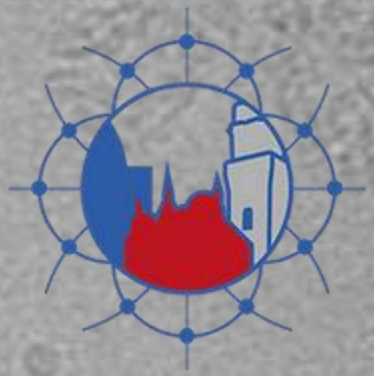
EYE-Lab-the Naples cryo EM Laboratory: what we can do for Life and Material Science

E. Esposito, G. Sanità, F. Donadio, D. Passaro, I. Rendina

Institute of Applied Science and Intelligent Systems "E. Caianiello" ISASI
National Research Council



ECM34
PADOVA 2024

26 August 2024

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EYE LAB

ISASI

The EYELAB journey

June 2023
Inauguration and starting activity

Octo 2022
Thermofisher
Final test

June 2022
Thermofisher
installation

Jan 2022
Lab work

Training on site 90 days

Glacios, cryo TEM 200kV
Aquilos 2, cryo SEM/FIB
Vitrobot

80 m^2

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LAB

ISASI

Emanuela Esposito Senior Researcher CNR-ISASI

2001
Researcher
CNR-ICIB-IMM

2000-2001
Researcher
INFM
Choerentia

1997-1999
Researcher INAF
Osservatorio
Astronomico di
Capodimonte

1994-1996
Researcher
Nuclear Physics
University of Oxford
(UK)

Development of:
superconducting photon and charged particle detectors
photonic sensors
advanced spectroscopy technology
Expertise:
Cryogenic technology up to mK
Micro and nano fabbrication
Imaging and metrology

Gennaro Sanità associate researcher

Development of:
- **Biofunctionalization of Nanoparticles**
- **Biofunctionalization of Photonic Biosensors**

Expertise:
- **Imaging and Metrology**
- **CryoEM: Single Particle Analysis**
  Thomography
  Proteins 3D reconstruction
  FIB cellular lamella production and nanofabrication

2024
Post-doc
@CNR ISASI
REMINDS

2022-2023
Post-doc
@CNR-ISASI
IMPARA

2020-2021
Post-doc
@ISASI
SHARID

2017-2020
Biology
PhD student
@UNINA

Federica Donadio associate researcher

2023-2024
Post-doc
@CNR-ISASI
IMPARA

2022-2023
Material And Process
Engineering
Visiting PhD student
@AALEN

2019-2023
Material And Process
Engineering
PhD student
@UNINA

Development of:
- Innovative Hybrid Configurations for Composite Materials
- Green Composites
Expertise:
- Materials Characterization
- Imaging and Metrology
- CryoEM: FIB cellular lamella production and nanofabrication

The current structure and governance

Funded by: Ministero della Ricerca - Potenziamento Infrastrutture di Ricerca EUBI, prog. *Imaging dalle Molecole alla Preclinica - 20 Mln€*
Obj 3: *Crio microscopia elettronica per biologia strutturale e cellulare - 4 Mln €*

Head of Unit. I. Rendina
Inf. Unit Scientific Manager E. Esposito

Main goal

- Support of national community:
  - Access to cutting edge technology
  - Technology development project
- Development of scientific collaboration:
  - sharing of expertise

Access Workflow

Cost estimate

Fixed Cost

- Service contract

Glscios+Aquilos+Vitrobot per day 650€

- Staff cost

Consumable Cost

LN glacios 40€ per day
aquilos 30€ per day

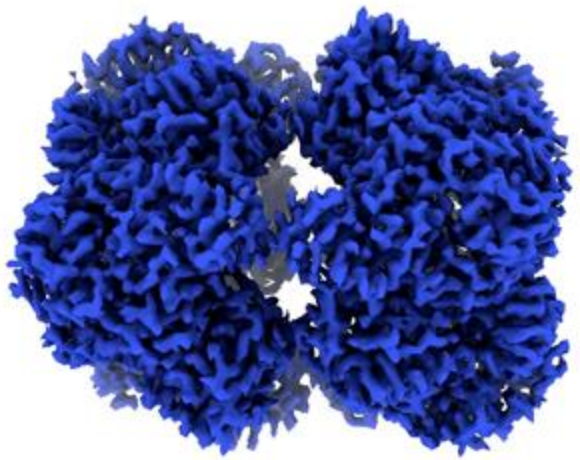
basic grid+clip and ring 25 each

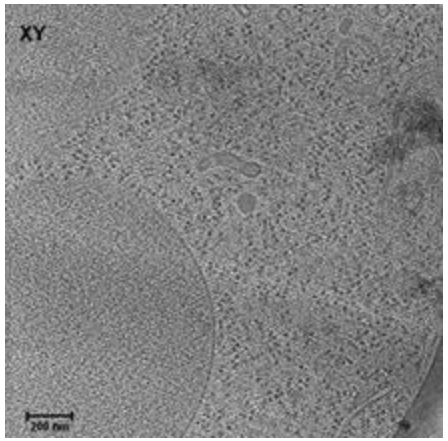
EYE Lab has full maintenance coverage for 3 years

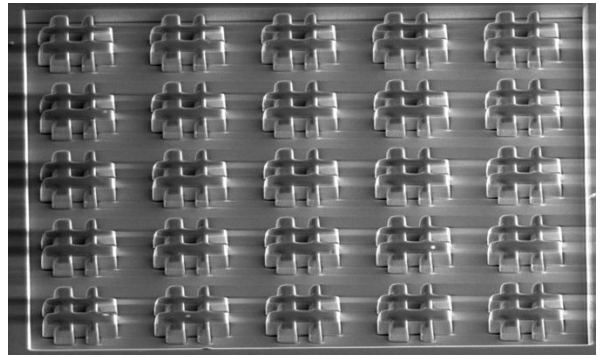
- Consumable cost for the duration of the experiment to the scientific community
- Full cost to private company

What we can do for Life and Material Science

The Lab is designed to efficiently combine
Single Particle Analysis (SPA) and Electron Tomography (ET),
to study in detail the structure of single macromolecules,
whole cellular compartments
&
Material Imaging, metrology and nanopatterning

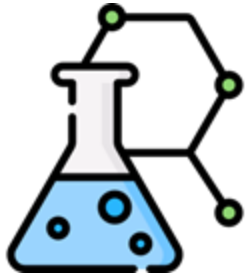






Workflows in modern structural biology

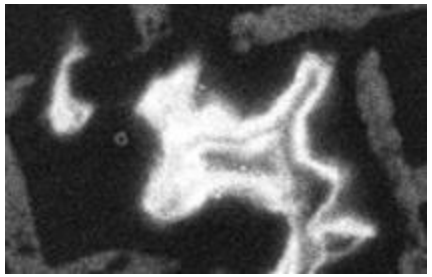
Single Particle Analysis



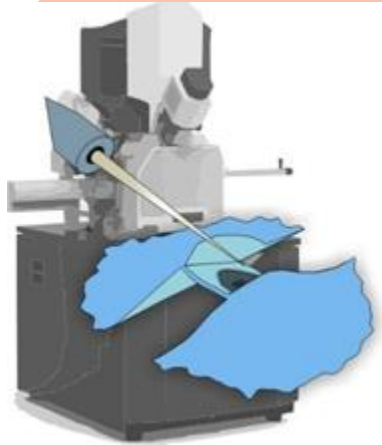
Sample vitrification → Low magnification screening → High magnification data acquisition → Data processing

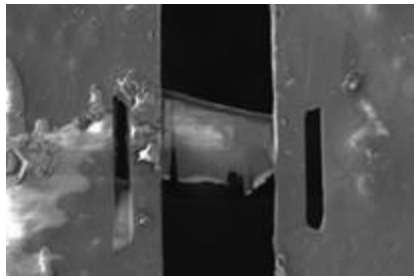
cryoSPARC

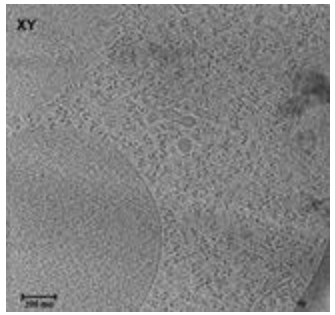
Electron Tomography



Sample vitrification → Thinning → Low to High magnification data acquisition → Data processing







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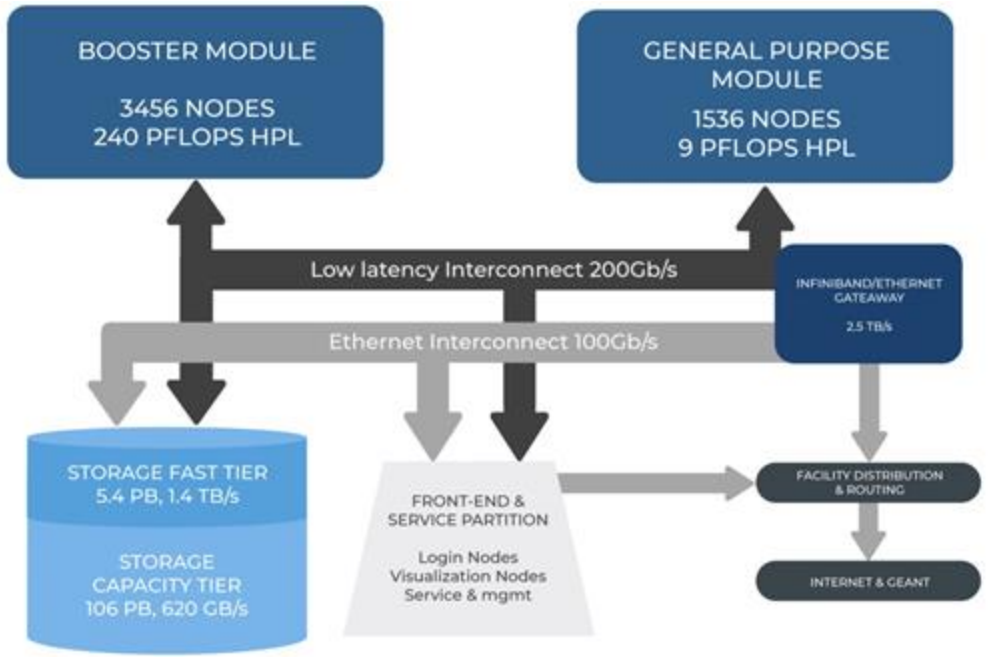
CINECA | UserDB

High Performance Computing

Dr. Cristiano Padrin

The pre-exascale Tier-0 EuroHPC supercomputer LEONARDO is classified in **6° position among the most powerful supercomputers in the** [Top500](Top500) List.
It is hosted by Cineca and is currently built in the Bologna Technopole.



Trial account x EYE Lab

CryoSPARC requirements

Quad-core or better Intel CPU
32GB+ CPU RAM
NVIDIA GPU with Compute Capability 3.5+ and 8 GB+ GPU RAM
256GB+ SSD drive

Leonardo HPC

Intel Sapphire Rapids CPUs, each with 56 cores
8x 64 GB DDR4 3200 MHz (512 GB) 4x NVIDIA custom Ampere A100 GPU 64GB HBM2e, NVLink 3.0 (200GB/s)
• High Performance Storage 5.7 PB, 1.4 TB/s Based on 31 x DDN Exascaler ES400NVX2

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Scientific Collaborations

1. Prof. Beatrice Vallone **La Sapienza, Biology Dep**
2. Dott. Luca De Stefano **CNR-ISASI** — paper published
3. Dott. Roberto Russo **CNR-ISASI** — paper published
4. Prof. Delia Picone **UNINA, Chemistry Dep.** — paper published
5. Prof. Patrizia Contursi **UNINA, Biology Dep.** — paper preparation/writing
6. Prof. Alfonso De Simone **UNINA, Pharmacy Dep.**
7. Dott.ssa Giuliana Fusco **CNR-IBB**
8. Dott.ssa Daniela Corda **CNR-IEOS** — paper preparation/writing
9. Prof Annalisa Lamberti **UNINA, Molecular Medicine Dep.**
10. Dott.ssa Rita Berisio **CNR-IBB**
11. Dott.ssa Claudia Tortiglione **CNR-ISASI**
12. Prof. Matteo Calvaresi **UNIBO, Chemisry Dep** — paper preparation/writing
13. Dott. Luigi Vitagliano, Dott. Ruvo Menotti **CNR-IBB**
14. Dott.ssa Candida Fasano, Dott.ssa Greta Varchi, Prof. Paola Sanese **CNR-ISOF(BO) - IRCCS (BA)**
15. Dott. Andrea Ilari, **CNR-IBPM (RM)**
16. Prof. Tillmann Page, **Uni Copenaghen, Biology Dep.**
17. Prof. Roberto Bassi, **Università di Verona**
18. Prof. Carla Zannella, **Università Vanvitelli**
19. Prof Annarita Falanga, **UNINA, Agriculture Dep.**

Project

CNR Multidisciplinary advanced platform for targeting neuronal and glial homeostasis

CNR-INVENIT

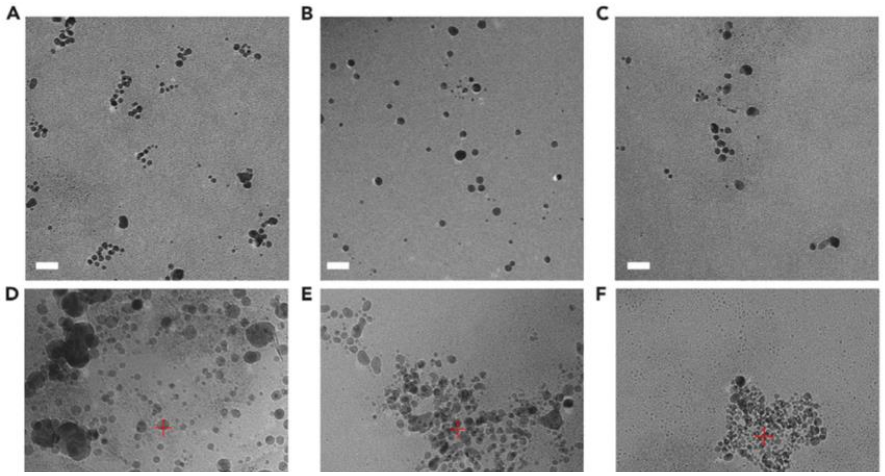
8 partner – founded 1,15Ml €- 12 m

Company

naturalis®
LIFE TECHNOLOGIES

Dr. L. De Stefano **ISASI -CNR**

Images and characterization of Ag-Tween NPs for new synthesis approach



STAR Protocols • *Open Access* • Volume 5, Issue 1 • 15 March 2024 • Article number 102920

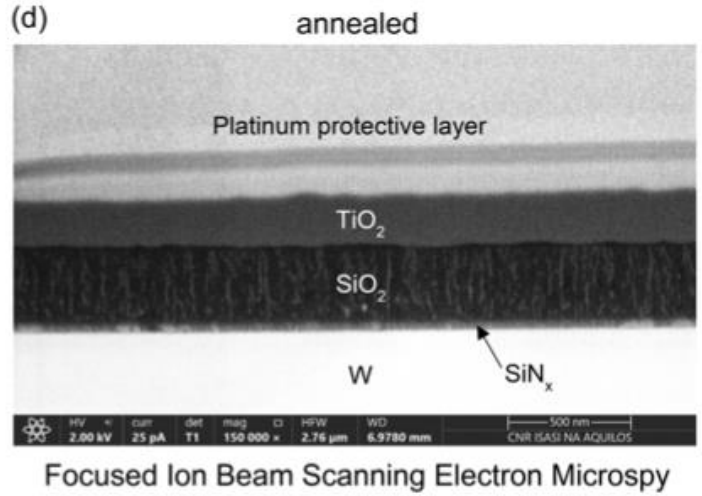
Protocol for synthesis of spherical silver nanoparticles with stable optical properties and characterization by transmission electron microscopy

Nocerino, Valeria[a, b]; Miranda, Bruno[a]; Dardano, Principia[a]; Sanità, Gennaro[c]; Esposito, Emanuela[c]; De Stefano, Luca[a]

Save all to author list

Dr. R. Russo **ISASI -CNR**

Cross section structural analysis



Solar RRL • Volume 8, Issue 10 • May 2024 • Article number 2400079

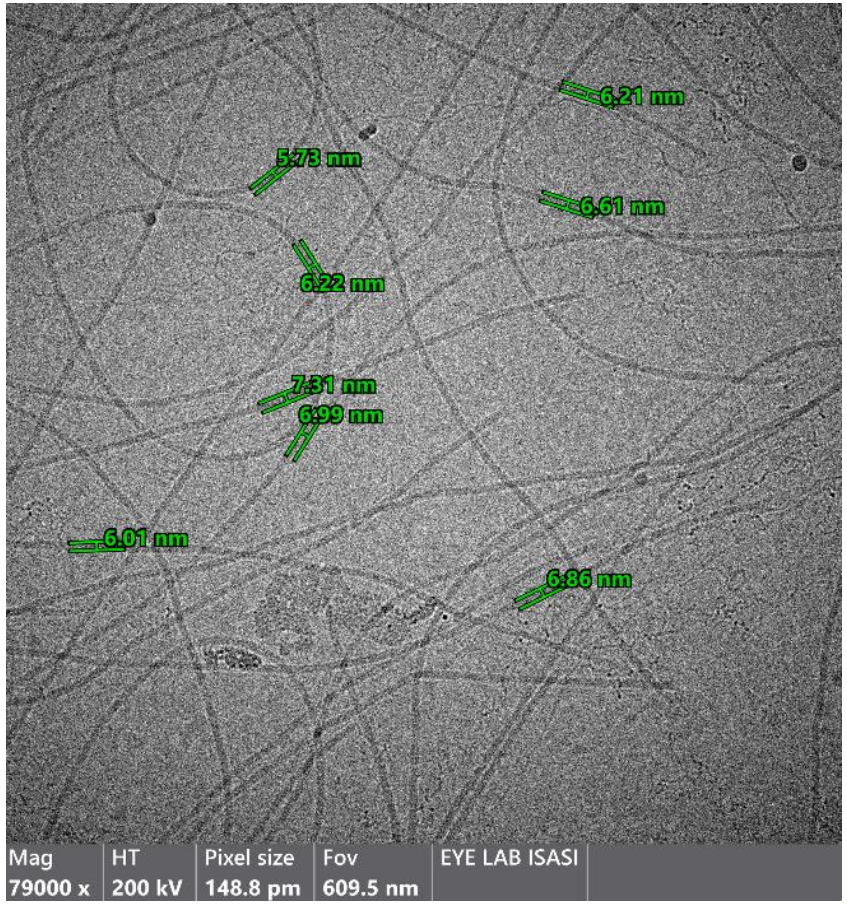
A Highly Efficient, Selective, and Thermally Stable Dielectric Multilayer Emitter for Solar Thermophotovoltaics

Caldarelli, Antonio[a, b]; De Luca, Daniela[b, c]; Lee, Changkyun[d]; Sanitá, Gennaro[e]; Esposito, Emanuela[e]; Musto, Marilena[a, b]; Russo, Roberto[b]; Bermel, Peter[d]

Save all to author list

Prof. M.Calvaresi **UNIBO, Chemisry Dep**

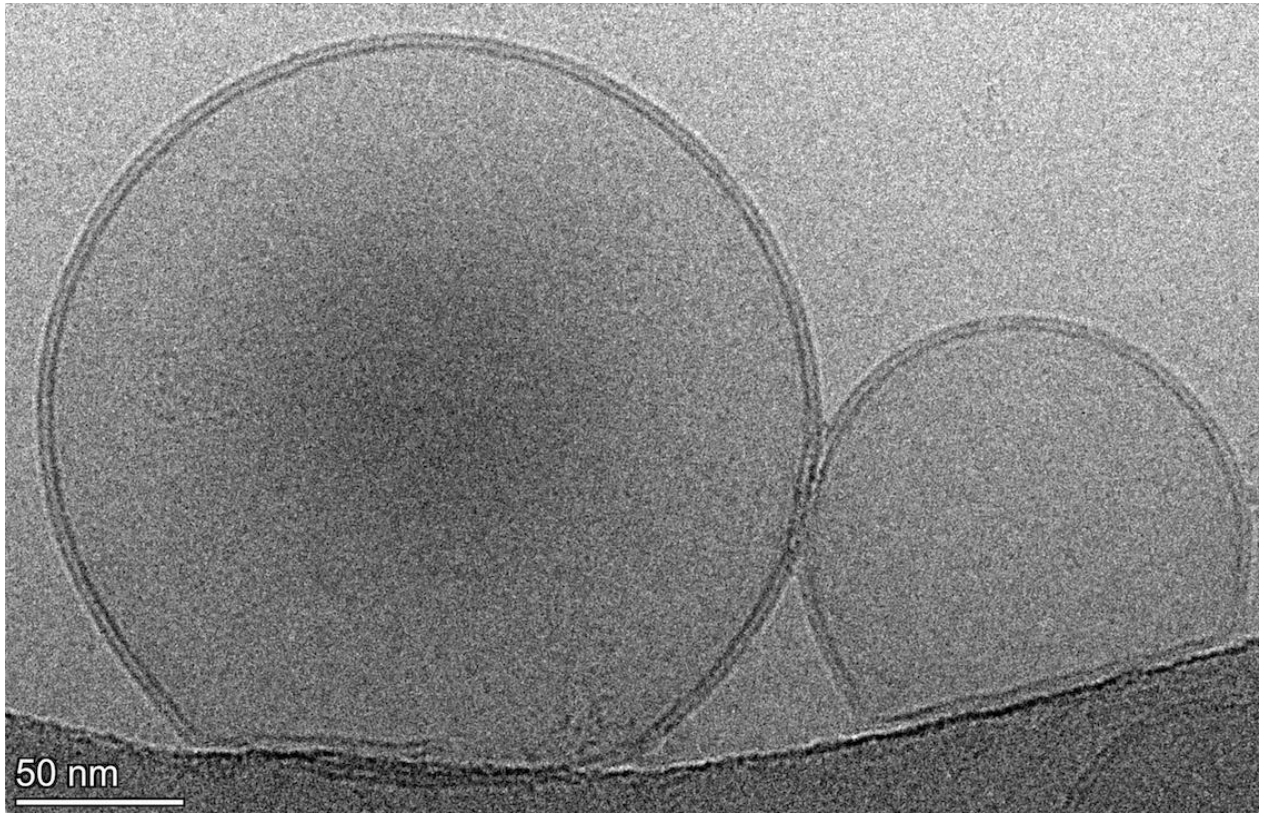
Study engineered M13 phages for bioimaging applications



*paper submitted

Dott.ssa Daniela Corda **CNR-IEOS**

Study of protein localization in liposomes and fission phenomena of Golgi-like liposomal membranes



*paper in preparation

Determination of the structure of empty and loaded Apoferritin with RuCO to evaluate any alteration in the structure of the protein after loading the drug

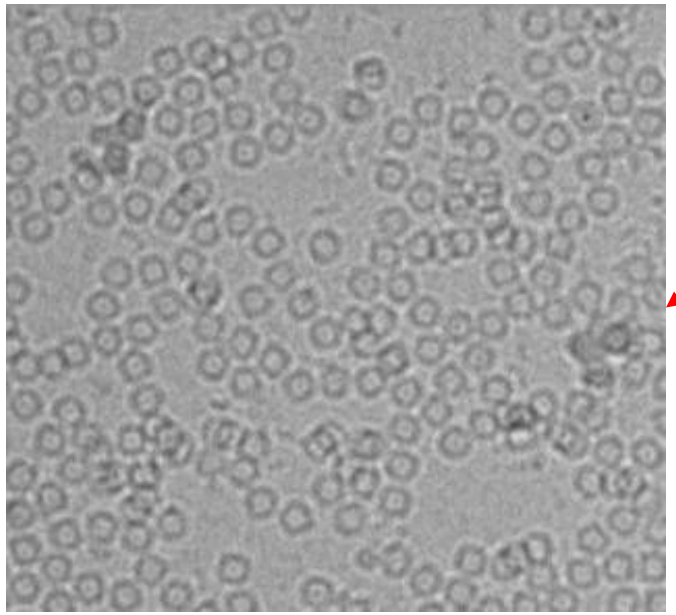
ELSEVIER

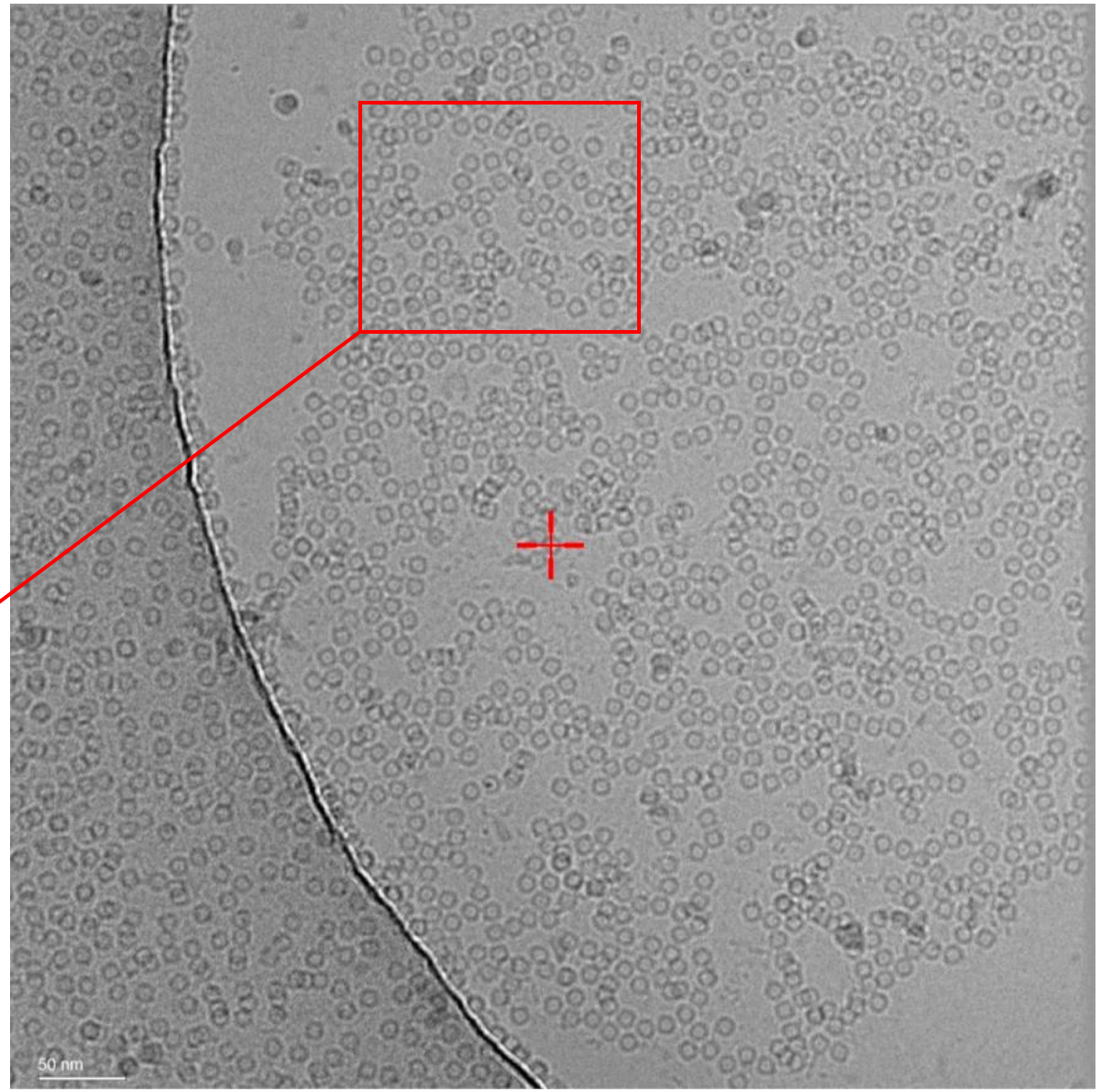
International Journal of Biological Macromolecules

Volume 277, Part 2, October 2024, 134373

Human ferritin nanocarriers for drug-delivery: A molecular view of the disassembly process

Rosanna Lucignano [a], Gennaro Sanità [b], Emanuela Esposito [b], Irene Russo Krauss [a c], Anna Maria D'Ursi [d], Michela Buonocore [a], Delia Picone [a]

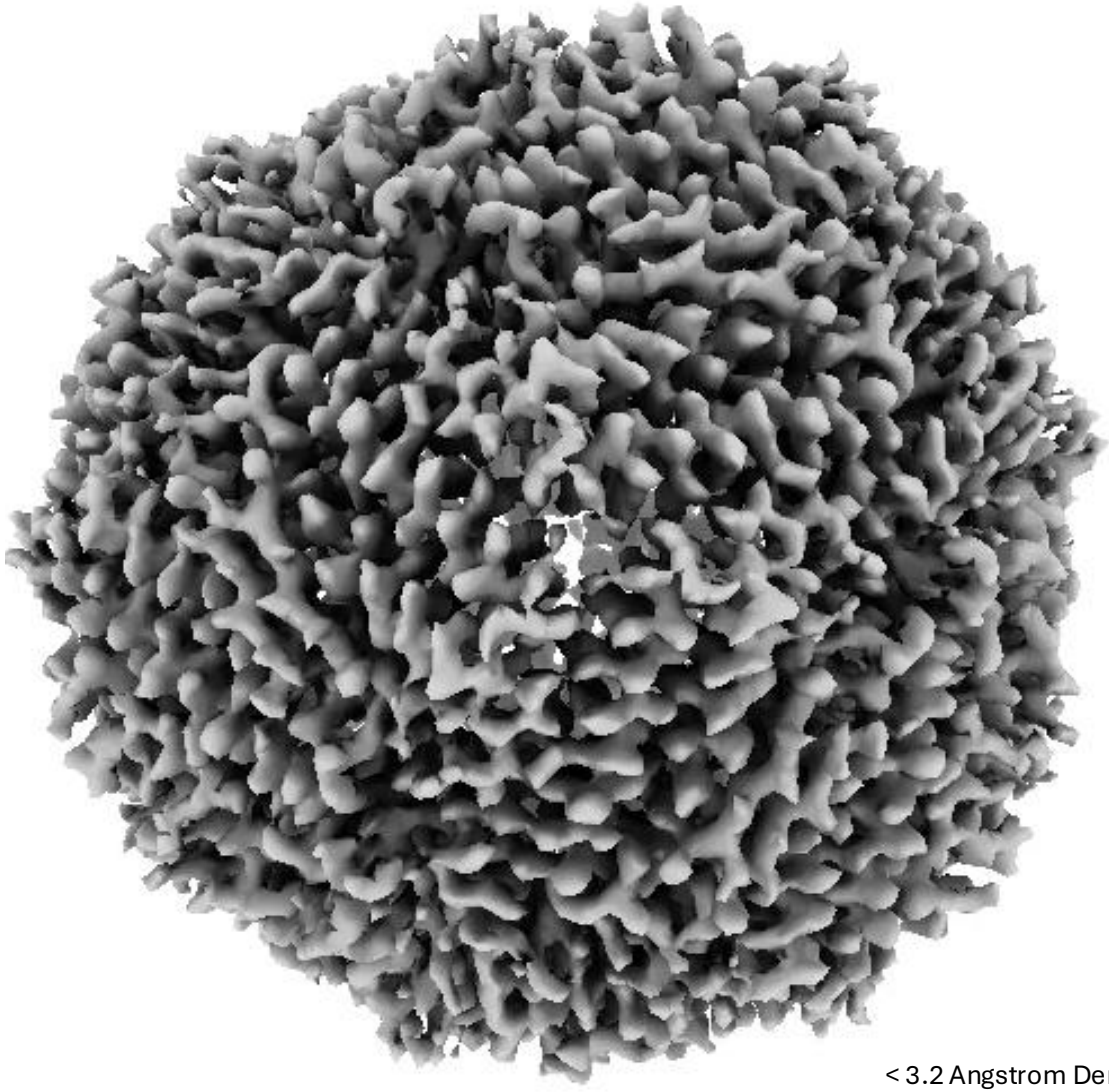




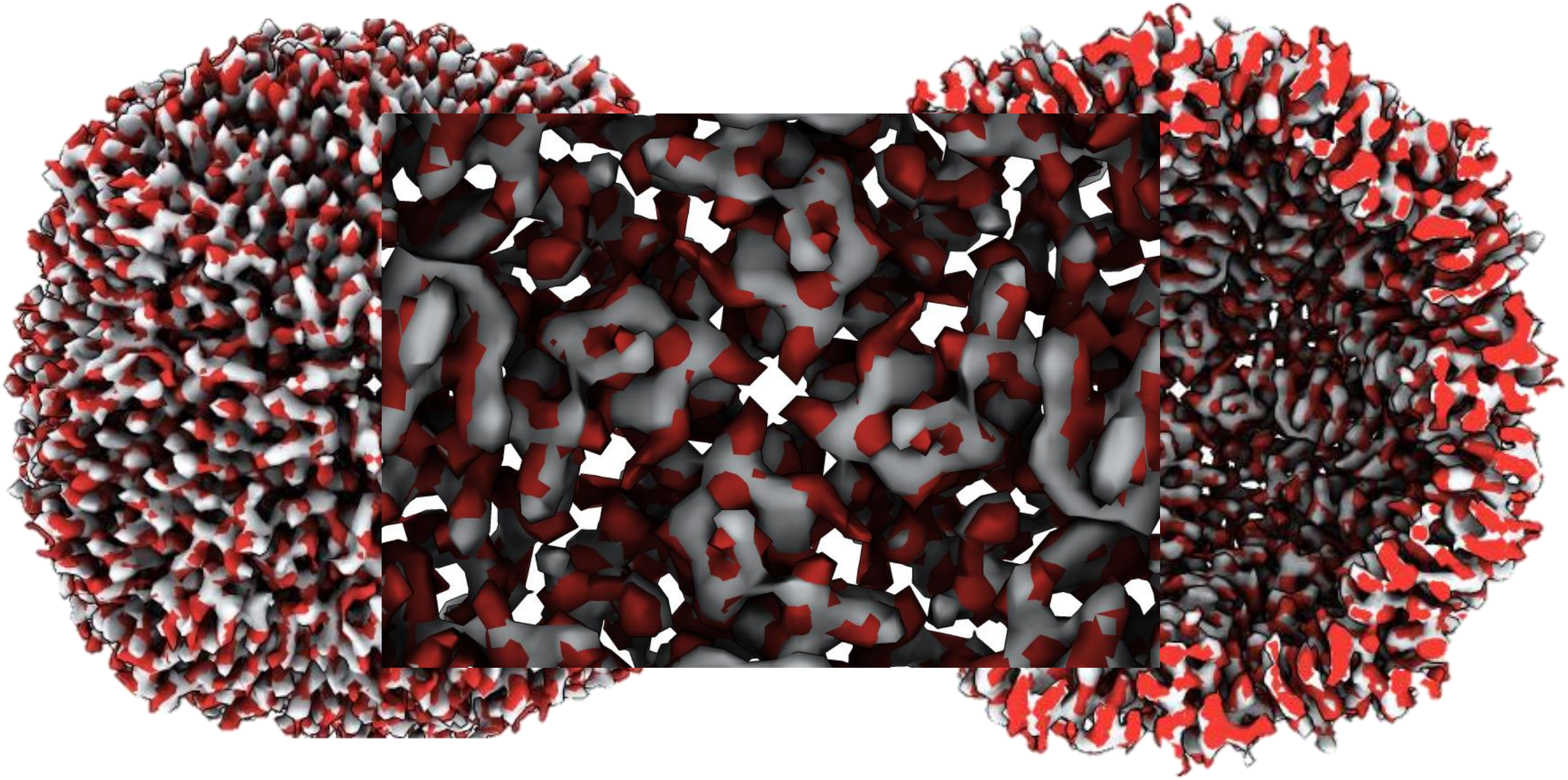
ApoF-RuCO loaded

CryoSPARC

ApoF-RuCO

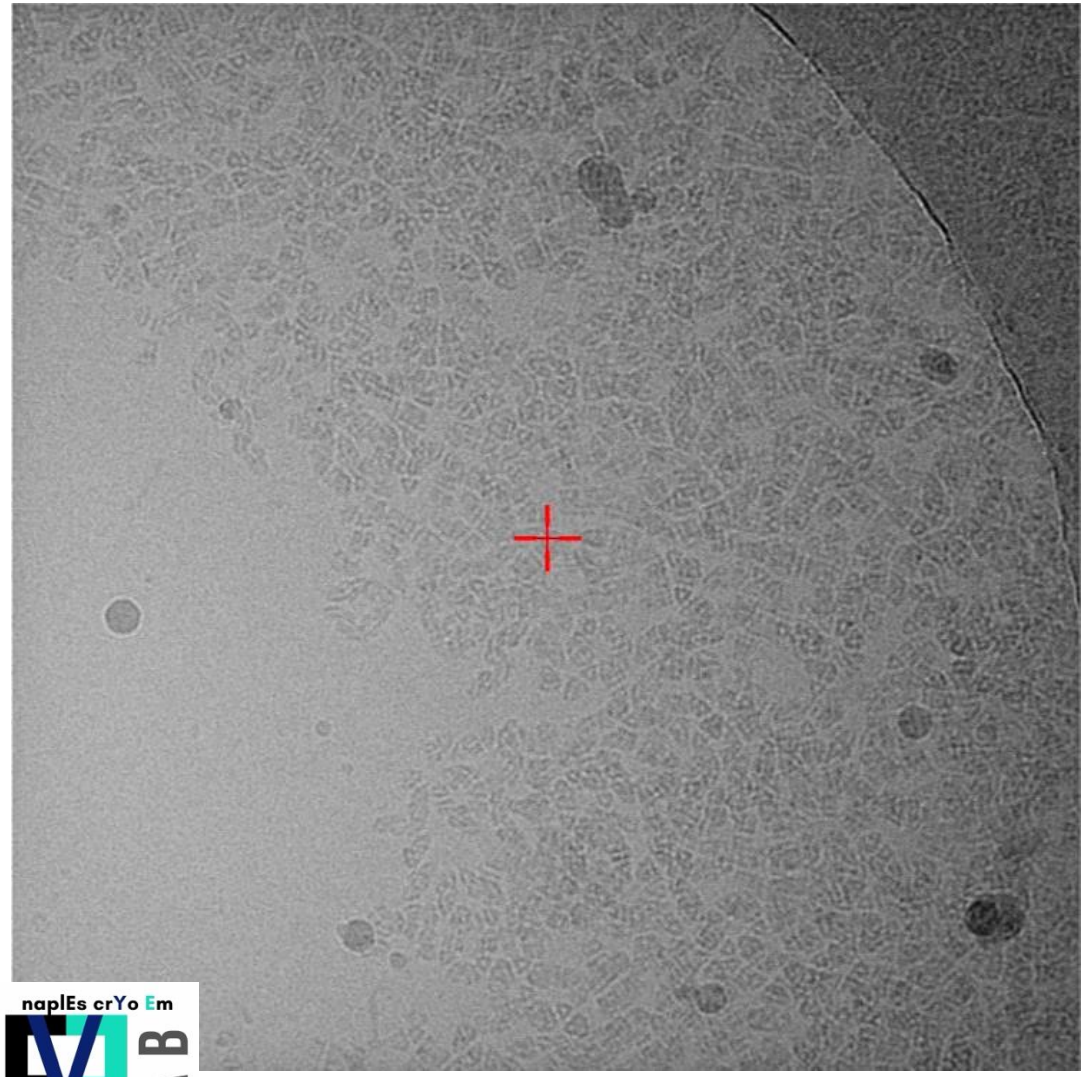


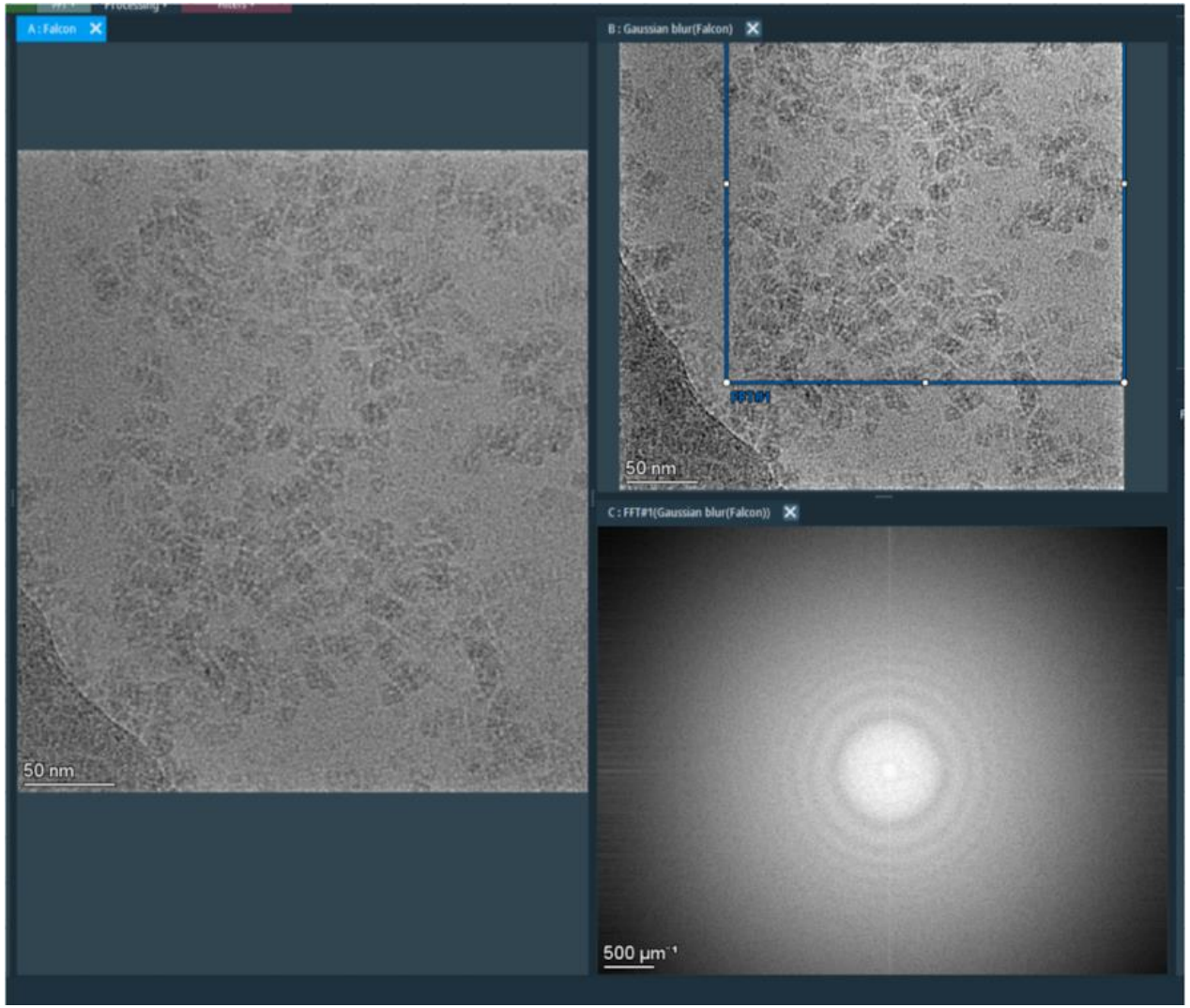
< 3.2 Angstrom Density Maps
Total Dose 35 e/A2
Pixel Size 1.5 Angstrom
High Tension 200 kV
CryoSPARC/ChimeraX data processing



Prof. P. Contursi **UNINA, Biology Dep**

Determination of the structure of the BcGALb enzyme for the localization of the active site and study of the trans-galactoxidative activity and direct mutation of the site





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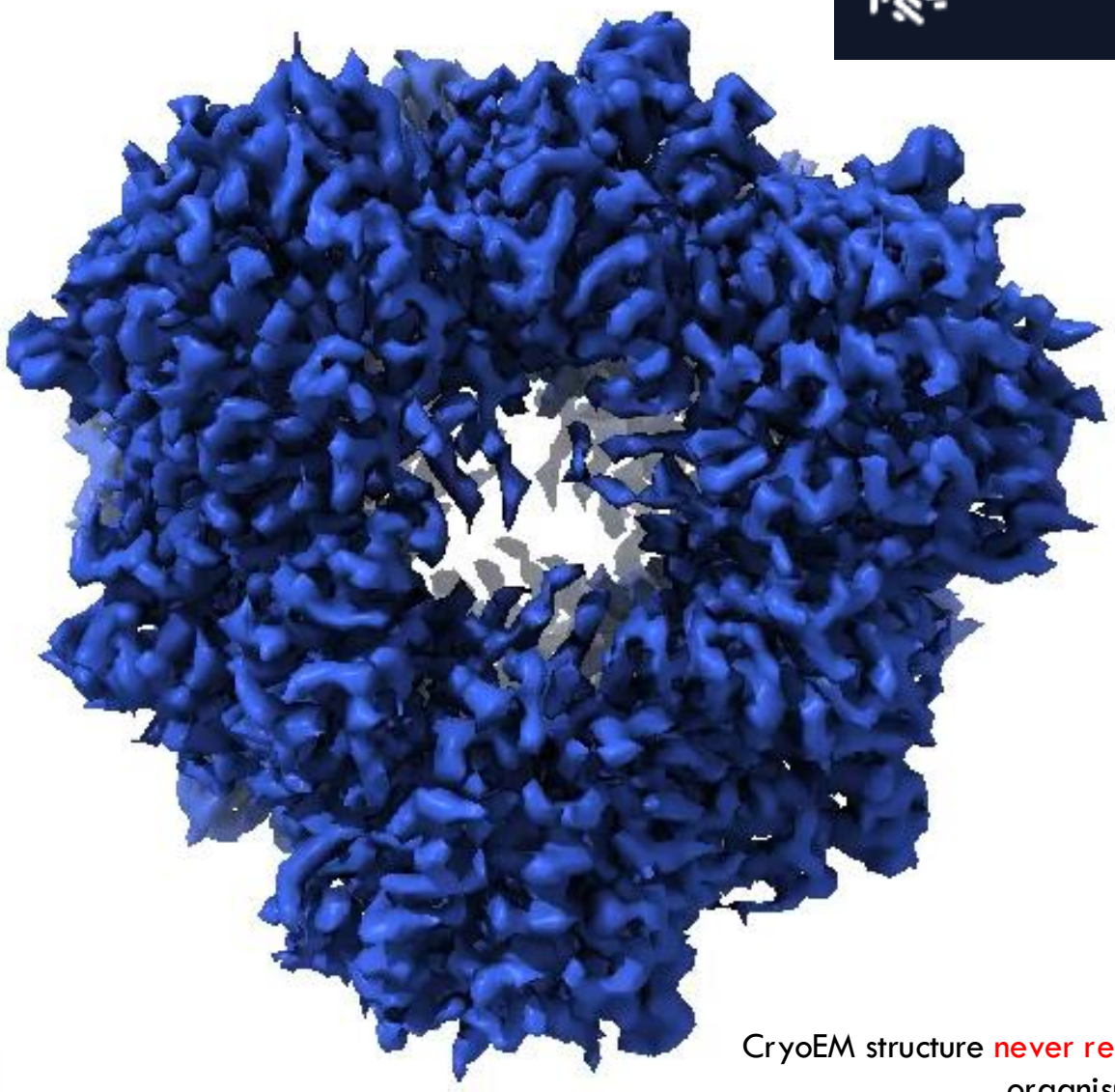
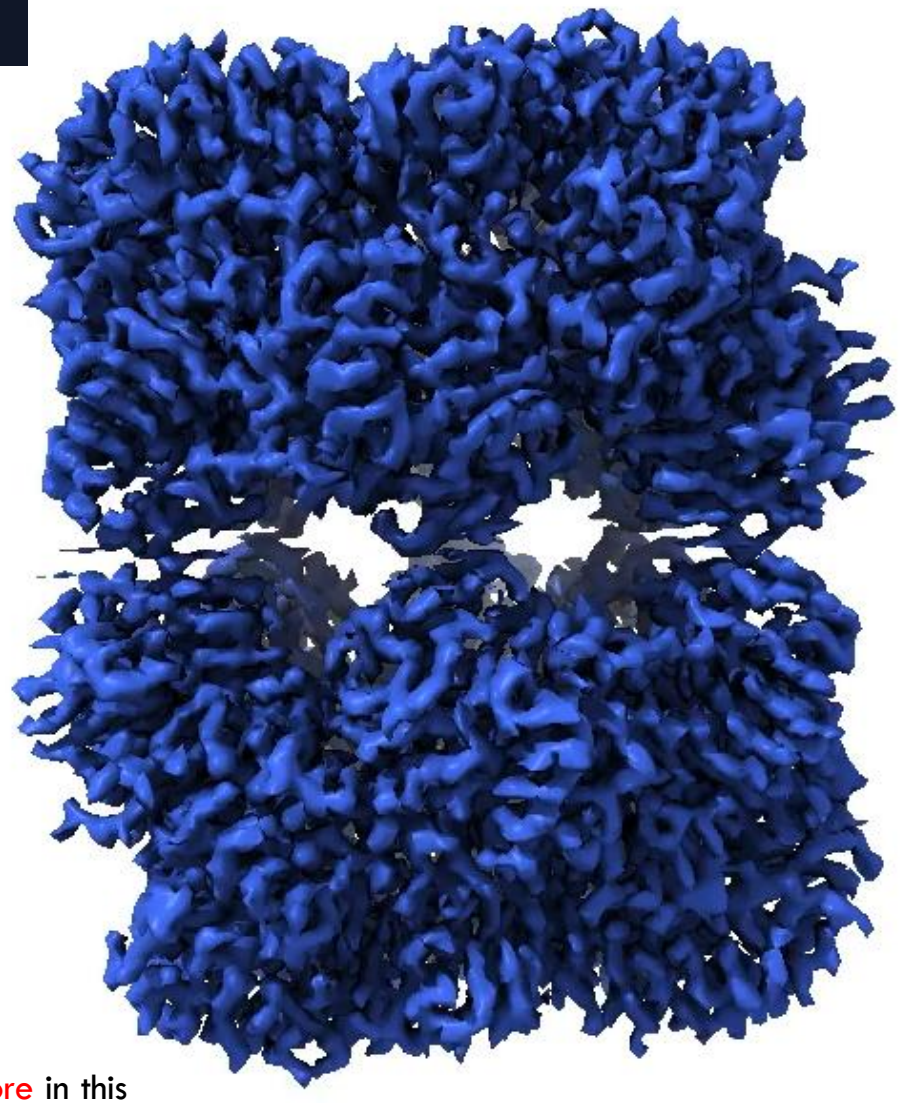
*paper in preparation

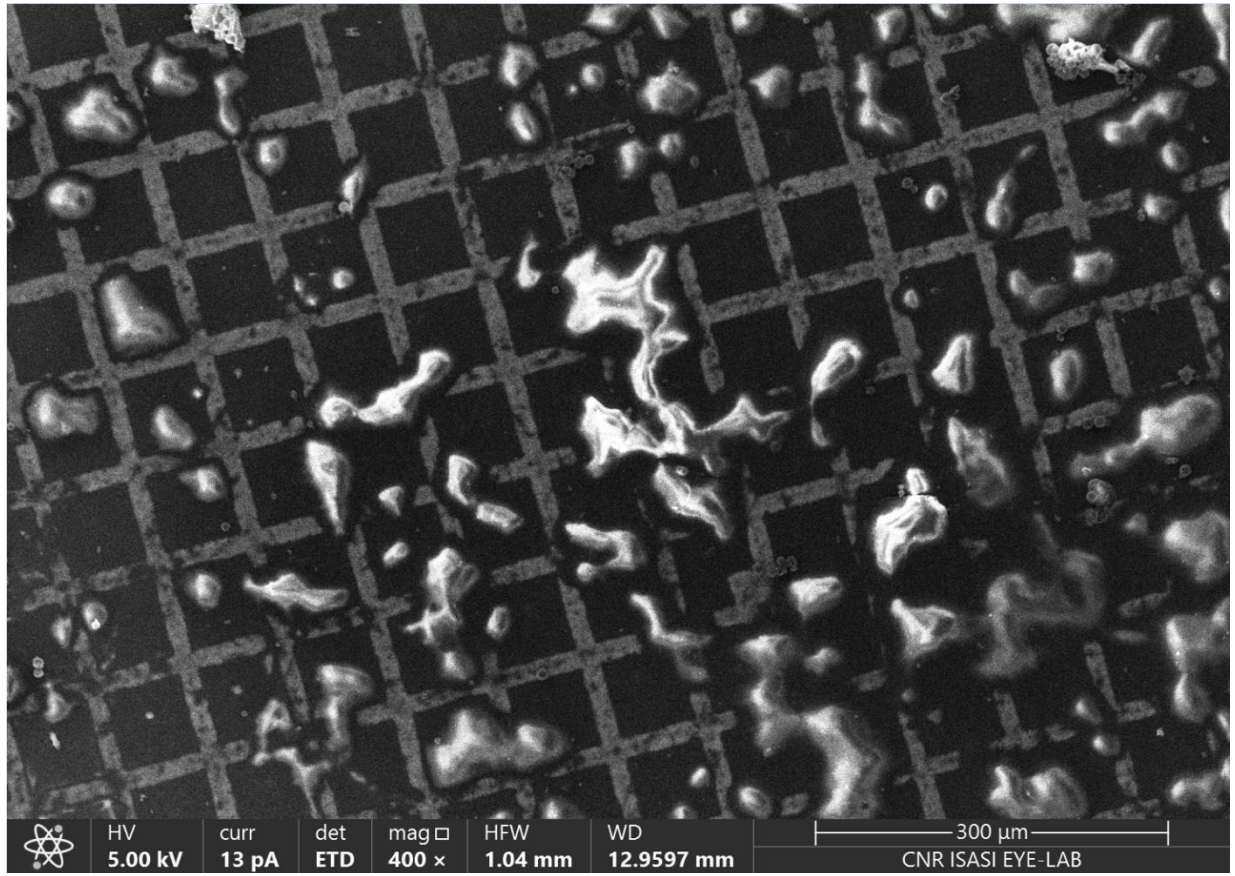


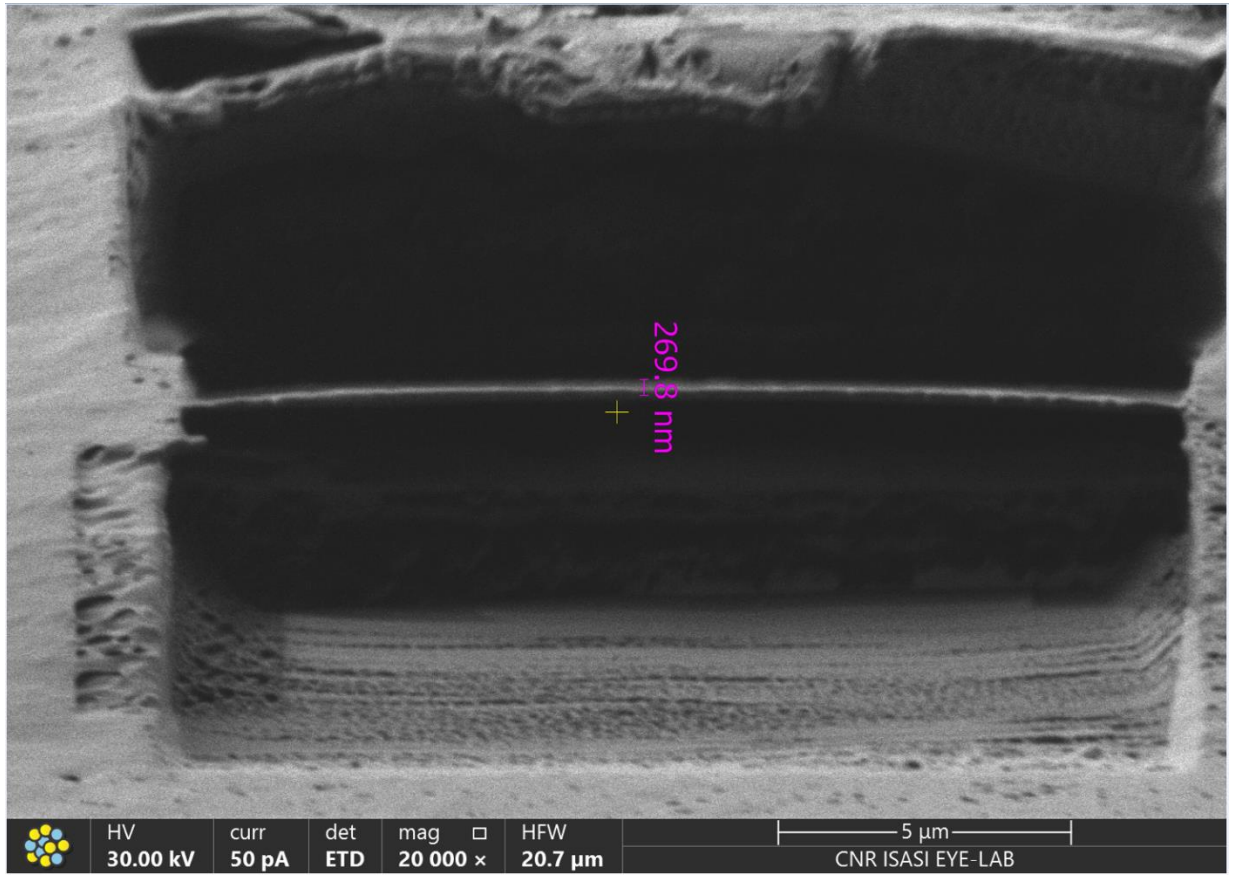


CryoEM structure never realized before in this organism

3.19 Angstrom Density Maps
Total Dose 45 e/A2
Pixel Size 1,5 Angstrom
High Tension 200 kV
CryoSPARC/ChimeraX data processing

Localization study of MelaSilAg-HSA@DOX NPs in cellular compartments





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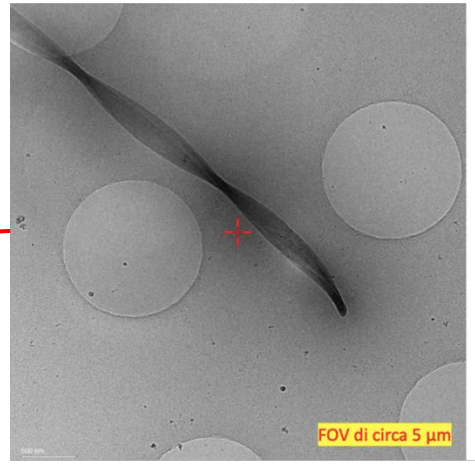
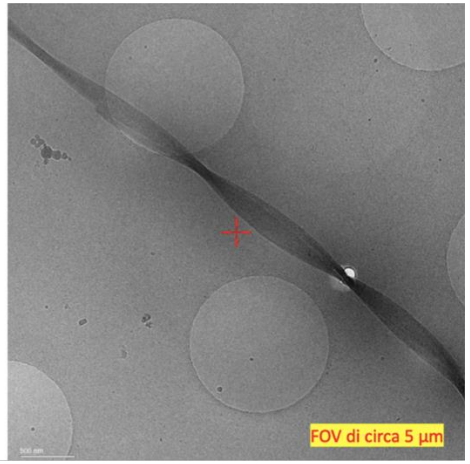
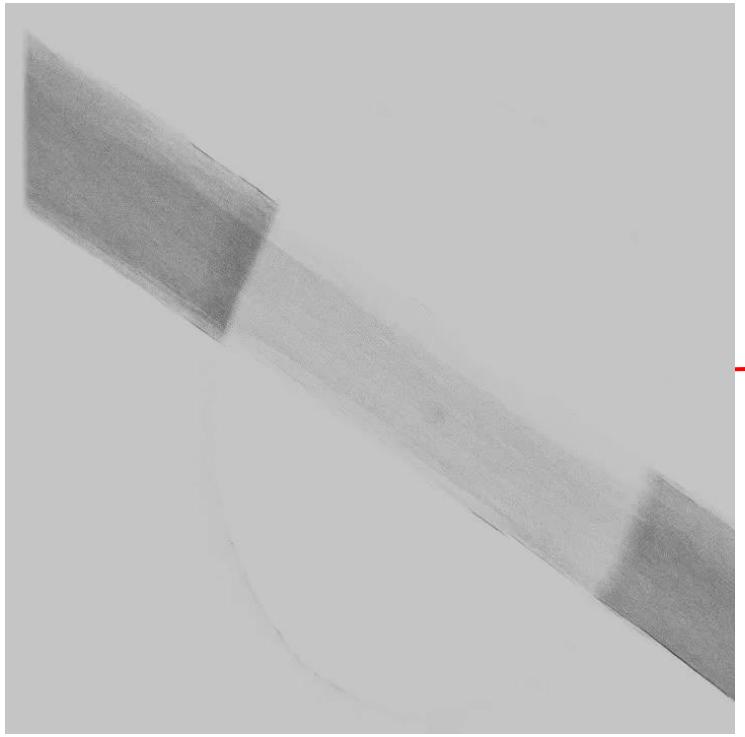
Dott.ssa C. Tortiglione **CNR-ISASI**

Study of DTTO fibers from Hydra vulgaris and cell lines

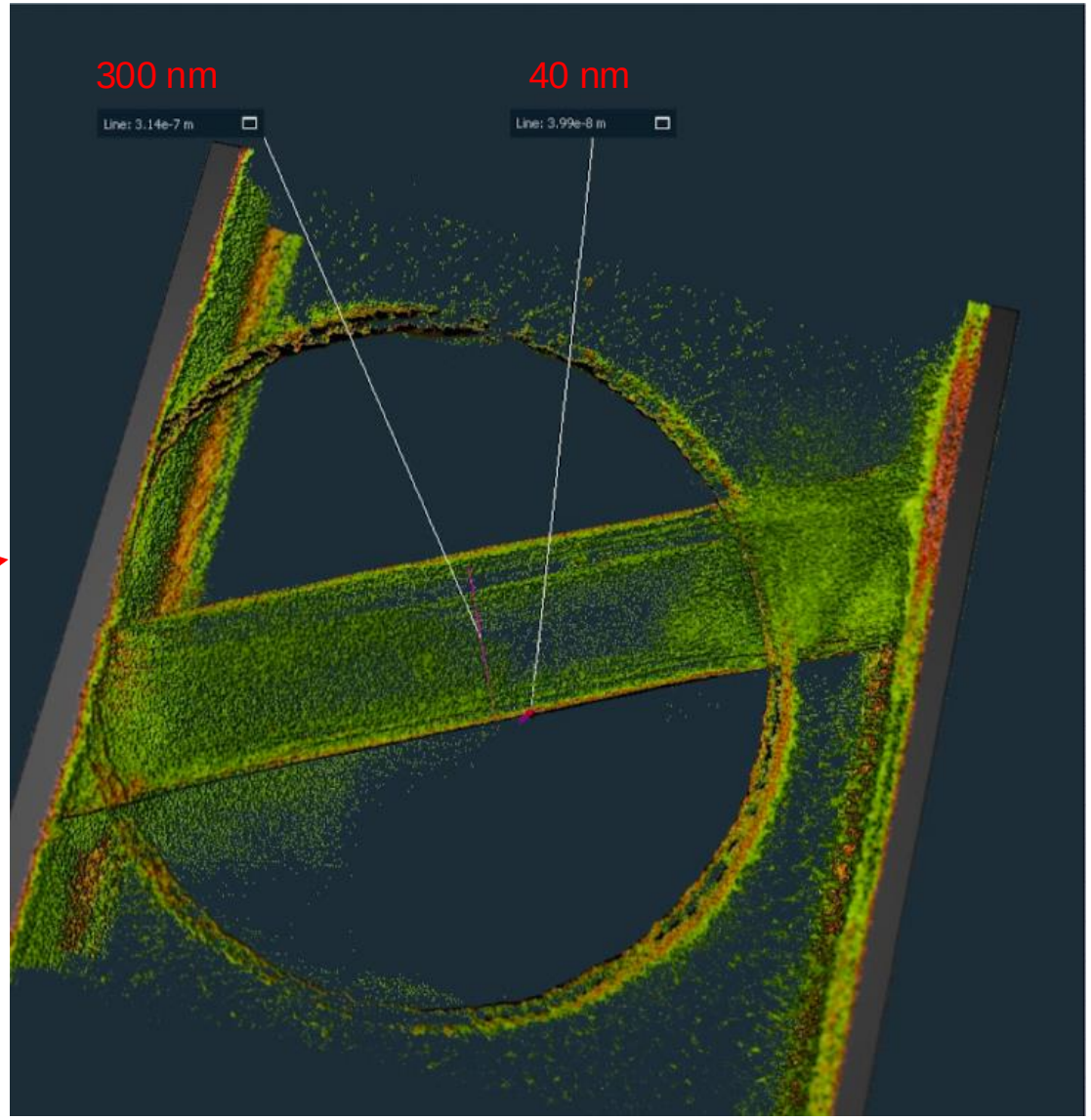




Tomography



AMIRA 3D



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THANK YOU for YOUR ATTENTION

Contact us:

www.isasi.cnr.it/scientific-instrumentation/eye-lab-em/

emanuela.esposito@cnr.it

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26 August 2024

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