

# Spatial-omics detection of neuroendocrine involvement in cancer tissue

Scott T. Clarke, Leticia Montoya, Mae Voeun, Chris Vonnegut, Nancie Mooney, Wenjun Zhou, Aleksey Rukavishnikov, Thermo Fisher Scientific, 29851 Willow Creek Road, Eugene, Oregon, USA, 97402

## Abstract

The study of neuroendocrine involvement observed in inflammation, neuronal damage, cancer, and neurodegenerative disease is an emerging field offering new opportunities of intervention and treatment of currently intractable disease. Cancer cells are known to invade the space surrounding neurons (perineuronal invasion) and derive pro-survival and anti-apoptotic benefits from the nerve fibers and their neurotransmitters, secreted amines and peptides<sup>1,2,3</sup>. Formation of new neural tissue is a characteristic of cancer with poor prognosis.

We present a spatial-omics approach of multiplexing immunohistochemistry (IHC) with mRNA *in situ* hybridization (ISH) detection to examine the microenvironment of neuroendocrine involvement and the biomarkers relevant to these tissues and infiltrating immune cells. We image both protein and mRNA abundance and distribution in FFPE tissue with neuroendocrine involvement. ISH detection uses single stranded branched DNA amplification scheme (ss-bDNA) for single molecule detection, while IHC detection uses direct antibody labeling for moderate and high abundant targets and enzymatic signal amplification for low abundant targets. We present protocol modifications with significant improvements minimizing incompatibility between IHC and ISH otherwise resulting in diminished or loss of signal. These modifications retain IHC and ISH signal and minimize auto-fluorescence required for multiplexed workflows. We show multiplex IHC labeling of neural biomarkers such as CD56, chromogranin A (CHGA) and synaptophysin (SYP) in relevant tissue which are sometimes detected in non-endocrine structures and tissues of some tumors, as well as in neurodegenerative disease tissue. The presence of neural mRNA expression (INSM1 and UCHL1), combined with immune markers such as CD68 and proliferation markers of Ki-67 in cancer tissue, reveal the neuroendocrine involvement in the tumor immune microenvironment.

## Introduction

The described workflow and images obtained using the Invitrogen™ EVOS™ S1000 Imaging System which uses spectral unmixing demonstrate the feasibility of combining ISH and IHC to obtain comprehensive analysis in complex tissue structure.

## Materials and methods

8 μm FFPE lung neuroendocrine tumor (NET) sections were deparaffinized, HIER retrieved for 40 minute with high pH buffer at 95° C. Invitrogen™ ReadyLabel™ Alexa Fluor™ 647 Antibody Labeling kit was used to label synaptophysin which was included in a tissue validated antibody cocktail in blocking buffer pre-treated with a RNase inhibitor (Table 1), incubated for 1 hour, cross-linked, then ISH labeled with Thermo Scientific™ ViewRNA Tissue Assay Fluorescence Kit. Spectral unmixing using single color controls for each of the markers and image acquisition was performed on the Invitrogen EVOS S1000 Imaging System. All reagents and equipment used were obtained from the Thermo Fisher Scientific catalog except the crosslinking aldehyde solutions.

Images acquired with 40x objective of the S1000 except where otherwise noted.

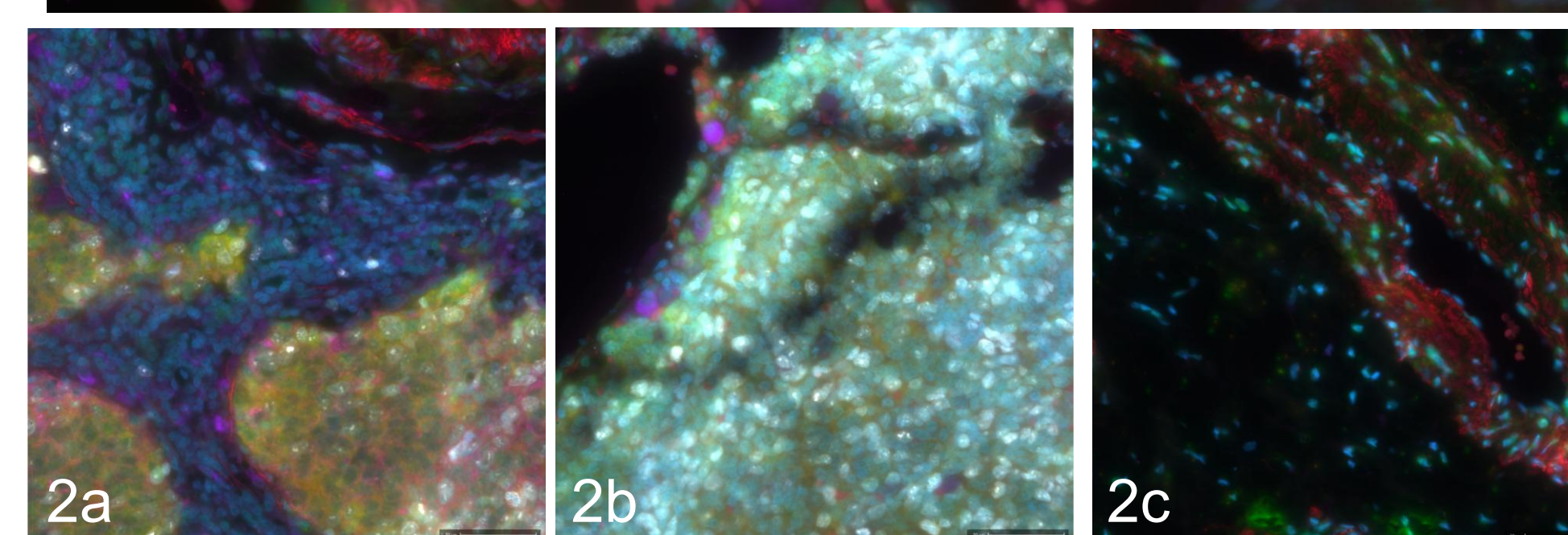
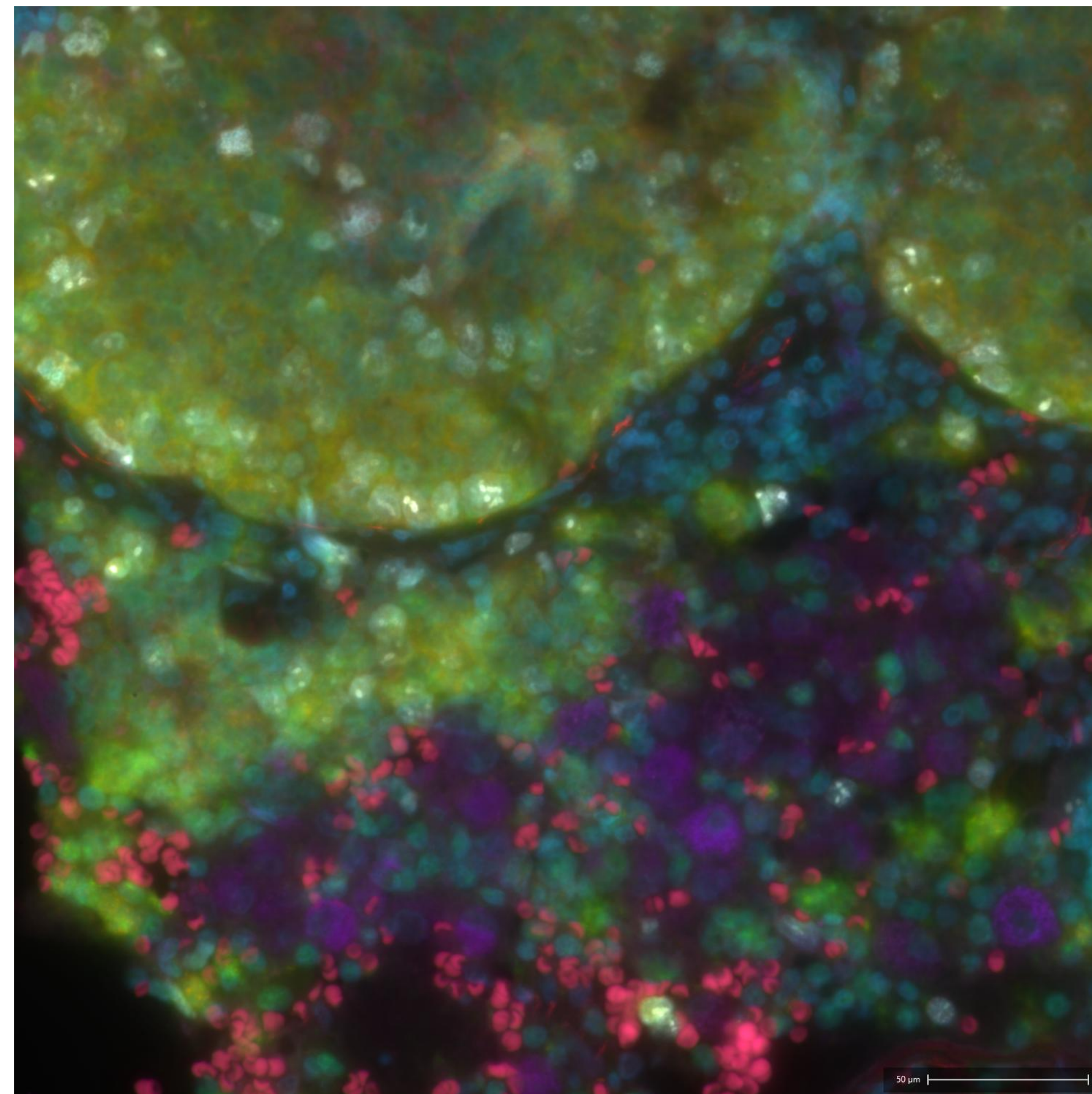
**Table 1** Panel Design

color	fluorophore	target	SKU	clone	conc. μg/mL
blue	DAPI	DAPI	D21490	N/A	1.4
cyan*	Alexa Fluor 420	Vimentin	758-9897-82	V9	2
green	Alexa Fluor 488	INSM1 RNA ISH	QV10700	N/A	N/A
orange	Alexa Fluor 555	UCHL1 RNA ISH	QV10700	N/A	N/A
red	Alexa Fluor Plus 594	α - smooth muscle actin	755-9760-82	1A4	1
magenta	Alexa Fluor Plus 647	CD56*	756-5603-94	123C3	5
magenta	Alexa Fluor 647	SYP*	R10710	7H12	5
magenta	Alexa Fluor 647	CHGA**	MA5-13096	LK2L10	12
violet	Alexa Fluor 700	CD68	56-0688-82	KP1	5
white	Alexa Fluor Plus 750	Ki-67	757-5698-82	SolA15	10

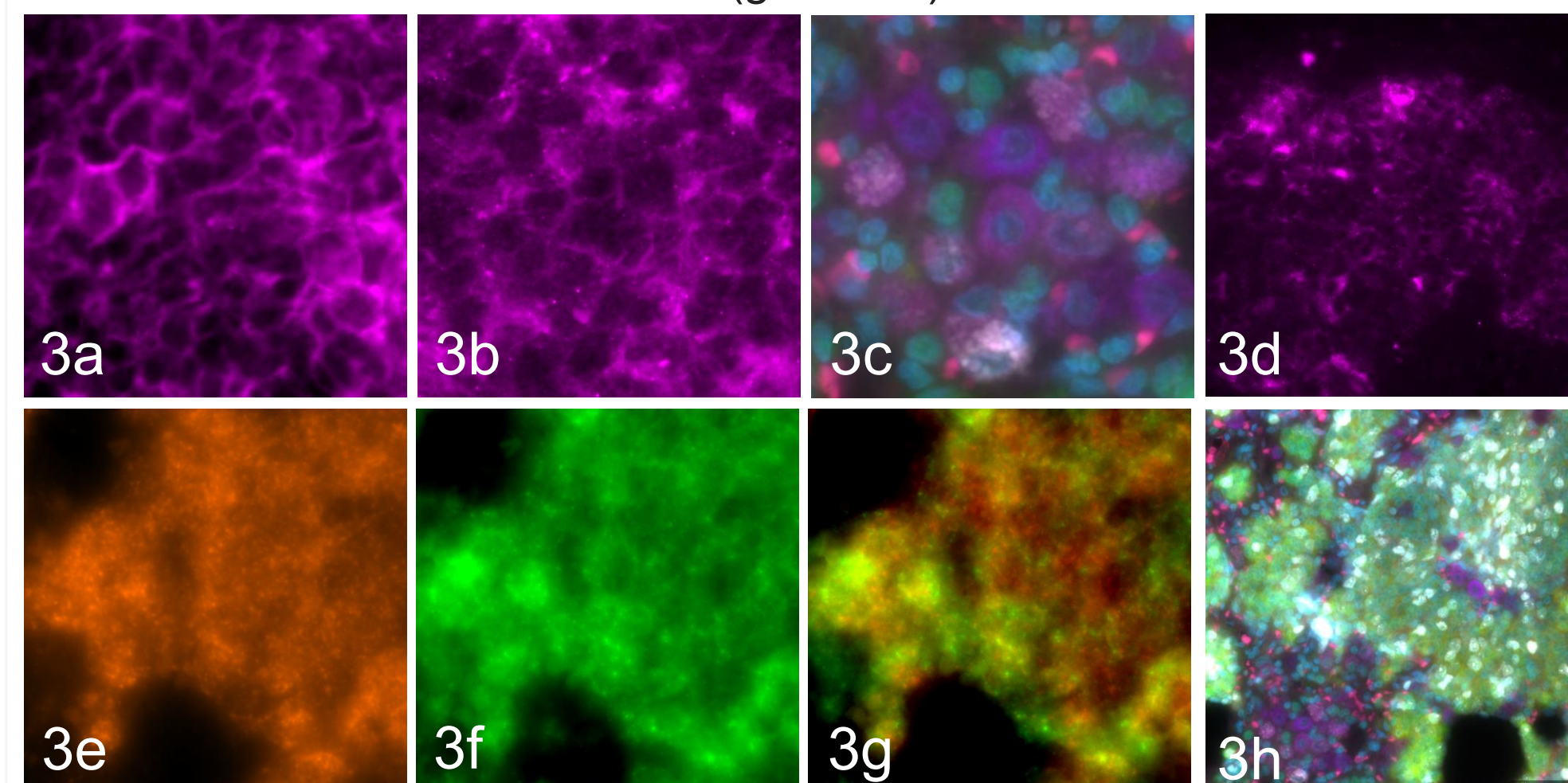
\* Not shown  
 # Stained as single color IHC image  
 \* Either CD56 or SYP but not both in one image  
 \* Labeled with ReadyLabel™ Antibody Labeling kit

## Results

**Figure 1.** Neuroendocrine lung tumor showing proliferating cells (white), tumor associated macrophages (TAMs) (violet), INSM1 and PGP9.5 RNA expression (green and orange) and red blood cells (carmine).



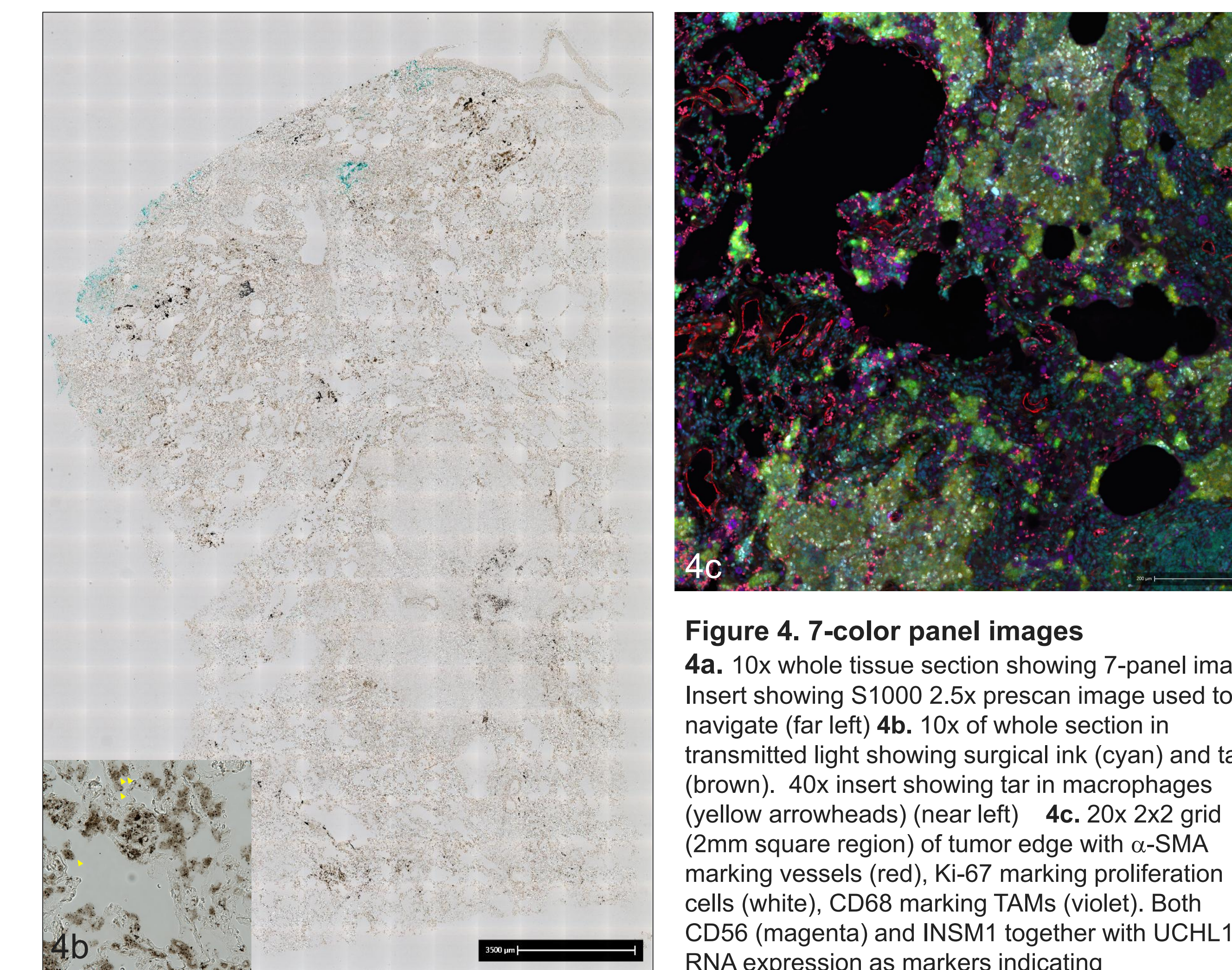
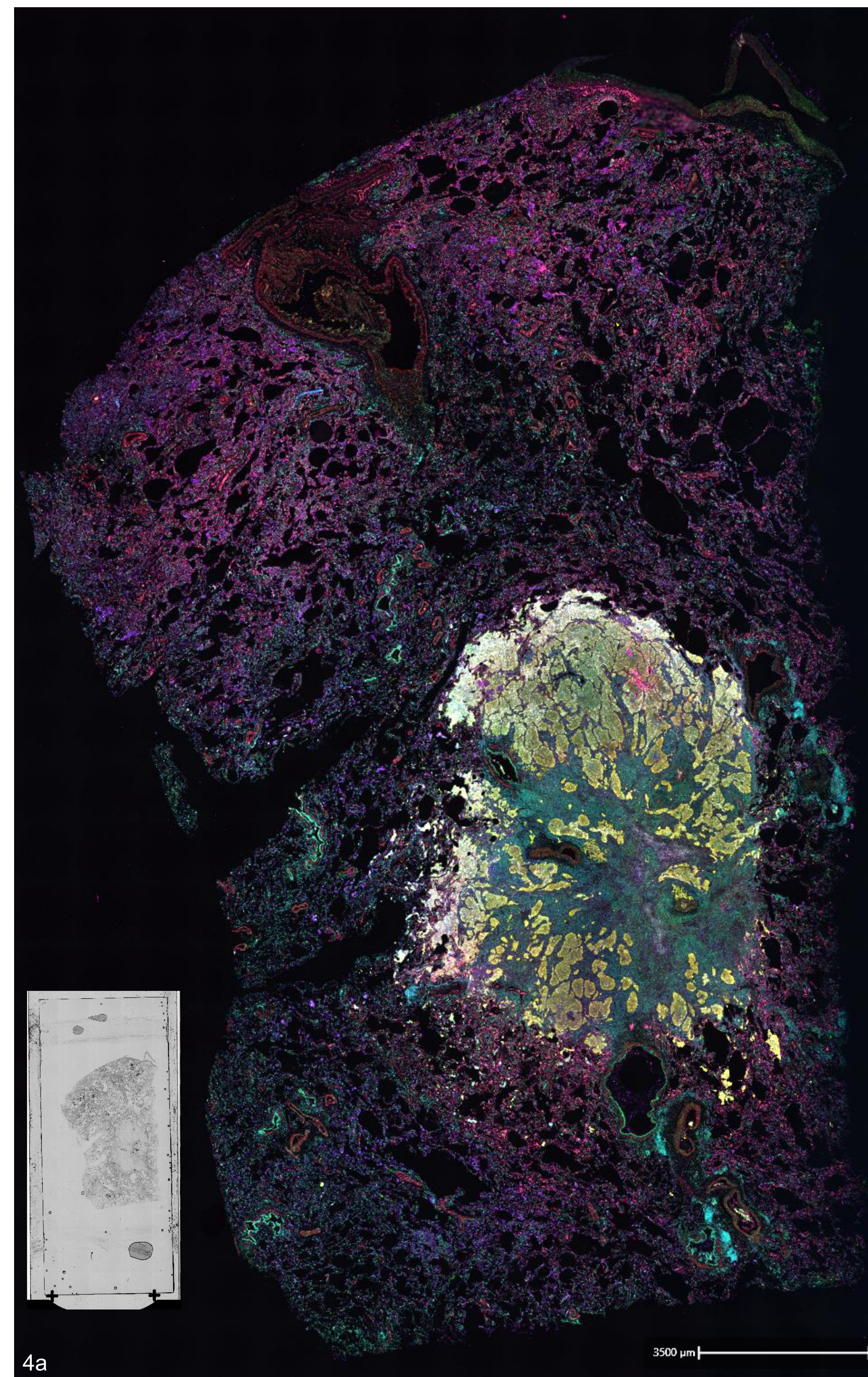
**Figure 2. Regions of the tissue** **2a** Tumor center showing abundance of SYP, INSM1 and PGP9.5 signal. **2b** Tumor edge showing dense regions of Ki-67 positive cells (white). **2c** Normal lung showing sparsely distributed nuclei (blue) along with vascular indicated by SMA (red) and some autofluorescence from tar (greenish)



**Figure 3. NET relevant markers**

**3a.** CD56 (NCAM) **3b.** synaptophysin (SYP) **3c.** TAM with Ki-67 cell fragments (violet cells containing white granules). **3d.** CHGA **3e.** UCHL1 RNA ISH **3f.** Insulinoma associated protein 1 (INSM1) RNA ISH **3g.** INSM1 + UCHL1 showing distinctive RNA puncta signal in both channels. **3h.** 7-color panel

## Panel of lung NET tissue section



**Figure 4. 7-color panel images**  
**4a.** 10x whole tissue section showing 7-panel image. Insert showing S1000 2.5x prescan image used to navigate (far left) **4b.** 10x of whole section in transmitted light showing surgical ink (cyan) and tar (brown). 40x insert showing tar in macrophages (yellow arrowheads) (near left) **4c.** 20x 2x2 grid (2mm square region) of tumor edge with α-SMA marking vessels (red), Ki-67 marking proliferation cells (white), CD68 marking TAMs (violet). Both CD56 (magenta) and INSM1 together with UCHL1 RNA expression as markers indicating neuroendocrine involvement within the tumor mass (green and orange) (above).

## Conclusions

- Invitrogen™ EVOS™ S1000 Spatial Imaging System with spectral unmixing used for multi-omics image acquisition of clinical FFPE NET lung tissue showed co-localization of IHC and ISH neuroendocrine markers within the tumor mass, high proliferation along the tumor edges and TAMs adjacent to and infiltrated within the tumor edges, and locally abundant angiogenesis as indicated by α-SMA. TAMs show evidence of diverse phagocytosed material including Ki-76 positive cell fragments and cigarette tar.
- Detection and treatment of cancers with neuronal involvement is an emerging approach to cancer immunotherapy treatment. Multiplexing relevant markers of IHC and ISH using spatial-omics is demonstrated here to reveal neuroendocrine involvement in tumor tissue. Using NET tissue demonstrates the feasibility of detecting neuroendocrine involvement in tumor tissue not normally recognized as having neuronal involvement.

## References

- Li J. The association between the neuroendocrine system and the tumor immune microenvironment: Emerging directions for cancer immunotherapy. *BBA - Reviews on Cancer.* (2023) 1878 189007
- Oronsky B. Nothing But NET: A Review of Neuroendocrine Tumors and Carcinomas. *Neoplasia.* (2017) 19(12), pp. 991-1002
- Mukherjee S. Various Markers of Neuroendocrine Tumor: A Narrative Review. *Cureus.* (2024) 16(8):e67493

## Acknowledgements

We would like to thank Drs. Clair Murphy and Deven Smith from Immunology Consultants for supplying tissue and expert advice.