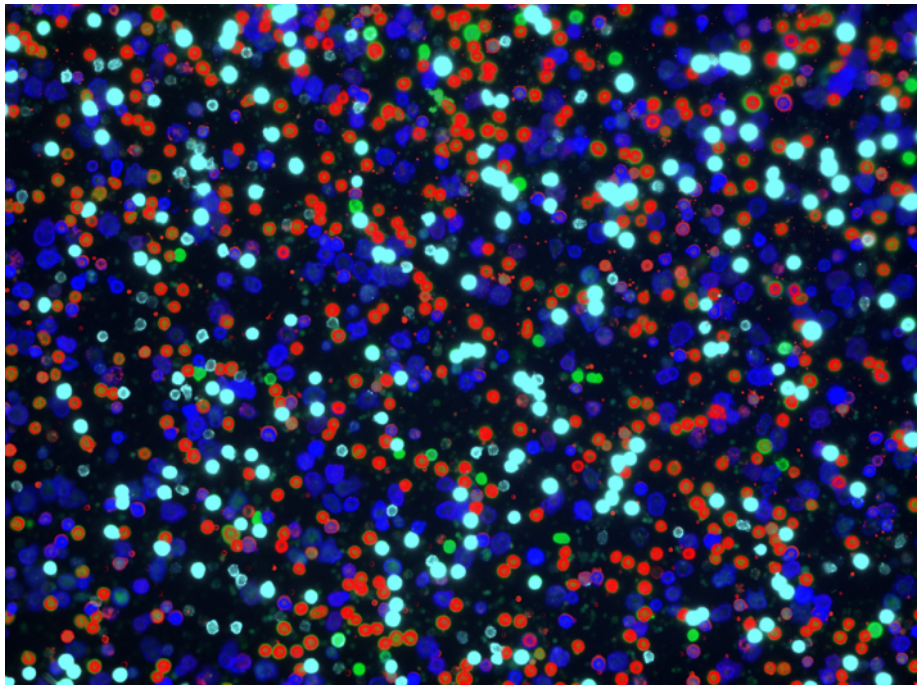
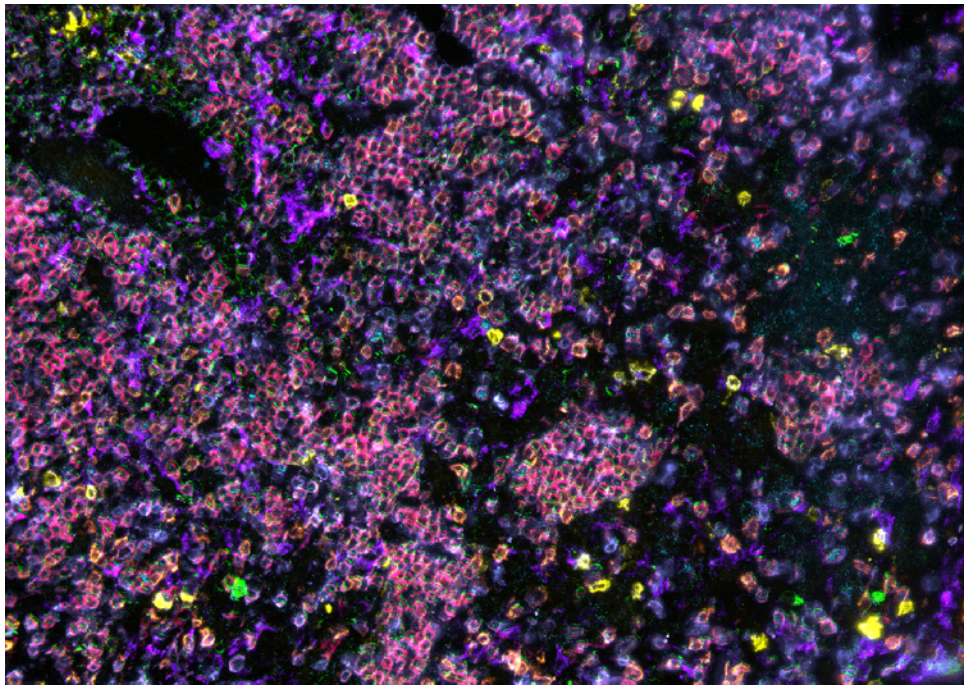
# High-Plex, Ultra-Deep Quantitation of Cellular Biomarkers

## Long-Term Sample Preservation and High-Content Cytometry on Cells and Tissues





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# A Powerful Tool for Immune Profiling

## How Quantitative Spatial High-Plex Ultra-Deep Phenotyping of Cellular Biomarkers Accelerates Immunotherapy Development from Pre-Clinical to Clinical Stages

Cancer immunotherapy is a market estimated at over $50 billion and expected to grow significantly over several years. Although approved products have shown great promise, there are still significant challenges to overcome, especially with respect to the limited number of patients who may benefit from monotherapy.

Today, over 2,500 clinical trials are underway incorporating checkpoint inhibitors, and 2 of the 3 largest selling cancer drugs are checkpoint-based immunotherapies. Combination therapies are seen as a way to improve the number of patient who could benefit, but there is a lack of biomarker signatures which could identify patient populations who might benefit from particular combinations.

Developing improved methods to identify patient subtypes who will benefit from combination therapies is key to improving outcomes for patients.

Quantitative ultra-deep spatial phenotyping of 120 or more cellular biomarkers enables the profiling of patients and drug response.

The Zellscanner One Chip Cytometer is unique is it's ability to quantify large panels of potential biomarkers in both bloods and tissues, humans and *in vivo* models, and is completely non-destructive, enabling the re-interrogation of samples for up to 2 years.

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# Single Cell Quantitation

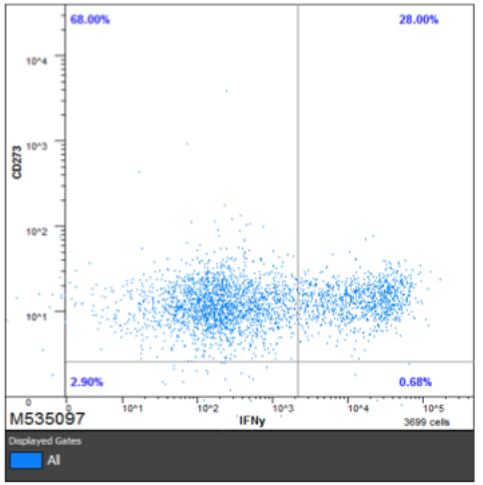
## It's all about the dynamics range and resolution…

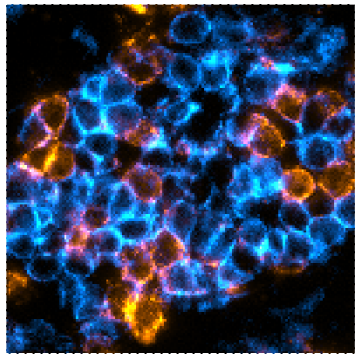
To be able to quantitate nuclear, intracellular and cell surface biomarkers requires extraordinary dynamic range. High-Plex, Ultra-Deep phenotyping of individual cells in cell suspensions and intact tissues requires cell by cell spatial resolution.

Chip Cytometry creates a HDR image with a dynamic range of greater than 8 logs. Being able to determine net fluorescence (signal minus noise) and still measure protein expression of common biomarkers such as interferon γ with an expression range of 6 logs, and a sensitivity greater than that of Flow Cytometry.

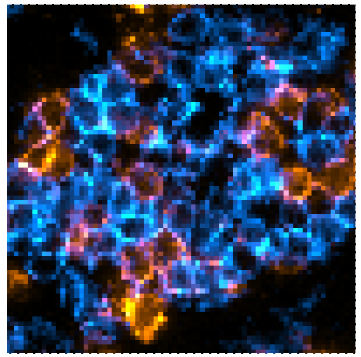
The Chip Cytometry camera has a resolution of 500nm/pixel in comparison to other platform with much lower resolution, which enables the clear resolution of cell boundaries, essential for quantitating biomarker expression, cell by cell.

**6 Log Expression of Interferon γ in T Cells**

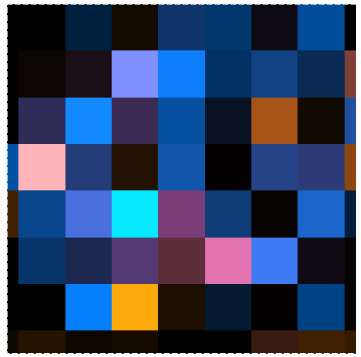


**500 nm/pixel**



**1,000 nm/pixel**



**10,000 nm/pixel**

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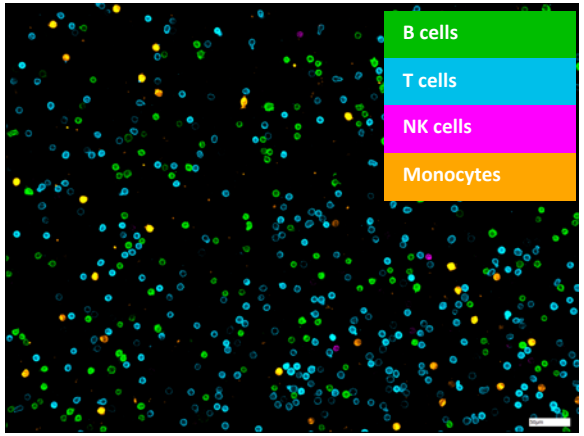
# A Complete Solution

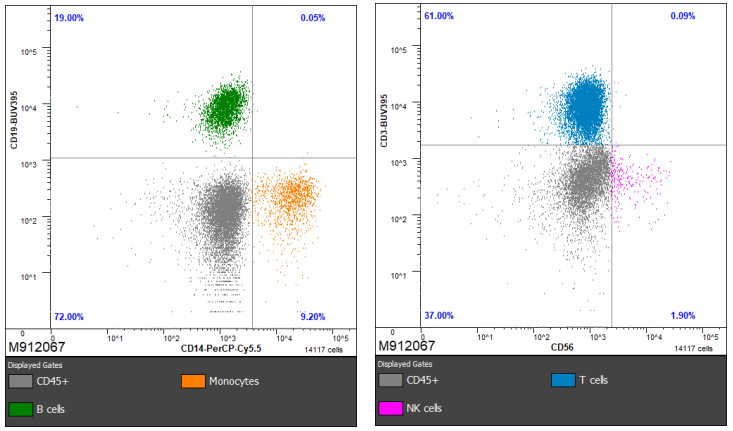
## It about the combined power of hardware and software

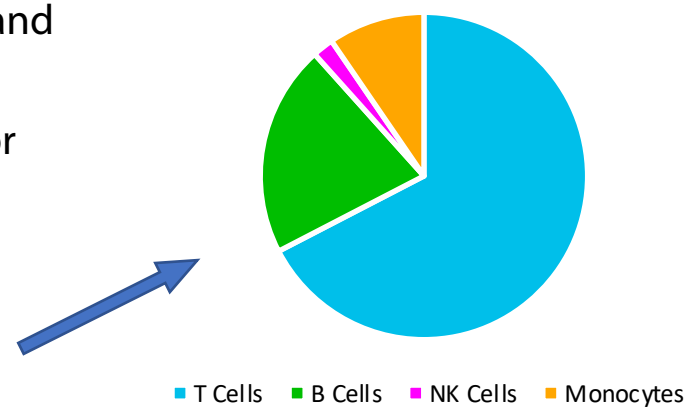
Being able to image at single cell resolution with an 8 log dynamic range is not the end of the story. Chip cytometry combines the power of leading image technology with the sciences of Immunology, Artificial Intelligence and Software Analytics.

Our fully integrated hardware and software, not only captures images, but managed the study design, protocols and antibodies, algorithmically segments the cells, quantifies the fluorescent values for each individual cell, and gates cells to characterise each cell and quantify key cell populations with single cell precision.

All high resolution image files are retained and data can be output in FCS format for further analysis in third party software.

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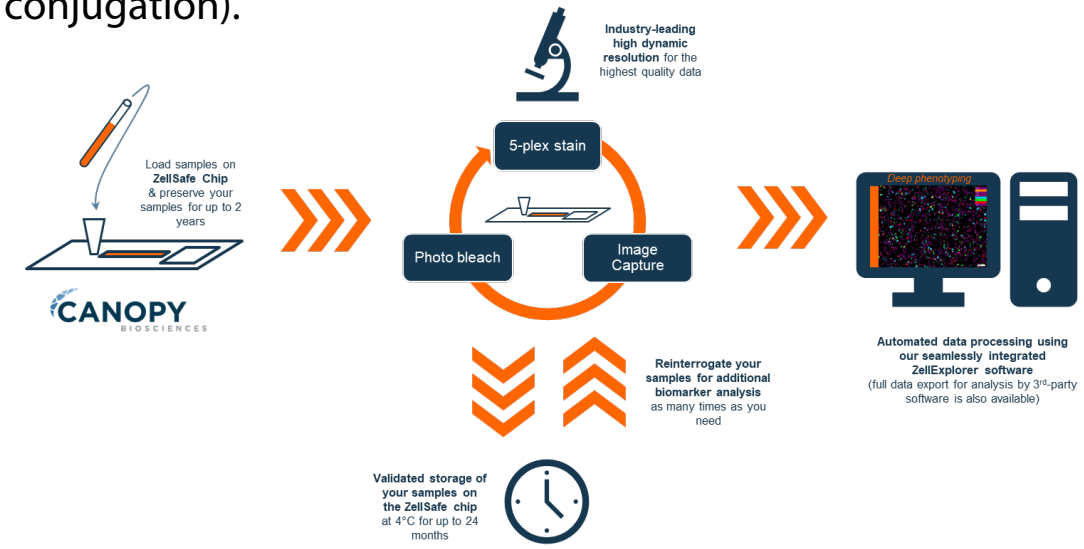
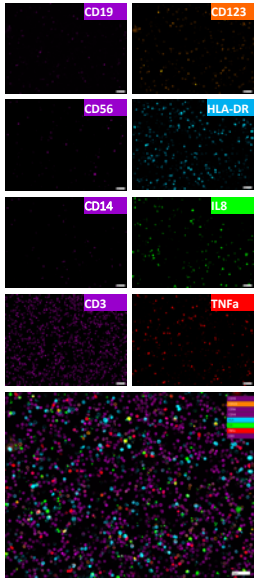
# Stable High Plex Biomarkers

## Iteratively interrogate over 120 validated biomarkers

Overcome the challenges with Flow Cytometry. Chip Cytometry is non-destructive. Once prepared on our chips, samples are stable for at least 2 years. Assay, store and re-interrogate for biomarkers as often as you want without loss of fidelity.

Our iterative stain, image and bleach process enables the measurement of many parameters without the spillover/compensation problems associated with Flow Cytometry. There is no limit to the number of biomarkers you could screen in a single sample, and we already provide a panel of 120 validated biomarkers. We use open source antibodies from any vendor (no requirement for expensive barcodes or metal ion conjugation).




| 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 | | | | |

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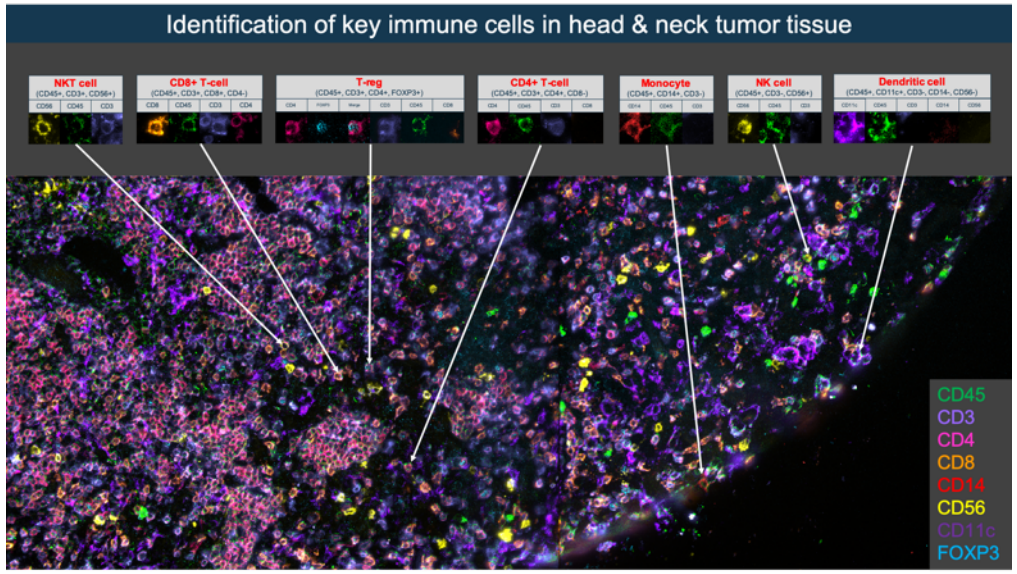
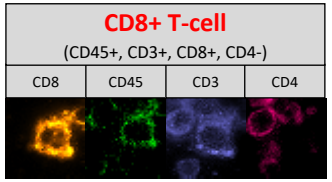
# Characterise and Quantitate in Tissues

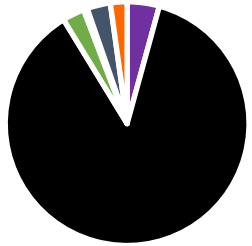
## Finally, a system that lives up to the promise of single cell, high-plex quantitation of the TME (tumor microenvironment)

Being able to characterise and quantify individual cells in a tissue is essential to accurately confirming both the composition of the TME and the true impact of drug therapy. For example, the Zellscanner can be used to identify and characterise individual immune cells in a tissue, quantify the exact number of each cell type, and quantify the exact number and location of PD-1 positive T cells in a tissue to differentiate between primary and metastatic tissue.



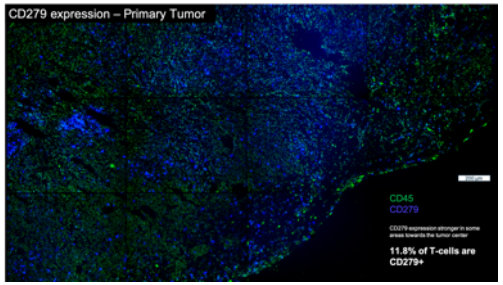



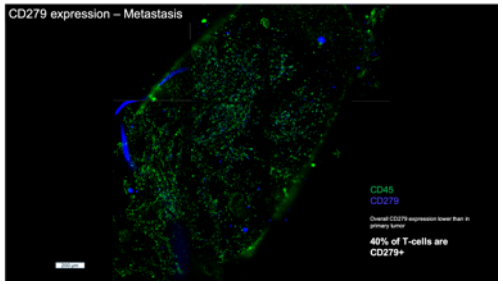

**Immune cell populations in primary tumor**



**Immune cell populations in metastasis**


**Quantify exact number and location of T cells which are PD-1 positive in primary vs. metastatic tissue**

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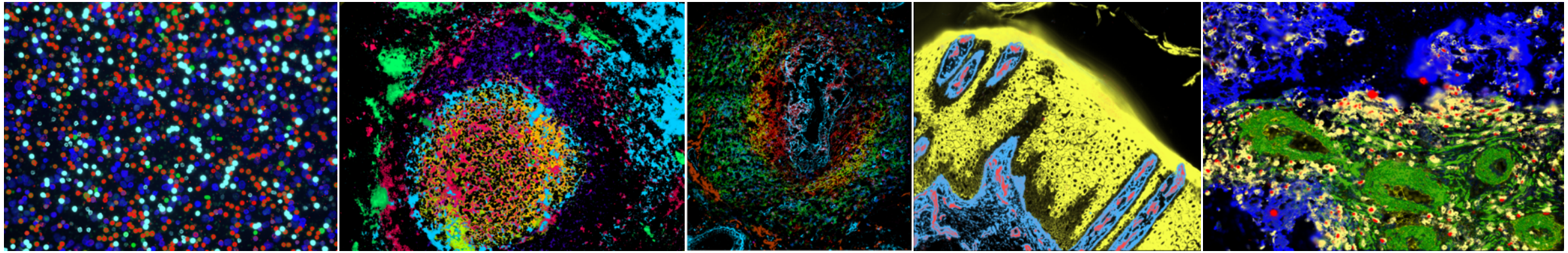
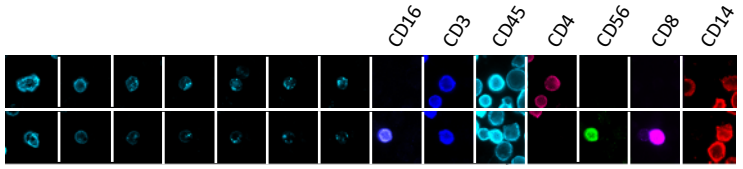
# Cell Suspensions and Tissues

## An all-in-one system for human, rodent and NHP samples

Many preclinical studies are run in humanised mouse models to study mechanism of action and efficacy. In clinical studies, samples are frequently limited to blood samples. For oncology you may have an initial tumor sample from surgery, but then longitudinal study patient samples are typically limited to bloods. Chip Cytometry works with cell suspension and tissue samples from human and animal models enabling continuity in studying disease and treatment progression, from research to the clinic, and preserves the integrity of biomarkers in samples to enable long-term studies.






CD4 T cell (CD3+ CD4+)

CD8 T cell (CD3+ CD8+)

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# References

Click on the links to review just some of the references about how chip cytometry is being used today

- Detection of PD-L1 and PD-L2 on Circulating Tumor Cells (CTCs) Using Chipcytometry (Merck & Co.)
- ChipCytometry identifies the presence of uncommon B cell subset in inflamed tonsils associated with autoimmunity (Novartis)
- Comparison between ChipCytometry and Flow Cytometry for Biomarkers and Immunophenotyping Applications (OncoMed)
- Validation of Treg, Th17 and Plasma Cell Assays (Ablynx)
- Comparison of Human Whole Blood Immunophenotyping by ChipCytometry and Flow Cytometry (MedImmune)
- High-Parameter Profiling of Psoriatic Tissue (Takeda)
- Cross-technology Comparison of Chipcytometry vs. Flow Cytometry for the Measurement of T Cell Phenotypic and Functional Markers (Pfizer)
- Imaging cytometry: the advantages of hybrid technology in support of drug discovery (GSK)
- Visit on website to see many more references and publications

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# Zellscanner One – Chip Cytometry

## True single cell quantitation

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