Rare Cell Discovery with ChipCytometry™

Highlights

- ChipCytometry is an all-in-one high-plex imaging platform for spatially resolved single-cell phenotyping
- ChipCytometry combines high-quality imaging with advanced analysis software to quantify protein expression and cell populations
- Commercially available, fluorescently conjugated antibodies are part of a flexible and practical solution to avoid tricky proprietary conjugation chemistry
- Studies in colon and tonsil tissues demonstrate ChipCytometry is a useful tool for the discovery of rare cells

Introduction

Investigation of therapeutically relevant cell types, cell states, and cellular interactions can inform the discovery of predictive biomarkers for disease. Multiplexing platforms present a unique opportunity to accelerate research through the collection of large single-cell datasets, and several large-scale projects to characterize healthy and diseased tissue are currently underway (HuBMAP Consortium, 2019; Rozenblatt-Rosen et al., 2020). These multiplexing technologies are likely to dominate the future of biological discovery and transform medicine as we know it.

Rare cells play pivotal roles in disease initiation, maintenance, and progression. Yet, identifying rare cells remains a difficult task for low-plex technologies, as it is not always clear a priori which biomarkers distinguish rare phenotypes from the remaining population. Available high-plex technologies – like flow cytometry and RNA-Seq – do not offer the spatial information necessary to understand how rare cells interact with neighboring cells in the tissue microenvironment (TME).

Furthermore, biomarker detection for translational research requires high-quality reagents. Many available spatial technologies currently use proprietary reagents with tricky conjugation chemistry, limiting ease-of-use and cross-platform compatibility. This poses a problem for researchers orchestrating projects designed to study novel predictive biomarkers.

To address these challenges, ChipCytometry offers spatially resolved multiplexing to detect dozens of biomarkers on the same sample with commercially available reagents. ChipCytometry leverages decades of histology and community consensus on antibody clones and enables spatial analysis of the TME (Figure 1).

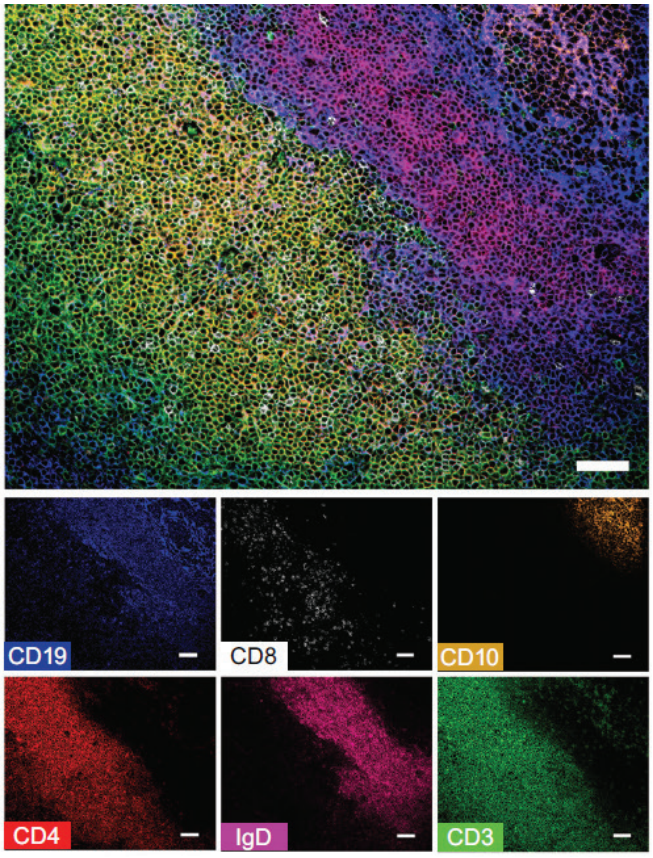

Figure 1. Spatial multiplexing with ChipCytometry. Top: Merged image of 6 markers. Bottom: Single marker images. (Source: Hagel et al. 2021)

ChipCytometry Overview

ChipCytometry is an iterative immunofluorescence workflow, using ZellScannerONE™ – a fully integrated and automated imaging system. ZellScannerONE uses high-resolution, high-dynamic range imaging to achieve true single-cell resolution and a greater range of protein expression data than is possible with standard imaging techniques.

Sample preparation is a critical step for acquiring high-quality data. With ChipCytometry, tissue sections or cell suspensions are loaded onto proprietary ZellSafe™ chips to preserve sample integrity during serial delivery of reagents. Prior to staining, ROIs are selected based on tissue autofluorescence. A solution containing up to five fluorescently conjugated antibodies is delivered in successive rounds of staining, imaging, and quenching (Figure 2). The chip technology enables sample storage for up to two years and reinterrogation with additional markers.

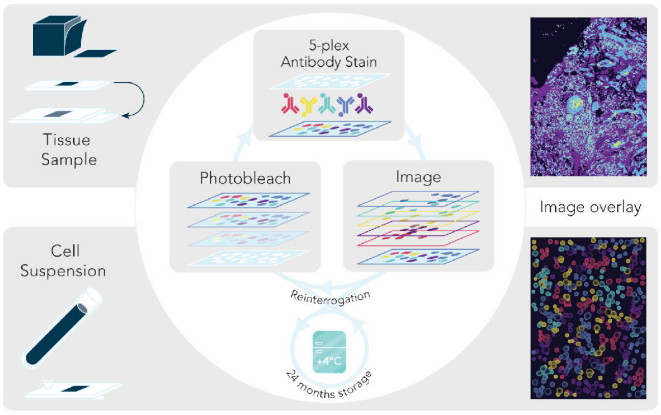

Figure 2. ChipCytometry workflow. Samples are loaded onto ZellSafe™ chips, and reagents are delivered in rounds of staining, imaging, and photobleaching. ZellScannerONE facilitates serial delivery of reagents and automated image analysis with the built-in ZellExplorer™ software.

Data analysis is performed using custom ZellExplorer™ software. Standard FCS files are generated from multichannel OME-TIFF images. In the software, the user can review the automatic cell segmentation and define cell phenotypes using a hierarchical gating strategy. The software is straightforward, easy to use, and does not require a computational background.

ChipCytometry Enables Rare Cell Discovery in Tissues

Investigation of therapeutically relevant cells in their native context requires a spatially resolved high-plex imaging approach. Studies designed by researchers at the University of Oxford used ChipCytometry to investigate rare cells in colon and tonsil tissues (Hagel et al., 2021; Leng et al., 2019). They focused on mucosal-associated invariant T (MAIT) cells, which play multiple roles in infection, inflammation, and cancer and have been previously investigated for their immunotherapeutic potential (Godfrey et al., 2019). Taken together, the data in these studies demonstrate the ability of ChipCytometry to drive the discovery of rare immune cells in the intact microenvironment with ready-to-use, commercially available reagents.

Panels were designed using different combinations of 24 markers to identify various immune cells, with a particular focus on T cell markers (Table 1).

Markers for MAIT Cell Analysis			
CD3	CD45RA	PLZF	Granzyme B
CD4	CD56	IL-18Ra	FoxP3
CD8	CD69	ICOS	CD10
CD19	CD161	PD-1	IgD
CD44	FASL	TCR Va7.2	Histone H3
CD45	CXCR5	BCL6	TCR-yd

Table 1. Markers used in ChipCytometry assays for the MAIT cell analysis.

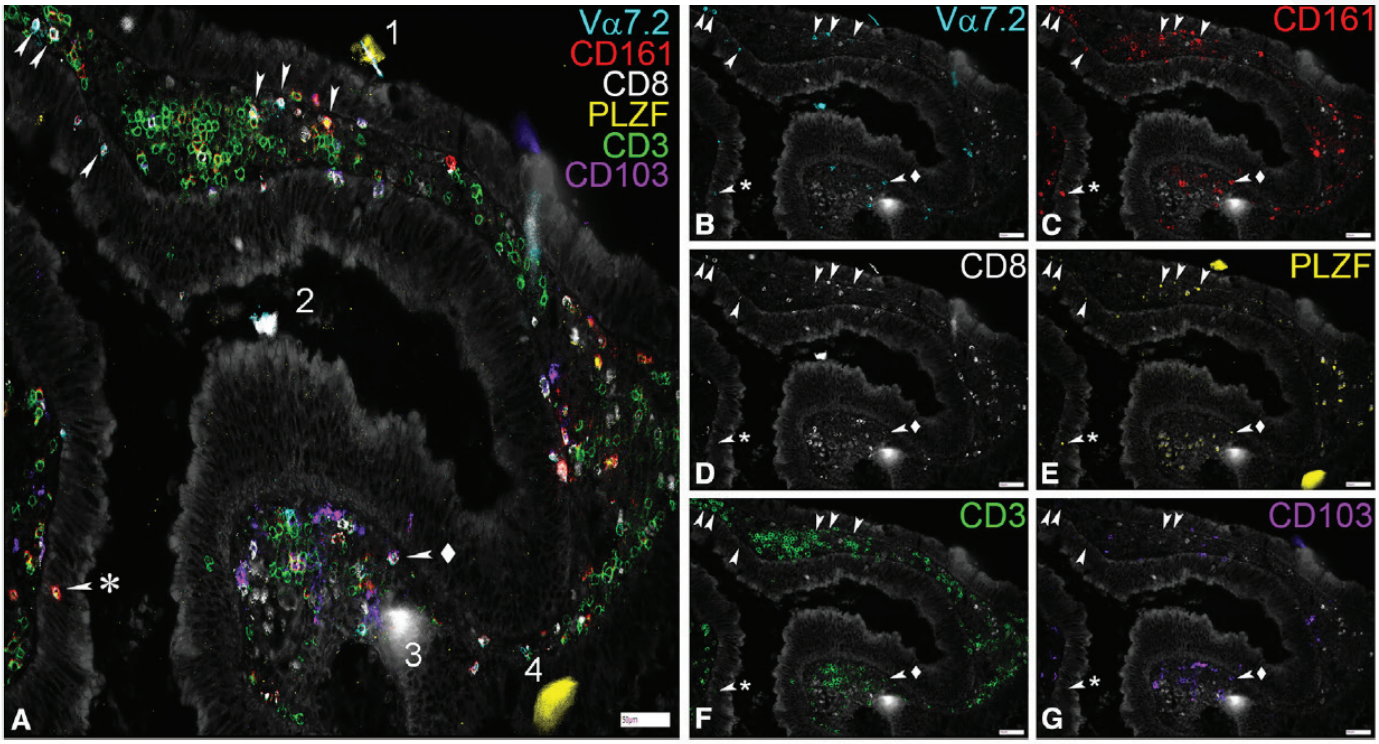

Figure 3. MAIT cells in colonic epithelium. Left: Merged image showing expression of Va7.2 (cyan), CD161 (red), CD8 (white), PLZF (yellow), CD3 (green), and CD103 (purple). MAIT cells co-express Va7.2, CD161, PLZF, and CD3 (white arrows) . A subset of MAIT cells express CD103 (white arrow with diamond). Right: Single marker images. (Source: Leng et al. 2019)

Human colon tissue is comprised of epithelial cells, which act as a protective barrier, and immune cells that combat infection and disease. In particular, MAIT cells have emerged as playing a critical role in the maintenance of tissue homeostasis at mucosal barriers (Nel et al. 2021). Researchers at the University of Oxford used ChipCytometry to study CD8+ MAIT cells, and their spatial organization with respect to neighboring cells, in intact colonic epithelium (Leng et al., 2019). Figure 3 shows the protein expression levels of various T cell markers. In this study, MAIT cells were defined as co-expressing Va7.2, CD161, PLZF, and CD3. MAIT cells expressing additional markers were also observed. For example, a subset of cells also expressed CD103, suggesting a functionally distinct CD8+ MAIT cell subtype.

With these results, the authors concluded that in tissues such as the gut, subpopulations of MAIT cells play a protective role. The study shows how ChipCytometry can be useful in an unsupervised, hypothesis-free approach to study rare cells within the TME.

Heterogeneous Expression Drives Quantification of Cell Phenotypes

MAIT cells are named after their preferential location in the mucosal tissue of the gut but are abundant in other peripheral and lymphatic organs. Recent work by Hagel and colleagues (2021) expanded on the previous study and used ChipCytometry to profile CD8+ MAIT cells in other organs, focusing on tonsil tissue.

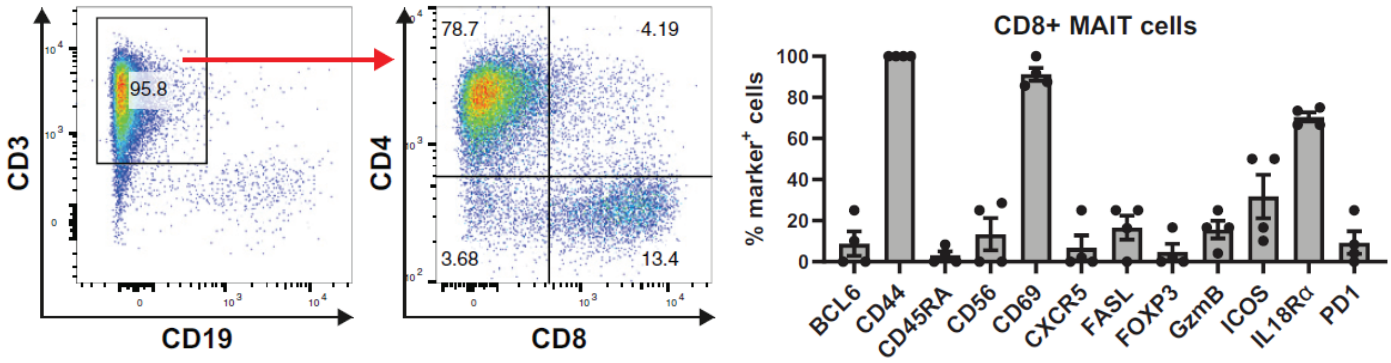

Figure 4. Left: Gating strategy for MAIT cells. Right: Percent of MAIT cells expressing various markers. (Source: Hagel et al. 2021)

The authors performed ChipCytometry on tonsil-derived CD3-enriched cell suspensions to quantify CD8+ MAIT cells (Figure 4). The data reveal that ChipCytometry is sufficiently sensitive to quantify cell types based on heterogenous expression of single markers. Subsequent ChipCytometry profiling of intact tonsil tissue provided spatial context of MAIT cells in the TME (Figure 5).

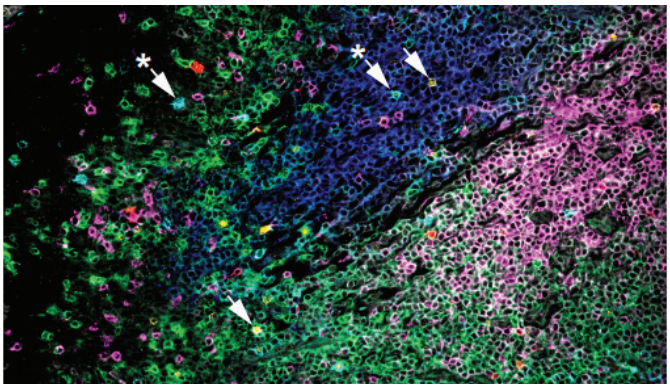

Figure 5. MAIT cells (arrows) in tonsil tissue co-express Va7.2 (red), CD8 (white), CD161 (magenta), PLZF (yellow), CD3 (green), and CD19 (blue). (Source: Hagel et al. 2021)

Summary

Spatial biology has recently exploded, as scientists recognize its potential to transform disease research and medicine. This report summarized several key features of ChipCytometry – chiefly the ability to spatially profile and quantify rare cell types in tissue and cell suspensions – and show how these aid research aimed to uncover mechanisms of disease. Leng et al. (2019) used ChipCytometry to determine protein expression profiles of MAIT cells in colon tissue, while Hagel et al. (2021) applied the technique to tonsil tissue and cell suspensions. Insights from these studies reveal how MAIT cells are implicated in immune and inflammatory pathologies.

In summary, ChipCytometry is an all-in-one high-plex imaging solution for rare cell discovery. ChipCytometry offers a wealth of data, an important feature given little is known about the role of rare cells in disease progression.

References

1. Godfrey et al. (2019). The biology and functional importance of MAIT cells. Nature Immunology, 20(9), 1110–1128.
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