

# TEM study of Alzheimer's disease lesion proteins

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**Alzheimer's disease (AD) is ranked as the seventh leading cause of death in the United States and is the most common cause of dementia among older adults.**

**The impact of AD to public health is going to get worse as the population ages: AD is predicted to affect almost 15 million Americans by 2050.**

**The hallmark neuropathological lesions of AD are amyloid-beta ( $A\beta$ ) plaques and hyperphosphorylated tau tangles. Other proteins, including Apolipoprotein E (ApoE), are also known to be aberrantly aggregated.**

**Not much study has been done on the ultrastructure, subcellular localization, and development of these aggregates in human brain.**

**Our goal is to study the ultrastructural feature of these aggregates focusing on detecting early stage of aggregates and protein interactions.**

**Amyloid-beta plaques, tau tangles, and ApoE**

## **Immunogold-labelling Transmission Electron Microscopy (TEM)**

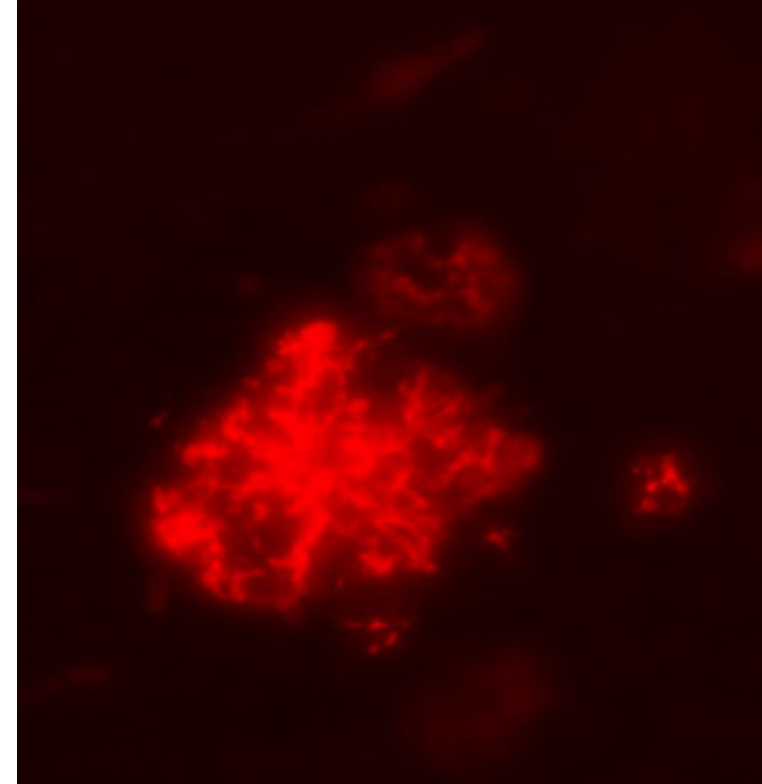
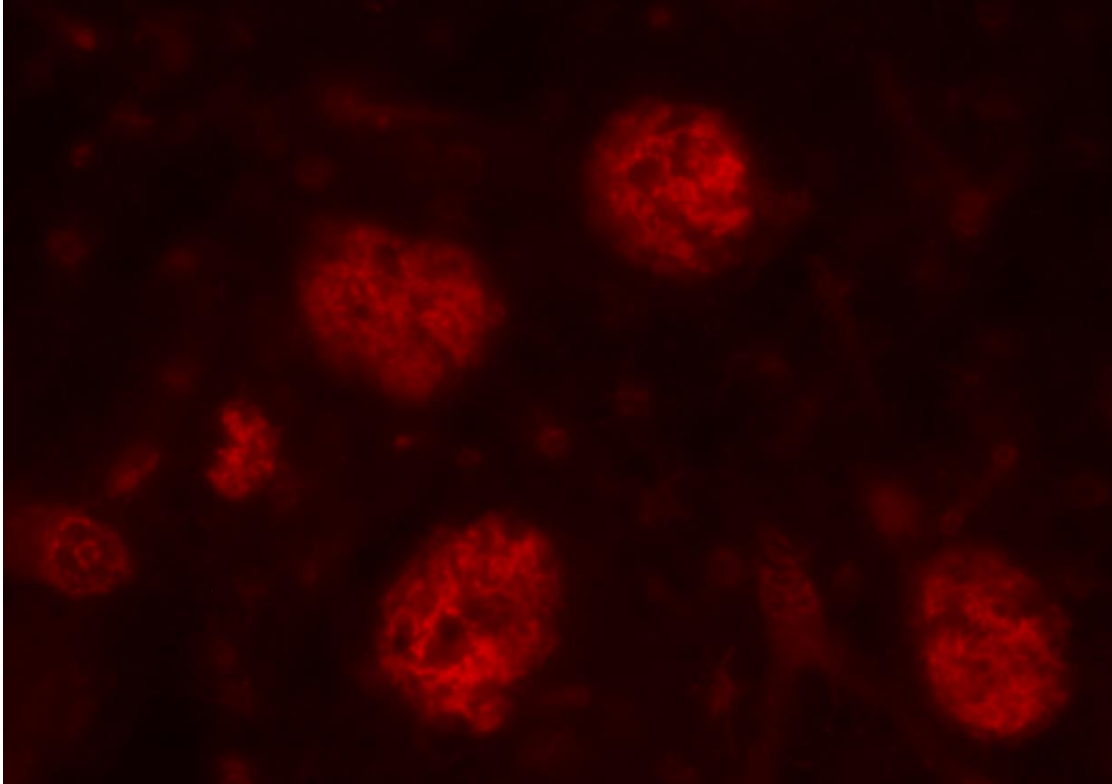
**Immunogold EM is a powerful technique that combines the ability of an antibody-labeling and ultrastructural resolution of EM, providing detailed information on the subcellular localization of a labeled protein**

**Ultra-small (0.8 nm) gold labeling in conjugation with silver enhancement**

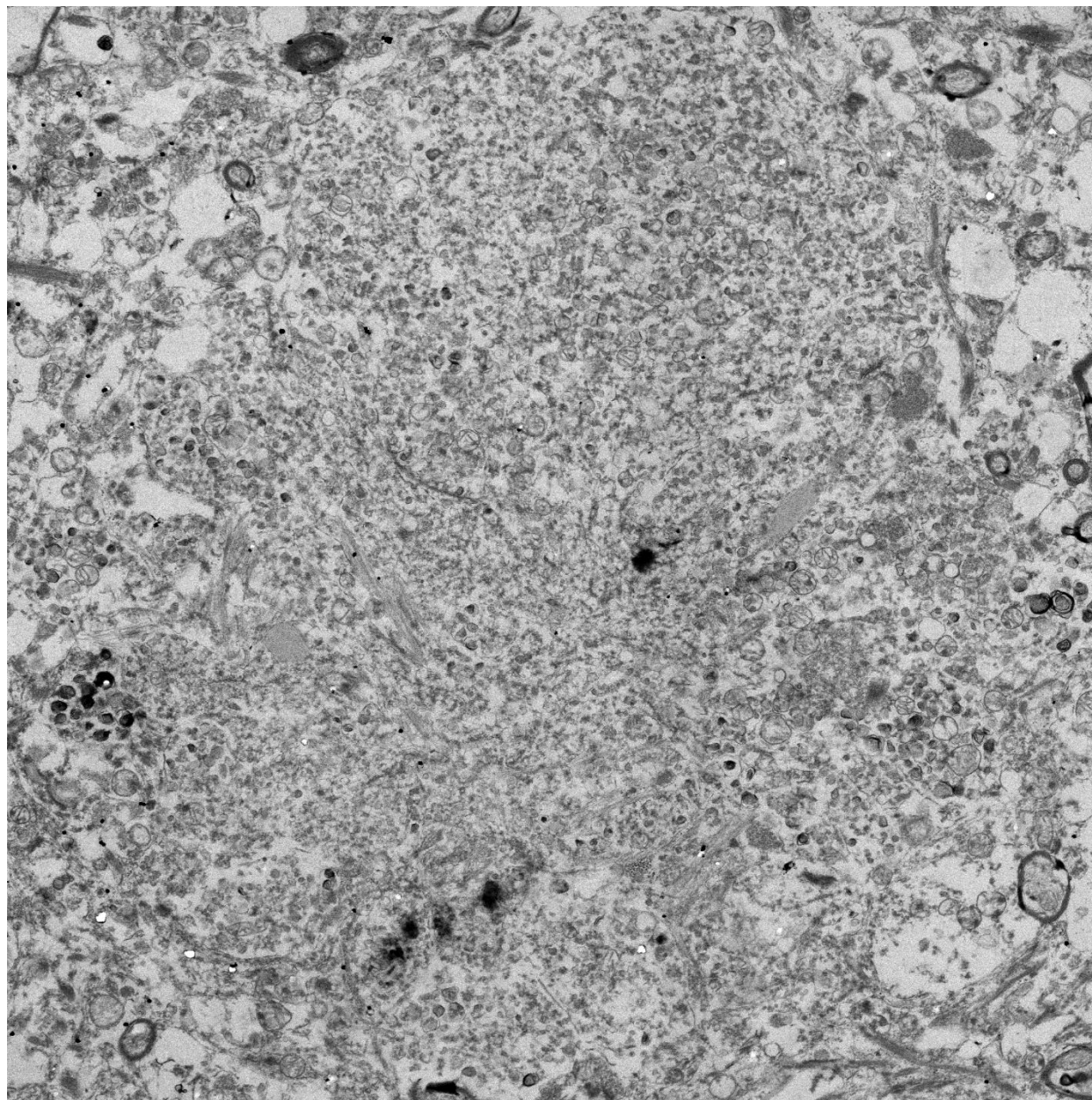
**The ultra-small gold particles allow for better tissue penetration, and the silver enhancement makes them visible under EM**

**Immunogold EM can be done pre-tissue fixation or post-tissue fixation**

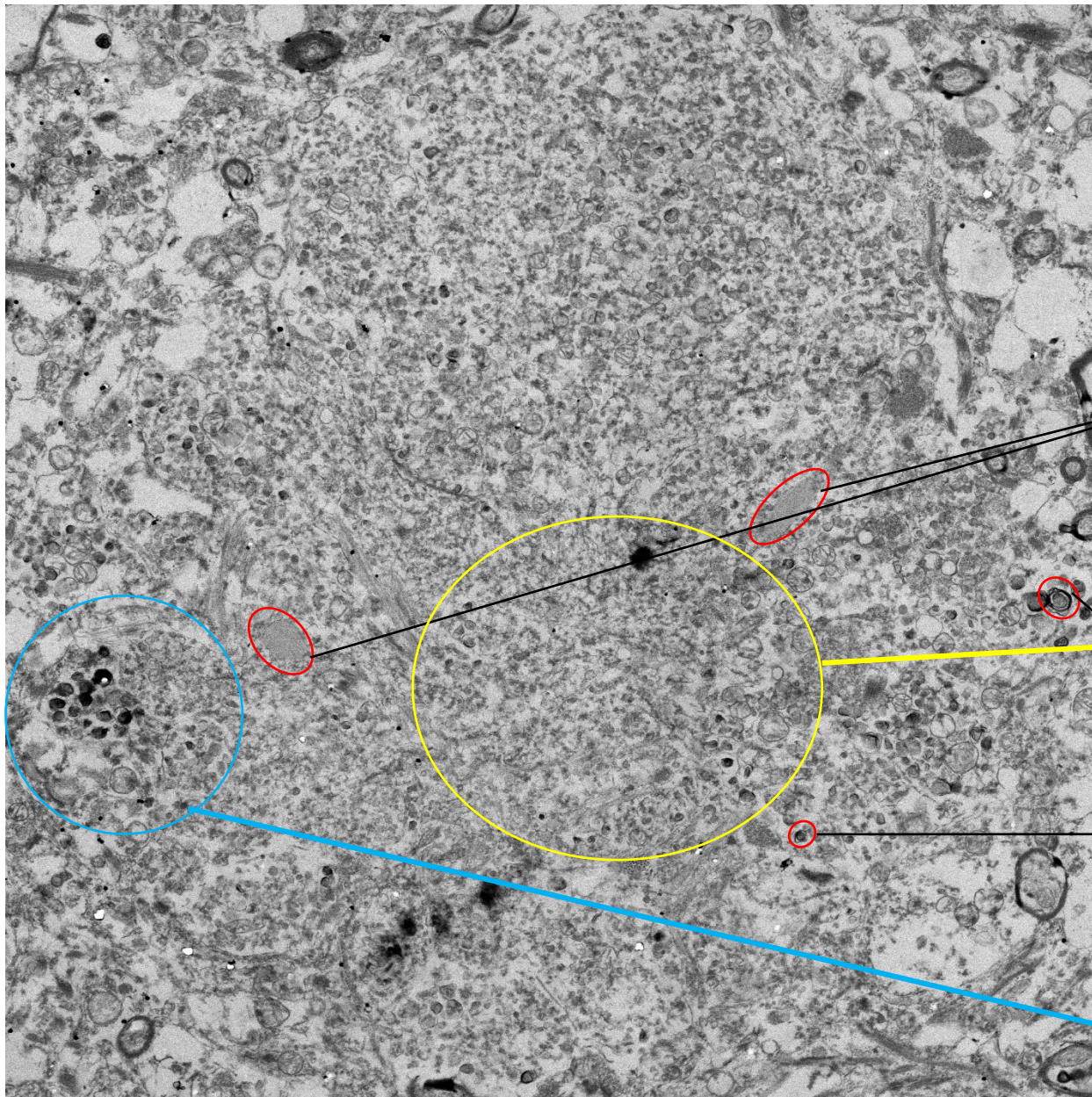
## Plaques in human brain-immunofluorescence-light microscope



# A plaque imaged by TEM



Size	Dwell	HT	Scan fov	STEM Mag	Pixel size	5 $\mu$ m
2048	5.00 $\mu$ s	200 kV	22.7 $\mu$ m	4.41 kx	11.06 nm	<hr/>



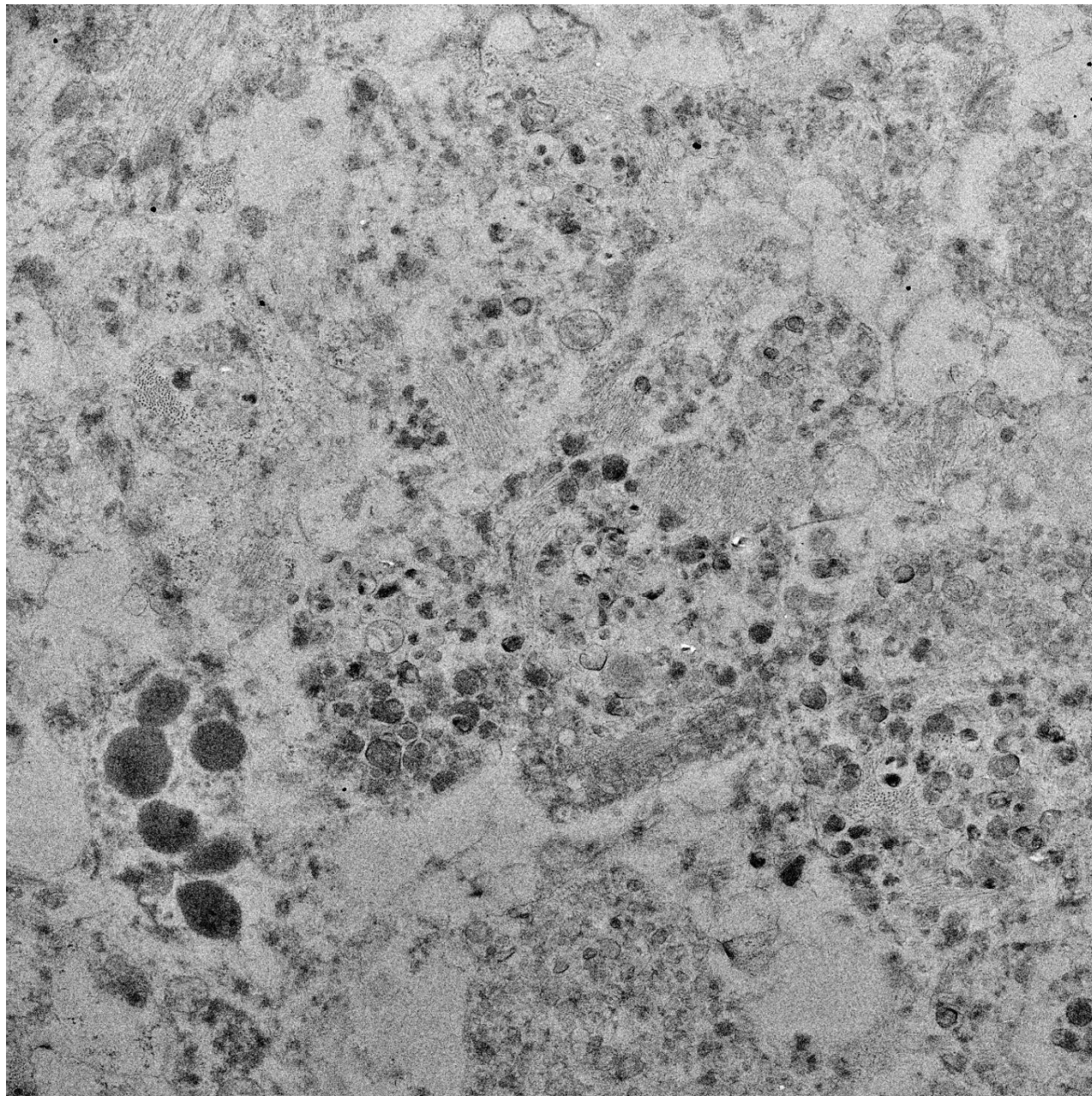
**Neurofilaments**

**Amyloid plaque core**

**Laminated body**

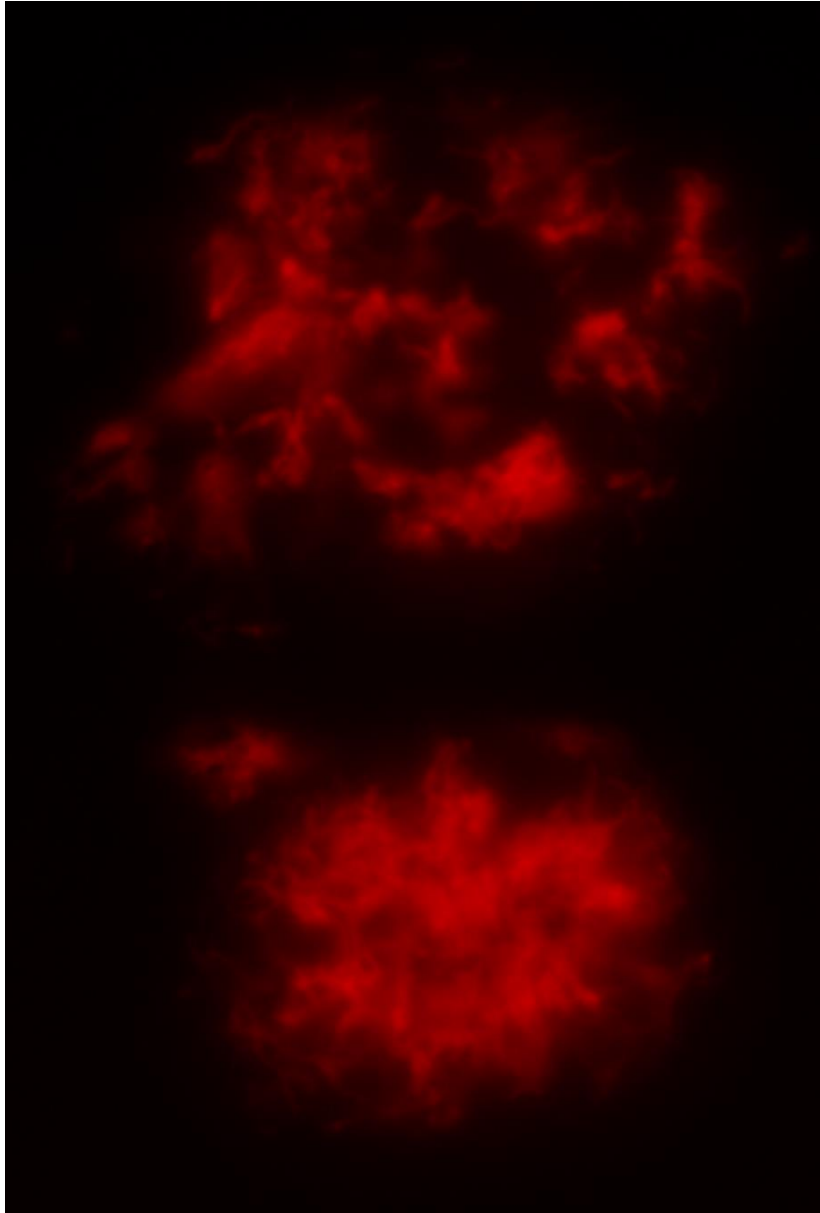
**Degenerated neurites**

Size	Dwell	HT	Scan fov	STEM Mag	Pixel size	5 $\mu$ m
2048	5.00 $\mu$ s	200 kV	22.7 $\mu$ m	4.41 kx	11.06 nm	

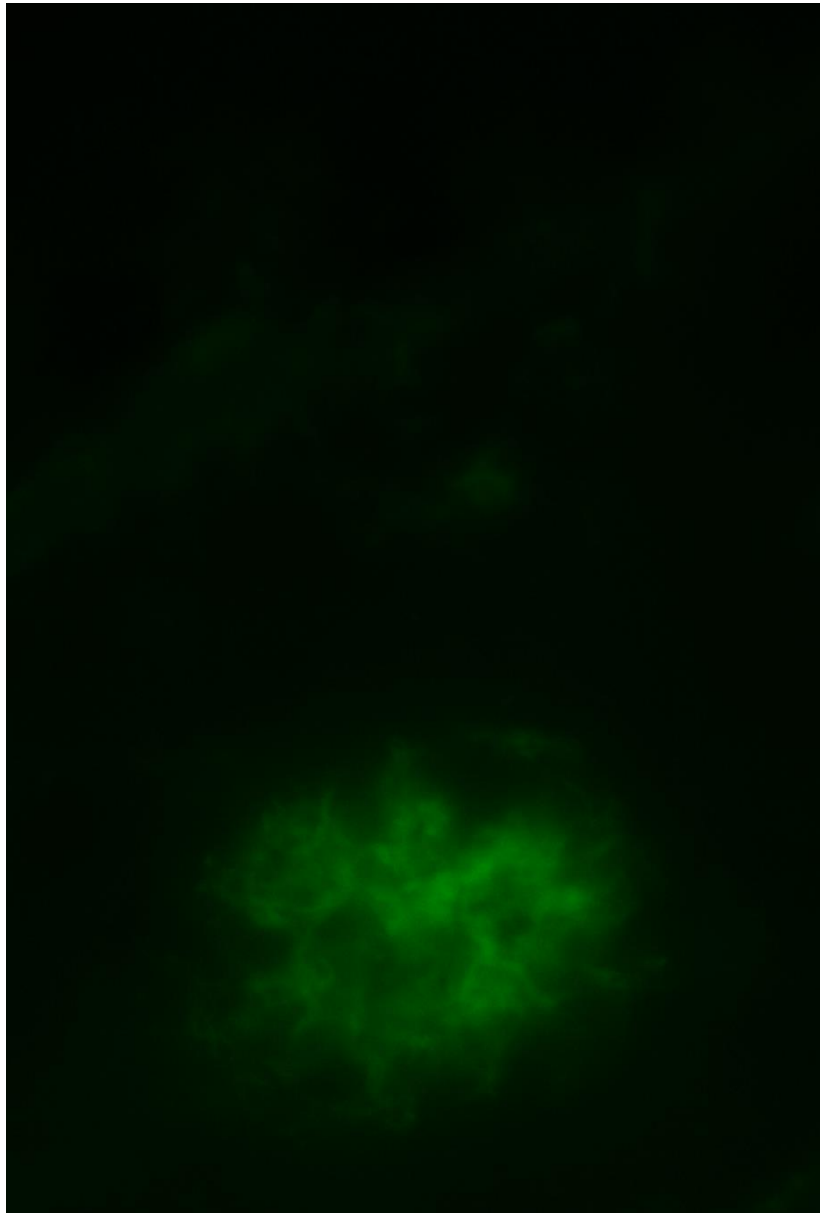


Size	Exp	Mag	Spot	HT	Pixel size	Fov	2 $\mu$ m
4096	5 s	4300 x	6	200 kV	3.477 nm	14.24 $\mu$ m	Ceta

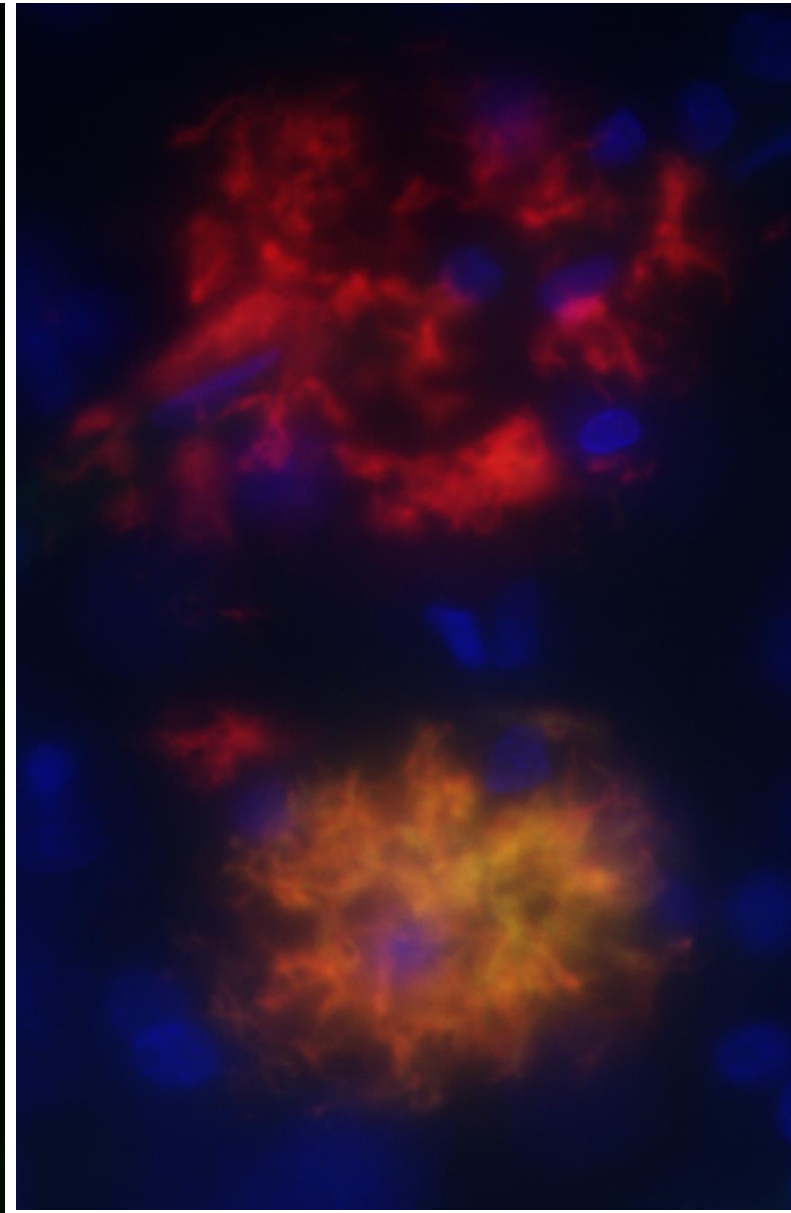
# ApoE proteins are often co-aggregated with plaques



**NAB228 (staining plaques)**



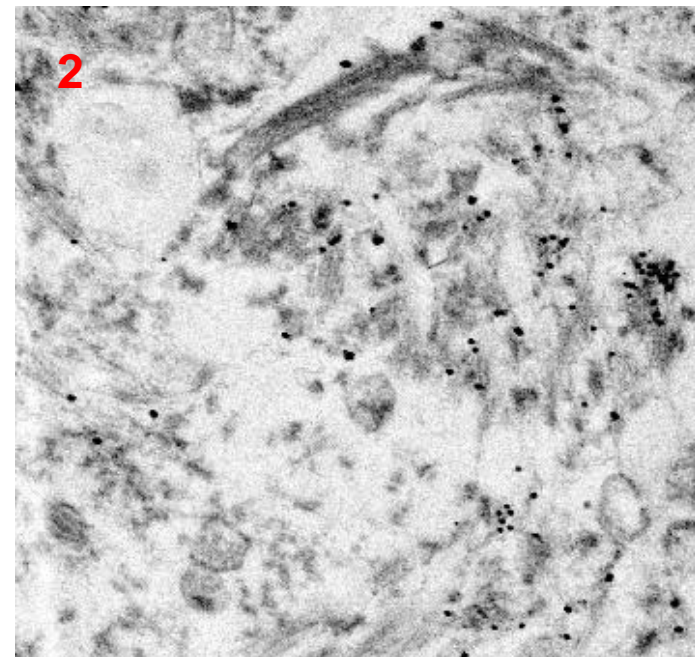
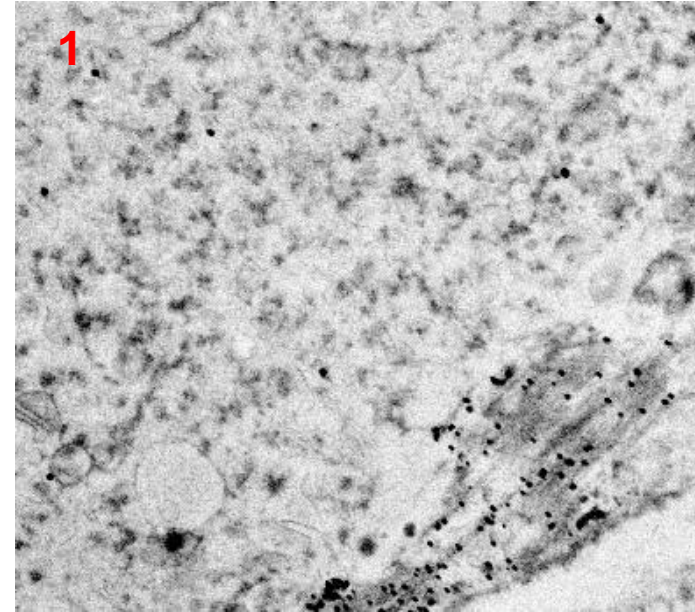
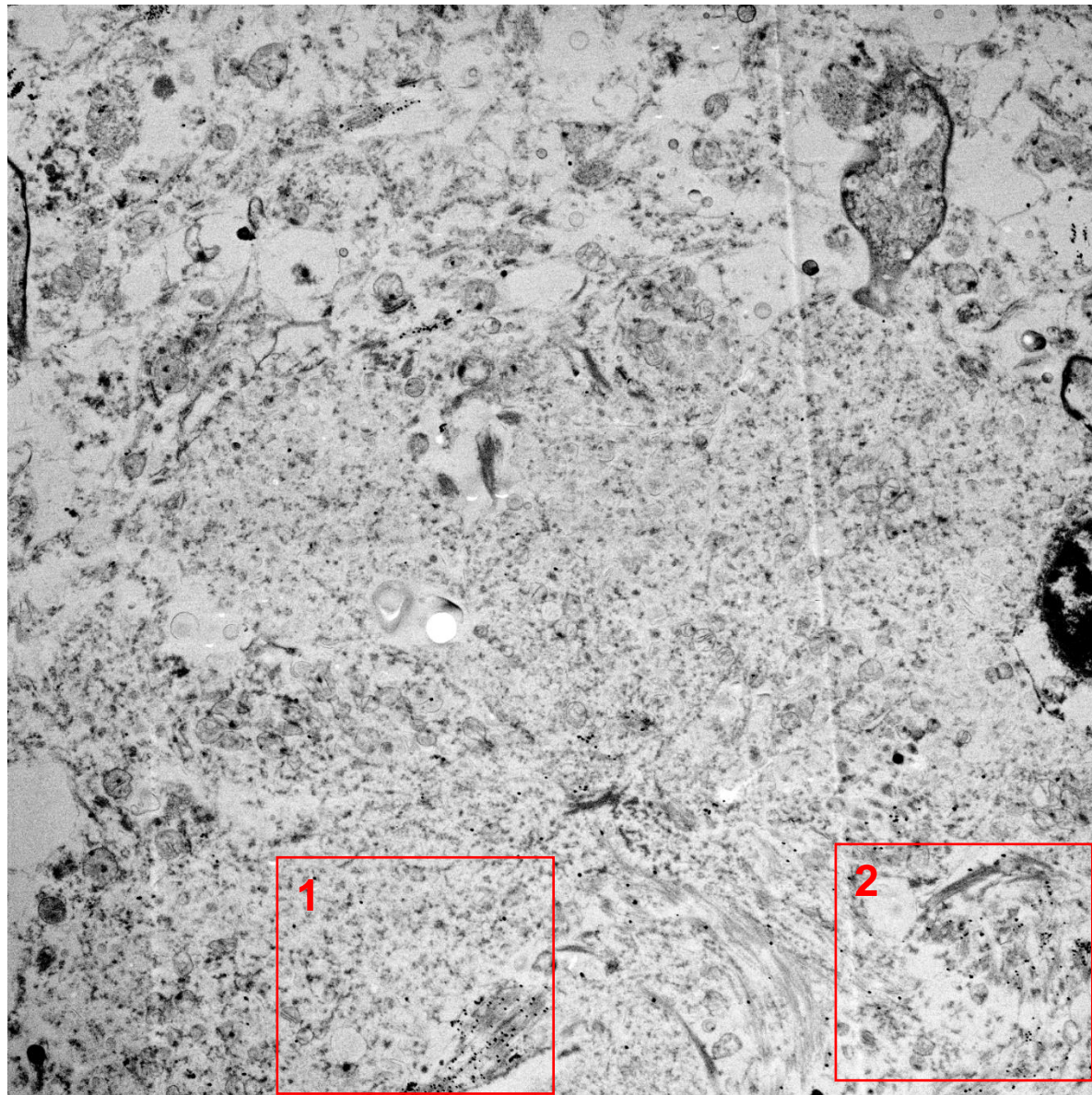
**ApoE (staining ApoE)**



**Overlap**

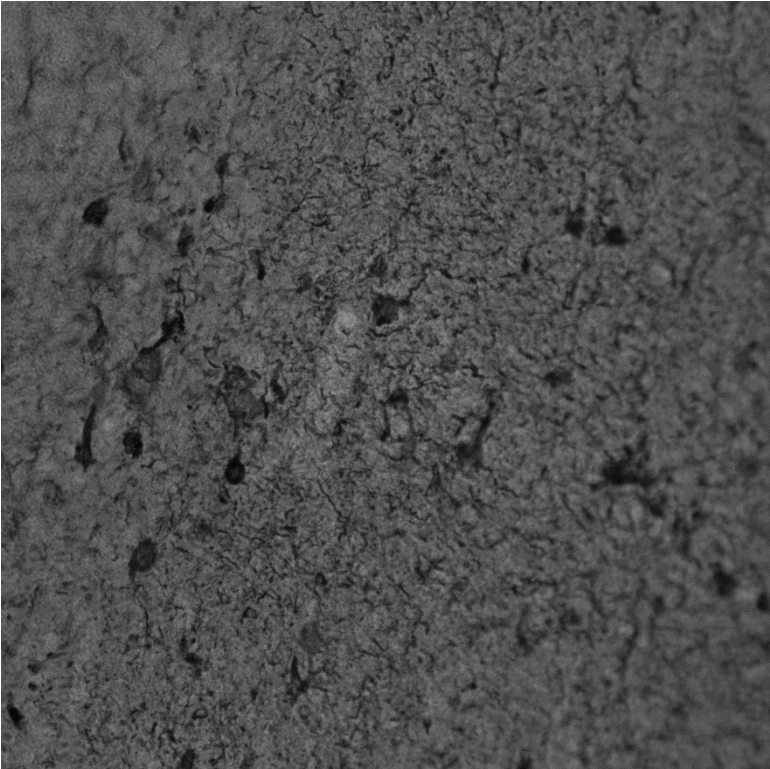


# Human Brain\_APOE\_Immunogold\_Amyloid Plaque

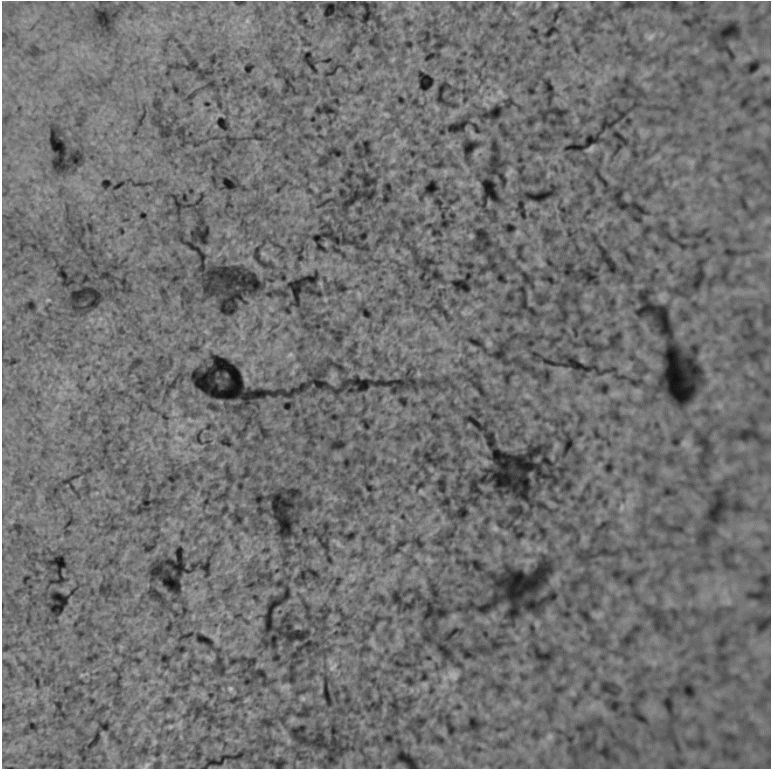


Size	Dwell	HT	Scan fov	STEM Mag	Pixel size	5 $\mu$ m
2048	50.0 $\mu$ s	200 kV	22.7 $\mu$ m	4.41 kx	11.06 nm	HAADF

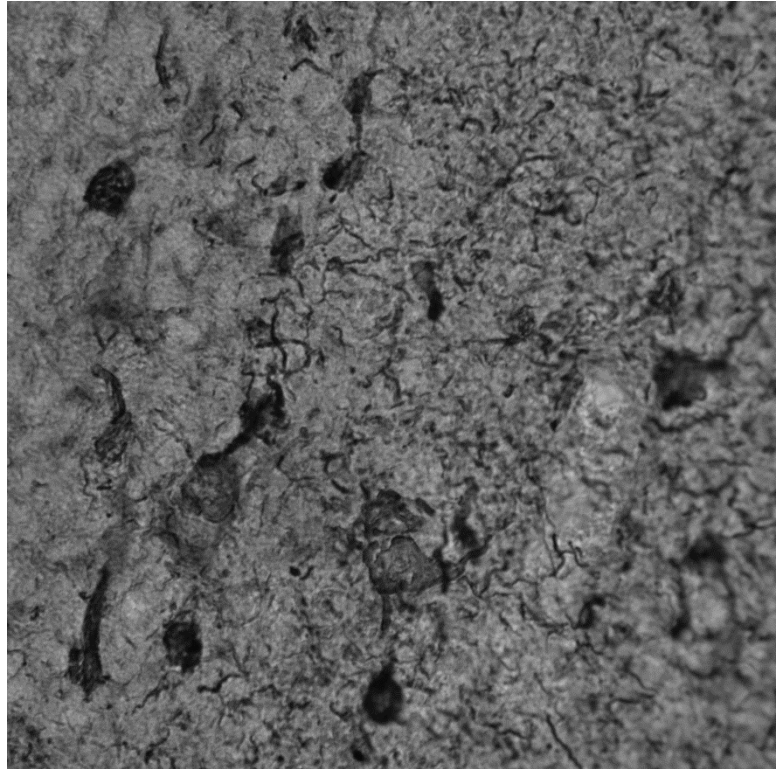
# Immunohistochemistry of tau tangles (anti-PHF1)



**20X**

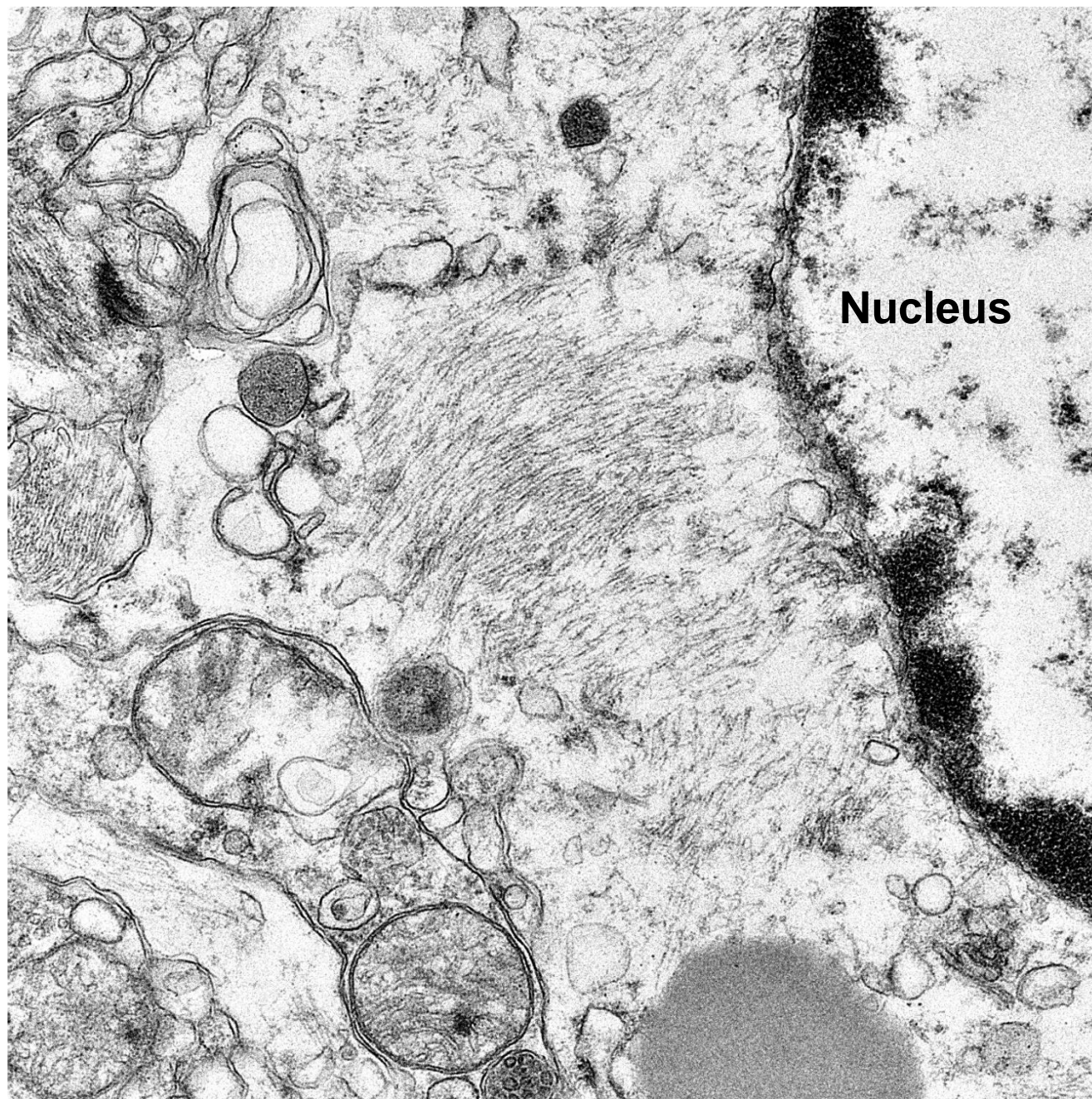


**40X**



**40X**

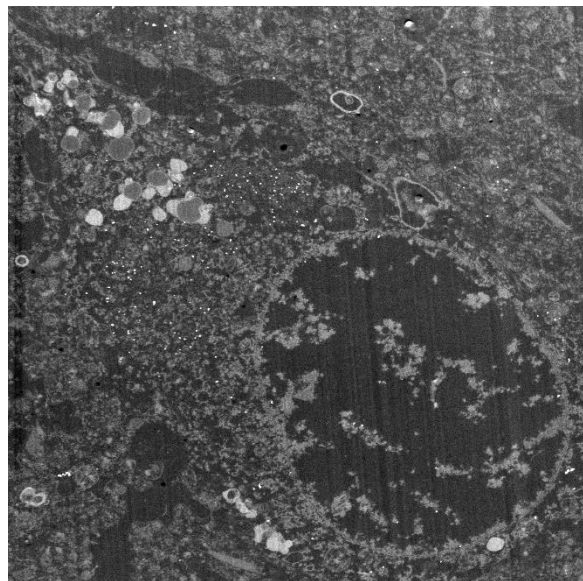
# Tau tangles in a neuron



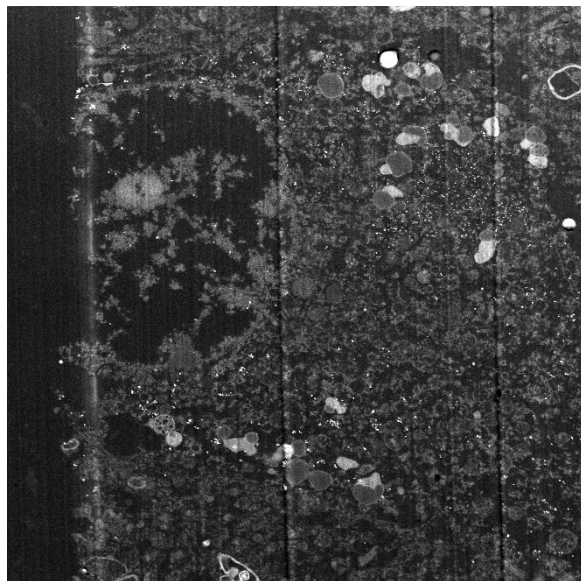
**Nucleus**

Size	Exp	Mag	Spot	HT	Pixel size	Fov	Defocus	500 nm
4096	4 s	14000 x	6	200 kV	1.085 nm	4.446 $\mu$ m	-38 $\mu$ m	Ceta

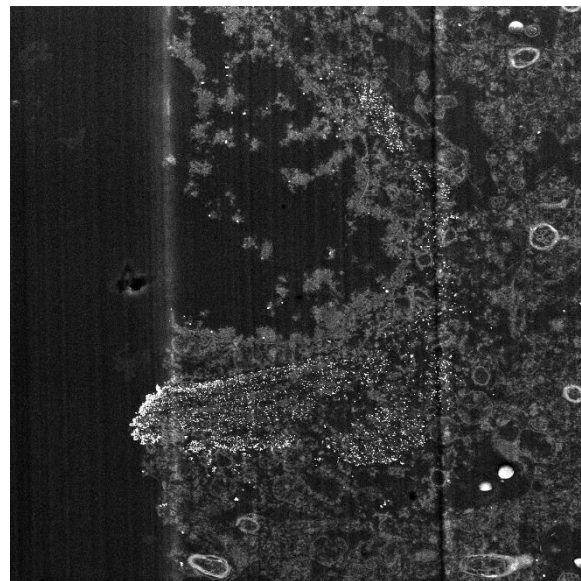
# ApoE protein were seen to co-localize with tau tangles



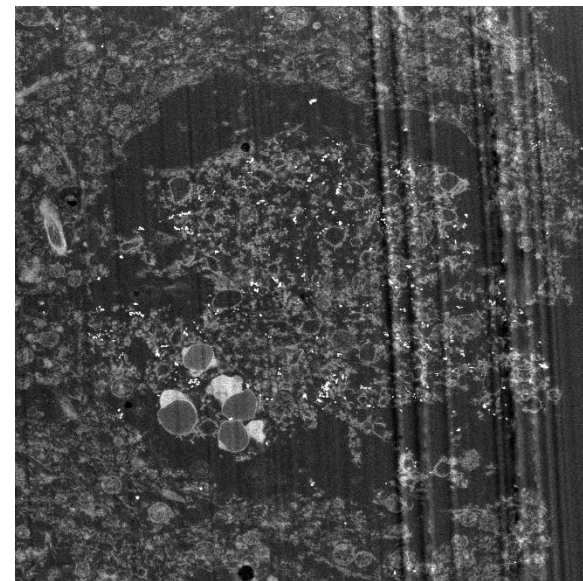
Size	Dwell	HT	Scan fov	STEM Mag	Pixel size	CL
2048	2.00 μs	200 kV	22.7 μm	4.41 kx	11.06 nm	5 μm



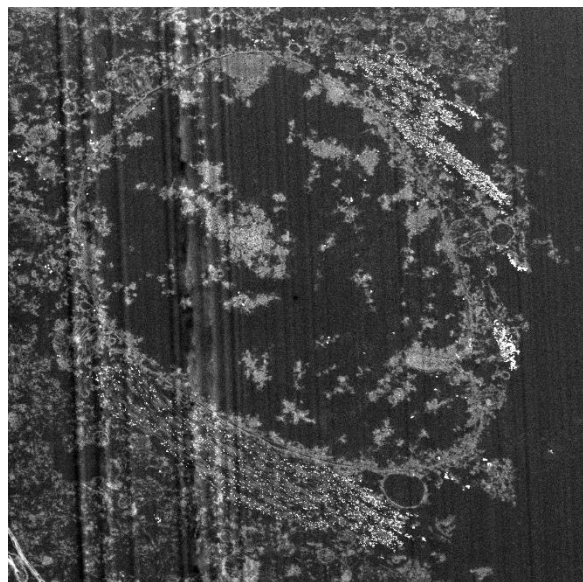
Size	Dwell	HT	Scan fov	STEM Mag	Pixel size	CL
2048	2.00 μs	200 kV	22.7 μm	4.41 kx	11.06 nm	5 μm



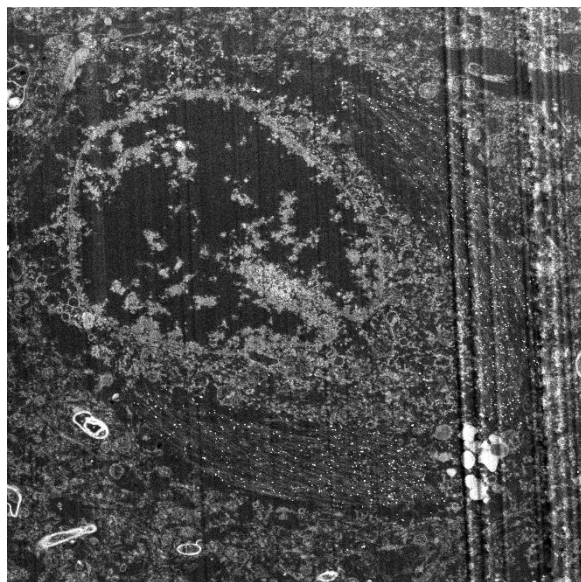
Size	Dwell	HT	Scan fov	STEM Mag	Pixel size	CL
2048	2.00 μs	200 kV	16 μm	6.24 kx	7.823 nm	205 mm



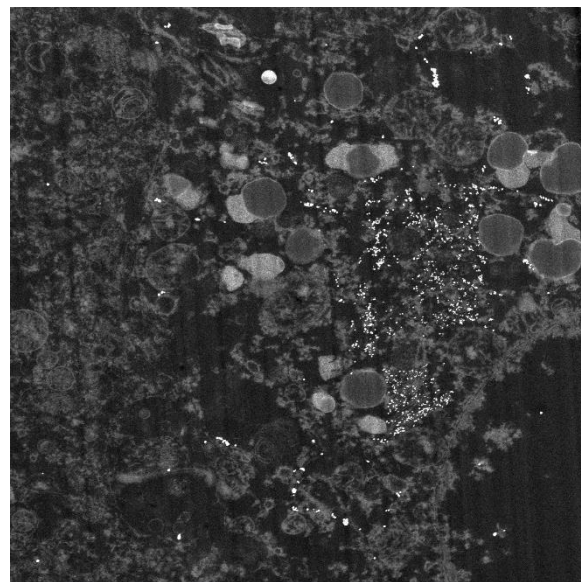
Size	Dwell	HT	Scan fov	STEM Mag	Pixel size	CL
2048	2.00 μs	200 kV	16 μm	6.24 kx	7.823 nm	205 mm



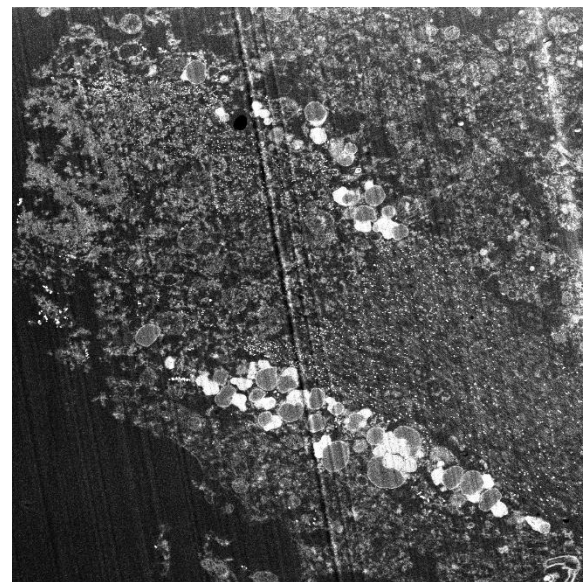
Size	Dwell	HT	Scan fov	STEM Mag	Pixel size	CL
2048	2.00 μs	200 kV	16 μm	6.24 kx	7.823 nm	205 mm



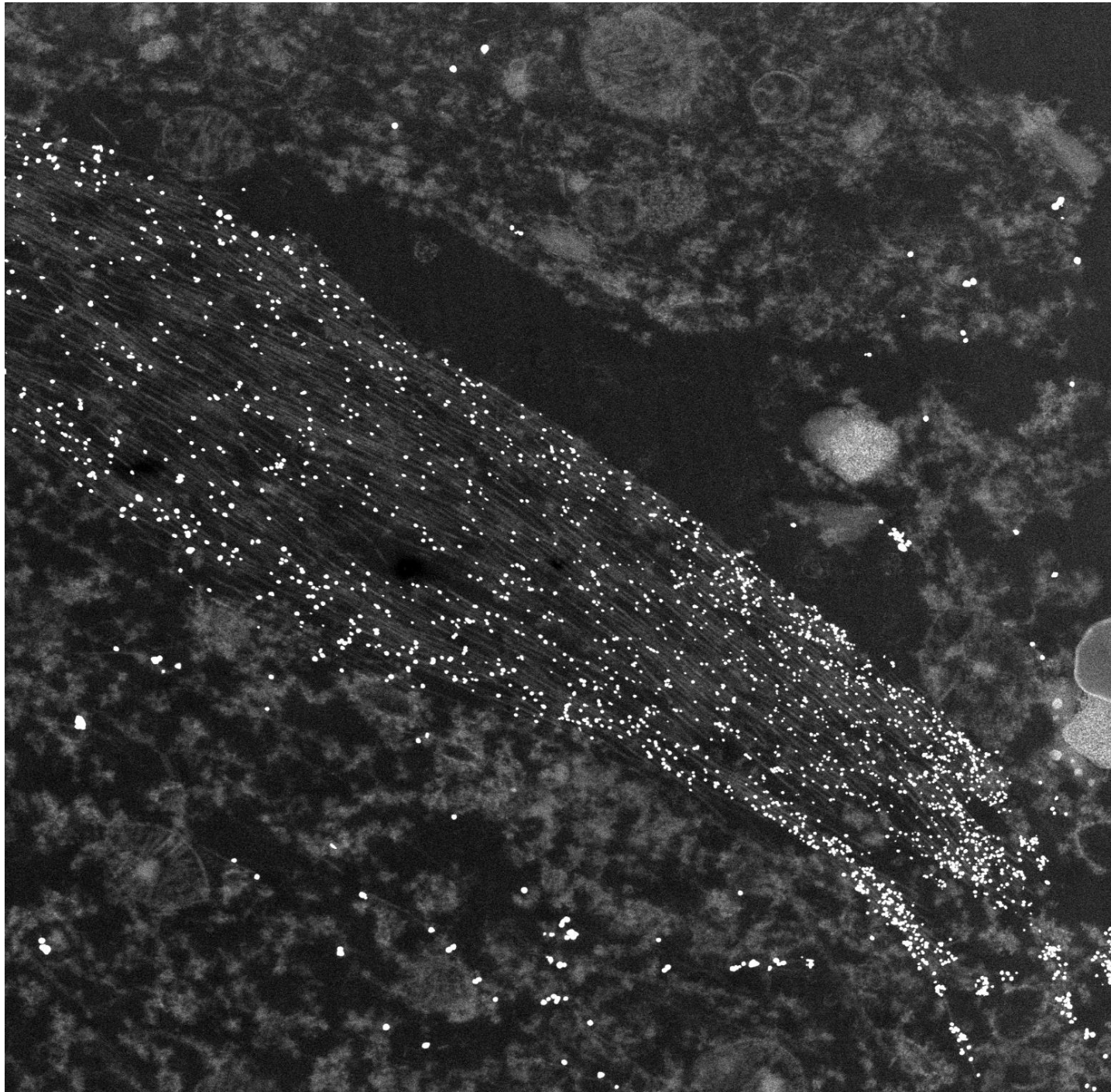
Size	Dwell	HT	Scan fov	STEM Mag	Pixel size	CL
2048	2.00 μs	200 kV	22.7 μm	4.41 kx	11.06 nm	5 μm

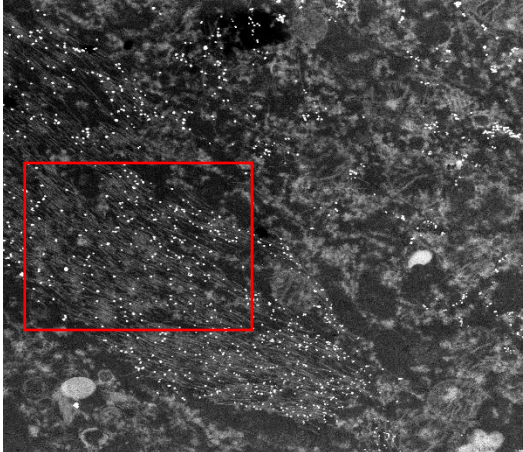
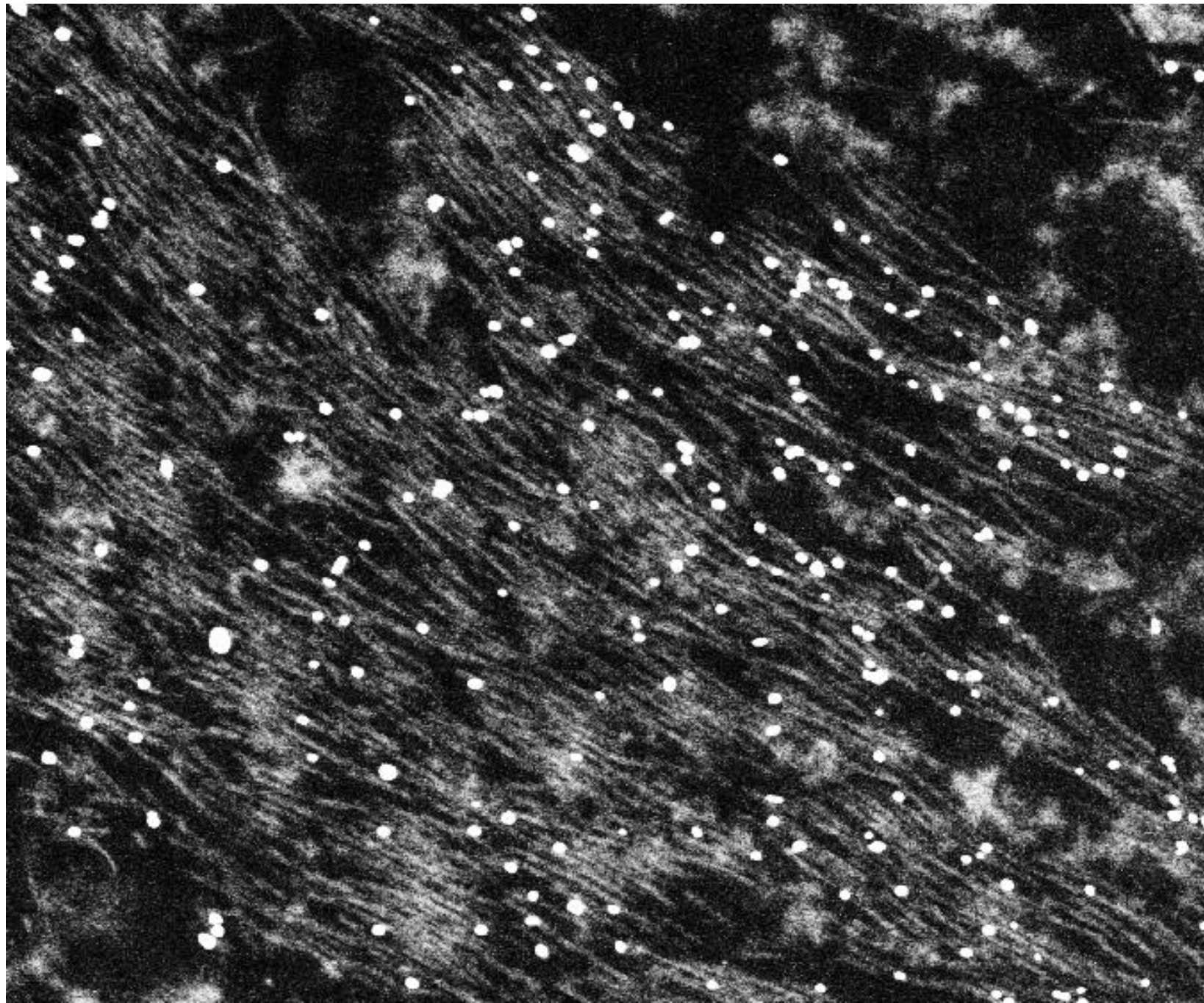


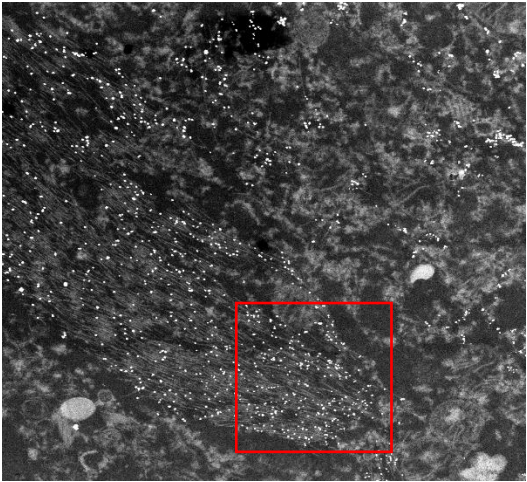
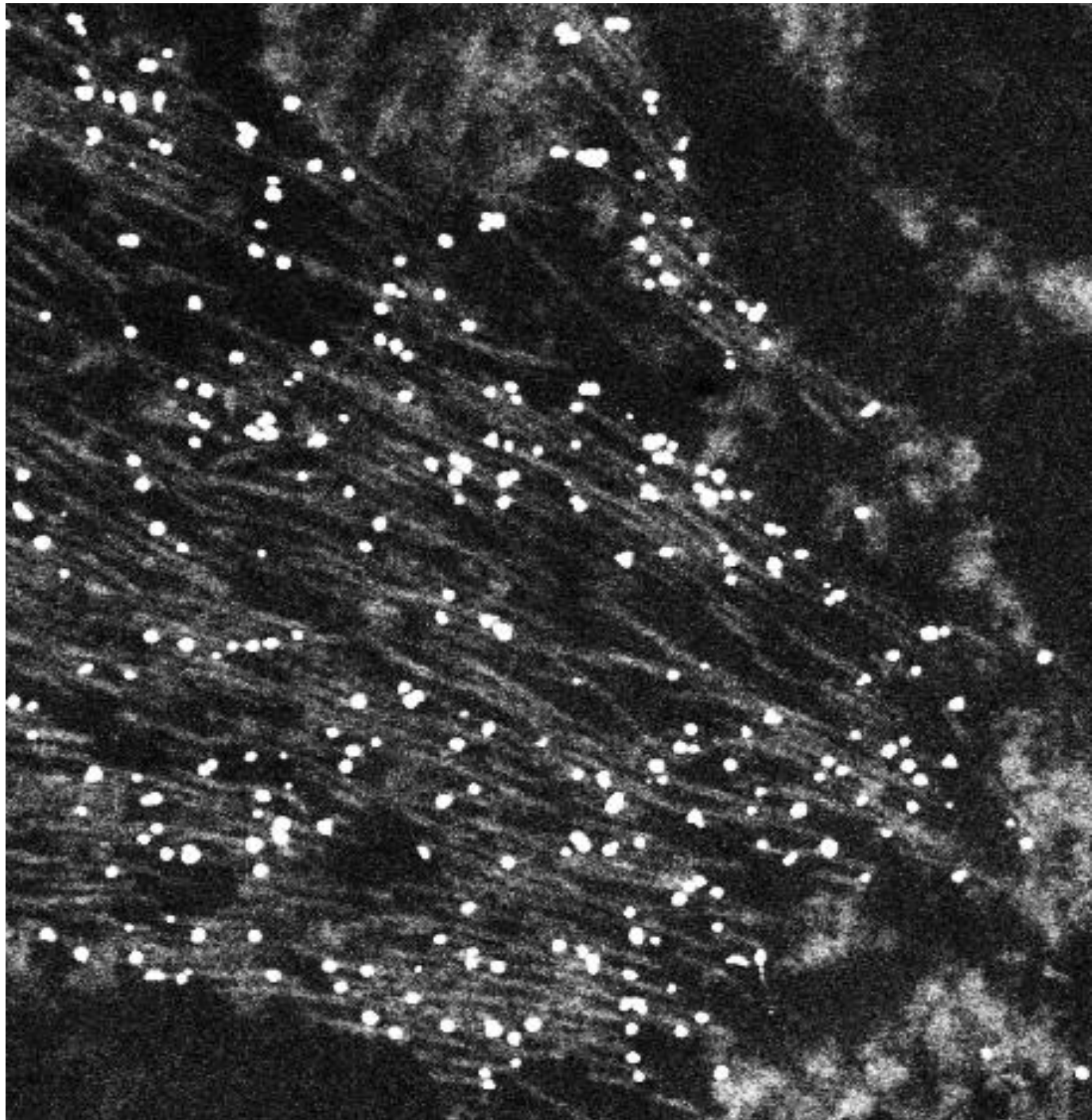
Size	Dwell	HT	Scan fov	STEM Mag	Pixel size	Spot
2048	2.00 μs	200 kV	11.3 μm	8.83 kx	5.532 nm	6



Size	Dwell	HT	Scan fov	STEM Mag	Pixel size	CL
2048	2.00 μs	200 kV	22.7 μm	4.41 kx	11.06 nm	5 μm







**Major challenges:**

**Antibody/gold particle penetration – maintaining ultrastructure of tissue**

**Specificity of antibody/gold labeling**

**Thank you!**