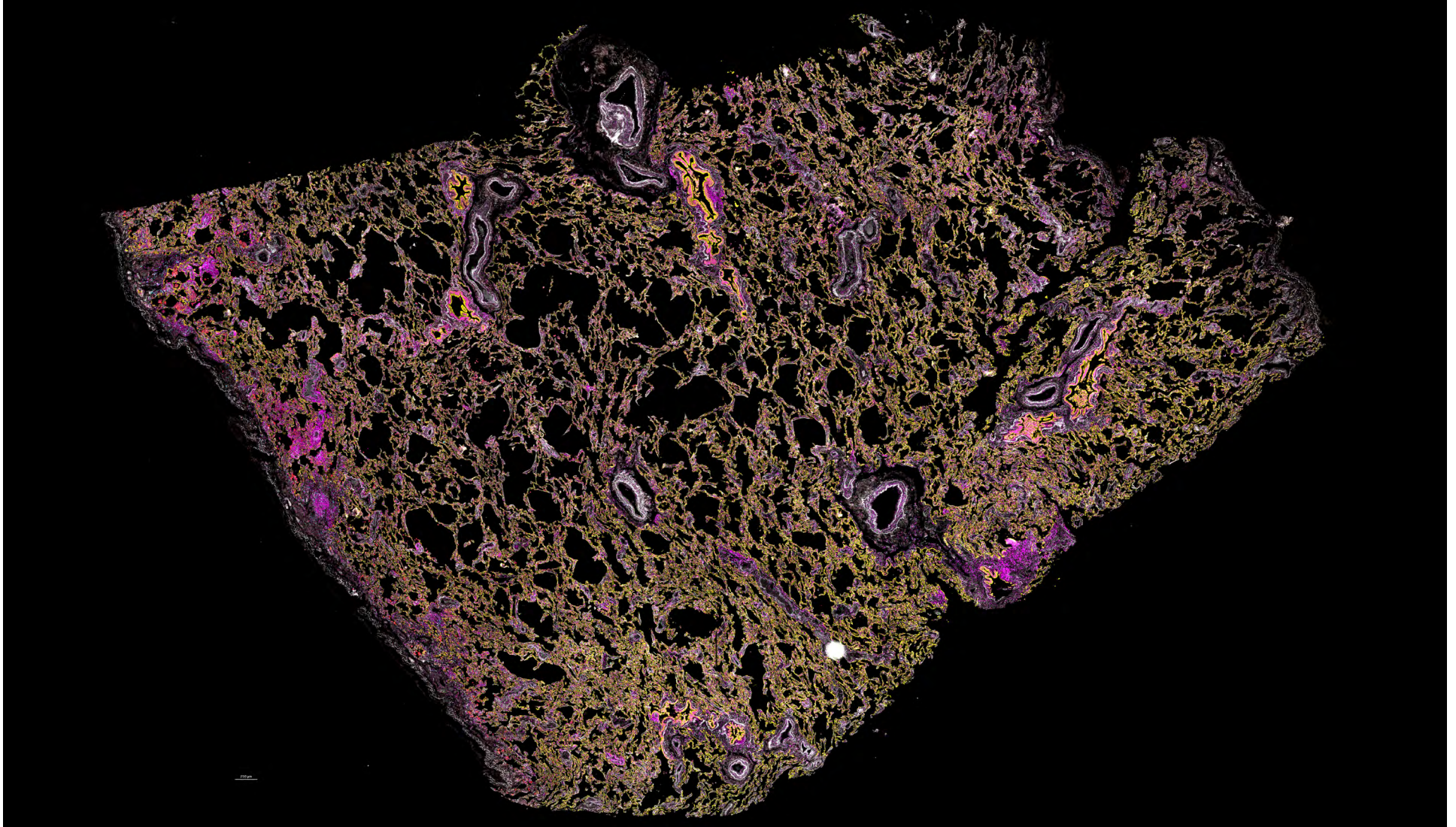


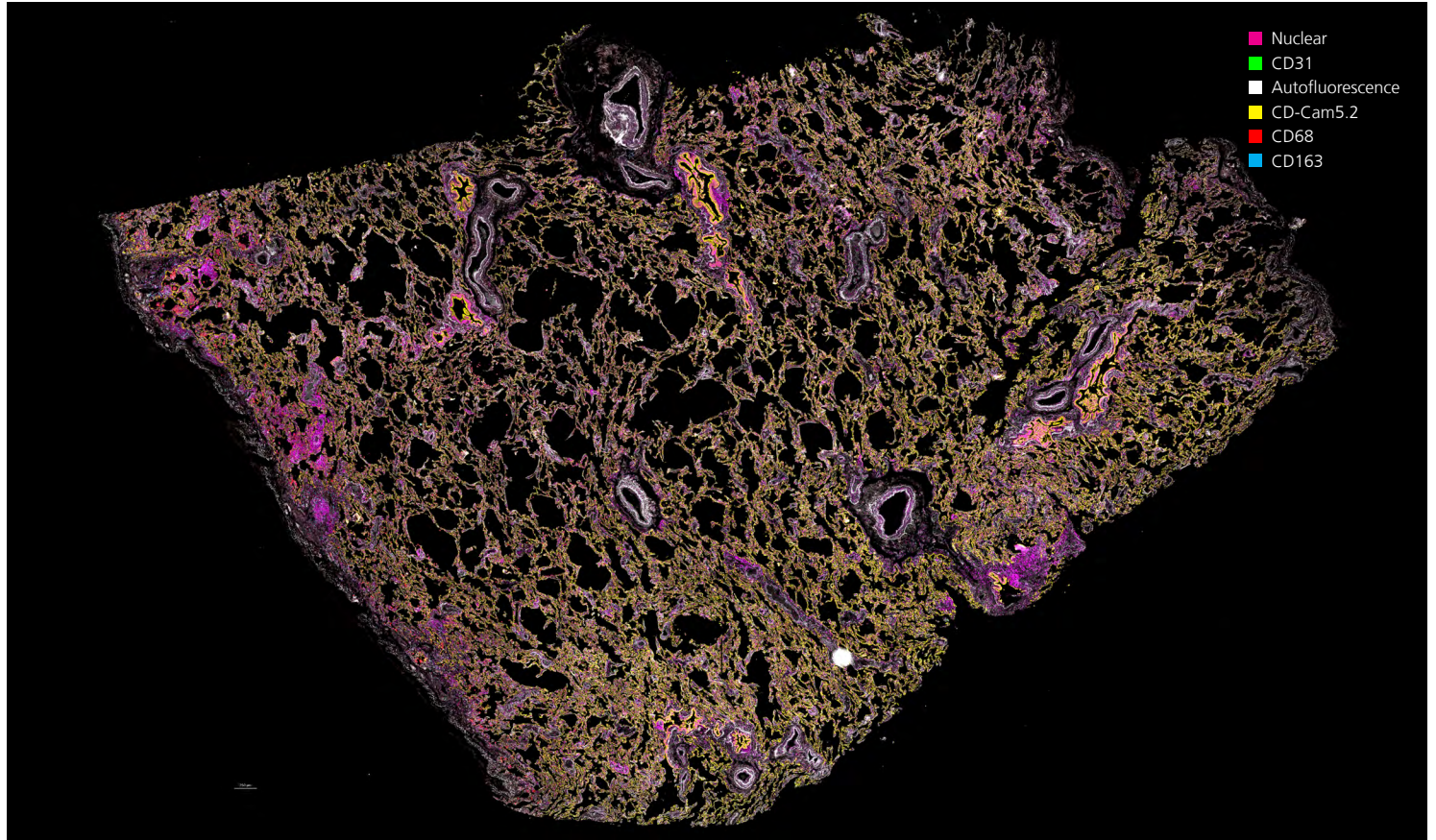
# RARECYTE<sup>®</sup>

## Orion<sup>™</sup> Tissue Investigation | Normal Lung Tissue



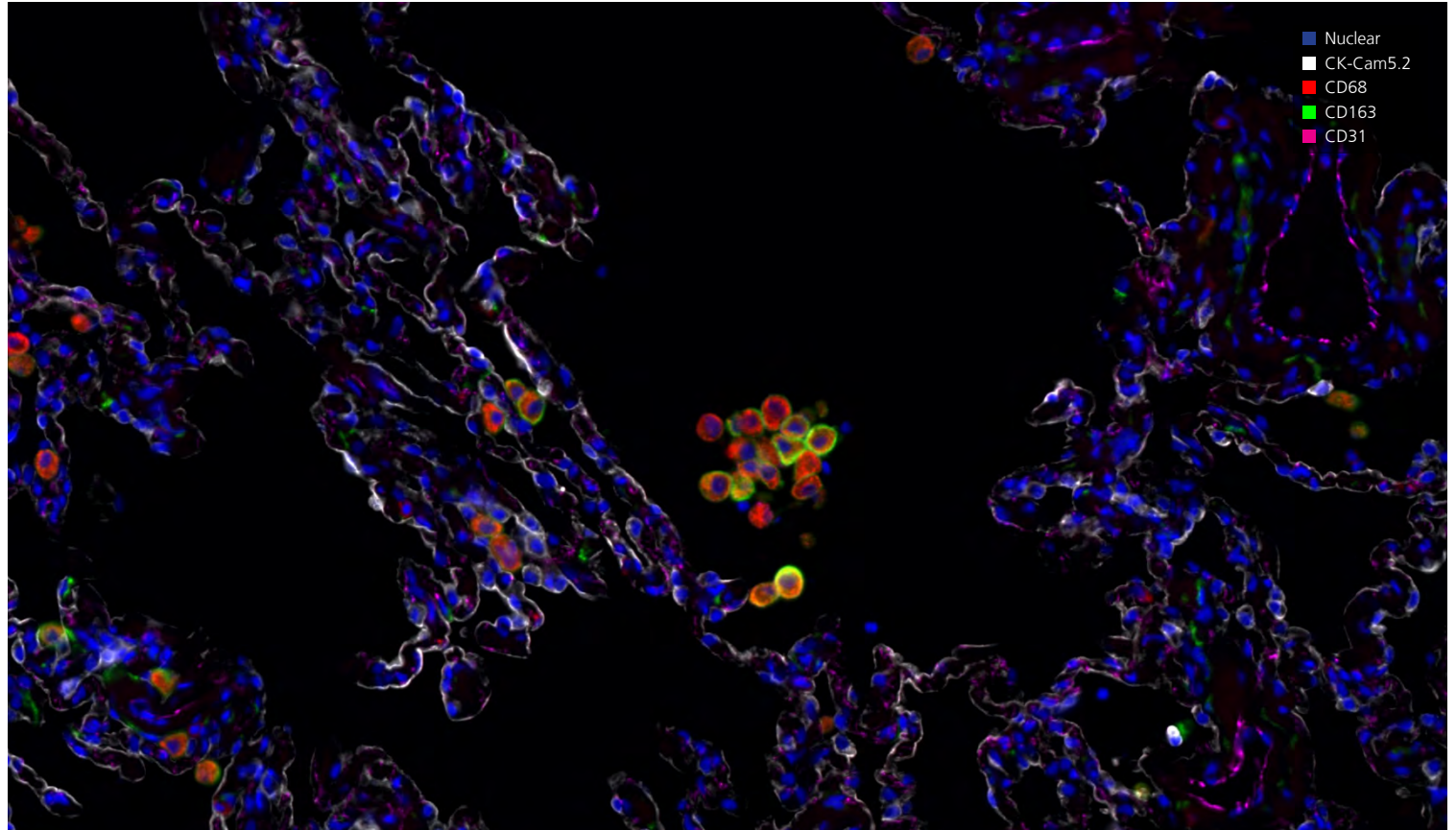
## 17-plex Orion™ imaging of lung section

This is a 17-marker whole-slide scan of a single section of lung from a region adjacent to a non-small cell lung carcinoma. It has intact normal lung alveolar architecture, with scattered immune cell infiltrates. The slide was stained with Orion reagents and imaged using the Orion Instrument. All markers were stained in a single staining procedure and imaged in a single scan.



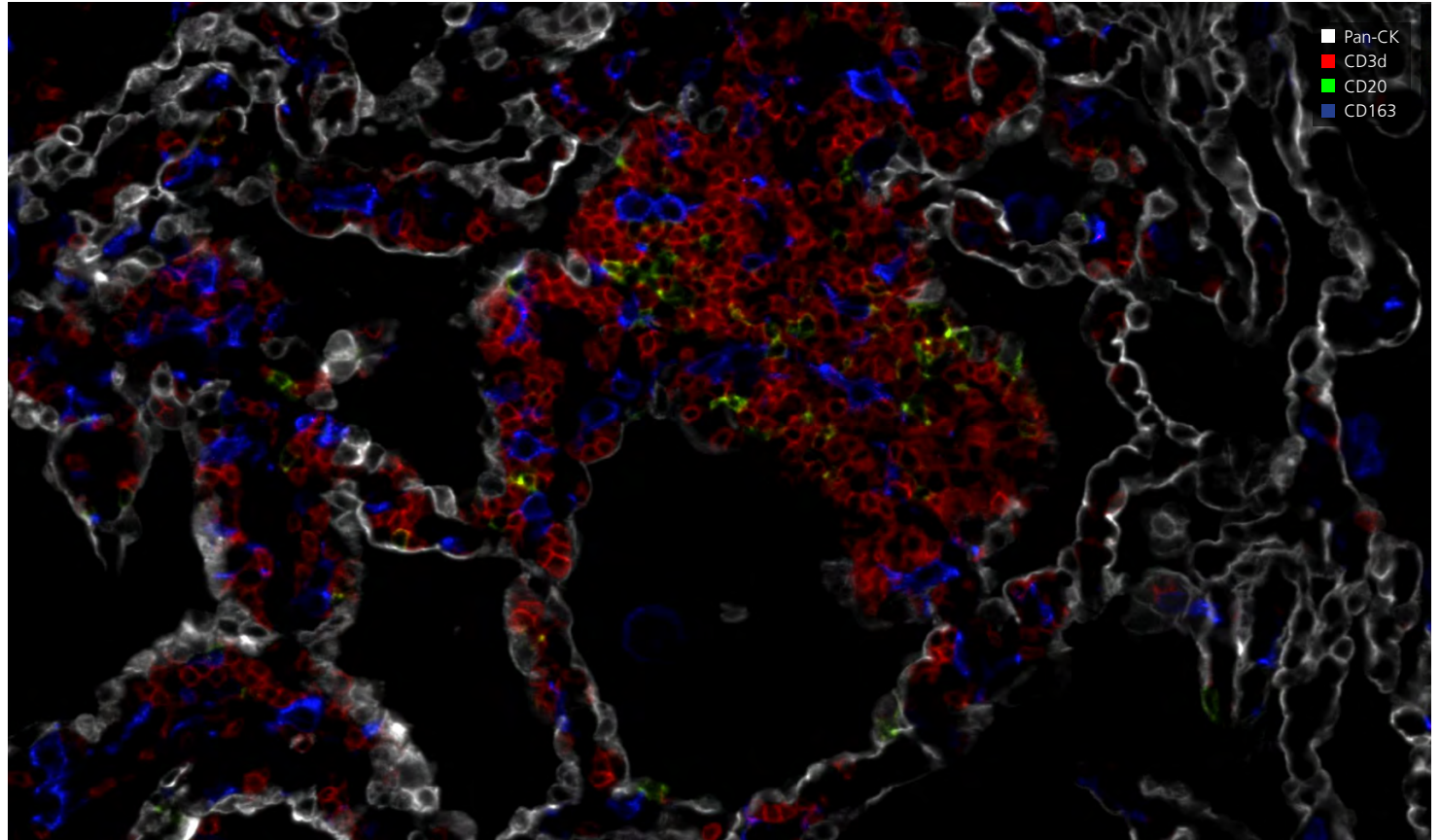
## The alveolus

The functional unit of the lung is the alveolus, an air-filled sac lined by epithelial cells that express cytokeratin (**CK-CAM5.2**, white, cytokeratin 7/8). Within the alveolar septum are fine **CD31** positive capillaries where blood is oxygenated. The alveolar space frequently has collections of macrophages; **CD68** stains the cytoplasm of the alveolar macrophages, and **CD163** marks the cell membrane.



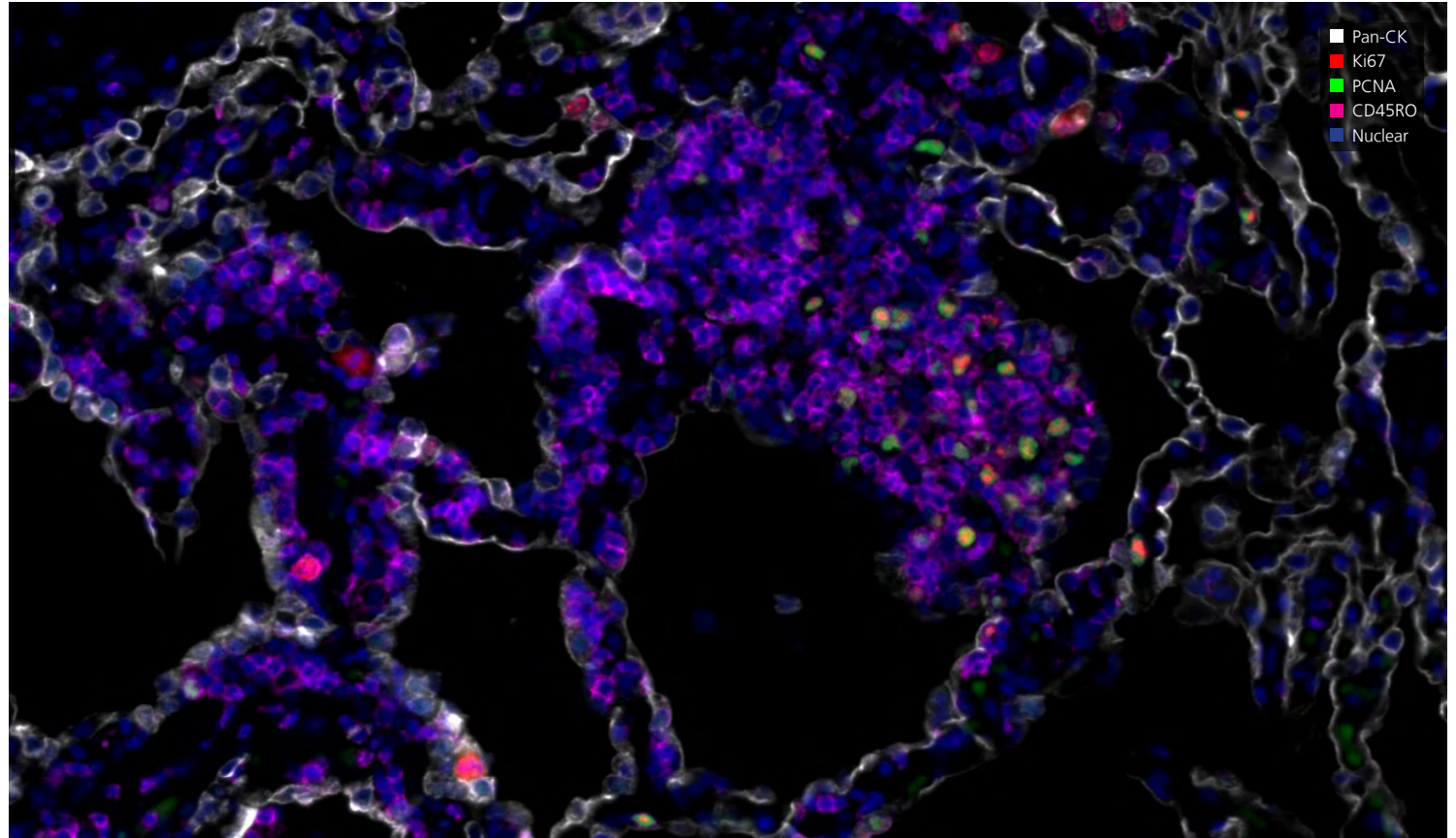
## Immune infiltrate

A collection of immune cells is present in the alveolar interstitium, which expresses cytokeratin (**Pan-CK**, white). It contains predominantly T cells (**CD3d**), with interspersed B cells (**CD20**) and macrophages (**CD163**). T cells infiltrate adjacent alveolar septae, widening them but they remain intact.



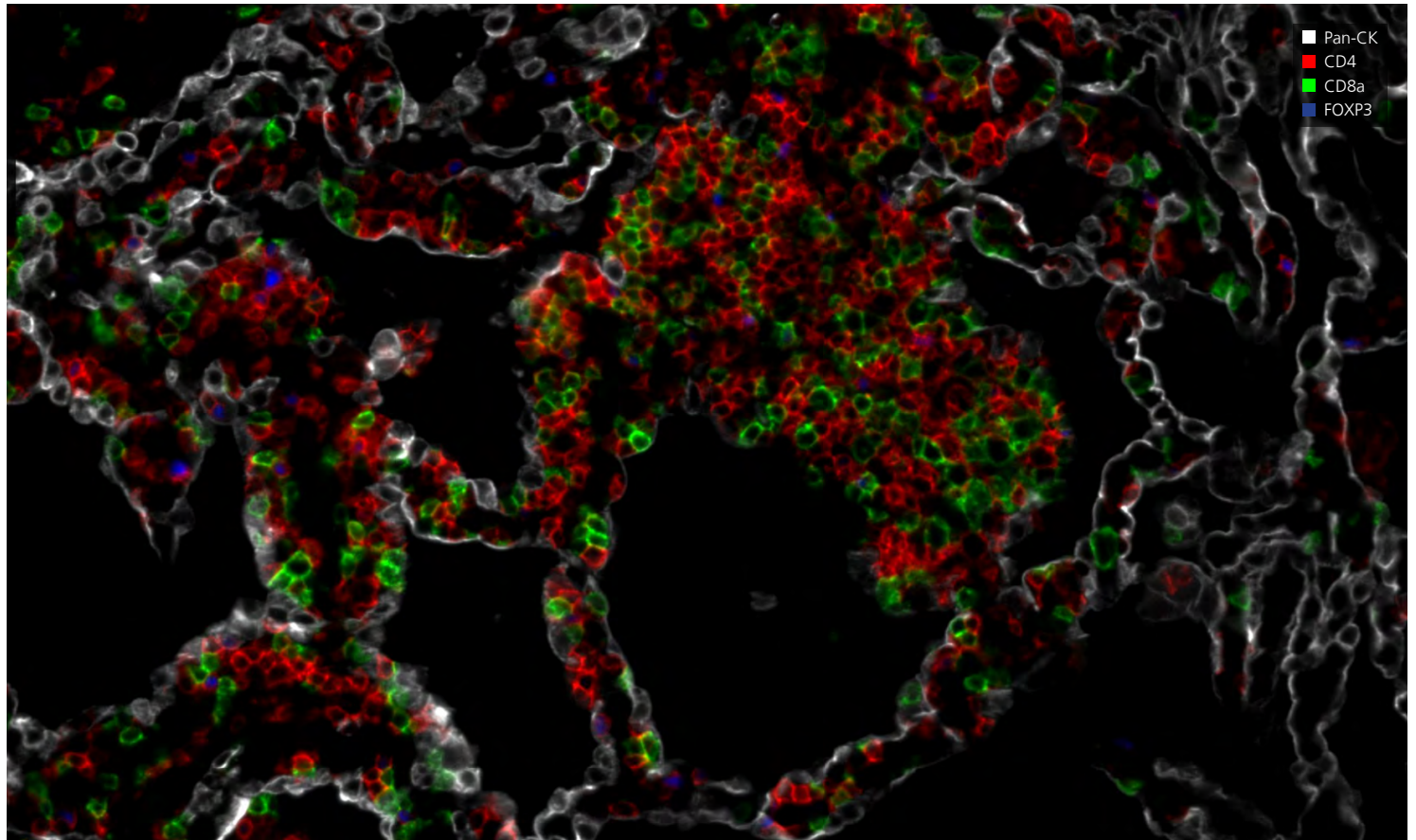
## Immune cell activation

The immune infiltrate has proliferating immune cells that express the cell cycle markers **PCNA** and/or **Ki67**. The activation/memory marker **CD45RO** is present in many of the cells.



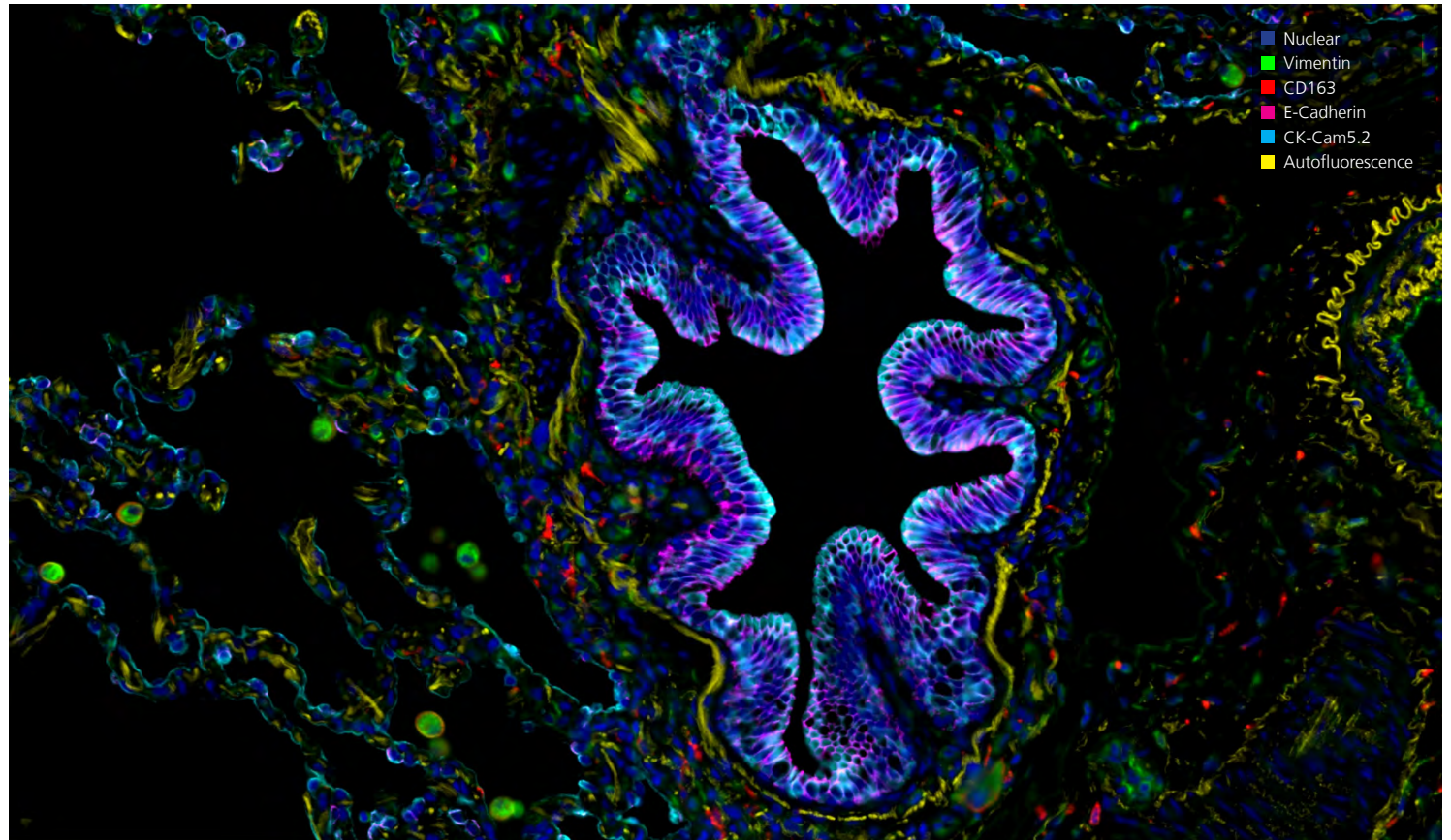
## T cell subsets

The T cells in the infiltrate are a mixture of helper (**CD4**) and cytotoxic (**CD8a**) cells. Scattered cells express nuclear **FOXP3**, a marker of T regulatory cells; most FOXP3-positive cells are **CD4** T cells.



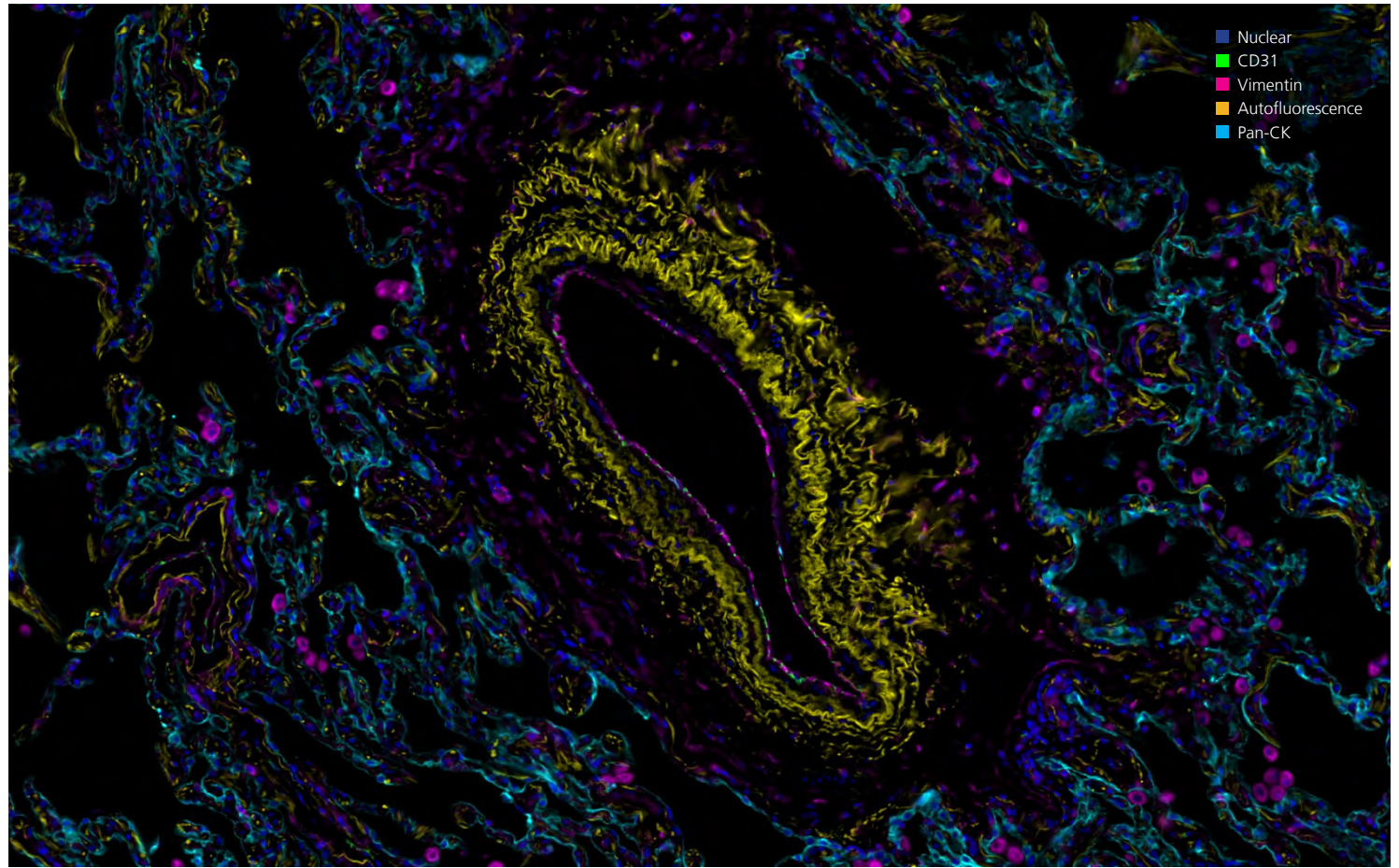
## The bronchiole

The bronchiole is a branch of the bronchus that leads to the alveolus. It is lined by a columnar epithelium. This epithelium is polarized, with basal and apical parts of the cell having different functions – mucous is secreted in the apical portion for instance. Polarization can be seen in the location of epithelial markers as well. Note that **E-Cadherin**, a cell adhesion molecule, is prominently expressed in the basal part of the epithelium. In contrast, cytokeratin 7/8 detected by **CK-CAM5.2** is more prominent in the apical part. Stromal cells surrounding the bronchus express the intermediate filament Vimentin as do the **CD163** positive macrophages. Orion technology allows isolation of autofluorescence signals; here the signal that is present from extracellular matrix has been visualized as if it were stained; this matrix can be seen surrounding the bronchiole.



## Blood vessel microanatomy

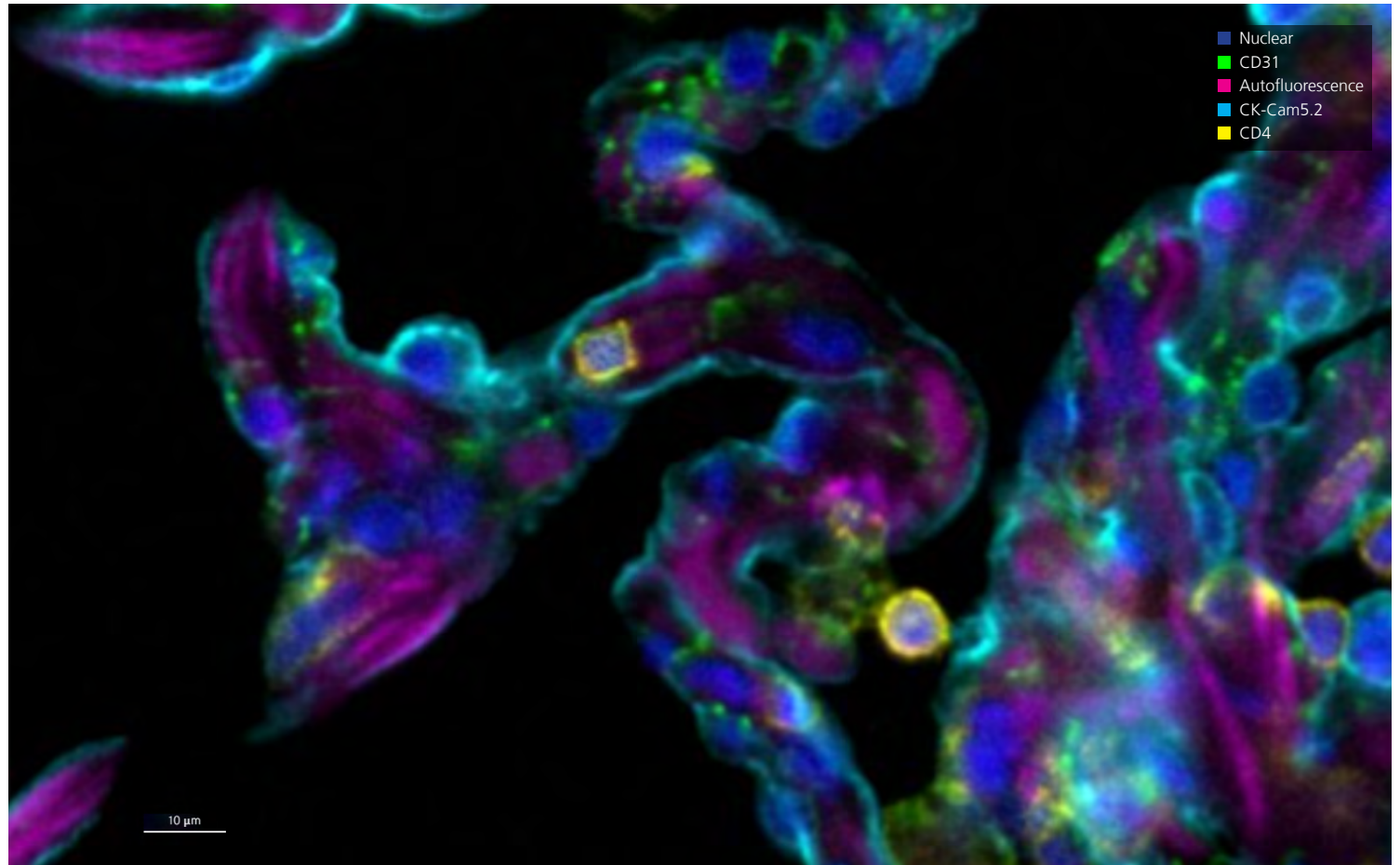
This cross section of an artery reveals expression of both **CD31** and **Vimentin** by the endothelial lining cells. The extracted **Autofluorescence** signal clearly defines the wavy elastic lamina of the artery wall. The alveolar macrophages also express Vimentin.





## T cell in alveolar wall capillary

Fine detail of a **CD4** T cell's spatial orientation can be seen as it travels through an alveolar septum. A T cell is also seen squeezing through the capillary, as well as the alveolar wall layers: epithelial (**CK-Cam5.2**), matrix (**Autofluorescence**), and endothelial (**CD31**).



Marker
PCNA
Nuclear (SYTOX Blue)
CD31
CAM5.2
CD68
CD4
FOXP3
CD8a
CD20
CD3d
CD163
E-Cadherin
CD45RO
Ki67
Pan-CK
Vimentin
Autofluorescence

## Orion Normal Lung Data Set – Sample Information

The FFPE lung section was stained with a 17-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

## 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



2601 Fourth Ave  
Seattle, WA 98121

855.727.3298  
206.455.9092

[www.rarecyte.com](http://www.rarecyte.com)

© 2023 RareCyte, Inc. All rights reserved.

RareCyte, the stylized logo, Orion, and ArgoFluor are trademarks or registered trademarks of RareCyte, Inc. in the United States and other countries. All other brand and product names are trademarks or registered trademarks of their respective owners.

For Research Use Only. Not for use in diagnostic procedures.

MC-4-01



**View interactive  
data sets**