

# Orion™: 17-Plex Single-Step Stain & Imaging of Colorectal Adenocarcinoma

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Precision Biology for Life Sciences

## Overview

This whole-slide tissue section of an invasive colorectal adenocarcinoma is stained with a 17-plex immunology biomarker panel and imaged with the Orion system in a single staining and scanning process (figure 1).

The mucosal surface of the tumor is at the far right of the image. Epithelial markers denote tumor cells (cytokeratins, Pan-CK & 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).

In the hematoxylin and eosin (H&E) stained whole-slide tissue image, the surface of the tumor is visible at the far right of the image (figure 2). Tumor cells invade the bowel wall into the pink smooth muscle layer seen in the center. At the far left is adipose tissue.

## Normal Colonic Epithelium

Colonic glandular structures called crypts (cytokeratins, E-Cadherin) are encircled by myofibroblasts in the lamina propria between the crypts (SMA). 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; part of the normal regenerative process within the mucosa (figure 3).

## 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 (figure 4).

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 arrow).

## Interstitial Immune Cell Phenotyping

The same collection of immune cells observed in figure 4 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+ (in light blue) 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 (figure 5).

## 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 (SMA). Note that the budding cells are surrounded by T cells (CD3) as part of the host response to these invasive cells (figure 6).

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 (figure 7).

Co-localization of PD-L1 on macrophages expressing both CD68 and CD163 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 arrow) cells are present in both neighborhoods.

## Autofluorescence Reveals a Neurovascular Bundle in the Colon Wall

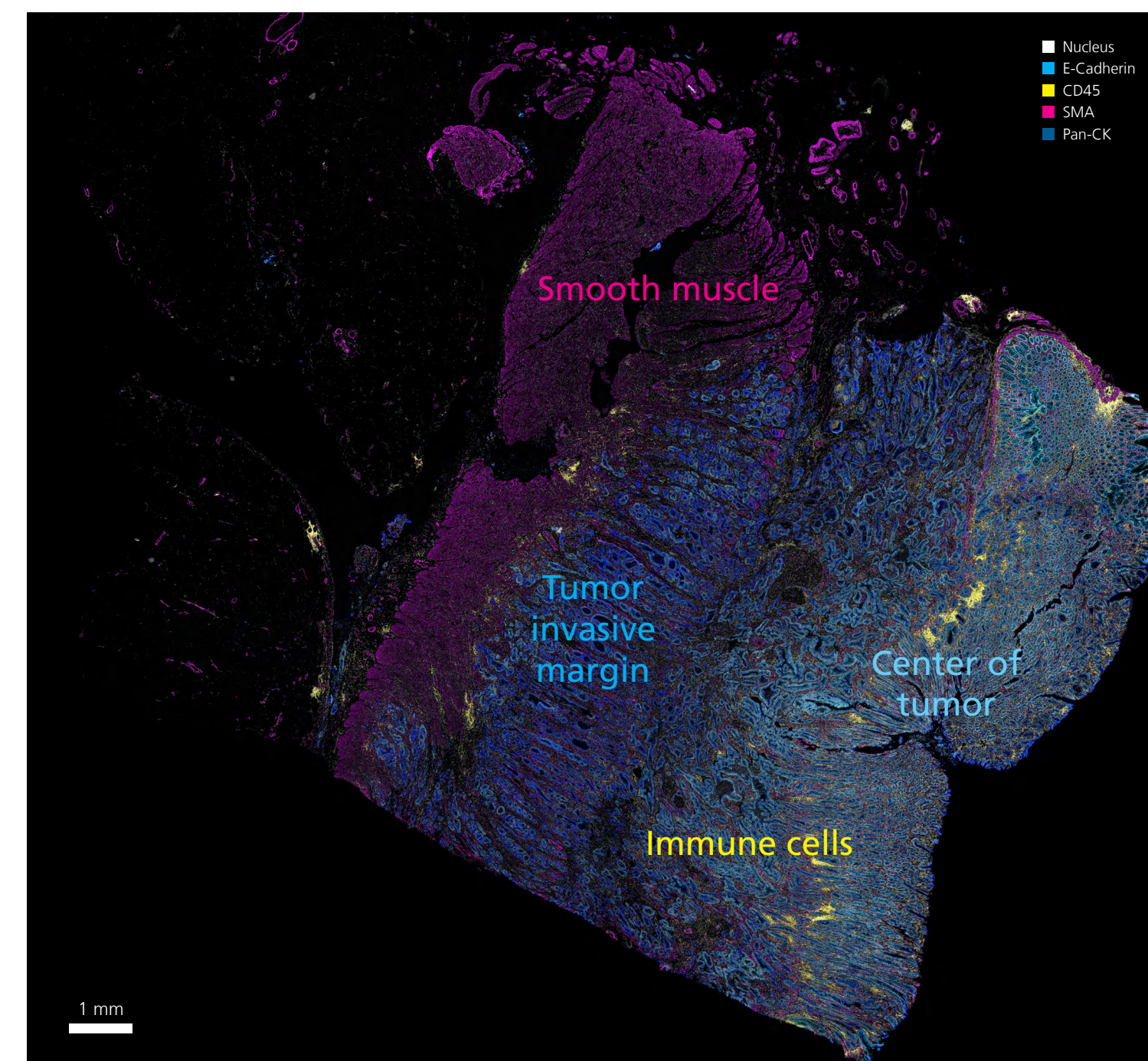
Elastin and nerve fibers can be identified by autofluorescence detected and visualized by Orion as its own fluorescence channel. The lower autofluorescent structure is an artery, just below a collapsed large caliber vein, both of which have prominent elastin fibers. Sparse nerve fiber remnants are seen next to the upper part of the vein. Endothelial cells are identified by CD31. The neurovascular bundle is surrounded by cancer cells (cytokeratin, E-cadherin) (figure 8).

## Summary of Orion Workflow

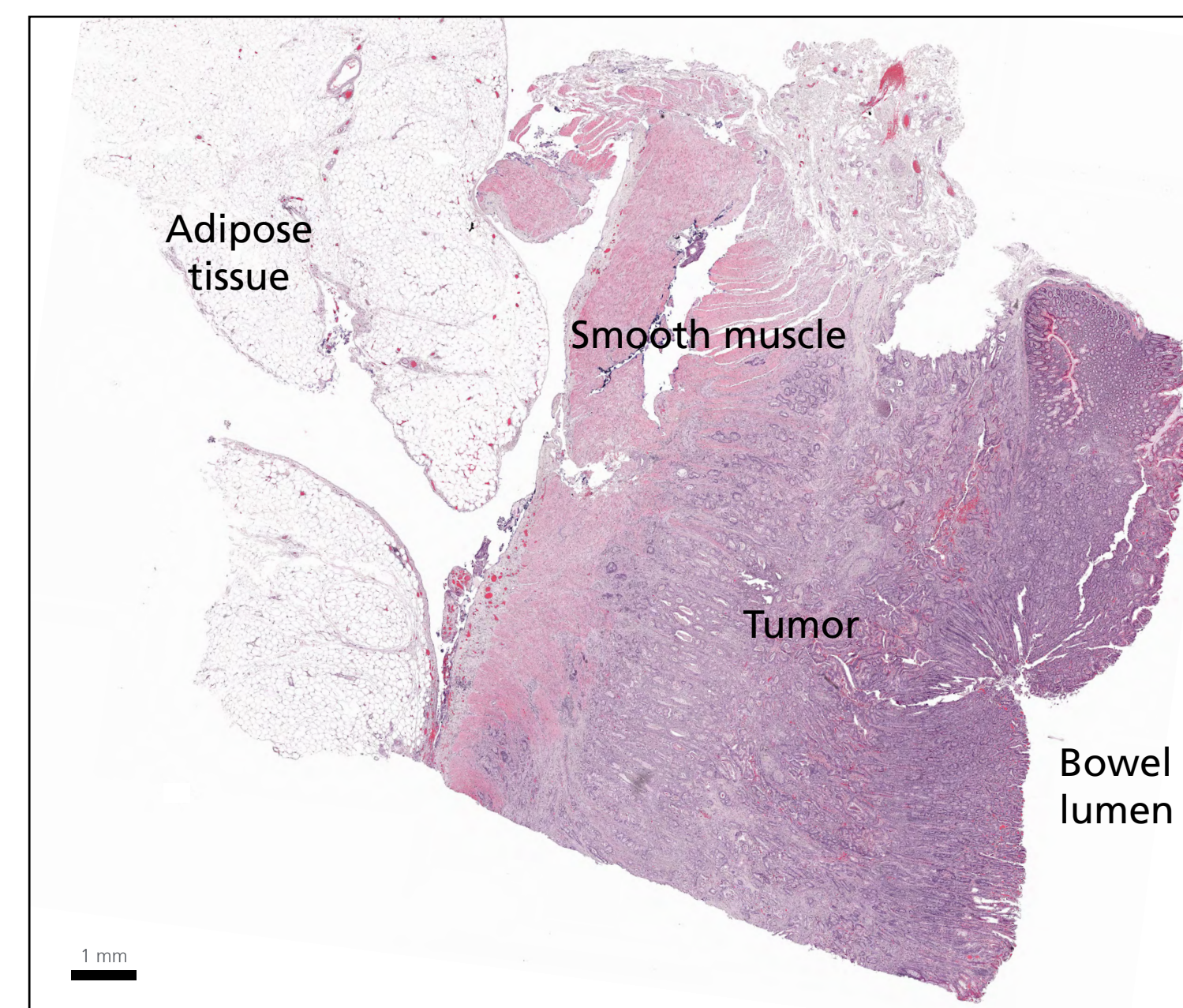


## Tissue Staining and Scanning Protocol

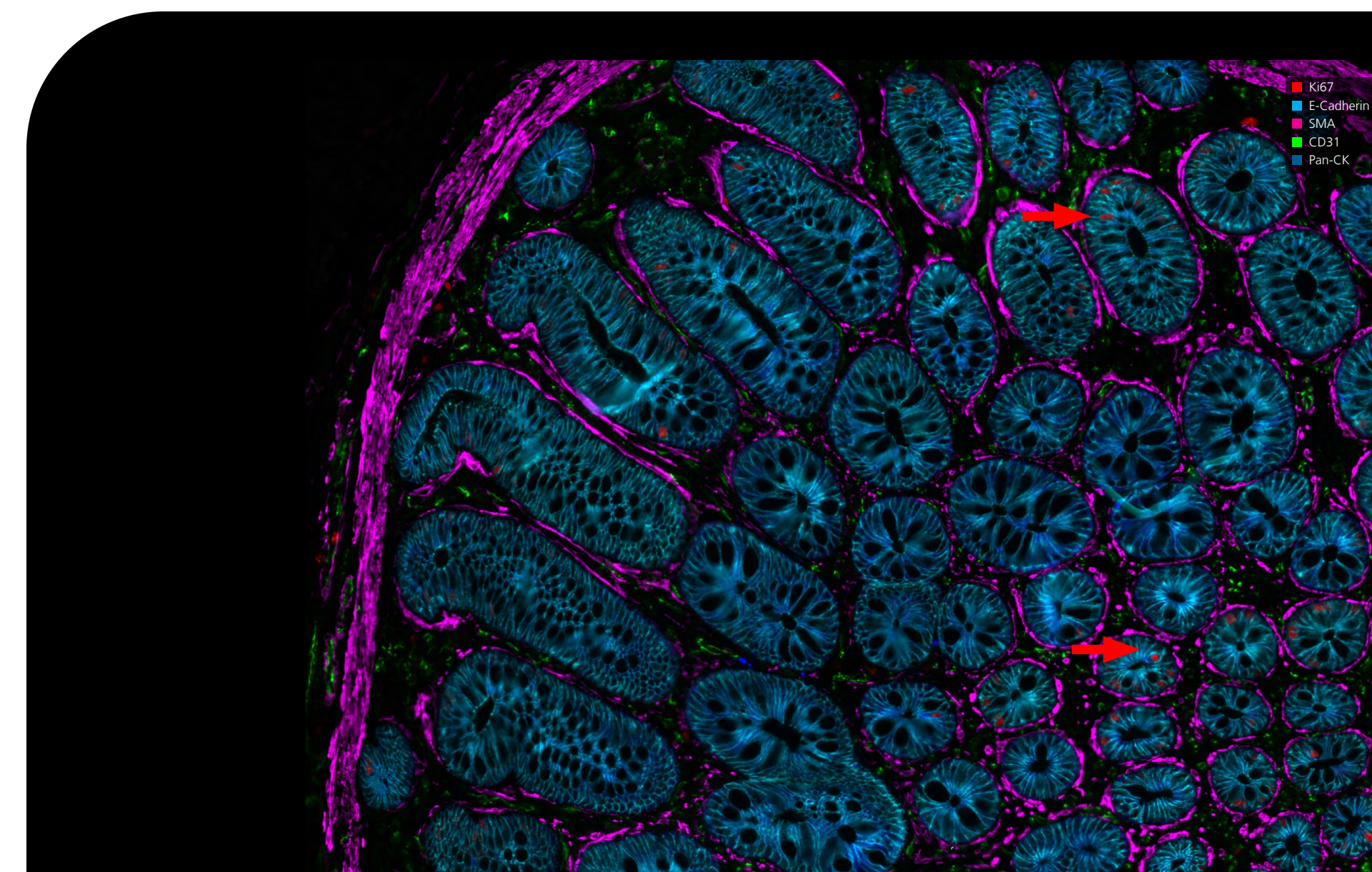
- Mount sections on glass slides
- De-paraffinize and perform antigen retrieval
- Quench autofluorescence
- Stain slides with panel of ArgoFluor™ conjugated antibodies
- Coverslip with ArgoFluor Mounting Medium and cure overnight
- Image whole slides at 20X magnification using Orion instrument
- Process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



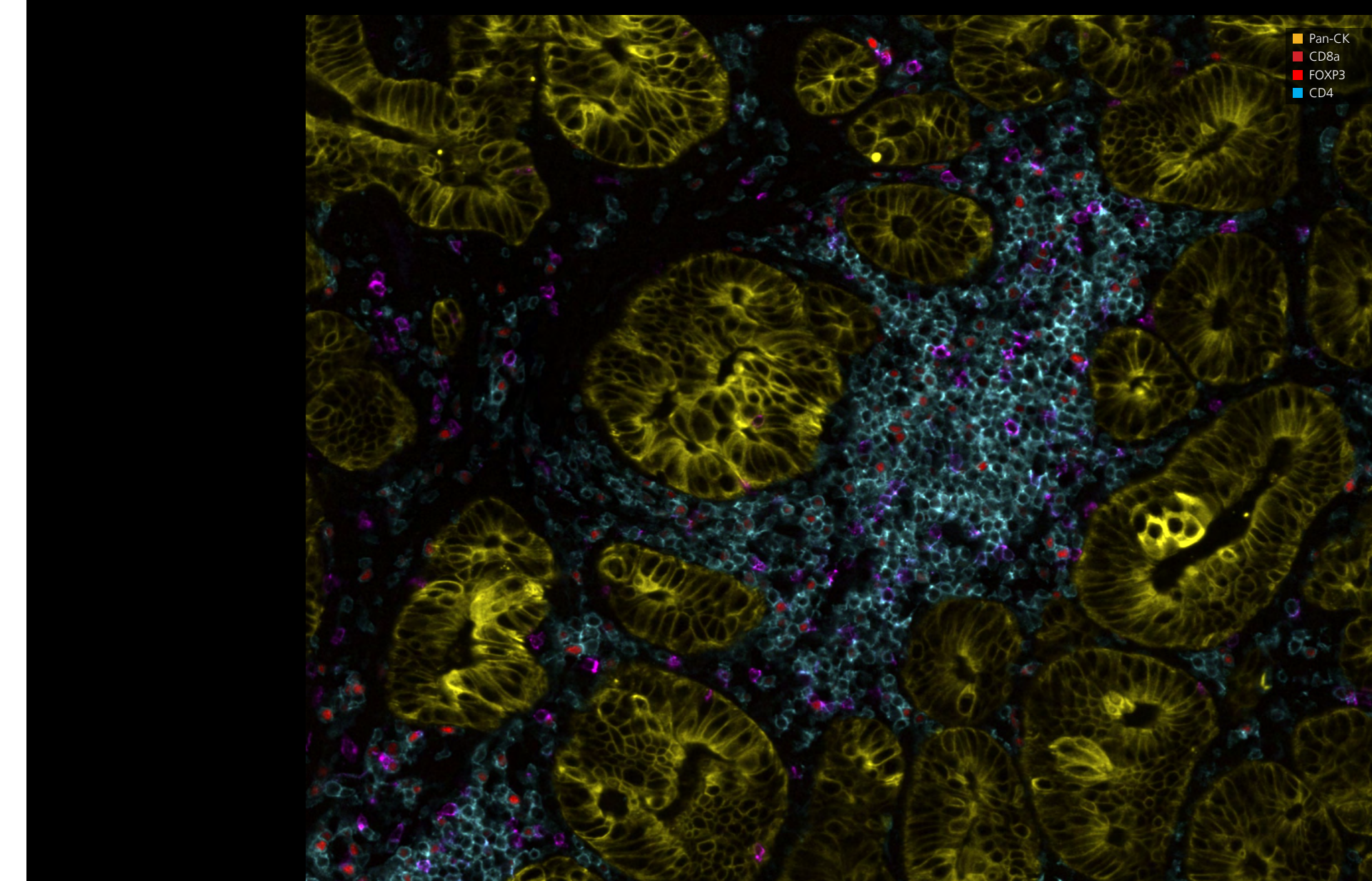
**Fig 1.** Whole-slide tissue section of an invasive colorectal adenocarcinoma stained with a 17-plex immunology biomarker panel.



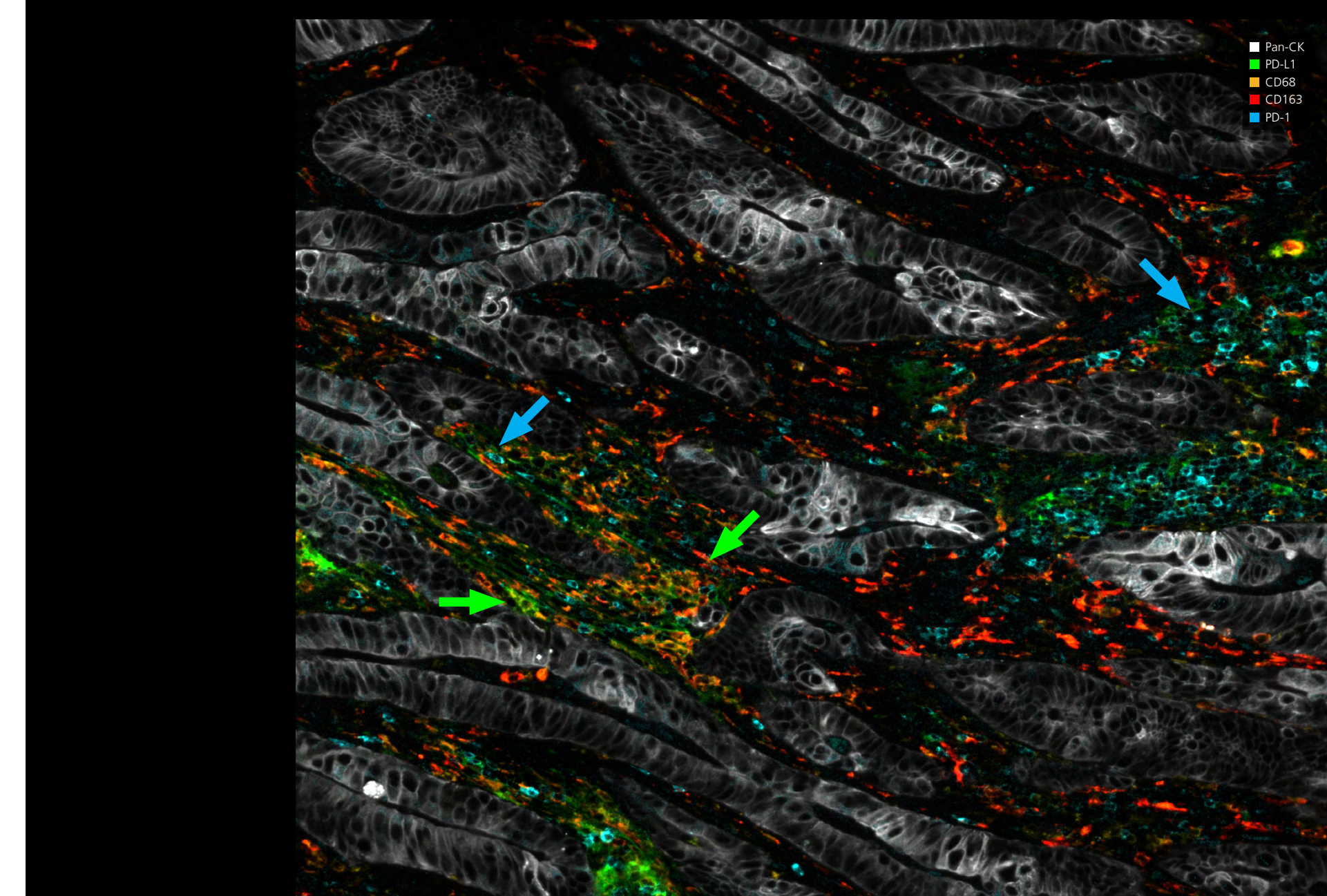
**Fig 2.** H&E stained whole-slide tissue section of an invasive colorectal adenocarcinoma.



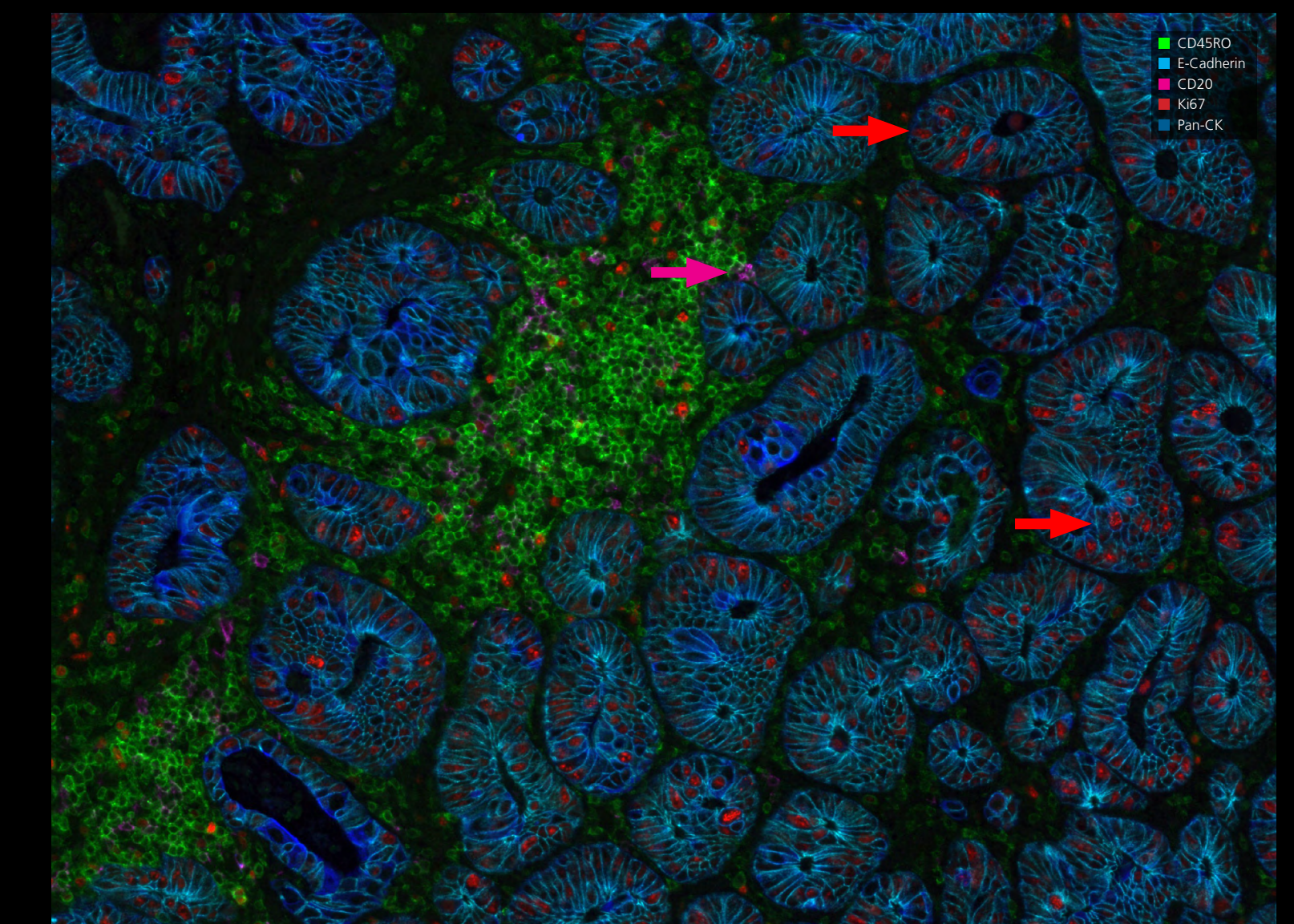
**Fig 3.** Colonic glandular structures called crypts (cytokeratins, E-Cadherin) are encircled by myofibroblasts in the lamina propria between the crypts. Also present within the crypts are Ki67+ proliferating cells (red arrows).



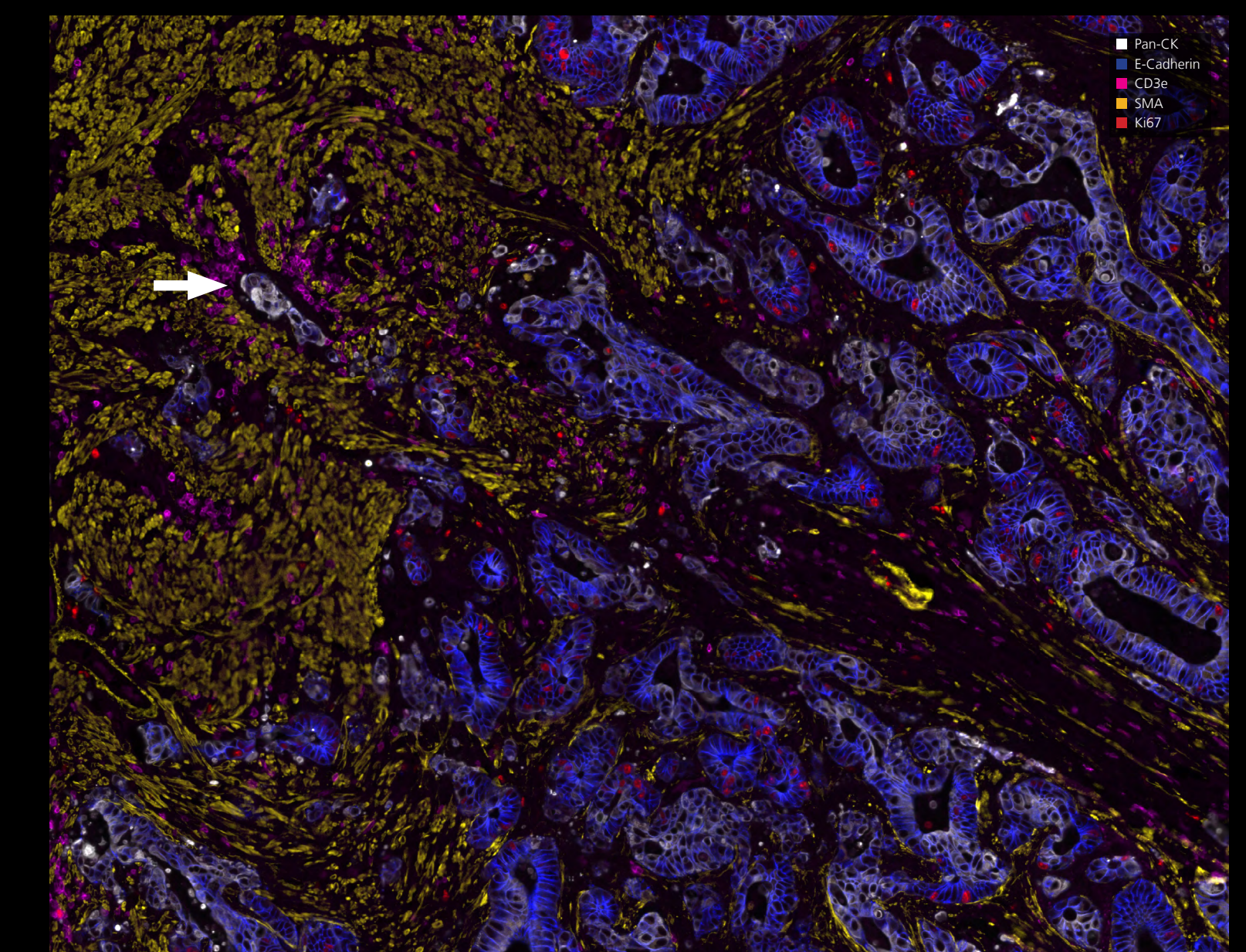
**Fig 5.** Dense collection of CD4+ (light blue) T helper cells with regulatory T cells (FOXP3, red) scattered within.



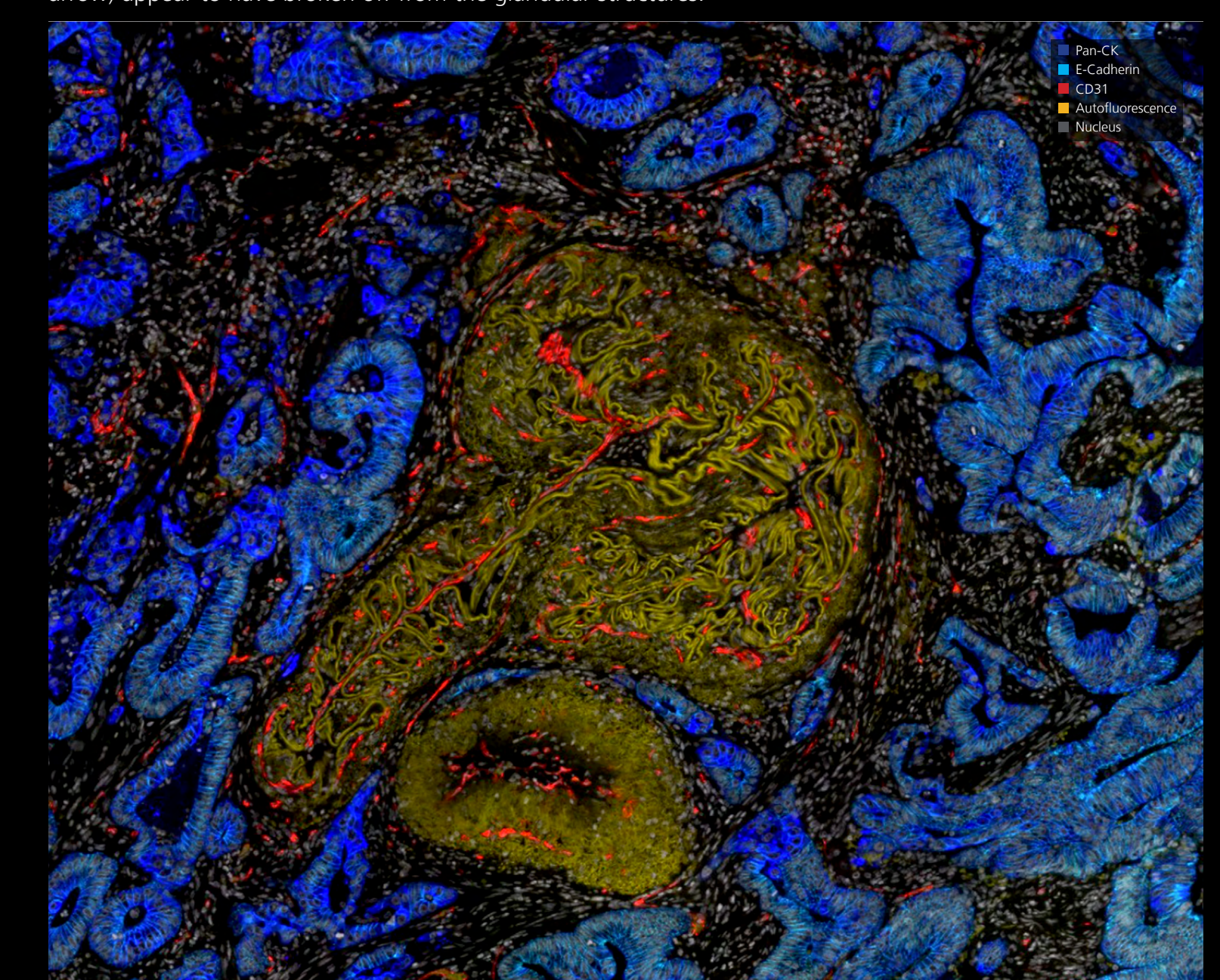
**Fig 7.** Co-localization of PD-L1 on macrophages expressing both CD68 and CD163 (green arrows) and PD-L1+ cells (light blue arrow).



**Fig 4.** Adenocarcinoma cells express membrane cell-cell adhesion glycoprotein E-Cadherin. Tumor cells in the proliferative phase (Ki-67, red arrows) and B cells (CD20, magenta arrow).



**Fig 6.** Multiplexed imaging reveals the invasive border of the tumor where clusters of malignant cells (white arrow) appear to have broken off from the glandular structures.



**Fig 8.** The colon contains nerves controlling peristaltic contraction of the bowel. Nerve fibers identified by autofluorescence, surrounded by cancer cells (cytokeratins, E-cadherin).

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