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Three-Dimensional Spatial Characterization of Tumor Spread Through Air Spaces by Tissue Clearing and Volumetric Imaging

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Introduction

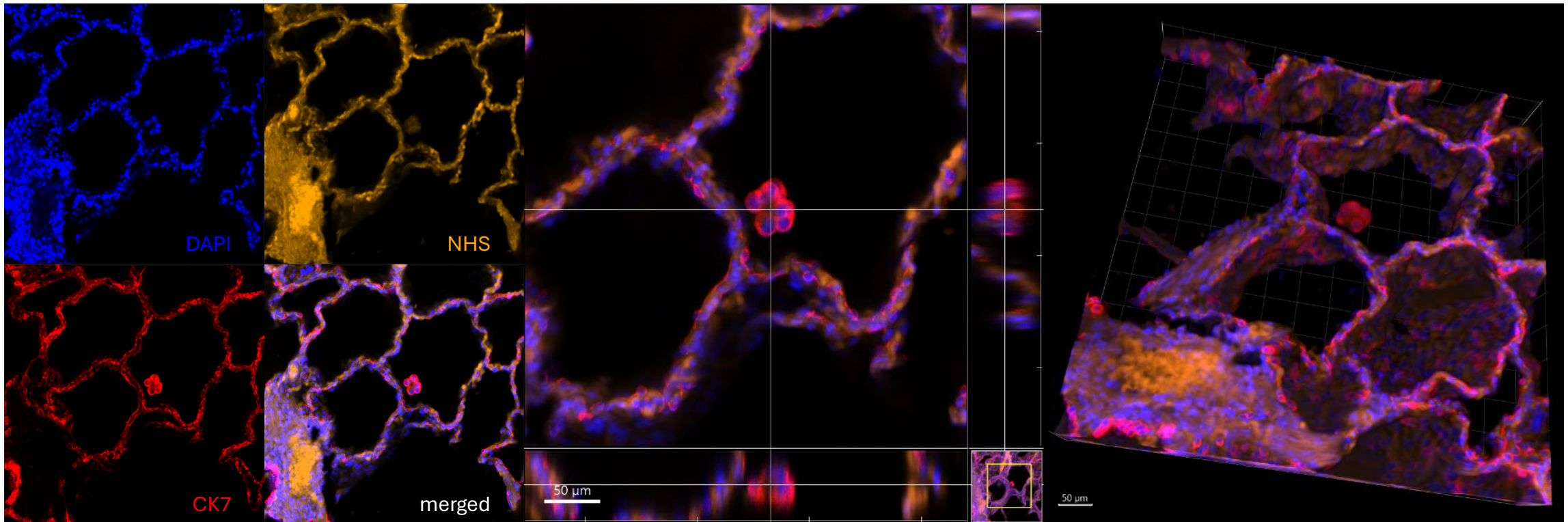
- **STAS (spread through air spaces):** tumor cell clusters observed in the alveolar spaces beyond the main tumor edge.
- How STAS clusters are spatially organized and interact with surrounding structures has not been fully characterized.
- **Aim of this study:**
 - To develop a method for directly visualizing STAS as a continuous three-dimensional volume at cellular resolution.
 - To examine its structural relationships within the alveolar microenvironment.

Discussion and Conclusion

- This study demonstrates the feasibility of **tissue clearing-based 3D imaging for visualizing STAS** in lung adenocarcinoma, enabling direct spatial observation within a continuous tissue volume.
- Detailed **characterization of the contact patterns** between STAS clusters and the alveolar wall may provide a basis for further understanding of the mechanisms underlying tumor cell survival within the airspace.

Results

◆ Three-dimensional visualization of a STAS cluster in a continuous tissue volume



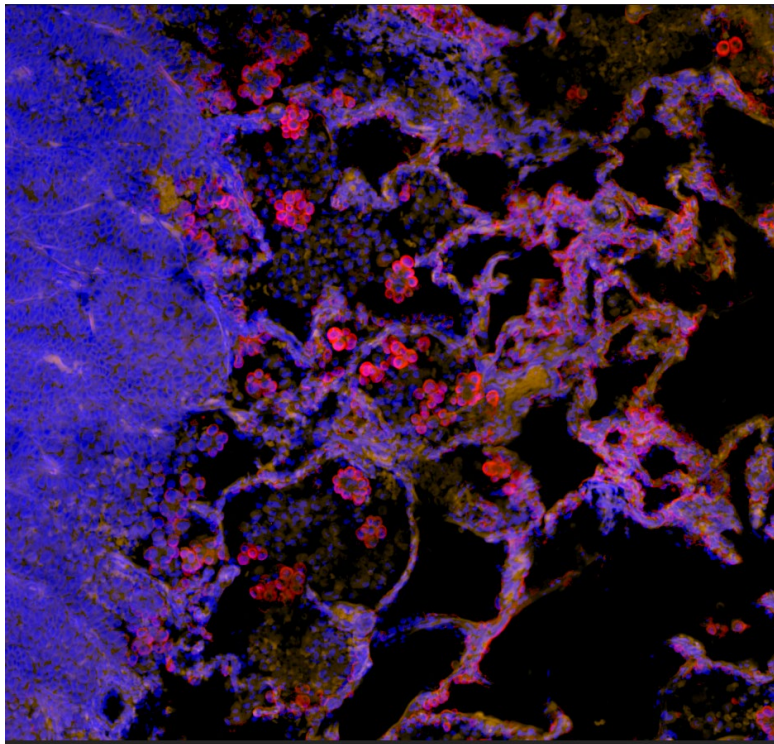
Cross-sectional images of each channel

Orthogonal section view (XY, XZ, YZ)

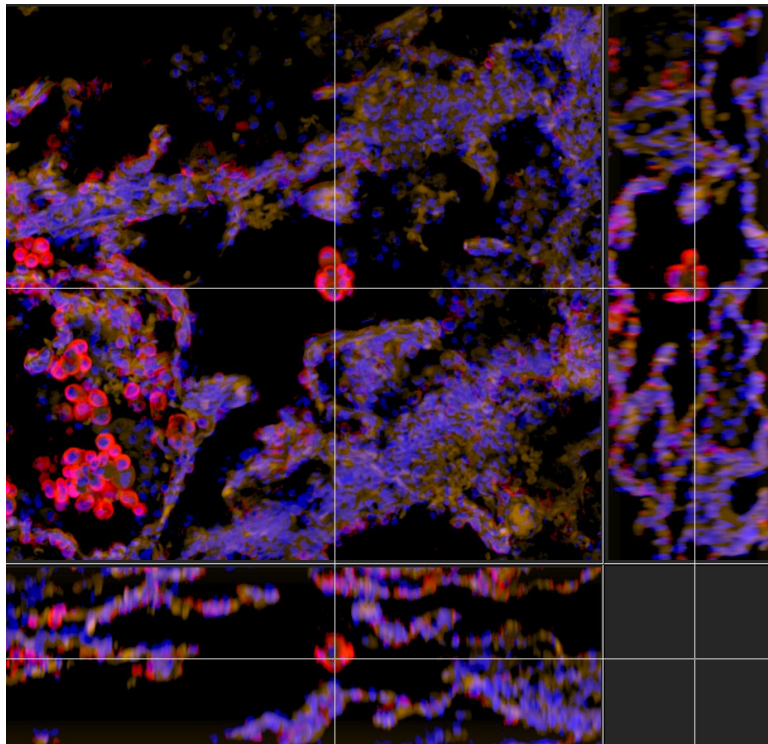
Three-dimensional view

Results

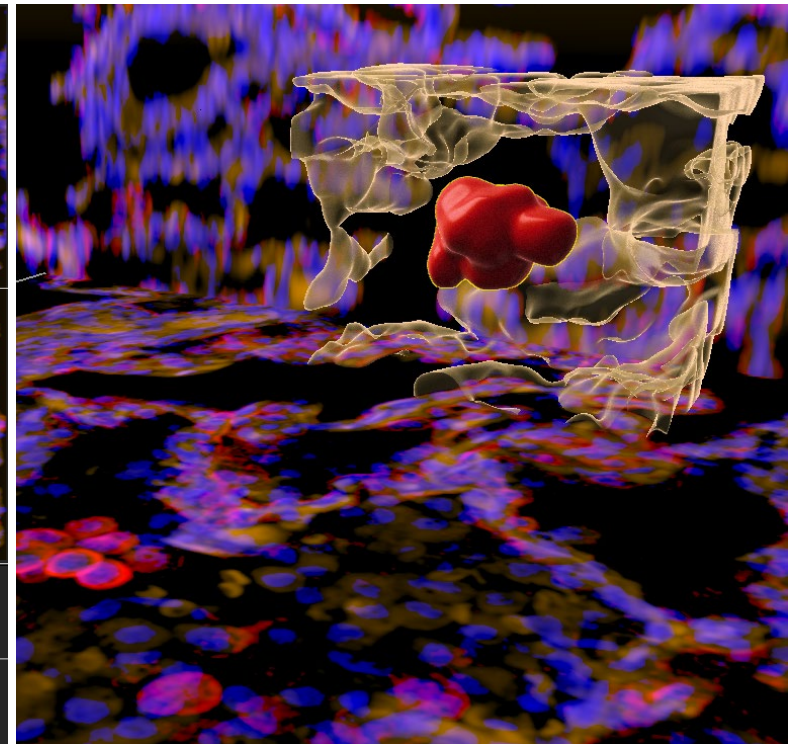
◆ Three-dimensional observation of STAS clusters in the alveolar spaces



XY-section of a volumetric image
revealing multiple STAS clusters



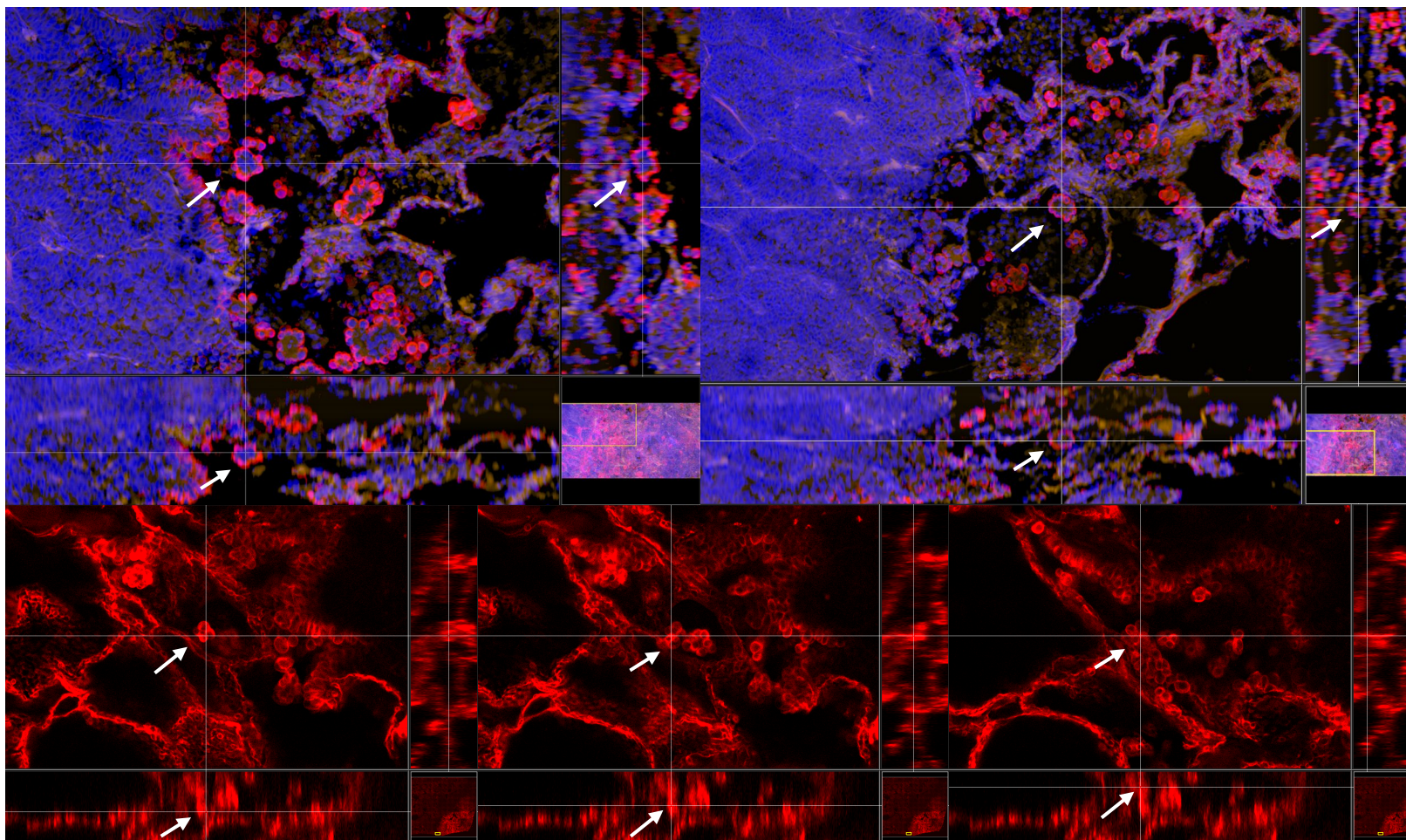
Observation of a STAS cluster in three
dimensions (orthogonal section view)



Three-dimensional view of STAS and its
neighboring alveolar wall
(surface rendering)

Results

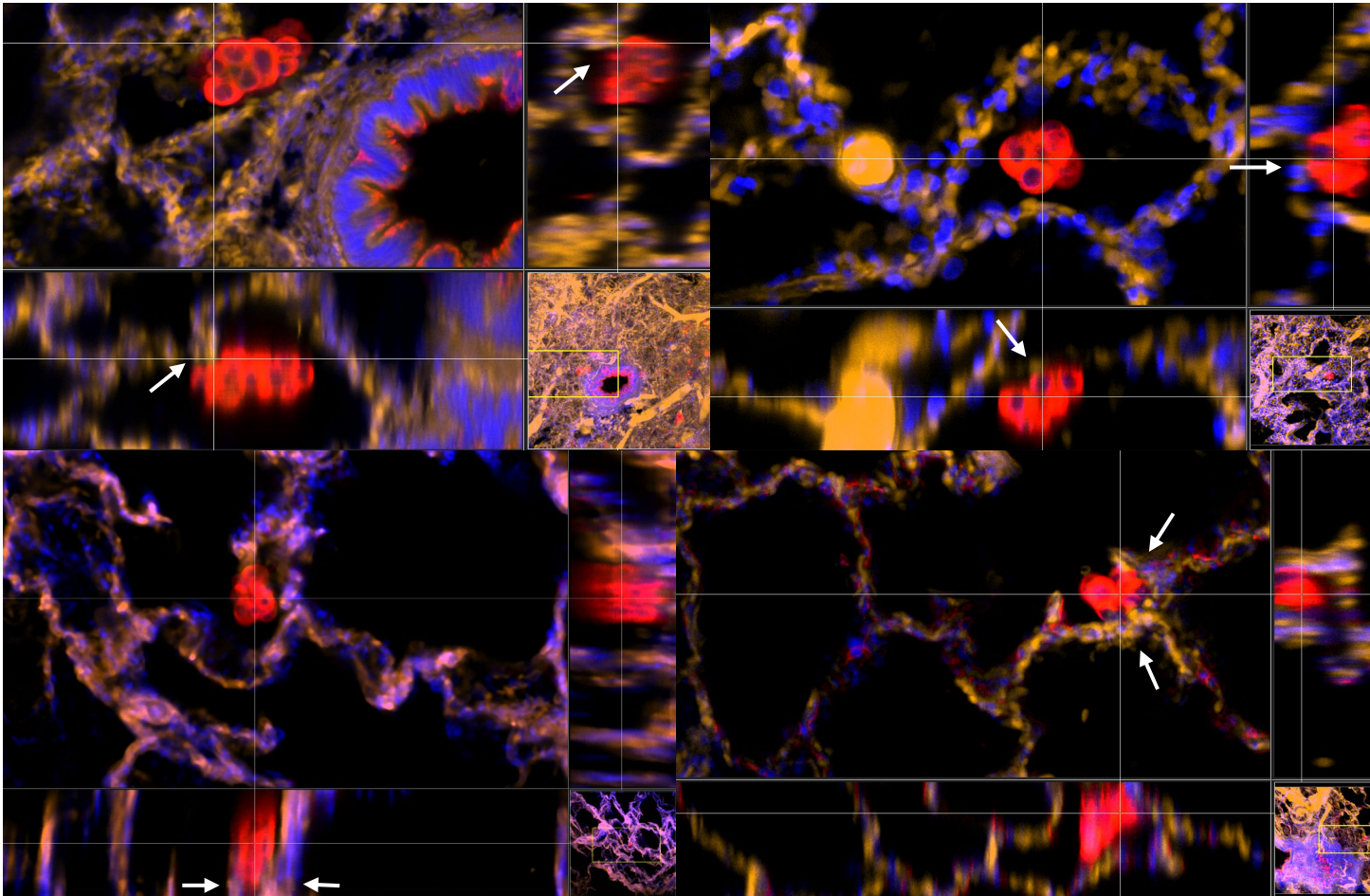
◆ Three-dimensional analysis of STAS near the main tumor



- Examples of STAS clusters (arrows) located near the main tumor without direct continuity
- A tumor cluster (arrows) that appear as discrete STAS near the main tumor on a cross-section (left) is found to be directly continuous with the main tumor at different z-levels (right)

Results

◆ Spatial relationship between STAS and the alveolar wall



➤ STAS clusters demonstrating focal contact with the alveolar wall (arrows)

➤ STAS clusters exhibiting broader areas of contact with the alveolar wall

Methods

❖ Specimens

- Surgically resected lung adenocarcinoma with STAS ($n=6$)
- 500 μm -thick tissue slabs from representative formalin-fixed paraffin-embedded blocks

❖ Tissue clearing and staining

- Modified SCARF¹ (Sodium Cholate Assisted Removal of lipid for Fluorescent imaging of deep biological tissues) protocol
- Additional passive delipidation using OptiMuS-Prime² solution
- DAPI (nuclear visualization), NHS-ester 647 (total protein staining), CK7-Alexa Fluor 594 conjugate (epithelial cell labeling)
- Refractive index matching using OptiMuS³ solution (1.47)

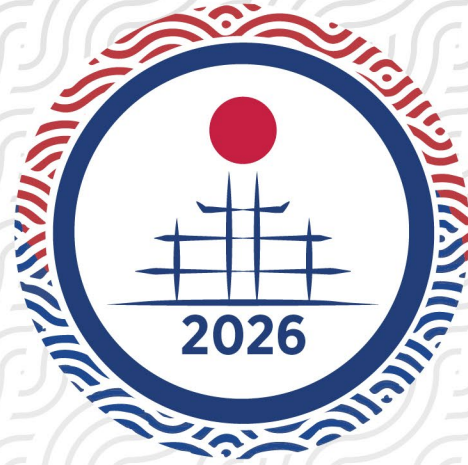
❖ Volumetric imaging

- Confocal microscopy (Nikon C2Si and Andor BC43) with 10X objective
- Three-dimensional rendering and analysis using Imaris software

References and Acknowledgements

1. Na M, Kim K, Oh K, Choi HJ, Ha C, Chang S. Sodium cholate-based active delipidation for rapid and efficient clearing and immunostaining of deep biological samples. *Small Methods*. 2022;6(1):2100943.
2. Kim K, Lee K, Kang T, et al. A novel protein-preserving passive tissue clearing approach using sodium cholate and urea for whole-organ imaging. *Exp Mol Med*. 2025;57(10):2292–2304.
3. Kim K, Na M, Oh K, Cho E, Han SS, Chang S. Optimized single-step optical clearing solution for 3D volume imaging of biological structures. *Commun Biol*. 2022;5(1):431.

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Thank You.●