電子顕微鏡による細胞内微細空間構造の解析：膜細胞骨格から核膜骨格へ

Spatial structure of membrane and peri-nuclear cytoskeletons revealed by electron microscopy

My research works

Early part

University of Tokyo, School of Medicine

UCLA Nagoya University, School of Medicine

Structures related to the photo-transduction from the outer segments to synapses

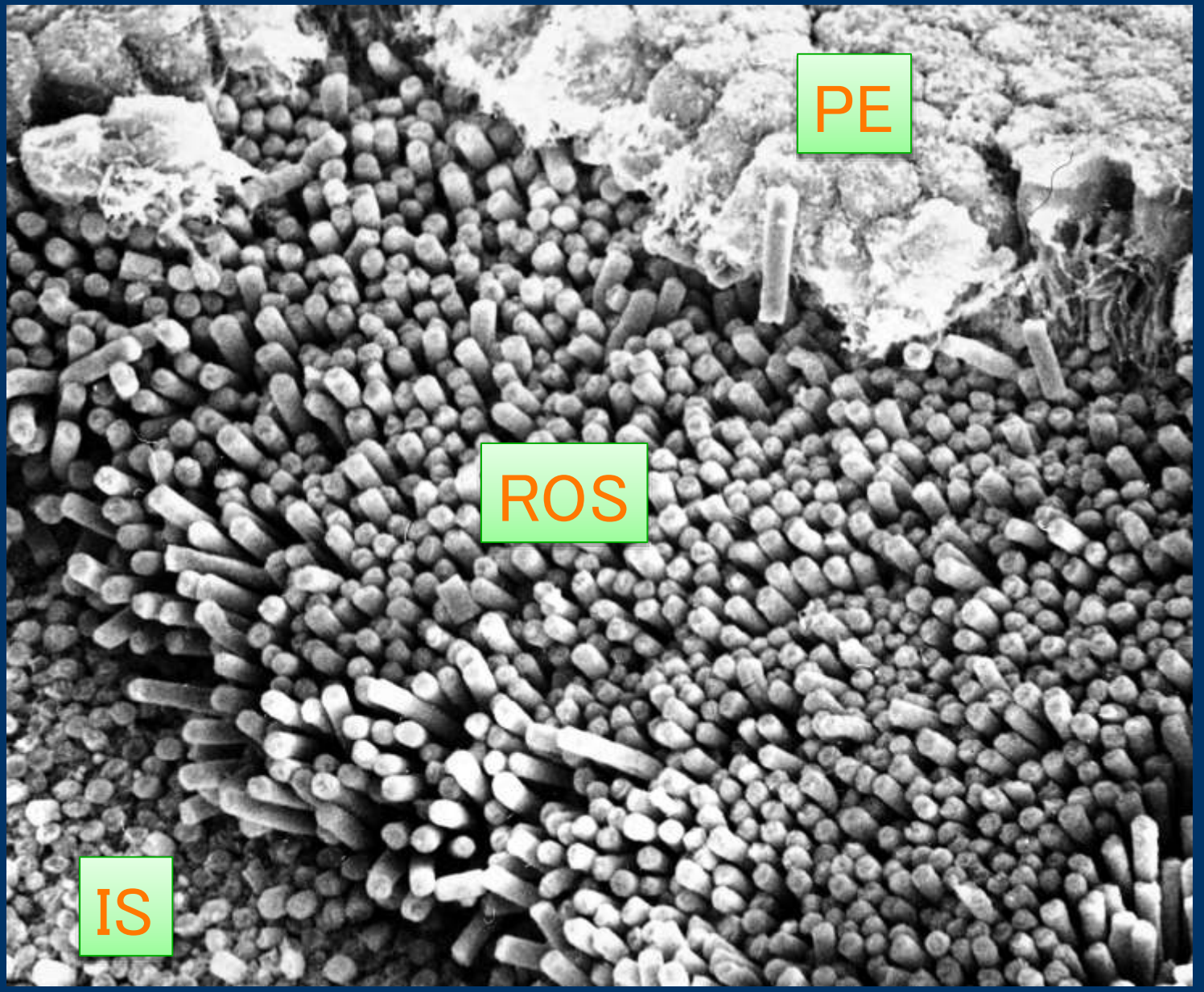
Later part

Nagoya University, School of Medicine

CRAST EcoTopia SI

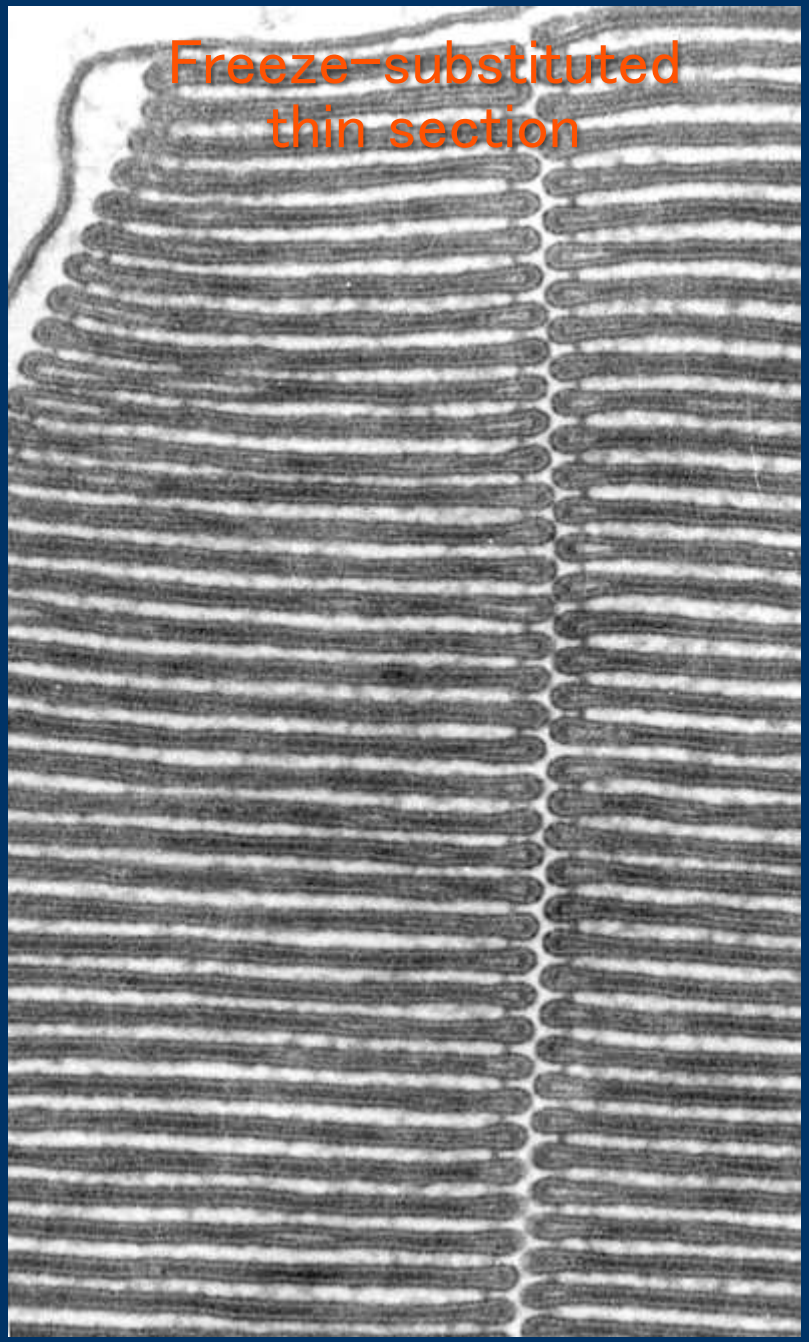
Spatial structure of actin cytoskeletons from membrane undercoat to peri-nucleus

Bird's-eye view of frog retina by SEM

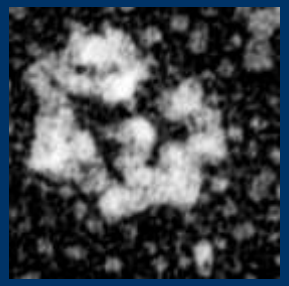


視細胞外節の超微構造

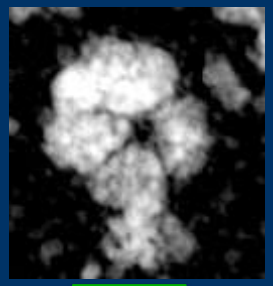
Fine structure of rod outer segments



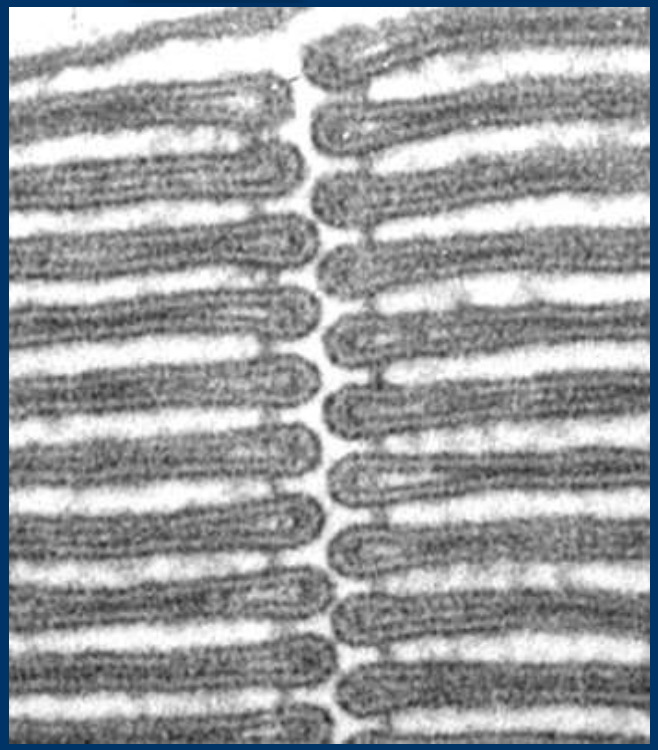
Supra molecular organization of rod outer segments

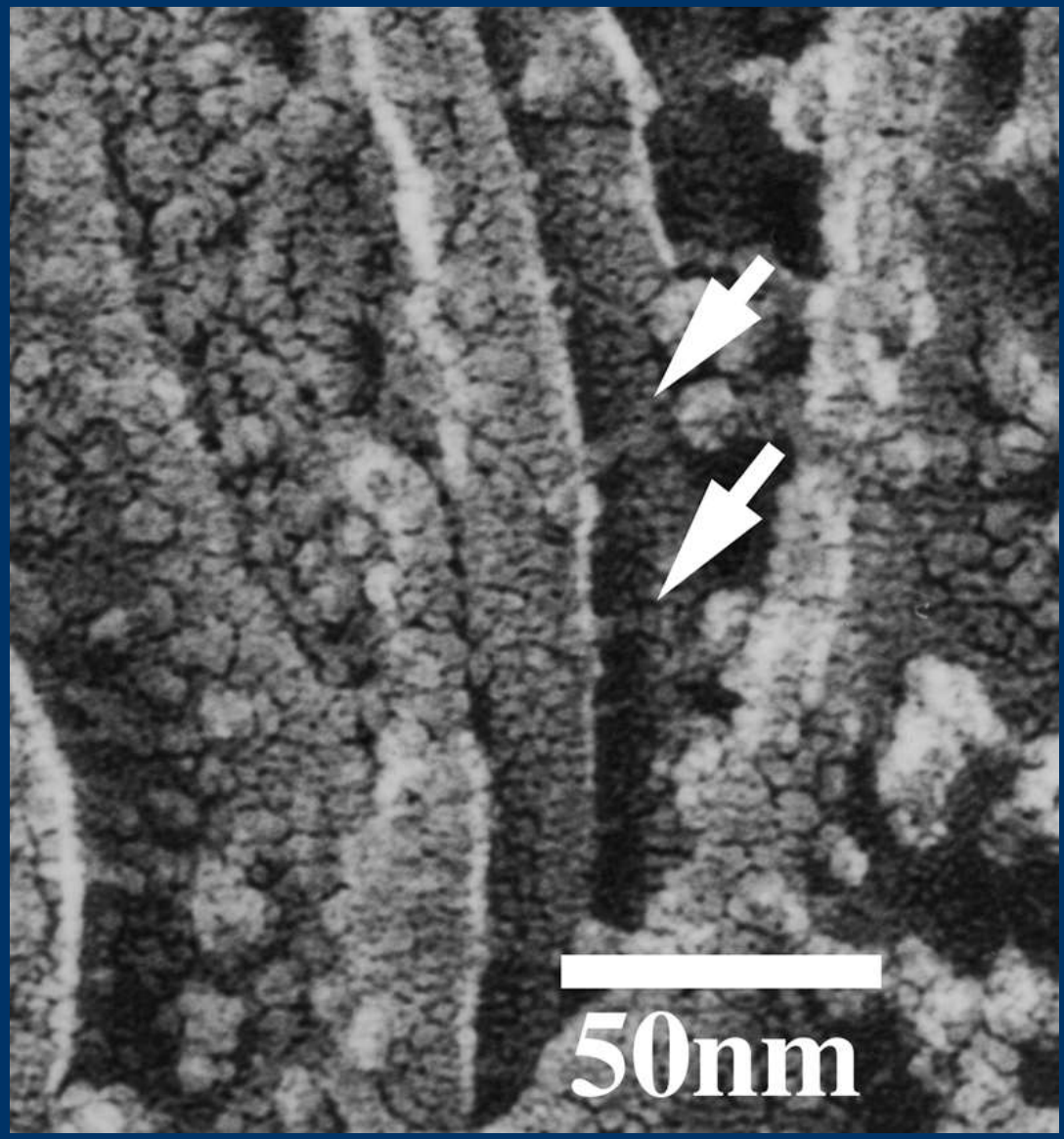


PDE

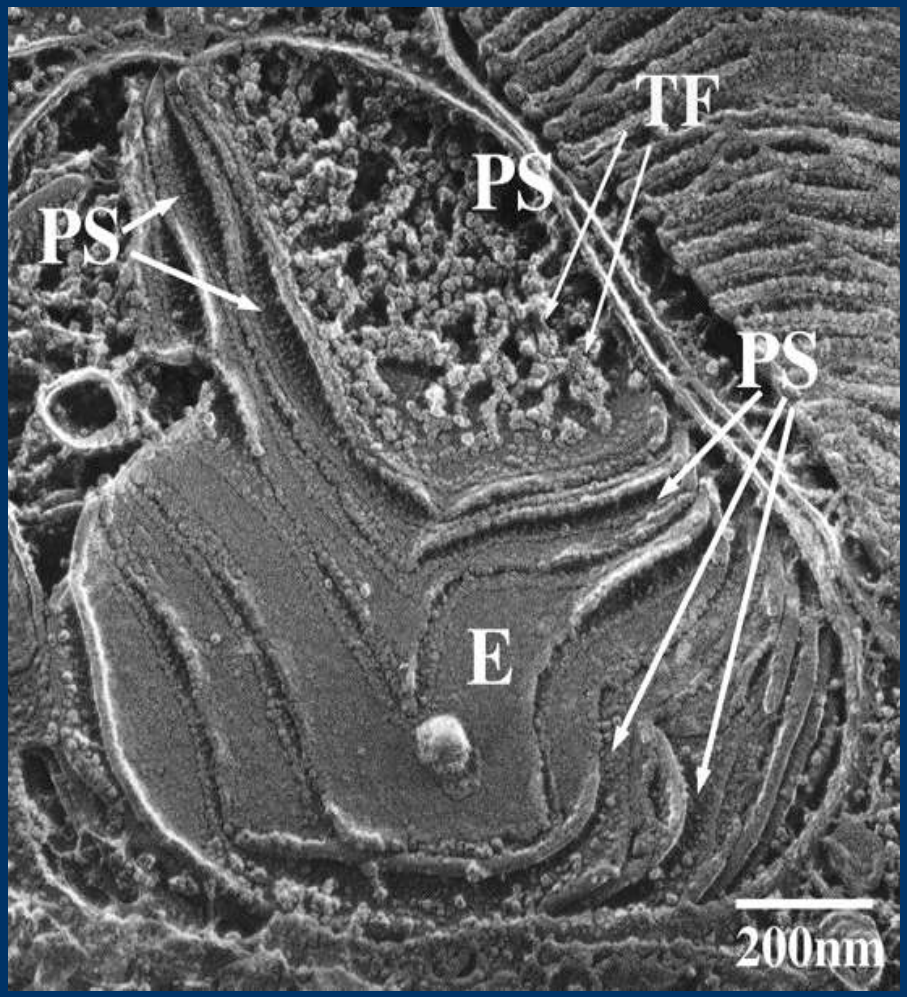


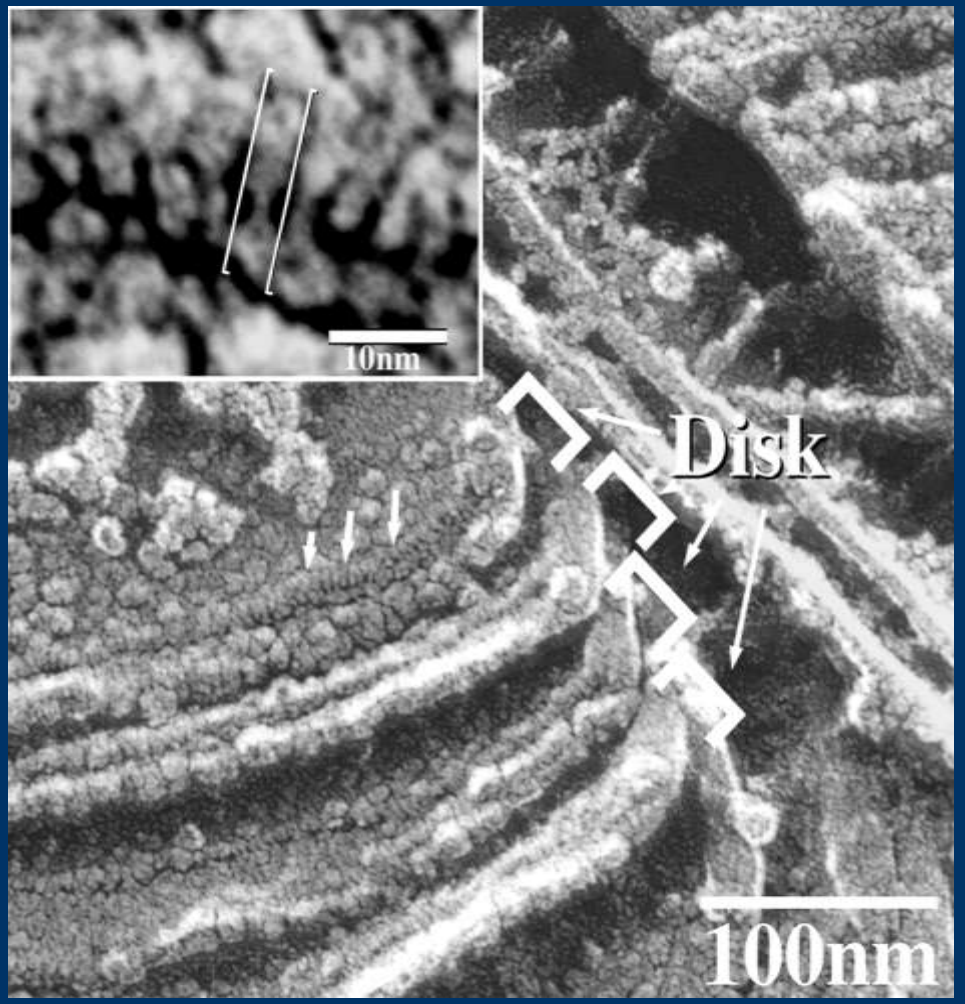
Tβ



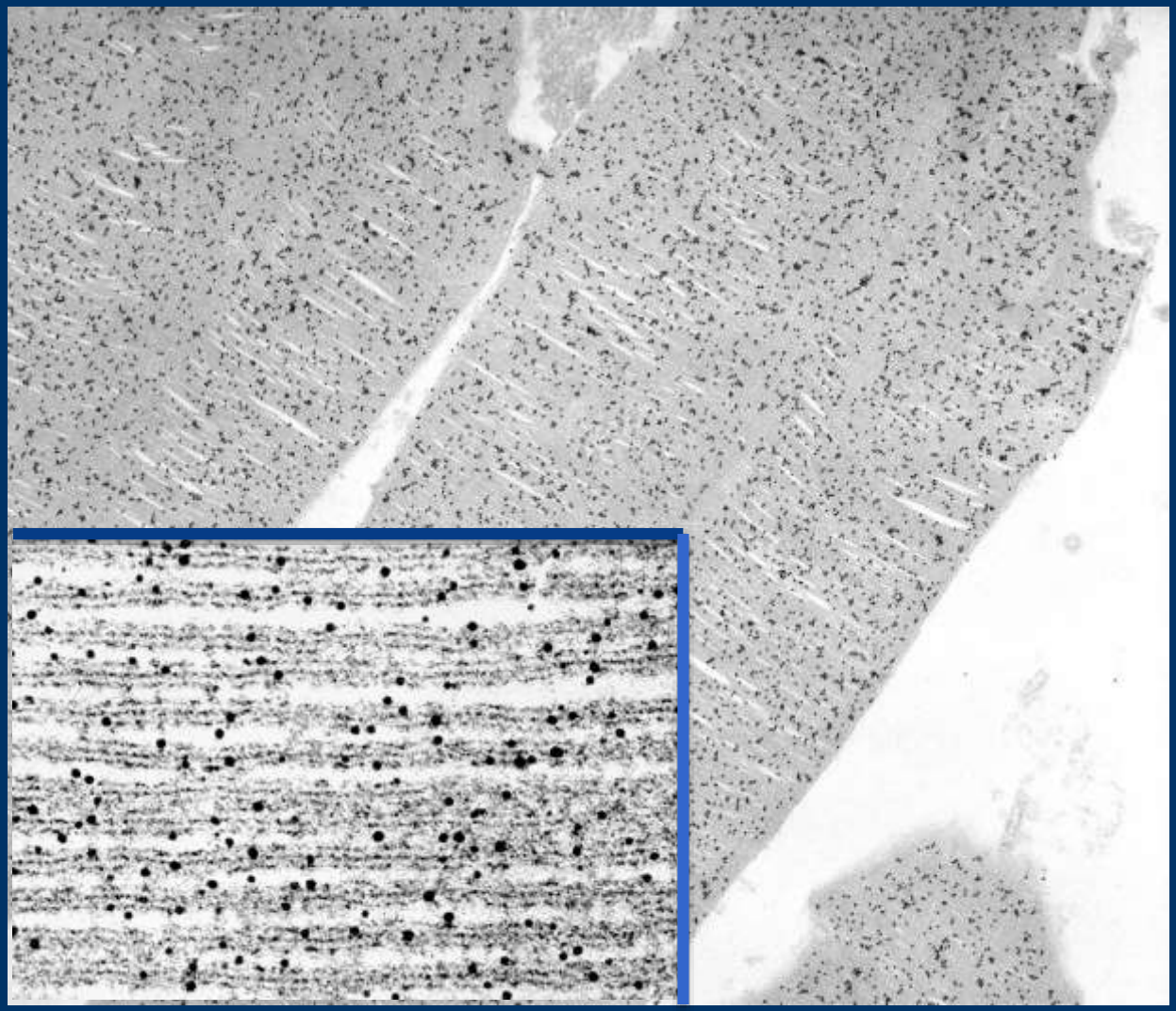


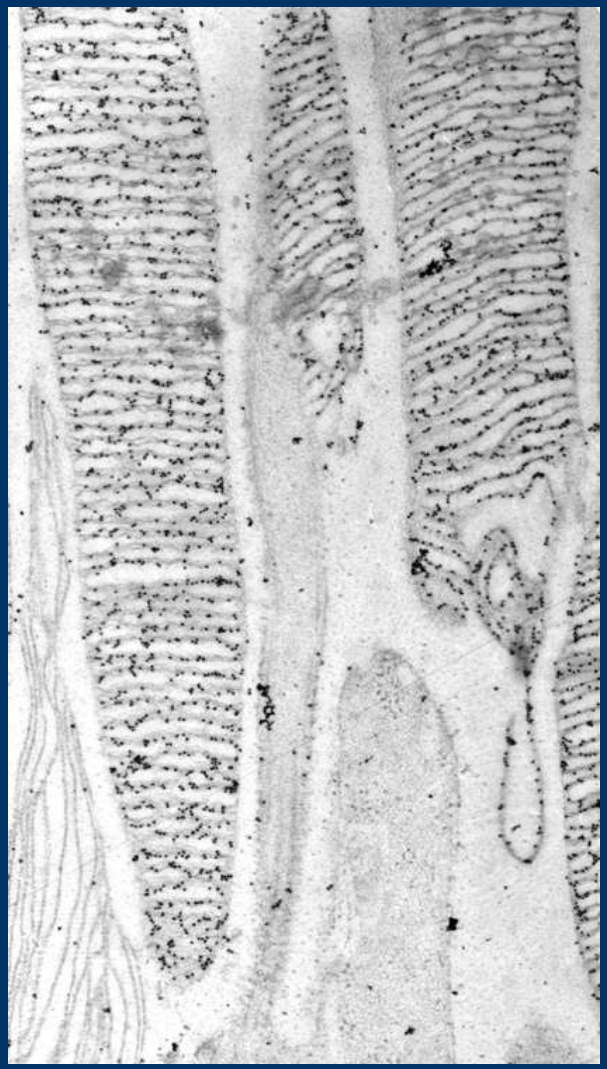
Freeze-etched replicas of rod outer segments of photoreceptor cells



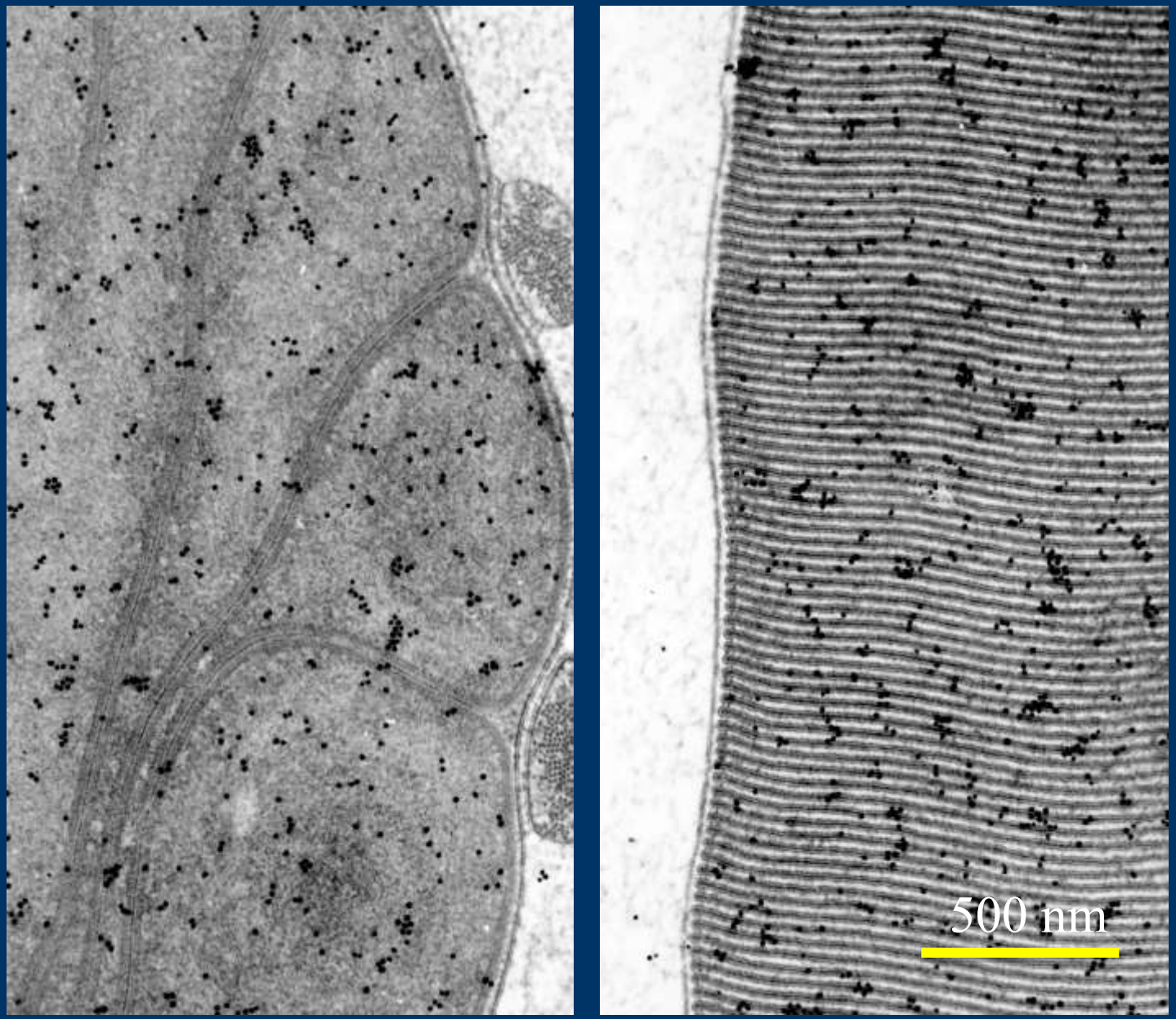


Localization of anti-opsin antibodies : Difference in fixation

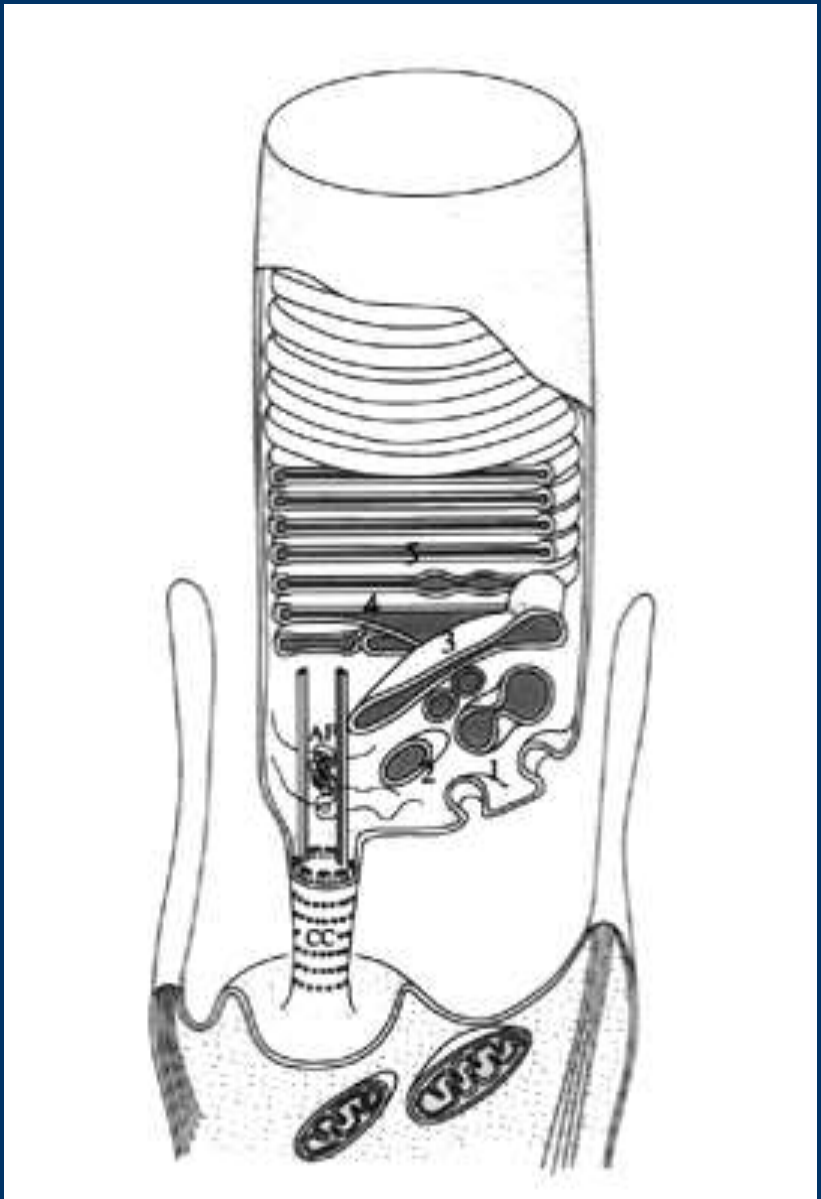


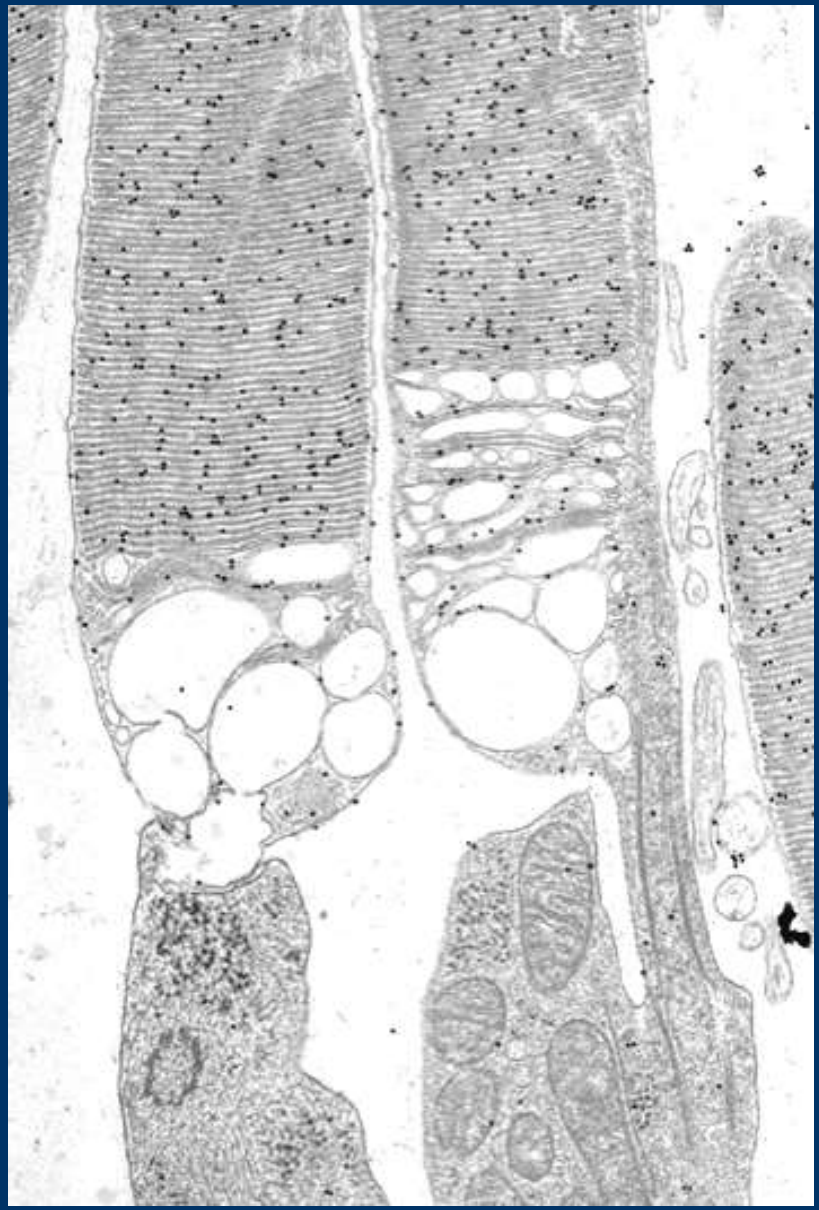


Immuno-localization of anti-opsin

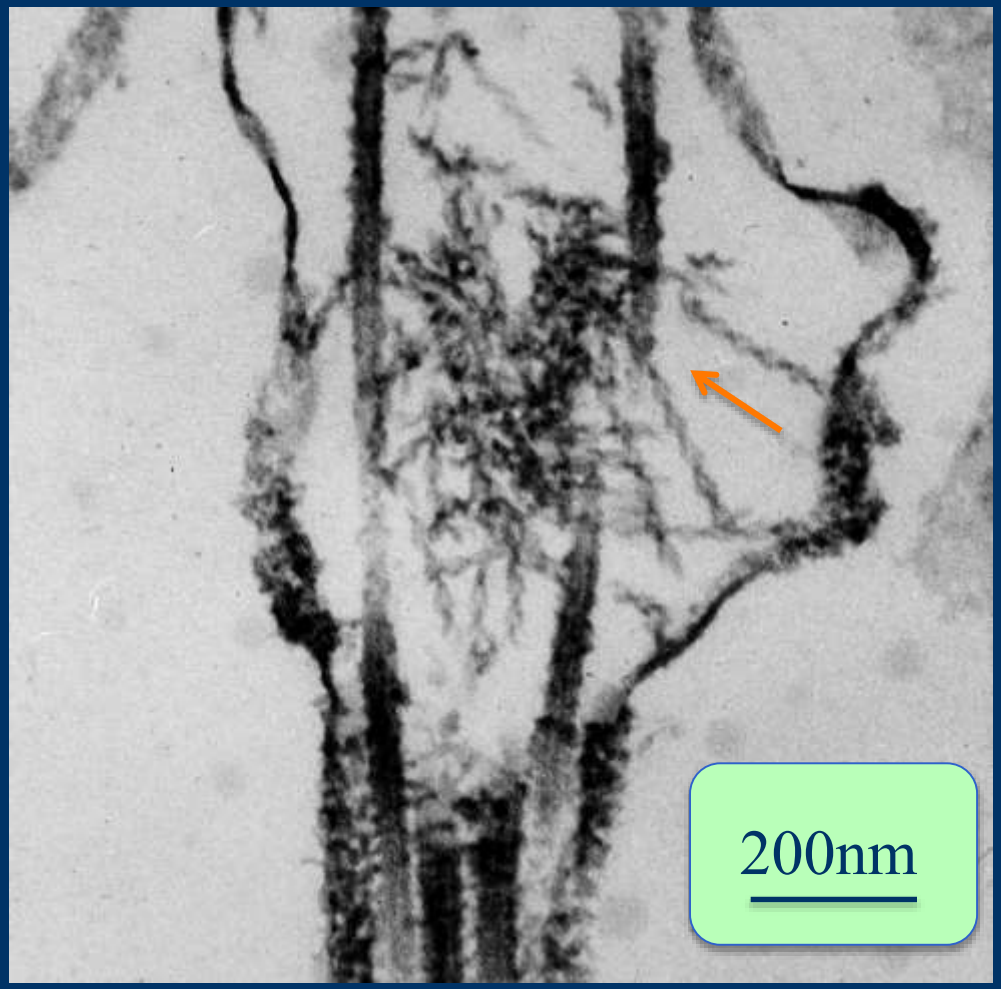


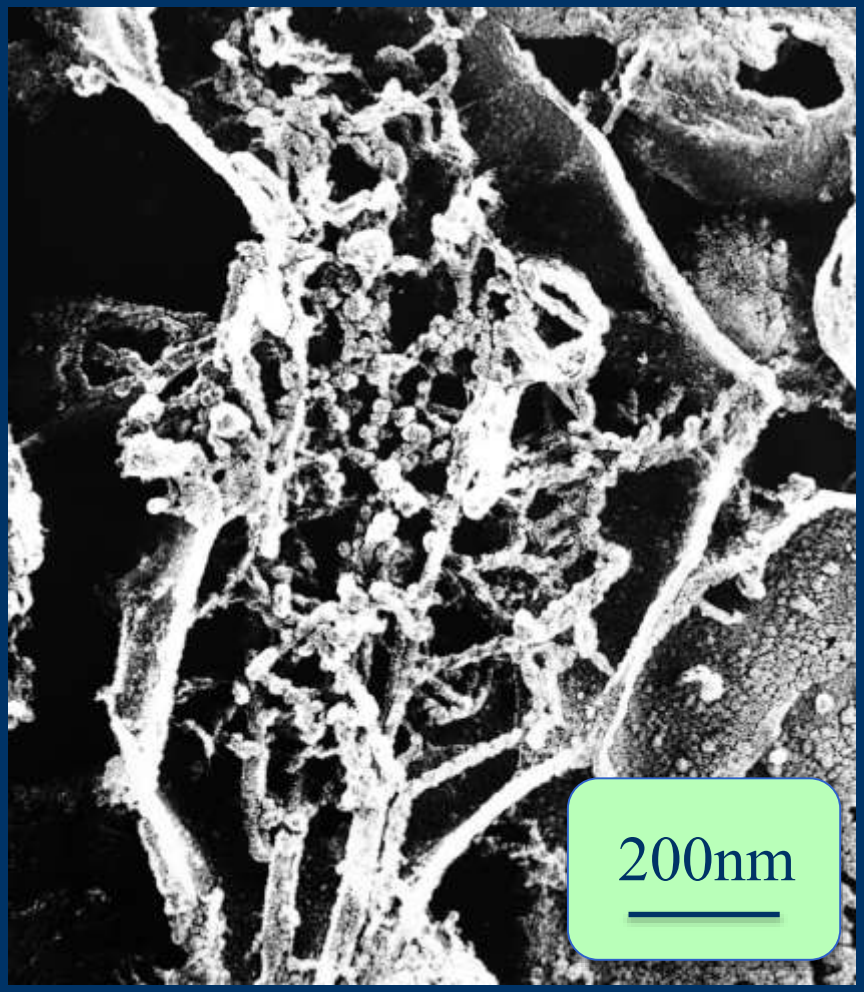
Morphogenesis of rod outer segments



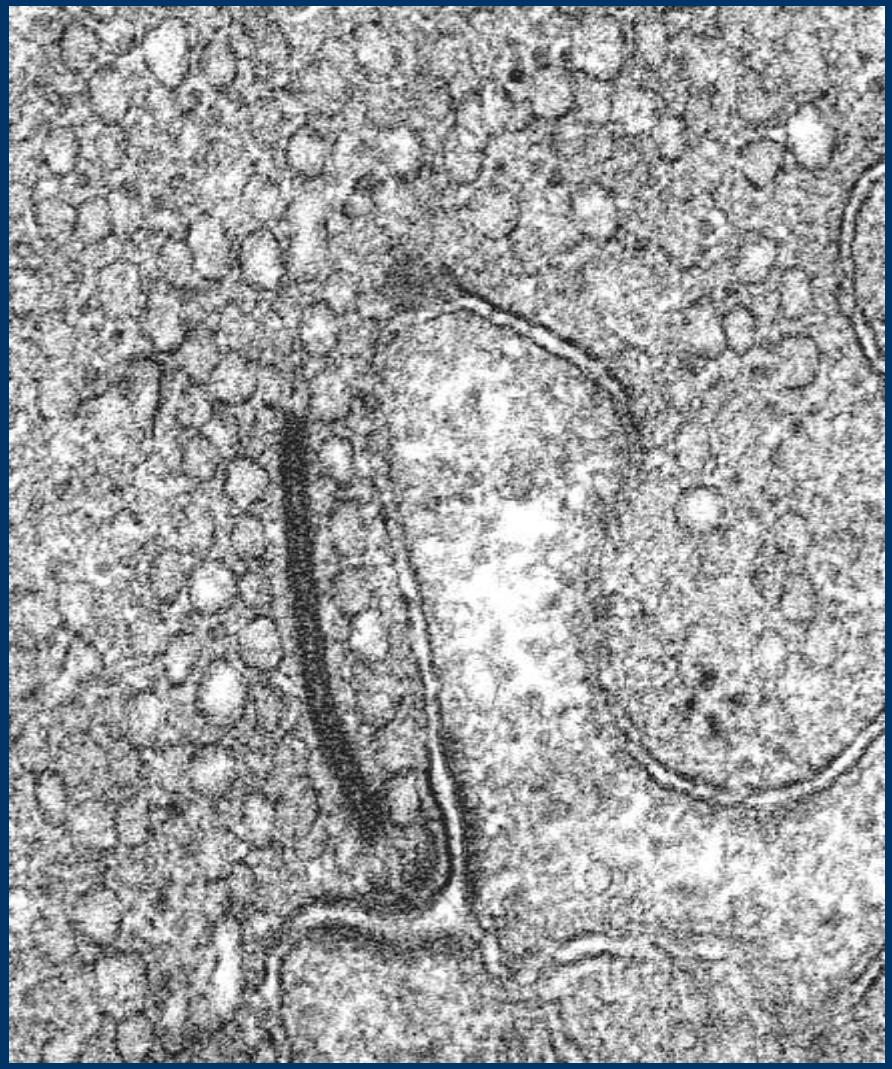


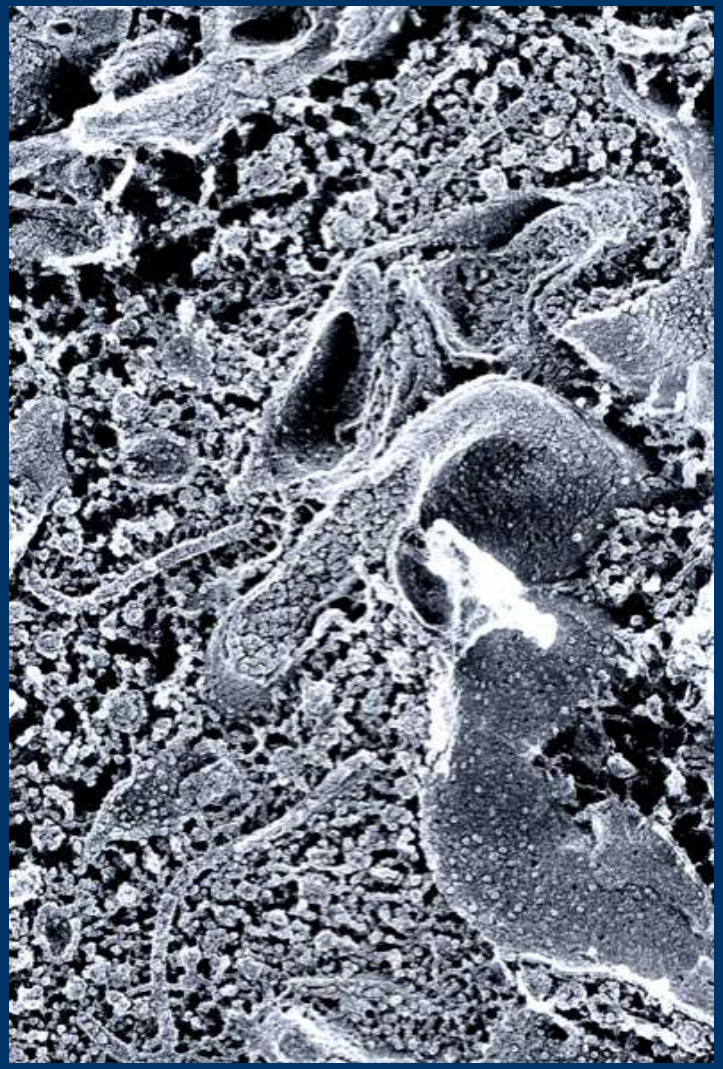
Polarity of actin filaments that are found at the tip of the connecting cilium : S1 decoration



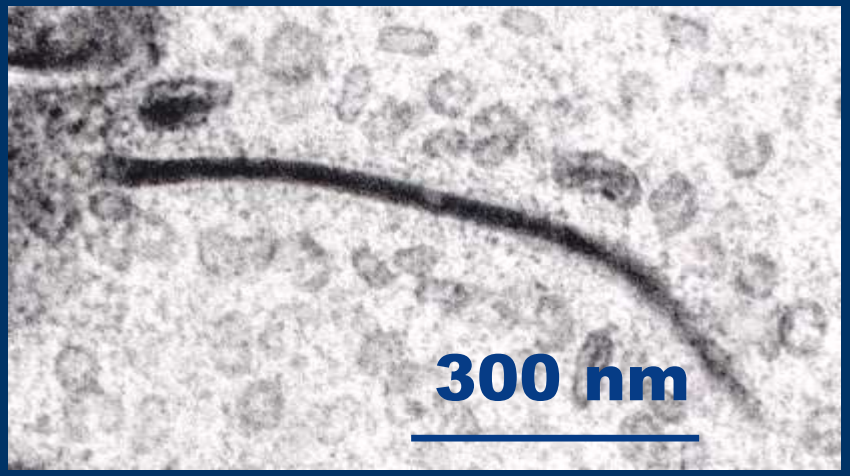


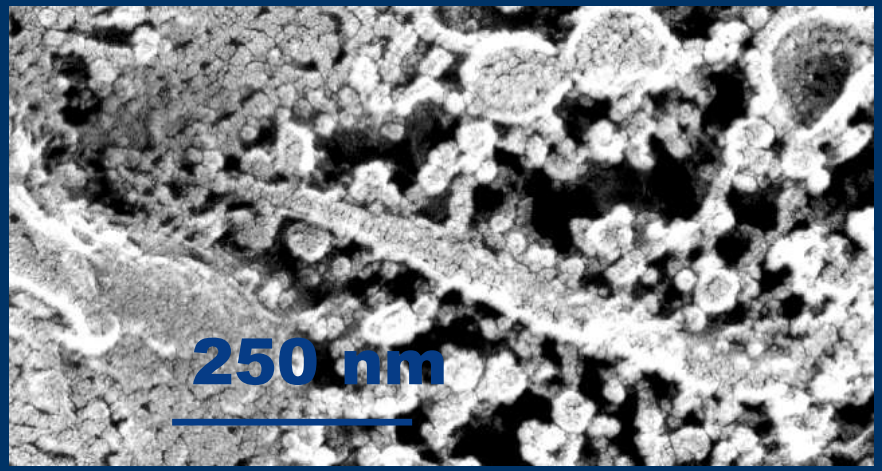
Electron micrographs of synaptic ribbons

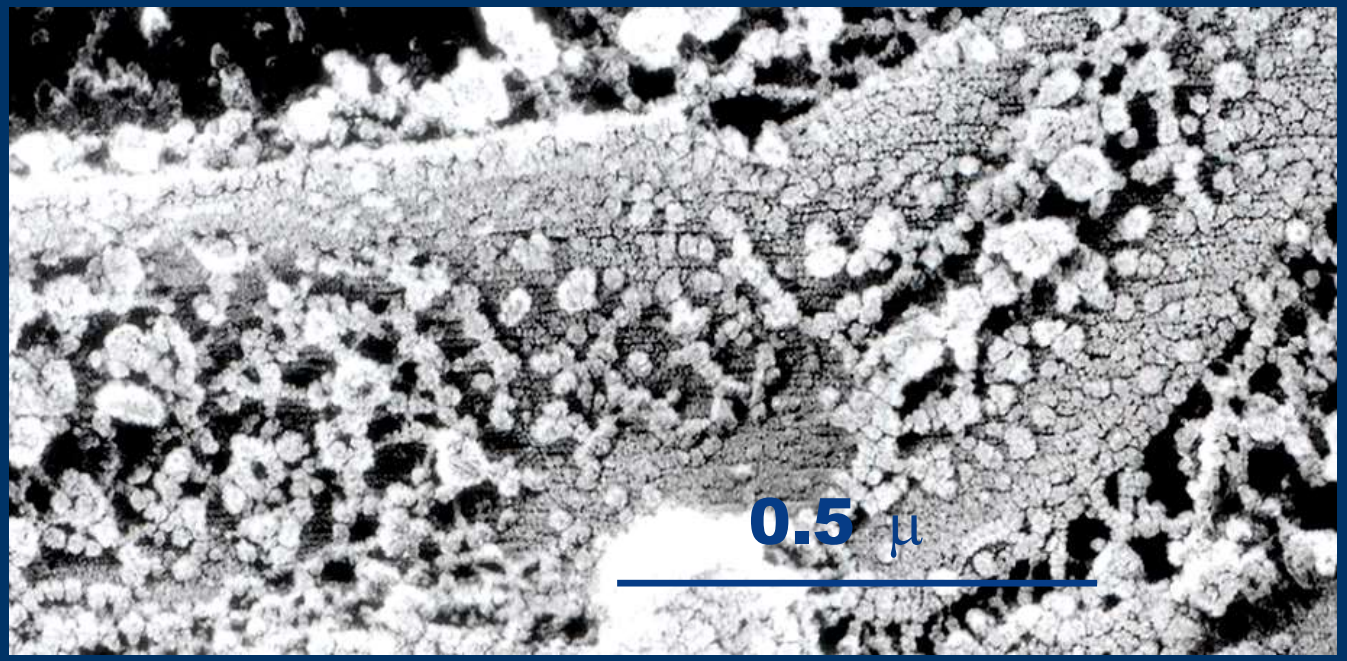




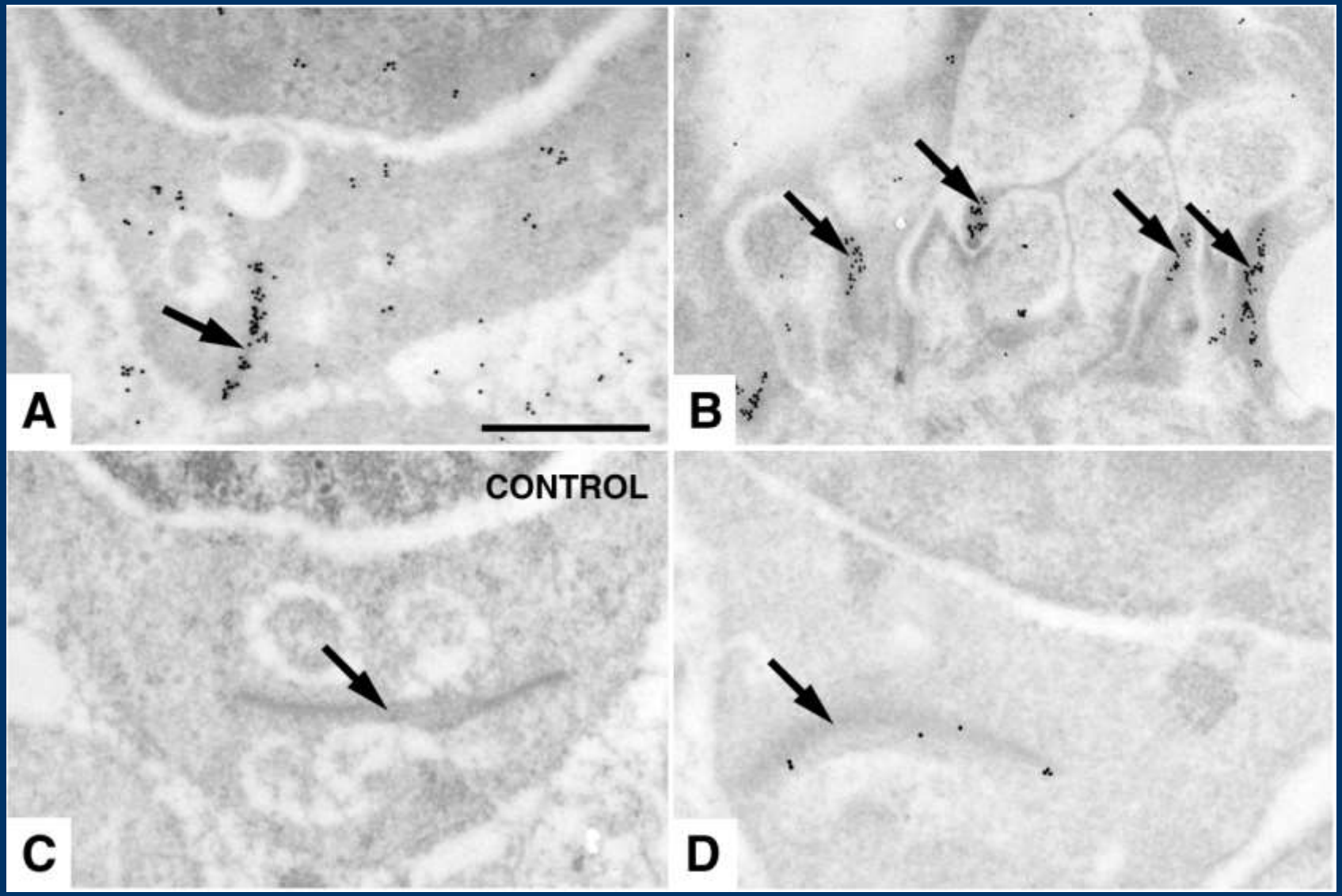
Synaptic ribbons observed from various points of view at mesoscale



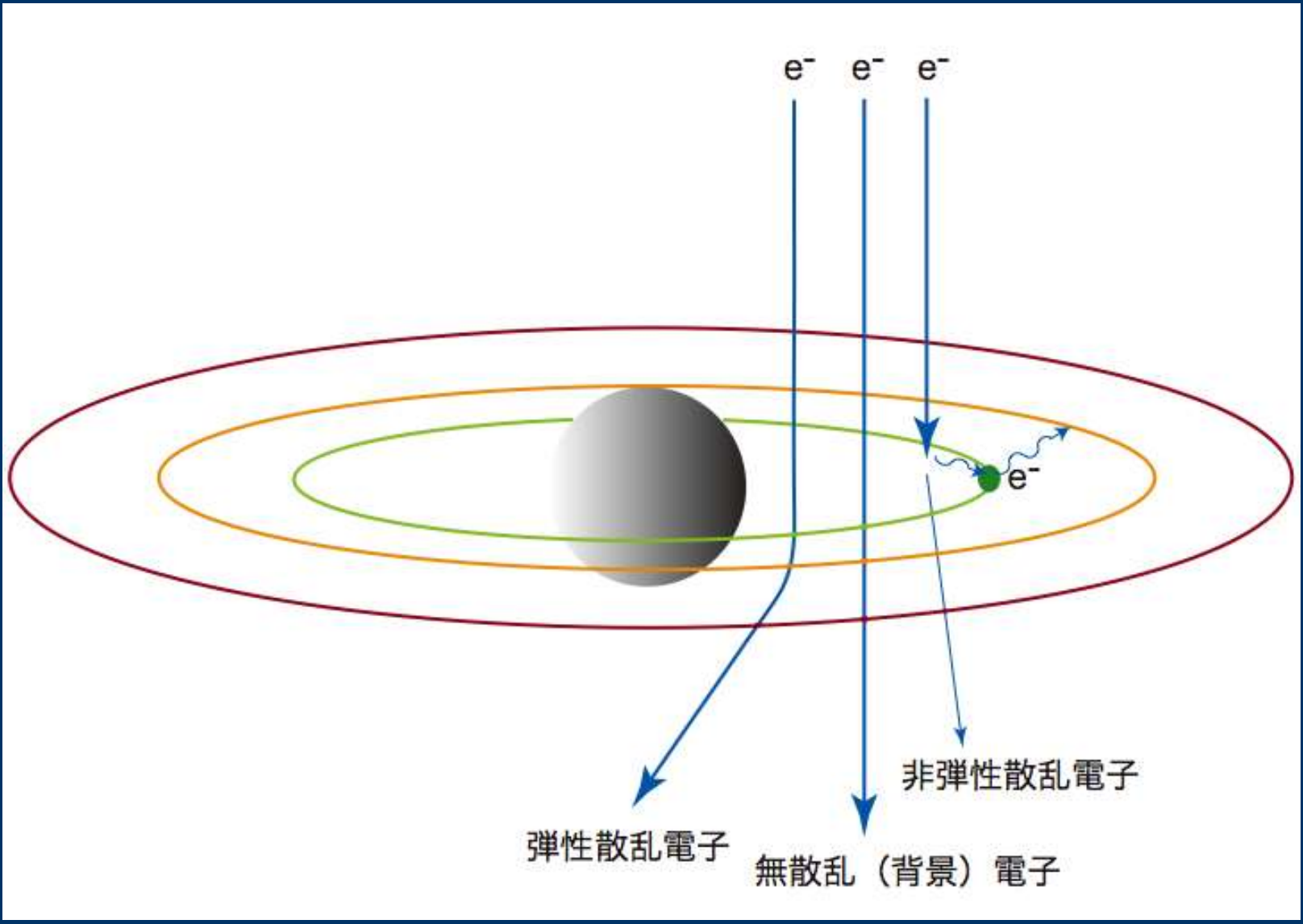




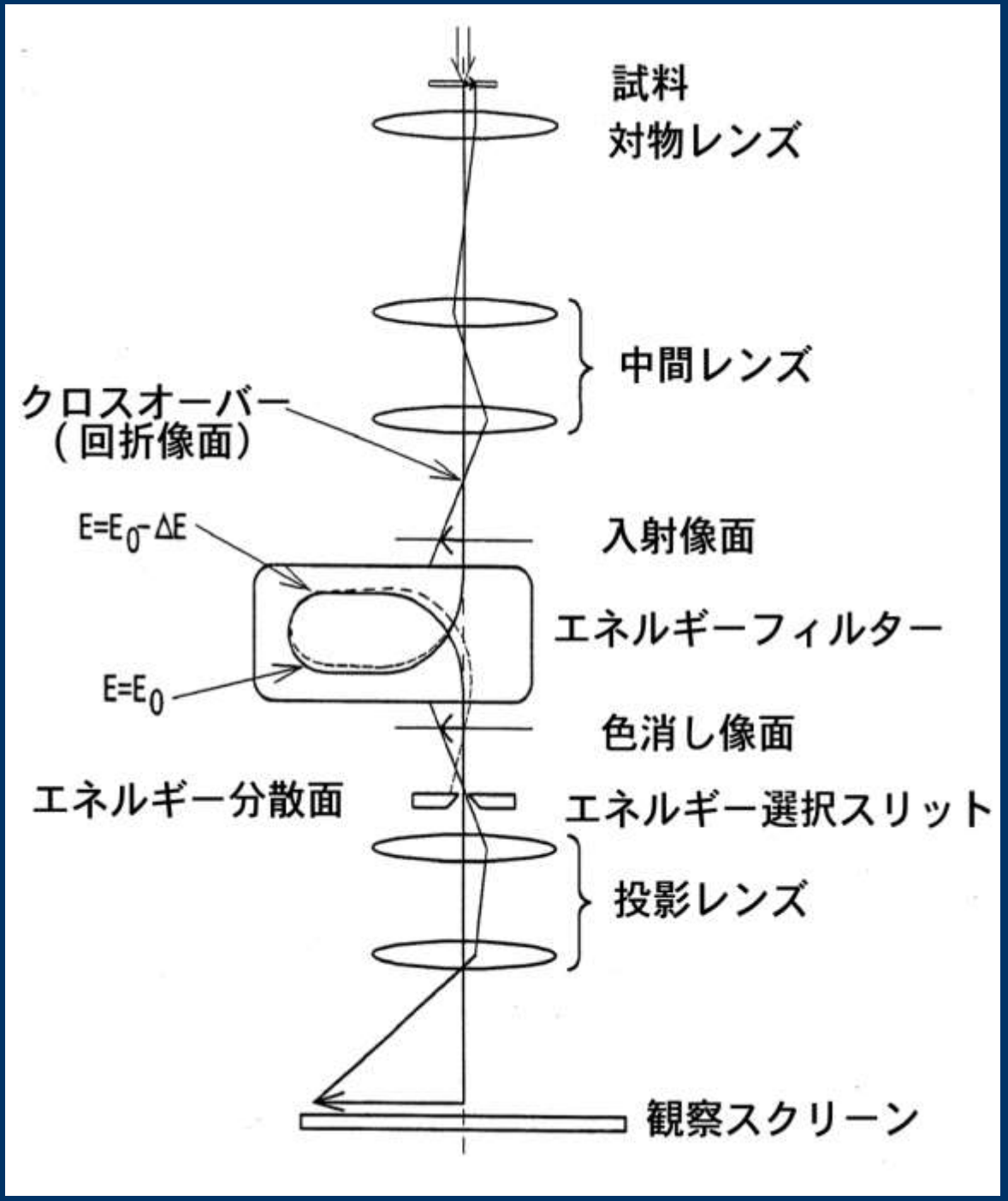
Anti-Caveolin 1 antibodies are found on the synaptic ribbon



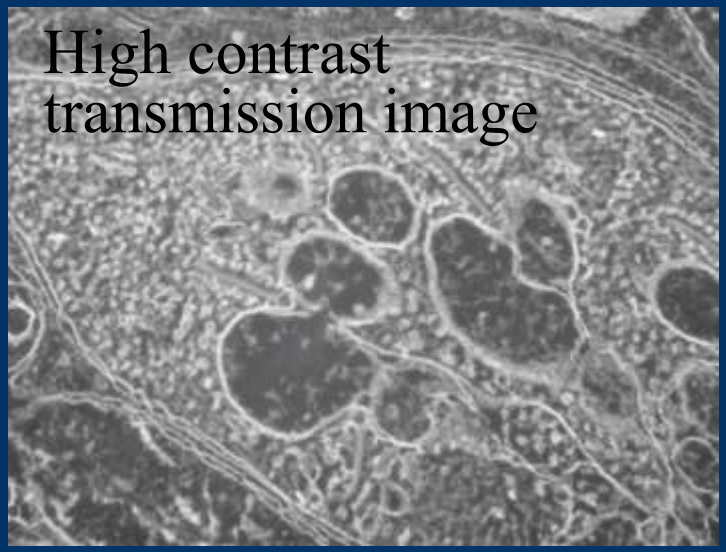
Elastic and inelastic scattered electrons

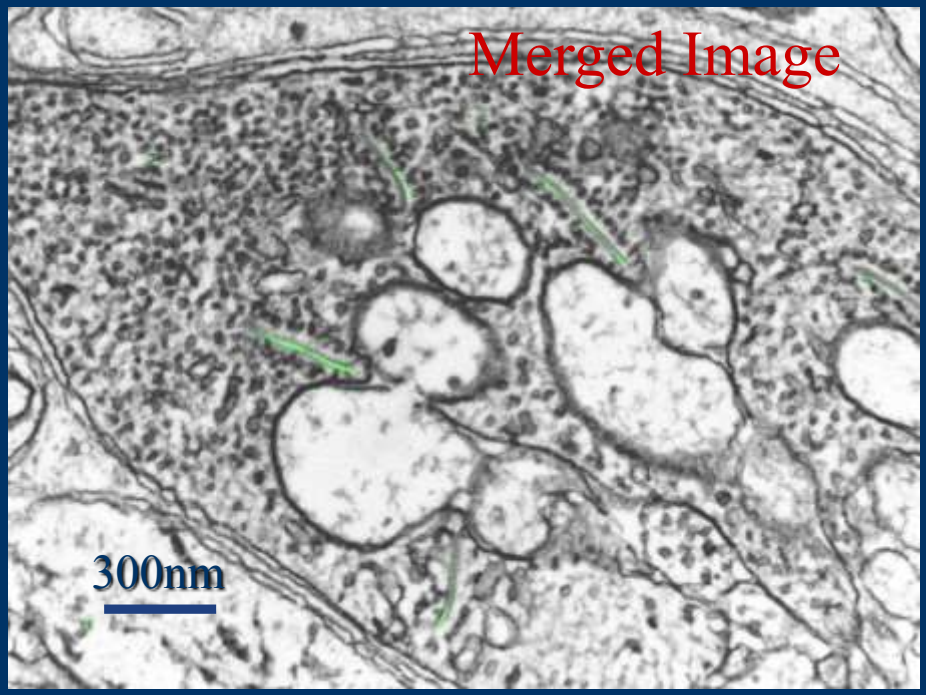


Optical pass way in the electron microscope equipped with γ type energy filter
(co-worked with Taya and Taniguchi in Hitachi)



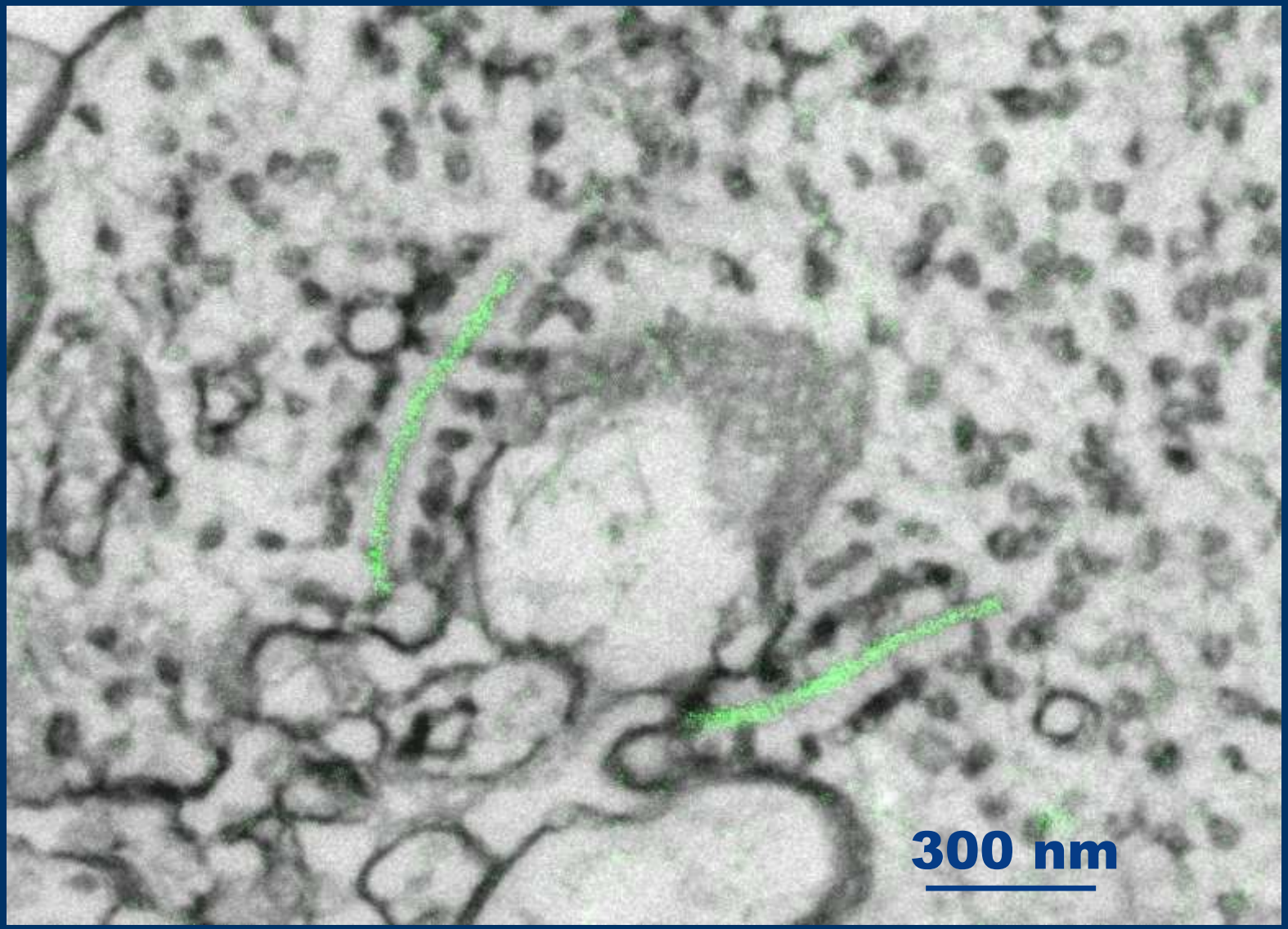
EELS imaging of Ca in cone synaptic pedicle





Ca is found on the synaptic ribbon predominantly.

Ca localization determined by EELS imaging



アクチン細胞骨格の空間構造と空間特異性

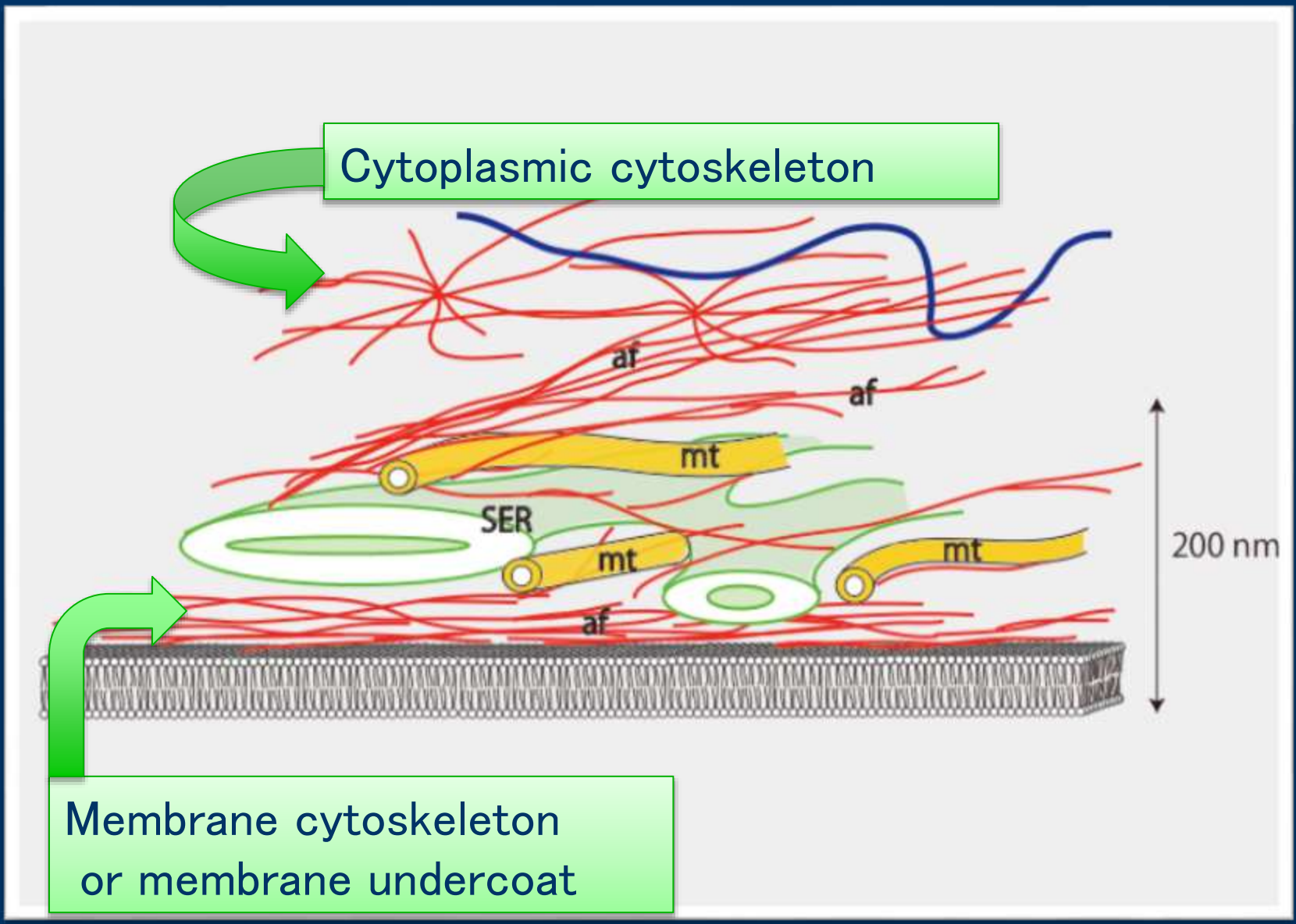
Spatial structure and specificity of actin cytoskeleton

Purpose

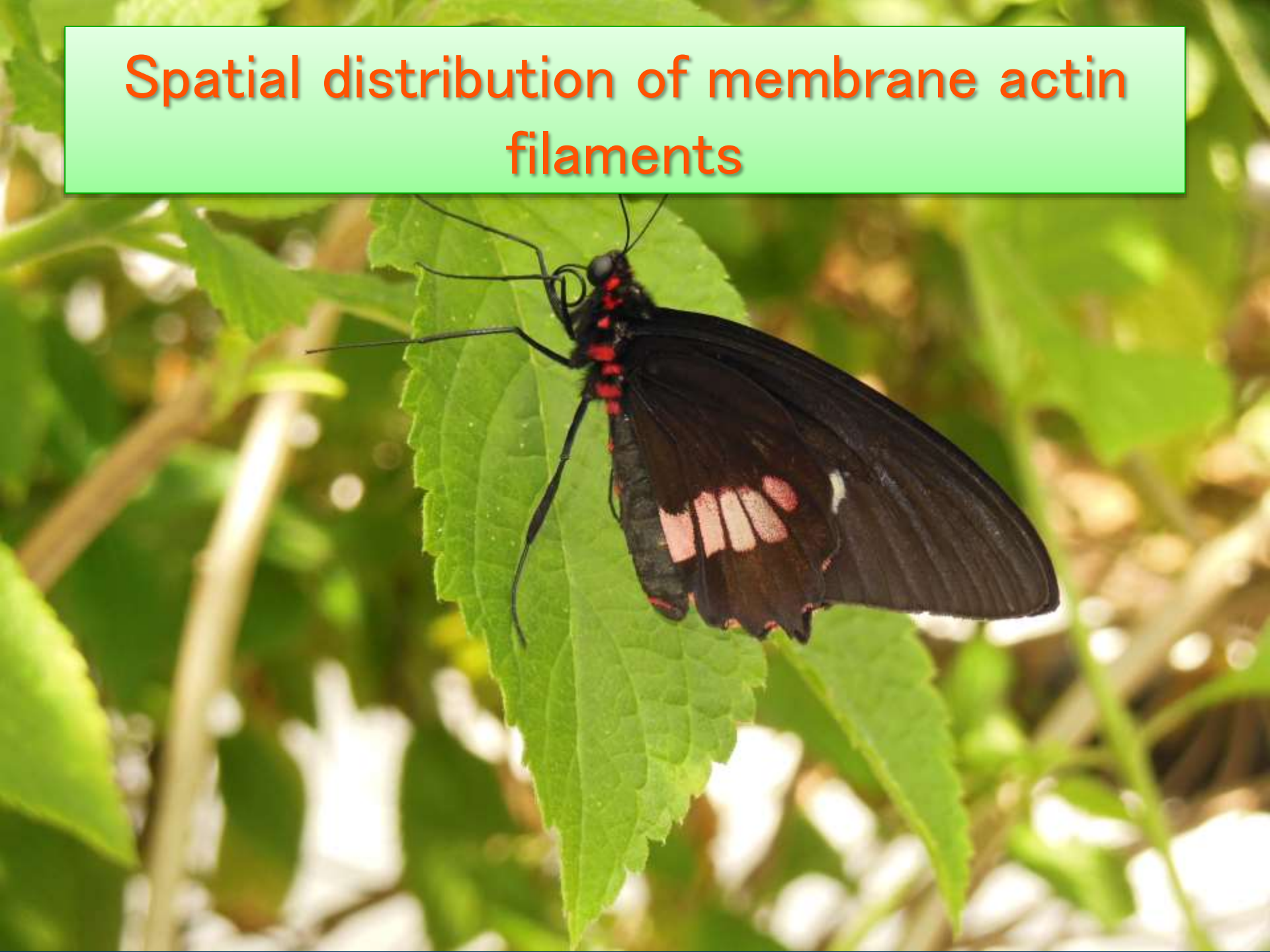
We investigate spatial structure of actin filaments on the membrane and in cytoplasm by various methods including cryo-electron microscope, immuno-freeze-etching, scanning electron microscope and atomic force microscope.

We are shedding the light on real actin filaments within cells from various points of view.

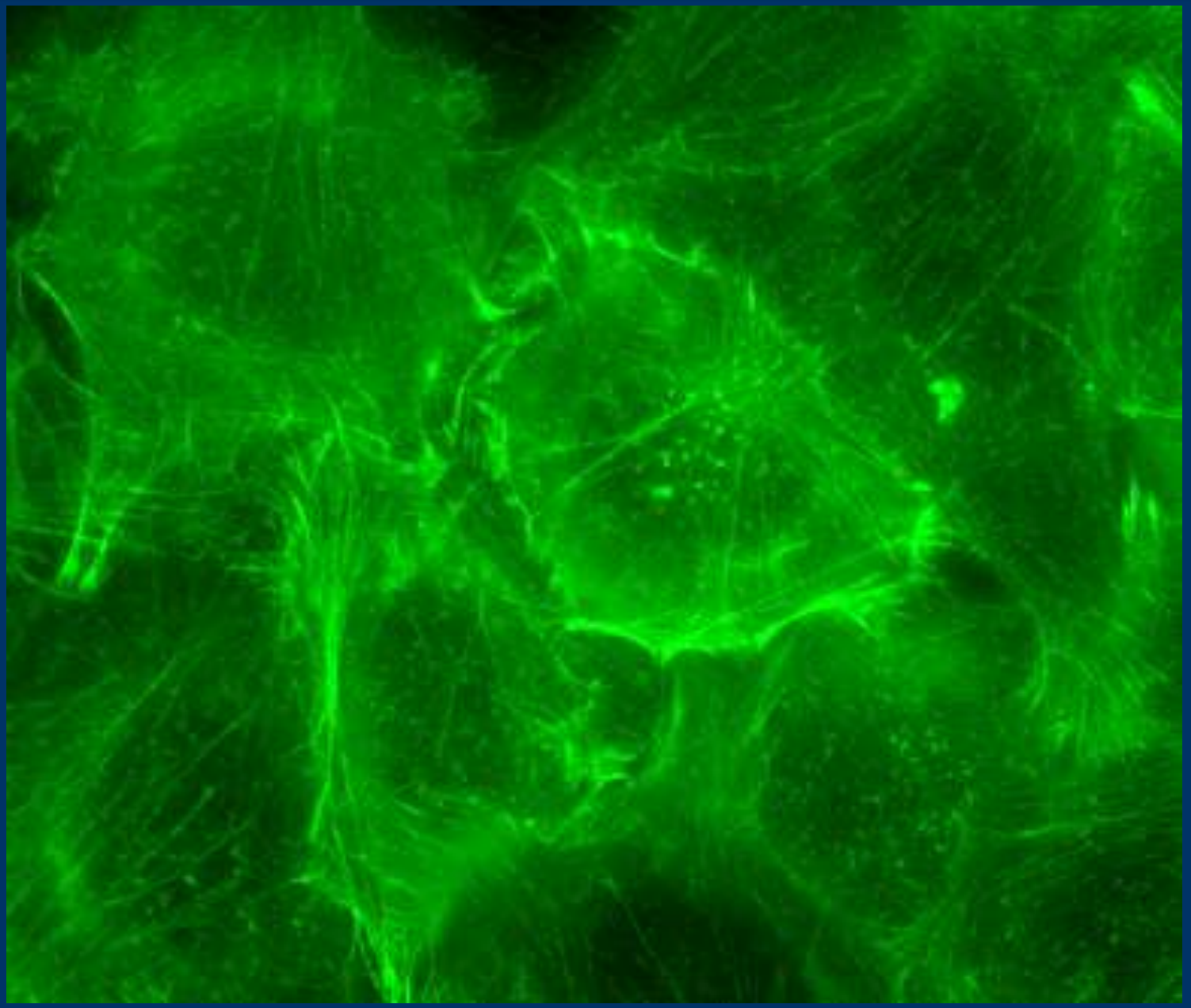
Membrane and cytoplasmic cytoskeleton



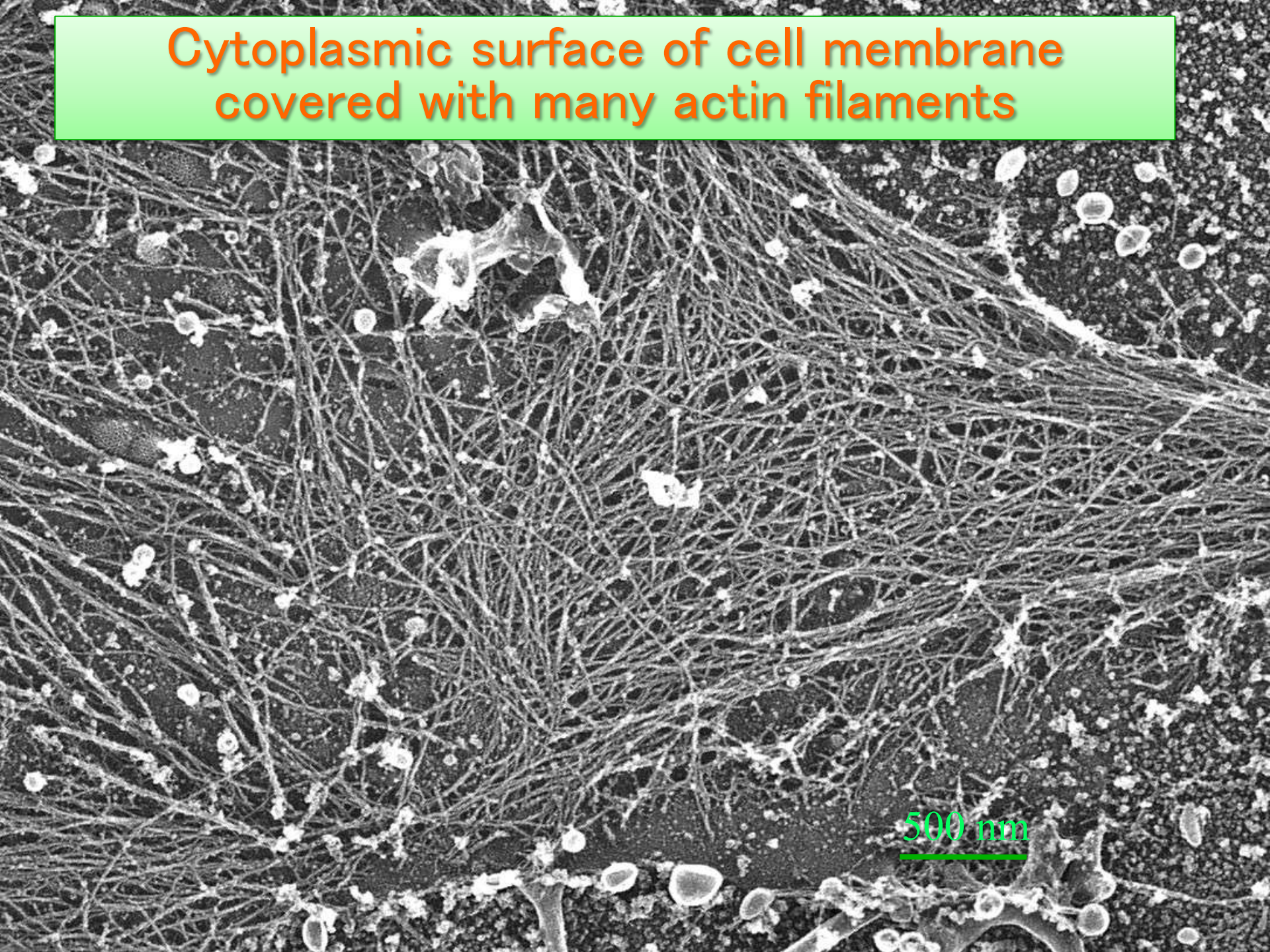
Spatial distribution of membrane actin filaments



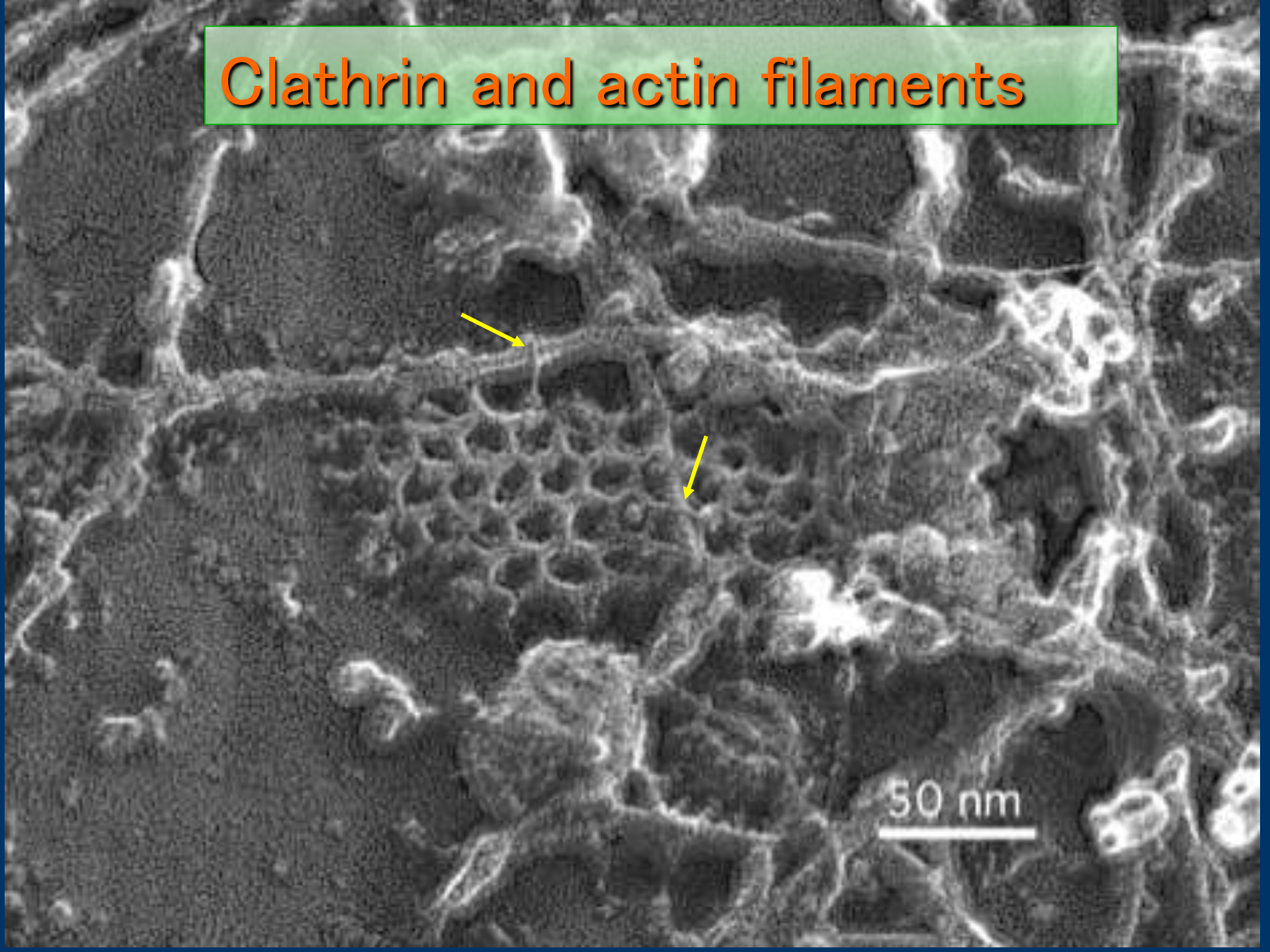
Live cell imaging of GFP-actin in NRK cells

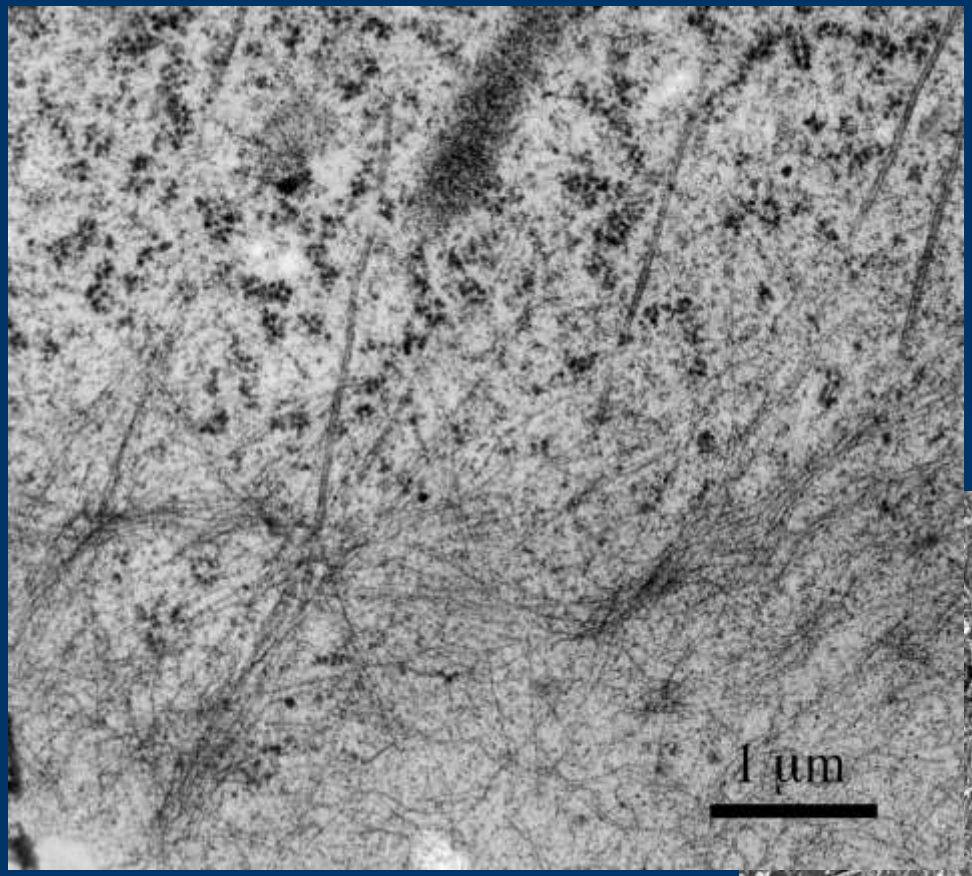


Cytoplasmic surface of cell membrane covered with many actin filaments



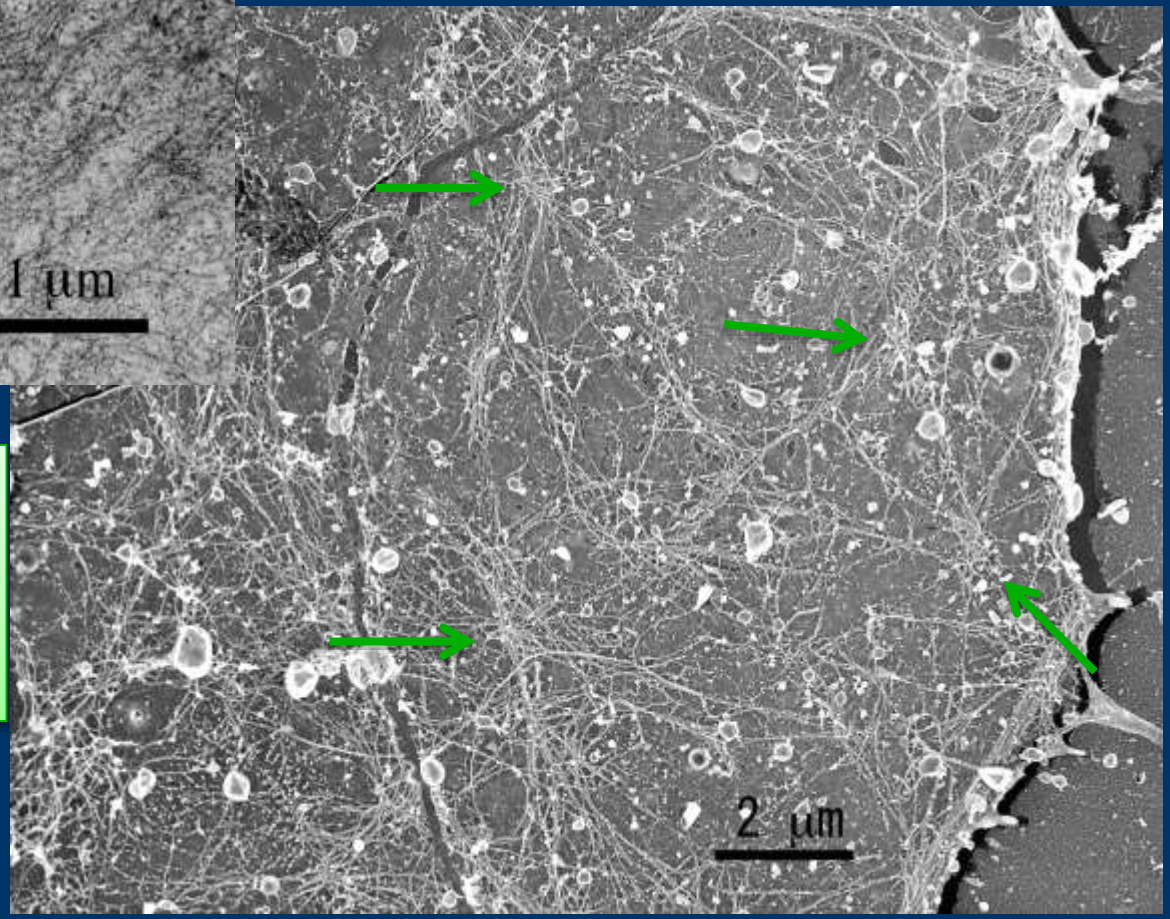
Clathrin and actin filaments





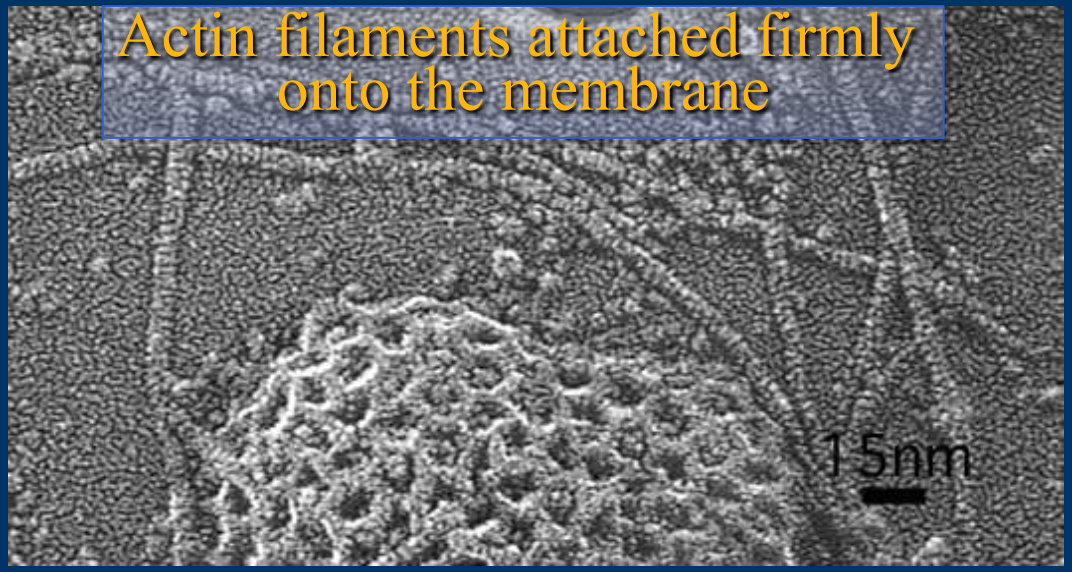
Few membrane cytoskeletons are detected even in grazing thin section,

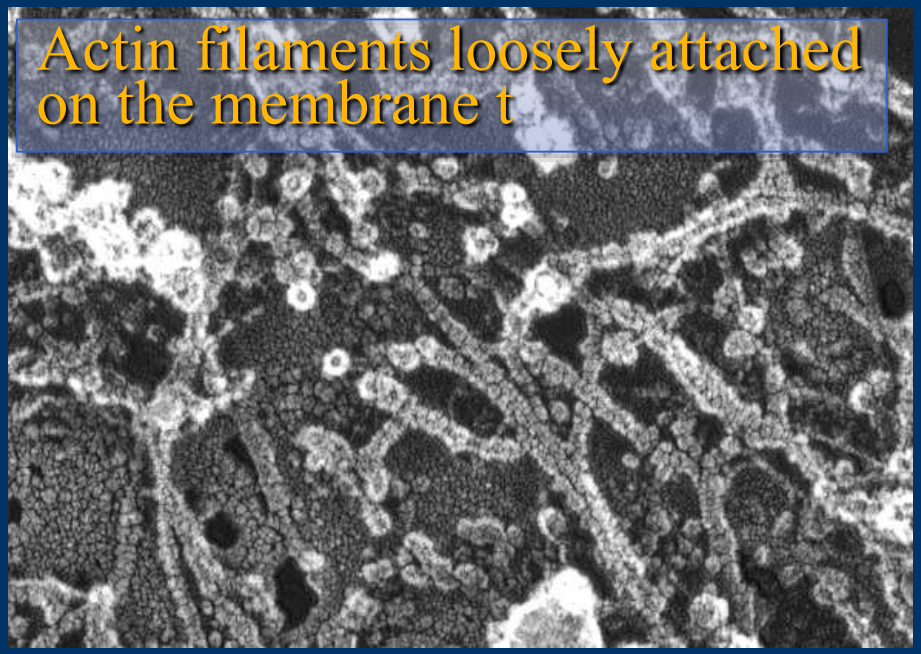
but observed in unroofed freeze-etched samples.

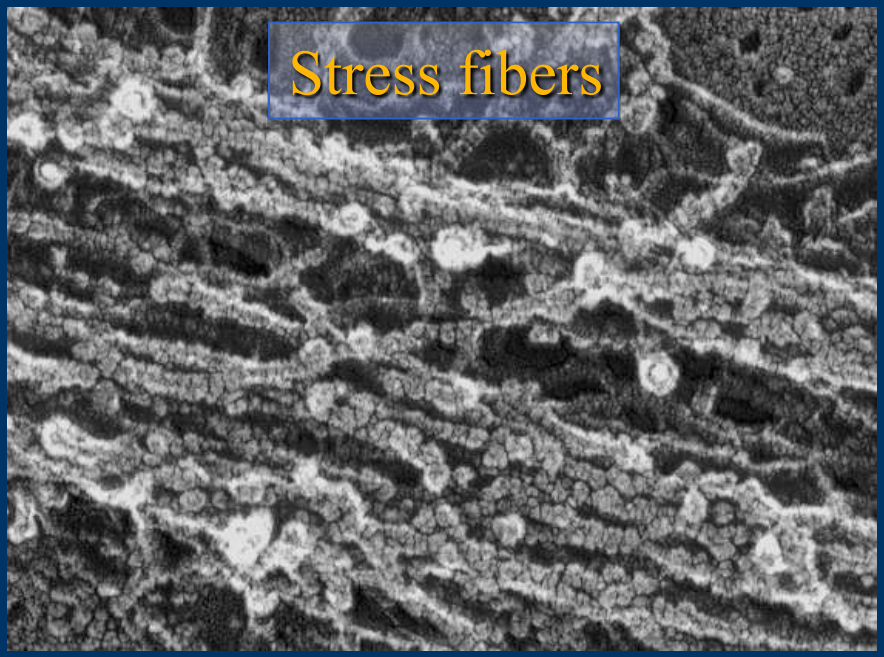


Tomography of membrane cytoskeleton

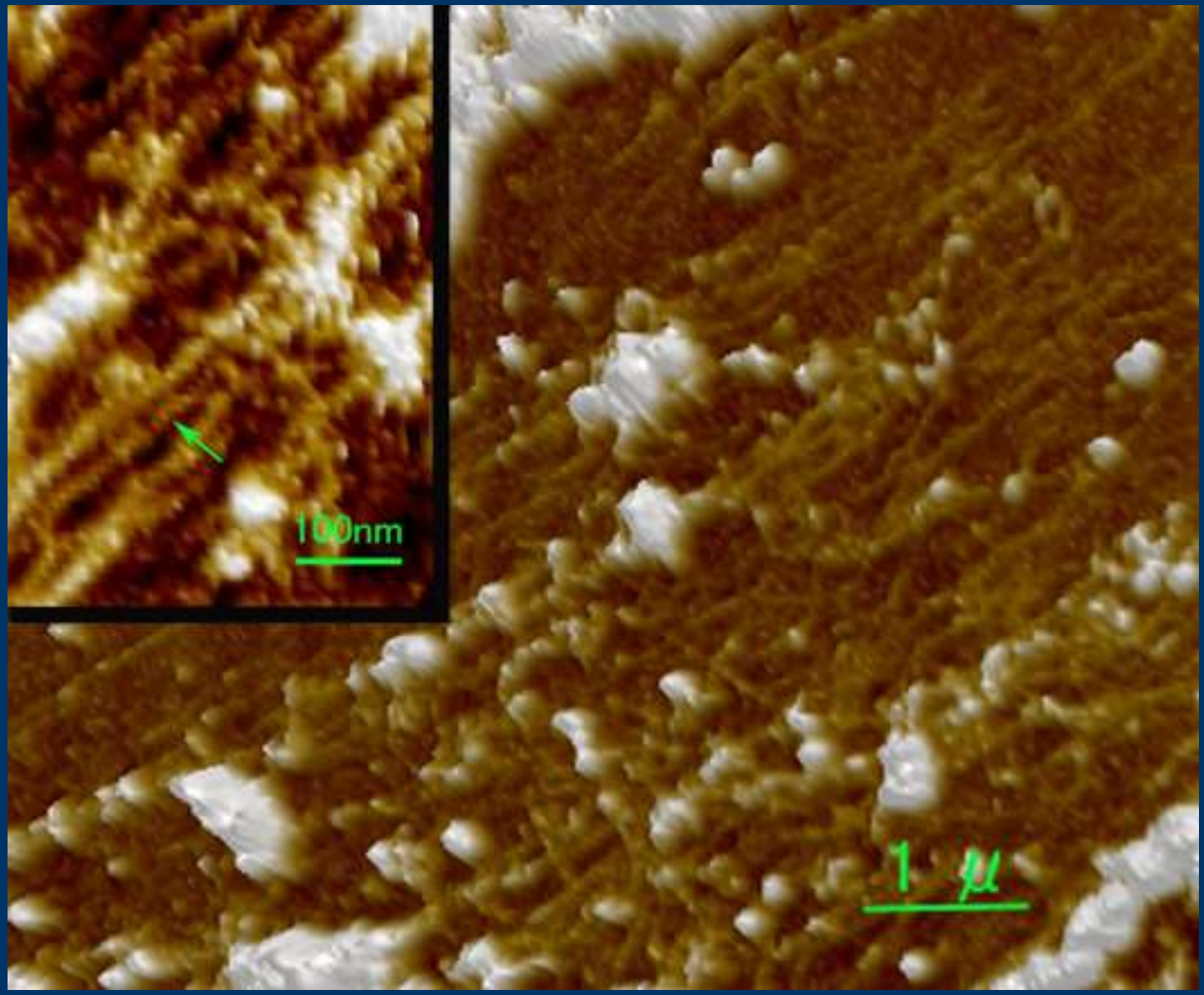
Actin filaments are classified into three types based on spatial distribution



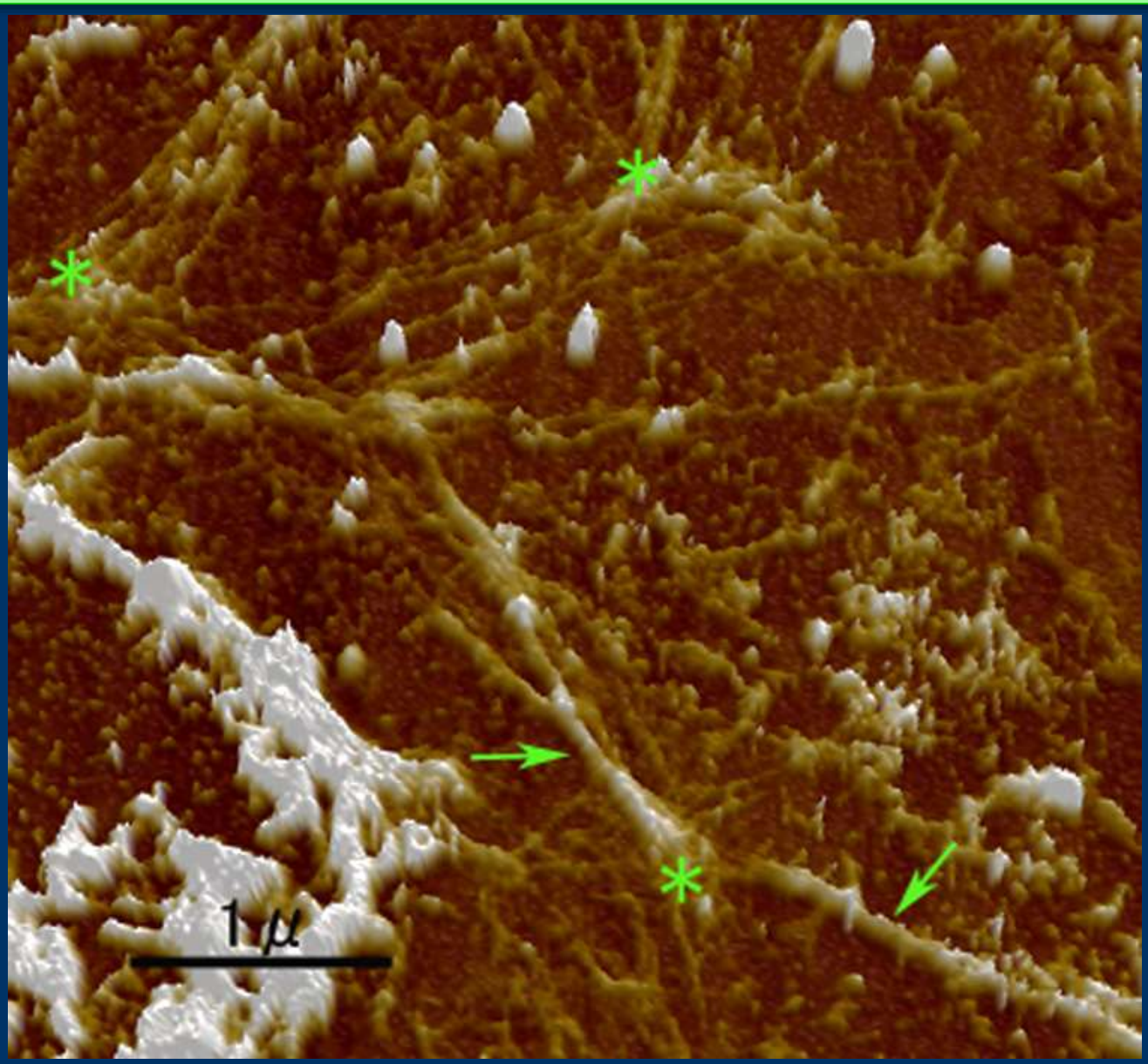




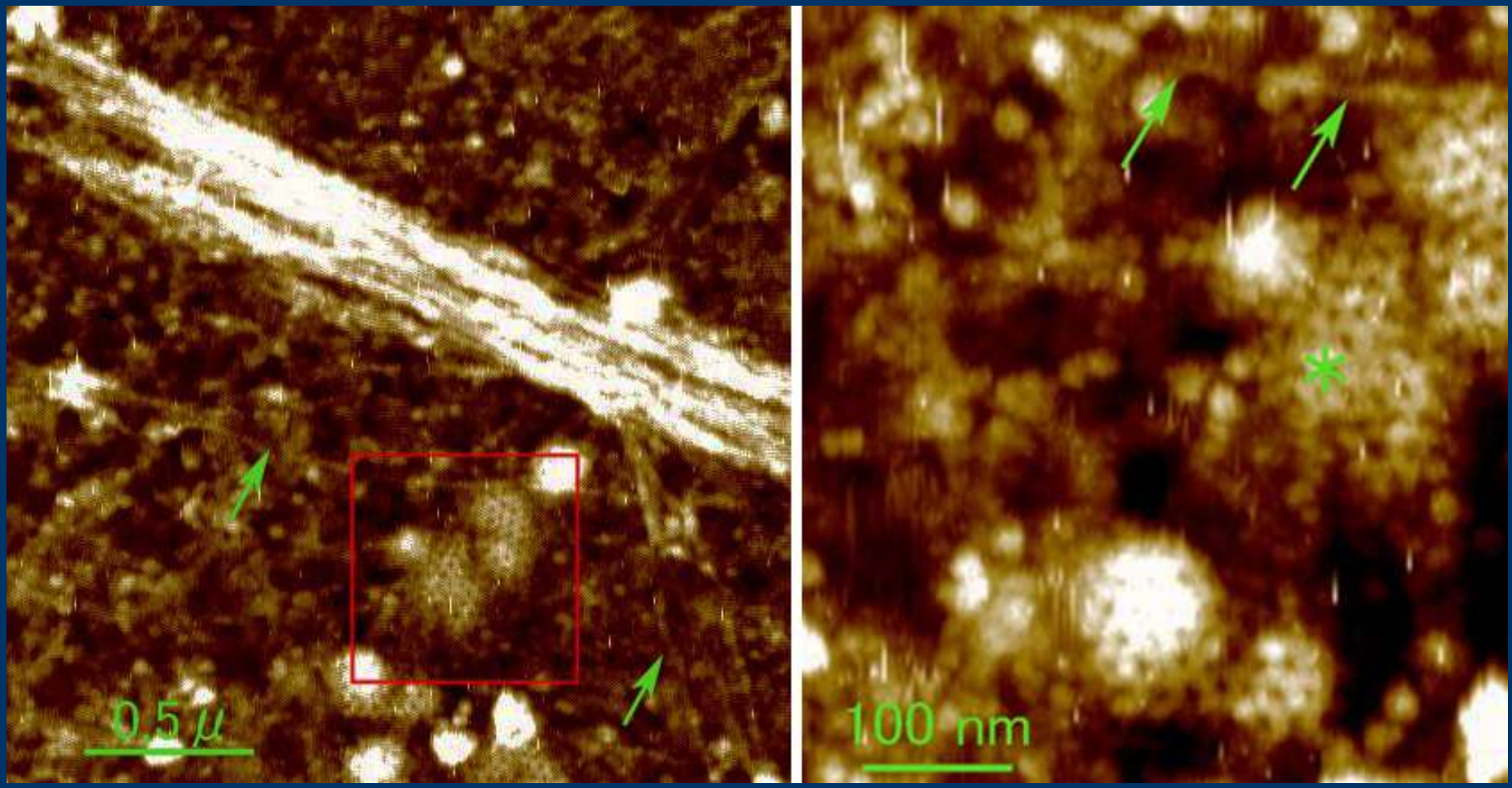
AFM imaging of membrane cytoskeleton in water



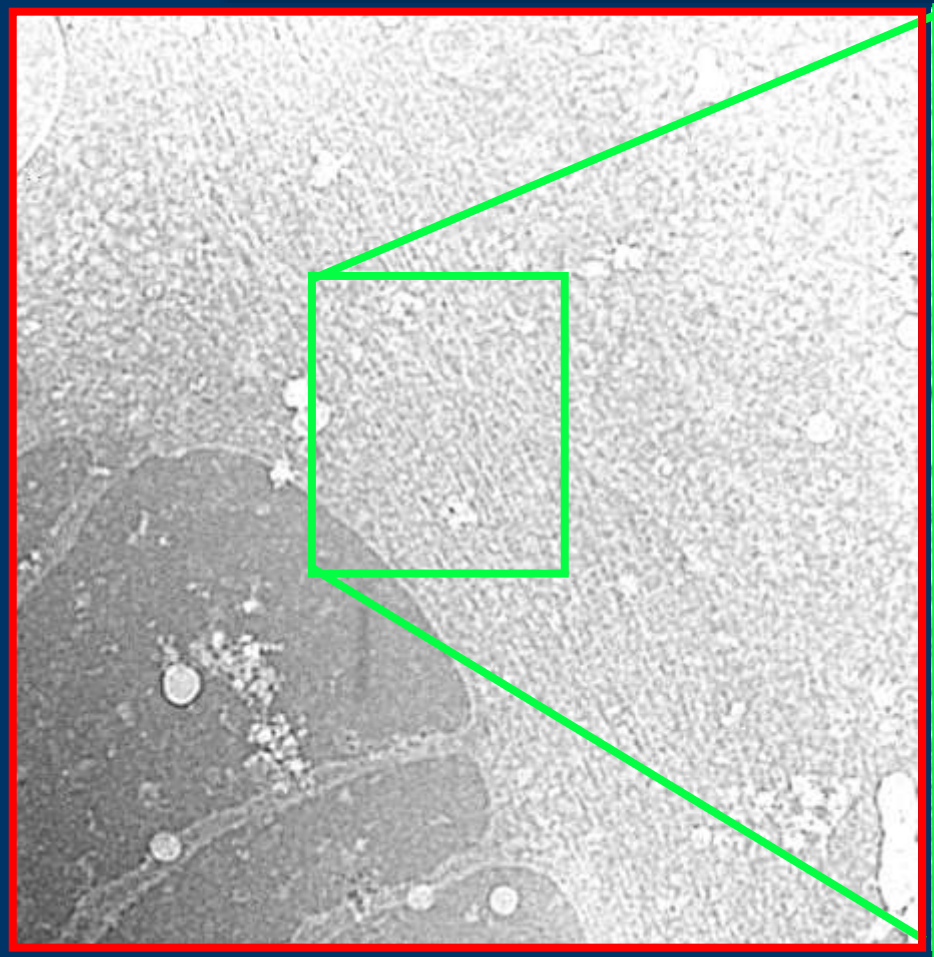
AFM imaging of membrane cytoskeleton in water

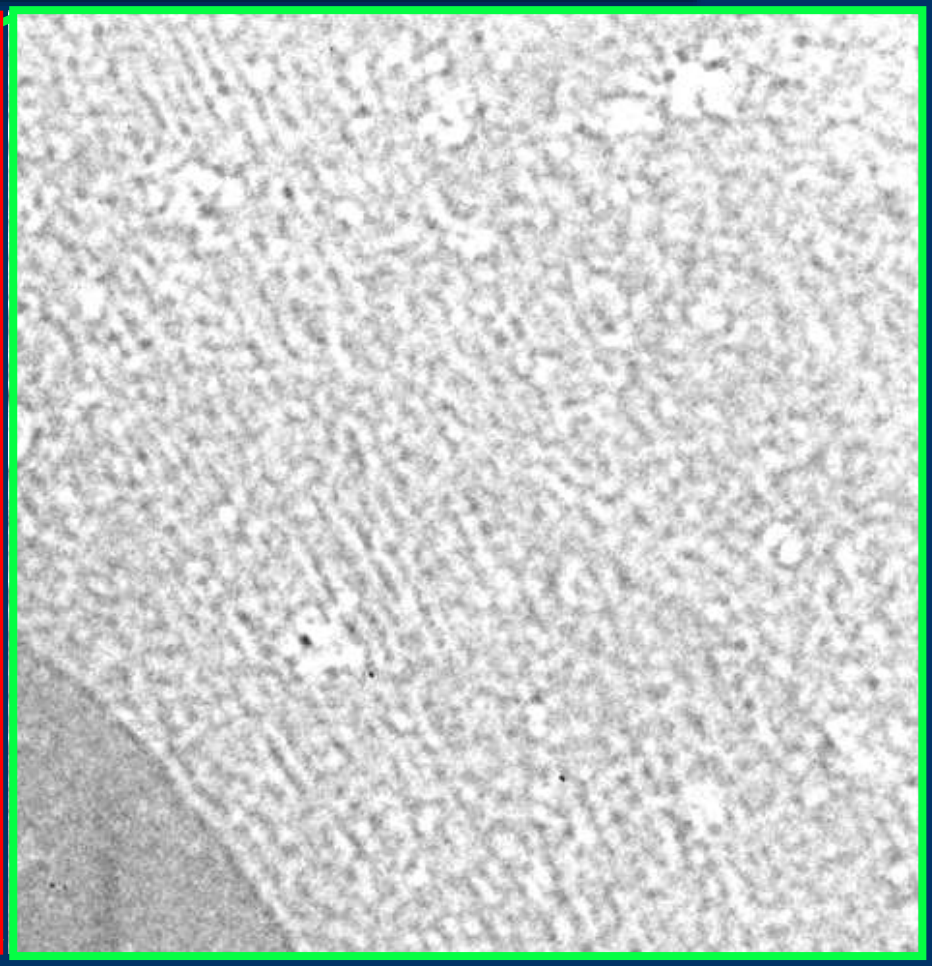


AFM imaging of clathrin coats in water



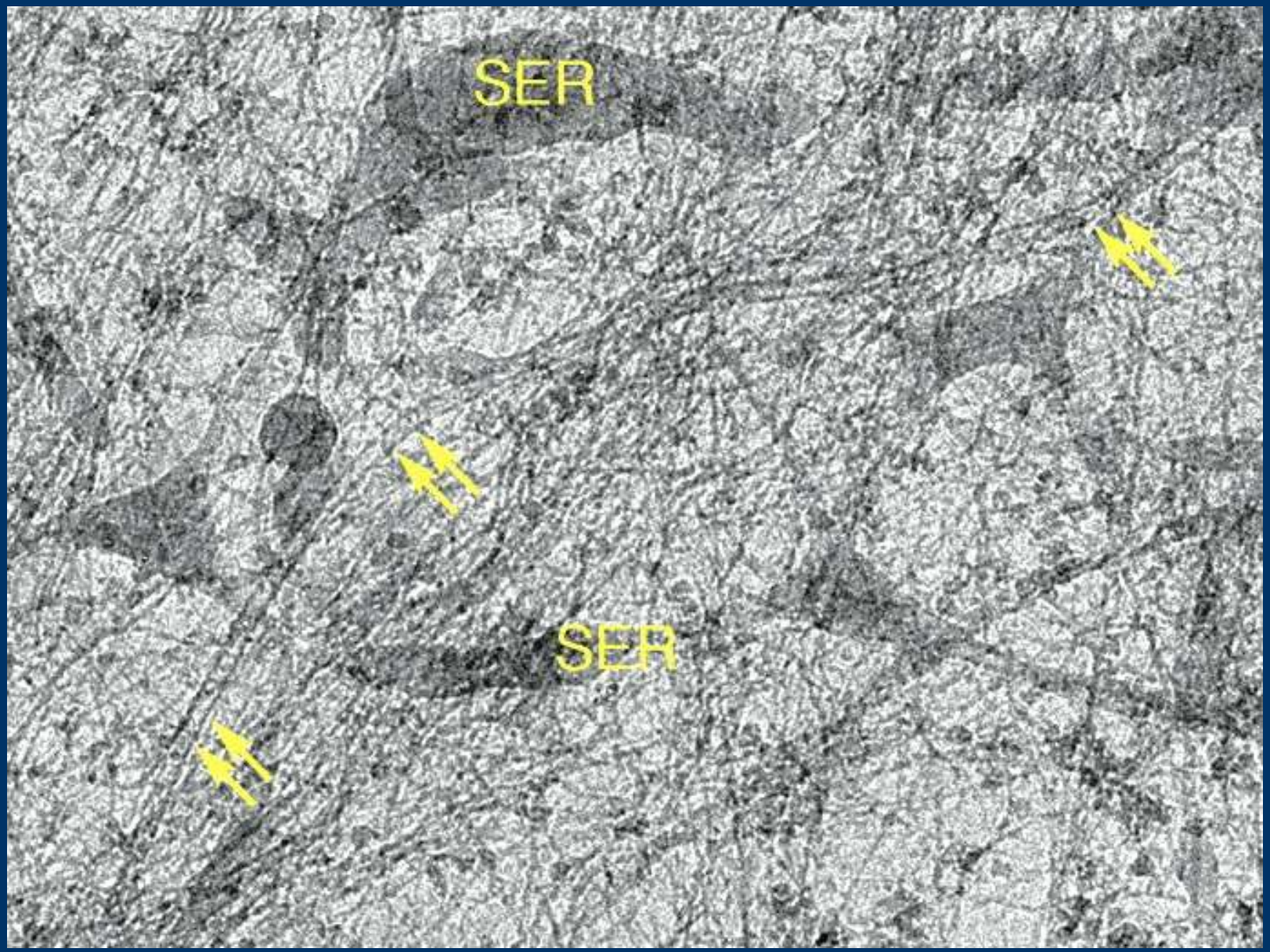
Whole cell observation



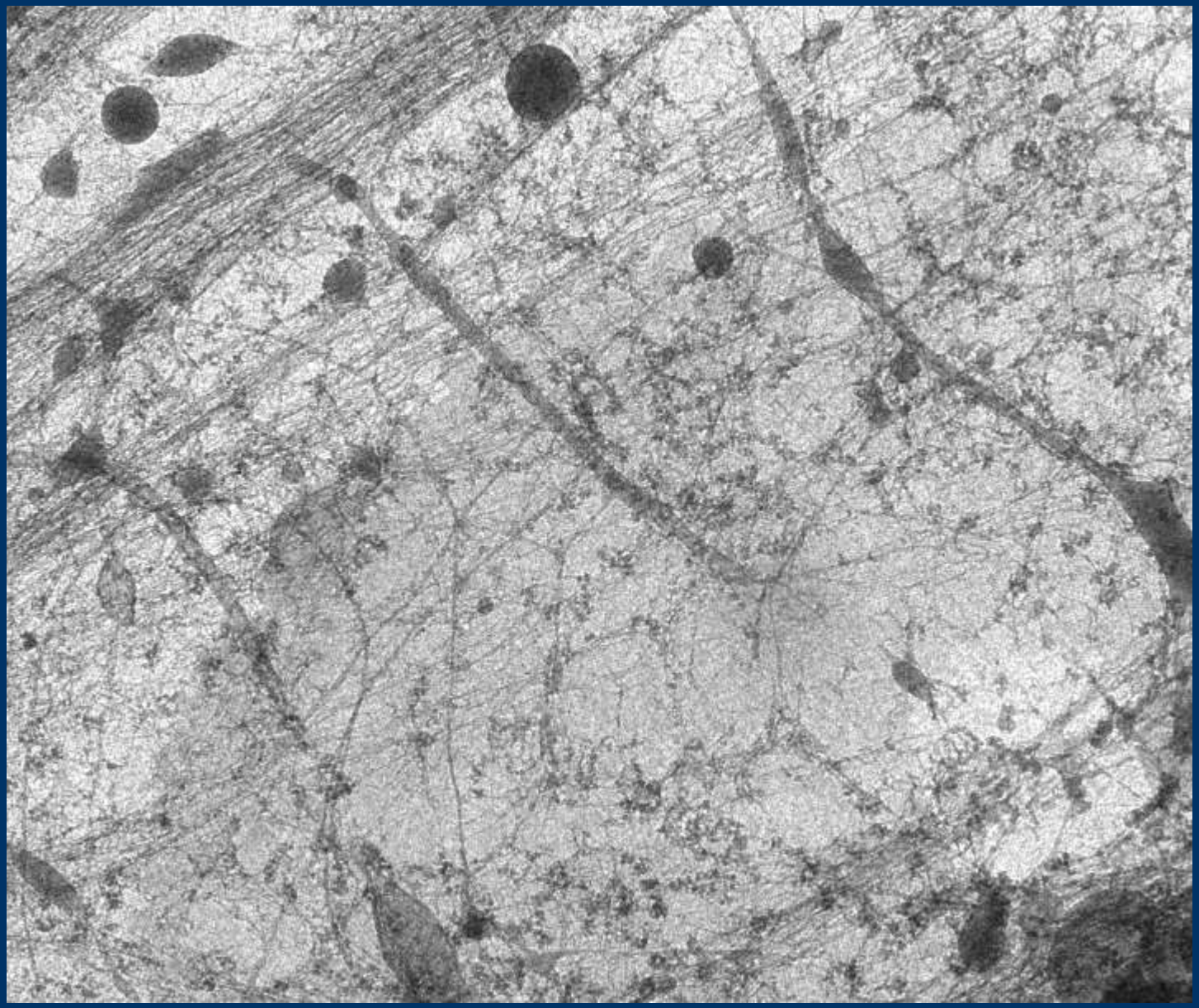


It is difficult to observe fine structures due to thick cytoplasm.

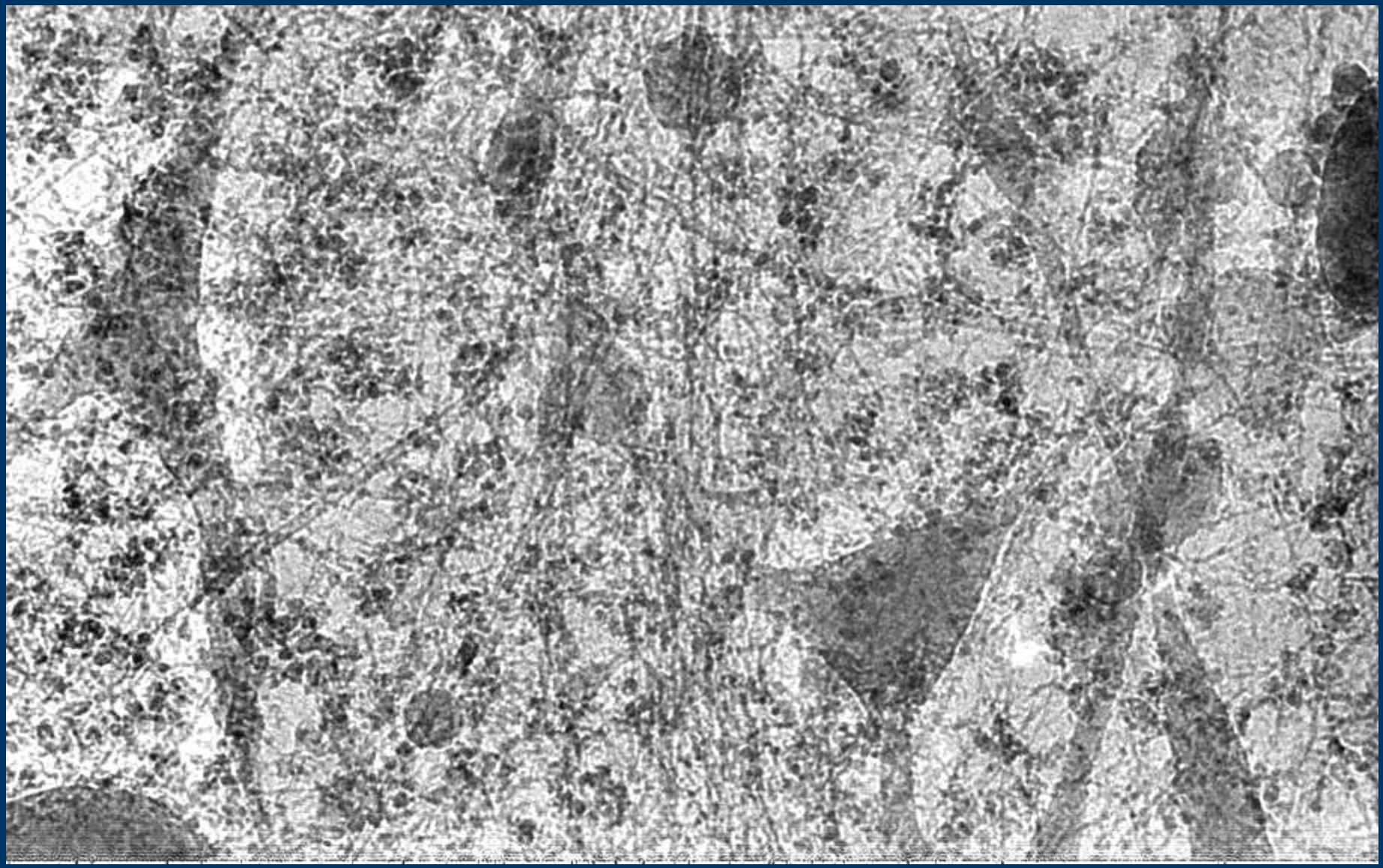
Cryo-electron micrograph of native cytoskeleton beneath the cell membrane



Cryo-electron micrograph of native cytoskeleton beneath the cell membrane

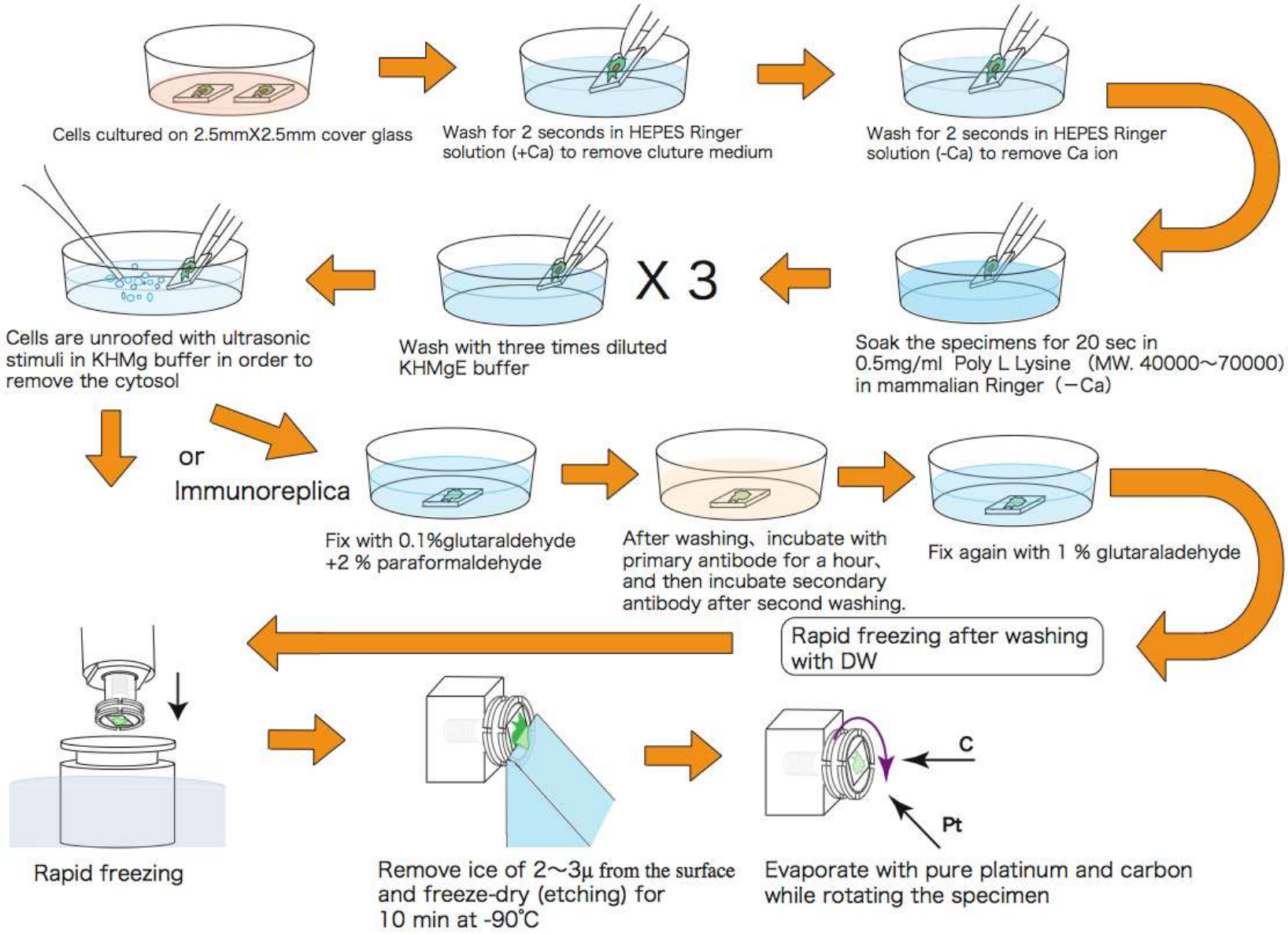


Cryo-electron micrograph of native cytoskeleton beneath the cell membrane

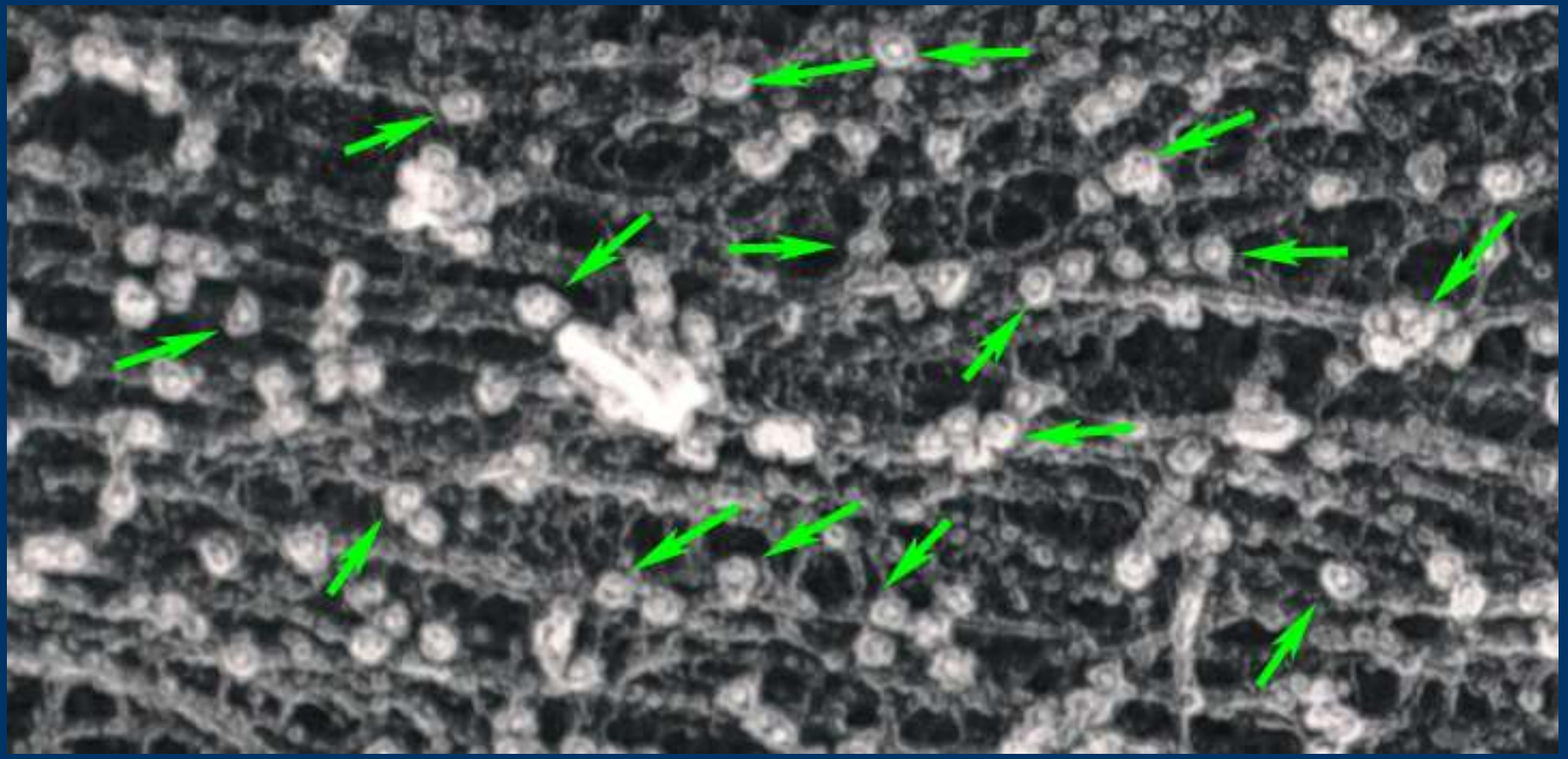


Spatial Specificity of Actin Cytoskeletons found by immuno-labeling

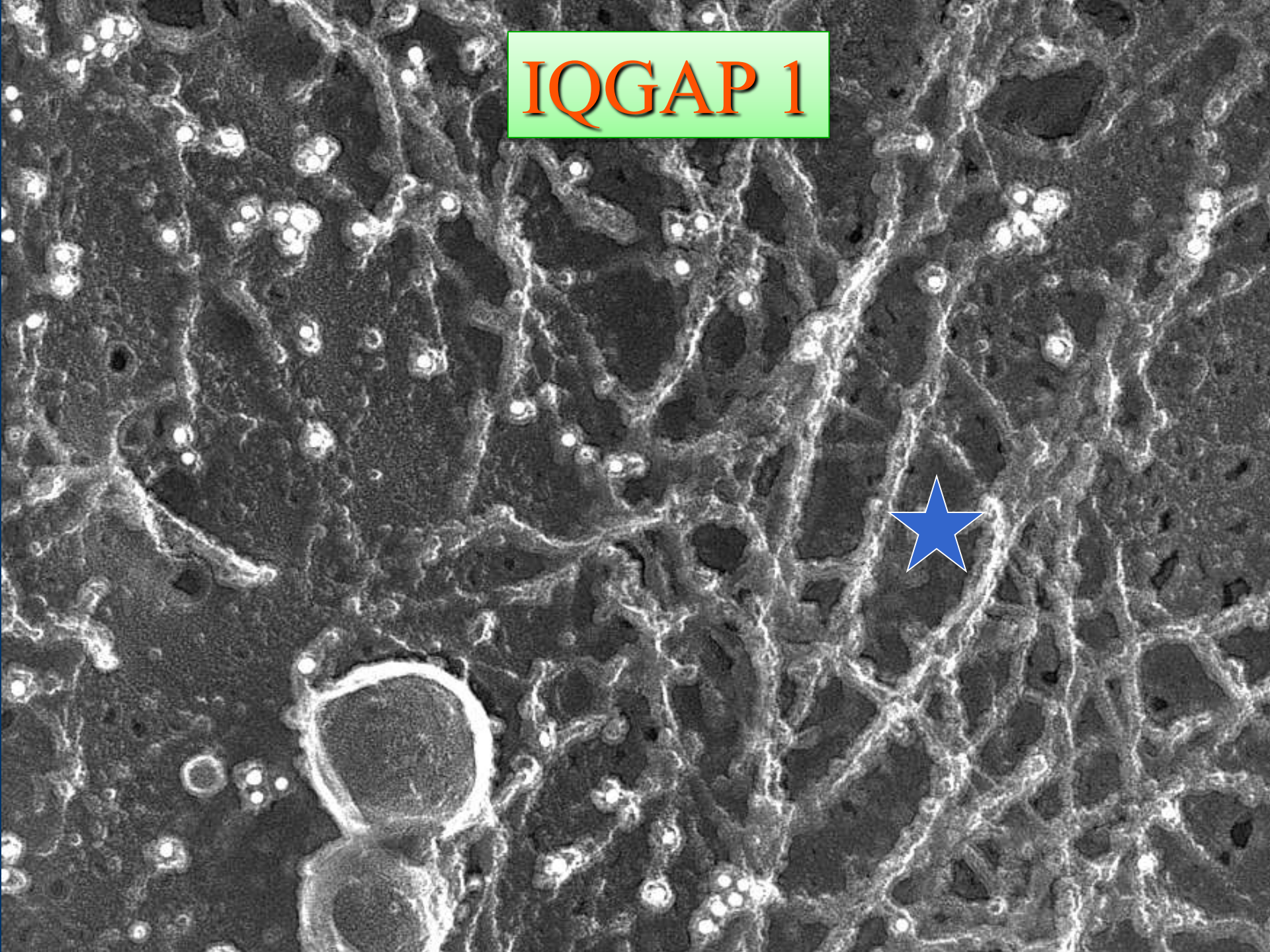
Protocol of immuno-freeze-etching

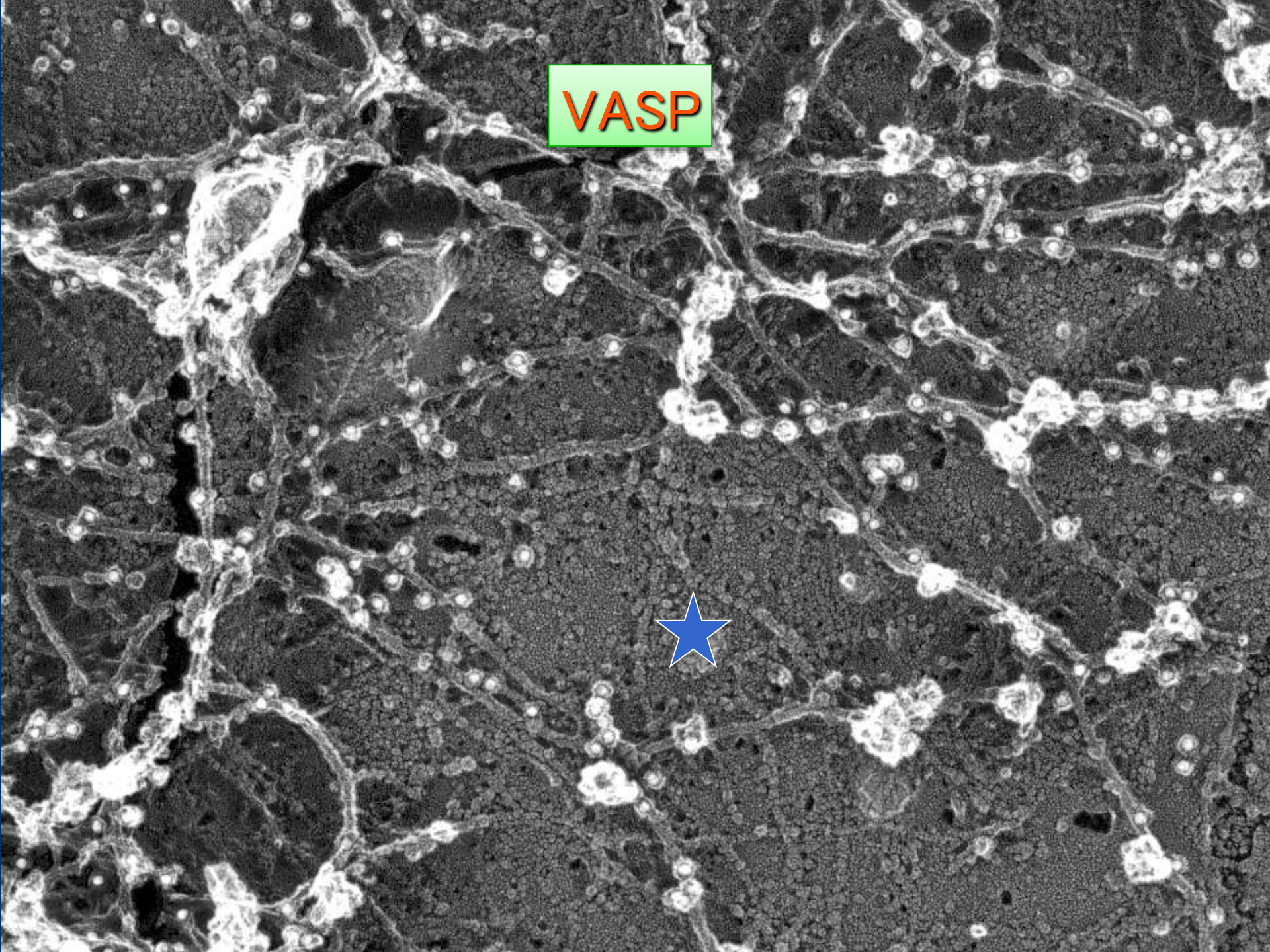


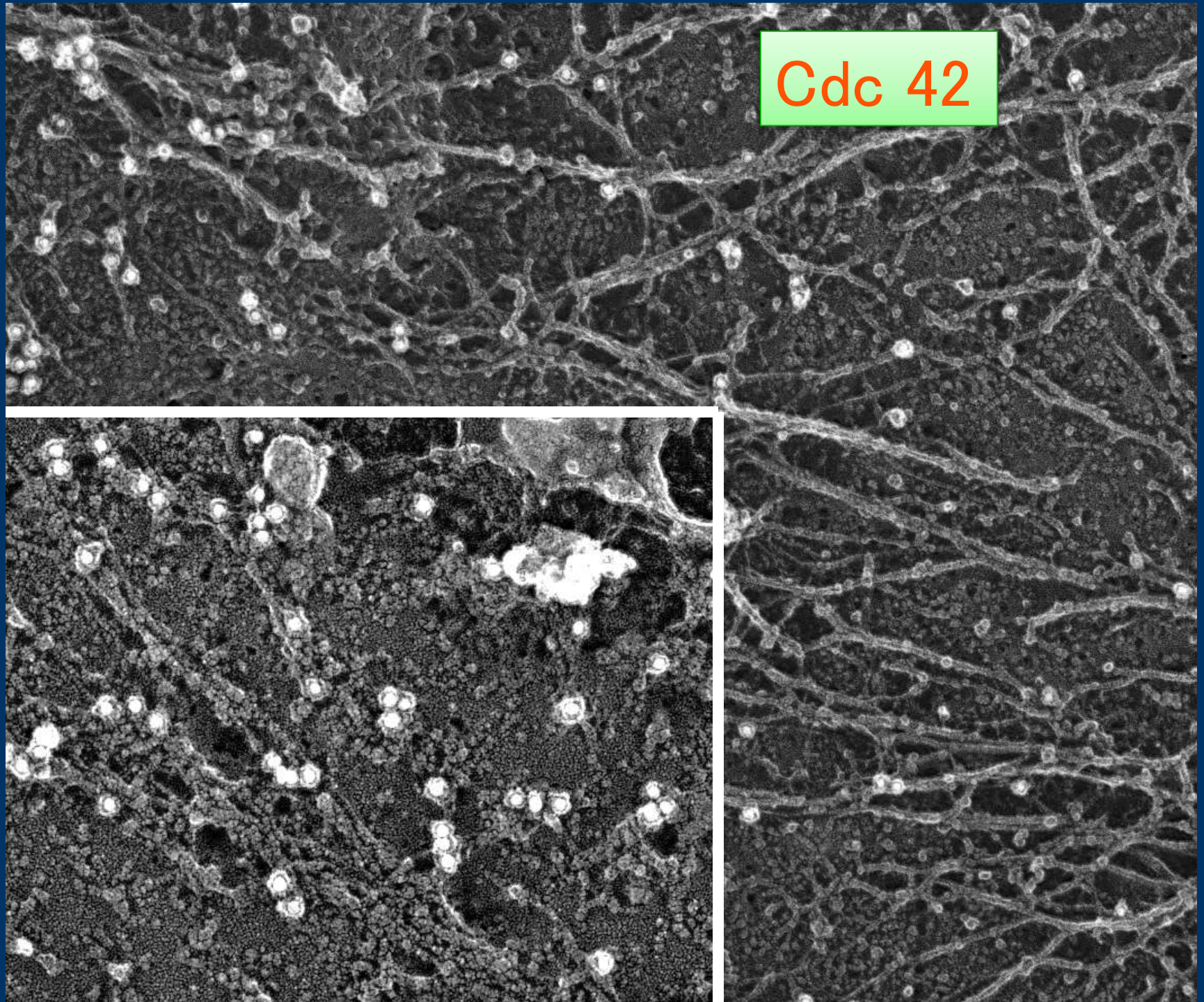
Actin filaments are identified by labeling with colloidal gold conjugated antibody

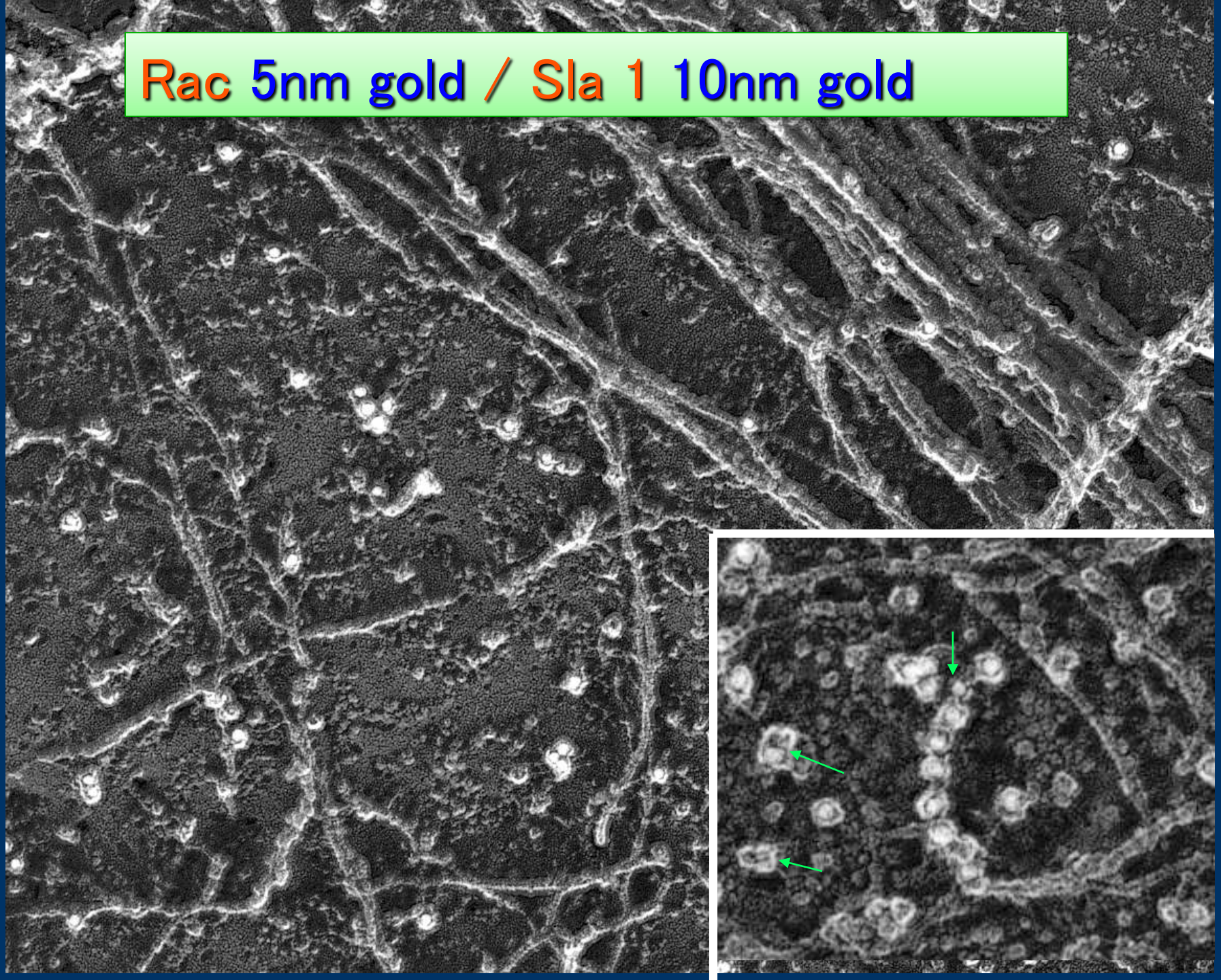


Colloidal gold particles are shown here as white dots because of reversal contrast.

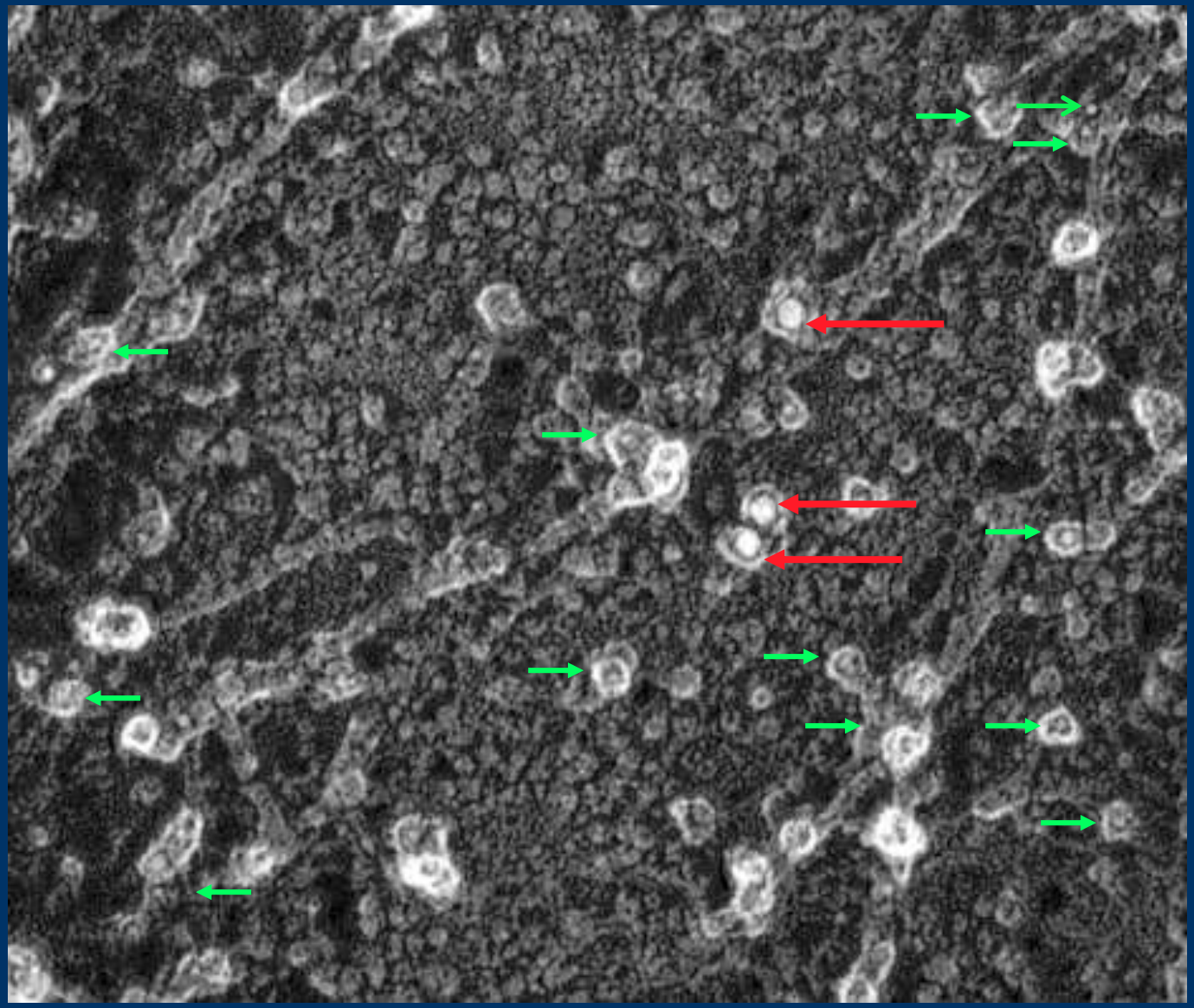

IQGAP 1


VASP

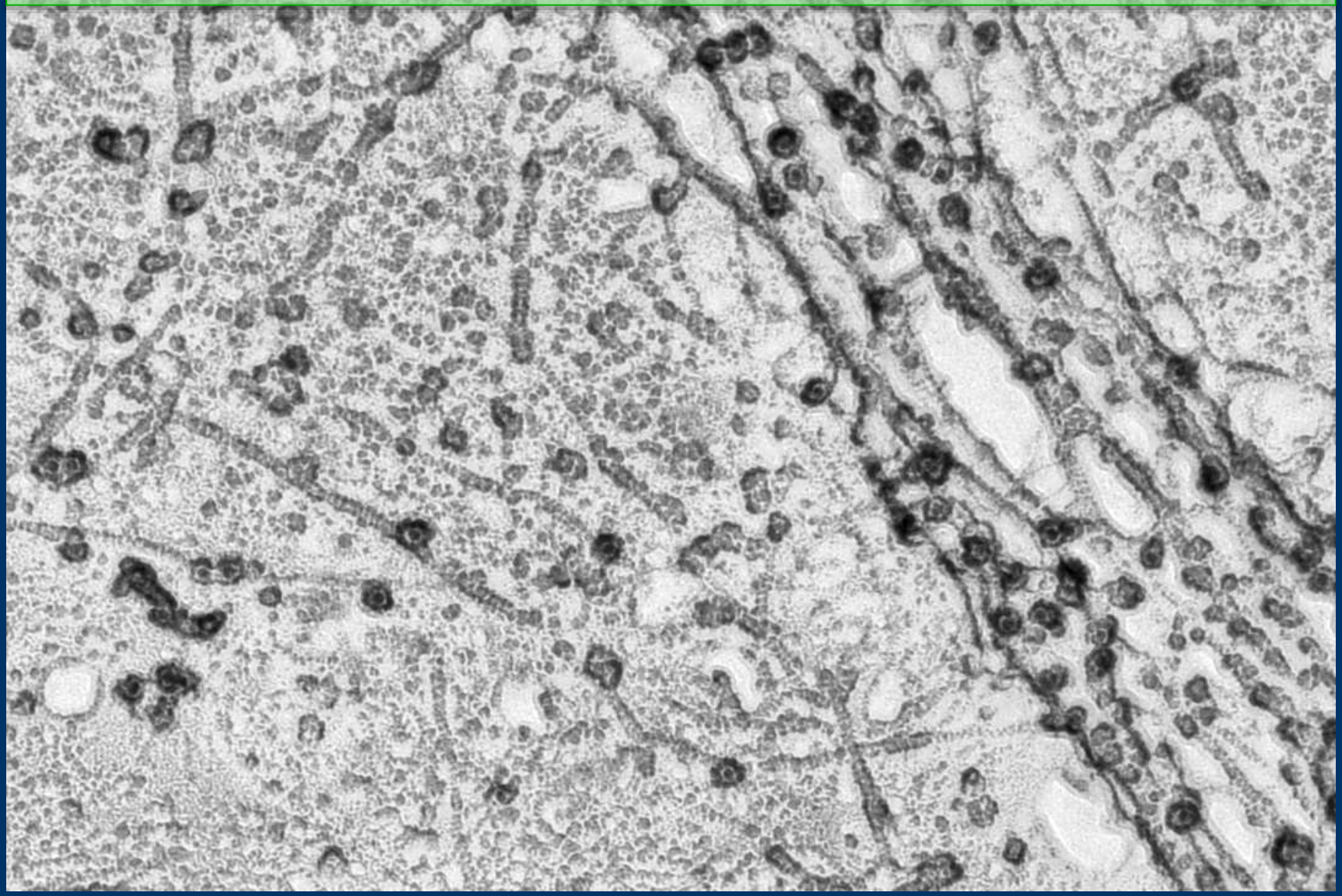

Cdc 42


Rac 5nm gold / Sla 1 10nm gold

N WASP 10nm gold / IQGAP 1 5 nm gold

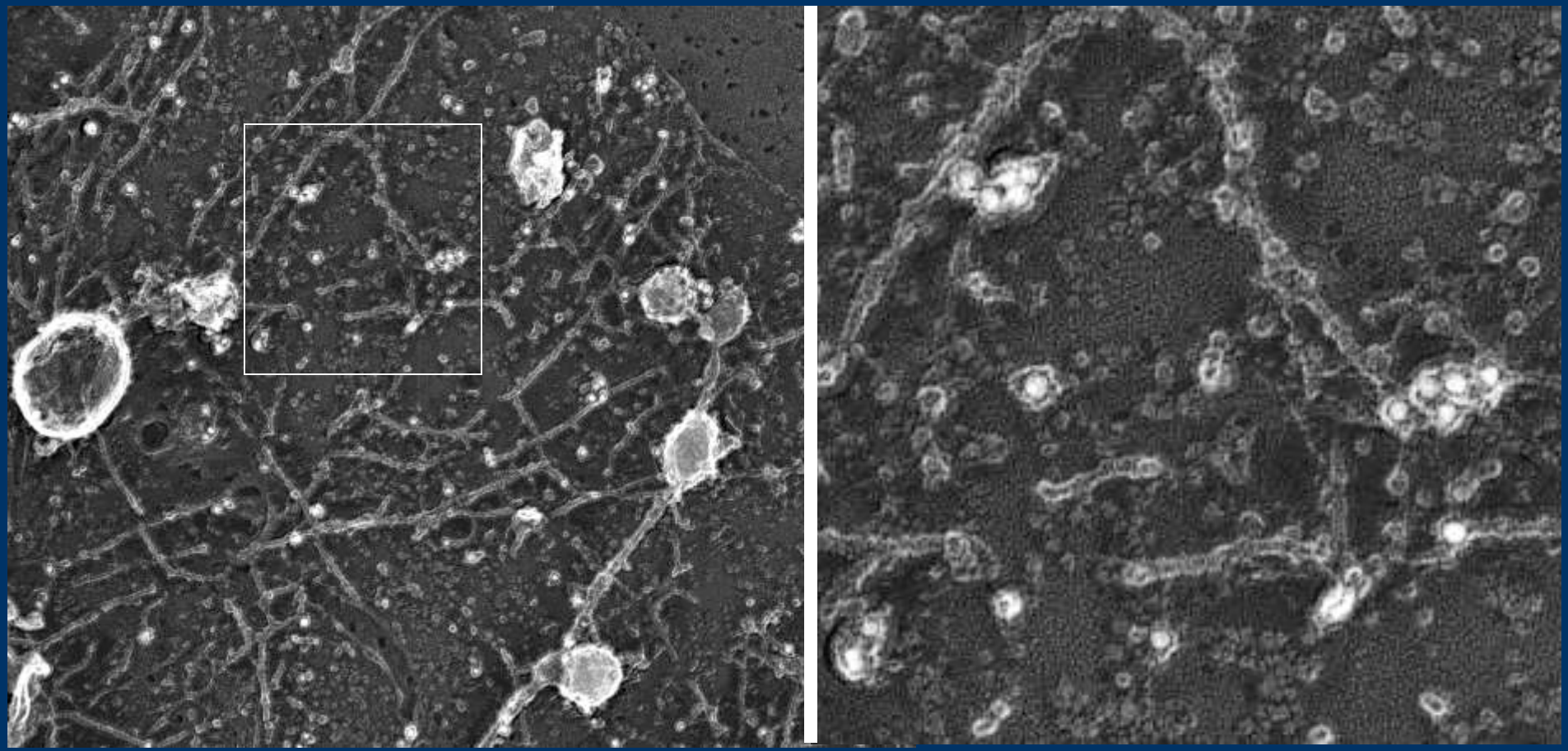


Myosin II appeared to be more abundant on stress fibers than the other type of actin filaments on the membrane.



Focal contact related proteins

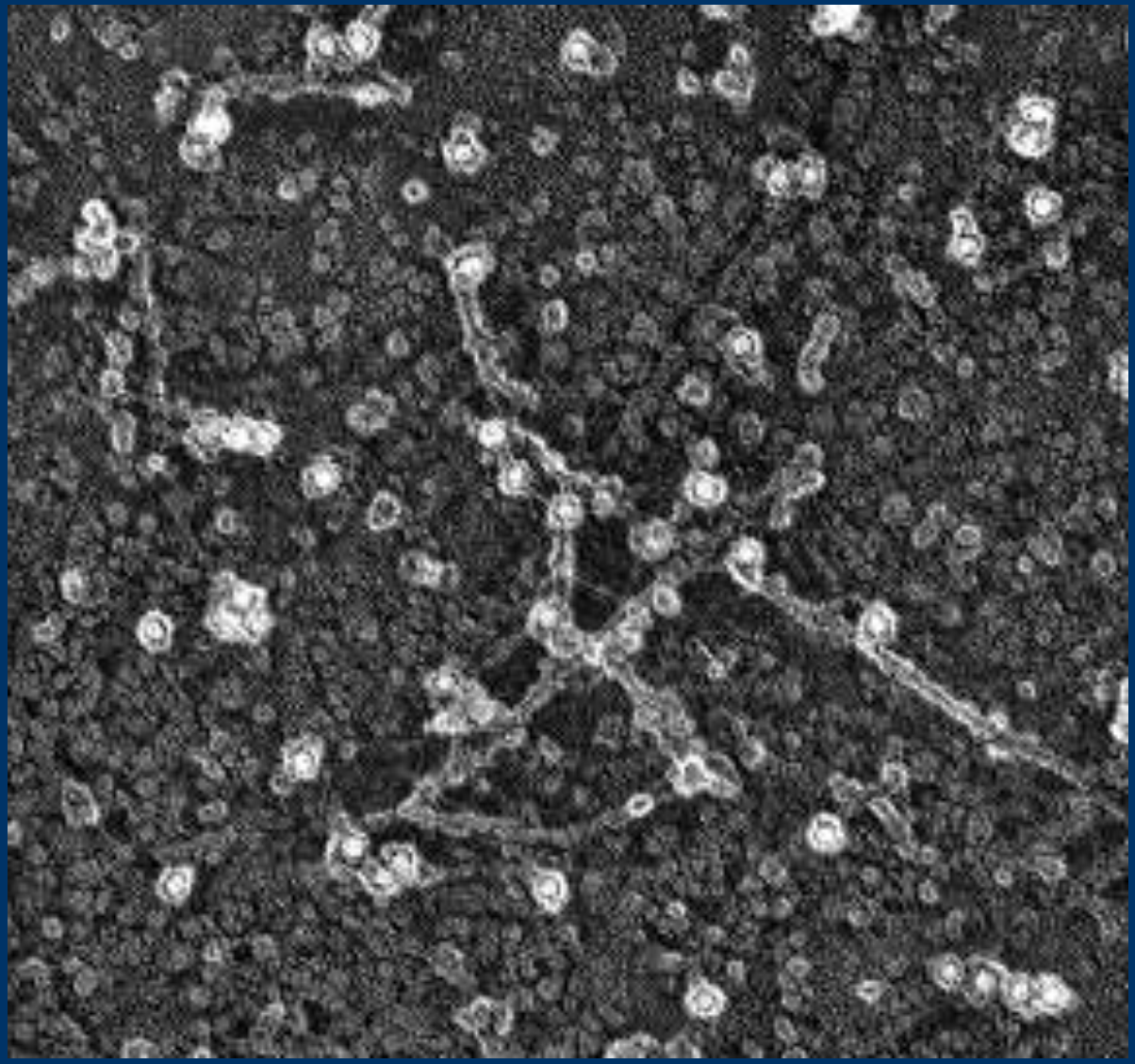
Integrin β1



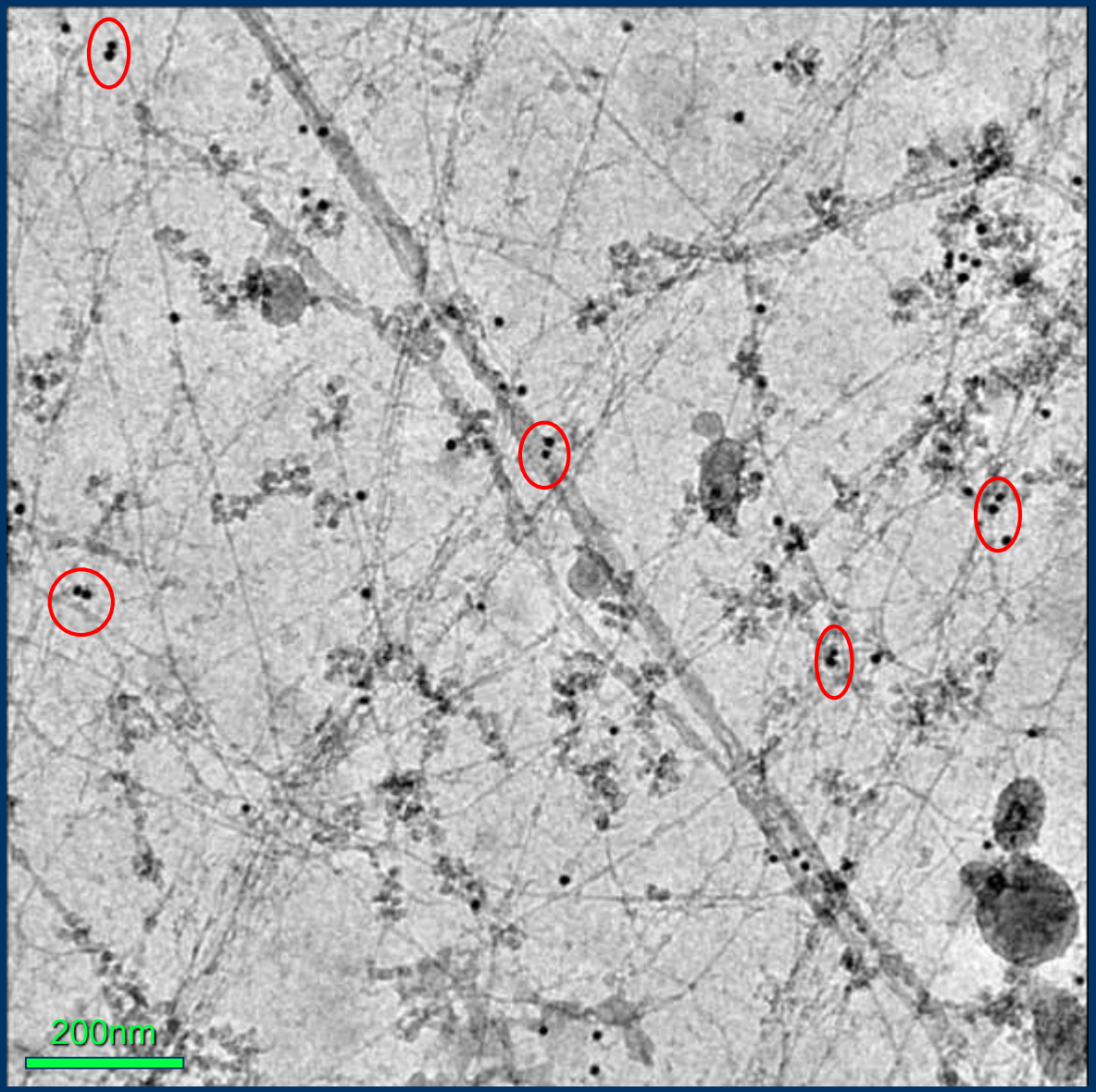
High magnification of boxed area

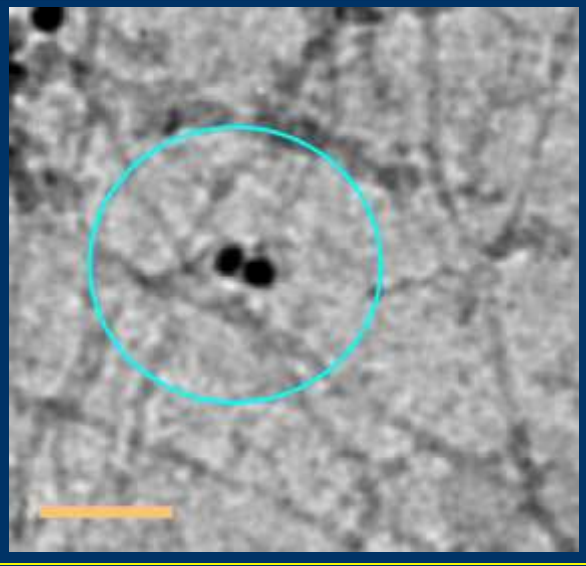
Focal contact related proteins

Zyxin



Immuno-gold labeling (IQCAP-1) in cryo-electron microscopy

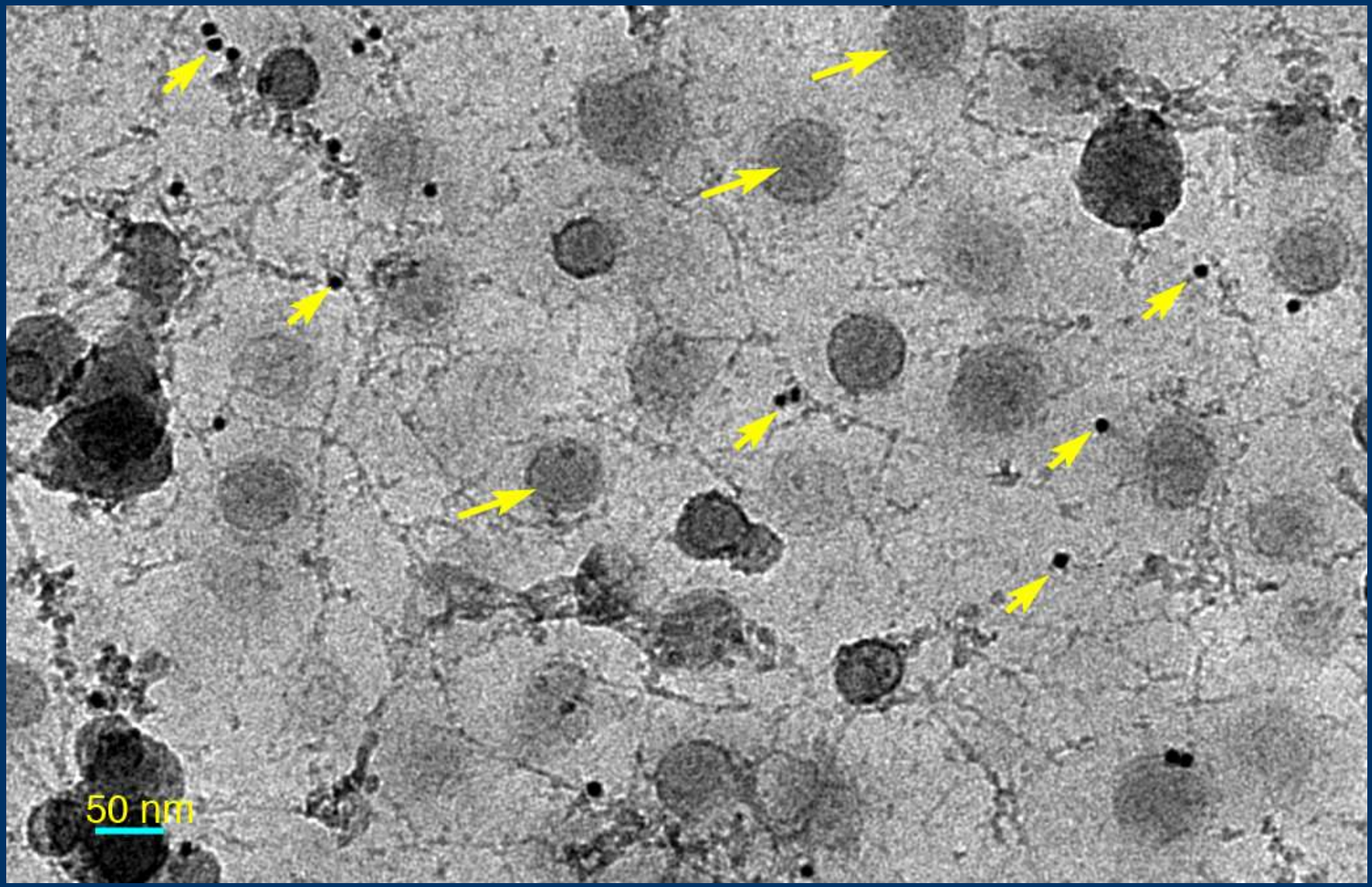




IQCAP-1 is found on actin filaments located at the cytoplasmic surface of the membrane as a dimer.

Dimer

Cryo-electron micrograph showing the dominant area of caveoli labeled with anti-IQGAP1 immunogold



Summary 1

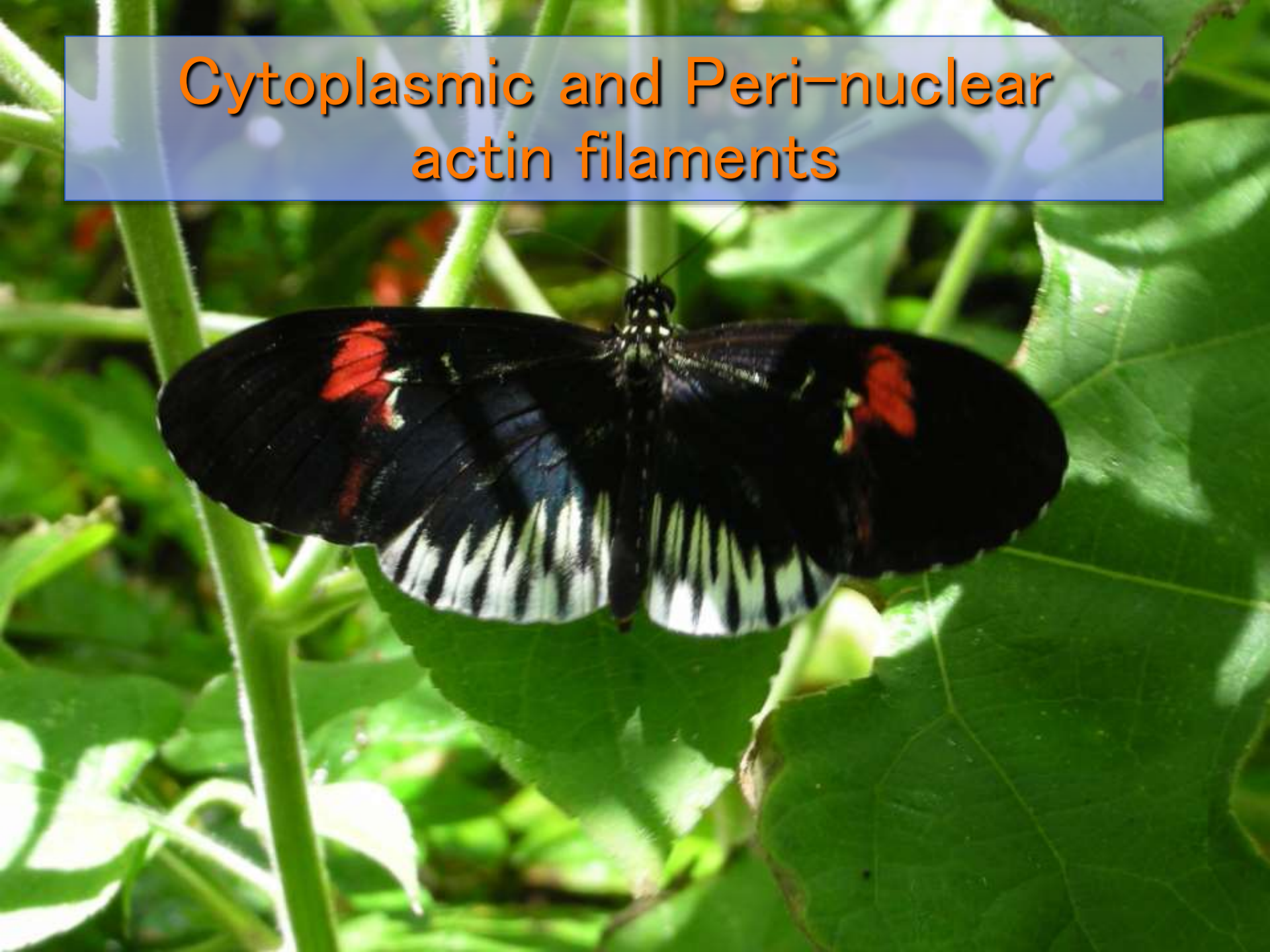
1. Actin filaments on the membrane were clsaafied into 3 types based on spatial disposition. First type of actin filaments are tightly bound to membrane. Second type is the filaments attached loosely to the membrane and form complicated mesh work at cortical area. Third type is so called stress fibers.

2. Binding of effecter proteins indicated spatial specificity. Effecter proteins, IQGAP1,WASP,VASP bound predominantly on type 2 actin filaments, but not on type 1. Type 3, stress fiber, also contained their effecter proteins, but less than Type 2.

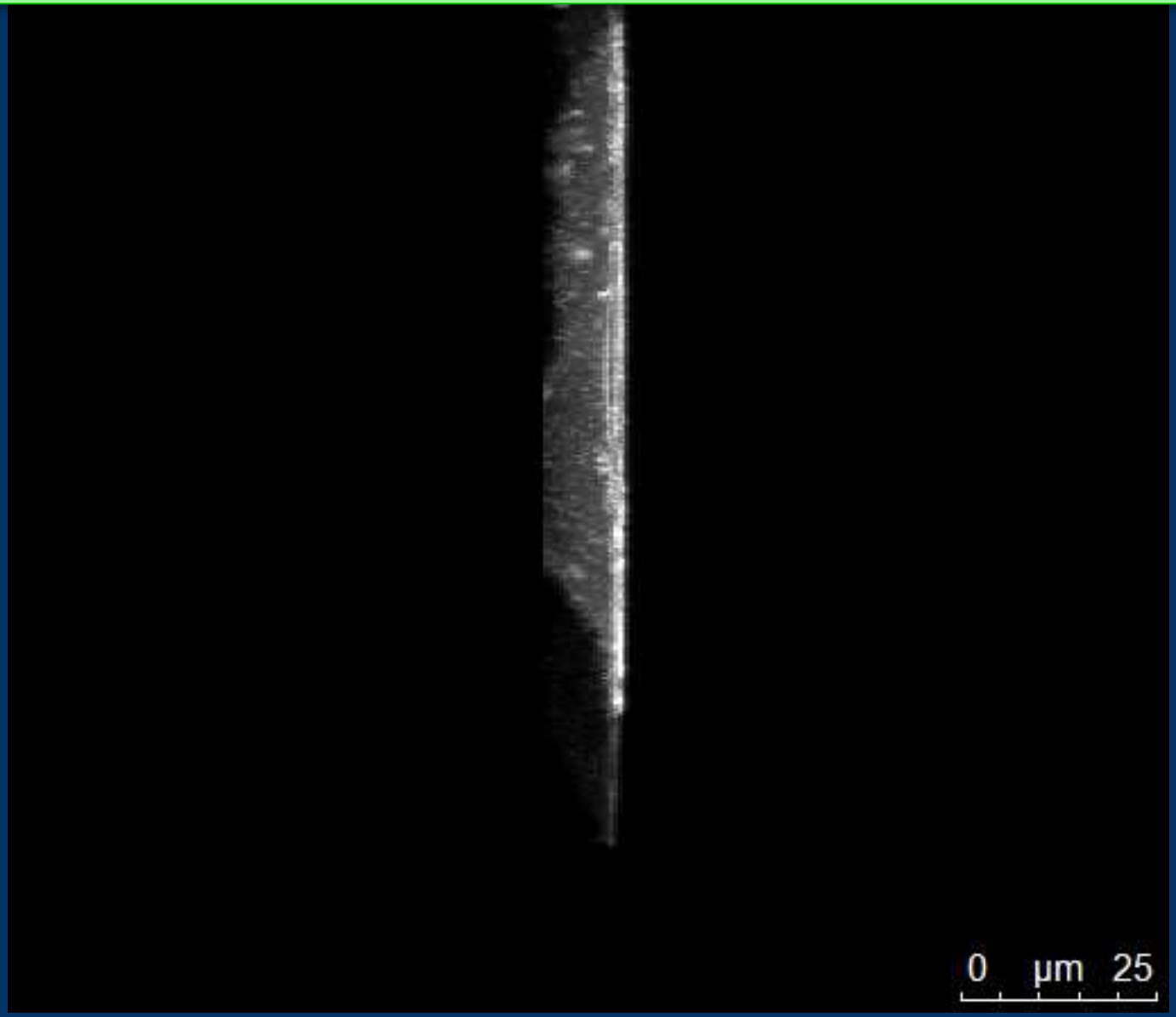
1. Anti-Myosin 2 antibodies was bound on every actin filaments, but a little on type 1.

1. After all, type 1 actin filaments was decorated with few actin binding proteins.

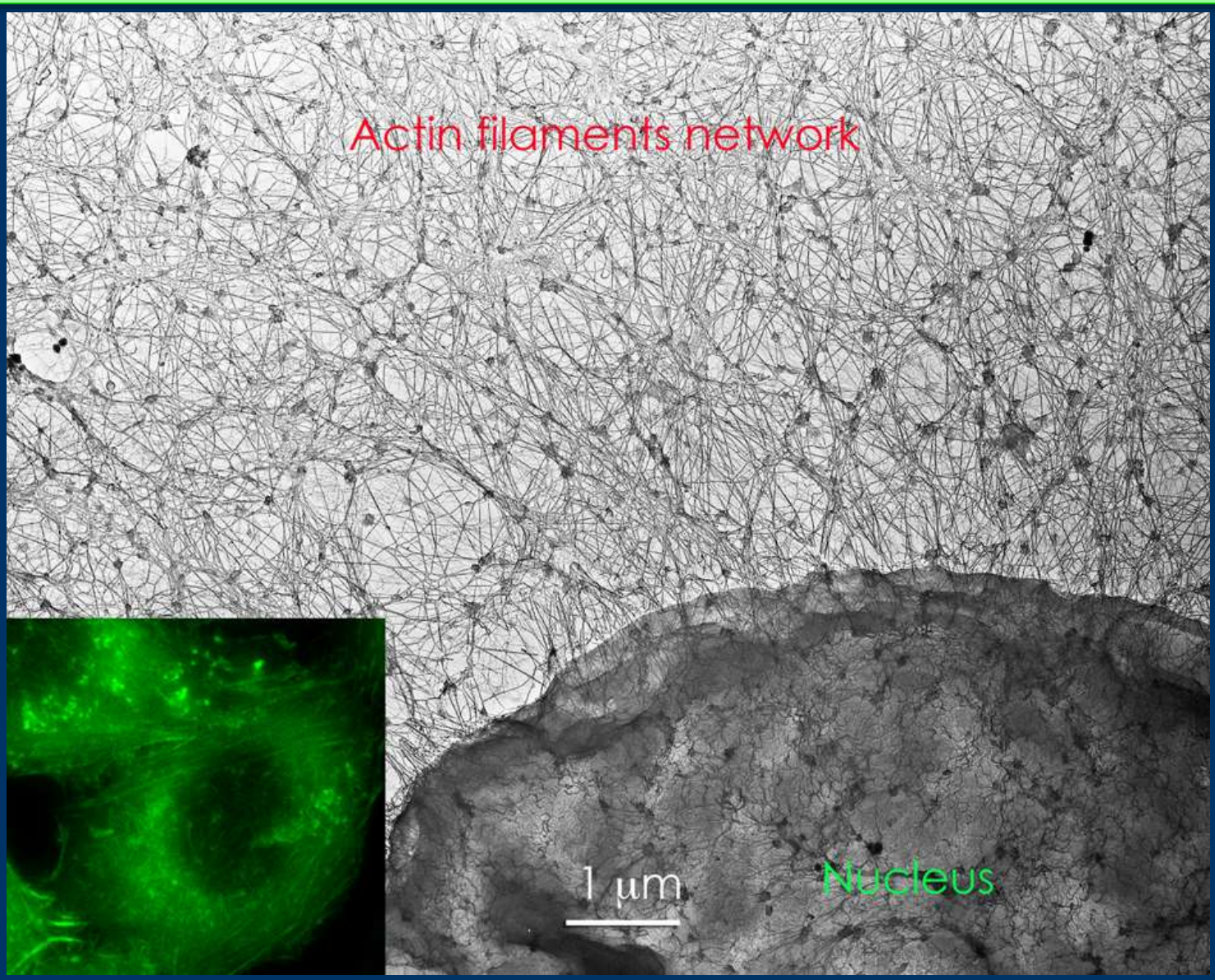
Cytoplasmic and Peri-nuclear actin filaments



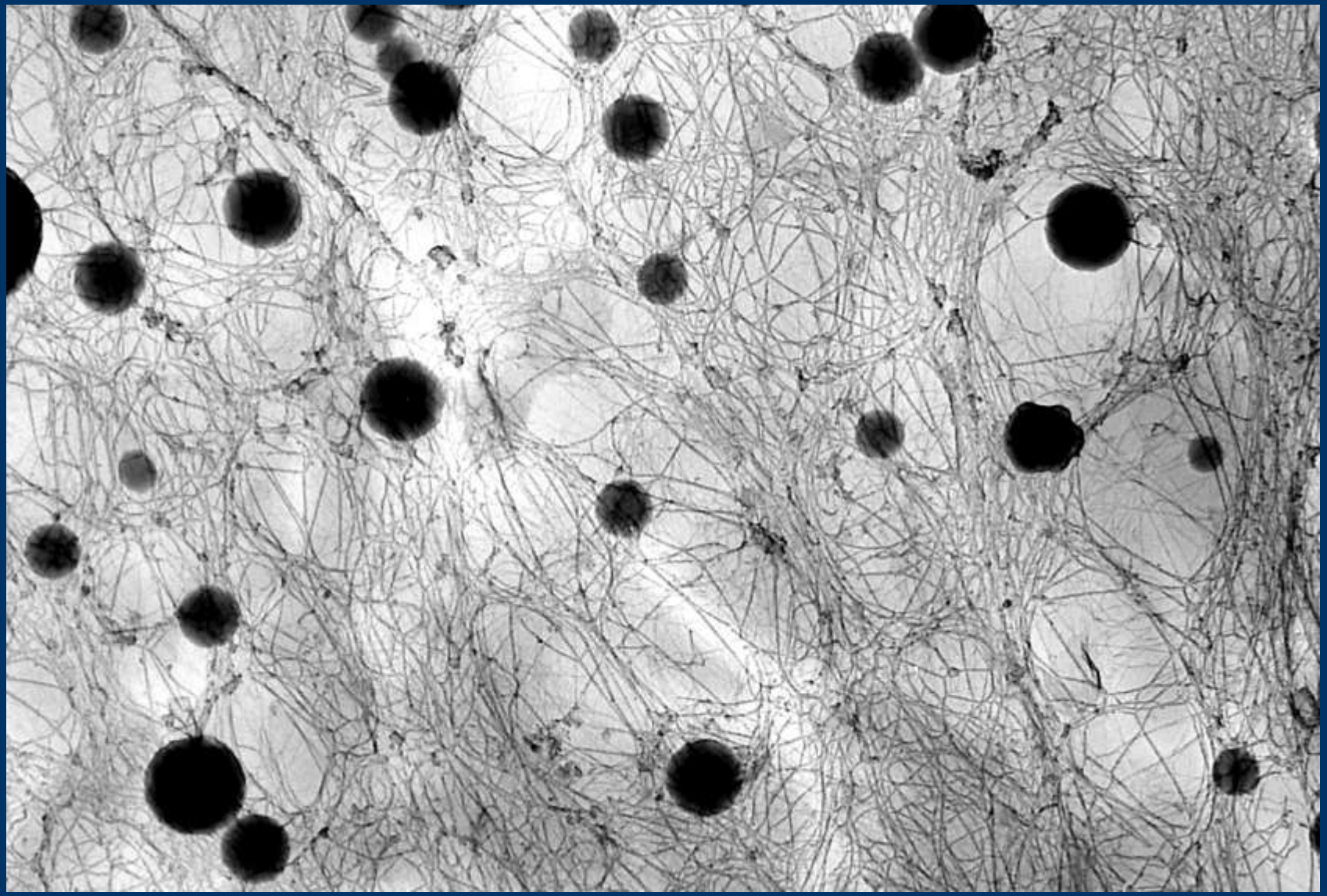
CLSM visualize the cytoskeleton mainly at a bottom plane.



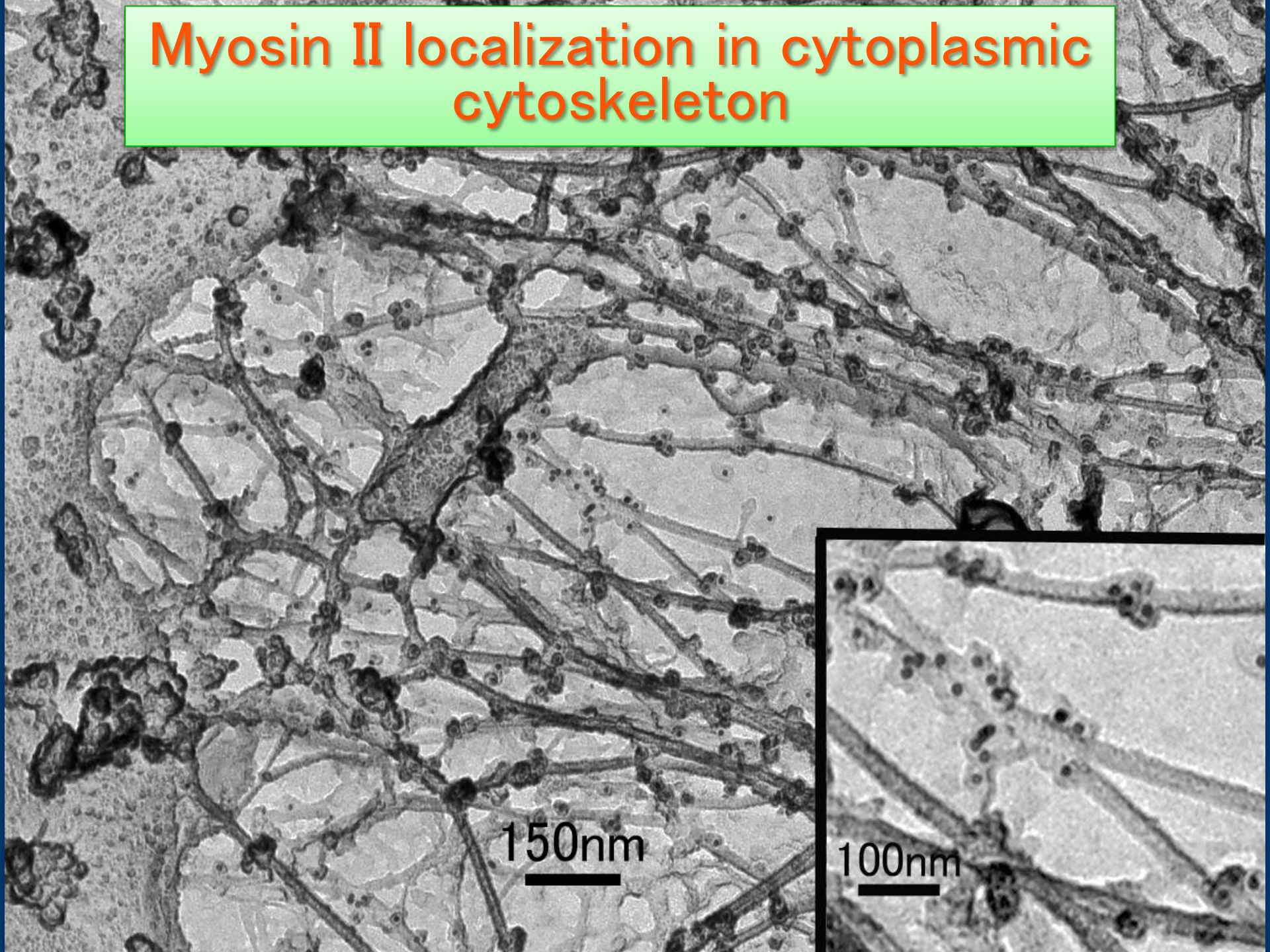
Cytoplasmic cytoskeleton observed by extremely high voltage (1000KV) TEM.



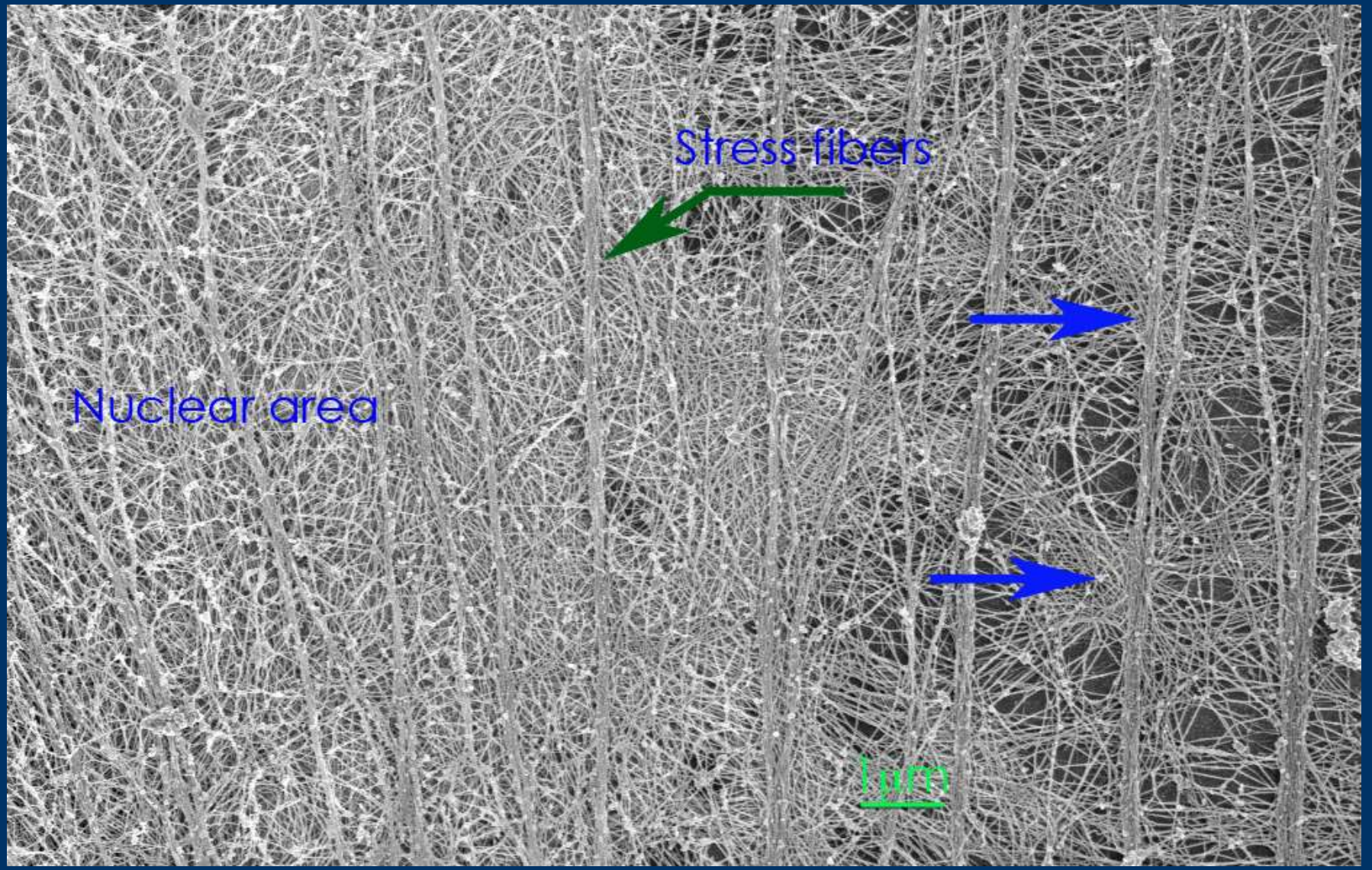
High voltage electron microscopic image of unroofed pigment epithelial cells.



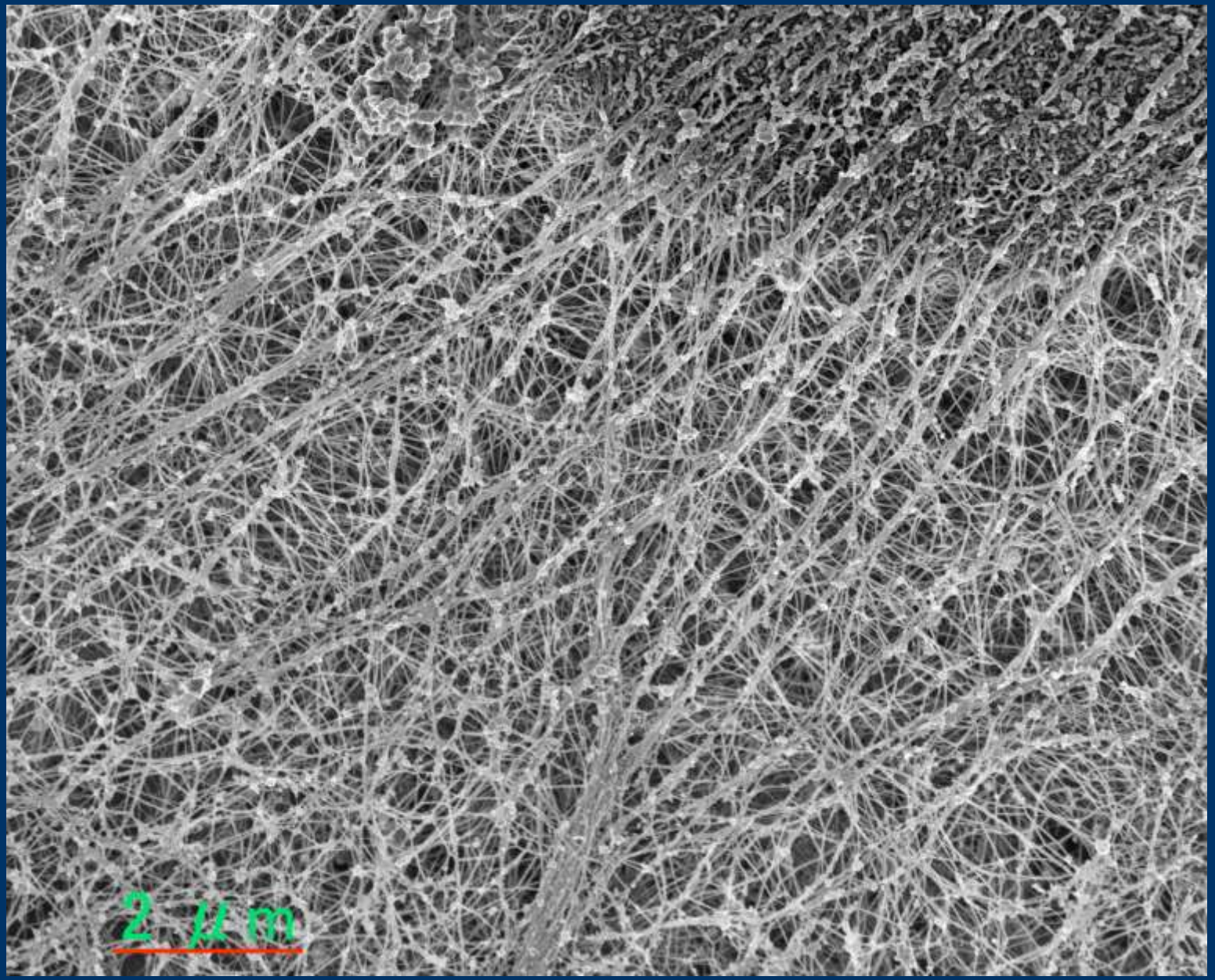
Myosin II localization in cytoplasmic cytoskeleton



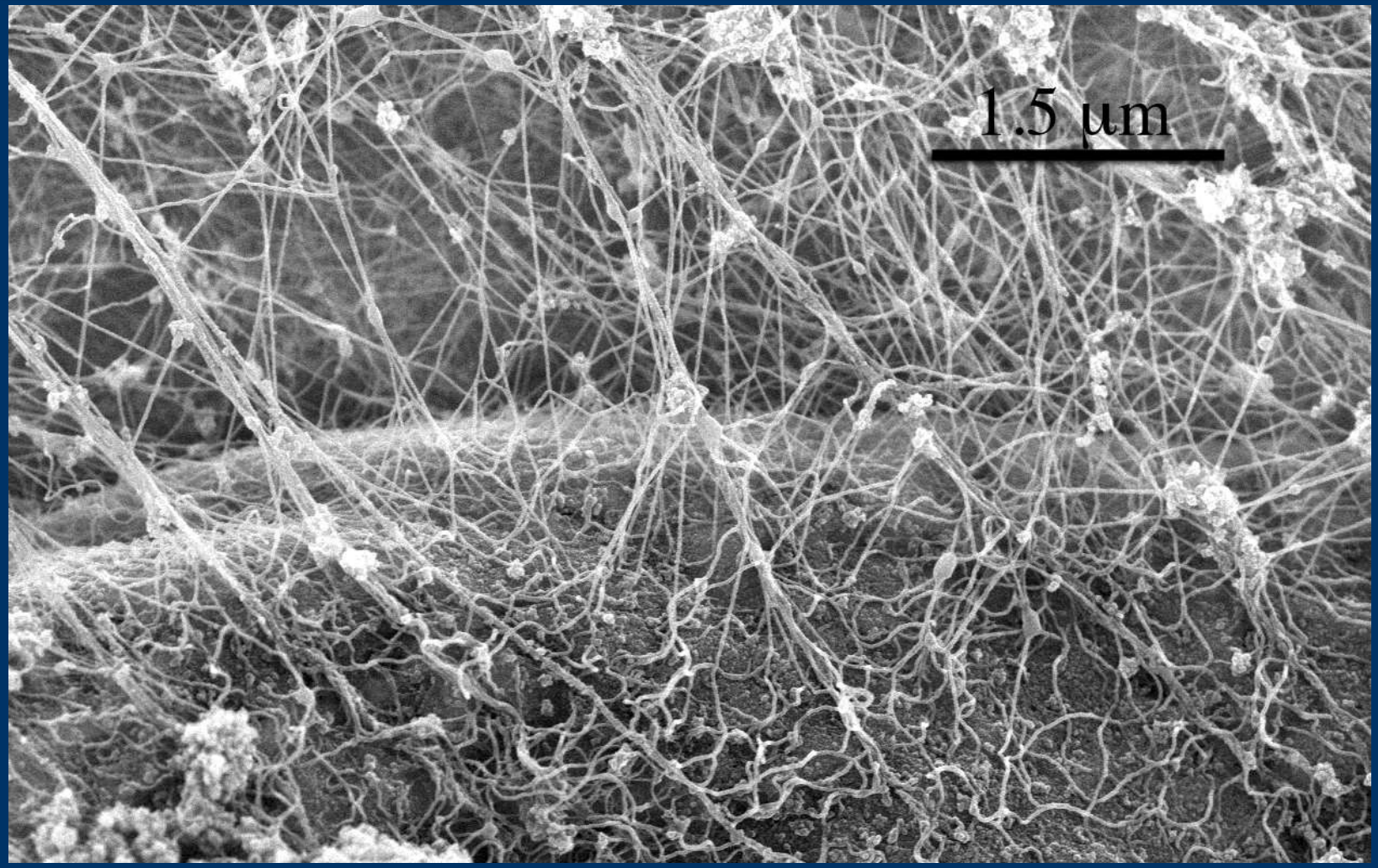
High resolution SEM image of cytoplasmic cytoskeleton



Actin filaments in the periphery of nucleus

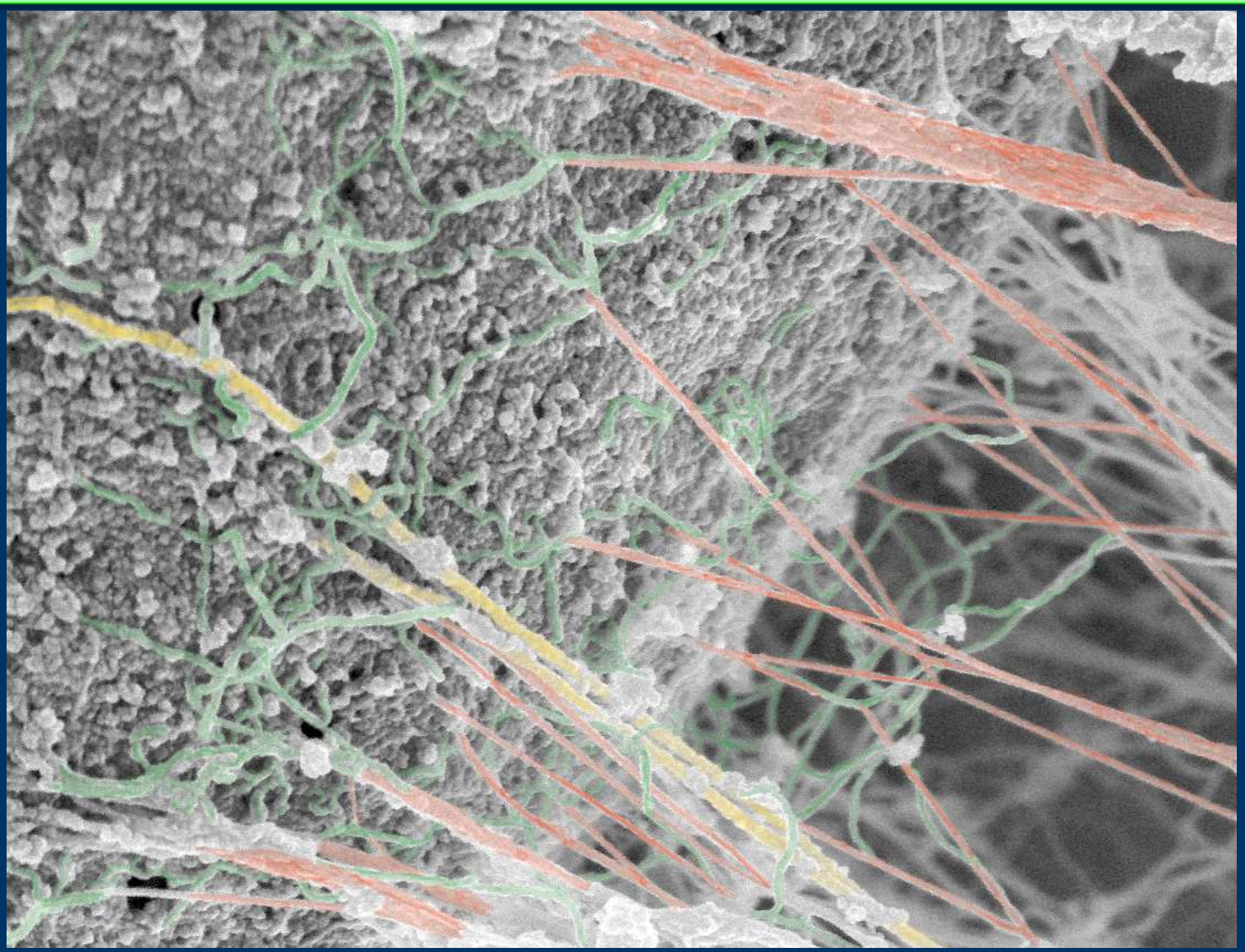


Many actin filaments are extending from the nuclear envelope

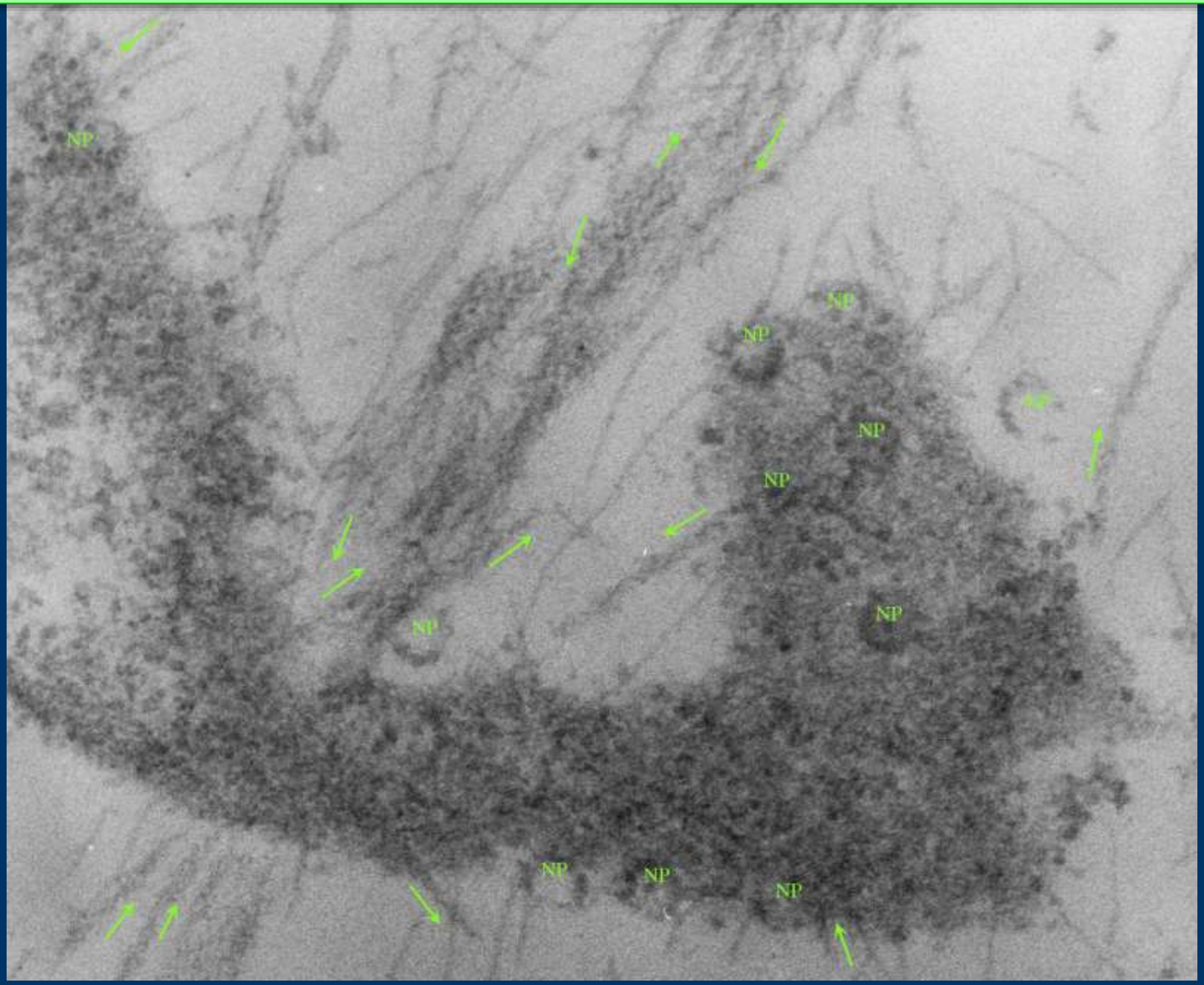


Actin filaments are associated with intermediate filaments in periphery of the nuclear envelope.

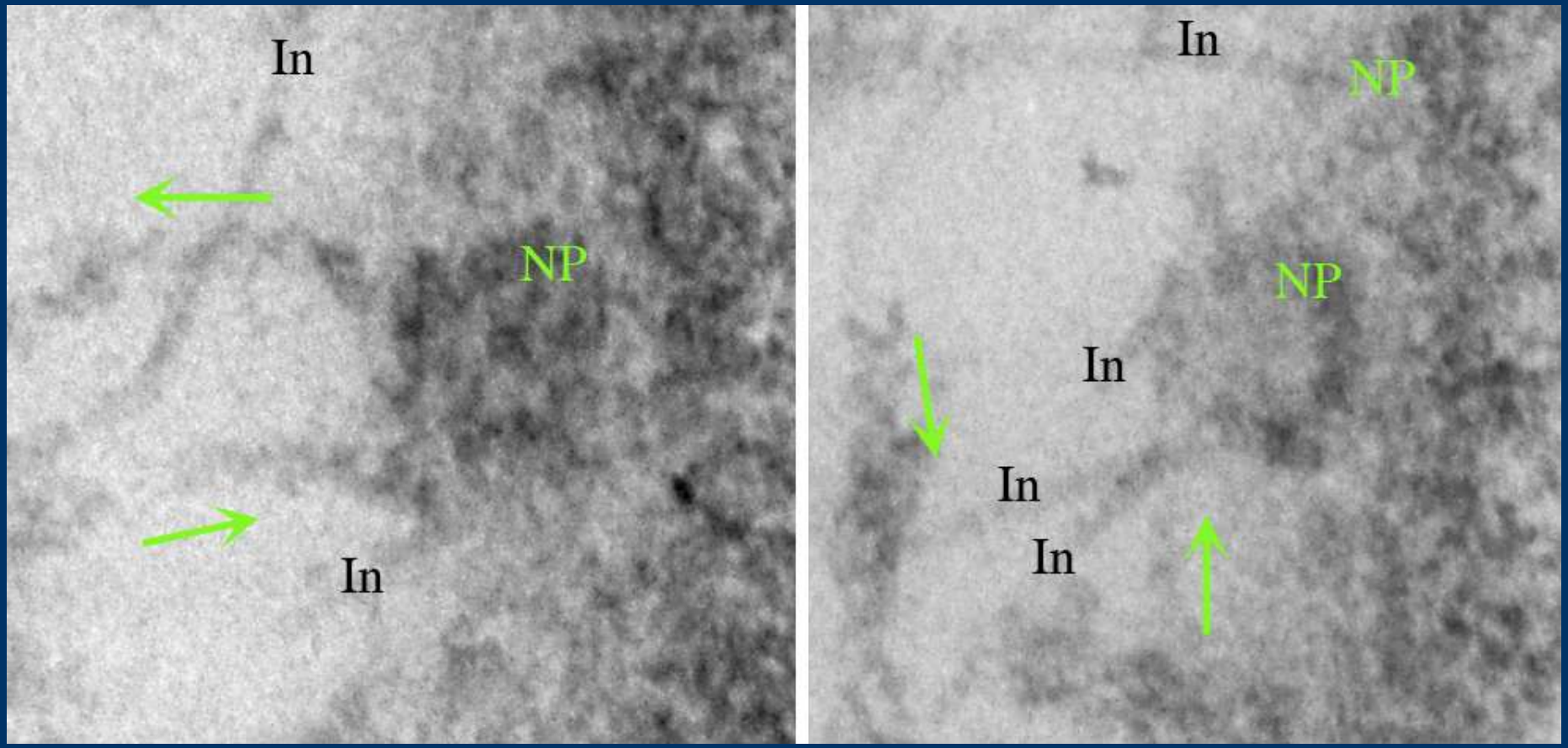
Actin filaments are associated intimately with intermediate filaments on the nuclear envelope.



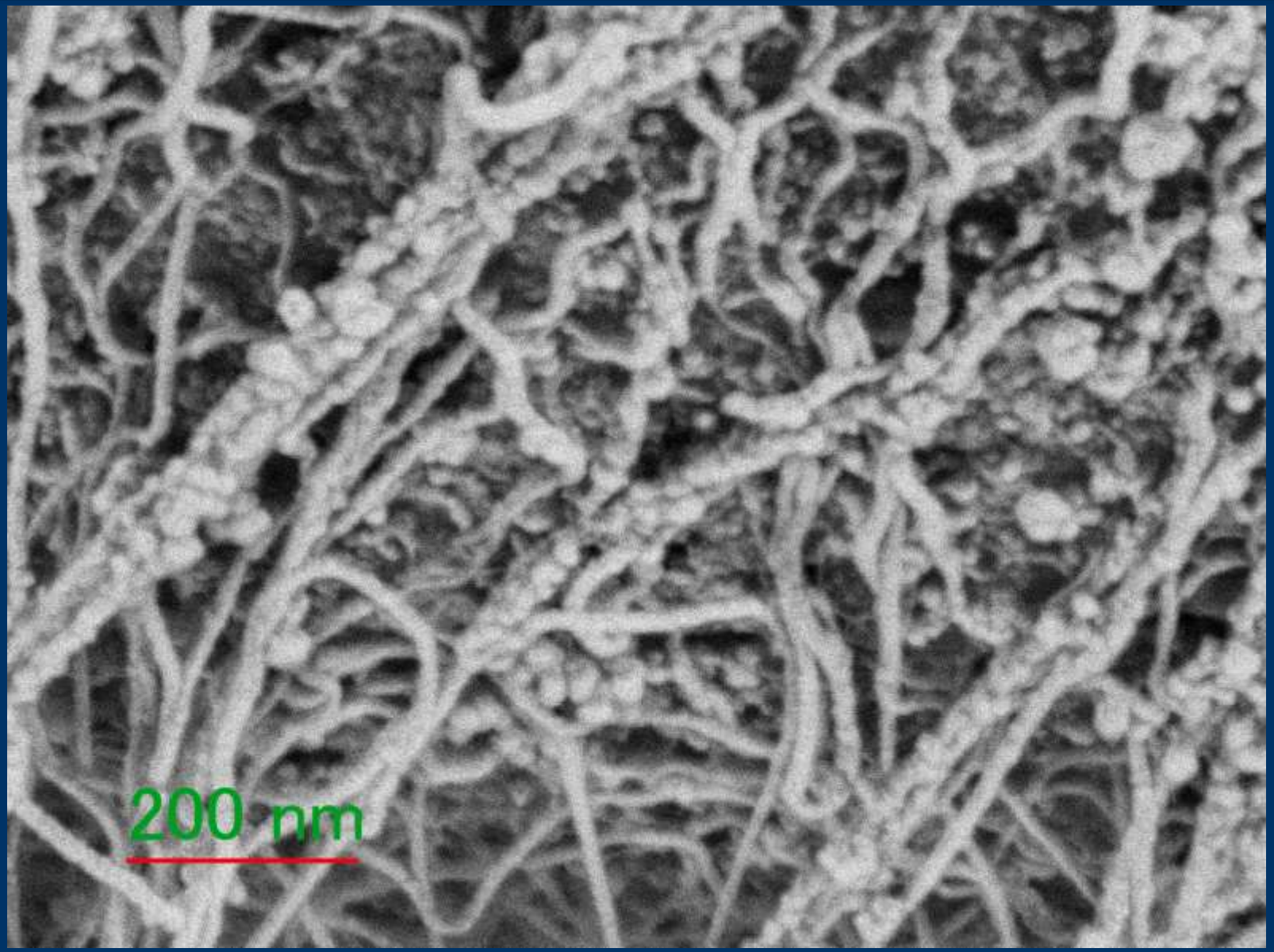
Grazing section of the nucleus labeled with SI



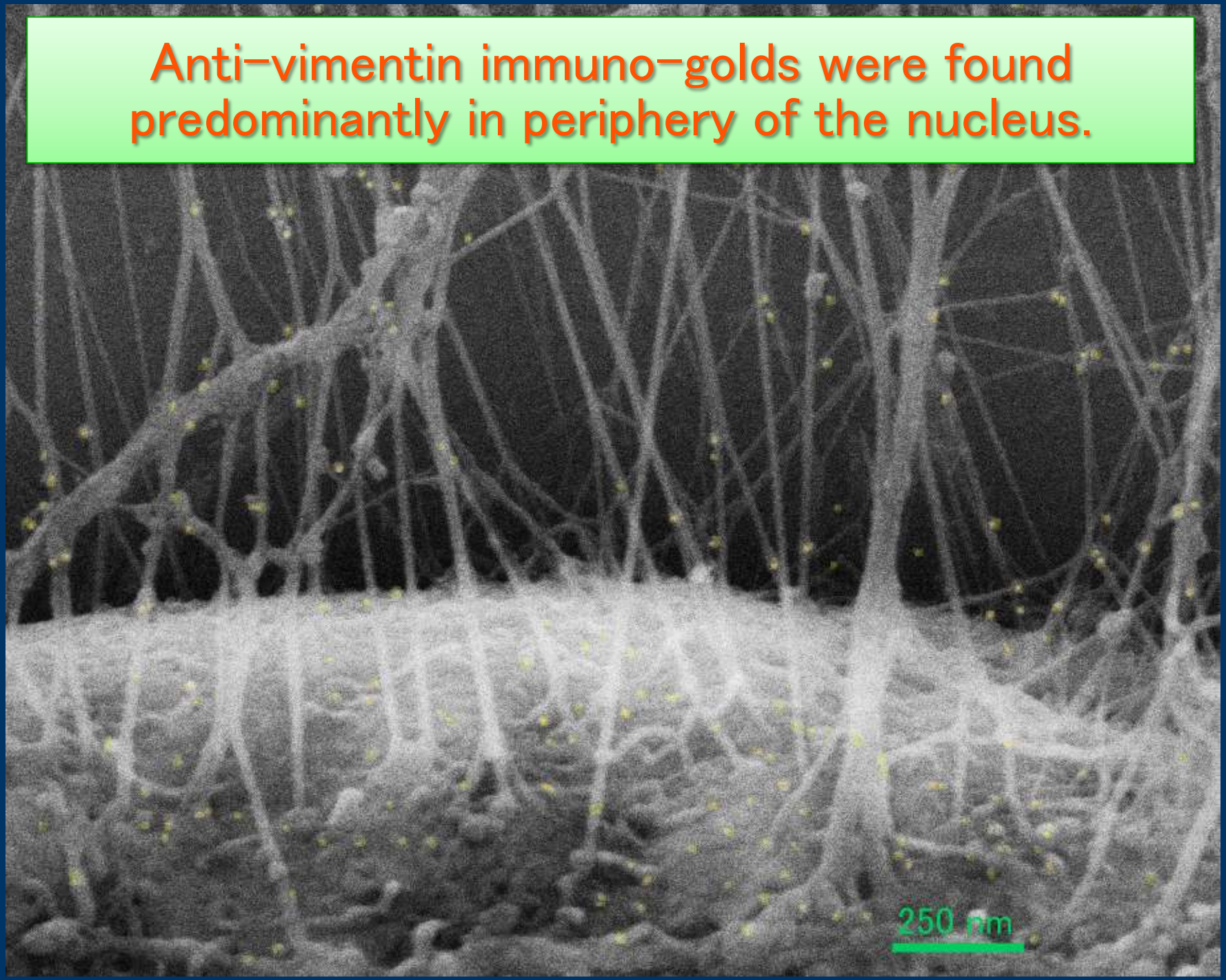
Actin and intermediate filaments originating from nuclear pores (periphery)



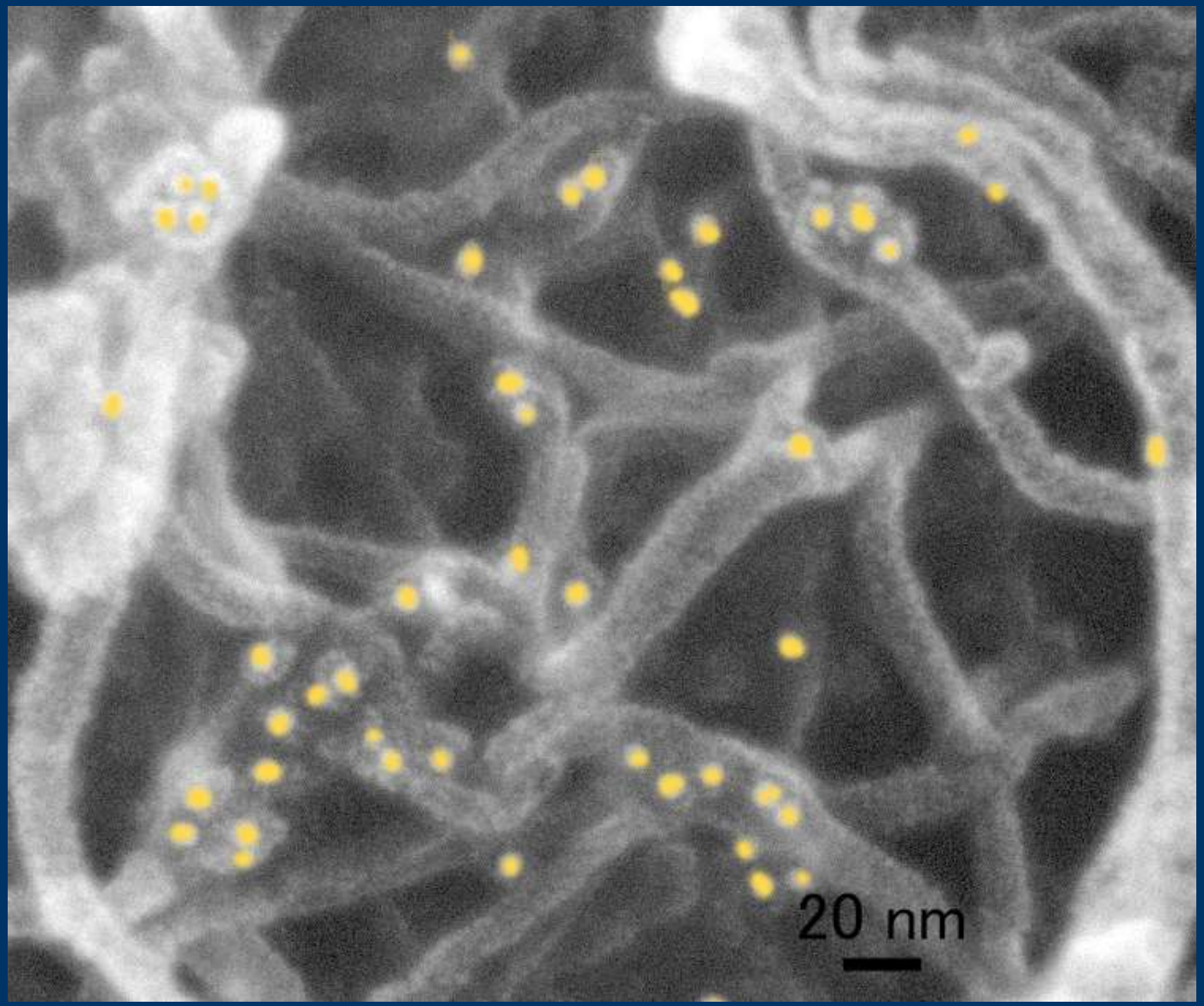
Back scattered electron image showing vimentin filaments extending from nuclear pores



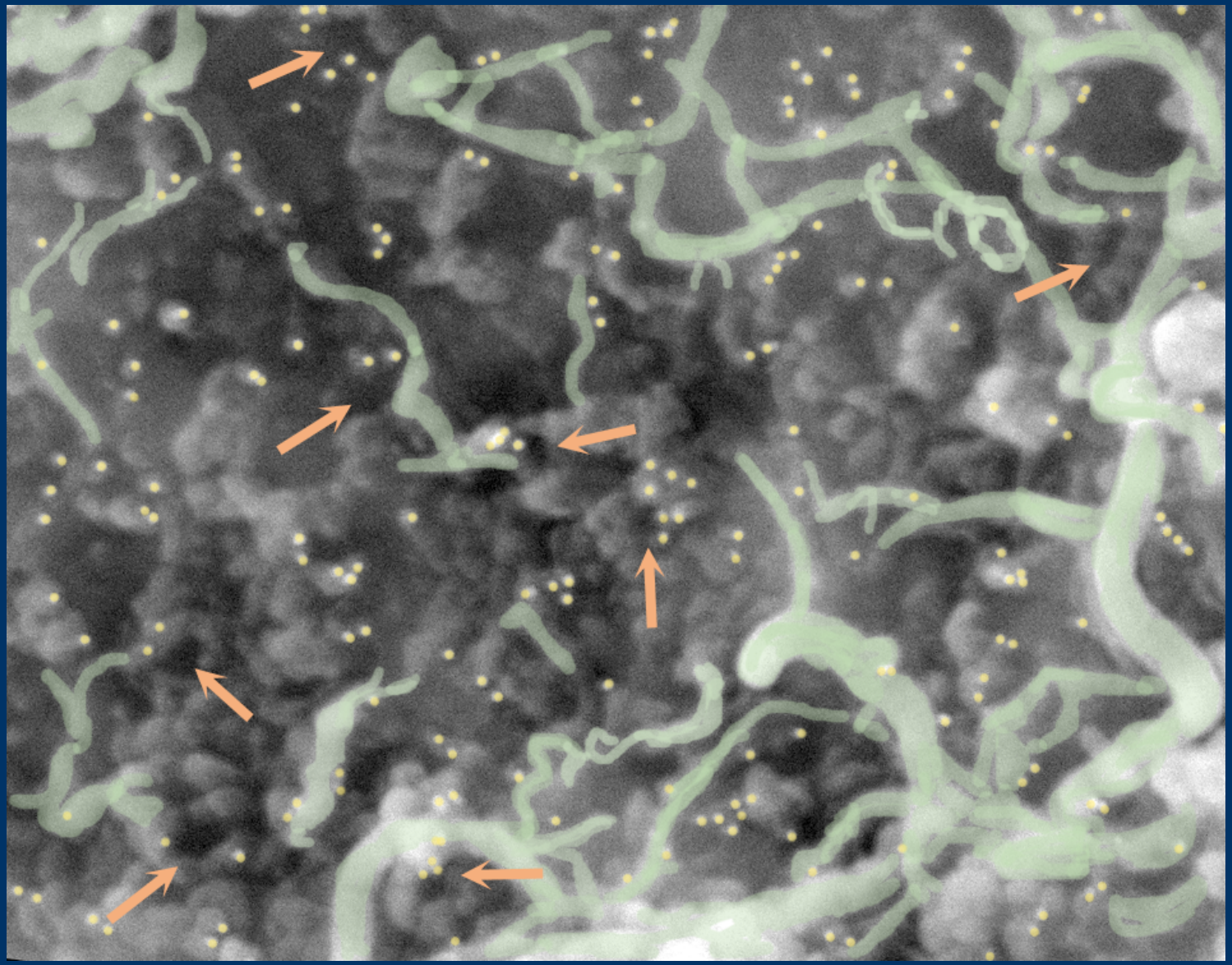
Anti-vimentin immuno-golds were found predominantly in periphery of the nucleus.



Intermediate filaments covering the outer nuclear envelope were identified as Vimentin.



Anti-Nesprin 1 antibodies are detected predominantly near nuclear pores.



Summary 2

1. Cytoplasm was comprised of abundant actin filaments that were not observed under light microscope. Many actin filaments passed over each other at some points and thereby divided the cytoplasm into several domains.
2. Many actin filaments were attached to nuclear envelope as well. Since S1 decoration showed both pointed and barbed ends clearly in the surface of nucleus, there seemed to be the terminations and origins of actin filaments on the nuclear envelope.
3. Nuclear envelope was covered with thick layer of intermediate filaments, vimentin.
4. Vimentin filaments extended from nuclear pore like a rosette shape and formed complicated mesh work.
5. Vimentin filaments associated or covered with almost all actin filaments near the nuclear envelope.
6. Nesprin 1 was detected in the periphery of nuclear pores as well.


ご静聴ありがとうございました。
Thank you very much for your attention.