RNA sequencing of rare antigen-specific T cells and tissue micro-regions using the RareCyte platform

Rebecca Podyminogin, Nolan Ericson, Eric Kaldjian, Tad George, Lance U'Ren
RareCyte, Inc., Seattle, WA

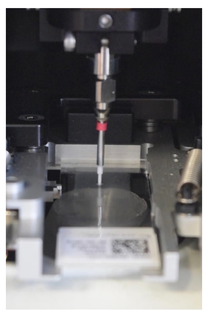
RARECYTE

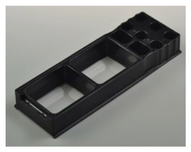
Abstract

The immune system provides antigen-specific protection against pathogens as well as malignancies, both of which evolve strategies to evade immune surveillance and containment. Effective immune response often depends on activation of rare immune cell sub-types, whose function are influenced by the tissue micro-environment, the pathogen or cancer, and other factors. The RareCyte platform provides integrated multi-parameter imaging and retrieval capabilities that allow phenotypic identification and isolation of rare cells and microscopic regions of interest (ROI) for sequence and transcript level analyses, and is therefore uniquely suited to study the complexity of host defense. We demonstrate that the platform can be used to identify and retrieve rare antigen-specific T cells by using tetramers against influenza-specific T cell receptors. We confirm the identity of the TCR by RNA sequencing and validate T cell activation by gene expression analysis. Additionally, we show that the system can be used for 6-color imaging of tissues and for the picking of tissue micro-regions, with confirmation of the process by RNA sequencing.

RareCyte Technology

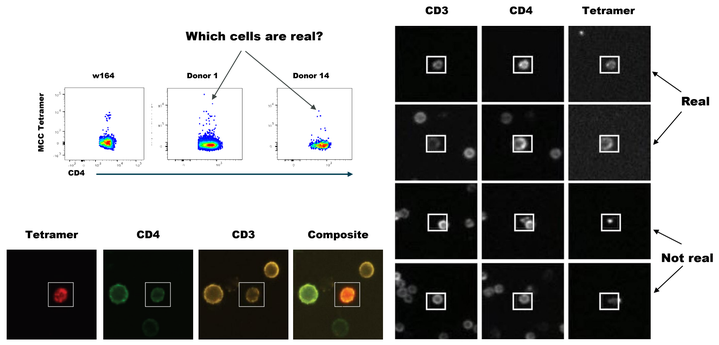
6-color imaging and retrieval from live cell, blood smear, and tissue preparations





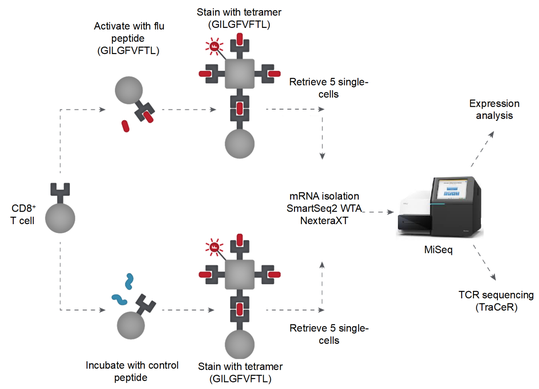
Rare single antigen-specific T cell expression analysis and TCR sequencing

A. Identification of rare CD4+ T cells by tetramer

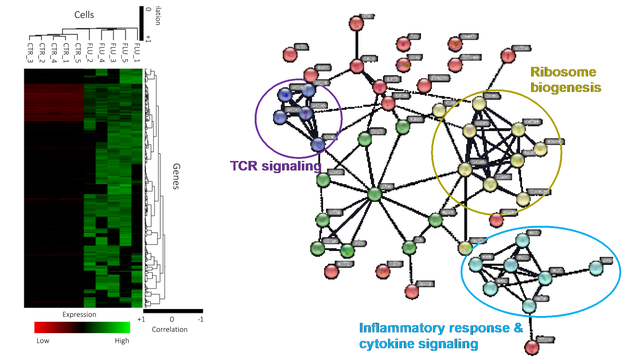


Paul Ngheim, University of Washington

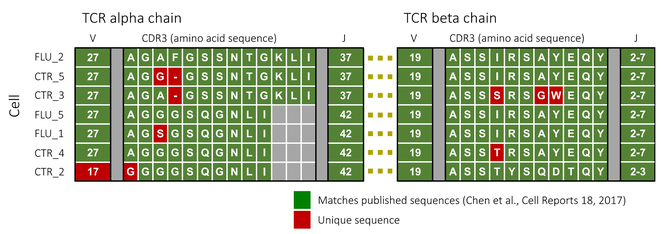
B. Influenza model to validate T cell specificity



C. Expression analysis confirms specificity



D. T cell receptors match known flu sequences

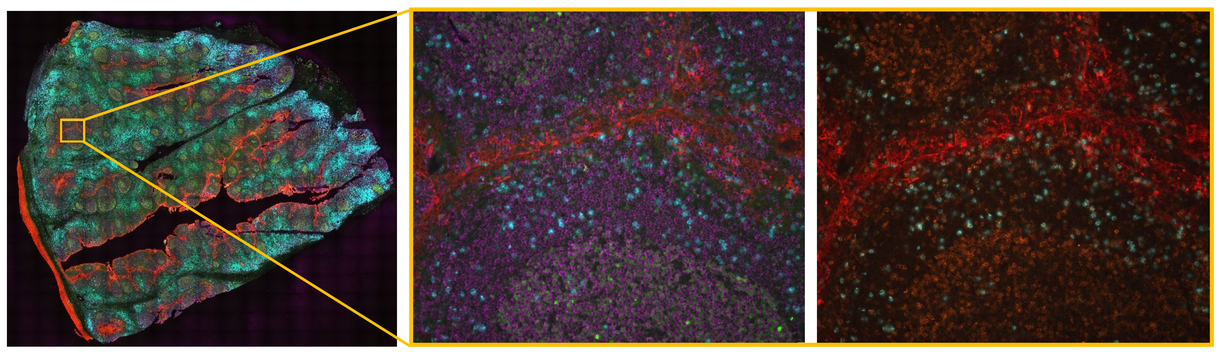


- 10/10 cells generated productive TCR α/β pairs
- 7/10 had exact or near-exact CDR3 matches against published flu-specific TCR sequences

Figure 1: Merkel carcinoma patient samples were stained with CD3-AF488, CD4-AF647, exclusion marker in BV-421 (CD14, CD15, CD20, CD8, DAPI viability), and MCPyV-specific tetramer-PE, then analyzed by flow cytometry and using the CyteFinder instrument (A). Schematic of the influenza model system used to validate the specificity of single antigen-specific T cells by both expression analysis and TCR sequencing (B). RNA from single cells isolated from control or flu peptide stimulated groups was amplified with the SMART-Seq® v4 (Takara Bio) and sequenced. The heatmap represents the 760 differentially expressed genes. The STRING cluster diagram represents the protein networks of the top 50 hits, with Gene Ontology Consortium (GOS) identifying the clusters (C). Single-cell RNA seq FASTQ files were analyzed with the TraCeR computational method to identify TCR alpha and beta chains in both control and flu peptide stimulated cells (D).

Multi-parameter tissue imaging and micro-region isolation for RNA sequencing

A. 6-color high-resolution whole tissue scanning of FFPE tonsil



CD8 / Ki67 / Cytokeratin / CD20 — CD8 / CD3 / Cytokeratin

B. 6-color high-resolution identification of ROI in FFPE breast carcinoma sections

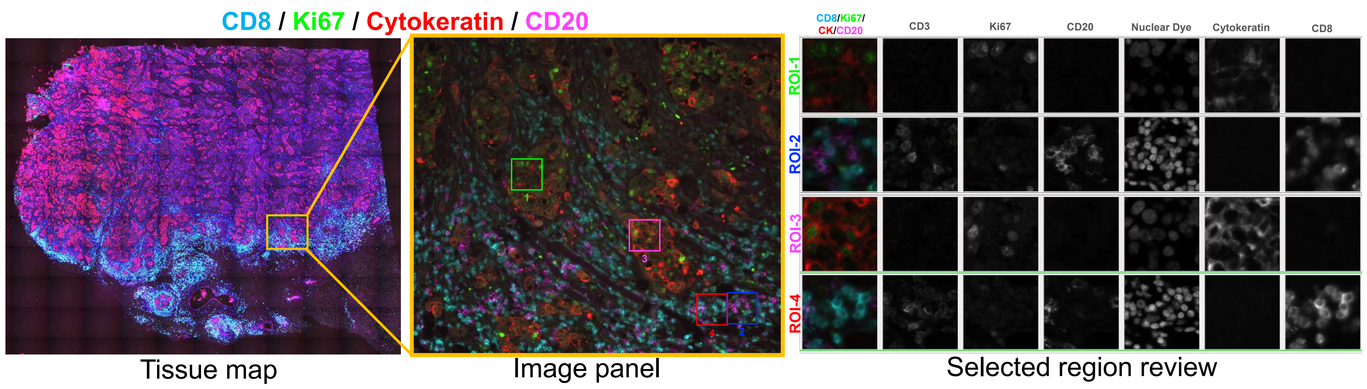


Figure 2: FFPE tonsil 5 um section stained with a 6-color panel consisting of Qdot®625 anti-CD3, CF®488 anti-Ki67, BV421™ anti-CD20, SYTOX® Orange, CF647 anti-Cytokeratin, and Qdot800 anti-CD8. The slide was imaged at 20X magnification using the CyteFinder instrument (A). FFPE breast carcinoma section stained with the same panel. Micro-regions of interest (ROI) were marked using CyteFinder software for detailed interrogation using composite and single color review panels (B).

RNA sequencing of tonsil micro-regions of interest

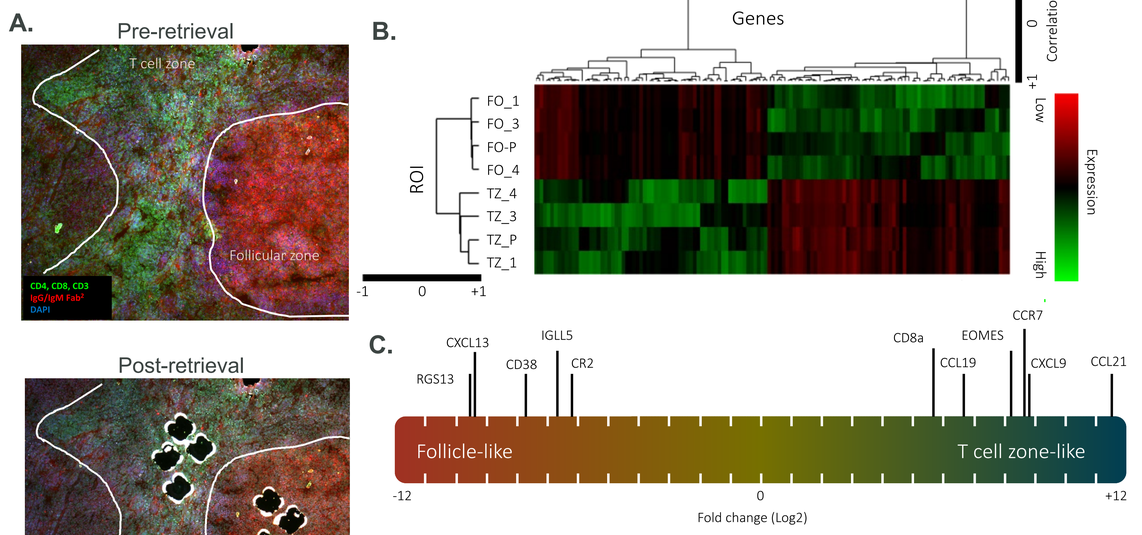


Figure 3: Frozen human tonsil (OCT) sections were stained with a cocktail of AF488-conjugated antibodies to T cell markers (CD3, CD4, CD8), Fab² anti-IgG/IgM AF647 and DAPI. Micro-regions from T cell zones or follicular zones, containing between 8-16 cells, were retrieved using the CytePicker module and deposited into PCR tubes (A). Differential RNA expression of T cell zone vs follicular zone micro-regions was obtained following whole transcriptome amplification using the SMART-Seq v4 kit, Nextera XT kit (Illumina) library preparation, and RNA sequencing using the MiSeq system (Illumina) (B). Differentially expressed genes include T and B cell phenotypic markers as well as a signaling molecules important for the formation and maintenance of tonsil germinal center architecture (C).

Conclusions

- The RareCyte CyteFinder instrument's combination of high-speed and high-quality imaging with automated rare cell candidate identification enable highly specific identification of rare antigen-specific T cells.
- TCR sequencing of single, live antigen-specific T cells retrieved with the CytePicker module resulted in paired α/β TCRs from all 10 cells isolated.
- Single cell differential RNA expression analysis of control vs stimulated antigen-specific T cells produced an expected pattern of stimulation-induced transcription.
- The CyteFinder instrument also generates high quality tissue images mapped across the entire tissue section, and enables identification and retrieval of micro-regions within the tissue.
- Differential RNA expression analysis of tissue micro-regions isolated from stained OCT tonsil tissue sections revealed the expected pattern of expression in the T cell and follicular zones.