24th ETH-Conference on Combustion Generated Nanoparticles
22.-24. June 2021, Online Conference

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Differentiating Translocated Exogenous vs Bio-generated Endogenous Nanoparticle Types in Olfactory Bulb of Humans with Neurodegeneration

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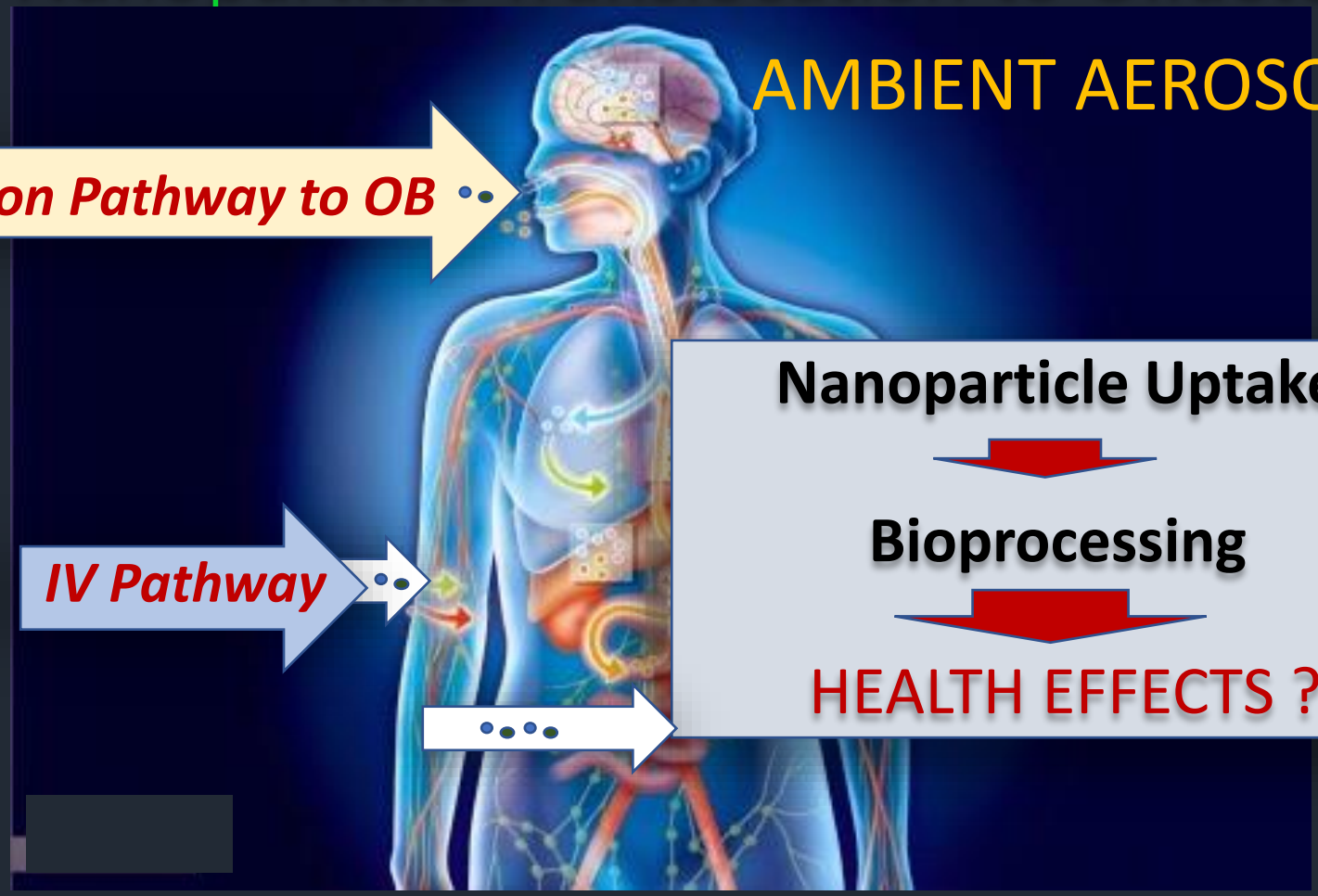
1R01AG067497-01

Air Pollution and Alzheimer's Dementia:
Neuropathologic and Olfactory Mechanisms in Multi-Ethnic Longitudinal Cohorts

J. Weuve — BOSTON UNIVERSITY
JJ Pinto — THE UNIVERSITY OF CHICAGO
DA Bennett — RUSH
J. Schneider — RUSH
G. Oberdorster — UNIVERSITY of ROCHESTER MEDICAL CENTER
UM Graham — University of Kentucky

OBJECTIVE

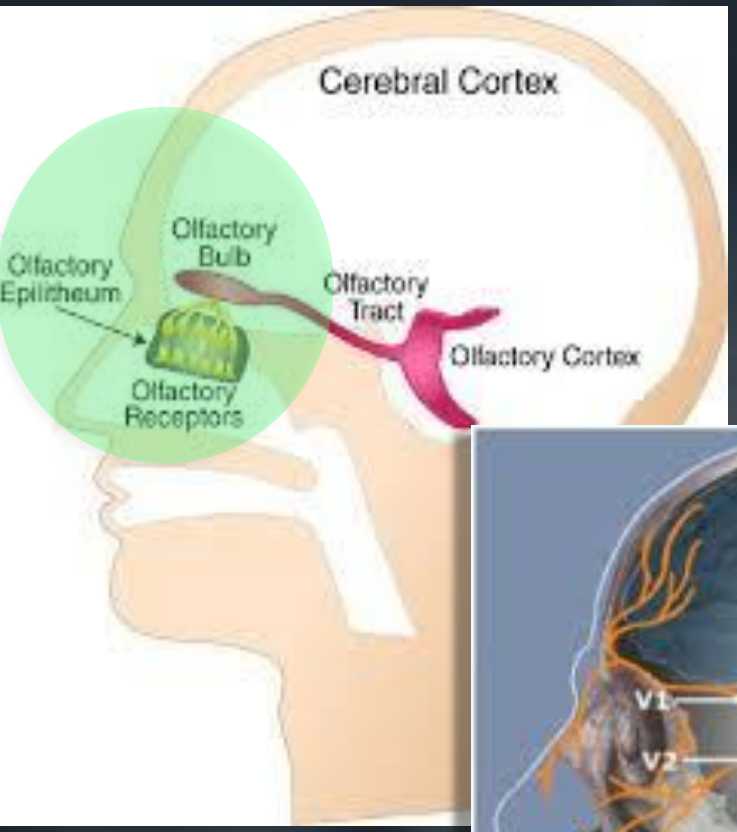
Determine: Nanoparticle Translocation to Olfactory Bulb

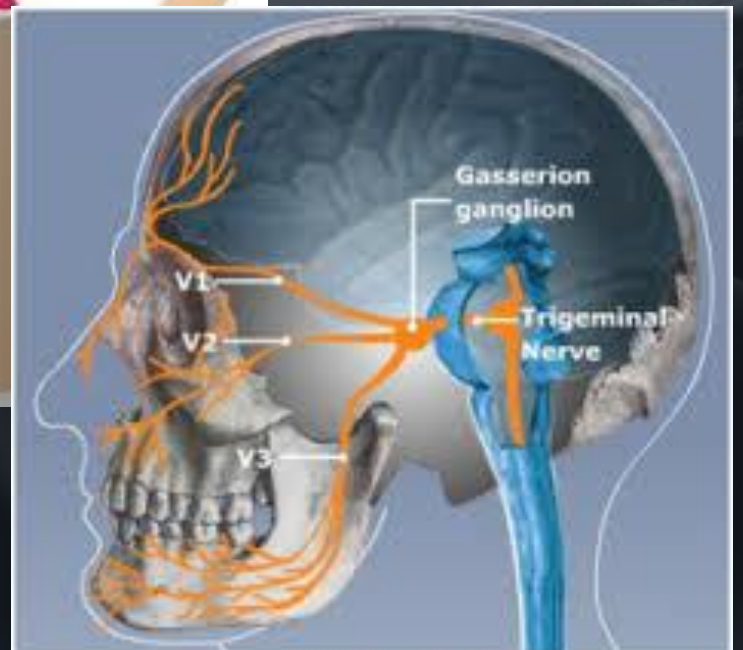


Exposure - Dose - Response

Confirming that inhaled airborne ambient NPs reach the CNS in Humans

NPs portals of entry to CNS: rostral ***(olfactory bulb)***
caudal ***(Trigeminal Ganglia)***





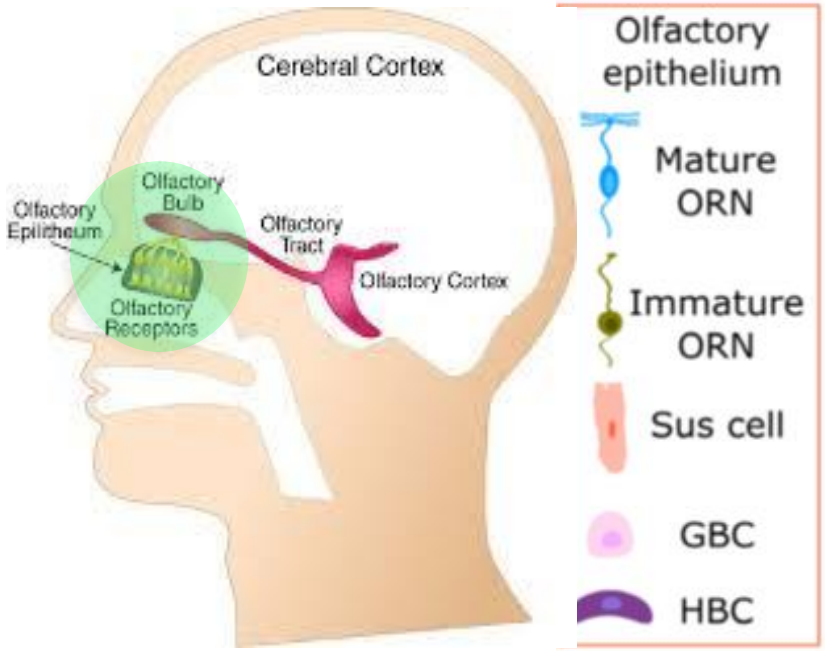
Environmental exposures as contributors to AD risk and pathogenesis

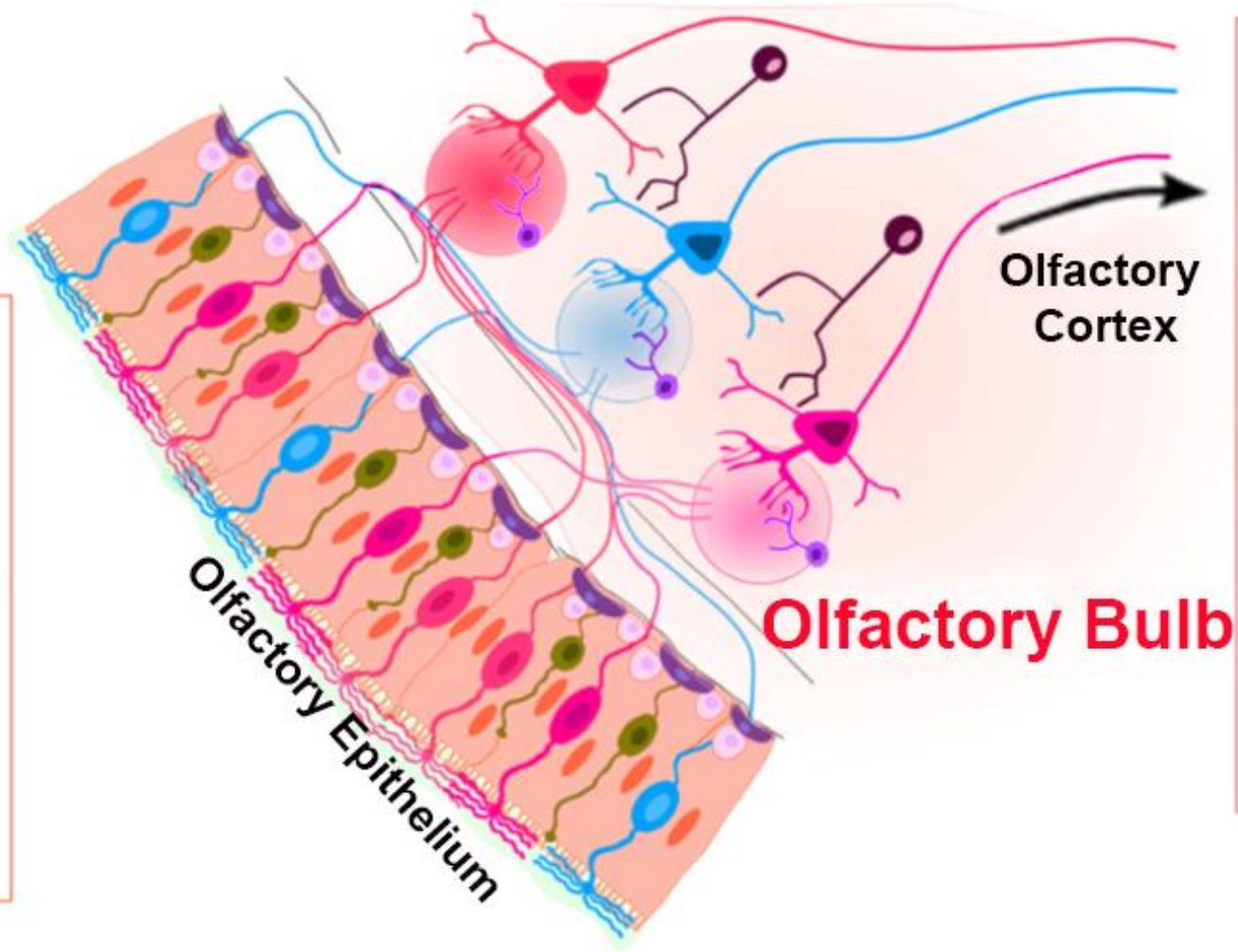
Previous Studies demonstrated:

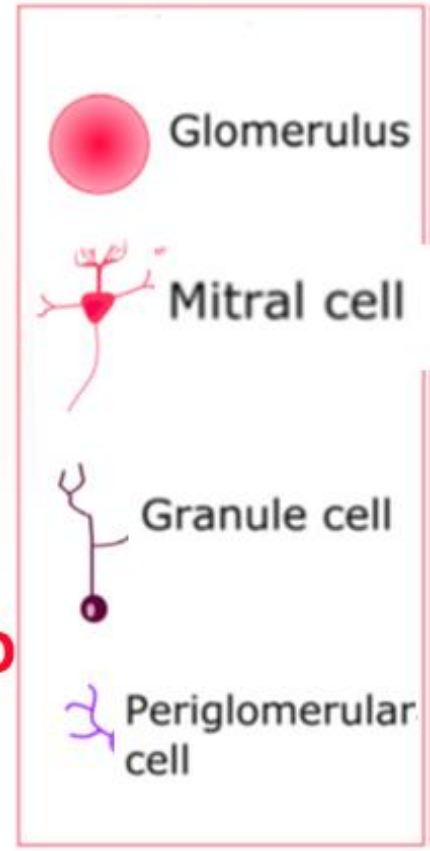
PM2.5 and Ultrafines (<100 nm) and O3: AD starts in the brainstem and OB.

Nasal breathing resulted in a much higher uptake of particles by the brain.

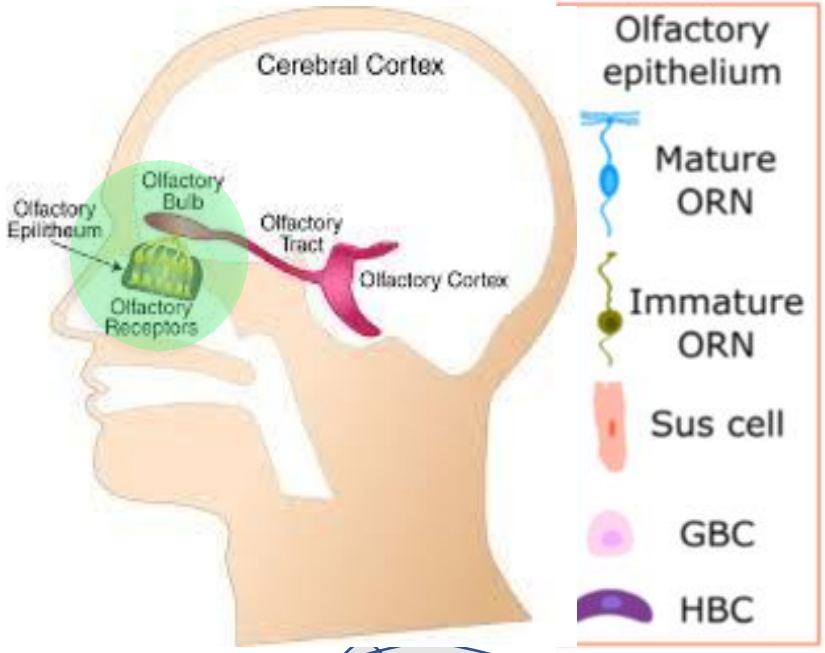
THE OLFACTORY SYSTEM



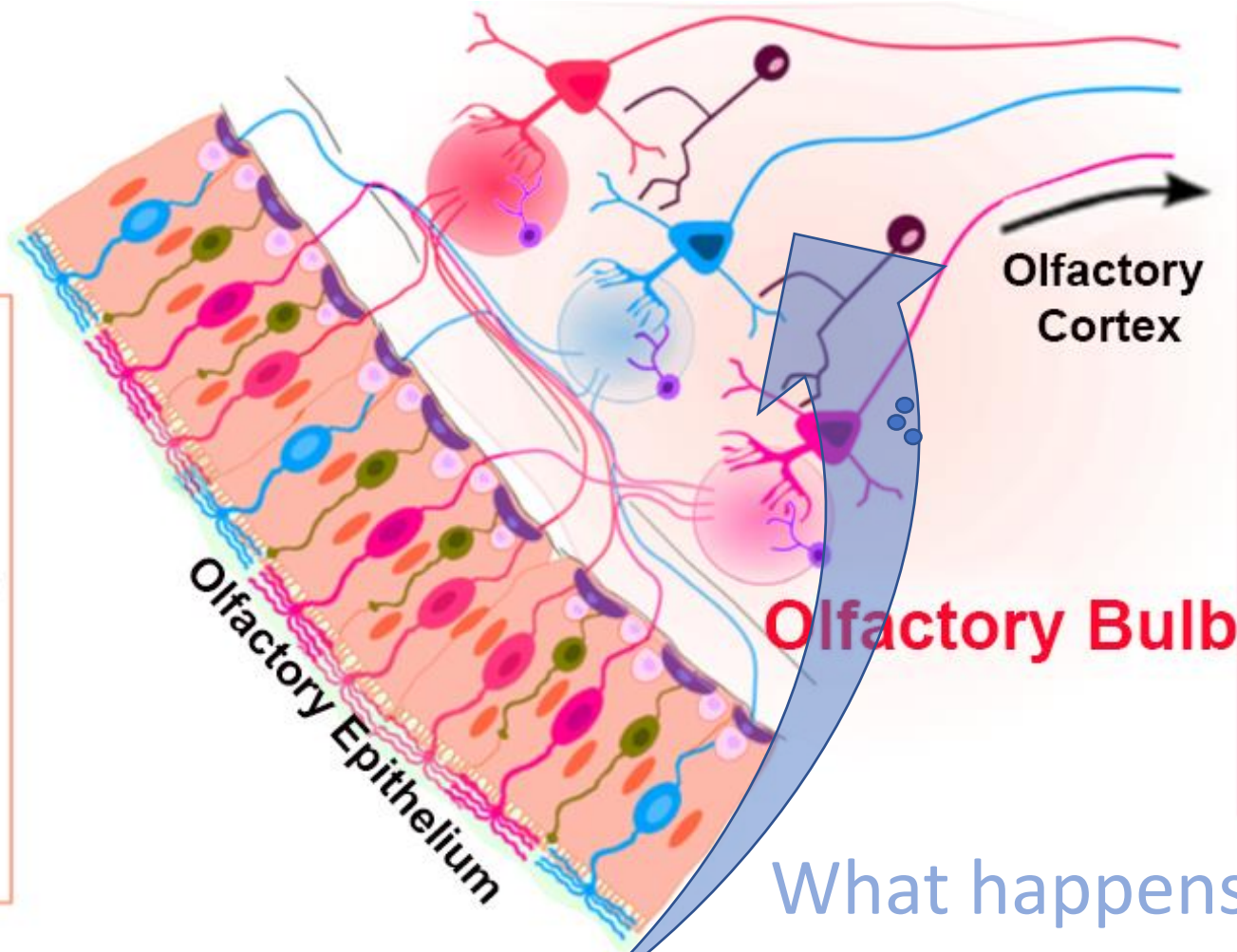


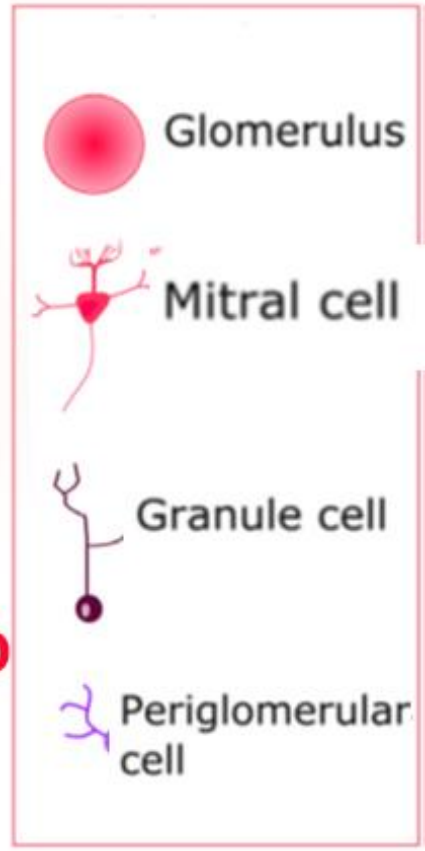


THE OLFACTORY SYSTEM



Nose Inhalation



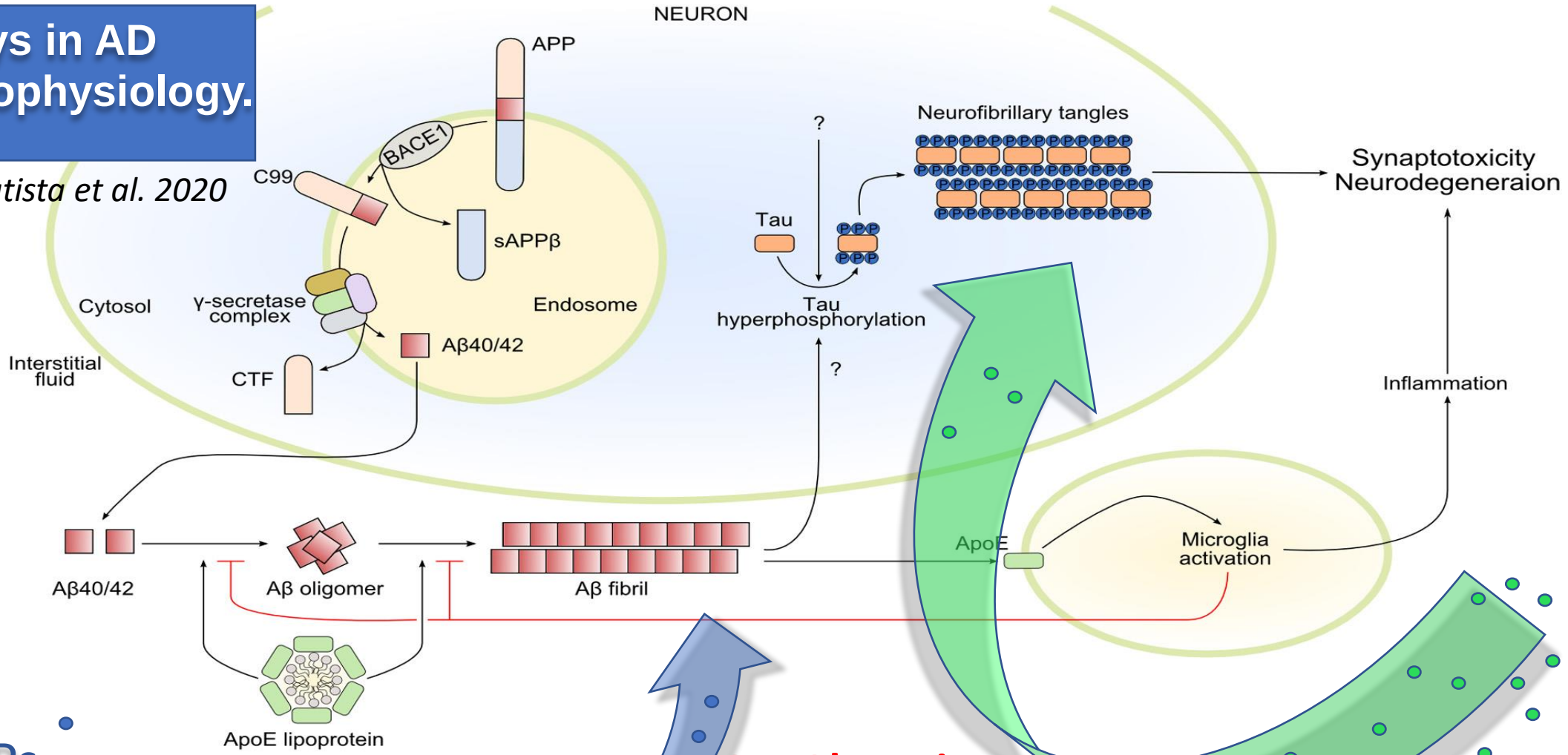


What happens to OB when Pollution NPs **invade?**

Is there a Link to AD?

Main Pathways in AD Disease pathophysiology.

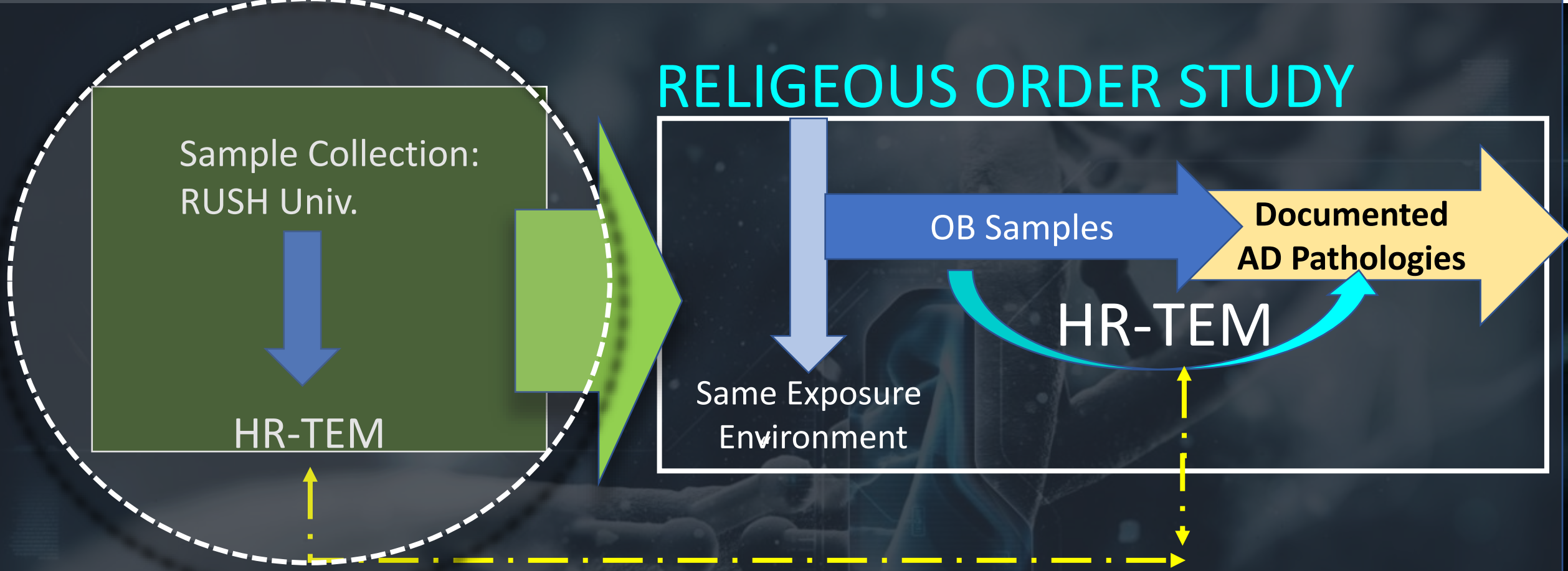
Modified after Dibatista et al. 2020



Aerosol NPs entering Interstitial Space

Are Alterations Caused by NPs?

Intracellular Aerosol NPs ?



Environmental PM in OB linked to AD?

Religious Order Study and Rush Memory and Aging Project

Subjects with different progression of AD.

PM < 100nm

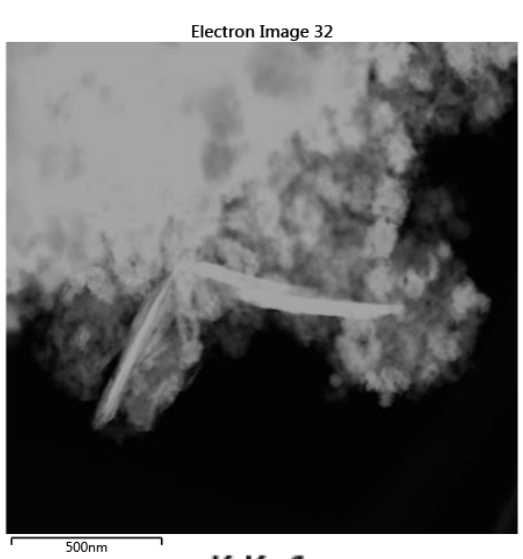
Exposures to fine including ultrafine particulate matter

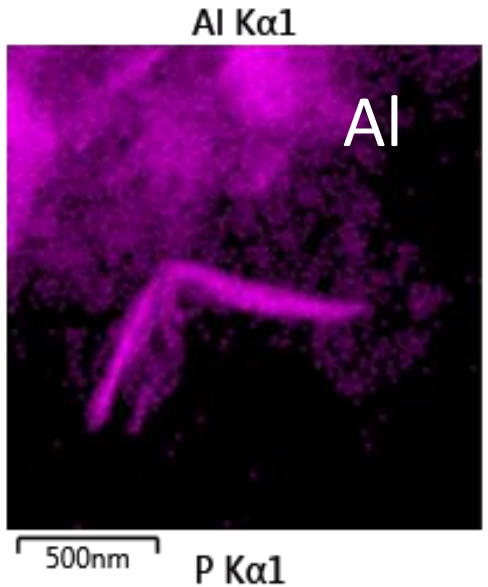
PM2.5

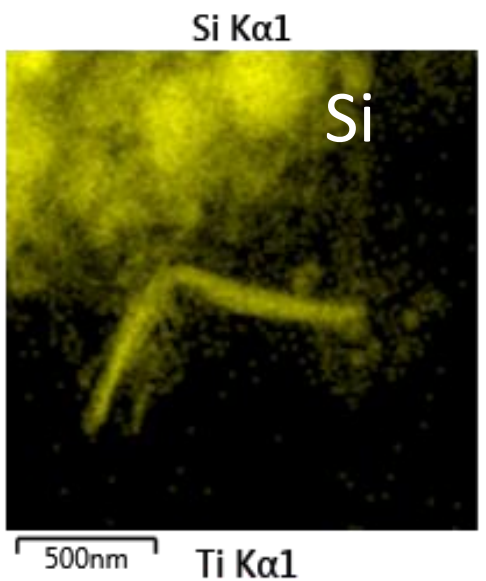
PM2.5 is associated with Alzheimer's disease (AD) risk.
The ultrafines are the only fraction of PM2.5 that can have access to the brain via neuronal translocation.

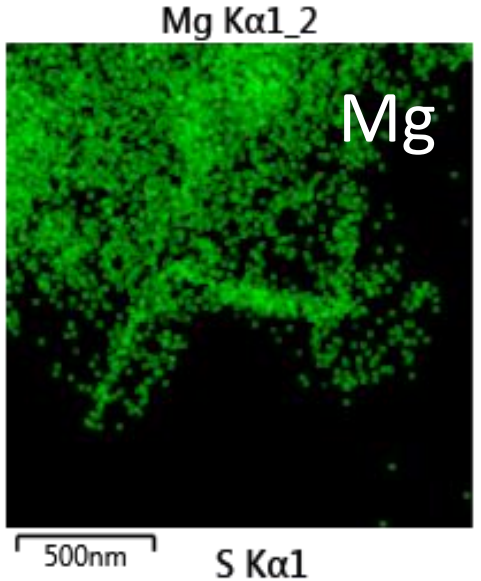
Characterize first the ambient aerosol samples and then compare the OB samples to look for NPs uptake and translocation.

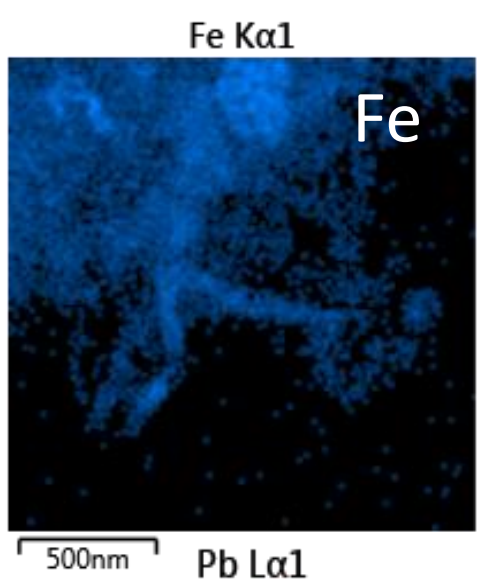
Environmental Sample:

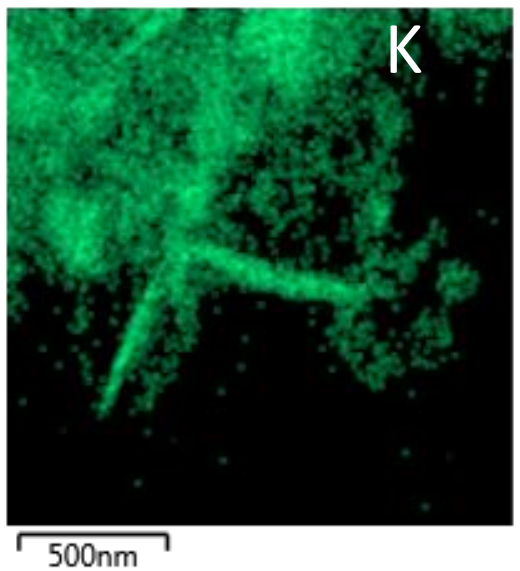


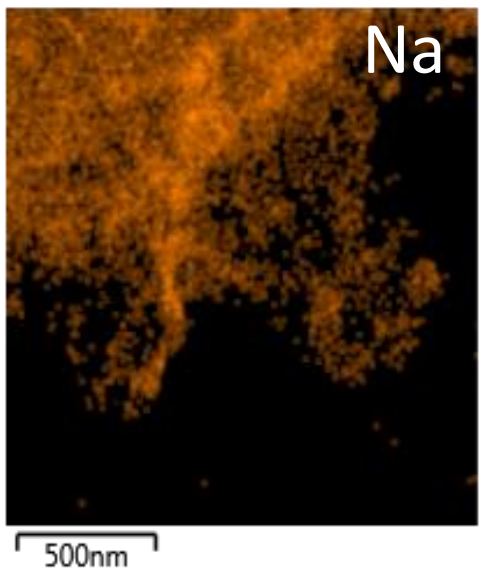


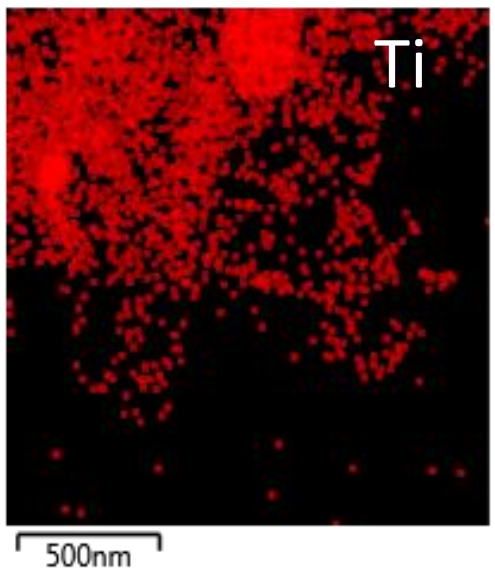


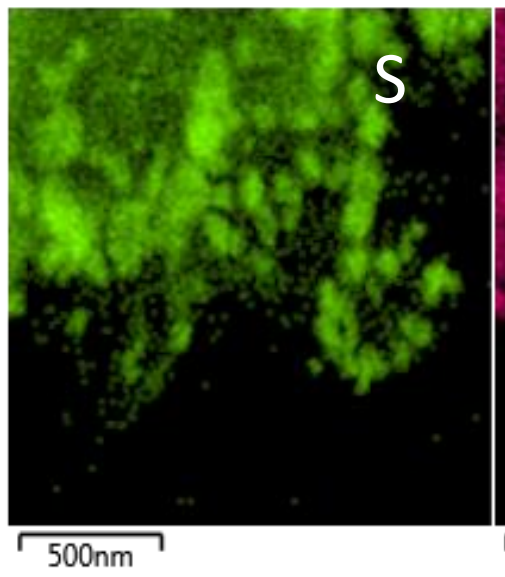


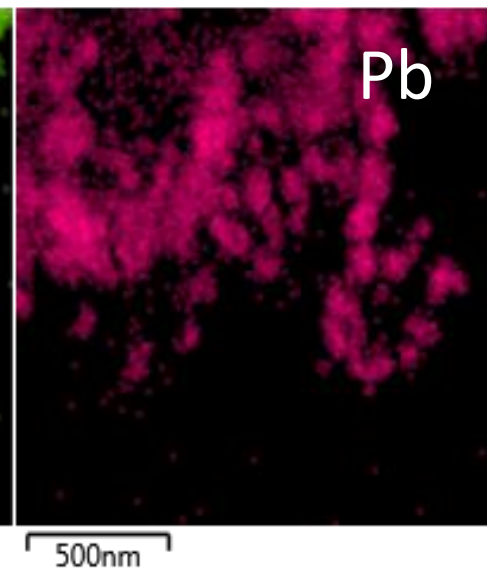


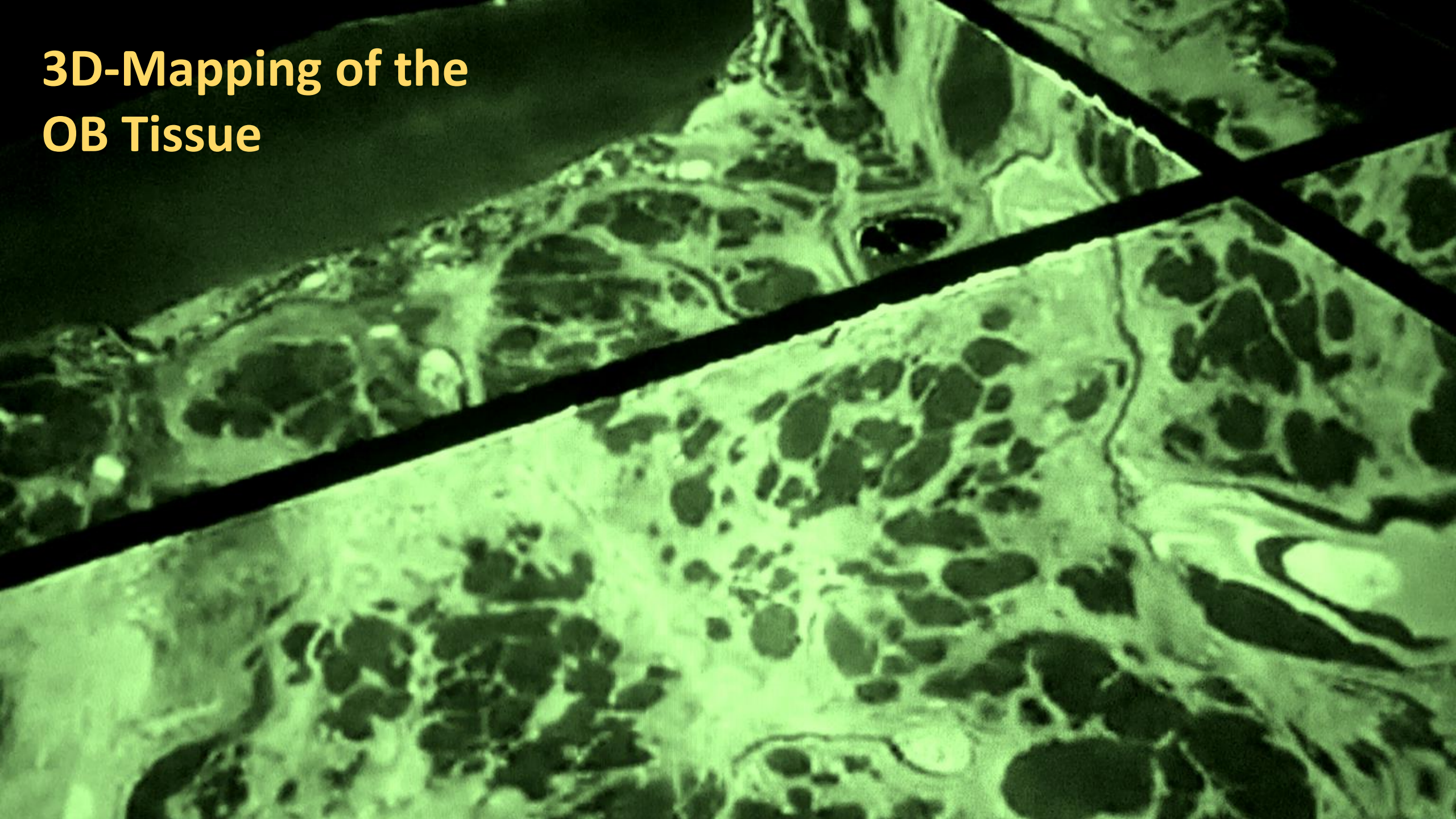











3D-Mapping of the
OB Tissue

Analytical Imaging: High Resolution TEM for Characterization of Nanoparticle-Cell Interactions

University of Kentucky

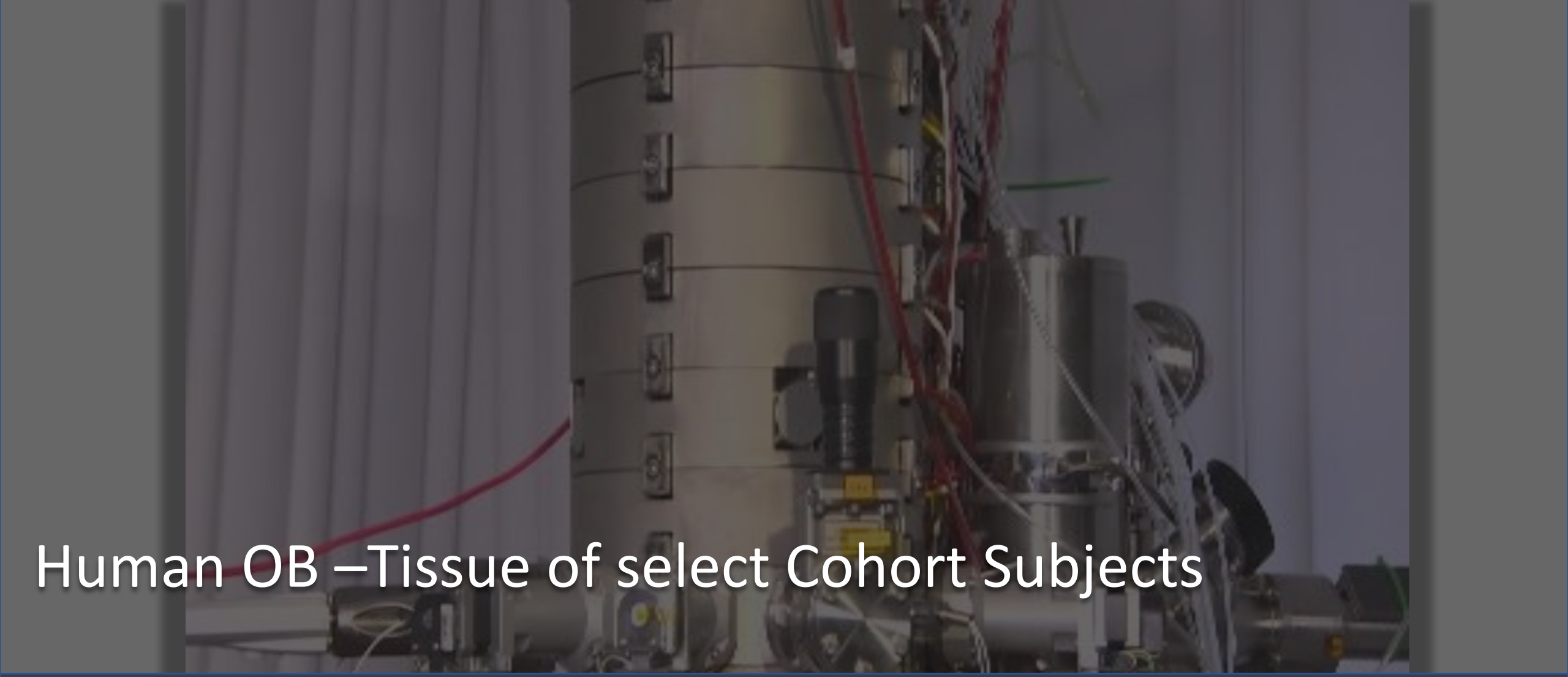
FARADAY ENERGY
New Frontiers in Material Synthesis

DEPARTMENT OF HEALTH AND HUMAN SERVICES · USA

CDC Workplace Safety and Health

NIOSH National Institute for Occupational Safety and Health

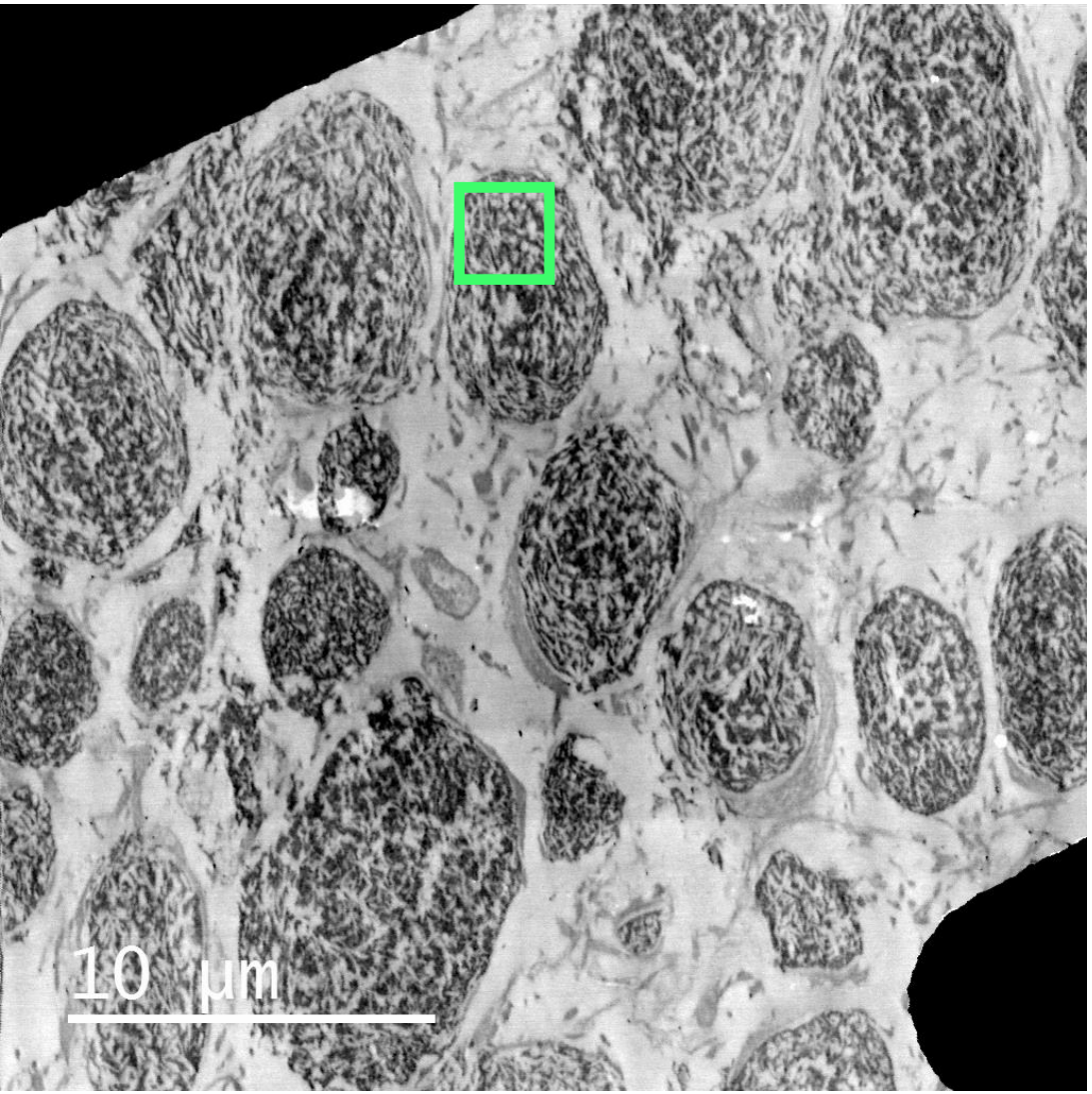
U.S. AIR FORCE

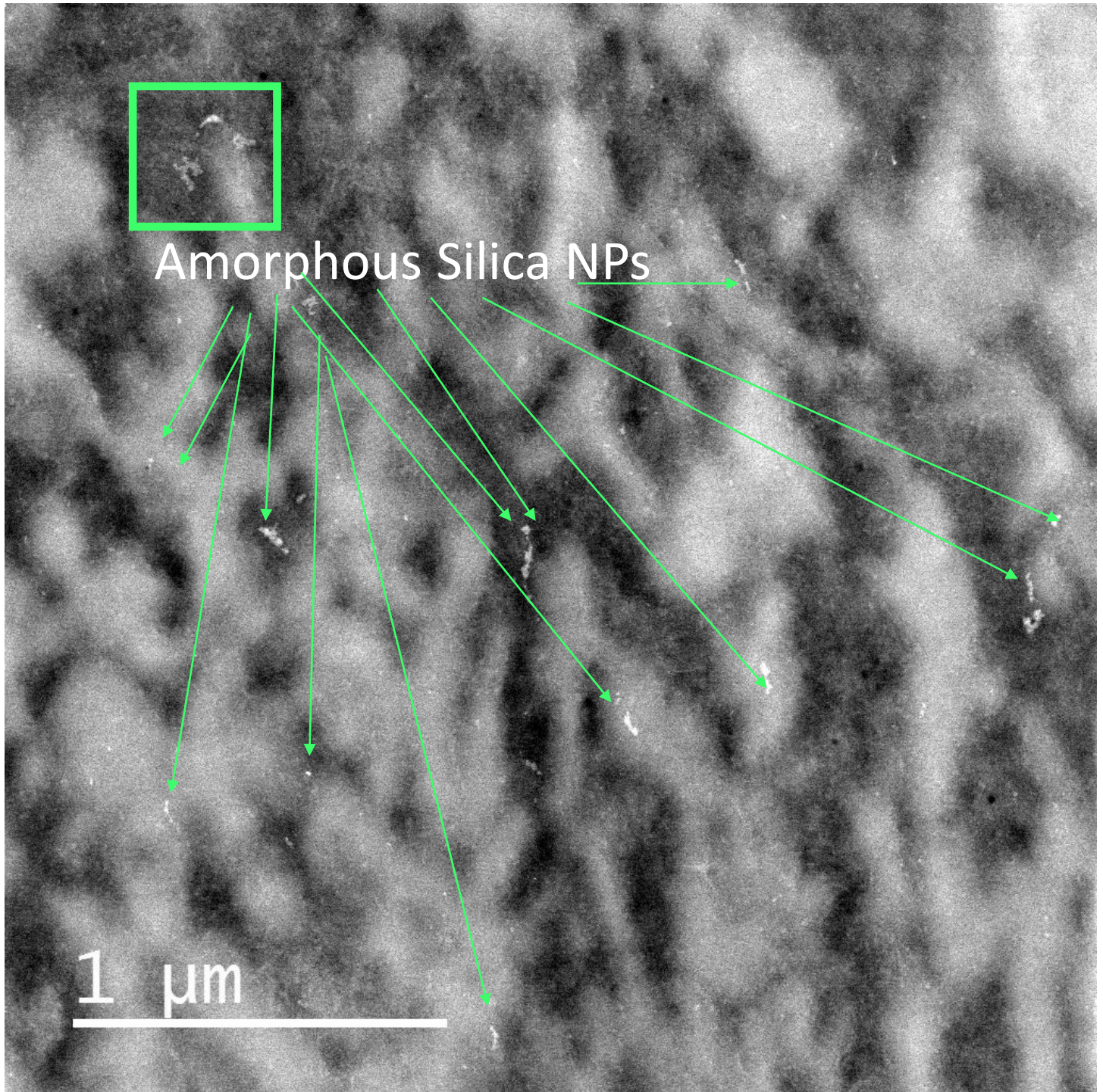


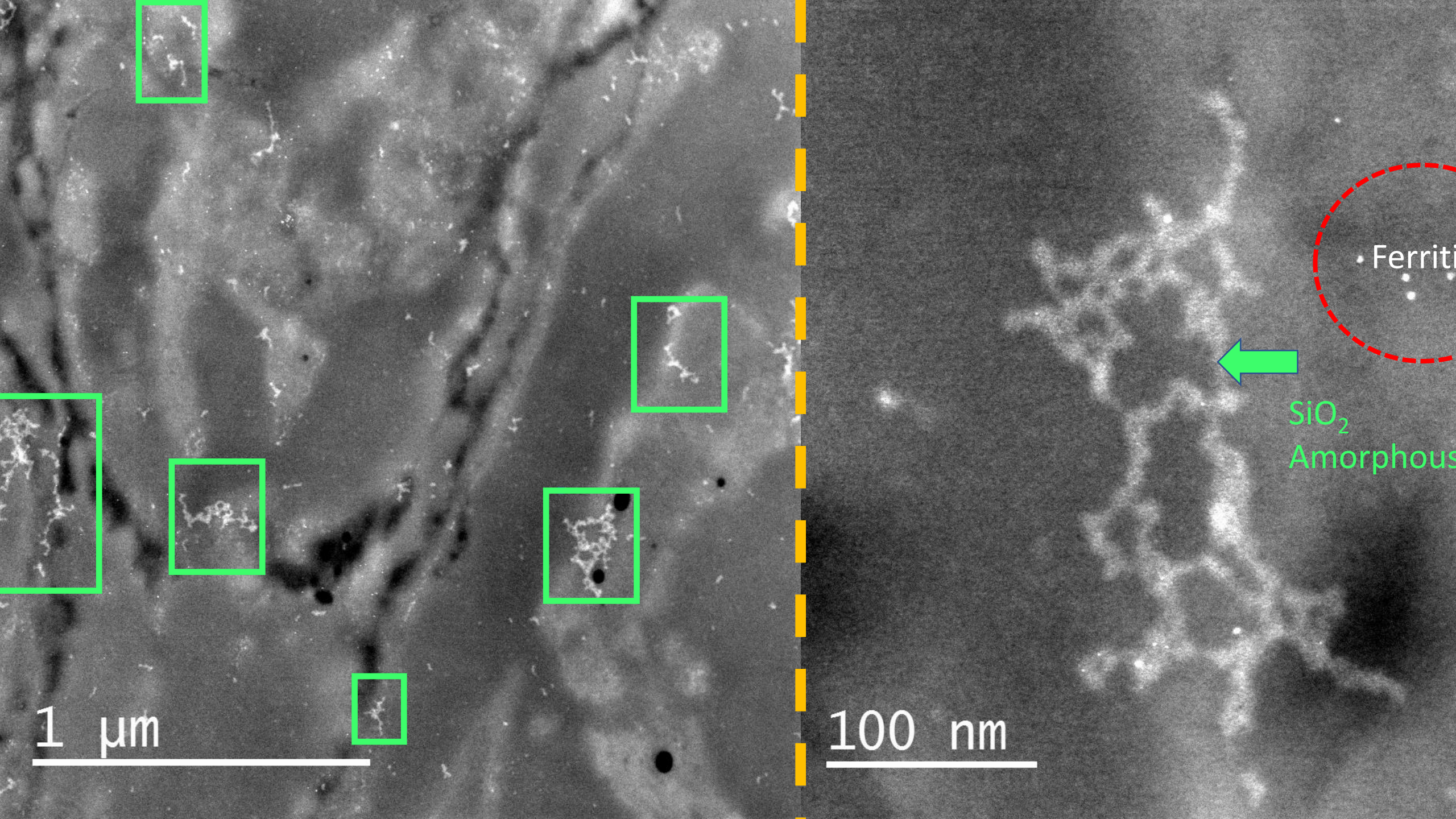
Human OB –Tissue of select Cohort Subjects

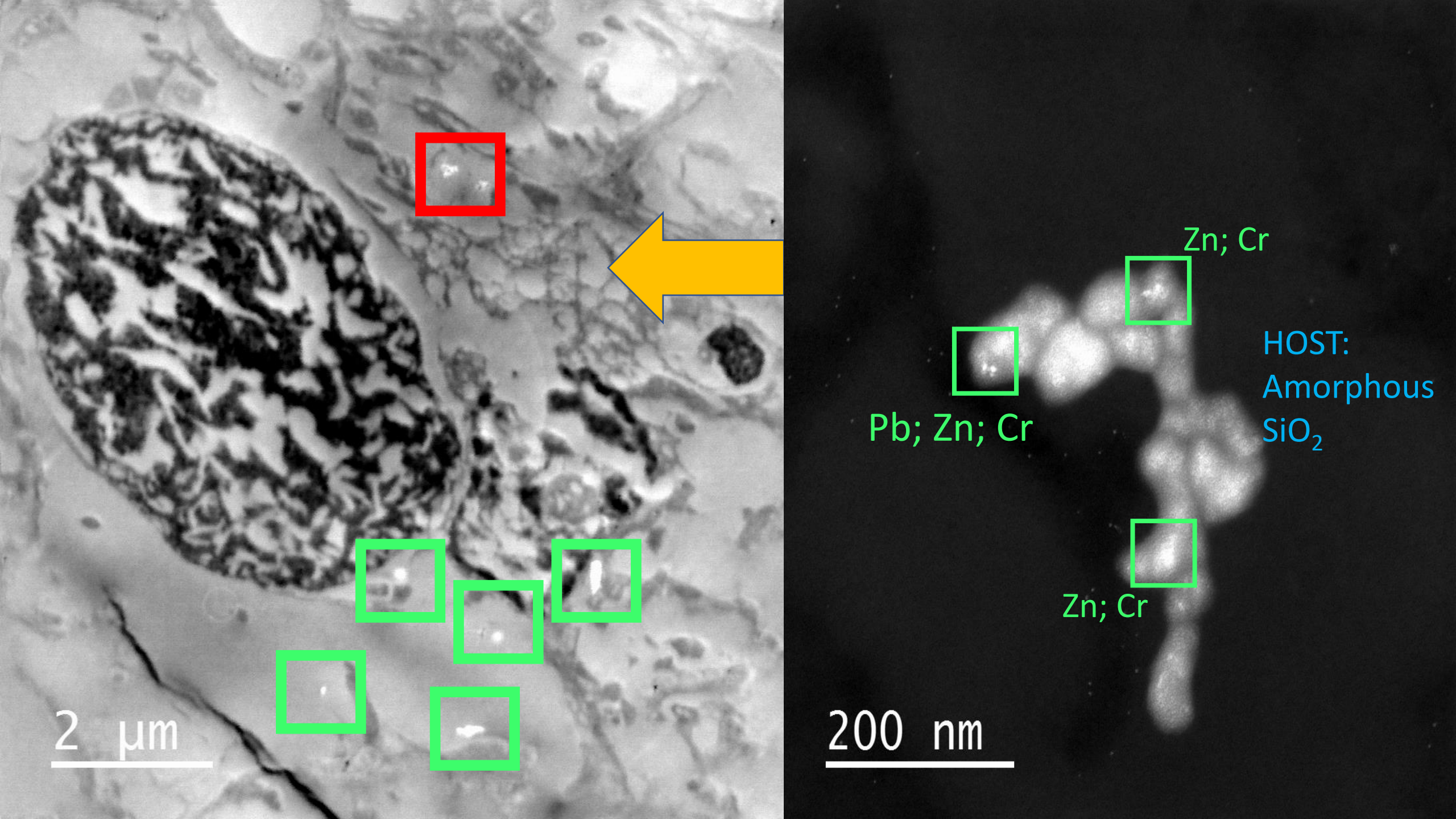
- Titan produce a spatial image and achieves ultra high resolution using aberration correction and can produces extremely narrow electron beam.
- Seeing ~1-10 nm NPs inside tissue without destroying the OB section.
- TALOS - for fast elemental mapping over larger tissue regions.

OB-STEM-F1






1 μm
100 nm
Ferriti
SiO2
Amorphous

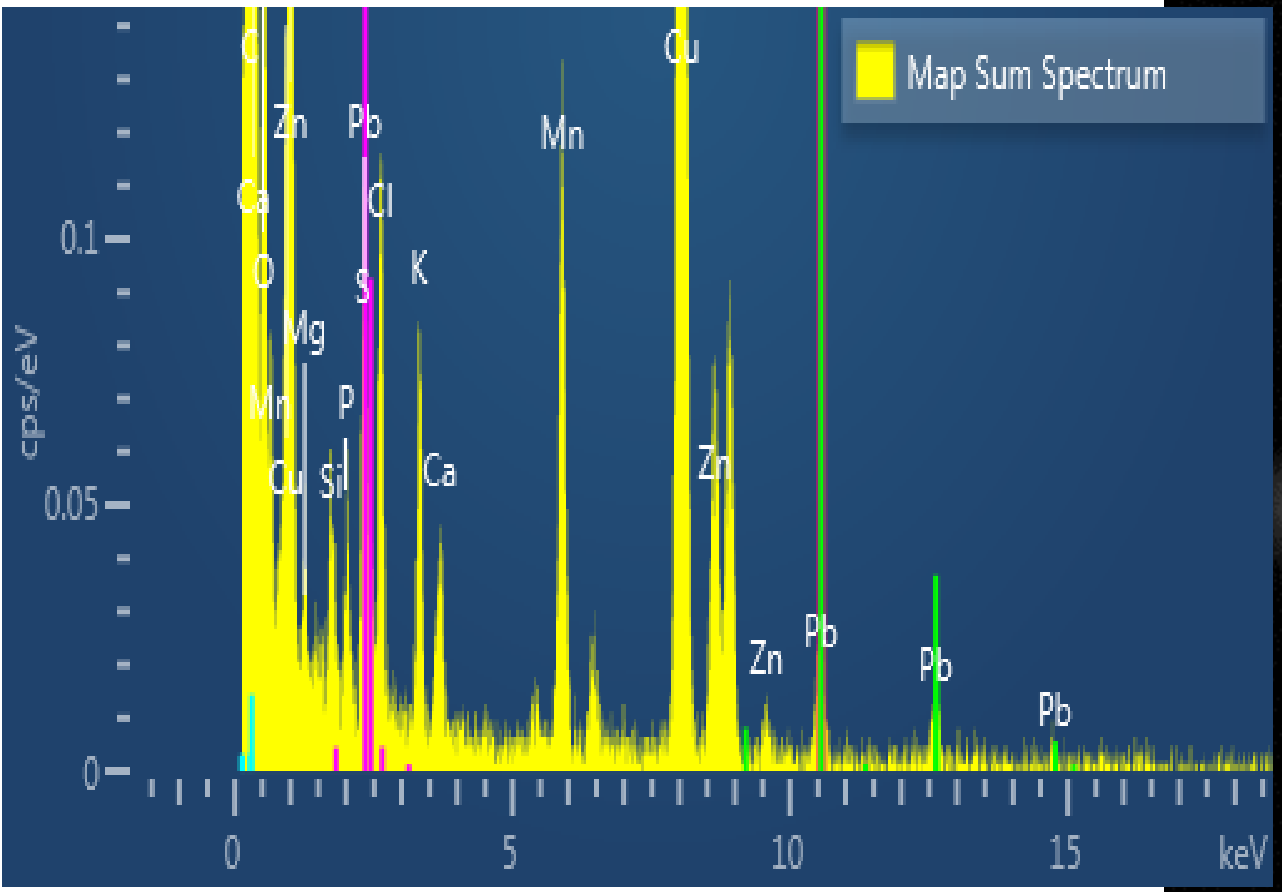

2 µm
Zn; Cr
Pb; Zn; Cr
HOST:
Amorphous
SiO2
Zn; Cr
200 nm

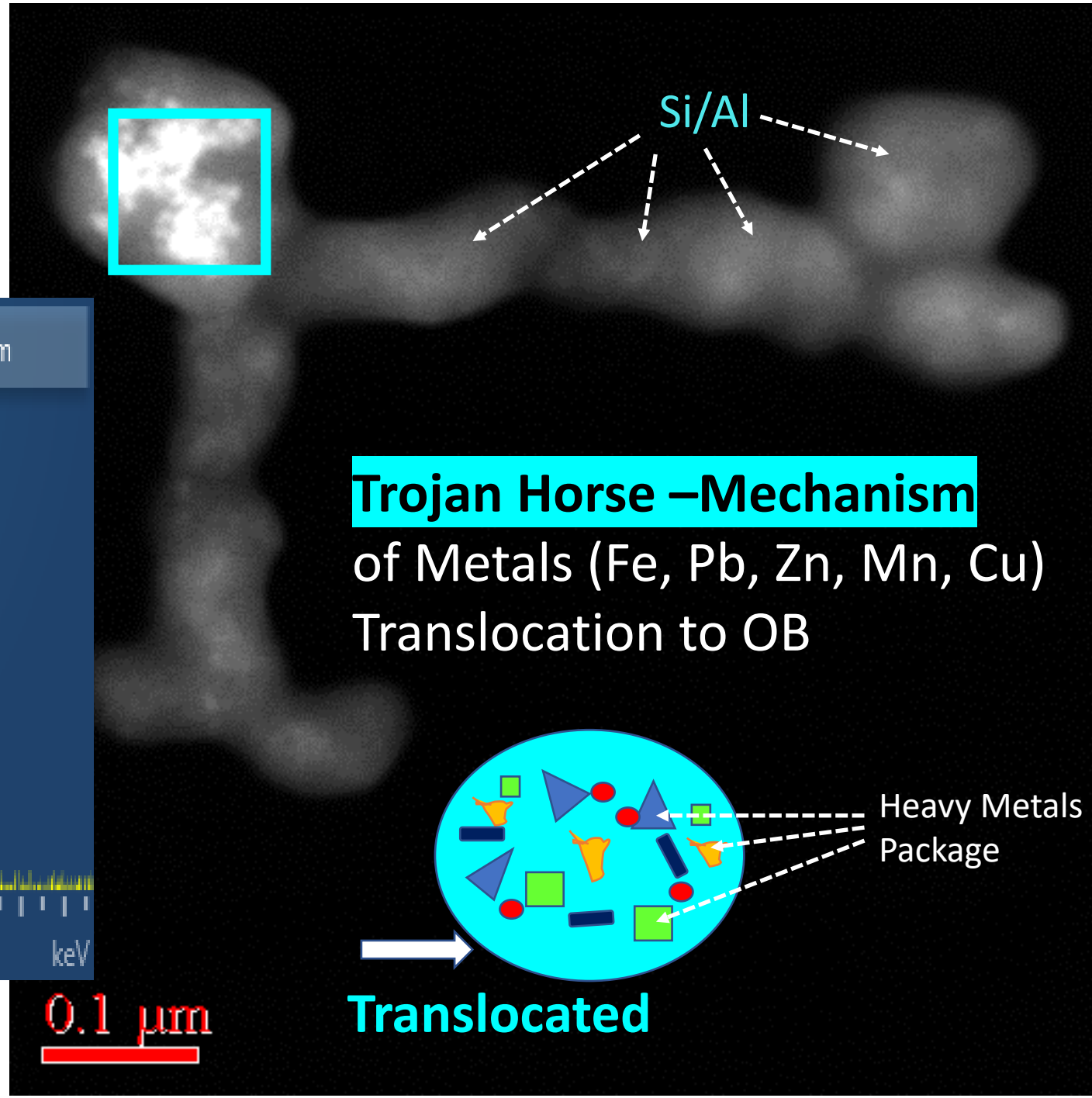

AZtec® - OB Project
File View Techniques Tools Help
EDS-TEM
Point & ID
Describe Specimen
Acquire and Confirm
Compare Spectra
Report Results
Guided
Custom
Scan Image START STOP Settings Second Image: None
Pb
Zin /Cr/Fe
P
Si/Al
100 nm
250nm
Acquire Spectrum START STOP Settings
Spectrum 54
Compare...
Atomic %
Powered by Tru-Q®
cps/eV
keV
Project Data
Current Site
Data Tree
OB Project
Specimen 1
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6
Site 47
Mini View
Input Count Rate (cps) Total:
770
Output Count Rate (cps) Total:
710
Dead Time
0%
Recommended WD 0.0 mm
Process Time: 3
Accelerating Voltage (kV): 200.0
Quant results Settings
Available Templates
Quant Result Details
Chosen Template: Summary Table - Single Spectrum
Requantify
Quant Results View
Powered by Tru-Q®
Viewed Data: Spectrum 54
Result Type: Atomic %
Processing Option Used: All Elements Processed
Element
Atomic %
Si 12.75
P 41.41
Confirm elements Settings
Periodic Table
Element List
Peak Labels
AutoID
Pre-defined
Clear all
Candidate Elements
Spectrum Height:
Double Click on a spectrum peak using the Show Candidate Elements tool.
Step Notes
This step allows you to:
Acquire an electron image.
Select points/regions on the image using the available beam control tools, Point, Rectangle, Ellipse, Freehand and Full Frame to acquire spectra.
Confirm elements identified by AutoID using markers, overlays and Show candidate elements tools to help you prepare a confirmed elements list for quantitative analysis.
Select a template from a list of quant templates to view the quant results.
Mag: 500000 x
AV: 200 kV
WD: 0 mm
Input Rate: 770 cps
Output Rate: 710 cps
Dead Time: 0%
Process Time: 3
3:05 PM
3/25/2021

Heavy Metal Uptake to the OB and potentially Deeper Brain via a Trojan Horse Nanoparticle Transport Mechanism

OB Sample: 20794261 - M1---A

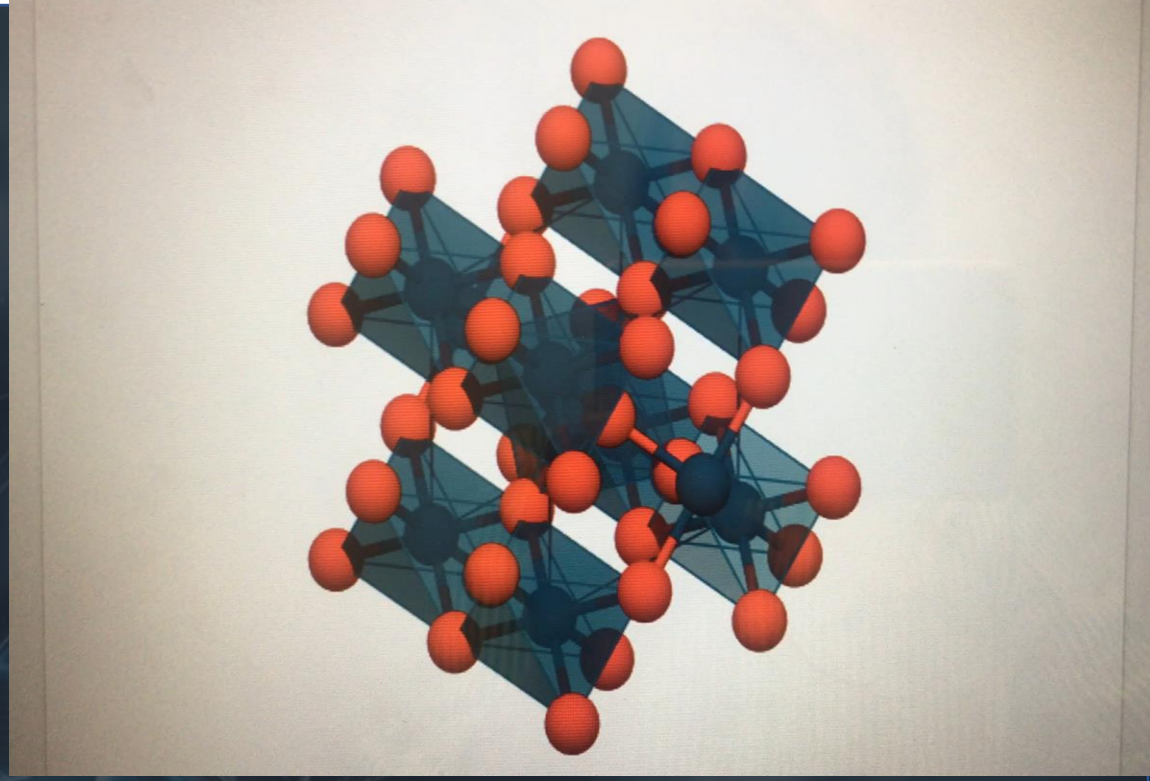
Translocated Si/Al NPs with heavy metal inclusions.



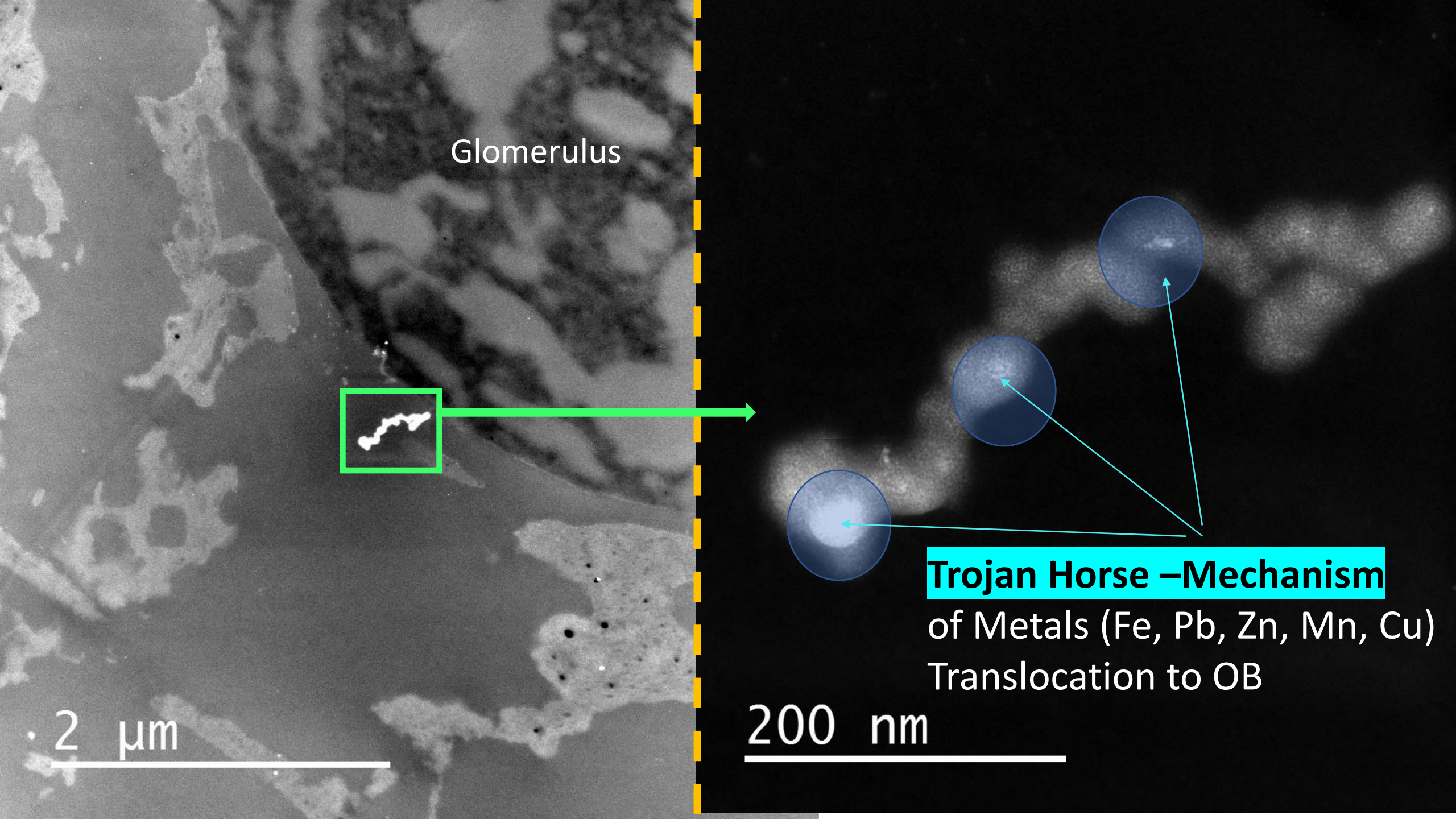


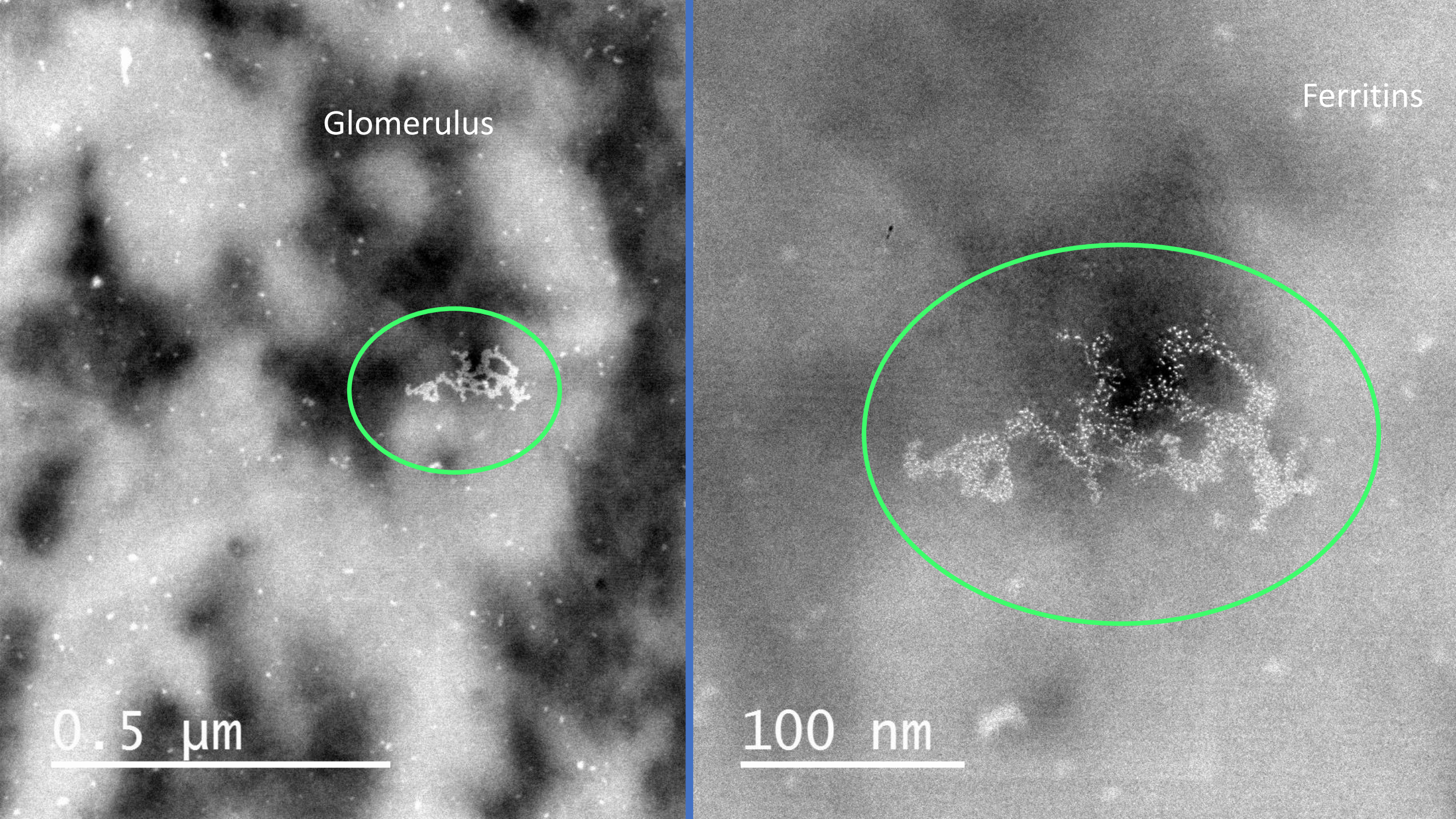
- Metallic Pb
- Nano-crystallites of PbO_2

Catalytic Properties



Olfactory Uptake of Lead (Pb) PM


Glomerulus
2 µm
200 nm
Trojan Horse –Mechanism
of Metals (Fe, Pb, Zn, Mn, Cu)
Translocation to OB


Glomerulus
0.5 µm
Ferritins
100 nm

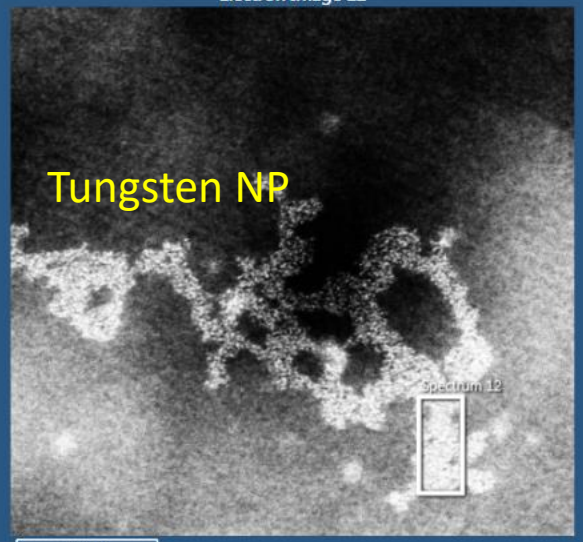
AZtec® - OB Project

File View Techniques Tools Help

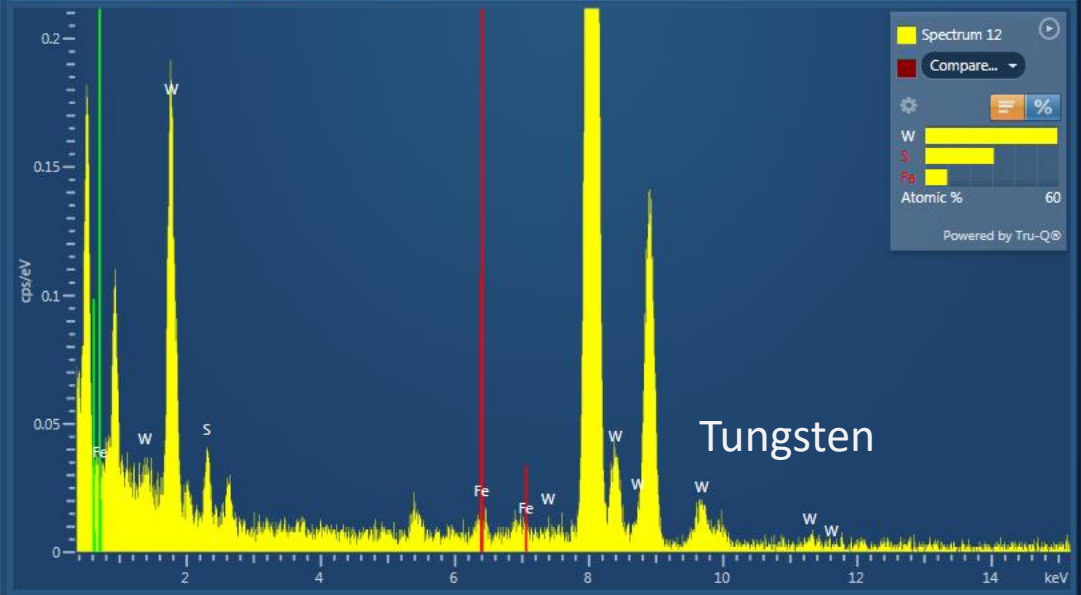
EDS-TEM | Point & ID | Describe Specimen | Acquire and Confirm | Compare Spectra | Report Results | Guided | Custom

Scan Image START STOP Settings Second Image: None

Electron Image 12



Acquire Spectrum START STOP Settings



Quant results Settings

Available Templates | Quant Result Details

Chosen Template: Summary Table - Single Spectrum

Requantify

Powered by Tru-Q®

Quant Results View

Viewed Data: Spectrum 12 Result Type: Atomic %

Processing Option Used: All Elements Processed

Element	Atomic %
S	30.78
Fe	9.81
W	59.40
Total	100.00

Confirm elements Settings

Periodic Table | Element List | Peak Labels

AutoID | Pre-defined | Clear all

Candidate Elements

Spectrum Height:

Double Click on a spectrum peak using the Show Candidate Elements tool.

Project Data

Current Site | Data Tree

- Electron Ima...
- Spectrum 9
- Spectrum 10
- Spectrum 11
- Site 10
  - Electron Ima...
  - Map Data 2
    - EDS Data
      - Map S...

Site 11

Mini View Rat...

Input Count Rate (cps) Total: 930

Output Count Rate (cps) Total: 900

Dead Time 0%

Recommended WD 0.0 mm

Process Time: 3

Accelerating Voltage (kV): 200.0

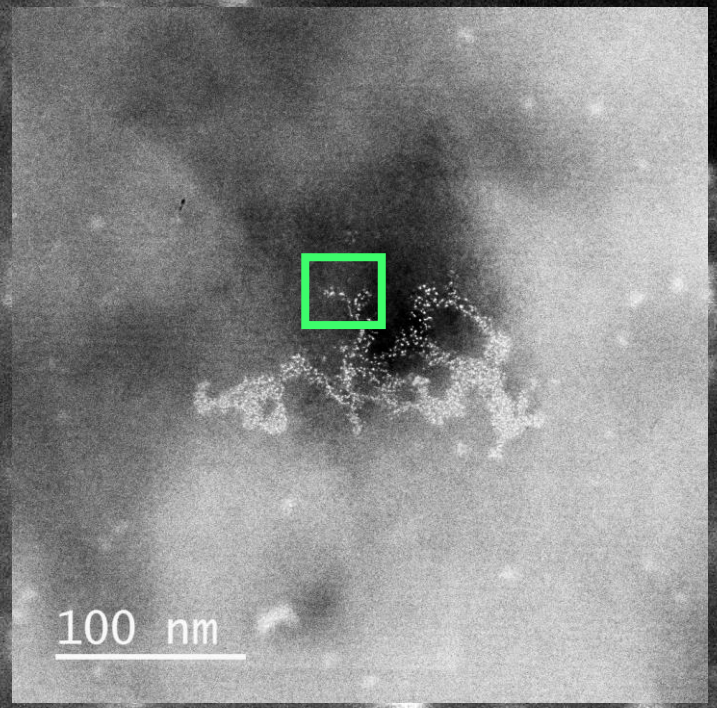
Step Notes

This step allows you to:

- Acquire an electron image.
- Select points/regions on the image using the available beam control tools, Point, Rectangle, Ellipse, Freehand and Full Frame to acquire spectra.
- Confirm elements identified by AutoID using markers, overlays and Show candidate elements tools to help you prepare a confirmed elements list for quantitative analysis.
- Select a template from a list of quant templates to view the quant results.

Mag: 800000 x | AV: 200 kV | WD: 0 mm | Input Rate: 930 cps | Output Rate: 900 cps | Dead Time: 0% | Process Time: 3

11:55 AM 3/11/2021



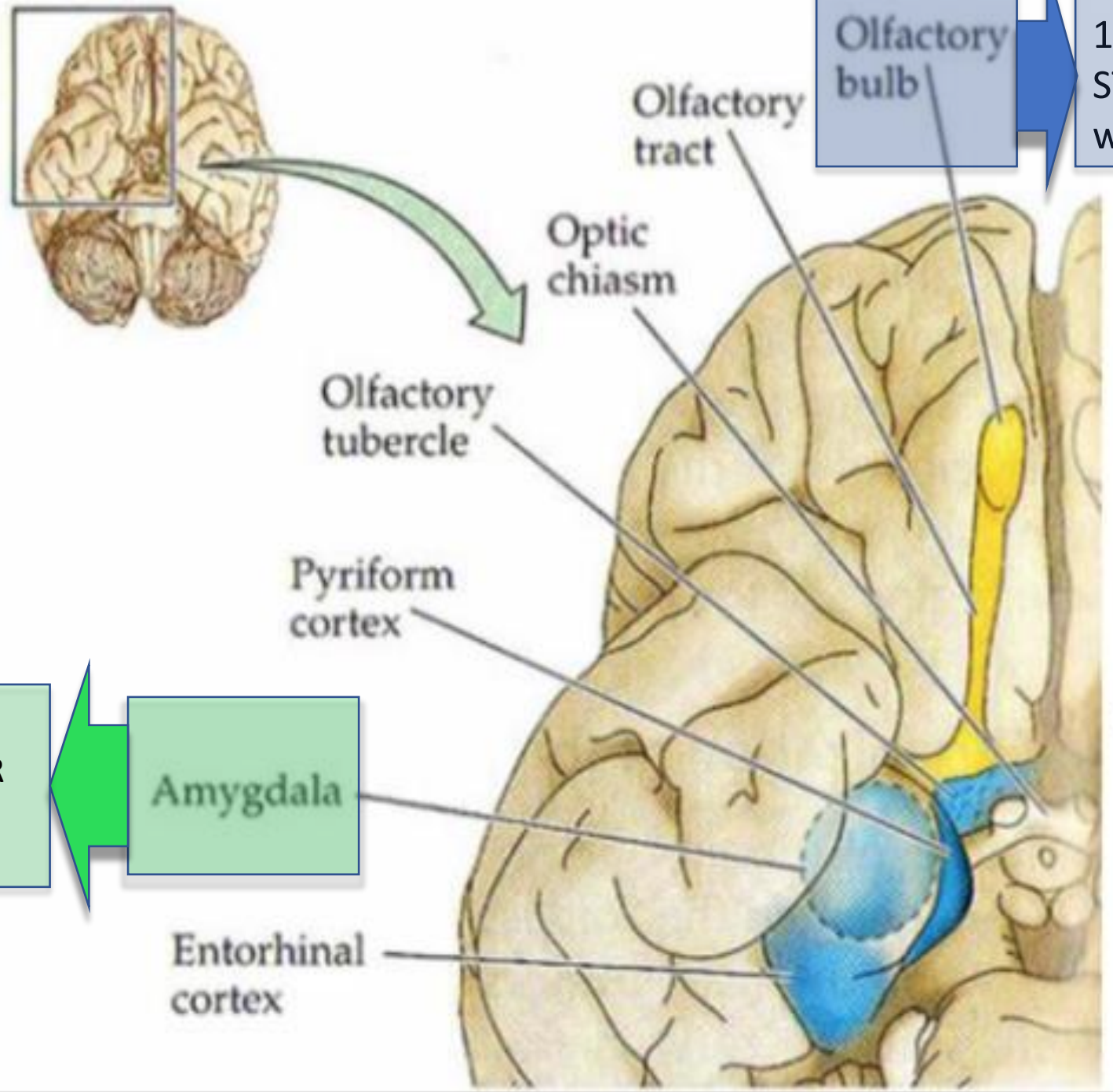
10 nm

Frozen – Vapor “W”
Tungsten

ATOMIC DISPERSION

1 nm

Indication of Incense Vapor Exposure?



Olfactory bulb → 12 OBs from RELIGEOUS ORDER STUDY COHORT with different progression of AD

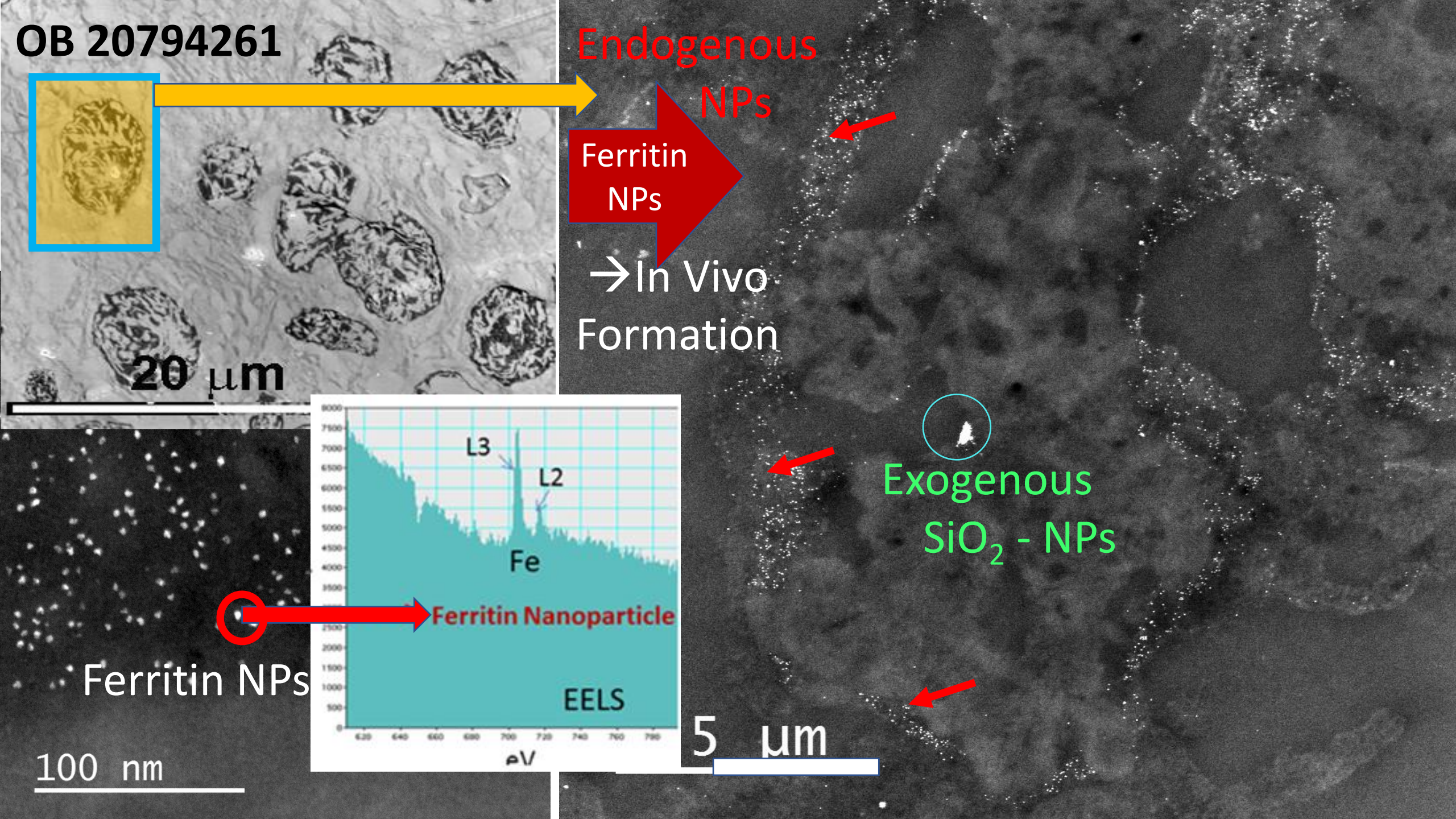
RELIGEOUS ORDER COHORT → Amygdala

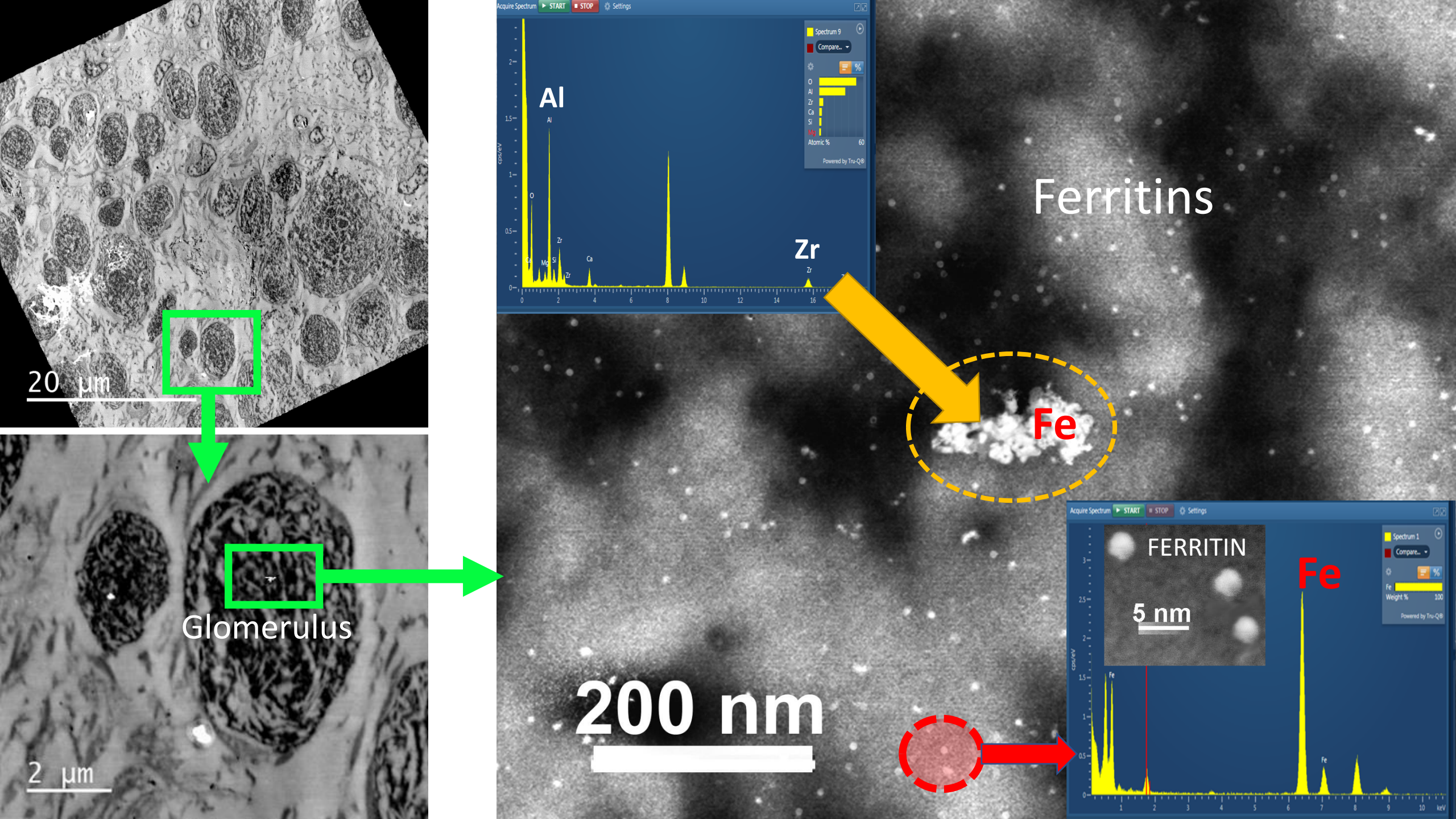
ONGOING STUDY

- NP Locations
- NP size, morphologies,
- Quantification
- Association of NP with cellular features
- NP association with ferritin enrichment

NEXT STUDY:

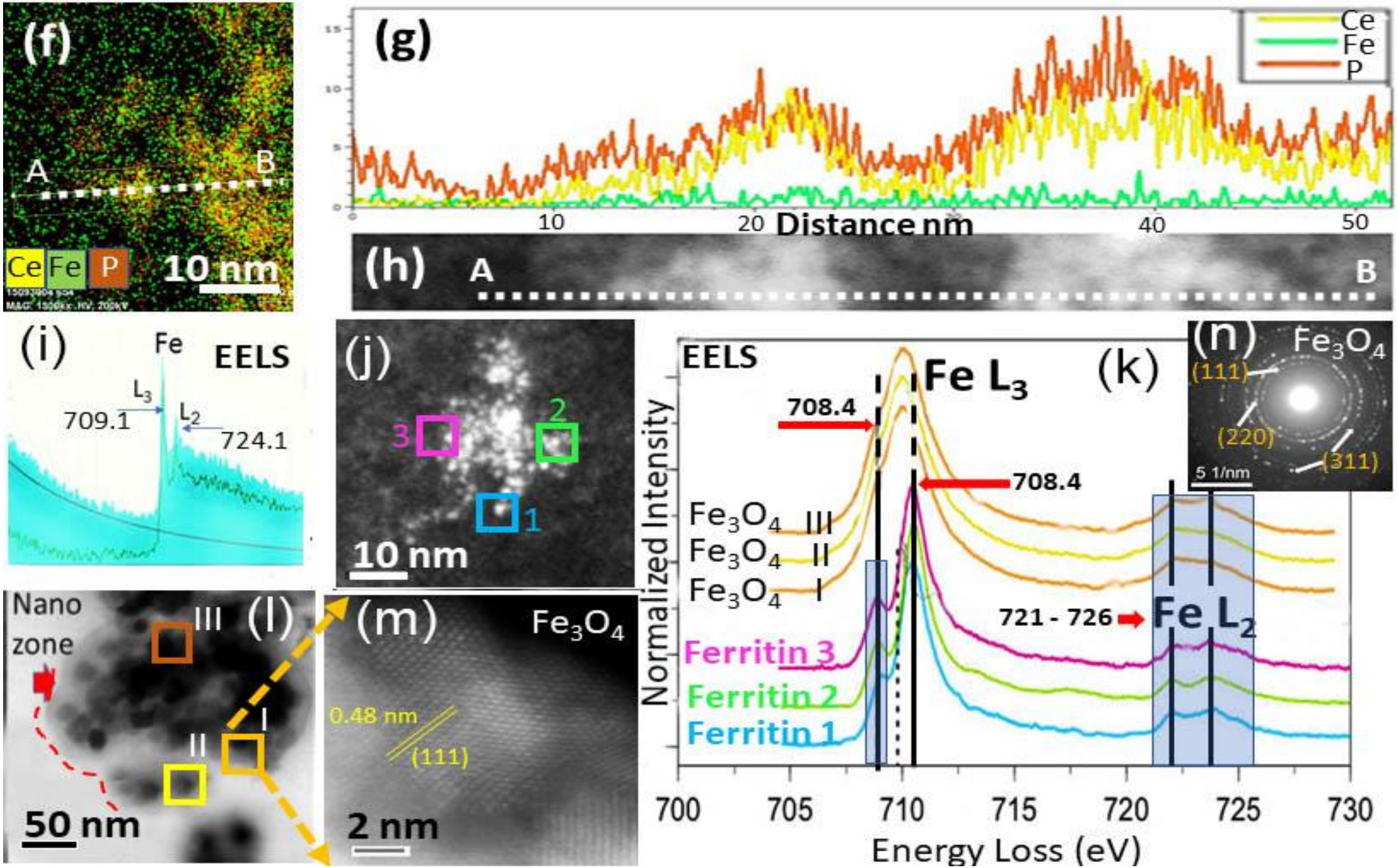
- Examine neuronal pathways from the OB glomeruli along the Olfactory tract to the amygdala and other deeper brain regions.


OB 20794261
20 µm
Endogenous NPs
Ferritin NPs
→In Vivo Formation
Exogenous SiO_2 - NPs
L3
L2
Fe
Ferritin Nanoparticle
EELS
eV
Ferritin NPs
100 nm
5 µm


20 μm
Glomerulus
2 μm
Al
Zr
Ferritins
Fe
200 nm
FERRITIN
5 nm
Fe

Fe - Nanoparticles in OB

Exogenous vs. Endogenous Fe


(f)
A
B
Ce
Fe
P
10 nm
(g)
Ce
Fe
P
10
20
Distance nm
40
50
(h)
A
B
(i)
Fe
EELS
L3
L2
709.1
724.1
(j)
3
2
1
10 nm
EELS
Fe L3
(k)
708.4
708.4
Fe3O4 III
Fe3O4 II
Fe3O4 I
Ferritin 3
Ferritin 2
Ferritin 1
721 - 726
Fe L2
Normalized Intensity
700
705
710
715
720
725
730
Energy Loss (eV)
(n)
Fe3O4
(111)
(220)
(311)
5 1/nm
Nano
zone
III
(l)
II
I
50 nm
(m)
Fe3O4
0.48 nm
(111)
2 nm

NPs and Neuronal Connections within the OB

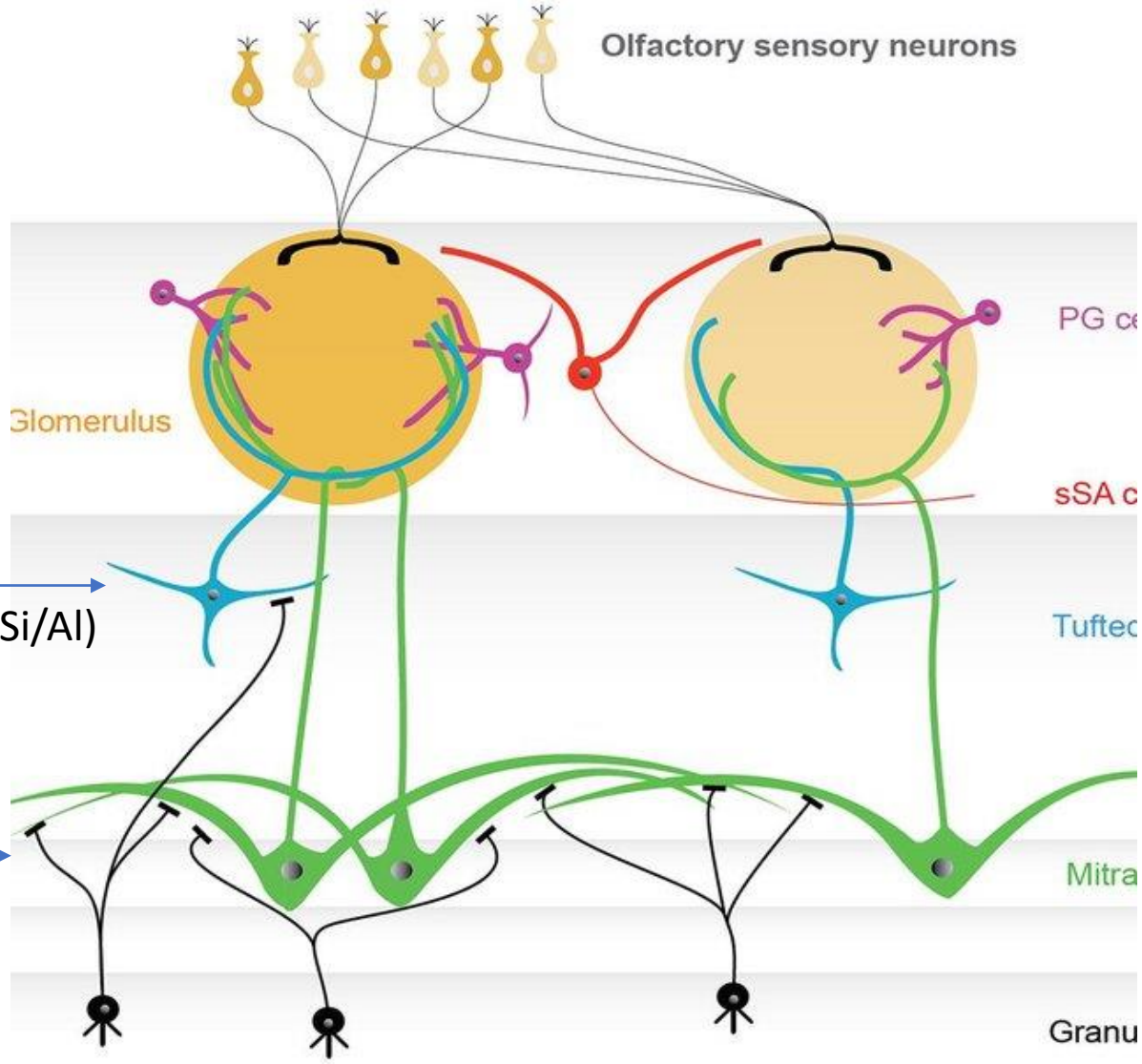
EXOGENOUS + ENDOGENOUS

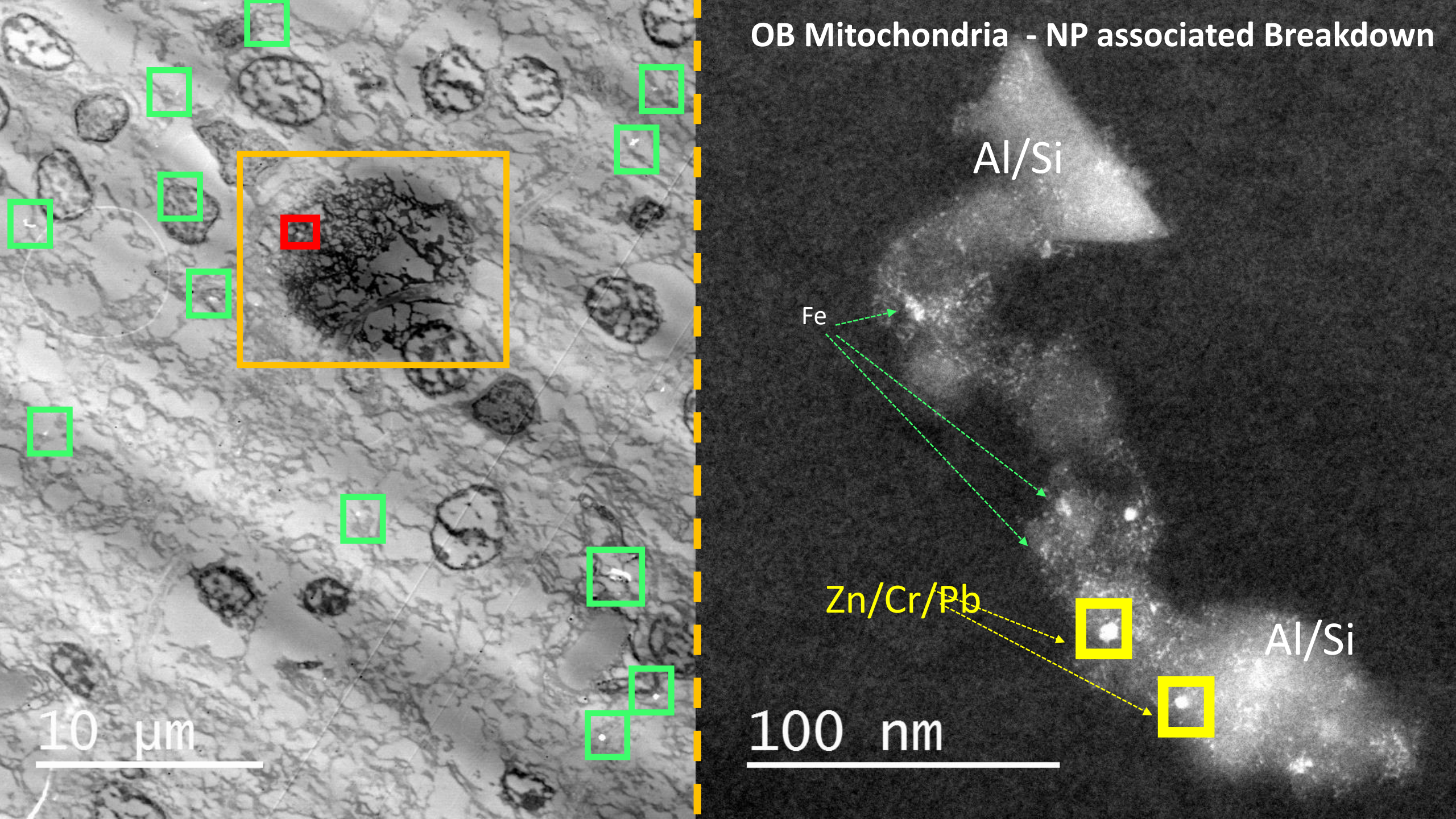
Si/Al; Trojan Horse NPs; Metal NPs (W; Pb, Zr)
Ferritins

EXOGENOUS + ENDOGENOUS (FeP; Fe3O4; Si/Al)

EXOGENOUS + ENDOGENOUS

Si/Al; Trojan Horse Metal NPs
Ferritins + FeP + Magnetite




OB Mitochondria - NP associated Breakdown
Al/Si
Fe
Zn/Cr/Pb
Al/Si
10 µm
100 nm

AZtec® - OB Project

File View Techniques Tools Help

EDS-TEM | Point & ID | Describe Specimen | Acquire and Confirm | Compare Spectra | Report Results | Guided | Custom

Search Help

Project Data

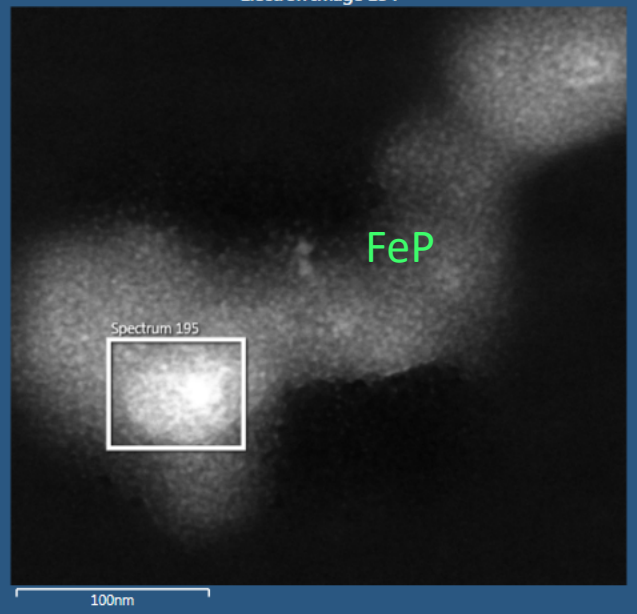
Current Site | Data Tree

- OB Project
  - Specimen 1
    - Site 1
    - Site 2
    - Site 3
    - Site 4
    - Site 5
    - Site 6
    - Site 7

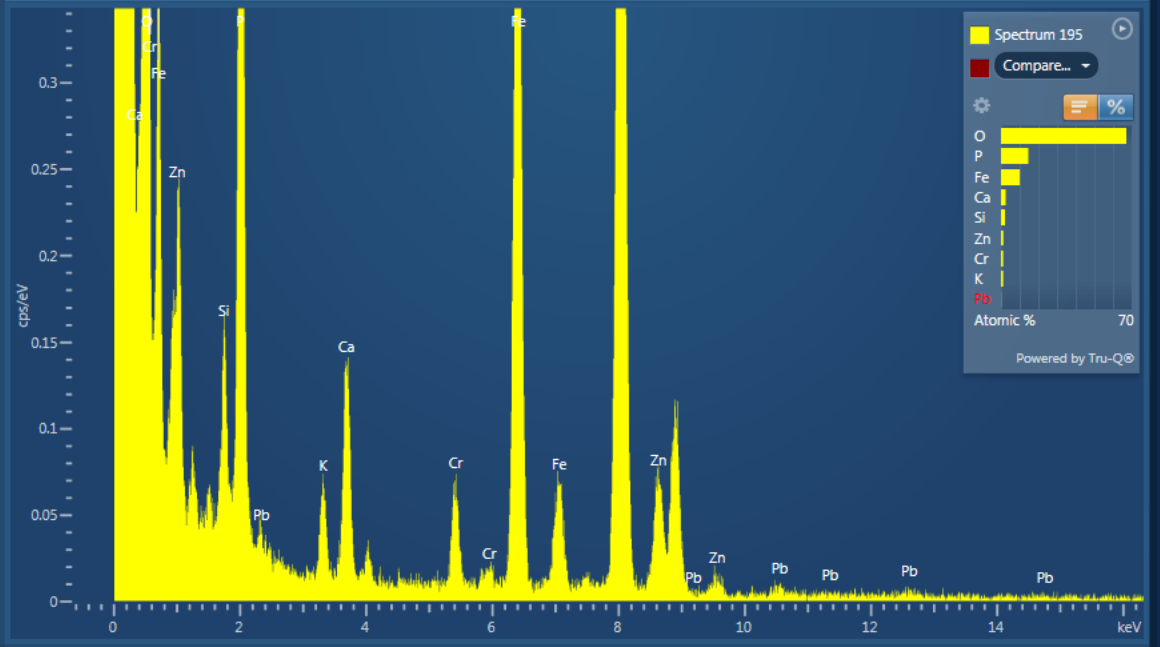
Site 159

Scan Image ▶ START ■ STOP Settings Second Image: None

Electron Image 154



Acquire Spectrum ▶ START ■ STOP Settings



Mini View Rat...

Input Count Rate (cps) Total: 1050

Output Count Rate (cps) Total: 1010

Dead Time 0%

Recommended WD 0.0 mm

Process Time: 3

Accelerating Voltage (kV): 200.0

Quant results Settings

Available Templates | Quant Result Details

Chosen Template: Summary Table - Single Spectrum

Requantify

Powered by Tru-Q®

Quant Results View

Viewed Data: Spectrum 195 Result Type: Atomic %

Processing Option Used: All Elements Processed

Element	Atomic %
O	67.14
Si	2.16
P	14.62
K	1.09
Ca	2.48
Cr	1.17

Confirm elements Settings

Periodic Table | Element List | Peak Labels

AutoID | Pre-defined | Clear all

Candidate Elements

Spectrum Height:

Double Click on a spectrum peak using the Show Candidate Elements tool.

Step Notes

This step allows you to:

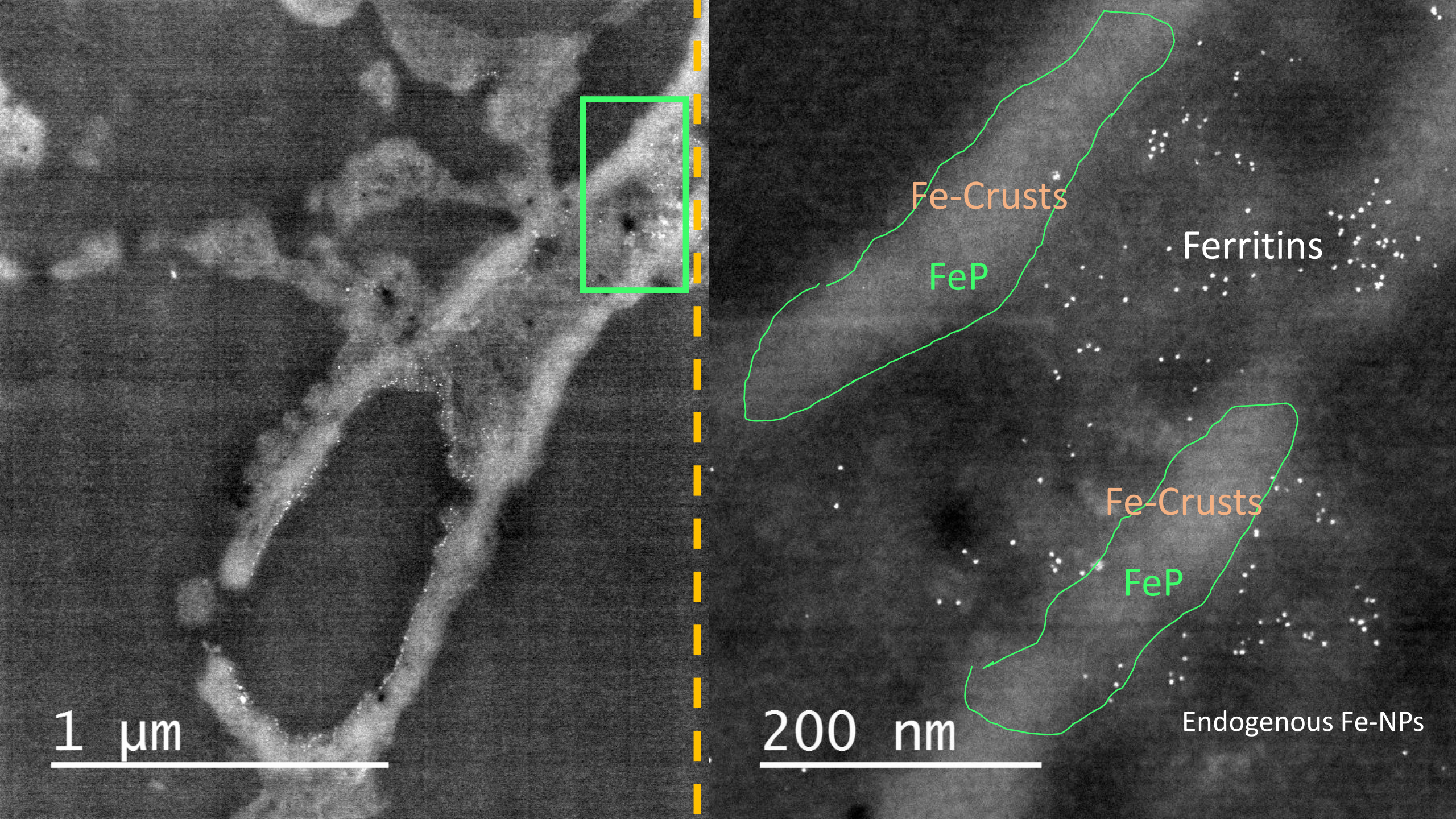
- Acquire an electron image.
- Select points/regions on the image using the available beam control tools, Point, Rectangle, Ellipse, Freehand and Full Frame to acquire spectra.
- Confirm elements identified by AutoID using markers, overlays and Show candidate elements tools to help you prepare a confirmed elements list for quantitative analysis.
- Select a template from a list of quant templates to view the quant results.

Information

Saved OB Project

Mag: 1000000x | AV: 200 kV | WD: 0 mm | Input Rate: 1050 cps | Output Rate: 1010 cps | Dead Time: 0% | Process Time: 3

12:13 PM 5/13/2021


Fe-Crusts
FeP
Ferritins
Fe-Crusts
FeP
1 μm
200 nm
Endogenous Fe-NPs

SUMMARY: Evidence for Air Pollution Particulates in OBs

- Heavy metals embedded in Al-Si particles in OB
- Trojan Horse transport mechanism is same for different Cohort Subjects!
- Biotransformation of translocated NPs in OB cells, including Fe-oxide to Fe-phosphate
- Presence of high levels of Ferritin (*acute phase protein*) due to Fe- biomineralization as indicator of inflammation.

- Does association mean causality?
- Heavy metal NPs in OB as indicator of environmental and occupational exposure (Pb;W) ?
- Need for studying NP - bioprocessing mechanisms in context with histopathology and molecular biology mechanisms

•Although very high numbers of exogenous NPs in OB tissue, what does it mean in terms of toxicity potential ?

•To be determined: Degree of association/correlation with pathology; plaques; A-β: Tau; and other marker proteins; activated microglial and oligodenedrocites etc.

Acknowledgements

1R01AG067497-01

ROS Cohort

Religious Orders Study and Rush Memory and Aging Project

Bennett DA, Buchman AS, Boyle PA, Barnes LL, Wilson RS, Schneider JA.
J Alzheimers Dis. 64(s1):S161-S189. PubMed PMID: 29865057
Published date: 2018 Jun 12

MAP Cohort

Overview and findings from the Rush Memory and Aging Project.

Bennett DA, Schneider JA, Buchman AS, Barnes LL, Boyle PA, Wilson RS.
Curr Alzheimer Res. 9(6):646-63. PubMed PMID: 22471867
Published date: 2012 Jul

The Minority Aging Research Study: ongoing efforts to obtain brain donation in African Americans without dementia.

Barnes LL, Shah RC, Aggarwal NT, Bennett DA, Schneider JA.
Curr Alzheimer Res. 9(6):734-45 PubMed PMID: 22471868
Published date: 2012 Jul

Instrumentation:

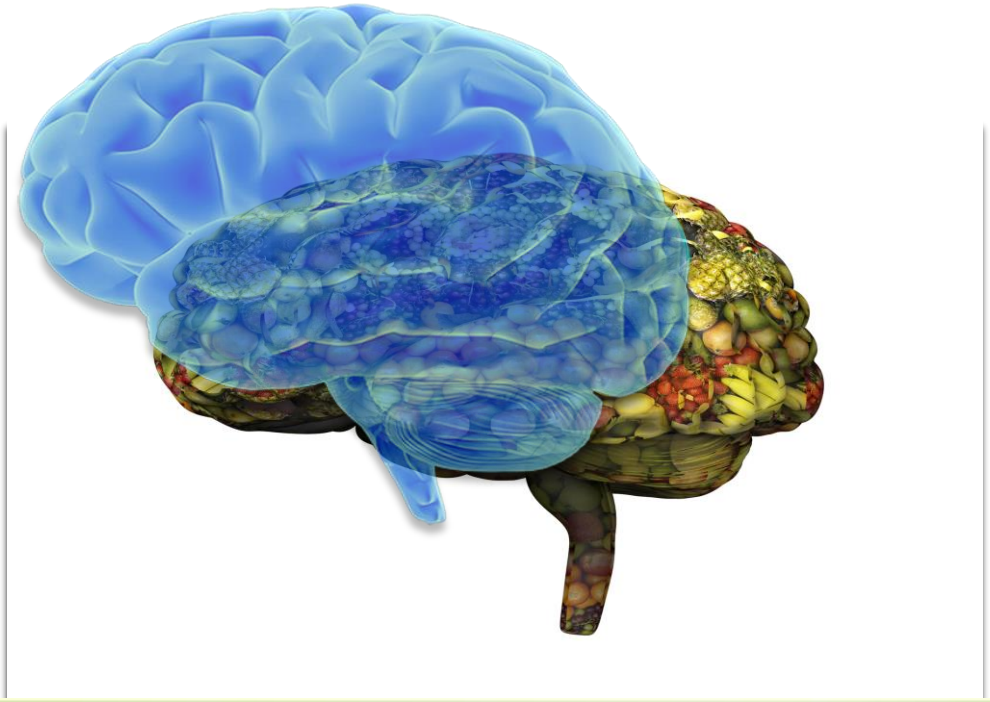
NIOSH, Cincinnati OH
Advanced Electron Microscopy Center
Dr. Alan K Dozier

AFRL
Right Patterson Air Force Base
Titan and Talos
Dr. Larry Drummy: Group Leader

In 1826, the French lawyer Anthelme Brillat-Savarin wrote, in *Physiologie du Gout, ou Meditations de Gastronomie Transcendante*:

"Dis-moi ce que tu manges, je te dirai ce que tu es."

Tell me what you eat and I will tell you what you are.




Tell us what you **breathe** ----- and we will tell you what happens in your brain.
Image Curtesy: from 'The Martian'