Orion™: 17-plex single-step stain and imaging of normal lung section adjacent to non-small cell lung carcinoma

RareCyte, Inc.

RARECYTE®
Precision Biology for Life Sciences

Overview

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 (figure 1).

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 in 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 (figure 2).

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 (figure 4).

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 (figure 5).

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 (figure 6).

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 (figure 3).

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 (figure 7).

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, light blue), matrix (Autofluorescence, magenta), and endothelial (faintly staining CD31, green) (figure 8).

Summary of Orion Workflow

PREPARE
Standard microscope slide and tissue section

STAIN
Single staining process, standard IF protocols

IMAGE
Highly multiplexed imaging in one scan

ANALYZE
OME-TIFF output for viewing and quantification

← ORION BENCHTOP FOOTPRINT →

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

Lung Tissue 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

Fig 2. The alveolus is an air-filled sac lined by epithelial cells that express CK-CAM5.2 (white). Capillaries within the alveolar septum express CD31+ (magenta), and in the alveolar space, collections of macrophages are seen in the cytoplasm (CD68, red) and cell membrane (CD163, green).
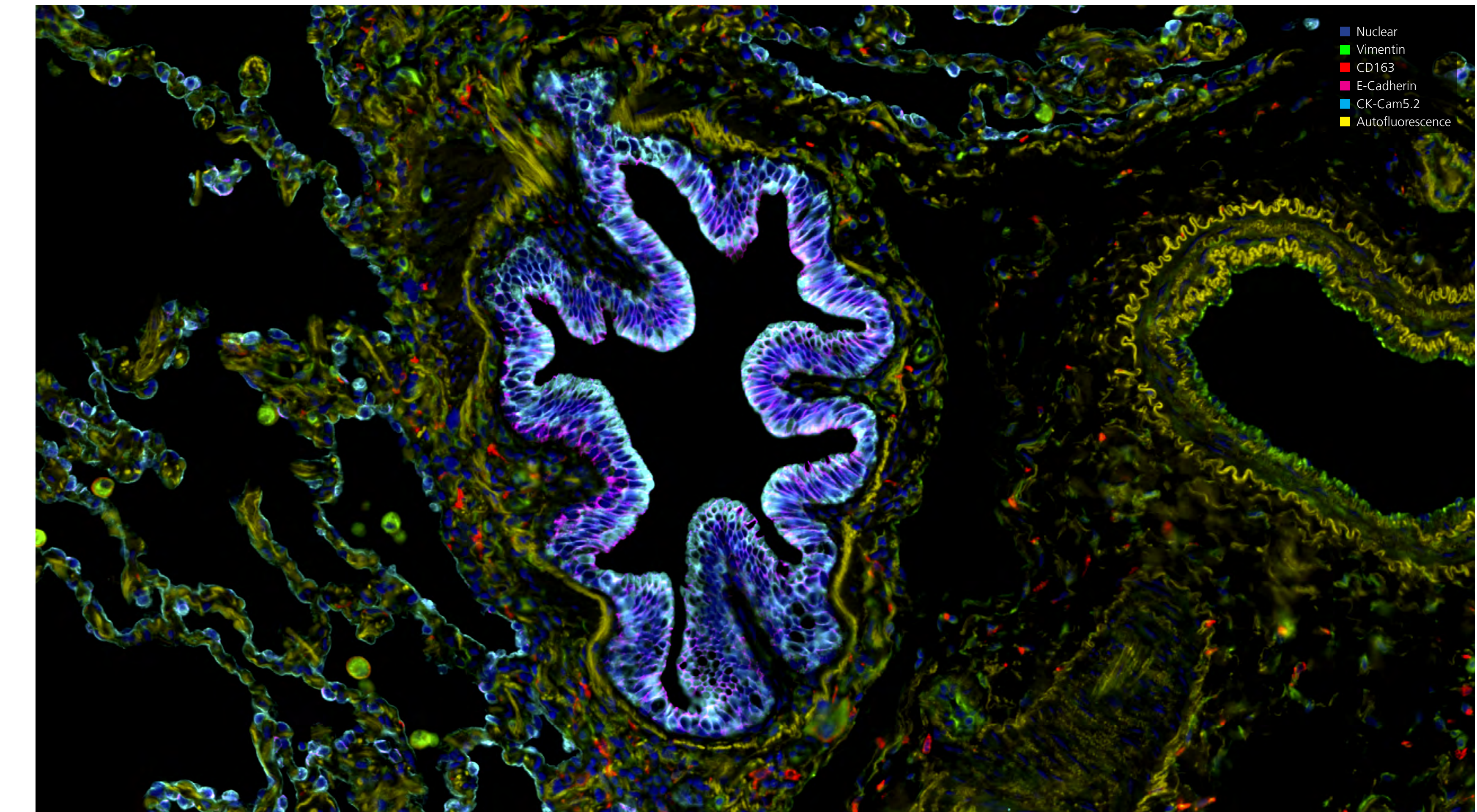
Fig 3. E-Cadherin (magenta), a cell adhesion molecule, is prominently expressed in the basal of the epithelium. In contrast, cytokeratin 7/8 detected by CK-CAM5.2 (blue) is more prominent in the apical part. Stromal cells surrounding the bronchus express the intermediate filament vimentin (green) as do the CD163 (red) positive macrophages.
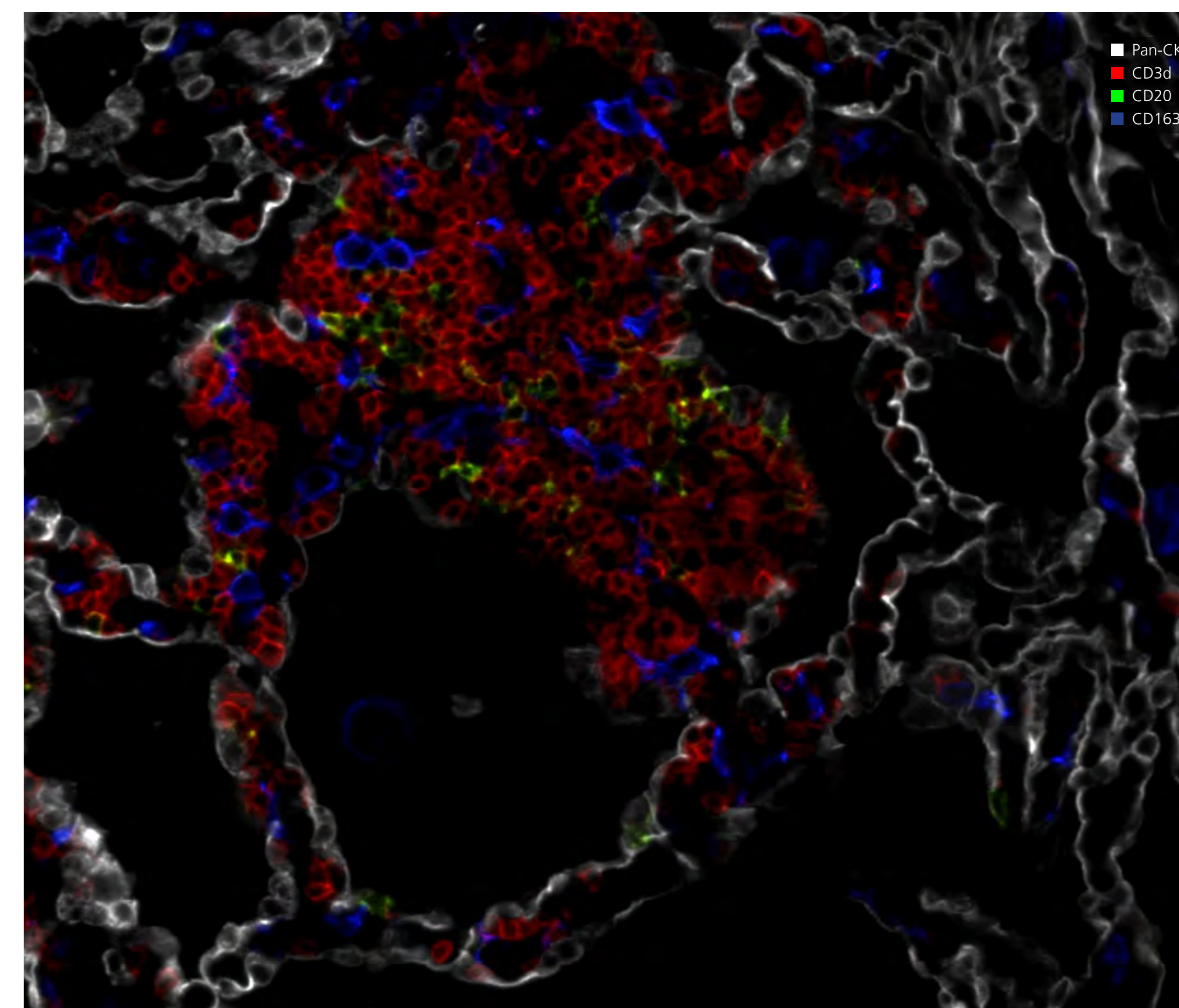
Fig 4. A collection of immune cells present in the alveolar interstitium expresses cytokeratin (Pan-CK, white), T cells (CD3d, red), interspersed B cells (CD20, green), and macrophages (CD163, blue).

Fig 5. Immune cell activation is shown by proliferating immune cells that express cell cycle markers PCNA (green) and/or Ki67 (red), and activation/memory marker CD45RO (magenta).

Fig 6. T cells shown in the infiltrate are a mixture of helper cells (CD4, red) and cytotoxic cells (CD8a, green). Scattered cells express nuclear FOXP3 (blue), a marker of T regulatory cells, while most FOXP3 positive cells are CD4 T cells.
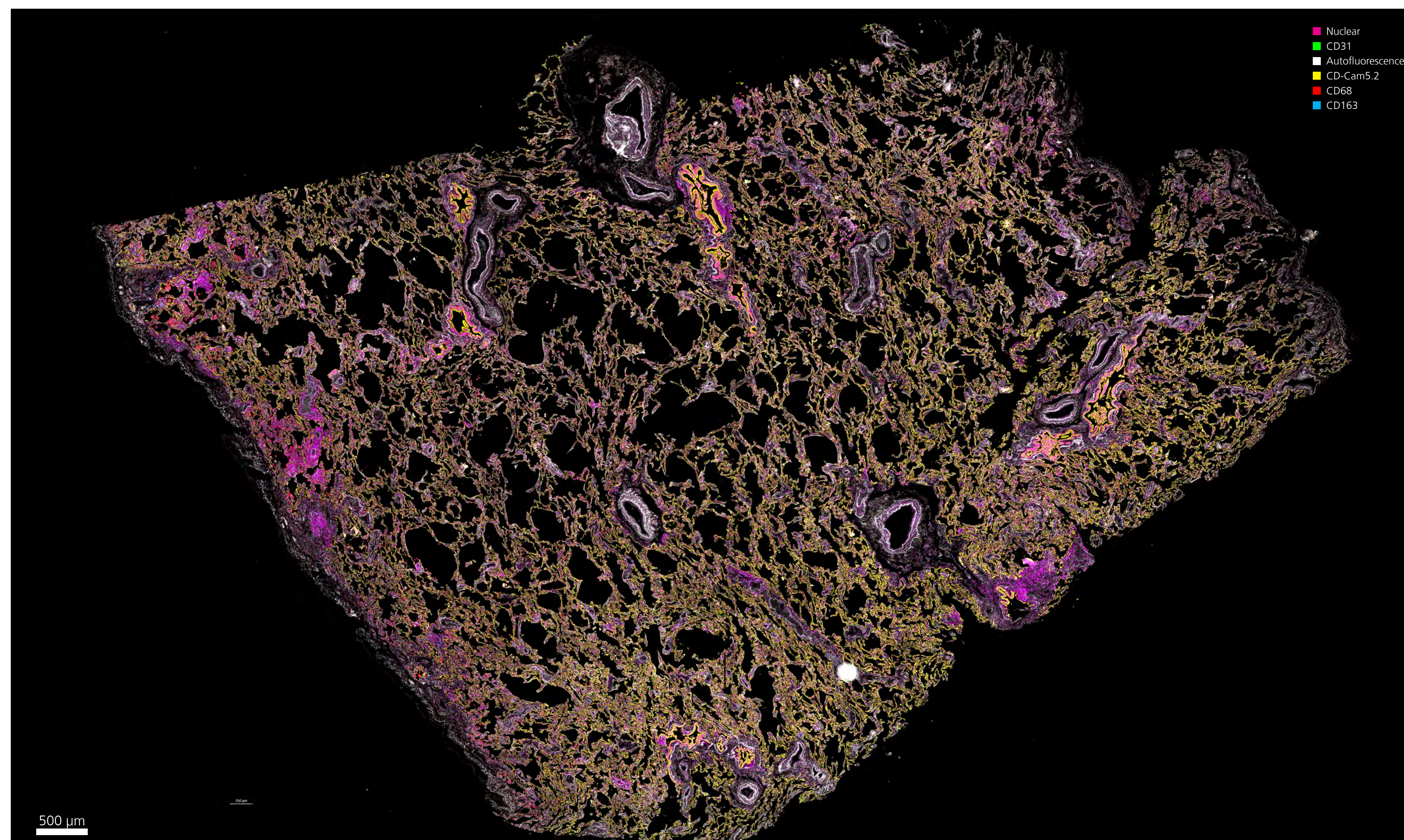
Fig 1. 17-marker whole slide scan of a single lung section from a region adjacent to a non-small cell lung carcinoma. All markers were stained in a single staining procedure and imaged in a single scan.
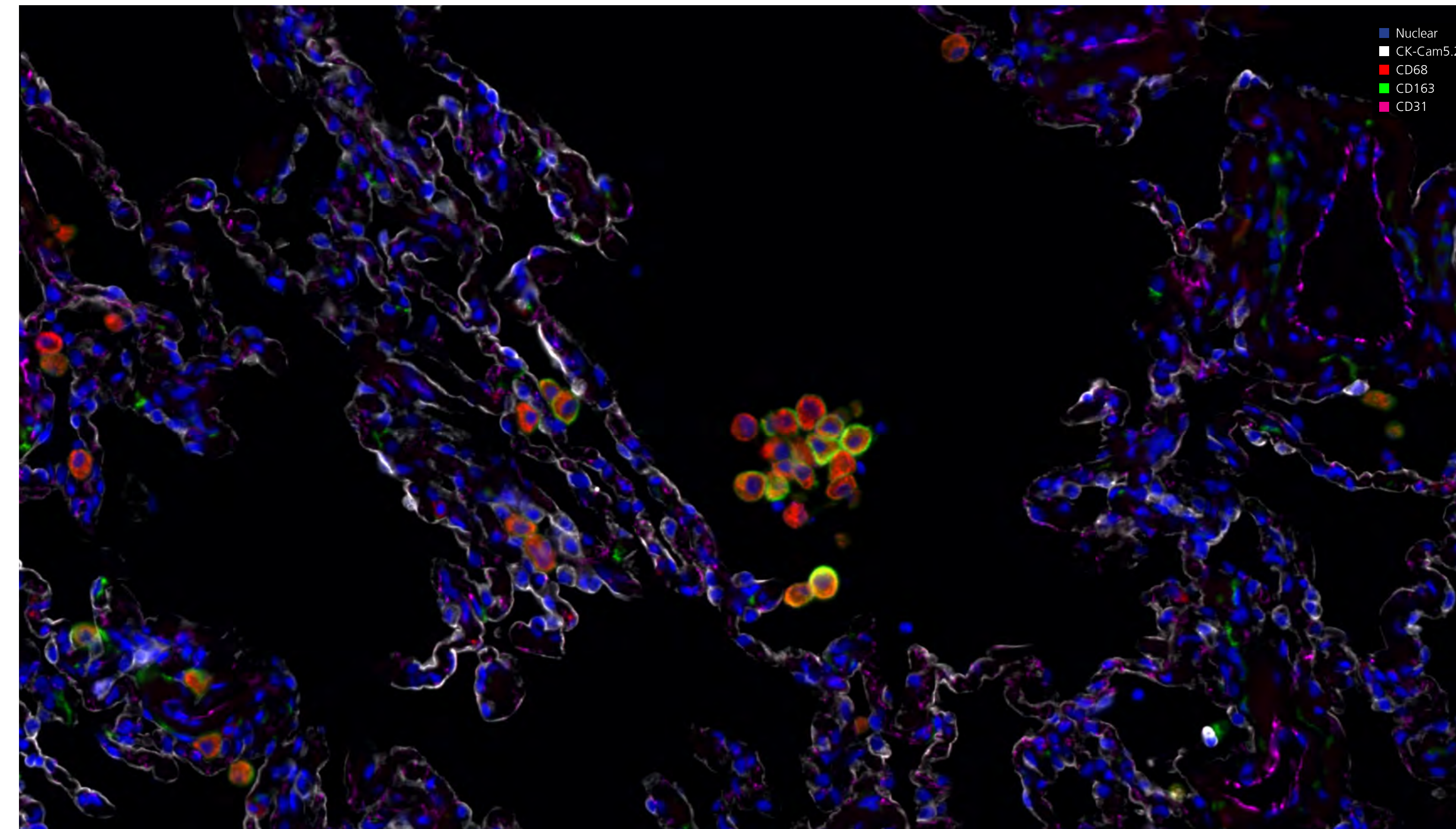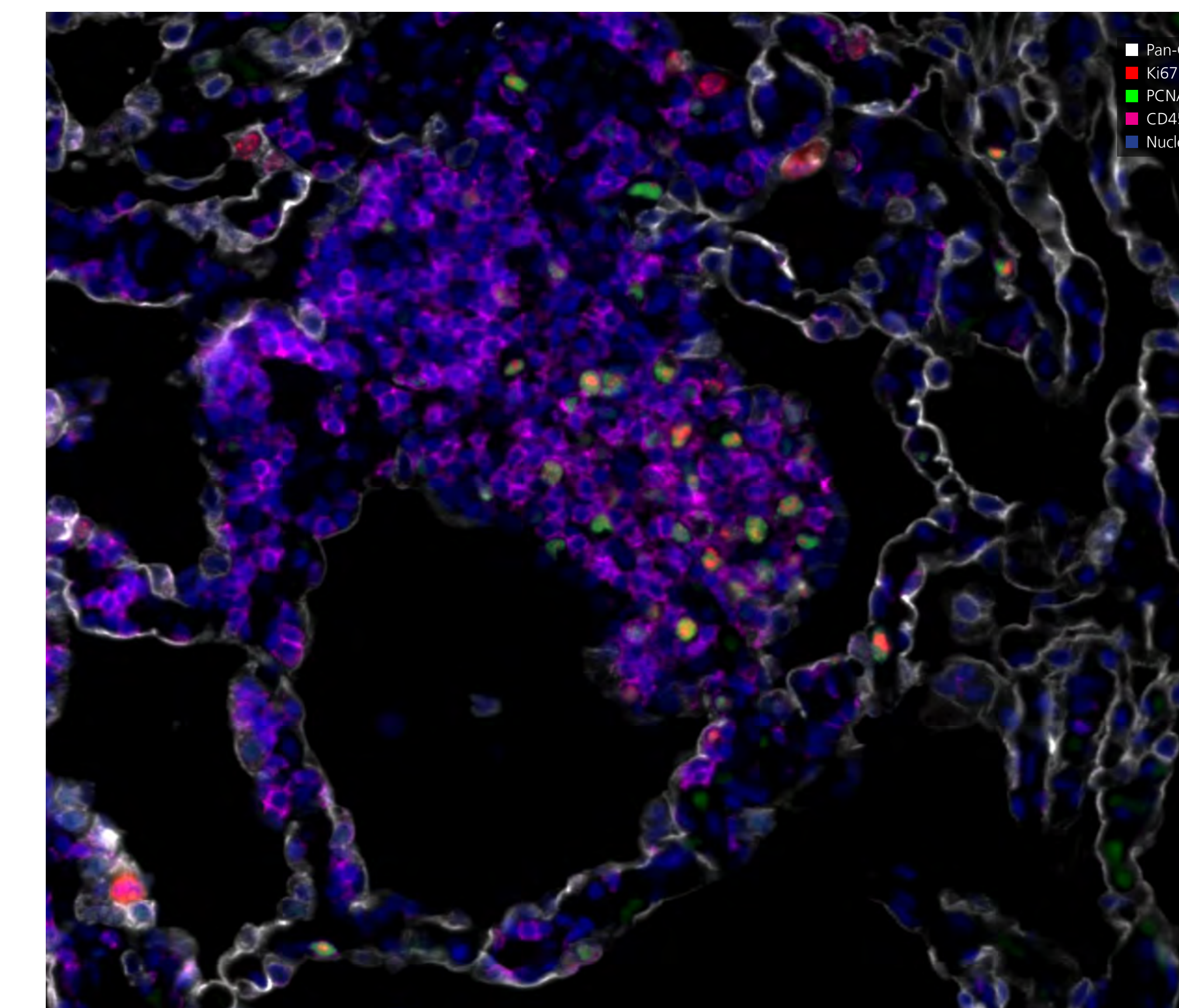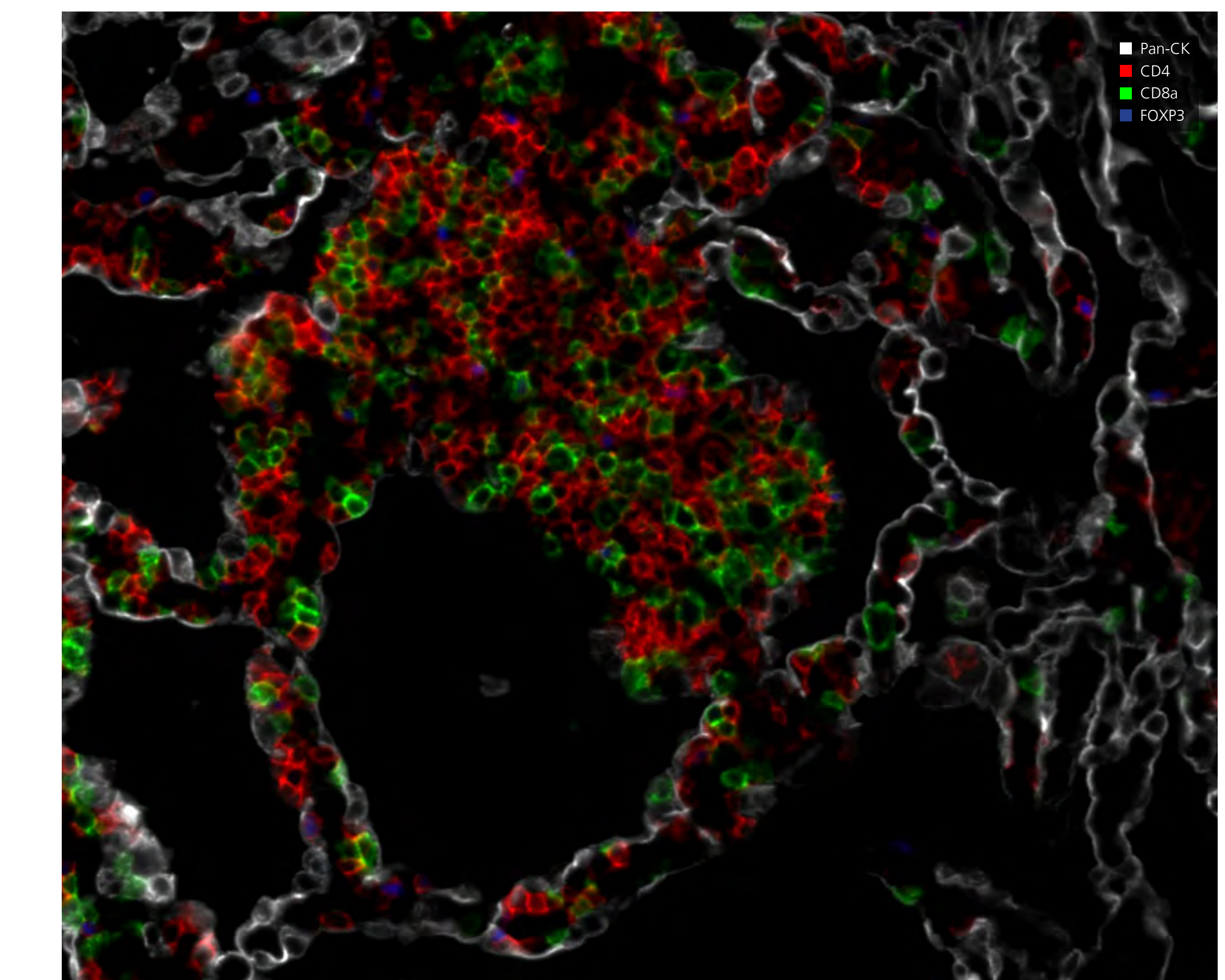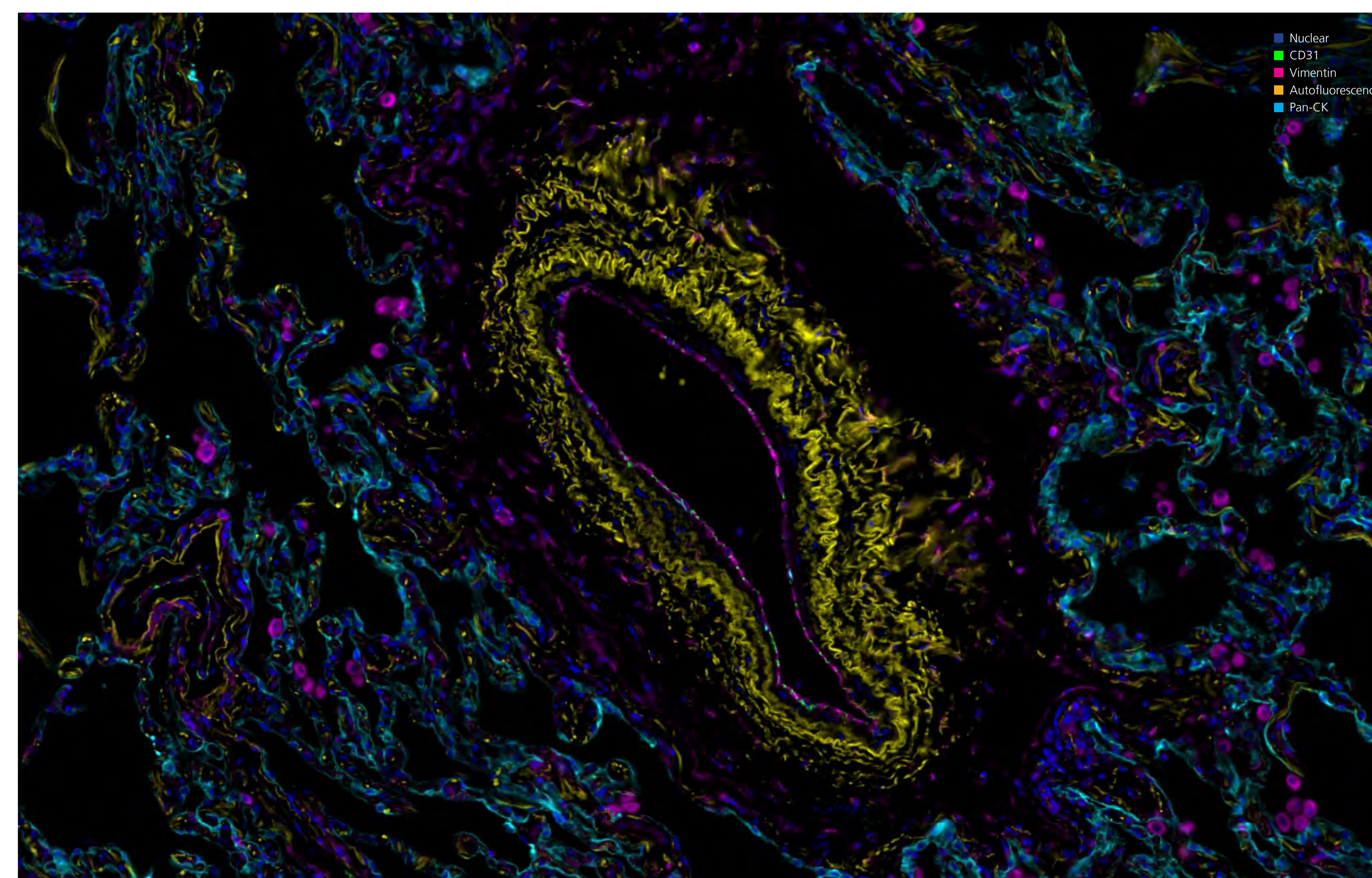
Fig 7. Cross section of an artery reveals expression of CD31 (green) and Vimentin (magenta) by the endothelial lining cells. The extracted Autofluorescence (yellow) signal clearly defines the wavy elastic lamina of the artery wall. Alveolar macrophages also express Vimentin.
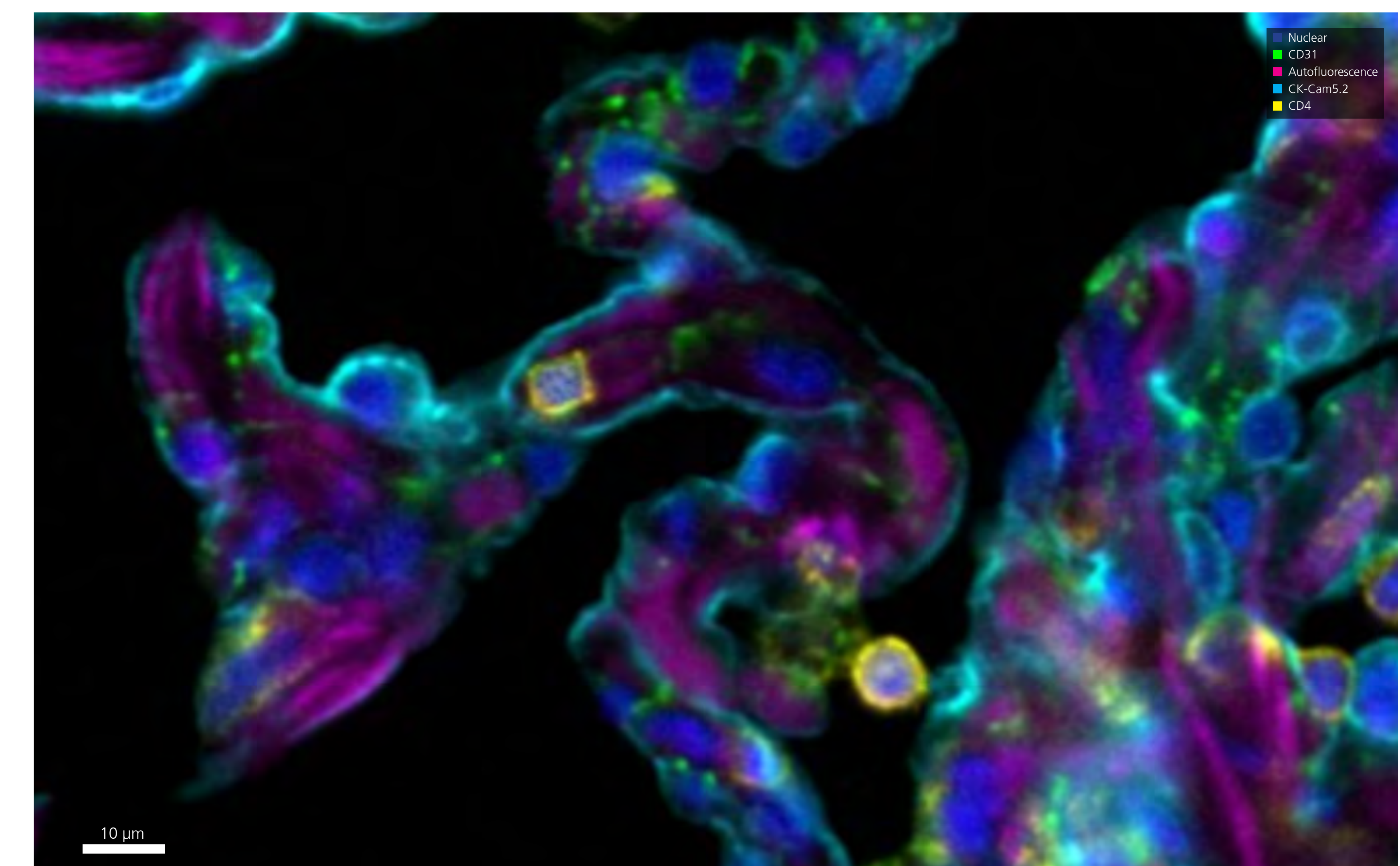
Fig 8. Fine detail of spatial orientation can be seen in this image of a CD4 T cell that is caught as it travels through an alveolar septum. Alveolar wall layers are also seen: epithelial (CK-Cam5.2, light blue), matrix (Autofluorescence, magenta), and endothelial (CD31, green).