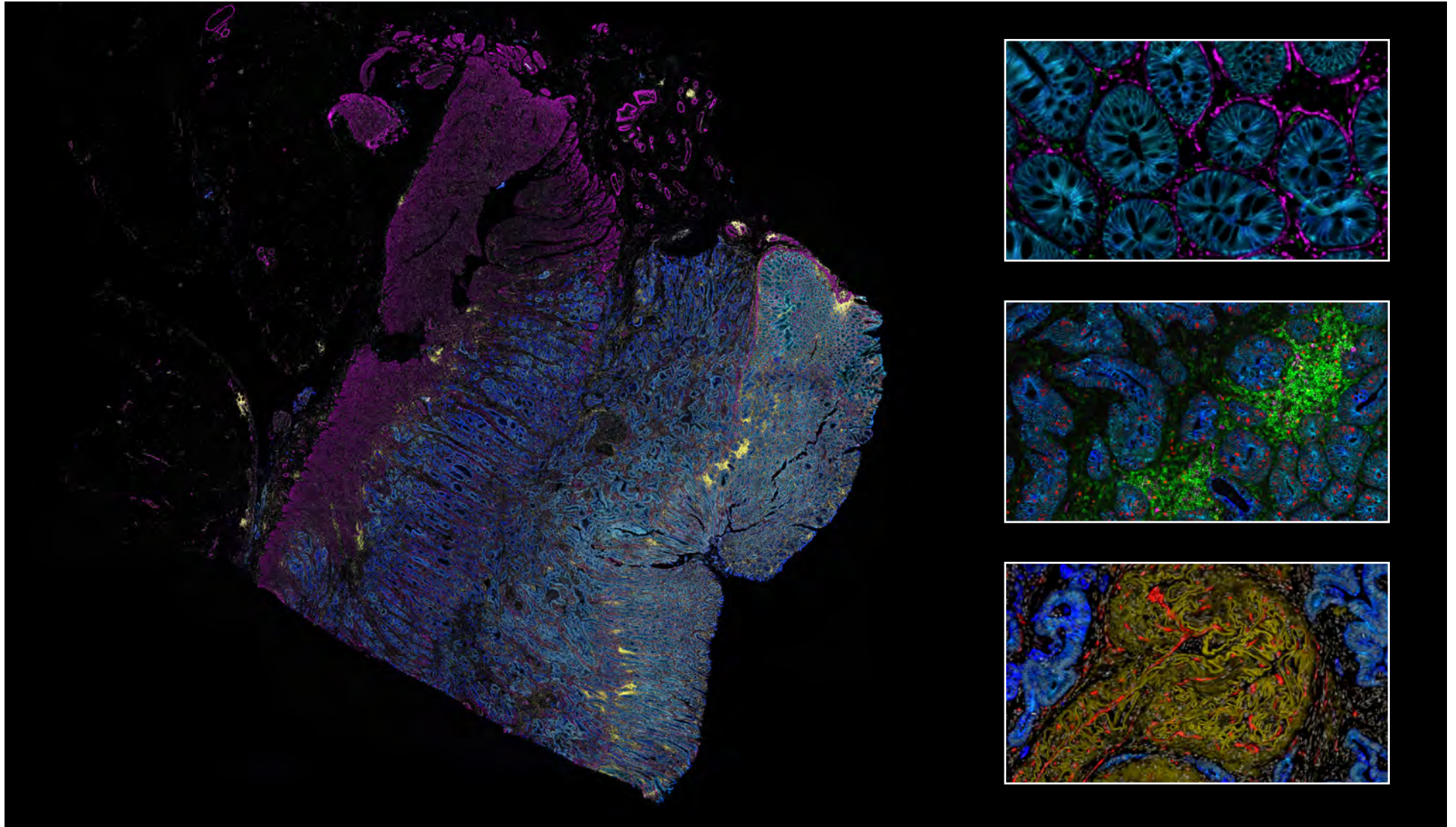


RARECYTE®

Orion™ Tissue Investigation | Colorectal Carcinoma



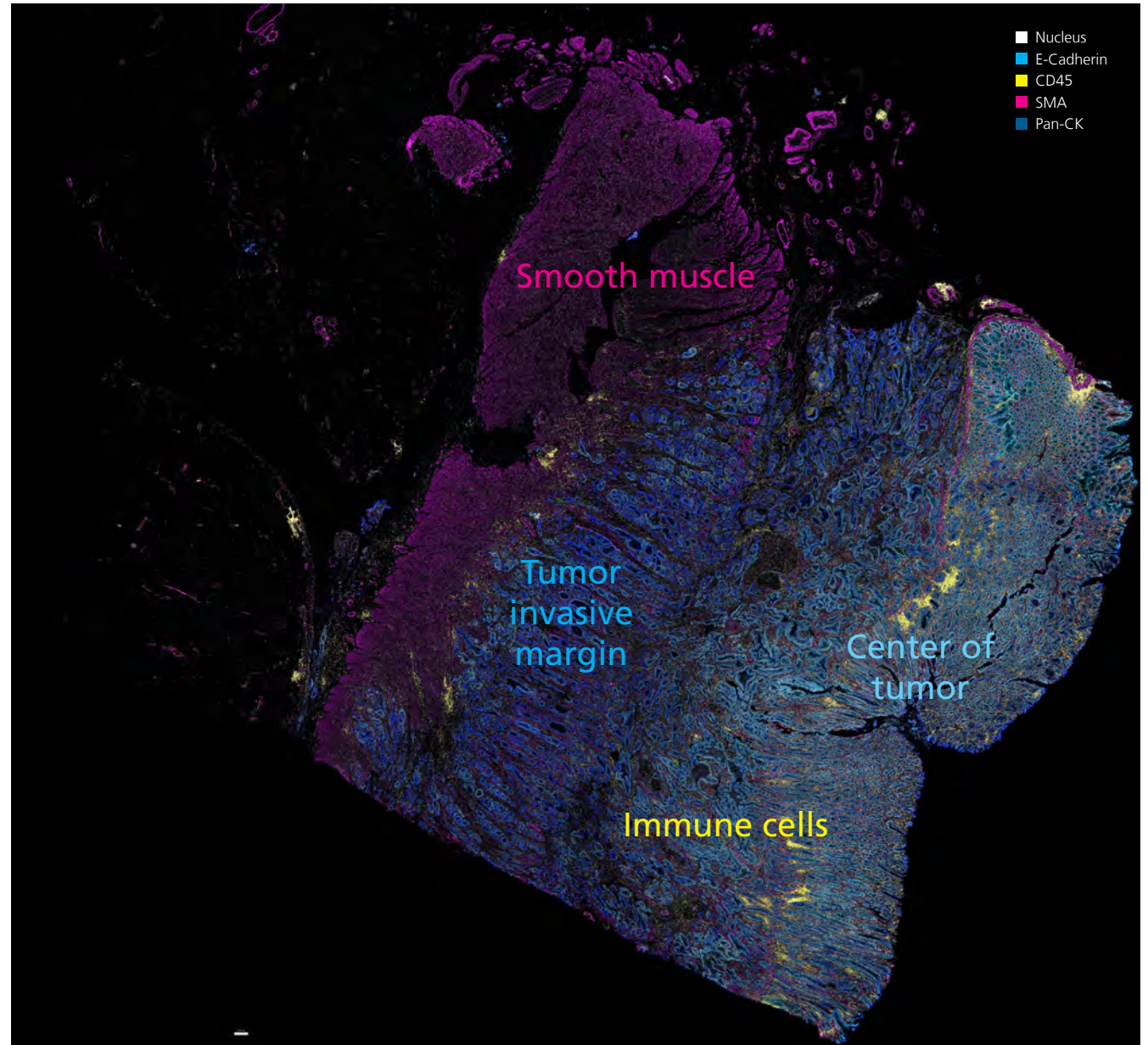
Overview

This is a whole-slide tissue section of an invasive colorectal adenocarcinoma stained with a 17-plex immunology biomarker panel and imaged with the Orion system in a single staining and scanning process.

Images in this presentation show smaller sets of markers that have been selected from the larger 17-plex panel.

The mucosal surface of the tumor is at the far right of the image. Epithelial markers denote tumor cells (**Cytokeratins – PanCK & E-cadherin**) invading beneath the mucosa into the smooth muscle layer seen in the center (**Smooth Muscle Actin - SMA**).

Dense collections of immune cells (**CD45**) can be seen at the base of the mucosa (right) and at the invasive margin of the tumor in the muscle wall (center).

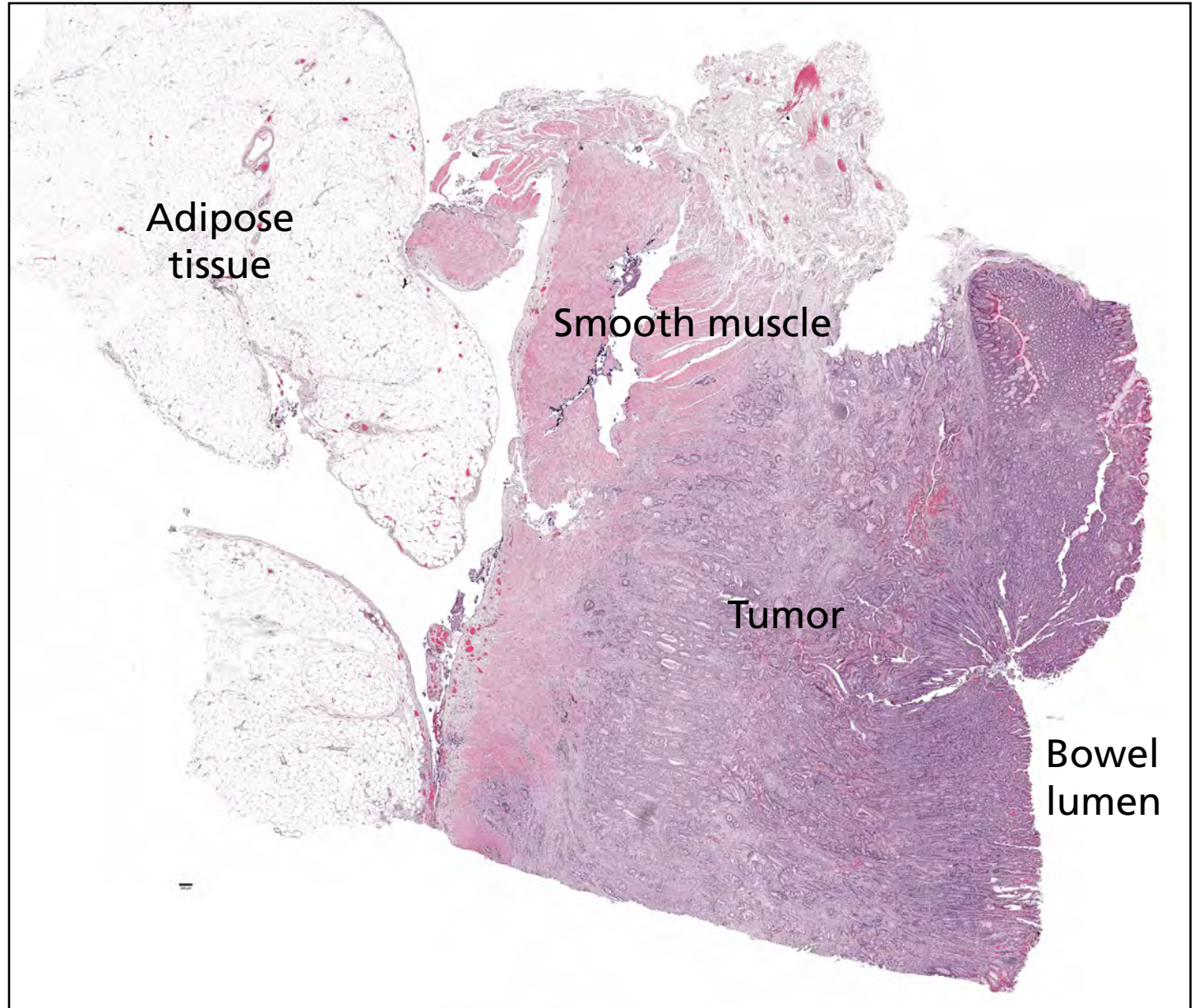


Invasive colorectal carcinoma

This is a hematoxylin and eosin stained whole-slide tissue section of an invasive colorectal adenocarcinoma.

The surface of the tumor is at the far right of the image. Tumor cells invade the bowel wall into the pink smooth muscle layer seen in the center. At the far left is adipose tissue.

In the following images, a serial section of this same tumor sample has been stained and imaged using the Orion system.



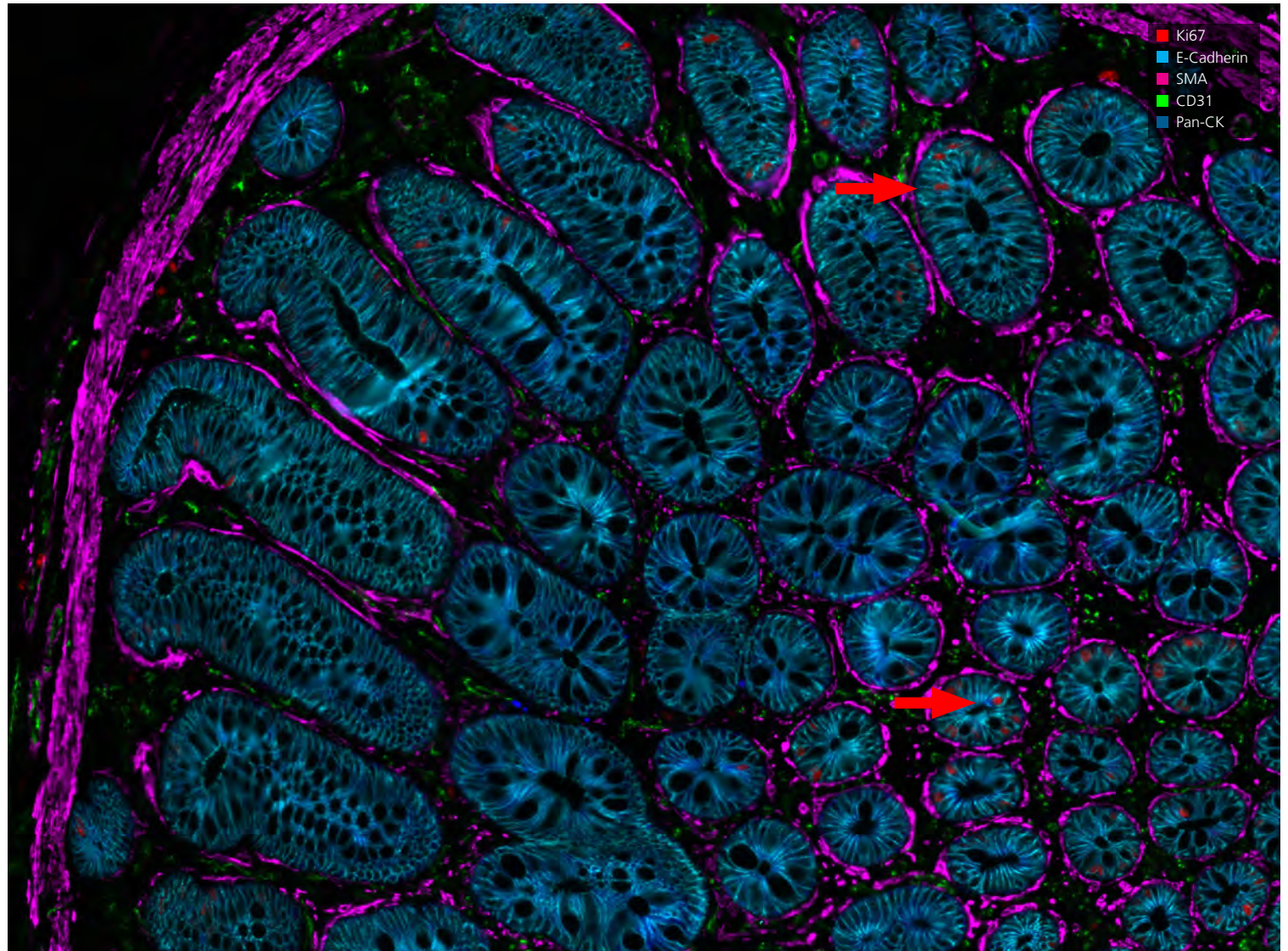
Normal colonic epithelium

Colonic glandular structures called crypts (**Cytokeratins**, **E-cadherin**) are encircled by myofibroblasts in the lamina propria between the crypts (**Smooth Muscle Actin**).

The smooth muscle of the muscularis mucosae, the boundary of the mucosa, is at far left.

The lamina propria also contains capillaries and small lymphatic vessels (**CD31**).

There are scattered **Ki67**+ proliferating cells (red arrows) present in the crypts; which are part of the normal regenerative process within the mucosa.

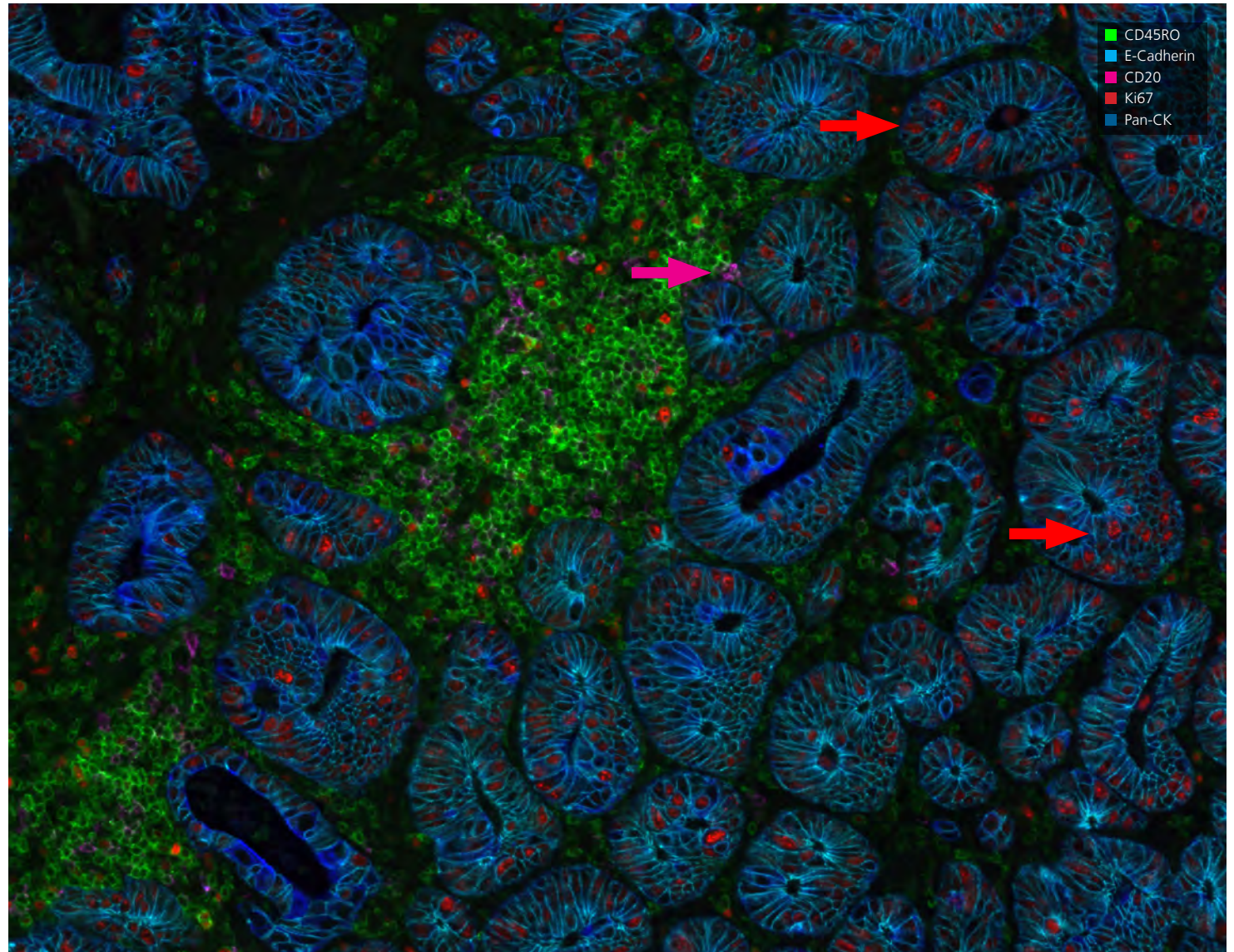


Well-differentiated adenocarcinoma with immune cell collection

Adenocarcinoma cells express membrane cell-cell adhesion glycoprotein **E-cadherin** uniformly in this area of cancer glands reminiscent of normal crypts. Cytoplasmic **cytokeratins** are prominent focally in groups of cells that generally appear larger and have less uniform shape within the malignant glands.

In contrast to normal crypt cells, a large fraction of tumor cells are in proliferative phase (**Ki-67**, red arrows).

A dense collection of activated immune cells (**CD45RO**) is prominent between the cancerous glands. Some of these cells are in proliferative phase. A few of these cells are B cells (**CD20**, magenta arrows).



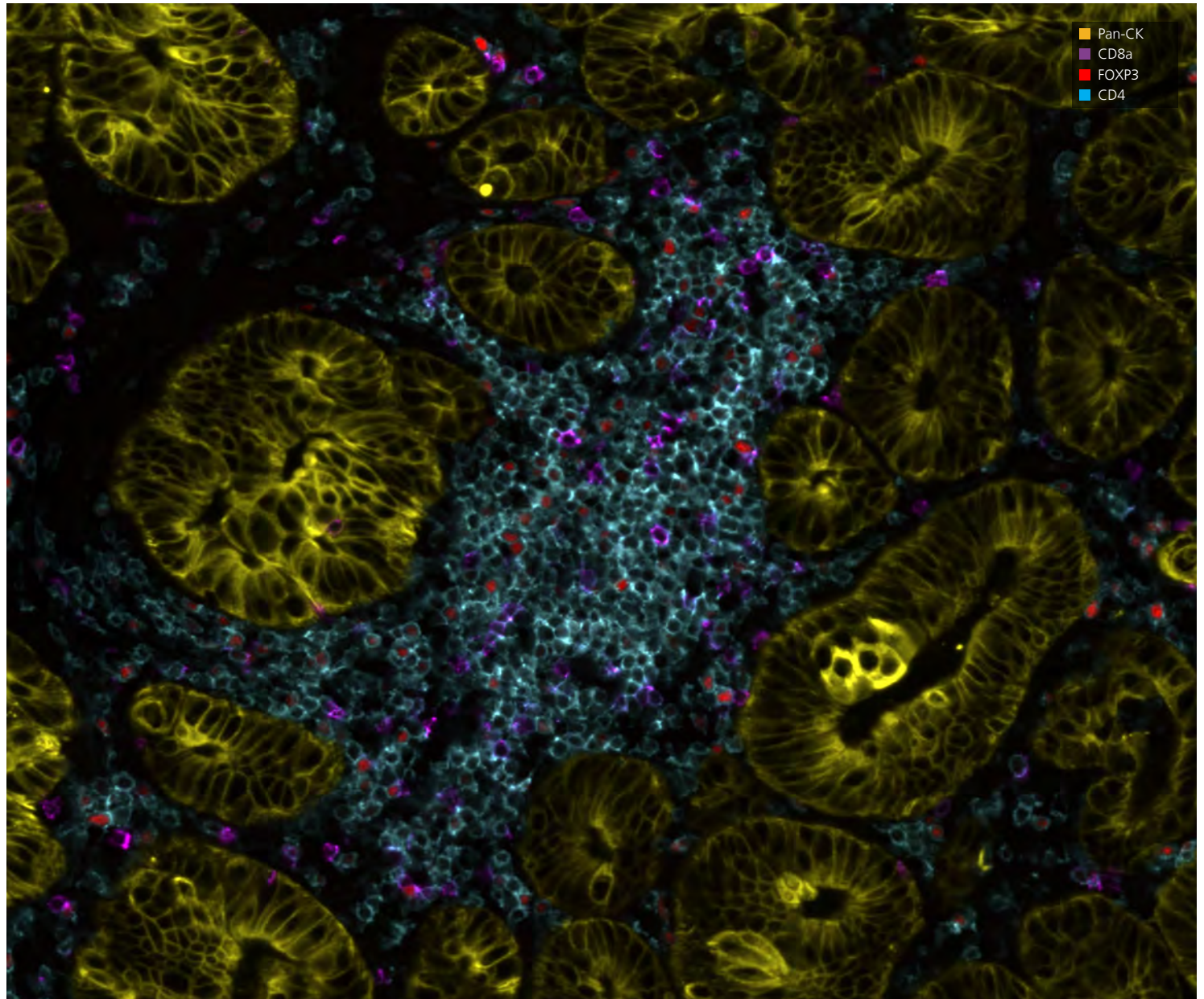
Interstitial immune cell phenotyping

The same collection of immune cells observed in the previous page is analyzed here with T cell sub-typing markers that are included in the staining panel.

Most of the cells in the dense collection are **CD4+** T helper cells. CD4 T cells can also be seen between tumor cells (**Cytokeratins**).

Scattered within the CD4+ population are regulatory T cells (**FOXP3**).

There are **CD8+** cytotoxic T cells present but they are much fewer in number than CD4+ cells.



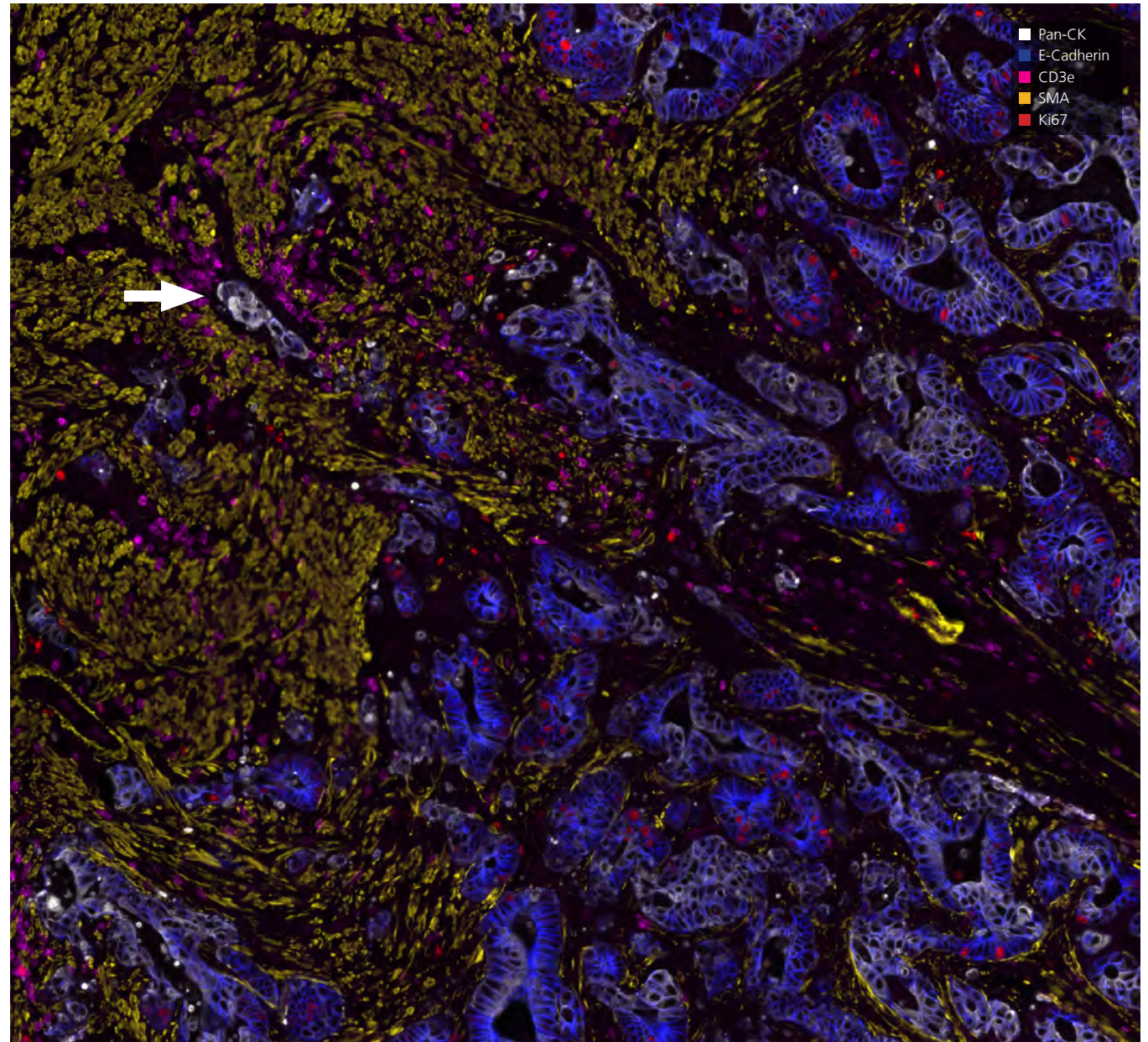
Infiltrating border of carcinoma

Multiplexed imaging distinctly reveals the invasive border of the tumor. Small clusters of malignant 'budding' cells seen in the upper left (**cytokeratins – PanCK**, white arrow) appear to have broken off from the glandular structures and infiltrated further into the smooth muscle (**smooth muscle actin**).

Note that the budding cells are surrounded by T cells (**CD3**) as part of the host response to these invasive cells.

The infiltrating border contrasts with other tumor regions in two ways.

1. The proliferative fraction is much lower (**Ki-67**, nuclear).
2. Staining of **E-cadherin** is reduced, and the presence of **cytokeratins** is more pronounced. This is consistent with a down-regulation of E-cadherin in the invasive cells that is part of epithelial to mesenchymal transition, leading to more aggressive tumor behavior.



Immune checkpoints on individual cells and in neighborhoods

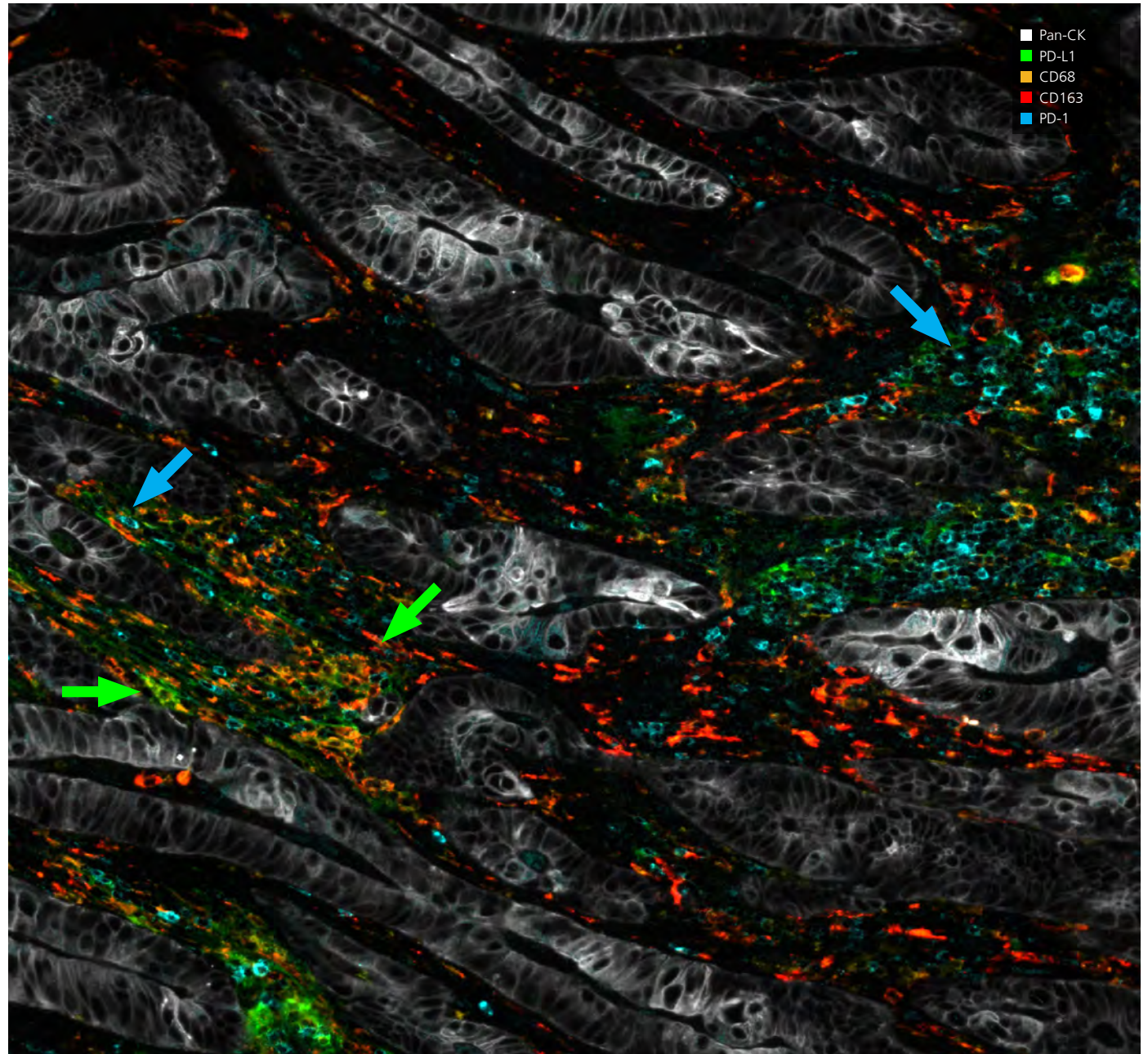
Macrophages are an important source of the checkpoint **PD-L1** in tumors. **PD-L1** binds **PD-1** on T cells to suppress anti-tumor immune response.

Interstitial macrophages surrounding tumor cells (**cytokeratins** – in white) in this region can be identified by the markers **CD68** and **CD163**.

Co-localization of **PD-L1** on macrophages expressing both markers can be seen prominently on the left (green arrows).

On the right **CD68+** macrophages are more prominent than **CD163+**, and the number of **PD-L1+** cells is lower, revealing a different “neighborhood” of expression.

PD-1+ (light blue arrows) cells are present in both neighborhoods.

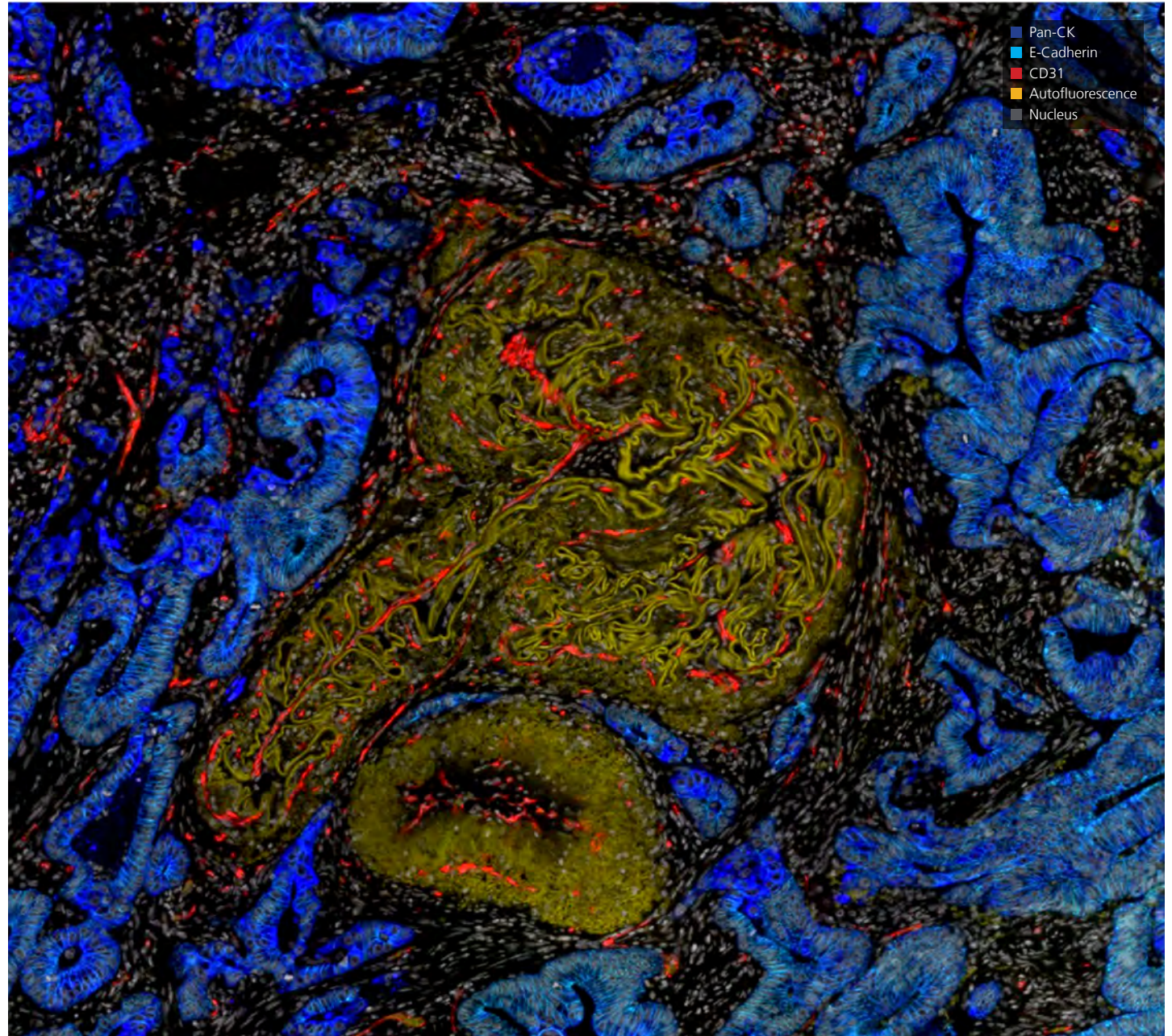


Autofluorescence reveals a neuro-vascular bundle in the colon wall

Elastin and nerve fibers can be identified by **autofluorescence** detected and visualized by Orion as its own fluorescence channel.

The lower autofluorescent structure is an artery, just below a collapsed large caliber vein, both of which have prominent elastin fibers. Sparse nerve fiber remnants are seen next to the upper part of the vein.

Endothelial cells are identified by **CD31**. The neurovascular bundle is surrounded by cancer cells (**cytokeratins**, **E-cadherin**).



Marker
Nucleus (Hoescht)
Autofluorescence
CD31
CD45
CD68
CD4
FOXP3
CD8a
CD45RO
CD20
PD-L1
CD3e
CD163
E-Cadherin
PD-1
Ki67
Pan-CK
Smooth Muscle Actin

Orion Colorectal Carcinoma Data Set – Sample Information

The FFPE colorectal 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
- 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



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**View interactive
data sets**