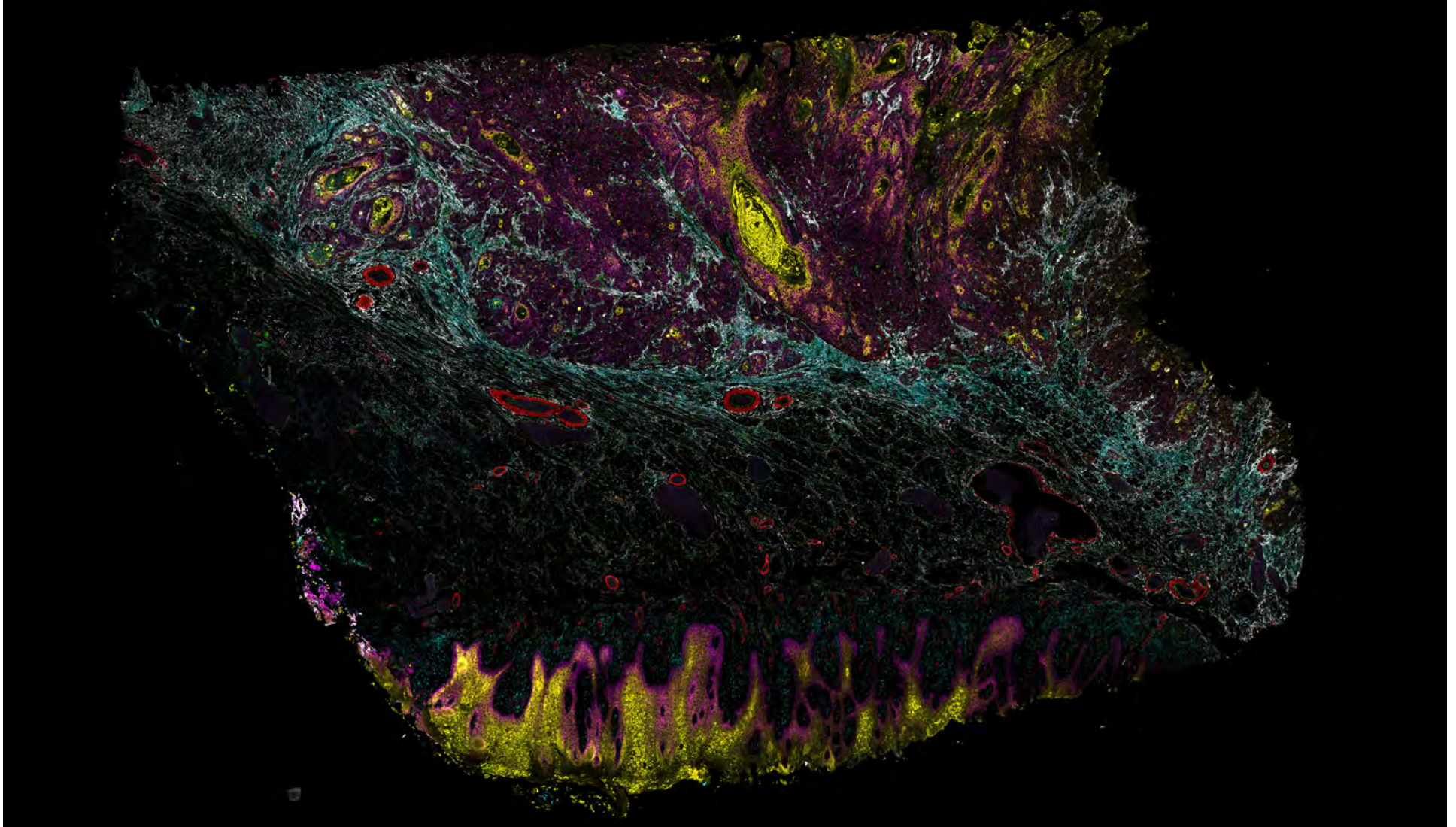


RARECYTE®

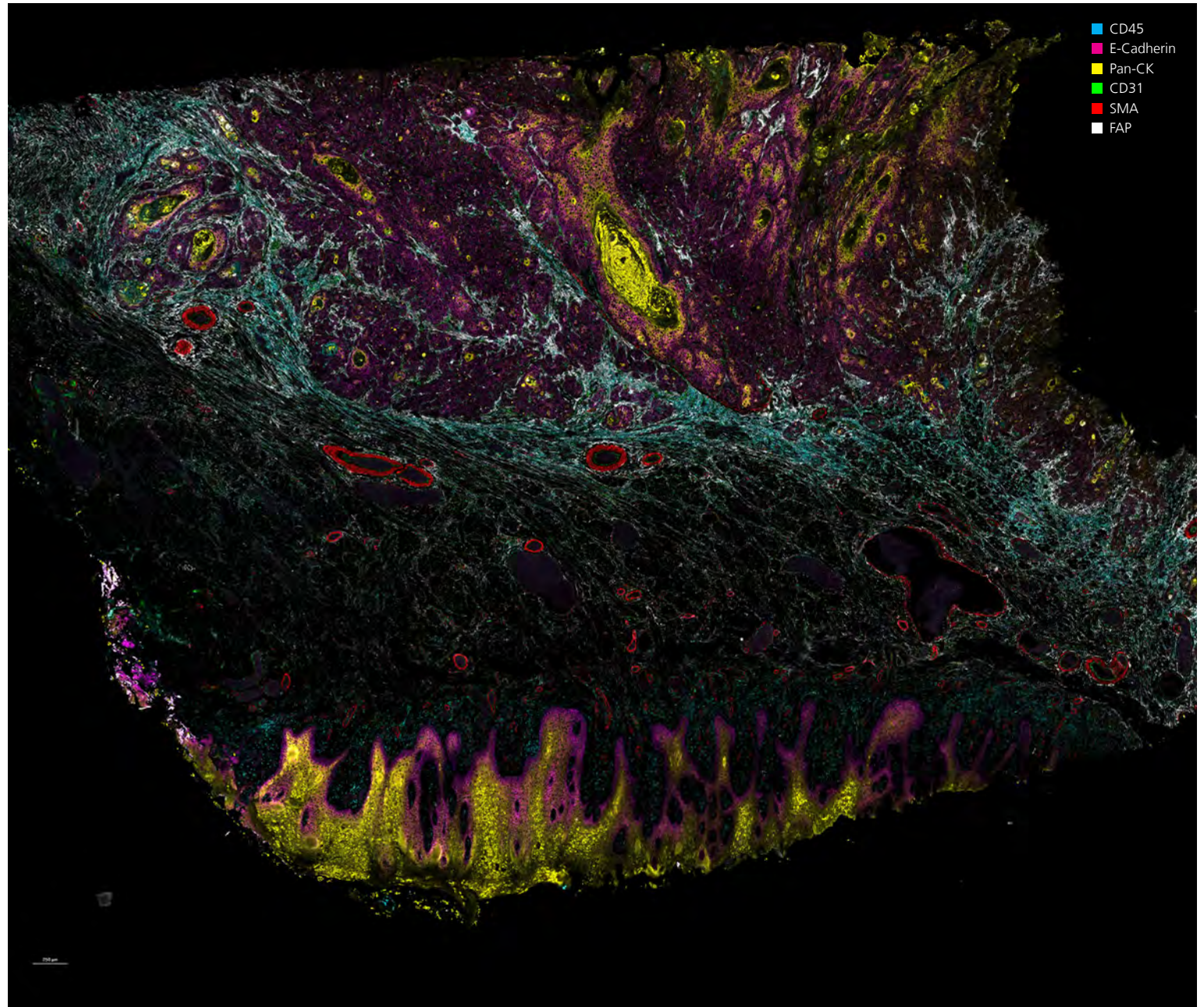
Orion™ Tissue Investigation | Oral Squamous Cell Carcinoma



Overview

This is a whole-slide section of an infiltrating squamous cell carcinoma of the tongue. The normal mucosal surface of the tongue is at the bottom of the image and the nodule of invasive cancer is seen in the top half, surrounded by fibrous reaction.

Note that at the lower left edge of the section is a brightly stained region. This is fluorescence of a marking pigment used to delineate the border of the tumor resection specimen.

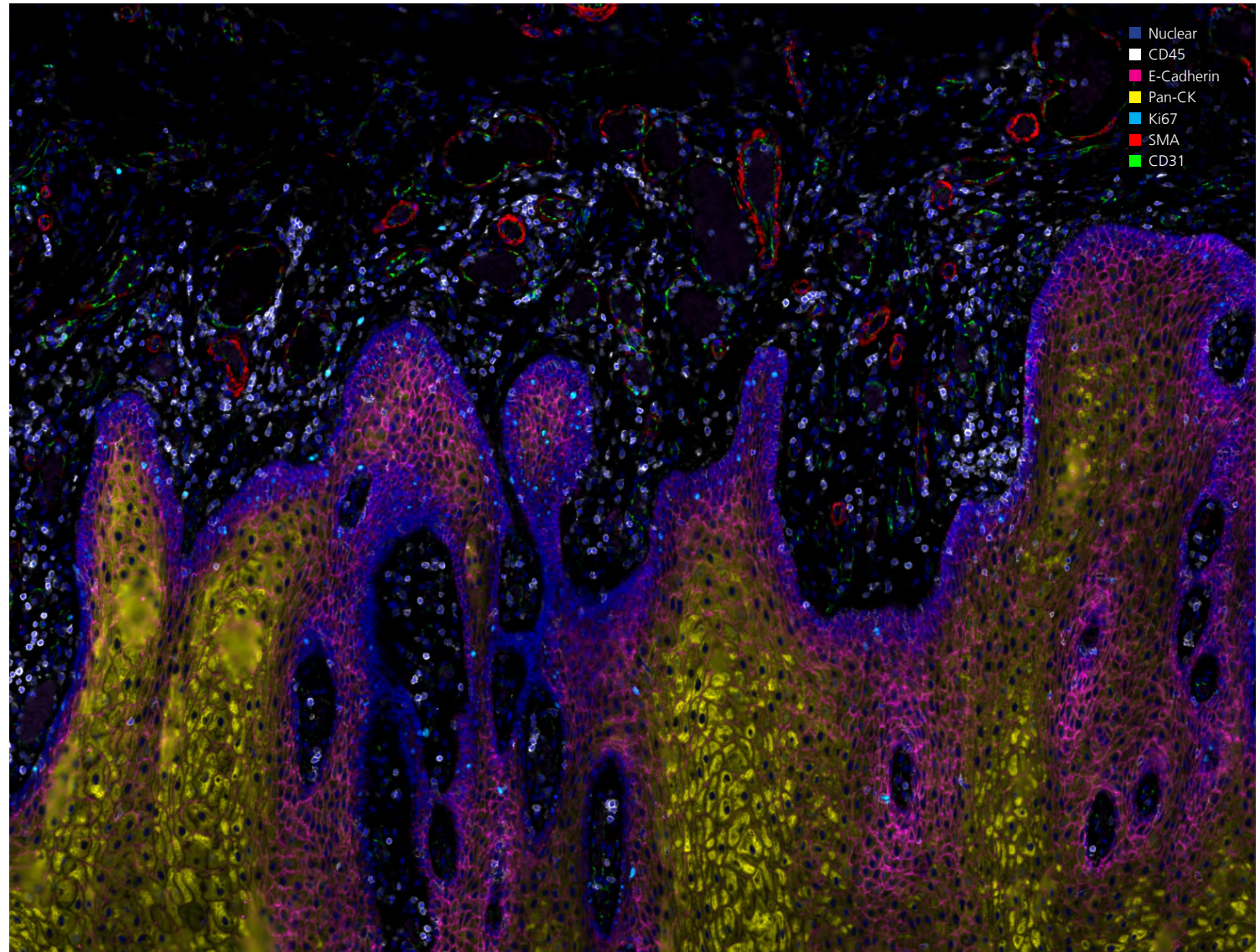


Normal tongue mucosa

The mucosa of the tongue contains a stratified squamous epithelium with extensions (rete pegs) that project into the underlying connective tissue (lamina propria).

The basal layer of the epithelium can be identified by the expression of **E-Cadherin**. Scattered proliferating cells can be identified in this layer by the nuclear expression of the marker **Ki67**. As the cells differentiate into squamous keratinocytes toward the surface, cytokeratins are prominently expressed.

Between the rete pegs are connective tissue papillae that contain **CD31+** capillary endothelial cells in venules, smooth muscle actin (**SMA**) in arterioles and a collection of immune cells (**CD45+**, white).

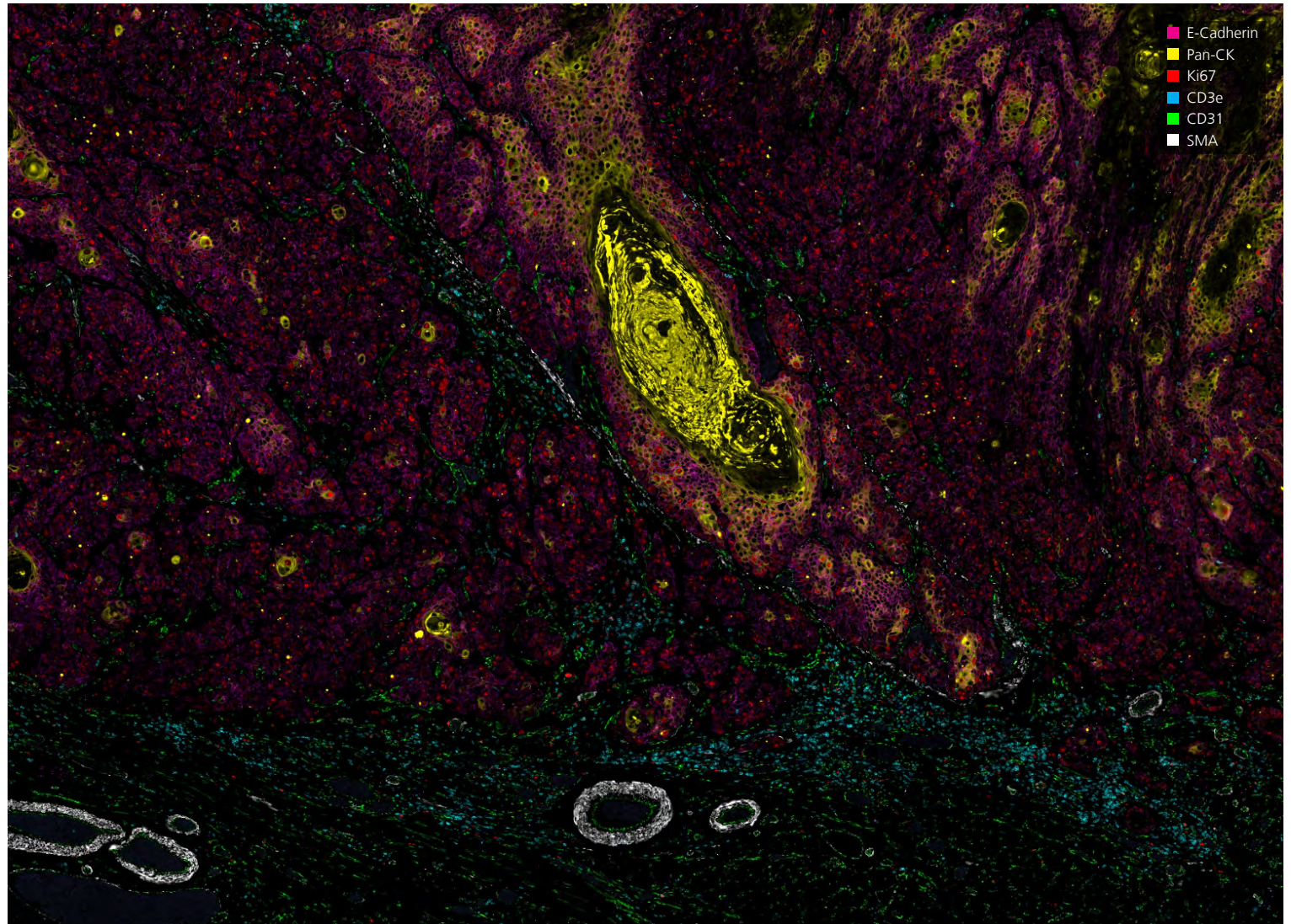


Infiltrating squamous cell carcinoma

The tumor cells recapitulate the organizational structure of the normal epithelium.

Most express **E-Cadherin** similar to basal mucosal epithelium. Yet in some regions, the cells differentiate into squamous cells and express **Pan-CK**.

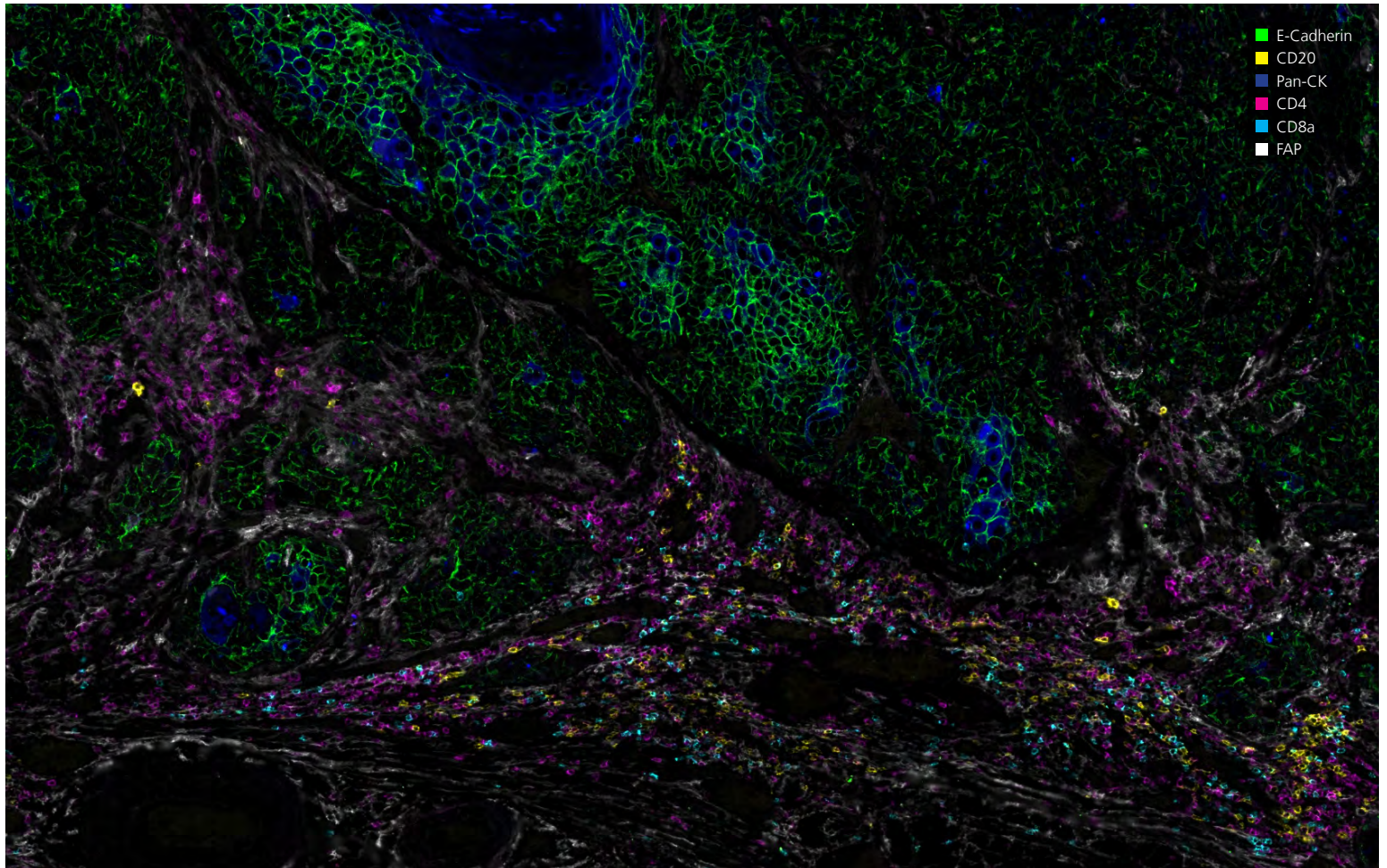
In contrast to the normal epithelium, the tumor is highly proliferative, with many **Ki67+** cells. Scattered T cells (**CD3**) surround the cancer cells.



Host response to tumor

The tumor stimulates a host fibrocellular immune response that is demonstrated by a mixture of immune cells at the border of the tumor nodule (**E-Cadherin** and **Pan-CK**).

In this image, collections of interspersed B cells (**CD20**), helper T cells (**CD4**) and cytotoxic T cells (**CD8+**) are present within an active area of cellular fibrosis (**FAP**, white).

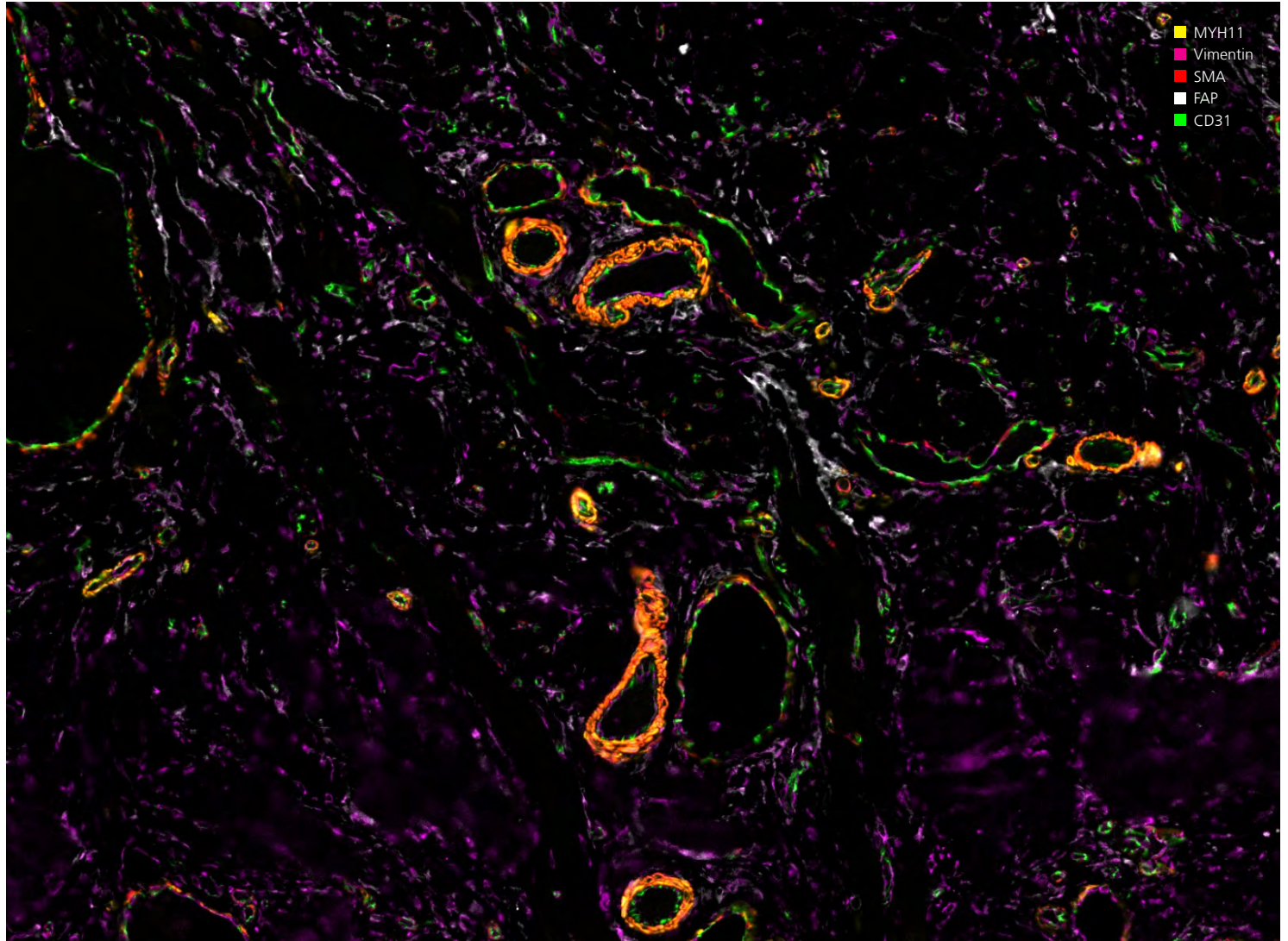


Submucosal stroma

In the submucosal region, there are stromal cells that include endothelial cells (**CD31**), vascular smooth muscle cells (**Smooth Muscle Actin**, **MYH11**), and fibroblasts (**FAP**, white).

In this image, the arteriolar blood vessel walls exhibit a bright orange color due to the co-expression of **SMA** and **MYH11**.

Vimentin is expressed in many different types of stromal cells.

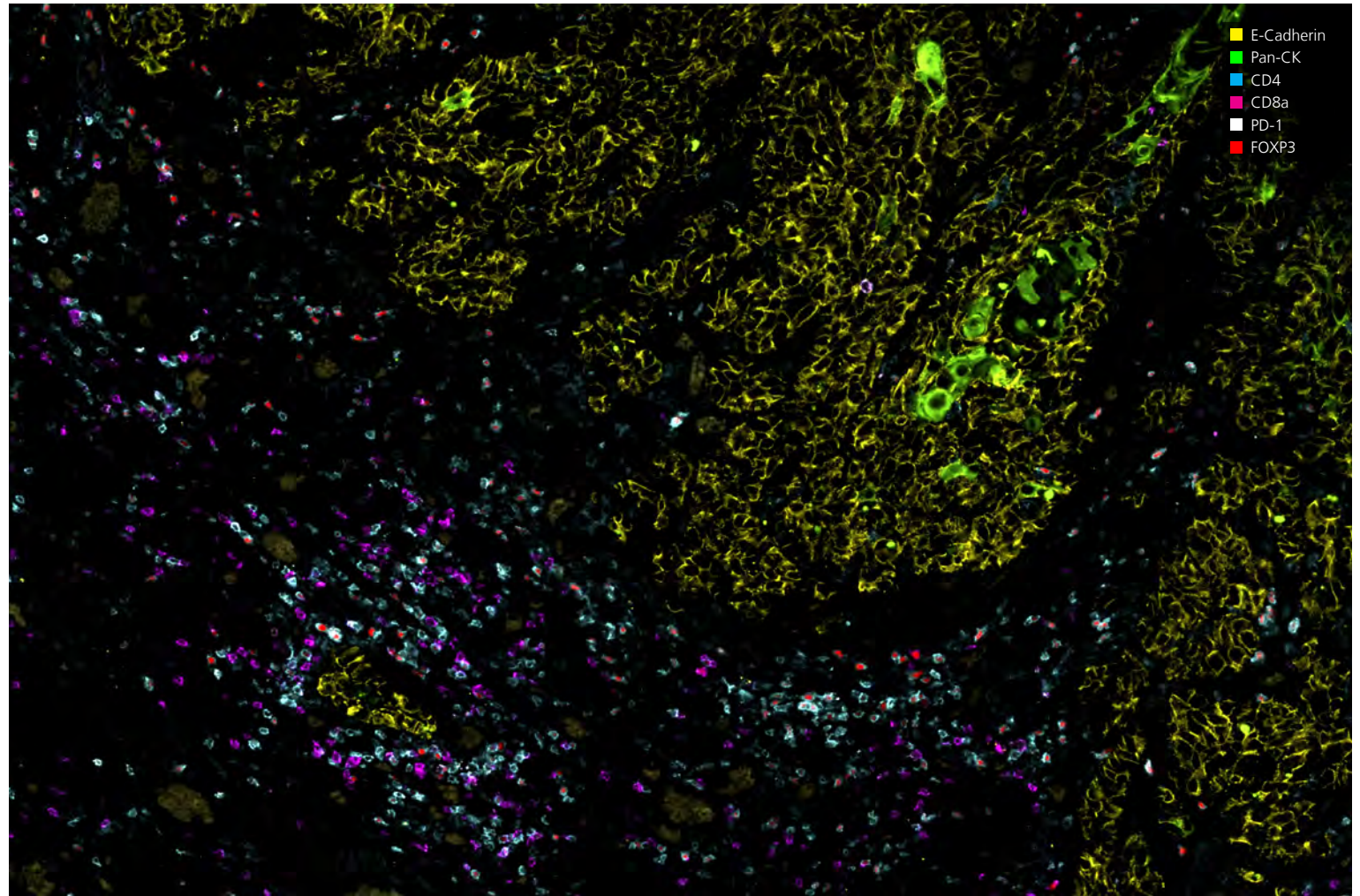


T cell subsets

Multiplexed biomarkers allow the study of a variety of T cell sub-types, including regulatory T cells and activated T cells. This region shows an aggregate of **CD4+** and **CD8+** T cells adjacent to nests of tumor cells (**E-Cadherin**, **cytokeratin**).

Regulatory T cells, typically CD4 T cells, are identified by **FOXP3** staining of the nucleus. **PD-1** (in white) marks activated cells.

Within the aggregate, the checkpoint marker **PD-1** is observed on **CD4** T cells, some of which are **FOXP3+** cells. There are lone **CD8** T cells (upper right) that strongly express **PD-1** within the tumor nests which may represent exhausted tumor infiltrating lymphocytes.



Marker
Nuclear (Hoechst)
CD31
CD45
Vimentin
CD4
FOXP3
CD8a
MYH11
CD20
FAP
CD3e
E-Cadherin
PD-1
Ki67
Pan-CK
Smooth Muscle Actin

Orion Squamous Cell Carcinoma Data Set – Sample Information

The FFPE squamous cell section was stained with a 16-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

RARECYTE®
Precision Biology for Life Sciences

2601 Fourth Ave
Seattle, WA 98121

855.727.3298
206.455.9092

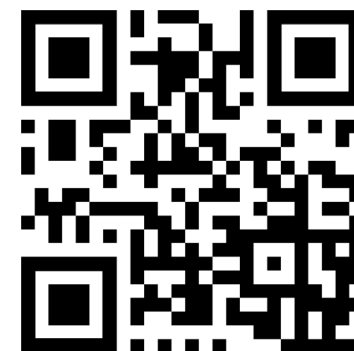
www.rarecyte.com

© 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-3-01



**View interactive
data sets**