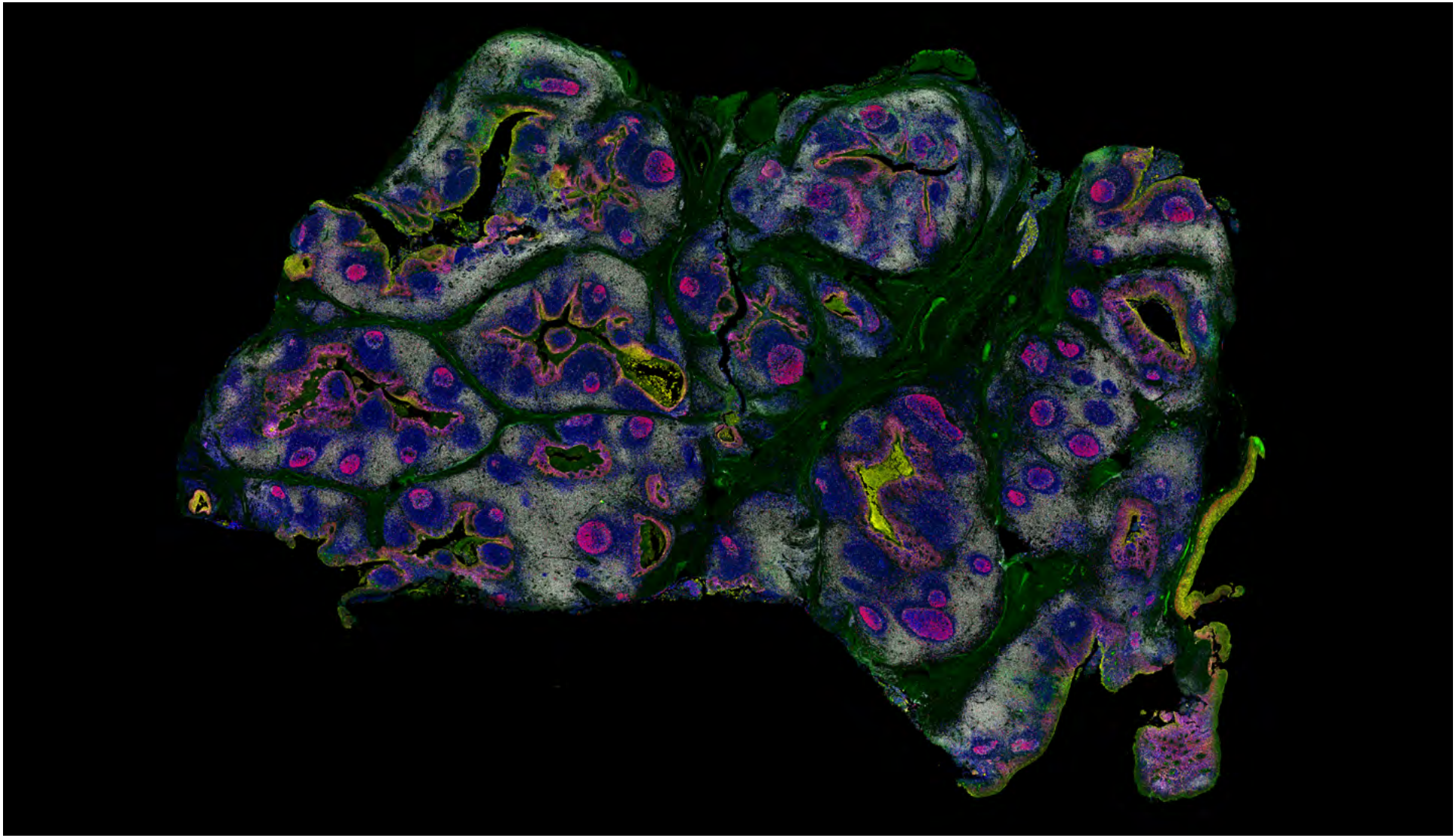
RARECYTE®

Orion™ Tissue Investigation | Tonsil Lymphoid Hyperplasia



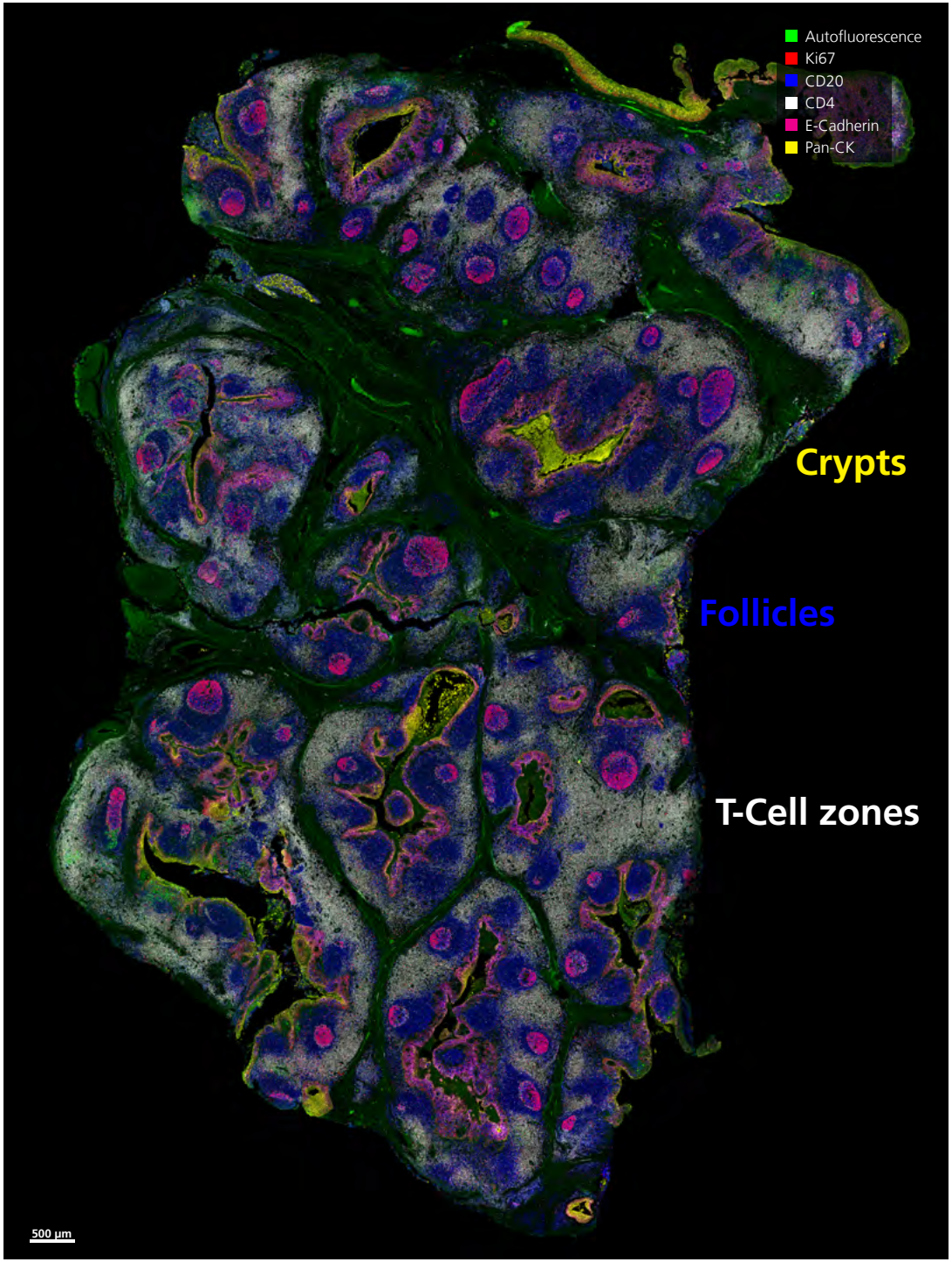
Overview

This is the same whole-slide tissue section of a tonsil with reactive lymphoid hyperplasia stained with a 15-plex immuno-oncology biomarker panel and imaged with the Orion system in a single staining and scanning process.

Images in this presentation show subsets of markers that have been selected from the larger panel.

In tonsil, B cell follicles with germinal centers and T cell interfollicular zones define the lymphoid compartment that is lined by epithelial crypts, which are invaginations of the squamous mucosa of the pharynx. Tonsillar architecture is illustrated here, with B-cell follicles (**CD20**), surrounding inter-follicular T-cell regions (**CD4**, in white), and epithelial cells of the tonsillar crypts (**Pan-Cytokeratin** and **E-Cadherin**).

Cell proliferation is indicated by the nuclear marker **Ki67** and tissue morphology is provided by measuring autofluorescence.

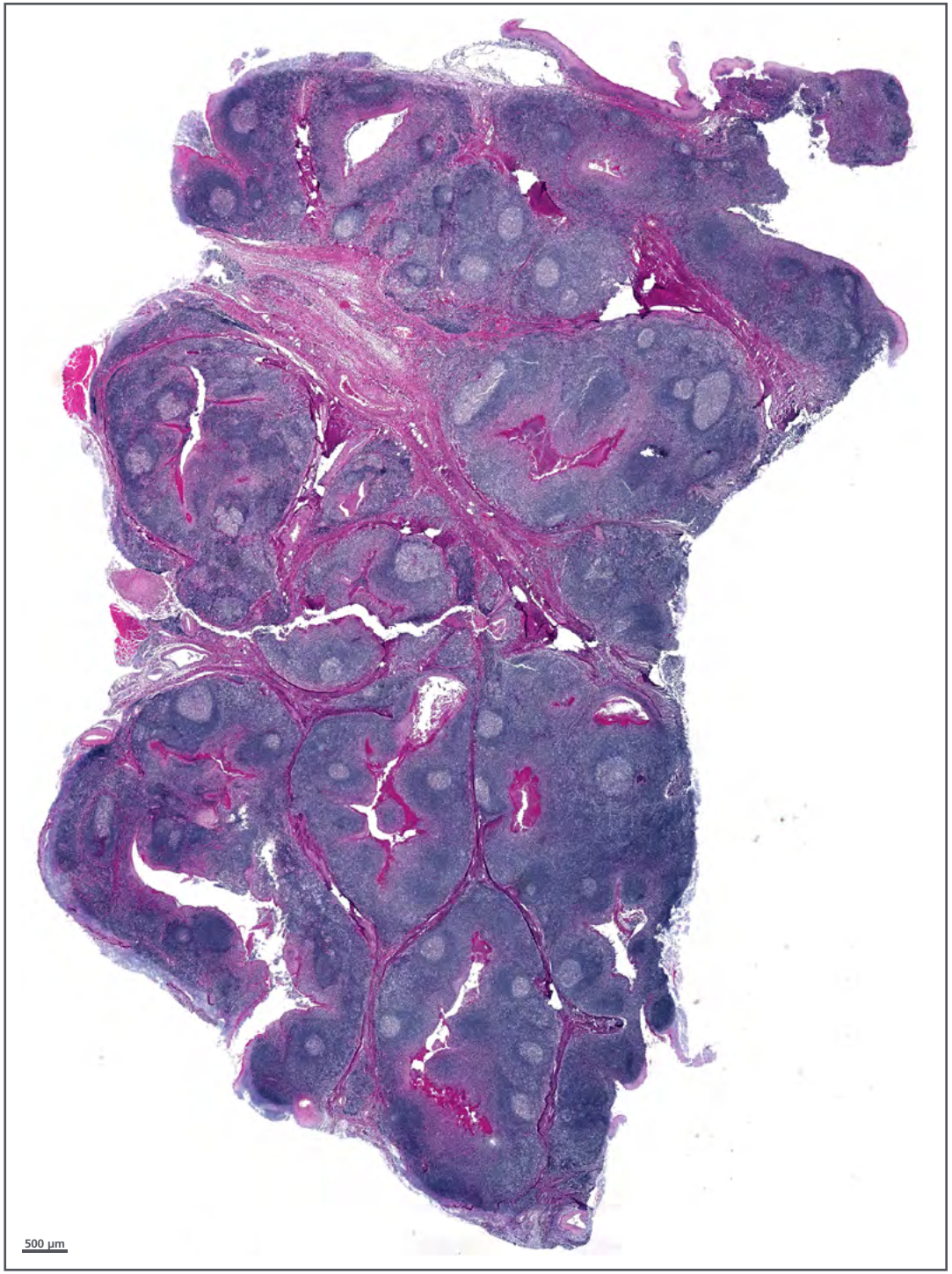


RARECYTE®

Reactive tonsil H&E-stained section

This is a hematoxylin and eosin (H&E) stained whole-slide tissue section of a tonsil with reactive lymphoid hyperplasia. Lymphoid hyperplasia is an increase in the number of lymphocytes that are contained in lymph nodes which often happens as part of the body's reaction to a chronic infection.

The following slides display this same tissue section stained using a 15-plex immunofluorescence biomarker panel and imaged using the Orion system.



RAREcyte®

Cell proliferation in the follicle

The follicle is the micro-anatomic site of clonal expansion of B cells in response to foreign antigens.

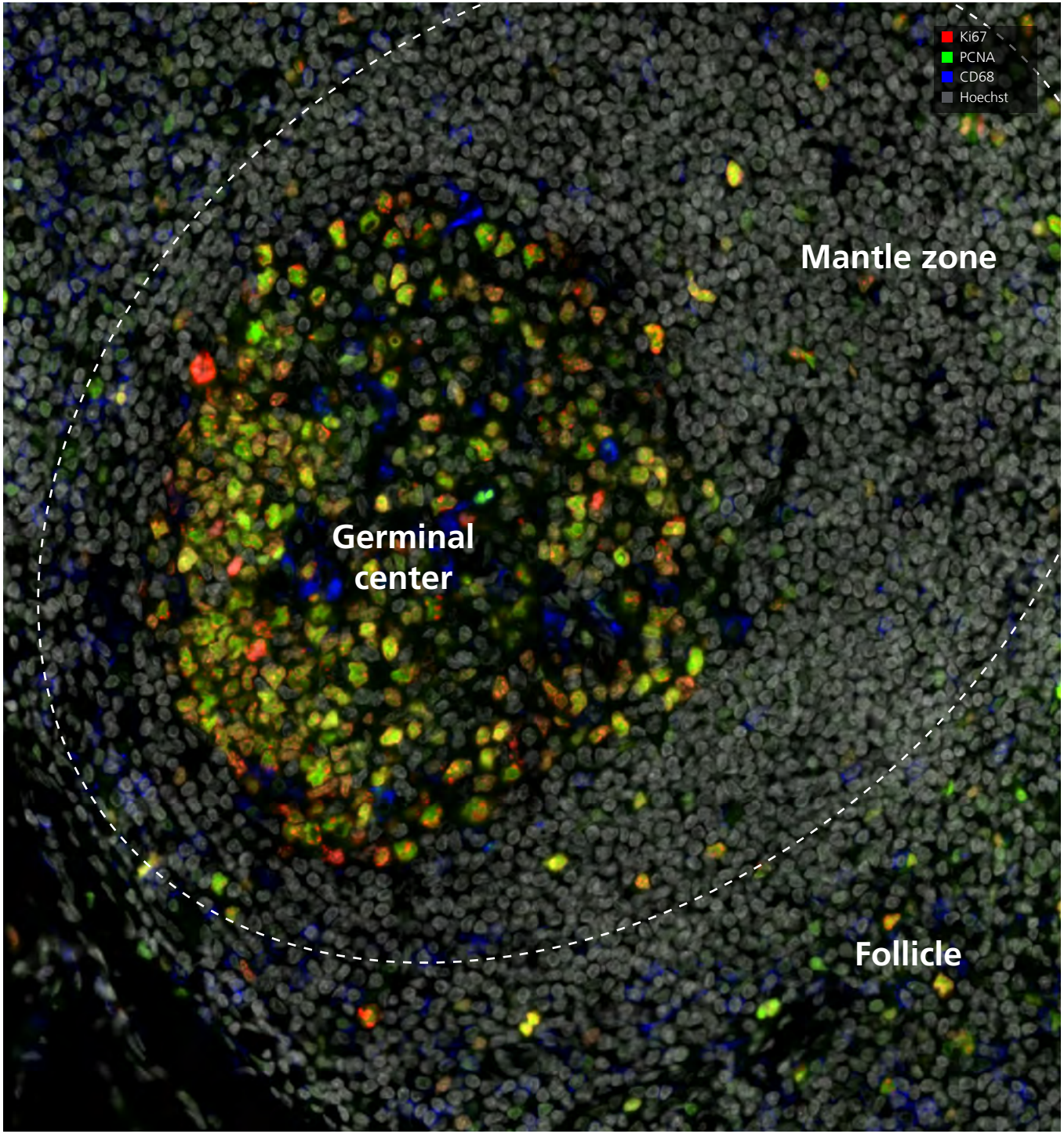
In reactive lymphoid tissues, these follicles are enlarged and have distinct compartments, including a highly proliferative germinal center and a surrounding mantle zone.

Nuclear staining (**Hoechst**) demonstrates the mantle zone (marked by the dotted line circle) is comprised of small, densely-packed cells surrounding the germinal center.

Germinal center cells are clearly visualized using cellular proliferation markers **Ki67** and **PCNA**. Indeed, in this view, many cells appear orange-yellow indicating **co-expression of both markers** within the cell nucleus.

A benefit of Orion collecting all markers in a single scan is that localization of individual markers may be robustly interrogated at subcellular resolution as singletons or in combination.

Macrophages (**CD68**) act to eliminate apoptotic B cells and, in this image, are found interspersed within the germinal center.

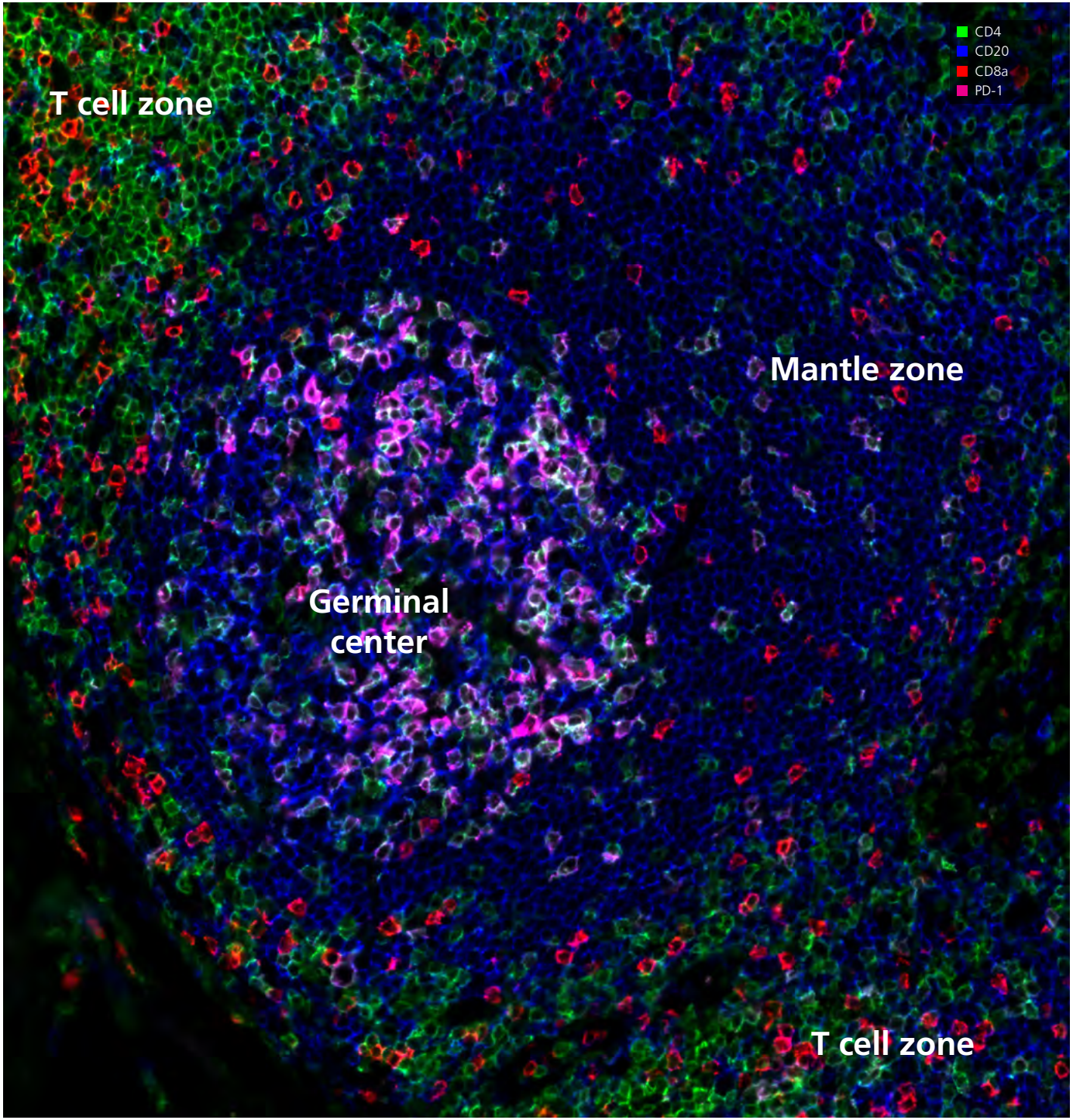


RARECYTE®

T cells in the follicle

Helper T cells (**CD4**) participate in the activation of B cell (**CD20**) expansion. Numerous helper T cells can be seen in the germinal center, with some seen in the mantle zone. Within the mantle zone scattered cytotoxic T cells (**CD8**) are also present.

Spatial multiplexing demonstrates that the immune checkpoint receptor **PD-1** is distinctly expressed by **CD4** helper T cells within the germinal center in this field, but not in helper T cells in the T cell zone, and not in **CD8** cytotoxic T cells.



RARECYTE®

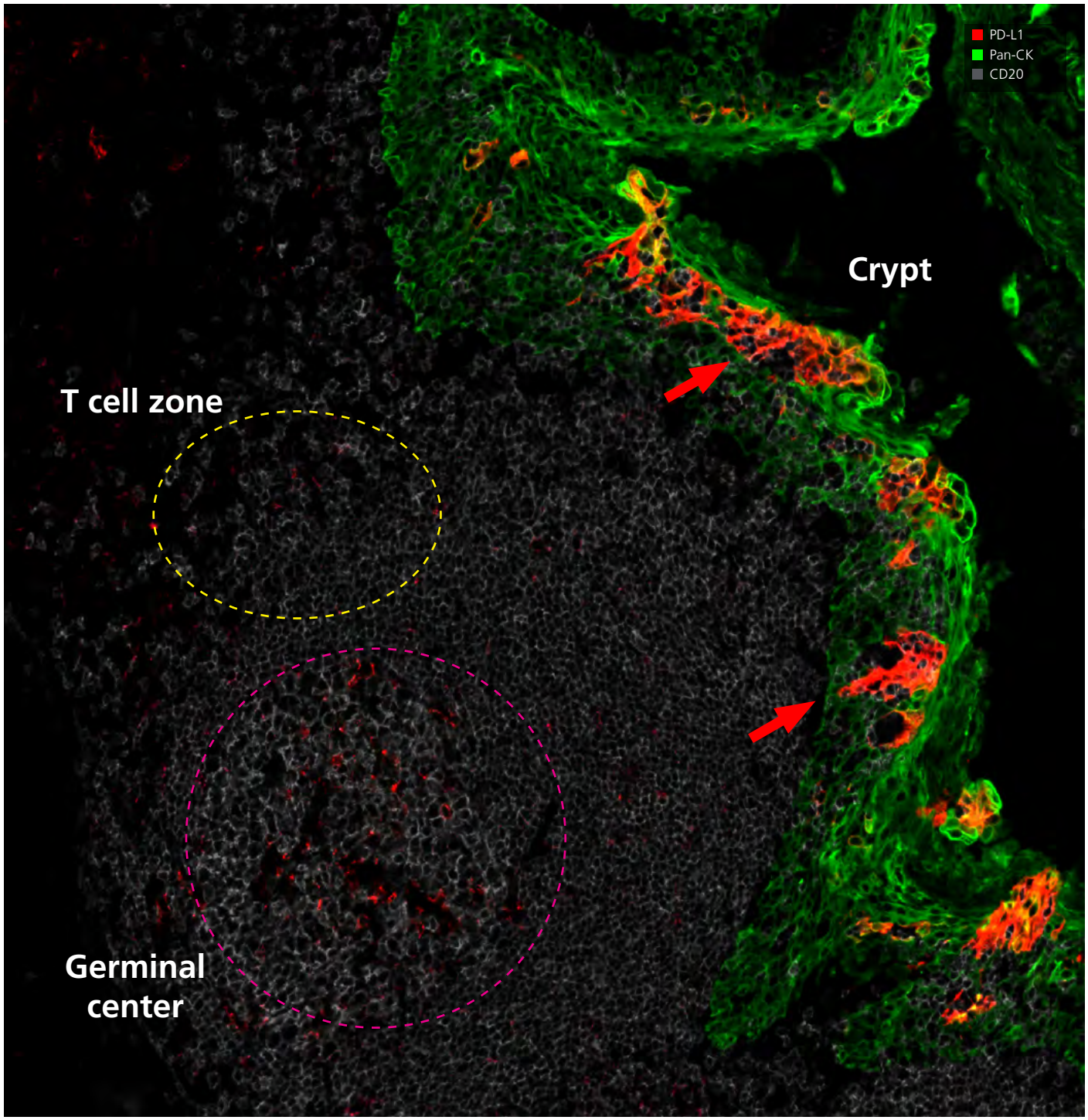
PD-L1 expression

PD-L1 and PD-1 are cognate immune checkpoint receptors involved in tumor evasion of the immune system, and their expression on tumor cells and within the tumor microenvironment impacts response to checkpoint inhibitor therapy.

PD-L1 is expressed at high levels (red arrows) in the tonsillar crypt epithelium (**Pan-CK**).

PD-L1 is expressed at moderate levels (magenta circle) in follicle germinal centers (**CD20**) to the left of the crypt.

PD-L1 is expressed at low levels (yellow circle) in stromal cells of the T cell zone, shown above the germinal center.



RARECYTE®

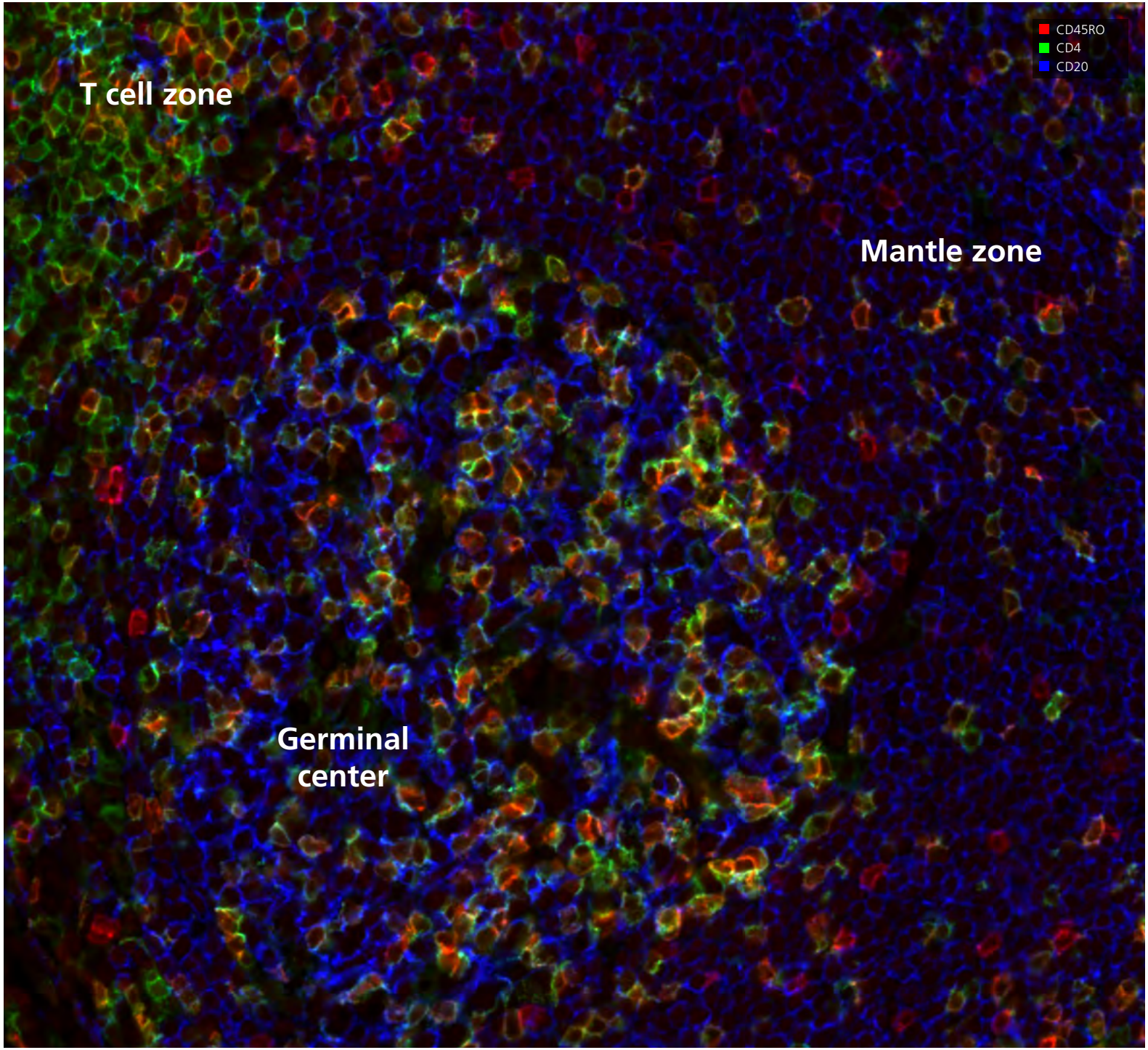
Memory cells in the follicle

Memory lymphocytes can be identified by the marker **CD45RO**.

In the follicle most of the memory cells co-express **CD45RO** and **CD4**, indicating that they are helper T cells.

Numerous memory helper T cells can be seen in the T cell zone, as well as **CD4** cells that are **CD45RO** negative.

Most B cells (**CD20**) are negative for **CD45RO**. This is most clearly seen in the mantle zone.



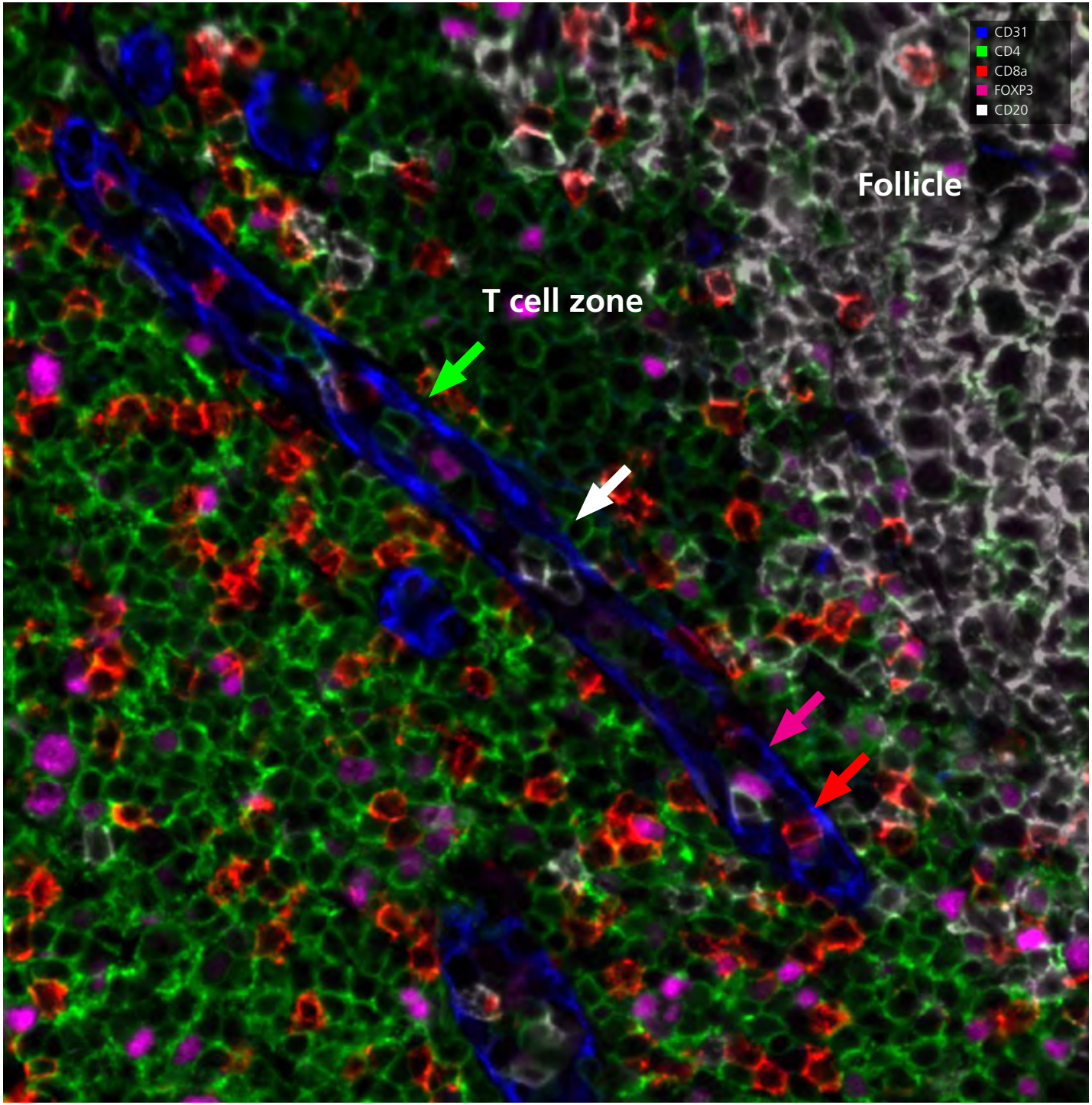
RARECYTE®

Lymphocyte trafficking in the high endothelial venule (HEV)

Recirculation of lymphocytes into lymphoid tissues occurs via HEVs: vessels specialized for the attachment and transmigration of circulating cells. HEVs are present in the T cell zone and contain characteristic endothelial cells which have ample cytoplasm as indicated by the marker **CD31**.

Arrows show the following cells within this longitudinal section of the HEV lumen:

- **CD4** T cells (including **FoxP3** T regulatory cells)
- **CD8** T cells
- **CD20** (in white) B cells



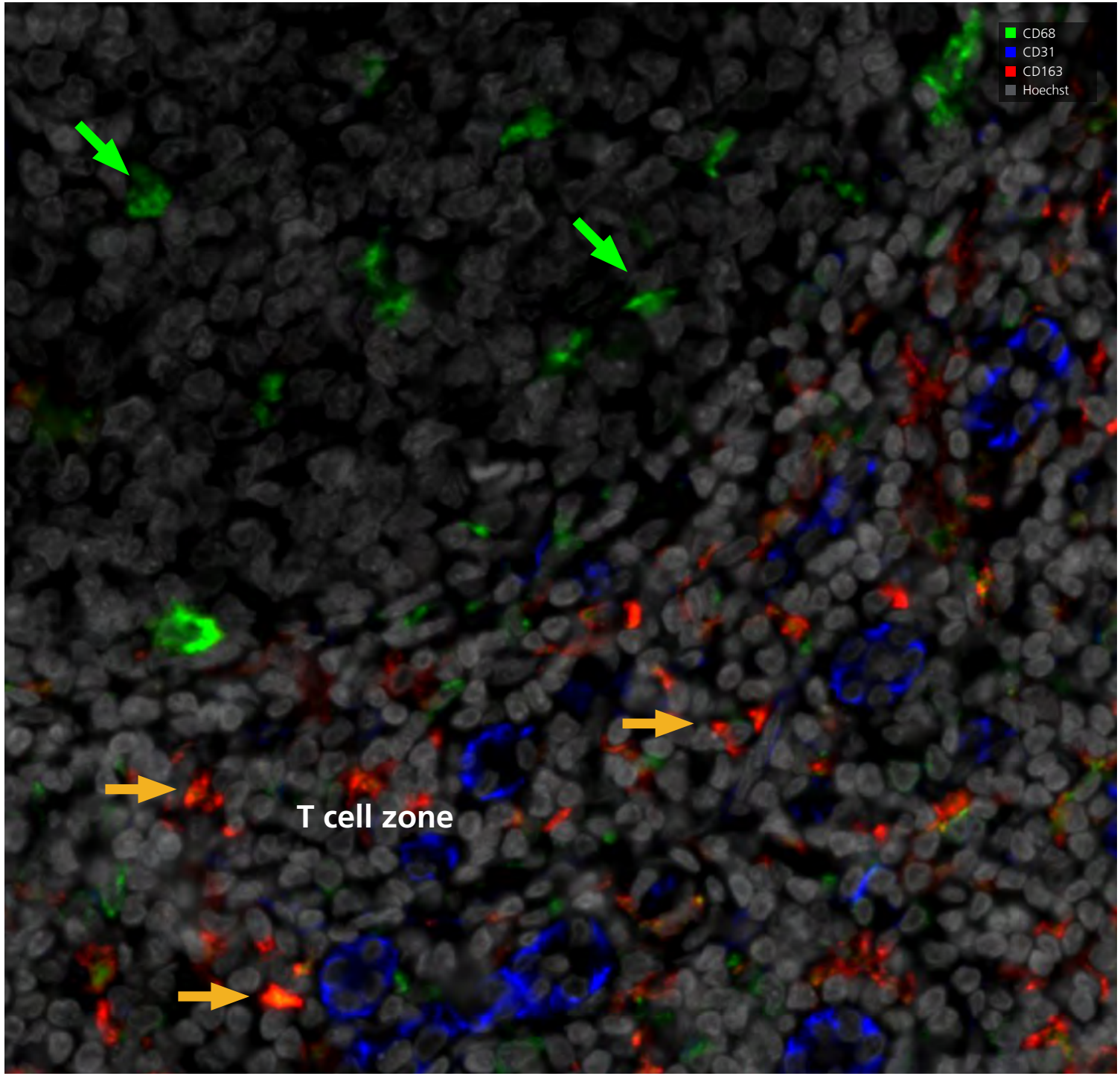
RARECYTE®

Macrophage subsets

Macrophages express different markers depending on their function, which may be mapped spatially to location within a tissue.

In the follicle, germinal center macrophages express **CD68** alone. Just outside the follicle in the T cell zone, HEV (**CD31**) **macrophages co-express** both **CD163** and **CD68** (orange arrows).

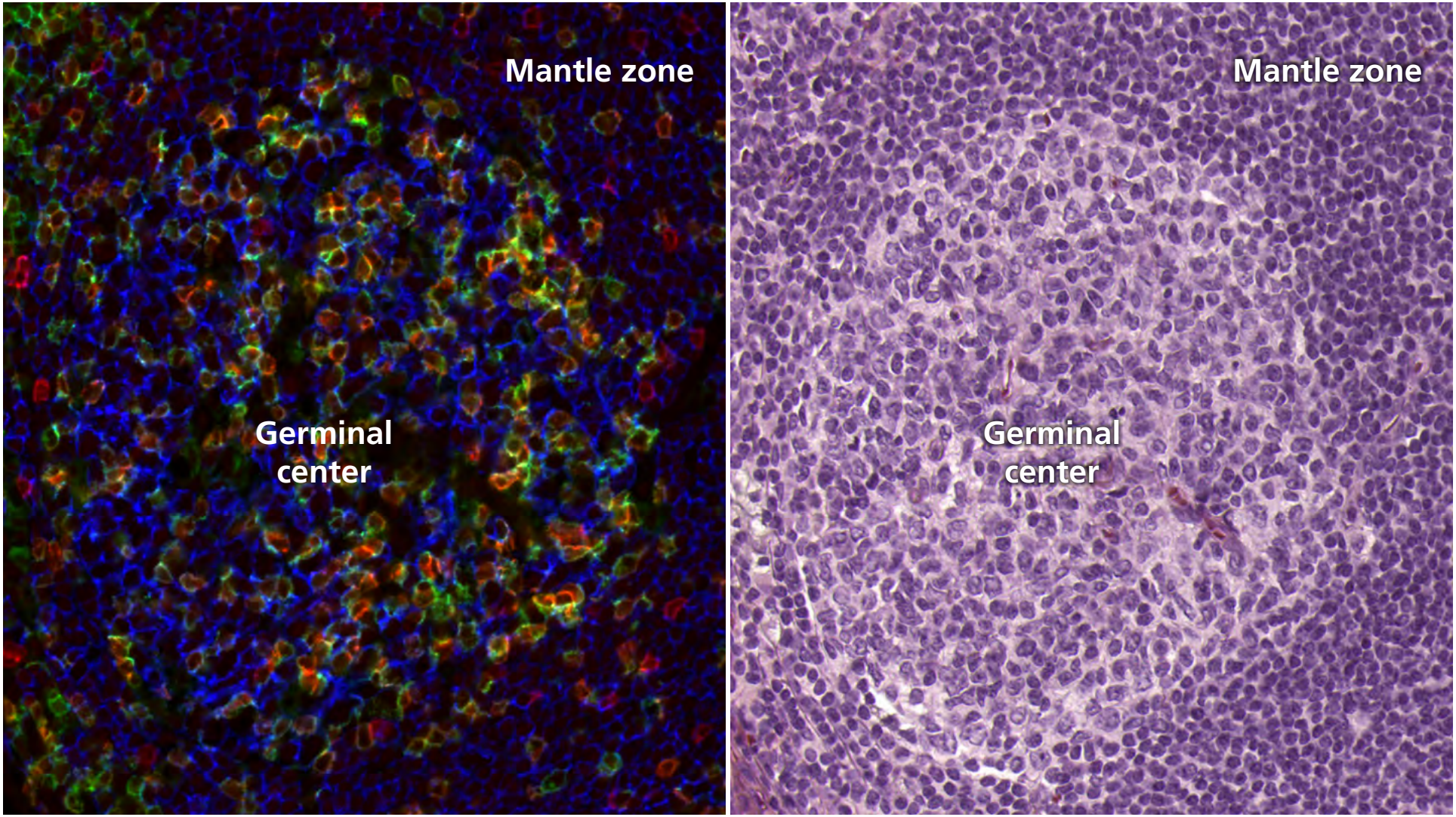
Note: Macrophages have processes that extend beyond the cell body. When these are cut in cross-section and stained the resulting image may appear as a point or wispy fragment.



RARECYTE®

H&E and IF on same section

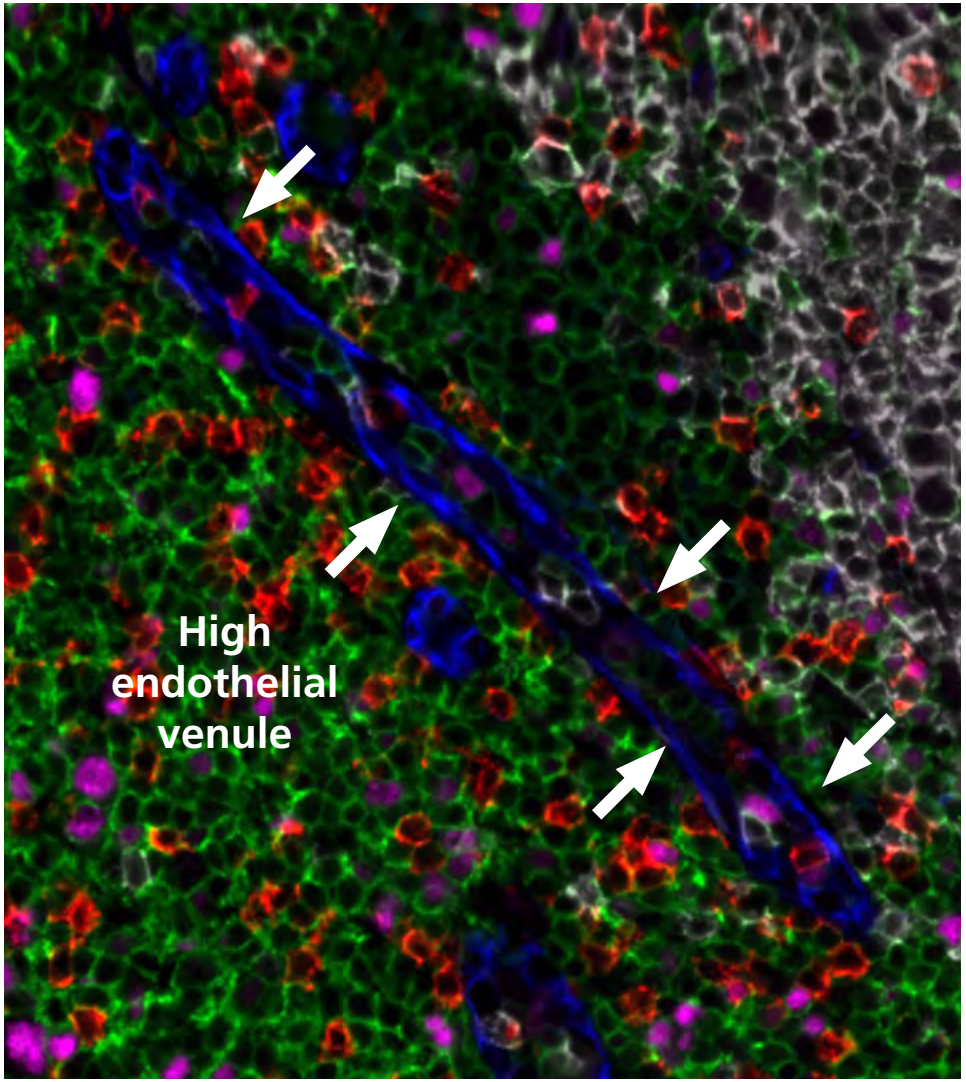
Morphology of cells and spatial location with biomarker phenotype may be correlated by staining the same tissue section with multiplexed IF and H&E.

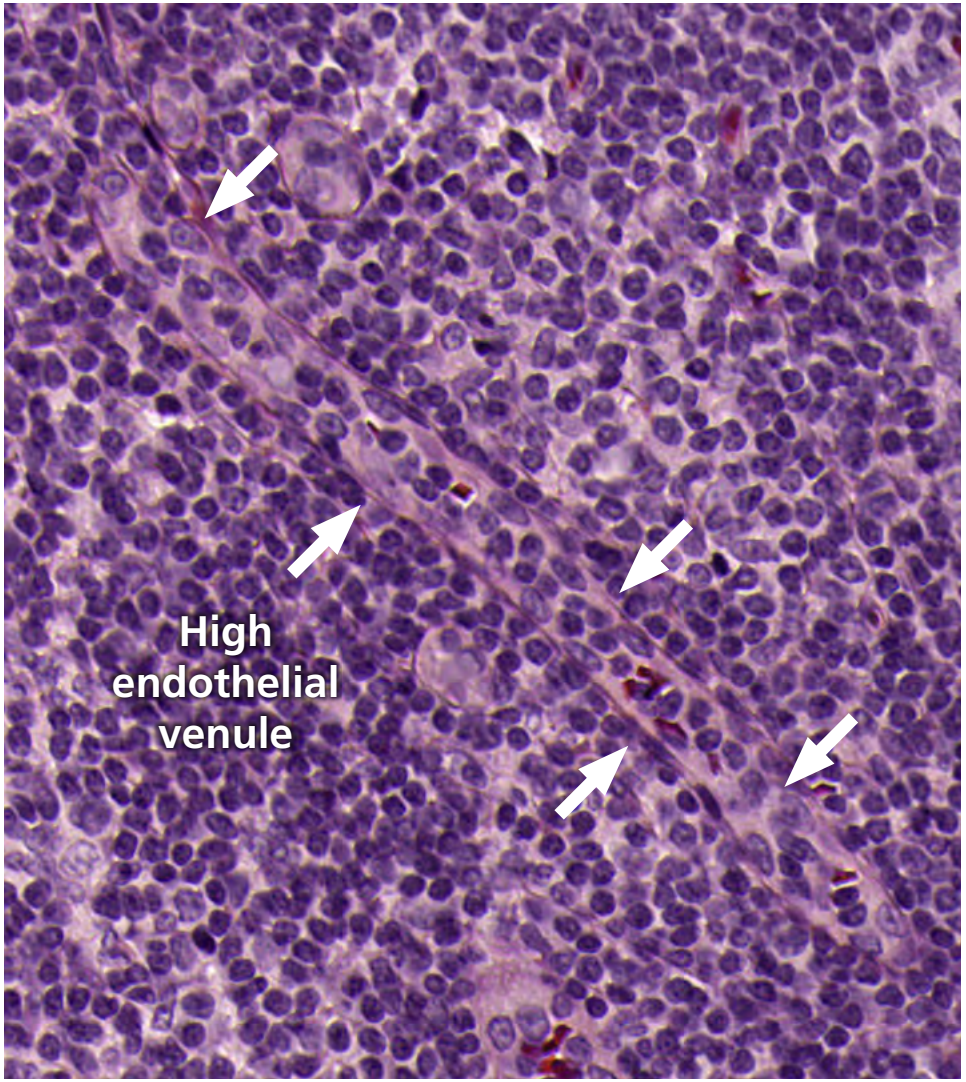


RARECYTE®

H&E and IF on same section

Morphology of cells and spatial location with biomarker phenotype may be correlated by staining the same tissue section with multiplexed IF and H&E.





Marker
Nucleus (Hoechst)
Autofluorescence
CD31
Ki67
CD68
CD163
CD20
CD4
CD8a
CD45RO
PD-L1
E-Cadherin
PD-1
FOXP3
Pan-CK
PCNA

Orion Tonsil Data Set – Sample Information

FFPE tonsil section was stained with 15-plex immunofluorescence (IF) panel in one staining round followed by whole slide imaging with the Orion instrument in one imaging round

- Tissue autofluorescence was imaged and isolated as an additional fluorescence channel
- H&E staining was performed after IF imaging on the same section and imaged by brightfield microscopy

Summary of Tissue Staining and Scanning Protocol

- Mount sections on glass slides
- De-paraffinize and perform antigen retrieval
- Quench autofluorescence
- Stain slides with panel of ArgoFluor™ conjugated antibodies
- Coverslip with ArgoFluor Mounting Medium and cure overnight
- Image whole slides at 20X magnification using Orion instrument
- Process to ome.TIFF and analyze
- De-coverslip in aqueous solution
- Perform H&E staining and scanning on same section

RARECYTE®
Precision Biology for Life Sciences

2601 Fourth Ave
Seattle, WA 98121

855.727.3298
206.455.9092

www.rarecyte.com

MC-1-01

View interactive data sets