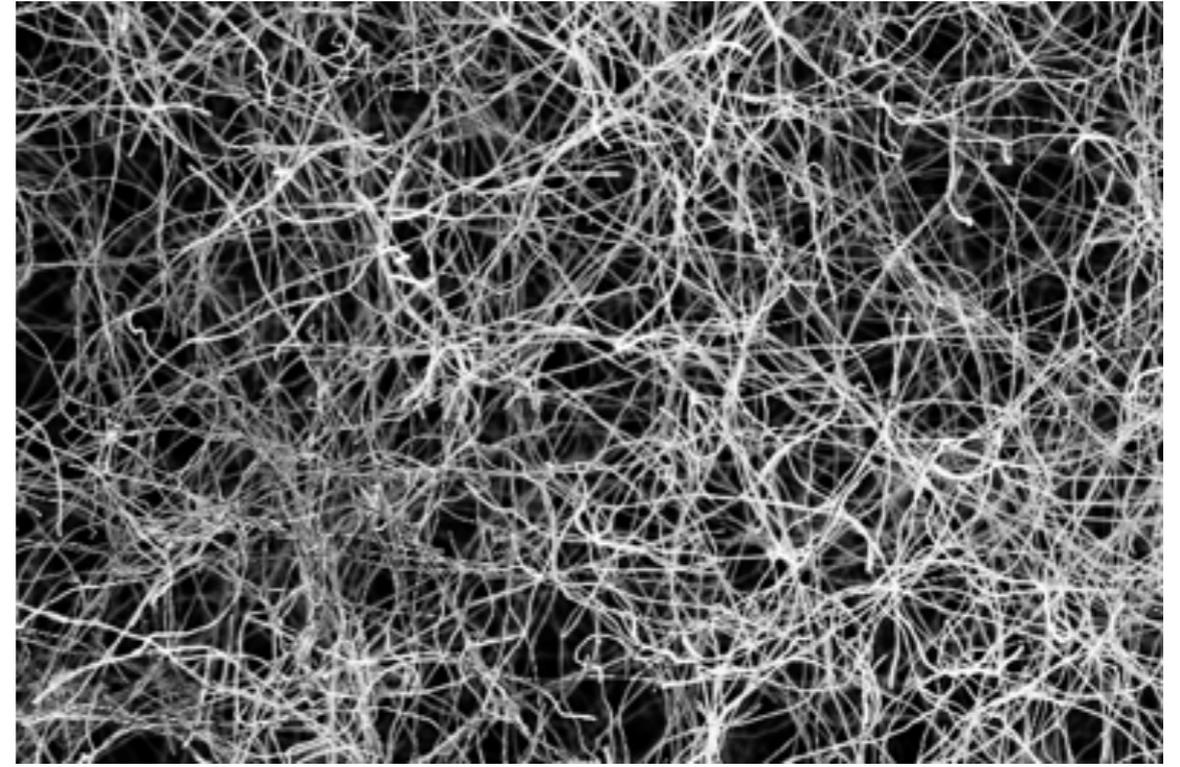
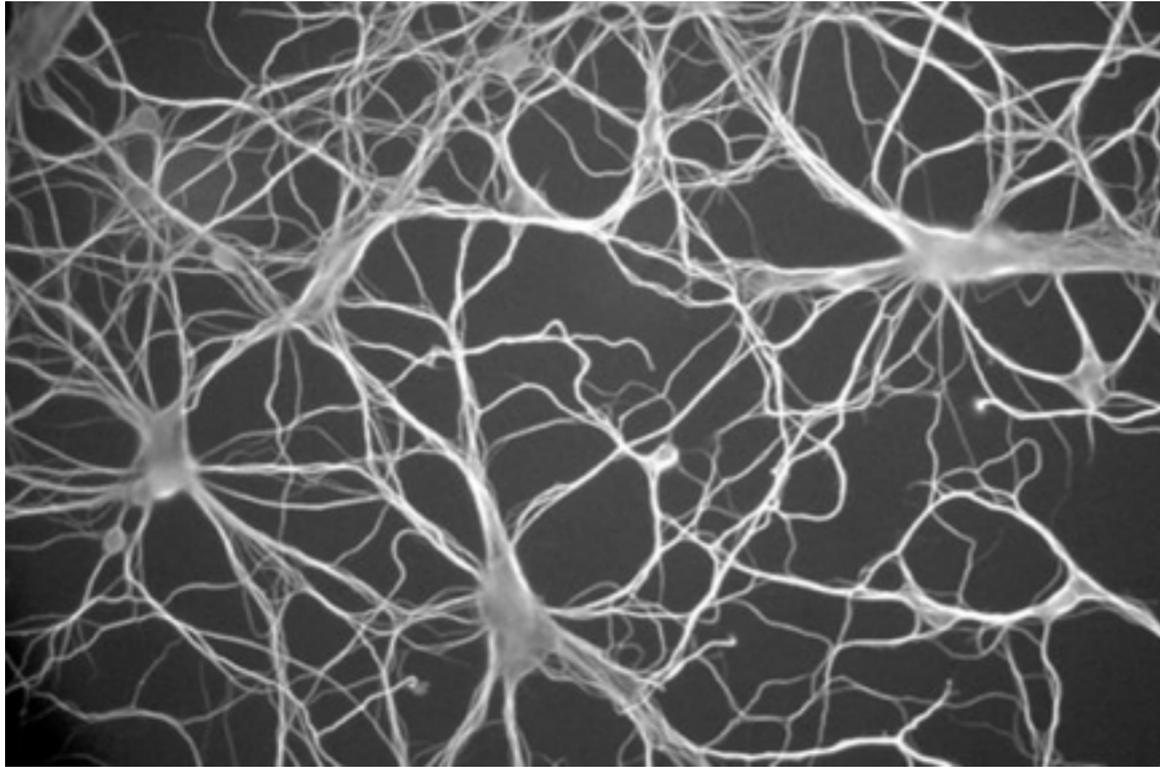


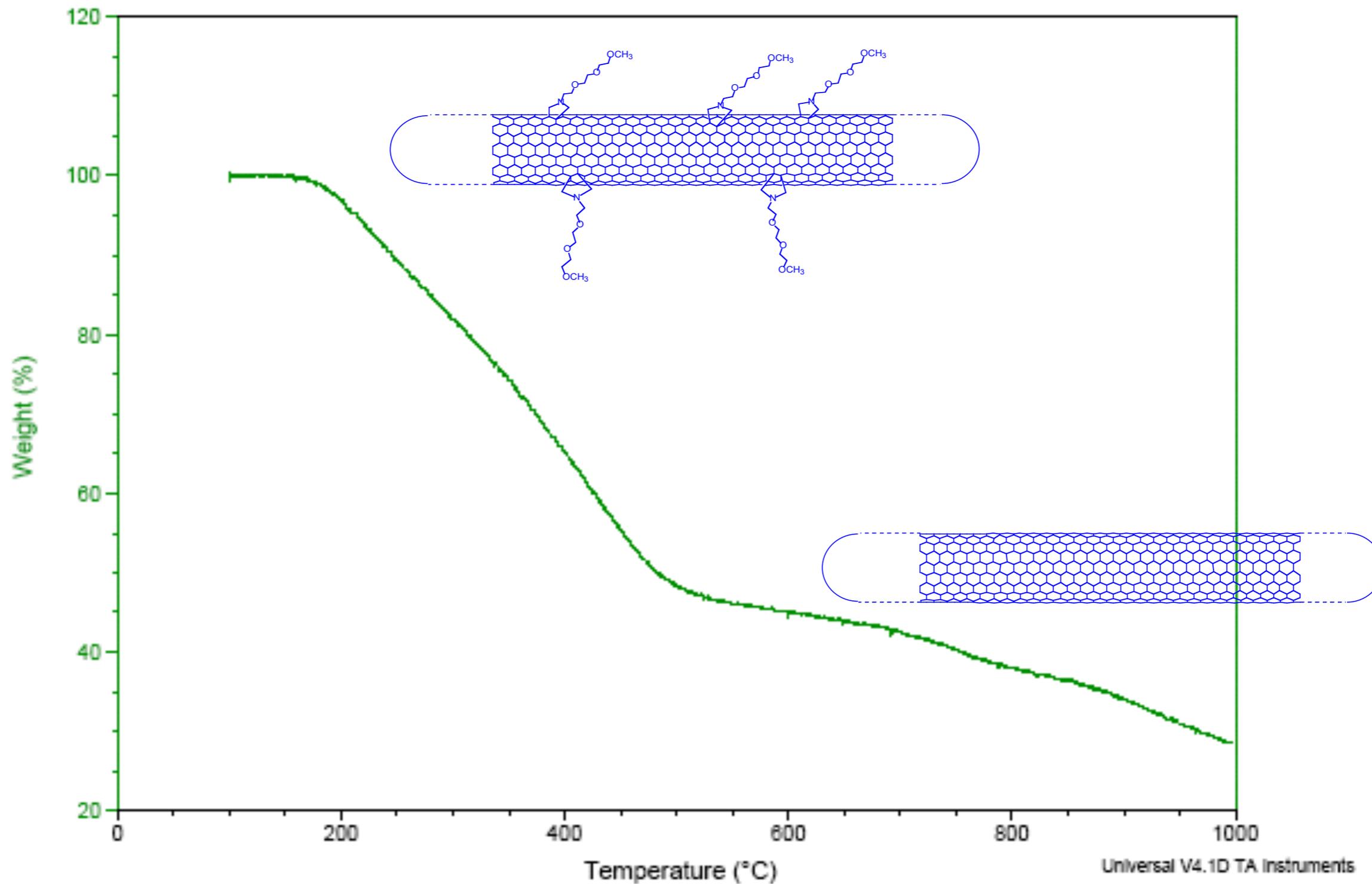
CNT as Substrates for Neuronal Growth



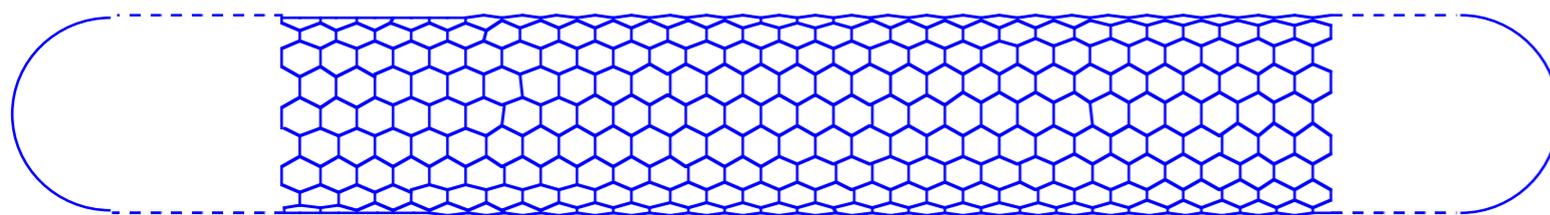
Disease Models & Mechanisms 6, 72-83 (2013) doi:10.1242/dmm.008946

As CNT are metallic or semiconducting, can they help bridging nerves that do not communicate anymore because of injury?

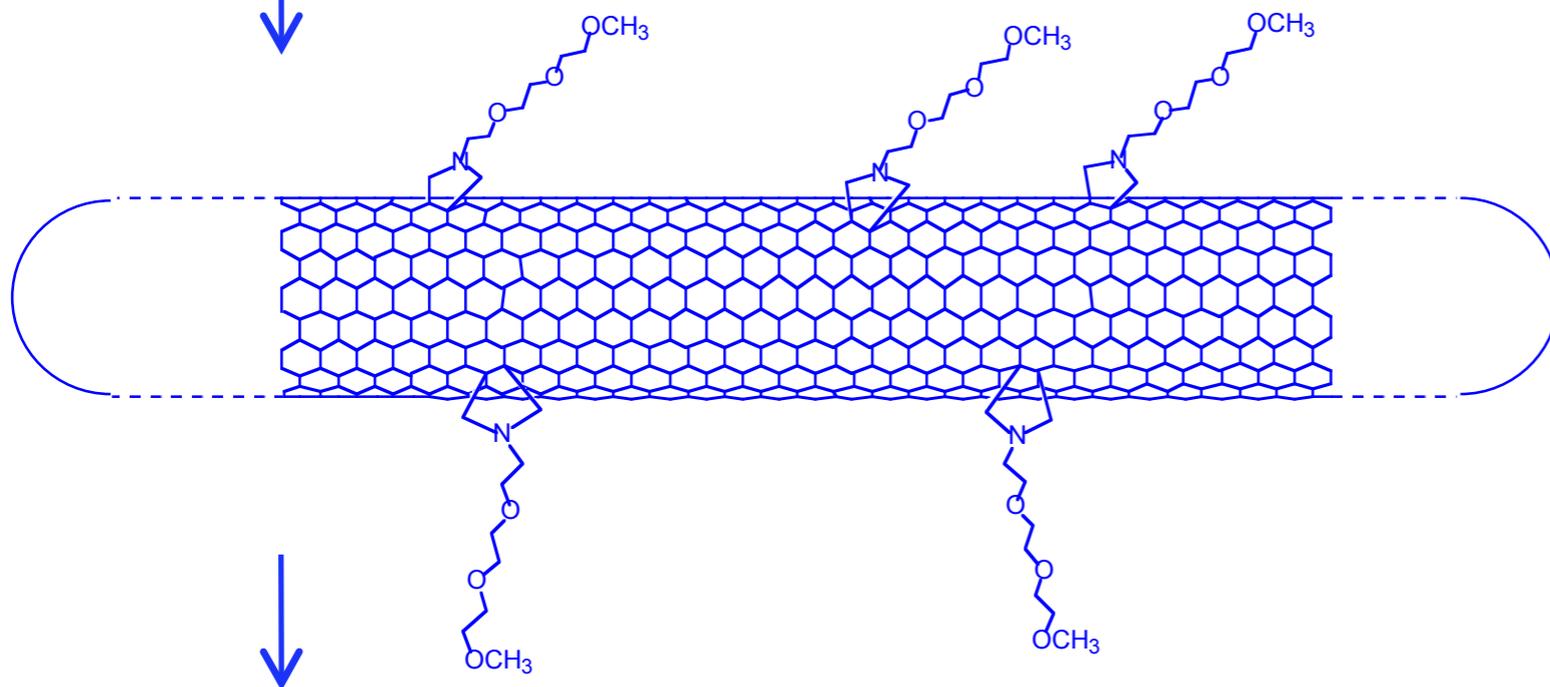
Thermogravimetric Analysis (TGA)



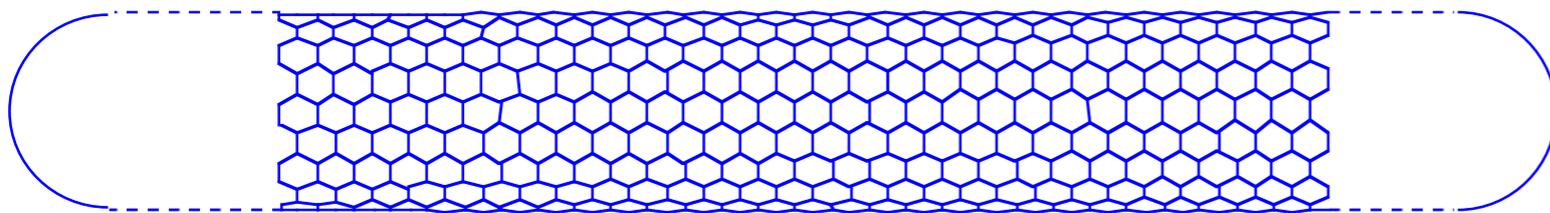
An Alternative Methodology for the Purification of SWNTs



+ amorphous carbon
+ metallic nanoparticles



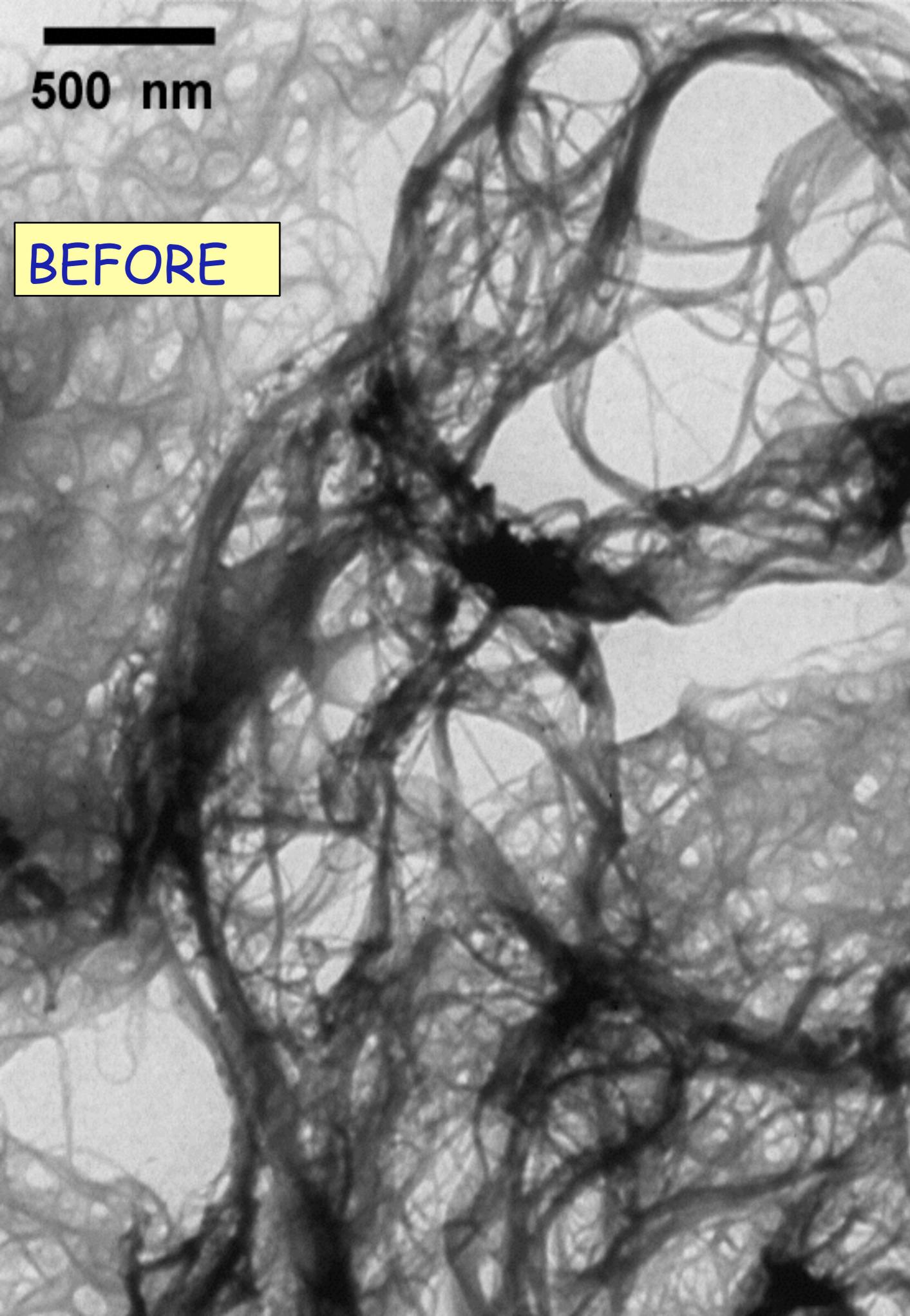
easy to handle, soluble
Can be purified!



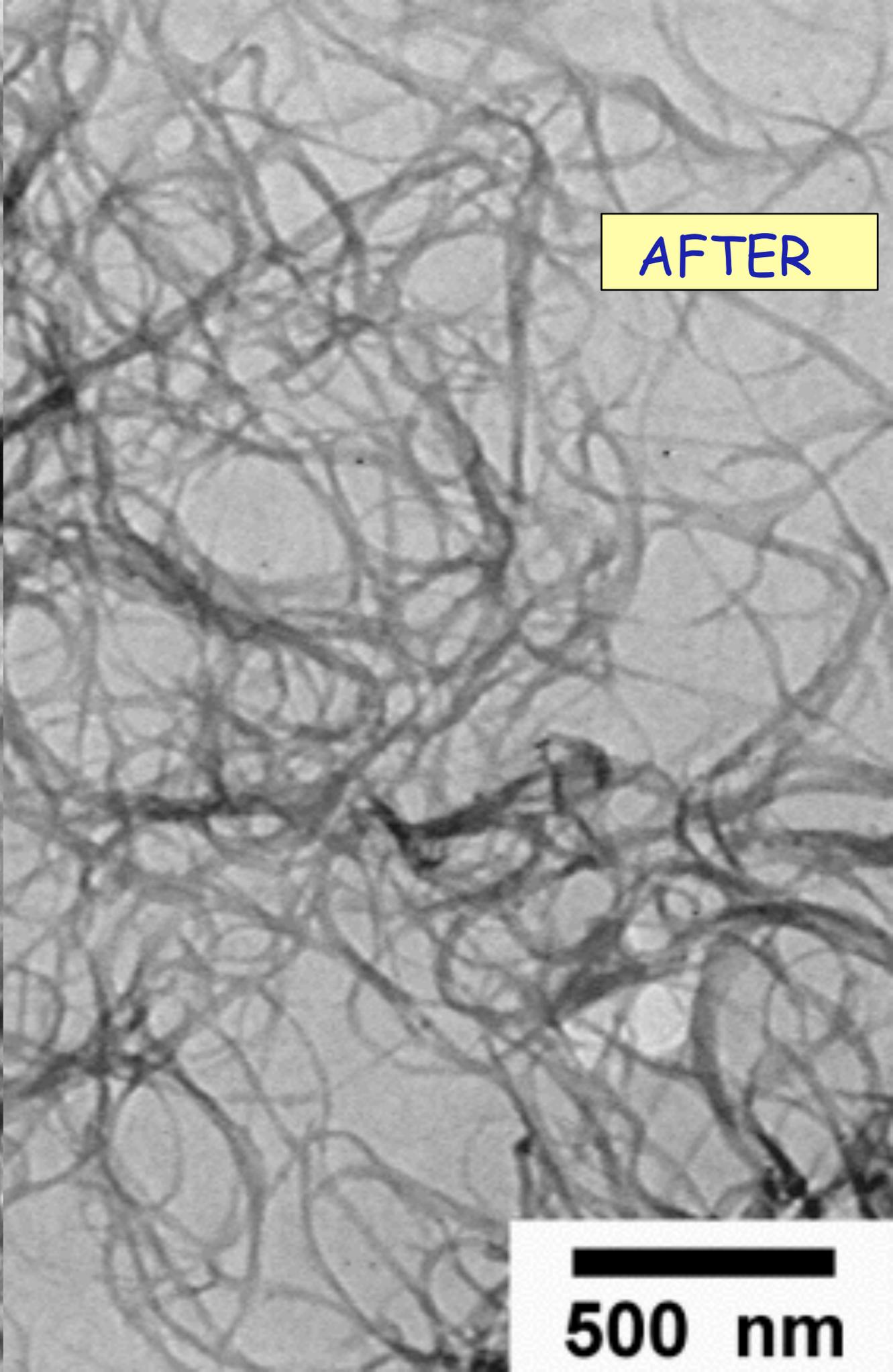
PURE! No amorphous
carbon, no metal

500 nm

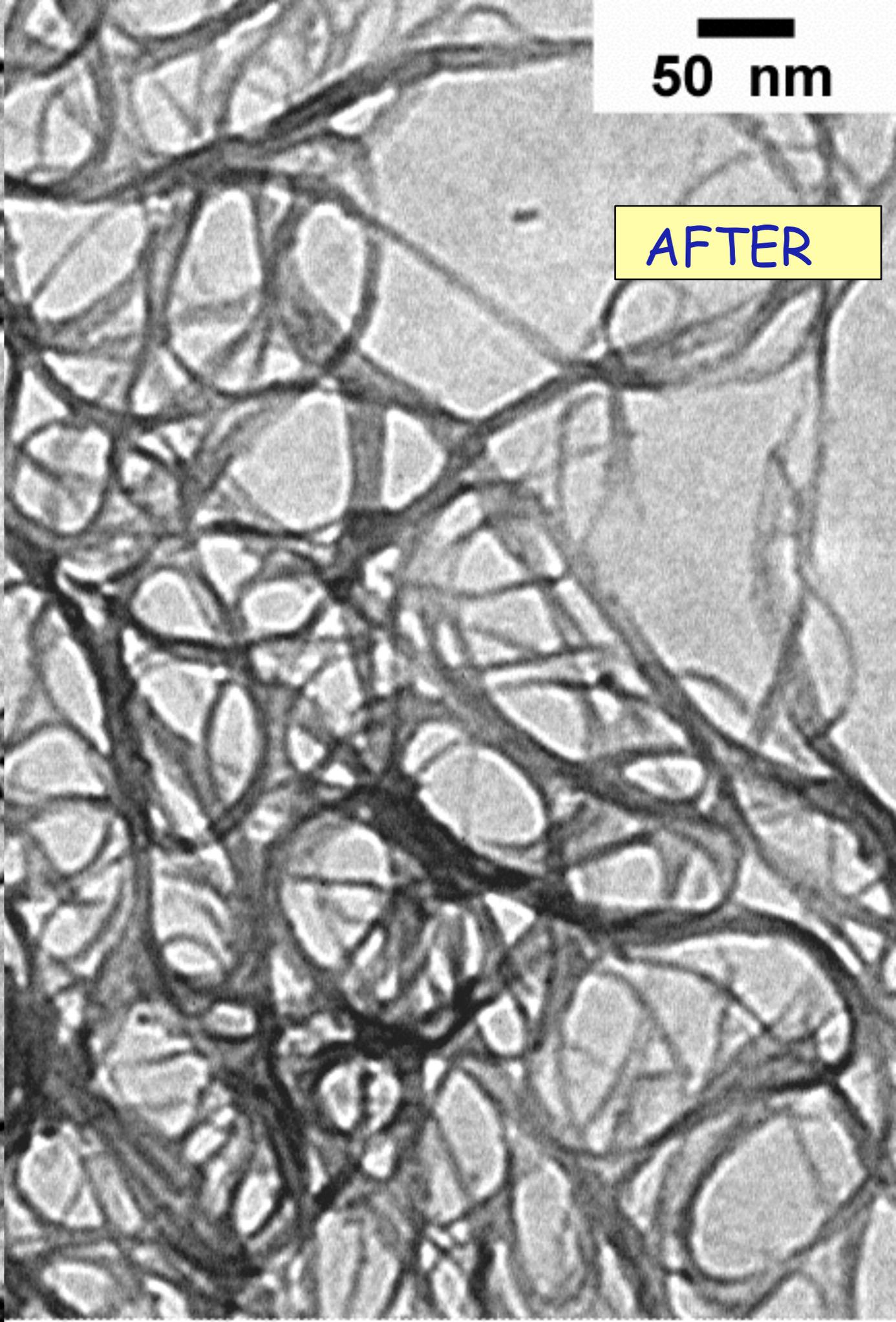
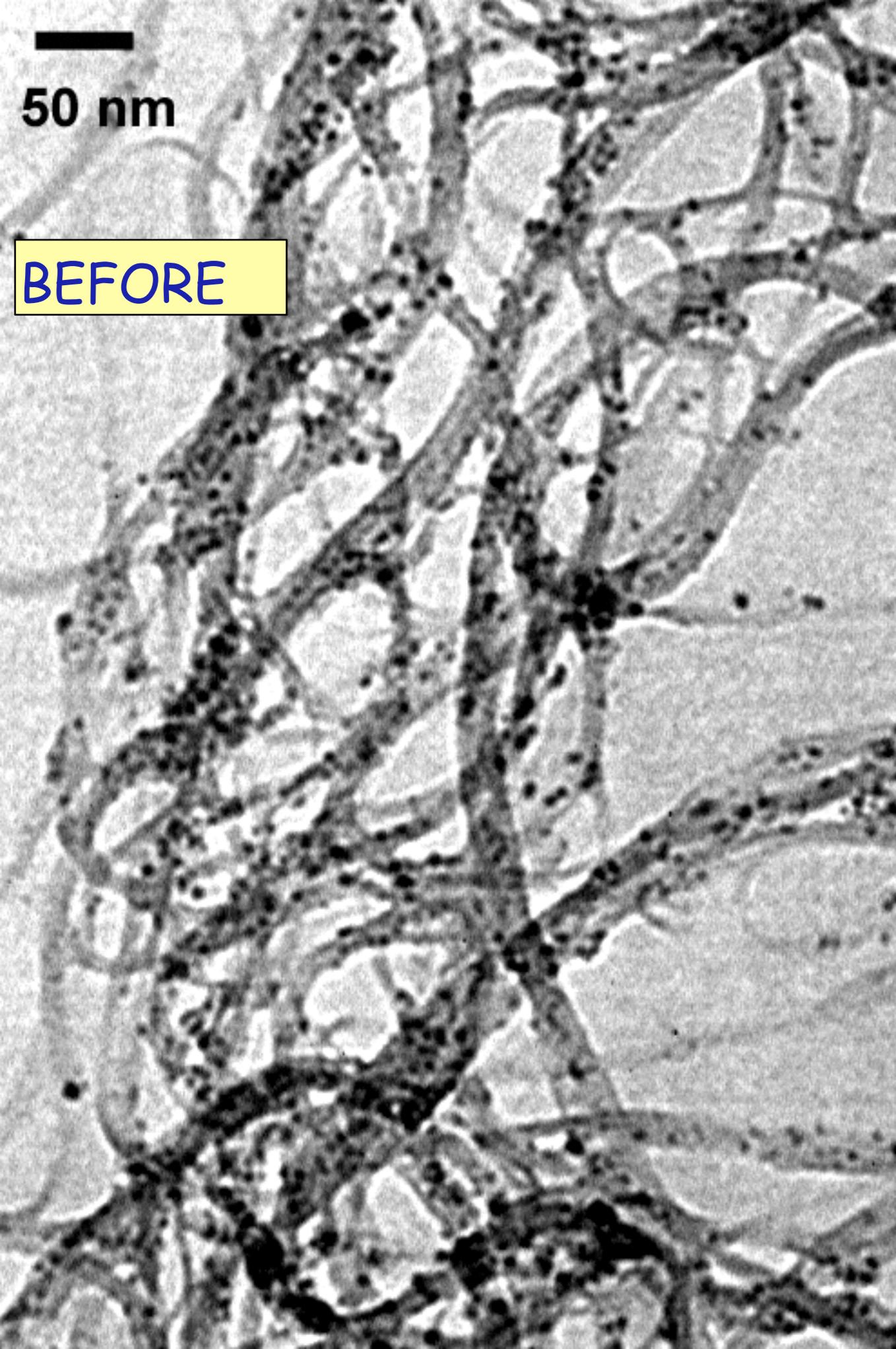
BEFORE



AFTER



500 nm



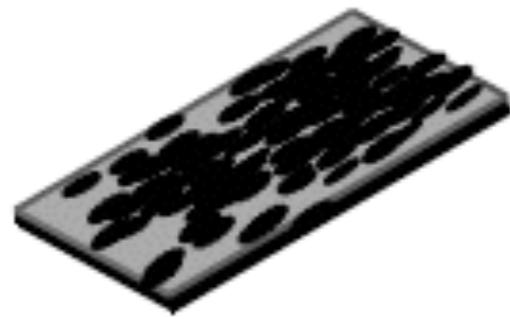
Solution containing
f-MWNT



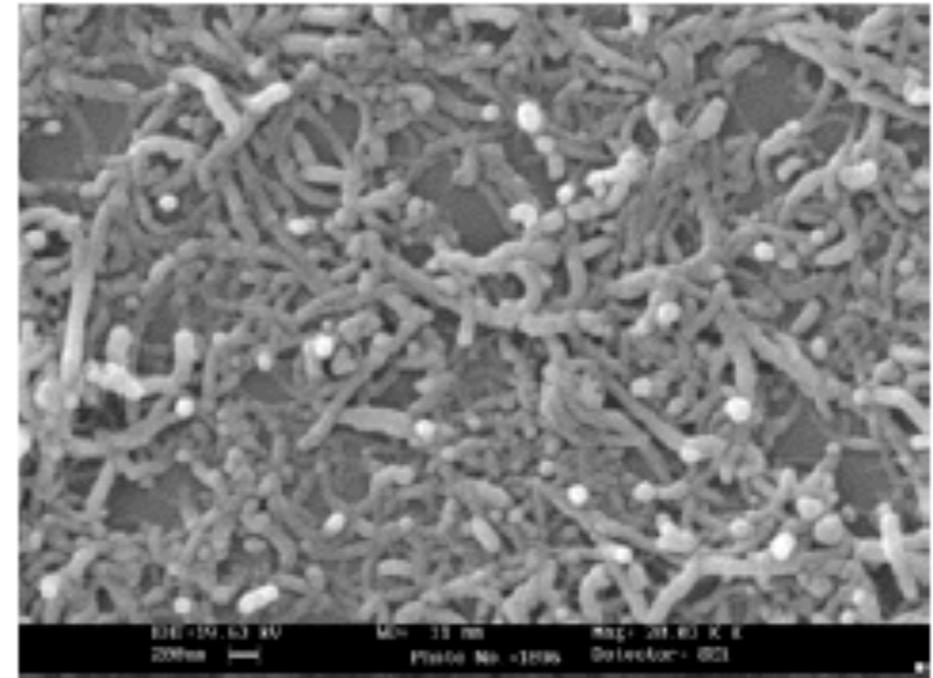
Glass coverslip

f-MWNT

Pure MWNT



SEM Analysis

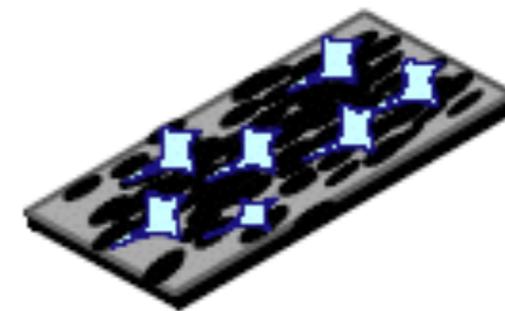




Pure MWNT



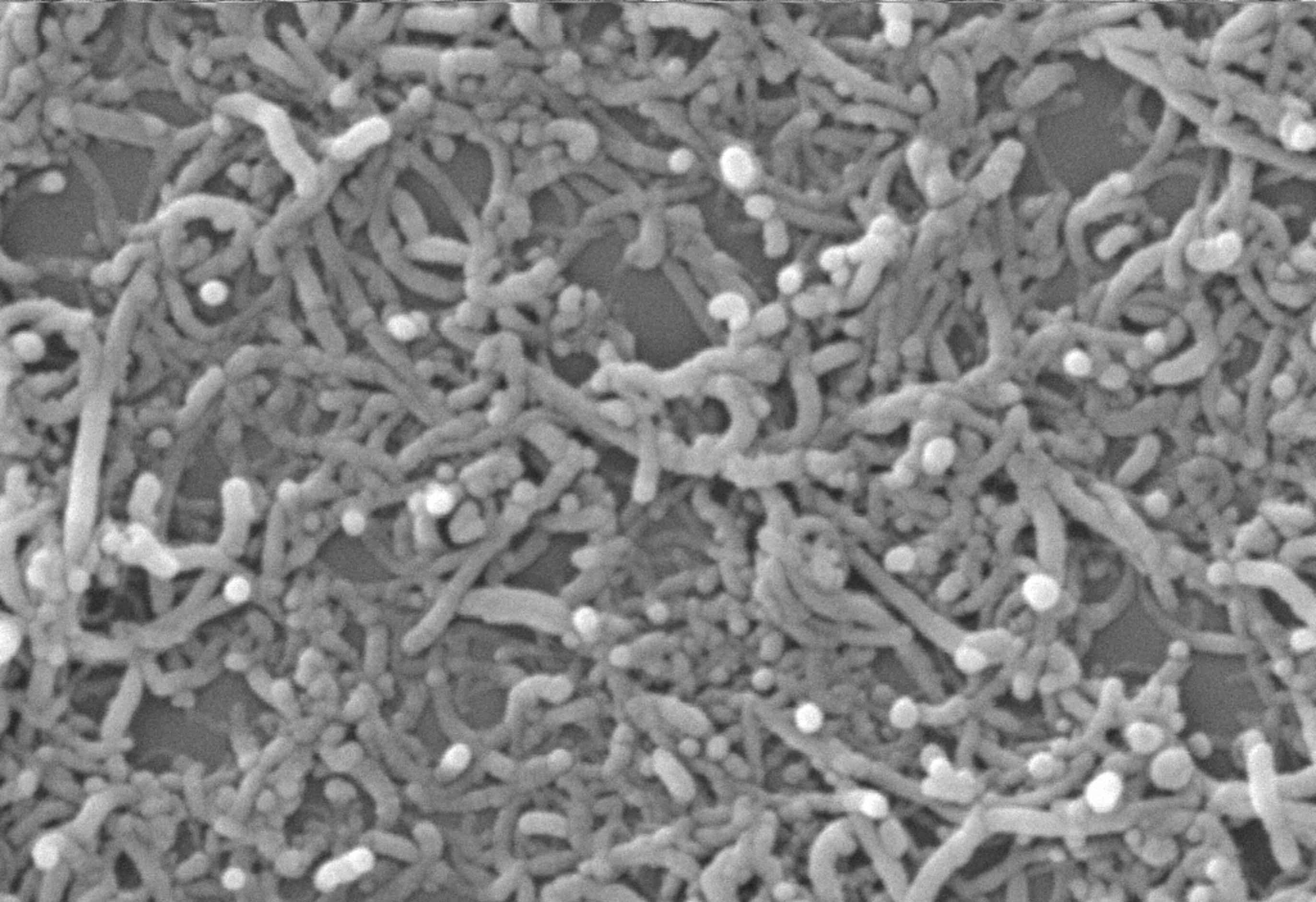
Neurons



Hypocampal neurons on pure MWNT



Incubation



EHT=19.63 kV

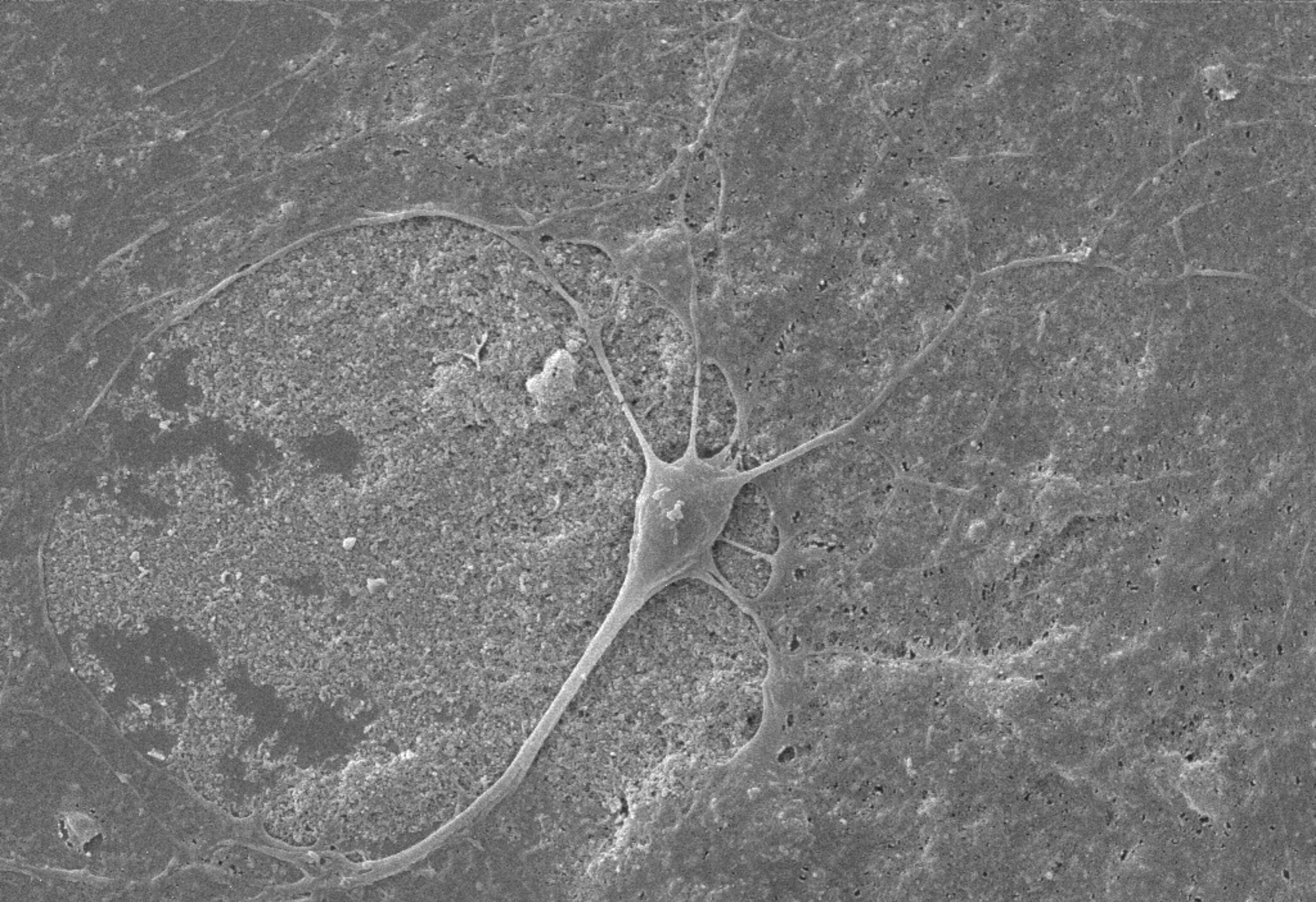
WD= 11 mm

Mag= 20.03 K X

200nm 

Photo No.=1896

Detector= SE1



EHT=15.00 kV

WD= 12 mm

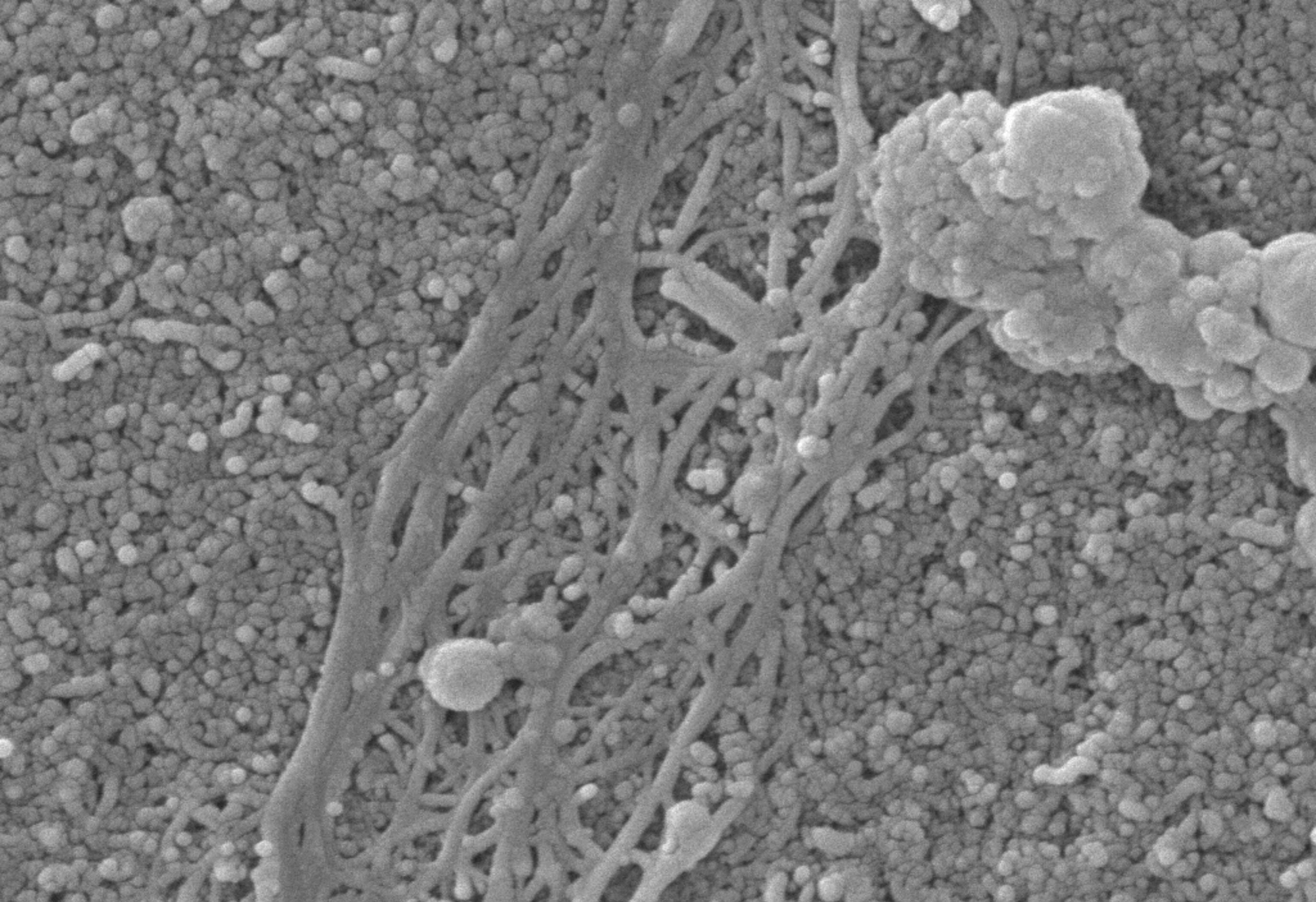
Mag= 1.49 K X

3 μ m



Photo No.=4946

Detector= SE1



EHT=15.00 kV

WD= 12 mm

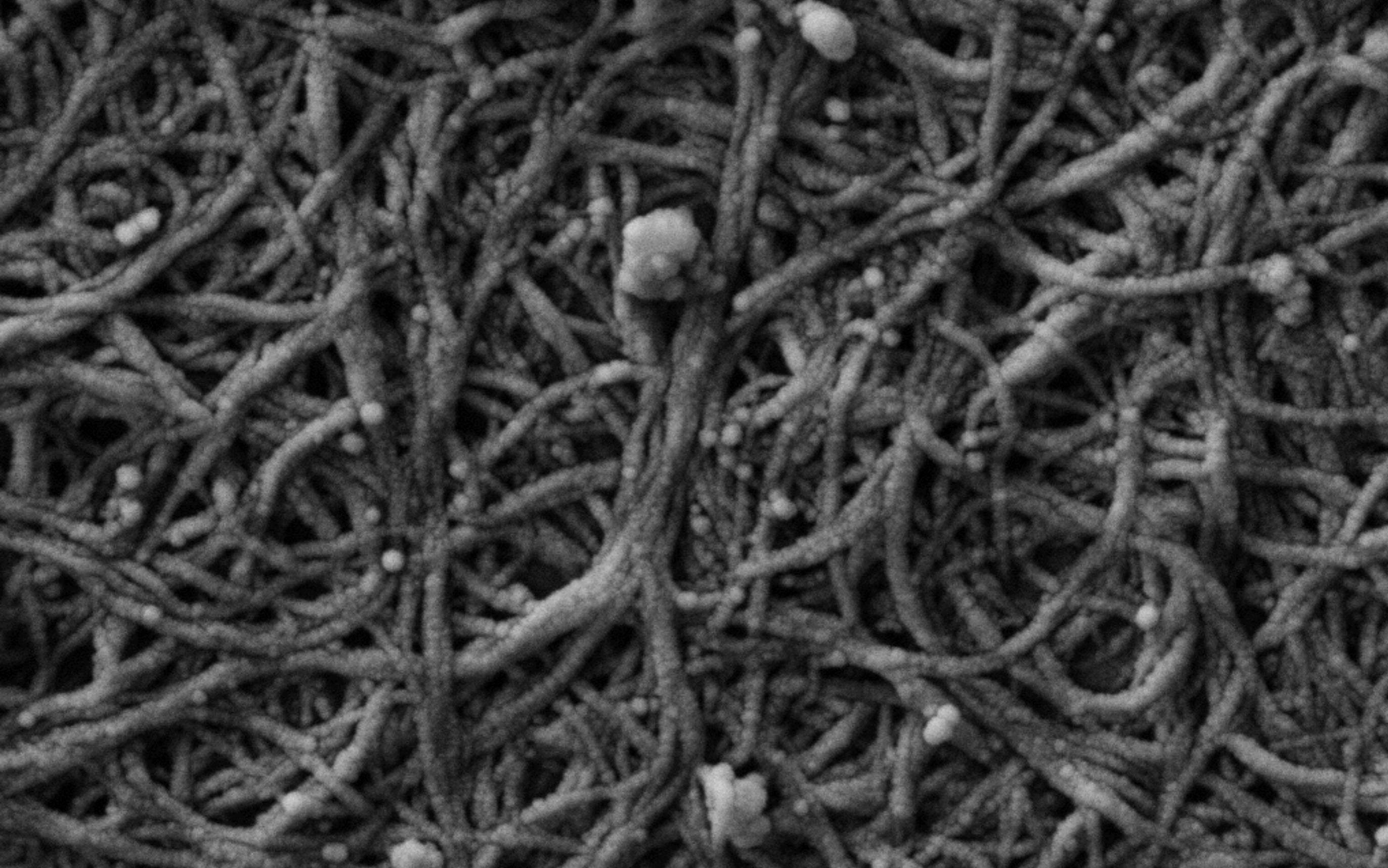
Mag= 10.09 K X

1 μ m



Photo No.=4933

Detector= SE1



LILIT

INFM-TASC

200nm



EHT = 5.00 kV

WD = 2 mm

Mag = 29.50 K X

FIB Mag = 323 X

FIB Lock Mags = No

FIB Probe = 100 pA

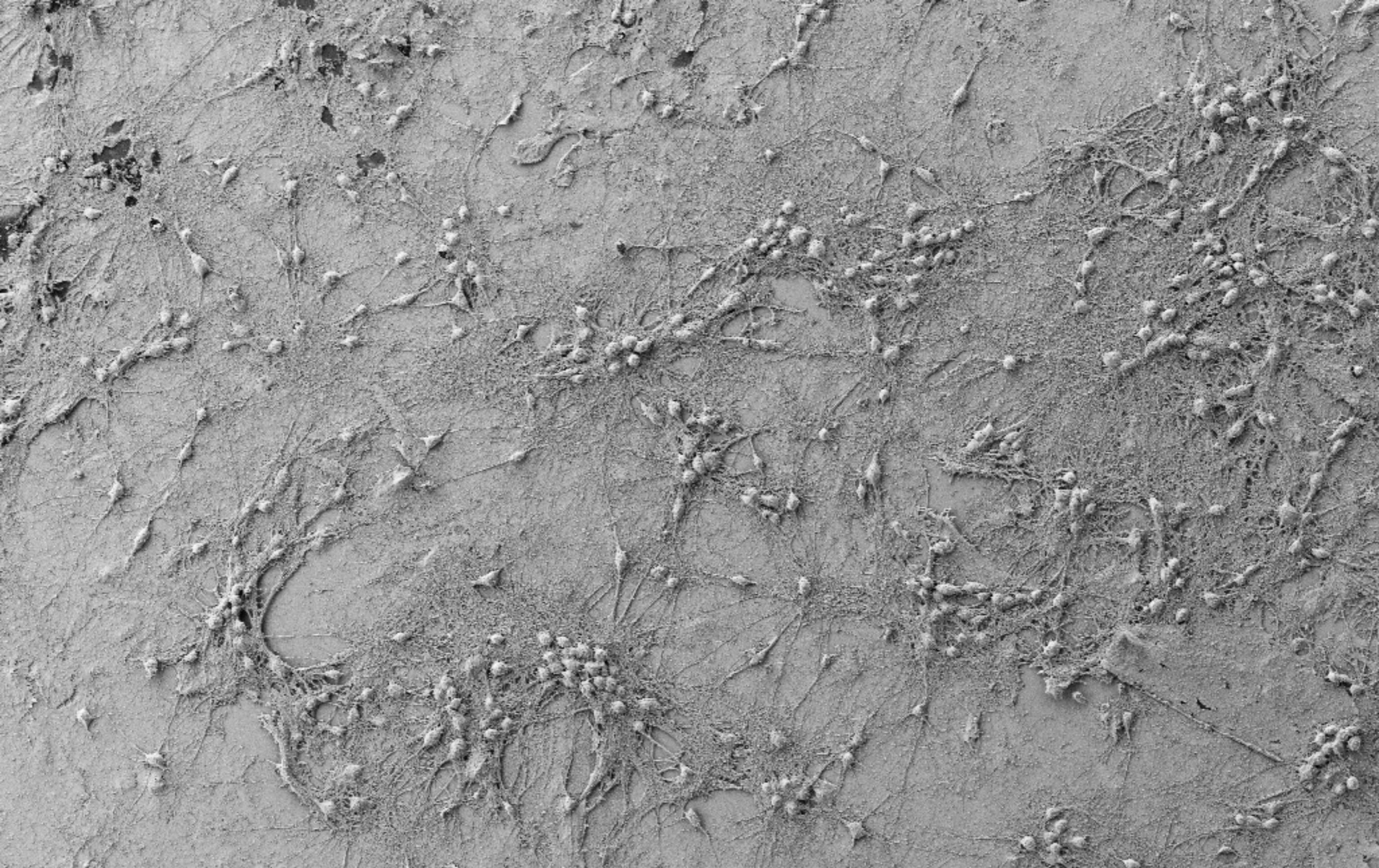
FIB Imaging = SEM

Signal B = SE2

Signal A = SE2

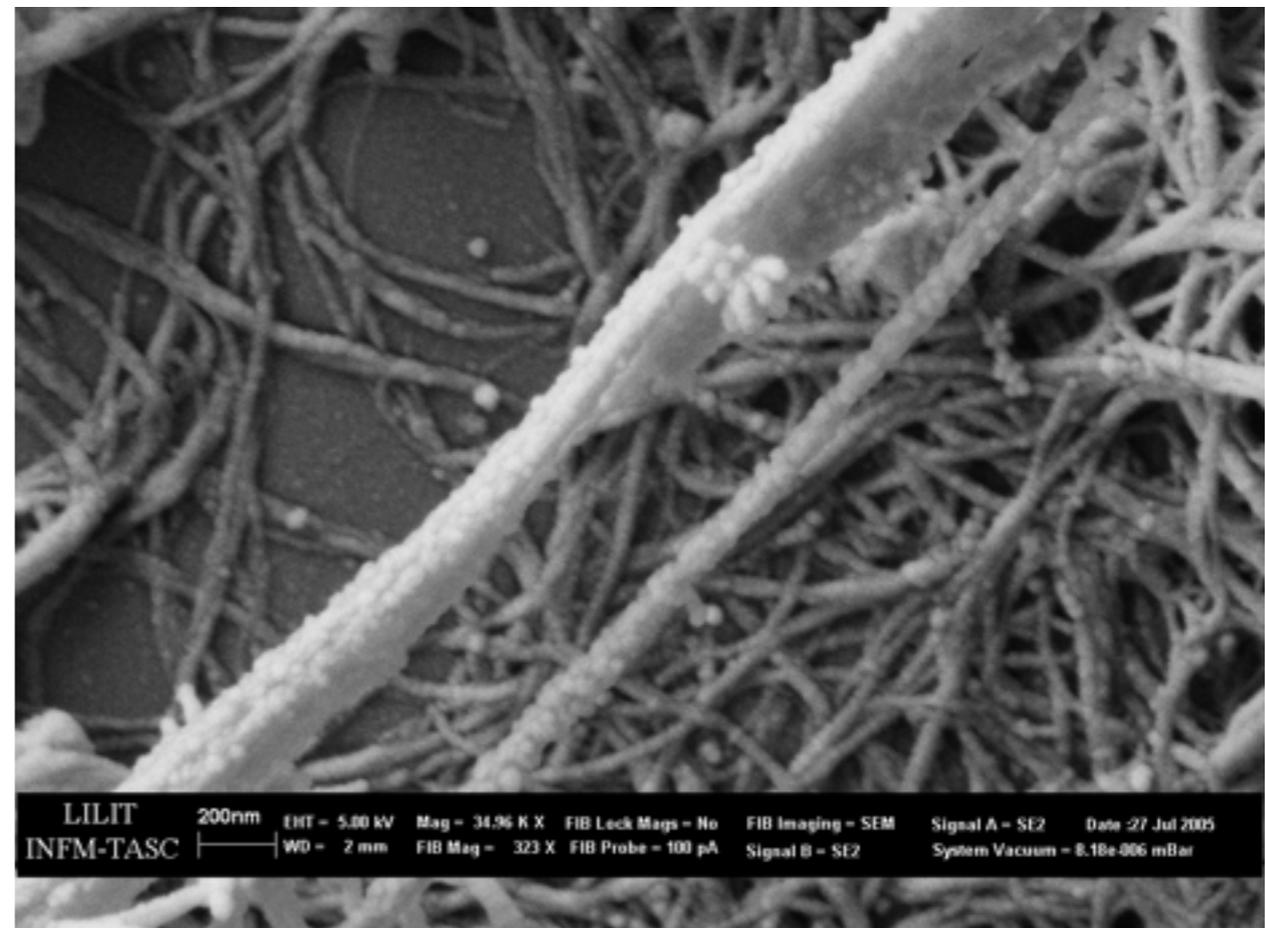
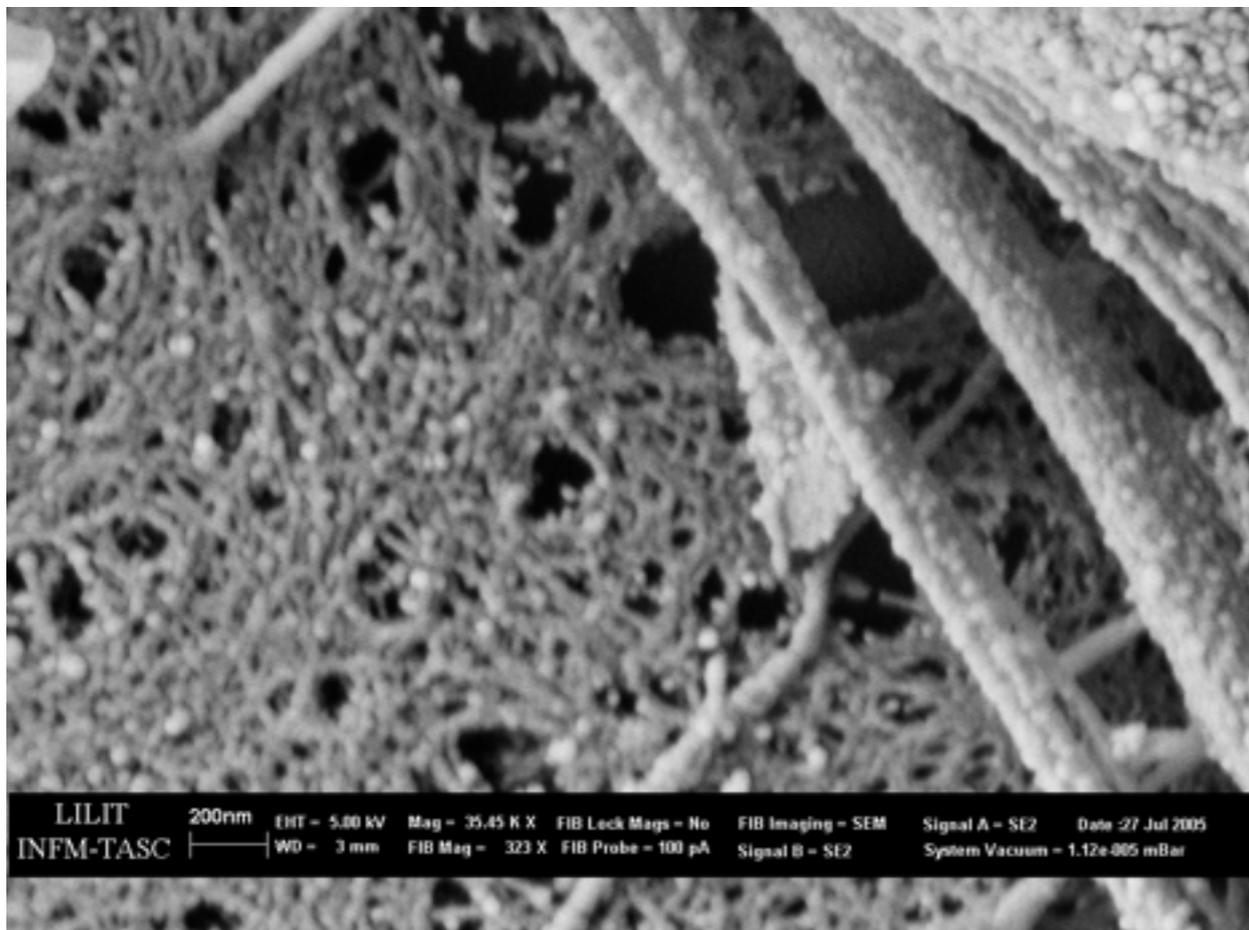
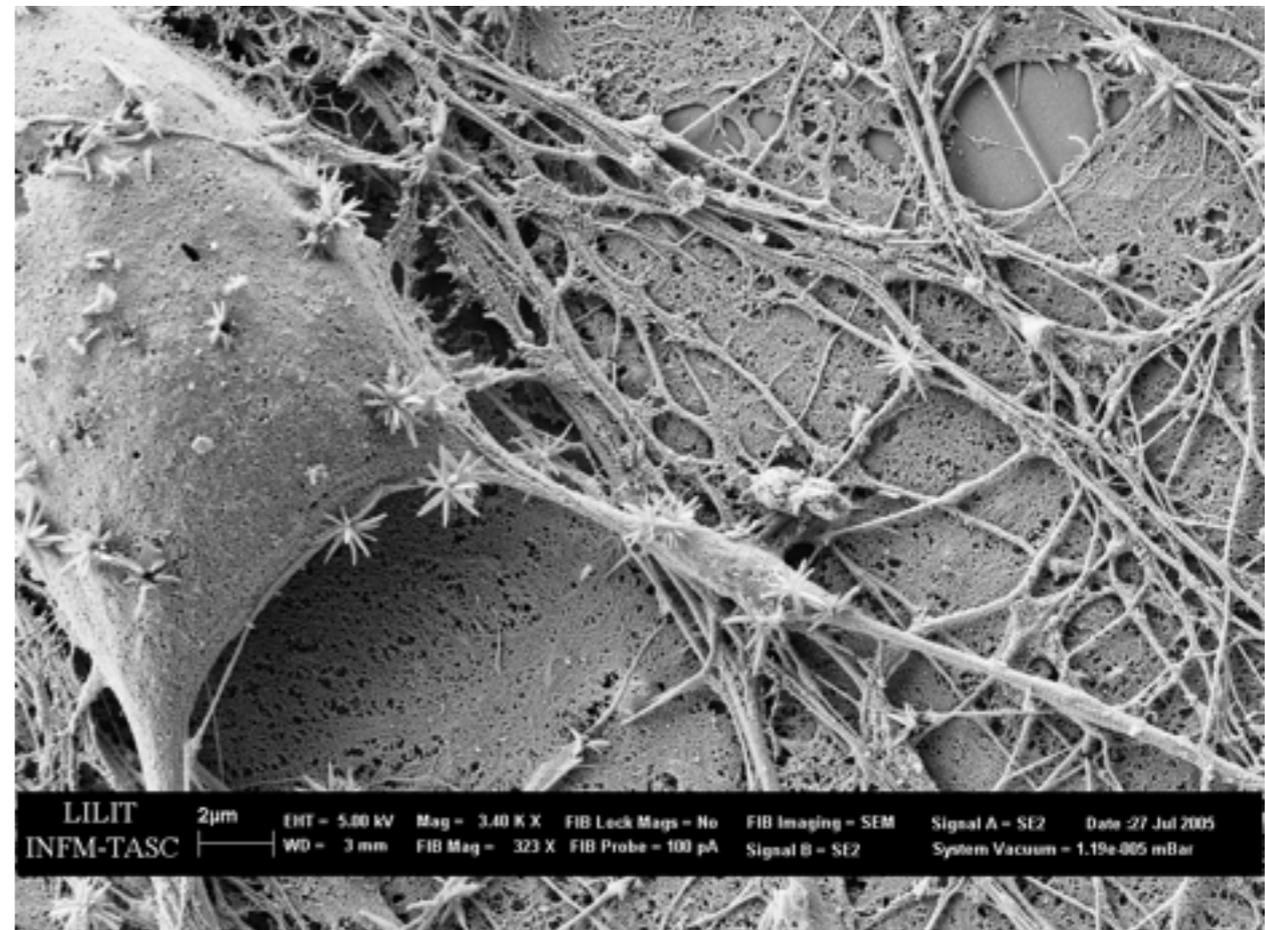
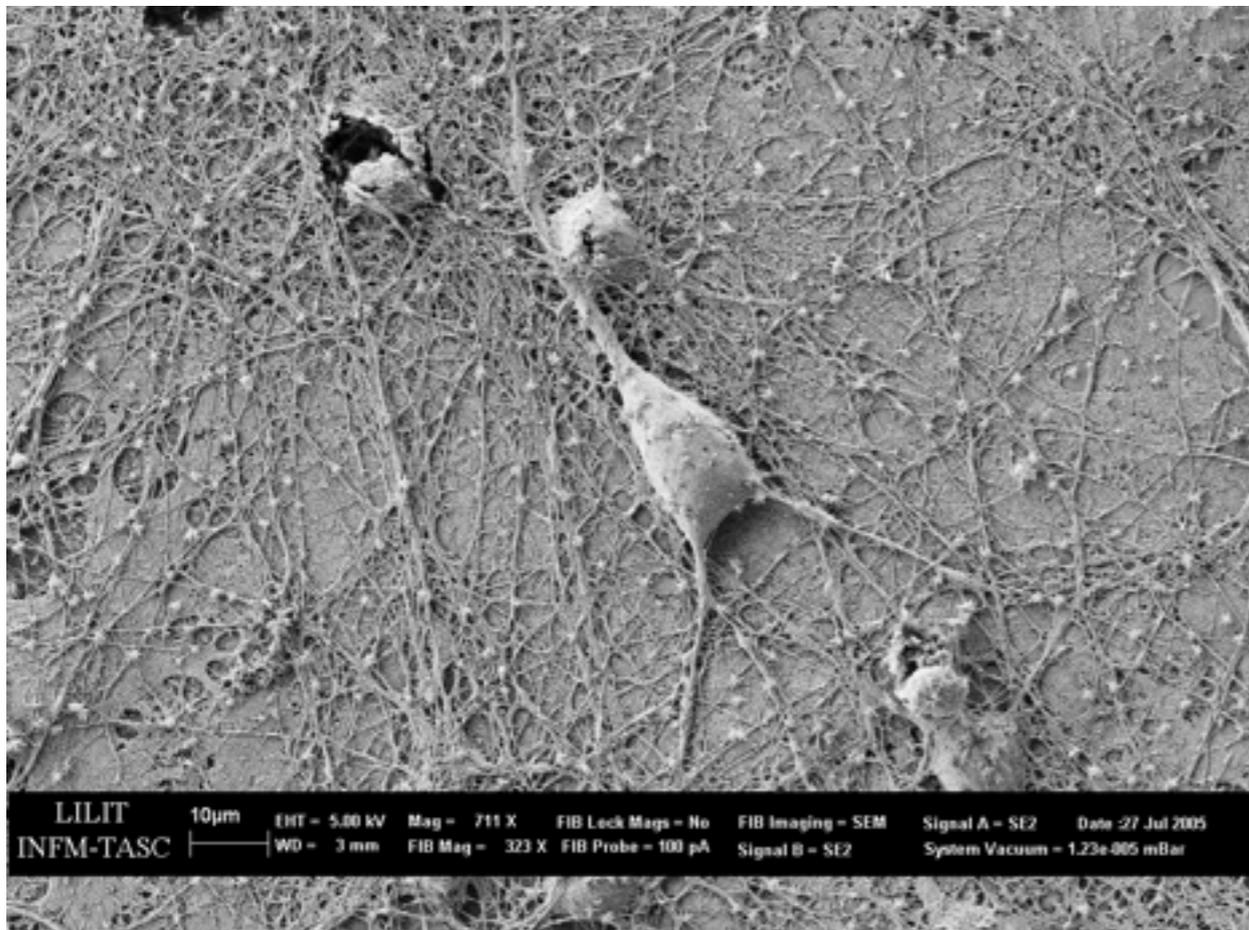
System Vacuum = 8.55e-006 mBar

Date :27 Jul 2005

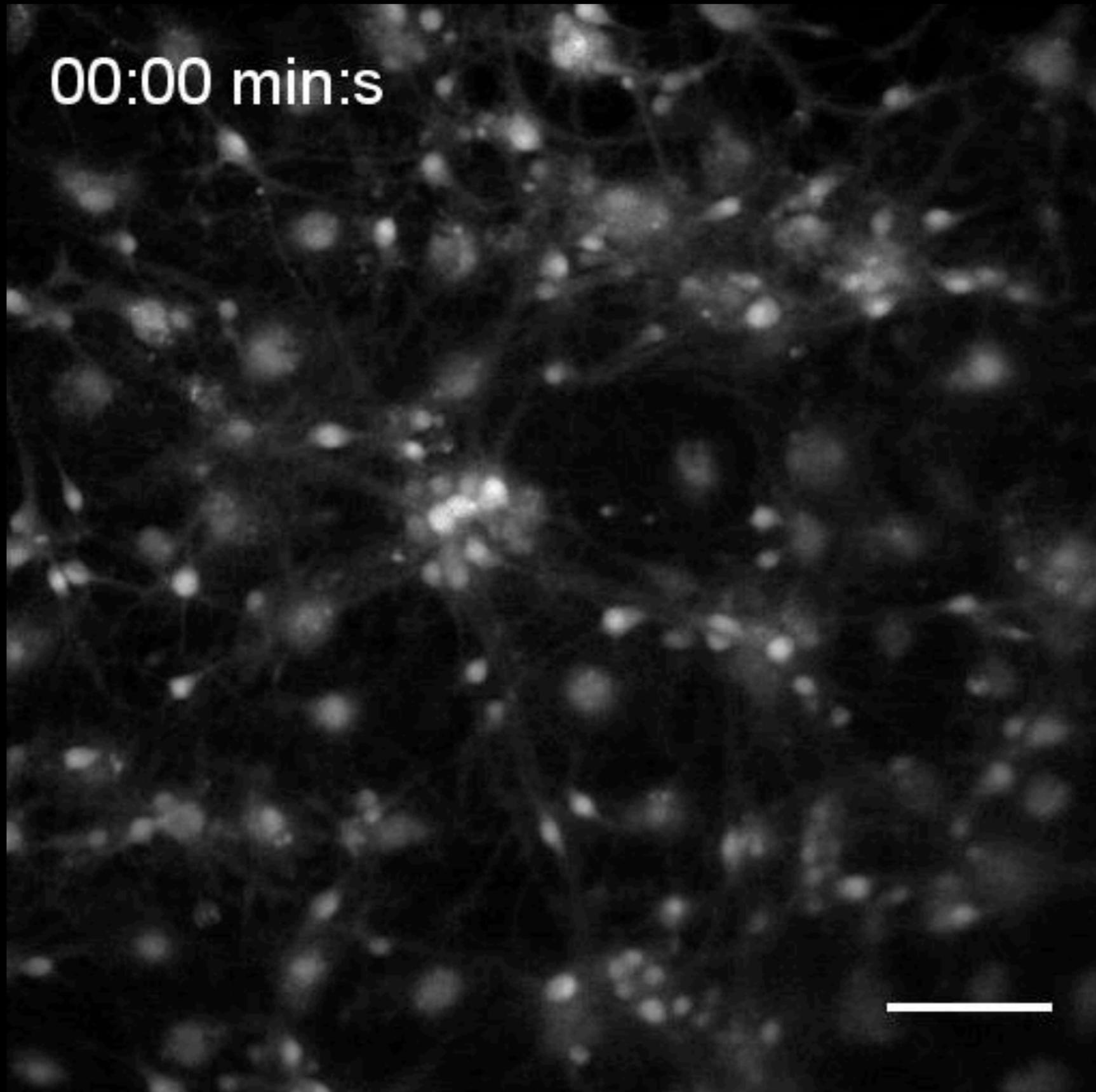


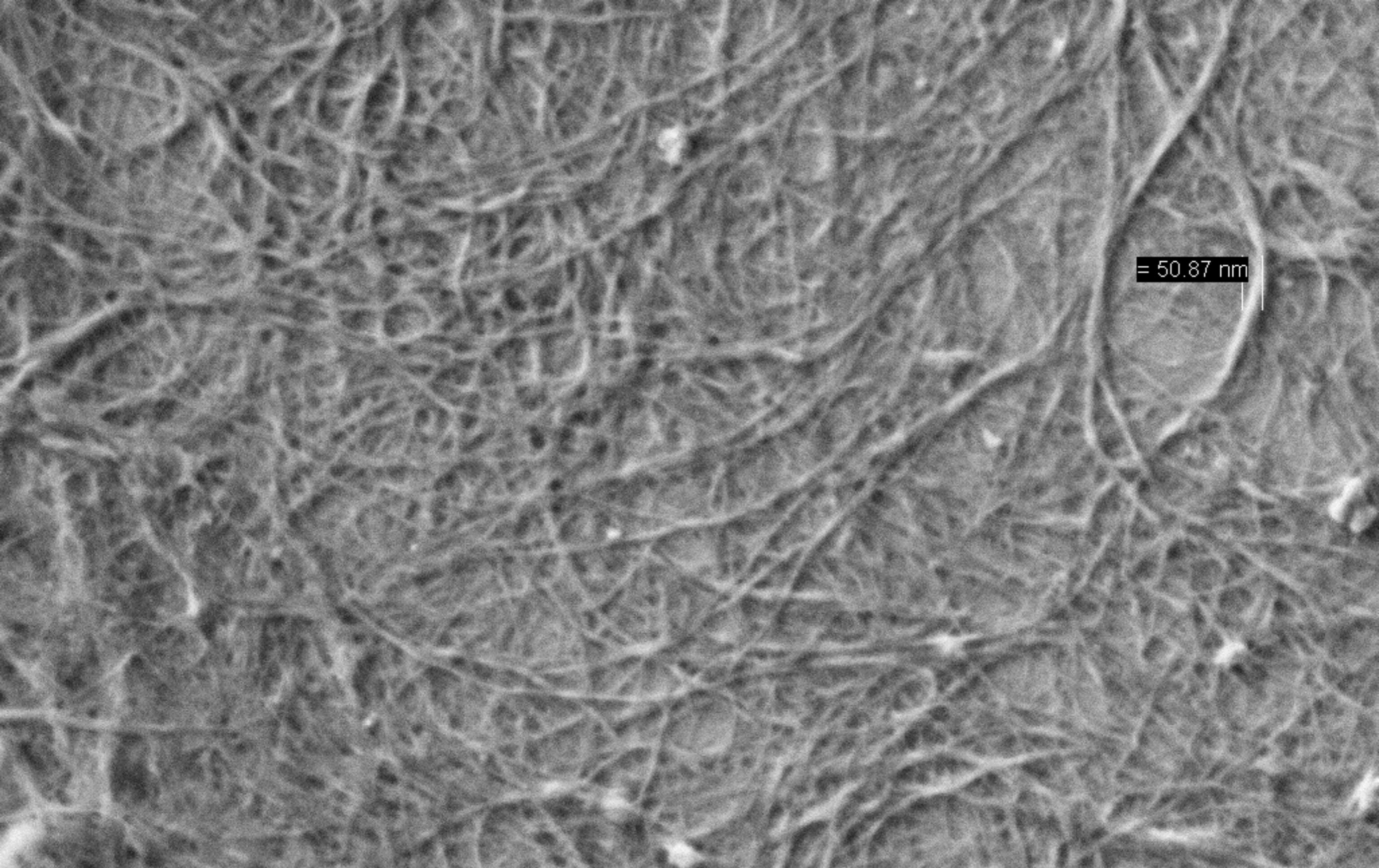
LILIT 20µm
INFM-TASC H

EHT = 5.00 kV	Mag = 88 X	FIB Lock Mags = No	FIB Imaging = SEM	Signal A = SE2	Date :27 Jul 2005
WD = 3 mm	FIB Mag = 323 X	FIB Probe = 100 pA	Signal B = SE2	System Vacuum = 1.48e-005 mBar	



Hippocampal culture on Polyornithine - 10 DIV





= 50.87 nm

LILIT
INFM-TASC

200nm

EHT = 1.00 kV
WD = 3 mm

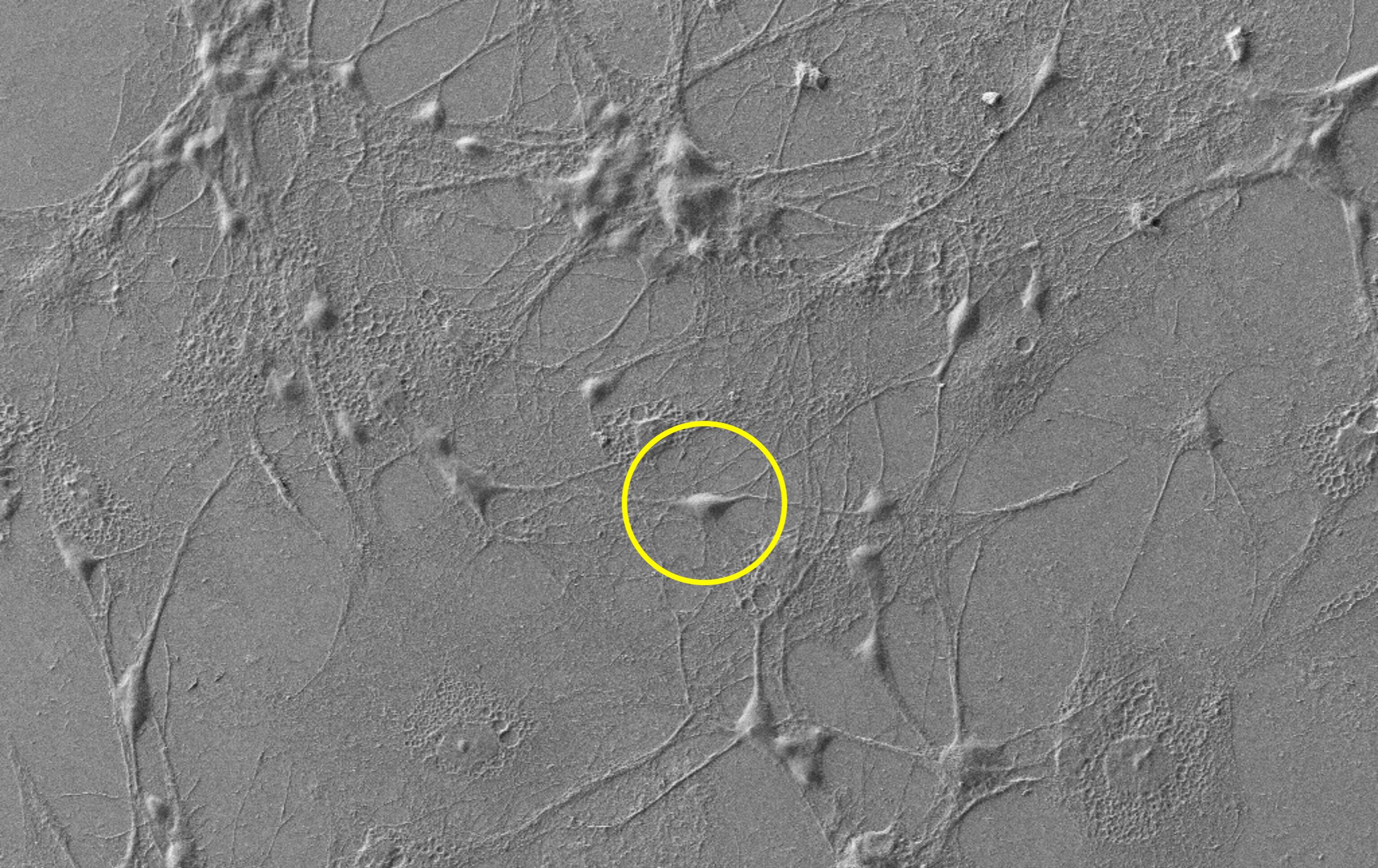
Mag = 32.92 K X
FIB Mag = 246 X

FIB Lock Mags = No
FIB Probe = 100 pA

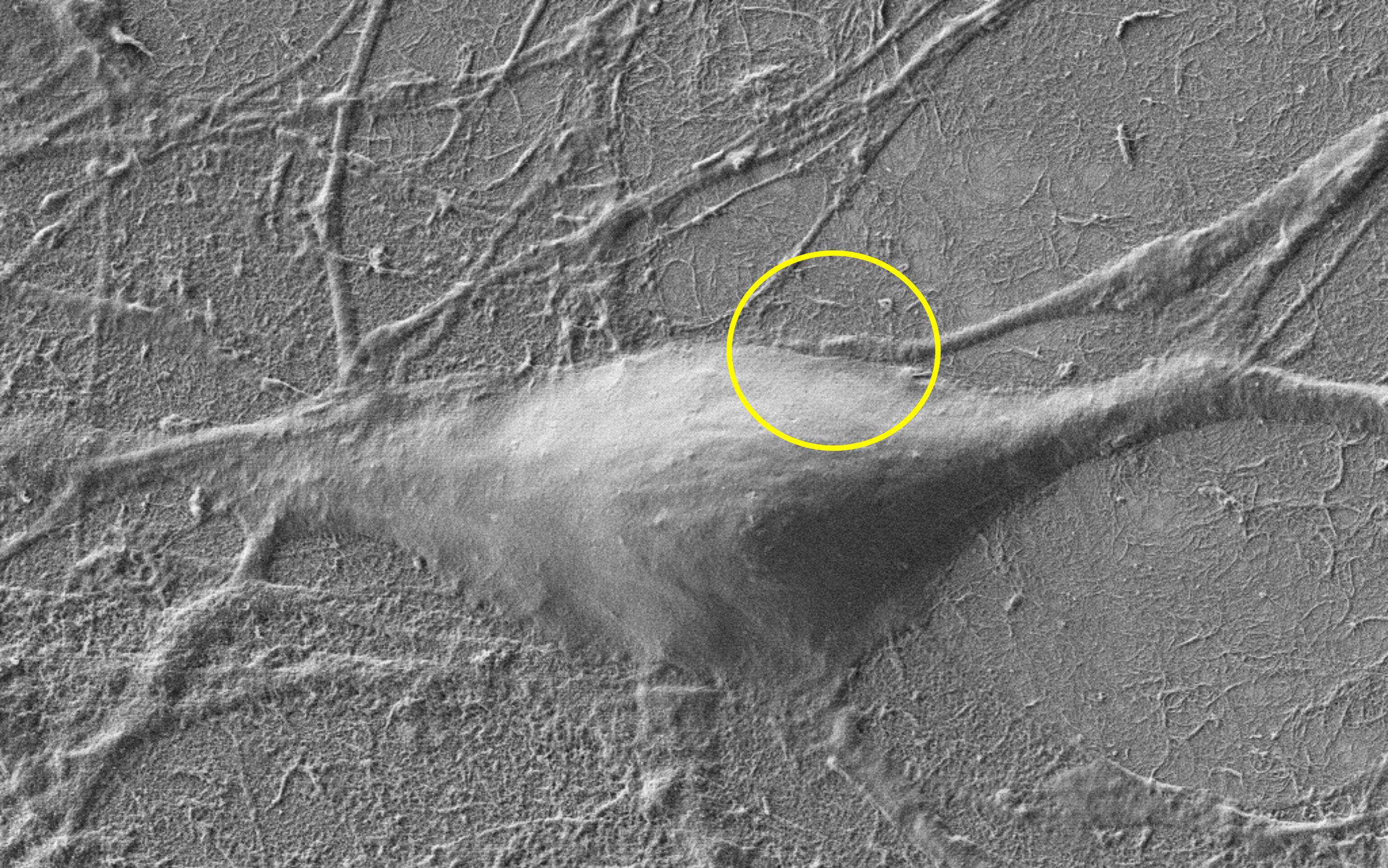
FIB Imaging = SEM
Signal B = SE2

Signal A = SE2
System Vacuum = 8.27e-006 mBar

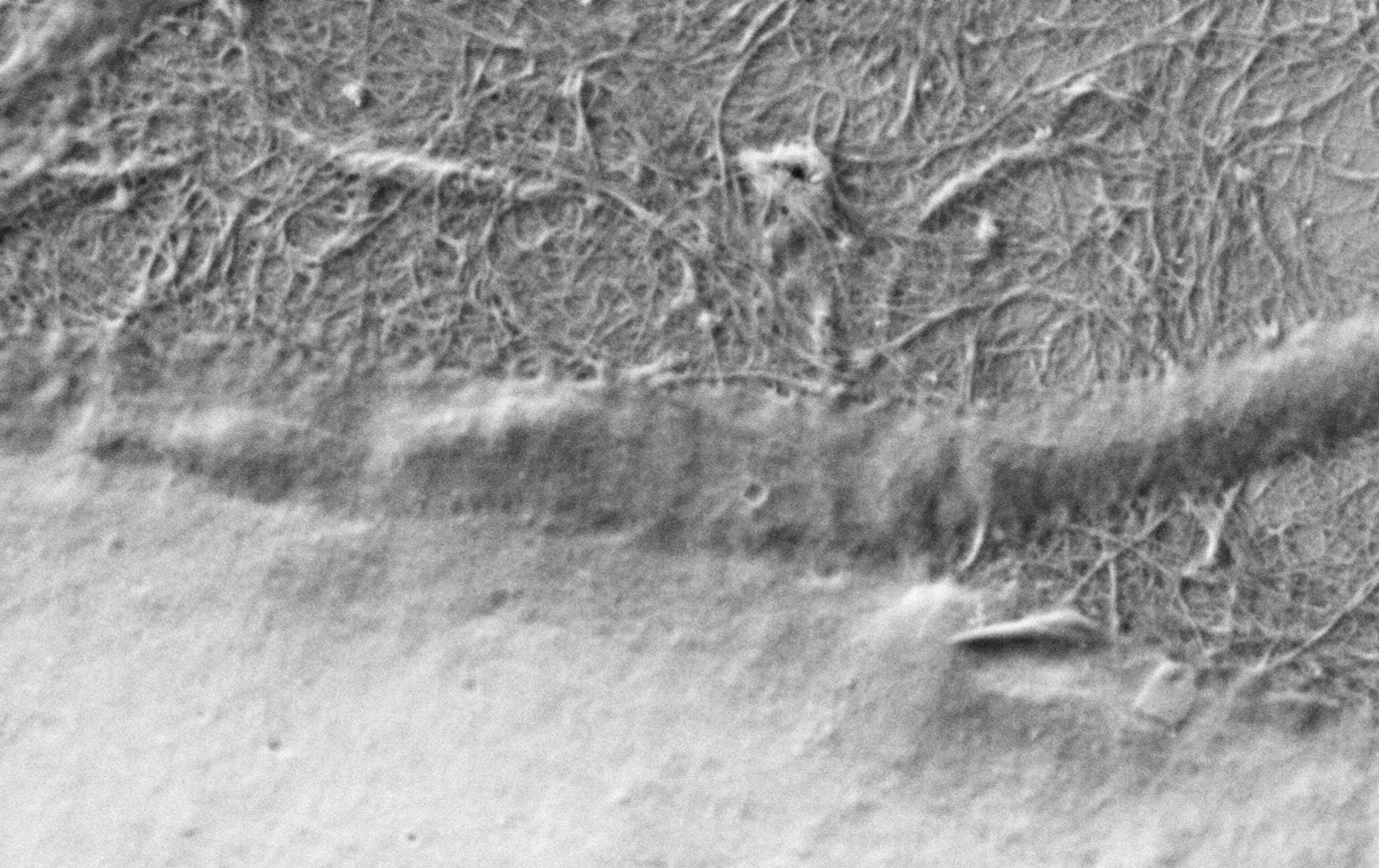
Date :19 Oct 2006



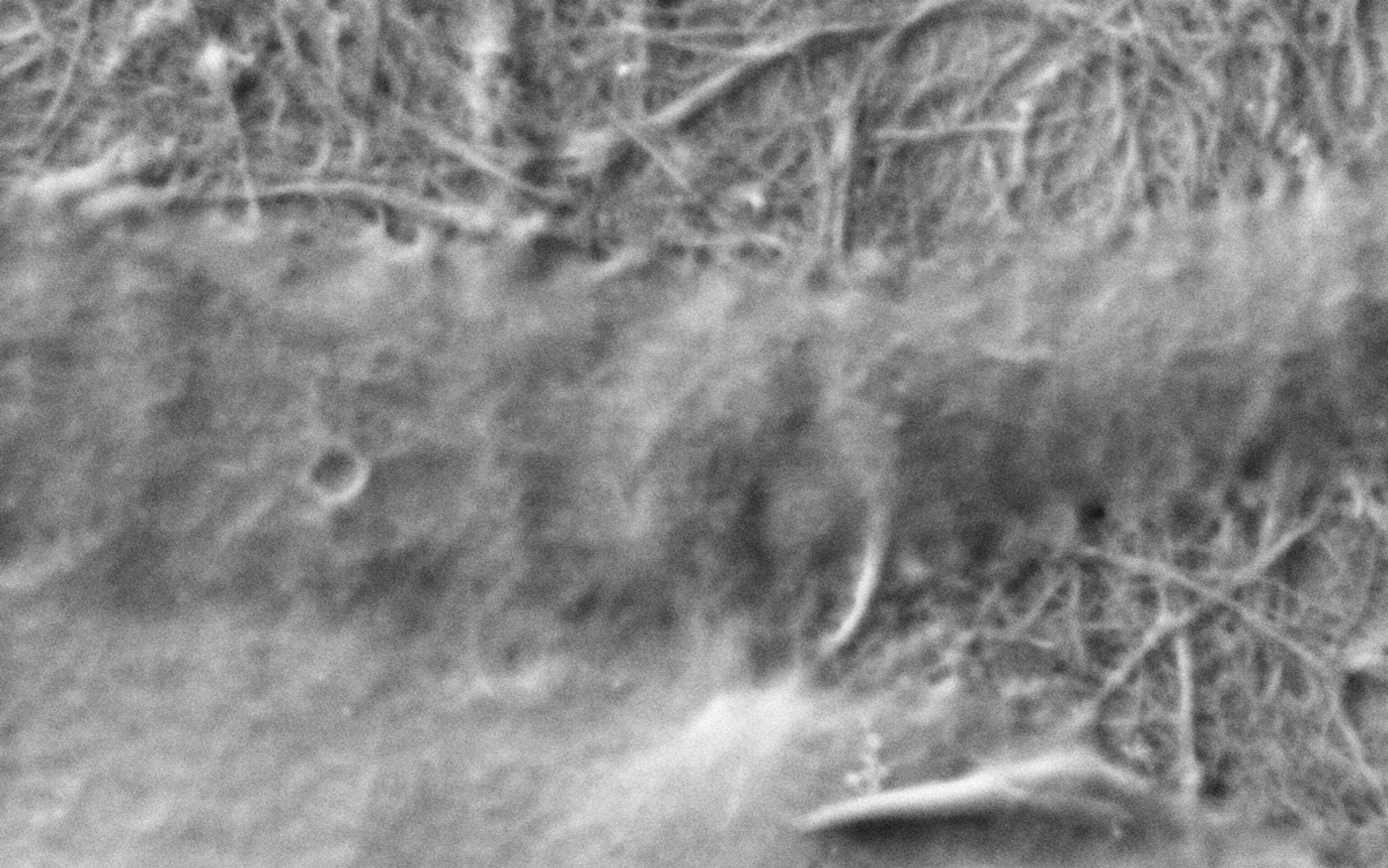
LILIT 10µm EHT = 1.00 kV Mag = 249 X FIB Lock Mags = No FIB Imaging = SEM Signal A = SE2 Date :19 Oct 2006
INFM-TASC ┆ WD = 3 mm FIB Mag = 246 X FIB Probe = 100 pA Signal B = SE2 System Vacuum = 1.07e-005 mBar



LILIT 2µm EHT = 1.00 kV Mag = 3.04 K X FIB Lock Mags = No FIB Imaging = SEM Signal A = SE2 Date :19 Oct 2006
INFM-TASC |————| WD = 3 mm FIB Mag = 246 X FIB Probe = 100 pA Signal B = SE2 System Vacuum = 9.57e-006 mBar



LILIT 200nm EHT = 1.00 kV Mag = 19.49 K X FIB Lock Mags = No FIB Imaging = SEM Signal A = SE2 Date :19 Oct 2006
INFM-TASC ───┬─── WD = 3 mm FIB Mag = 246 X FIB Probe = 100 pA Signal B = SE2 System Vacuum = 8.90e-006 mBar



LILIT 100nm EHT = 1.00 kV Mag = 45.64 K X FIB Lock Mags = No FIB Imaging = SEM Signal A = SE2 Date :19 Oct 2006
INFM-TASC |—| WD = 3 mm FIB Mag = 246 X FIB Probe = 100 pA Signal B = SE2 System Vacuum = 8.51e-006 mBar

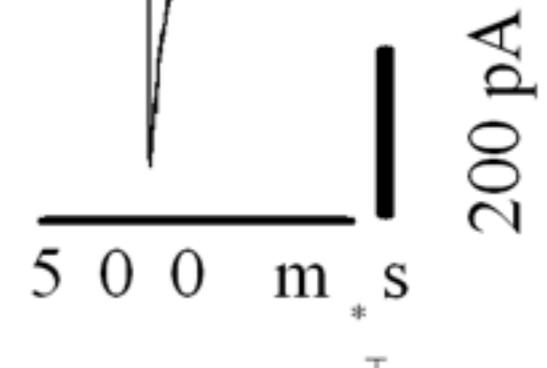
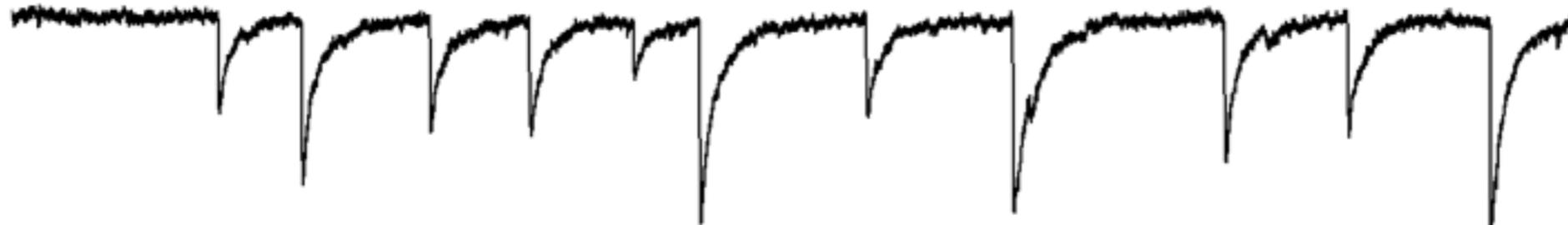
Patch-Clamp



c o n t r o l



C N T s



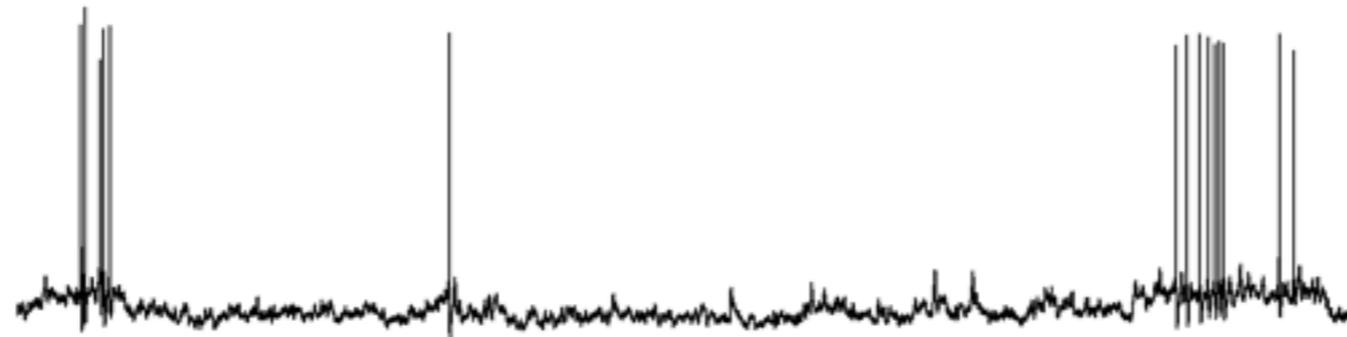
CNT substrate increases hippocampal neurons spontaneous synaptic activity and firing.

Spontaneous synaptic currents (PSCs) are shown in both control (top tracings) and in cultures grown on CNT substrate (bottom tracings).

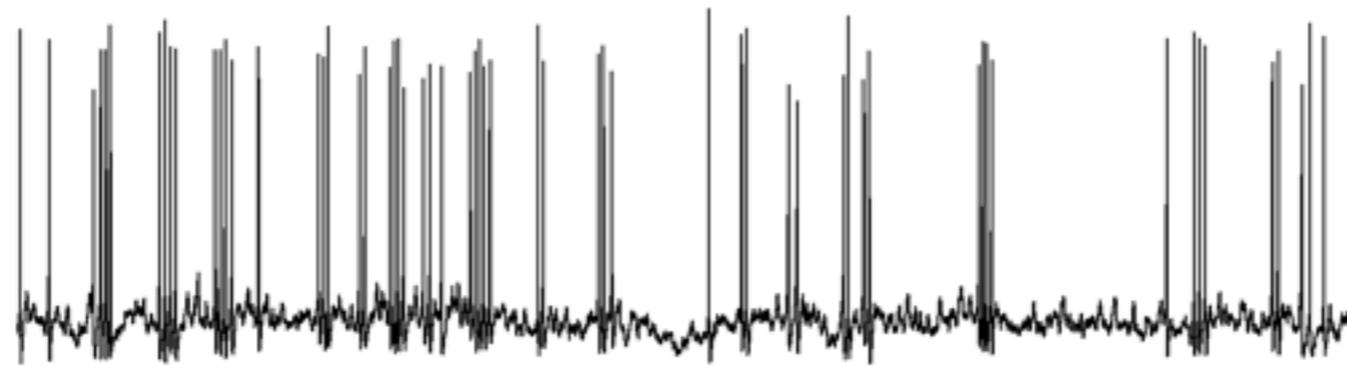
Note the increase in PSCs frequency under the latter condition.

Recordings were taken after 8 days in culture.

c o n t r o l



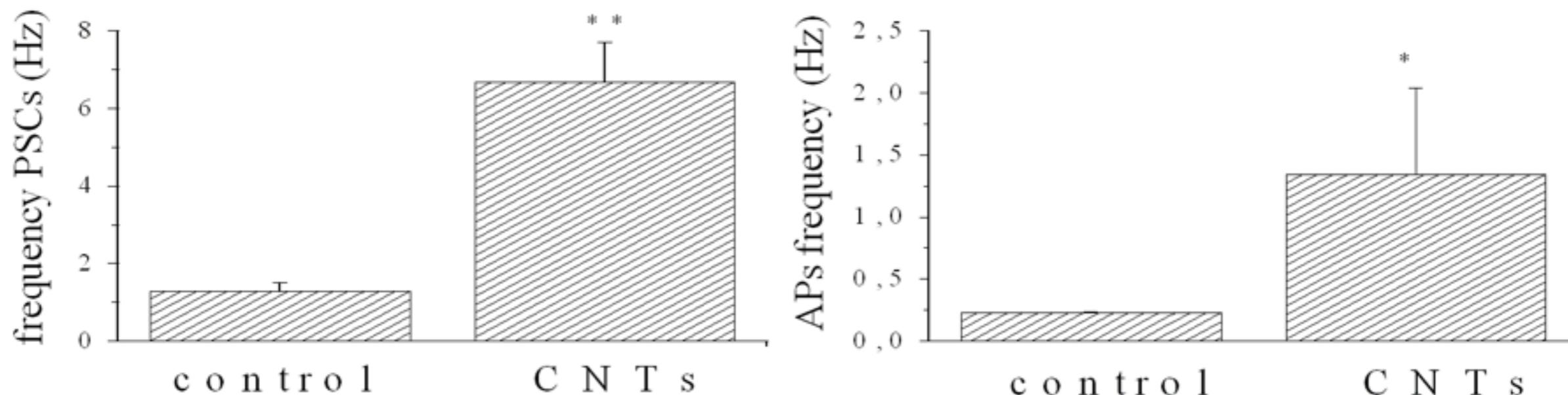
C N T s



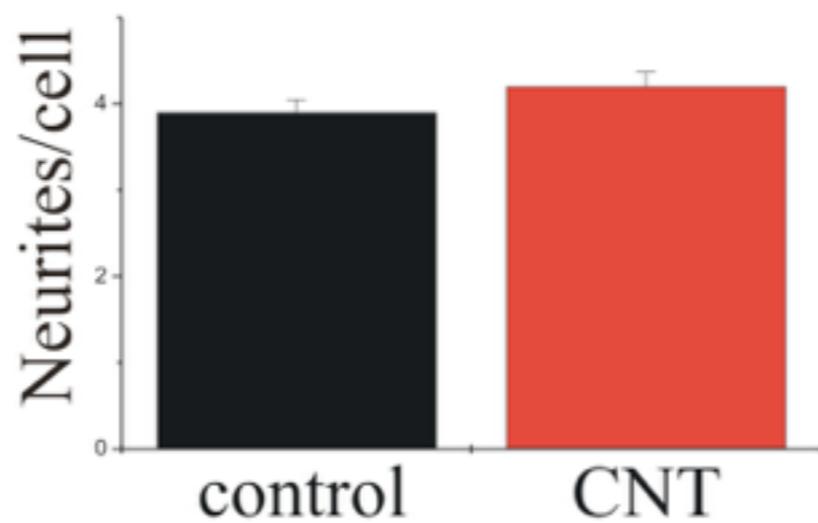
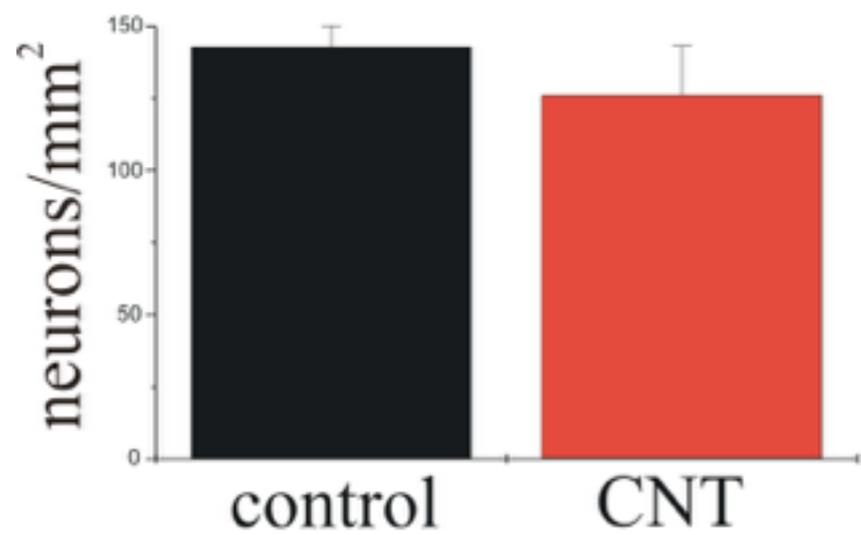
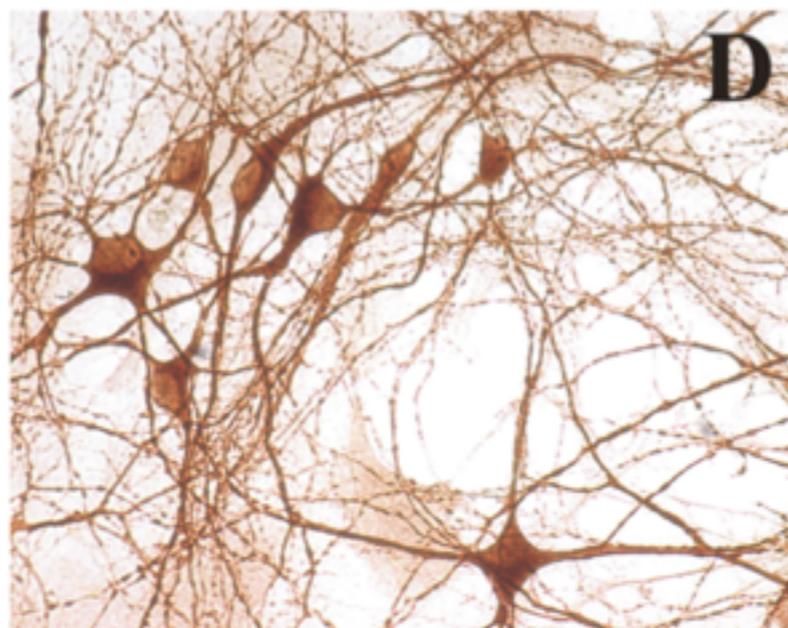
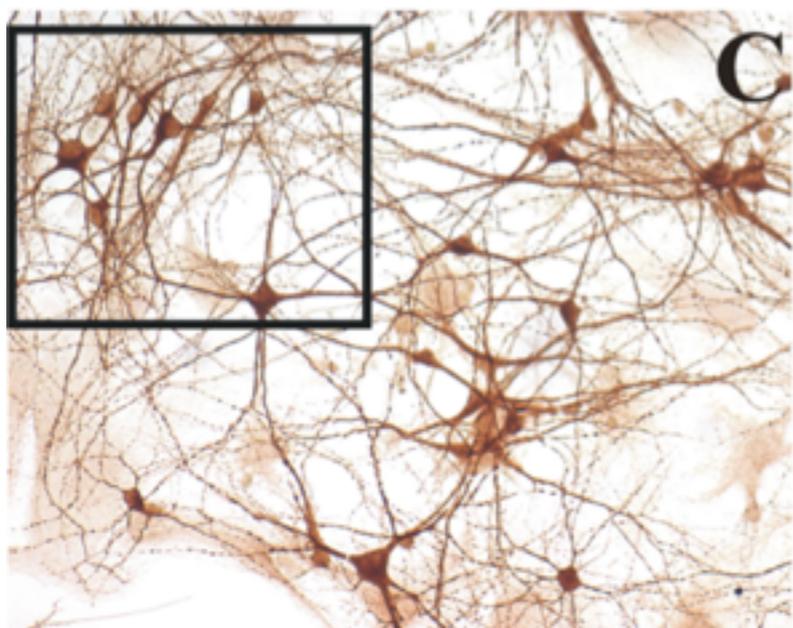
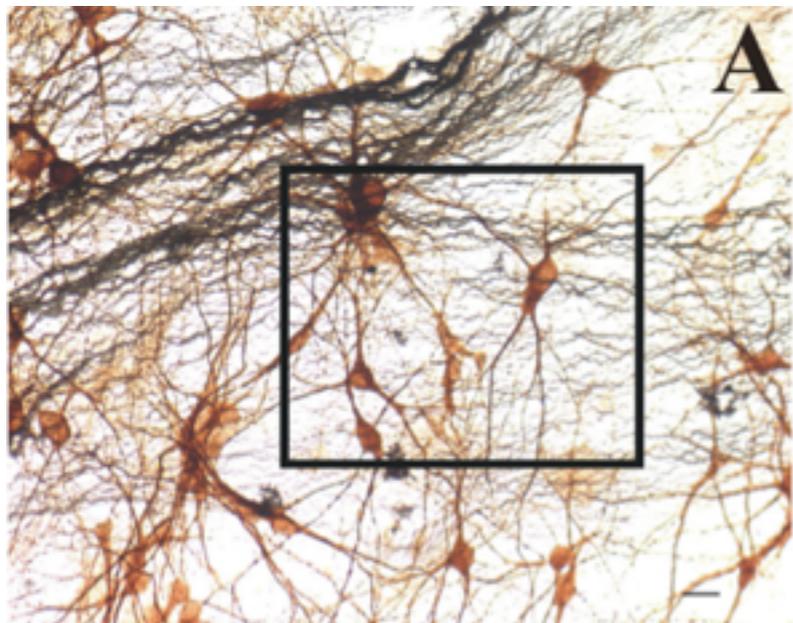
1 s

50 mV

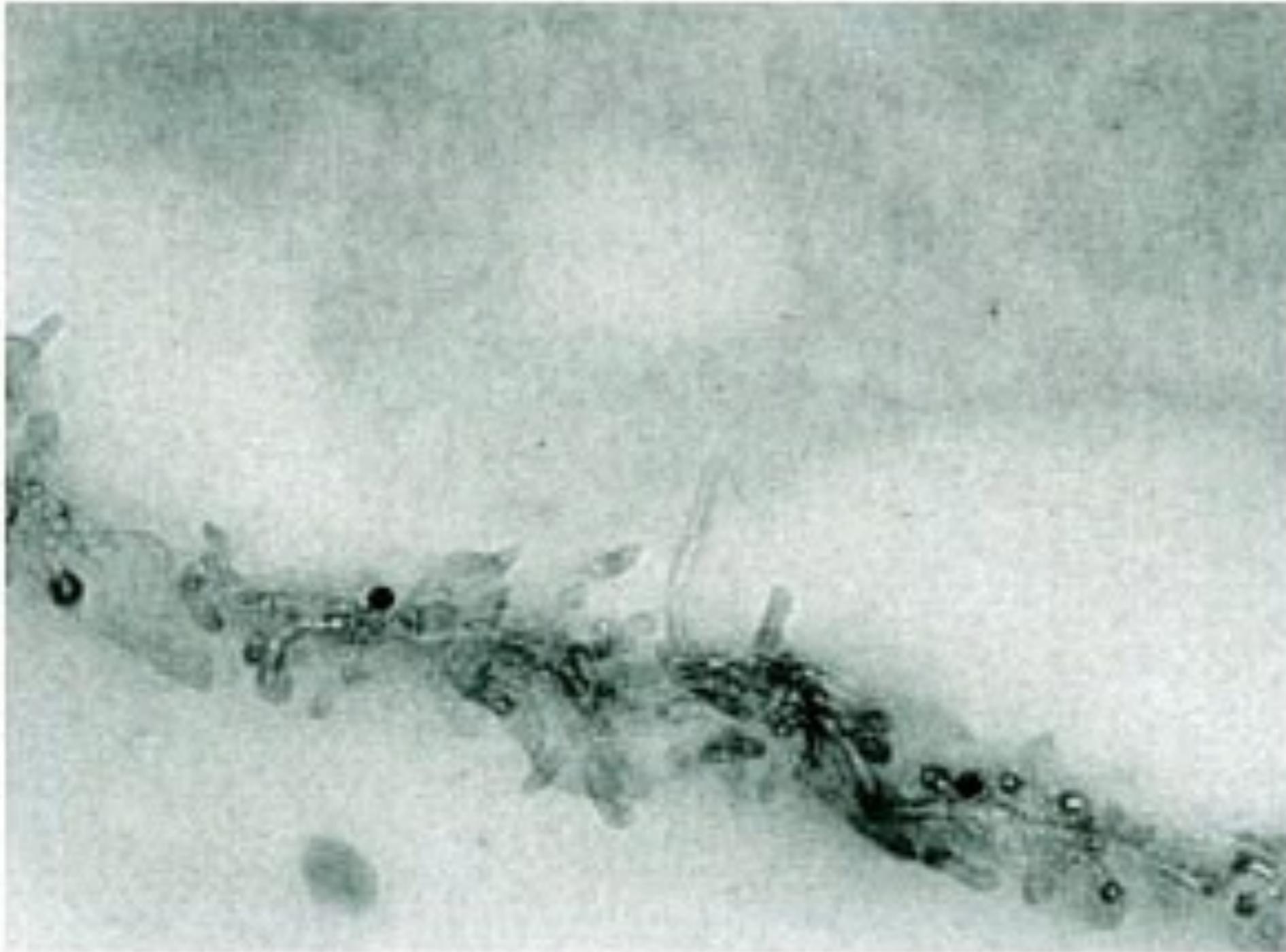
Current clamp recordings from cultured hippocampal neurons in control (top tracings) and CNT growth conditions (bottom tracings). Spontaneous firing activity is greatly boosted in the presence of CNT substrates.



Histogram plots of Post-Synaptic Currents (PSCs, left) and Action Potentials (Aps, right) frequency in control and CNT cells.
Note the significant increase in the occurrence of both events when measured in CNT cultures.

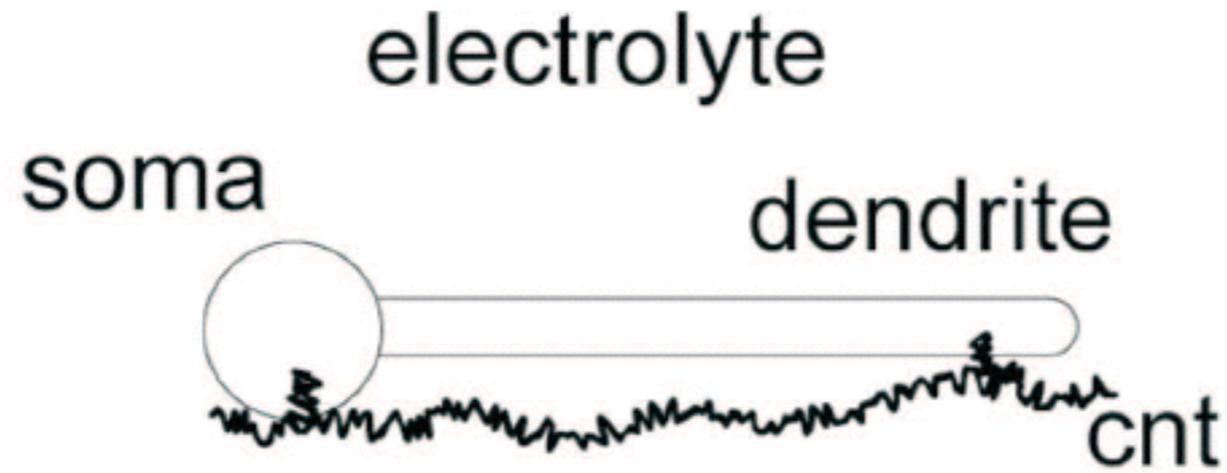


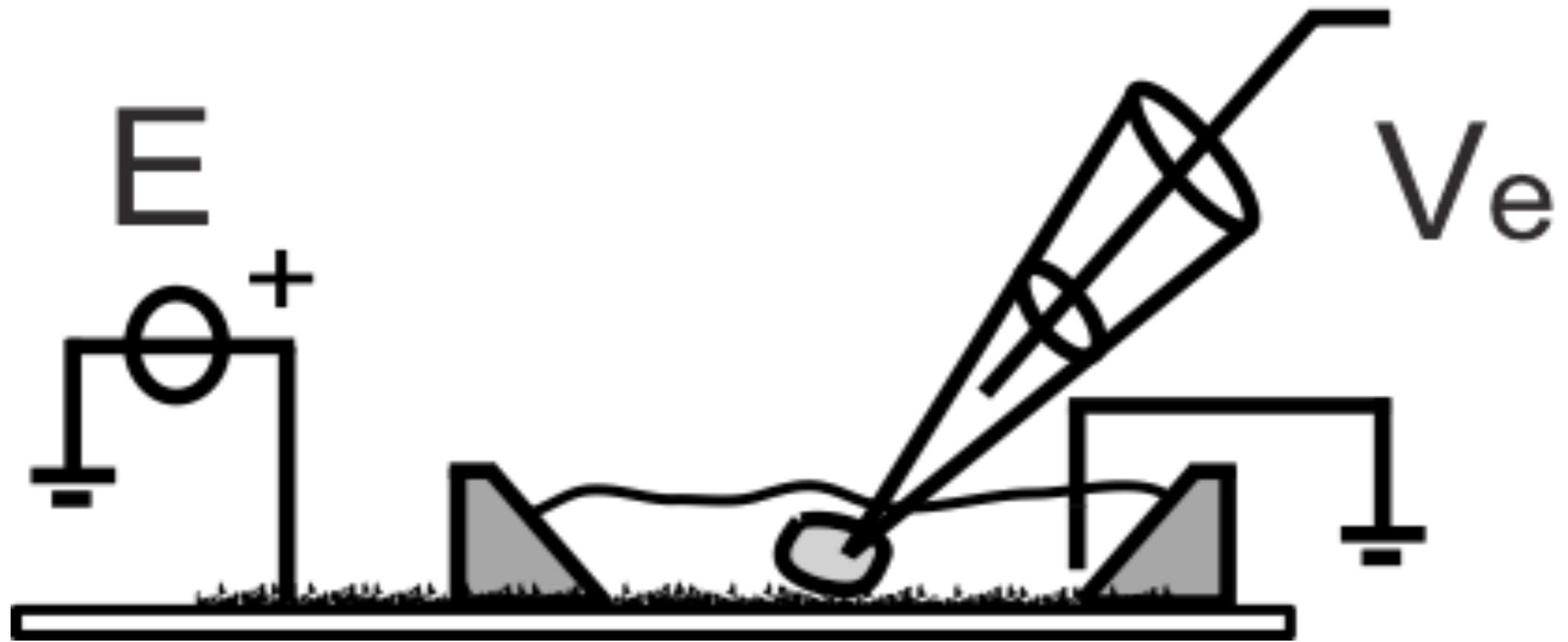
Transversal sections of the glass coverslips



Reasons for increased frequency of activity of neurons on CNTs

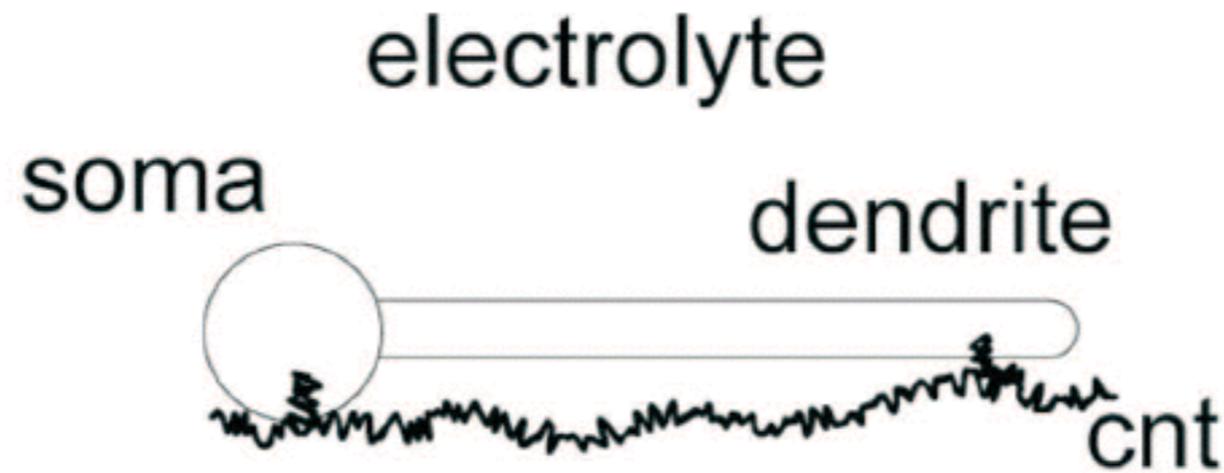
1) Direct electrical coupling between nearby dendritic compartments



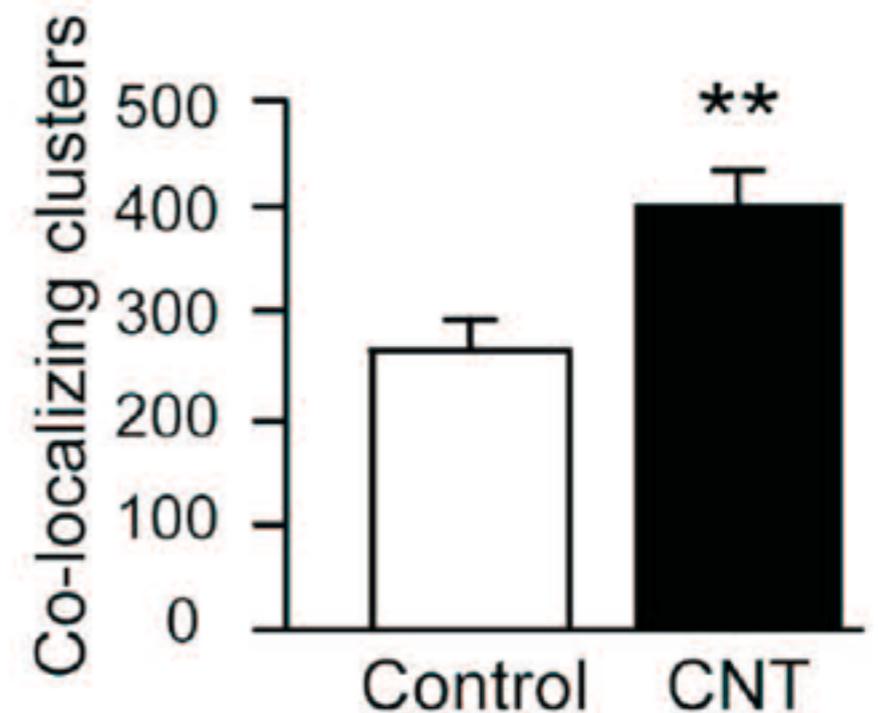
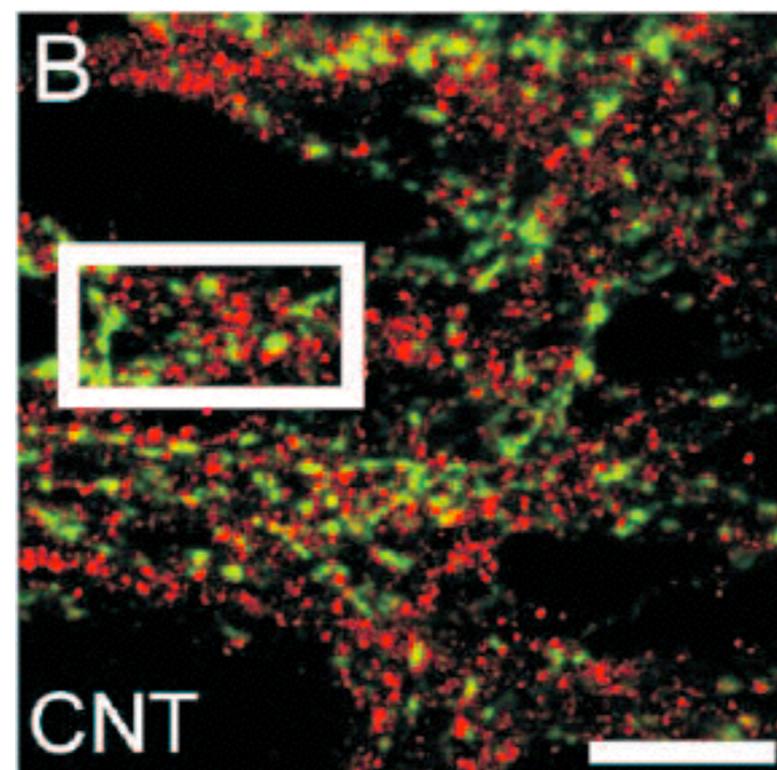
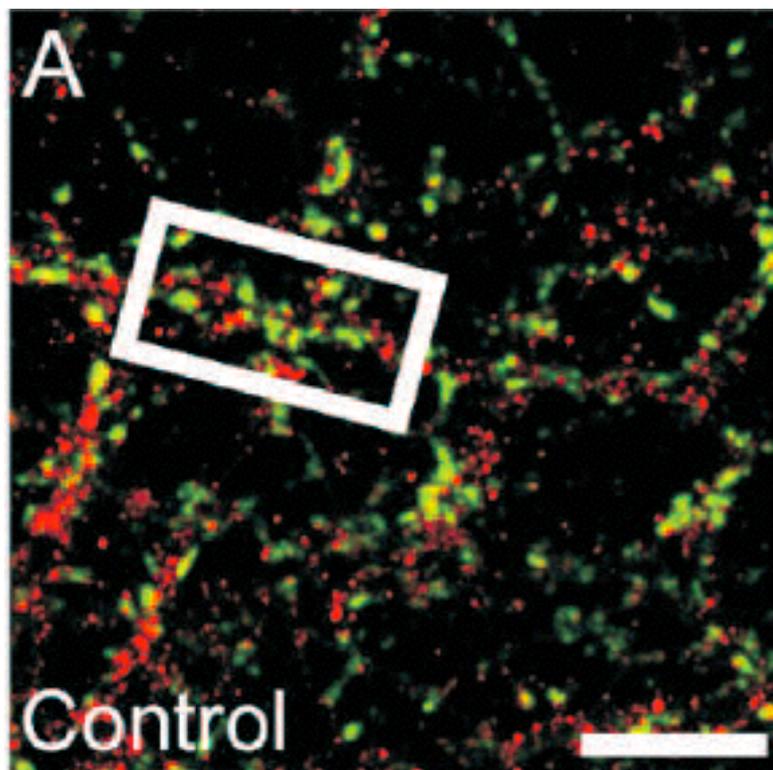


Reasons for increased frequency of activity of neurons on CNTs

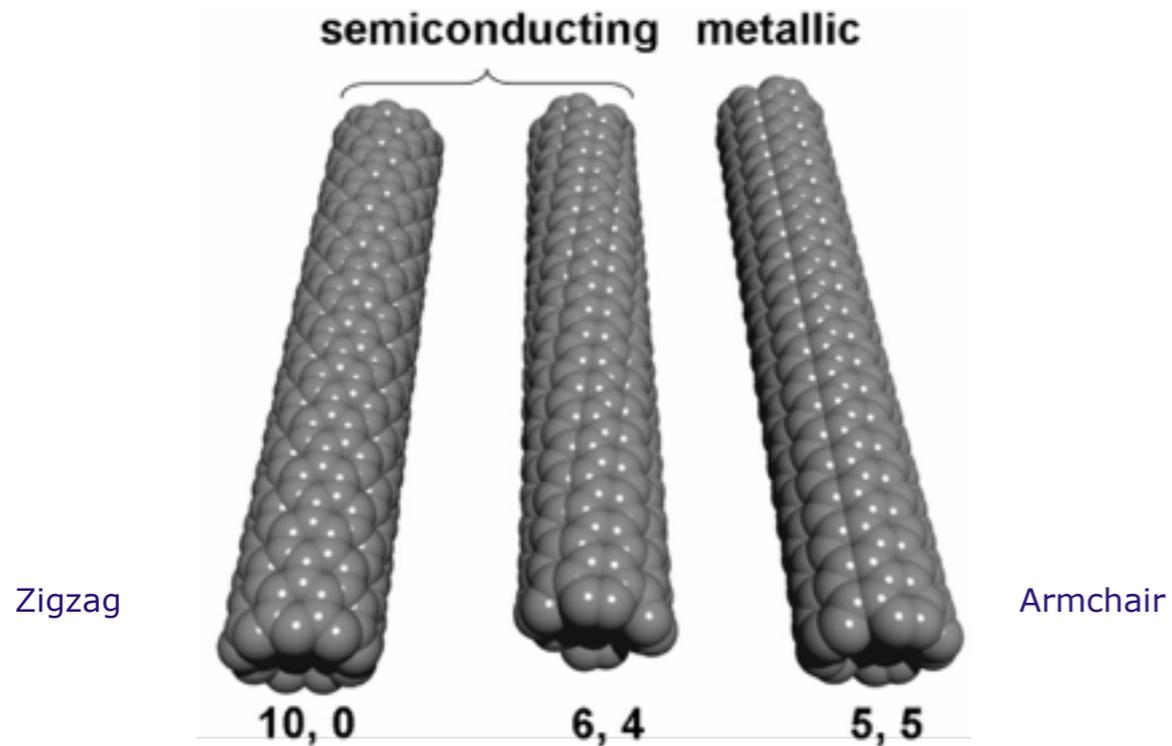
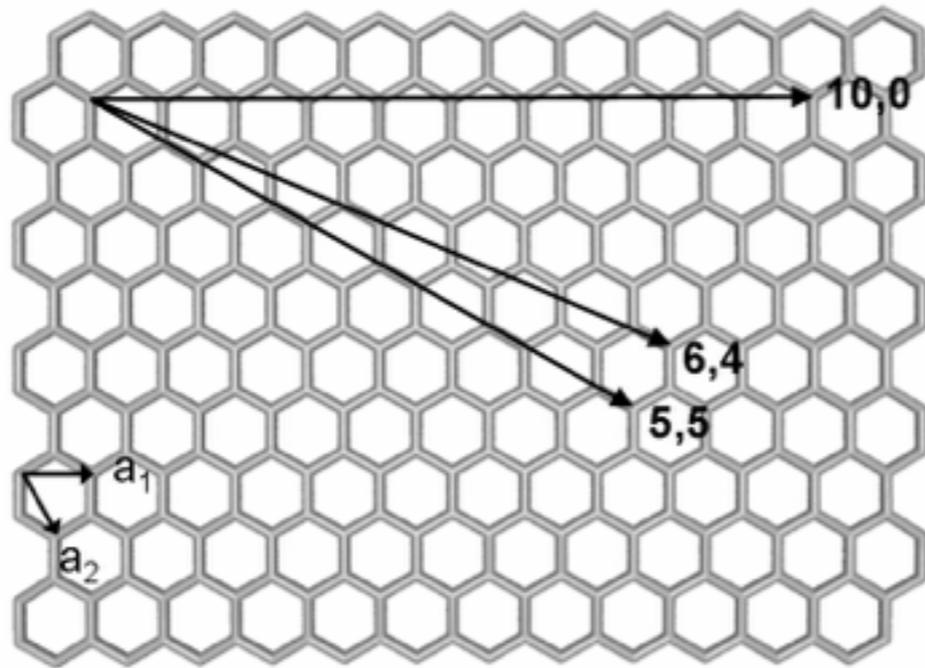
1) Direct electrical coupling between nearby dendritic compartments



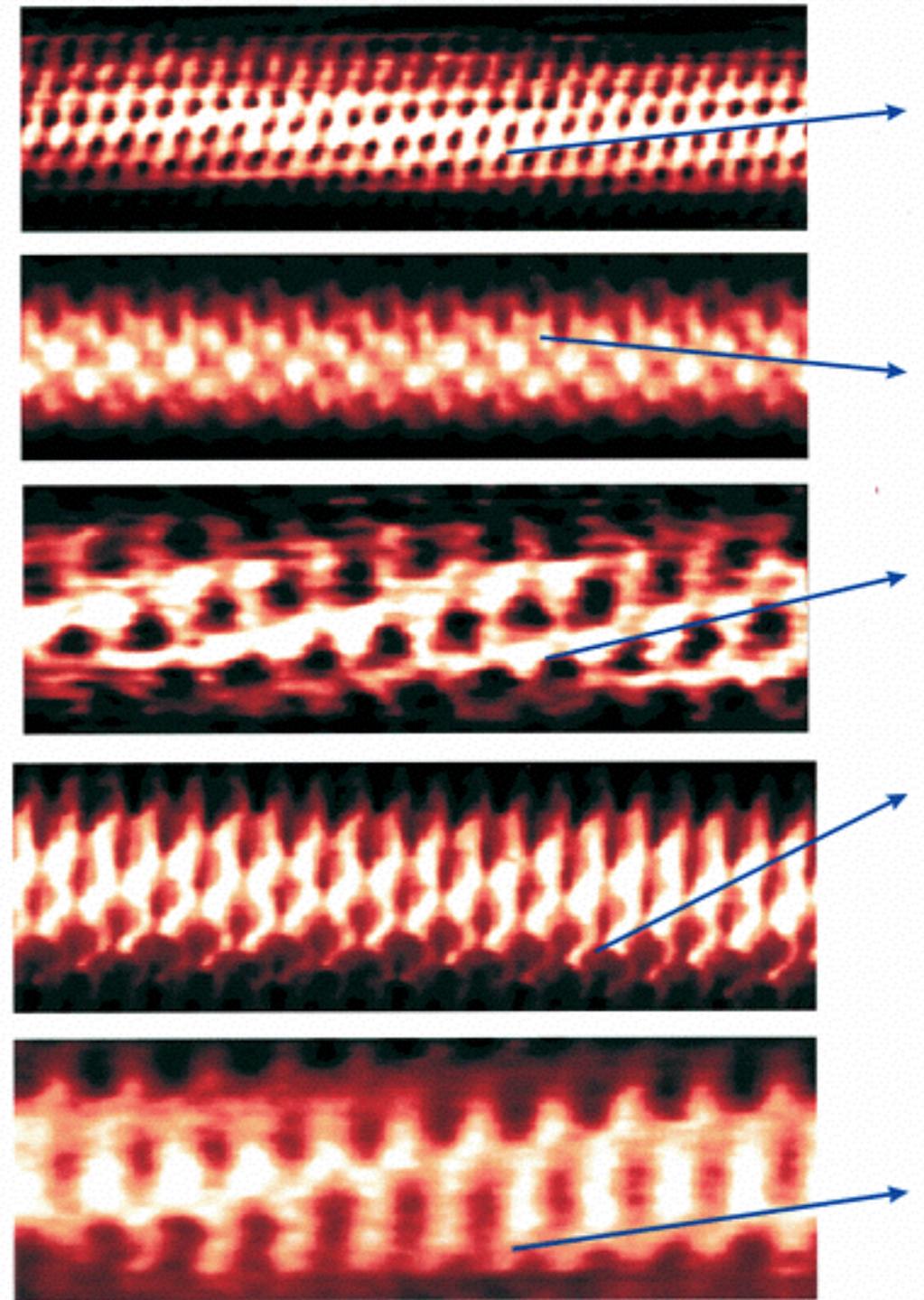
2) Immunofluorescence experiments show an increased number of synaptic contacts



Atomic resolution STM



Large variety of helicities



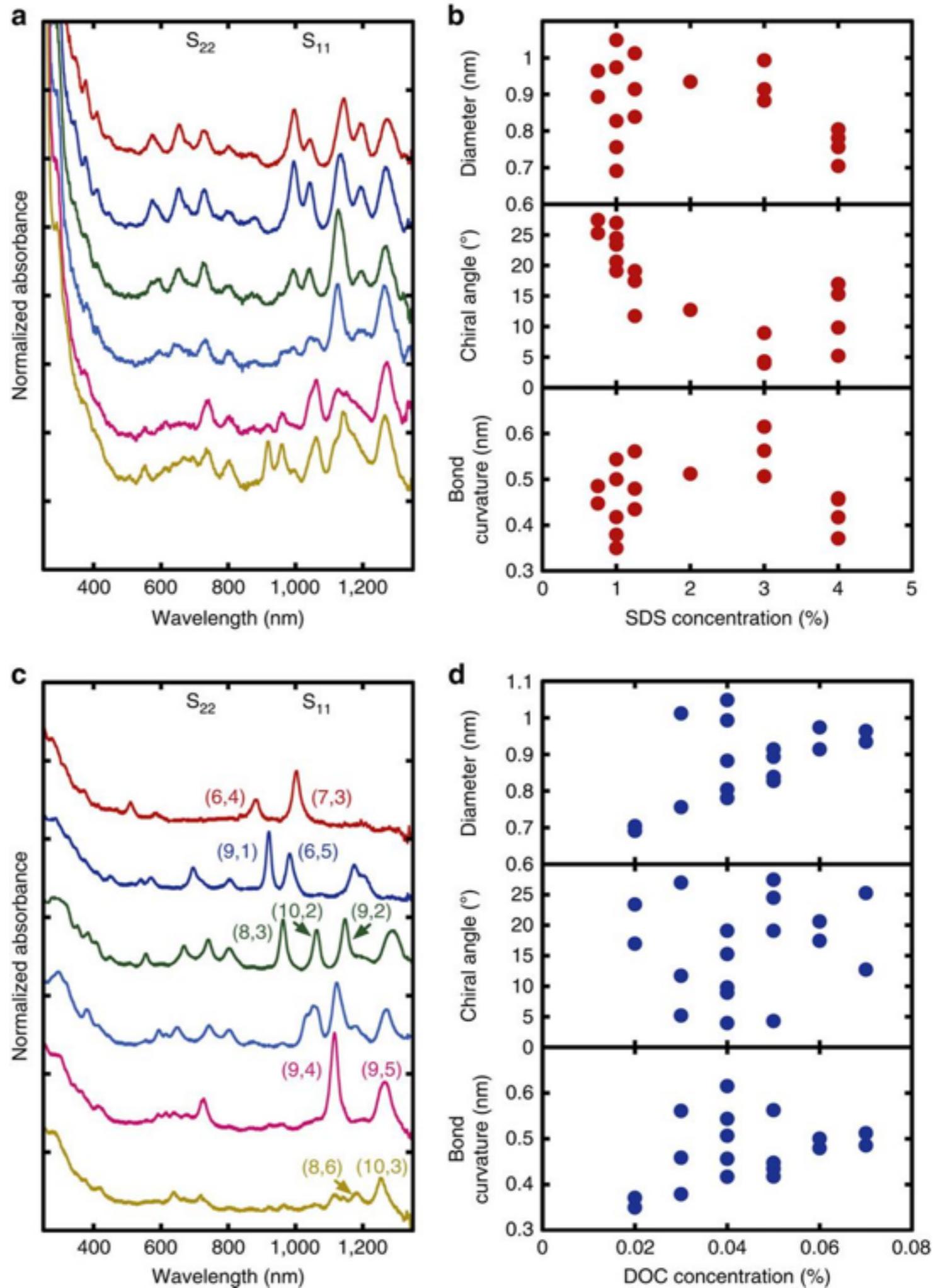
 **Tube axis**

Taken from Cees Dekker (Delft, NL) website

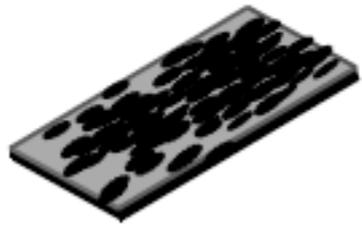
chiral index (n, m) : nanotubes in which $n = m$ are metallic and quasi-metallic (with tiny band gap) if $n-m$ is divisible by 3. All other tubes are semiconducting with band gaps of the order of 0.5 eV

Single-chirality, single-wall carbon nanotubes are desired due to their inherent physical properties and performance characteristics. Here, we demonstrate a chromatographic separation method based on a newly discovered chirality-selective affinity between carbon nanotubes and a gel containing a mixture of the surfactants. In this system, two different selectivities are found: chiral-angle selectivity and diameter selectivity. Since the chirality of nanotubes is determined by the chiral angle and diameter, combining these independent selectivities leads to high-resolution single-chirality separation with milligram-scale throughput and high purity.

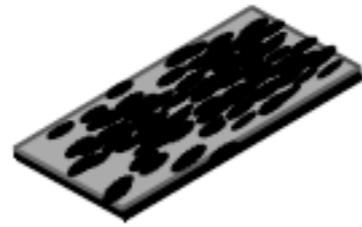
Nature Communications volume 7, Article number: 12056 (2016)



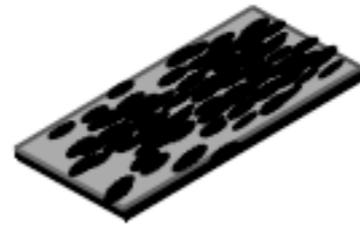
Comparison among different types of CNTs



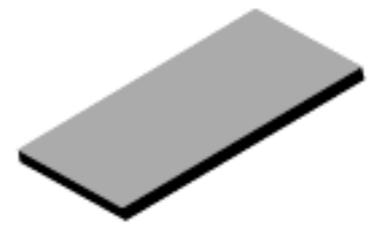
metallic SWCNTs



semiconducting SWCNTs



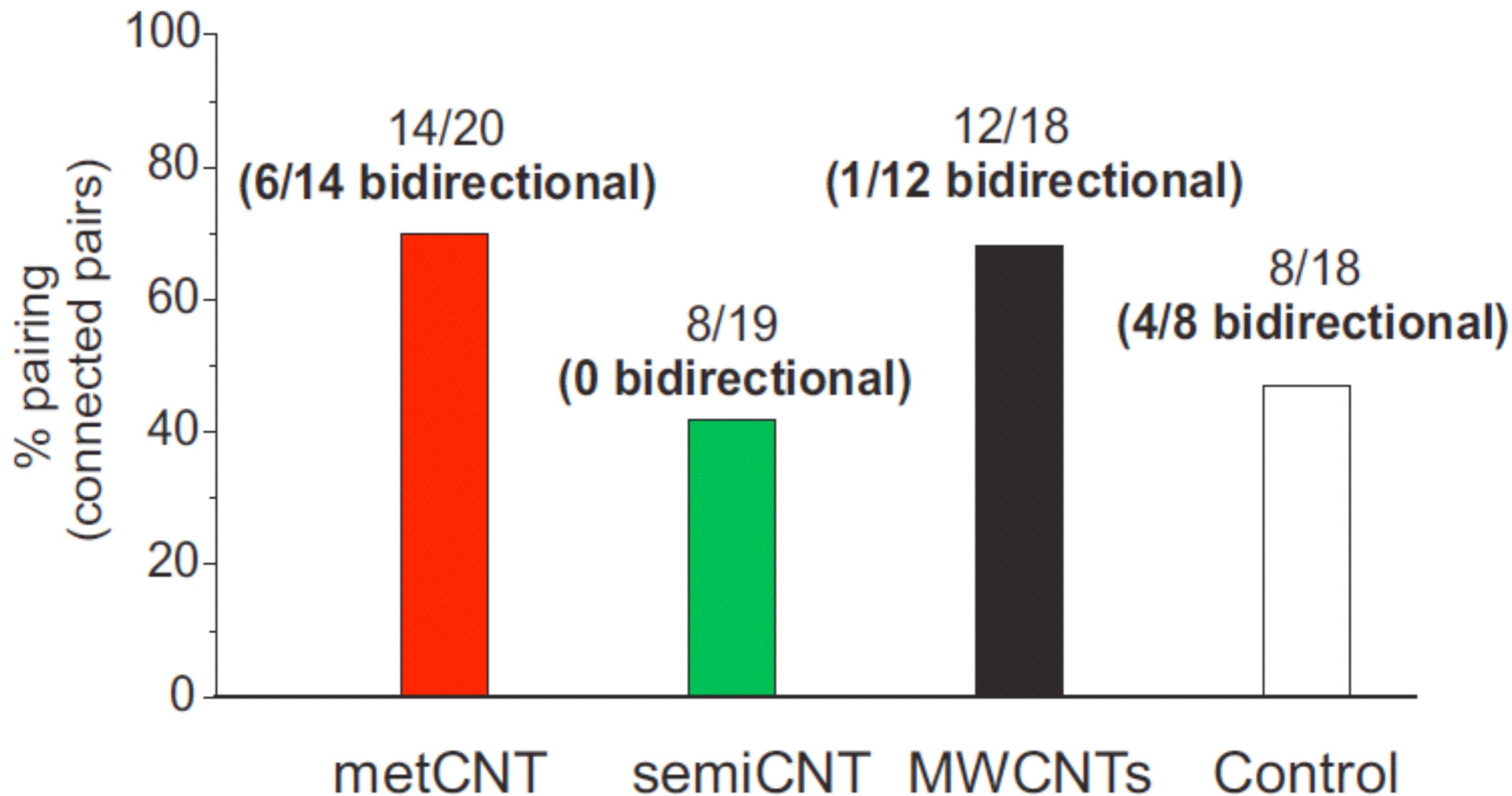
MWCNTs



control
(pure glass)

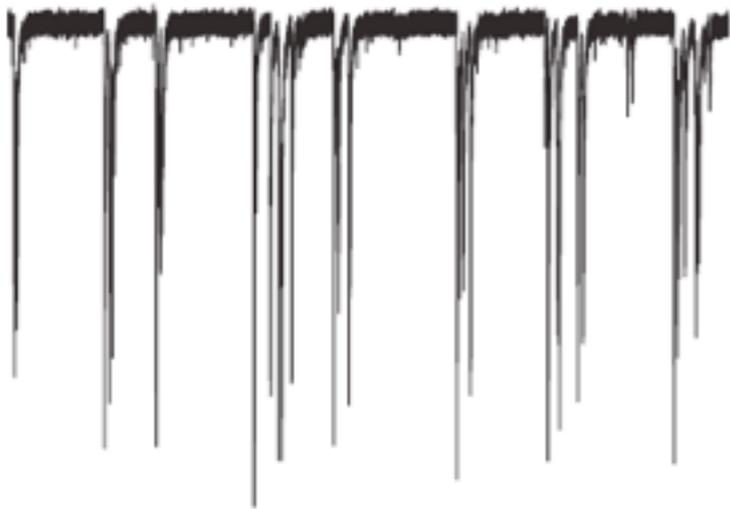
metallic and semiconducting SWCNTs were separated by Francesco Bonaccorso in Andrea Ferrari's laboratory in Cambridge, by density gradient separation

**Connectivity (probability of finding synaptically connected pairs)
for n=2 culture series:**

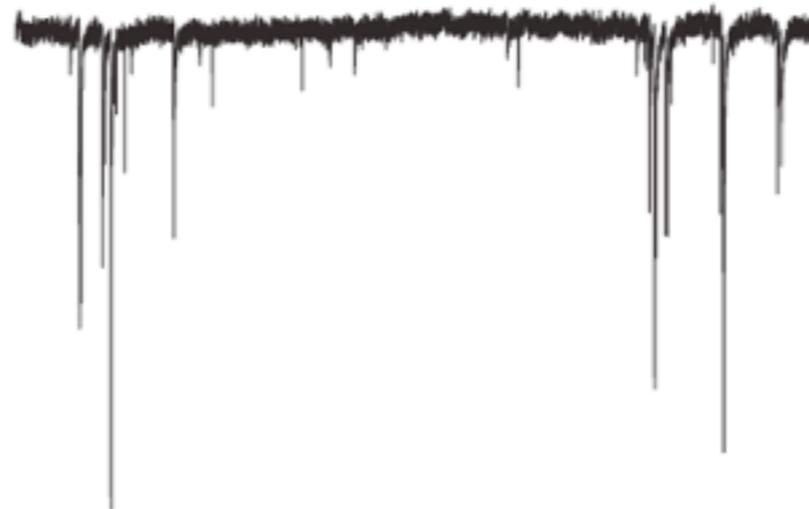


Substrate effect on spontaneous activity: frequency of the spontaneous postsynaptic currents (PSCs)

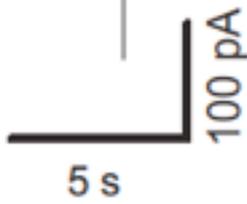
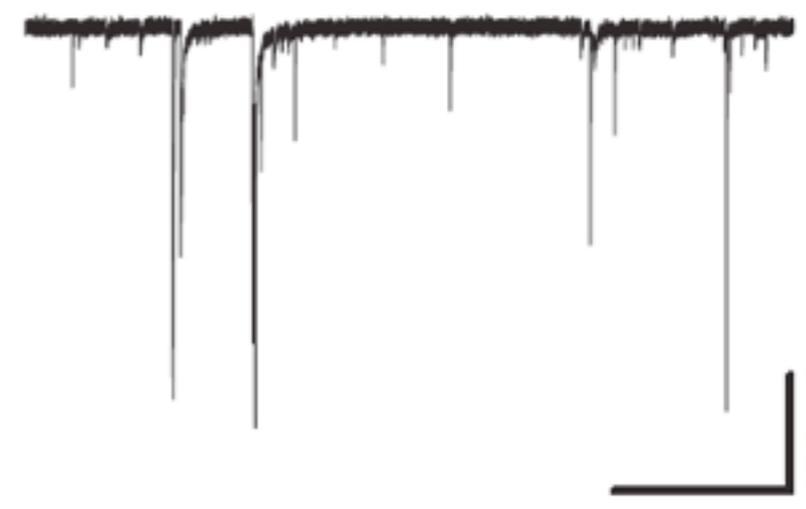
metCNT



semiCNT



Control



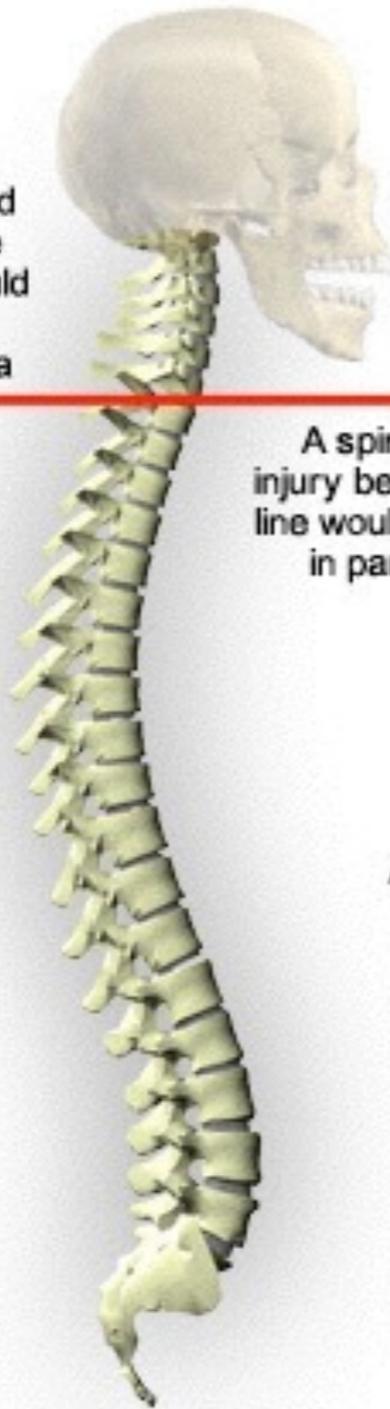


A spinal cord injury above this line would result in quadraplegia

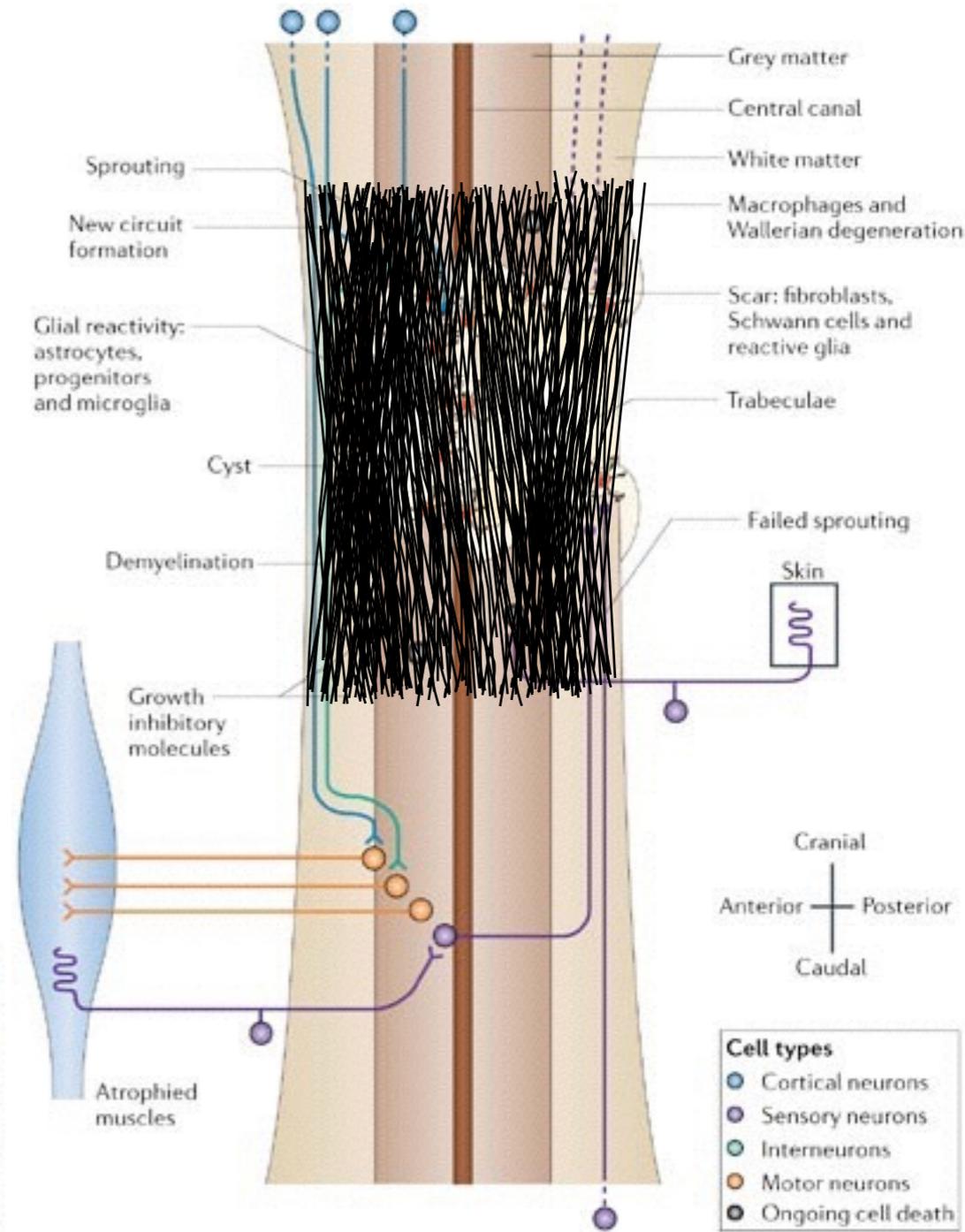
A spinal cord injury below this line would result in paraplegia

Posterior (Rear)

Anterior (Front)

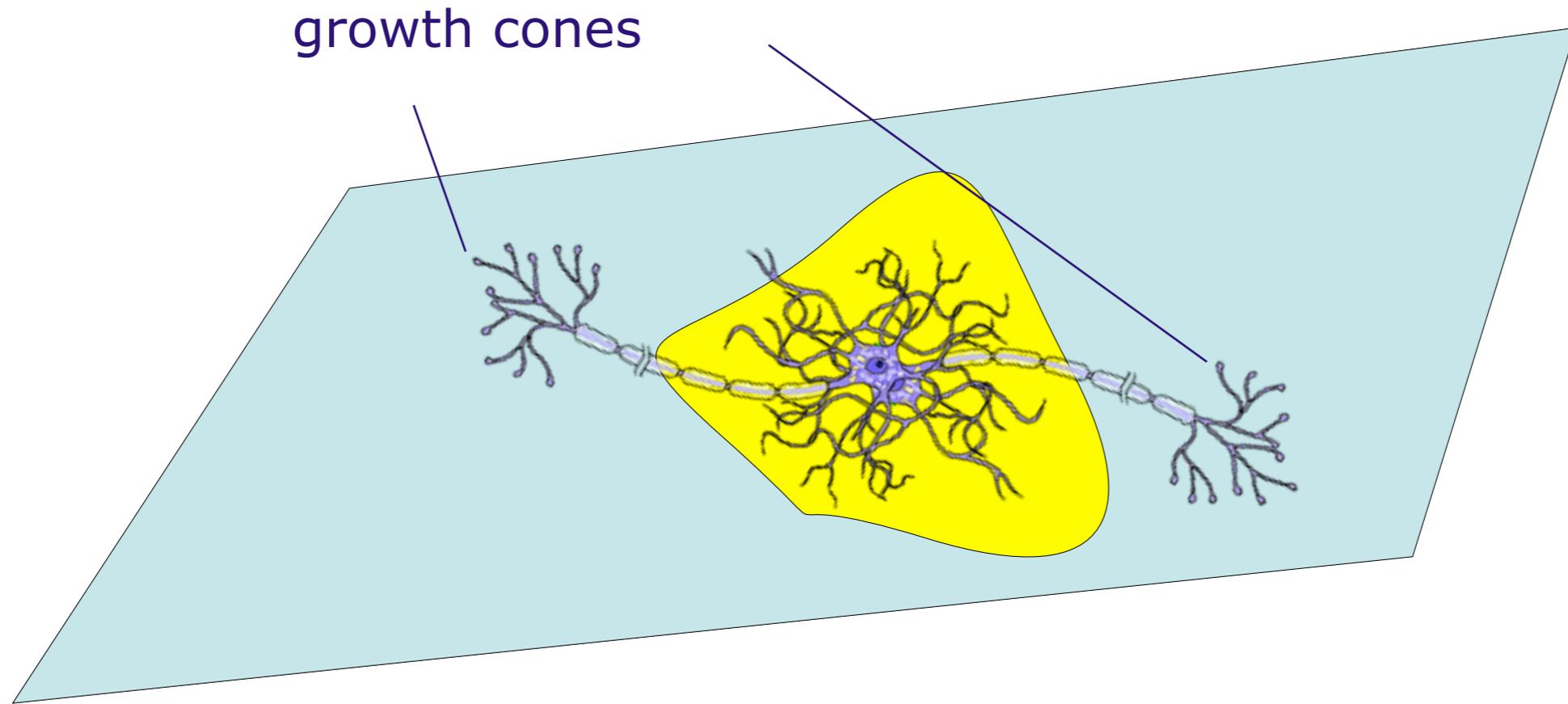


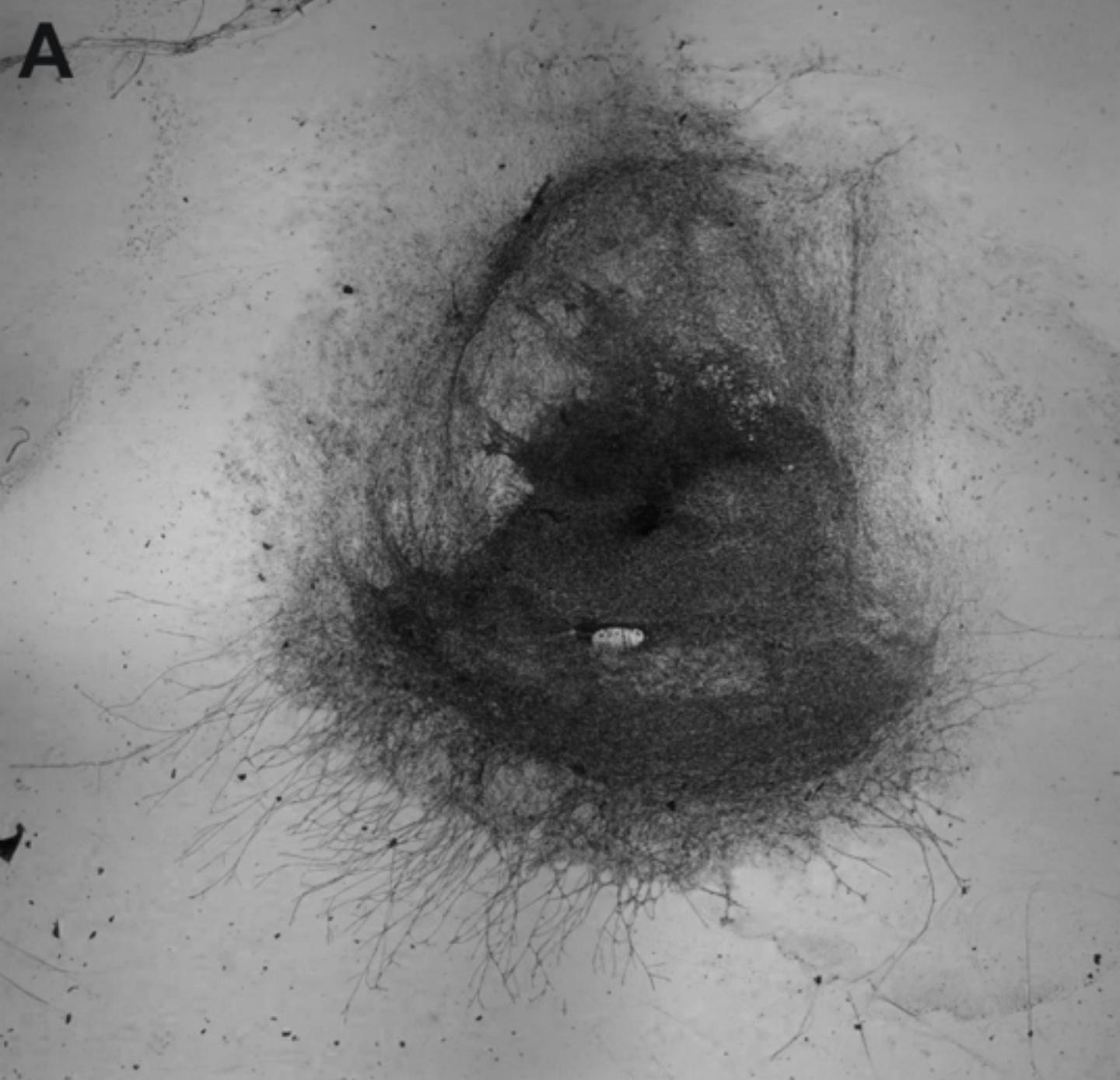
www.epiparealyzed.com



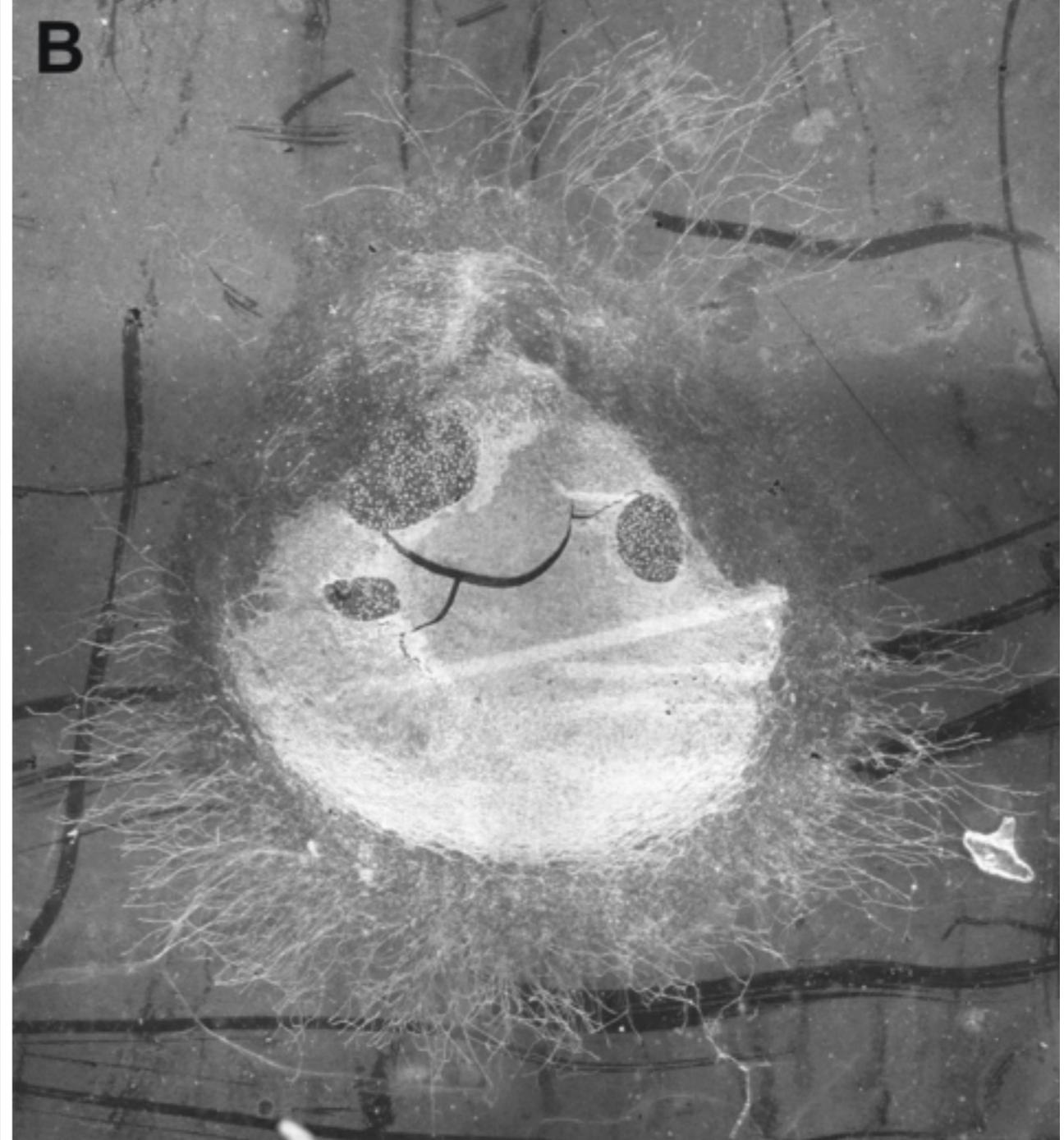
An entire slice of spinal cord tissue

slices of ca 150 μm





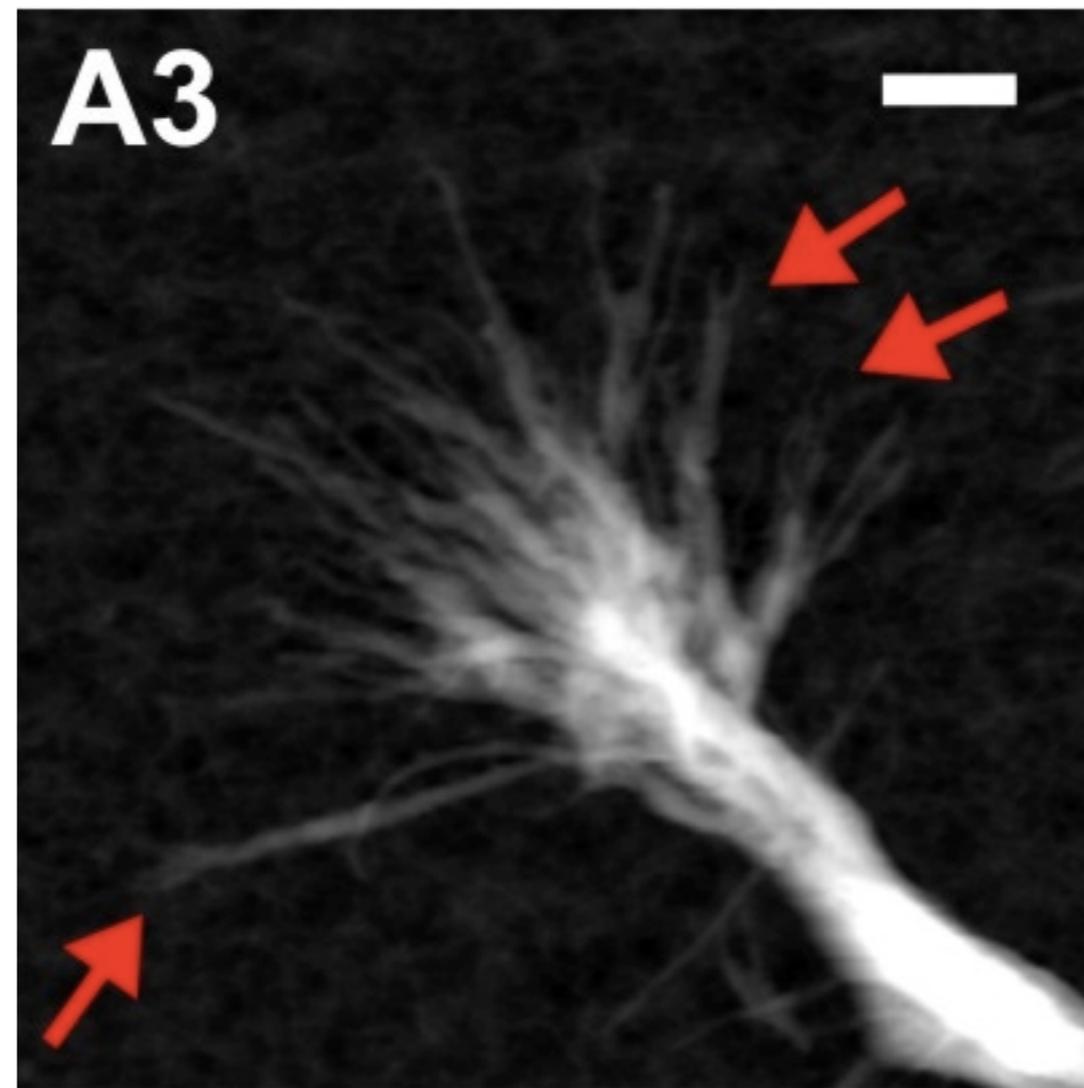
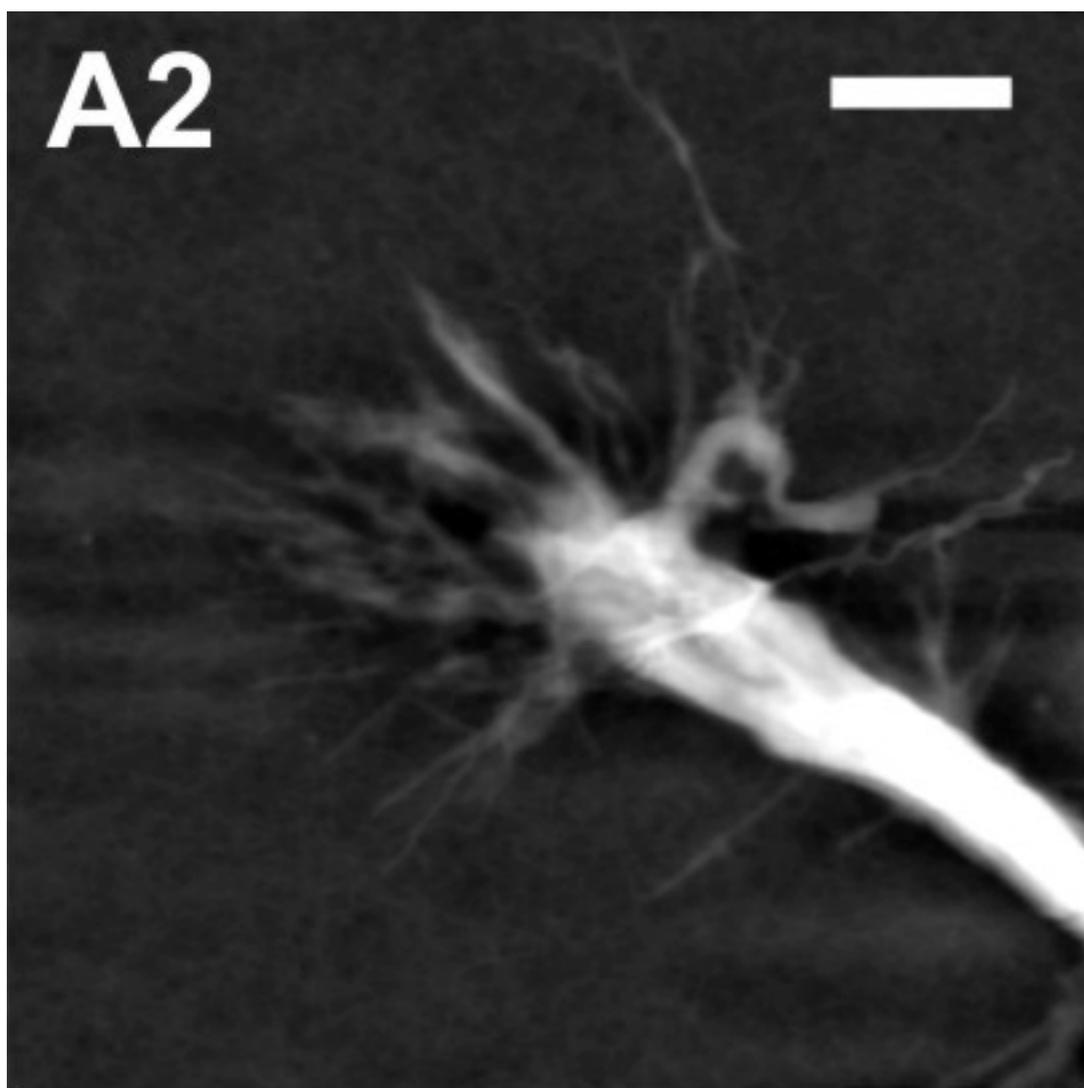
control



CNT (phase contrast)

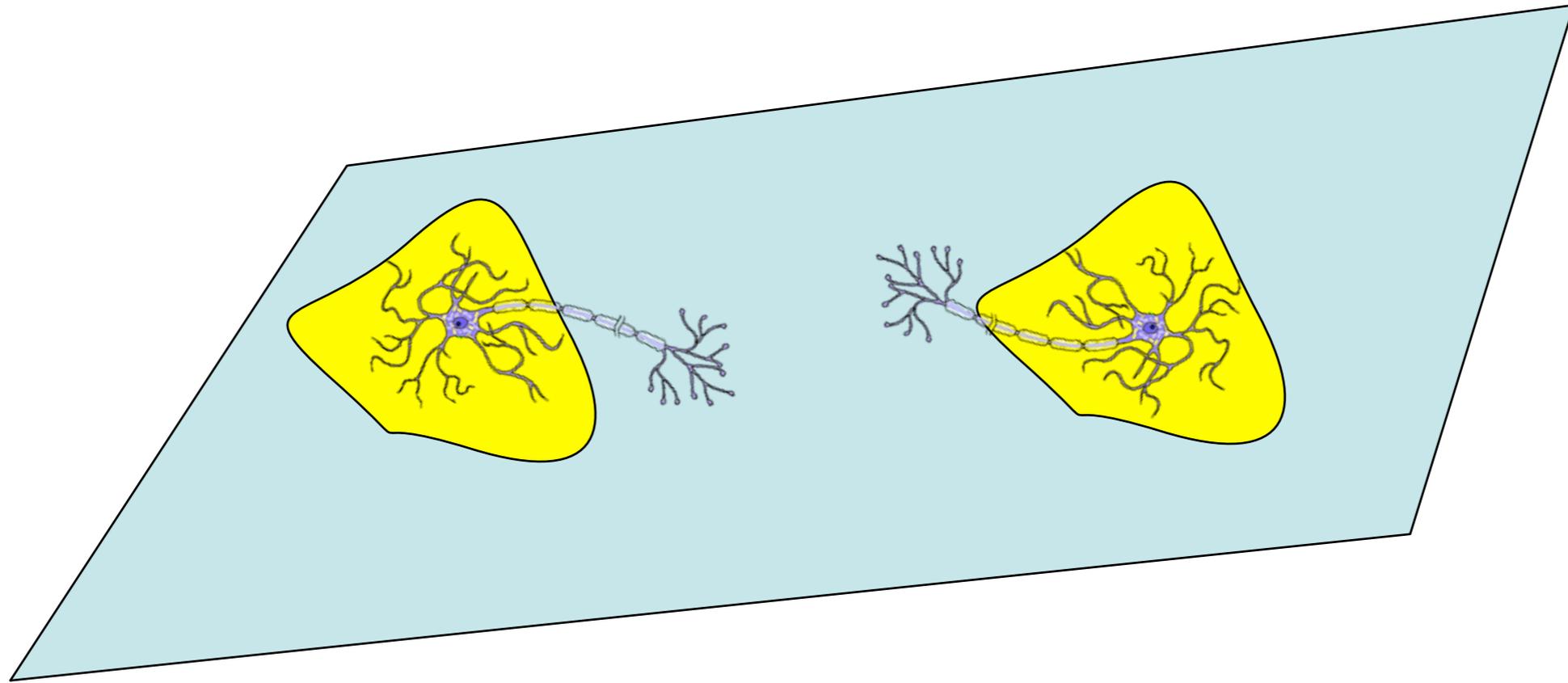
Spinal explants (slices of 150 μm ca)

On CNT, the number of fibers grow 40% more (15 samples), length increases by 25% ca

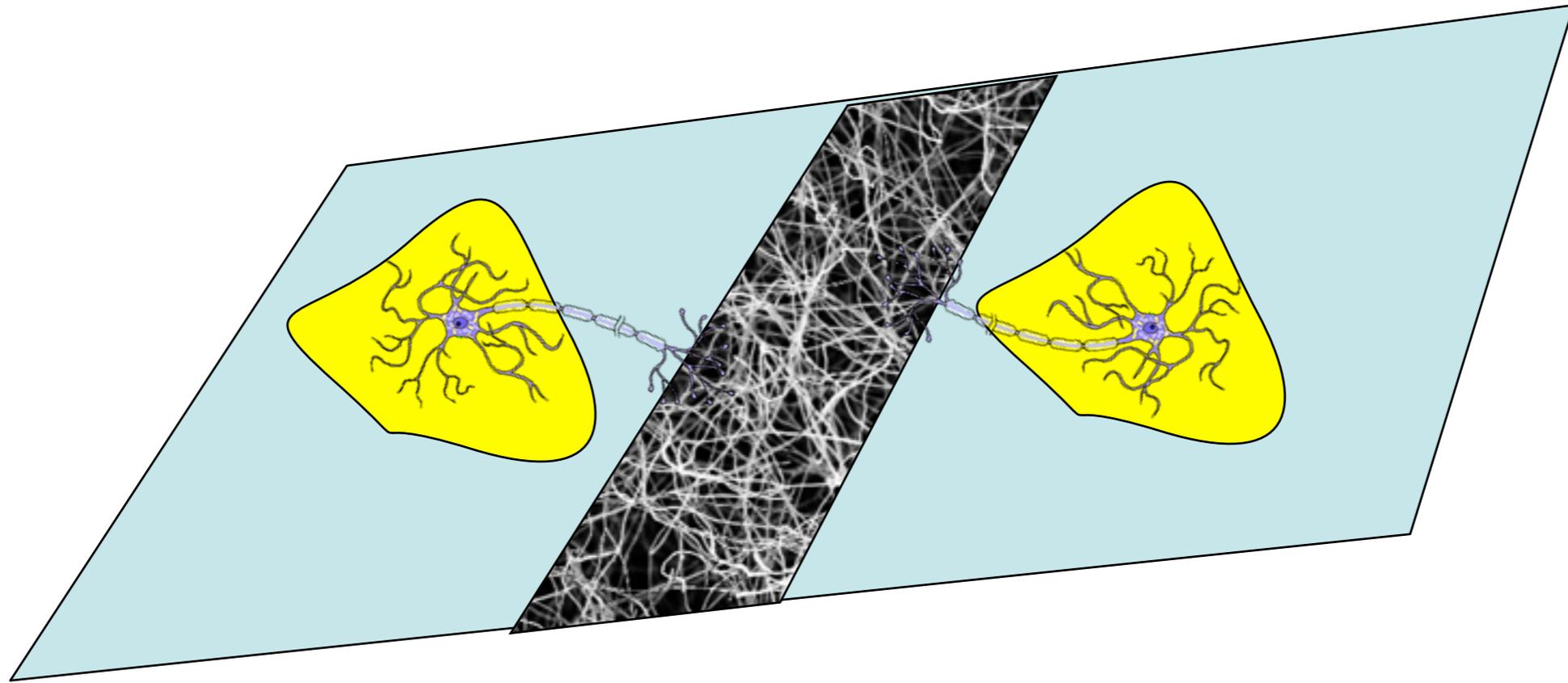


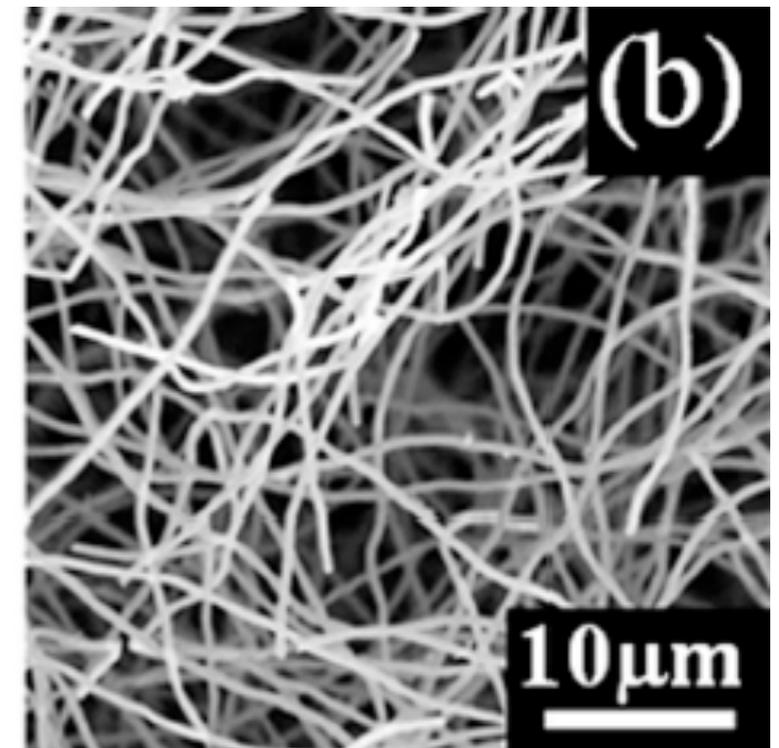
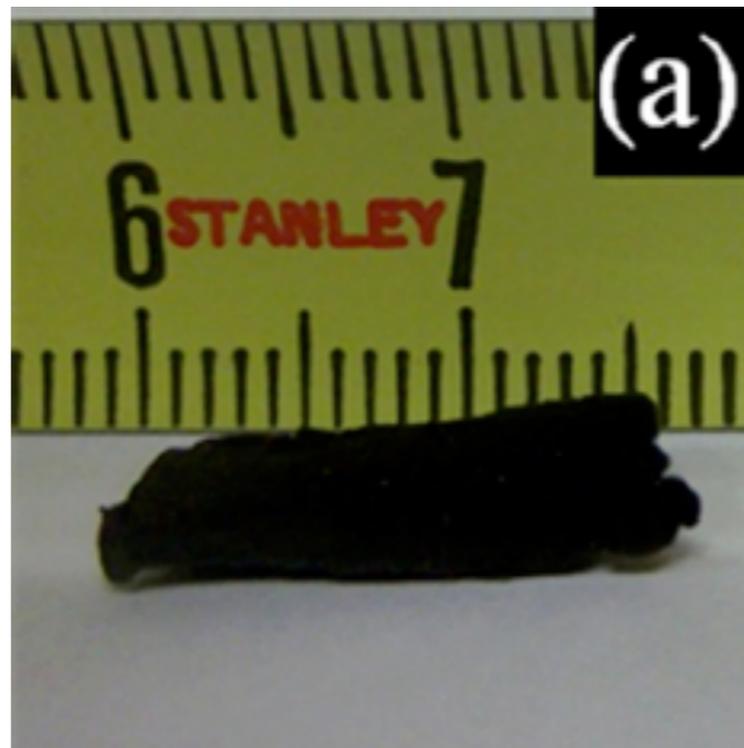
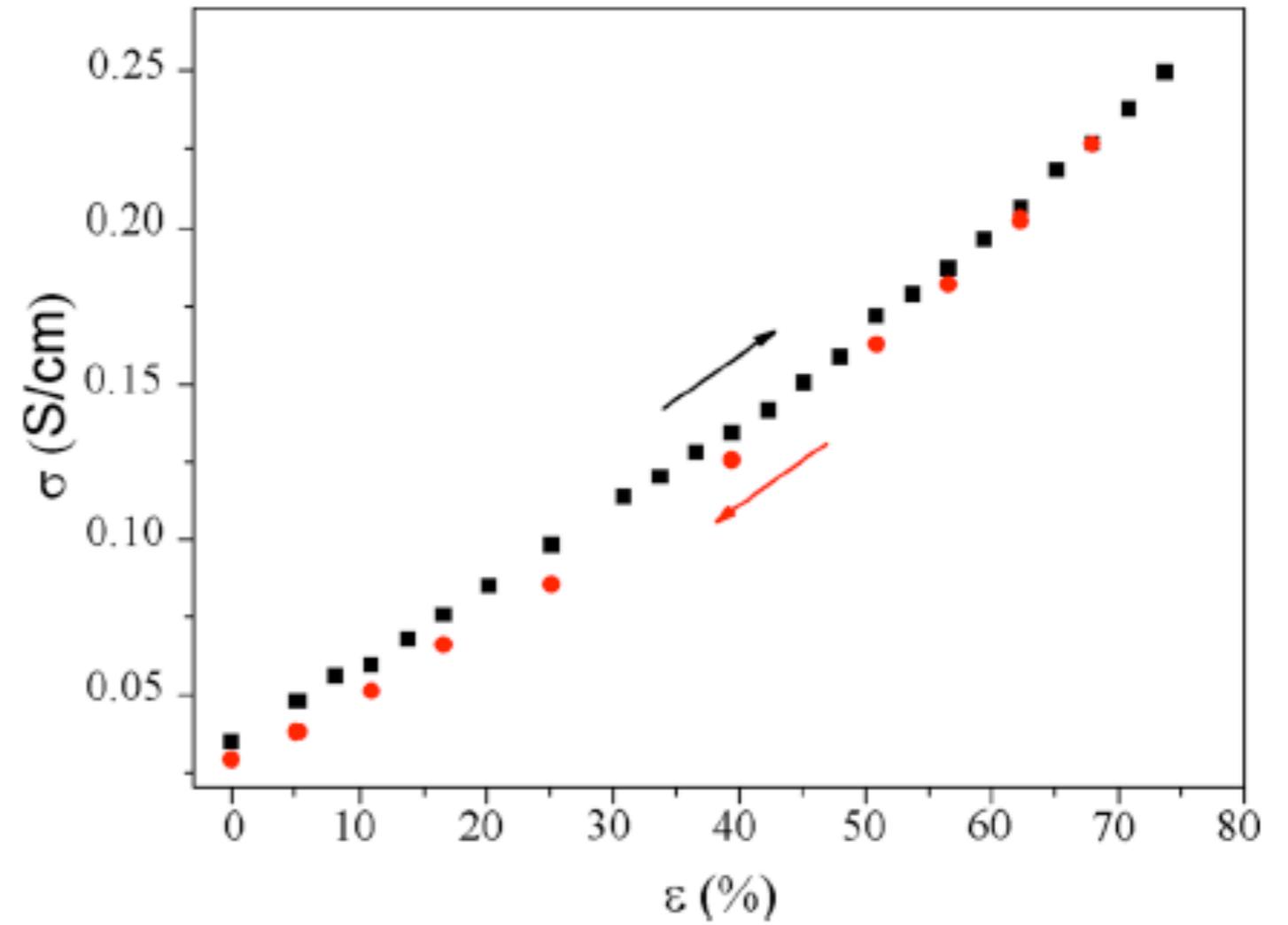
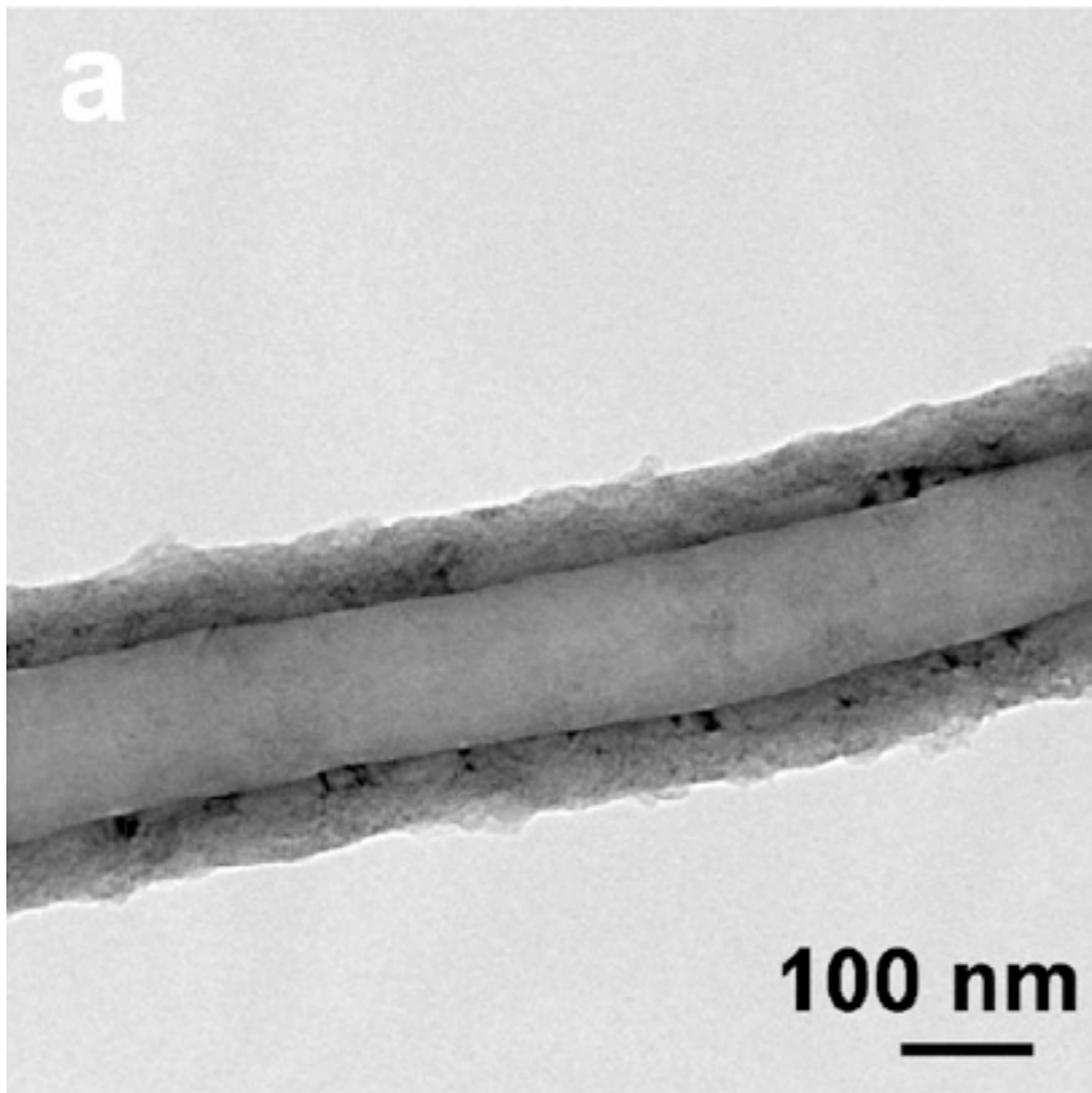
Growth cones grow more distant on CNT

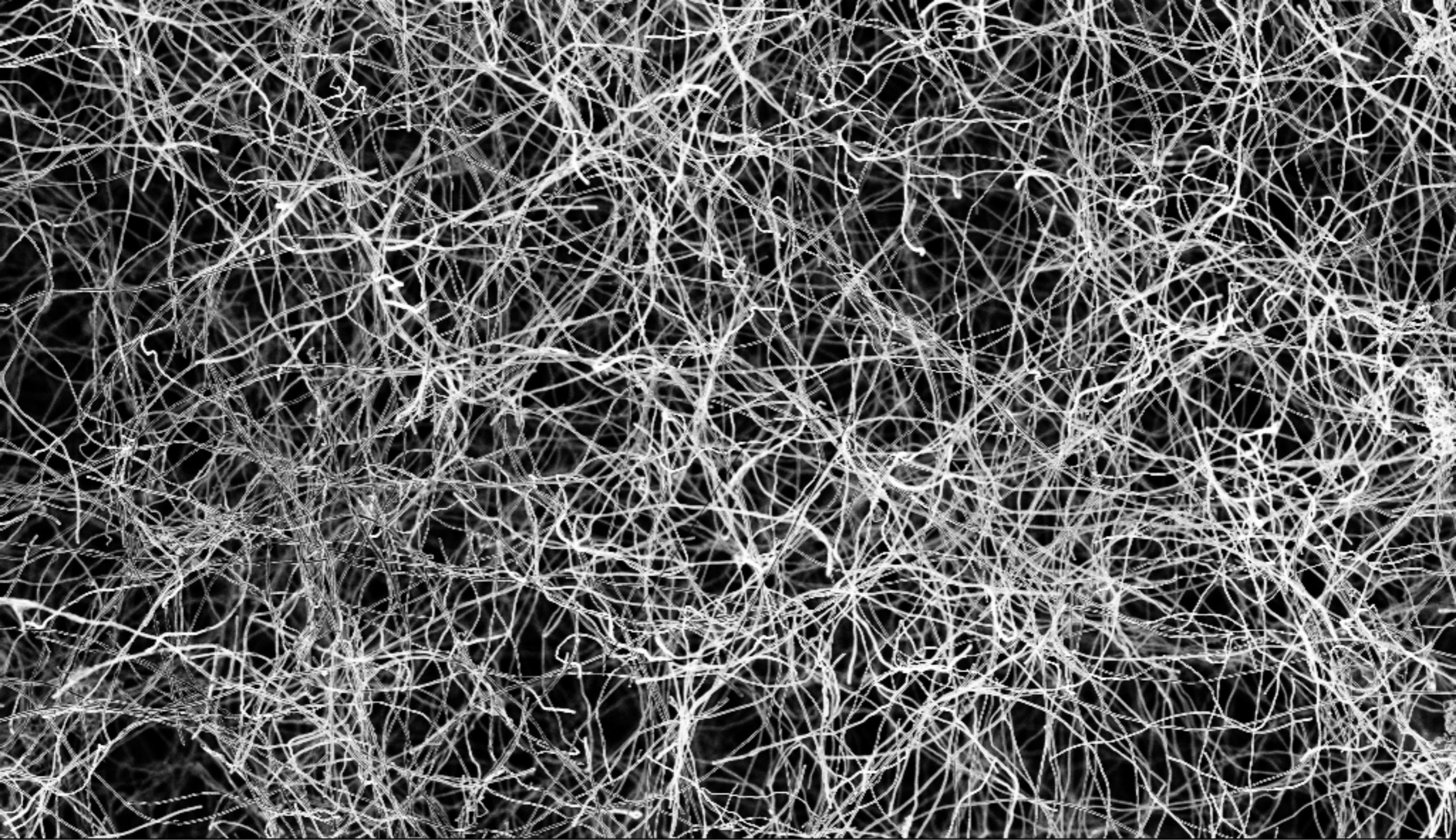
Looking for communication between two separated slices



Looking for communication between two separated slices

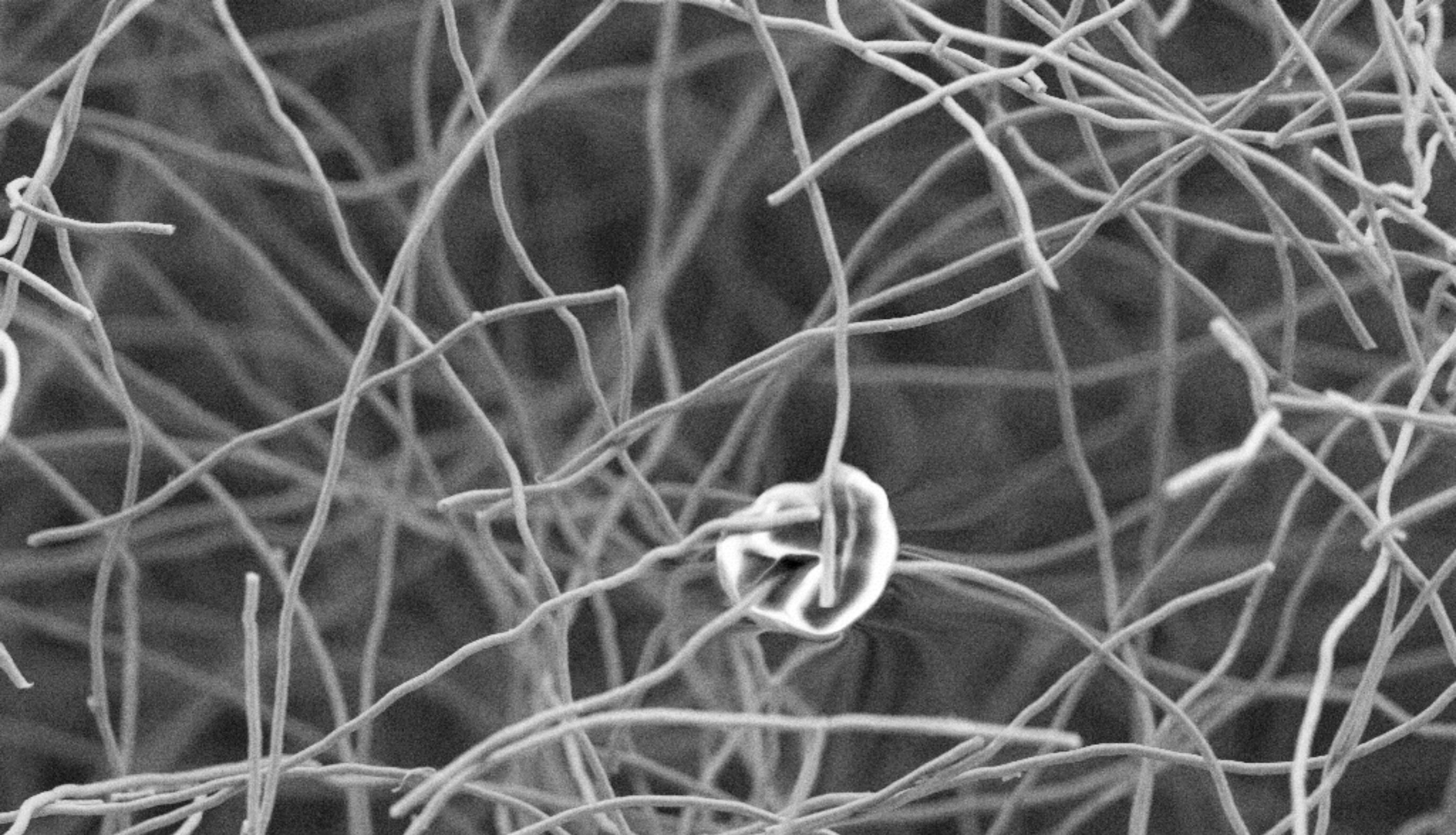






20 μm
Date :15 May 2013 EHT = 5.00 kV WD = 4.8 mm Brightness = 45.1 % Stage at T = 0.0 ° Stage at Z = 49.100 mm
Time :12:36:28 Mag = 1.00 K X Signal A = InLens Contrast = 36.1 % Tilt Angle = 45.0 ° Aperture Size = 30.00 μm
Tilt Corr. = Off





10 μm
Date :15 May 2013 EHT = 5.00 kV WD = 4.8 mm Brightness = 48.1 % Stage at T = 0.0 ° Stage at Z = 49.100 mm
Time :12:39:33 Mag = 5.00 K X Signal A = SE2 Contrast = 43.3 % Tilt Angle = 45.0 ° Aperture Size = 30.00 μm
Tilt Corr. = Off





10 μ m
Date :15 May 2013 EHT = 3.00 kV WD = 4.9 mm Brightness = 48.1 % Stage at T = 0.0 ° Stage at Z = 49.100 mm
Time :12:59:44 Mag = 5.00 K X Signal A = SE2 Contrast = 43.3 % Tilt Angle = 45.0 ° Aperture Size = 30.00 μ m
Tilt Corr. = Off





2 μ m

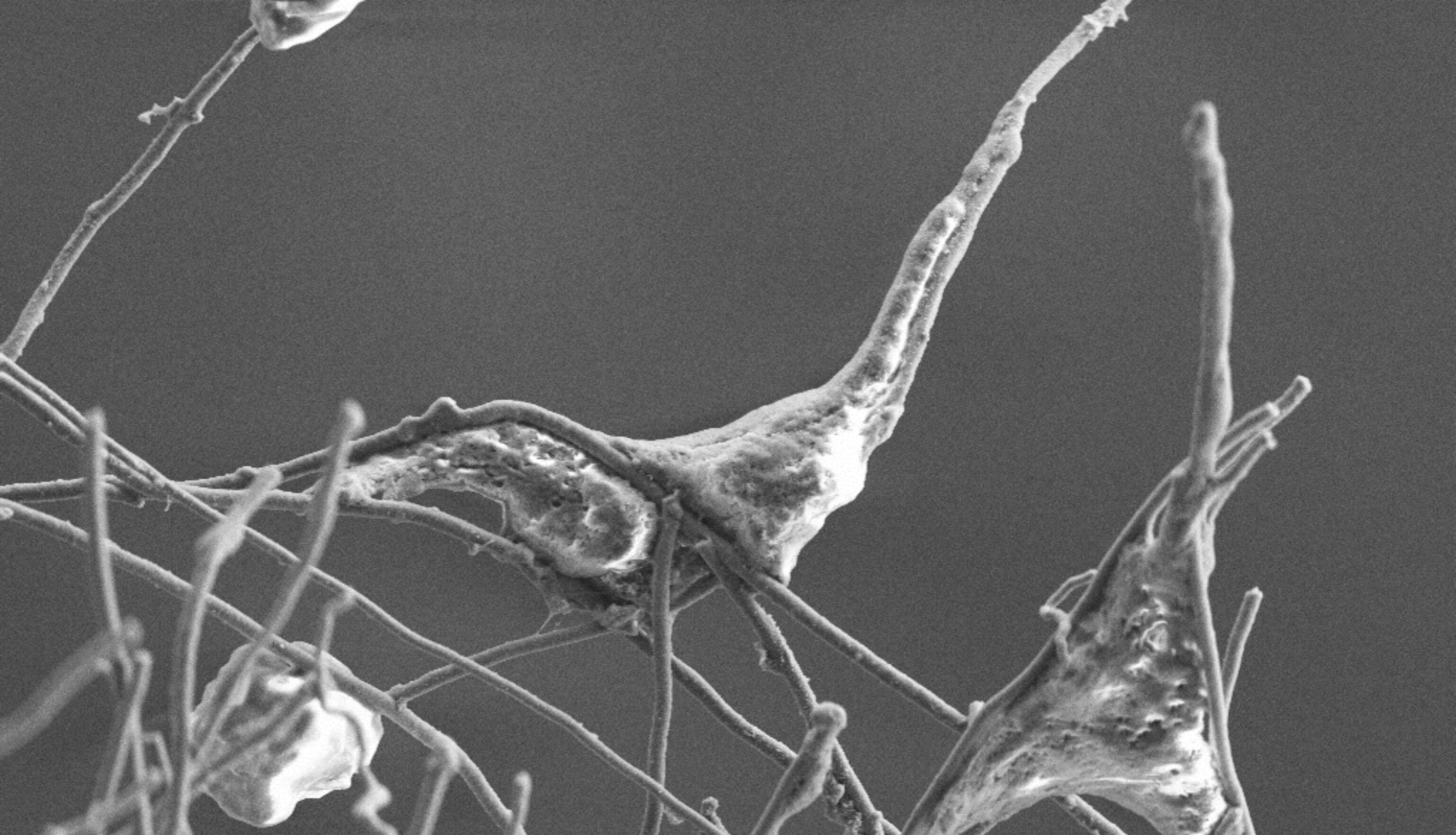

Date :15 May 2013	EHT = 3.00 kV	WD = 4.9 mm	Brightness = 48.1 %	Stage at T = 0.0 °	Stage at Z = 49.100 mm
Time :12:59:10	Mag = 10.00 KX	Signal A = SE2	Contrast = 43.3 %	Tilt Angle = 45.0 °	Aperture Size = 30.00 μ m
				Tilt Corr. = Off	





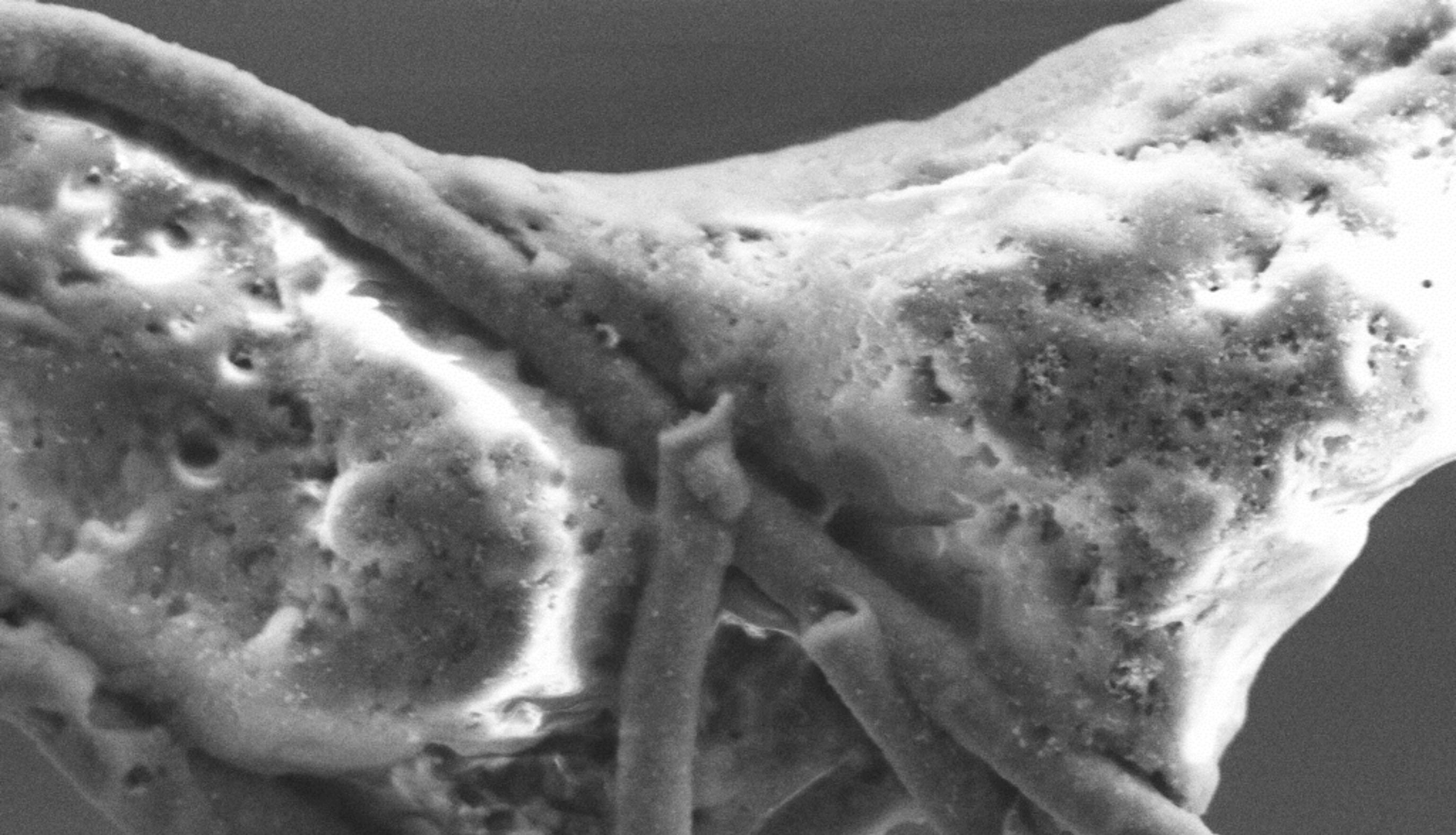
10 μm
Date :15 May 2013 EHT = 3.00 kV WD = 5.9 mm Brightness = 50.1 % Stage at T = 0.0 ° Stage at Z = 49.100 mm
Time :13:24:01 Mag = 5.00 K X Signal A = SE2 Contrast = 36.9 % Tilt Angle = 45.0 ° Aperture Size = 30.00 μm
Tilt Corr. = Off





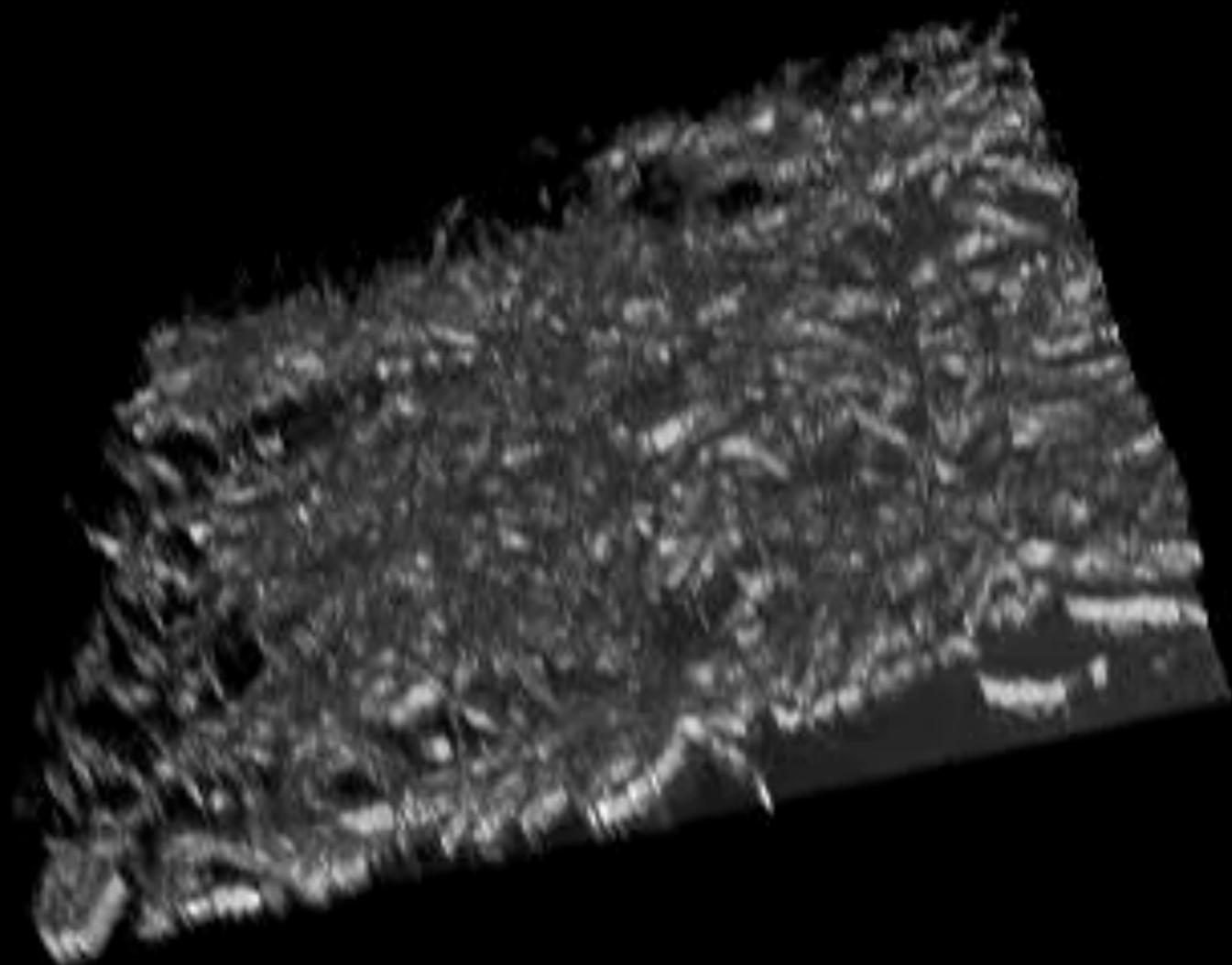
2 μ m
Date :15 May 2013 EHT = 3.00 kV WD = 5.9 mm Brightness = 50.1 % Stage at T = 0.0 ° Stage at Z = 49.100 mm
Time :13:24:39 Mag = 8.00 K X Signal A = SE2 Contrast = 36.9 % Tilt Angle = 45.0 ° Aperture Size = 30.00 μ m
Tilt Corr. = Off

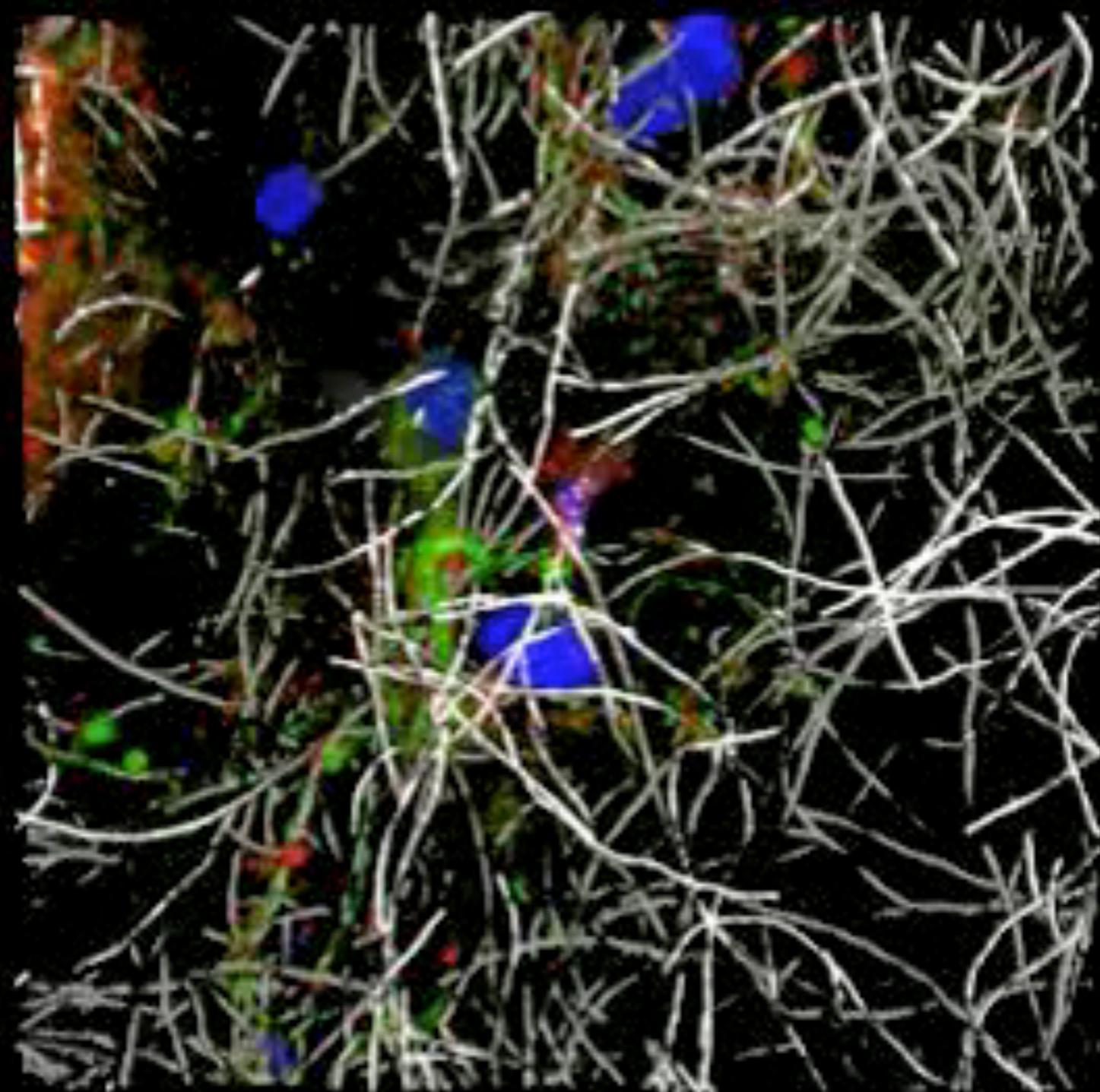


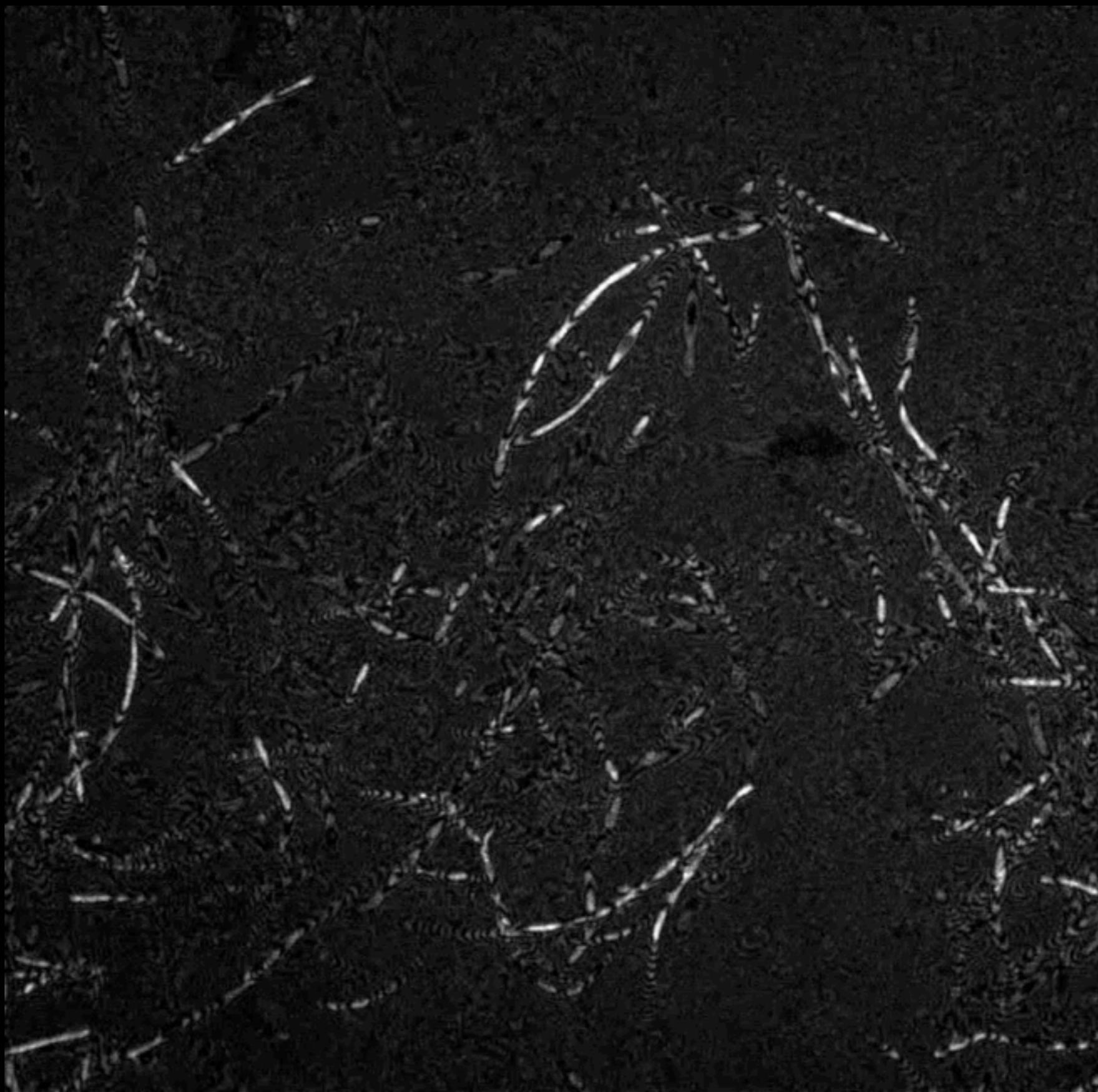


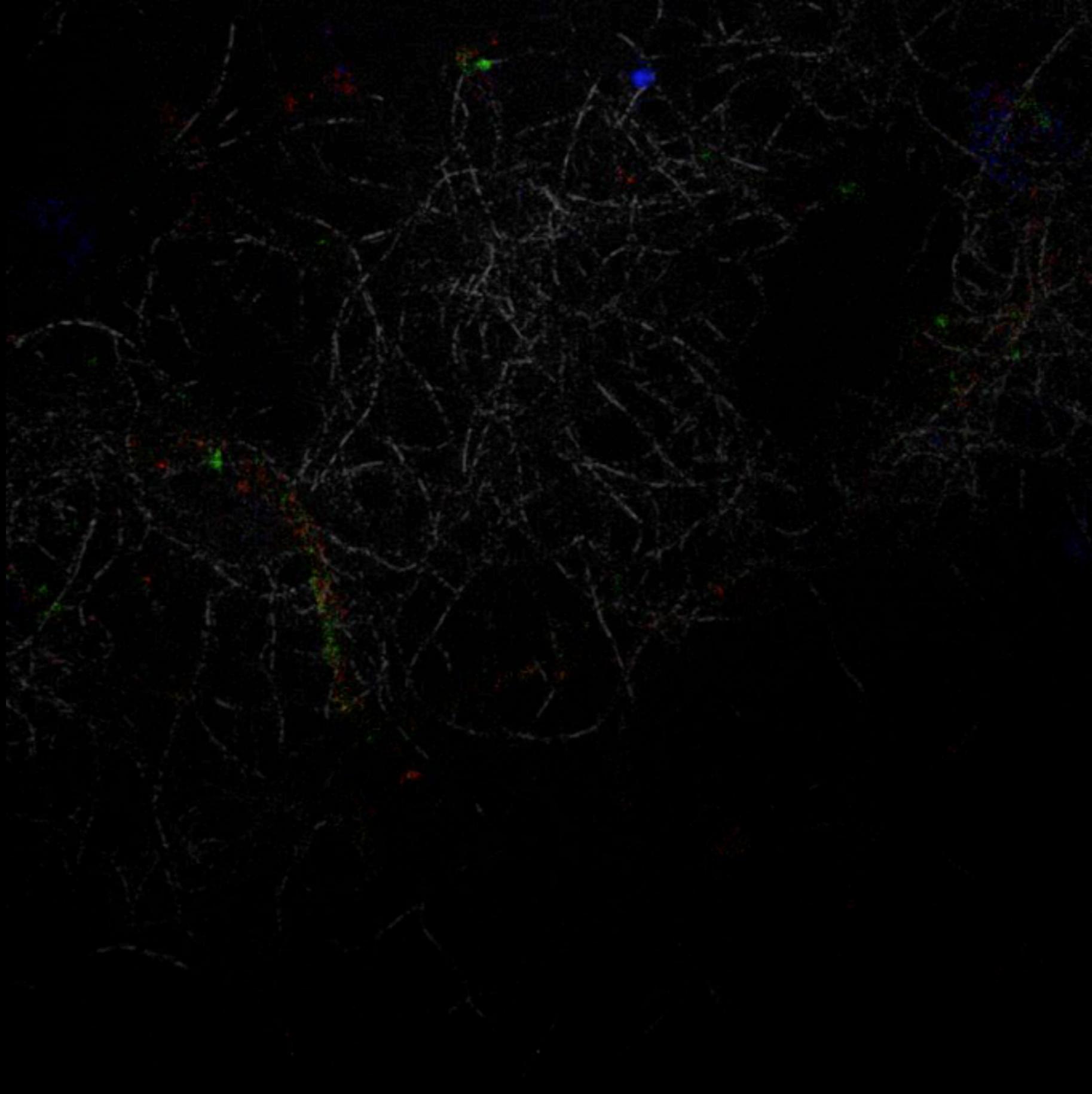
2 μ m | Date : 15 May 2013 EHT = 3.00 kV WD = 5.9 mm Brightness = 50.1 % Stage at T = 0.0 ° Stage at Z = 49.100 mm
Time : 13:26:25 Mag = 35.00 KX Signal A = SE2 Contrast = 36.9 % Tilt Angle = 45.0 ° Aperture Size = 30.00 μ m
Tilt Corr. = Off











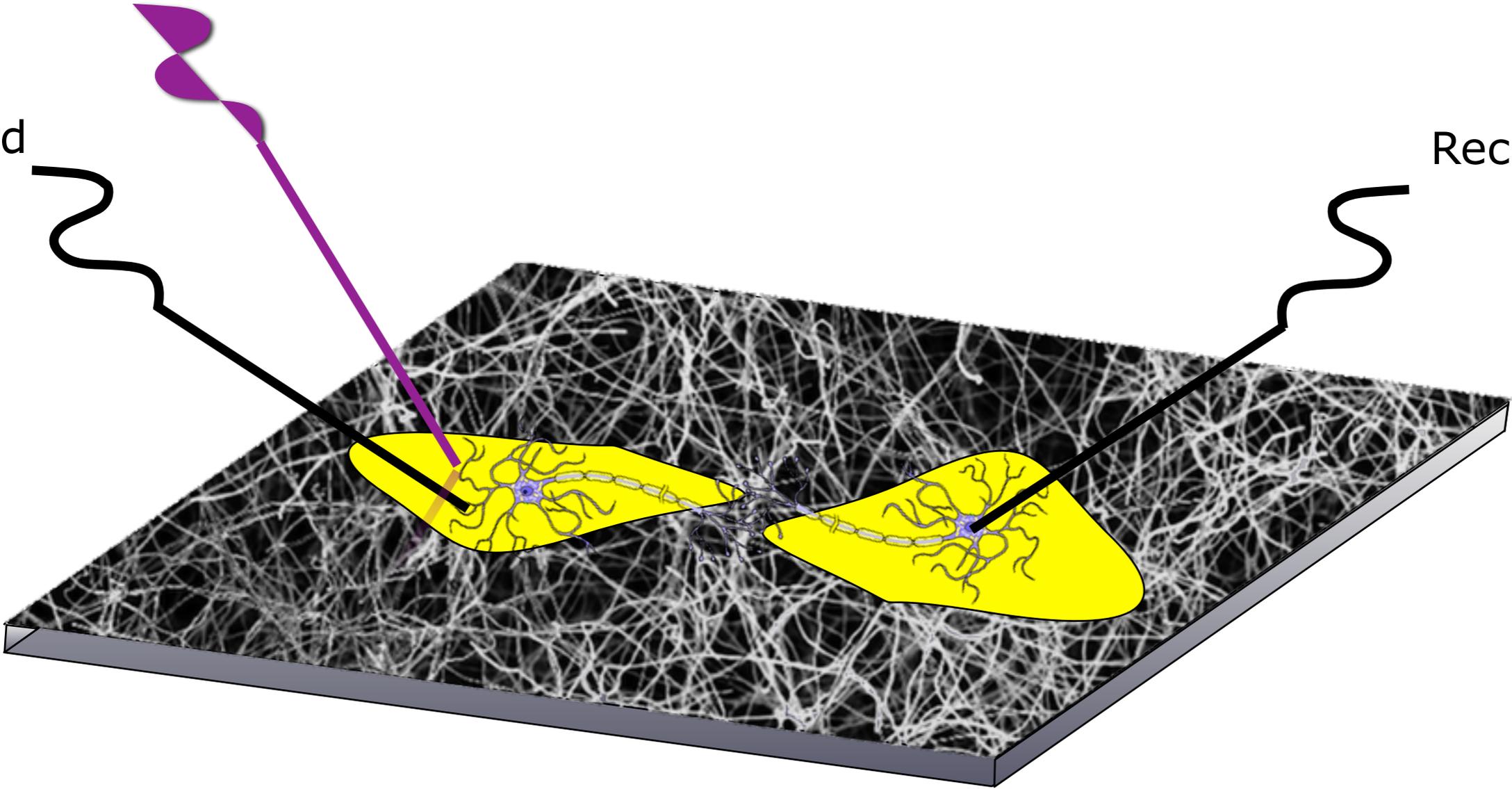
Color codes: blue (all nuclei, both neurons and glia); green (axons and dendrites); red (synapses)

Looking for communication between two separated slices

Stimulate

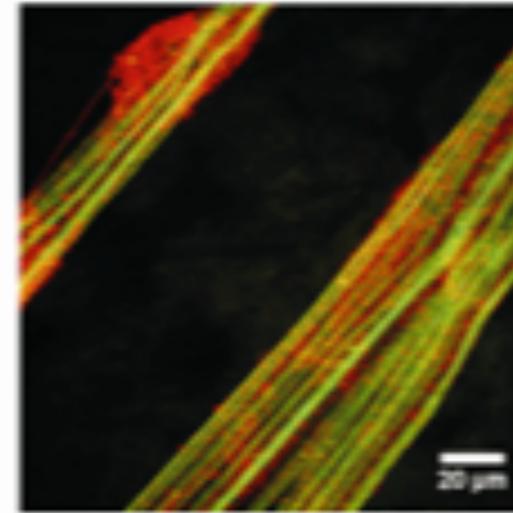
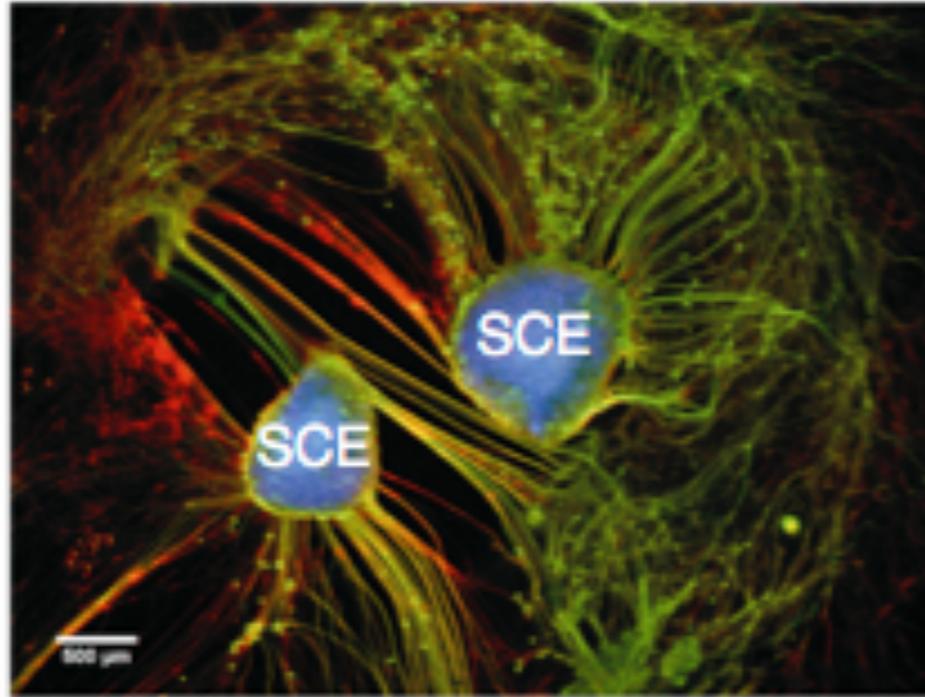
Record

Record

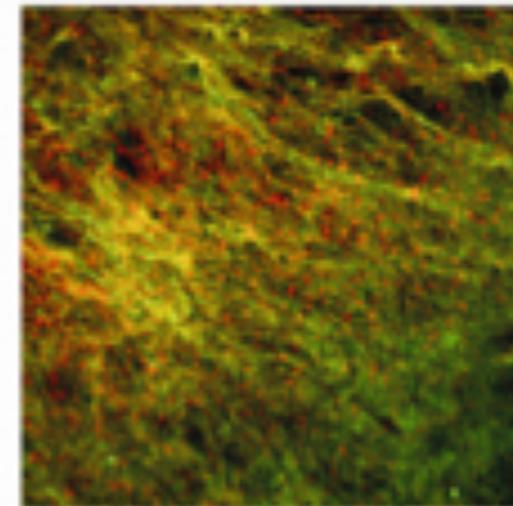
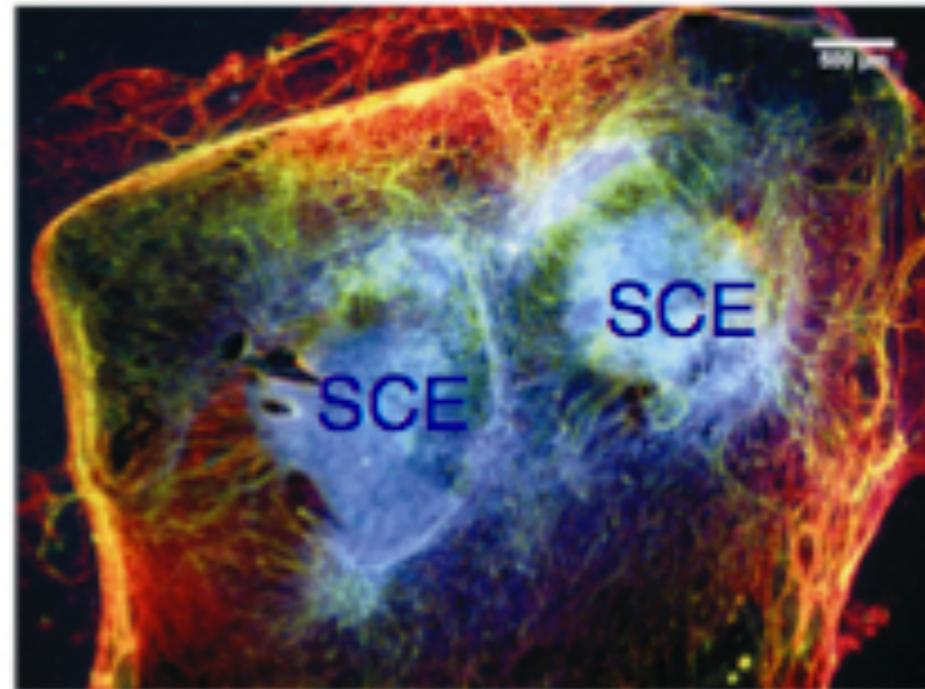


control

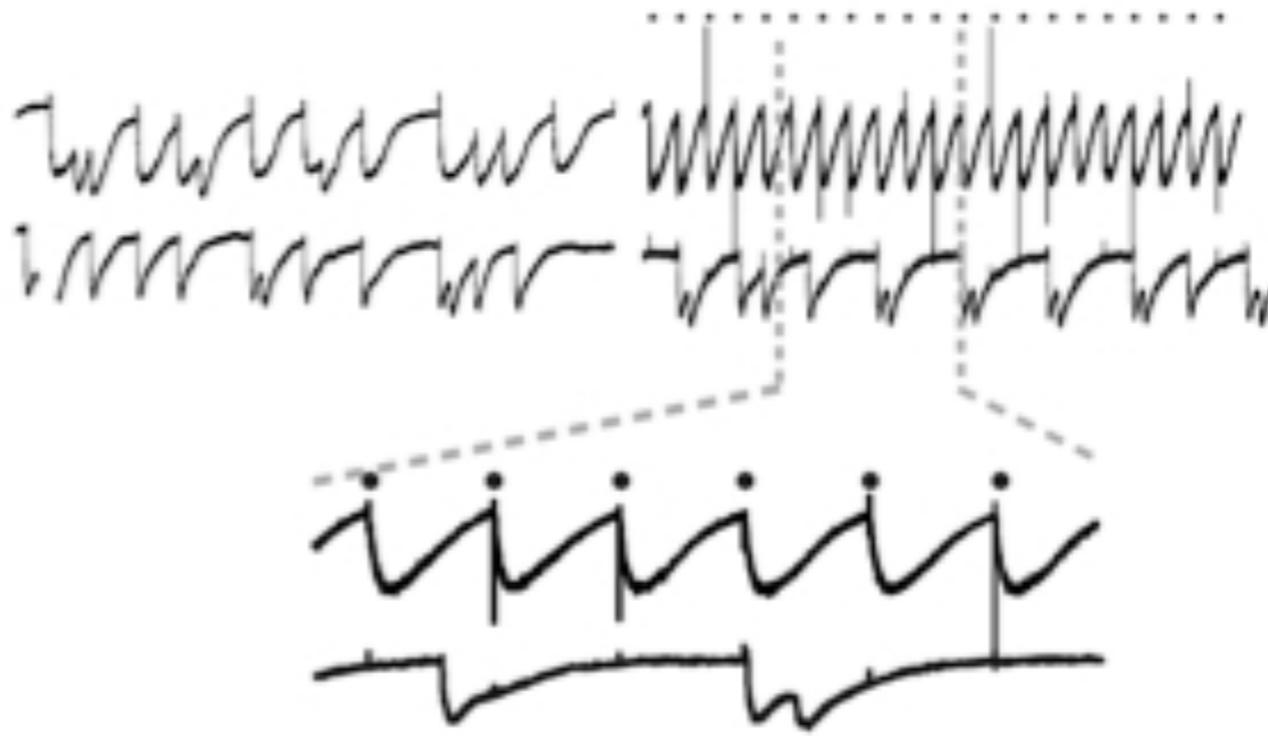
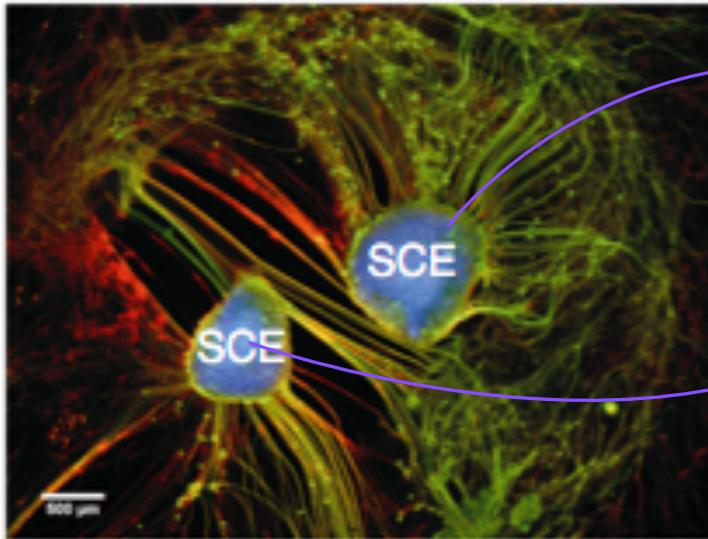
A



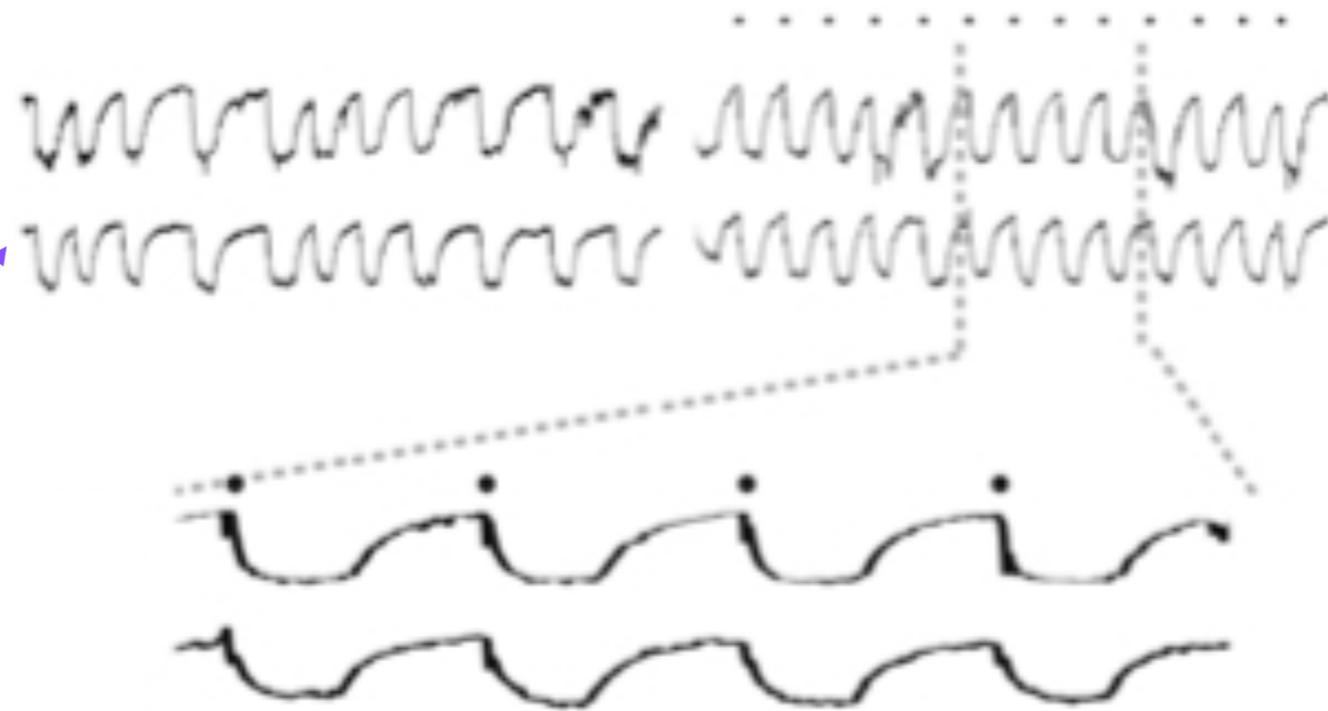
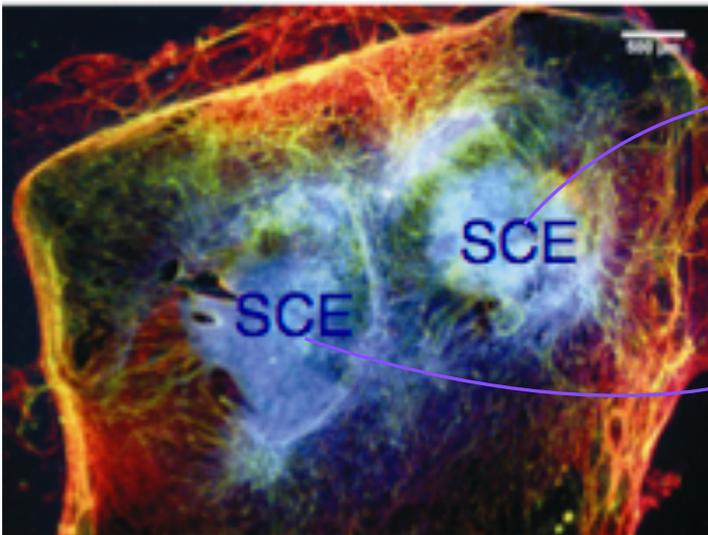
3D CNT



control

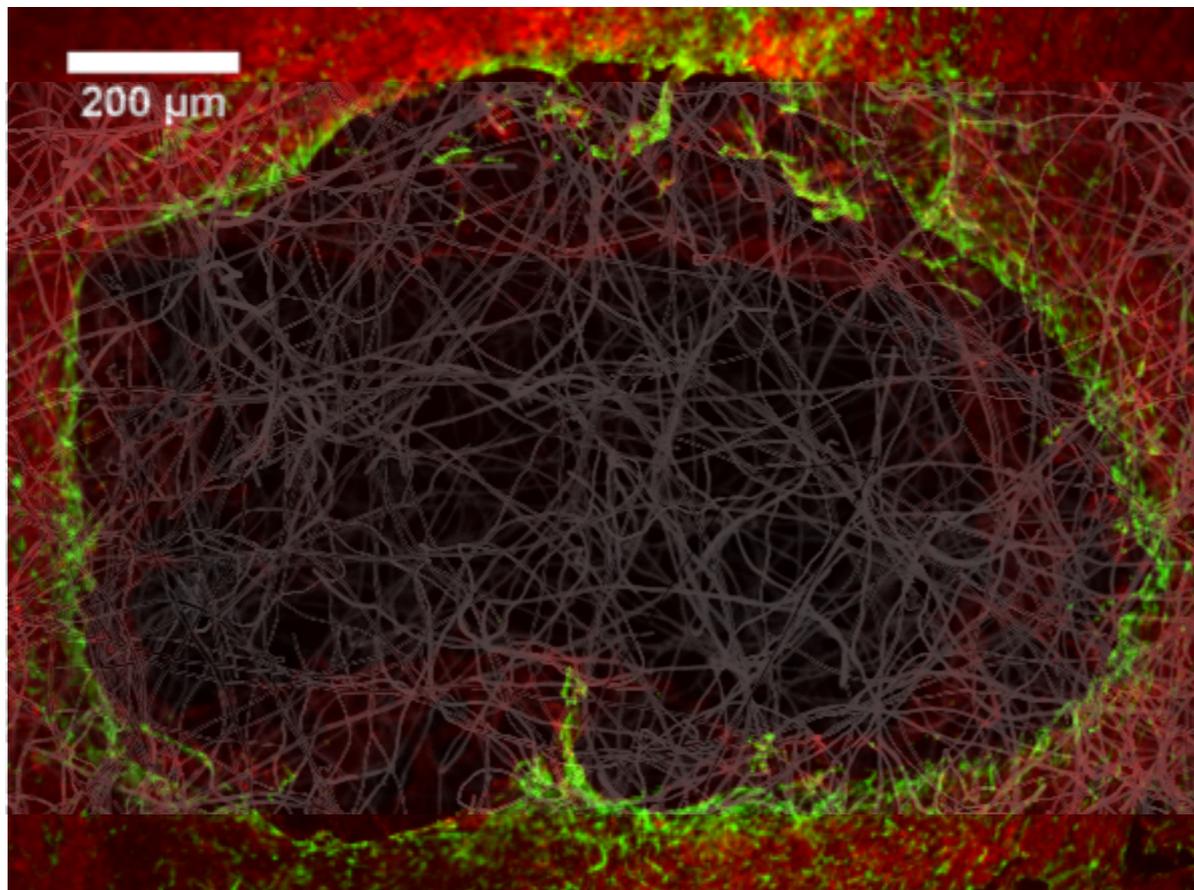
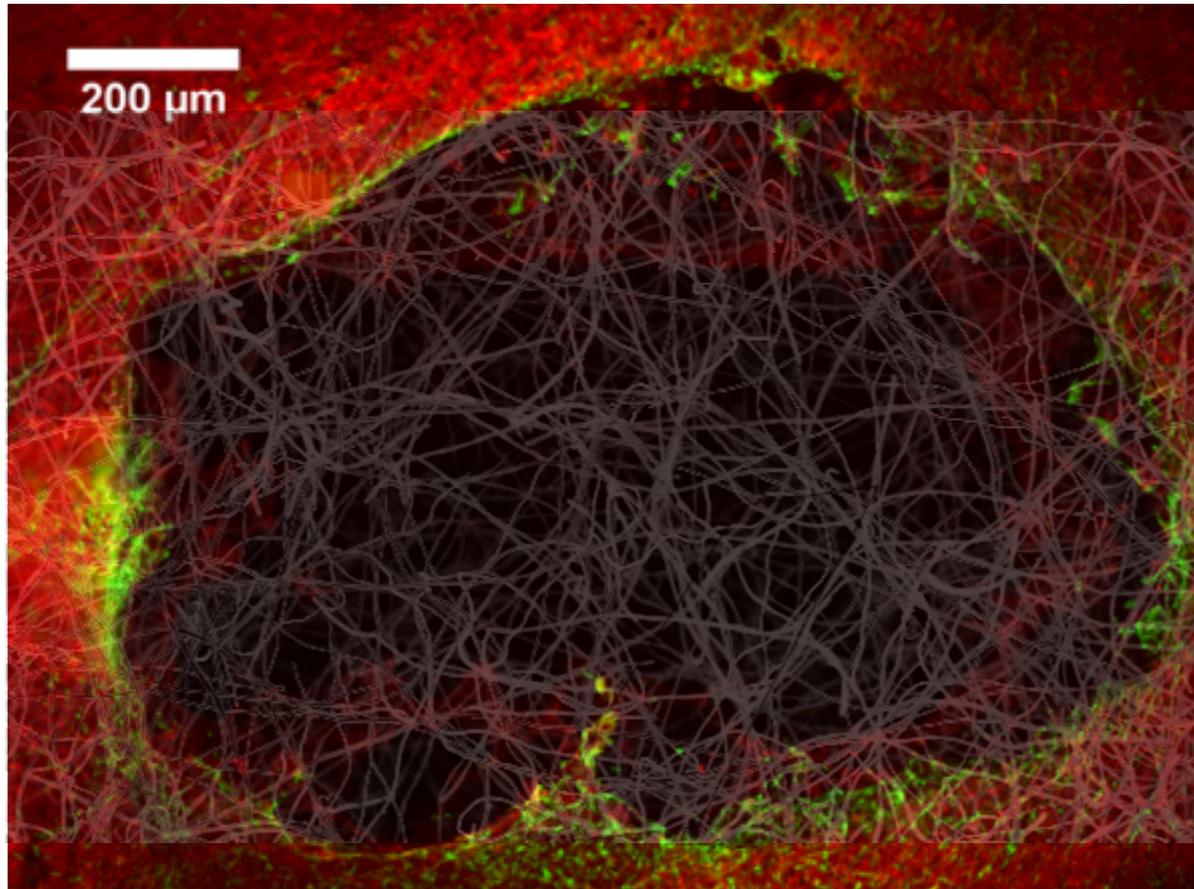


3D CNT

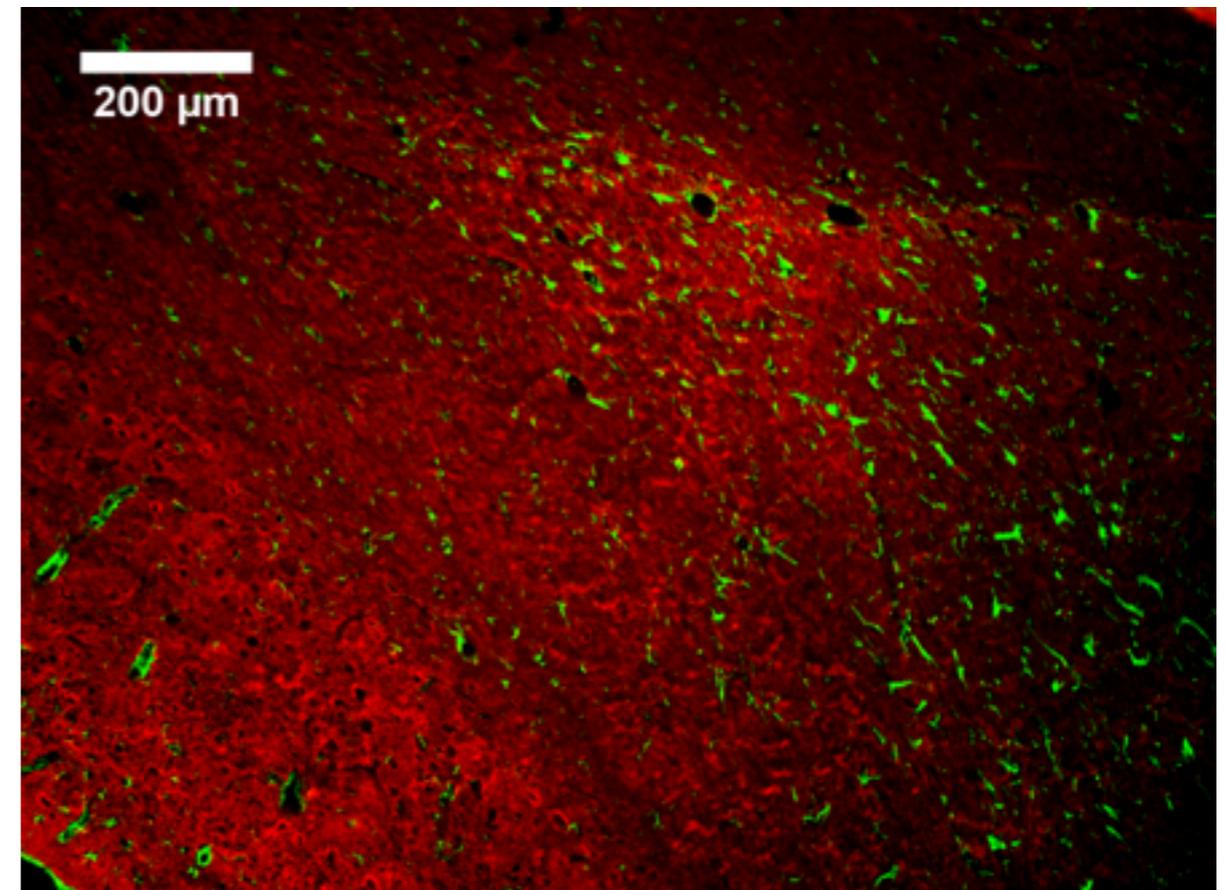
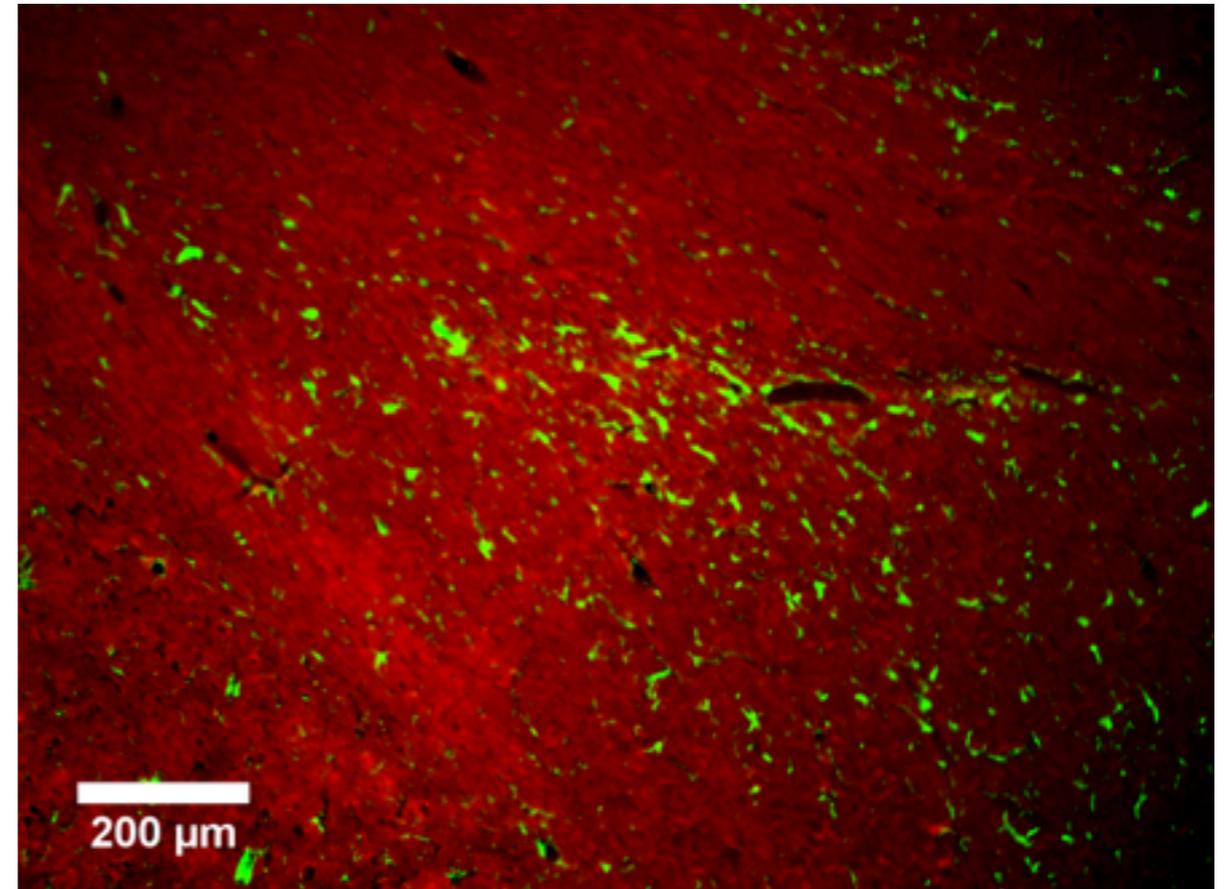




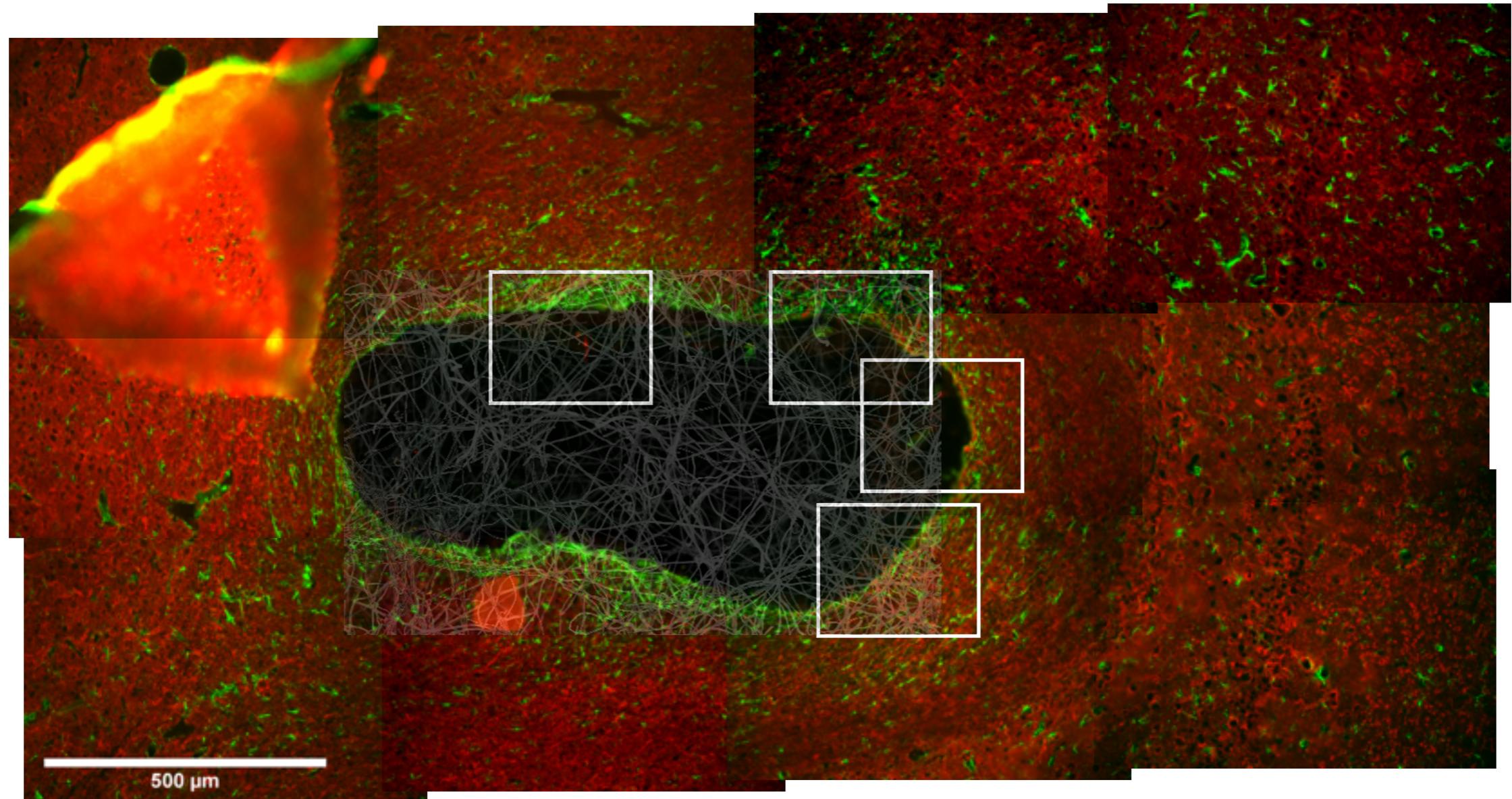
Sponge



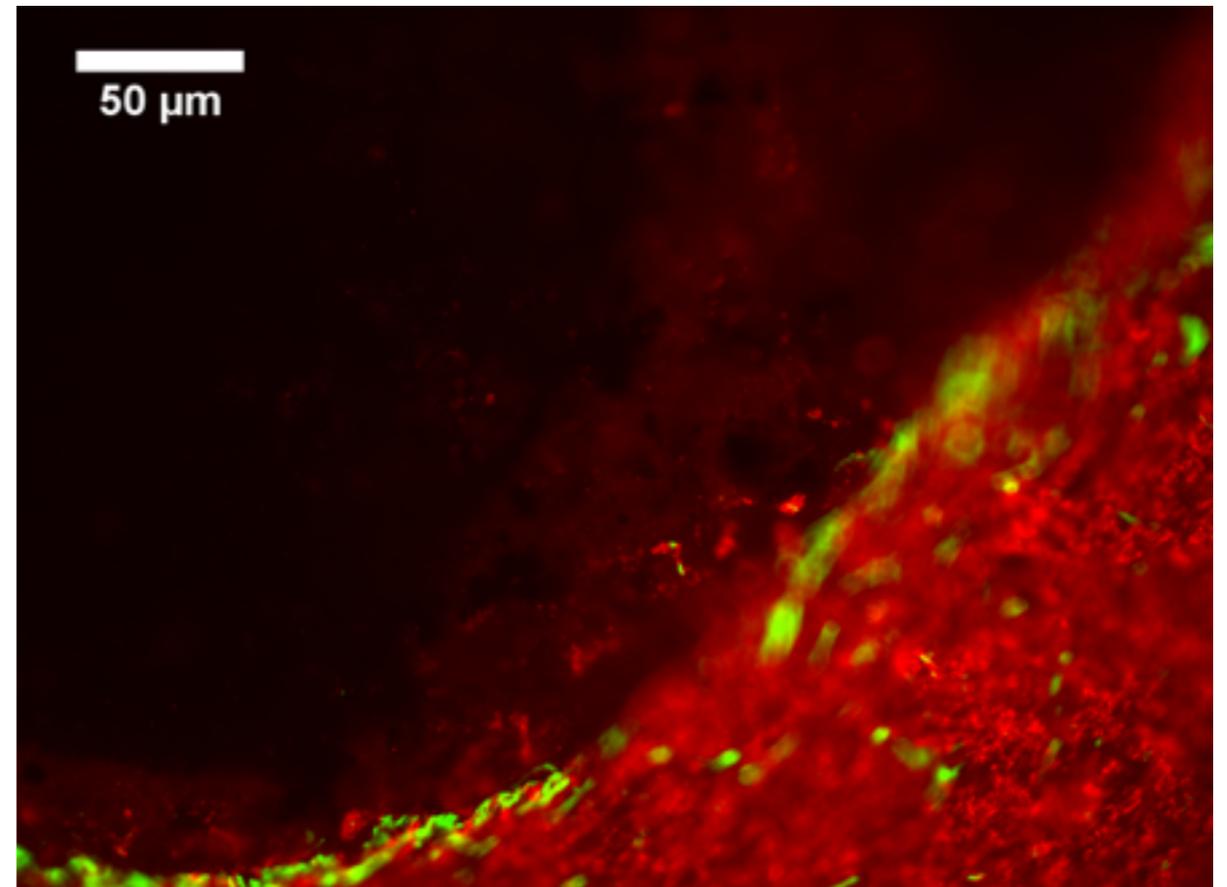
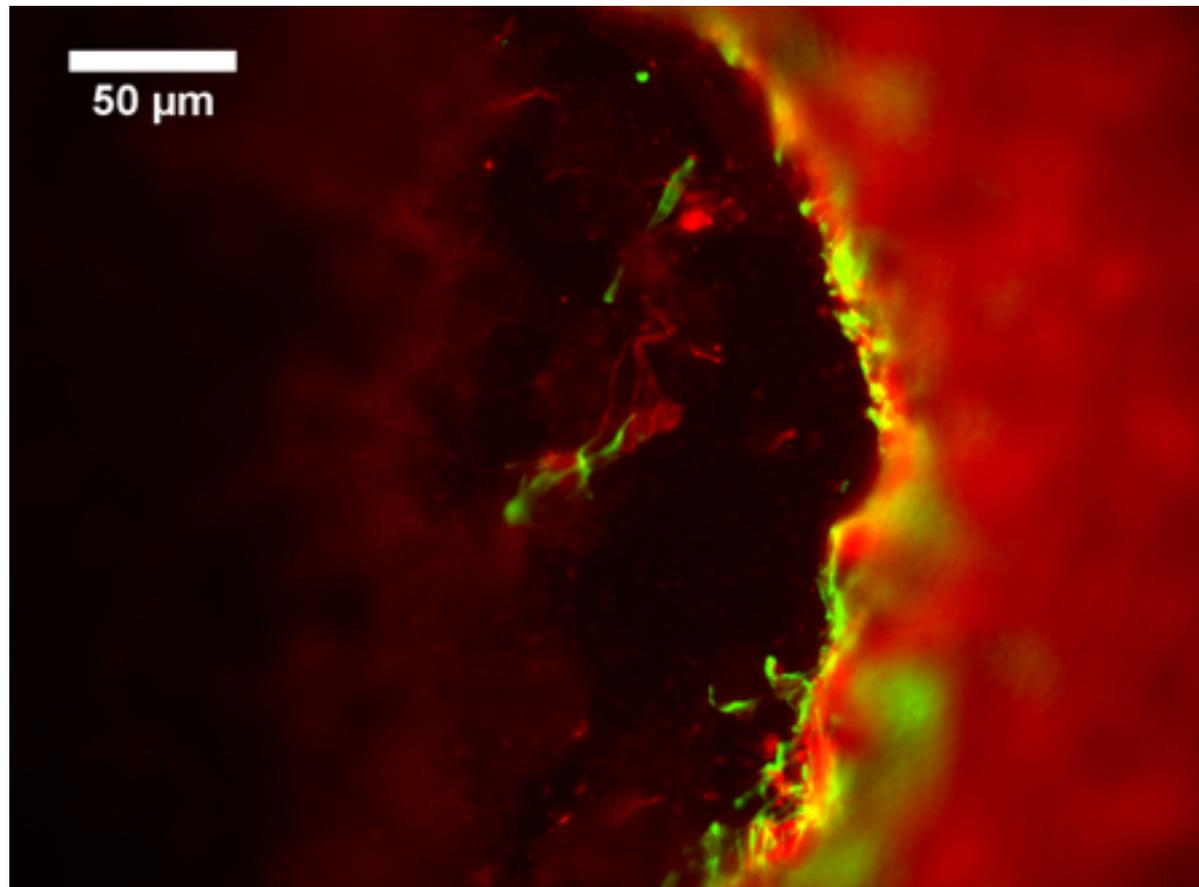
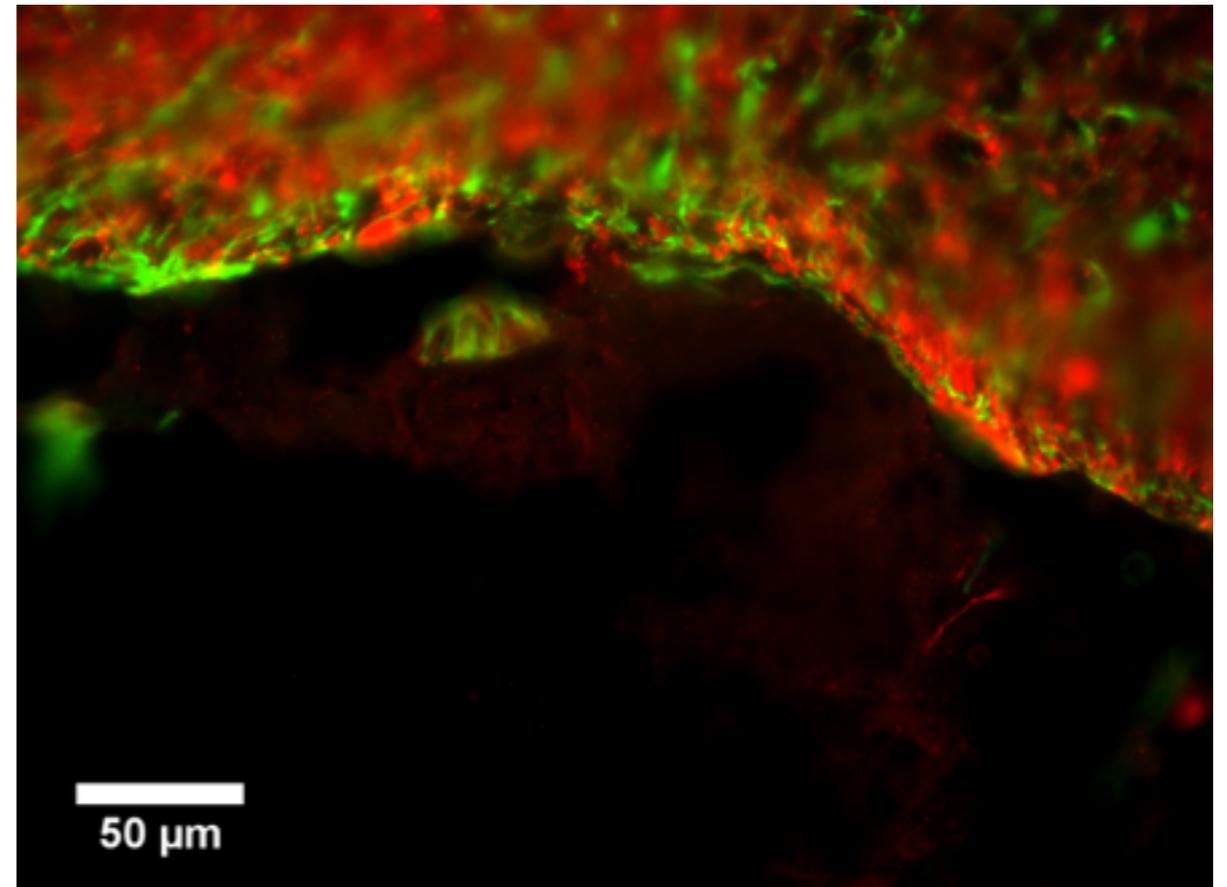
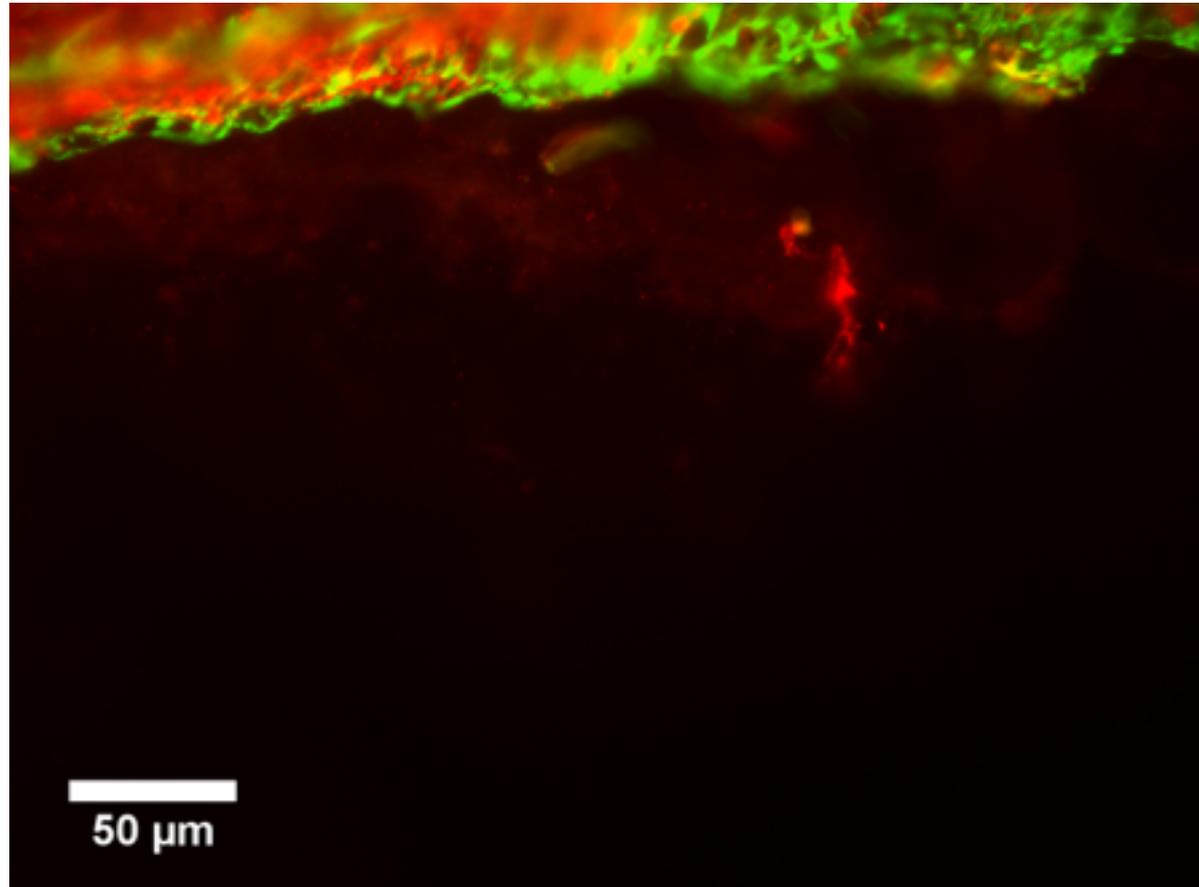
II hemisphere

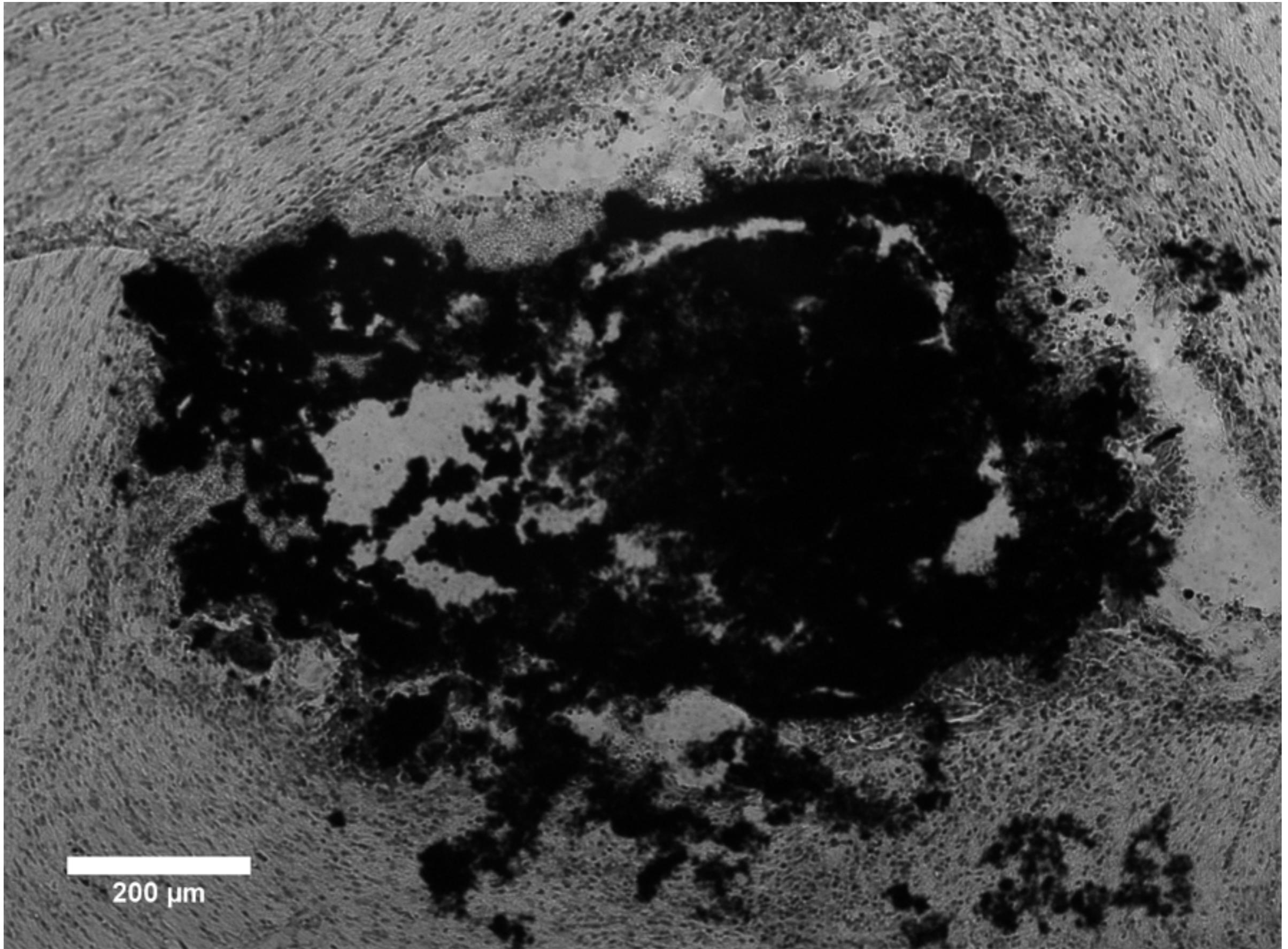


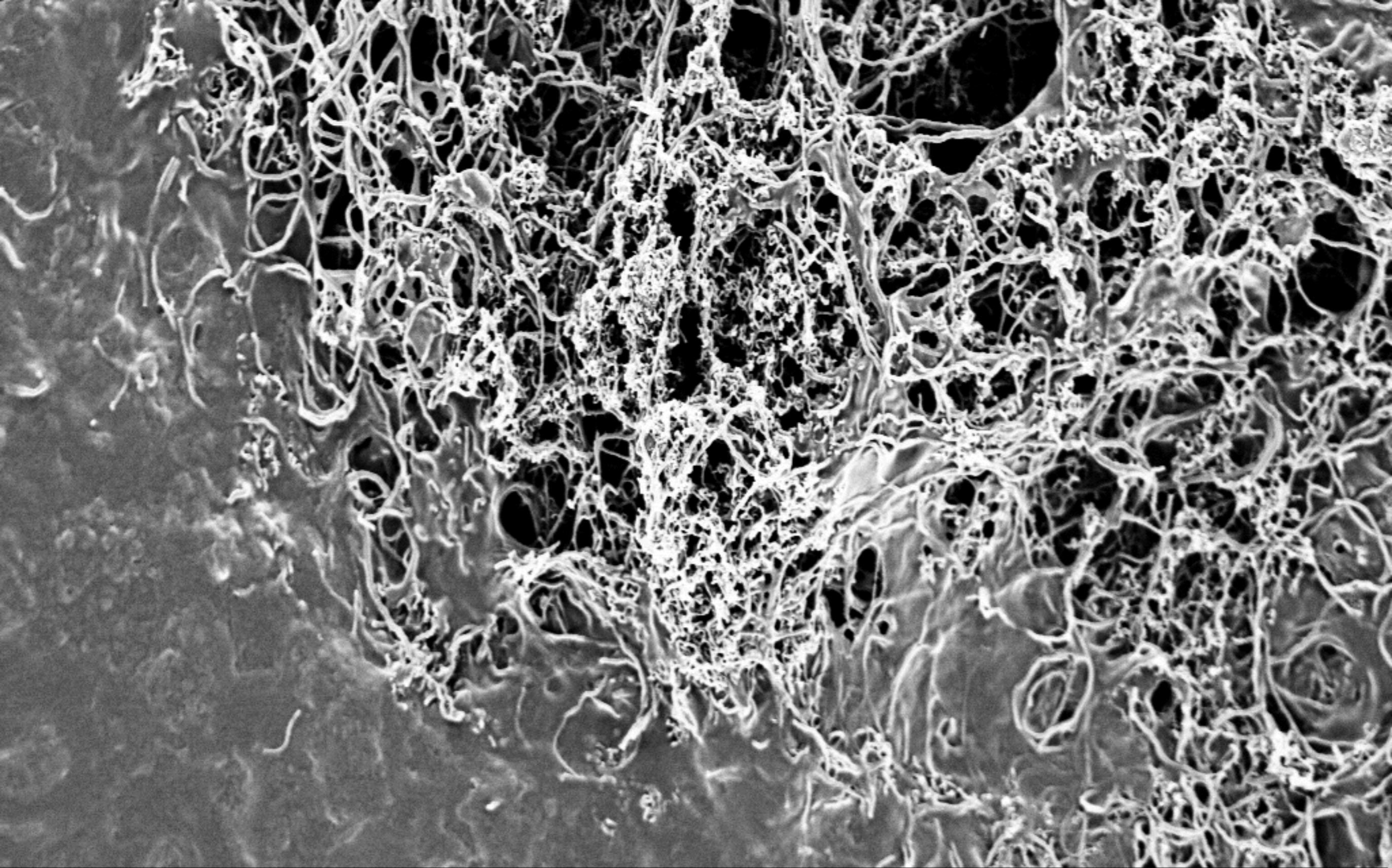
Coverslip 21 (III slice), objective 20x



Coverslip 21 (III slice), objective 40x







2 μm

Date :9 May 2014

Time :13:47:08

Mag = 10.00 K X

EHT = 2.00 kV
Brightness = 45.1 %
Contrast = 33.3 %

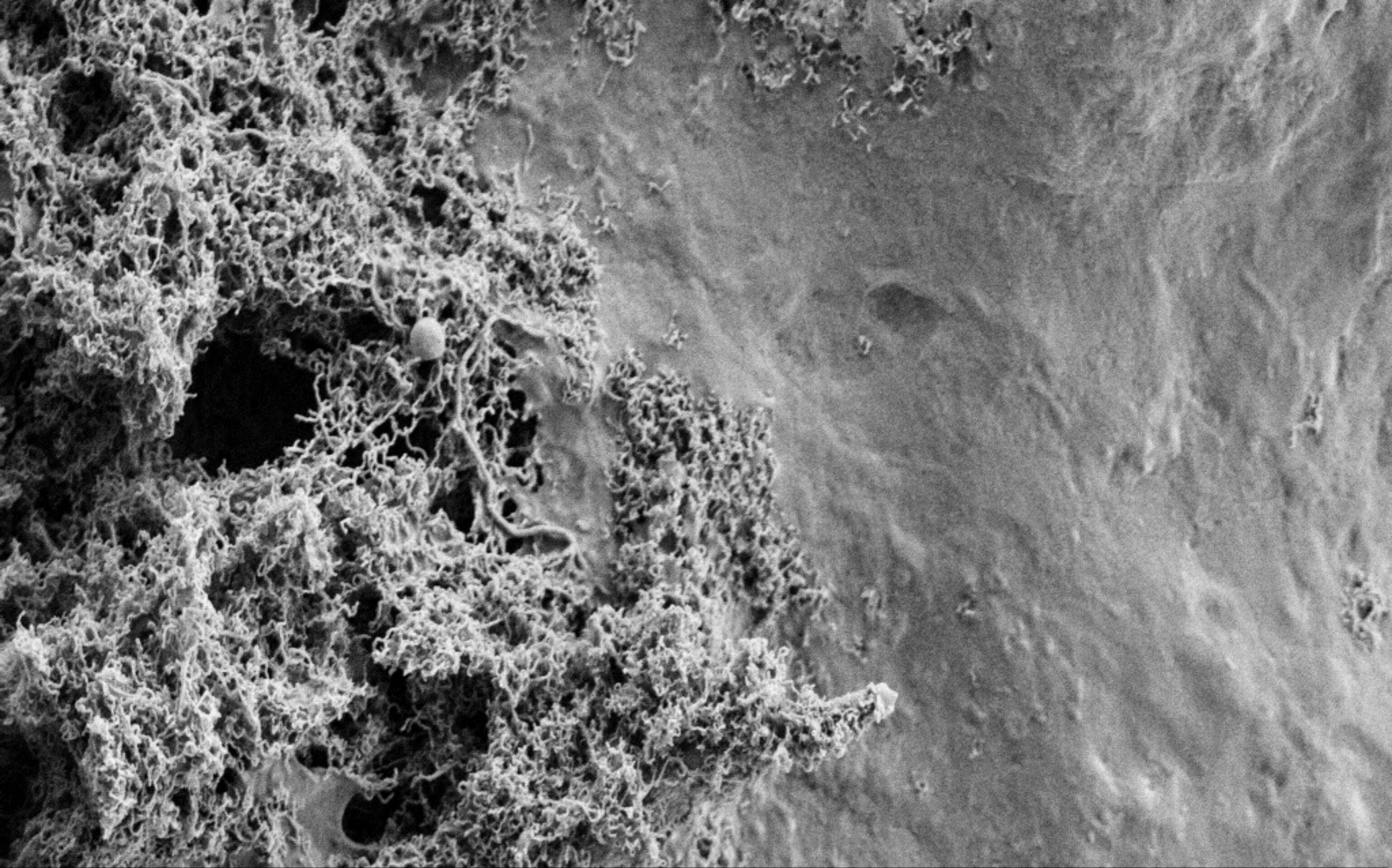
Signal A = InLens

Aperture Size = 30.00 μm

Stage at T = 0.0 $^{\circ}$

Stage at Z = 49.000 mm





2 μm

Date :9 May 2014

Time :13:51:10

Mag = 10.00 K X

EHT = 2.00 kV
Brightness = 48.9 %
Contrast = 42.2 %

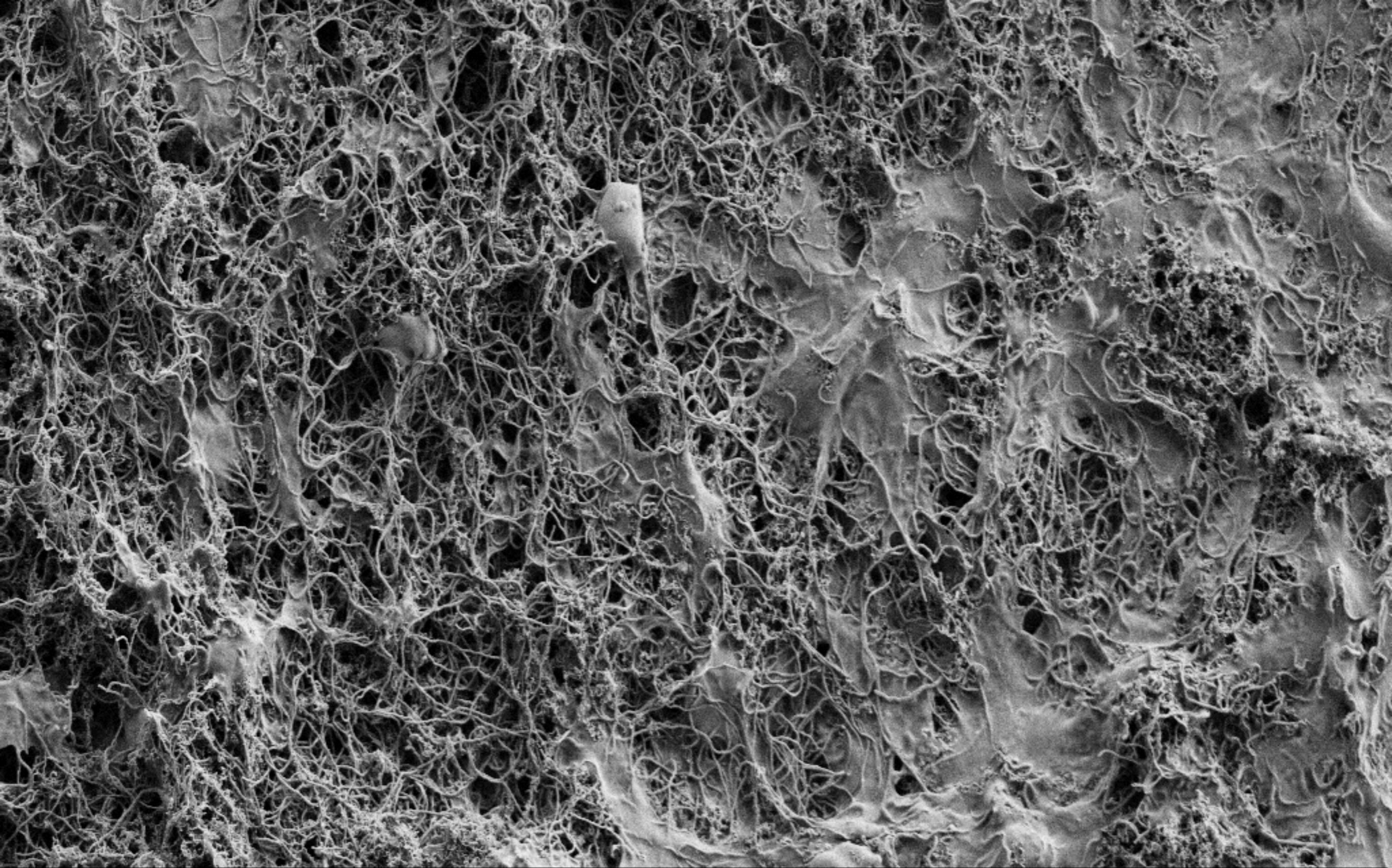
Signal A = SE2

Aperture Size = 30.00 μm

Stage at T = 0.0 °

Stage at Z = 49.000 mm





10 μm
Date :9 May 2014
Time :14:13:09
Mag = 5.00 K X
EHT = 2.00 kV
Brightness = 48.9 %
Contrast = 42.2 %
Signal A = SE2
Aperture Size = 30.00 μm
Stage at T = 0.0 °
Stage at Z = 49.000 mm



- The described approach is a system to evaluate the interactions between CNTs and spinal cord slice
- We need to fabricate a new device or to cover the surface of implantable electrodes!!!

