

MRT



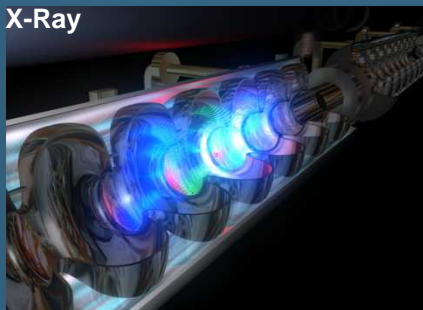
CLSM



TEM



X-Ray



# Imaging Infection of Biological Systems by Systemic Imaging Technologies

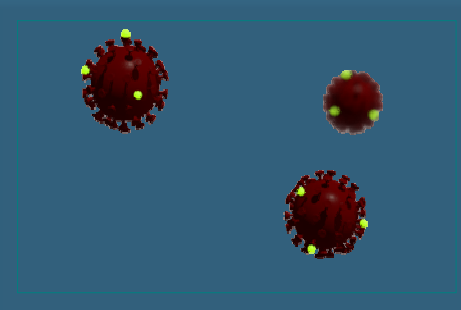
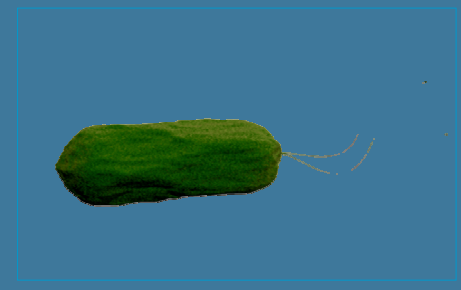
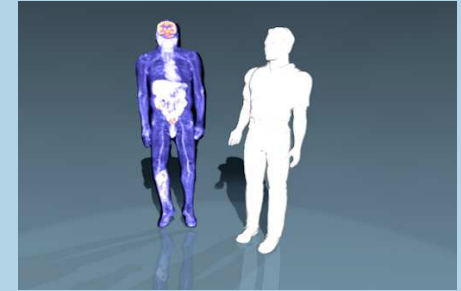
*Heinrich-Pette-Institute  
for Experimental Virology*

-

*DESY- PETRA III, Bio-Imaging Group  
German Synchrotron, Hamburg*

Heinrich Hohenberg

**Istituto Zooprofilattico, Roma,  
Workshop on Electron Microscopy,  
November 8<sup>th</sup>. 2013**



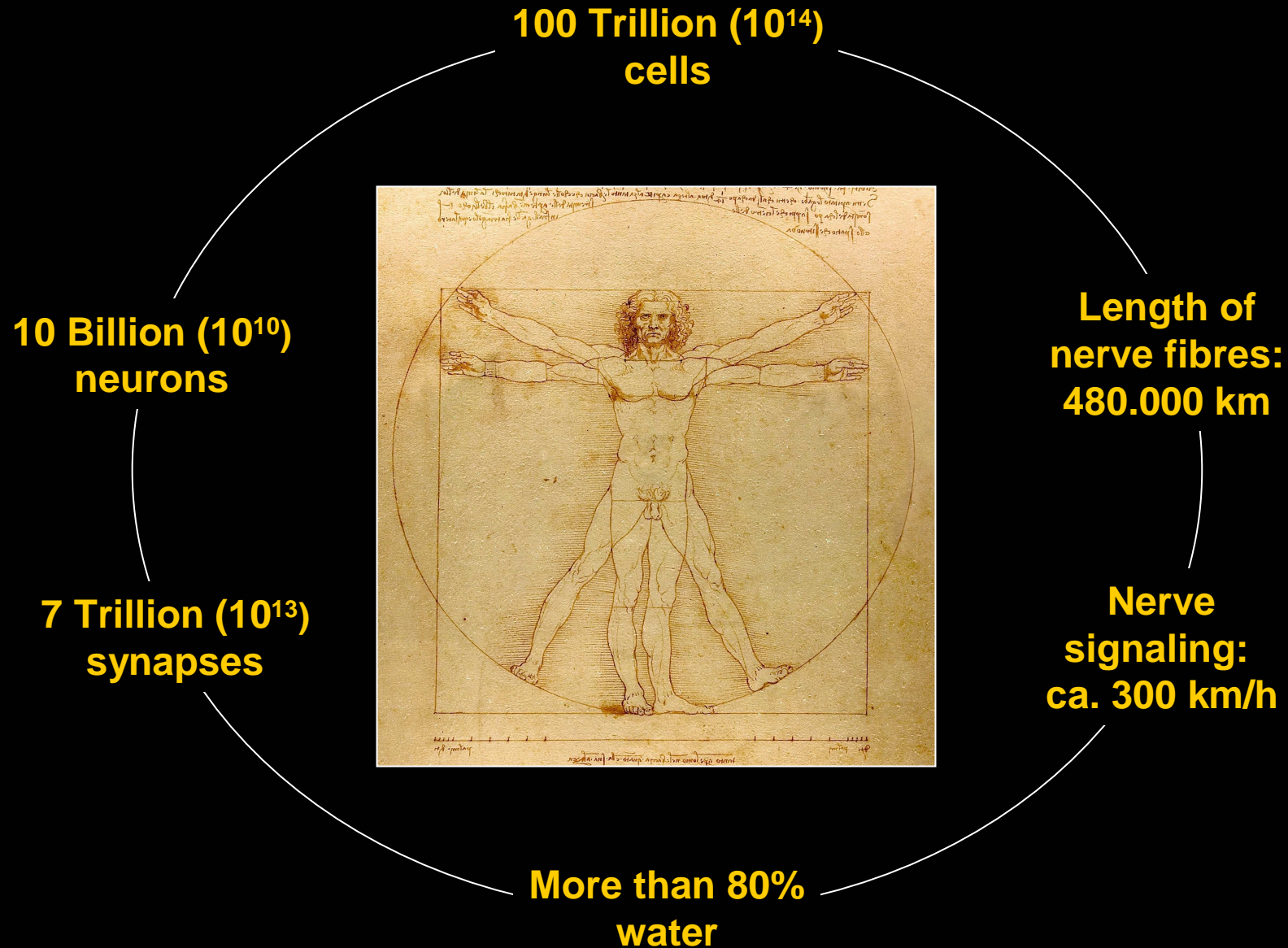
**Systemic Imaging?**



# From *multimodal* and *correlative* imaging to *Systemic* imaging in biomedical research:

- which involves the investigation of the same (labelled) specimen of a biological system with
- different imaging technologies,
- at different time, resolution and complexity levels,
- applying preparation methods, selected to guarantee the structural integrity and relocation possibilities.
- The different imaging technologies are connected by bridging or translational techniques and methods.

# ORGANISMS are highly complex information-driven systems



# CELLS are information-driven network systems

## Bio-Structures & Detection

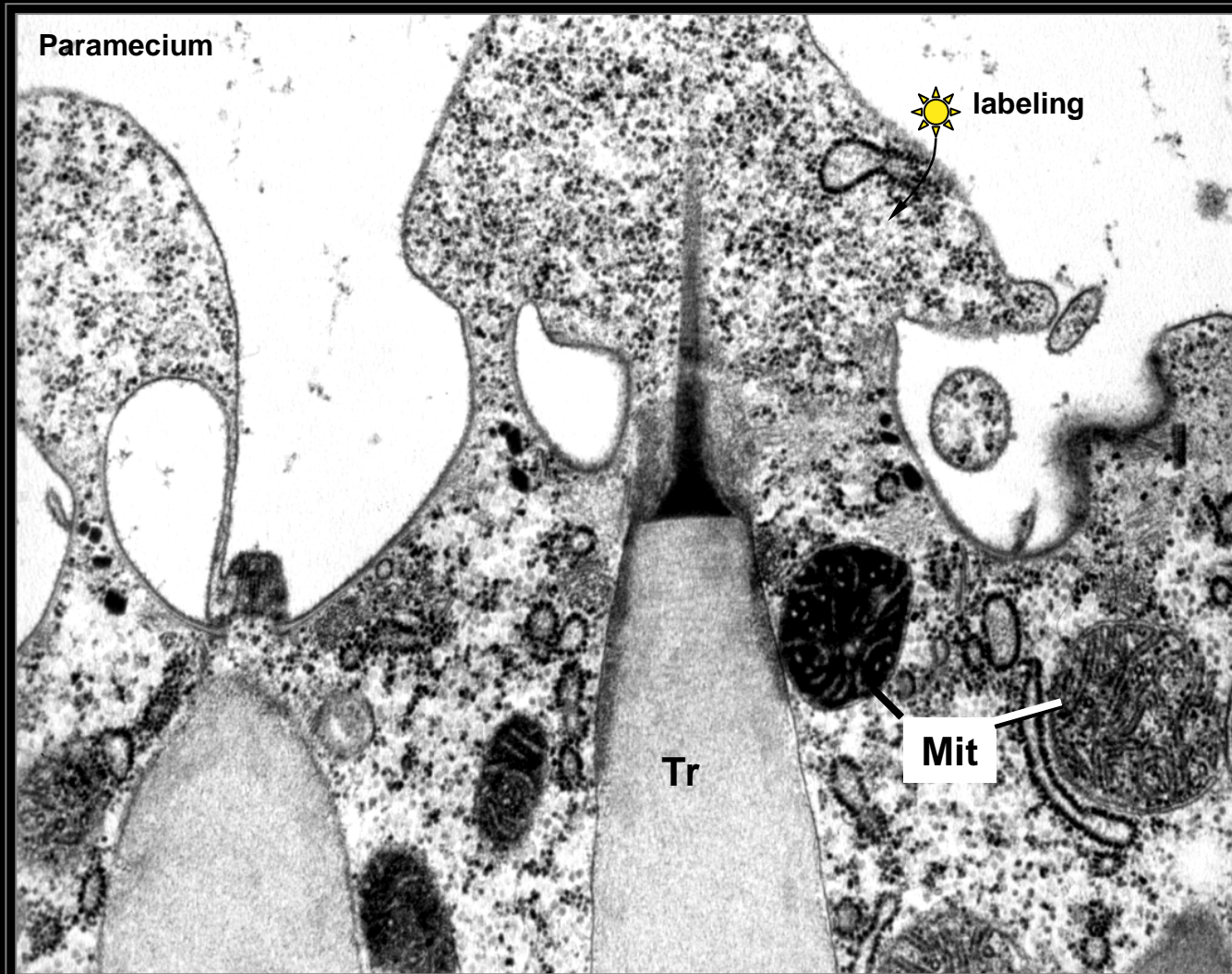
Molecules

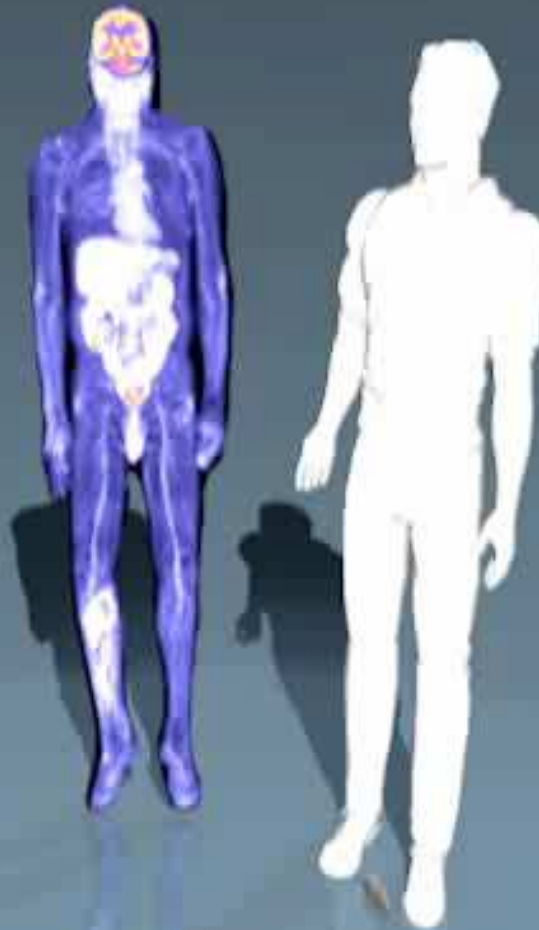
Position

Concentration

Structures

Time



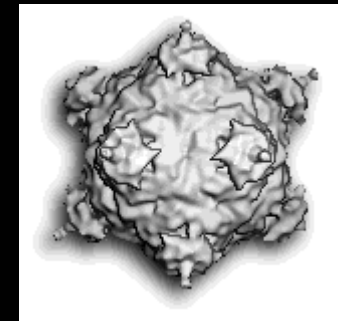
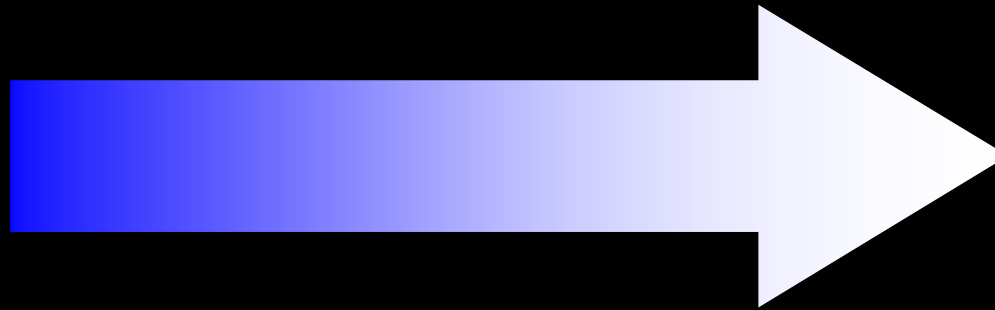
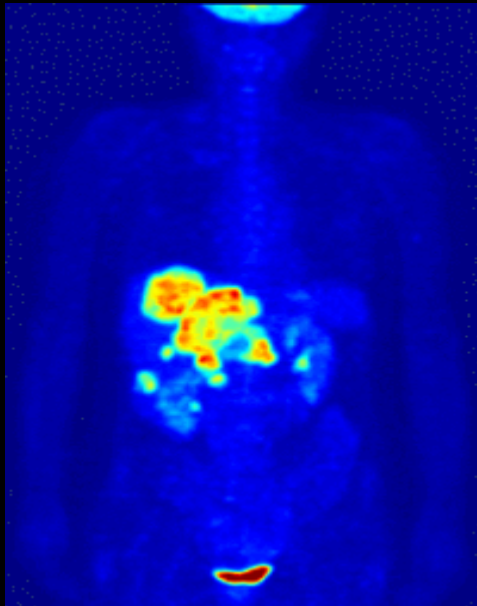


**Imaging in systemic infection research  
crosses a wide range of complexity and magnification scales**

**Basic considerations:**  
**Applied Imaging Technology**

# Imaging of the same bio-system?

From the **organism** to its **molecule complexes *in situ***



**Medical  
Tomography:**

**Intact Organisms**  
PET, MRT

**Light-  
Microscopy:**

**Cells, Tissues**  
Live Cell imaging,  
Intravital, CLSM

**Electron  
Microscopy of  
SURFACES:**

**Cells, Tissues**  
SEM  
ESEM

**Electron  
Microscopy of  
CELL INTERIOR:**

**Cells, Tissues**  
TEM  
TEM-Tomography

**X-ray  
Imaging and  
Diffraction:**

**Thick Sections,  
Single Molecules**

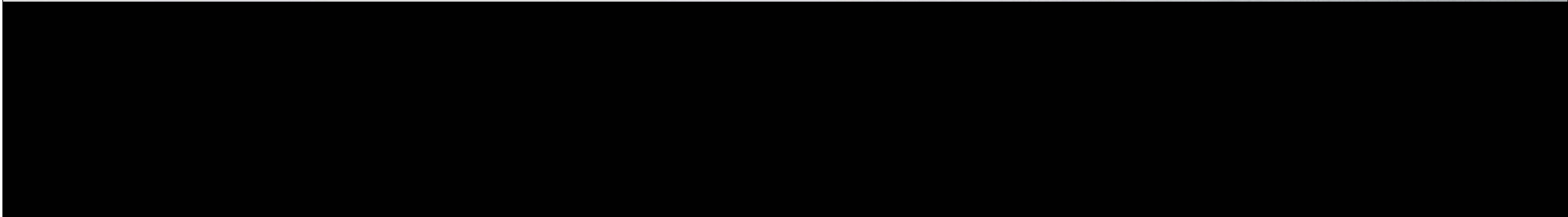




**Intact  
System?**

**or...**

## Systems components?

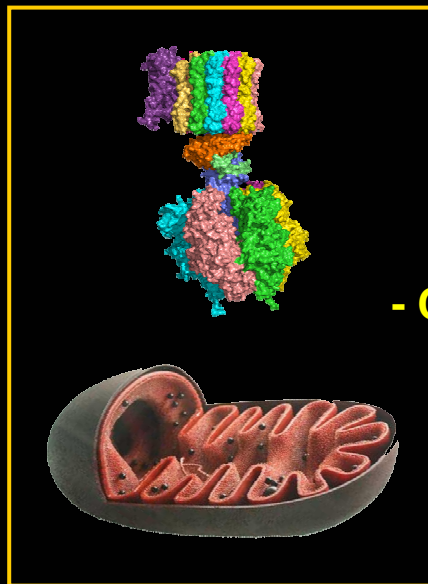




# Analysis of **isolated** or **integrated** structural elements ?

$$\text{Whole} = \Sigma \text{Elements?}$$

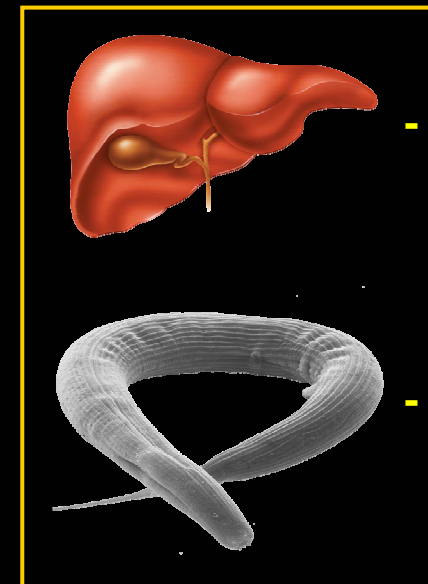
**Isolated** structural and functional elements without **information network**



- Cytoplasm

or

**Structural and functional elements integrated into a functional matrix**



- Organism

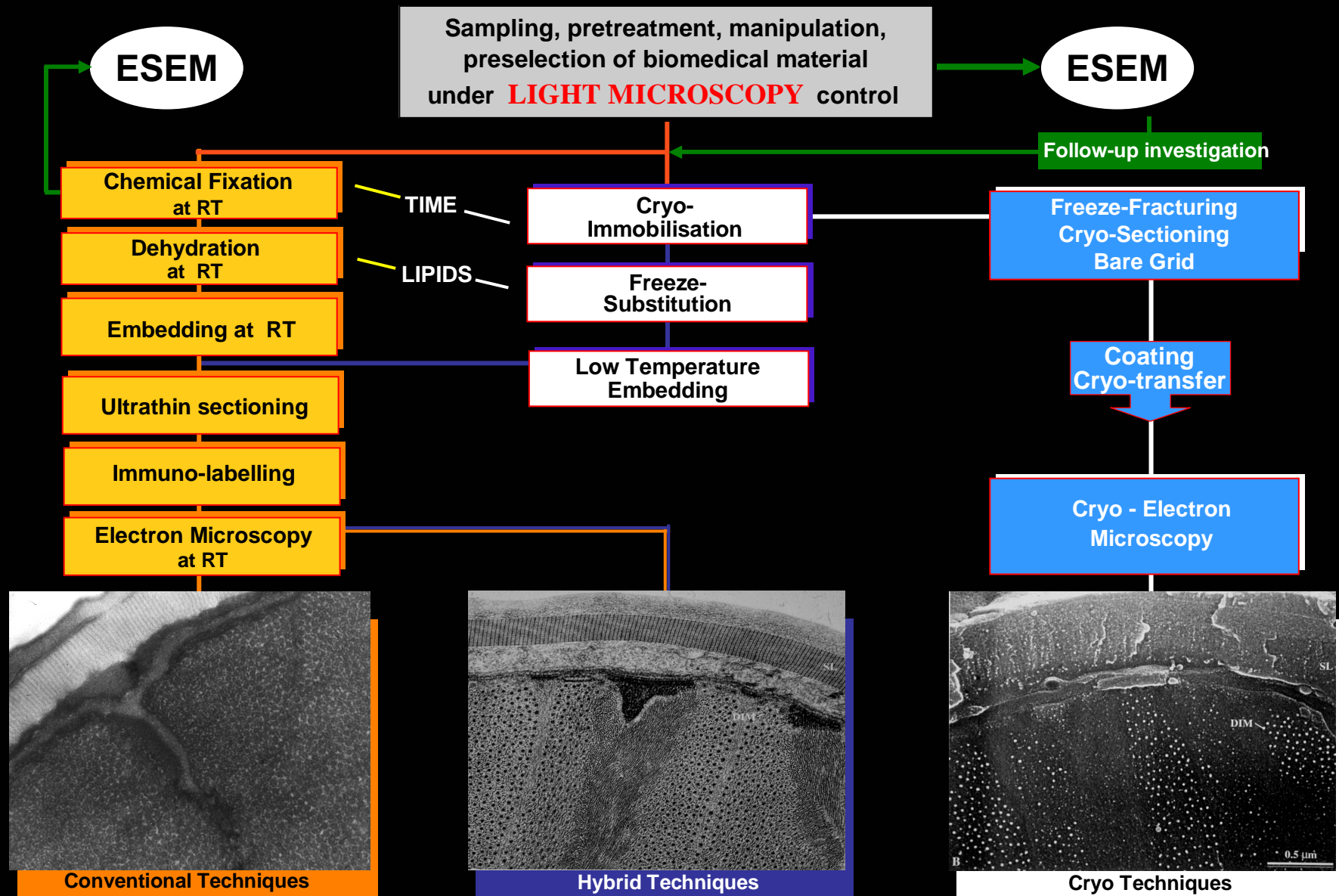
- Environment

**EM of the molecular nano-morphology**  
**of desintegrated systems elements**

**EM of the micro- and nano-morphology**  
**of integrated systems elements**

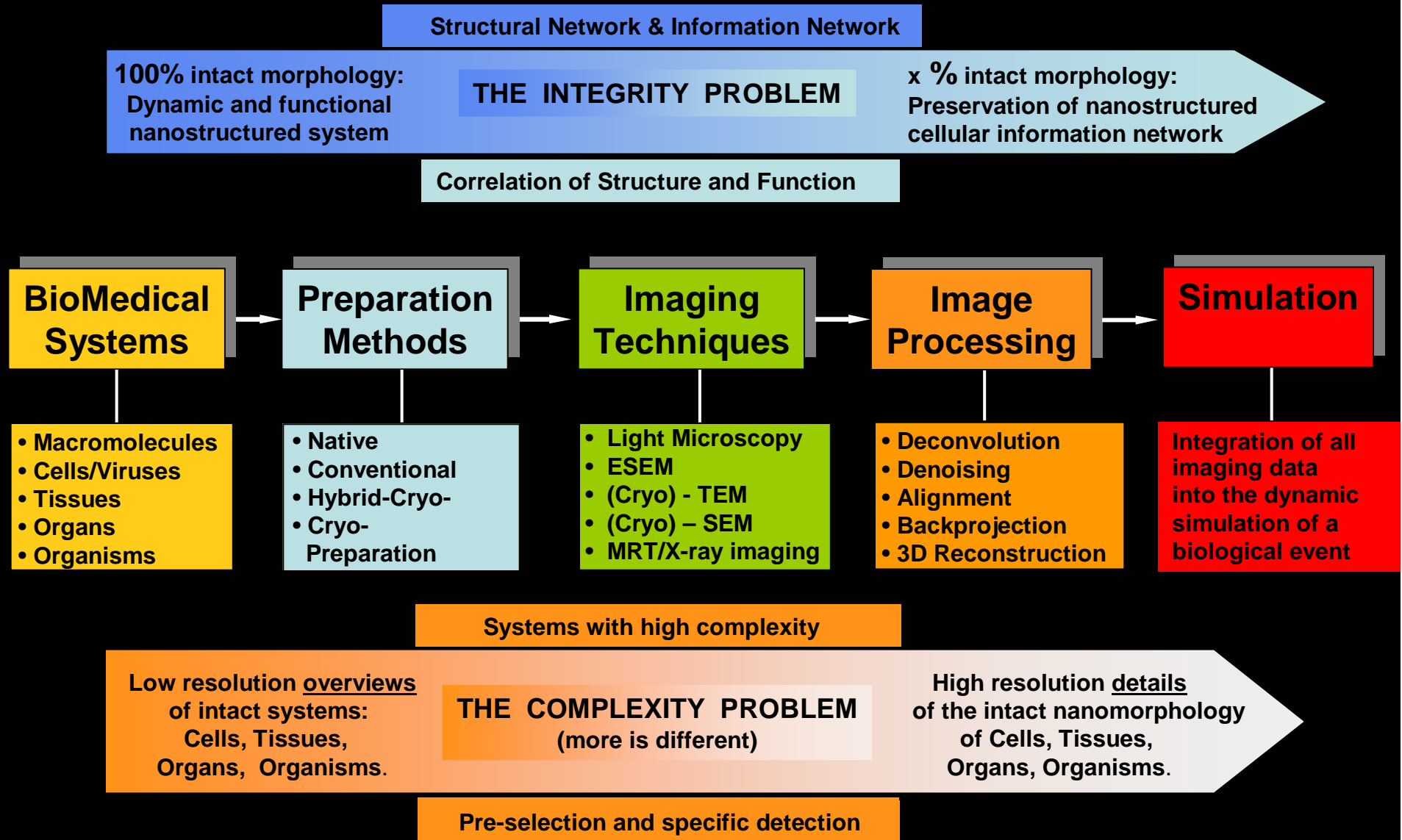
**Preparation techniques are interventions  
into the  
cellular structure and information network**

# Different EM preparation pathways: **You see what you do!**



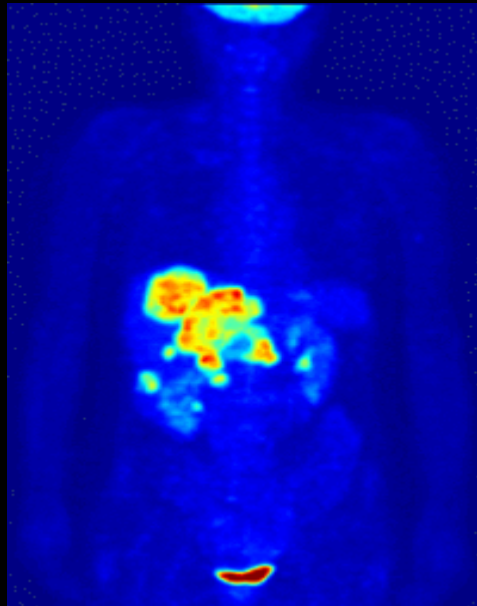
EM-Preparation methods **"manipulate"** the nanoarchitecture of molecules, cells and tissues

# Analysis of complex biosystems by imaging: Elements and Problems of Systems Imaging

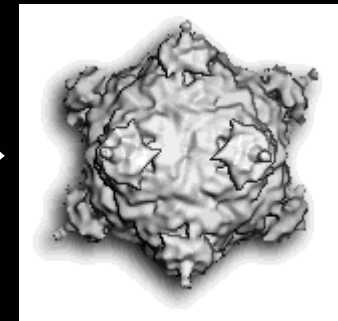


# Imaging of the same bio-system:

## Systemic preselection using specific detection and transfer technologies



**Systems Imaging  
by using new  
Methods & Technologies**



**Translational and Bridging Technologies**

**Medical  
Tomography:**

**Intact Organisms**  
PET, MRI

**Light-  
Microscopy:**

**Cells, Tissues**  
Live Cell imaging,  
Intravital, CLSM

**Electron  
Microscopy  
of surfaces:**

**Cells, Tissues**  
SEM  
ESEM

**Electron  
Microscopy  
of cell interior:**

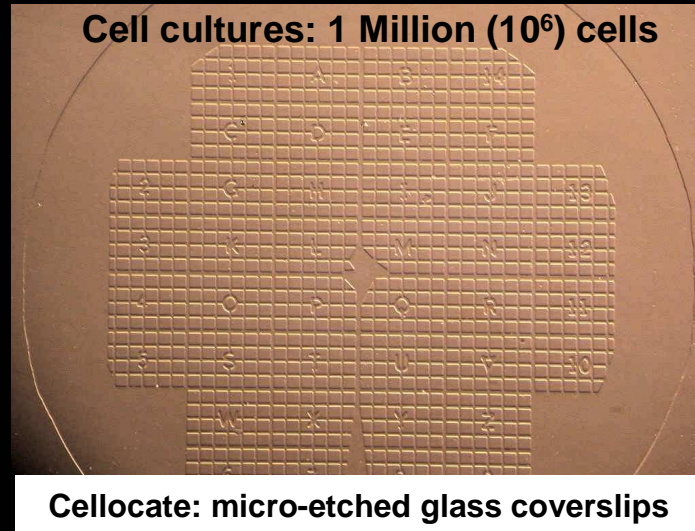
**Cells, Tissues**  
TEM  
TEM-Tomography

**X-Ray  
Diffraction and  
Imaging:**

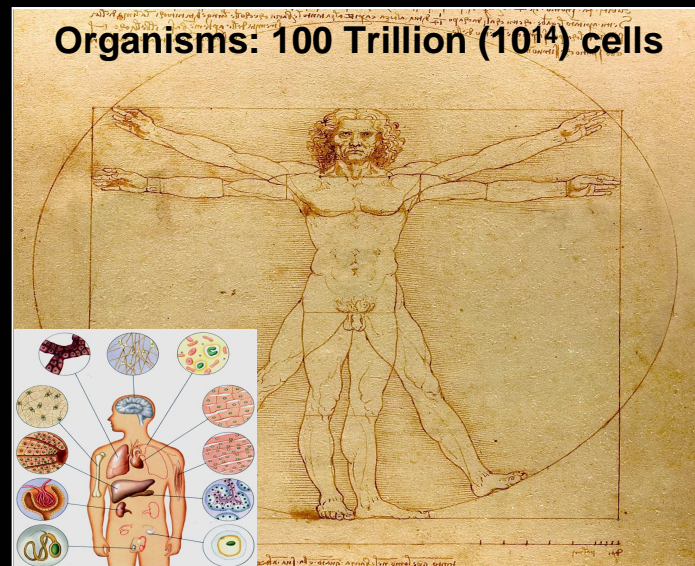
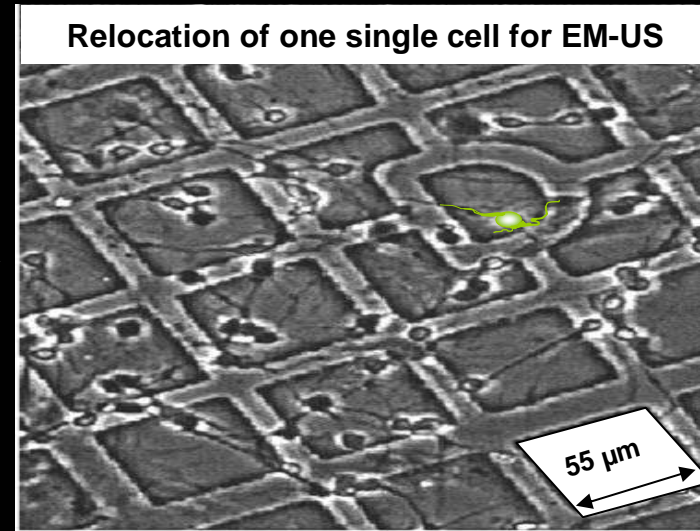
**Thick Sections,  
Single Molecules**



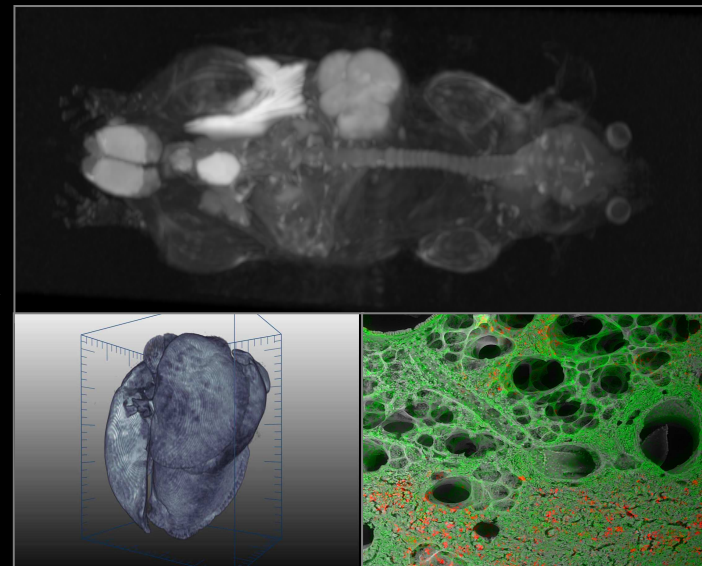
# How can we find the structures and cells of interest in complex systems?



Topology  
→  
based location

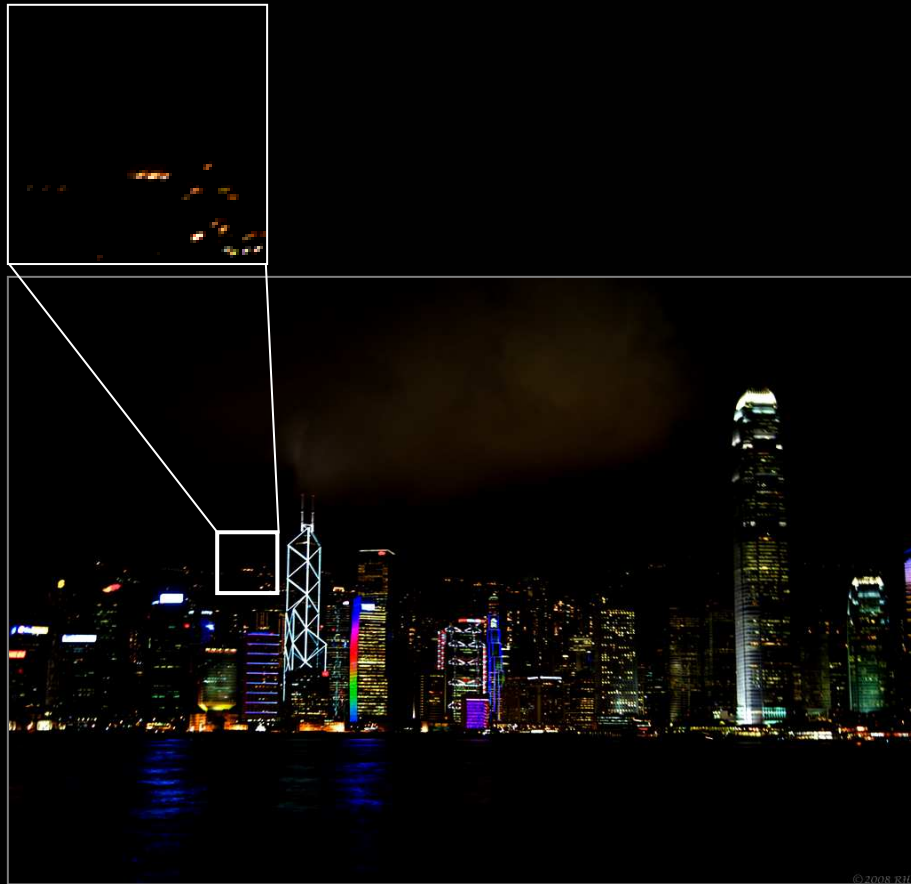


Marker-Signal  
→  
based location

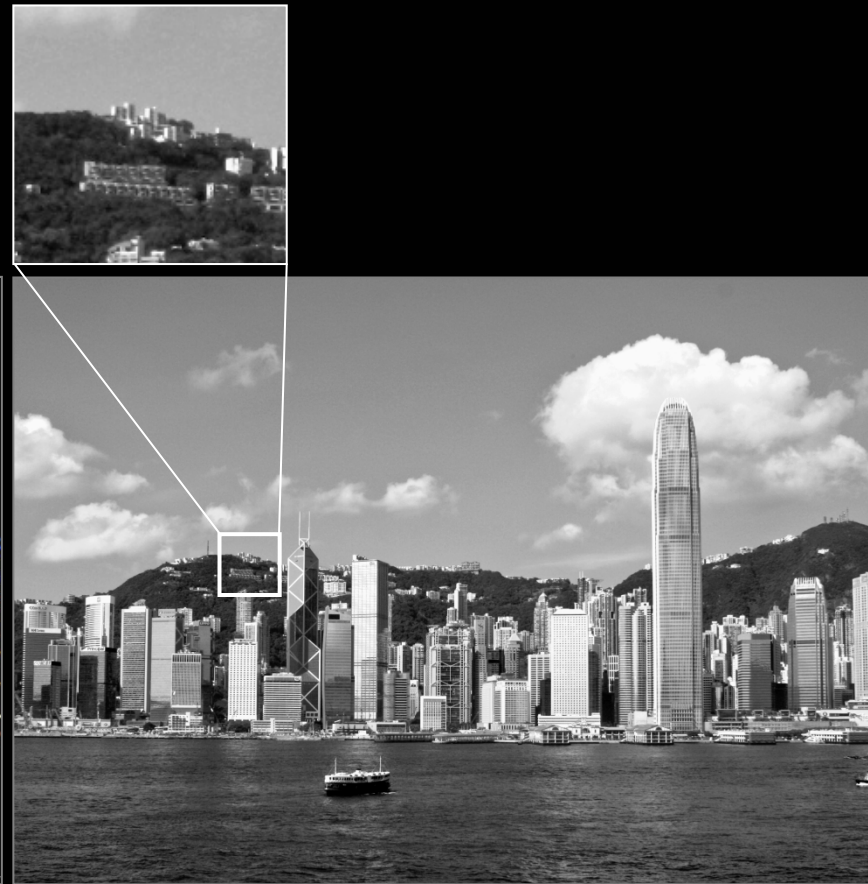


# Lights in the dark

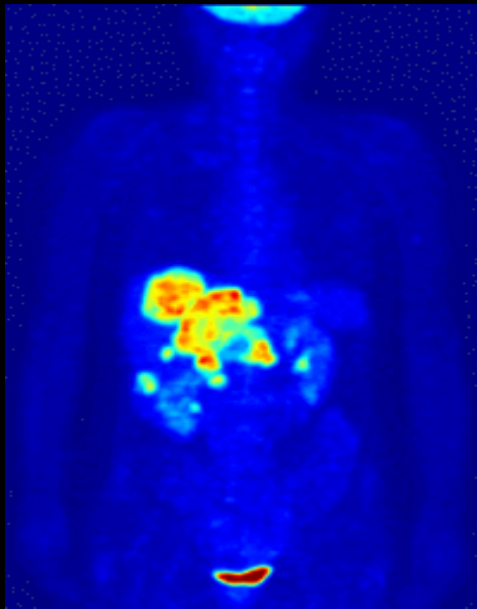
Labelled features



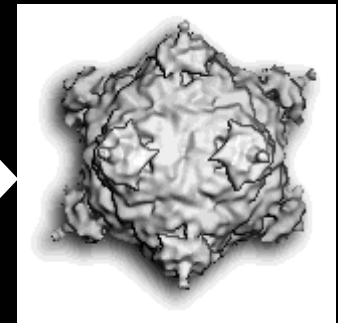
Structural information



From the intact organism to its molecule complexes *in situ*:  
**Nanoparticles**



Bridging Technologies:  
Nanoparticles as marker for **medical**  
**imaging, light** and **electron**  
**microscopy**





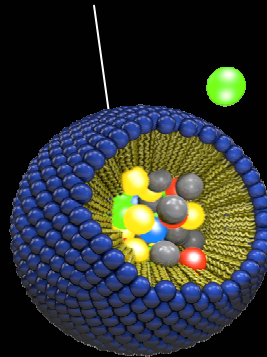
# Bridging MEDICAL IMAGING and MICROSCOPY:

## Nanoparticles (NP) allow specific detection and localisation

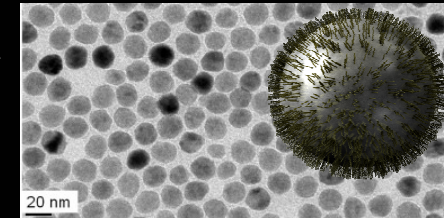
Quantum Dots



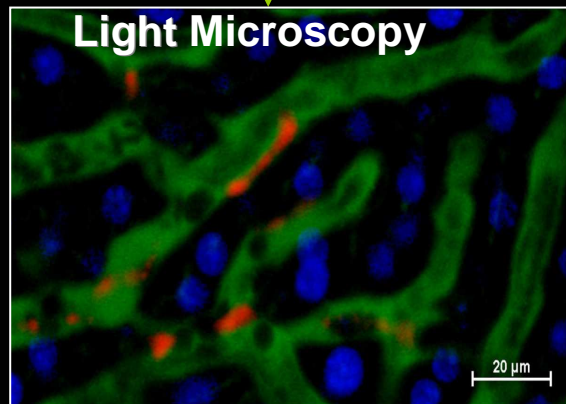
Nanosomes



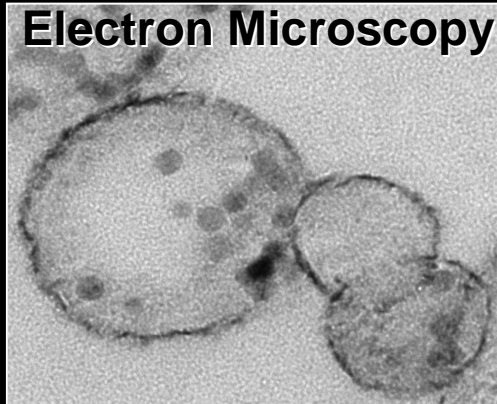
Super-Paramagnetic Iron-Oxide (SPIOs)



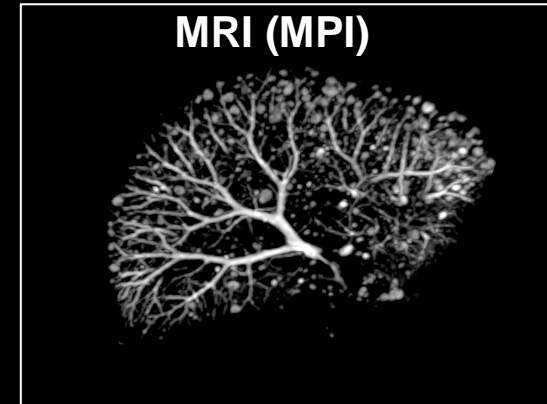
XRF



Electron Microscopy



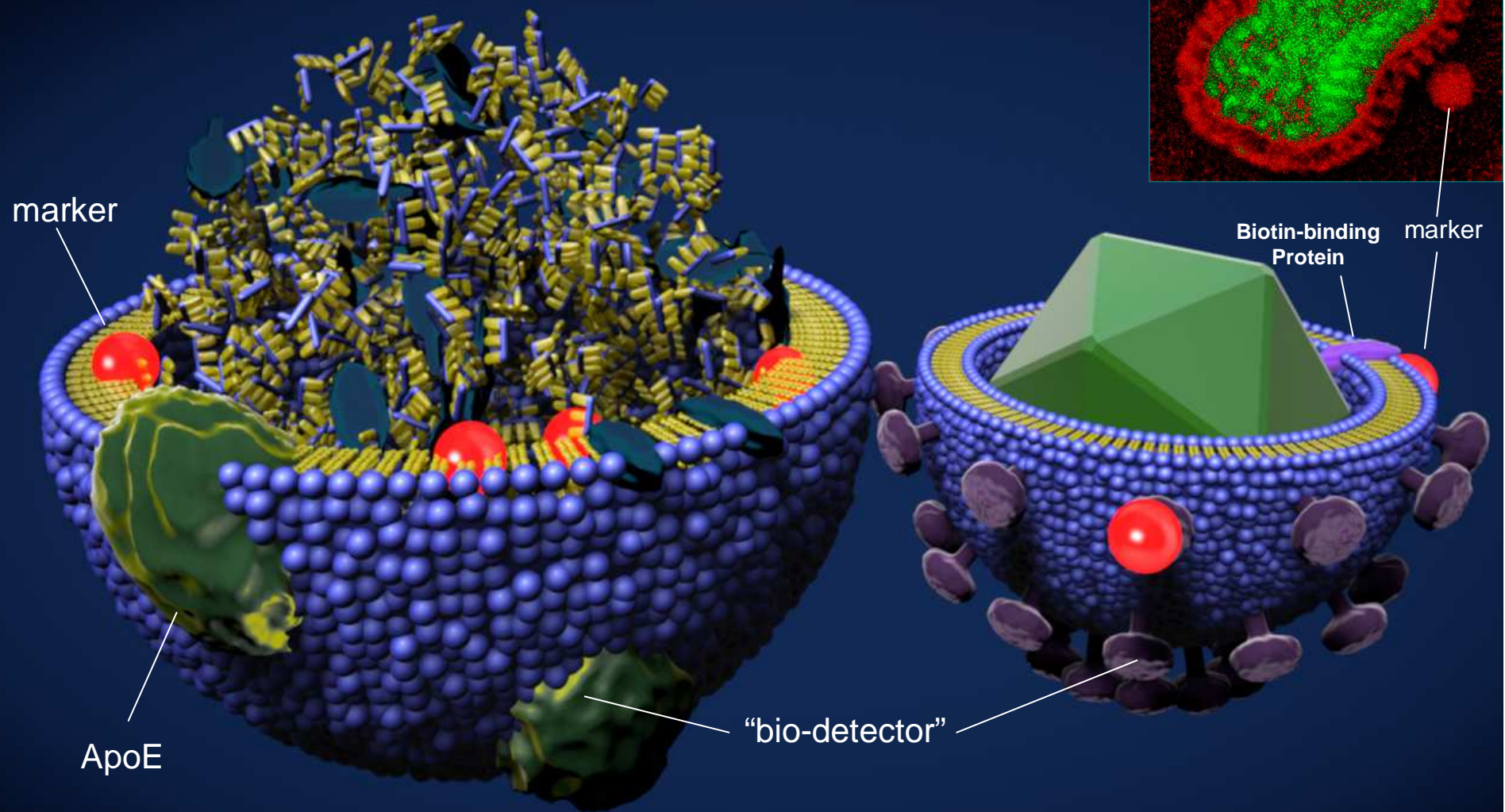
MRI (MPI)





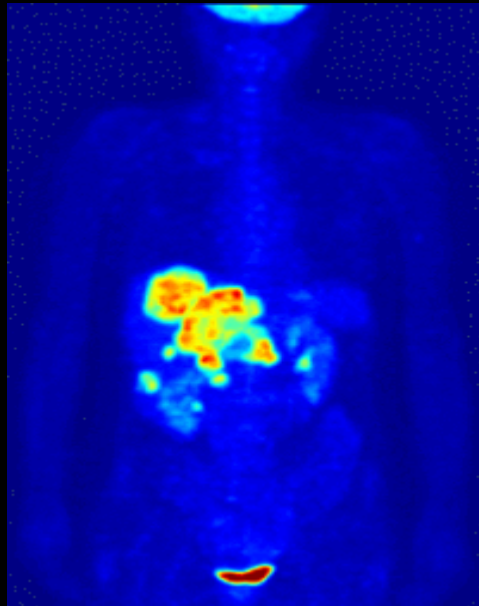
**Superparamagnetic iron oxide (SPIOs) nano-particles  
are inclosed into liposomes or micelles**



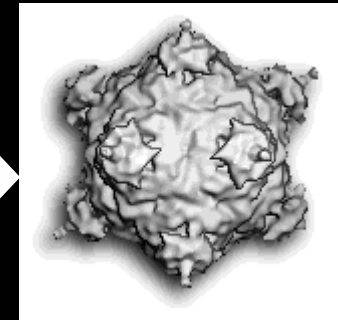


**Nanoparticles (contrast agent) enclosed into micelles (transport) or linked to viruses**

# From the intact organism to its molecule complexes *in situ*:



**Bridging Technologies:**  
Nanoparticles as marker for **medical imaging** and **EM**



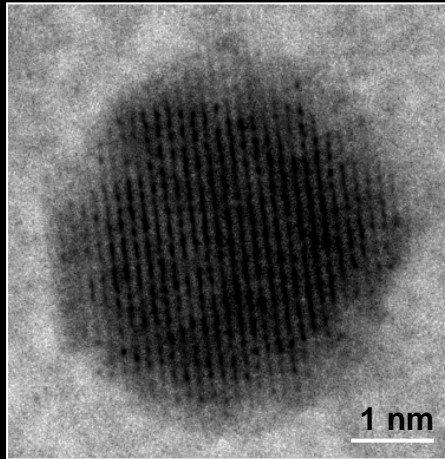
**Medical Tomography:**  
**Intact Organisms**  
MRI

**Electron Microscopy of surfaces:**  
**Cells, Tissues**  
SEM  
ESEM

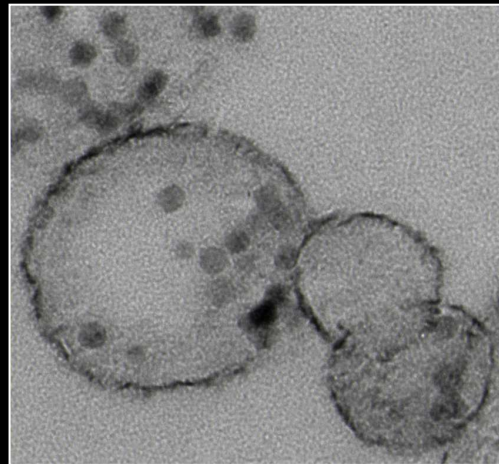
**Electron Microscopy of cell interior:**  
**Cells, Tissues**  
TEM  
TEM-Tomography

# Analysis of complex biosystems:

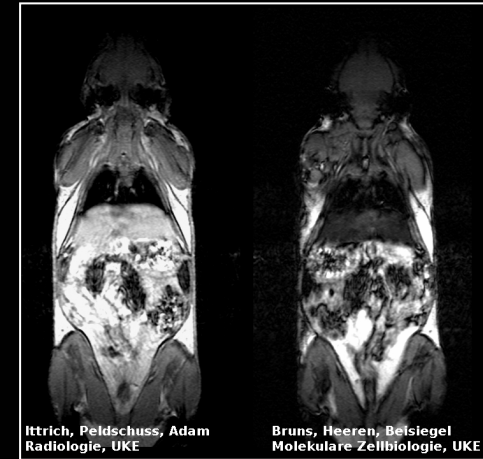
From organisms to molecules: Combined NP-based targeting and correlative EM methods



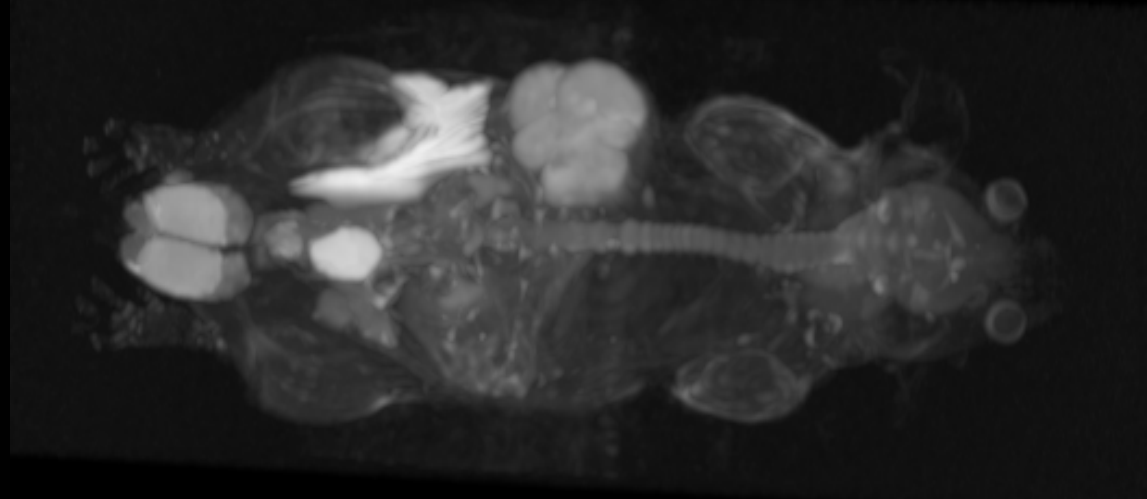
**Super Paramagnetic Iron Oxide (SPIO) Nanoparticle**



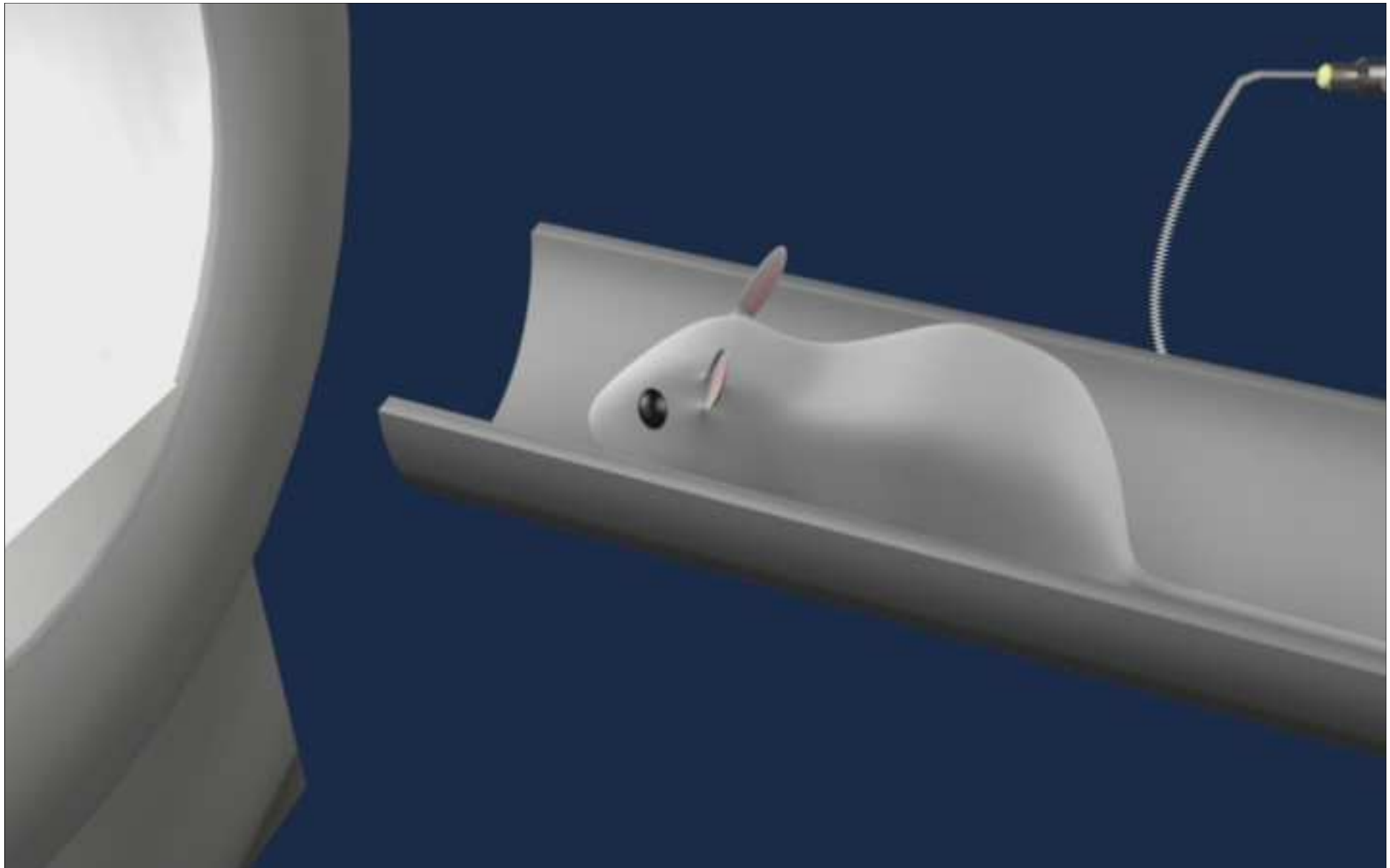
**SPIOs integrated into functionalized Nanosomes**



**Injected Nanosomes in the liver of a mouse detected by MRI**



**3D-Reconstruction of MRI data**



**Simulation of the workflow:**

**Injection of nanosomes > MRI-localisation > biopsy > EM specific preparation > TEM**

Bartelt *et al.* (2011), Nature Medicine

**Systemic micro- and bridging techniques:**

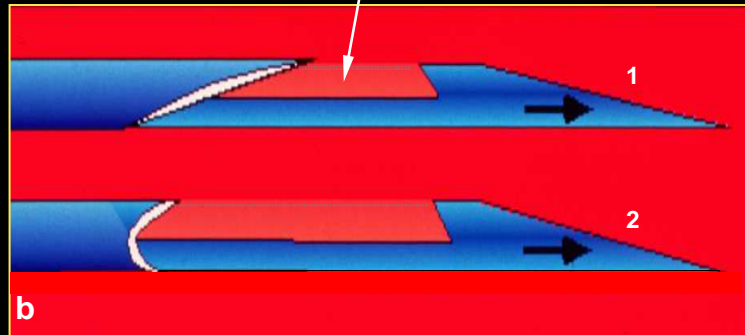
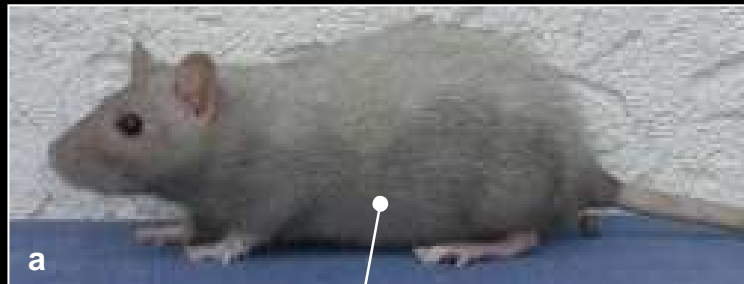
**Targeted minimal-invasive microbiopsy**

**Micro-transfer of vital  
patient and laboratory animal tissues  
for direct freezing or recultivation**

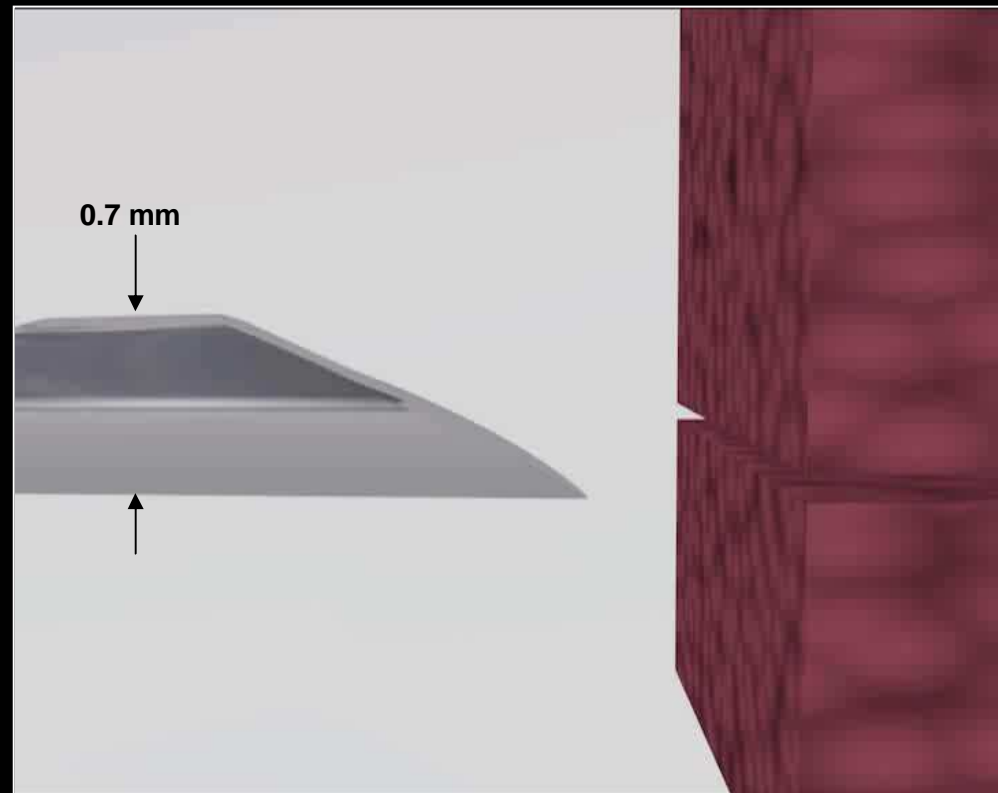


Targeted (e.g. ultrasound) minimal-invasive microbiopsy of laboratory animals and human patients, as a fast micro-transfer and bridging technology for tissues.

**Gateway to the inner organs of patients and laboratory animals**



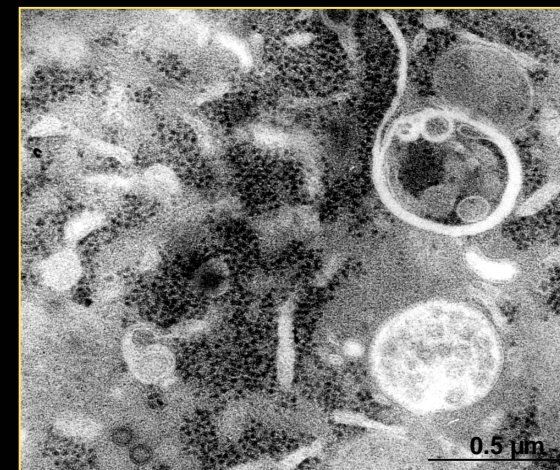
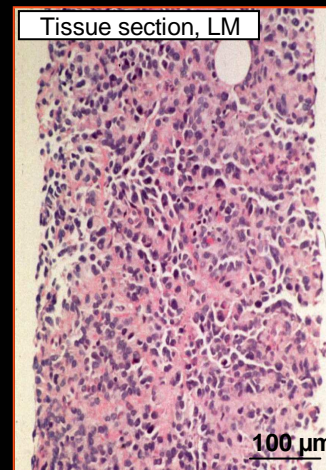
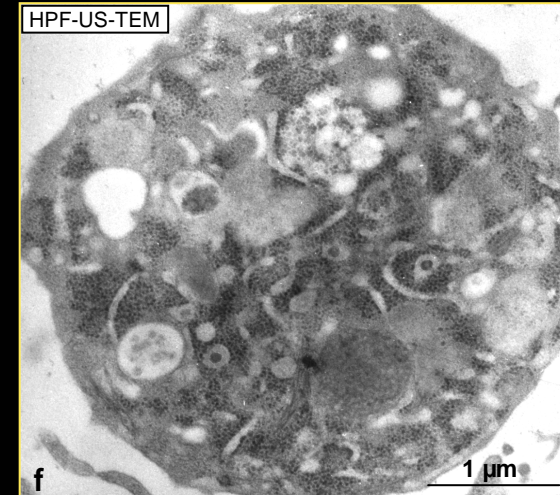
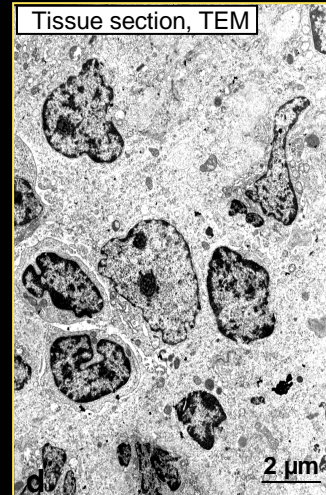
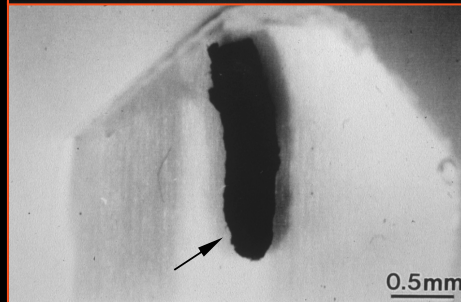
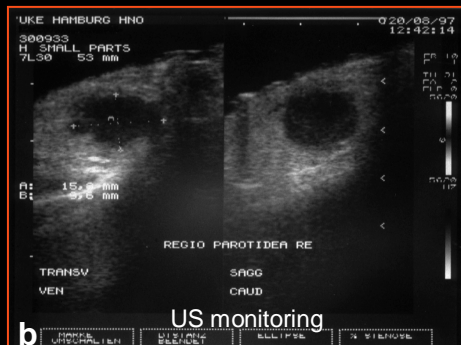
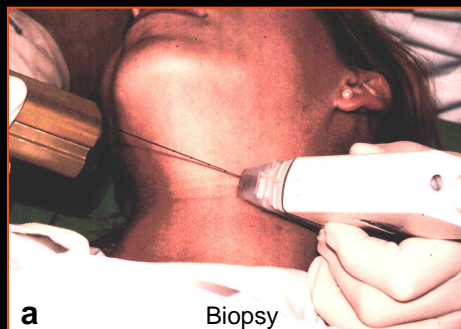
A high velocity cutting needle (b) is integrated into an automatic high velocity biopsy gun



In 4 ms an automatic biopsy-needle cuts a tissue piece out of an organ (40 cell layers high), without any compression damage. The cells are encapsulated inside of the needle .

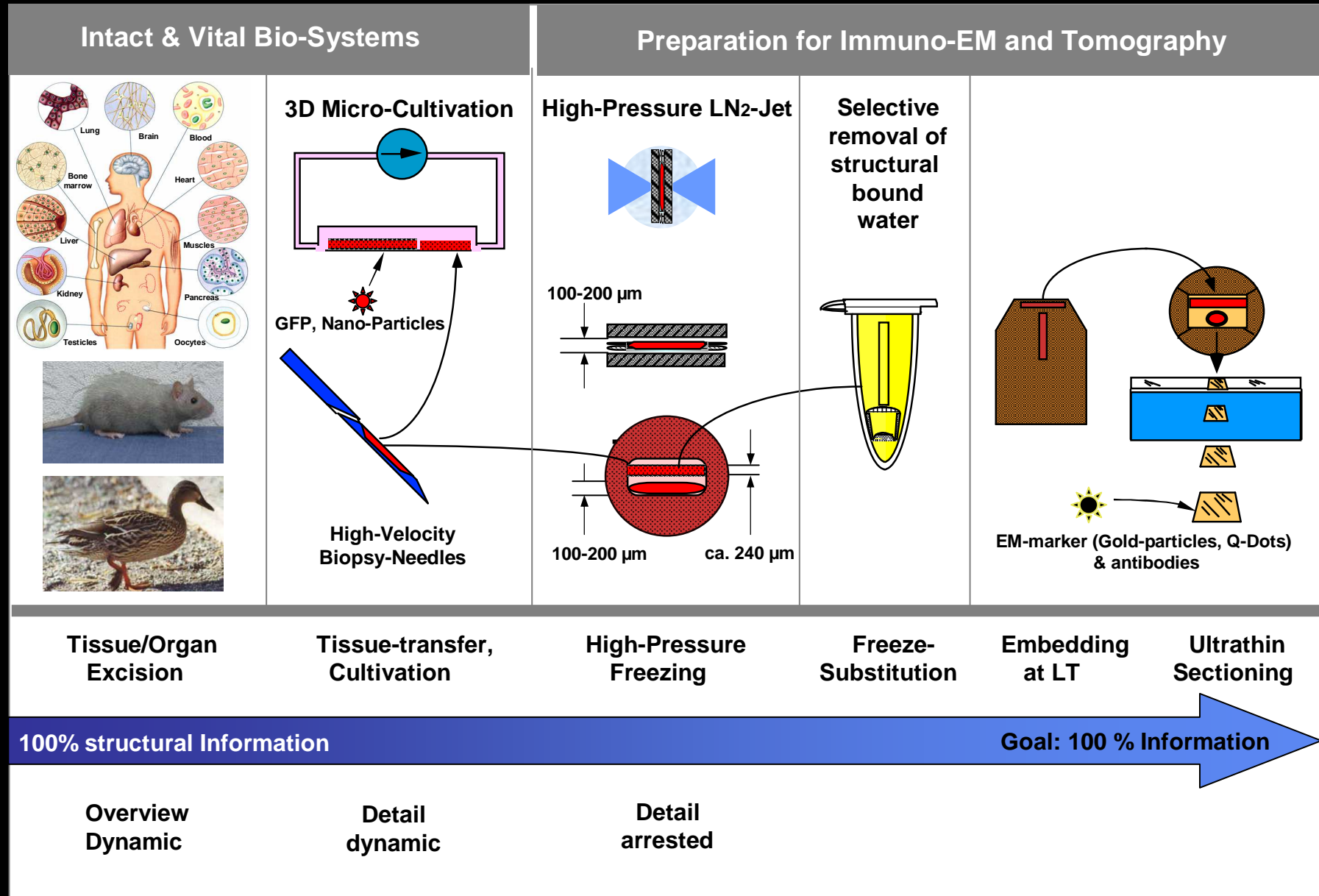


# Ultrasound-guided micro-biopsy of human tissue (lymphatic node)



**Minimal-invasive micro-biopsy of pinpoint detected human tissue for EM- diagnostics or organoid cultivation**

# Preparation of complex bio-systems based on micro-, nano- and cryo-techniques





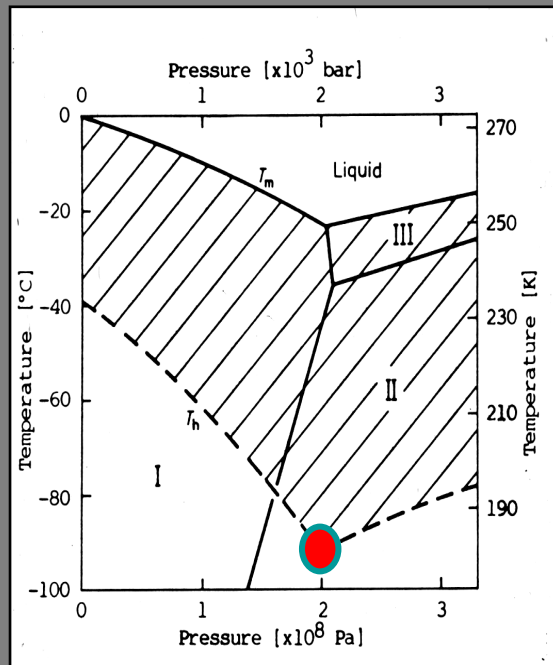
**Cryotechniques:**  
**Essential prerequisite for a life-like preserved cellular structure  
and its molecular-morphological analysis**



# A high-tech freezer for vital cells and tissues: **The High-Pressure Freezing Machine**

Cryofixation at high pressure allows to freeze:

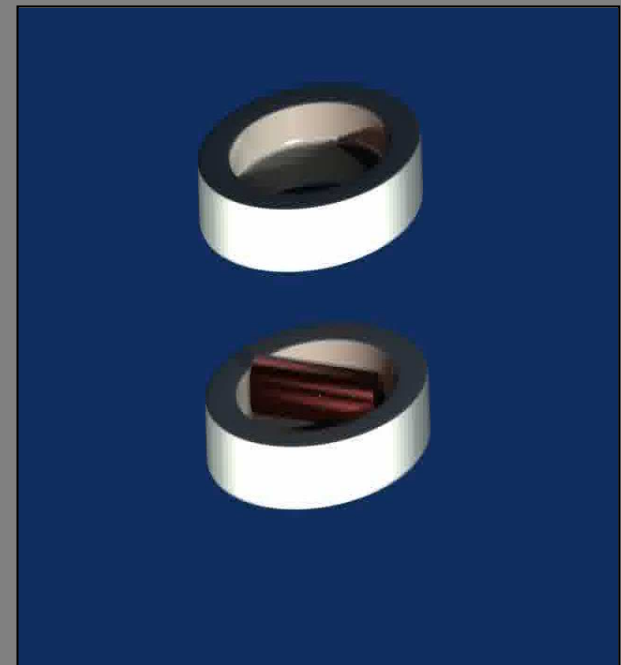
- native infected cells and tissues without ice crystals (vitrification)
- up to a thickness of 200  $\mu\text{m}$  (limit)



P/T phase diagram



HPM 010



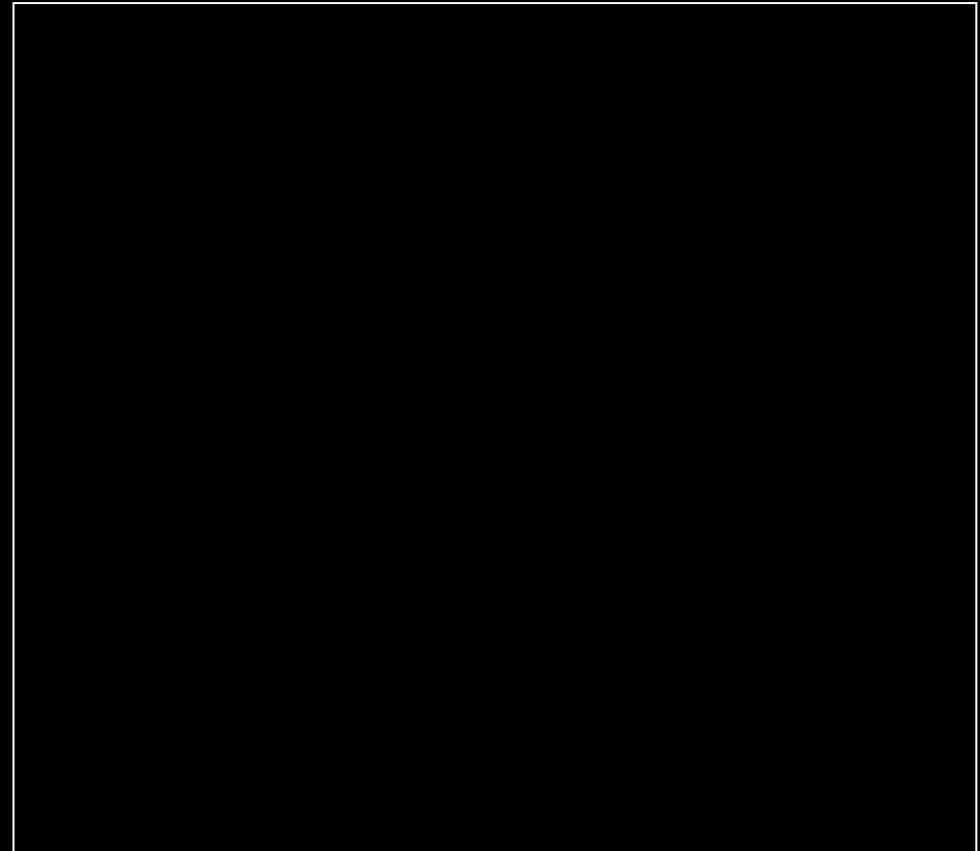
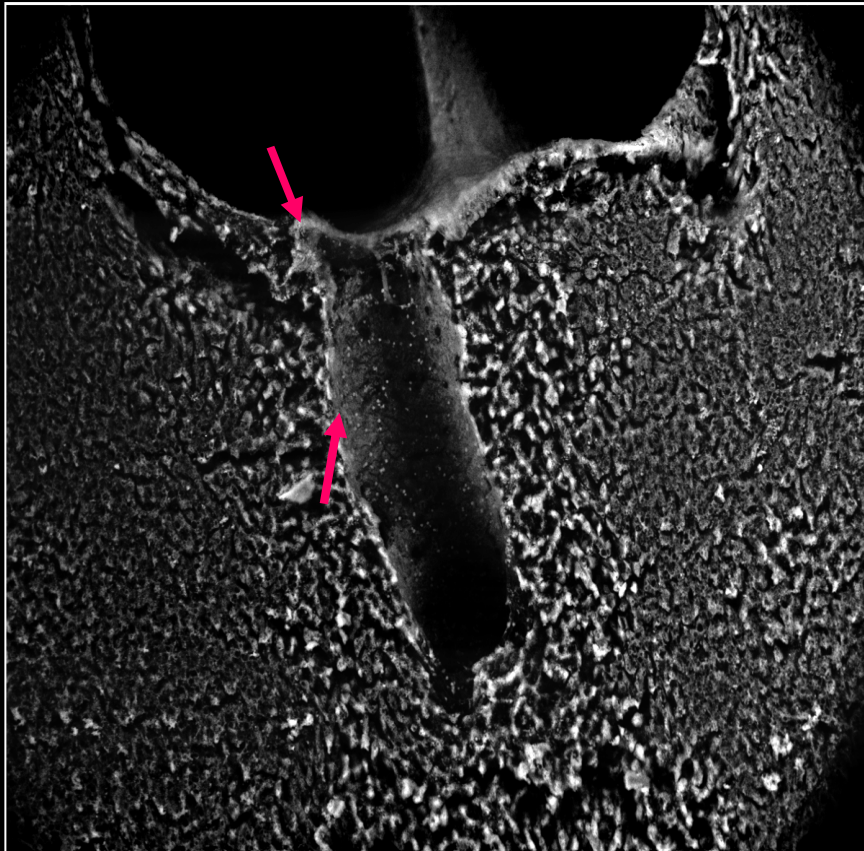
2040 bar Cryo-Jet (LN<sub>2</sub>)

**The only and optimal freezing technique for the cryofixation  
of complex bio-systems**

**From organisms to molecules: preselection is absolutely necessary!**

**Combined NP-based preselection and correlative EM methods:**

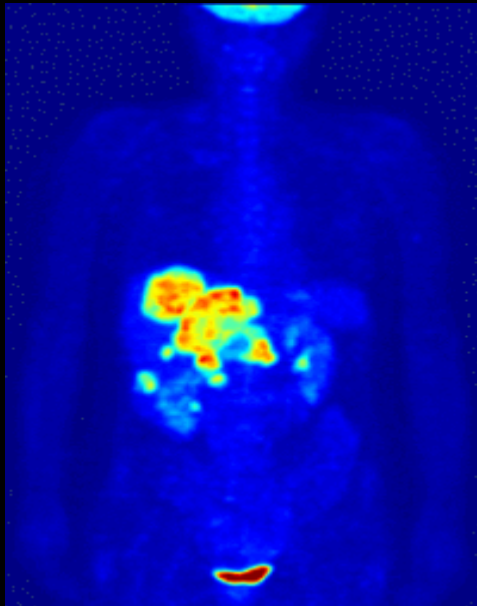
**From ESEM to TEM**



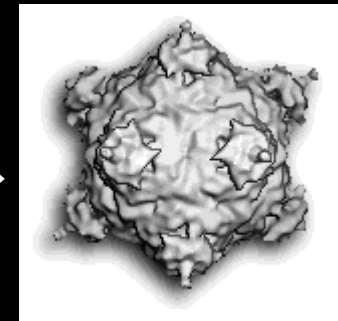
**Biopsies are investigated in the ESEM, areas with a high NP BSE-Signal are preselected (left) and processed for ultrathin sectioning and investigation in the TEM (right)**

## Imaging of the same bio-system:

### Nano-Particle (SPIOS & Qdots) as preselection-markers for LM-EM transfer



**Systemic Imaging:**  
Magnetic NP and Qdots as marker  
for LM and EM-Microscopy



**Light-  
Microscopy:**

**Cells, Tissues**  
Live Cell imaging,  
Intravital, CLSM

**Electron  
Microscopy  
of surfaces:**

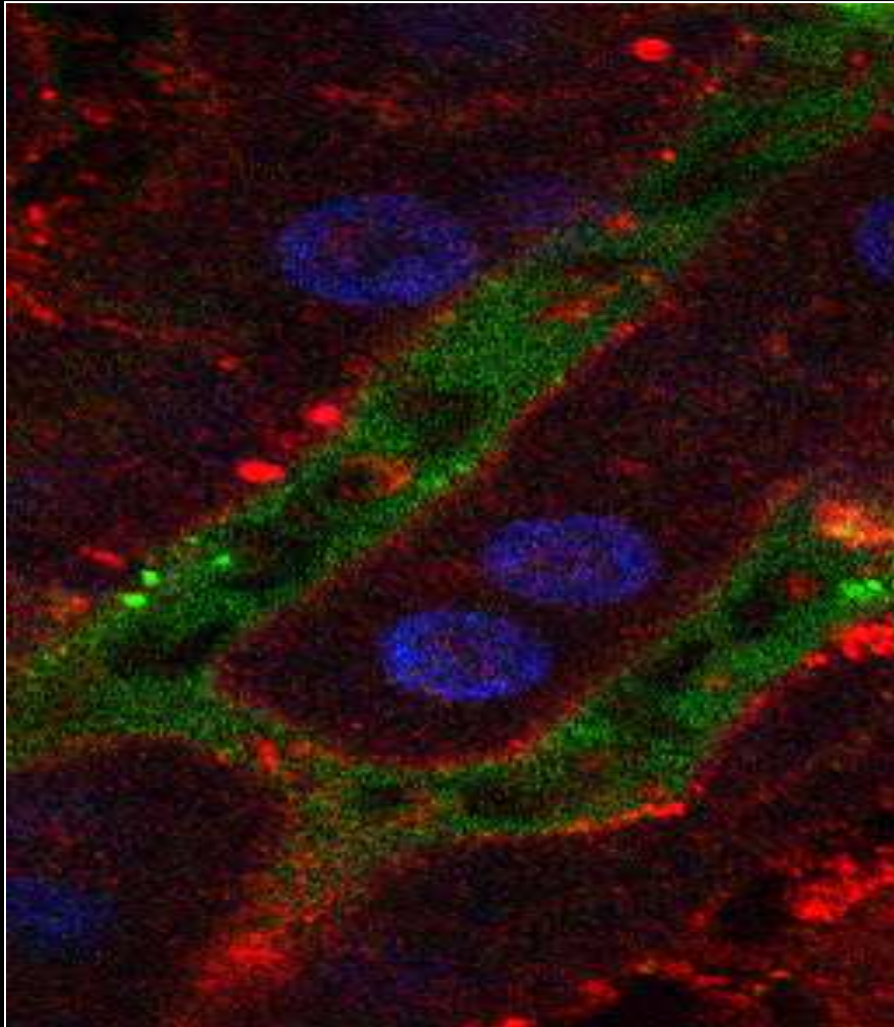
**Cells, Tissues**  
SEM  
ESEM

**Electron  
Microscopy  
of cell interior:**

**Cells, Tissues**  
TEM  
TEM-Tomography

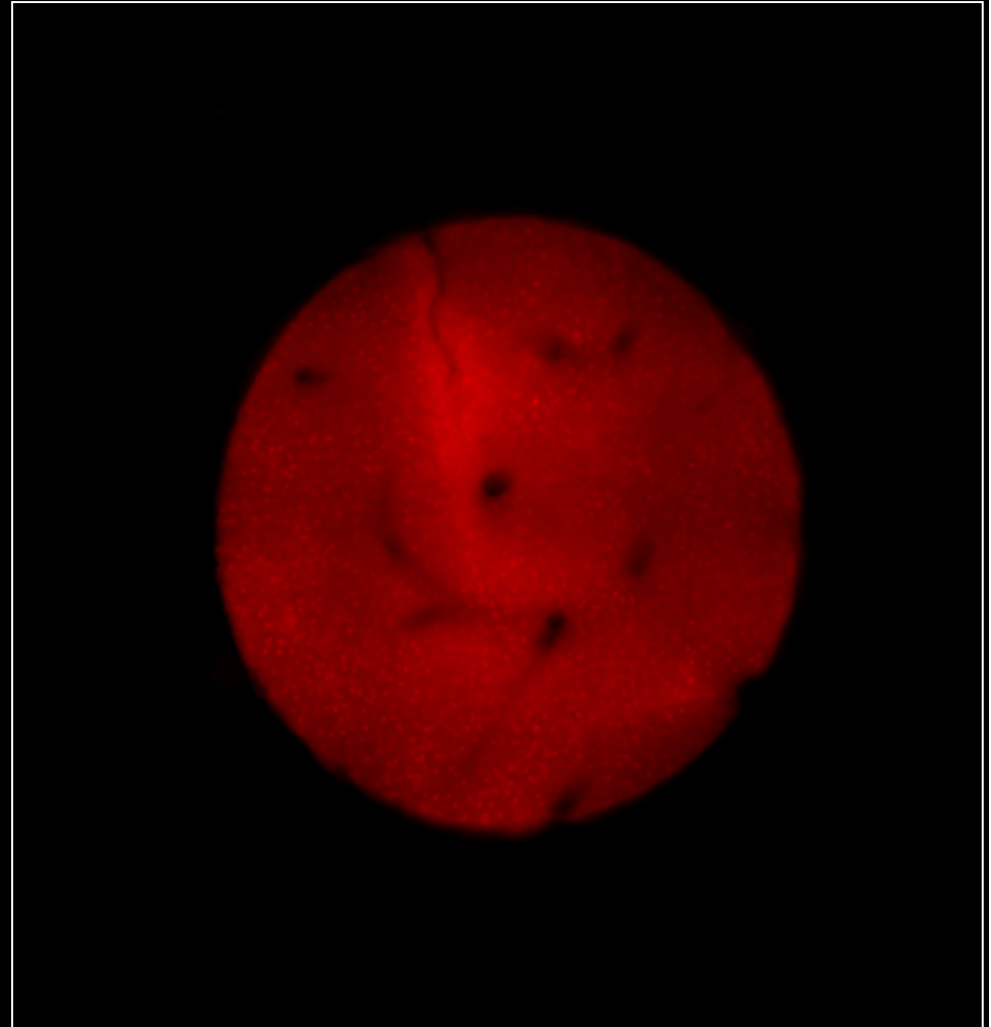


# Nanoparticles (QDots) for the microscopy of bio-systems: From in vivo microscopy to 3D tomography



Mouse liver in real-time **intravital CLSM**

Nucl.: Hoechst, green:FITC-Dextran, red:NP



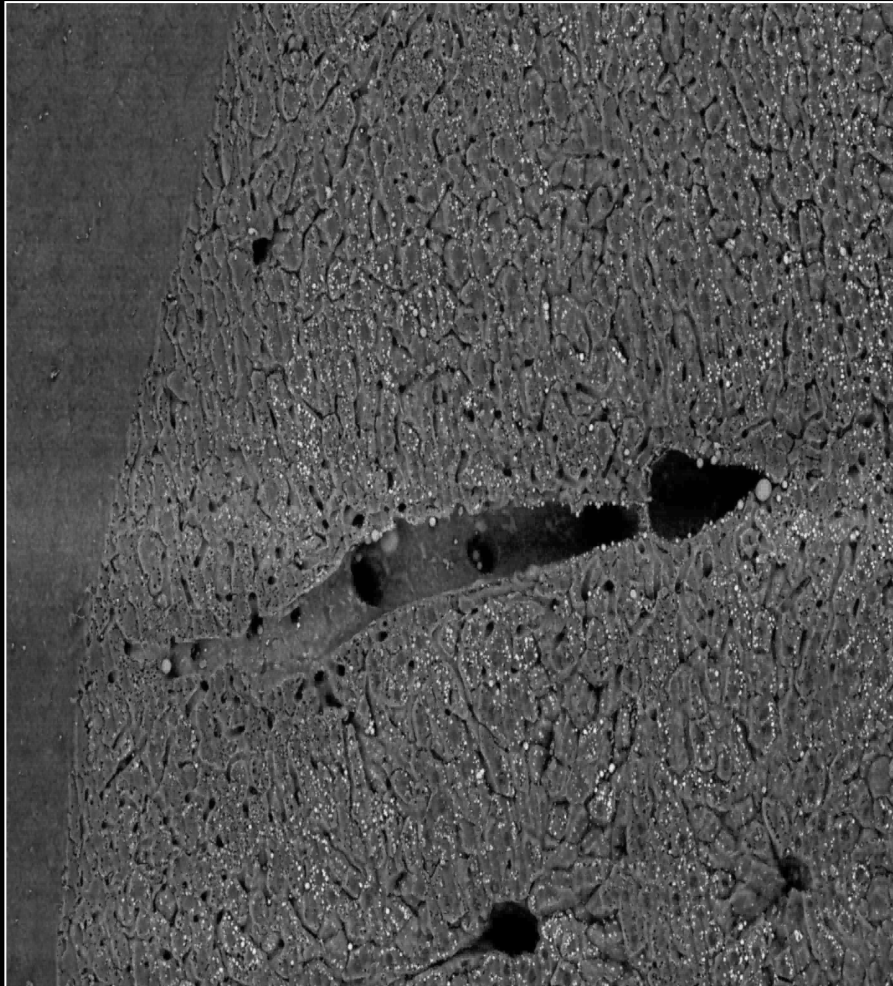
Section of a mouse liver tissue,  
preselected by intravital **CLSM**

**Cultivation of organotypic serial liver slices in thin foil containments:**  
**Vital tissue in defined states for cryofixation**

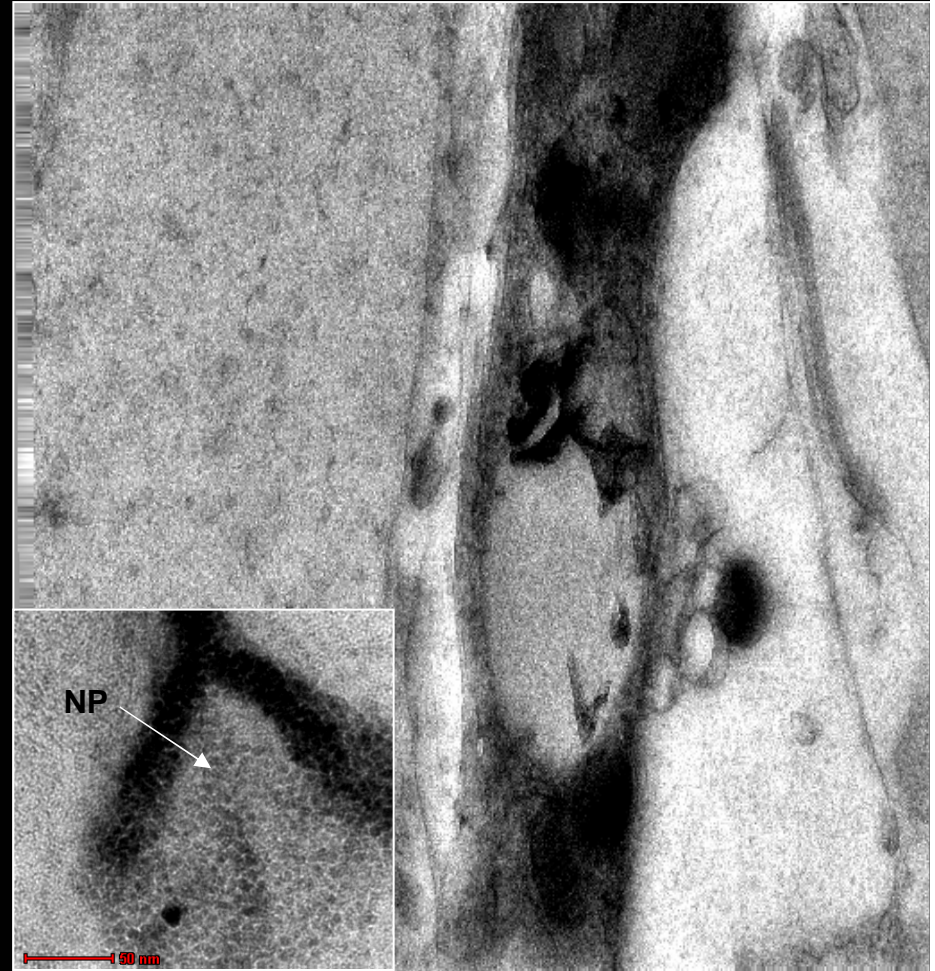
**Fast sectioned liver slices (200  $\mu\text{m}$  universal thickness) are cultivated in a membrane containment. MWCO of the highly porous membrane: 5-10 kD**



# Nanoparticles for the microscopy of bio-systems: From in vivo to 3D tomography



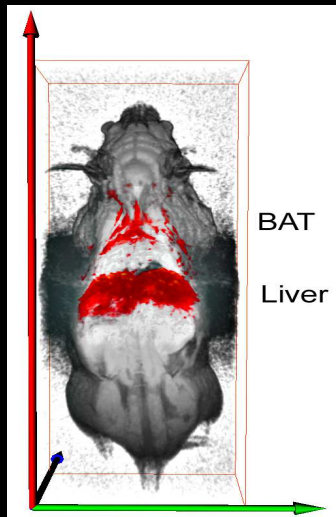
Cultivated mouse liver section  
selected by means of intravital  
CLSM in the ESEM



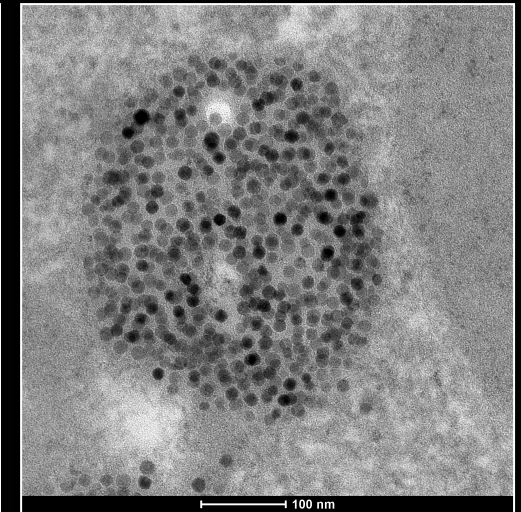
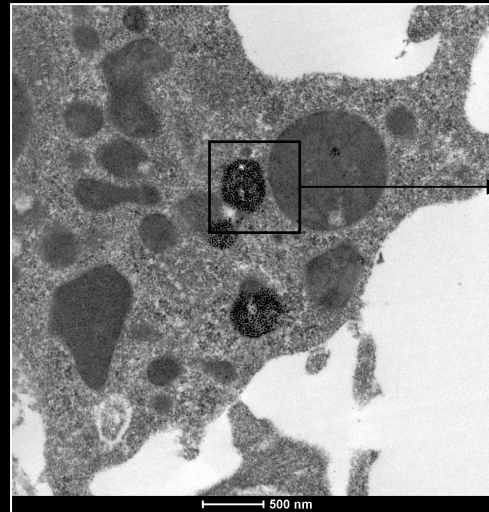
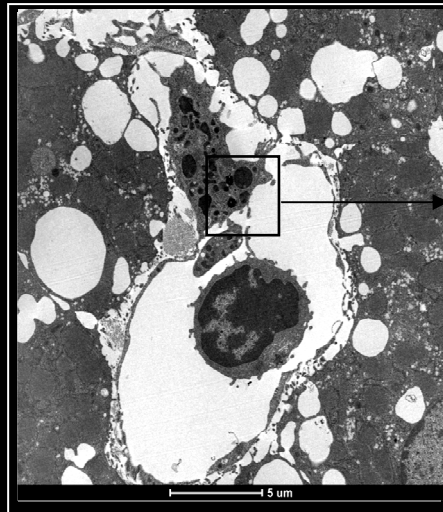
Biopsy of a mouse liver tissue after  
high pressure freezing in the TEM.  
Accumulation of NP in macrophages



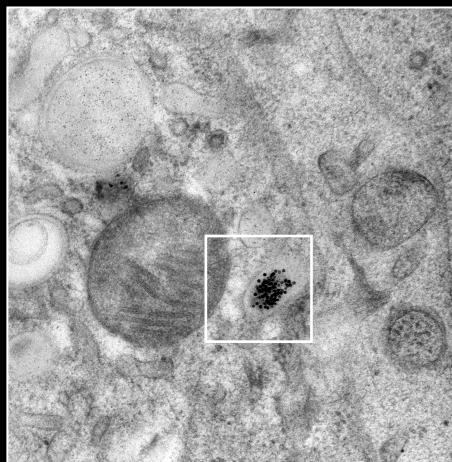
# Strategies for the microscopical analysis of complex bio-systems: From whole organisms to molecules: Combined targeting & correlative microscopy methods



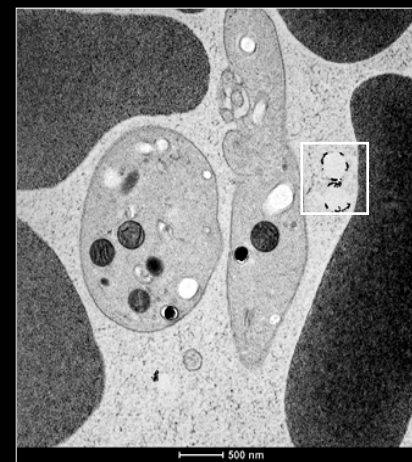
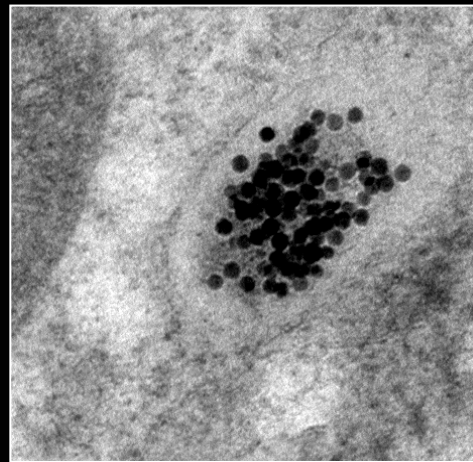
3D reconstruction of SPIO-Nanosomes labelled BAT (brown adipose tissue) and liver of a mouse in the MRI



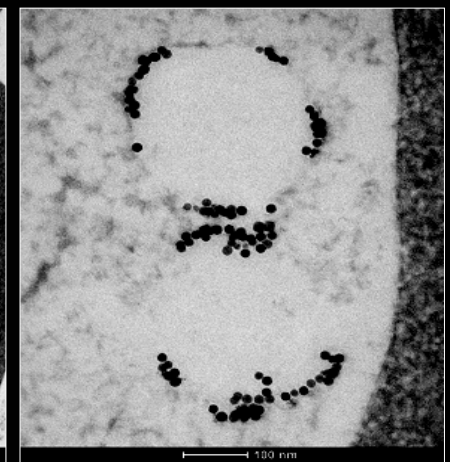
Nanosomes in macrophages in the liver, **one week after uptake**  
A. Bartelt *et al.*, *Nature Medicine*, 2011



Nanosomes in **brown adipose tissue 2h after uptake** (microbiopsy, HPF in carbocell tubes, freeze-substituted, HM20 embedded)



Unprocessed nanosomes in **blood** (high pressure frozen in carbocell tubes, freeze-substituted, HM20 embedded)

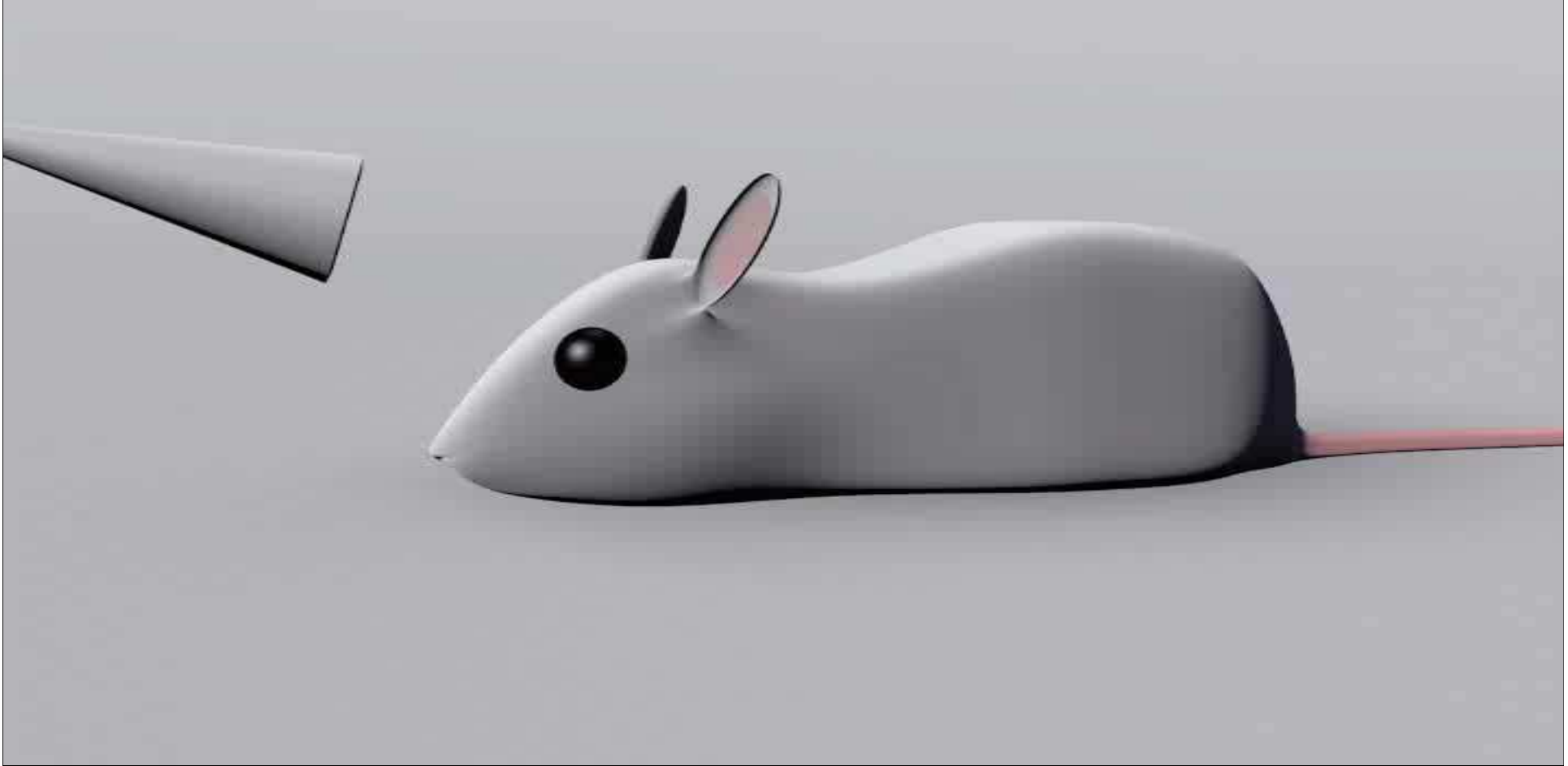


**Translational and systemic application:**

**Infected mouse lung**

**From MRT, LM, CLSM, ESEM to the TEM**

## Systemic Imaging of Infections

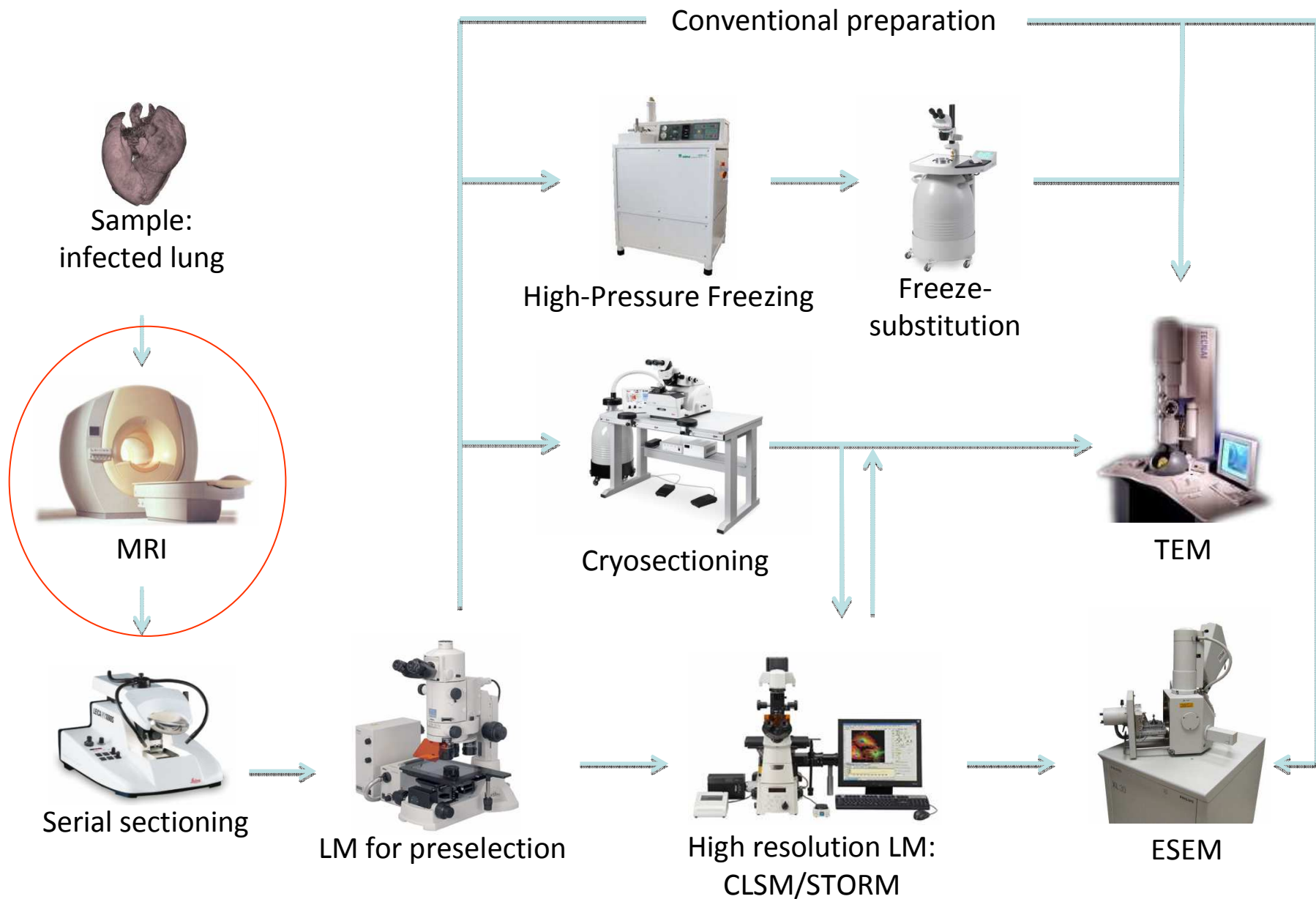


**Tracking the pathway/routes of infectious pathogens *in vivo***

- *how pathogens overcome biofilms and other barriers*
- *blood transport*
- *uptake and processing in specific organs, tissues etc.*



# Integrative Microscopy of infected tissue



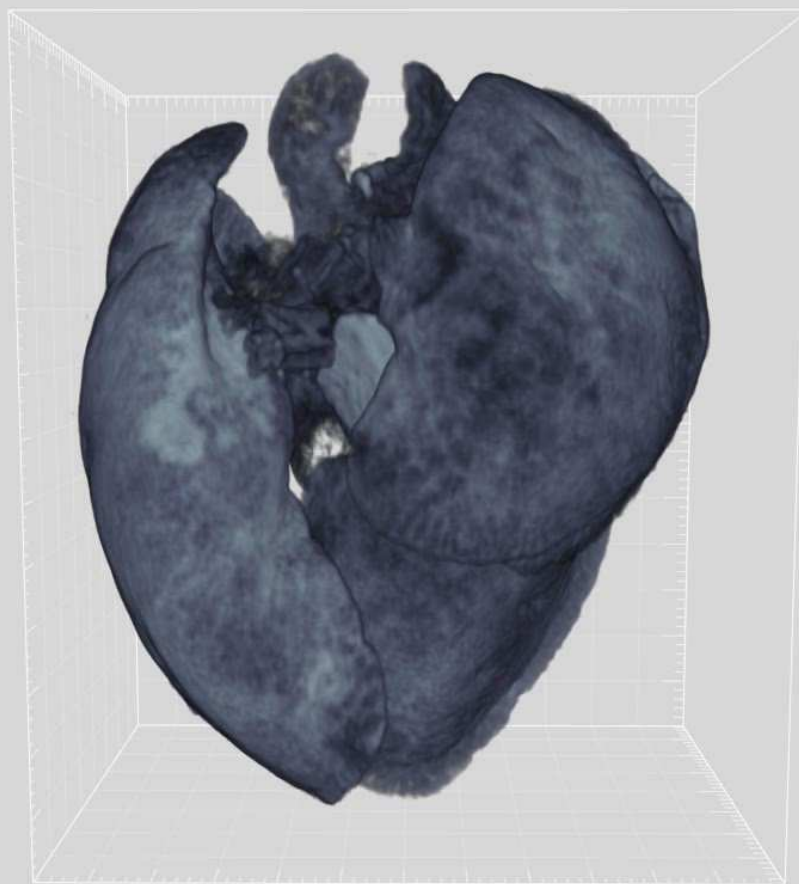
## MRI as a 3D Microscope

- 50  $\mu\text{m}$  isotropic resolution
- No special preparation or staining necessary



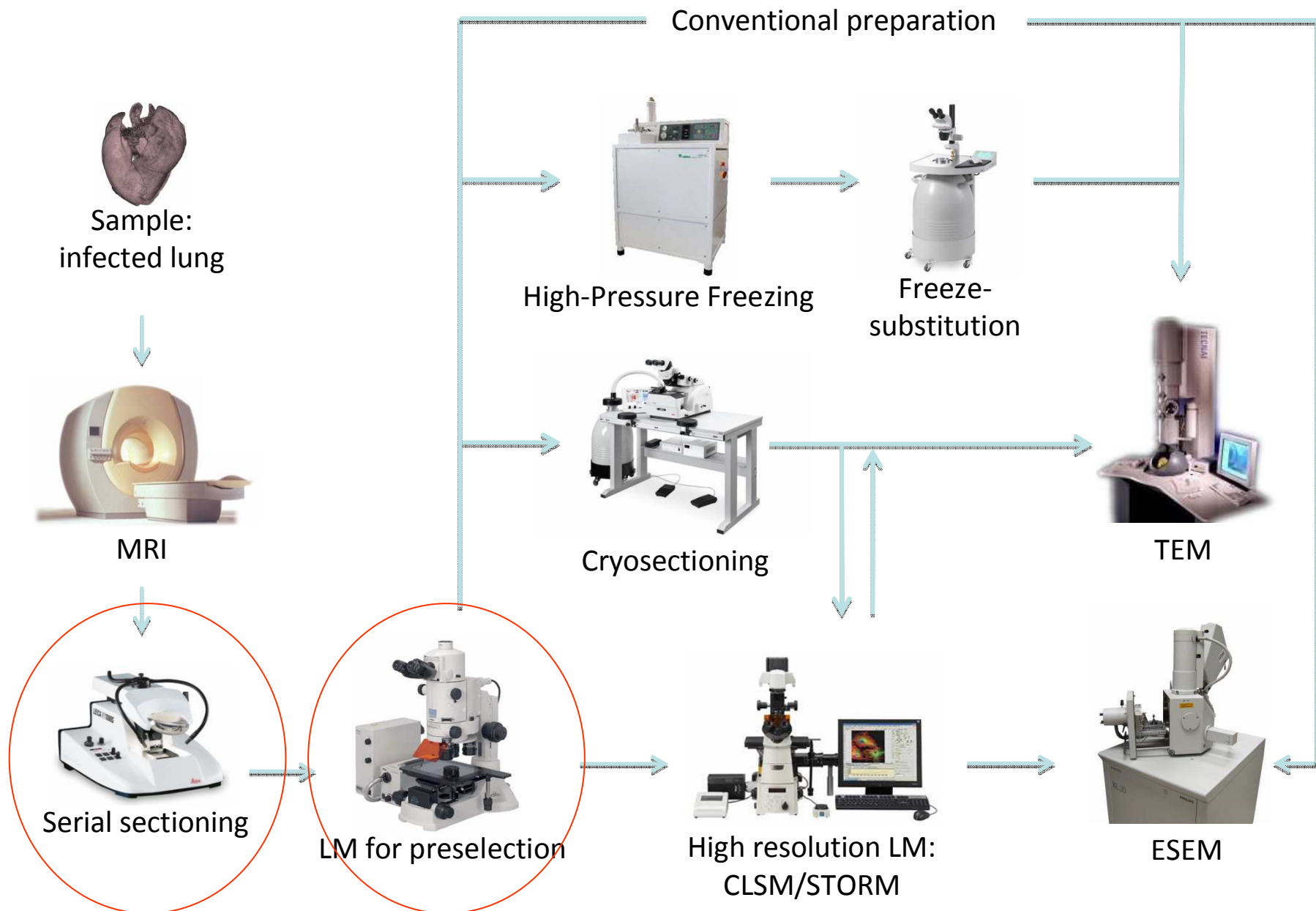
*Mycobacterium tuberculosis* infected mouse lung, PFA-fixed, LMP-agarose embedded. Imaged with a Philips Intera 3T MRI equipped with a small animal coil

## MRI as a 3D Microscope



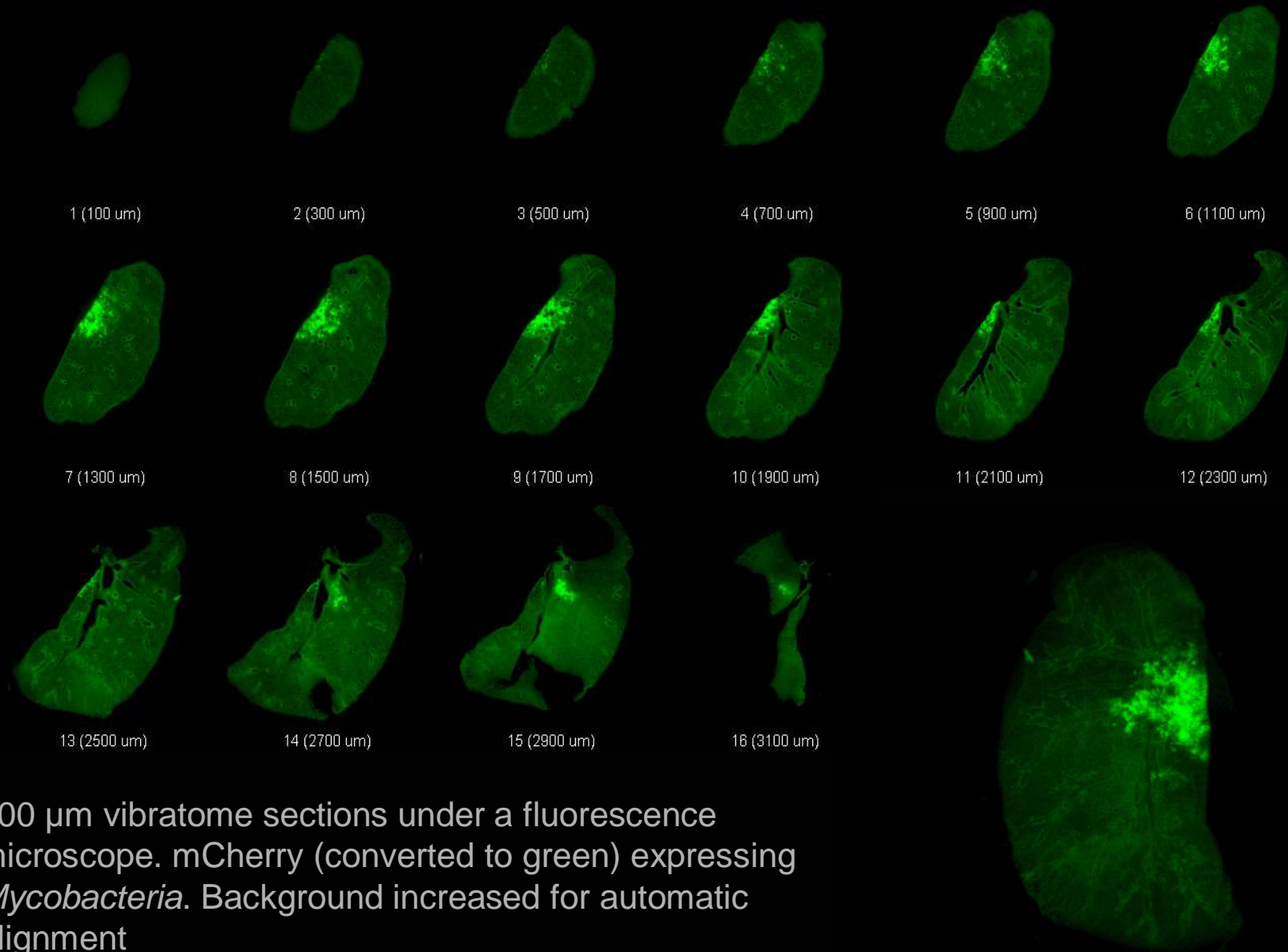
3D reconstruction of the MRI dataset with Imaris. Blend mode faded to maximum intensity projection with a two colour LUT

## Integrative Microscopy of infected tissue

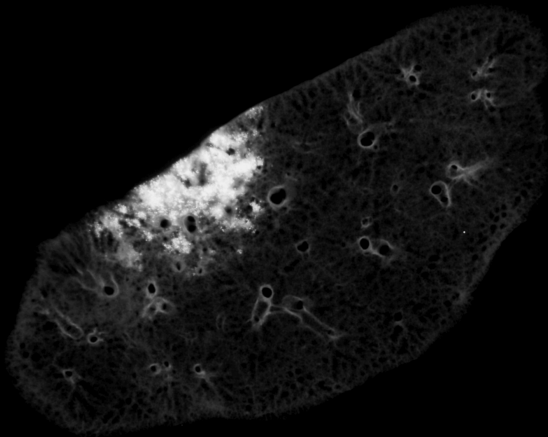
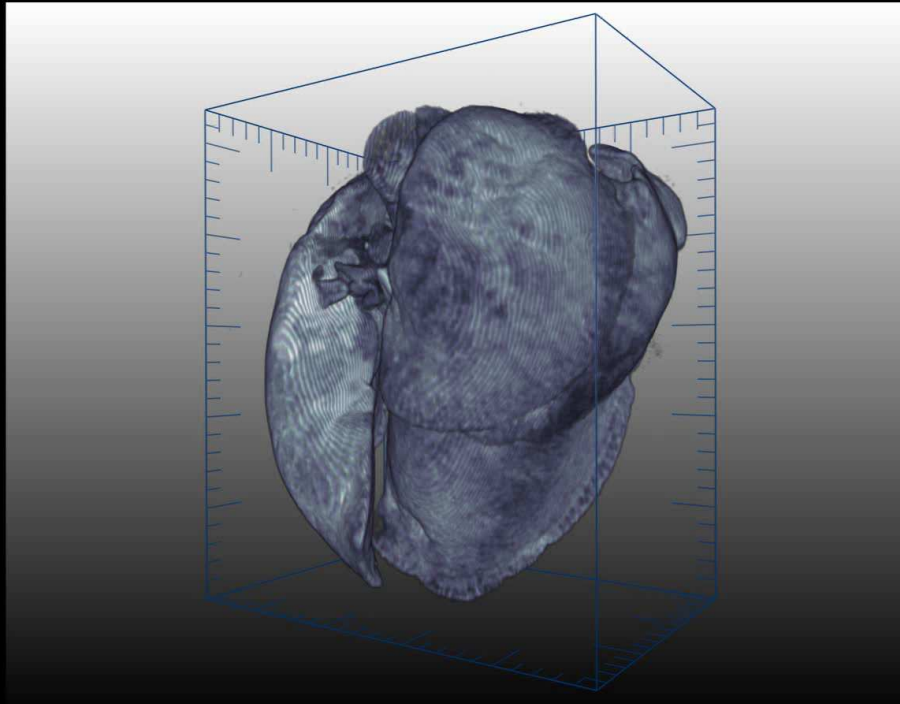




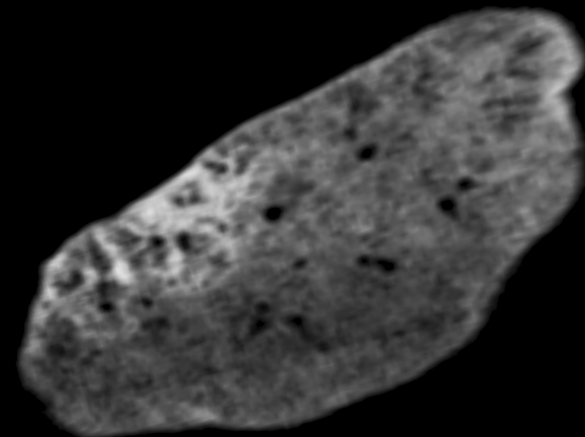
## Vibratome serial sectioning



## Relocalisation of vibratome sections in the MRI dataset

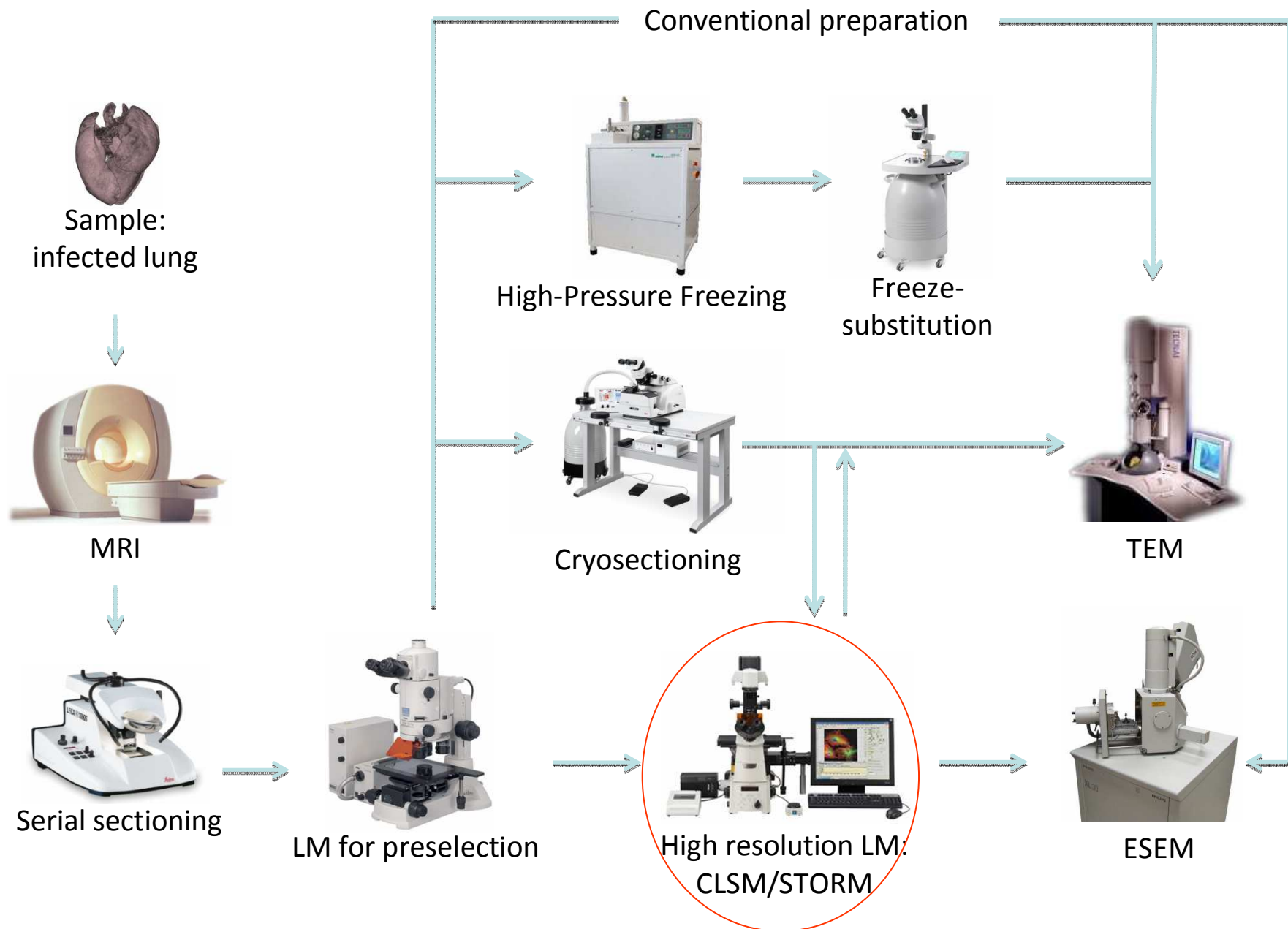


Vibratome section #7

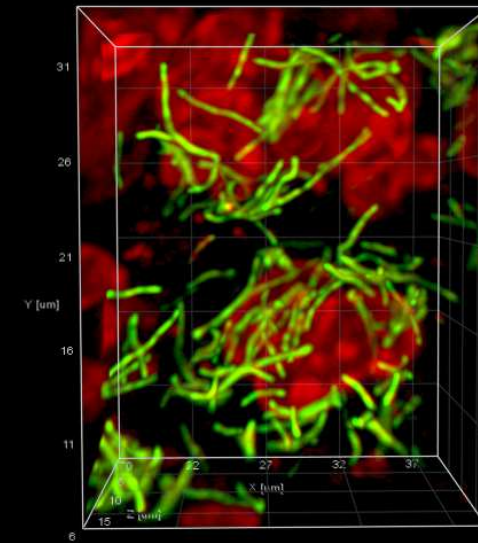
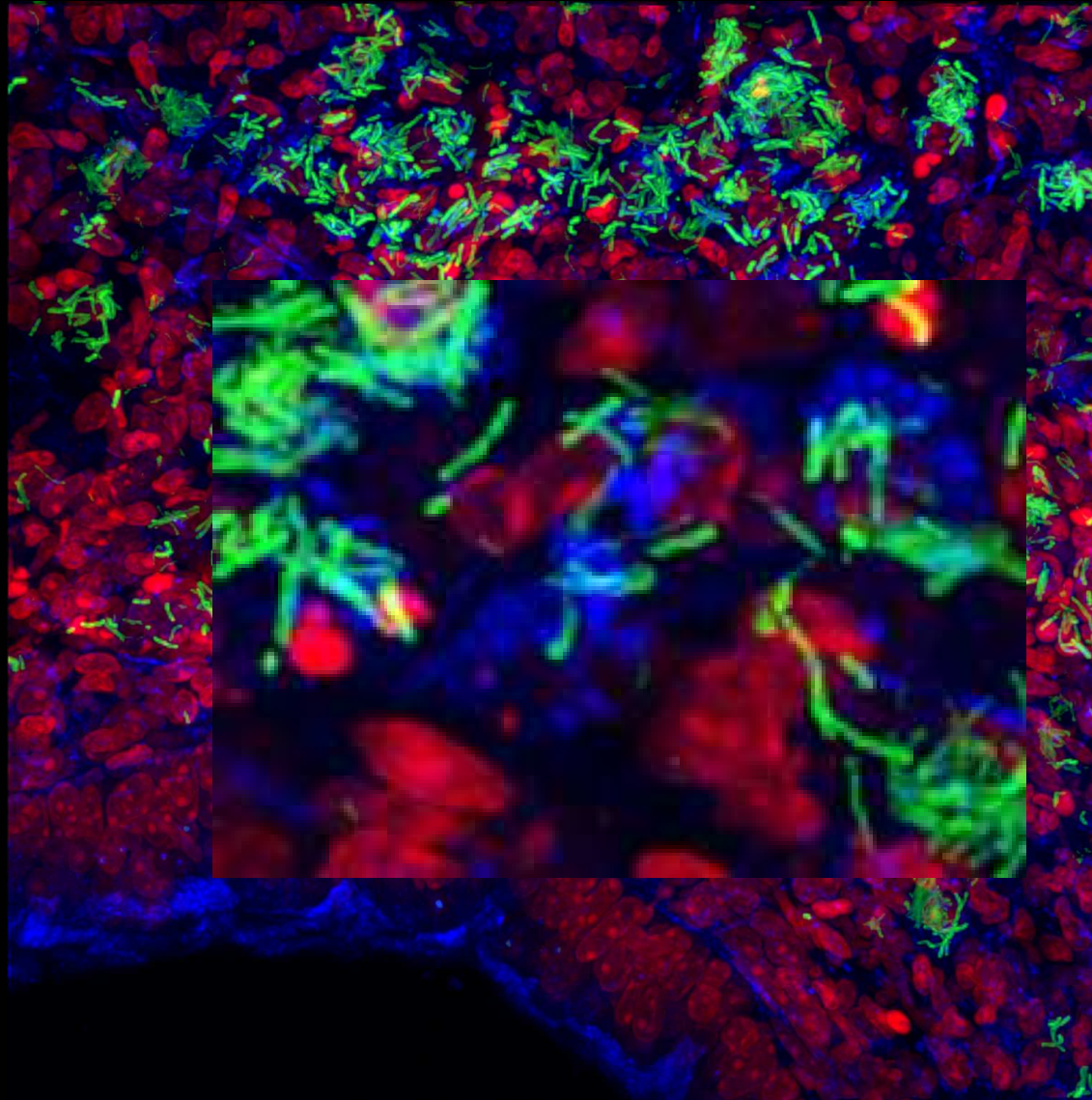


Virtual MRI section

# Integrative Microscopy of infected tissue



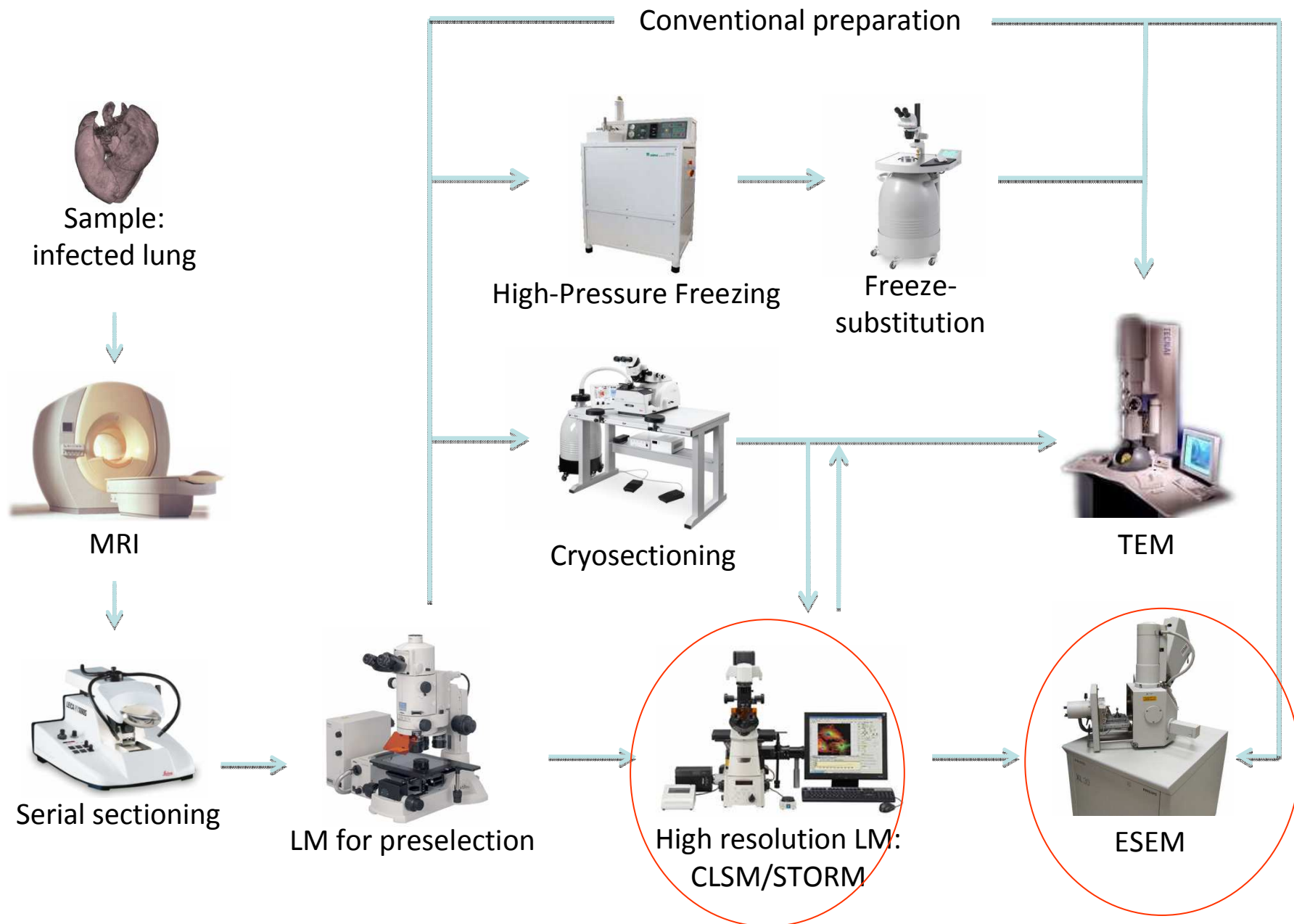
# Confocal Laser Scanning Microscopy



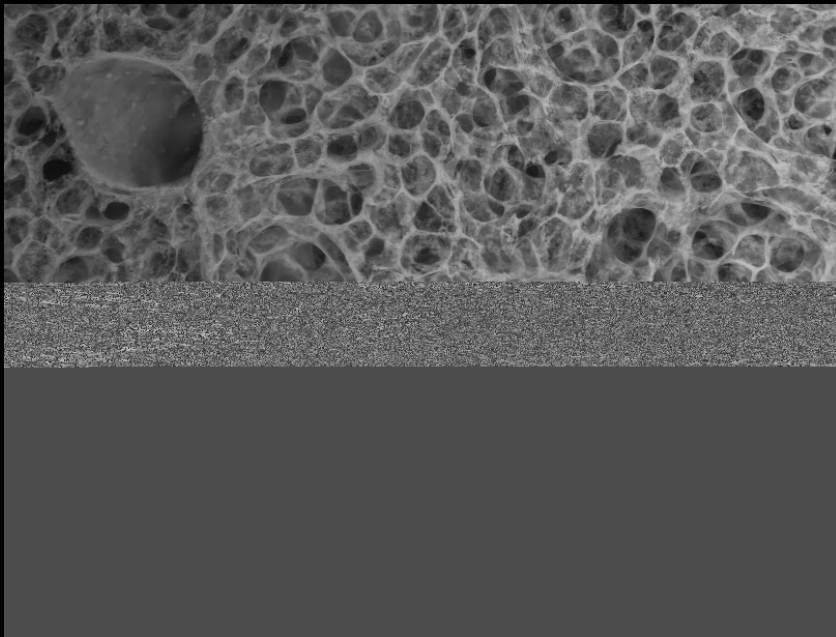
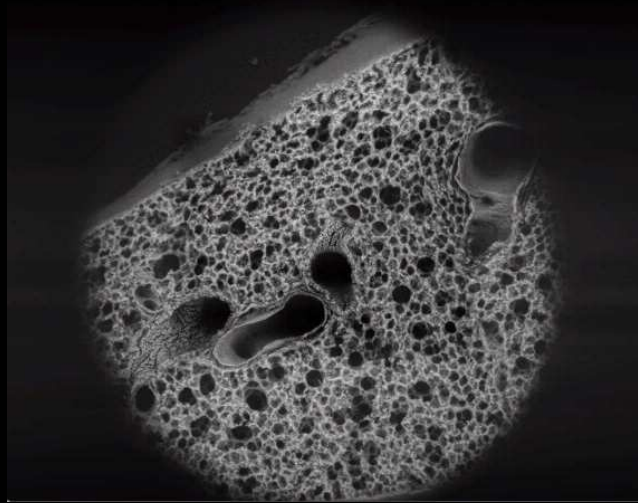
- Mycobacteria (mCherry)
- DNA (DRAQ5)
- Reflex channel



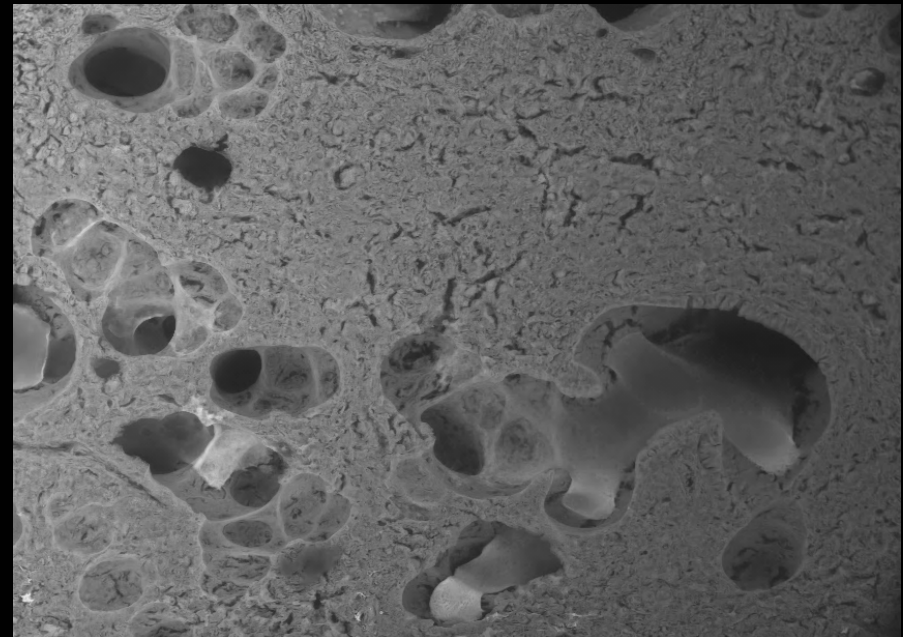
## Integrative Microscopy of infected tissue



## Environmental SEM



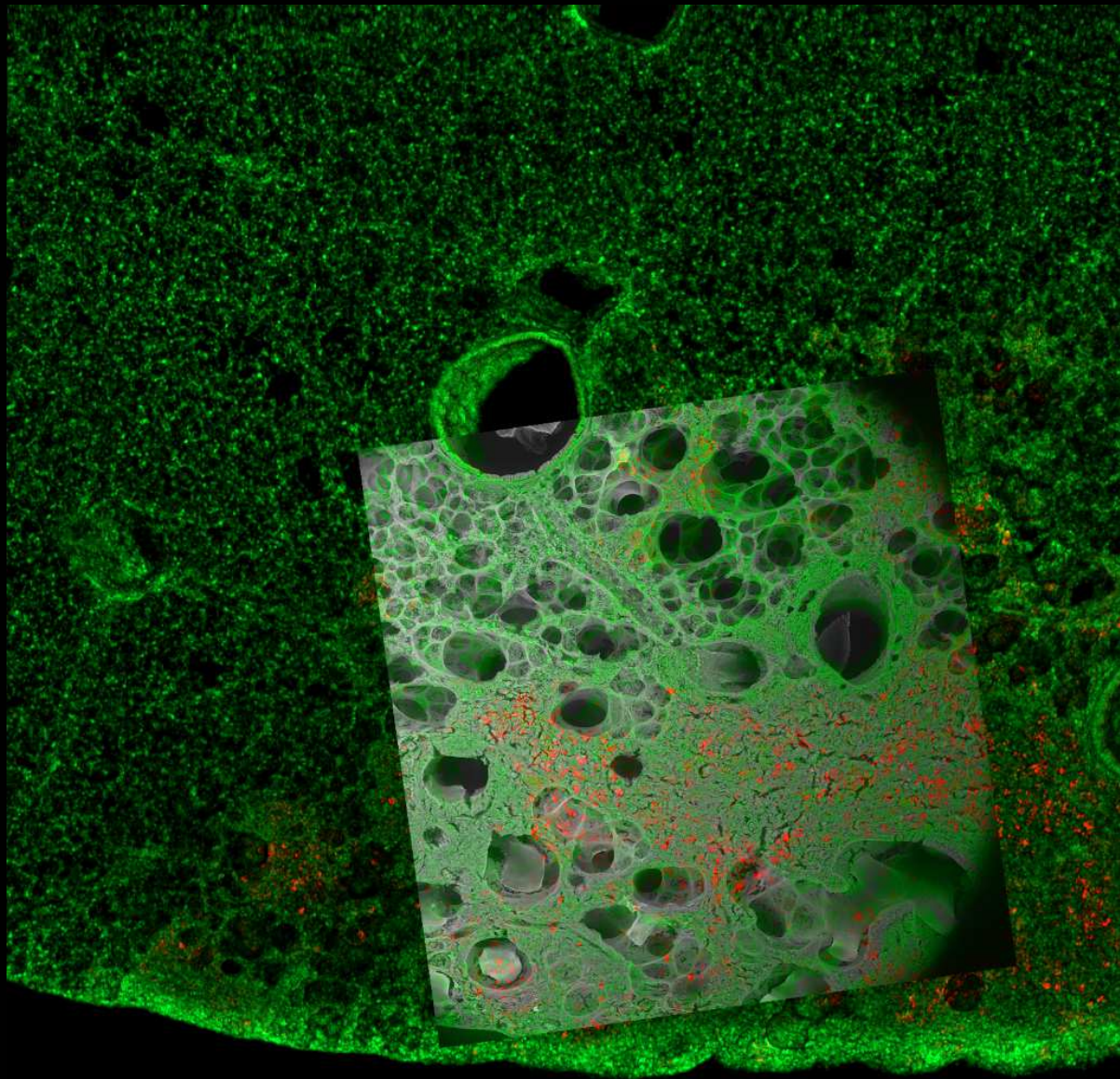
Normal lung tissue



Lesion



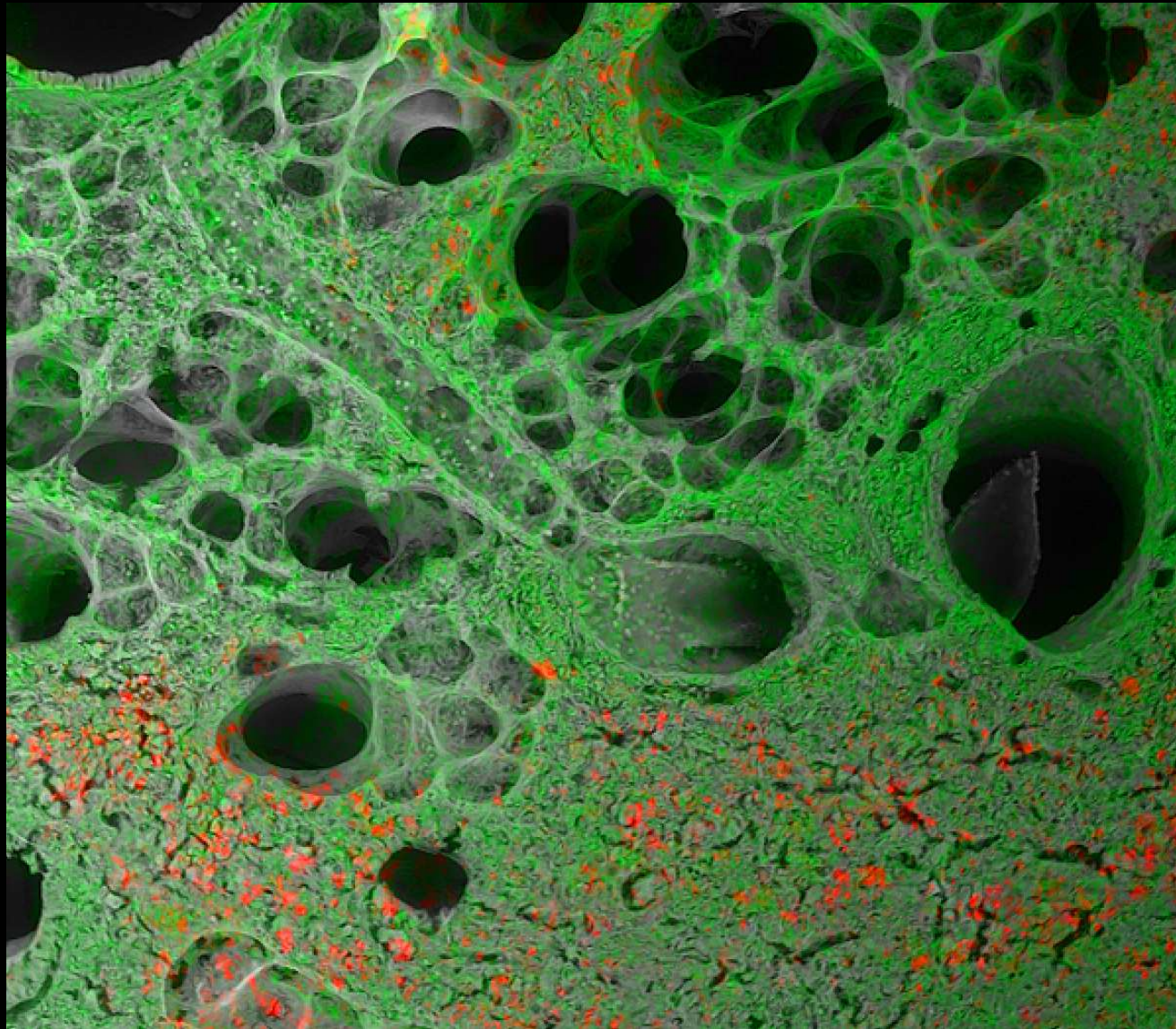
## Correlative CLSM-ESEM



- Reflex channel
- Mycobacteria (mCherry)
- Environmental SEM



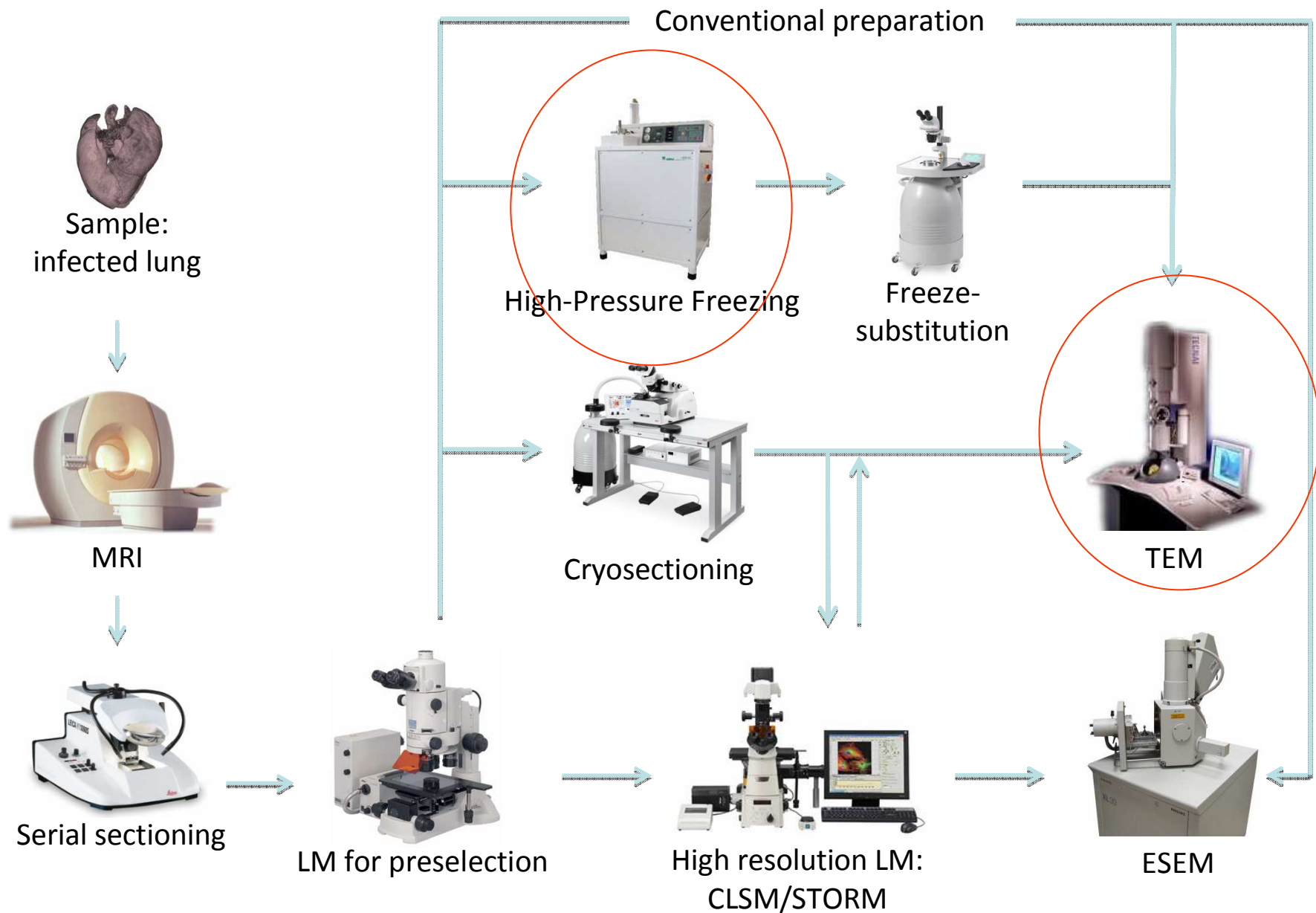
## Correlative CLSM-ESEM



- Reflex channel
- Mycobacteria
- Environmental SEM



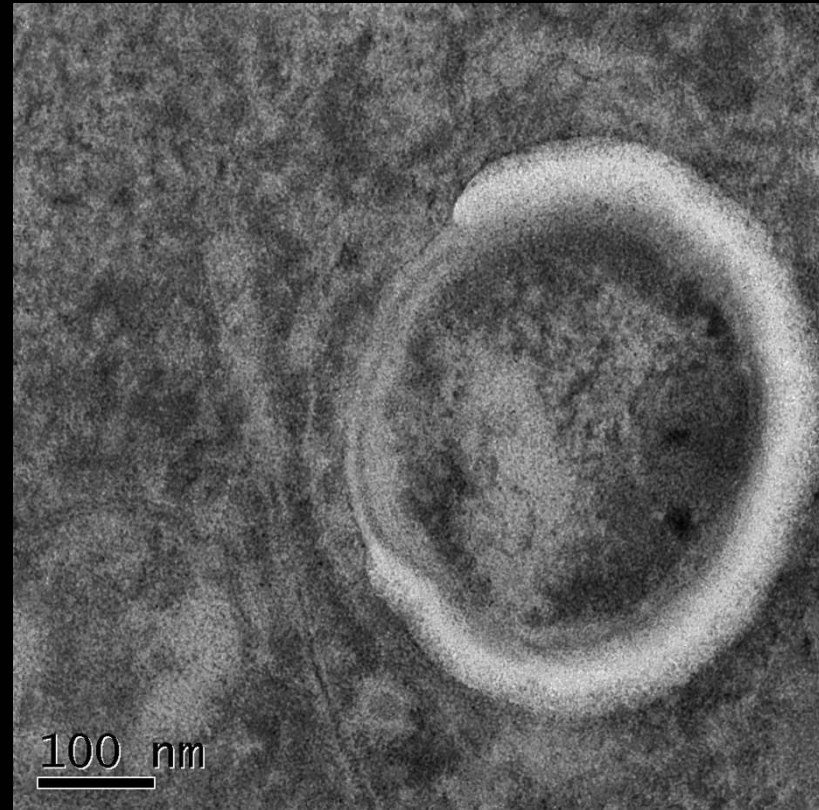
# Integrative Microscopy of infected tissue



## Comparison conventional /cryo preparation for TEM

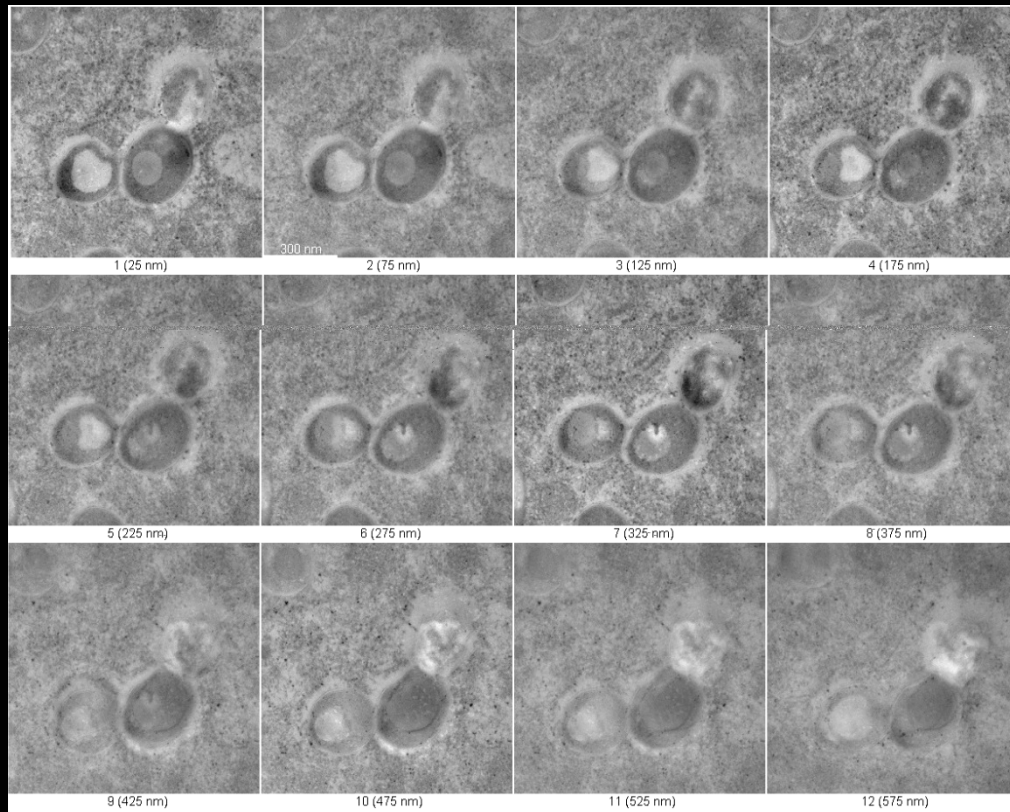


Room temperature processing



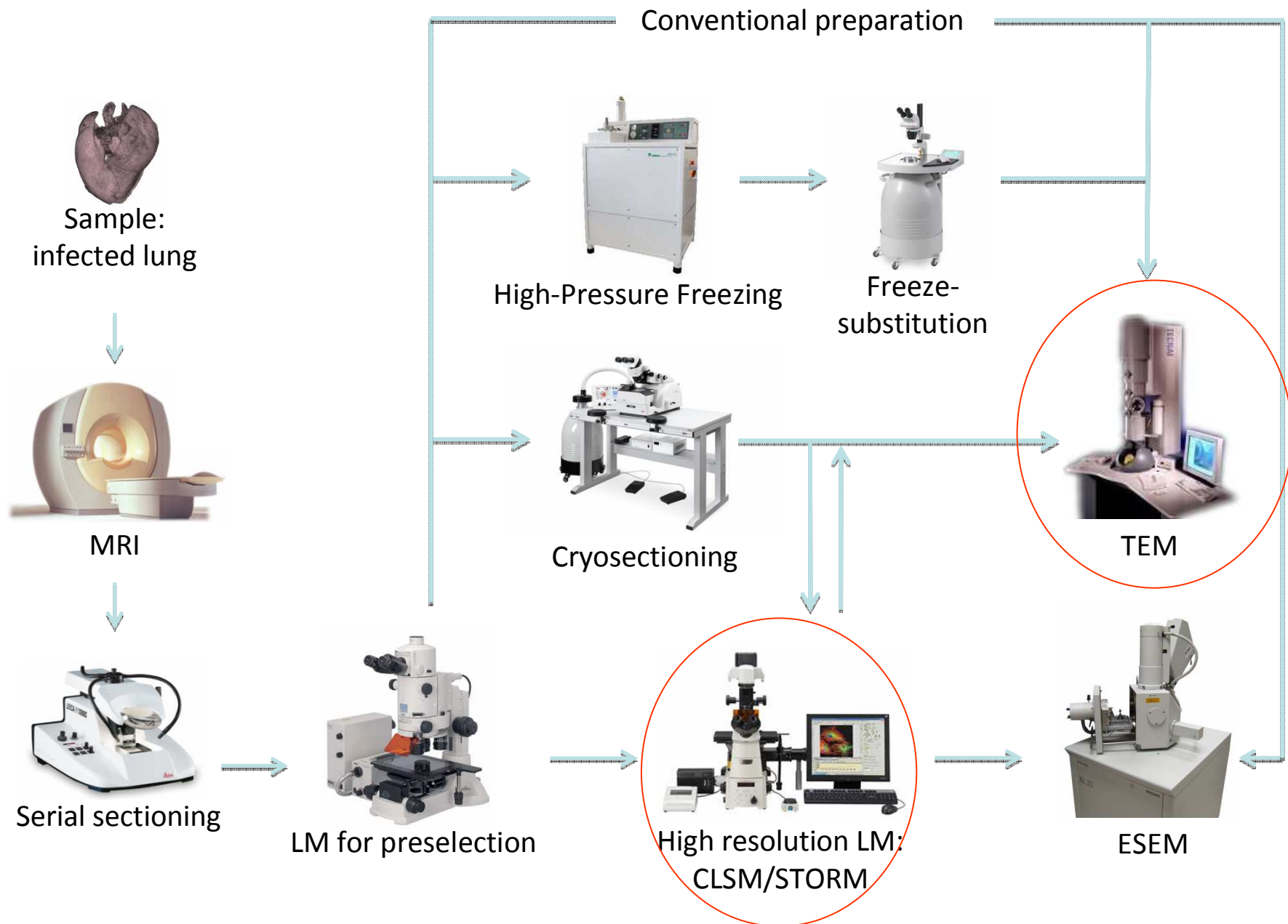
High-pressure freezing /  
freeze-substitution

# TEM of serial sections



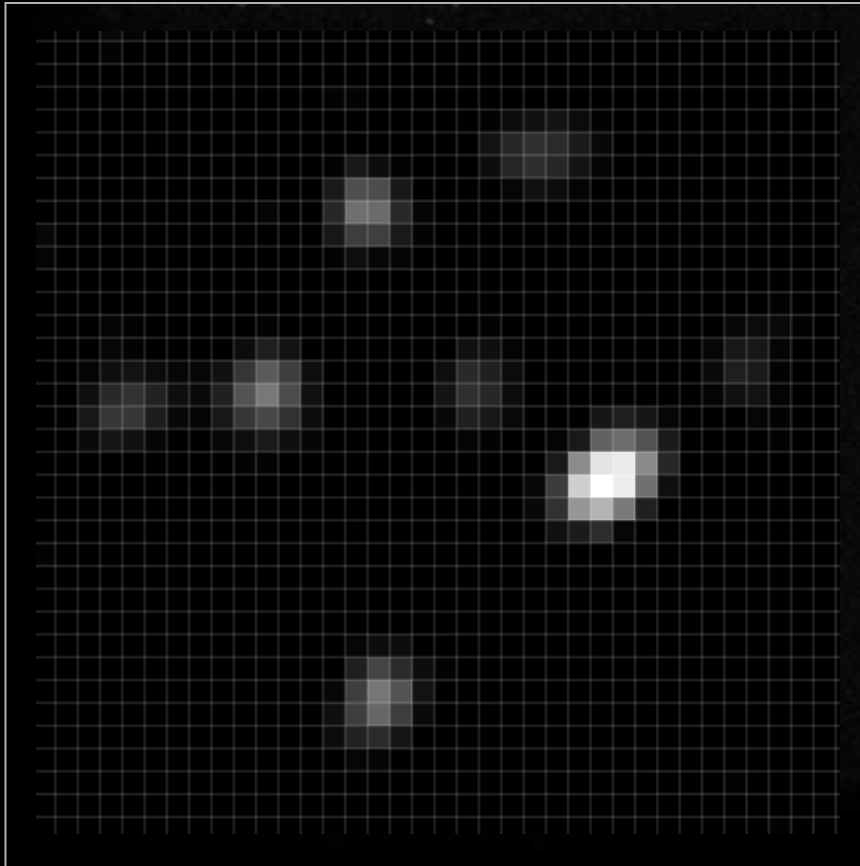


# Integrative Microscopy of infected tissue

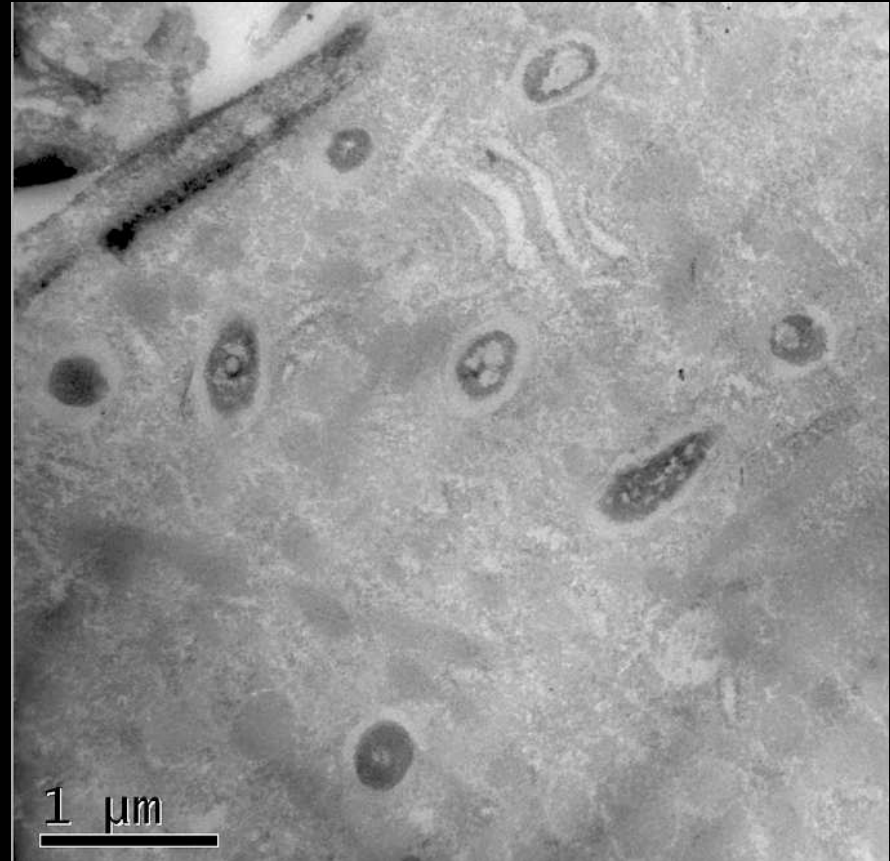




## Correlative fluorescence LM / transmission EM with Tokuyasu cryosections

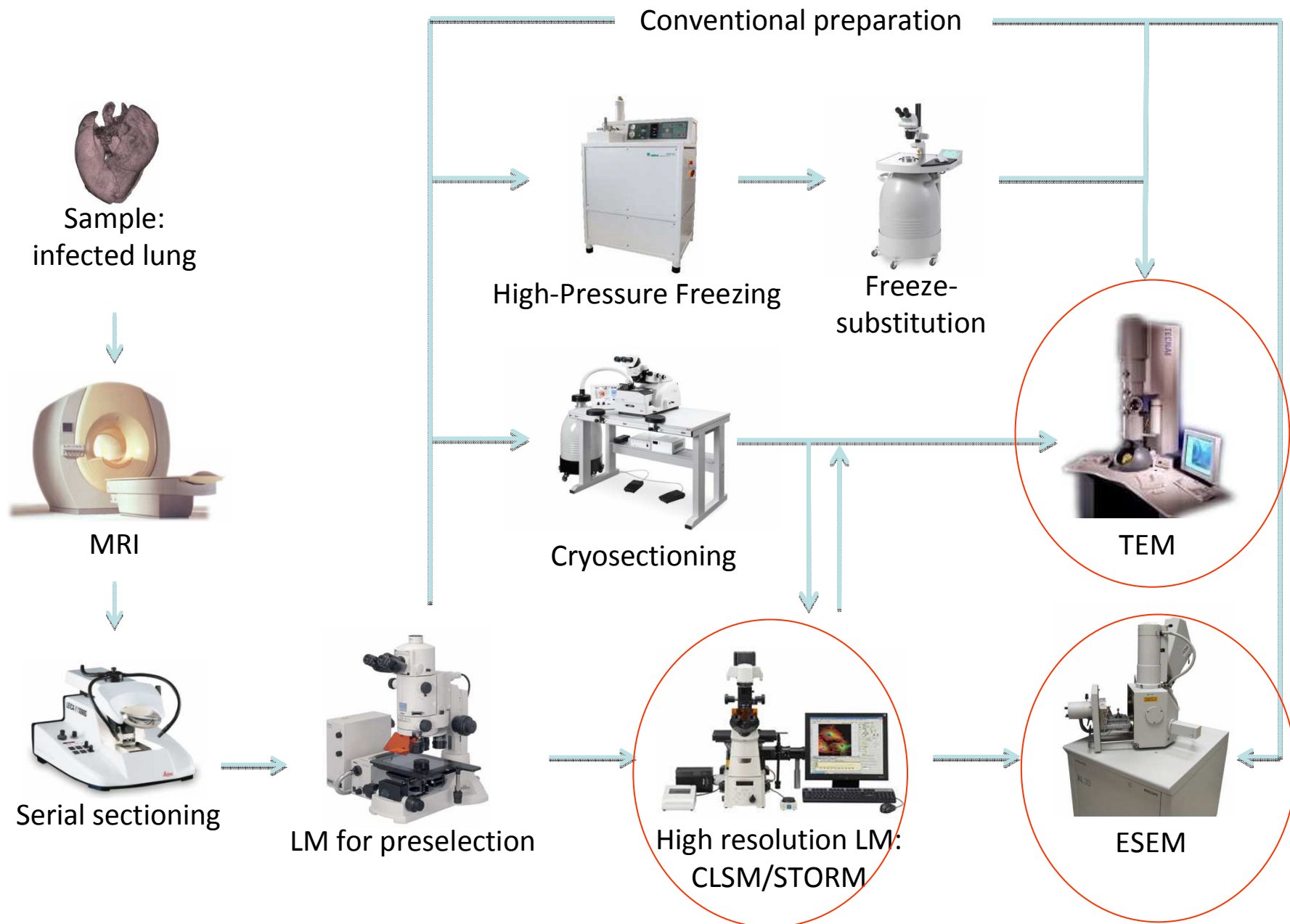


Mycobacteria (mCherry)  
fluorescence in LM

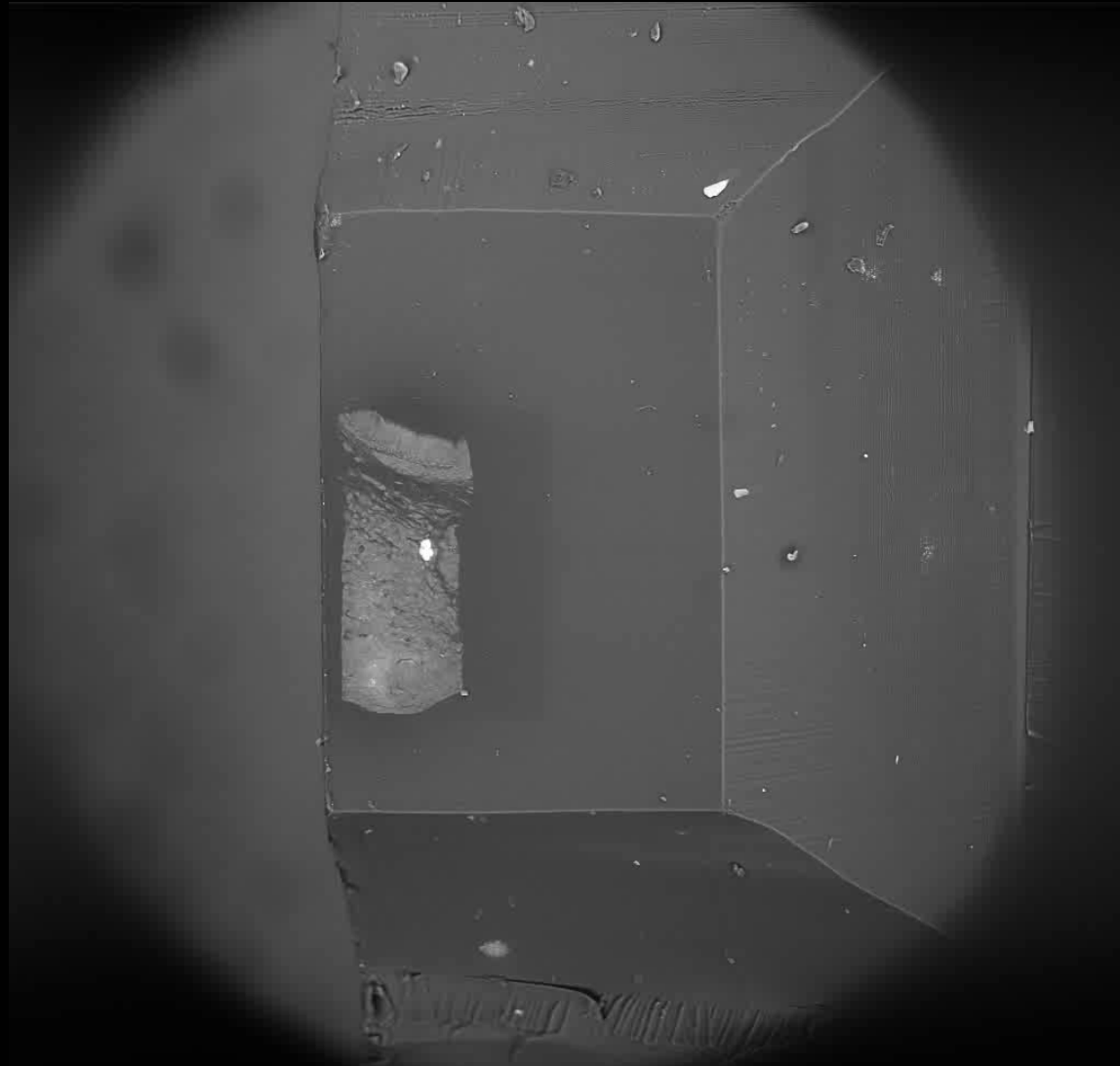


Mycobacteria in TEM

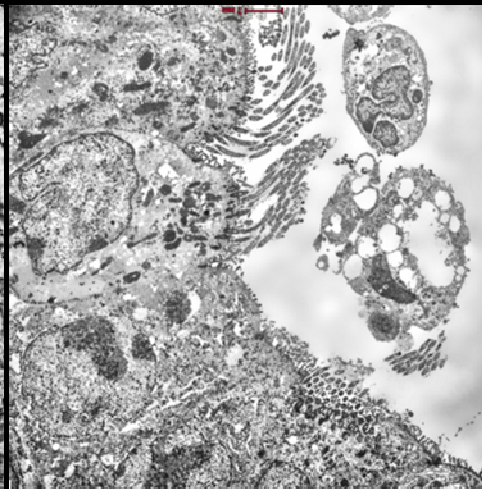
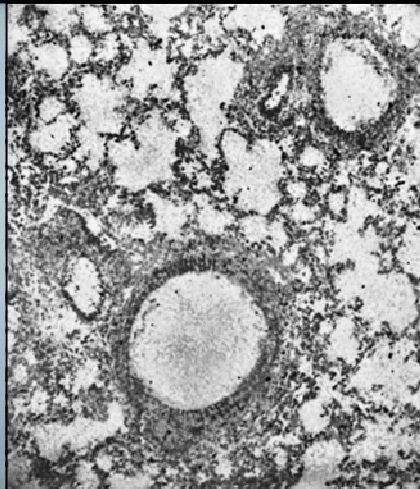
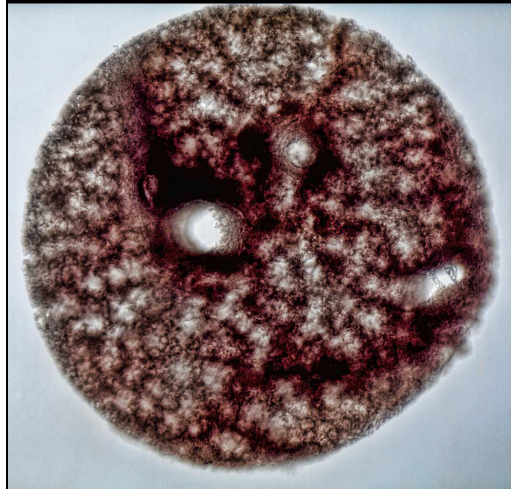
# Integrative Microscopy of infected tissue



## Blockface Imaging in the ESEM



Pinpoint localisation and preselection of structures of interest prior to ultrathin sectioning for TEM





**Systemic micro- and bridging techniques:**

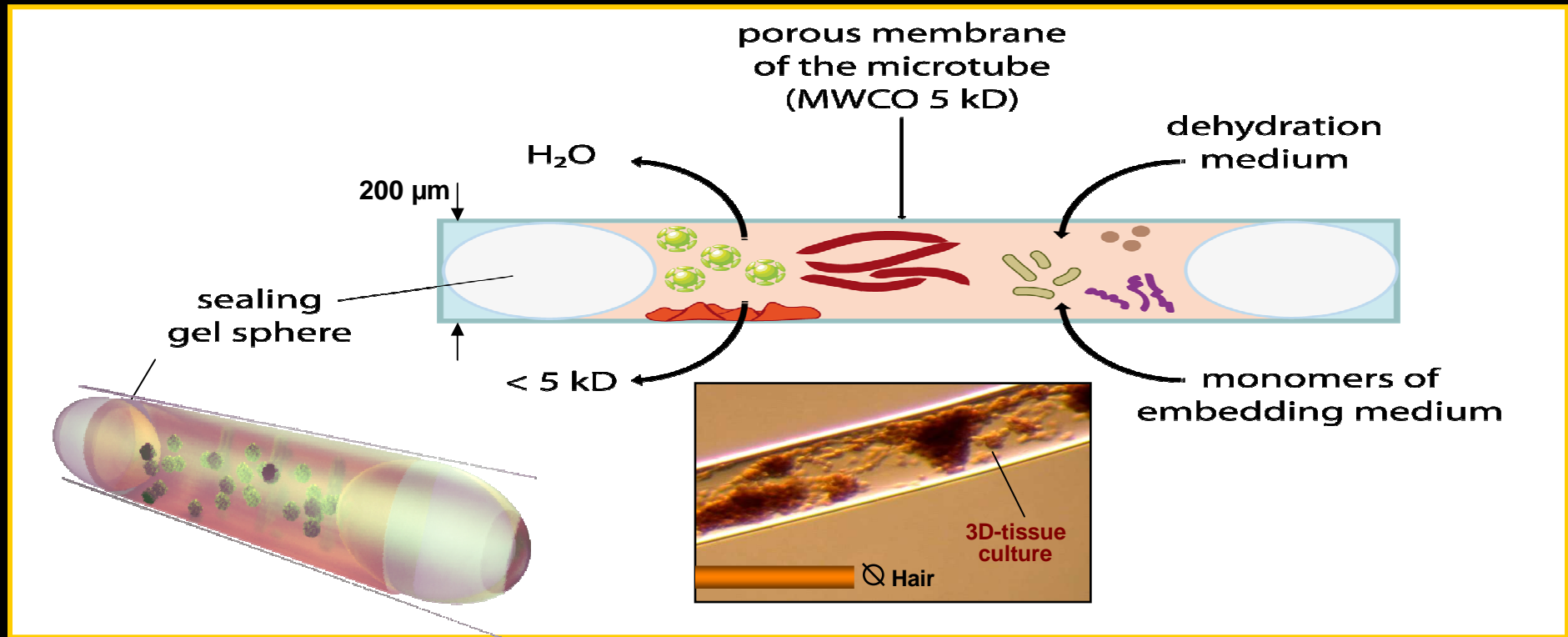
**The Micro-Reactor Technologie**

**Micro-bioreactors for the handling and preparation of suspended molecules, organelles, viruses, parasites and micro-organisms**

**and**

**the 3D-cultivation of cells & tissues**

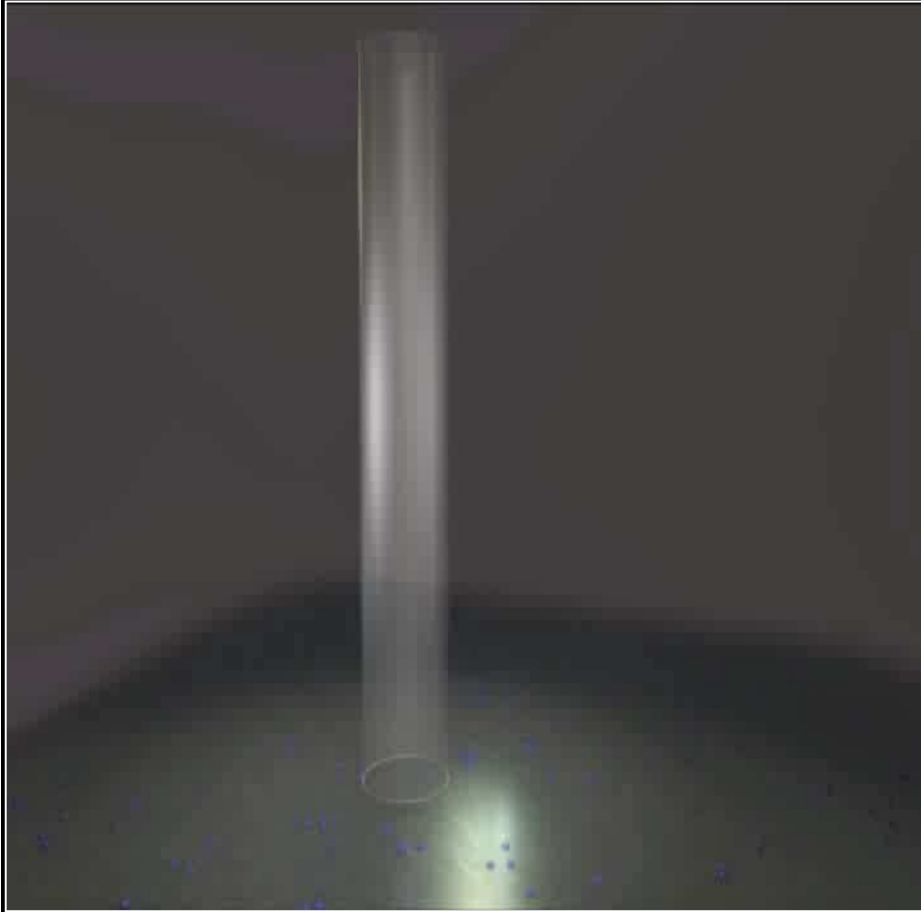
## CarboCell micro-reactors for the preparation of bio-medical material



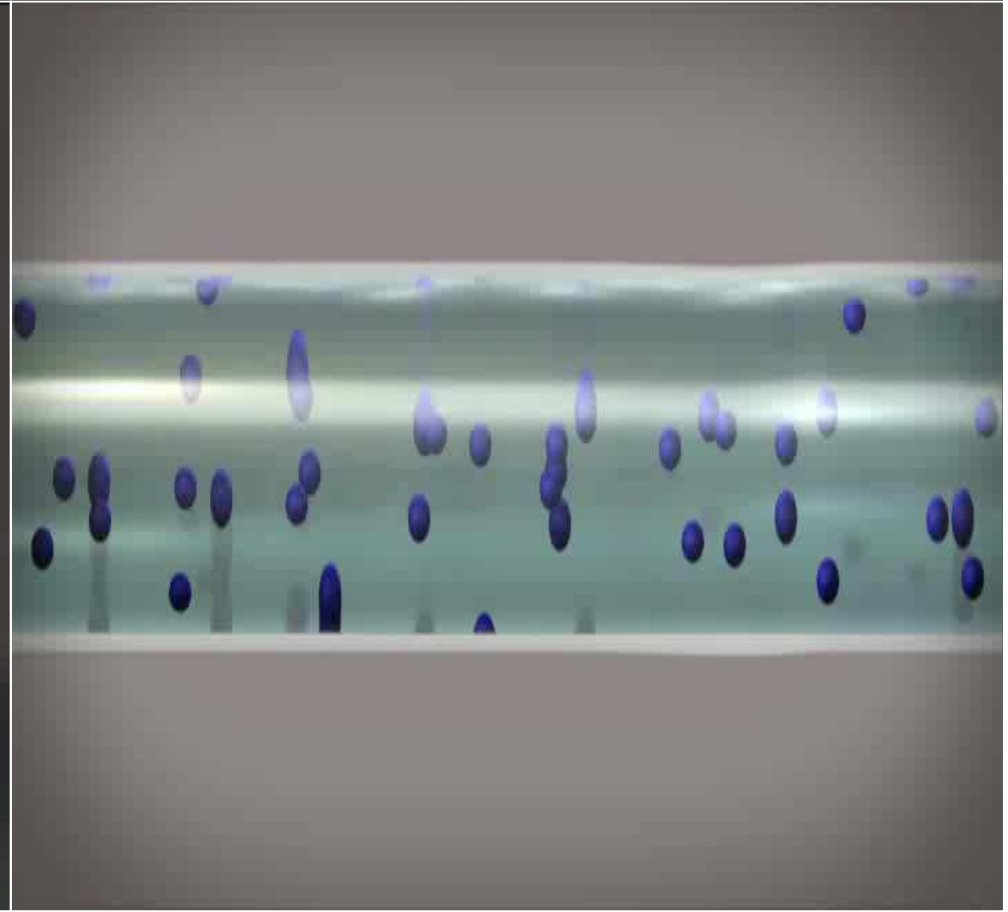
### *CarboCell* micro-reactors with nano-filtration properties

- Sealed micro-bioreactors to prepare suspended molecules, organelles, viruses, organisms
- 3D-cultivation of cells & tissues
- Bio-compatible and transparent, mechanical stable, minimal volume (nano-liter range)
- Material is concentrated & encapsulated in its cultivation medium (environmental preparation)
- Chemical- and freeze-resistant containments with high porosity and variable MWCO.

## Environmental preparation of suspended material in its mother liquid



**Filling by capillary force**



**Concentration by evaporation or dialysis  
Sealing by crushing the ends**

**From macro- to nano-range:**

**LM, ESEM preselection and TEM investigation: Micro-containments as bridging technology**



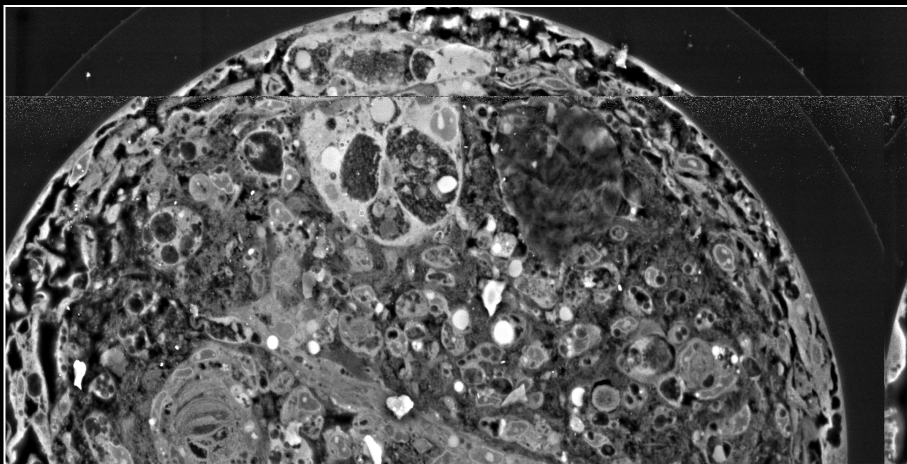
**Preparation of a selected tube segment with infected neuronal STEM cells, 3D cultivation in micro-containments (ca. 3 days, time lapse) for ultrathin sectioning and TEM-investigation**



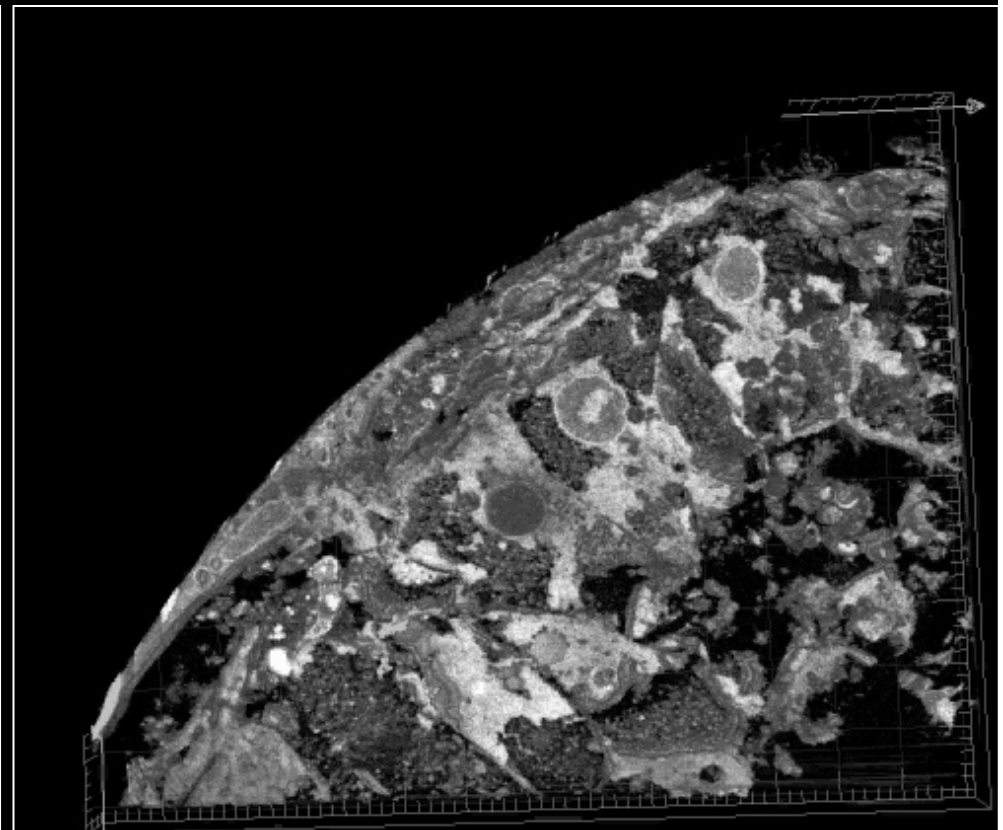
## Cultivation of organotypic tissue biopsy cylinder in a micro-tube container and 3D reconstruction of cryo-processed 3D liver tissue



LM-monitored cultivation of a liver biopsy (movies of tissue self organisation with time lapse LM)



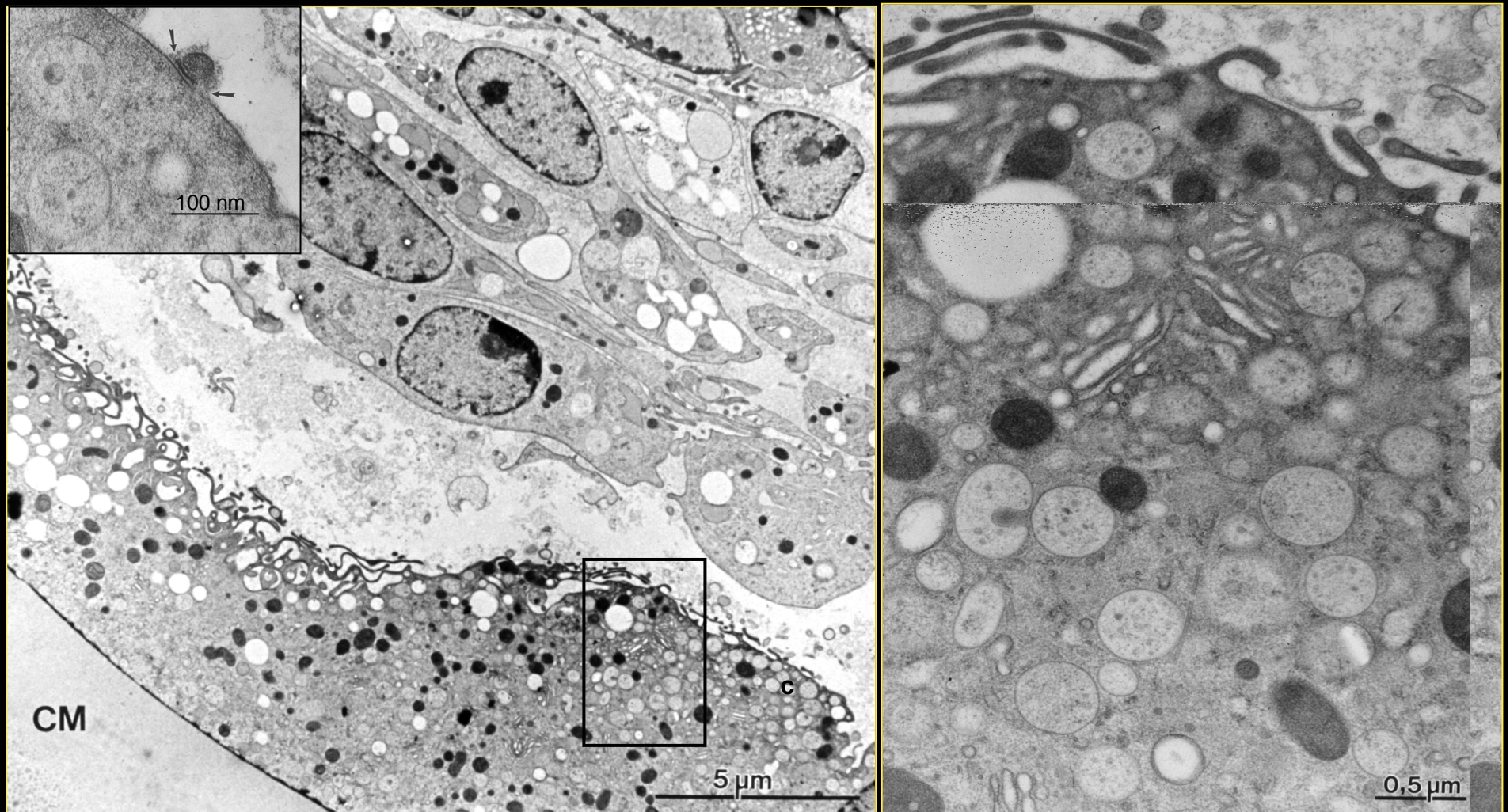
ESEM



3D reconstruction (30 serial sections) of a high-pressure frozen and freeze-substituted infected organotypic liver tissue applying an HRSEM BSE-detector. Analysis of crosstalk molecules *in situ*.

## Vital “Micro-Organs”

### 3-D liver organoid systems and the morphogenesis of Duck Hepatitis viruses

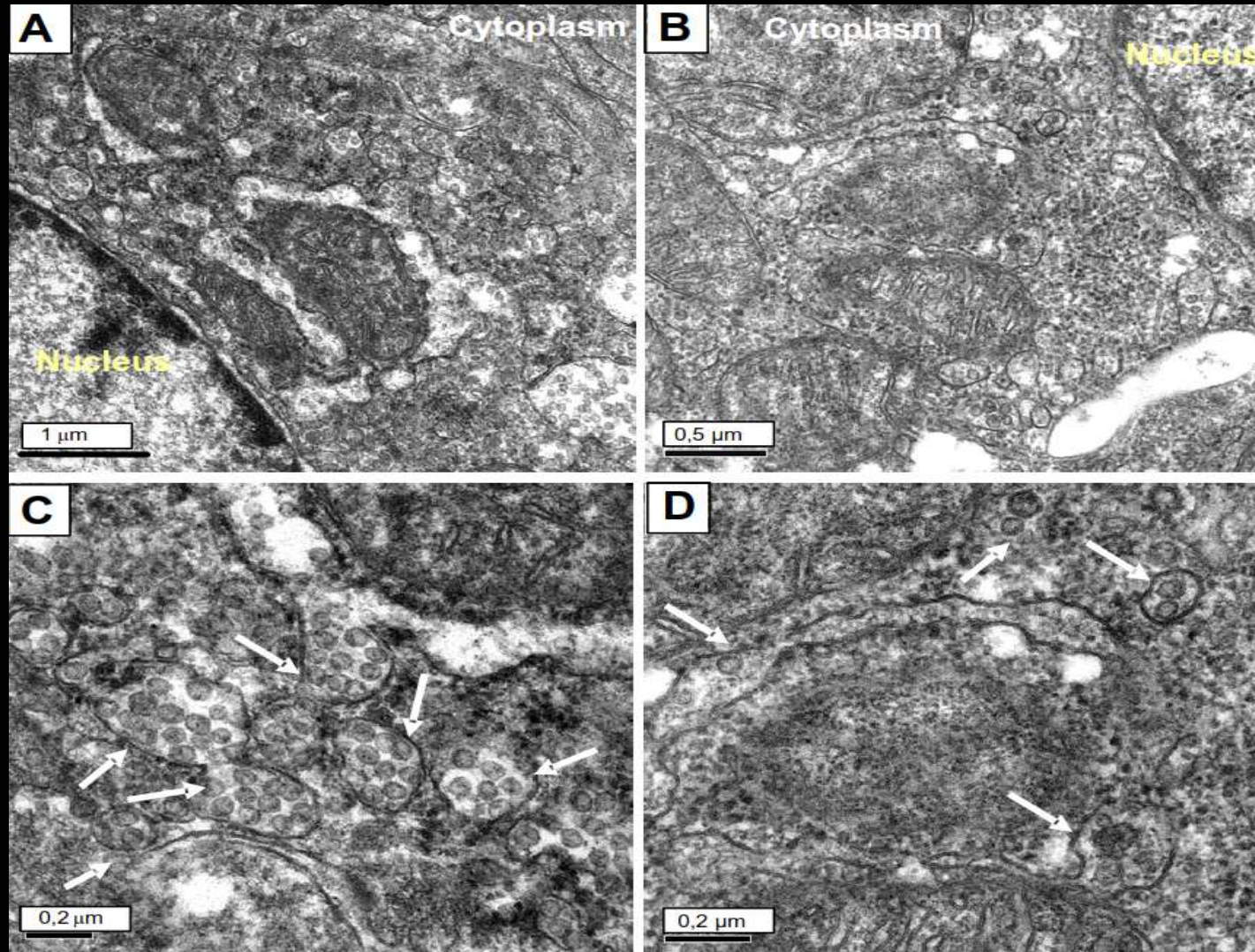


Duck liver organoid 9 days after cultivation inside of the micro-tube.  
High-pressure frozen, deep-temperature dehydrated and embedded.



## Vital “Micro-Organs”

### 3-D liver organoid systems and the morphogenesis of Hepatitis viruses



Cultivated biopsy of an infected **duck liver**

3 D cultivated infected **PDHs**

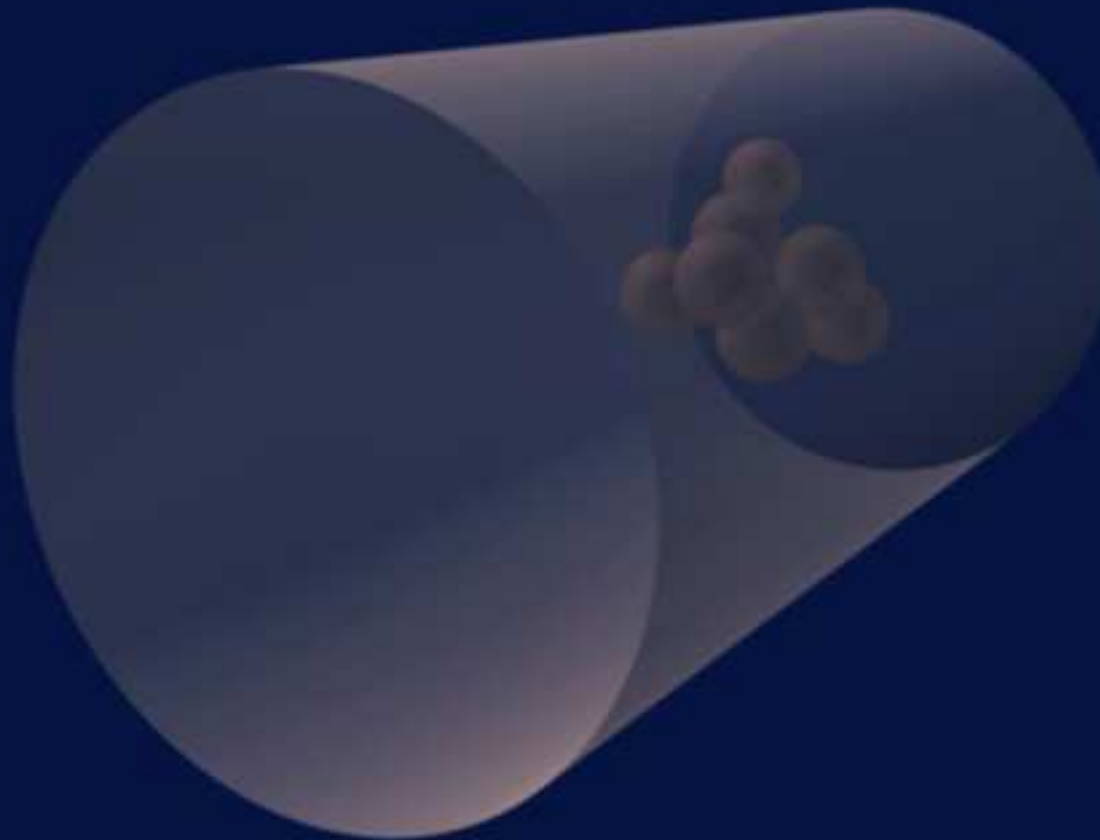
**Systemic micro-methods:**

**Applying the preselection and  
correlation methods:**

**Analysis of 3D cell cultures  
infected with Cytomegalovirus (CMV)**

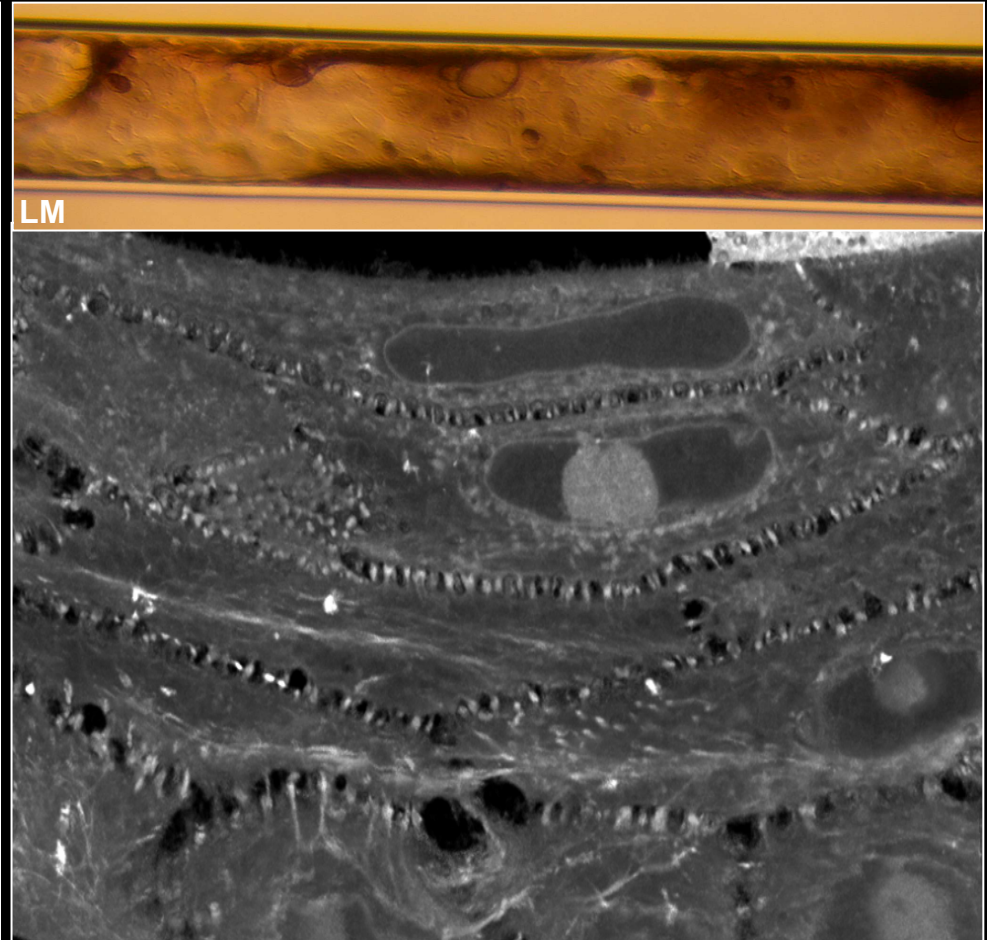
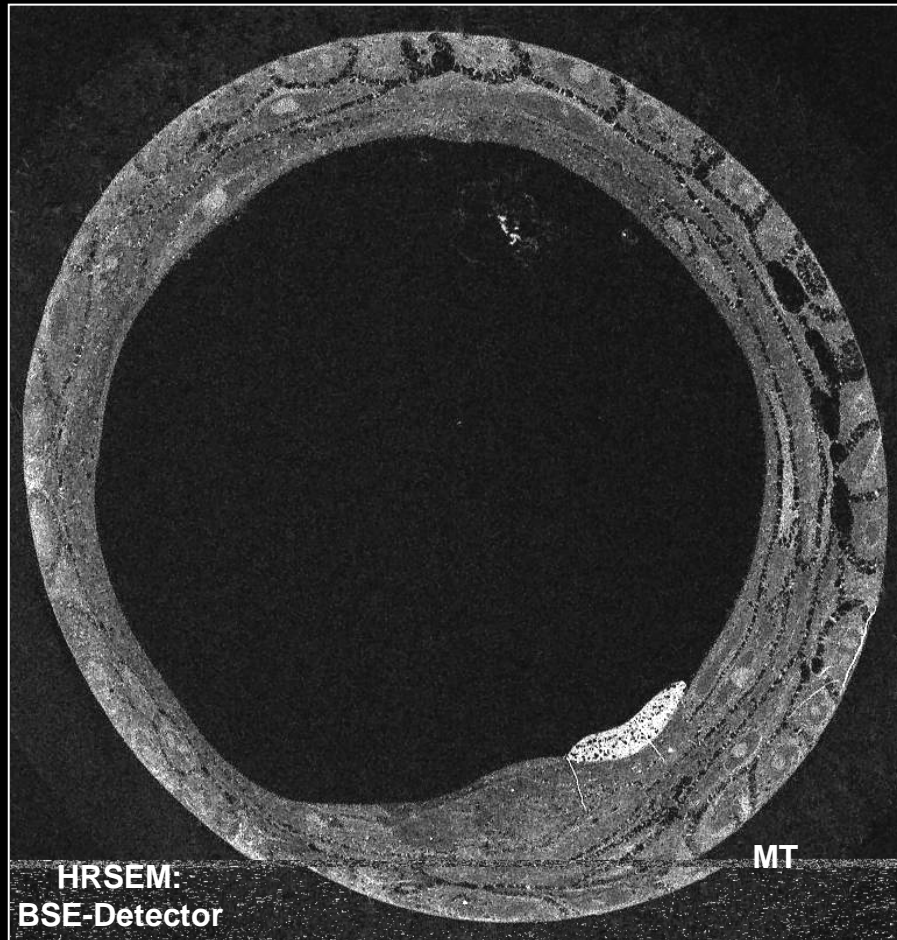


**Analysis of 3D cell cultures infected with CMV:**  
**Cultivation of skin cells in a microtube container**



**Preservation of the structural network & the cell specific information network**

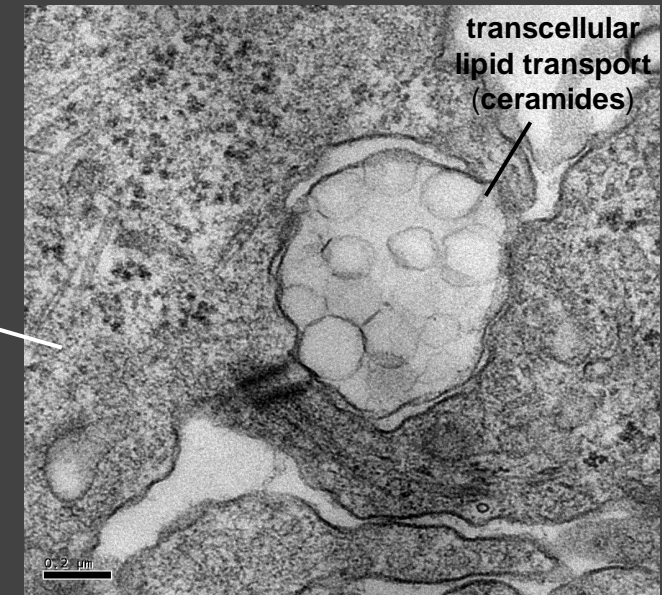
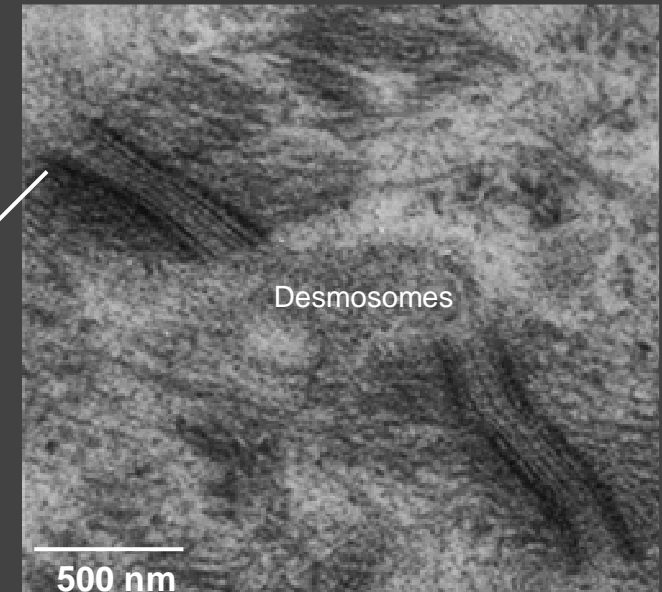
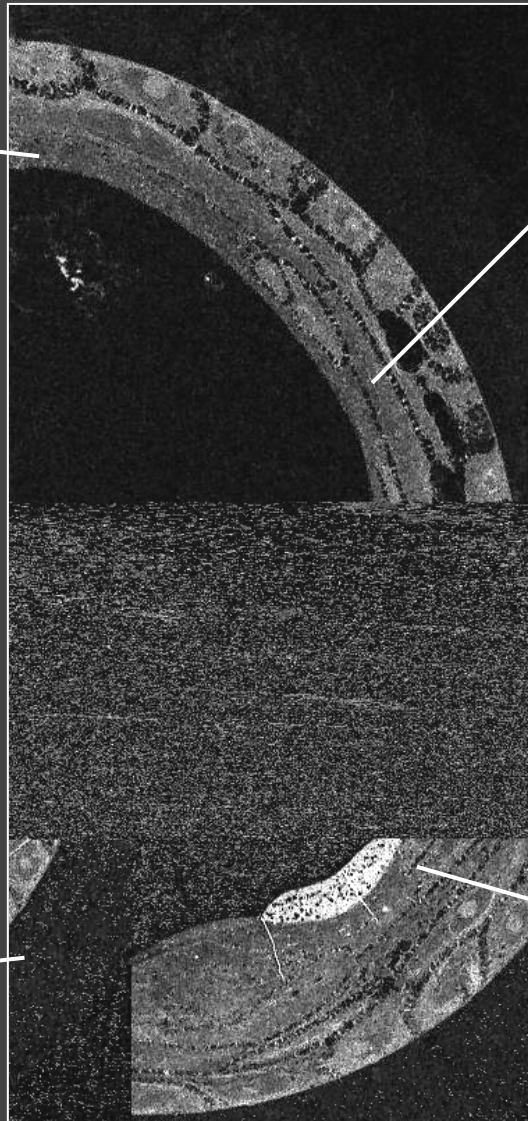
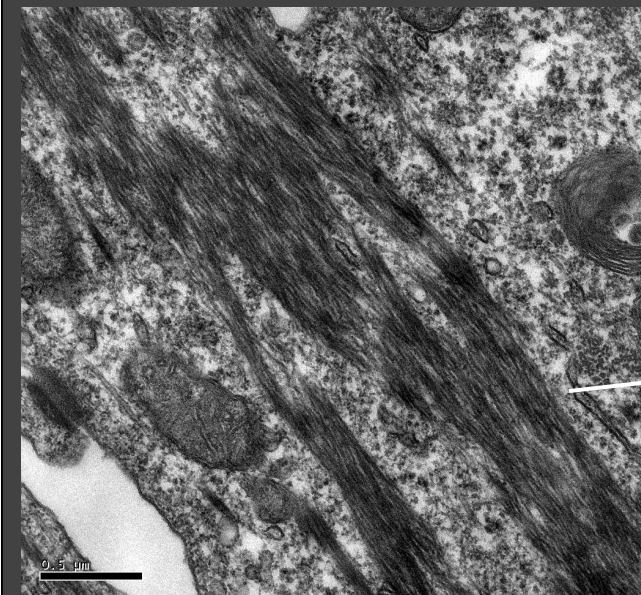
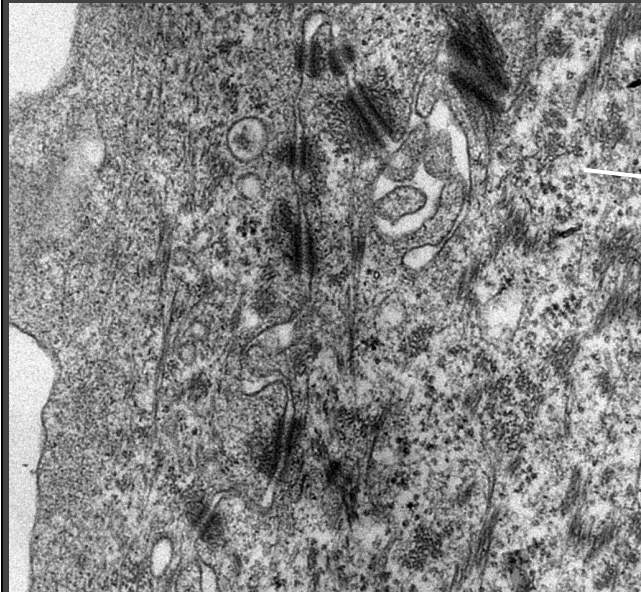
**Cultivation of human skin cells in a tubular container**  
**3D-cell culture in a surface modified micro-tube (MT) container**



**3D human skin culture (4 layers) in a micro-tube (MT) container tailor-made for high-pressure freezing and cryo-processing.**



# Cultivation of human skin cells in a tubular container



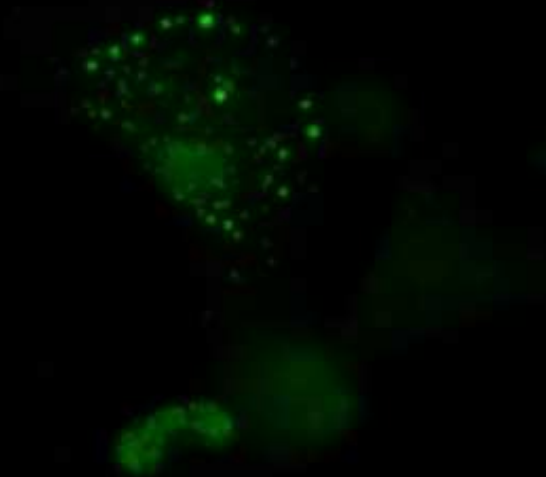
**Monitoring the dynamics of a virus infection at different time resolution  
by controlled injection of GFP-tagged CMV into a 3D pseudo-tissue culture**

**LM-controlled micro-injection of a virus suspension (nl-range) into the lumen of a micro-  
bioreactor, sealed at both ends**



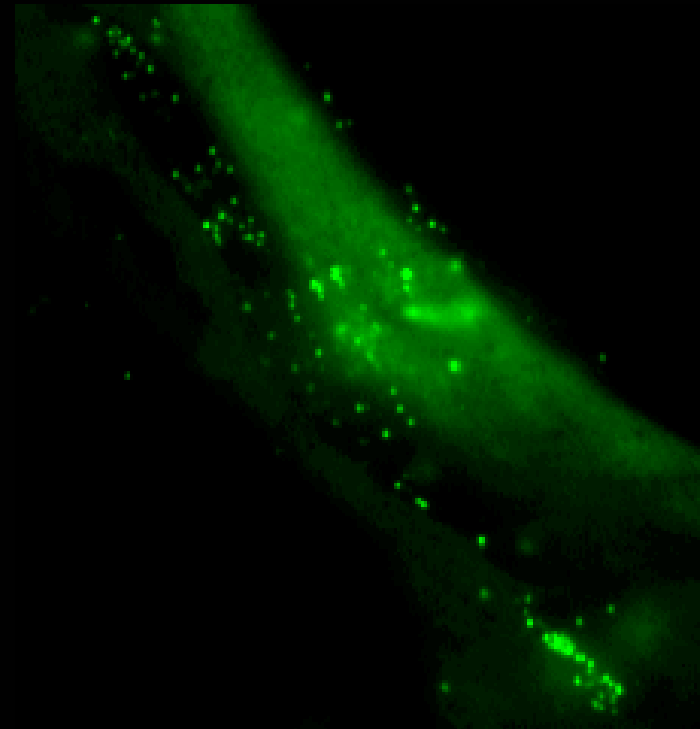
**GFP-tagged CMV at different time resolution inside of living cells:  
Imaging the dynamic process of virus assembly and movement**

1 F/min



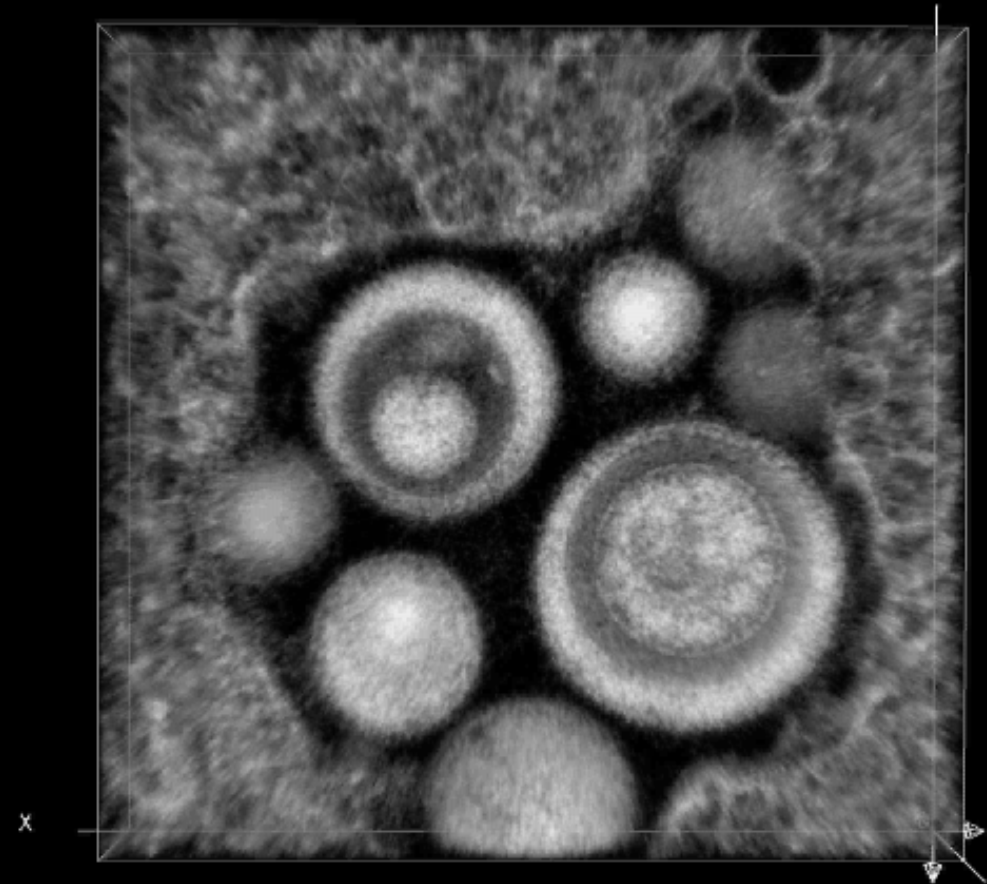
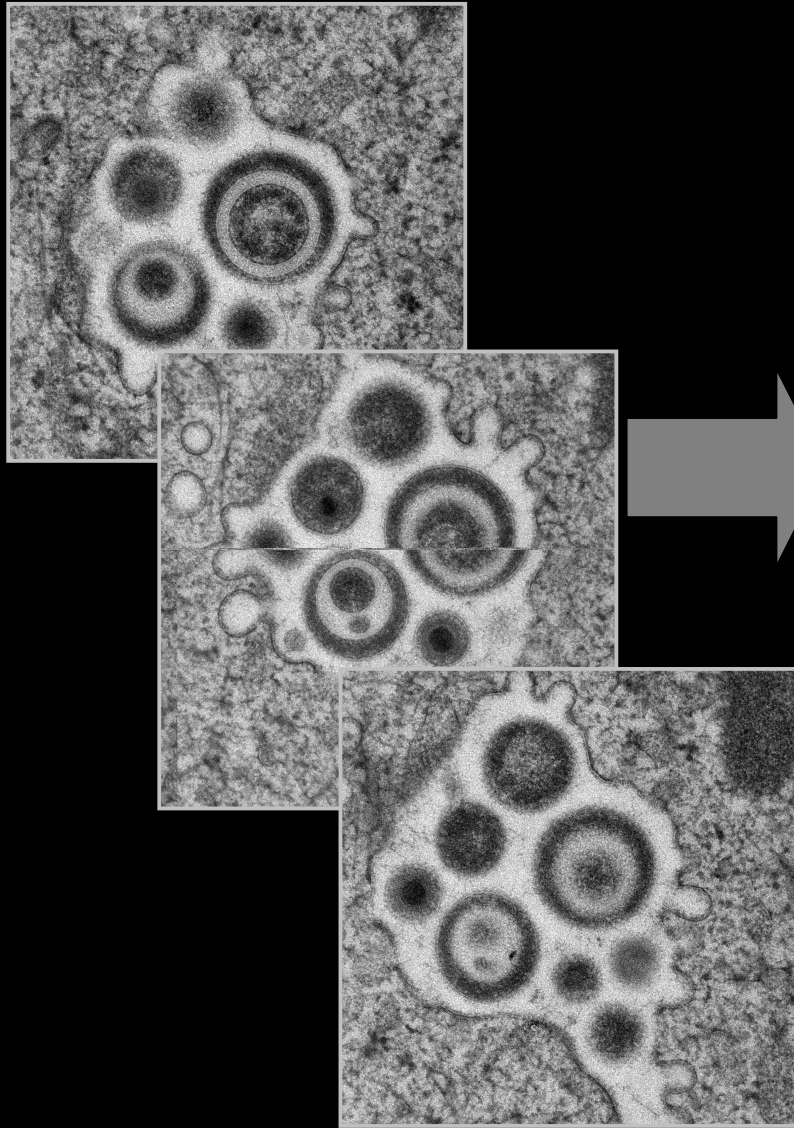
*Fusion of CMV-filled vacuoles*  
Time lapse microscopy 48h with  
1F/min, Bar = 10  $\mu\text{m}$

30 F/sek



*Single virus particles inside a  
vacuole realtime TIRF microscopy*  
Bar = 2  $\mu\text{m}$

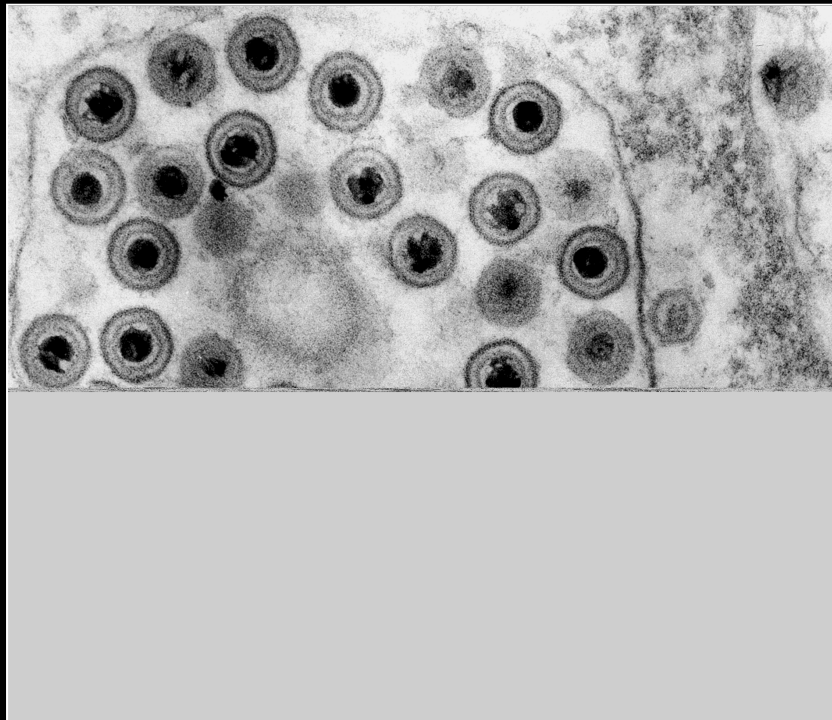
## 3D Reconstruction of cryoprocessed infected 3D CMV morphogenesis *in situ*



**3D reconstruction of a „virus factory“:**  
The different assembly and maturation  
sequences of CMV in the cytoplasm

# EM analysis of intracellular vesicles filled with Cytomegalovirus

## Conventional Preparation for TEM

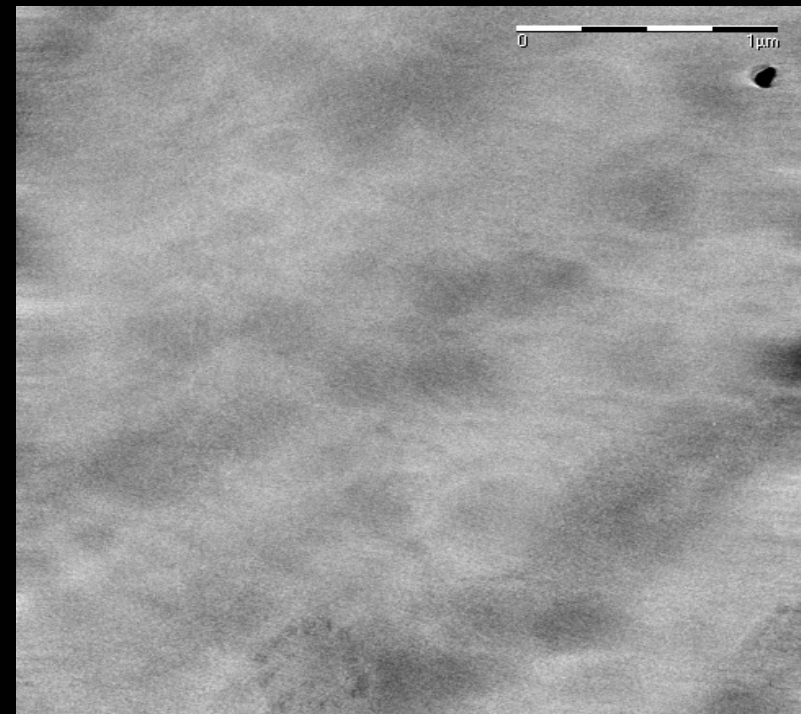


*Aldehyde-Fixation*

x 25.000

## Cryo-Preparation for TEM

Tomogram of a 200 nm thick section



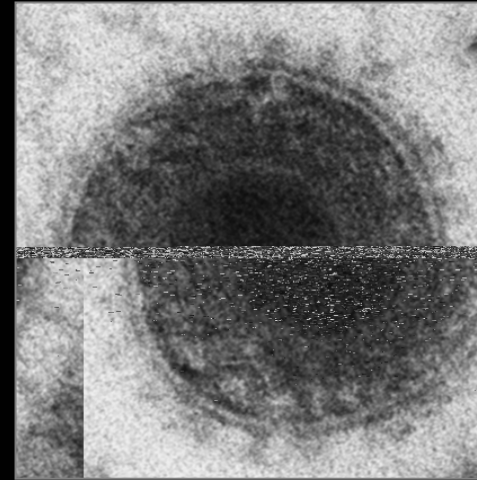
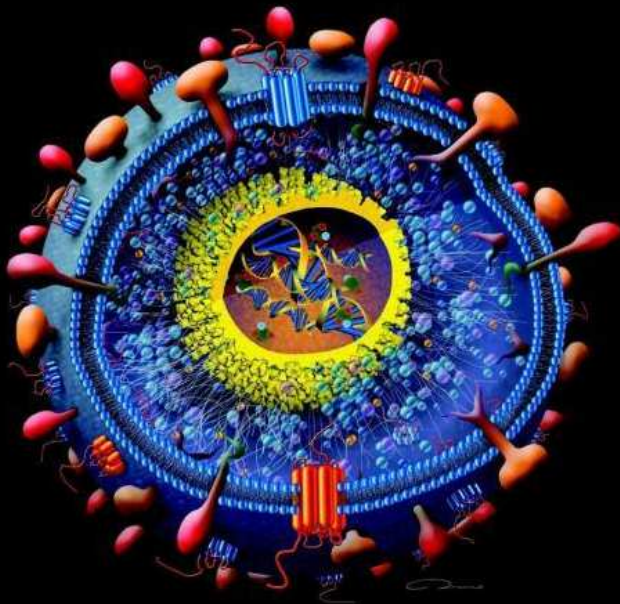
*High-Pressure Freezing*



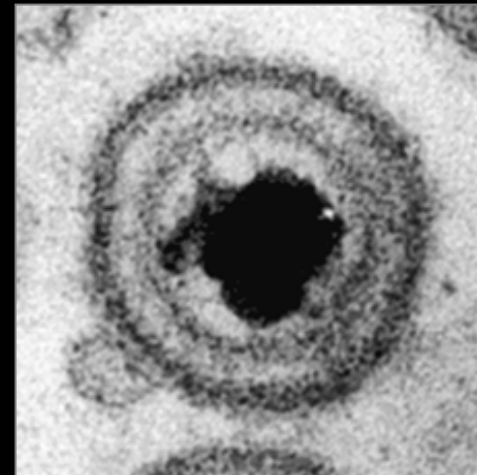
# CYTOMEGALOVIRUSES

Molecular Biology and Immunology

edited by Matthias J. Reddehase



Cryoprocessed CMV



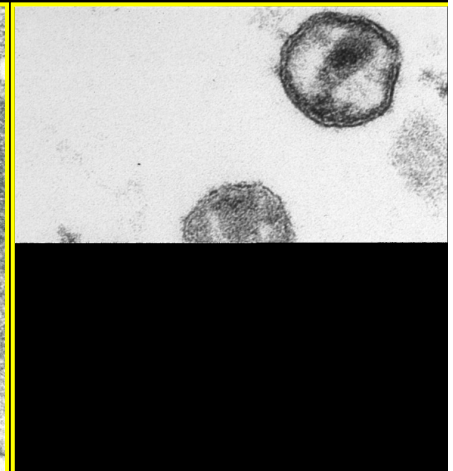
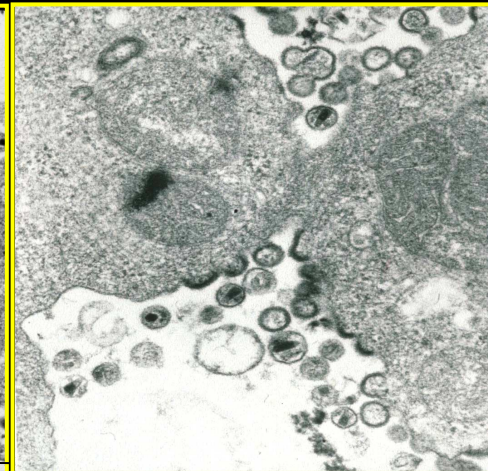
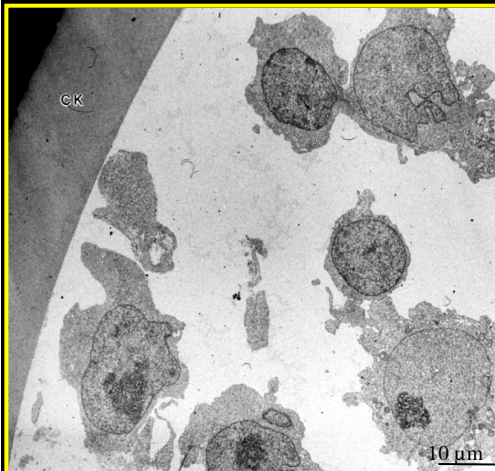
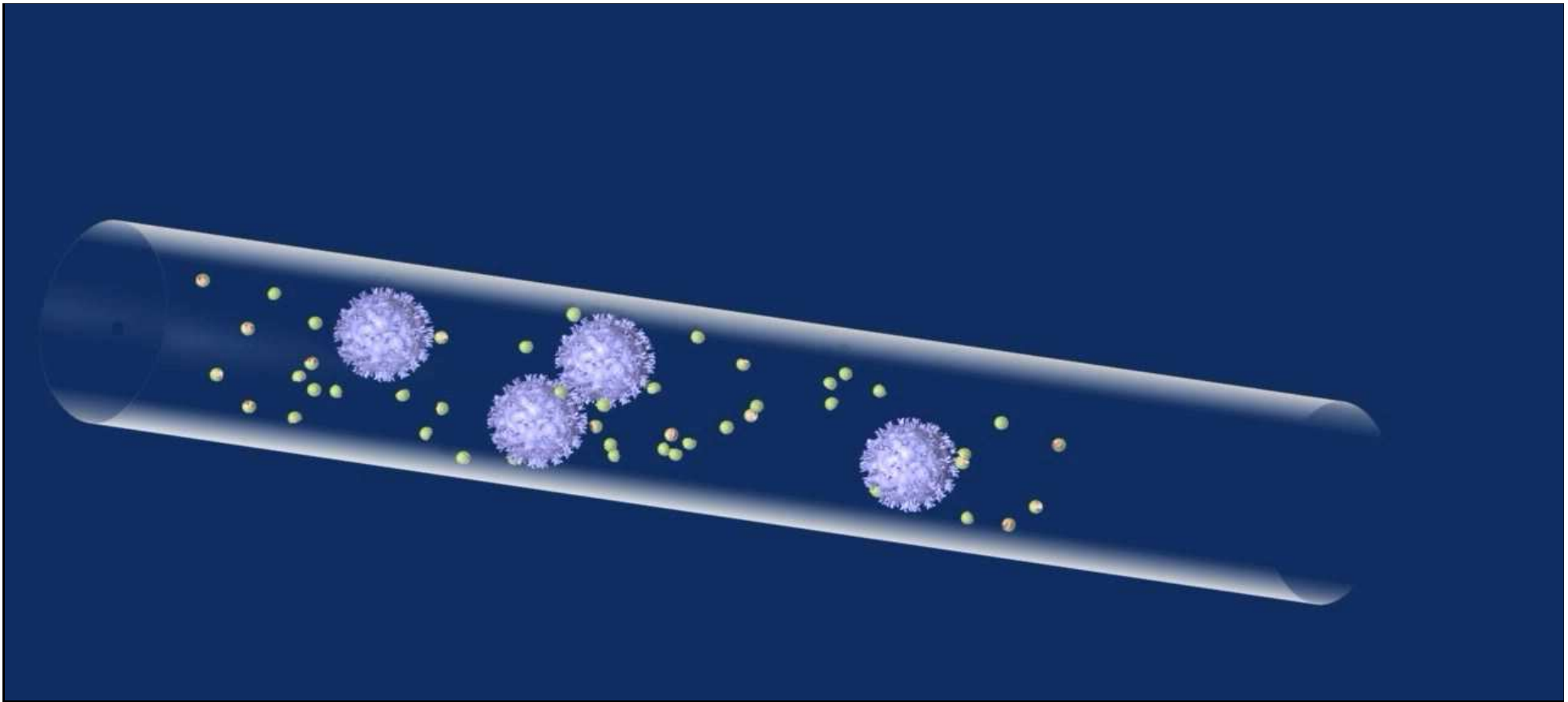
Conventionally processed  
CMV



## **Micro-containments as bridging technology:**

### **Closed cell suspension systems for *in vivo*-like virus production**

- no loss of released viruses (no centrifugation)
- analysis of all viruses and virus-induced cell components
- quantification of virus production
- analysis of extracellular virus maturation

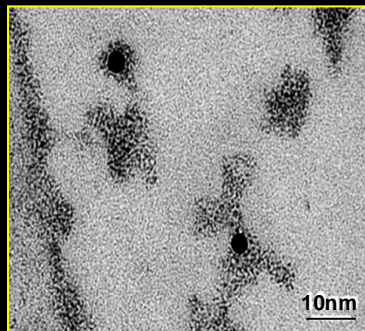
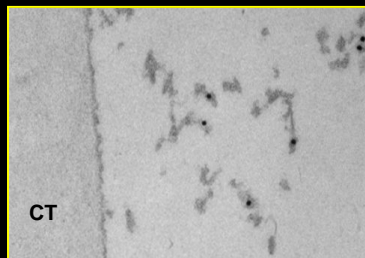


In situ EM-preparation of HIV-1 producing MT- 4 cells

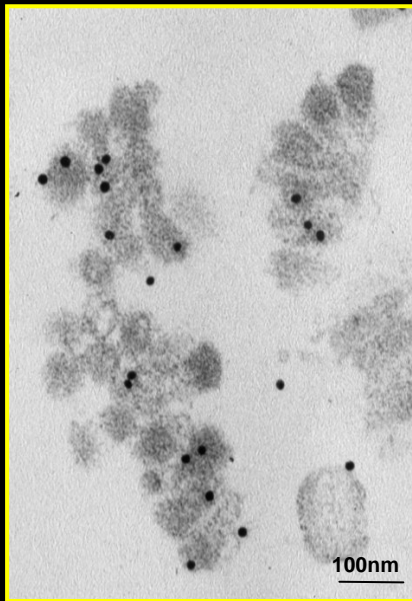
Analysis of all released extracellular viruses



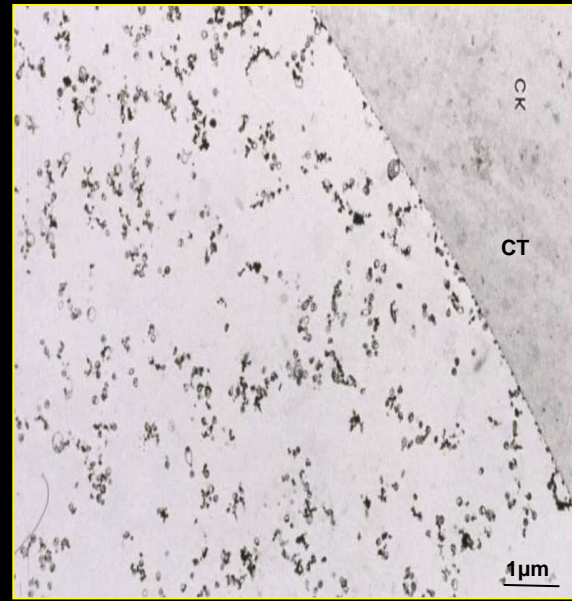
**Molecular filtration of molecules > 5 kD**



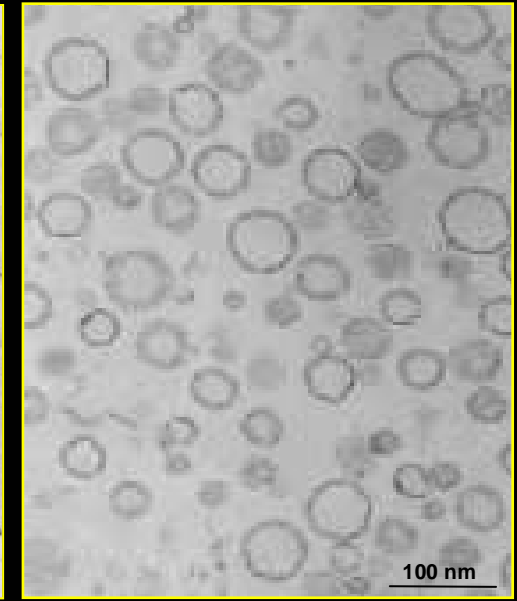
**IgG-molecules 5nm pAg**



**Isolated HIV capsids**



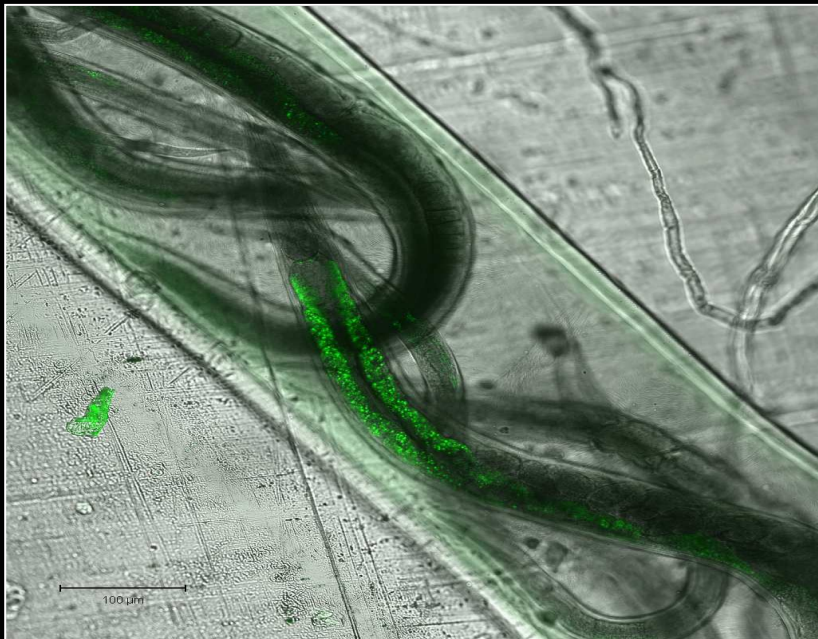
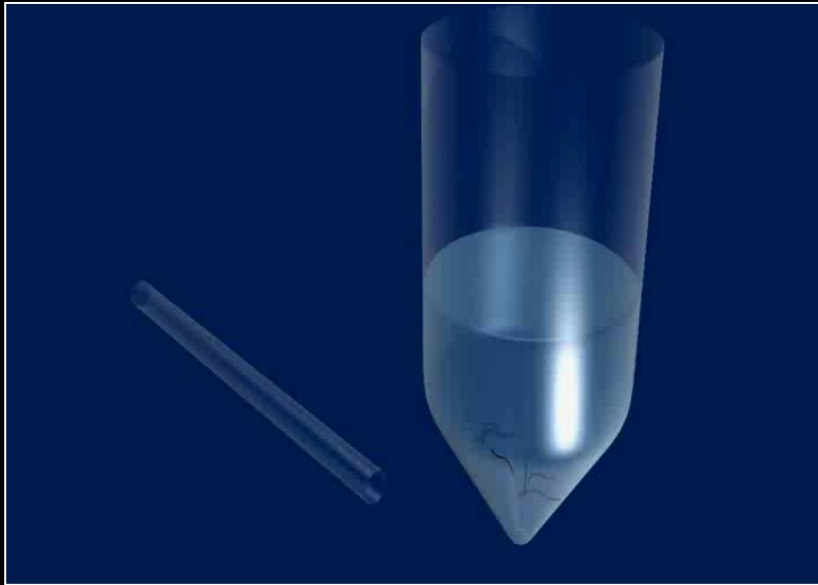
**HIV suspension**



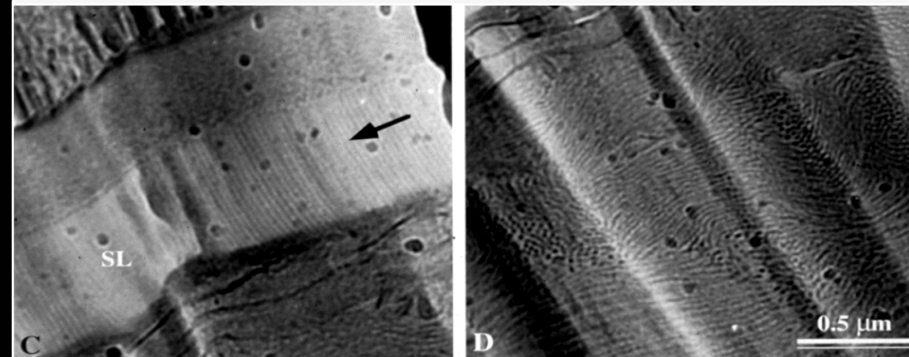
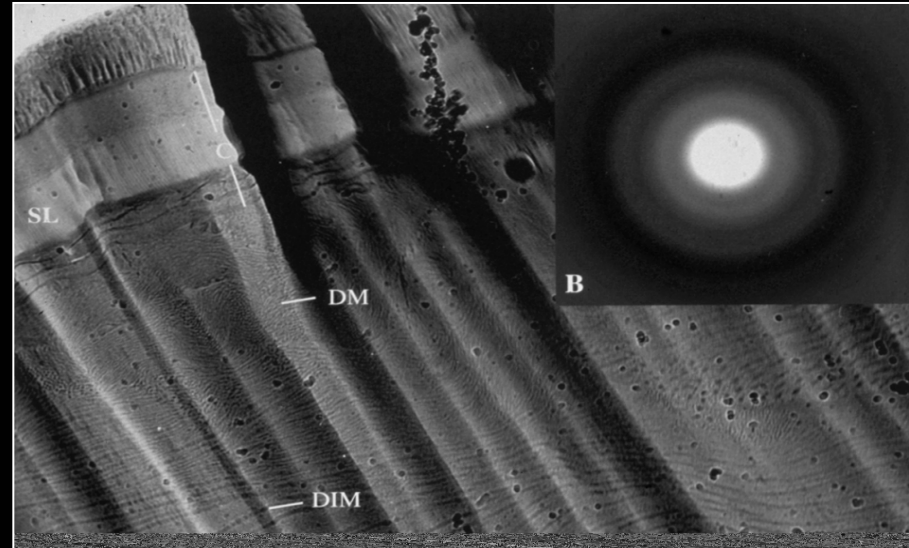
**Suspended liposomes or micelles**



# Environmental preparation of parasites in their cultivation medium



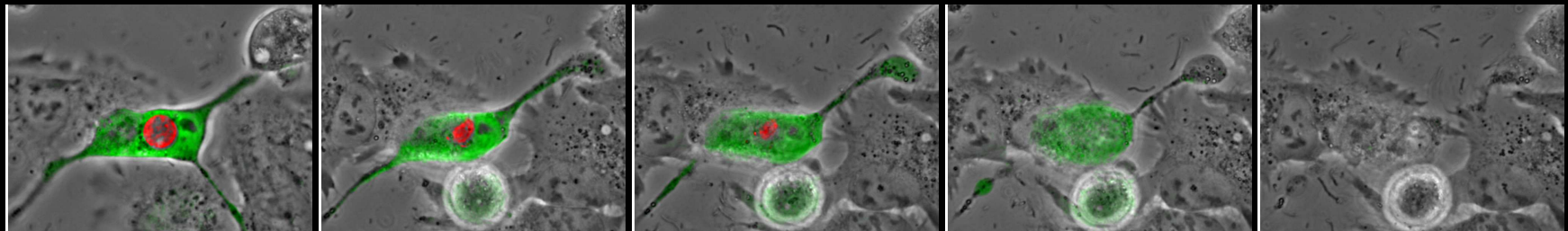
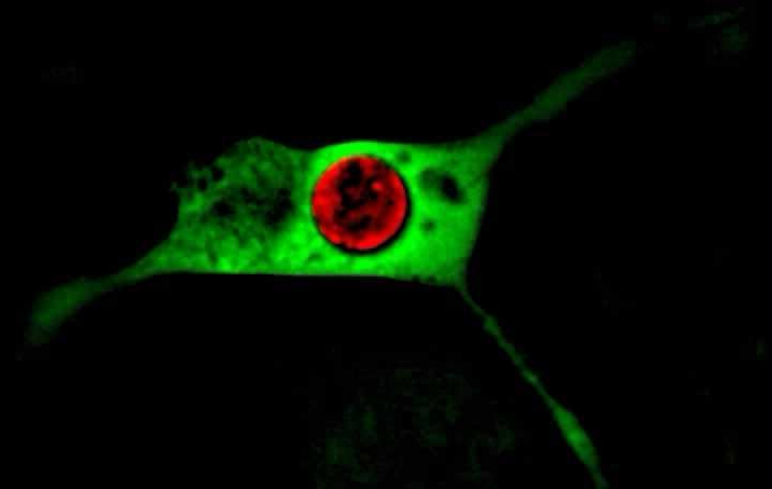
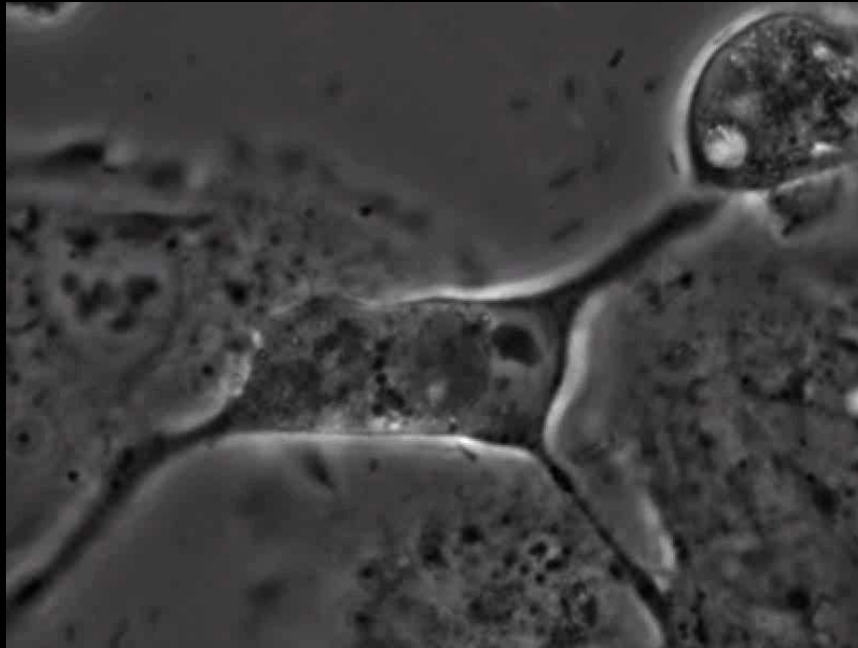
GFP-labelled cryo-processed Nematodes



Ultrathin cryosection in the Cryo-TEM



## Monitoring the *Plasmodium berghei* release from a HepG2 cell in a LM-time lapse Biostation



0:00:00

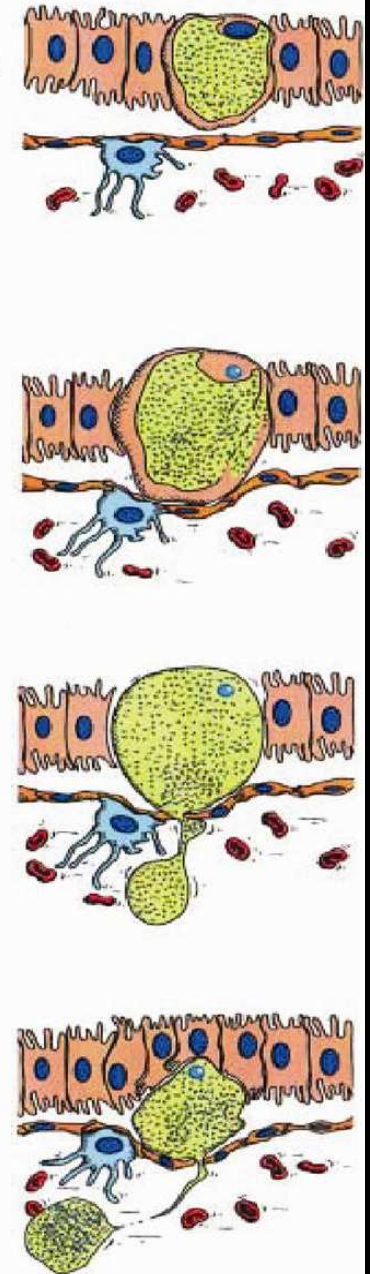
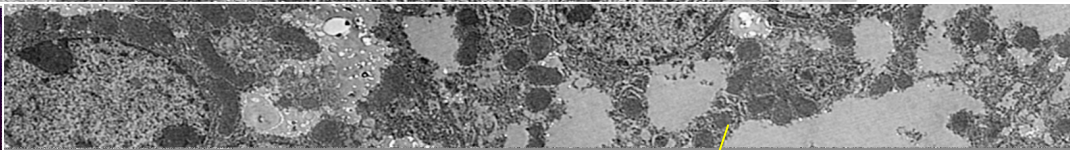
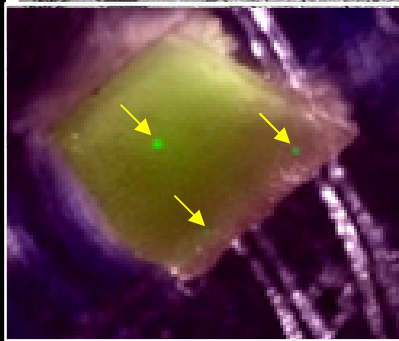
6:26:59

6:32:48

6:35:35

6:46:25

LCI-collaboration HPI with BNI (Malaria-Group: Heussler)

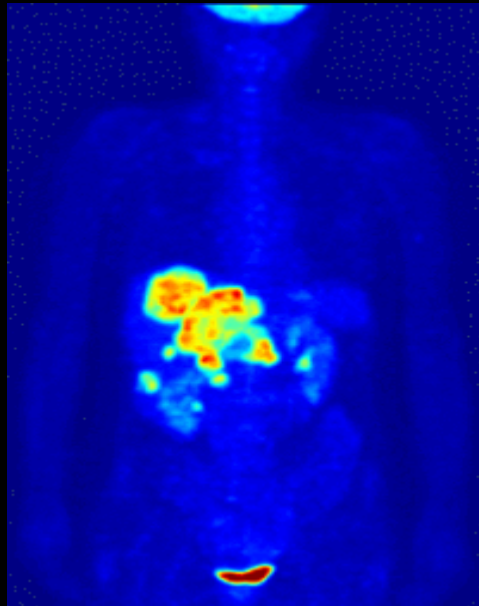


Targeted preparation of a Schizont out of mouse liver tissue after high pressure freezing in the TEM

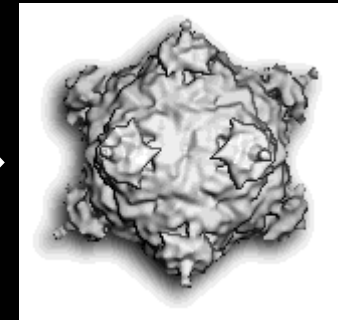
# **Systemic imaging and the translational shift of methods:**

**From biological light and electron microscopy  
to synchrotron radiation**

# Translational technology Transfer: EM Cryo-preparation methods for native protein crystals:



**Systemic Imaging:**  
X-ray-analysis of crystallized viruses  
X-ray-fluorescence of thick sections



Transfer- and Bridging Technologies

DESY (German Synchrotron, Hamburg)

Medical  
Tomography:

**Intact Organisms**  
PET, MRI

Light-  
Microscopy:

**Cells, Tissues**  
Live Cell imaging,  
Intravital, CLSM

Electron  
Microscopy  
of surfaces:

**Cells, Tissues**  
SEM  
ESEM

Electron  
Microscopy  
of cell interior:

**Cells, Tissues**  
TEM  
TEM-Tomography

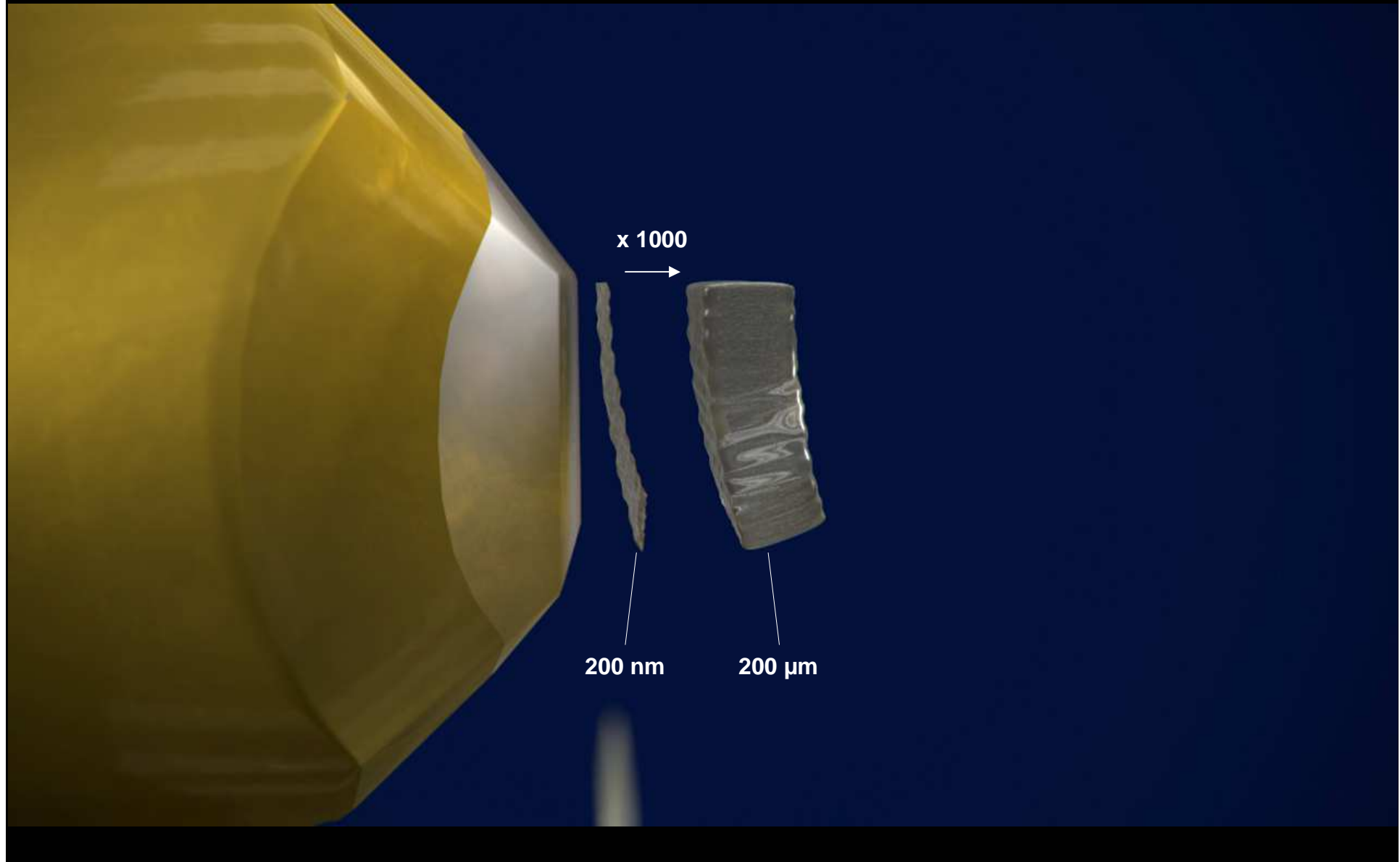
X-Ray  
Imaging and  
Diffraction:

**Thick Sections,  
Single Molecules**



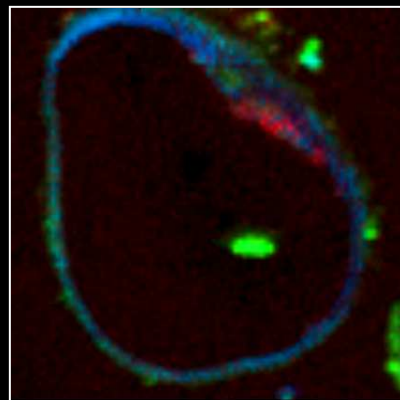
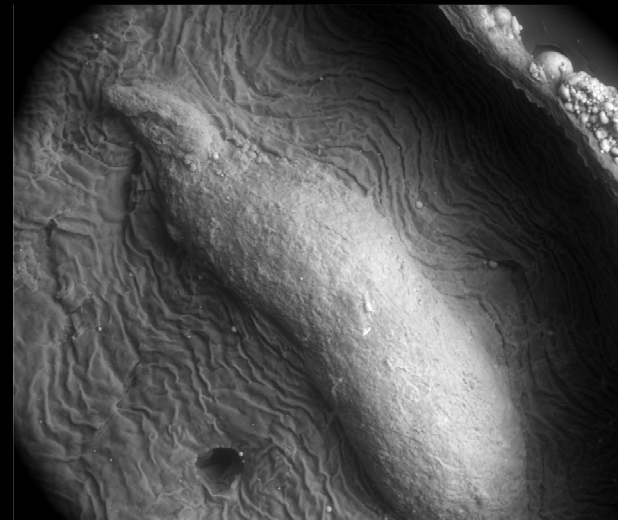
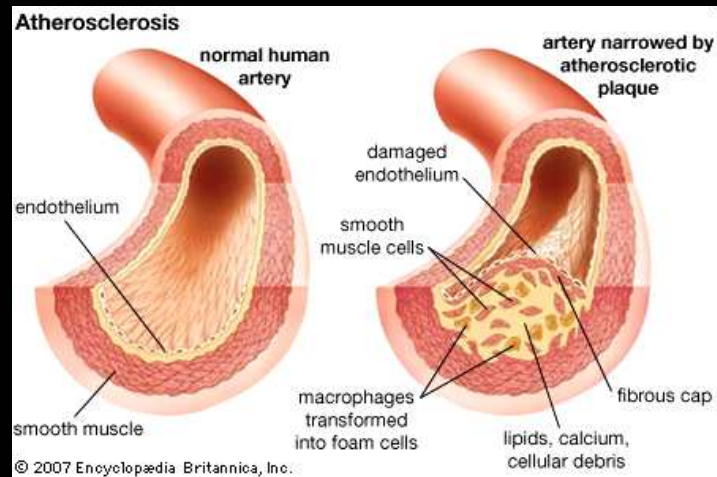
# Medical application: Nanoparticles as X-ray Fluorescent Dyes

Element mapping in the nanometer range in thick cryo-sections

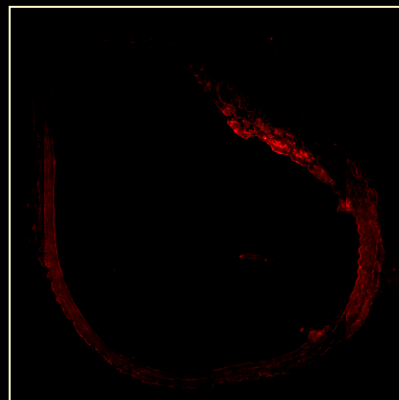


## Medical application: Nanoparticles as X-ray Fluorescent Dyes

X-Ray imaging & element mapping in 200  $\mu\text{m}$  thick cryo-sections : Arteriosklerotic plaques



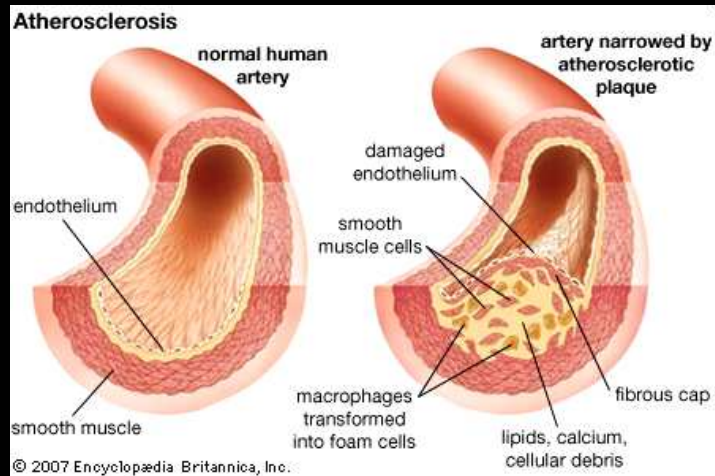
X-Ray Fluorescence at  
DESY, DORIS-L Beamline  
Fe- green, Se- red, Zn- blue



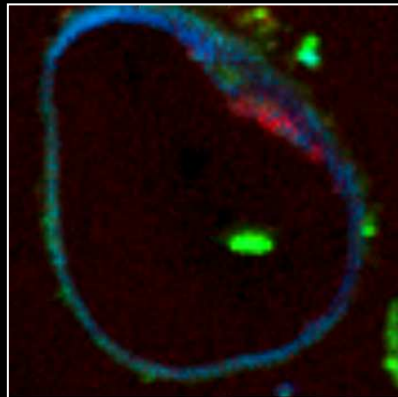
QD655-Nanosomes  
fluorescence signal

## Medizinische Anwendung: Nanopartikel für die Röntgen-Fluoreszenz

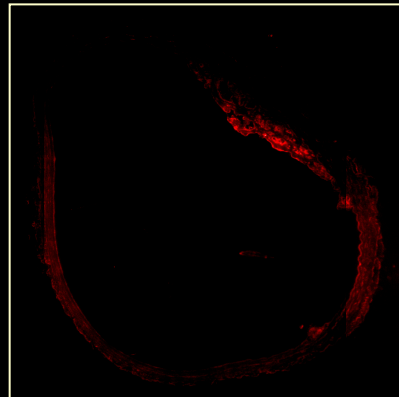
Röntgen-Imaging und Element-“mapping“ in 200 µm dicken Kryoschnitten von arteriosklerotischen Plaques



CdSe-Quantum dots in arteriosklerotischen Plaques im Kryoschnitt einer Mauseorta

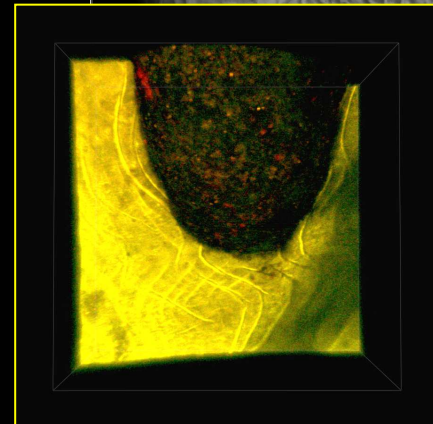
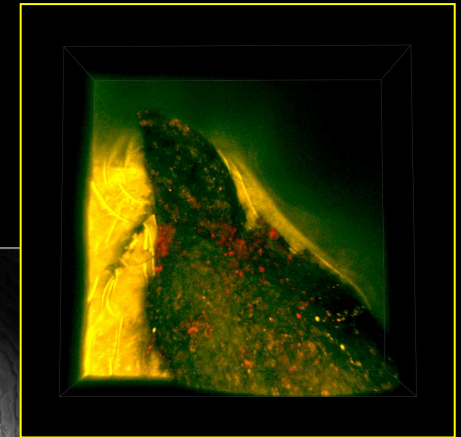
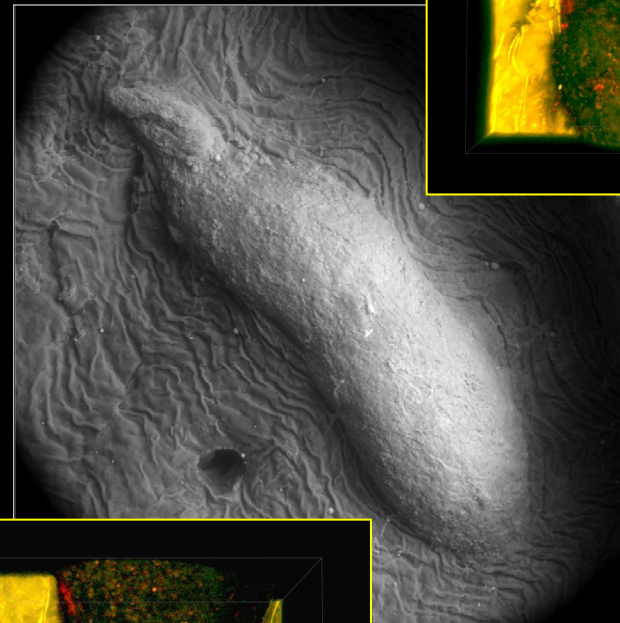


Röntgen-Fluoreszenz am  
DESY, DORIS-L Beamline  
Fe- grün, Se- rot, Zn- blau



QD655-Nanosomen  
(Fluoreszenzsignal)

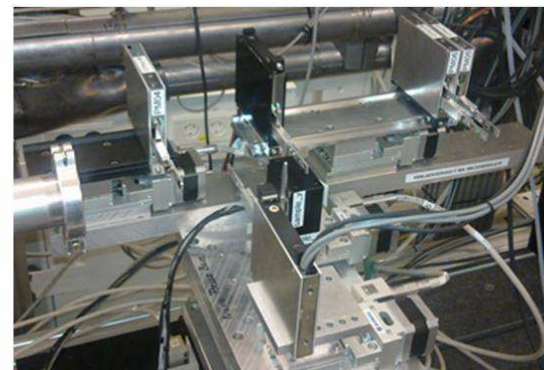
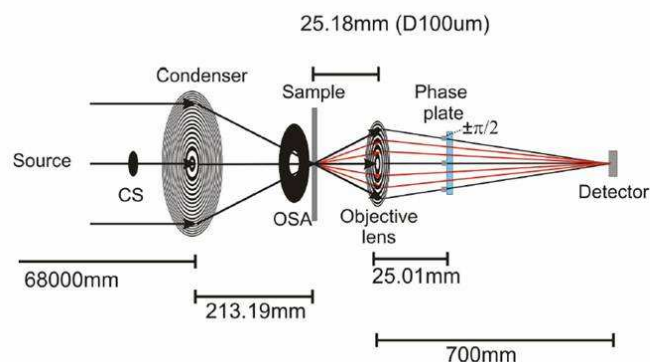
Endothel einer Mauseorta  
mit einem Plaque im ESEM



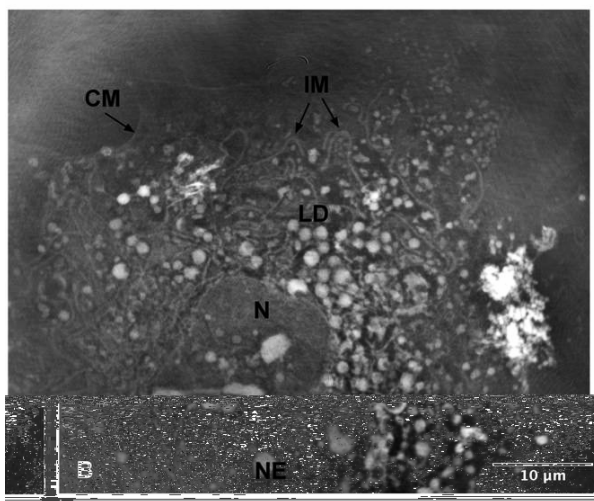
3D Rekonstruktion  
eines Plaques mit **QD-**  
**Nanosomen** im CLSM



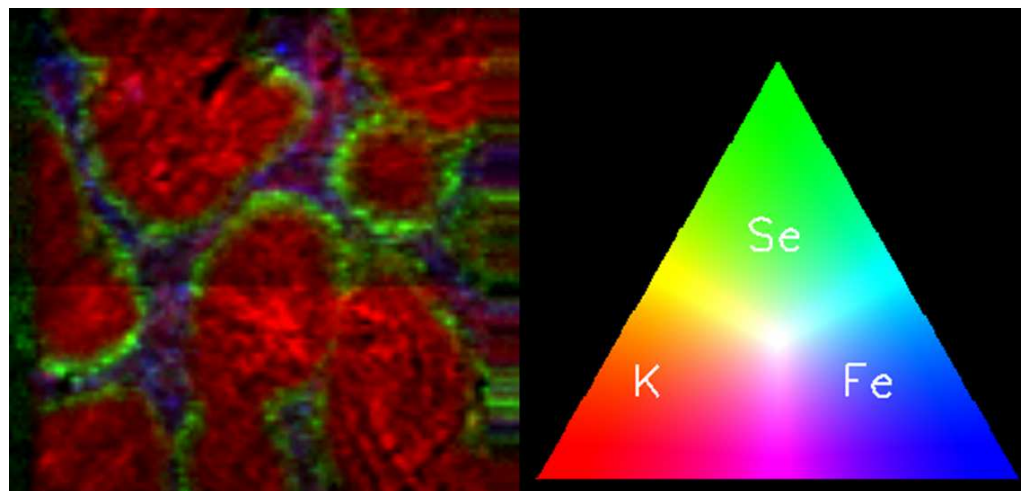
# Outlook: Combined X-Ray Fluorescence and Zernicke Phase Contrast at P11/PETRA III



Zone plate phase contrast optics at P11/PETRA III



Huh7 cell in X-Ray phase contrast

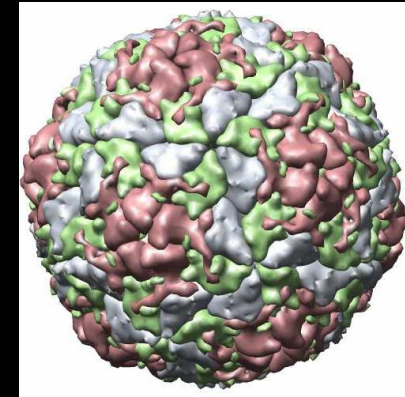
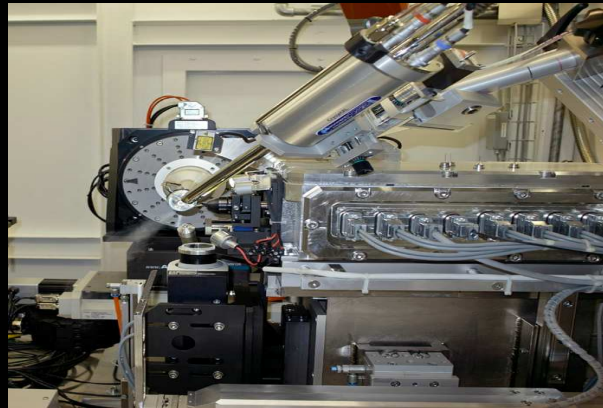
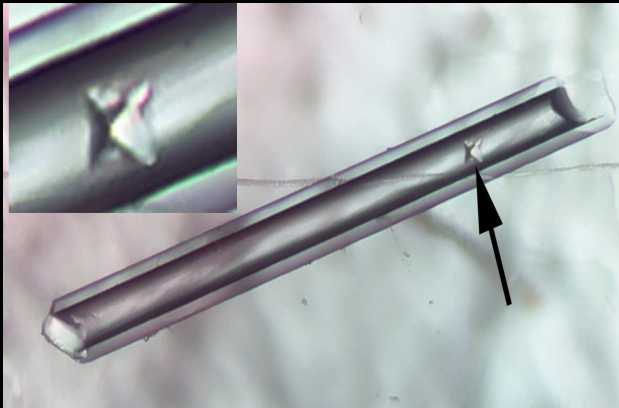


CdSe Quantum Dots in a mouse spleen by X-Ray fluorescence



## Translational technology transfer: EM Cryo-preparation methods for native protein crystals:

High pressure freezing: a sensitive method for virus crystals



Structure of  
bovine enterovirus

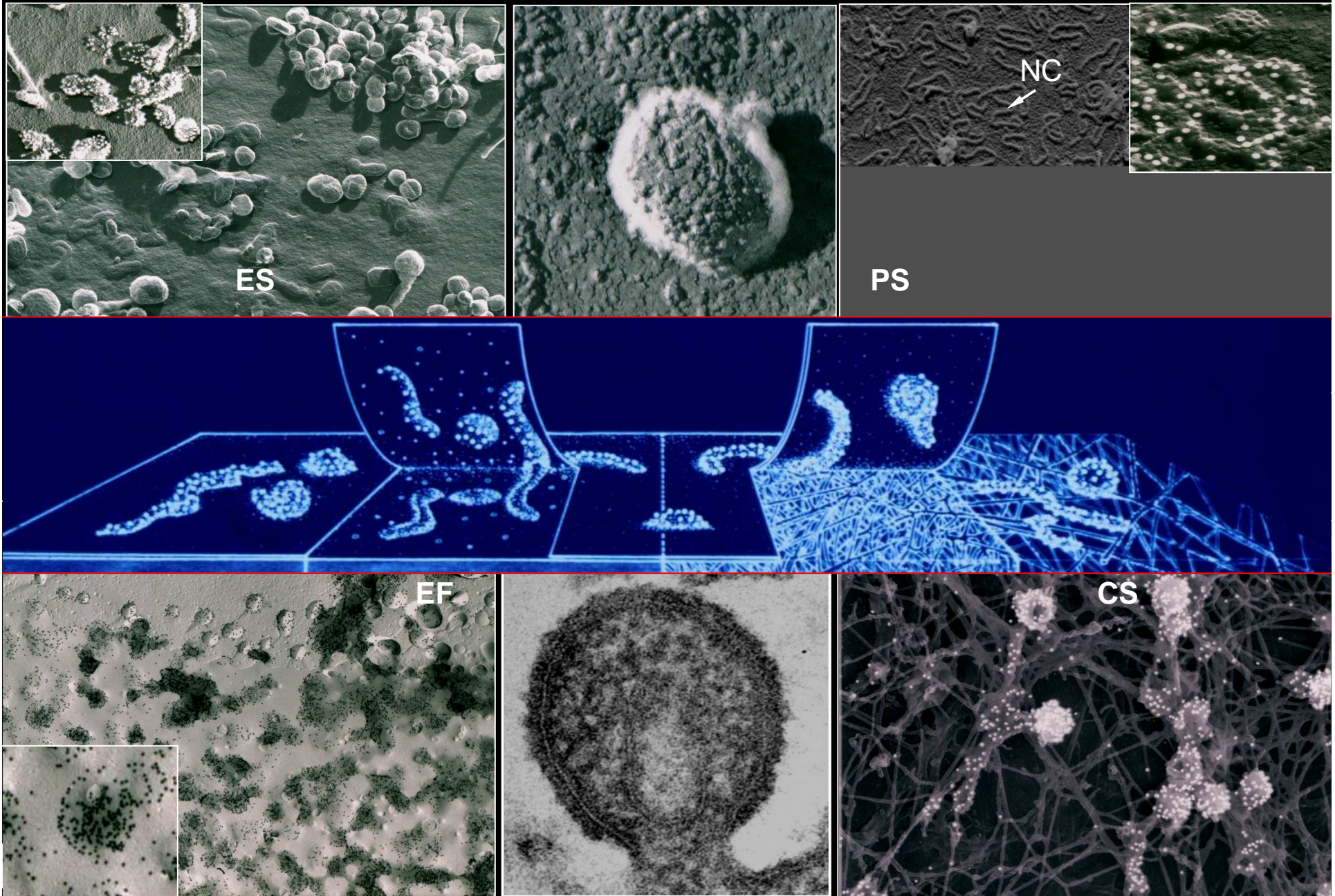
- BEV is a member of the picornavirus family and closely related to the human rhinoviruses (HRVs).
- BEV has a high susceptibility to cryoprotectants, therefore we use HPF.
- In cooperation with the group of **David Stuart** (university of Oxford) we are applying a modified high pressure freezing method to bovine enterovirus BEV- and EV71 crystals.  
Only **1! measured small HPF crystal** (single position measurement) had better quality than **28 large crystals** (76 position measurement)

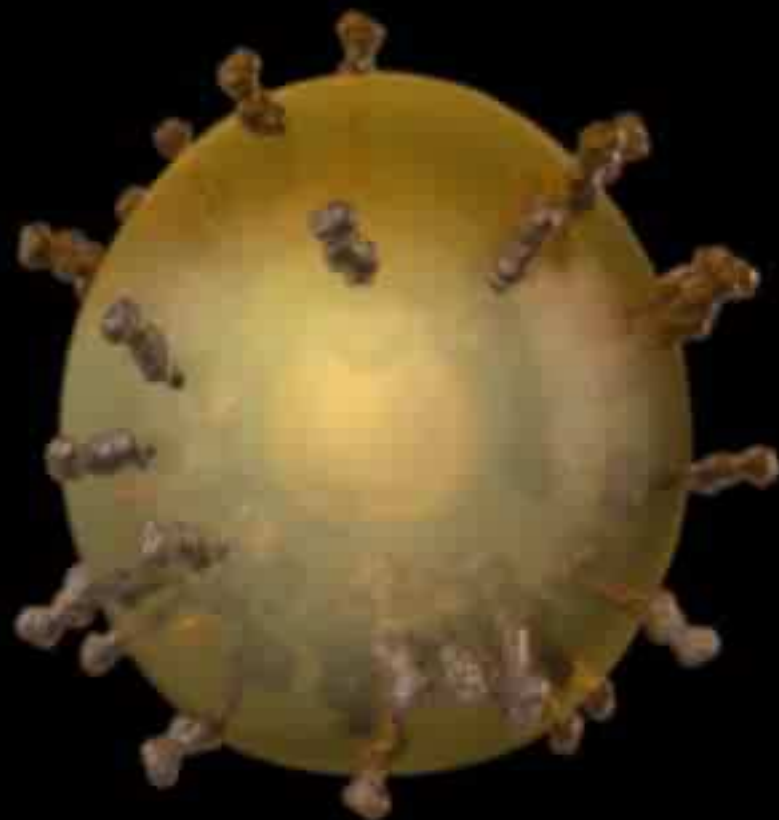
**Simulation of imaging data:**

**Integration of all imaging data  
into the dynamic simulation of a biological event**



# High-Resolution Immunogold Stereo Surface-Replica-Technique of Measles-Virus Infected Cells

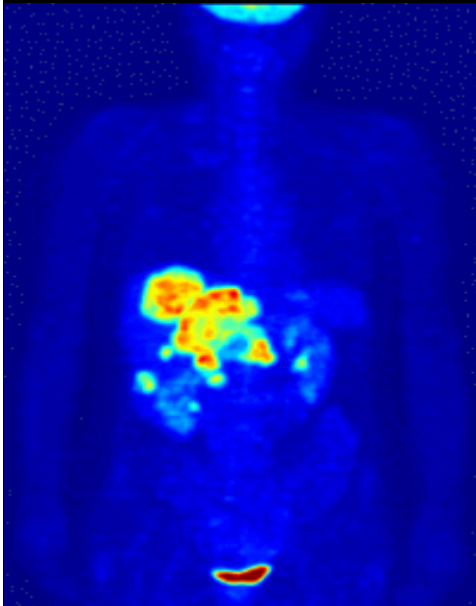




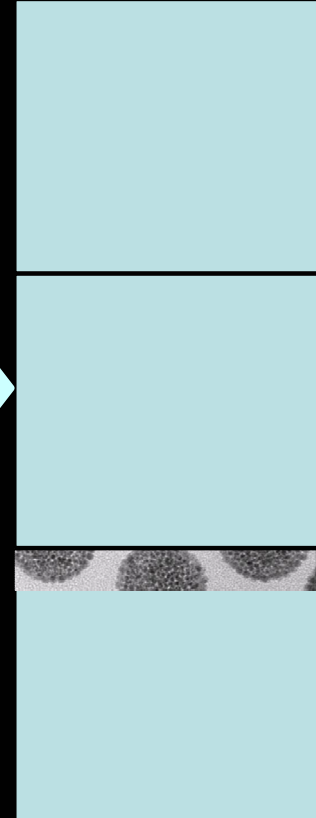


# Systemic imaging of near to life Systems

From the intact organism to its molecule complexes *in situ*



- More sensitive detection of small NP (MPI)
- Efficient labelling of viruses and bacteria with NP
- Higher signal strength / new nano-particle



**Medical  
Tomography:**  
**Intact Organisms**  
PET, MRI

**Light-  
Microscopy:**  
**Cells, Tissues**  
Live Cell imaging,  
Intravital, CLSM

**Electron  
Microscopy  
of surfaces:**  
**Cells, Tissues**  
SEM  
ESEM

**Electron  
Microscopy  
of cell interior:**  
**Cells, Tissues**  
TEM  
TEM-Tomography

**X-Ray  
Imaging and  
Diffraction:**  
**Thick Sections,  
Single Molecules**



Photosynth is a software technology (MS Live Labs) that analyzes digital images to build a three-dimensional point cloud of an user-defined object. Pattern recognition components compare portions of images to create points, which are then compared to convert the image into a model, (Proposal BILL & MELINDA GATES foundation).

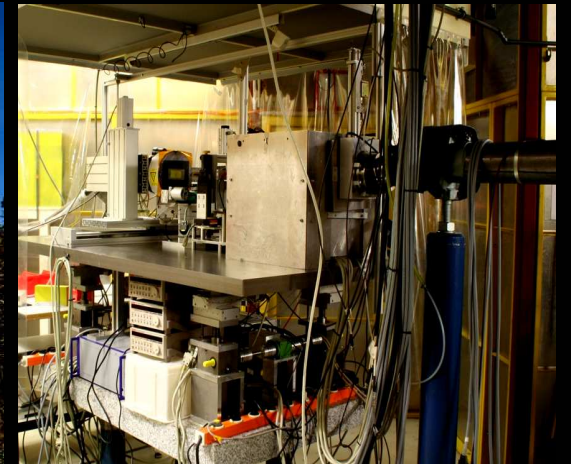


## Collaborators

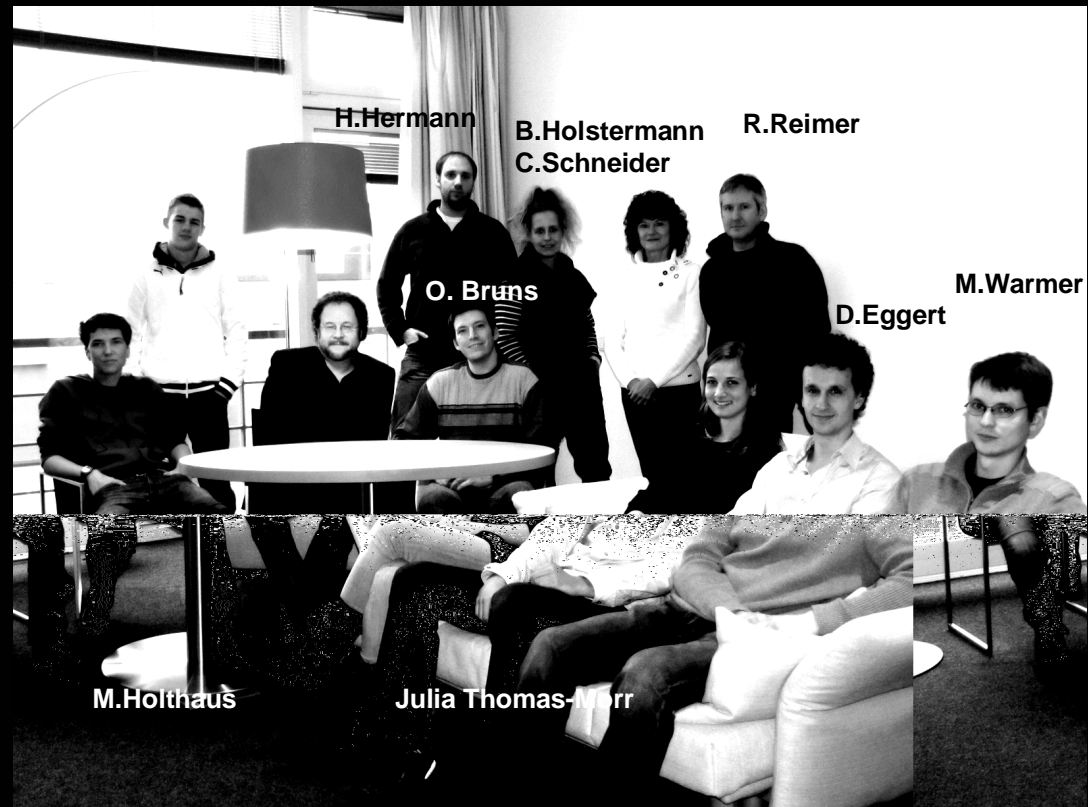
Horst Weller (Uni Hamburg)  
 Alexander Eychmüller (TU Dresden)  
 Edgar Weckert (HASYLAB, DESY)  
Alke Meents (HASYLAB, DESY)  
 Anja Burghard (HASYLAB, DESY)  
 Tanja Ducic (HASYLAB, DESY)  
Gerhard Adam (Radiologie, UKE)  
 Harald Ittrich (Radiologie, UKE)  
Jörg Heeren (Biochemie, UKE)  
 Alexander Bartelt (Biochemie, UKE)  
 Peter Nielsen (Biochemie, UKE)  
 Markus Heine (Anatomie, UKE)  
Ulrich Schaible (FZB)  
 Volker Heussler (Uni Bern)  
Udo Schumacher (Anatomie, UKE)  
 Hüseyin Sirma (Pathologie, UKE)  
 Michael Winkler (Uni Kiel)  
 Oliver Bruns (MIT Boston)  
 Hans-Georg Kräusslich (HPI)  
 Gülsah Gabriel (HPI)  
 Nikon Applikationszentrum (HPI)



EM and Micro-Technology - Department for Structural Virology



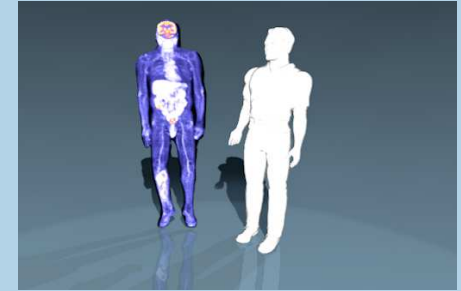
DESY: Bio-Imaging Beamline



MRT



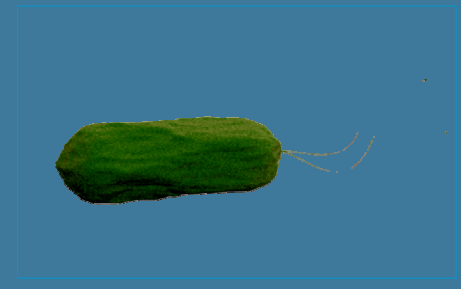
Thank you  
for your attention  
and patience!



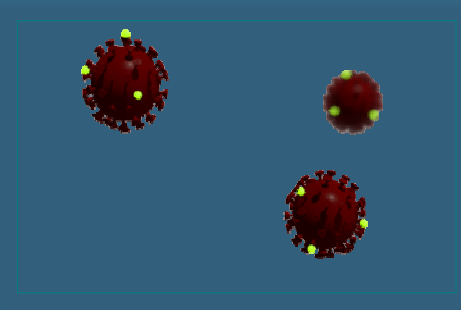
CLSM



TEM



X-Ray



Hamburg Harbour