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

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**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.

**Antigen-specific T cells**

Identification of rare CD4+ T cells by tetramer

**RareCyte Technology**

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

**Multi-parameter immunofluorescence tissue imaging**

6-color high-resolution identification of ROI in FFPE tonsil sections

**Serial section stains of regionally expressed cell markers**

Cytokeratin / Ki67 / IgG / CD20

CD8 / CD68 / IgG / PDL1

**RNA sequencing of tonsil T and B cell ROI (frozen)**

**Pre-retrieval**

**Post-retrieval**

• RNA expression from T cell zone ROI were referenced to follicular zone ROI
• Key follicular and T cell zone genes appeared in the proper ends of the differential expression scale, validating the approach