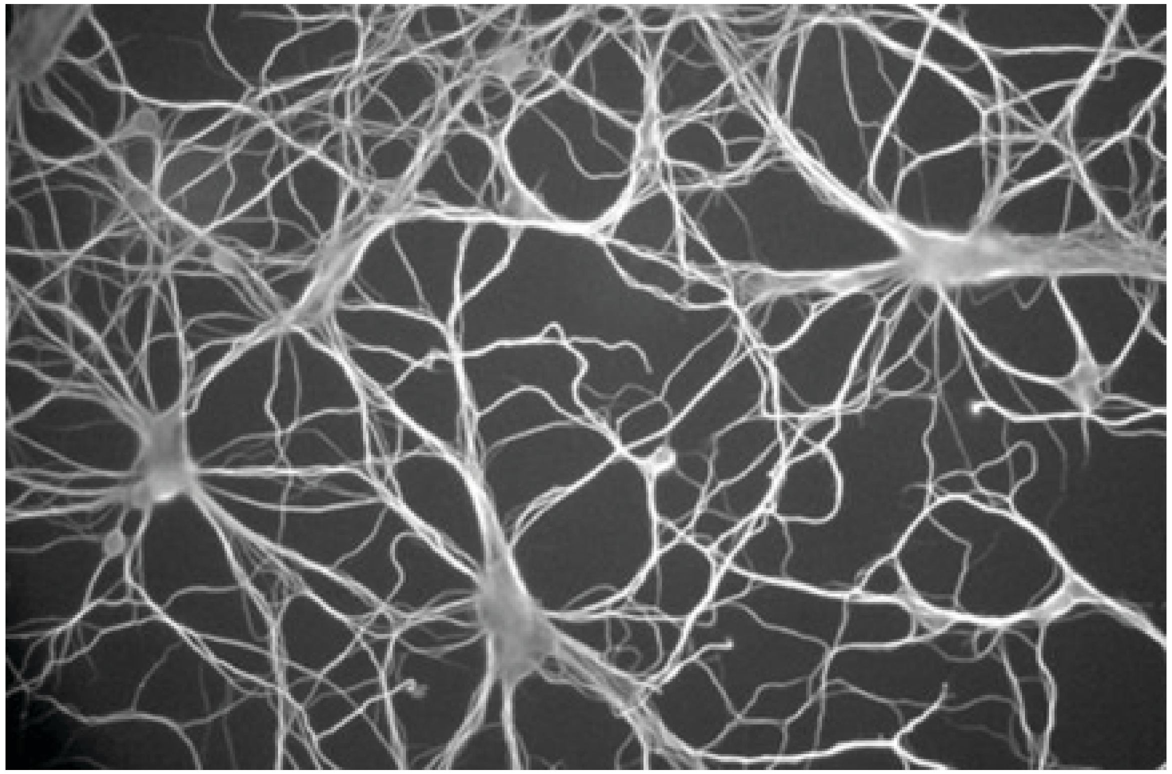
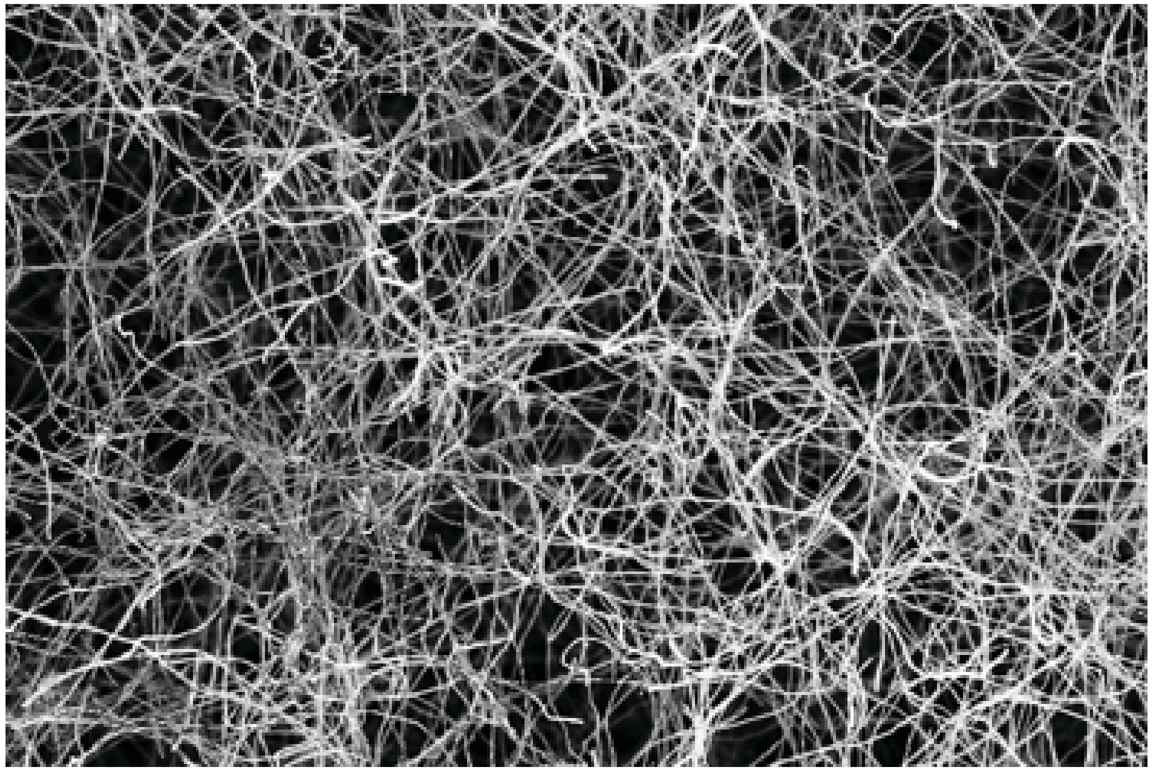
CNT as Substrates for Neuronal Growth

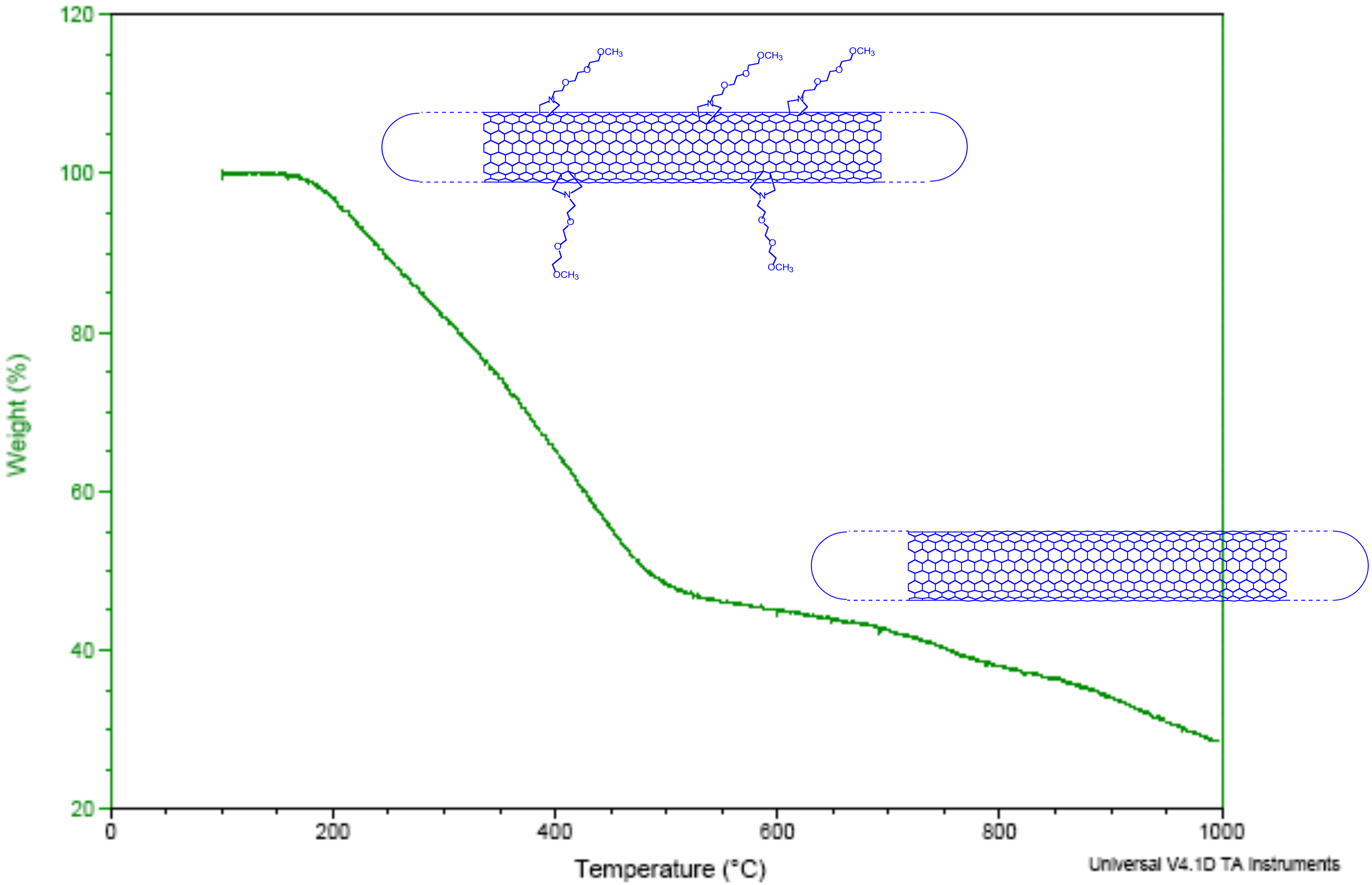




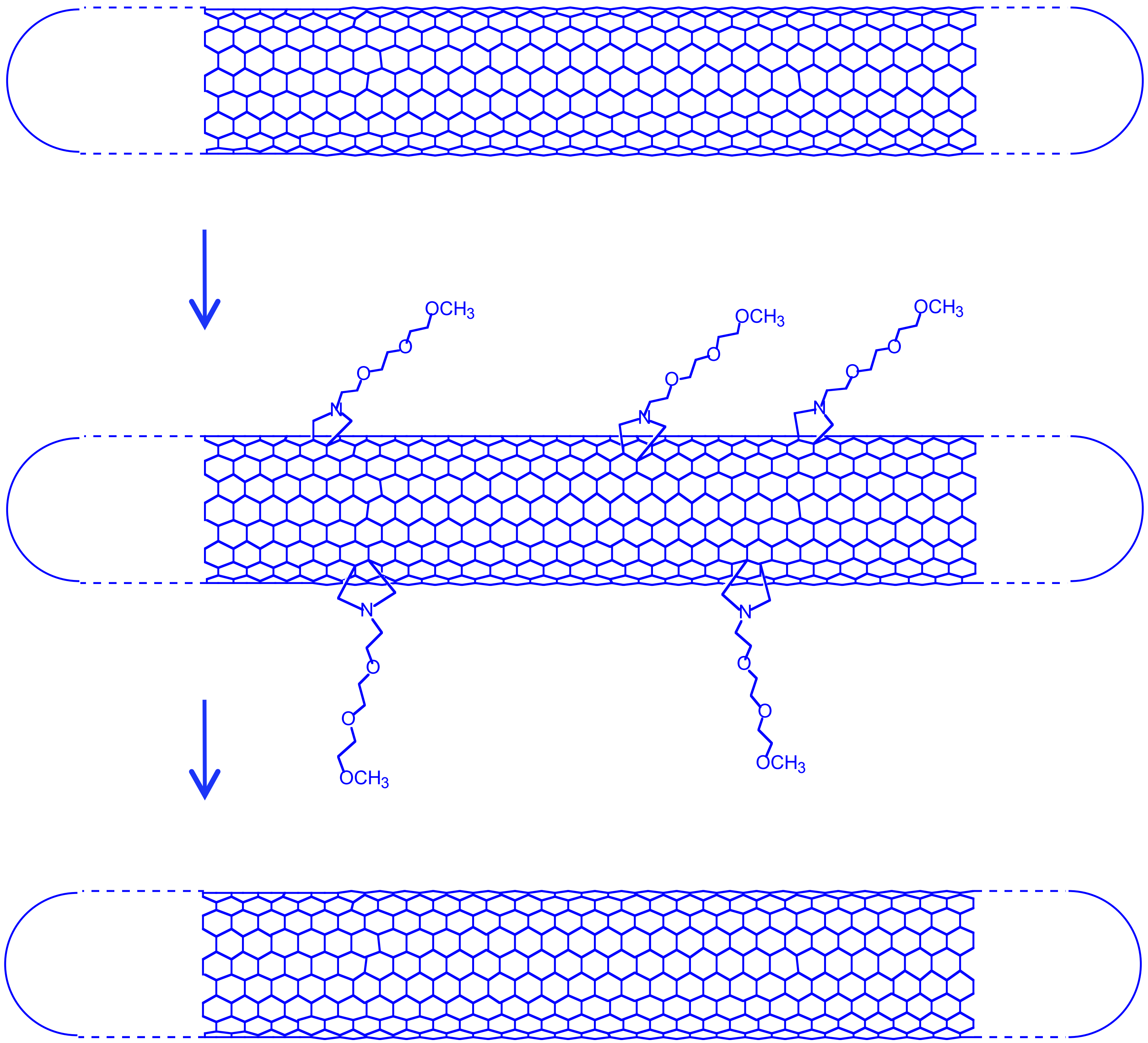
Disease Models & Mechanisms 6, 72-83 (2013) doi:10.1242/dmm.008946

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

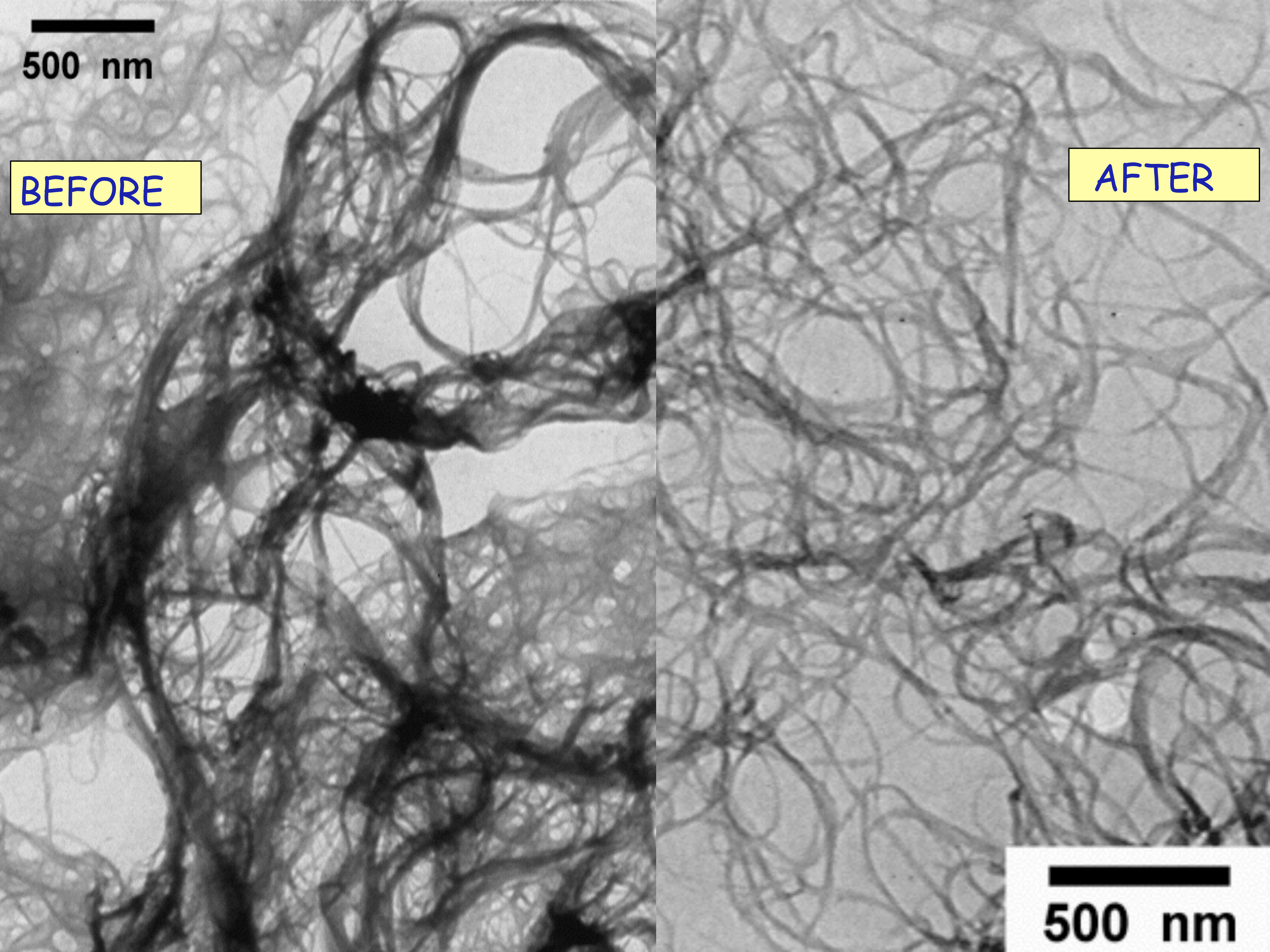


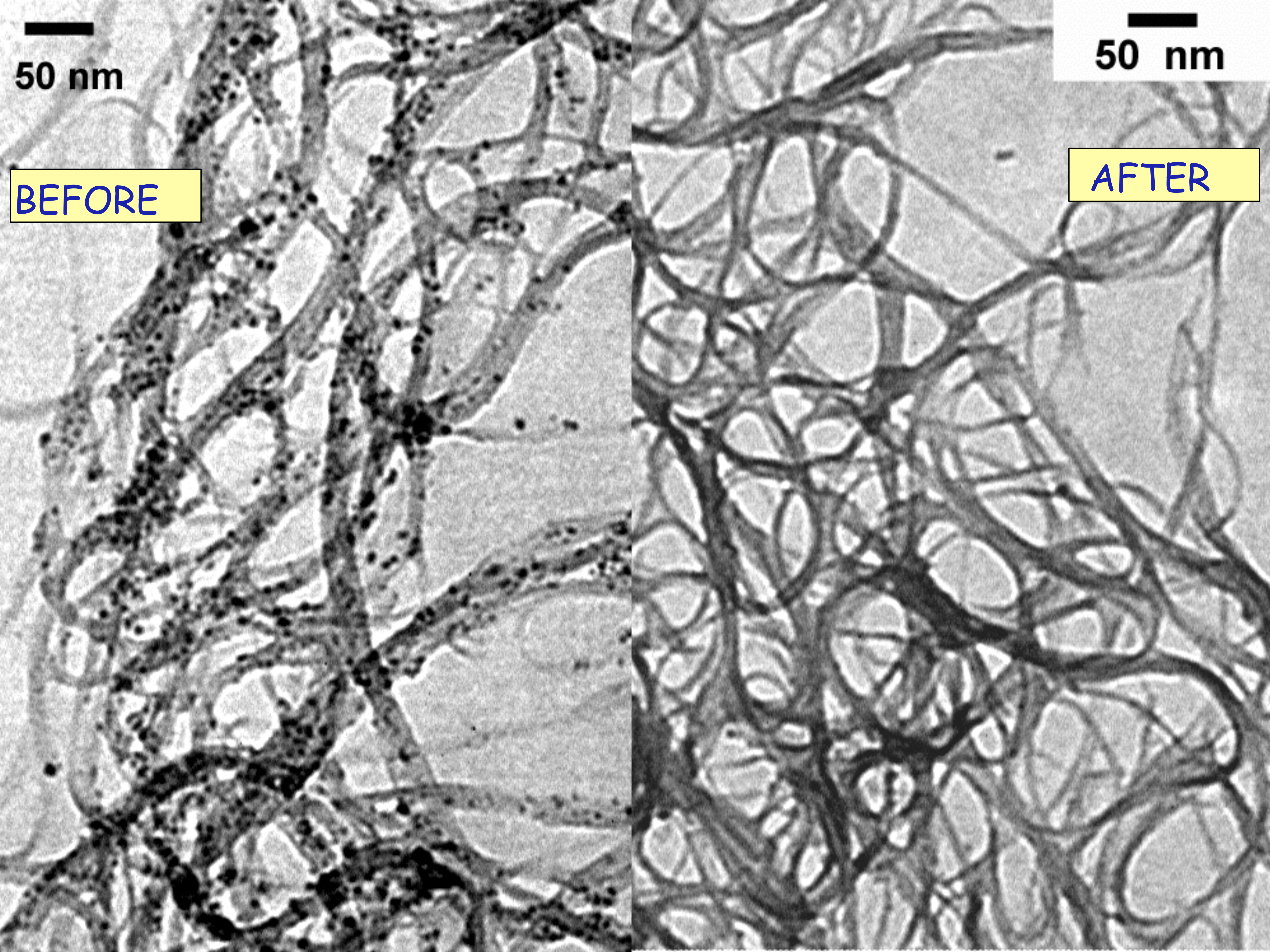
+ amorphous carbon
+ metallic nanoparticles

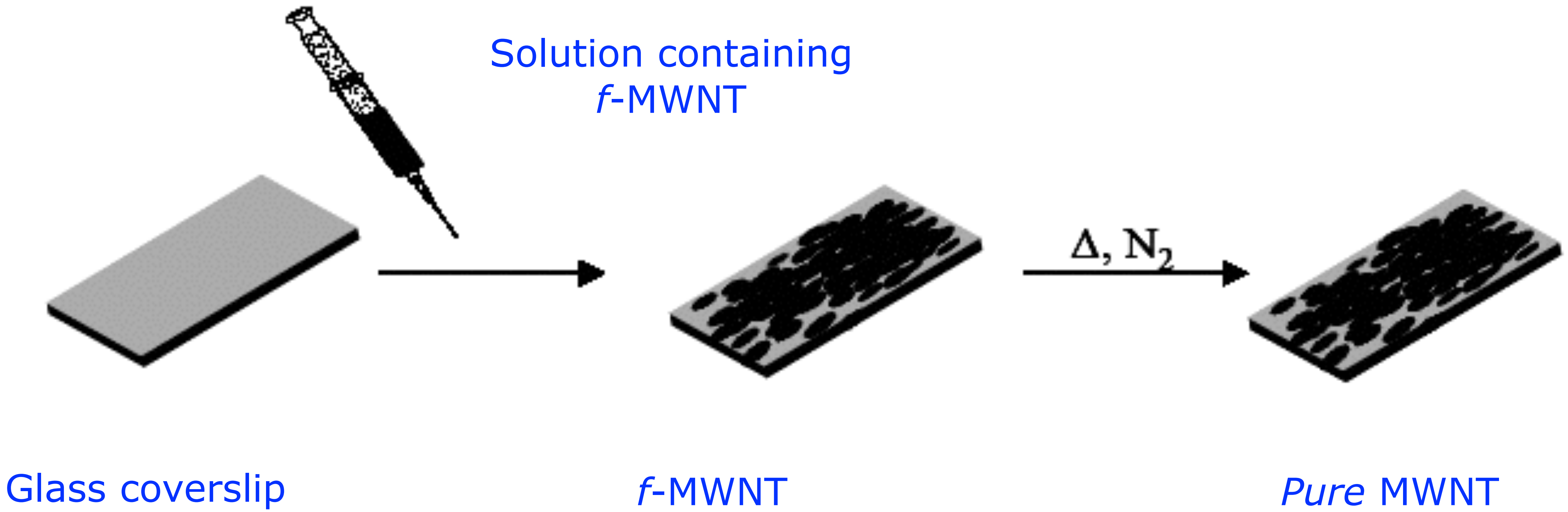
easy to handle, soluble
Can be purified!

PURE! No amorphous carbon, no metal

J. Am. Chem. Soc. **2002**, 124, 14318-14319

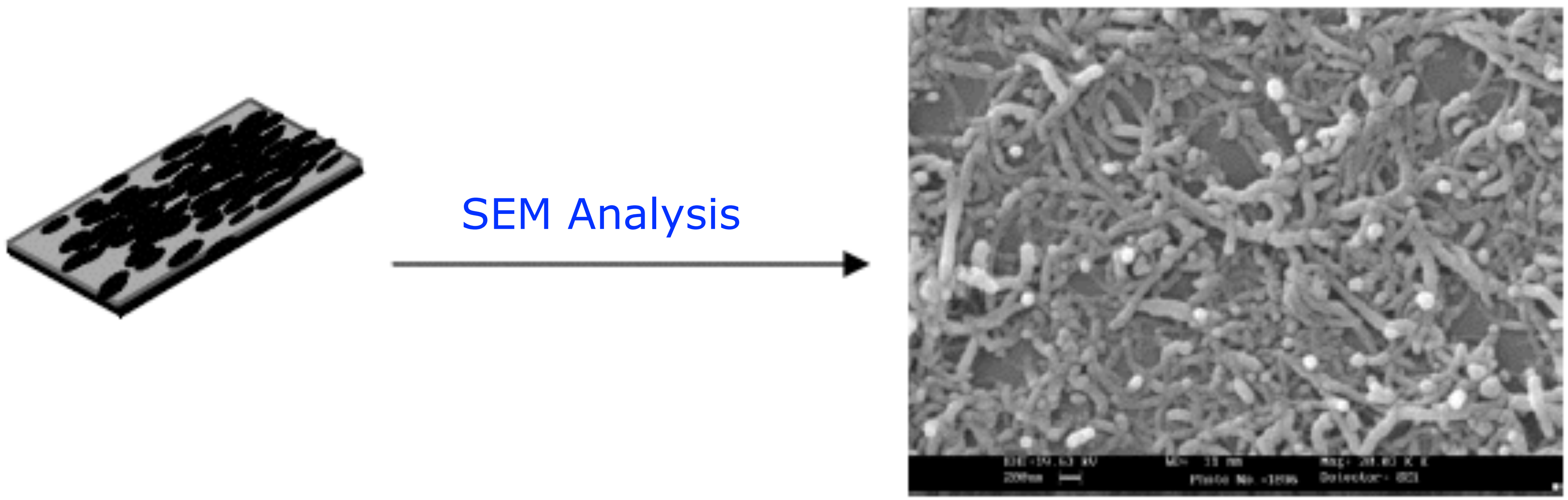

500 nm
BEFORE
AFTER
500 nm

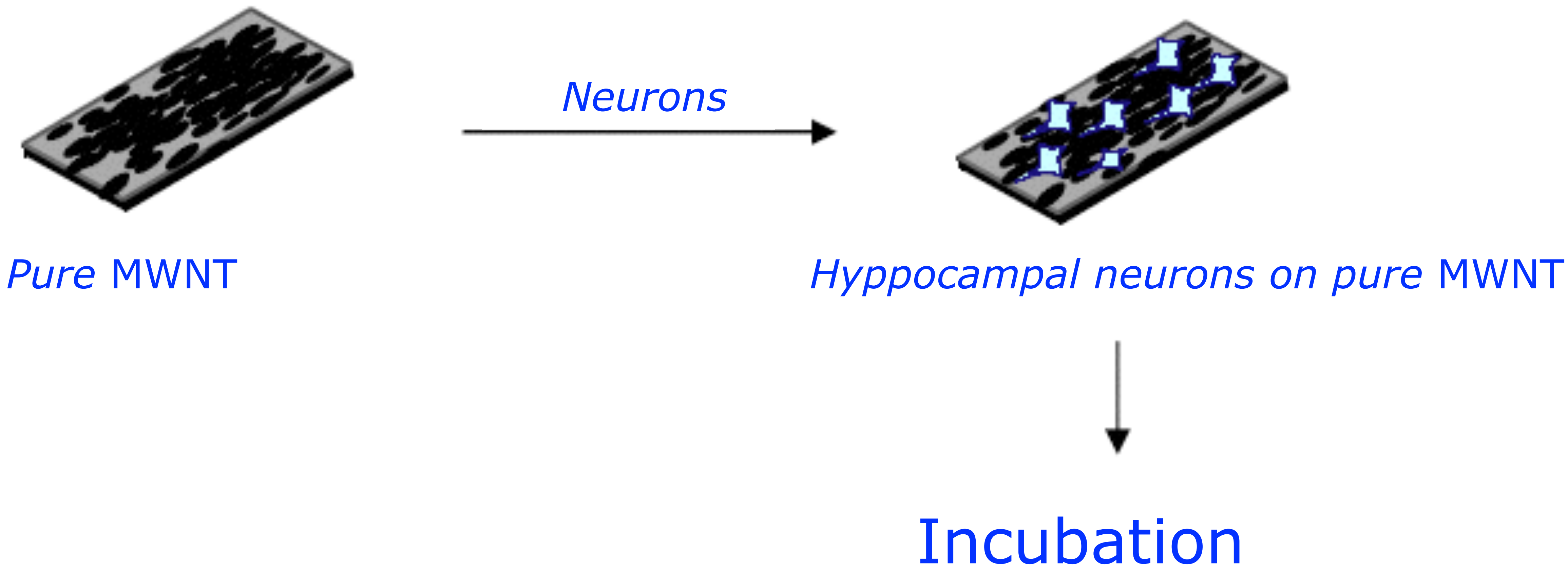

50 nm
BEFORE
50 nm
AFTER

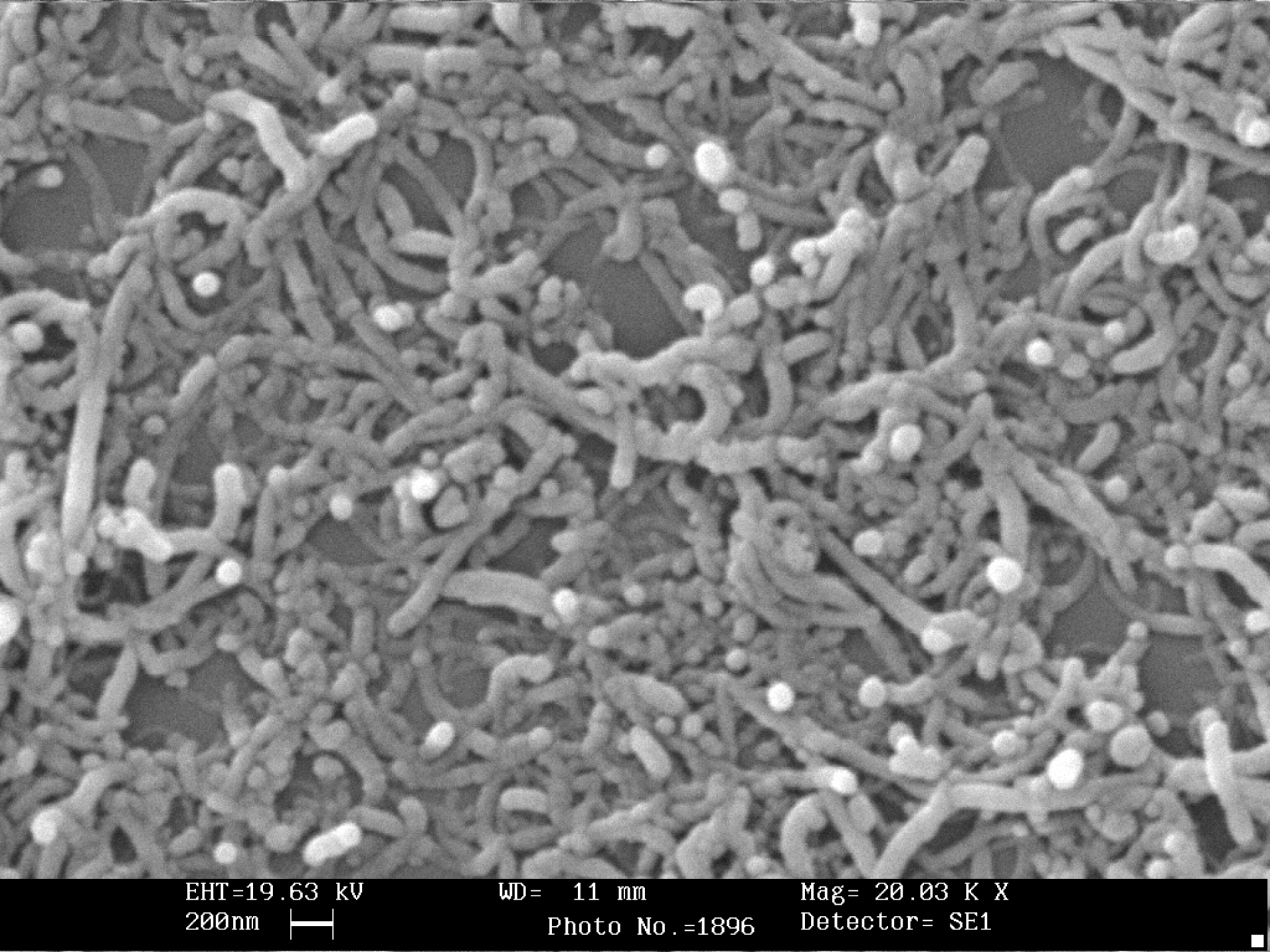


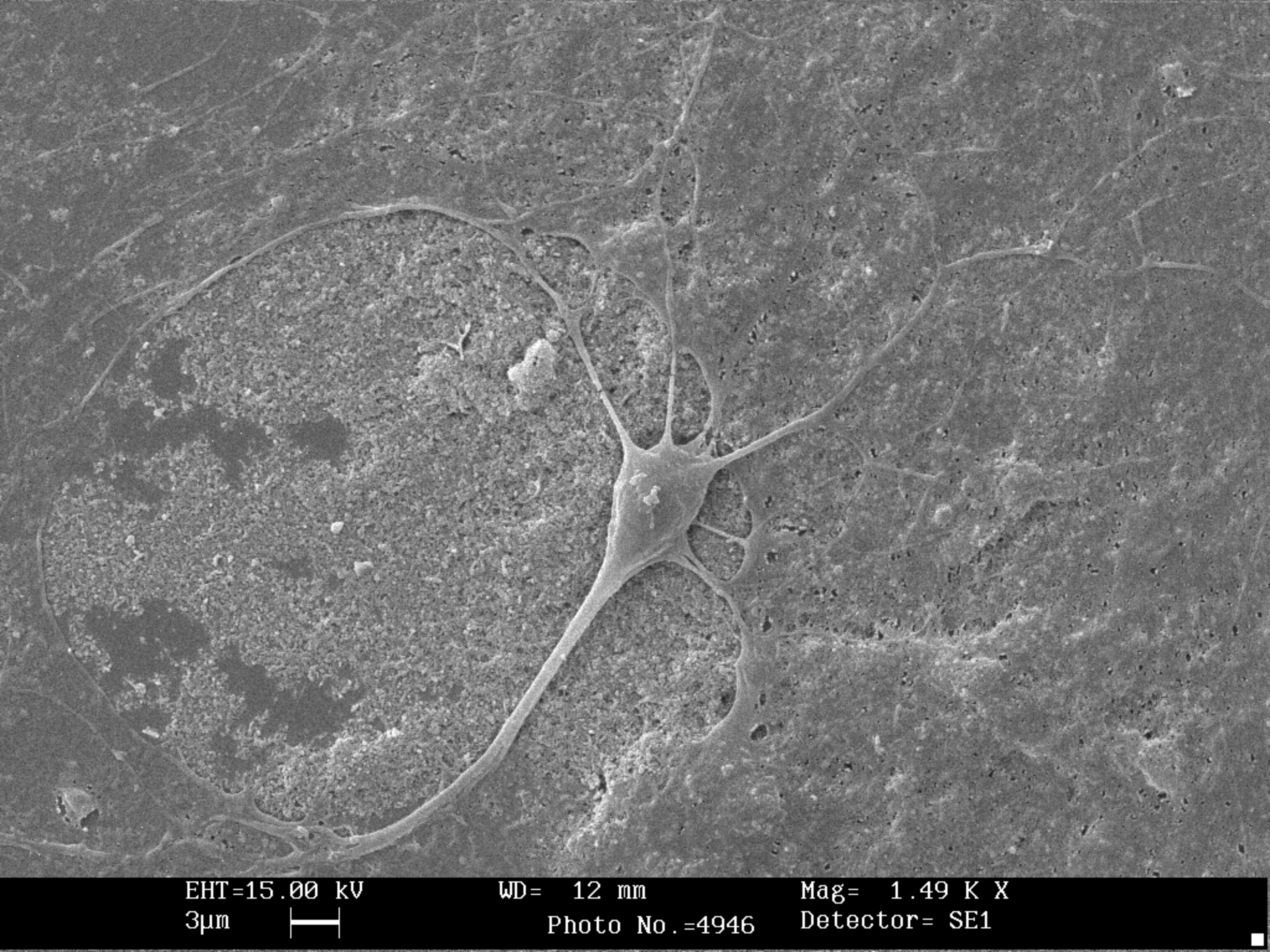
Solution containing
f-MWNT
Δ, N2
Glass coverslip
f-MWNT
Pure MWNT

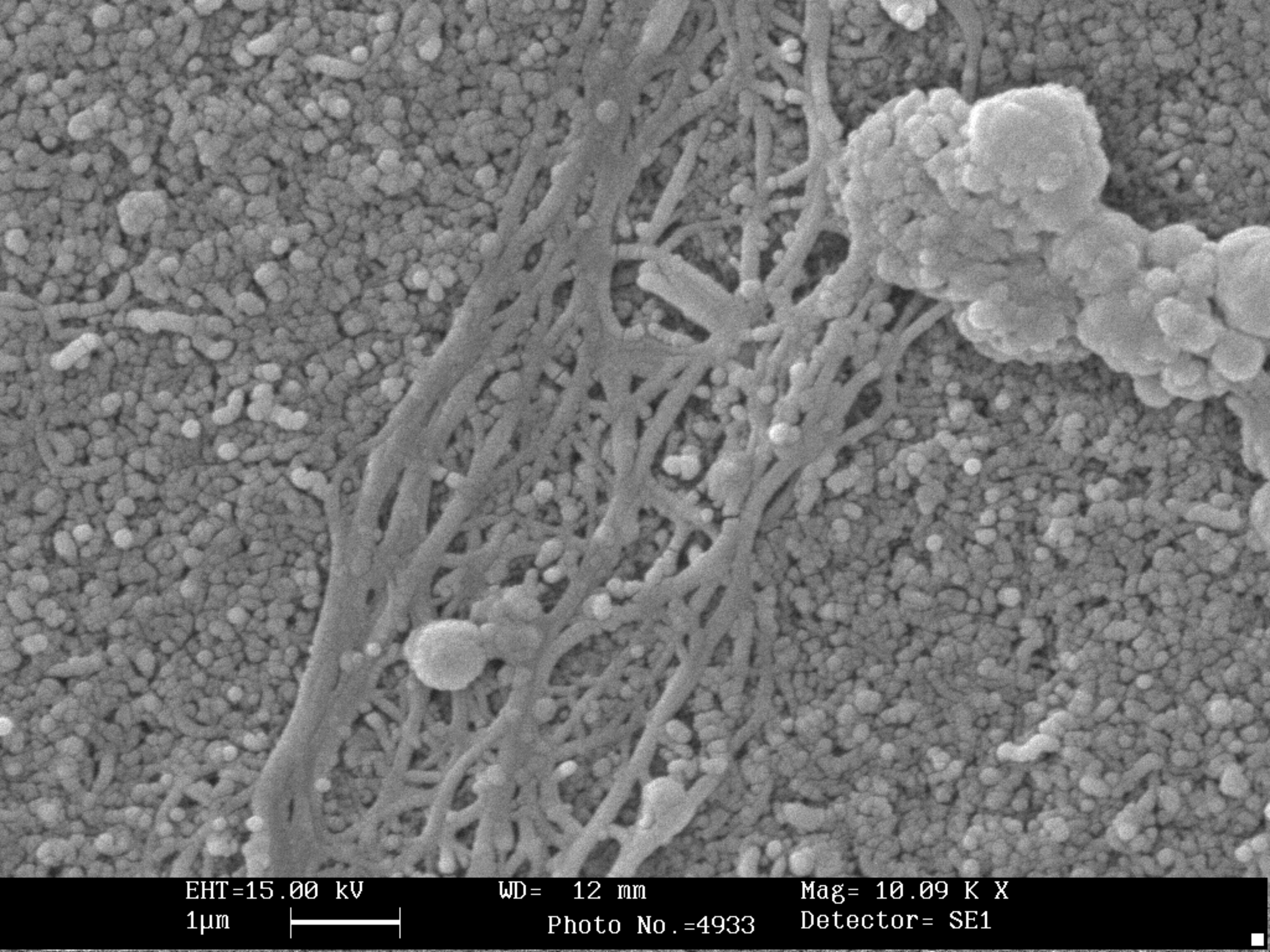
V. Lovat

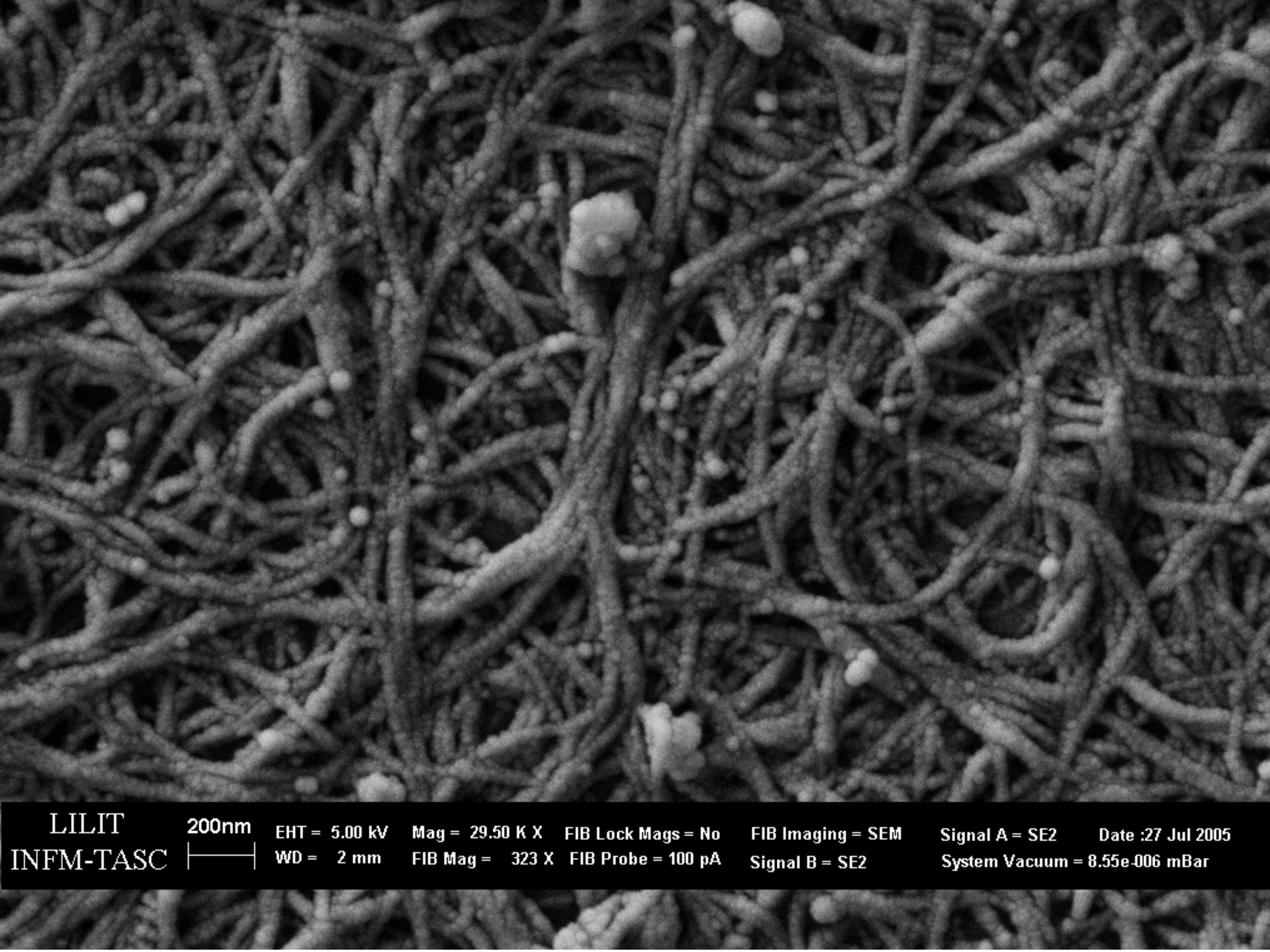

SEM Analysis

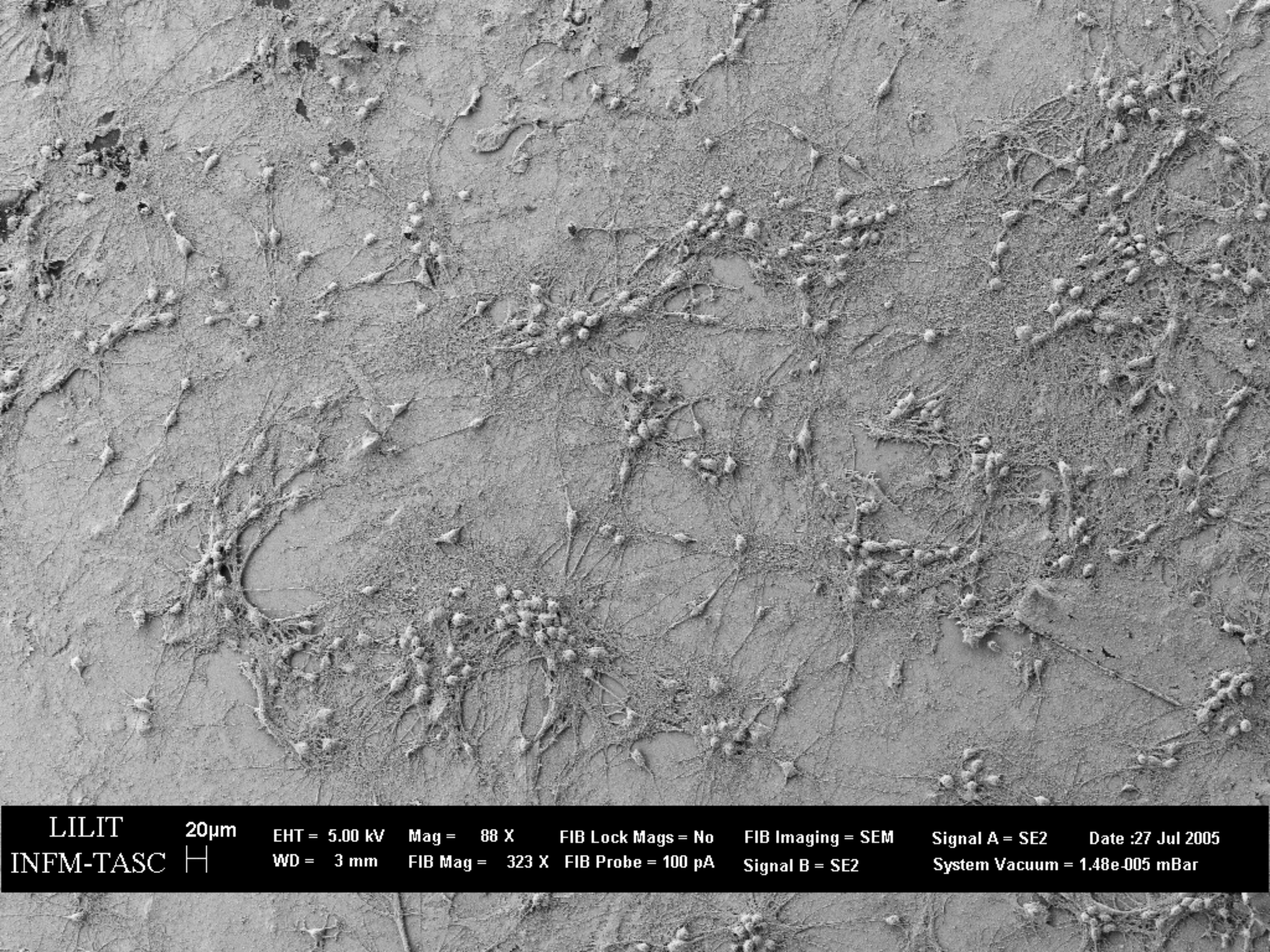

Neurons
Pure MWNT
Hyppocampal neurons on pure MWNT
Incubation


EHT=19.63 kV
WD= 11 mm
Mag= 20.03 K X
200nm
Photo No.=1896
Detector= SE1

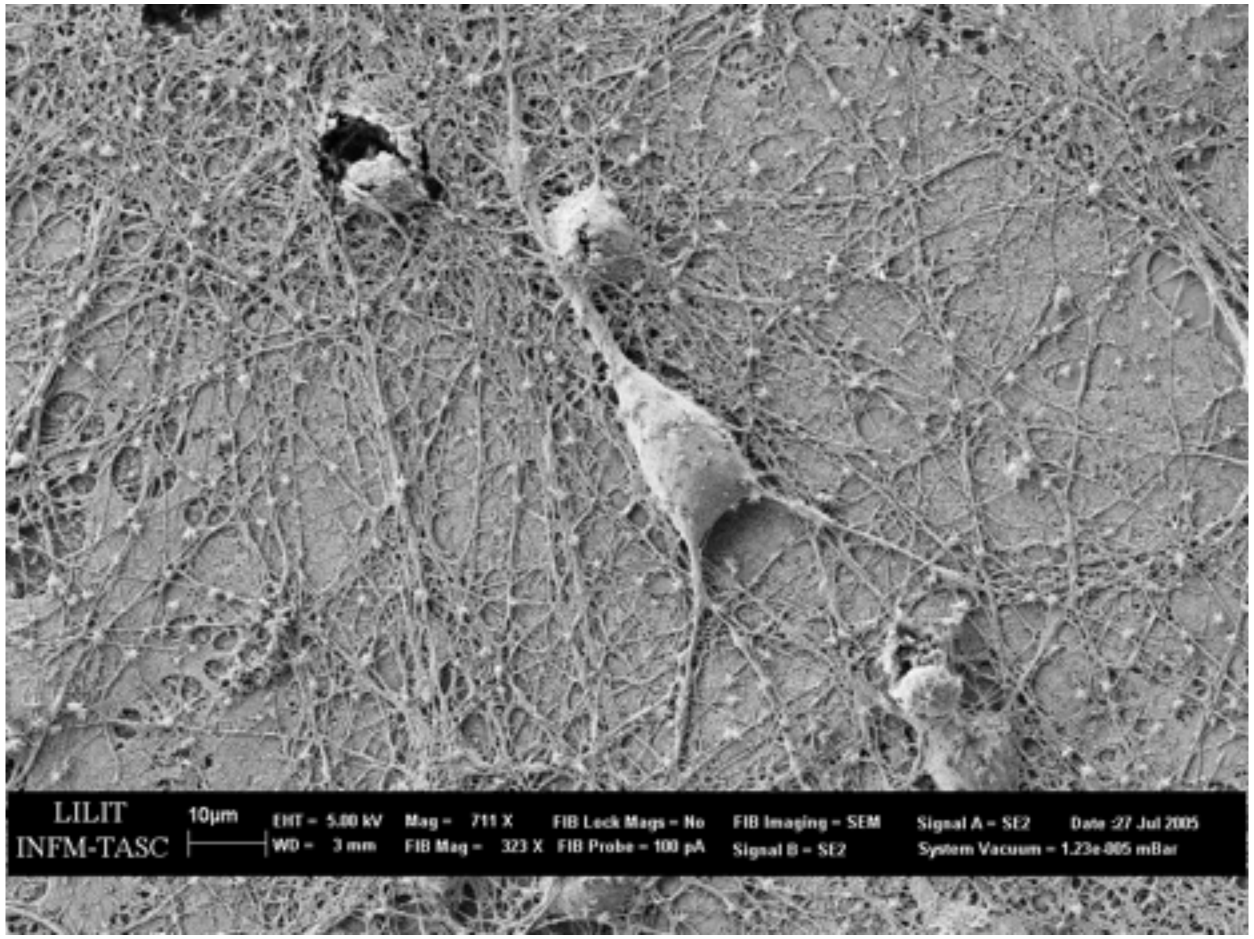

EHT=15.00 kV
WD= 12 mm
Mag= 1.49 K X
3µm
Photo No.=4946
Detector= SE1


EHT=15.00 kV
WD= 12 mm
Mag= 10.09 K X
1µm
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
INFM-TASC
20µm
EHT = 5.00 kV
WD = 3 mm
Mag = 88 X
FIB Mag = 323 X
FIB Lock Mags = No
FIB Probe = 100 pA
FIB Imaging = SEM
Signal B = SE2
Signal A = SE2
Date :27 Jul 2005
System Vacuum = 1.48e-005 mBar


LILIT
INFM-TASC
10μm
EHT = 5.00 kV
WD = 3 mm
Mag = 711 X
FIB Mag = 323 X
FIB Lock Mags = No
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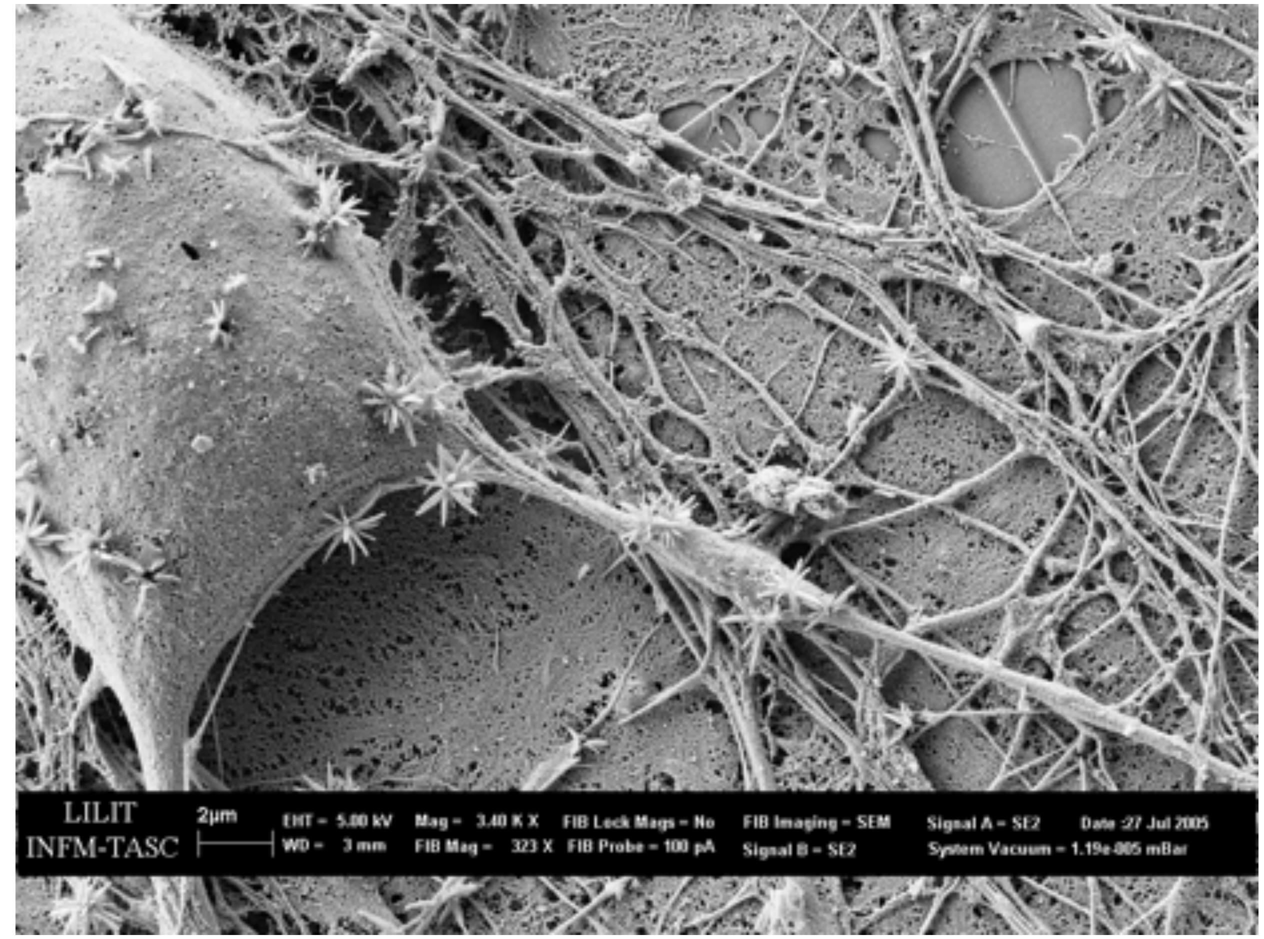
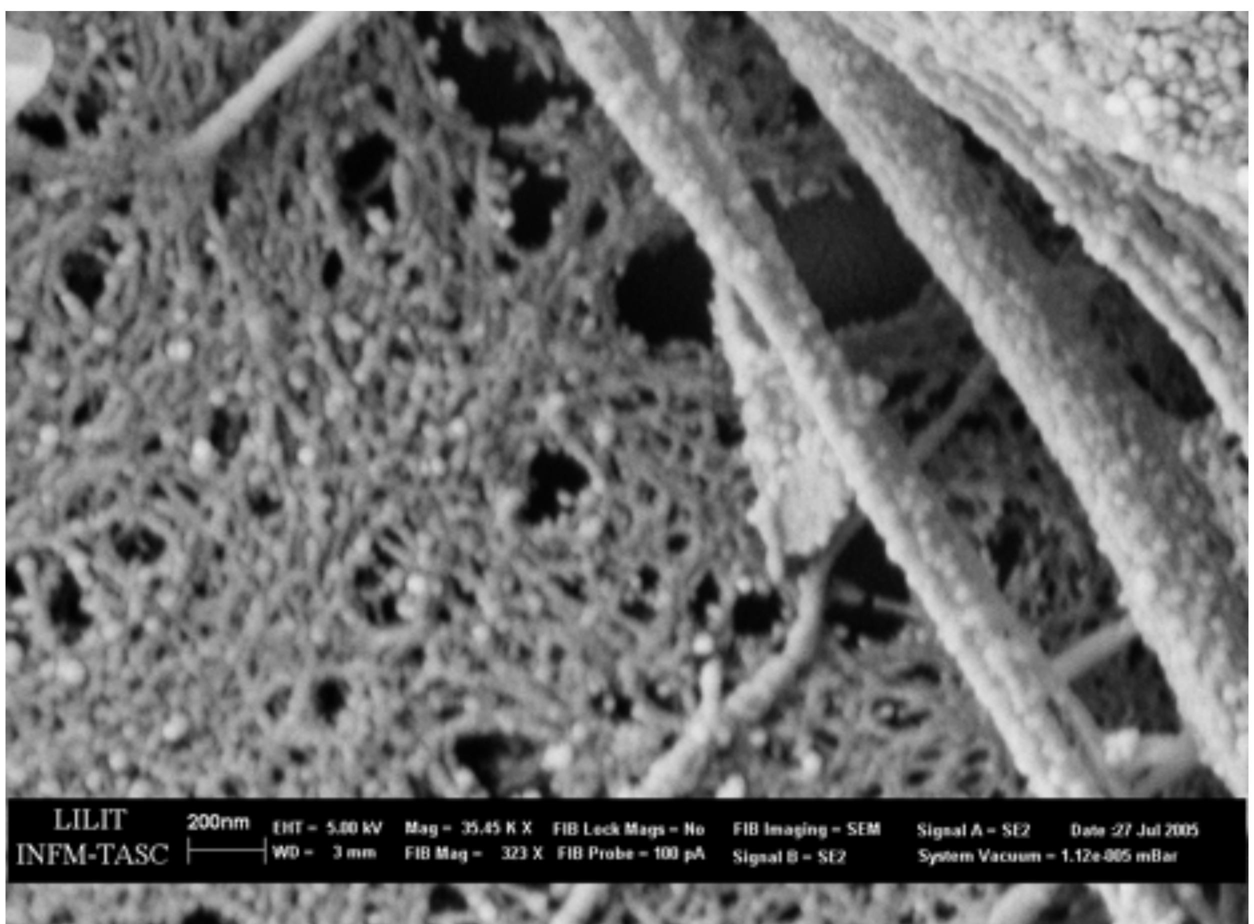
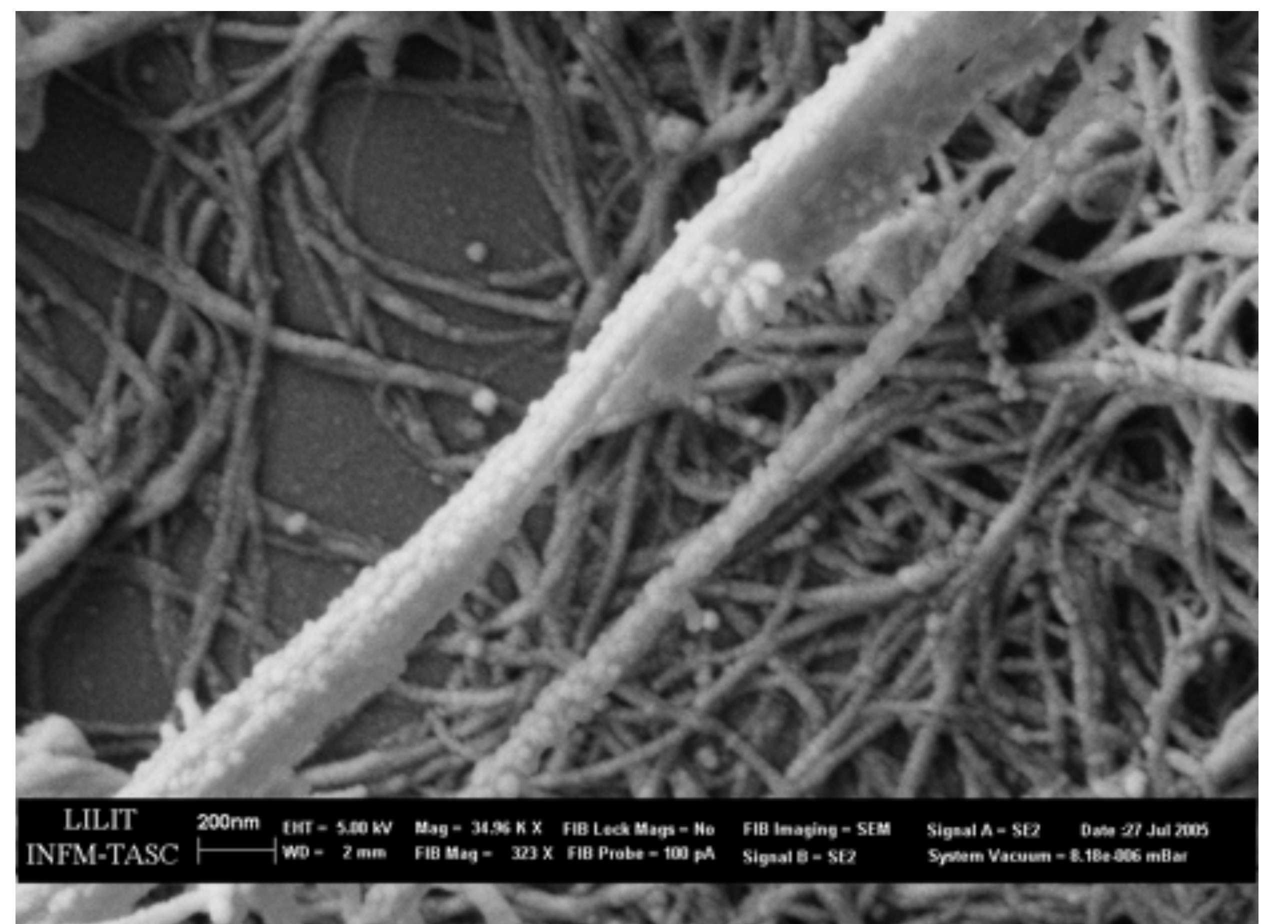
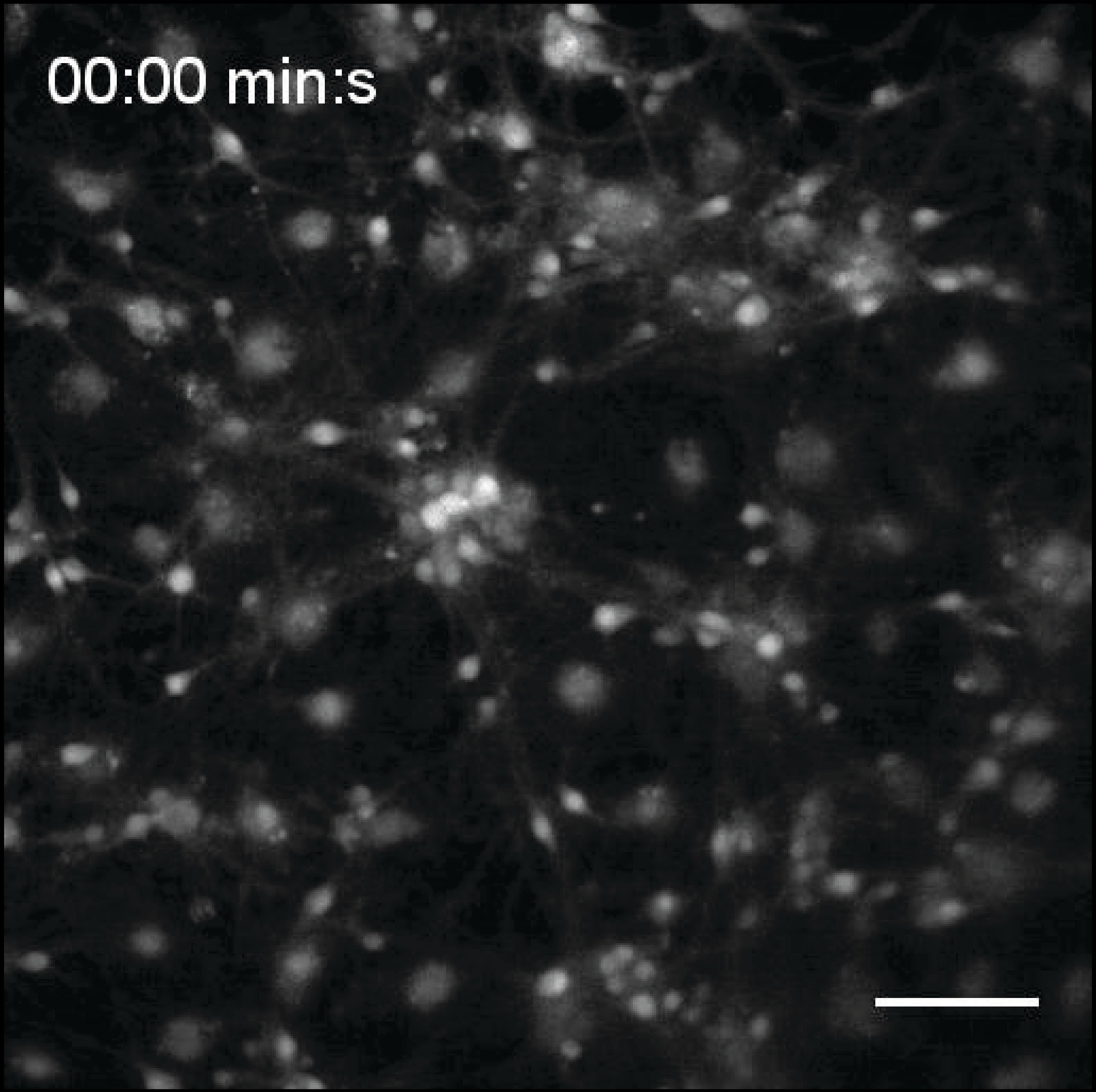
FIB Imaging = SEM
Signal B = SE2
Signal A = SE2
System Vacuum = 1.23e-005 mBar
Date :27 Jul 2005

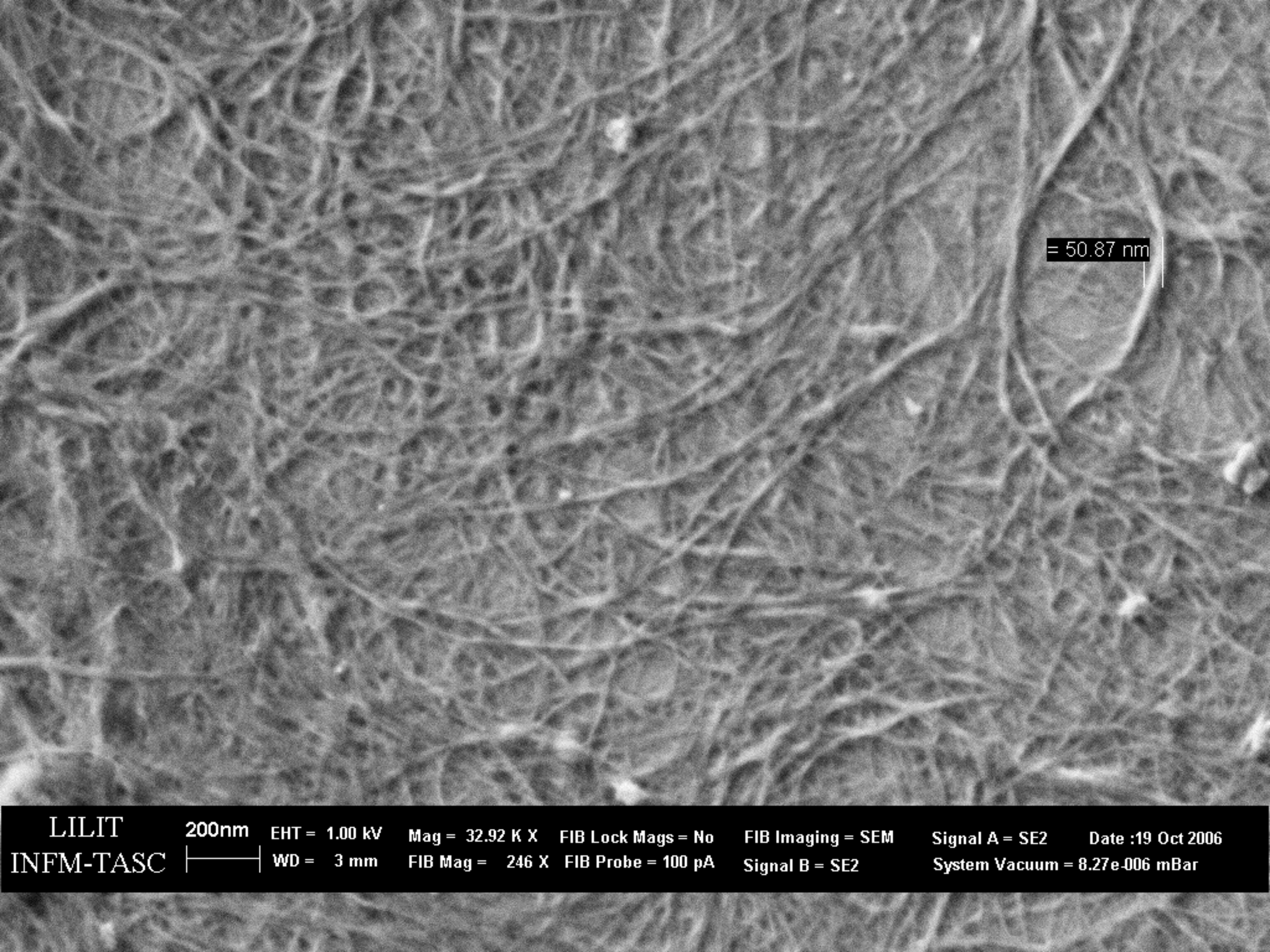

LILIT
INFM-TASC
2μm
EHT = 5.00 kV
WD = 3 mm
Mag = 3.40 K X
FIB Mag = 323 X
FIB Lock Mags = No
FIB Probe = 100 pA
FIB Imaging = SEM
Signal B = SE2
Signal A = SE2
System Vacuum = 1.19e-005 mBar
Date :27 Jul 2005


LILIT
INFM-TASC
200nm
EHT = 5.00 kV
WD = 3 mm
Mag = 35.45 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 = 1.12e-005 mBar
Date :27 Jul 2005


LILIT
INFM-TASC
200nm
EHT = 5.00 kV
WD = 2 mm
Mag = 34.96 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 = 8.18e-006 mBar
Date :27 Jul 2005

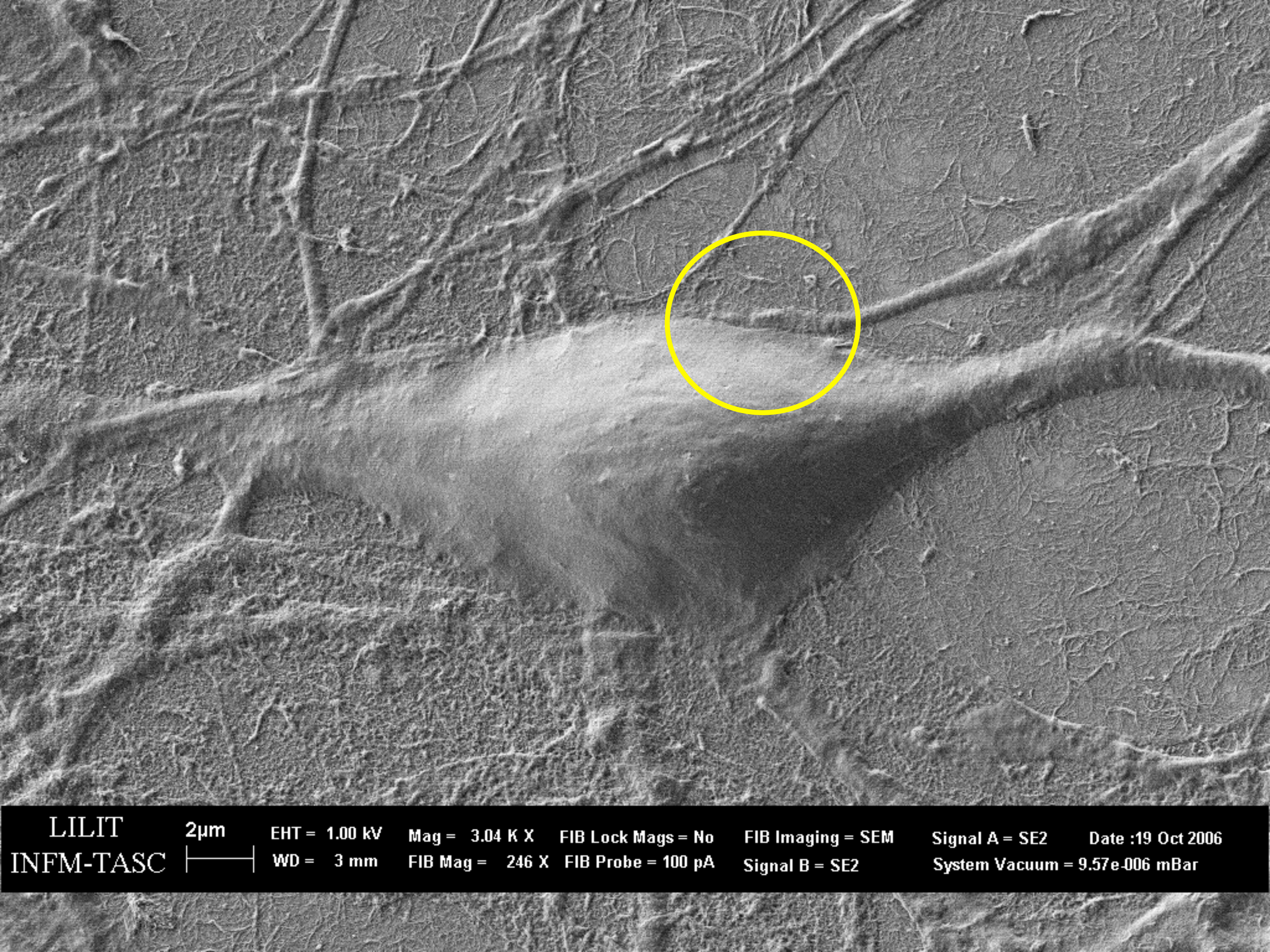
Hippocampal culture on Polyornithine - 10 DIV

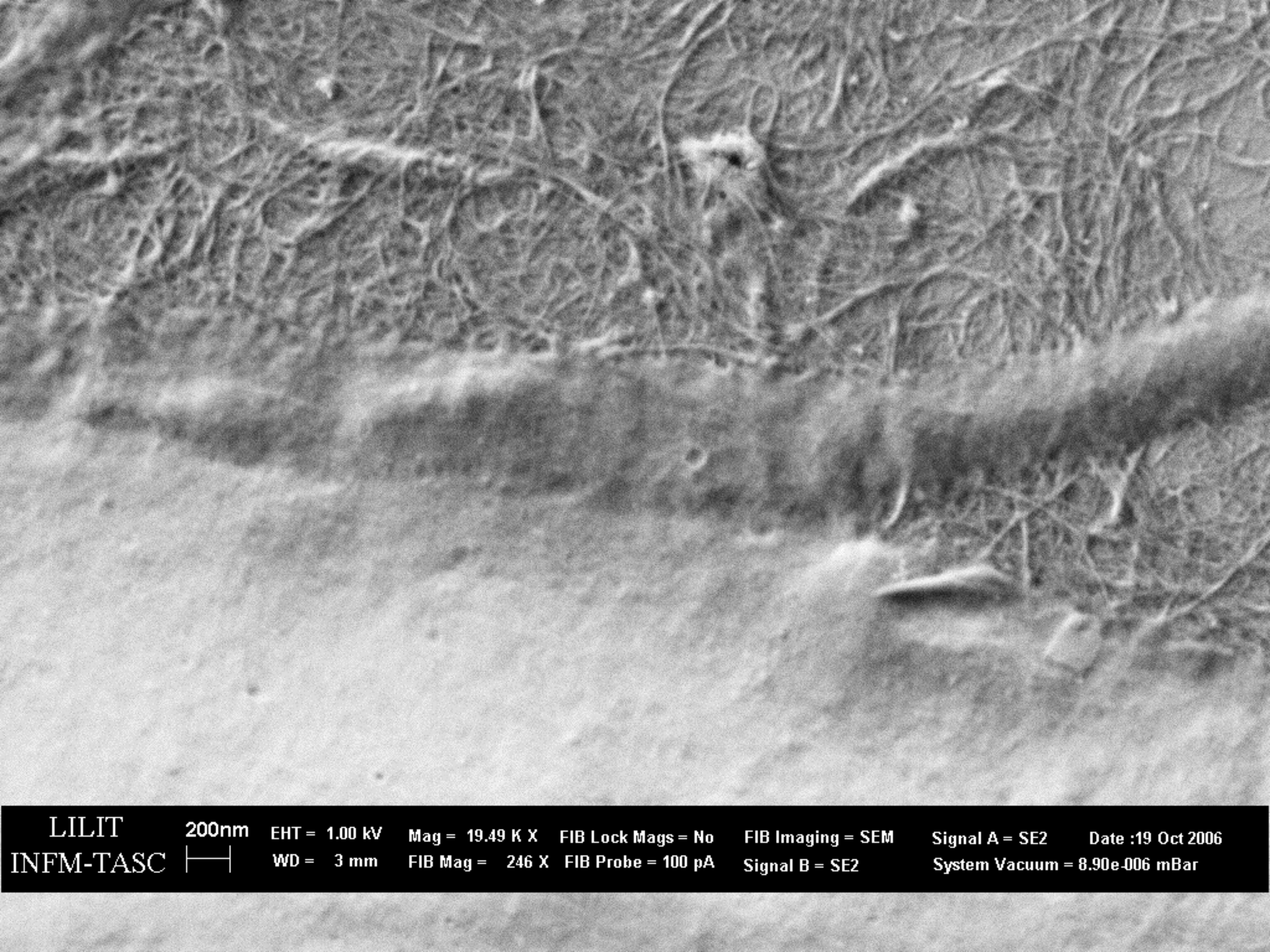


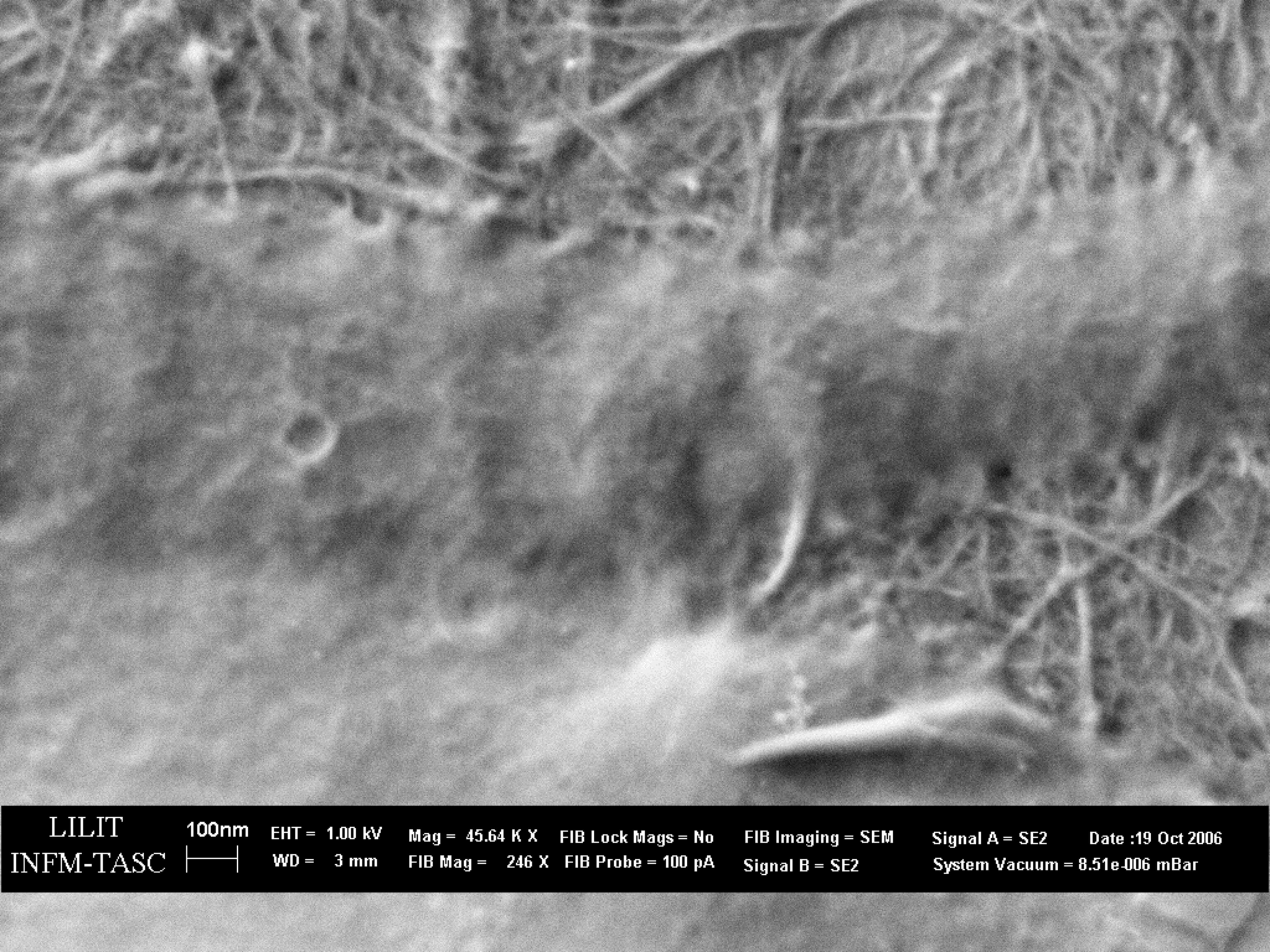


= 50.87 nm
LILIT
INFM-TASC
200nm
EHT = 1.00 kV
WD = 3 mm
Mag = 32.92 K X
FIB Mag = 246 X
FIB Lock Mags = No
FIB Probe = 100 pA
FIB Imaging = SEM
Signal B = SE2
Signal A = SE2
System Vacuum = 8.27e-006 mBar
Date :19 Oct 2006

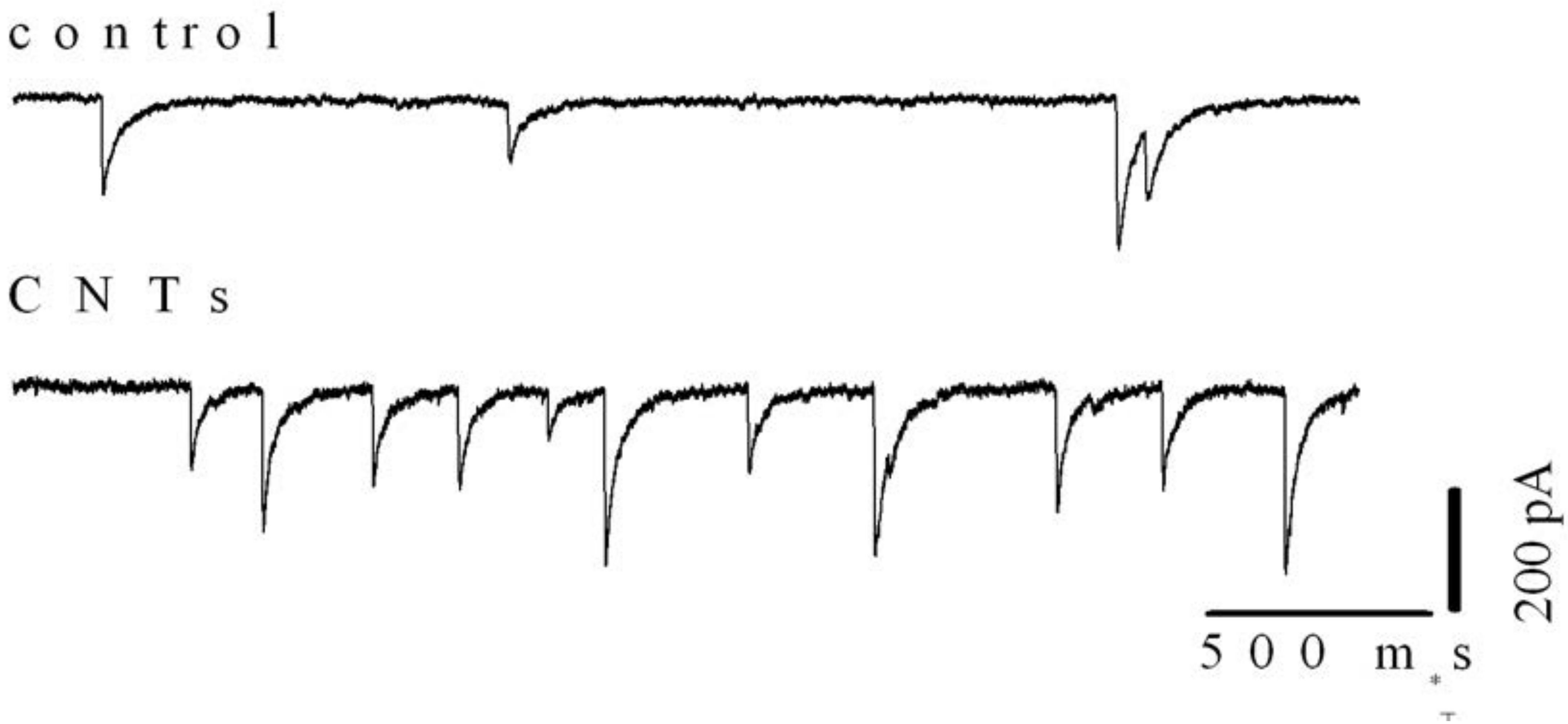

LILIT
INFM-TASC
10µm
EHT = 1.00 kV
WD = 3 mm
Mag = 249 X
FIB Mag = 246 X
FIB Lock Mags = No
FIB Probe = 100 pA
FIB Imaging = SEM
Signal B = SE2
Signal A = SE2
Date :19 Oct 2006
System Vacuum = 1.07e-005 mBar


LILIT
INFM-TASC
2μm
EHT = 1.00 kV
WD = 3 mm
Mag = 3.04 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 = 9.57e-006 mBar
Date :19 Oct 2006

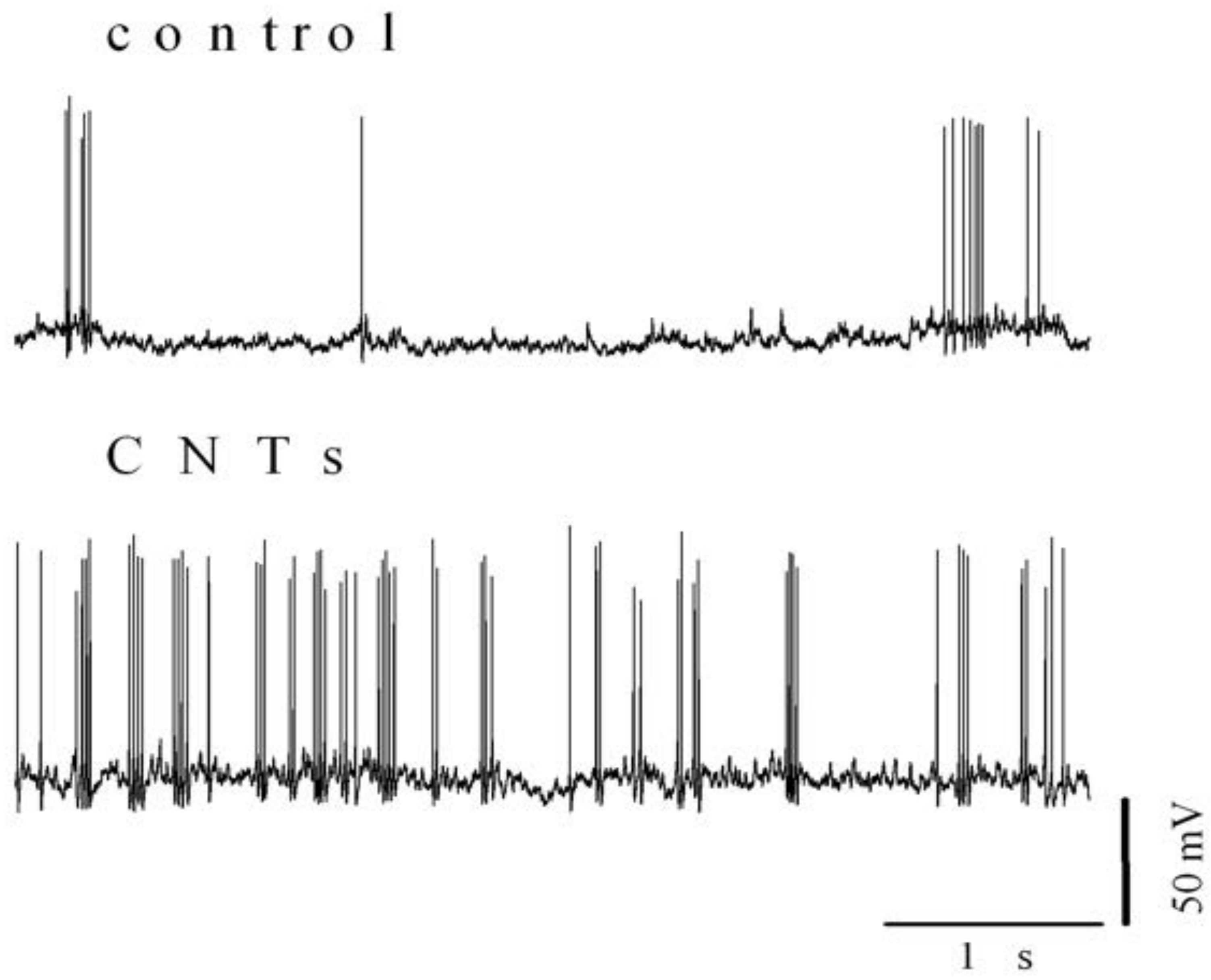

LILIT
INFM-TASC
200nm
EHT = 1.00 kV
WD = 3 mm
Mag = 19.49 K X
FIB Mag = 246 X
FIB Lock Mags = No
FIB Probe = 100 pA
FIB Imaging = SEM
Signal B = SE2
Signal A = SE2
System Vacuum = 8.90e-006 mBar
Date :19 Oct 2006


LILIT
INFM-TASC
100nm
EHT = 1.00 kV
WD = 3 mm
Mag = 45.64 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 = 8.51e-006 mBar
Date :19 Oct 2006

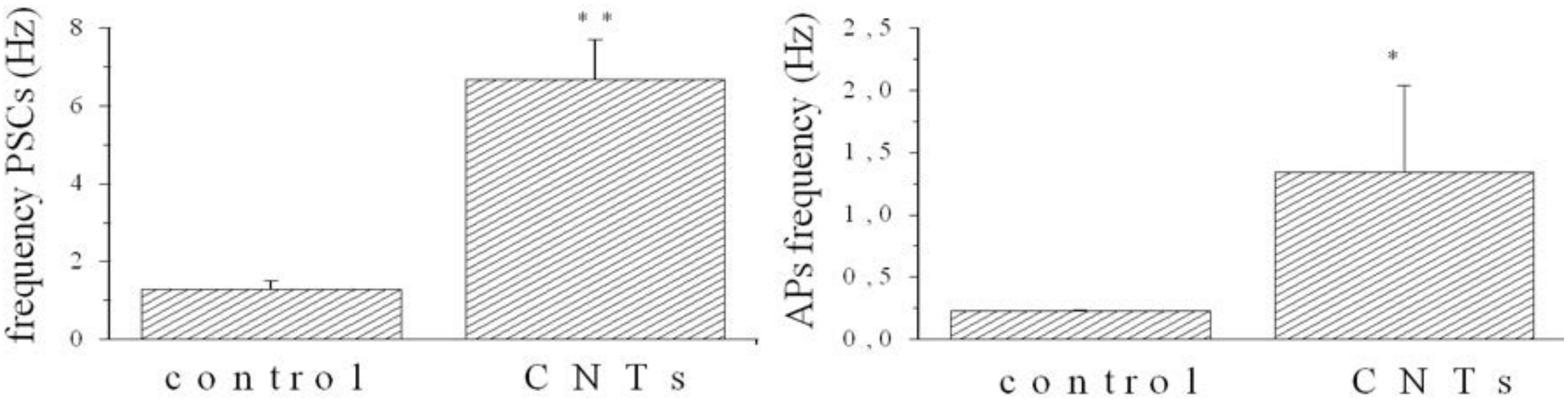
Patch-Clamp



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.

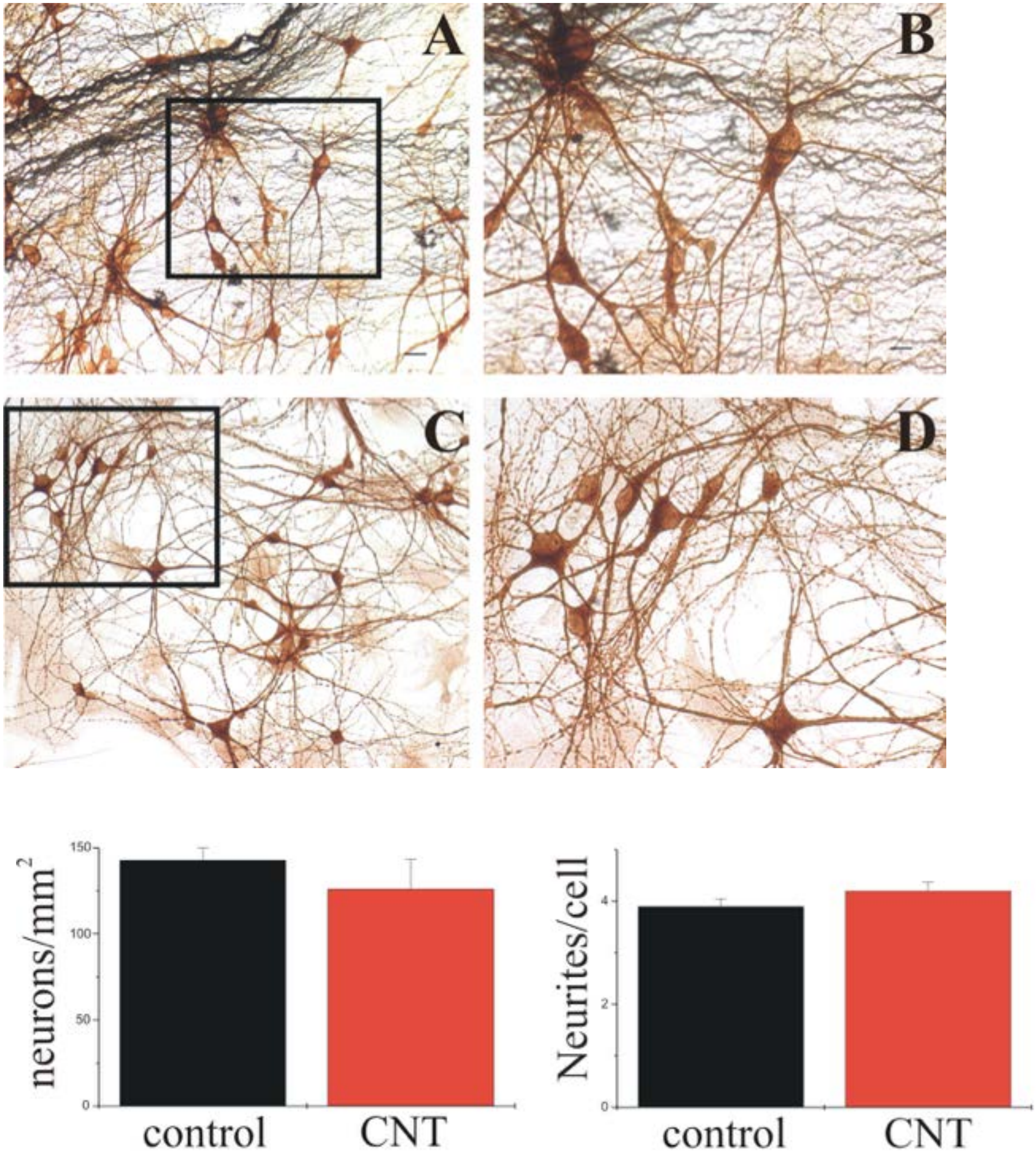


Current clamp recordings from cultured hippocampal neurons in control (top tracings) and CNT growth conditions (bottom tracings). Spontaneous firing activity is greatly boosted in the presence of CNT substrates.



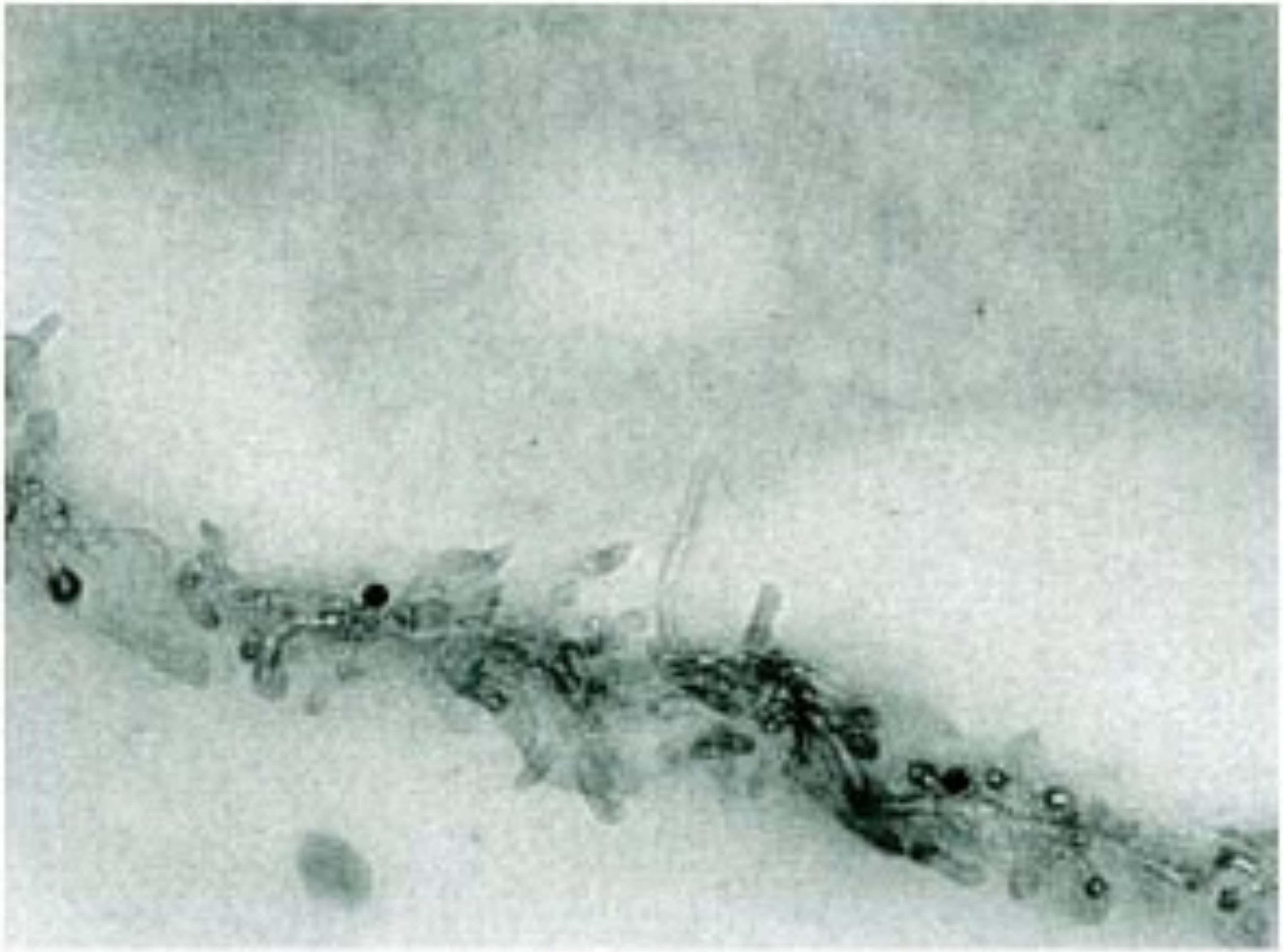
Histogram plots of Post-Synaptic Currents (PSCs, left) and Action Potentials (Aps,right) frequency in control and CNT cells.
Note the significant increase in the occurrence of both events when measured in CNT cultures.

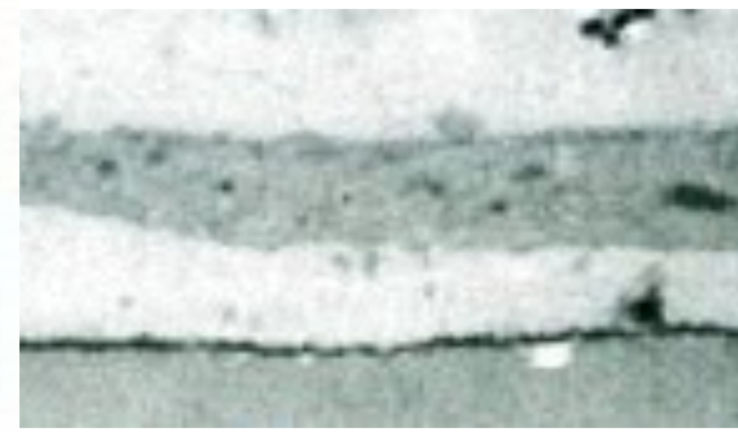
Nanoletters 2005



A
B
C
D
neurons/mm2
150
100
50
0
control
CNT
Neurites/cell
4
2
0
control
CNT

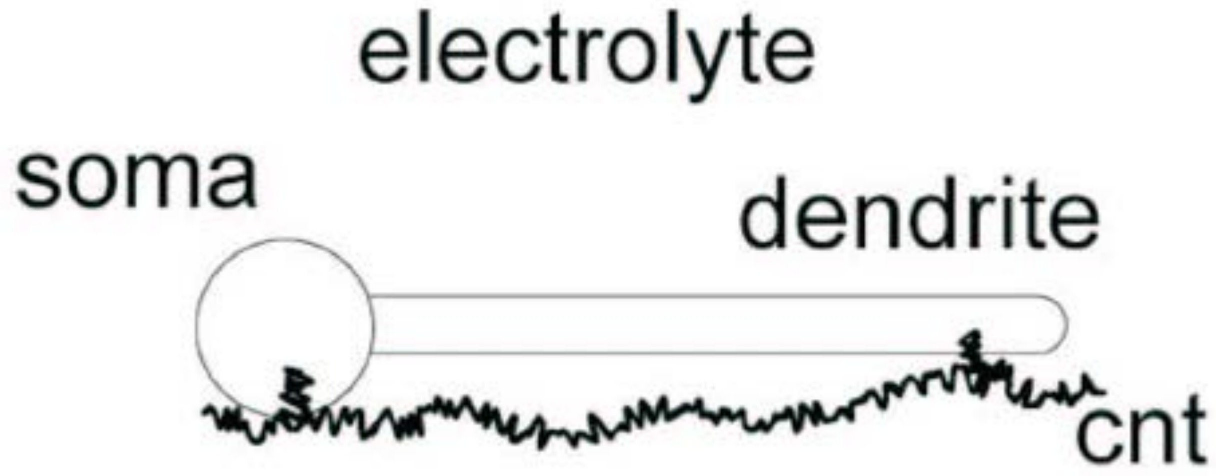
Transversal sections of the glass coverslips

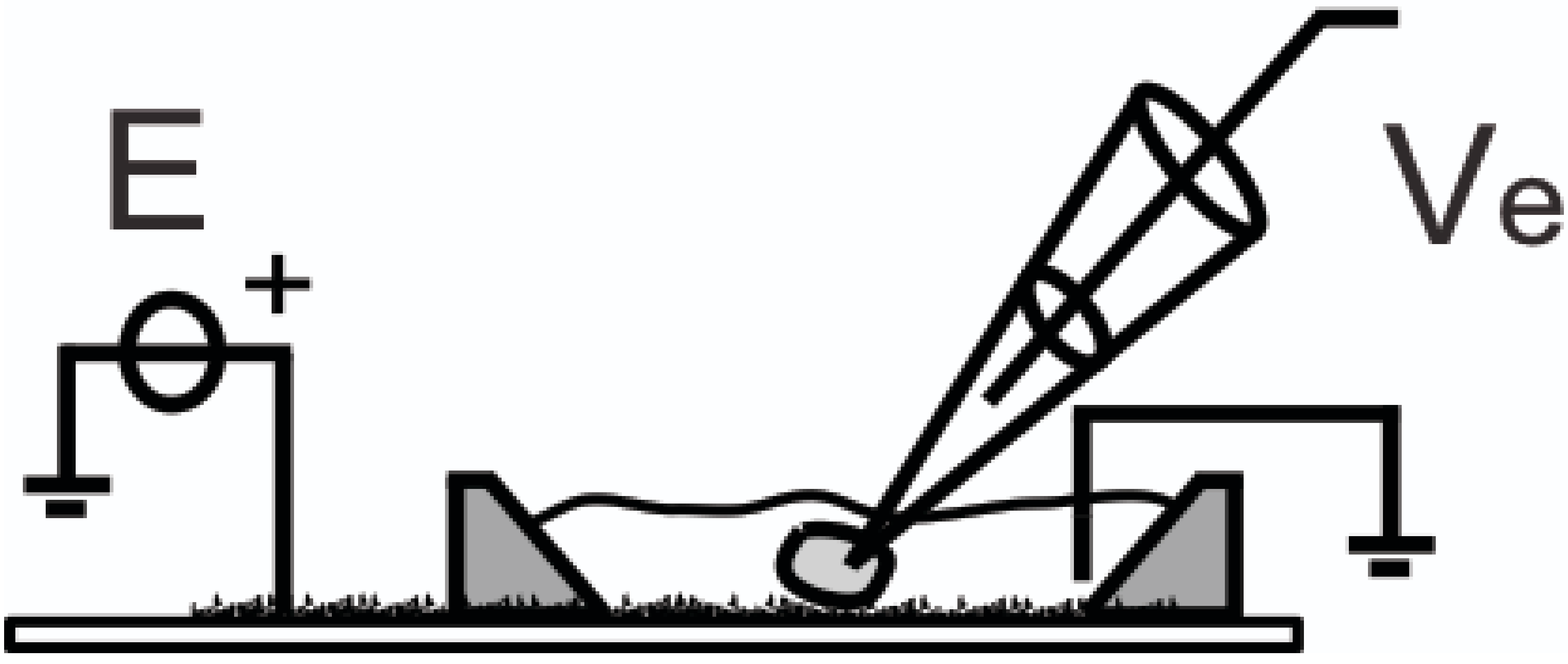




Reasons for increased frequency of activity of neurons on CNTs

1) Direct electrical coupling between nearby dendritic compartments

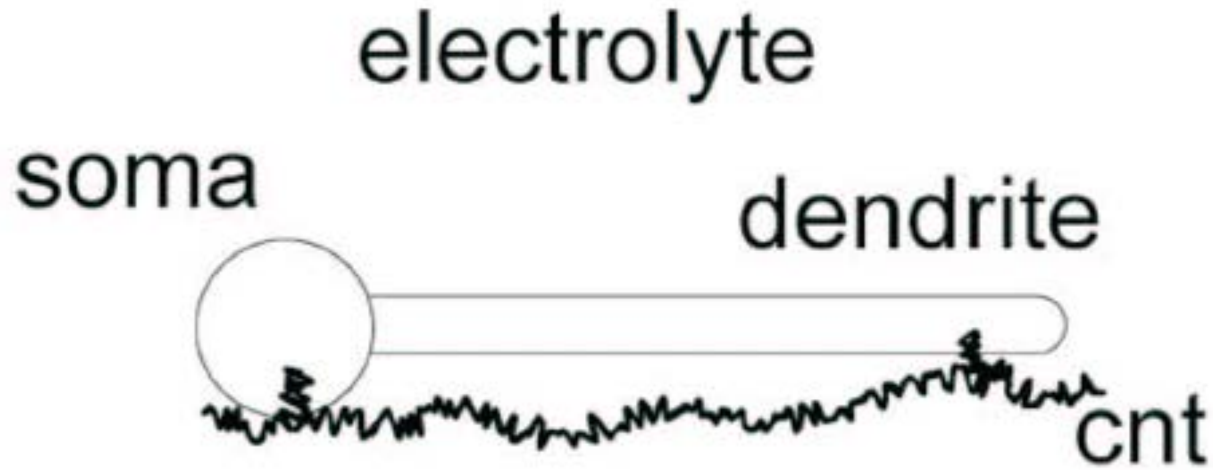




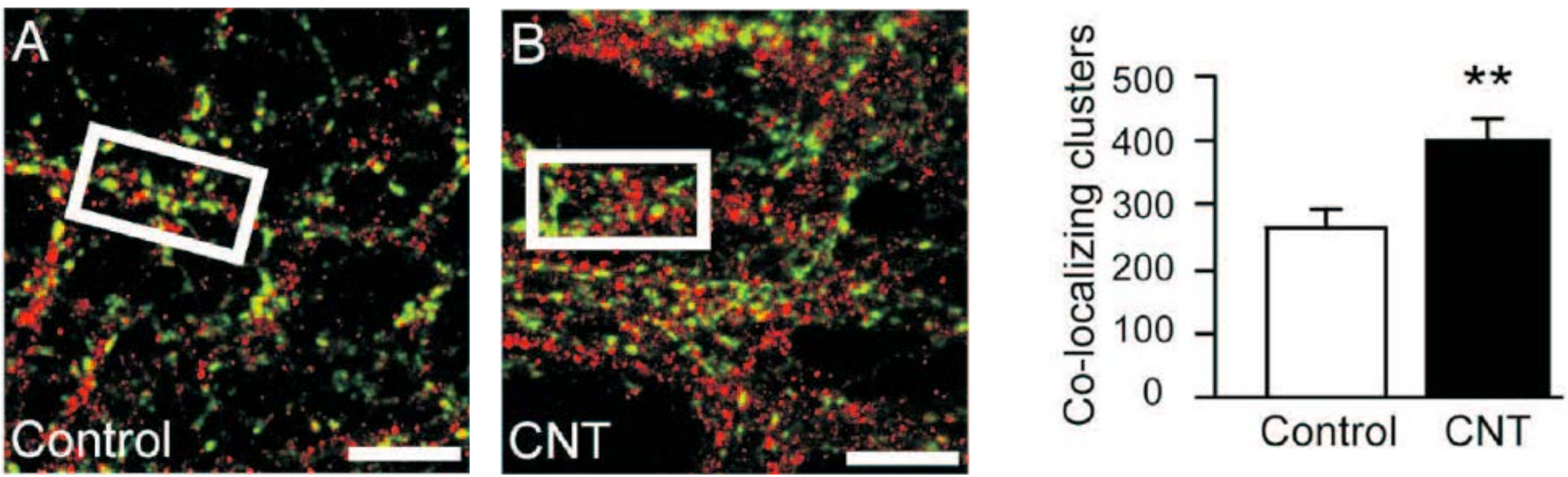
J. Neurosc. 2007

Reasons for increased frequency of activity of neurons on CNTs

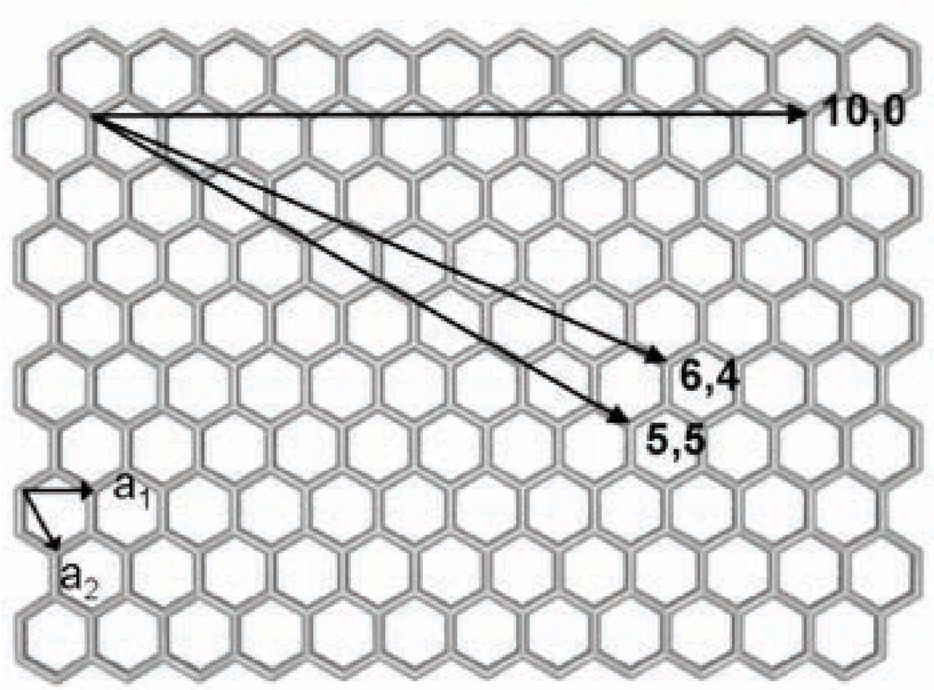
1) Direct electrical coupling between nearby dendritic compartments



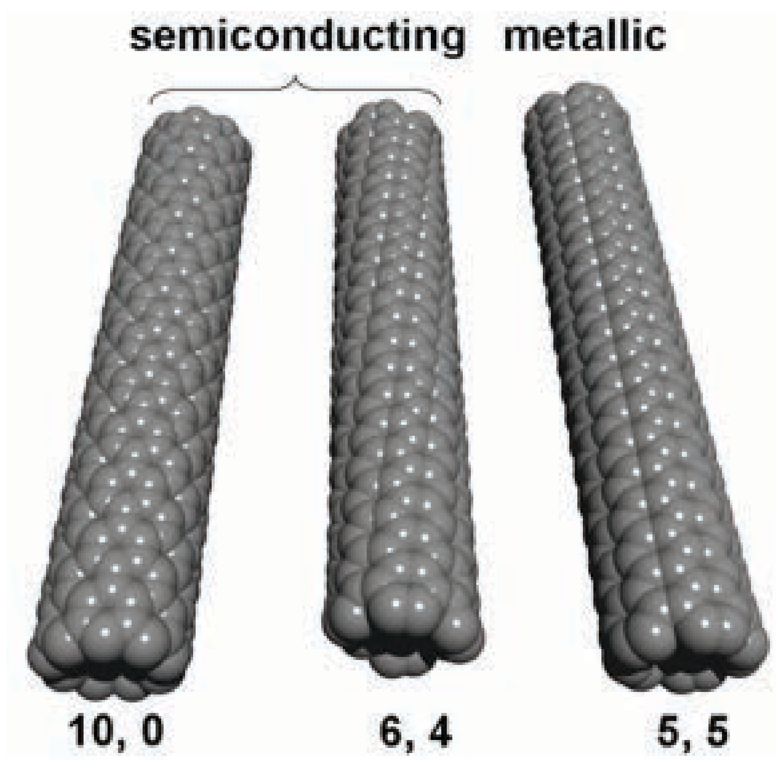
2) Immunofluorescence experiments show an increased number of synaptic contacts



Atomic resolution STM



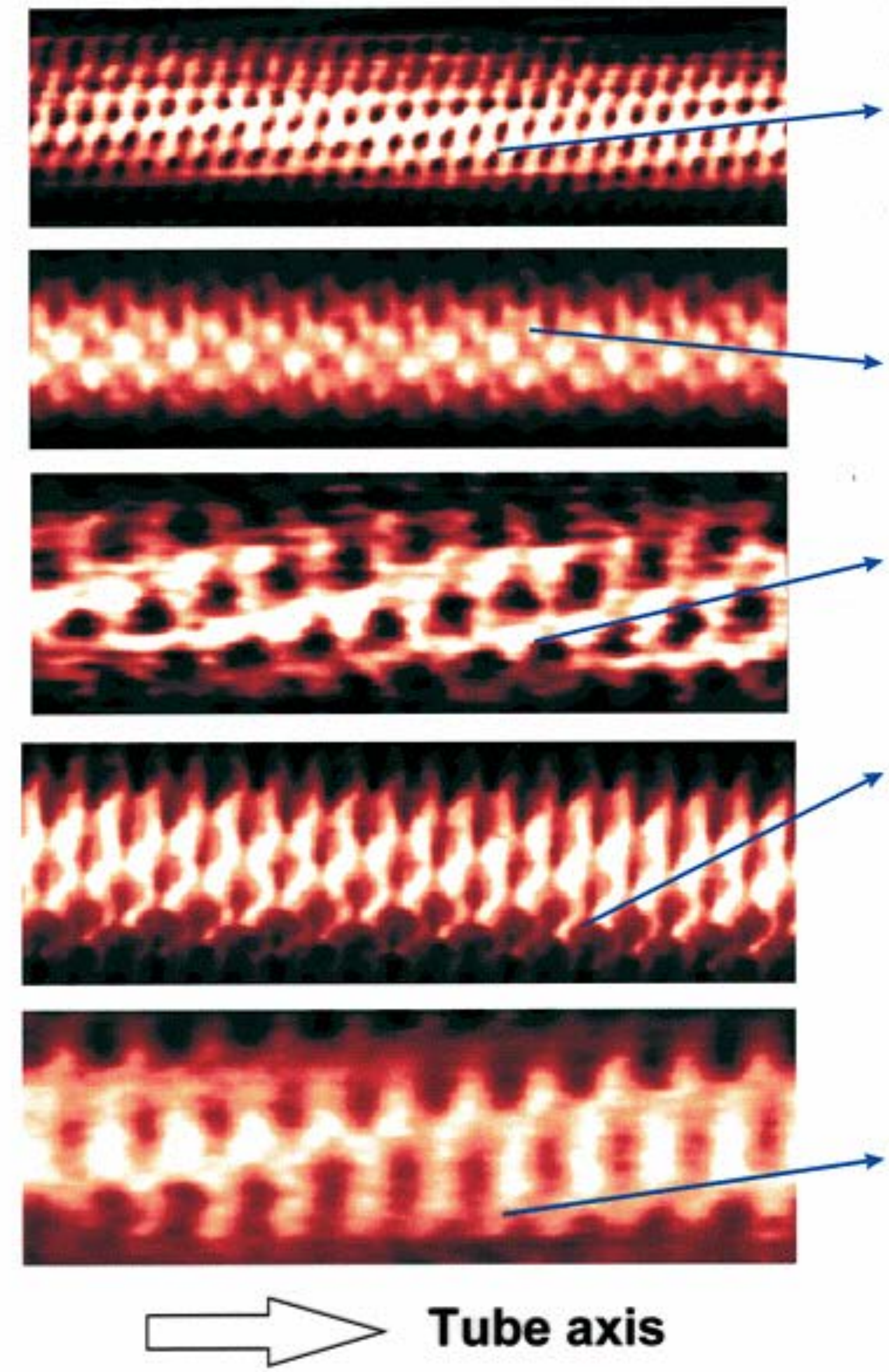
Zigzag



Armchair

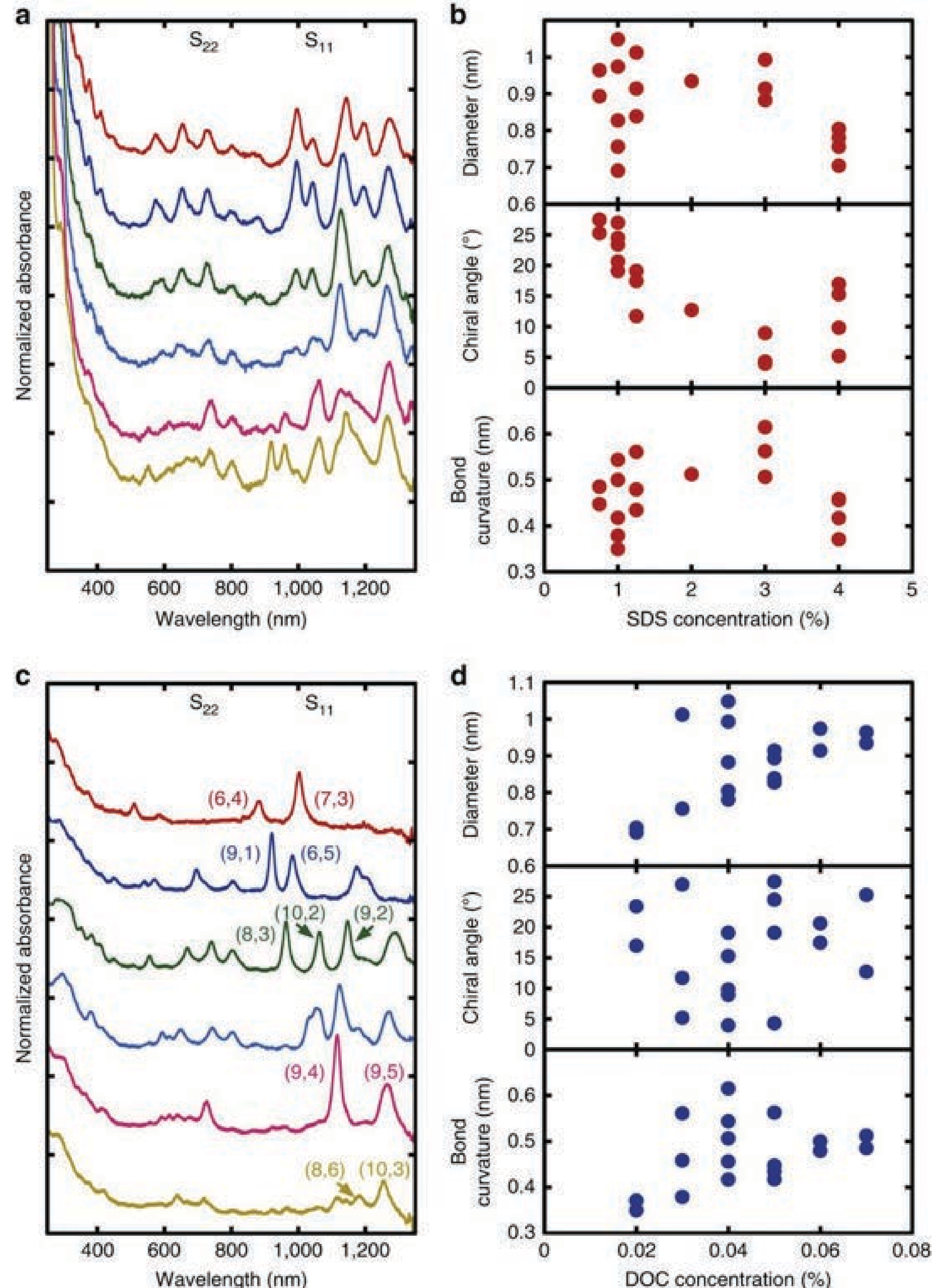
chiral index (n, m):. nanotubes in which n = m are metallic and quasi-metallic (with tiny band gap) if n-m is divisible by 3. All other tubes are semiconducting with band gaps of the order of 0.5 eV

Large variety of helicities



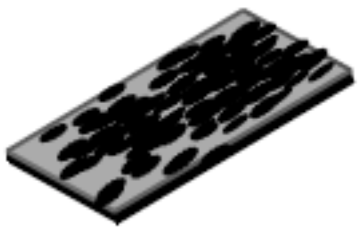
Taken from Cees Dekker (Delft, NL) website

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.

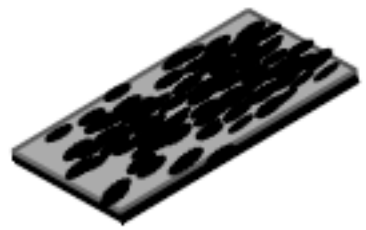


Nature Communications volume 7, Article number: 12056 (2016)

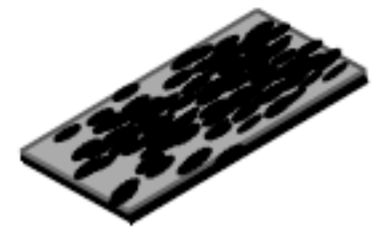
Comparison among different types of CNTs



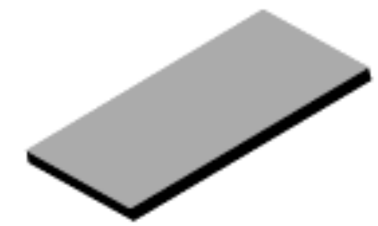
metallic SWCNTs



semiconducting SWCNTs



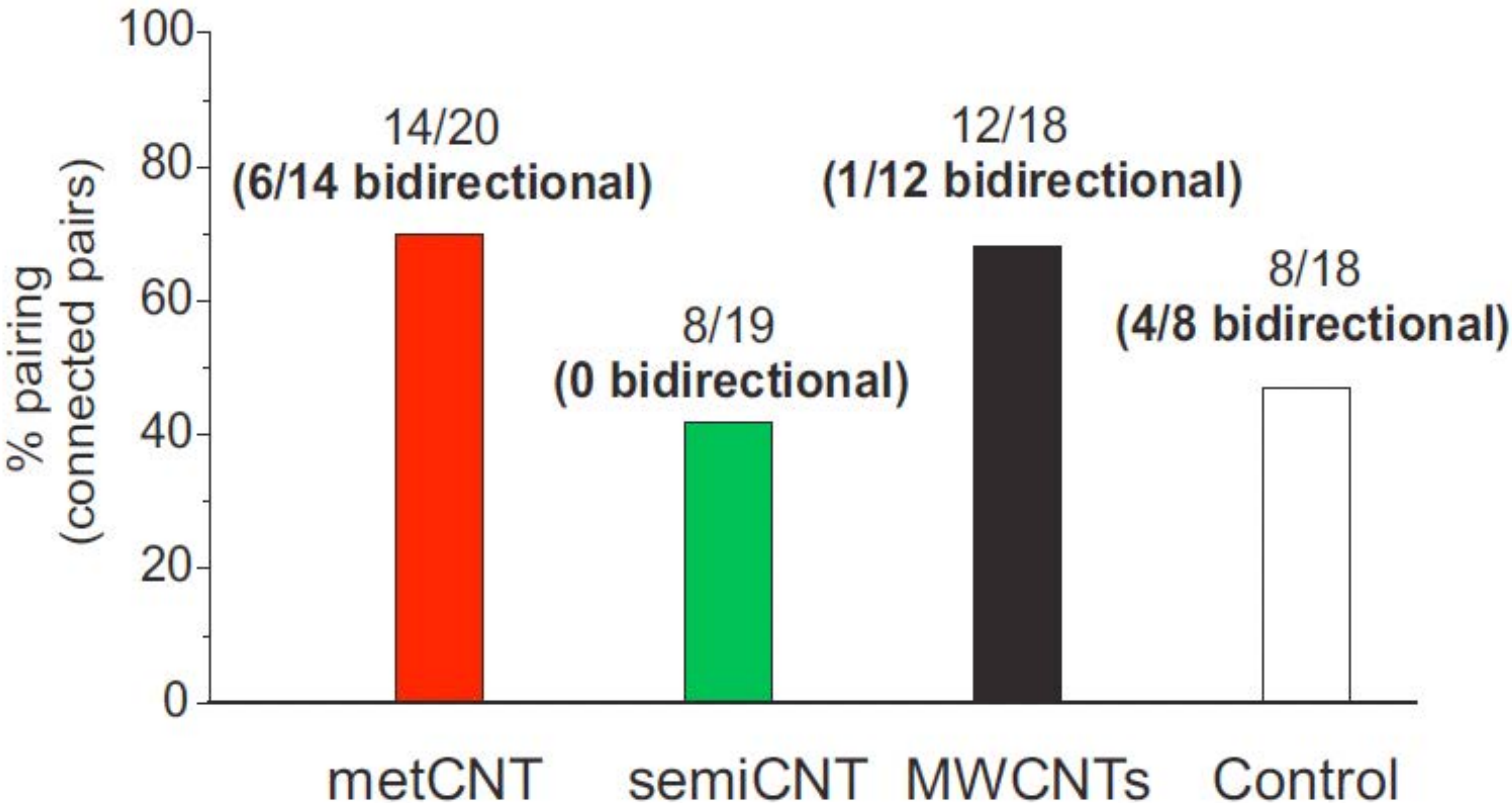
MWCNTs



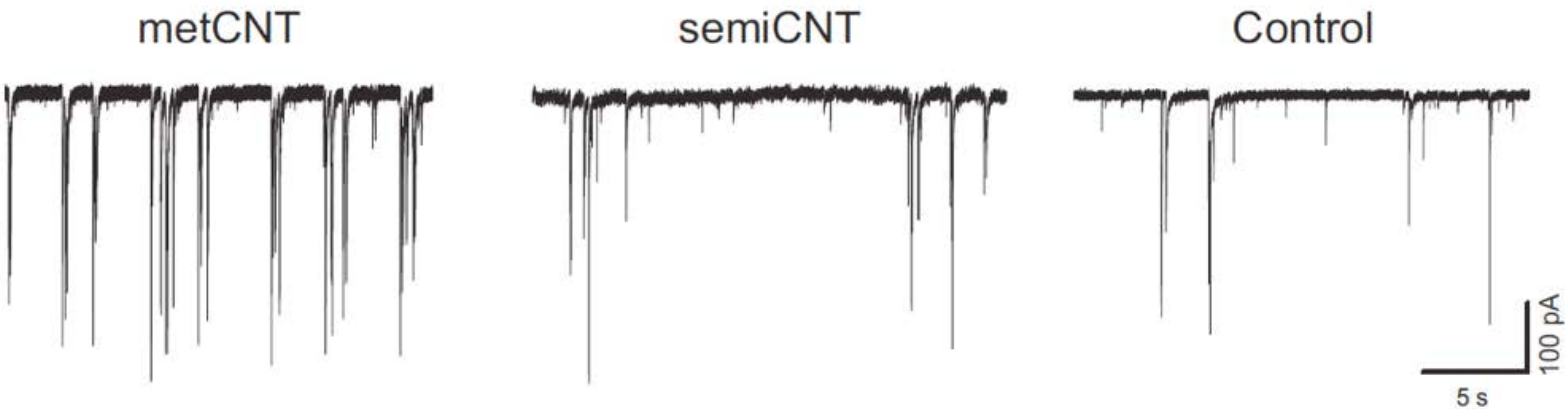
control
(pure glass)

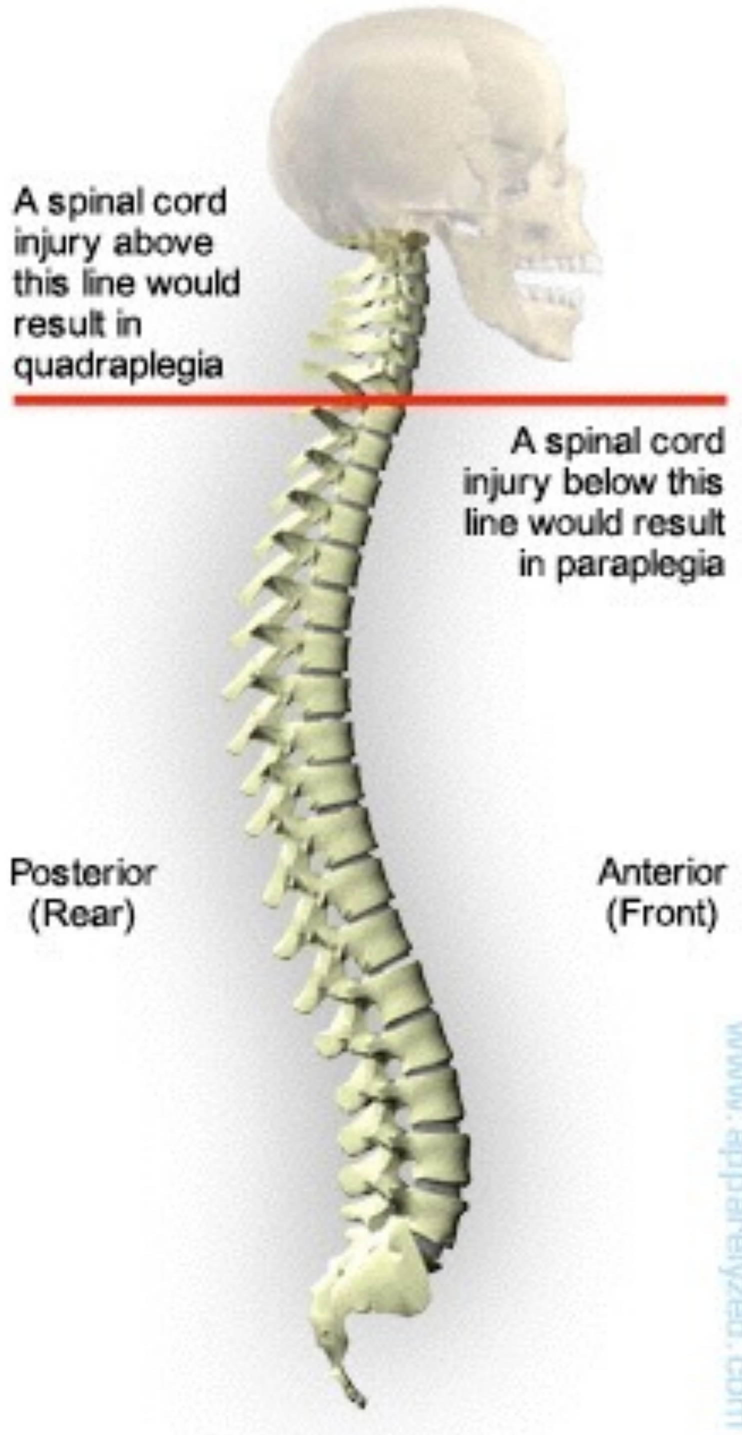
metallic and semiconducting SWCNTs were separated by Francesco Bonaccorso in Andrea Ferrari's laboratory in Cambridge, by density gradient separation

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

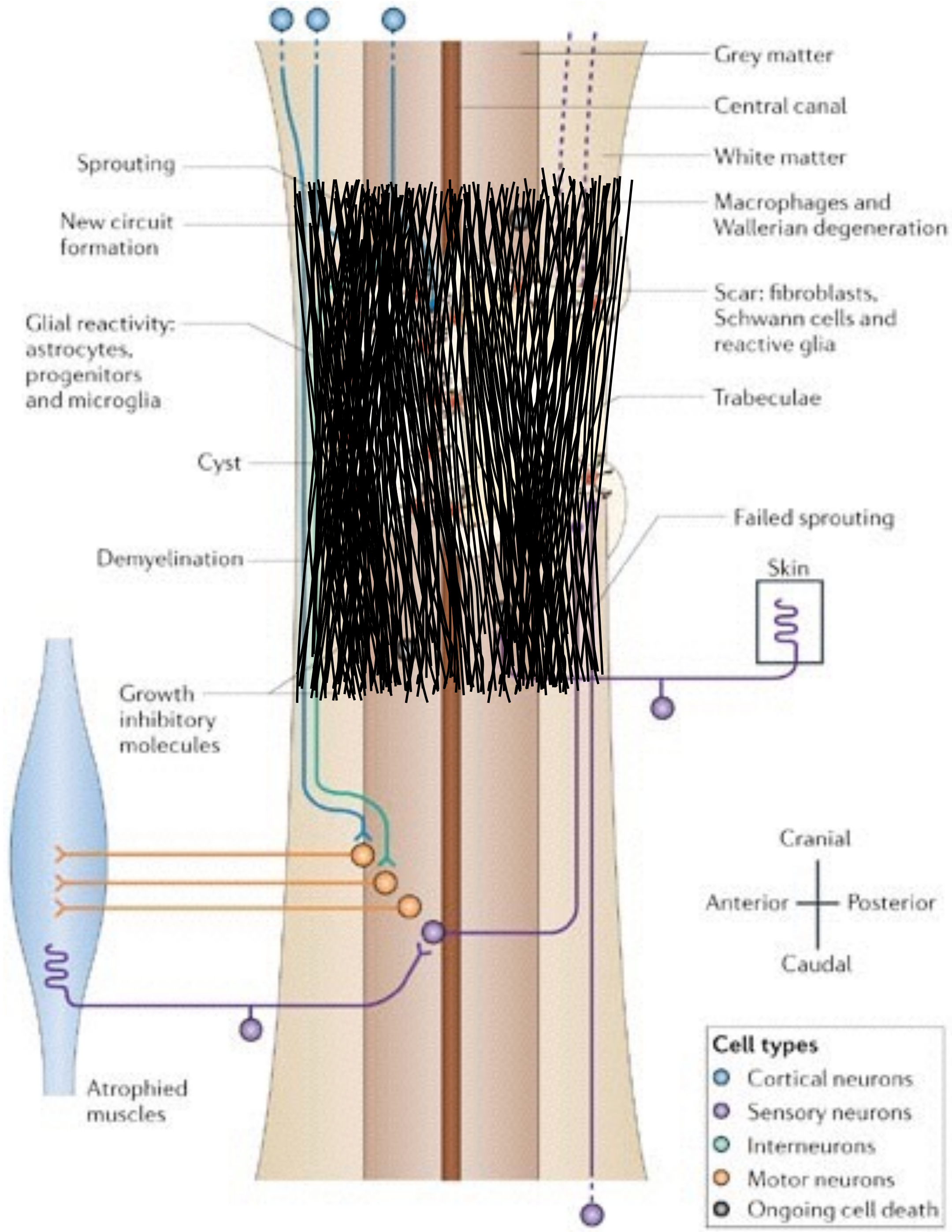


Substrate effect on spontaneous activity: frequency of the spontaneous postsynaptic currents (PSCs)





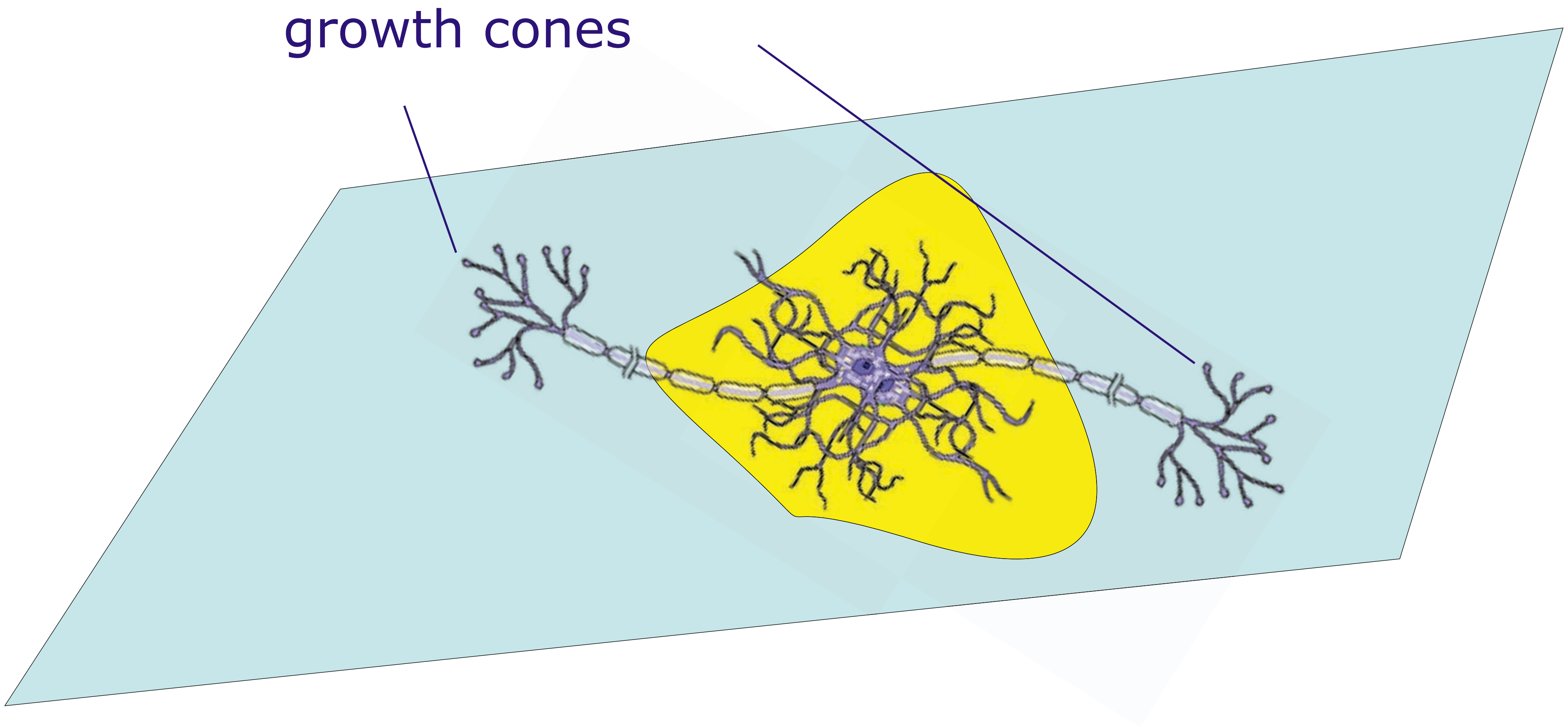
A spinal cord injury above this line would result in quadraplegia
A spinal cord injury below this line would result in paraplegia
Posterior (Rear)
Anterior (Front)
www.apparelyzed.com

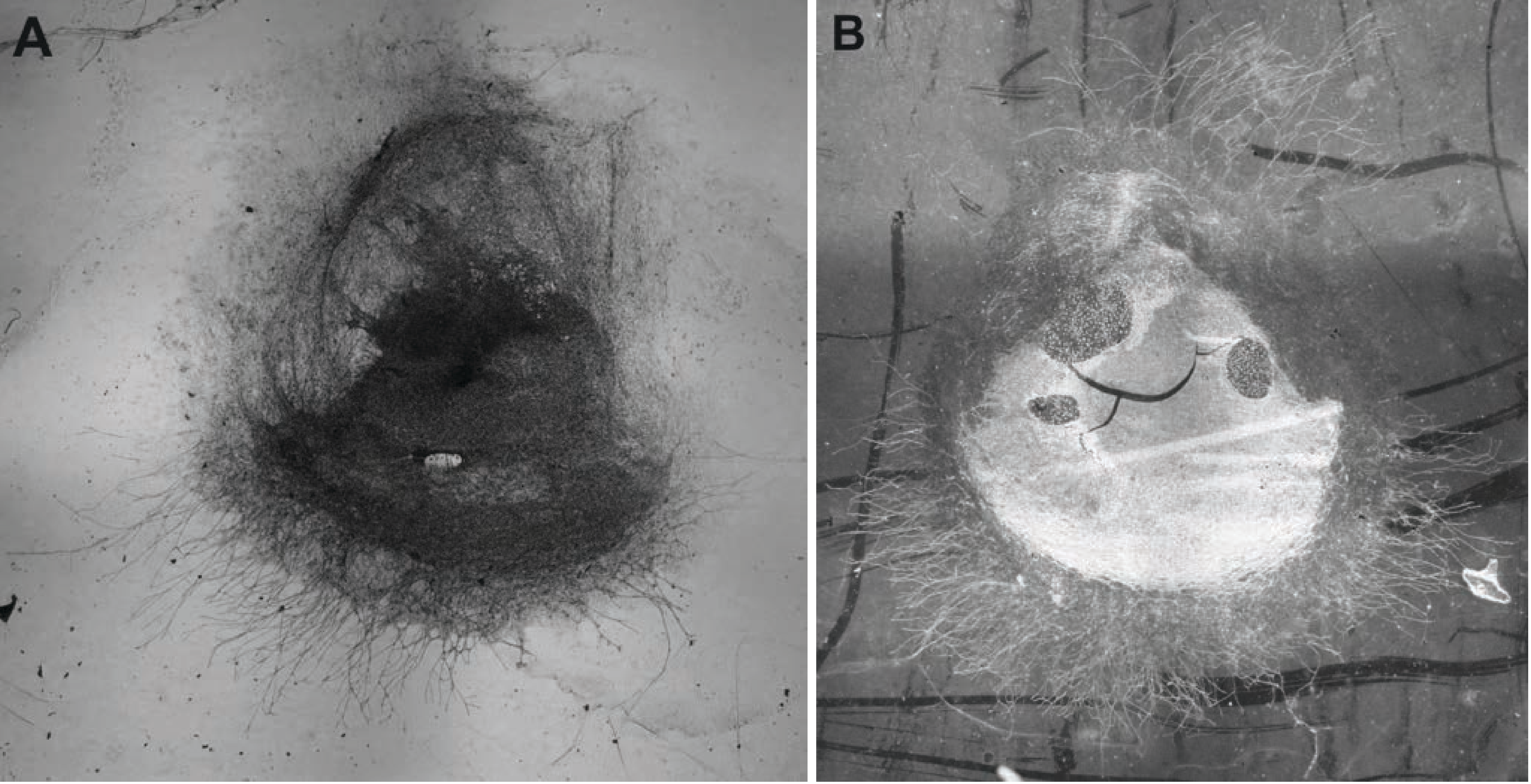


Grey matter
Central canal
White matter
Macrophages and Wallerian degeneration
Scar: fibroblasts, Schwann cells and reactive glia
Trabeculae
Failed sprouting
Skin
Sprouting
New circuit formation
Glial reactivity: astrocytes, progenitors and microglia
Cyst
Demyelination
Growth inhibitory molecules
Atrophied muscles
Cranial
Anterior
Posterior
Caudal
Cell types
Cortical neurons
Sensory neurons
Interneurons
Motor neurons
Ongoing cell death

An entire slice of spinal cord tissue

slices of ca 150 μm



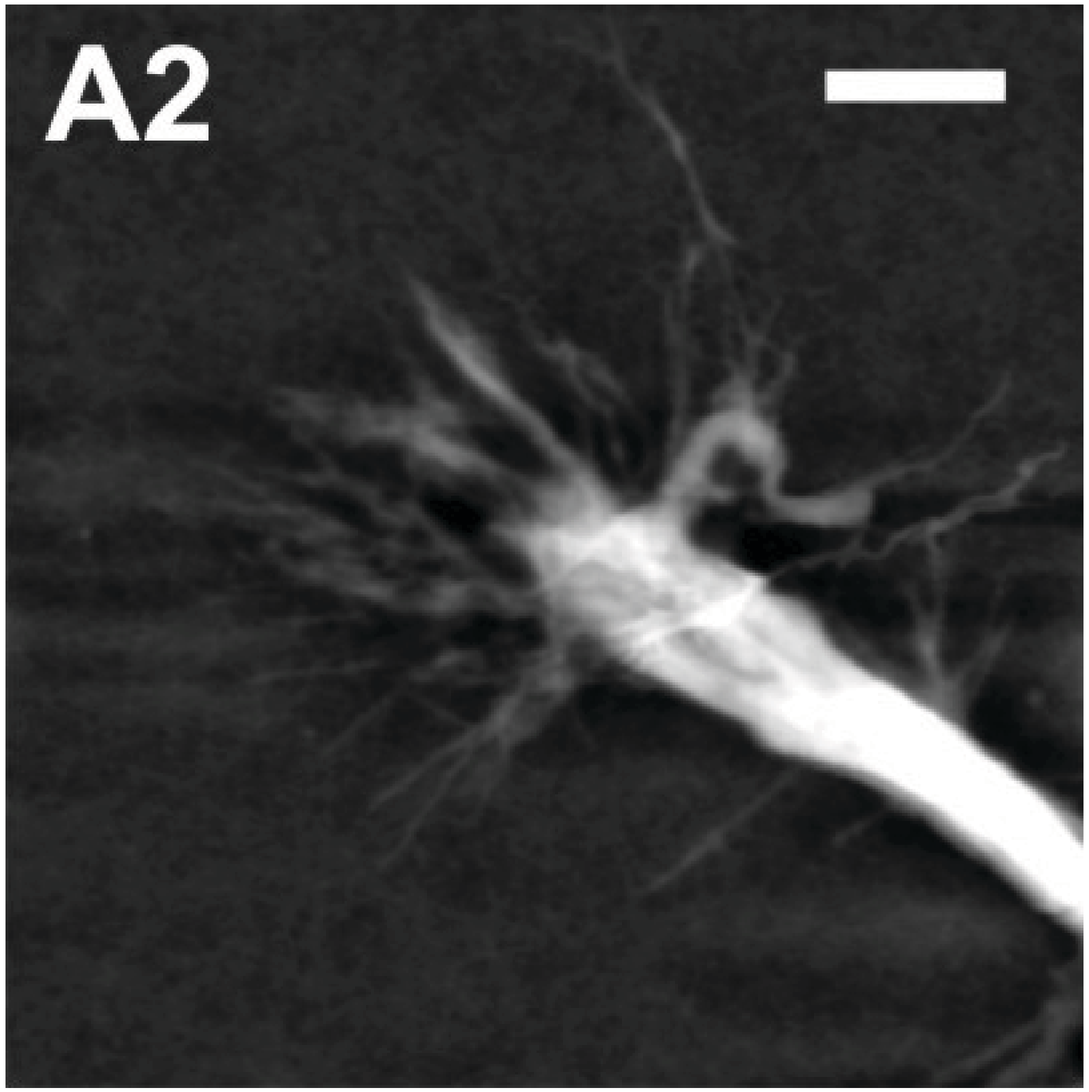


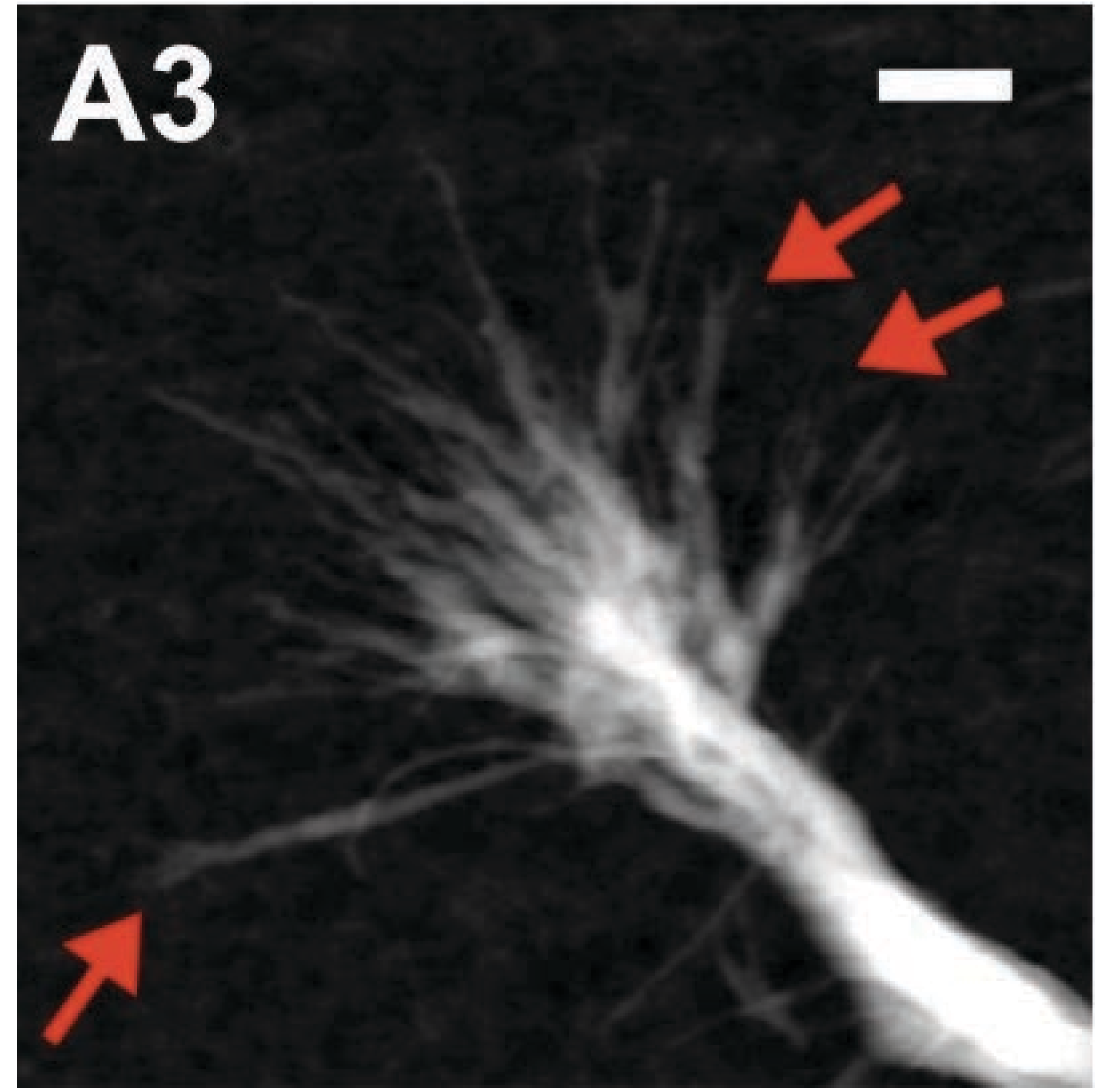
control

CNT (phase contrast)

Spinal explants (slices of 150 um ca)
On CNT, the number of fibers grow 40% more (15 samples), length increases by 25% ca

Fabbro, A. er al, *ACS Nano* **2012**, *6*, 2041-2055.

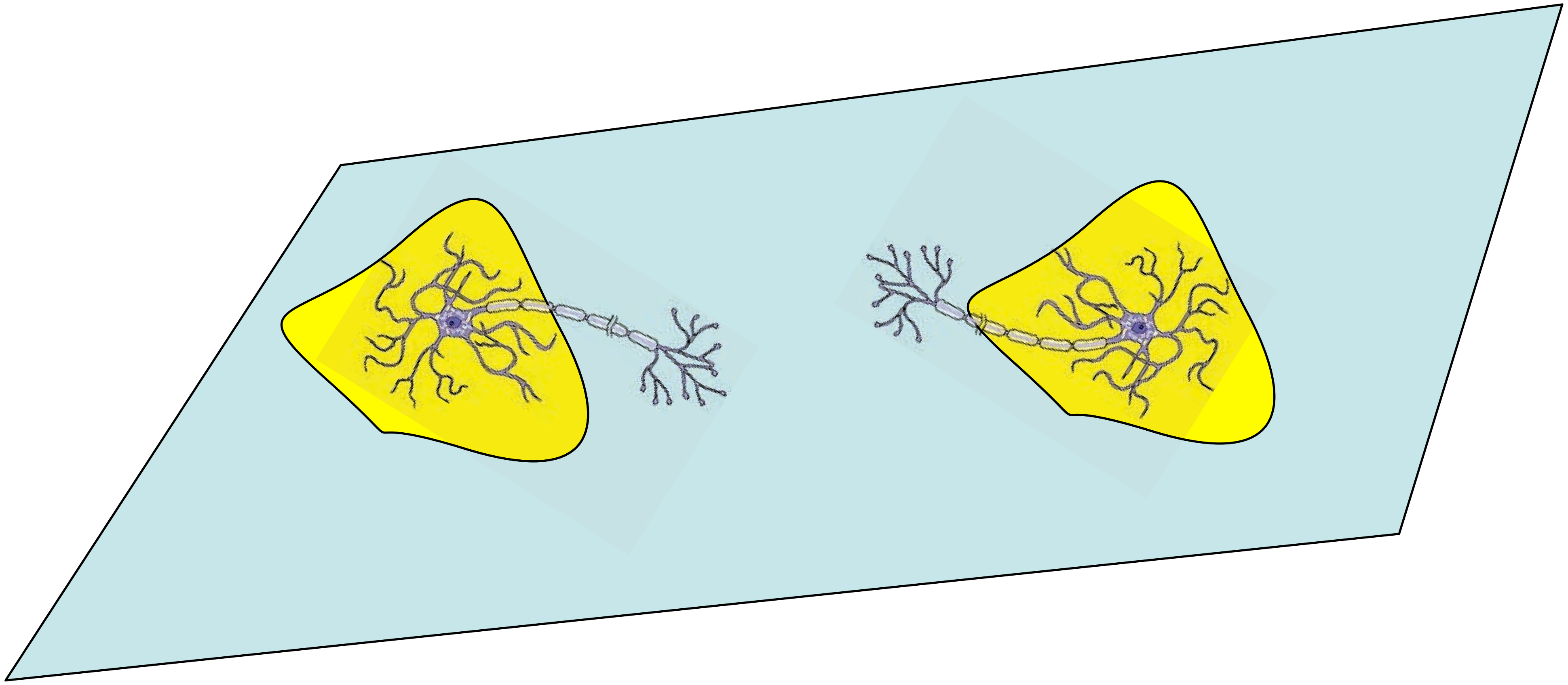




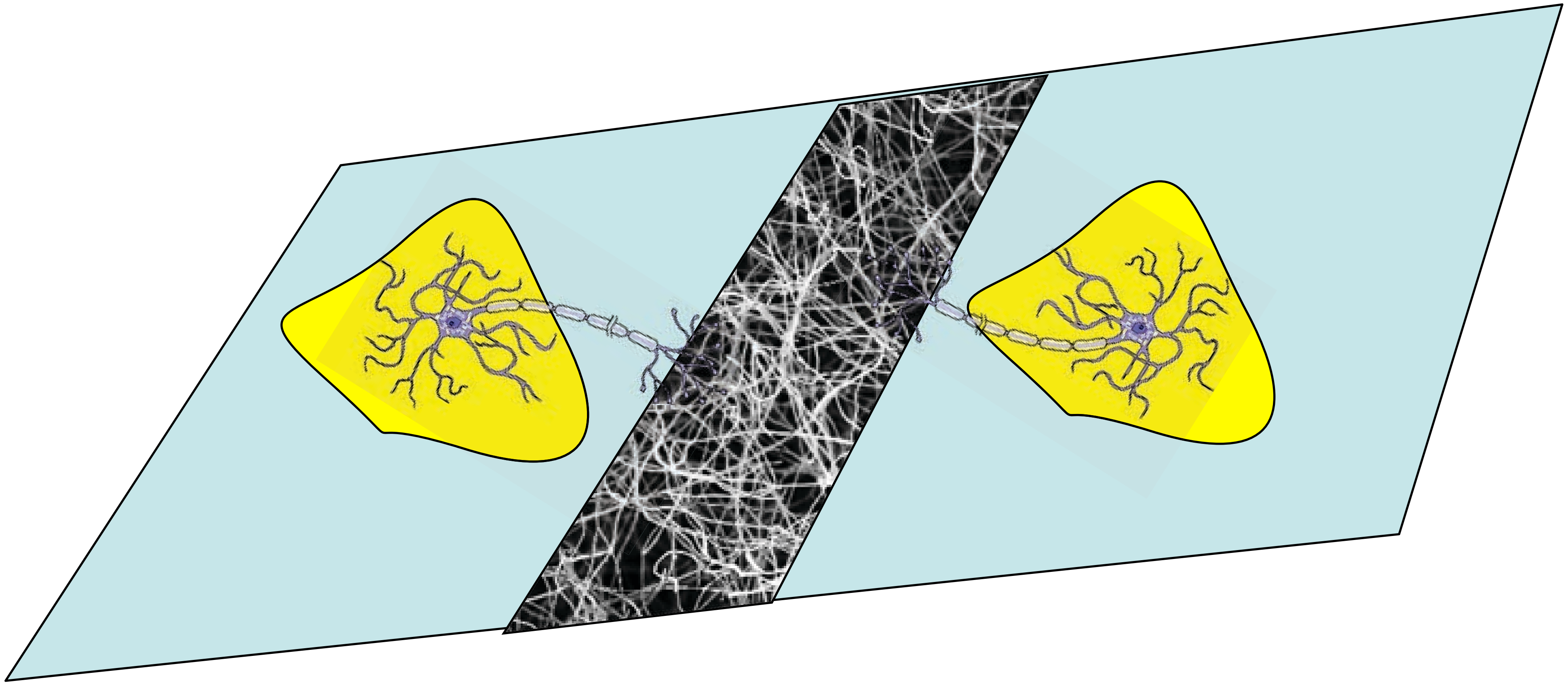
Growth cones grow more distant on CNT

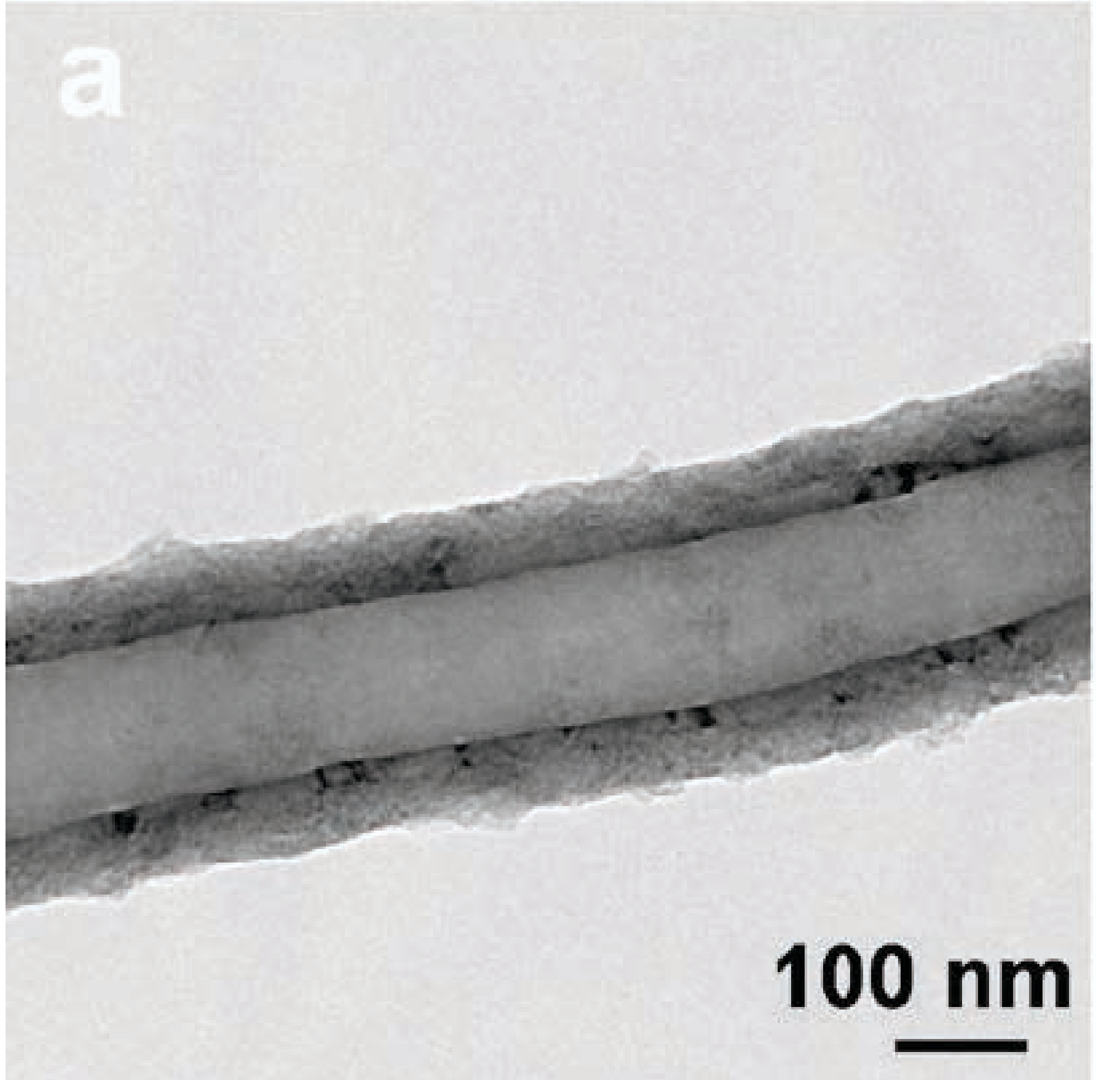
Fabbro, A. er al, *ACS Nano* **2012**, *6*, 2041-2055.

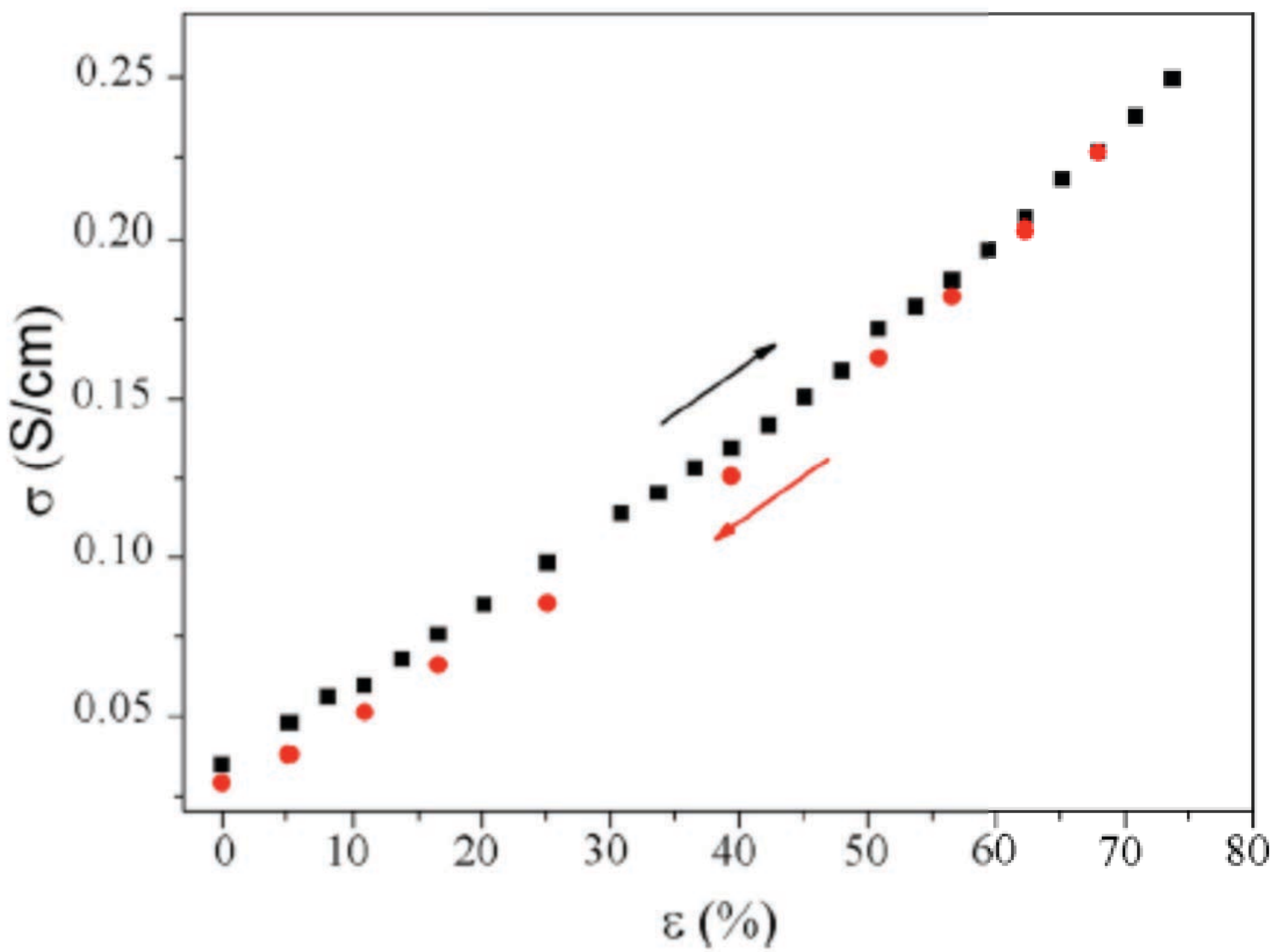
Looking for communication between two separated slices

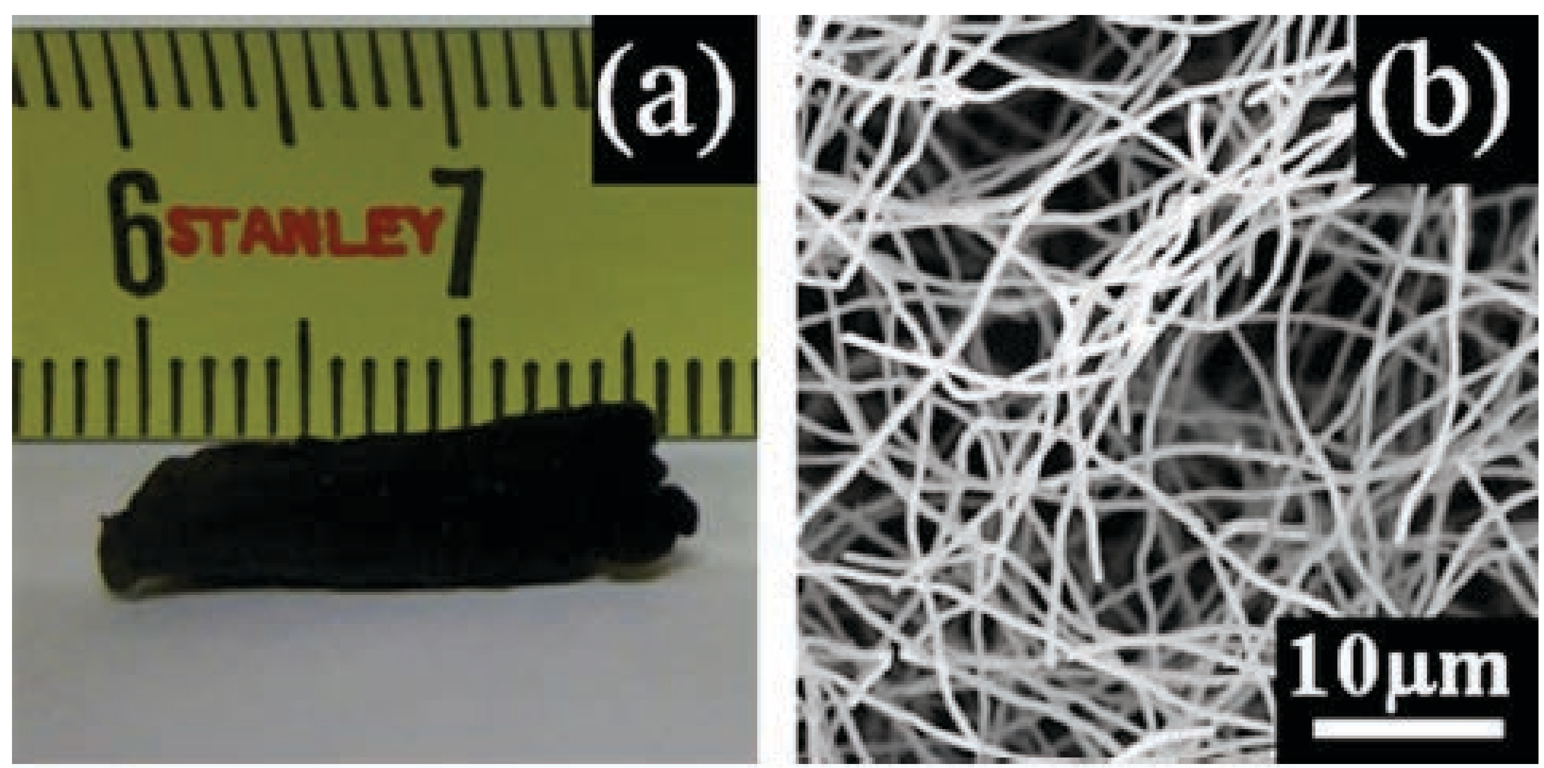


Looking for communication between two separated slices

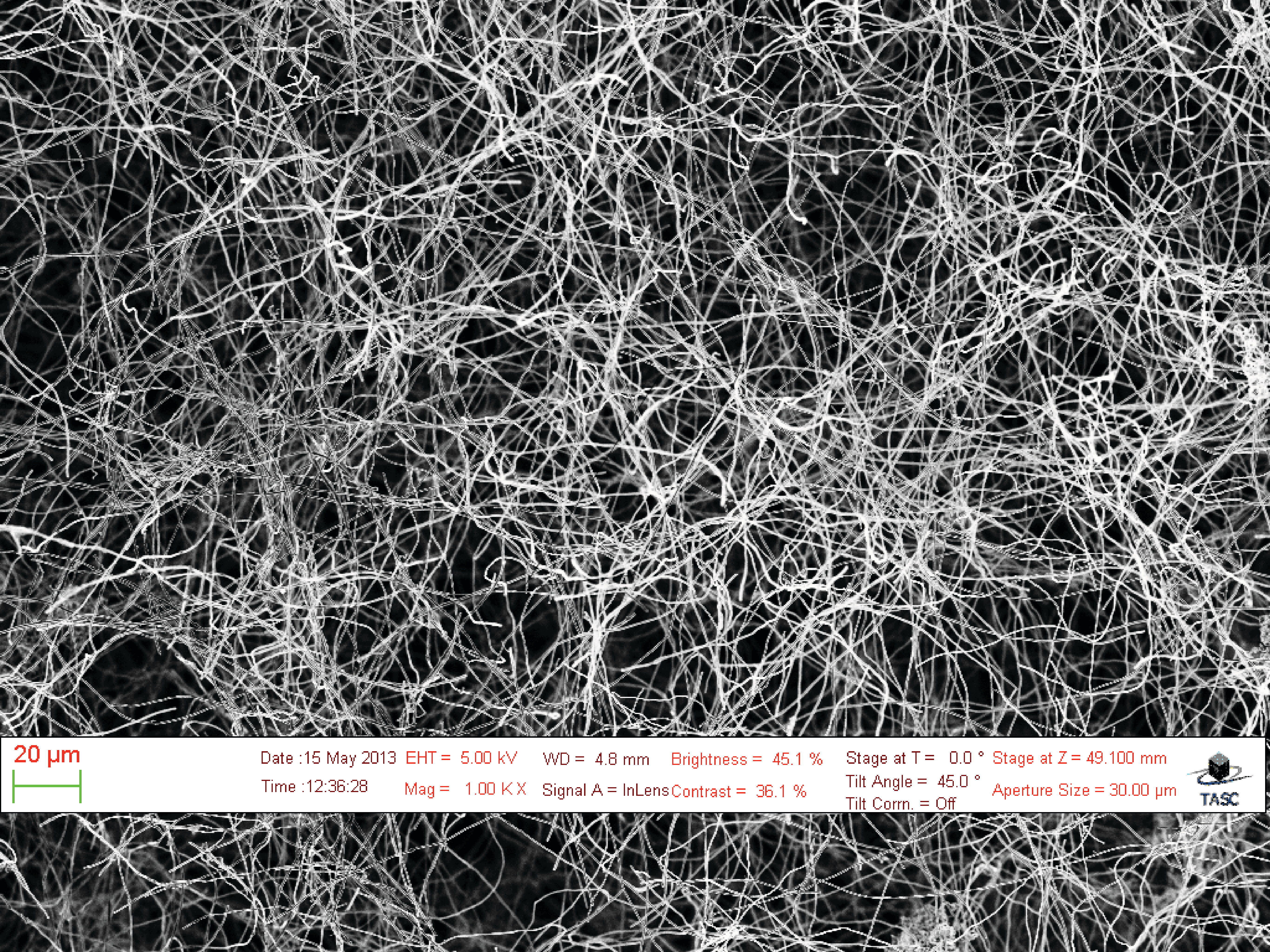


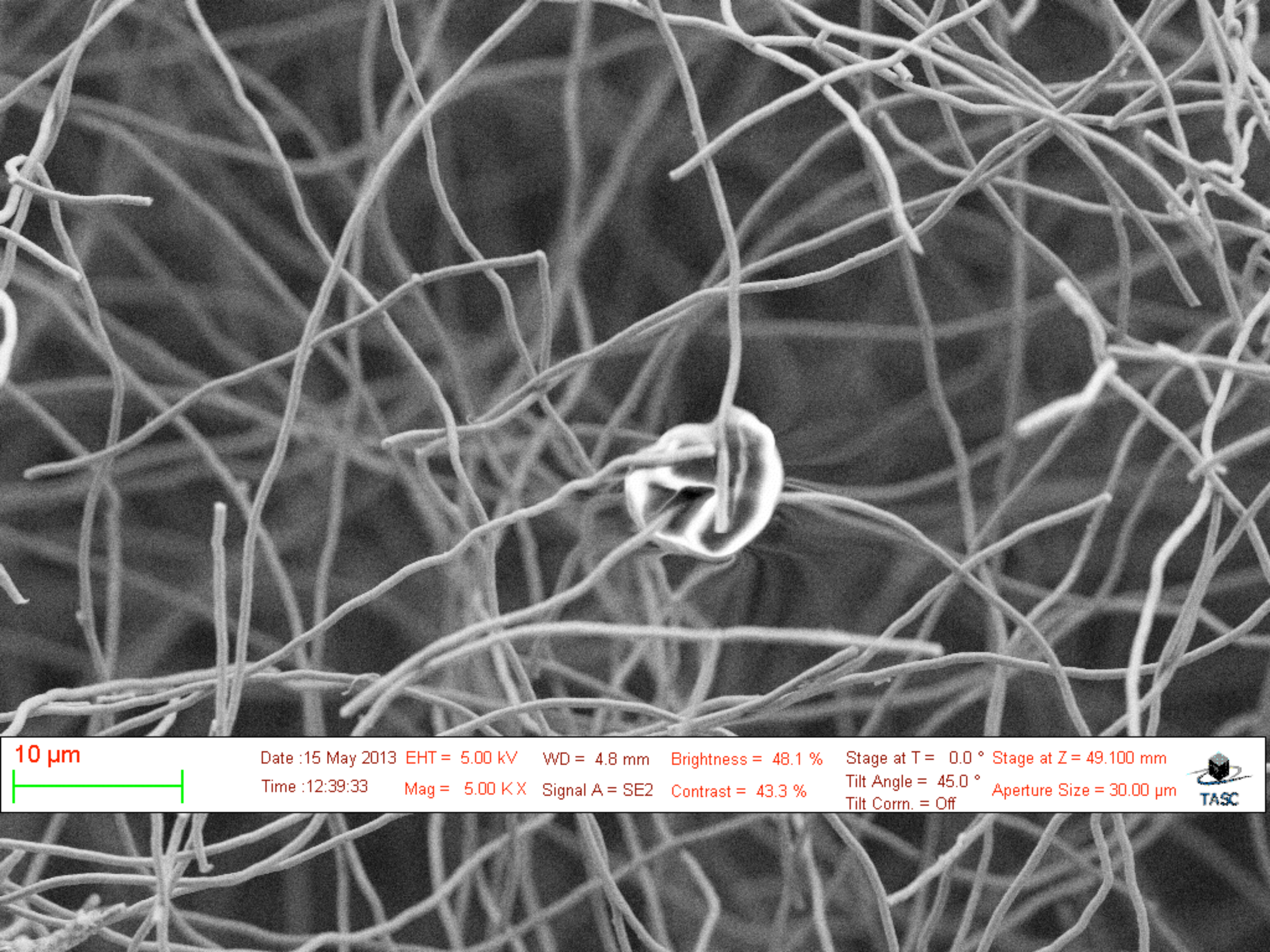


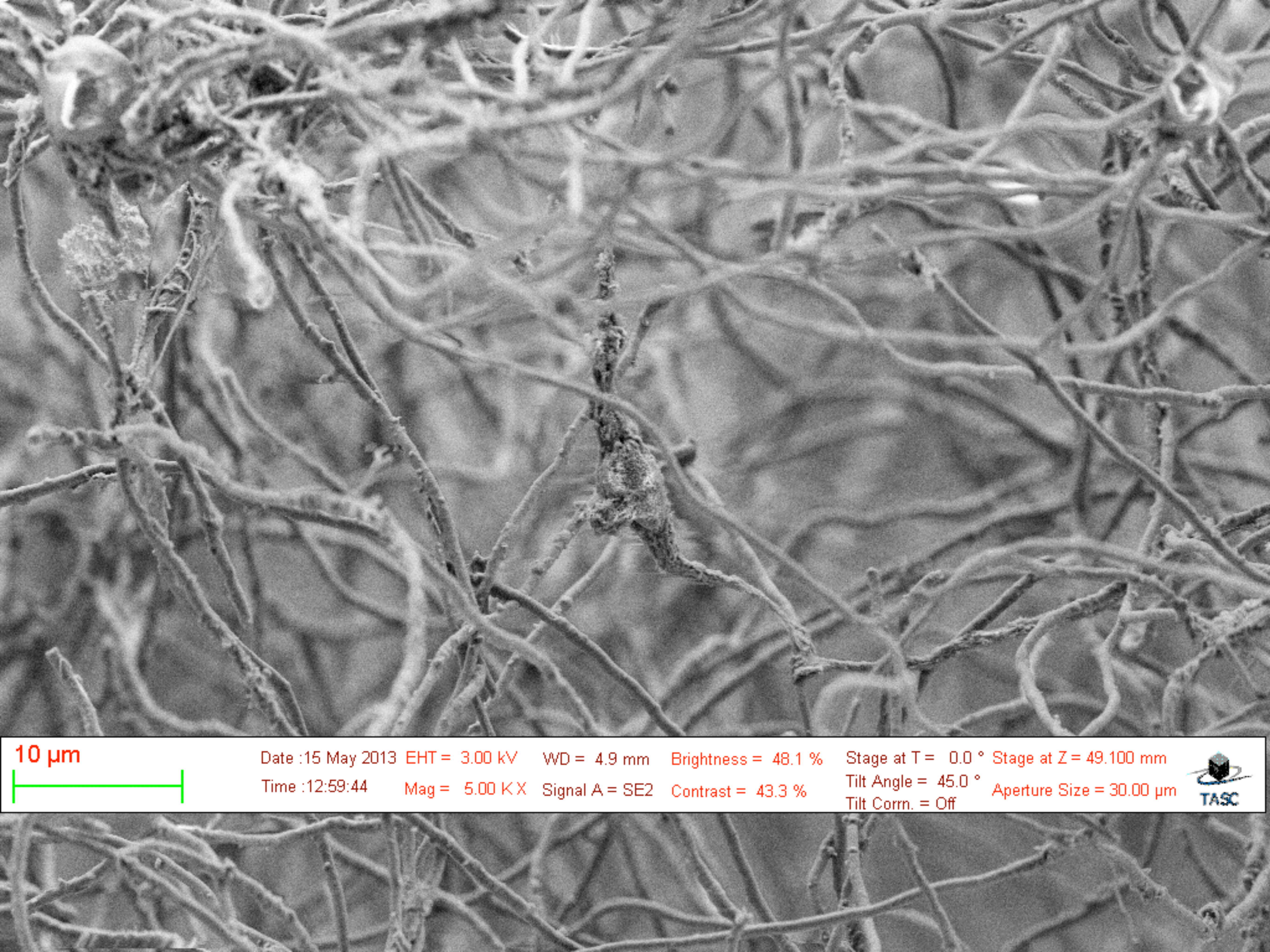


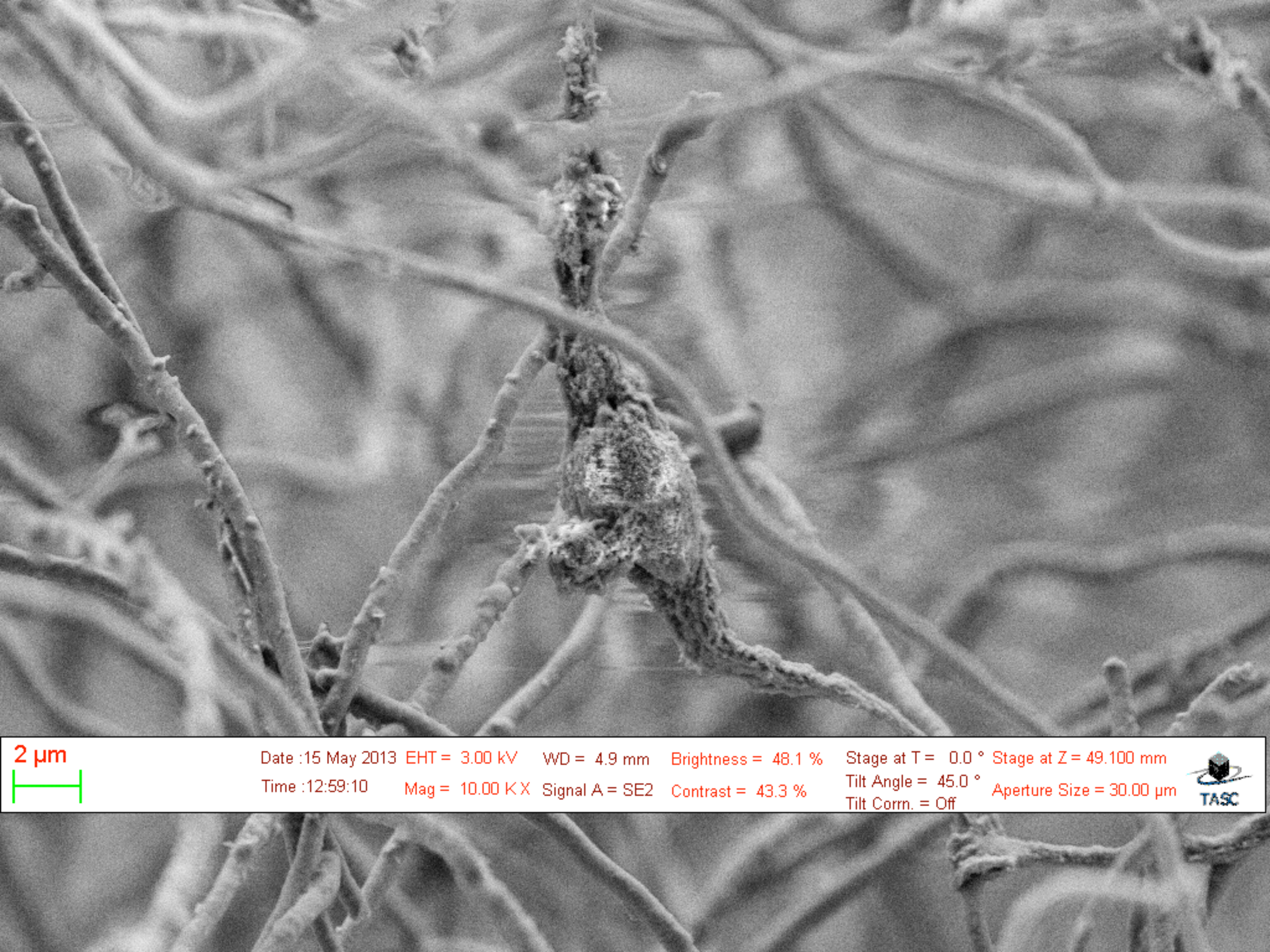


M. De Crescenzi and coll., APPLIED PHYSICS LETTERS 102, 183117 (2013)


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

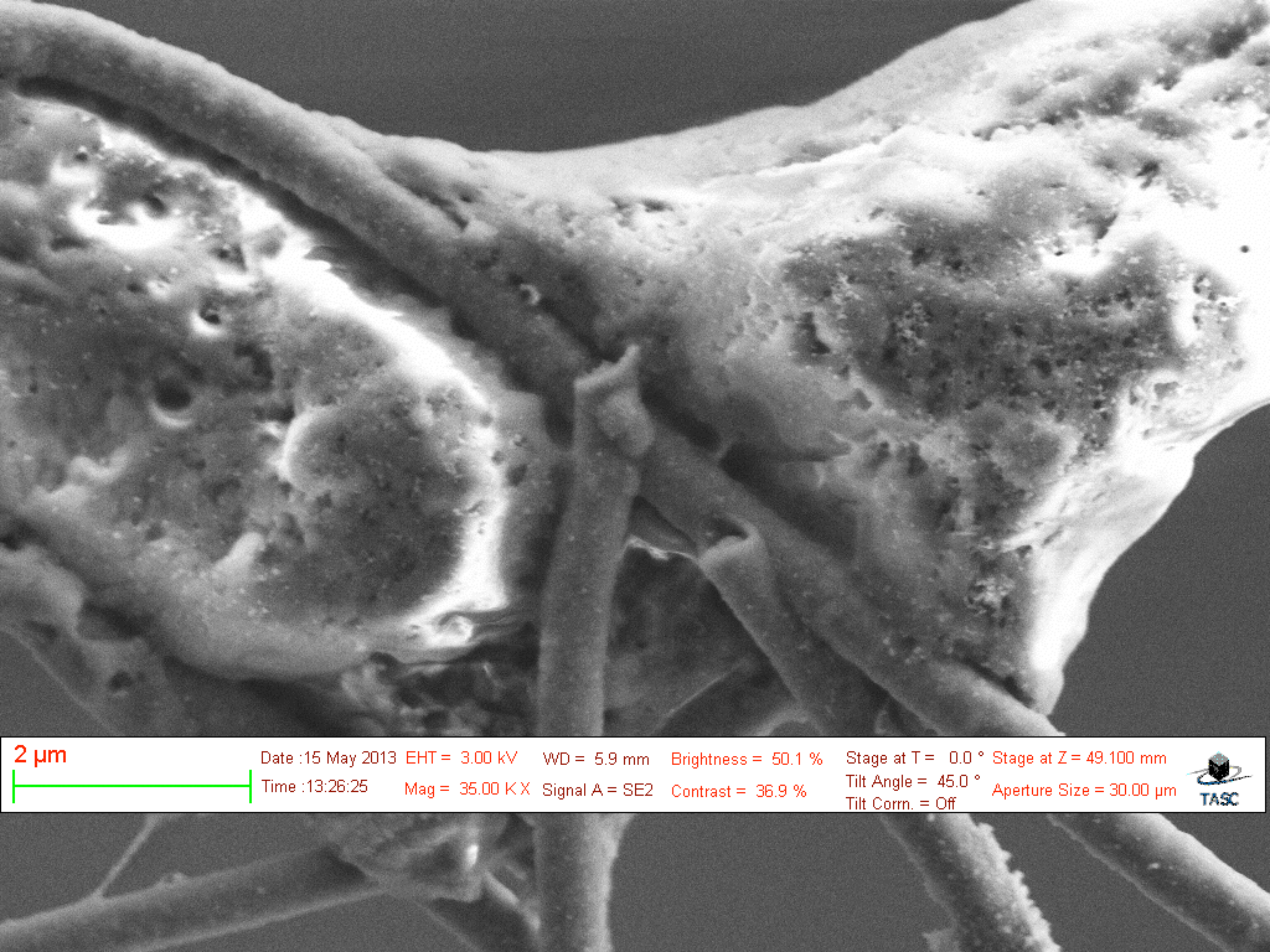

10 µm
Date :15 May 2013
Time :12:39:33
EHT = 5.00 kV
Mag = 5.00 K X
WD = 4.8 mm
Signal A = SE2
Brightness = 48.1 %
Contrast = 43.3 %
Stage at T = 0.0 °
Tilt Angle = 45.0 °
Tilt Corrn. = Off
Stage at Z = 49.100 mm
Aperture Size = 30.00 µm
TASC

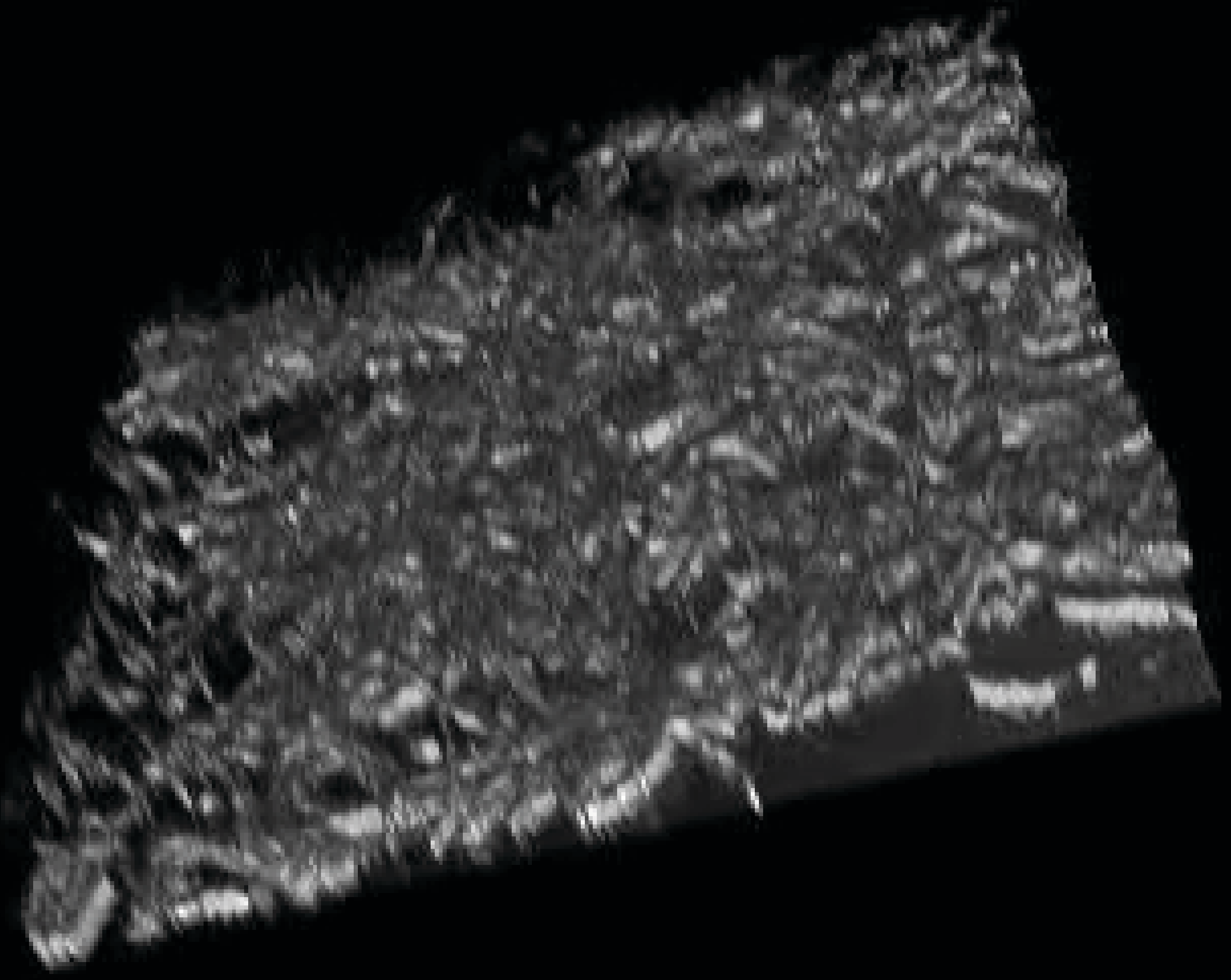

10 µm
Date :15 May 2013
Time :12:59:44
EHT = 3.00 kV
Mag = 5.00 K X
WD = 4.9 mm
Signal A = SE2
Brightness = 48.1 %
Contrast = 43.3 %
Stage at T = 0.0 °
Tilt Angle = 45.0 °
Tilt Corrn. = Off
Stage at Z = 49.100 mm
Aperture Size = 30.00 µm
TASC

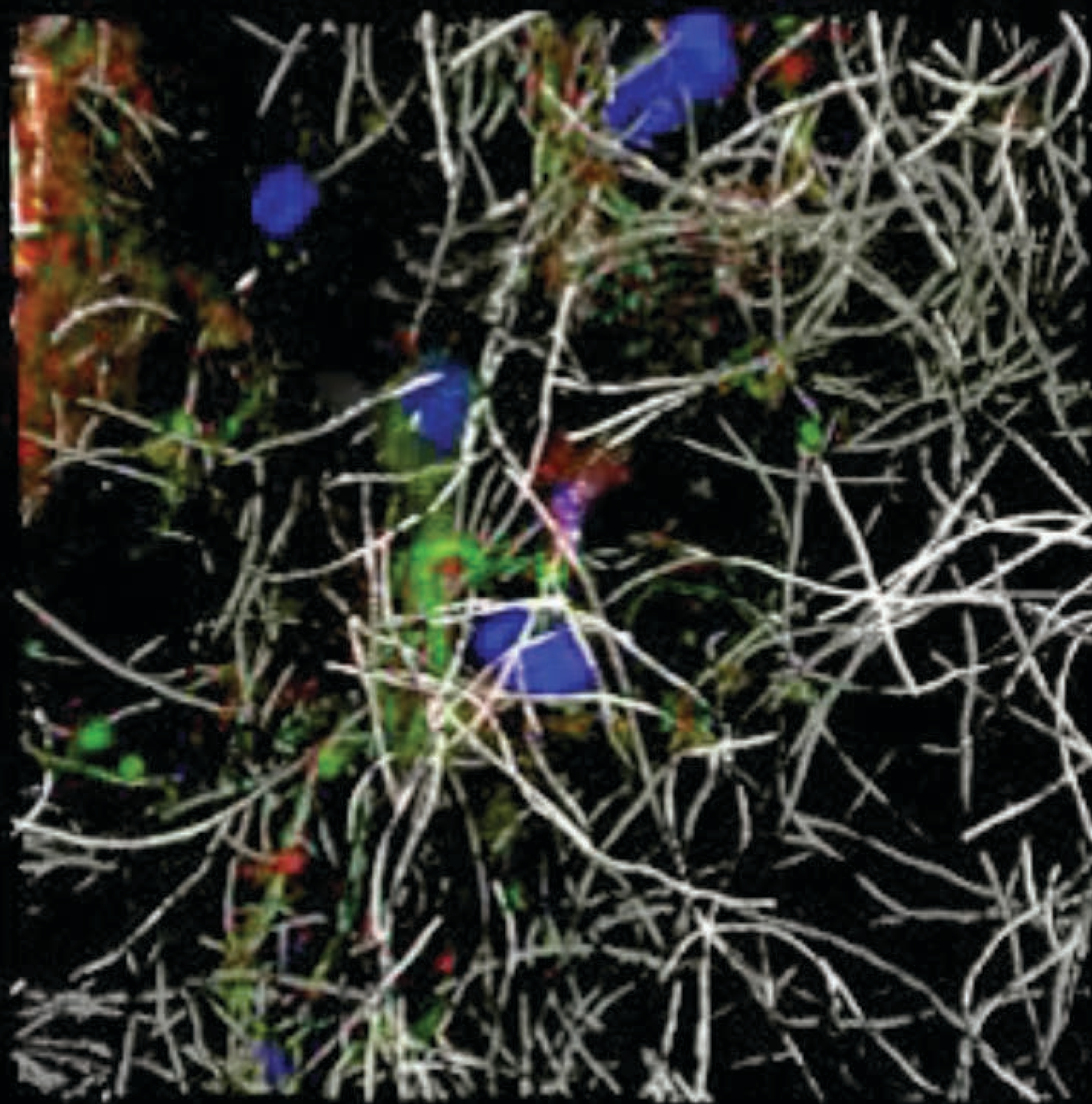

2 µm
Date :15 May 2013
Time :12:59:10
EHT = 3.00 kV
Mag = 10.00 K X
WD = 4.9 mm
Signal A = SE2
Brightness = 48.1 %
Contrast = 43.3 %
Stage at T = 0.0 °
Tilt Angle = 45.0 °
Tilt Corrn. = Off
Stage at Z = 49.100 mm
Aperture Size = 30.00 µm
TASC

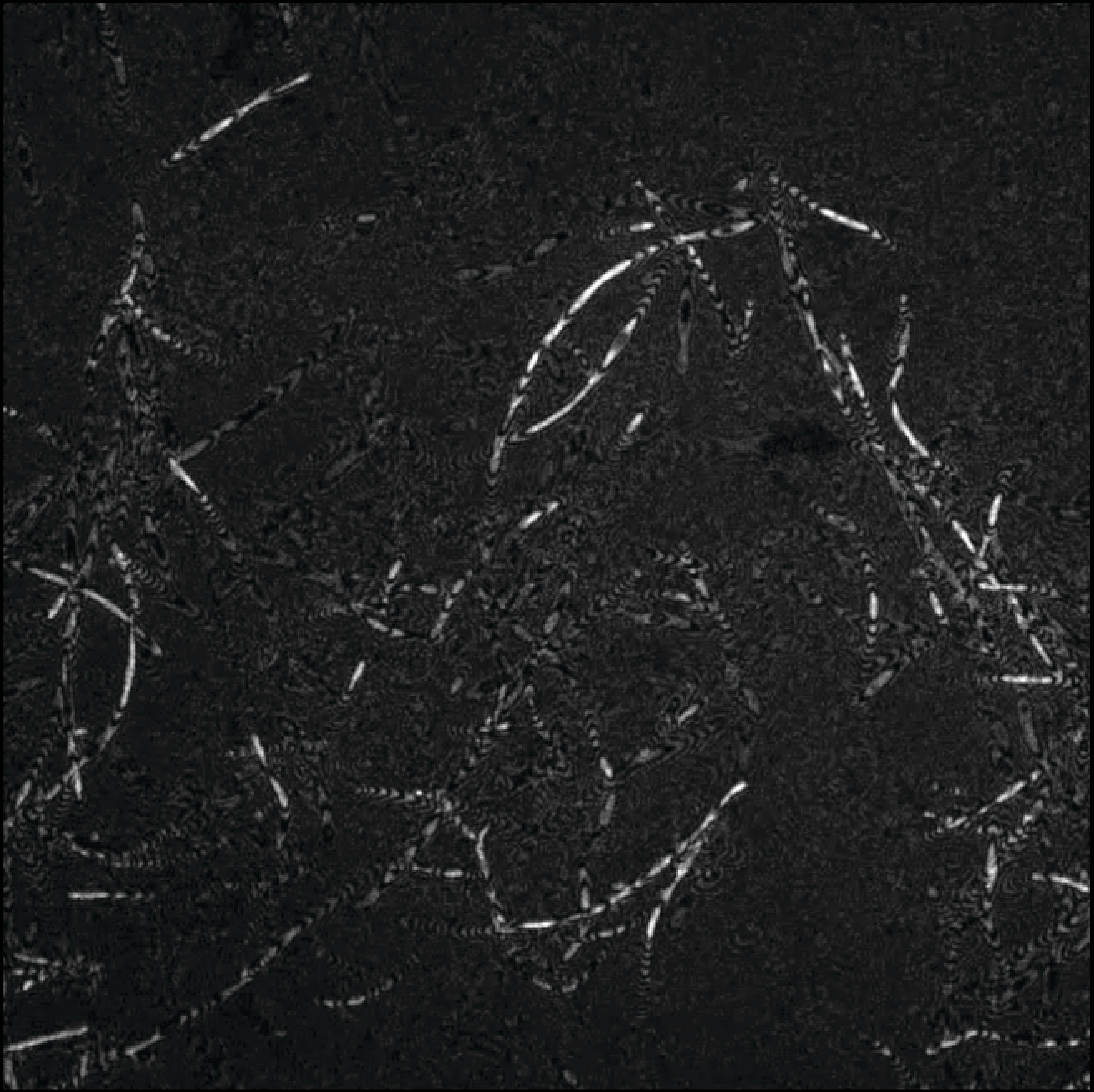

10 µm
Date :15 May 2013
Time :13:24:01
EHT = 3.00 kV
Mag = 5.00 K X
WD = 5.9 mm
Signal A = SE2
Brightness = 50.1 %
Contrast = 36.9 %
Stage at T = 0.0 °
Tilt Angle = 45.0 °
Tilt Corrn. = Off
Stage at Z = 49.100 mm
Aperture Size = 30.00 µm
TASC

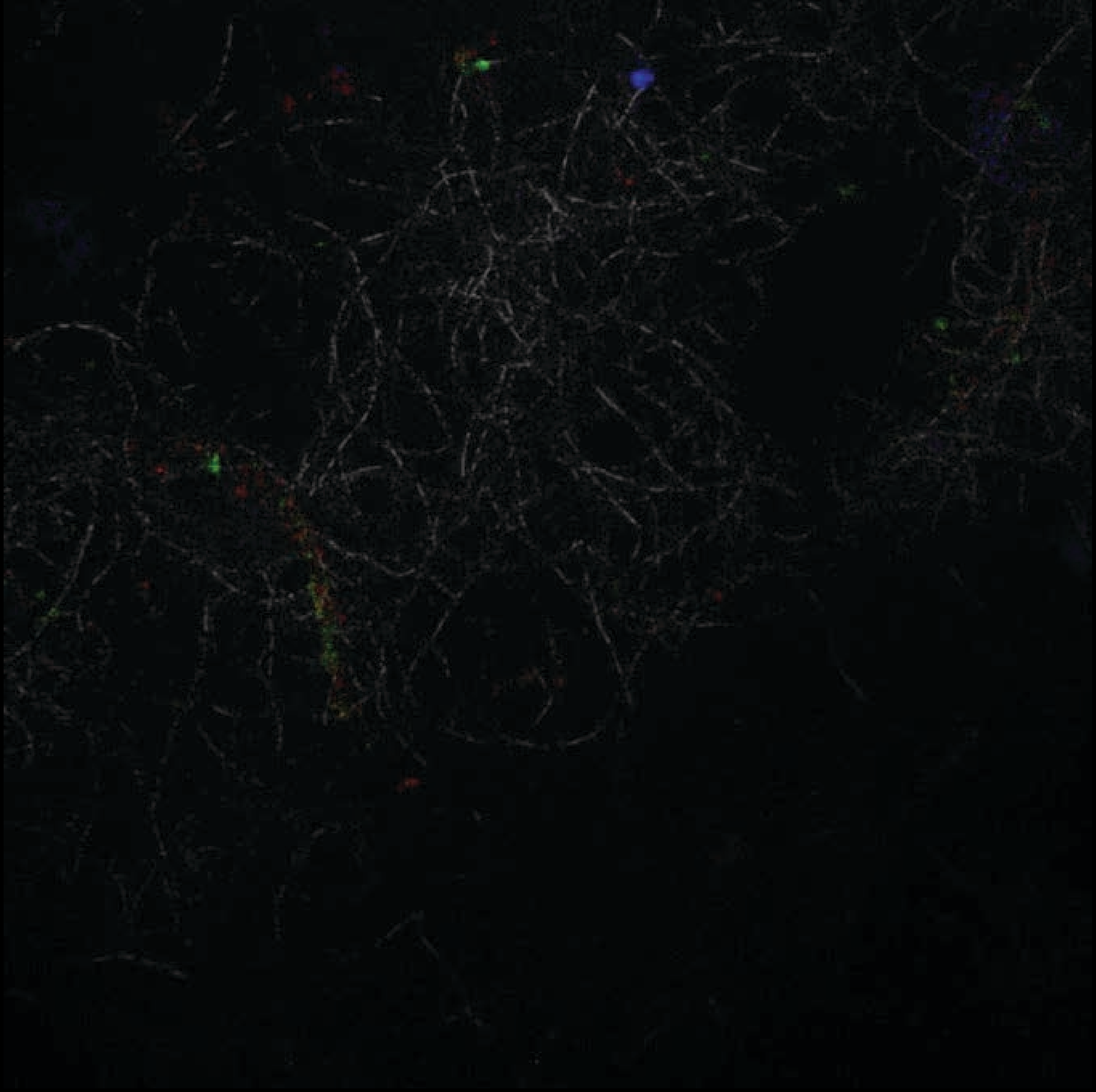

2 µm
Date :15 May 2013
Time :13:24:39
EHT = 3.00 kV
Mag = 8.00 K X
WD = 5.9 mm
Signal A = SE2
Brightness = 50.1 %
Contrast = 36.9 %
Stage at T = 0.0 °
Tilt Angle = 45.0 °
Tilt Corrn. = Off
Stage at Z = 49.100 mm
Aperture Size = 30.00 µm
TASC


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



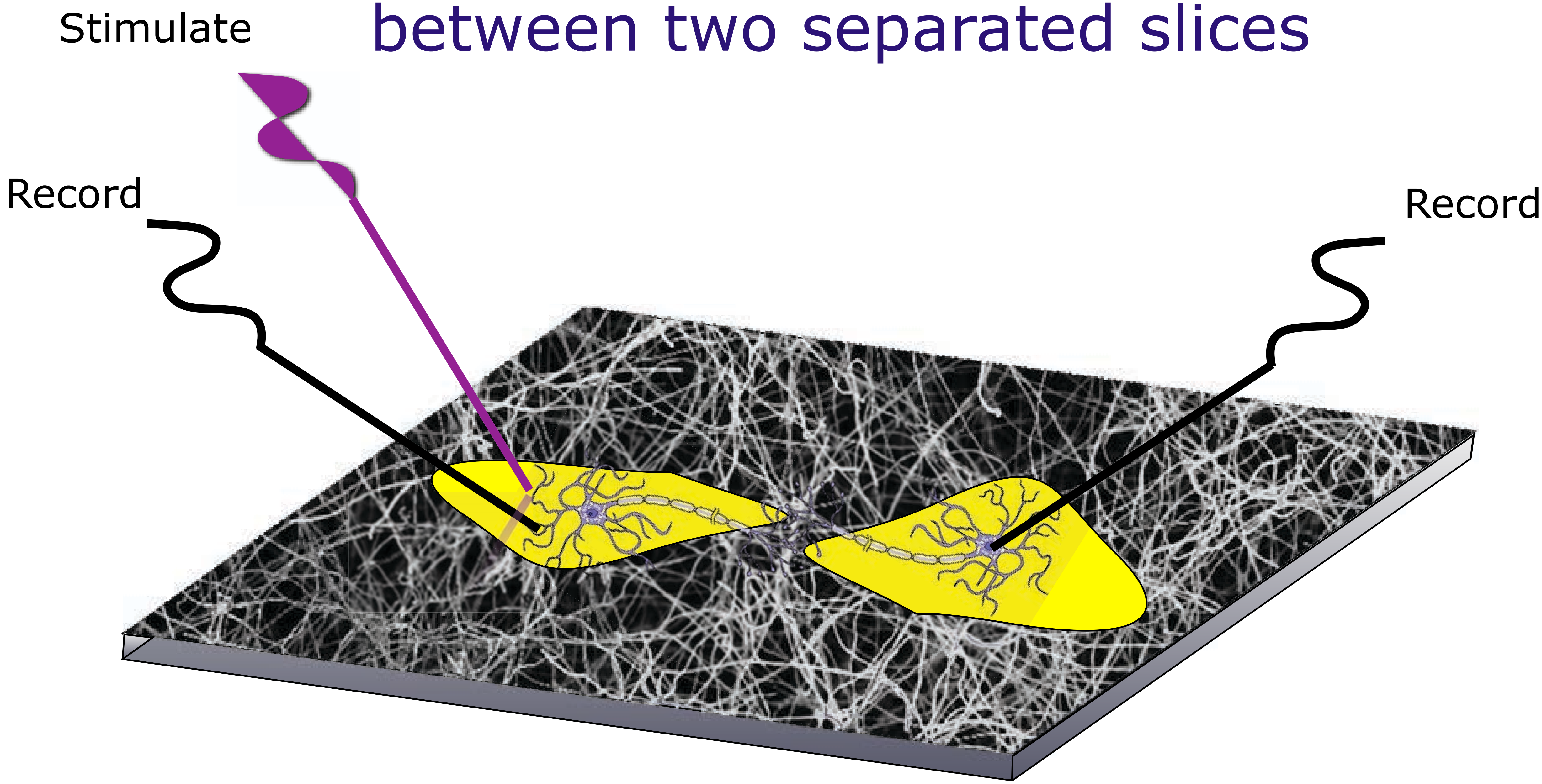


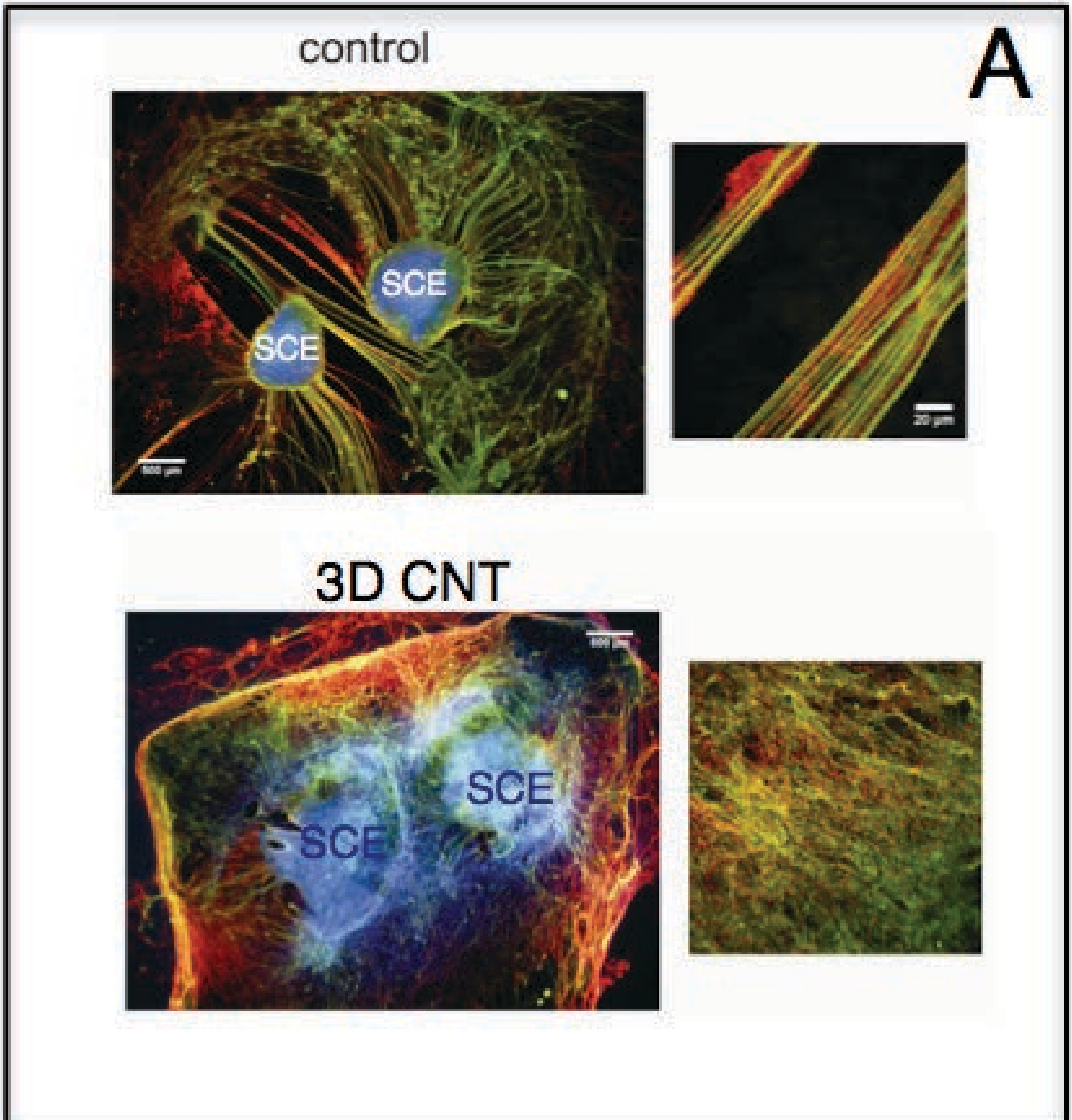




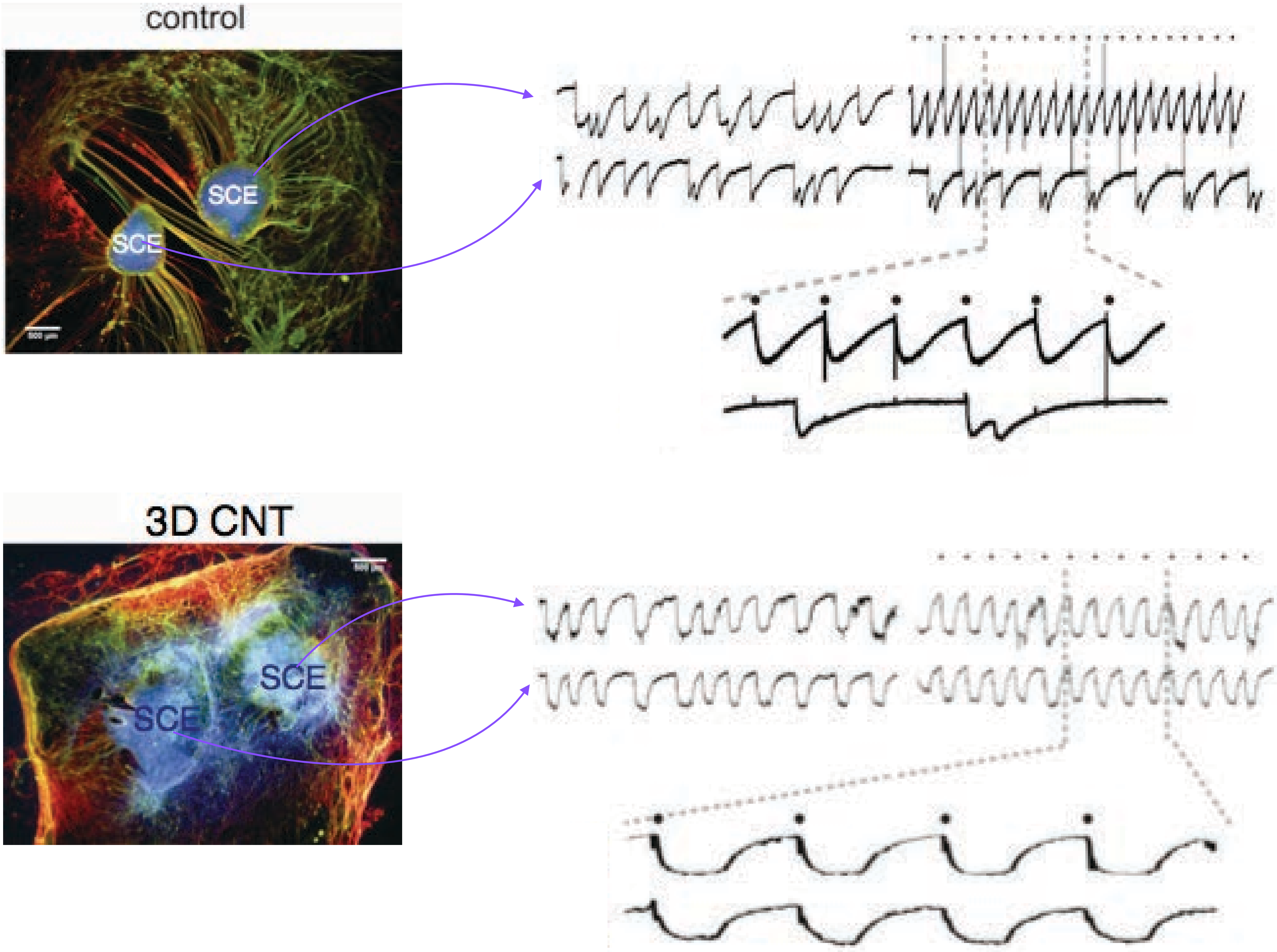
Color codes: blue (all nuclei, both neurons and glia); green (axons and dendrites); red (synapses)

Looking for communication between two separated slices





A
control
SCE
SCE
500 µm
20 µm
3D CNT
500 µm
SCE
SCE



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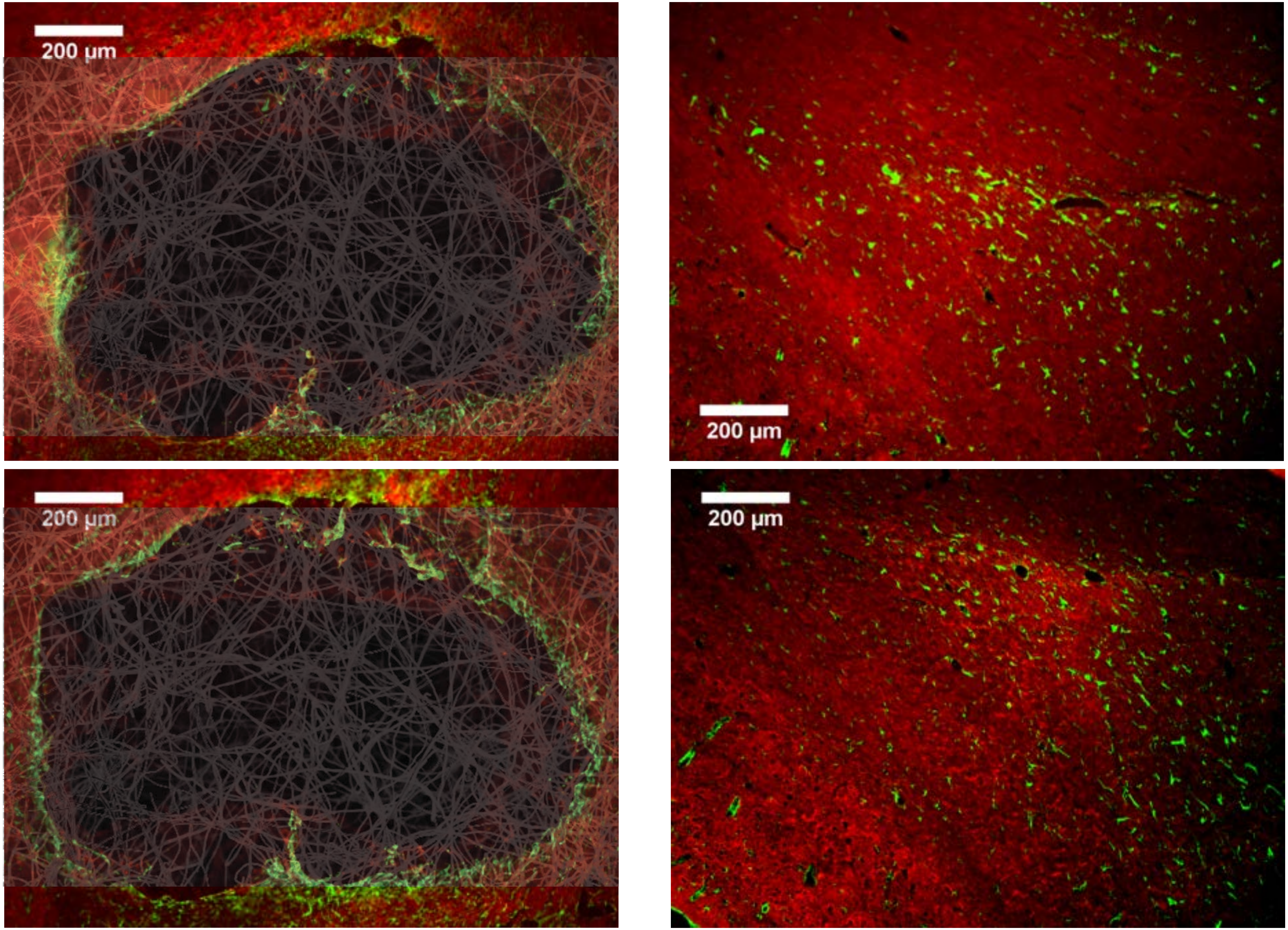
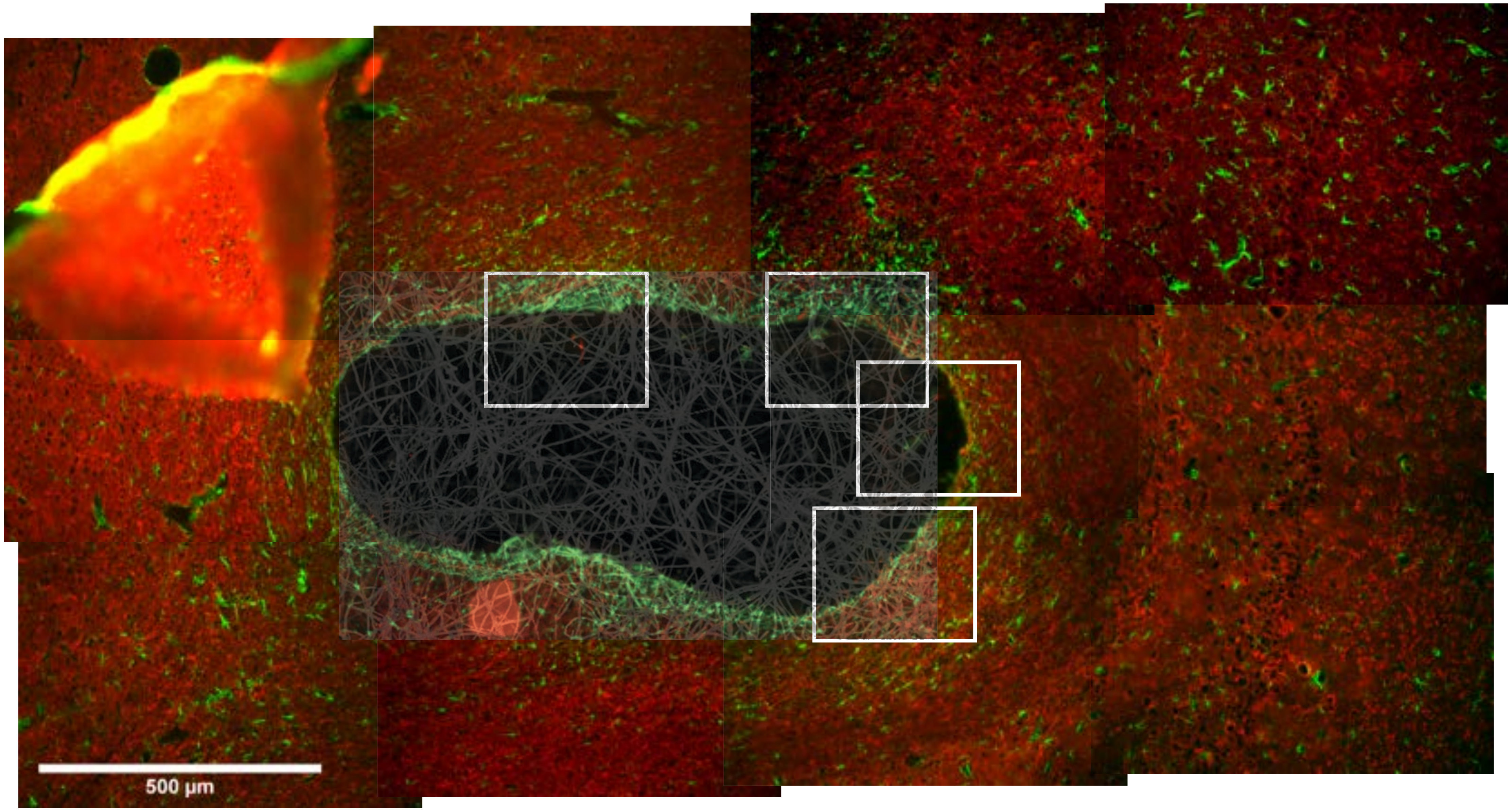
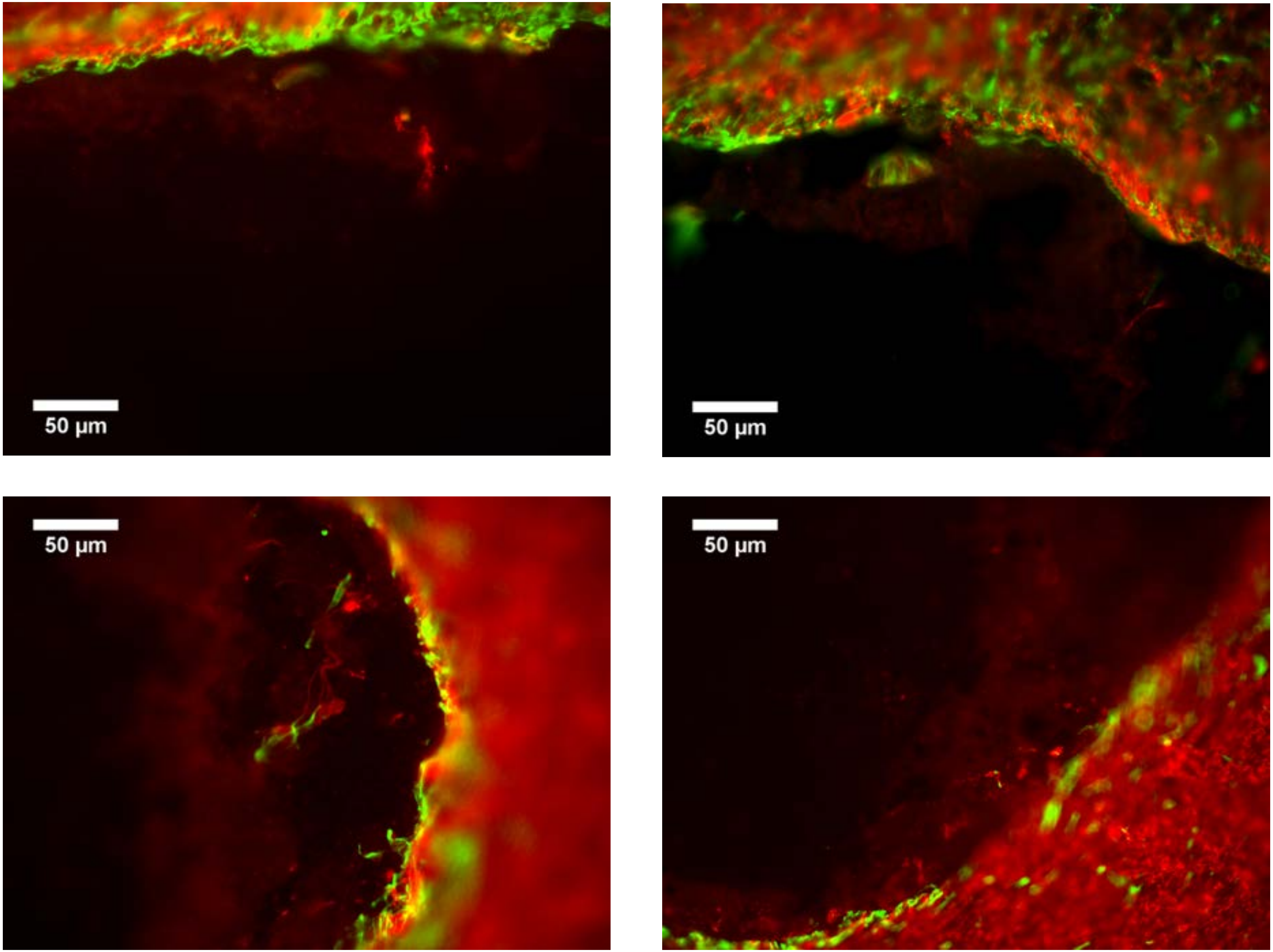
Sponge | II hemisphere

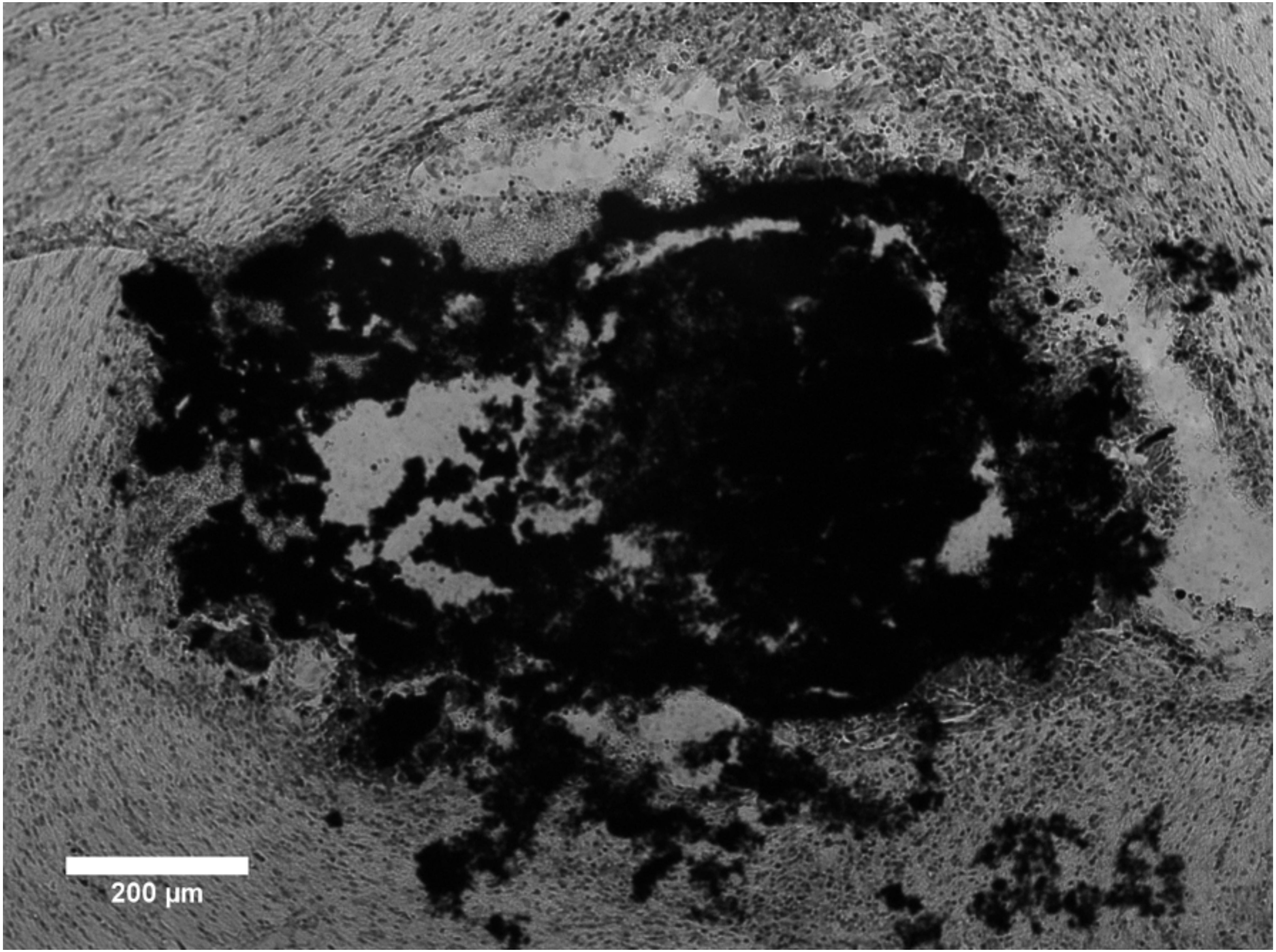


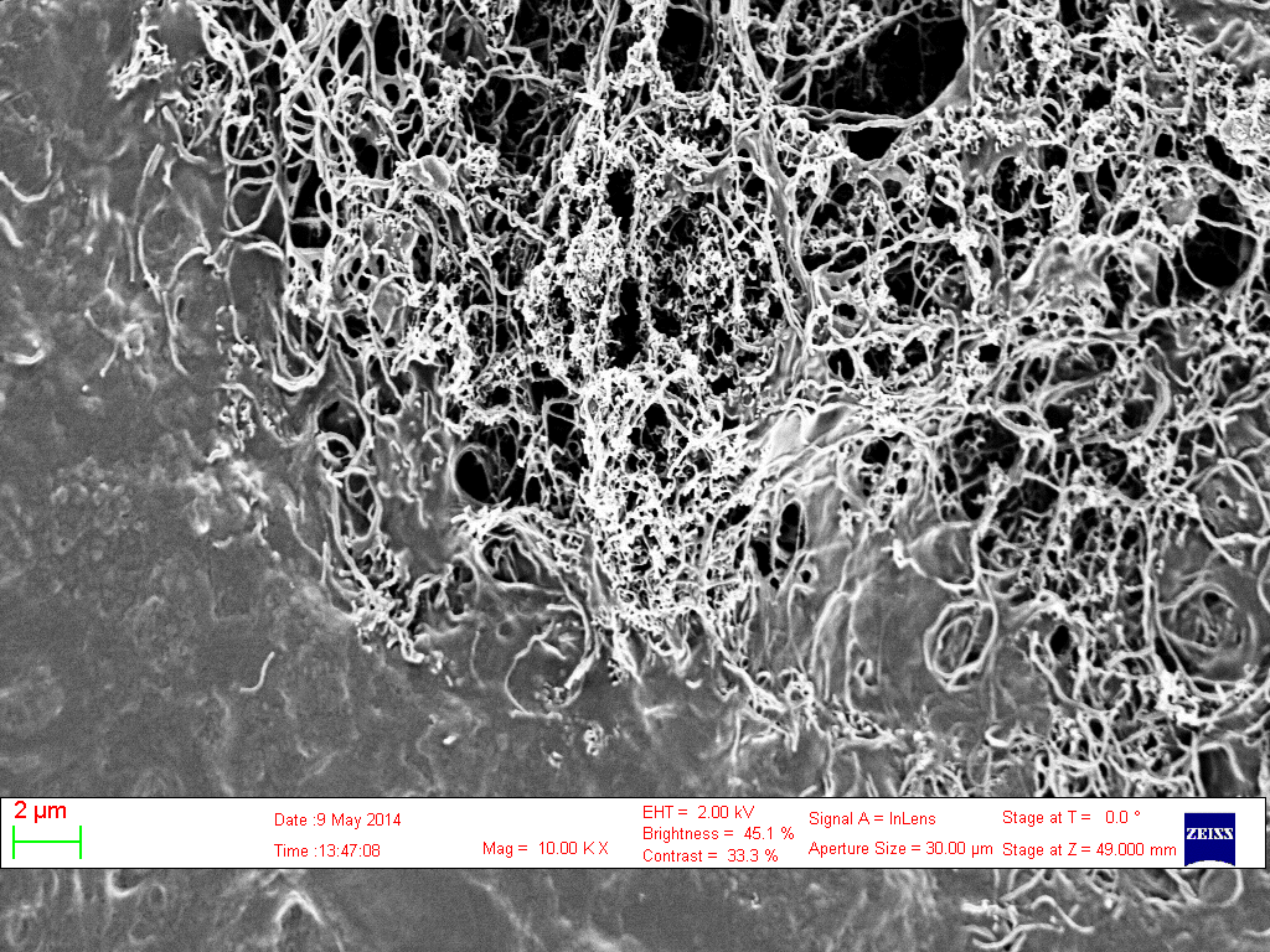
Coverslip 21 (III slice), objective 20x



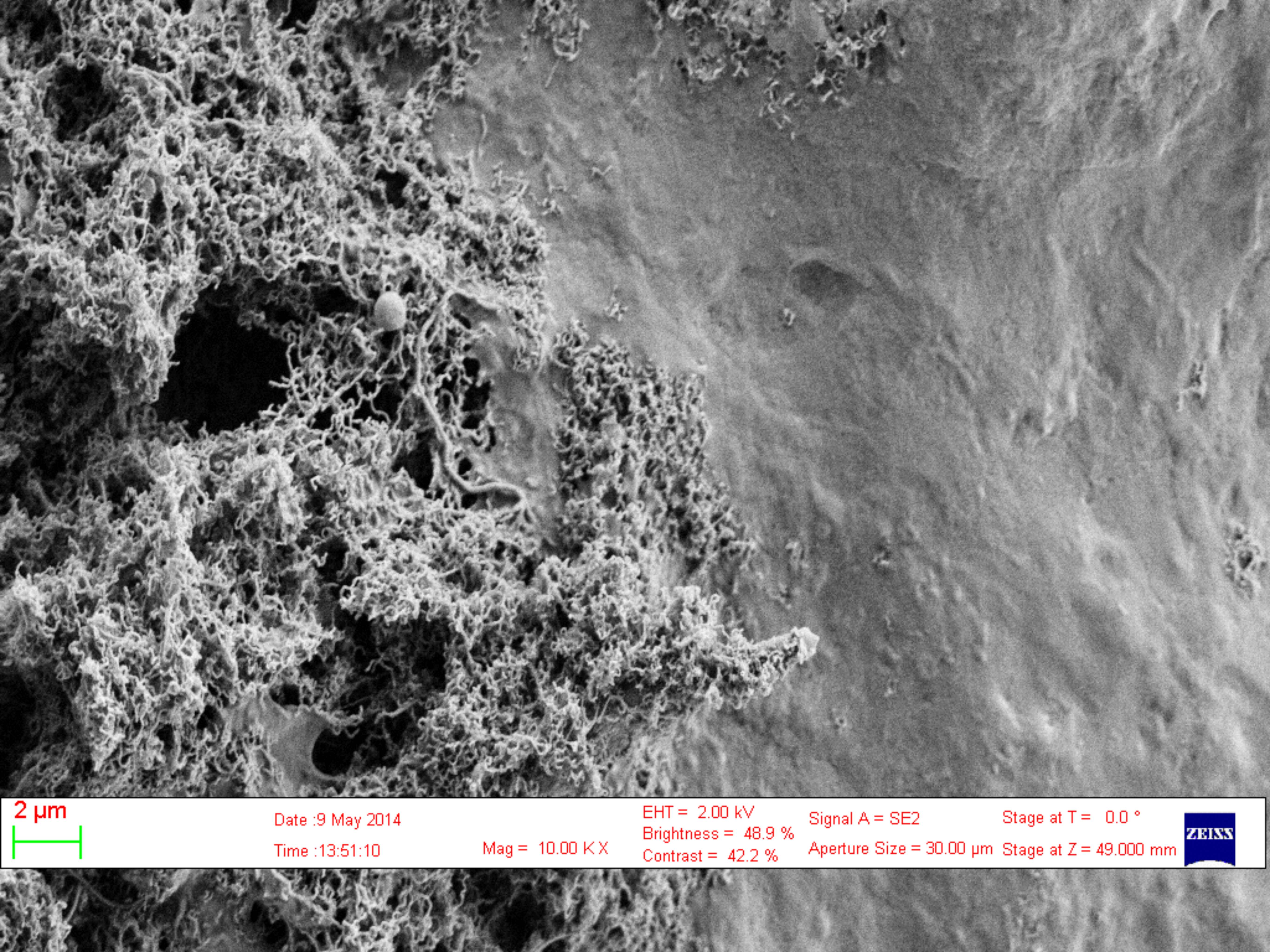
Coverslip 21 (III slice), objective 40x

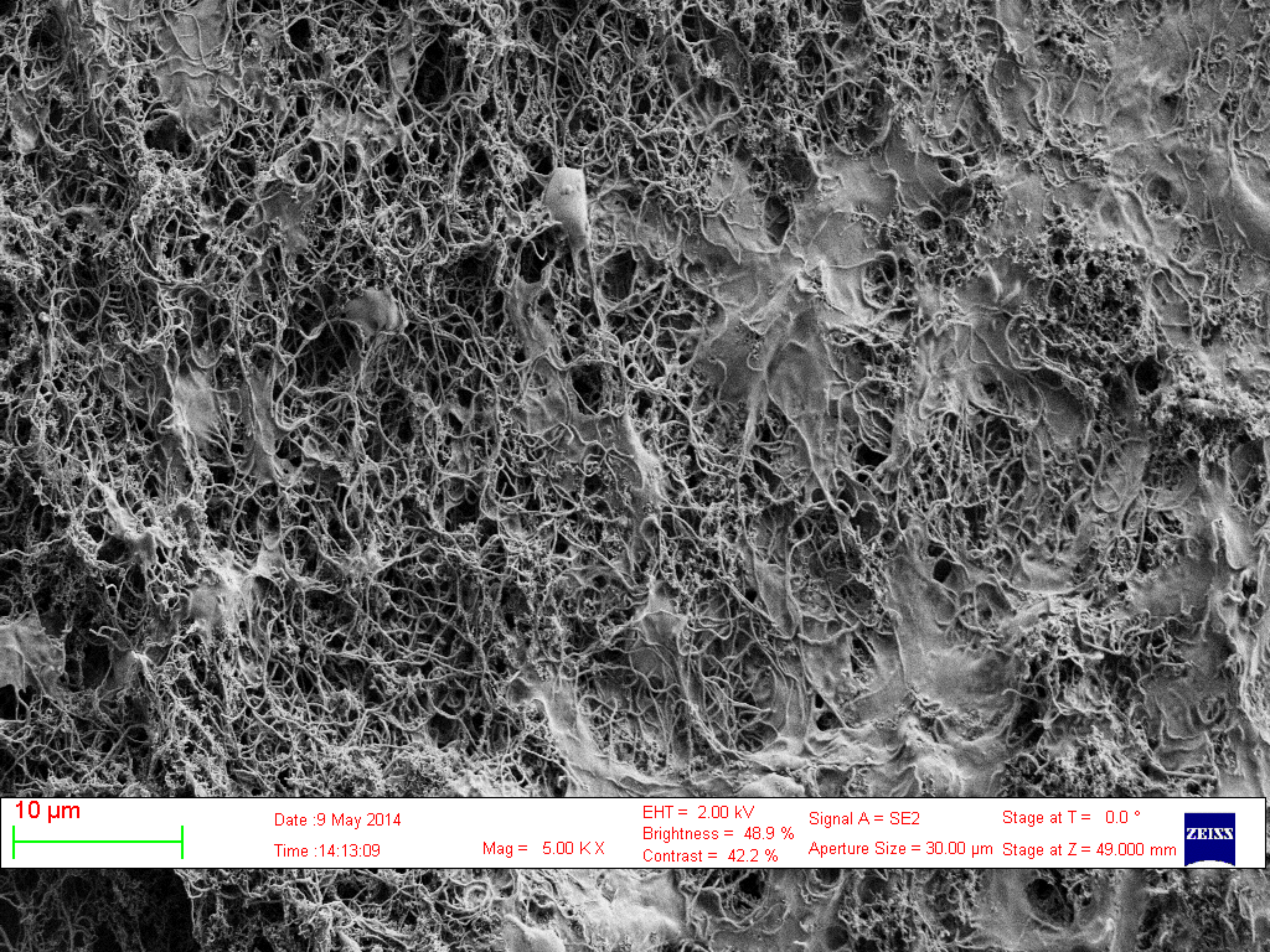



200 µm



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 °
Stage at Z = 49.000 mm
ZEISS


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
ZEISS

- 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!!!