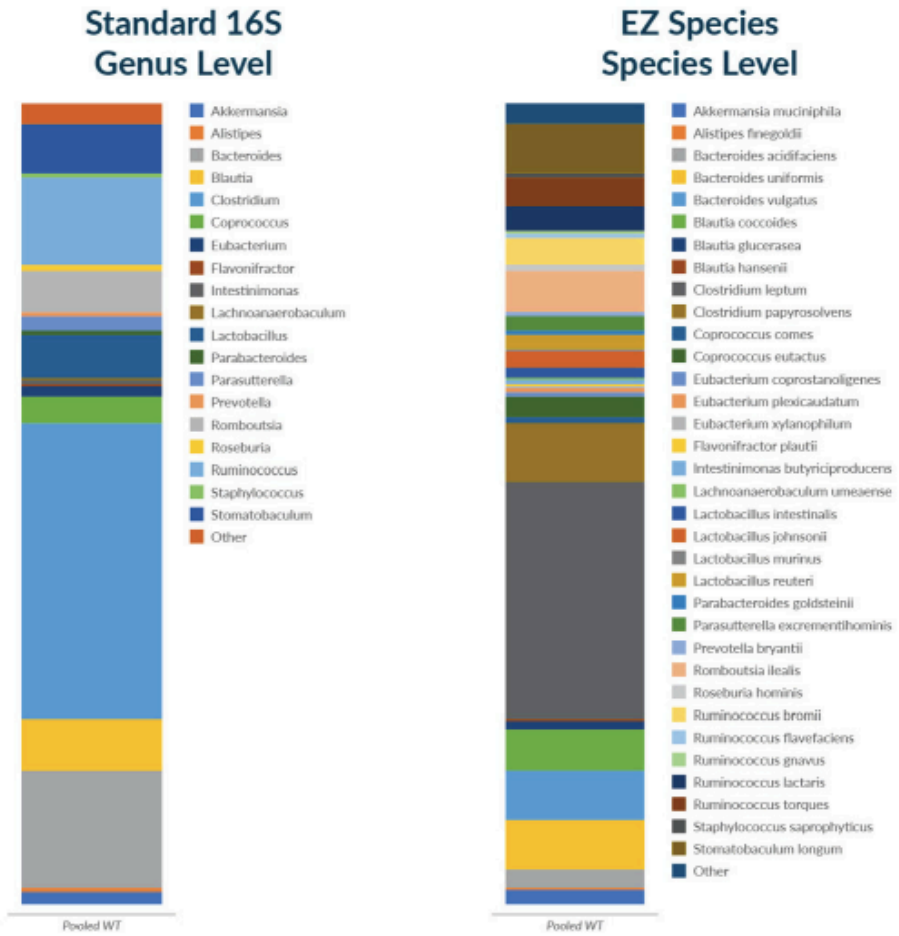
Pre-leukemic subclone identification



Main Menu

EZ Species: Microbiome Sequencing Service

- Easily ID the bacterial composition of your sample, down to the species level
- Achieve shotgun sequencing quality data with the efficiency of 16S sequencing
- Standard 16S rRNA hypervariable region sequencing and analysis is only specific enough to yield genus level sample identification, leaving important taxonomic information on the table
- Comprehensive report available



Main Menu

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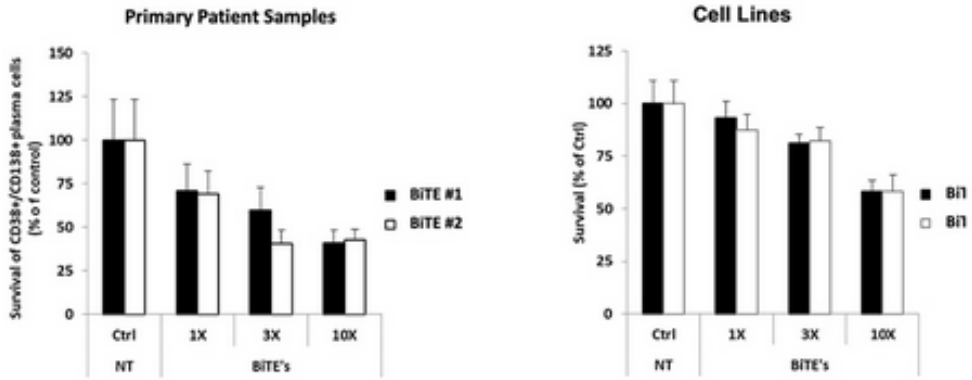
Cellatrix 3D Cell Culture systems

- **Human Bone 3D Matrix**: A 3D-matrix derived from human bone marrow. No exogenous polymers. Promotes cell proliferation for hematopoietic cells and stem cells, especially hematologic malignancies.
- **Human Peripheral Blood 3D Matrix**: A 3D-matrix derived from human peripheral blood. No exogenous polymers. Promotes cell proliferation especially primary human cells, especially solid tumors.
- **Mouse Peripheral Blood 3D Matrix**: A 3D-matrix derived from mouse peripheral blood. No exogenous polymers. Promotes cell proliferation.
- **3D Efficacy Studies:** Screening service to assess the efficacy of your immunotherapies on patient-derived cells grown in our 3D matrix.

Translational efficacy studies for immunotherapies

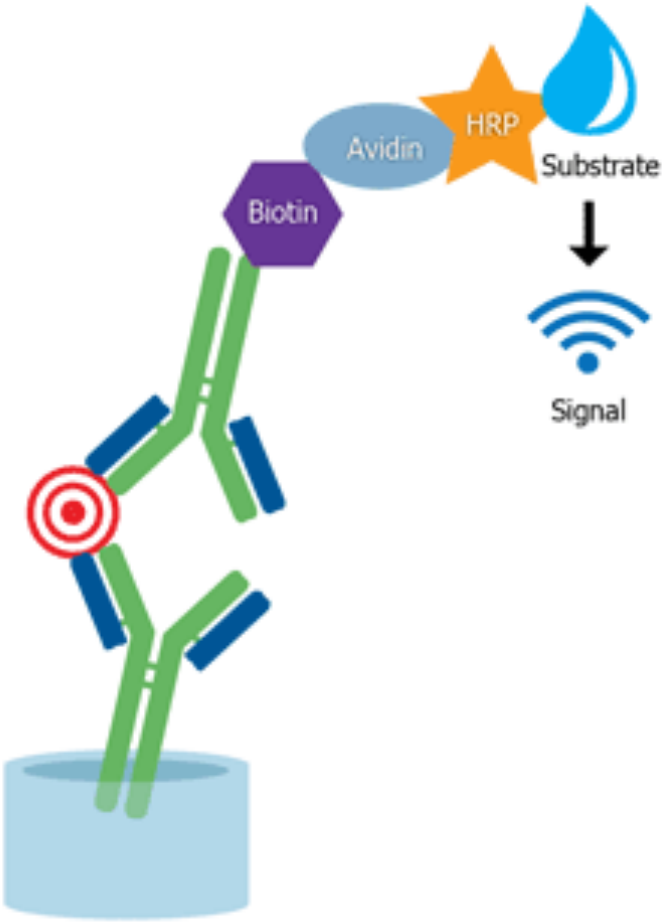
Our 3D efficacy studies have demonstrated highly translational results for a variety of emerging immunotherapies, including CAR-T, BiTE, antibody-drug conjugates, and immunomodulatory drugs.



Main Menu

Host Cell Protein ELISA Kit

- Detect and quantify HCP concentrations at any point in your purification process
- ELISA-based: quick, familiar format with high sensitivity
- Available for E. coli, Pichia pastoris, CHO, Protein A and HEK293T
- 96-well plate coated with capture antibody
- HCP standards
- Reporting antibody Streptavidin-HRP conjugate
- TMB substrate 5x dilution buffer, 10x PBS-T, stop solution and plate sealers also included



Main Menu

Chip Cytometry: Quantitative High-Plex Proteomics

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Main Menu

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Agenda

- Key challenges
- Case Studies
- Principles of Chip Cytometry

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Key Challenges

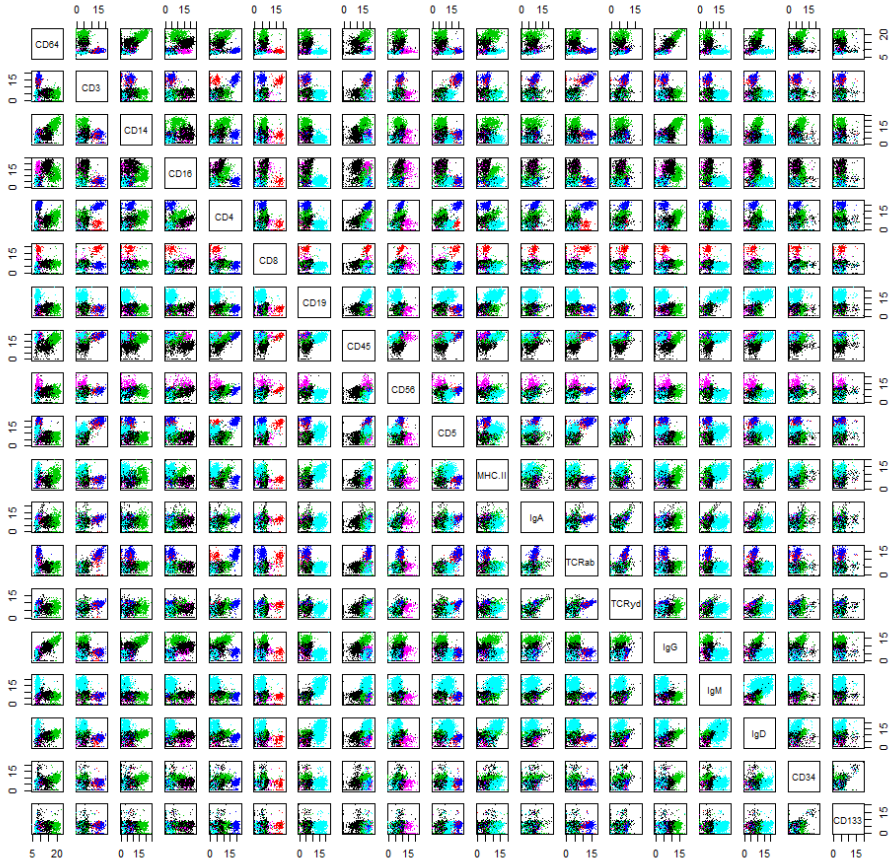
Why are researchers looking for alternatives to traditional approaches?

- **Single Cell Flow Cytometry**
- **Visual Pathology**
- **Biomarkers & Clinical Trials**

Single Cell Precision
Spatial Deep Phenotyping
Checkpoint inhibitors
CAR-T Therapy
Chip Cytometry

Single Cell Flow Cytometry

- Large capacity
- Wide dynamic range
- Well-used tool for multiplexed data
- Tissue sample must be digested
- Sample stable for 2-3 Days
- Must select the right biomarkers at the start of the study
- Significant spillover/compensation challenges for high-plex panel
- No spatial information, limited morphological information



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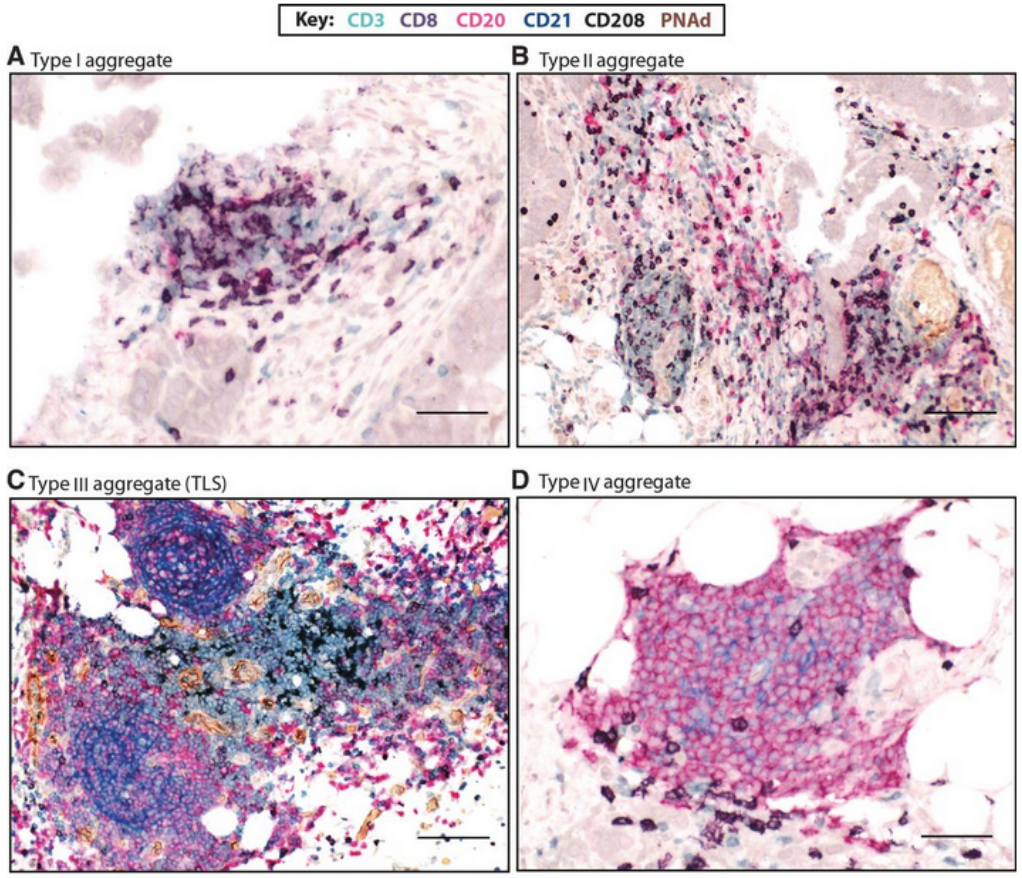
Visual Pathology

- Intact Spatial Relationships
- Intact Cell Morphology
- **Limited Multiplexing Capability**
- **Semi-quantitative**



Kroeger, Biology of Human Tumors 2016

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Biomarkers & Clinical Trials

- **Sample Stability:** Limited ability to answer all the questions your have about patient longitudinally across a clinical trial (miss objectives?):
  - New Biomarkers?
  - Re-interrogate?
- **Standardisation:** Limited ability to standardise clinical trial sample collection across multiple clinical research sites resulting in a lack of reliable, reproducible data which ultimately negatively impacted the performance and outcome of the clinical trial.
- **Biomarker Discovery & Validation:** Lack of biomarker signature to select patients for recruitment onto studies which reduced the success rate in the trial and ultimately may lead to a negative outcome.
- **Multi-omics:** Not able to maximise the extraction as much omics data as possible from precious *in vivo* or patient samples to support discovery and development programs, resulting in reduced confidence.

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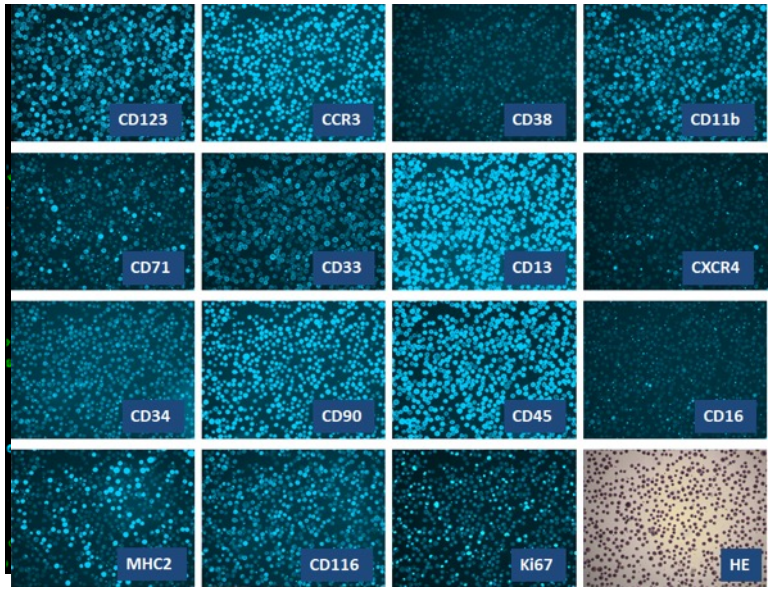
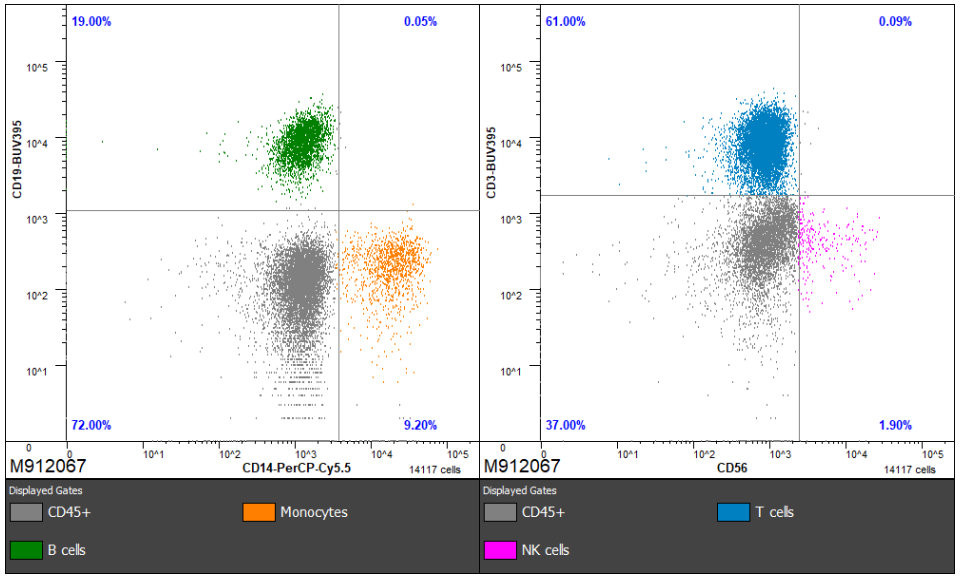
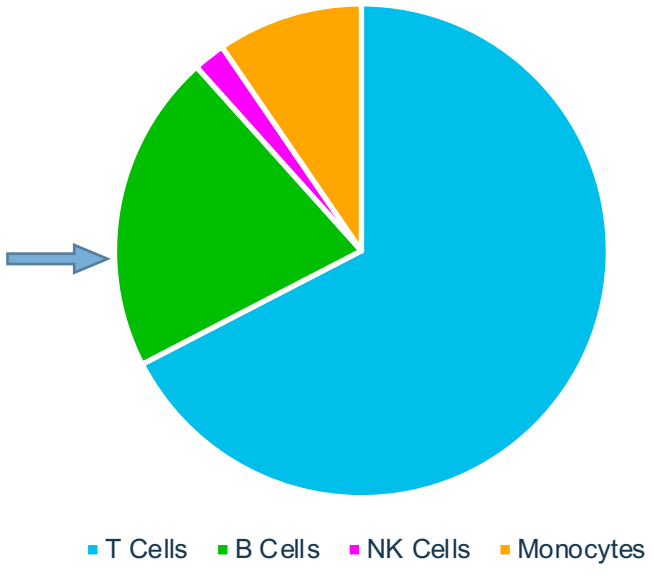
ChipCytometry: Convert images to quantitative flow-like data







Image

Index Sorting

Quantify Populations

- FCS files are generated from high-resolution images allowing for the phenotyping and of each individual cell in your sample
- We also retain all the original images

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Case Studies

Single Cell Precision

Checkpoint inhibitors

Spatial Deep Phenotyping

CAR-T Therapy

- **Quantitative deep-profiling of the immune compartment and protein expression in monitoring disease progression**
- **Building a biomarker strategy for checkpoint inhibition/combination therapy**
- **Understanding the spatial relationship of cell types in tissues & distance metrics**
- **Characterising CAR-T products and measuring engraftment kinetics**

Single Cell Precision

Quantitative deep-profiling of the immune compartment and protein expression in monitoring disease progression

Single Cell Precision Spatial Deep Phenotyping

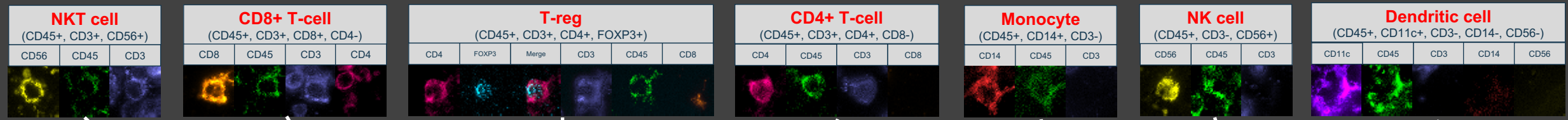
Checkpoint inhibitors CAR-T Therapy Chip Cytometry

Comparing Primary with Metastatic Disease in Head and Neck Cancer

- Evaluate the difference in immune population between primary and metastatic tumors.
- Exploratory 12-plex Chipcytometry assay.
- Data processing: FL-quantification and gating.

Antibody	Fluorochrome
CD45	FITC
CD3	BUV395
CD4	PerCP-Cy5.5
CD8	PE
CD56	PE
FOXP3	PE
CD14	PerCP-Cy5.5
CD27	PE
CD279	PE
CD11c	PE
CD39	BV421
CD45RA	BUV395

Identification of key immune cells in head & neck tumor tissue



CD45
CD3
CD4
CD8
CD14
CD56
CD11c
FOXP3

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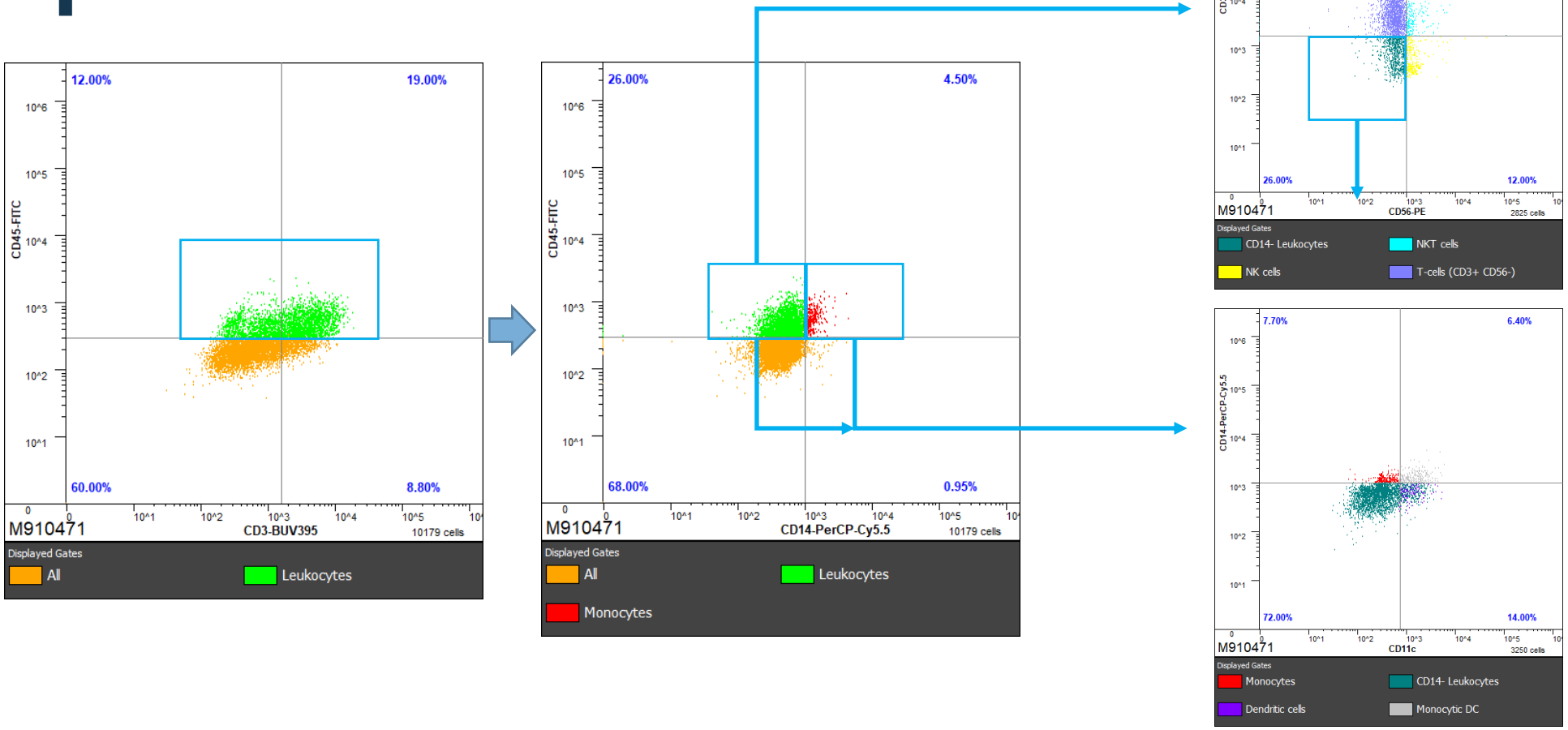
Gating: Immune cell quantification



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Absolute Cell Numbers

Population	Primary Tumor	Metastasis
All	29170	10179
Leukocytes	17454 (59.8% of all)	3291 (32.3% of all)
Monocytes	745	460
T-cells	15191	1576
CD8+ T-cells	681	914
CD4+ T-cells	7788	235
T-reg	827	82
NKT-cells	511	220
NK cells	87	320
CD279+ T-cells	1700	634
CD27+ T-cells	4230	1069
Dendritic cells	534	145
Monocytic DC	541	209

Cell number by mm^2

Population	Primary Tumor	Metastasis
All	8334	2908
Leukocytes	4987	940
Monocytes	213	131
T-cells	4340	450
CD8+ T-cells	195	261
CD4+ T-cells	2225	67
T-reg	236	23
NKT-cells	146	63
NK cells	25	91
CD279+ T-cells	486	181
CD27+ T-cells	1209	305
Dendritic cells	153	41
Monocytic DC	155	60

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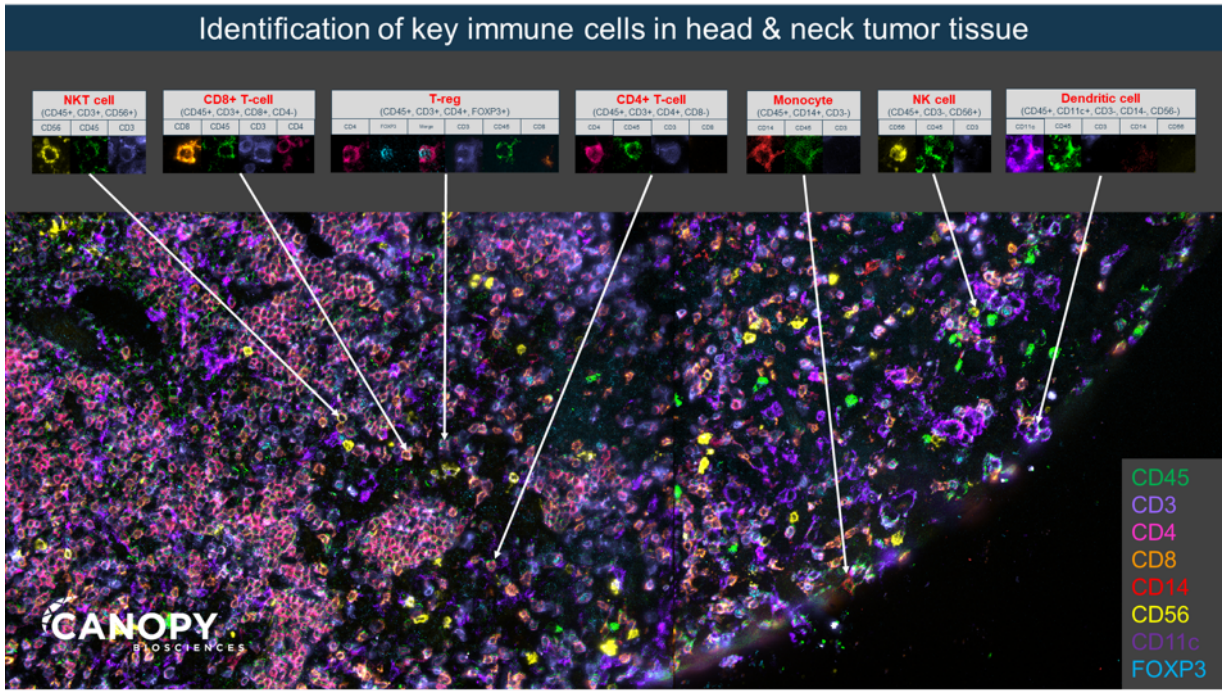
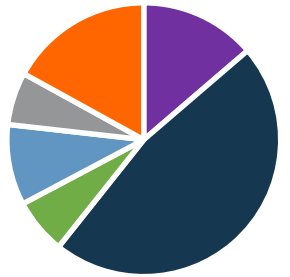
Single-cell resolution in tissue samples



- Algorithmic segmentation of single cells enables identification and quantification of fluorescence values for each individual cell.
- Key cell populations are quantified with single-cell precision.

Immune cell populations in primary tumor

Immune cell populations in metastasis



Single Cell Precision Spatial Deep Phenotyping

Checkpoint inhibitors CAR-T Therapy Chip Cytometry

CONFIDENTIAL

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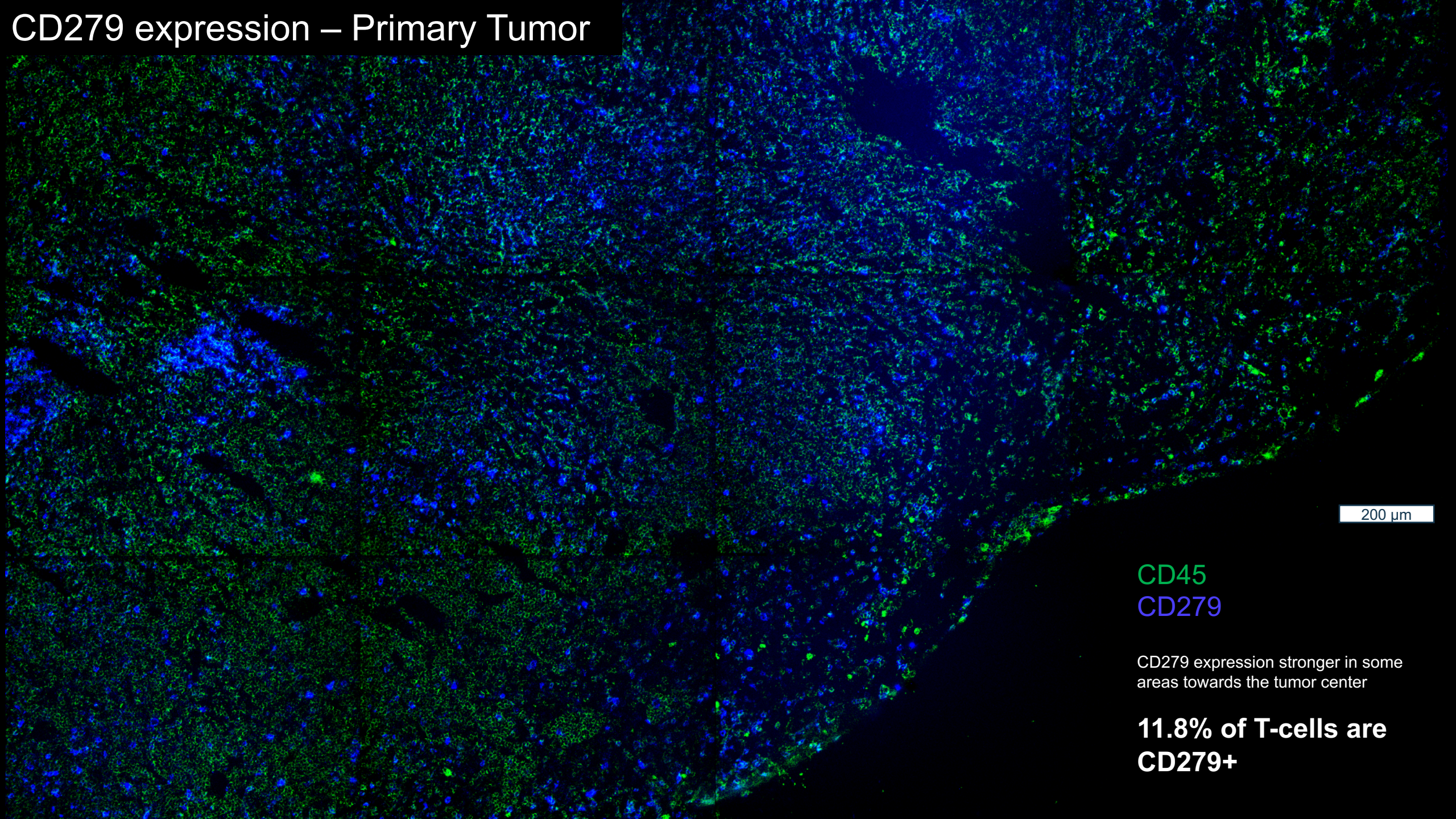
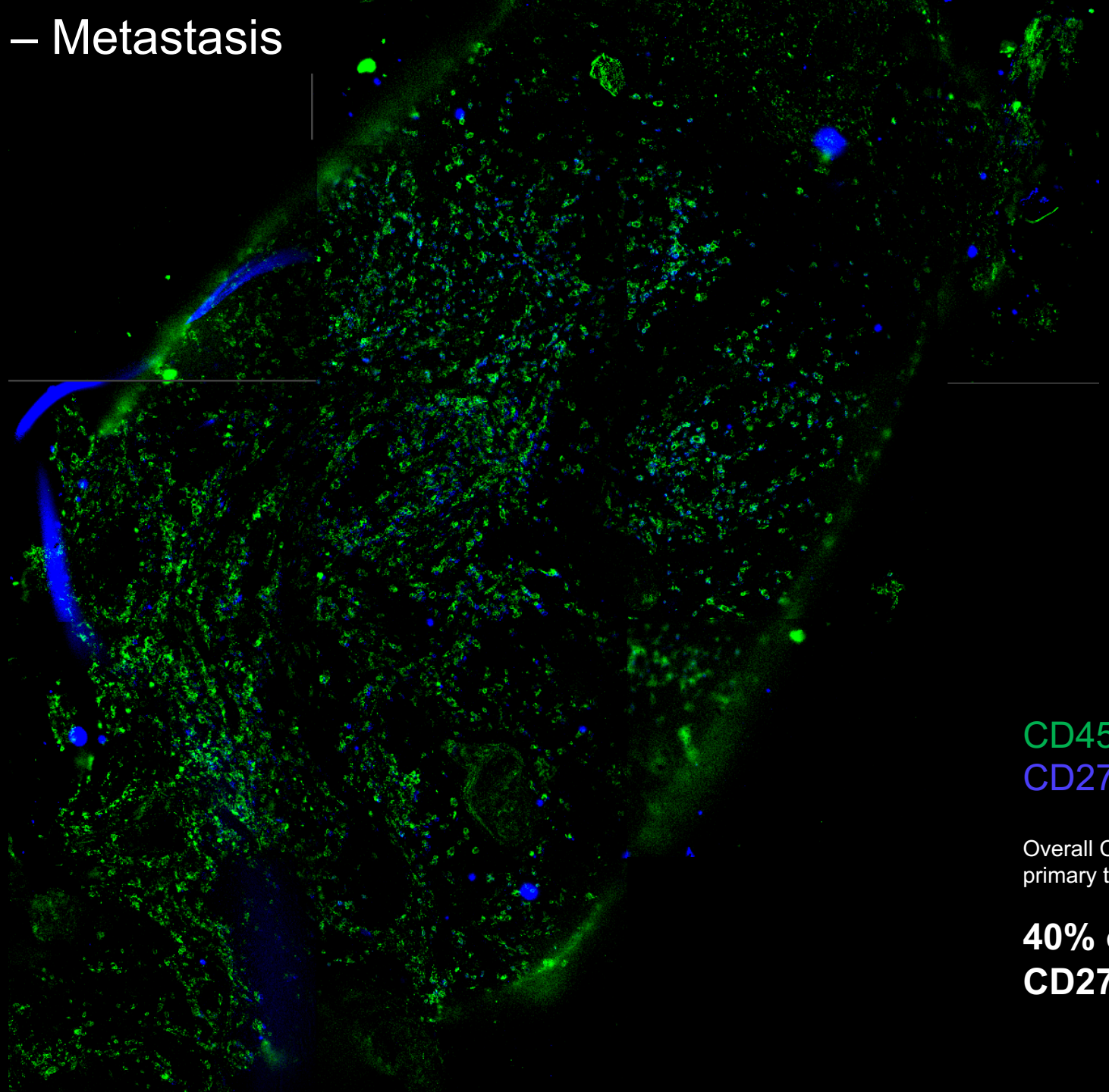
CD279 expression – Primary Tumor



CD279 expression stronger in some areas towards the tumor center

11.8% of T-cells are CD279+

CD279 expression – Metastasis



CD45
CD279

Overall CD279 expression lower than in primary tumor

40% of T-cells are CD279+

Conclusions

- Metastatic tumor has much lower overall cell density than primary tumor (~35% of primary tumor)
- Metastatic tumor has a differing composition of infiltrating immune cells compared to primary tumor:
  - Overall lower immune cell infiltration (primary tumor: 59.8% of all cells; metastasis: 32.3%)
  - Lower T-cell infiltration (but higher CD8+ T-cell percentage)
  - Higher NK cell and NKT-cell percentage
  - Higher monocyte infiltration
- The 12-plex panel for Head and Neck Cancer assay enables clinical trial support.

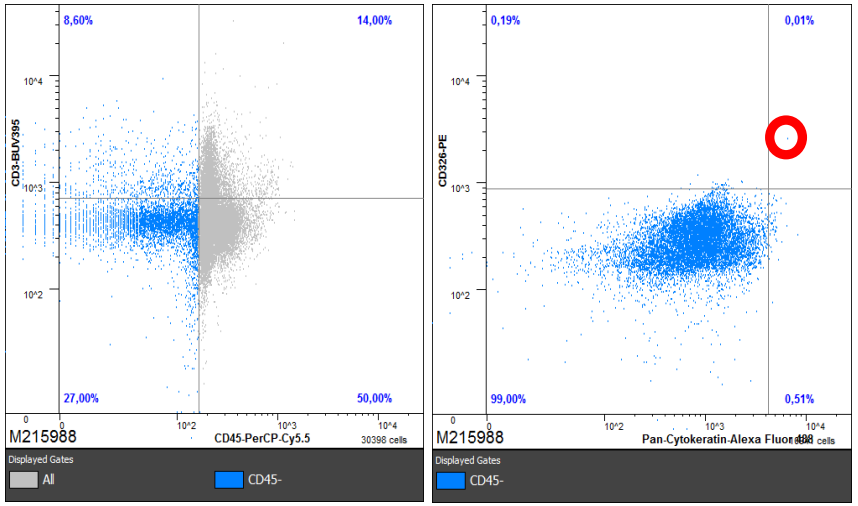
Checkpoint inhibitors

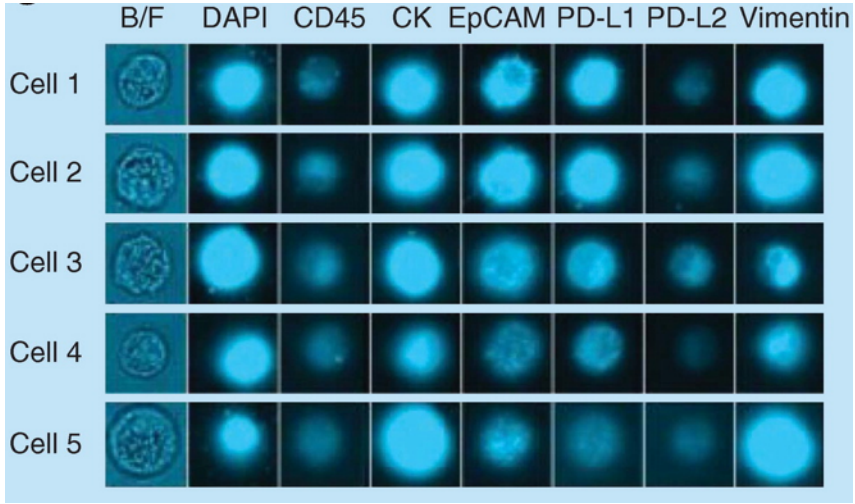
Building a biomarker strategy for checkpoint inhibition/combination therapy

Enabling frequent longitudinal monitoring during cancer therapy

- Obtaining tumor biopsies for PD-L1 interrogation is invasive and not suited for frequent longitudinal monitoring during cancer therapy.
- Tumor heterogeneity for PD-L1 expression may not accurately capture the PD-L1 status of the whole tumor burden in a single biopsy.
- An alternative approach - the analysis of Circulating Tumor Cells (CTCs).

Chipcytometry enabled the phenotyping of CTCs in Breast Cancer





- CTC phenotyping by Chipcytometry
- CTC candidates identified by CK (Cytokeratin) and EpCAM (Epithelial cell adhesion molecule CD326)
- PD-L1 & PD-L2 expression quantified on CTCs

Teo et al. (2017): A preliminary study for the assessment of PD-L1 and PD-L2 on circulating tumor cells by microfluidic-based Chipcytometry. Future Science OA; Published Online:4 Sep 2017 https://doi.org/10.4155/fsoa-2017-0079

MSD

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Ultra-deep phenotyping of cellular biomarkers to support patient selection and combination therapy

- For downstream analysis of the isolated CTCs, we believe the main advantages of chipcytometry lie in the iterative staining process that allows retrospective evaluation of additional markers and the potential to measure a large number of parameters without the spillover/compensation problems encountered with flow cytometry.
- This approach allows the analysis of additional immunomodulatory targets on tumor cells beyond PD-L1 and PD-L2, which is particularly critical, considering high dimensional analysis of these markers is likely to become increasingly relevant as immunotherapy moves beyond the administration of single immunomodulatory agents toward combinations that synergize in their antitumor immune response.
- In addition, the possibility of including more tumor and immune markers (positive and negative) will increase confidence that the identified cells are indeed CTCs.

Teo et al. (2017): A preliminary study for the assessment of PD-L1 and PD-L2 on circulating tumor cells by microfluidic-based Chipcytometry.
Future Science OA; Published Online:4 Sep 2017 https://doi.org/10.4155/fsoa-2017-0079

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Conclusions

- Chip cytometry enables the iterative, retrospective evaluation of additional biomarkers, supporting long term studies.
- Chip cytometry enables the evaluation of new biomarkers that may be identified post sample collection.
- Chip cytometry is non-destructive and suitable for both cells and tissues.
- Chip cytometry enables ultra-deep phenotyping of immune cells supporting the evaluation of combination therapies.

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Spatial Deep Phenotyping

Understanding the spatial relationship of cell types in tissues & distance metrics

Oxford University

featured on the cover of Cell Reports (Sept 2019)

- Several types of unconventional T lymphocytes sit at the bridge between innate and adaptive immunity, including mucosal-associated invariant T (MAIT) cells.
- To achieve sufficient activation, TCR signaling is supported by other costimulatory signals, such as CD28, and by cytokines, such as interleukin (IL)-18 and IL-12.

TCR and Inflammatory Signals Tune Human MAIT Cells to Exert Specific Tissue Repair and Effector Functions
Leng et al., 2019, Cell Reports 28, 3077–3091 September 17, 2019 ª 2019 The Author(s).
https://doi.org/10.1016/j.celrep.2019.08.050

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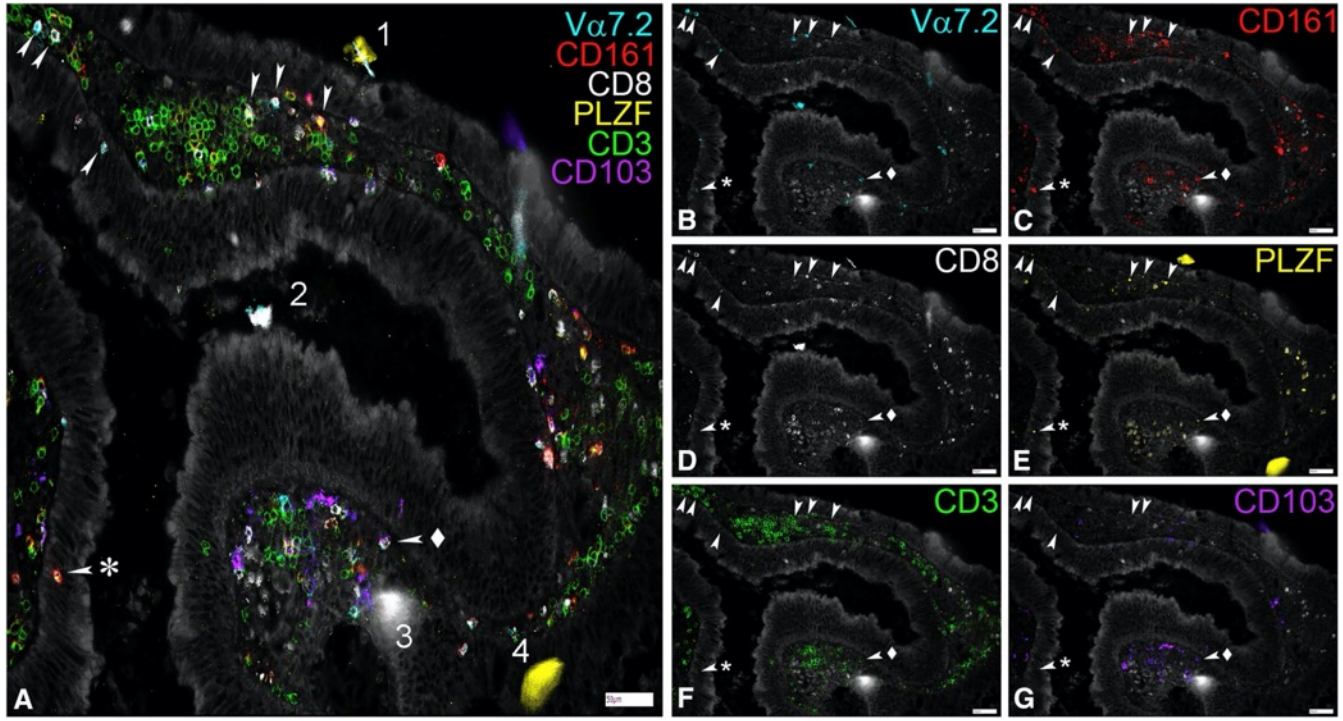
High Content and Spatial Imaging reveals importance of localisation

- MAIT cells (indicated as white arrows) show co-expression of Va7.2, CD161, PLZF, and CD3.
- By co-staining for multiple relevant markers in colonic tissue, apposition is observed between MAIT cells and intact epithelium, suggesting two-way cross talk is possible under homeostatic conditions.
- TCR triggering of MAIT cells reveals a transcriptional program linked to tissue-repair functions seen *in vivo*, consistent with a homeostatic role for these cells in epithelia.
- Chip Cytometry revealed the importance of the spatial relationship between cell types.



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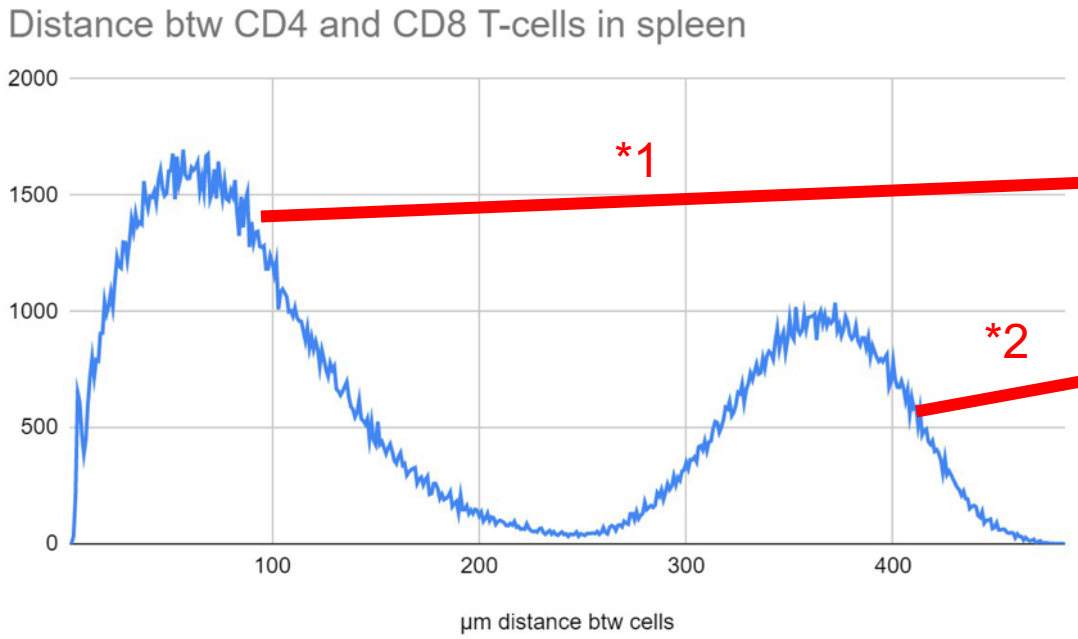
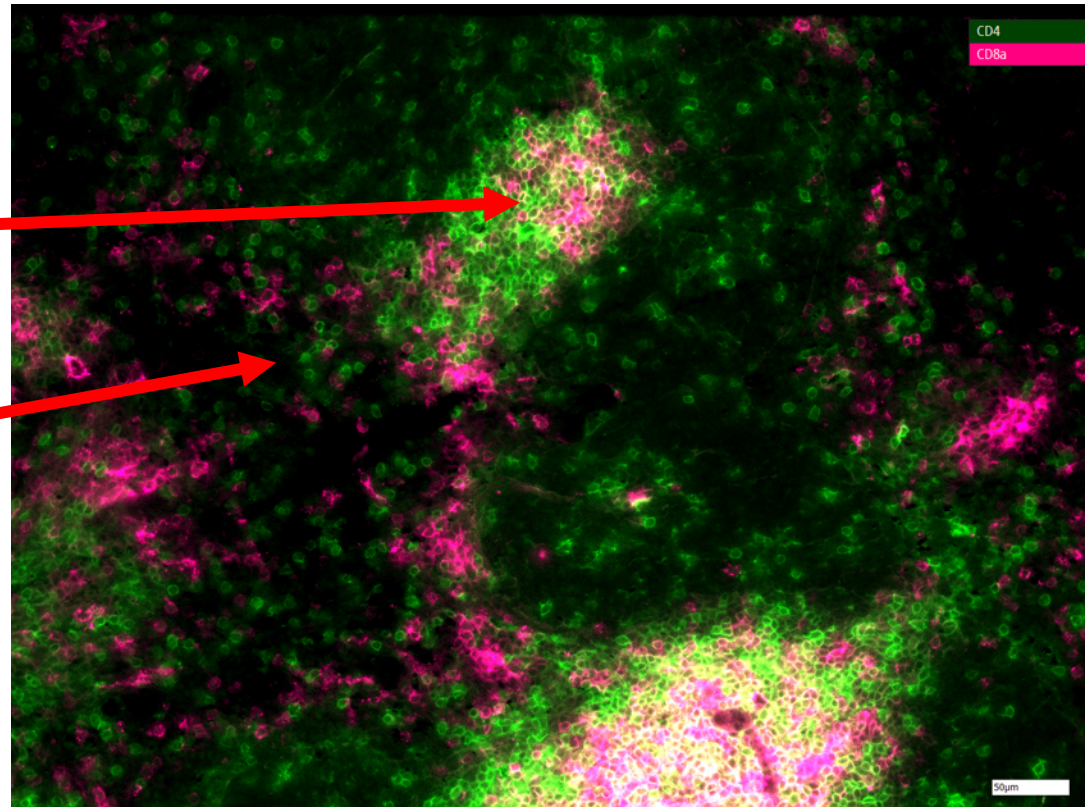
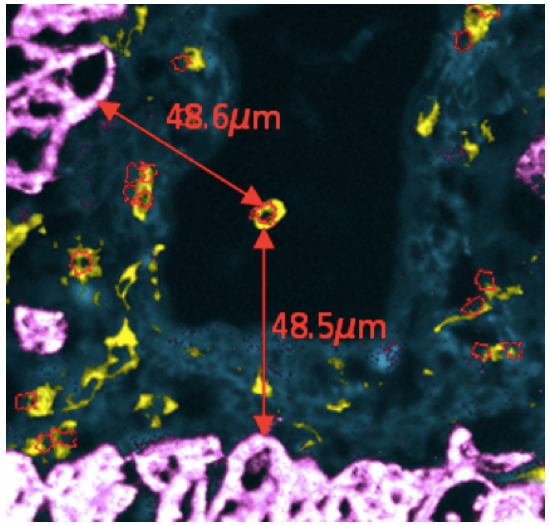
Automated measurement of distances between different cell populations

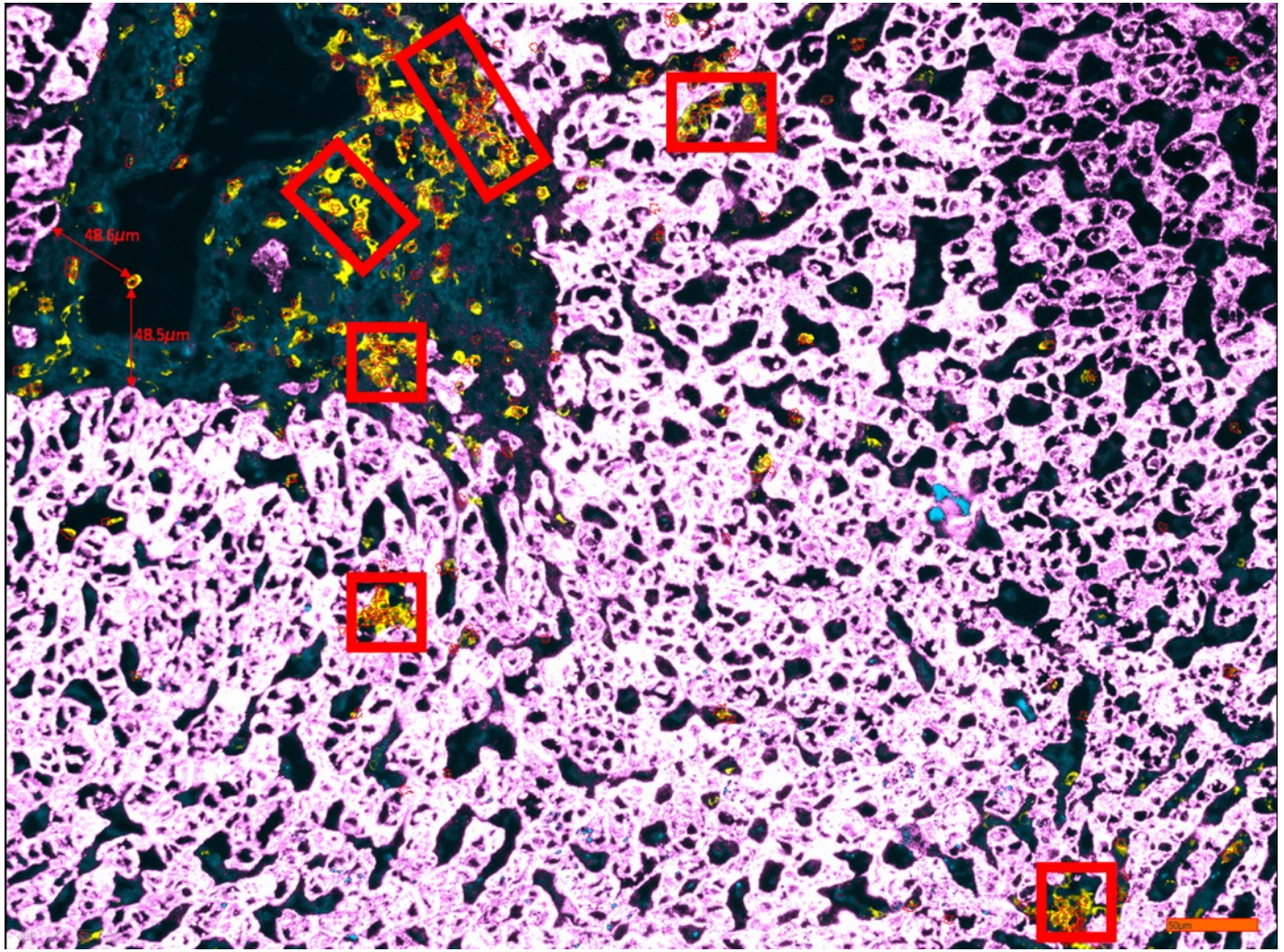




- *1: Population 1: germinal center interacting CD4/CD8 T-cells
- *2: Population 2: CD4/CD8 T-cells not belonging to the same germinal center

Cellular Agglomeration detection in Liver





Cytokeratin 18

Autofluorescence

CD45

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Conclusions

- Chip Cytometry reveal the importance of spatial relationships between cell types.
- The exact distances between cell populations can be determined.

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CAR-T Therapy

Characterising CAR-T products and measuring engraftment kinetics

Validated biomarkers
for characterizing CAR-Ts

- Characterise phenotype using 26+ validated biomarkers
- Custom assay development for any additional biomarker

Biomarkers validated for CAR-T samples				
CD3	CD19	CD45RA	CD152	Granzyme B
CD4	CD25	CD45RO	CD184	Ki-67
CD8a	CD27	CD56	CD197	
CD14	CD28	CD57	CD278 (ICOS)	
CD15	CD34	CD95	CD279 (PD-1)	
CD16	CD45	CD127	FoxP3	
***Other biomarkers available on a custom basis**				

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Receptor internalisation

Video Cytometry

Single Cell Precision

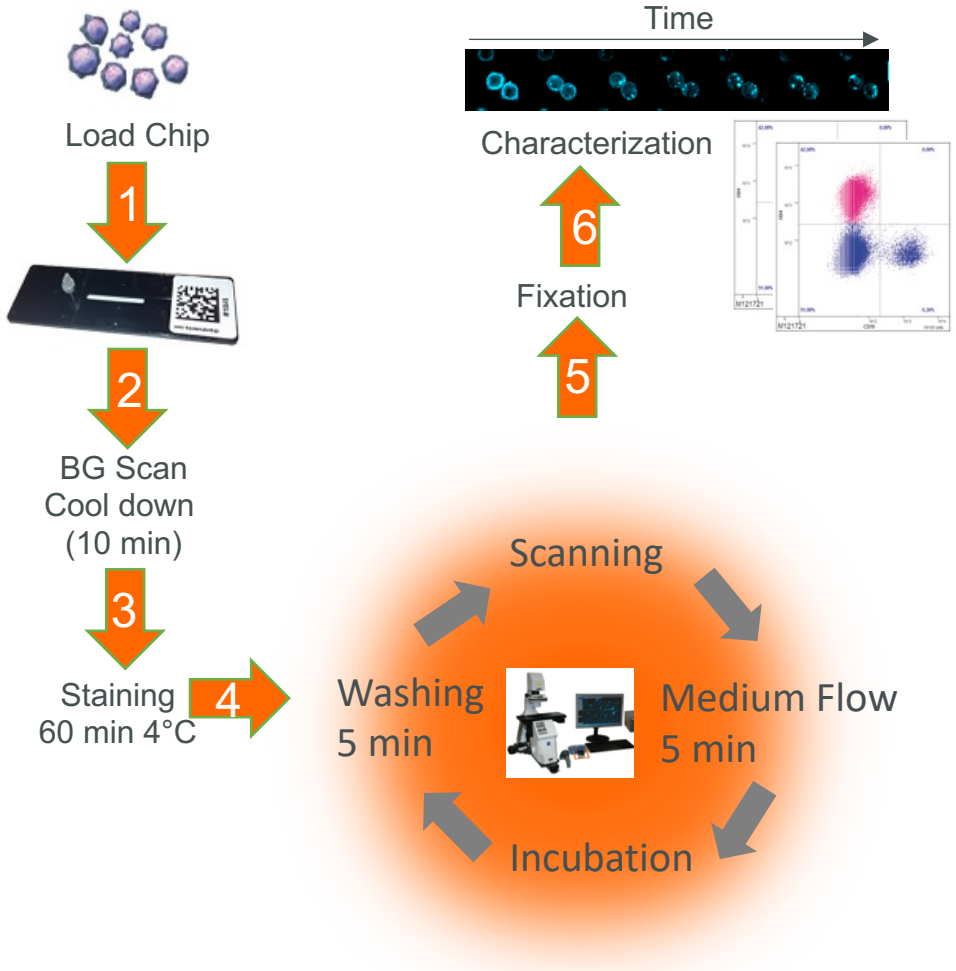
Spatial Deep Phenotyping

Checkpoint inhibitors

CAR-T Therapy

Chip Cytometry

Receptor internalization study



- 1) PBMC preparation due to SOP
- 2) BG images
- 3) Cool down for 10 min
- 4) Incubation with CD127 1h, 4°C
  - Cycle (total 6h):
  - Washing (5 min)
  - Image acquisition
    - Medium flow for 5 min
    - Incubation in the videocytometry chipholder (37°C)
- 5) Fixation
- 6) Post-characterization

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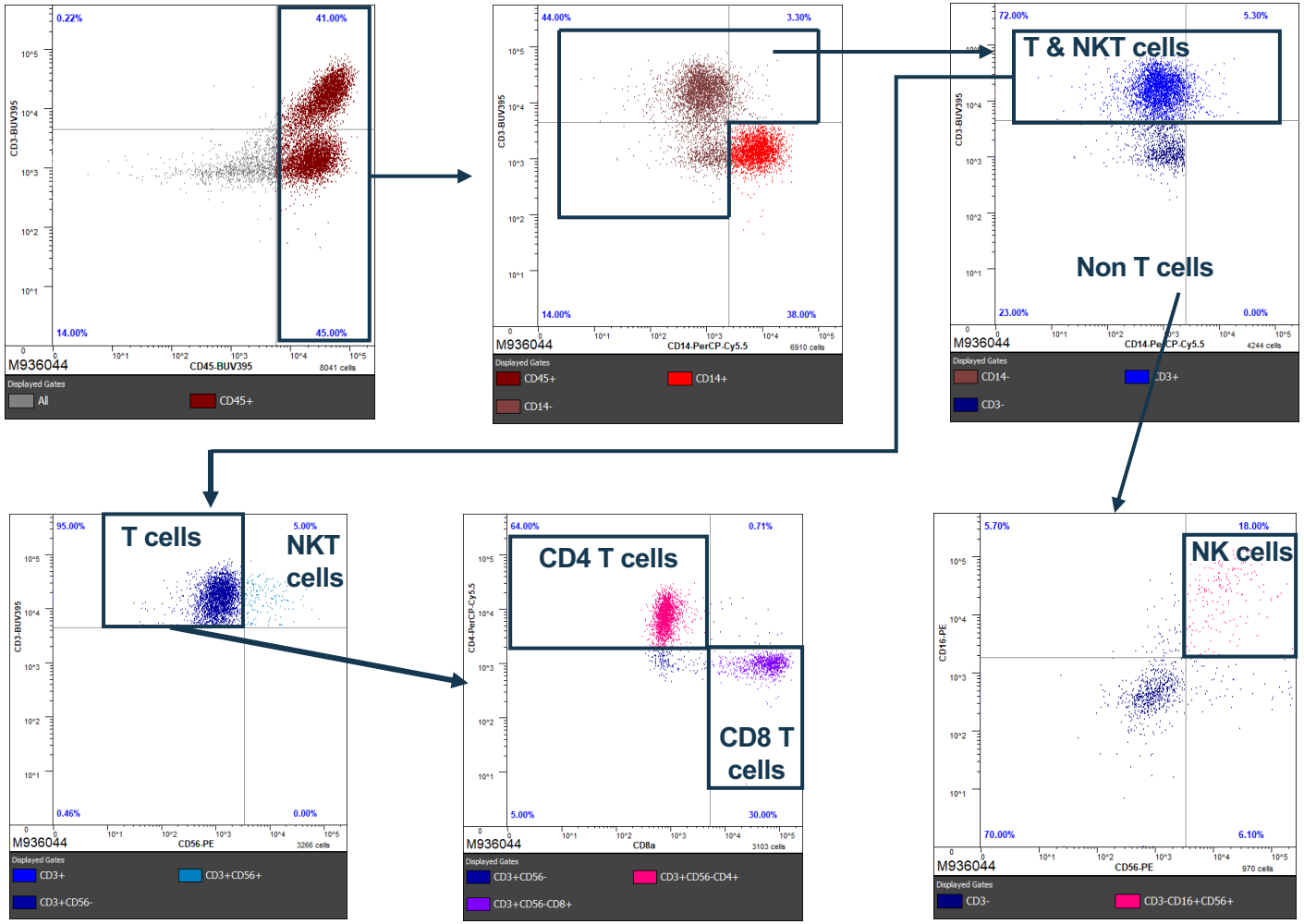
Gating Strategy Post Characterisation



[Single Cell Precision](#) [Spatial Deep Phenotyping](#)

[Checkpoint inhibitors](#) [CAR-T Therapy](#) [Chip Cytometry](#)

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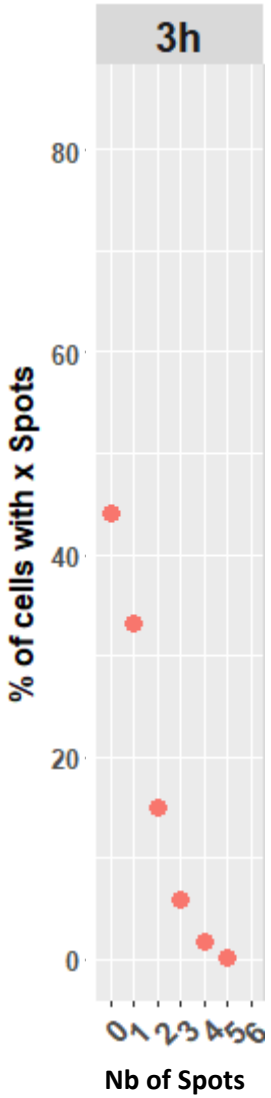
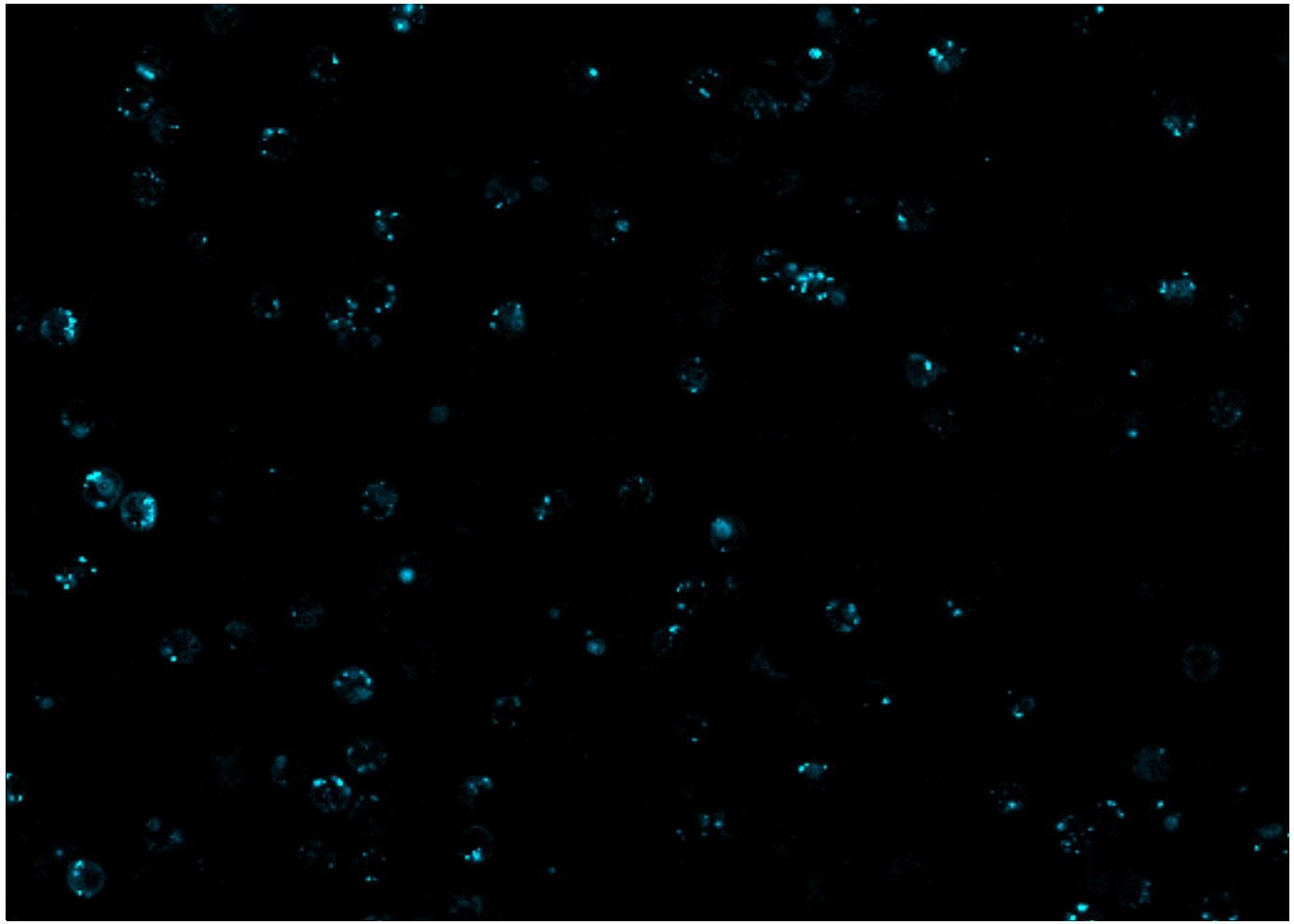
Internalisation of CD127 (IL-7 Receptor) over 3h





Gating on CD127+ T cells for quantification

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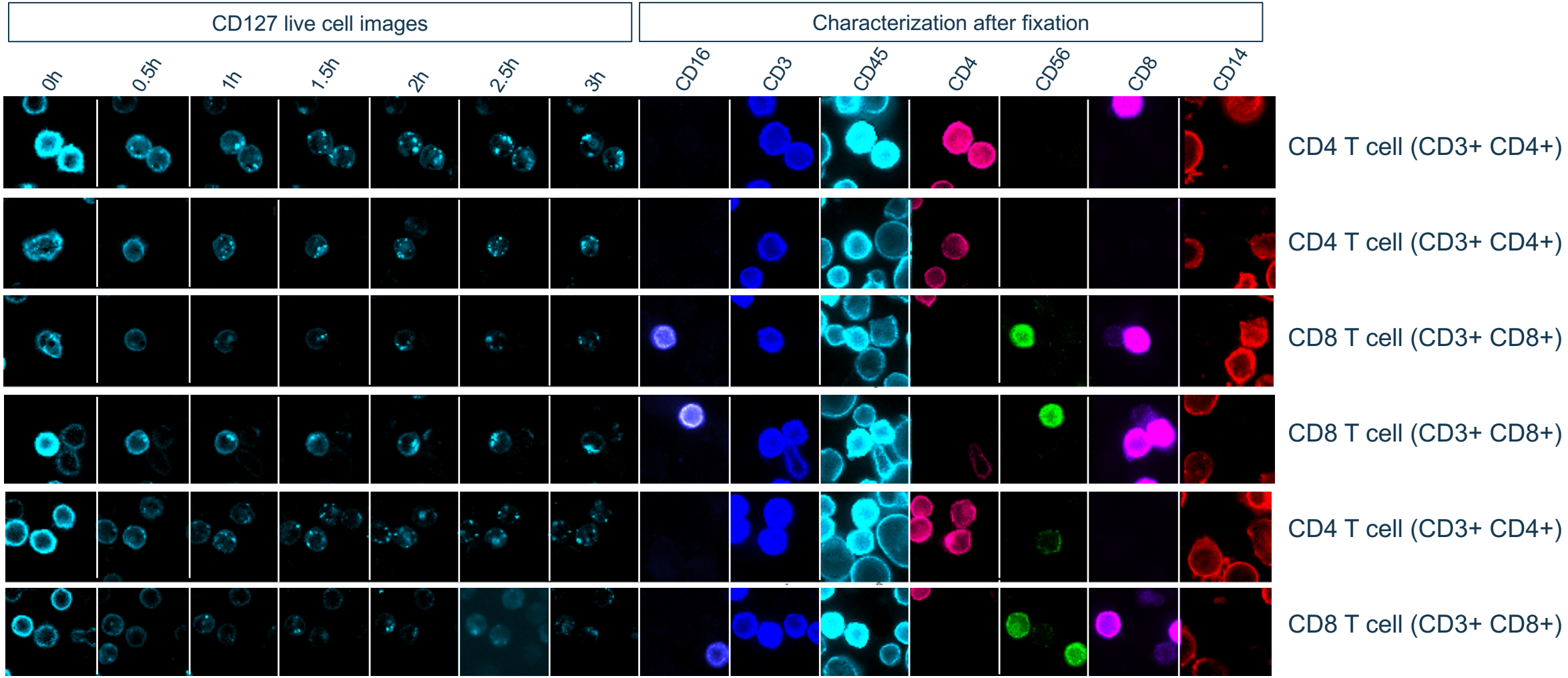
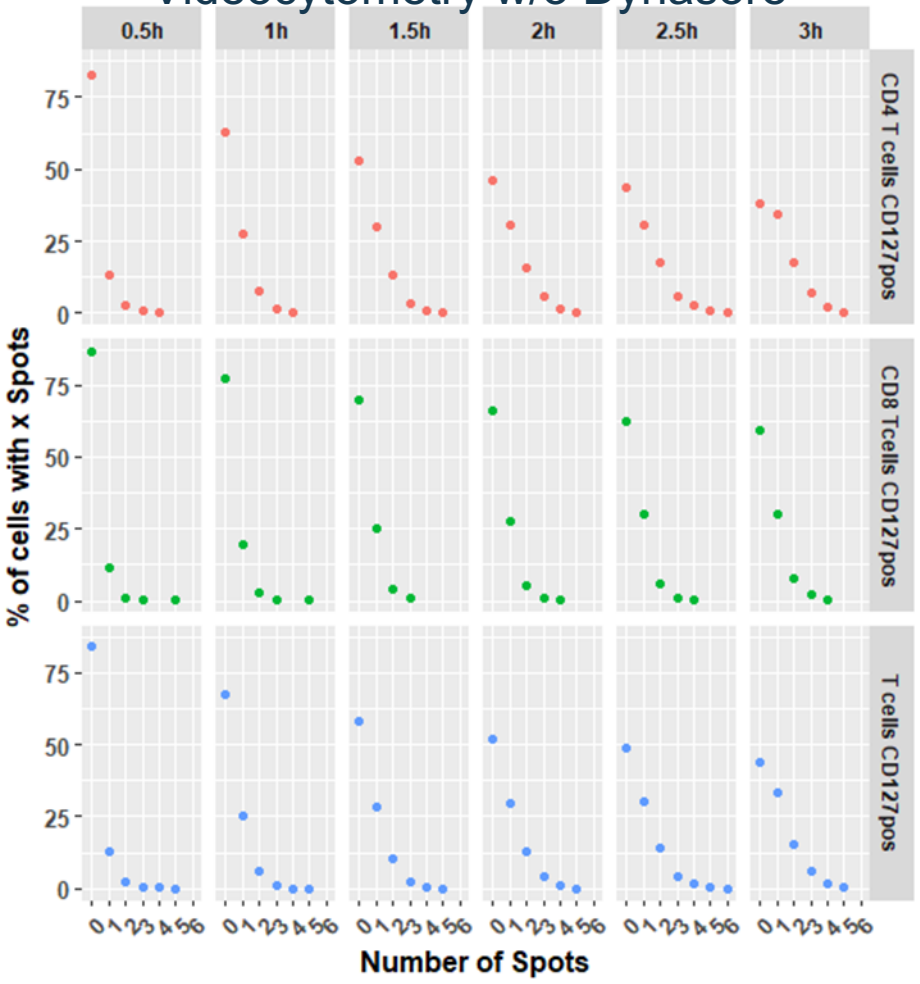
Internalization of CD127 (IL-7 Receptor) and characterization of cells after fixation

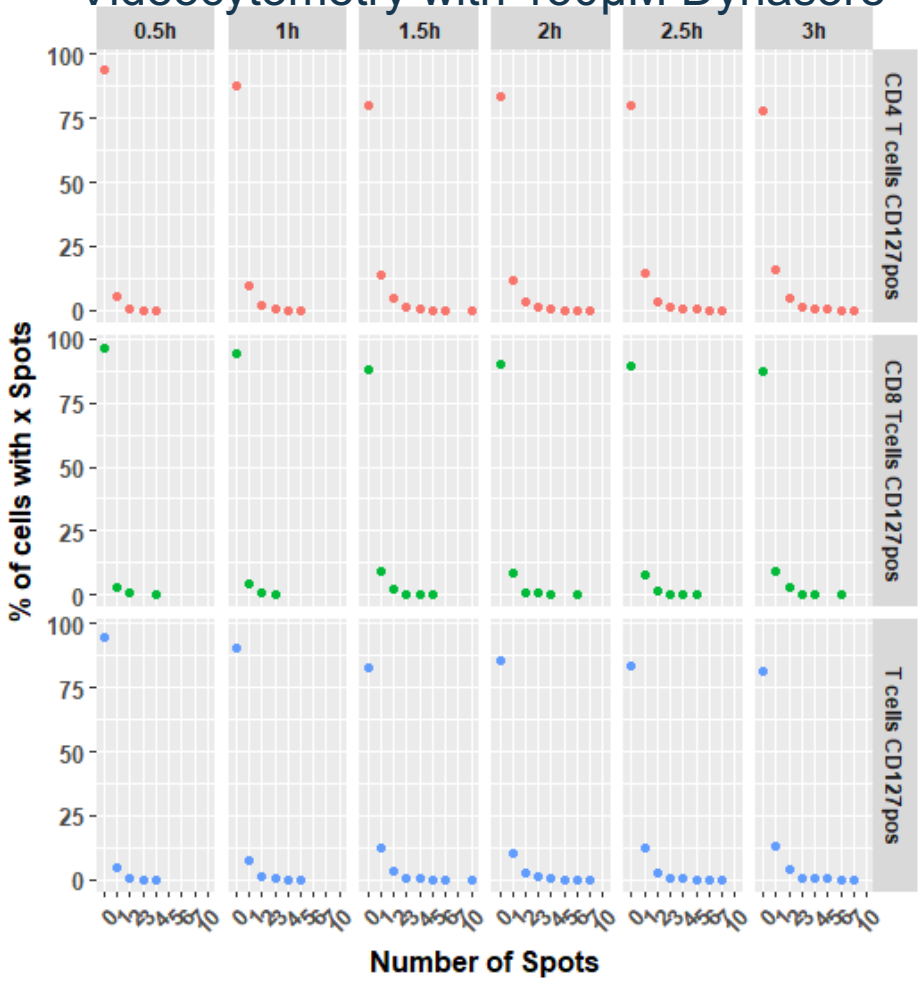


Internalisation of CD127 (IL-7 Receptor) - Quantification

Videocytometry w/o Dynasore



Videocytometry with 150µM Dynasore



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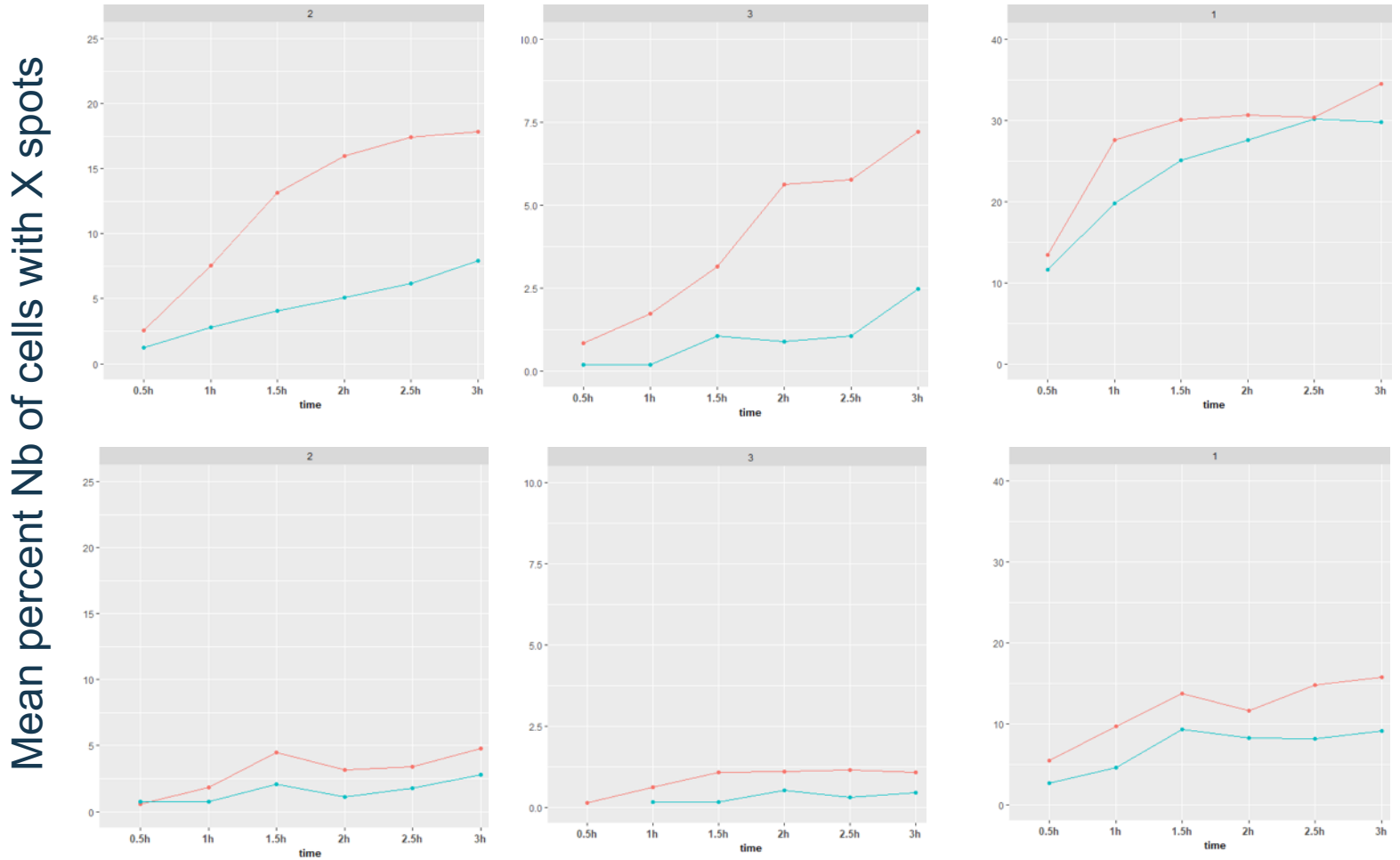
Internalisation of CD127 (IL-7 Receptor) - Comparison of CD4 and CD8 T cells



Videocytometry w/o Dynasore

Gate
CD4 T cells CD127pos
CD8 Tcells CD127pos

Videocytometry with 150µM Dynasore

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Conclusions

- Chip Cytometry enables the characterization of CAR-T cells.
- Engraftment kinetics can be determined for each patient to optimise dosing schedules.

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Single Cell Precision Chip Cytometry: what is it?

Single Cell Precision
Checkpoint inhibitors
Spatial Deep Phenotyping
CAR-T Therapy
Chip Cytometry

Ultra-Deep Proteomics & Phenotyping

- Preclinical
  - Investigate and validate target selection
  - Elucidate mechanism of action
  - Study cell cycle, signaling and intracellular cytokine secretion
- Clinical
  - Long-term sample preservation, storage and reinterrogation
  - Chip Cytometry enables sample stability for 24+ months
- Precision Medicine
  - Identify predictive biomarkers
  - Define patient cohorts

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Chip Cytometry

ZellScanner ONE™

- Benchtop instrument
- Semi-automated
- Exploratory / Phase I trial application

Cytobot™

- Stand alone, automated system
- Fully automated, 24/7
- Phase II/III trial applications

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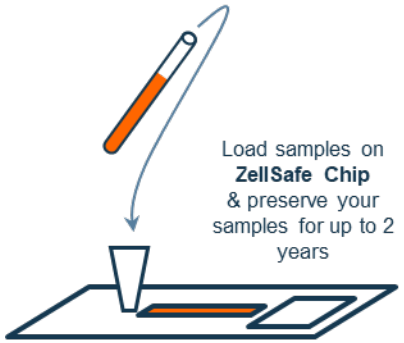
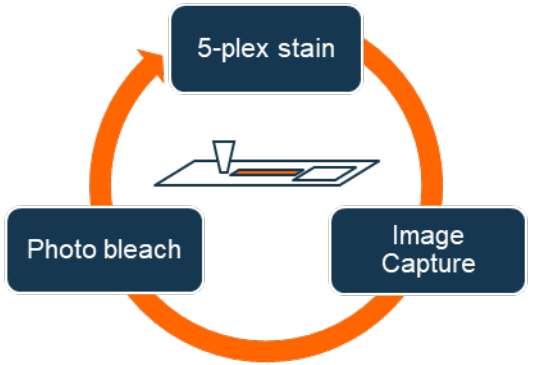
The sample workflow



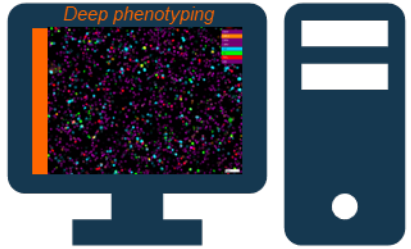
Load samples on **ZellSafe Chip** & preserve your samples for up to 2 years

Industry-leading high dynamic resolution for the highest quality data

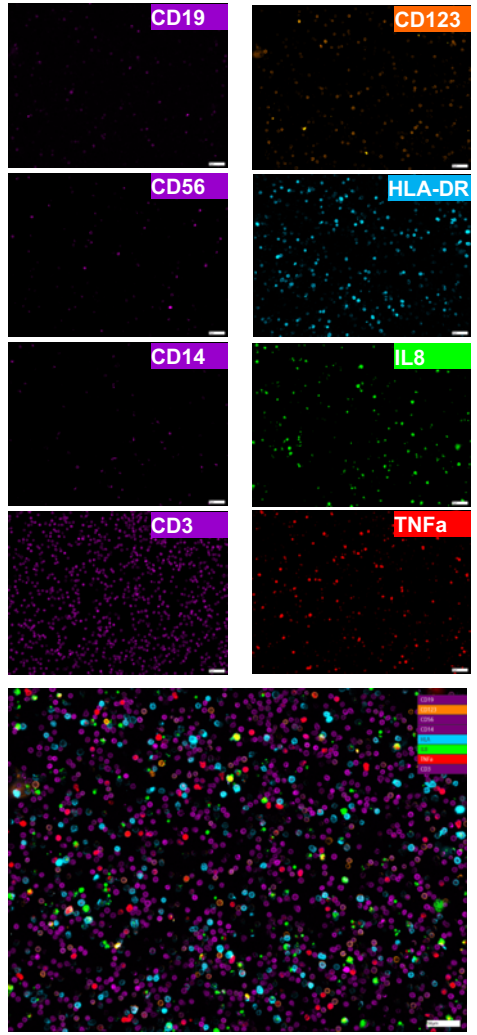


Reinterrogate your samples for additional biomarker analysis as many times as you need

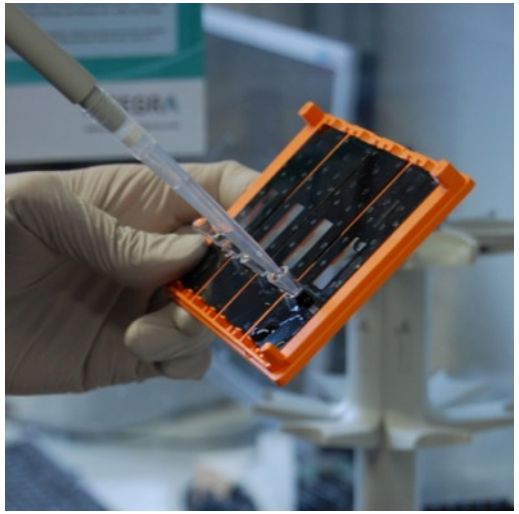
Validated storage of your samples on the ZellSafe chip at 4°C for up to 24 months

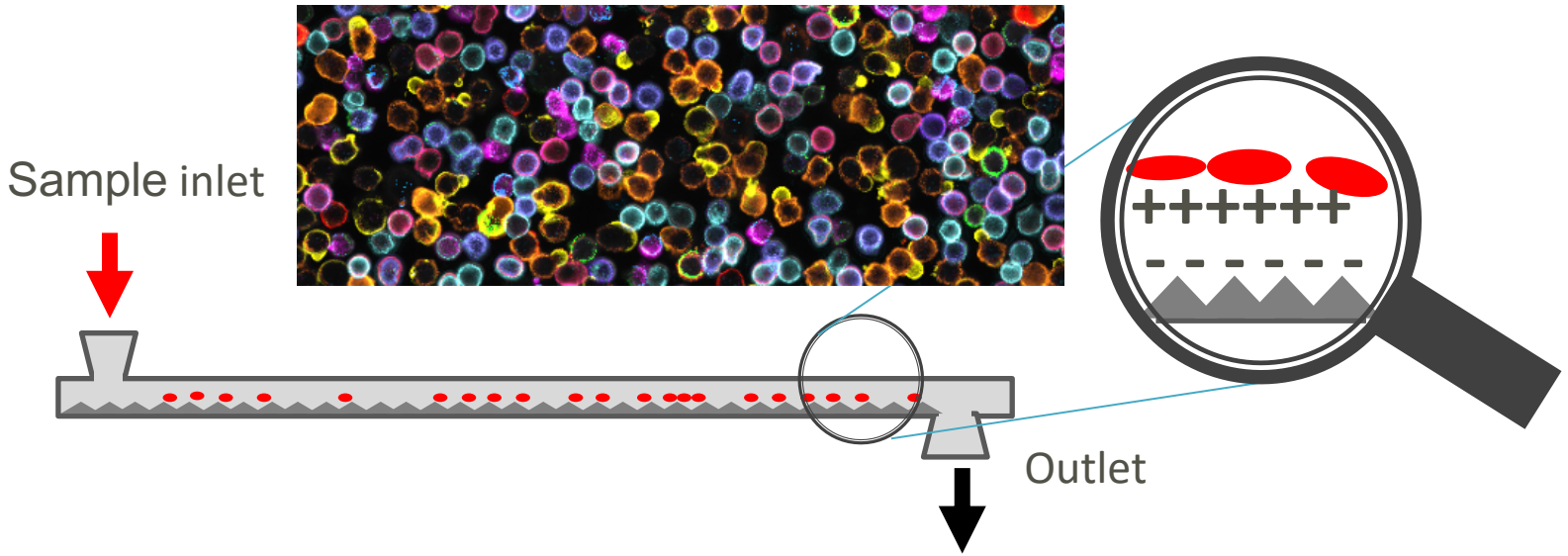


Automated data processing using our seamlessly integrated ZellExplorer software
(full data export for analysis by 3rd-party software is also available)



Sample Collection and Storage





Product	ZellSafe™ Cells	ZellSafe™ Rare	ZellSafe™ Tissue
Specimen	cell suspension	rare cells (<0.02%)	Tissue sections
Loading capacity	40-100µl	40-300µl	6 sections
Cell number	typically 250,000	typically 1,000,000	tissue-dependent

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Store your valuable samples for 2 years+

- **Sample integrity is conserved** throughout data collection
- **Reinterrogate** your Biobanked samples for additional biomarkers at any time
- Target Choice **AFTER** Sample Collection

Capture and secure all your precious samples and enable re-interrogation

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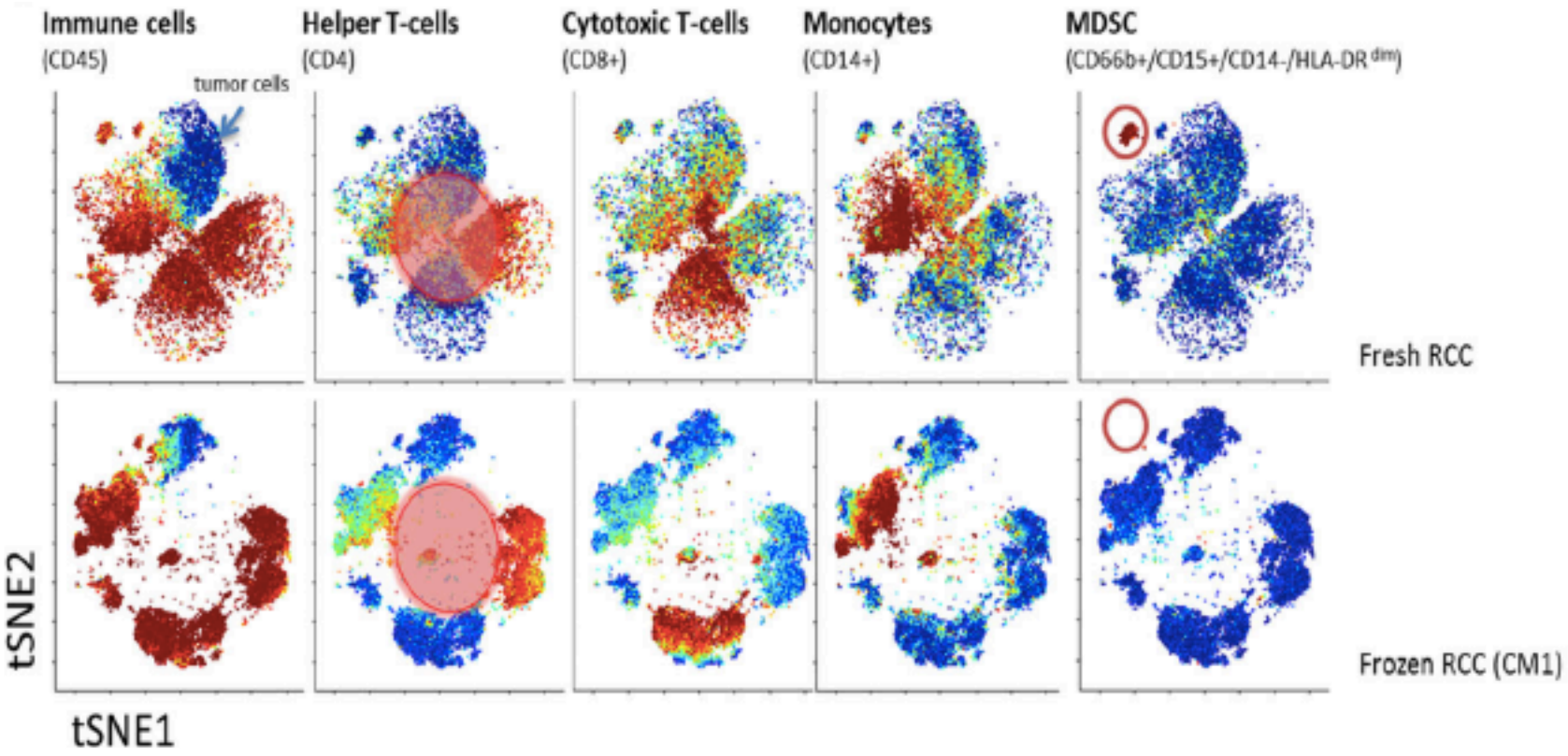
Avoid the Effects of Cryopreservation



"… 62% of analyzed markers show decreased median intensities upon cryopreservation."

Source: Kadić et al. BMC Immunology (2017) 18:6
DOI 10.1186/s12865-017-0192-1

MERCK

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To quantify with single cell precision requires…

- The dynamic range of flow cytometry
- The resolution of single cells
- Stable samples for 2 years or more
- A large repertoire of biomarkers for blood cells and tissues
- The ability to work with living cells for kinetic assays
- An integrated hardware and software solution to precisely identify and quantify expression in individual cells

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Compare what's important

Feature	Chip Cytometry	Flow Cytometry	Mass Cytometry	Spatial Transcriptomics
Sample stability	2 years +	1-3 days	1-3 days	?
Non-destructive	Yes	No	No	No
Spatial Resolution	500nm/pixel	N/A	1,000nm/pixel	10,000nm/pixel
Dynamic Range	>8 logs	8 logs	4 logs	5 logs
Multiplexing	∞	≥48	≥96 theoretically	≥96 (not single cell)
Rare Cells (>5000)	Yes	No	No	N/A
Video Cytometry	Yes	No	No	No

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Zellscanner ONE – quantitative cytometry

- 1392 x 1040 pixels high sensitivity grayscale camera
- 20x 0.8 NA very light sensitive Zeiss Apochromat objective
- designed filtersets for each color
  - no/low spillover
  - UV protection filter (no/low protein degradation)
- **HDR imaging – the only HDR microscope at the market**
  - 32bit = 4.3 billion intensity values (>8logs dynamic range)
- **Net fluorescence** (pre/post stain images)
  - corrects for illumination artefacts
  - corrects for autofluorescence

BUV395	BV421	FITC	PE	PerCP

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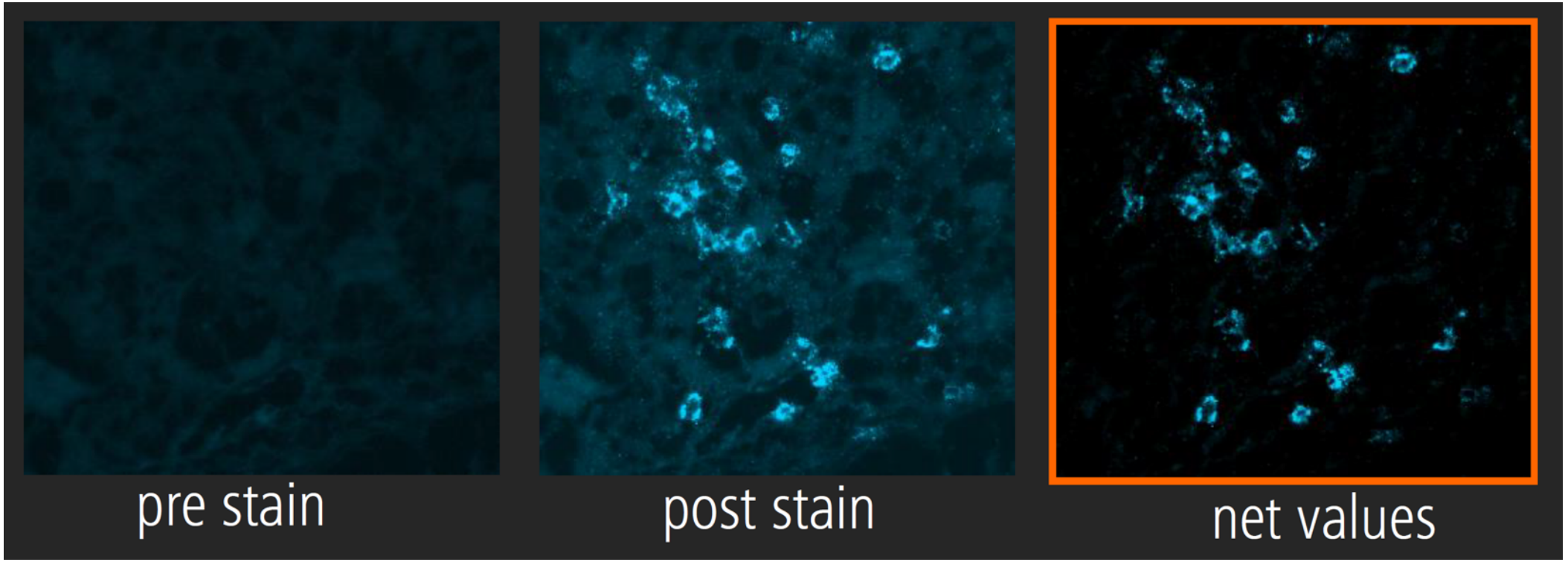
Net Fluorescence and Dynamic Range



To quantitatively calculate true net fluorescence values, both the pre- and post-stain values must lie within the linear dynamic range of the instrument.

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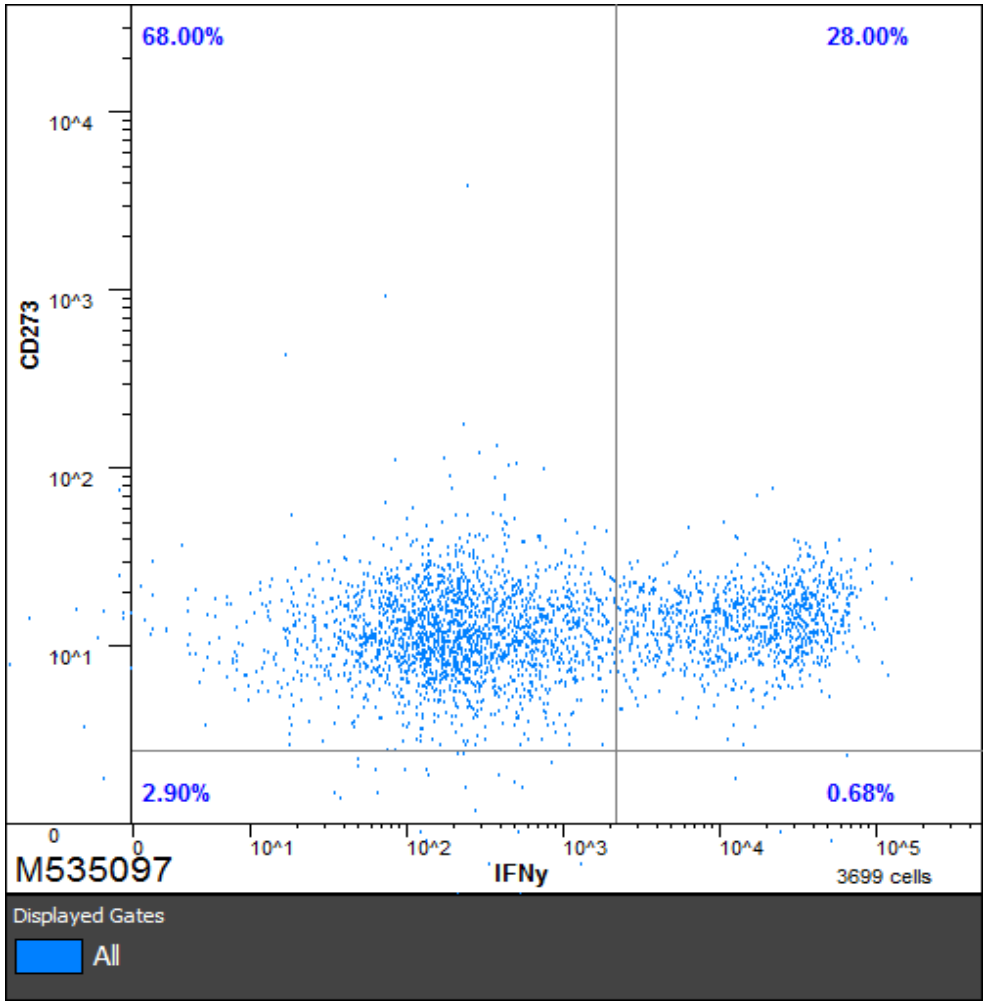
Dynamic Range

Why is dynamic range important?

Why is dynamic range important?



Protein expression levels:

- Expression of interferon-γ in T cells
- 6 logs of protein expression

> 6 logs dynamic range required

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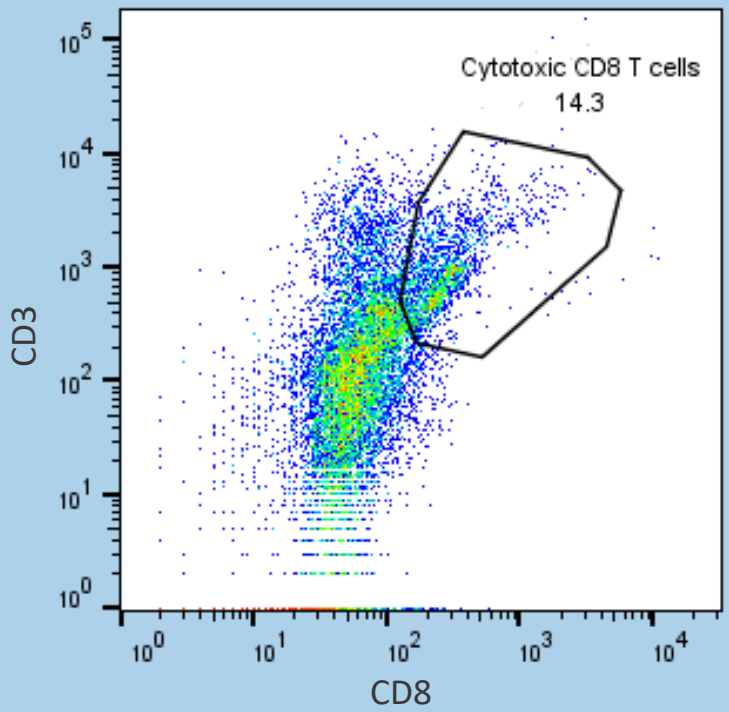
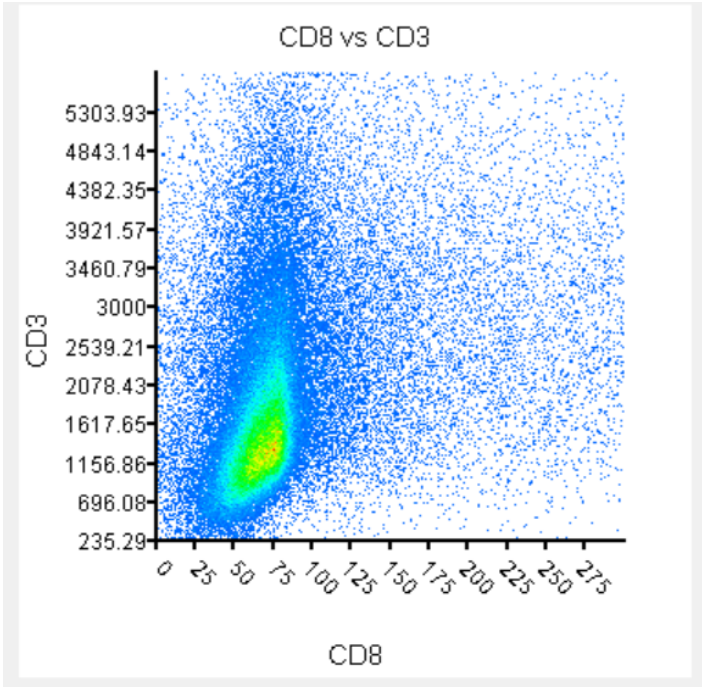
Dynamic Range: CD8 T-Cells in CRC

CD3: >4 logs of dynamic range



Source: Canopy Biosciences, 2019

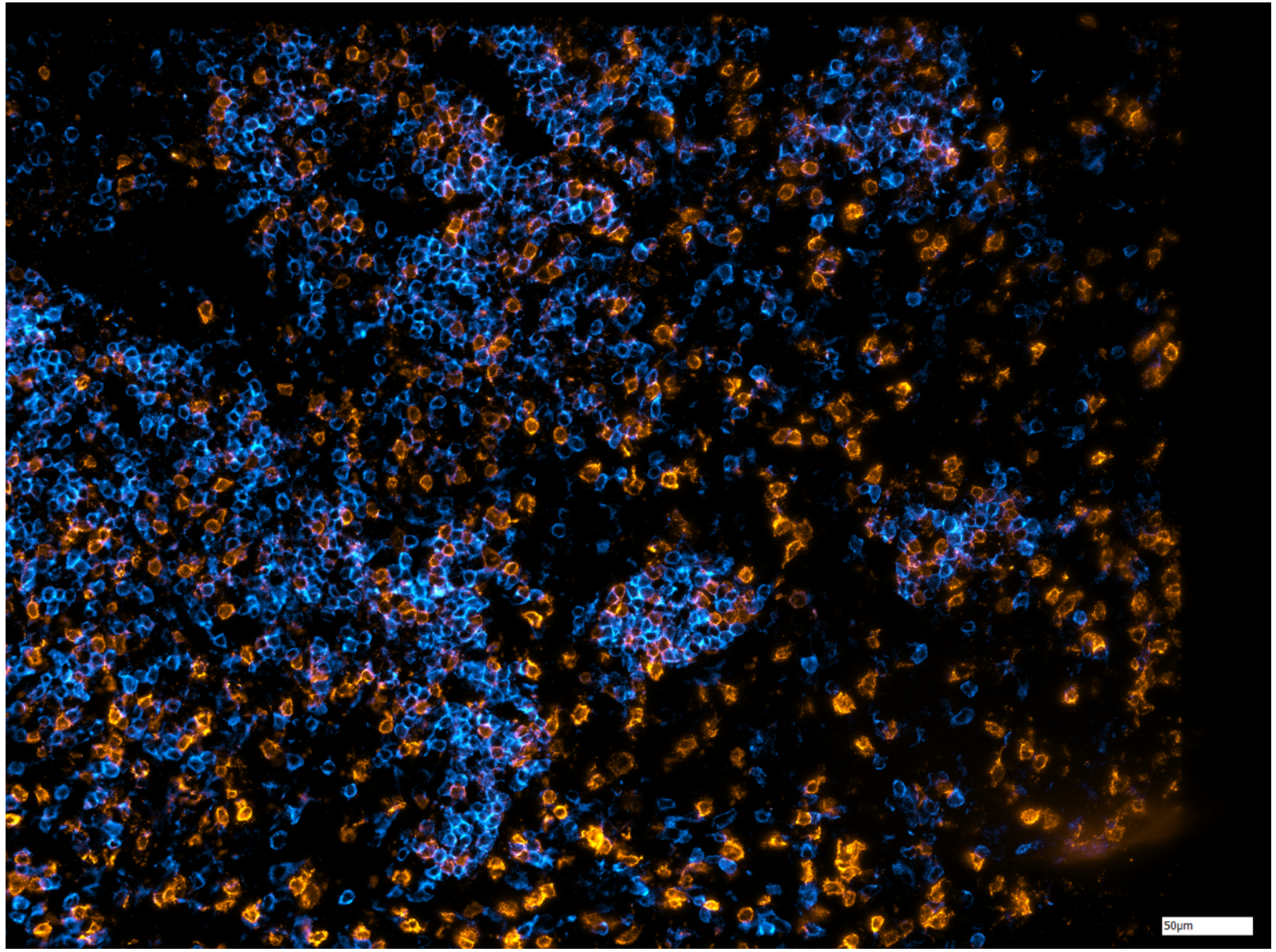
~1.5 log dynamic range



Source: Kim et al. 2018, AACR

CANOPY BIOSCIENCES

8 log
Dynamic range



CD4
CD8

Head & Neck
Cancer

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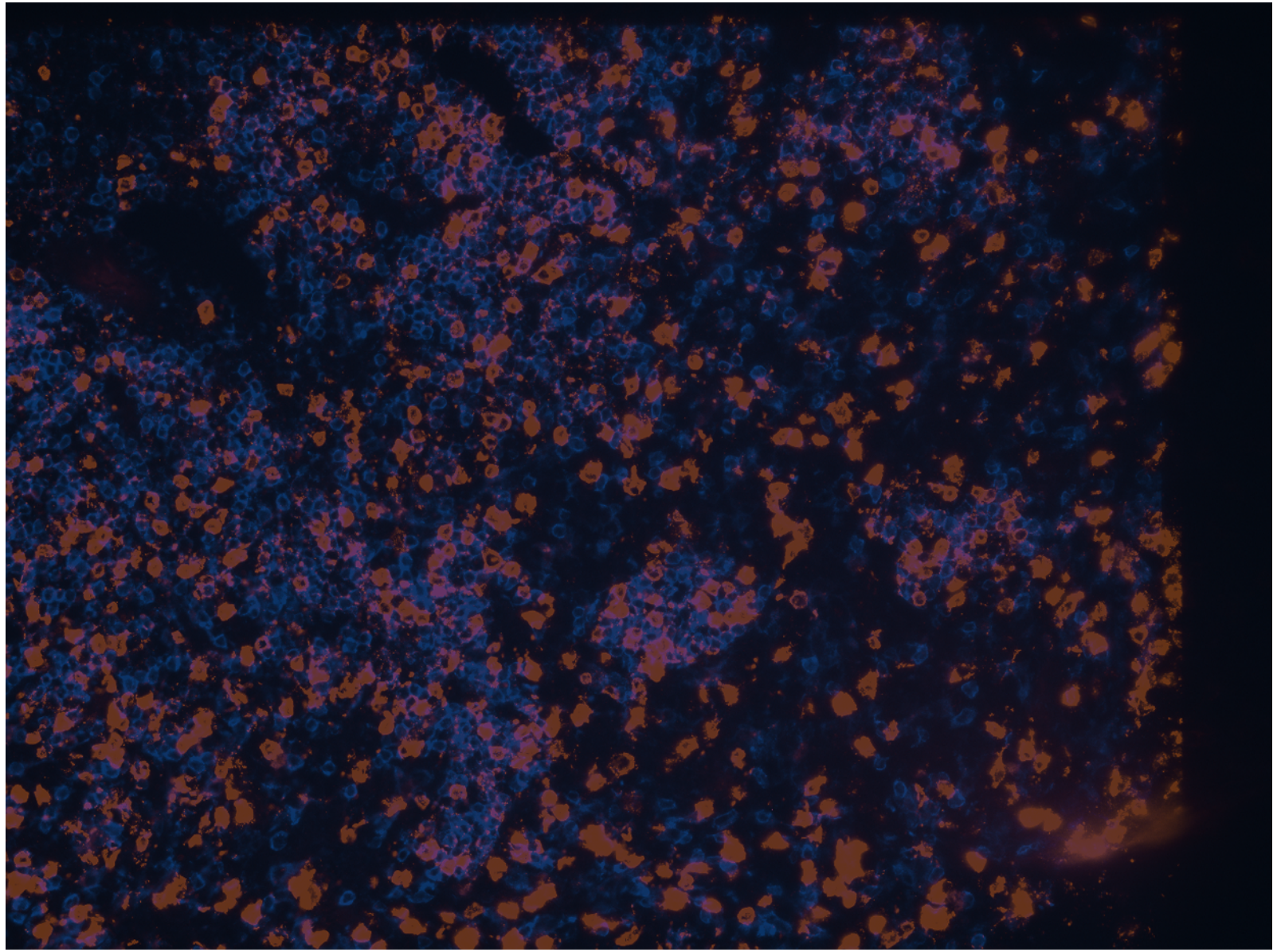
2.5 log
Dynamic range



CD4
CD8

Head & Neck
Cancer

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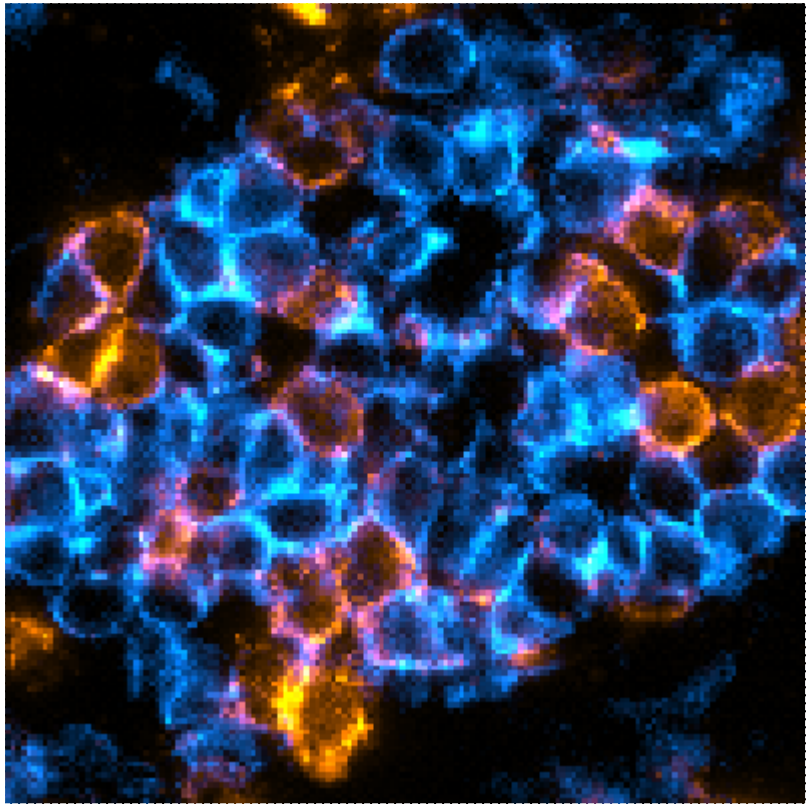
Resolution

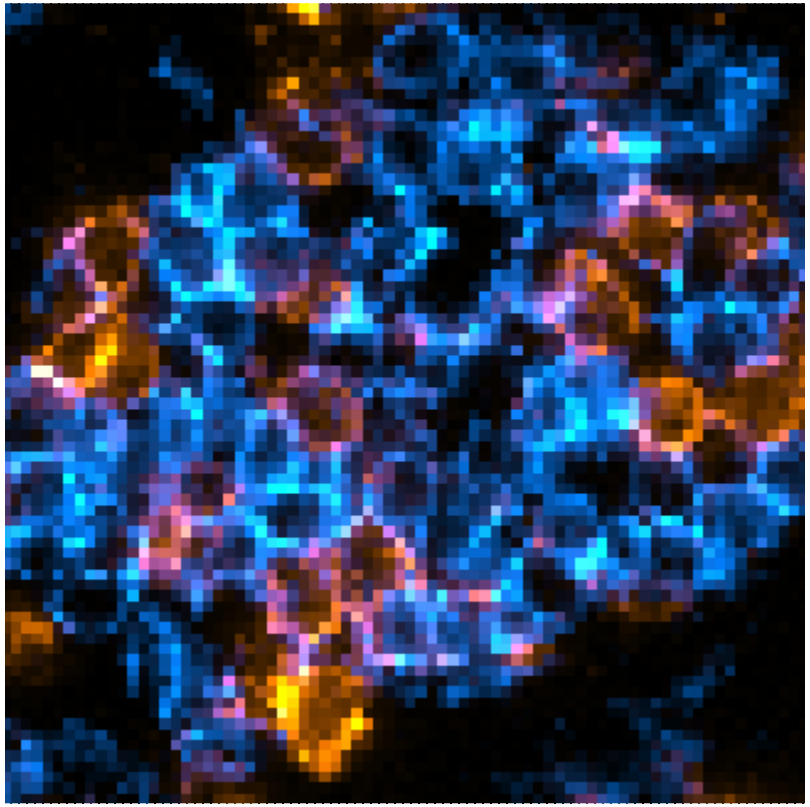
Why is Resolution important?

Enables True Single Cell Resolution

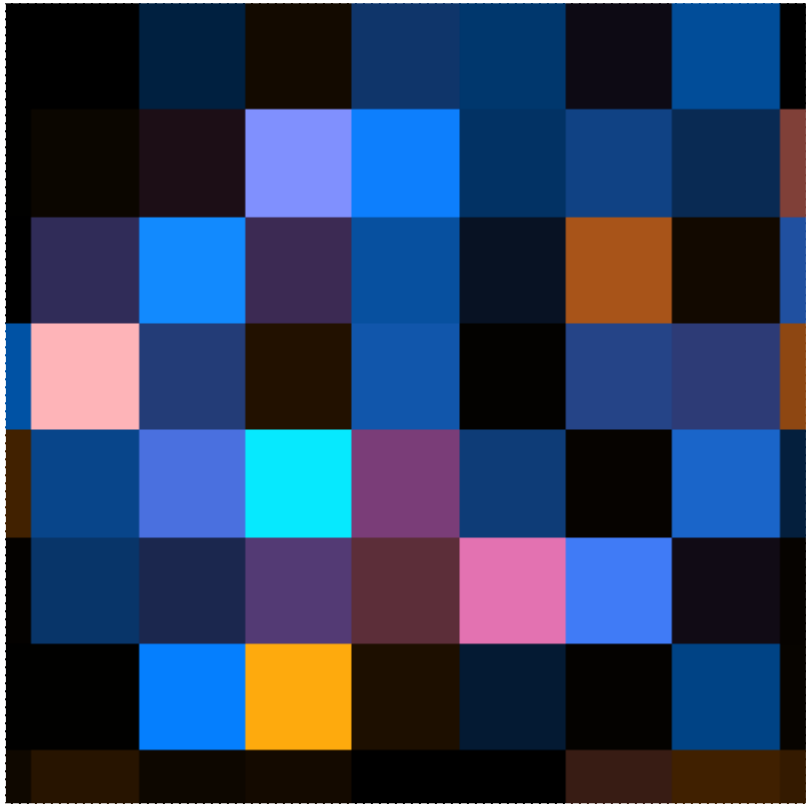
500 nm/px



1000 nm/px



10000 nm/px



Chipcytometry

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Multiplexing

A large, validated repertoire of protein markers

Validated biomarkers
Human Cell Suspension

- Gather complex phenotypic data by multiplexing among 120 validated biomarkers
- Custom assay development for any additional biomarker

Biomarkers validated for human PBMC samples				
CD2	CD39	CD123	CD273 (PD-L2)	IL5
CD3	CD40	CD127	CD274 (PD-L1)	IL8
CD4	CD45	CD134	CD278 (ICOS)	IL10
CD5	CD45RA	CD138	CD279 (PD-1)	IL12
CD8	CD45RO	CD141	CD294 (CRTH2)	IL17A
CD10	CD54	CD152 (CTLA-4)	CD319 (CRACC)	IL17F
CD11b	CD56	CD154 (CD40L)	CD326 (EpCAM)	IL23R
CD11c	CD57	CD161	CD366(TIM3)	Ki-67
CD14	CD61	CD163	AIOLOS (IKZF3)	Laminin
CD15	CD62L	CD172a/b	Bcl-2	LC (κ)
CD16	CD64	CD183 (CXCR3)	Collagen IV	LC (λ)
CD19	CD66b	CD184 (CXCR4)	FoxP3	pan Cytokeratin
CD20	CD68	CD185 (CXCR5)	GM-CSF	Perforin
CD21	CD69	CD193 (CCR3)	Granzyme B	RORγ(t)
CD22	CD71	CD194 (CCR4)	Helios	T-bet
CD24	CD73	CD195 (CCR5)	HLA-DR	TNFα
CD25	CD80	CD196 (CCR6)	IFNγ	Vimentin
CD27	CD81	CD197 (CCR7)	IgA	Zap-70
CD28	CD86	CCR10	IgD	Caspase-3
CD29	CD90	CD206	IgG	pATM (S1981)
CD30	CD95	CD223 (Lag-3)	IgM	pHistone H2A.X
CD31	CD105 (Endoglin)	CD235a (Glycph. A)	IL1b	pHistone H3
CD34	CD115	CD244	IL2	pStat1
CD38	CD117 (c-Kit)	CD257 (BAFF)	IL4	p21 Waf1/Cip1
*Other Custom Biomarkers Available				

CANOPY BIOSCIENCES

Validated biomarkers
Mouse Cell Suspension

- Gather complex phenotypic data by multiplexing among 39 validated biomarkers
- Custom assay development for any additional biomarker

Biomarkers validated for mouse PBMC samples			
CD3	CD86	CD202B	IL12
CD4	CD103	CD204	IL17a
CD5	CD105	CD206	Ly6C
CD8a	CD106	CD274 (PD-L1)	Ly6G (GR1)
CD11b	CD115	CD301	MHC-II
CD11c	CD124 (IL4Ra)	CD317	NK1.1
CD19	CD135	F4/80	SMA
CD25	CD154	FoxP3	T-bet
CD45R (B220)	CD163	IFNg	TNF alpha
CD62L	CD201	IL10	
*Other biomarkers available on a custom basis			

CANOPY BIOSCIENCES

Validated biomarkers
NHP Cell Suspension

- Gather complex phenotypic data by multiplexing among 31 validated biomarkers
- Custom assay development for any additional biomarker

Biomarkers validated for NHP cell suspension samples		
CD3	CD45	CD279
CD4	CD45RO	FoxP3
CD8	CD49d	Granzyme B
CD11b	CD56	HLA-DR
CD11c	CD80	Ki-67
CD14	CD86	Lambda LC
CD16	CD95	IL-2
CD20	CD107	IFNy
CD27	CD123	TNFa
CD39	CD161	
CD40	CD278	
*Other Custom Biomarkers Available		

CANOPY BIOSCIENCES

Validated biomarkers
in human tissue

- Gather complex phenotypic data by multiplexing among 54 validated biomarkers
- Custom assay development for any additional biomarker
- FFPE available, as well as F/F

Biomarkers validated for human FF tissue samples				
CD3e	CD27	CD69	CD193 (CCR3)	FoxP3
CD4	CD29	CD73	CD278 (ICOS)	HER2
CD8a	CD31	CD86	CD279 (PD-1)	HLA-A (MHC I)
CD10	CD38	CD90	CD299	HLA-DR
CD11c	CD39	CD95	CD326 (EpCAM)	Ki-67
CD14	CD40	CD105	CD335	Pan-cytokeratin
CD16	CD45	CD123 (IL3RA)	CD366 (TIM3)	SMA
CD19	CD45RA	CD141	Collagen IVa	SMAD1/2/3
CD20	CD45RO	CD152 (CTLA4)	Cytokeratin 18	TIGIT
CD21	CD56	CD155	DNA (Hoechst)	Vimentin
CD25	CD68	CD161	EGFR	
***Other biomarkers available on a custom basis**				

CANOPY BIOSCIENCES

Validated biomarkers
in human FFPE tissue

- Gather complex phenotypic data by multiplexing among validated biomarkers
- Custom assay development for any additional biomarker

Biomarkers for human FFPE tissue samples			
CD3	CD19	CD45RO	HLA-DR
CD4	CD20	CD68	Ki-67
CD8	CD27	CD123	
CD11c	CD45	CD279	
CD14	CD45RA	FOXP3	
*Other biomarkers available on a custom basis			

CANOPY BIOSCIENCES

Validated biomarkers
in mouse tissue

- Gather complex phenotypic data by multiplexing multiple validated biomarkers
- Custom assay development for any additional biomarker

Biomarkers validated for mouse FF tissue samples		
B220	CD45	GFAP
CD3e	CD64	Ly6c
CD4	CD68	Ly6G (GR1)
CD5	CD86	I-A/I-E (MHC II)
CD8a	CD160	NK1.1
CD11b	CD274 (PD-L1)	Pan-Cytokeratin
CD11c	CD326 (EpCAM)	
CD19	DAPI	
CD27	F4/80	
CD31	FoxP3	
***Other biomarkers available on a custom basis**		

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PBMC Immune Profiling Panel

Immune Cell Subsets	Defining markers
Immune Cells	CD45+
T cells	CD45+**CD3+**CD14-CD19-CD56-
CD4 T cells	CD45+**CD3+**CD14-CD19-CD56-**CD4+**CD8-
CD8 T cells	CD45+**CD3+**CD14-CD19-CD56-CD4-**CD8+**
Regulatory T cells	CD45+**CD3+**CD14-CD19-CD56-**CD4+**CD8-**CD25+FoxP3+**
Naive CD4 T cells	CD45+**CD3+**CD14-CD19-CD56-**CD4+**CD8-**CD27+CD45RA+**
Central memory CD4 T cells	CD45+**CD3+**CD14-CD19-CD56-**CD4+**CD8-**CD27+CD45RA-**
Effector CD4 T cells	CD45+**CD3+**CD14-CD19-CD56-**CD4+**CD8-**CD27-CD45RA+**
Effector Memory CD4 T cells	CD45+**CD3+**CD14-CD19-CD56-**CD4+**CD8-**CD27-CD45RA-**
Naive CD8 T cells	CD45+**CD3+**CD14-CD19-CD56-CD4-**CD8+CD27+CD45RA+**
Central memory CD8 T cells	CD45+**CD3+**CD14-CD19-CD56-CD4-**CD8+CD27+CD45RA-**
Effector CD8 T cells	CD45+**CD3+**CD14-CD19-CD56-CD4-**CD8+CD27-CD45RA+**
Effector Memory CD8 T cells	CD45+**CD3+**CD14-CD19-CD56-CD4-**CD8+CD27-CD45RA-**
B cells	CD45+CD3-CD14-**CD19+**CD56-
Memory B cells	CD45+CD3-CD14-**CD19+**CD56-**CD27+**
Naïve B cells	CD45+CD3-CD14-**CD19+**CD56-**CD27-**
Monocytes	CD45+CD3-**CD14+**CD19-CD56-
Non-classical monocytes	CD45+CD3-**CD14+**CD19-CD56-**CD16+**
Classical monocytes	CD45+CD3-**CD14+**CD19-CD56-**CD16-**
Dendritic cells	CD45+CD3-CD14-CD19-CD56-**HLADR+**
Myeloid dendritic cells	CD45+CD3-CD14-CD19-CD56-**HLADR+CD11c+CD123-**
NK cells	CD45+CD3-CD14-CD19-**CD56+CD16+**
NK cells	CD45+CD3-CD14-CD19-**CD56+CD16+**

Biomarkers validated for human PBMC samples		
CD3	CD16	CD45RA
CD4	CD19	CD56
CD8	CD25	CD123
CD11c	CD27	FOXP3
CD14	CD45	HLADR
***Other biomarkers available on a custom basis**		

- 15 biomarkers used for the identification of basic immune cell phenotyping
- Custom assay development for any additional biomarker

CANOPY BIOSCIENCES

Validated biomarkers
for characterizing CAR-Ts

- Characterise phenotype using 26+ validated biomarkers
- Custom assay development for any additional biomarker

Biomarkers validated for CAR-T samples				
CD3	CD19	CD45RA	CD152	Granzyme B
CD4	CD25	CD45RO	CD184	Ki-67
CD8a	CD27	CD56	CD197	
CD14	CD28	CD57	CD278 (ICOS)	
CD15	CD34	CD95	CD279 (PD-1)	
CD16	CD45	CD127	FoxP3	
***Other biomarkers available on a custom basis**				

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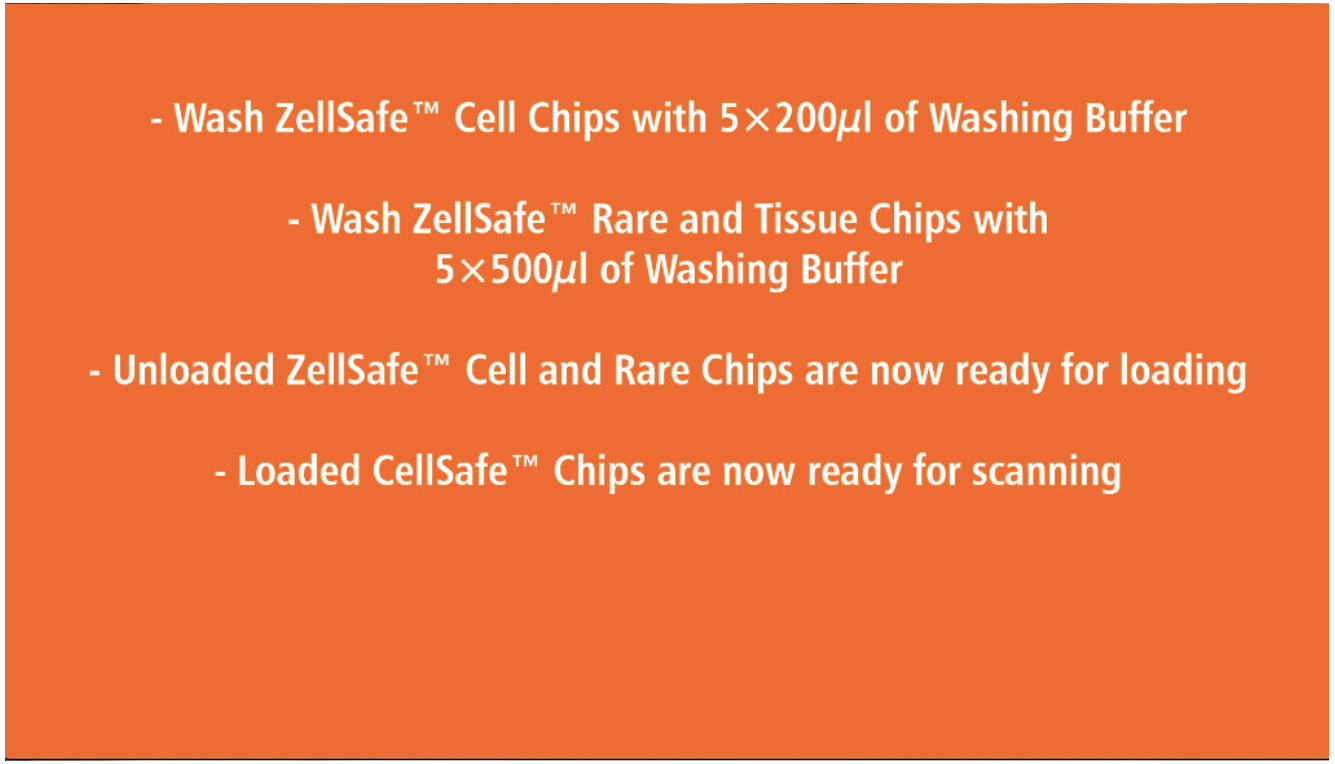
Standarisation

Multi-centre global trials

We provide training and accreditation



To help standardize the acquisition, storage and transportation of precious blood and tissue samples

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In conclusion…

- Chip Cytometry is a truly quantitative, non-destructive, ultra-deep phenotyping solution, available as an instrument or a service.
- Addresses key challenges from biomarker discovery to managing global clinical trials.
- Future proof your studies.

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Any questions?