ZEIM

Central Facility for Microscopy, ZEIM
Head: Prof. Dr. rer. nat. habil. Manfred Rohde

HZI HELMHOLTZ Centre for Infection Research

Our motivation and mission

The ZEIM team cooperates with the customer to establish tailored preparation protocols and imaging technique/s to ensure that the best methodology is applied for each project. For that purposes ZEIM has a portfolio of different methods available for preparing biological or material samples for light and electron microscopy.

A strength of ZEIM is applying

a variety of approaches/techniques

from light microscopy

to electron microscopy

to investigate biological mechanisms

Equipment at a glance (in D-building)

The platform provides access to several upright and inverse fluorescence microscopes, confocal microscopes, two transmission electron microscopes (TEM), a high resolution field emission scanning electron microscope (FESEM) in addition to the peripheral preparation equipment.

Ease of use for our customers

- We discuss the project together
- We fill in the order form (Bestellformular)
- Customer brings samples, for S3/S2-samples fixative is provided by ZEIM
- ZEIM staff is processing the samples
- ZEIM staff is performing imaging
- Image plates for publications icluding meth part are delivered or images are send to the customer

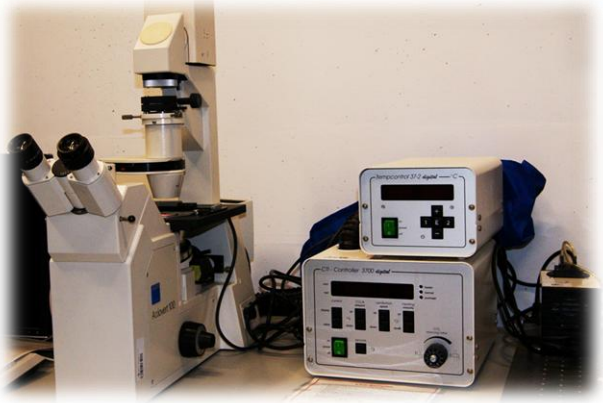
Equipment for light microscopy

Zeiss Imager Z2
D 0.43
Mathias Müsken

Zeiss Axio Imager A1
D 1.01
Gabriella Molinari

Zeiss Axio Imager A2
D 1.25
Gabriella Molinari

Zeiss Axiovert 200 M
D 0.43
Gabriella Molinari
Mathias Müsken



Zeiss Axiovert 100 M
D 1.25
Gabriella Molinari

Equipment for confocal microscopy

Leica Live imaging system
D 0.52
Mathias Müsken
Gabriella Molinari

Leica SP5 Confocal
D 0.52
Gabriella Molinari
Mathias Müsken

Zeiss LSM510 Meta
D 3.55
Ulfert Rand
Mario Köster

We can provide access to:

a confocal microscope (spinning disk)
in the S3 Facility

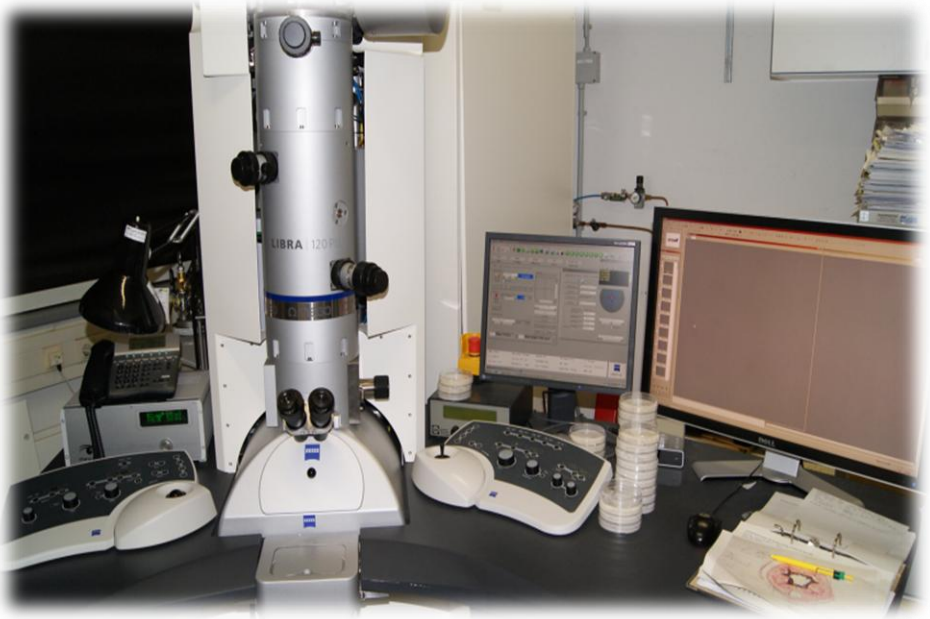
**Perkin Elmer UltraView
can also be used for
S2/S1 experiments**

Zeiss Apotome system with CO_2-chamber

**Zeiss Observer Z1 with Apotome and
incubation chamber**

Equipment for transmission electron microscopy

Zeiss EM 910 STEM
D 0.44
Manfred Rohde
Ina Schleicher



Zeiss Libra 120Plus
D 0.45
Mathias Müsken

Equipment for field emission scanning electron microscopy

Zeiss Merlin

D 0.42

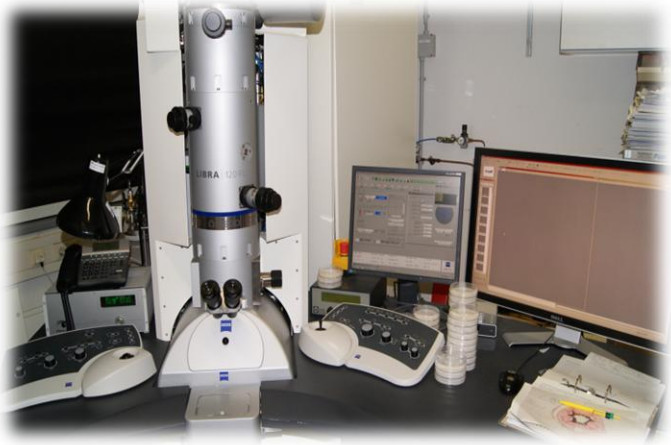
Manfred Rohde

Mathias Müsken

Ina Schleicher

Specifications for transmission electron microscopes, for insiders

Zeiss Libra120 Plus



1. 60-120 kV acceleration voltage
2. Slow Scan CCD 2K Sharp eye
3. Integrated energy filter Omega type
4. ITEM software
5. Small Cryo equipment
6. Tomography possibility, only one axis
7. ESI (electron spectroscopic imaging)
8. EELS (electron energy loss spectroscopy)
9. LaB_6 cathode

Zeiss TEM 910

1. 60-120 kV acceleration voltage
2. Slow Scan CCD 1K ProScan
3. ITEM software
4. Wolfram cathode

Specifications for FESEM, for insiders

ZEISS MERLIN

1. Smart SEM V 5.05 software
2. 200V-30 kV acceleration voltage
3. HE-SE2 SE-detector
4. InLens SE-detector
5. AsB BSE-detector
6. EsB detector
7. STEM detector
8. ATLAS system
9. Leica VLT100 Cryo system
10. Oxford AzTec-EDS(EDX) system
11. SDD X-Max detector with 50 mm^2 window
12. Shuttle&Find system for CLEM
13. Air lock system for sample exchange
14. Schottky cathode

Preparation equipment for transmission electron microscopes

1. Bal-Tec MED020 coating system with e-gun and freeze-drying unit

2. Balzers BAF400 freeze-fracture system

3. Leica EM-Pact for high pressure freezing (HPF)

4. Leica ultramicrotome Ultracut S

5. Leica ultramicrotome Ultracut S with Cryo system FCS

6. Reichert-Jung Freeze-substitution system CS Auto, Leica EM AFS

7. Leica Ultratrim for trimming resin embedded samples

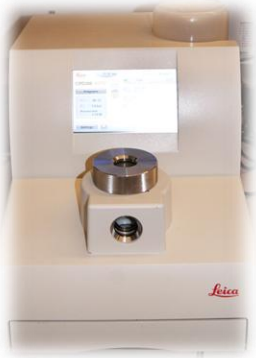
8. Reichert-Jung Knifemaker for glass knives

9. Reichert-Jung Immersion Cryofixation system KF80

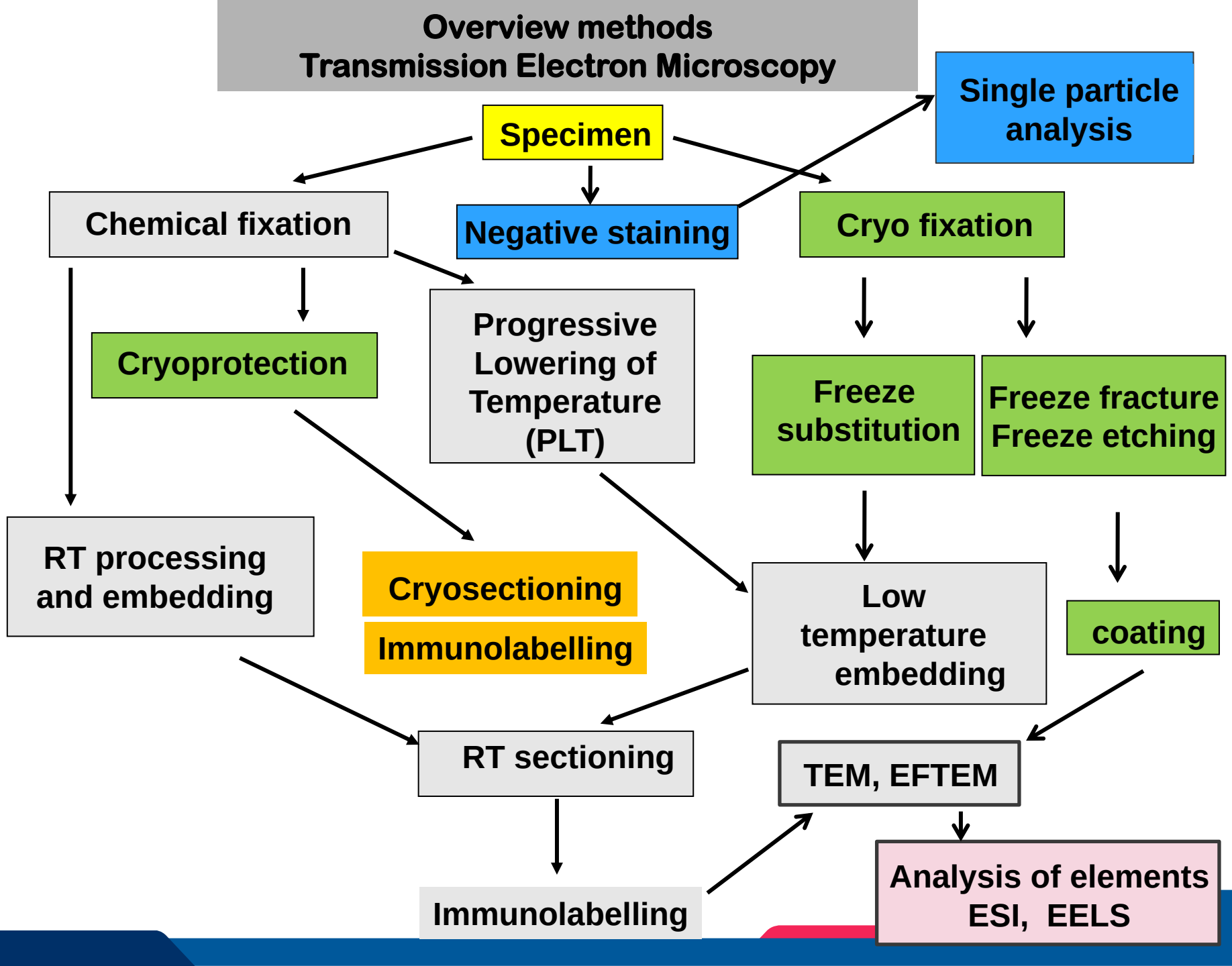
Preparation equipment for FESEM

1. Bal-Tec CPD030 Critical-point dryer

2. Leica CPD300 automatic critical-point dryer

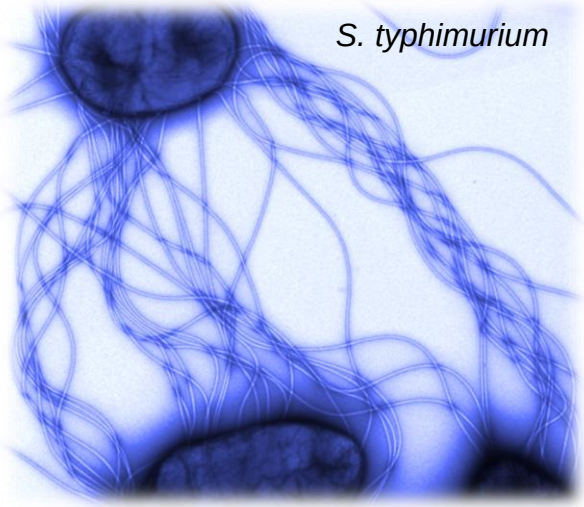


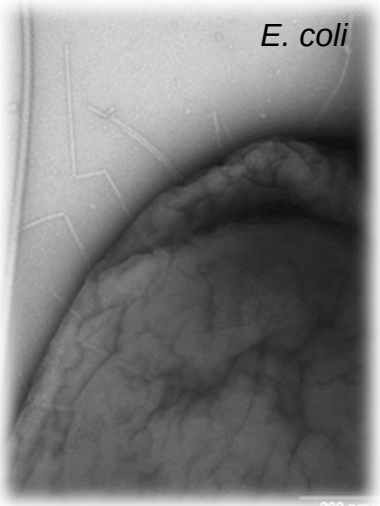
3. Bal-Tec sputter coater SCD 500 with carbon evaporation unit

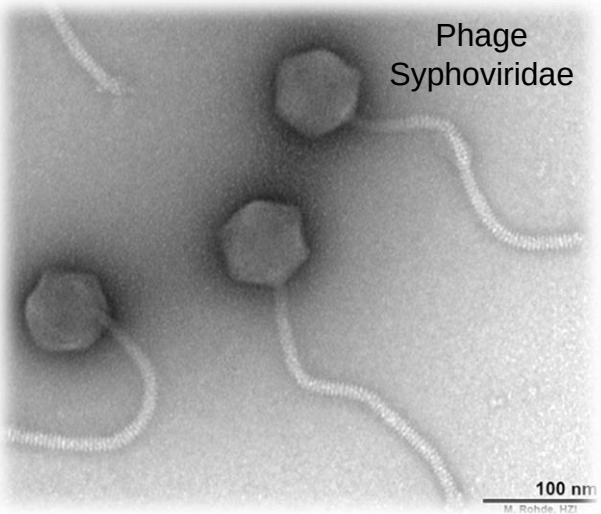

Overview methods
Transmission Electron Microscopy
Specimen
Single particle analysis
Chemical fixation
Negative staining
Cryo fixation
Cryoprotection
Progressive Lowering of Temperature (PLT)
Freeze substitution
Freeze fracture Freeze etching
RT processing and embedding
Cryosectioning
Immunolabelling
Low temperature embedding
coating
RT sectioning
TEM, EFTEM
Immunolabelling
Analysis of elements ESI, EELS

Methods for transmission electron microscopy

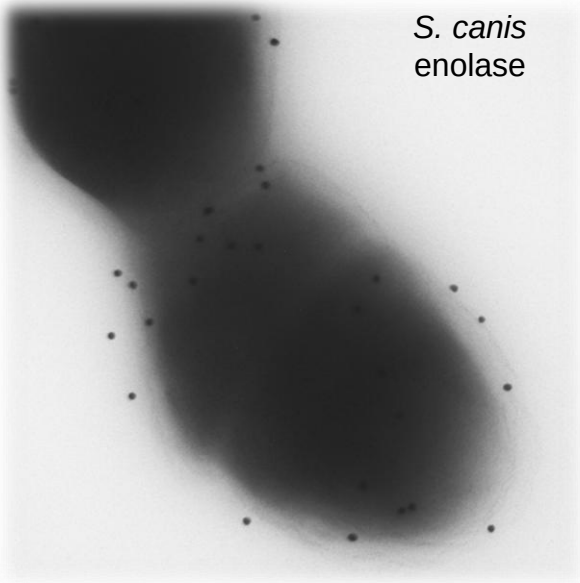
1. Negative-staining of bacteria, viruses, phages, cells etc.

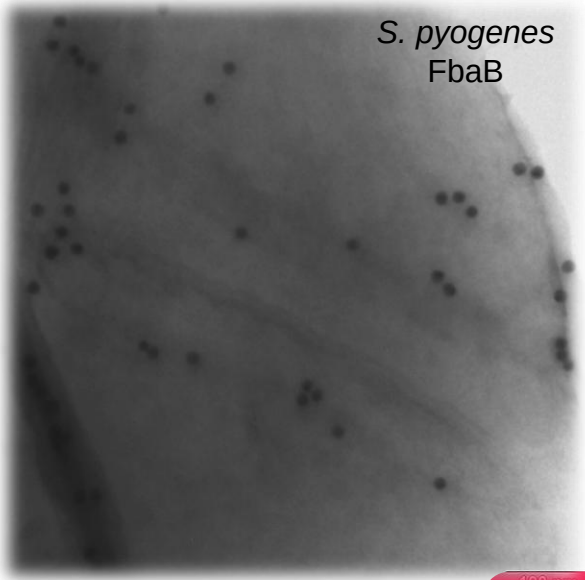


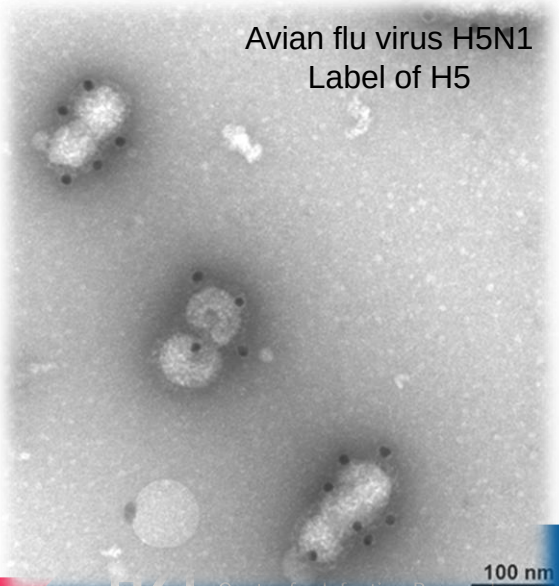




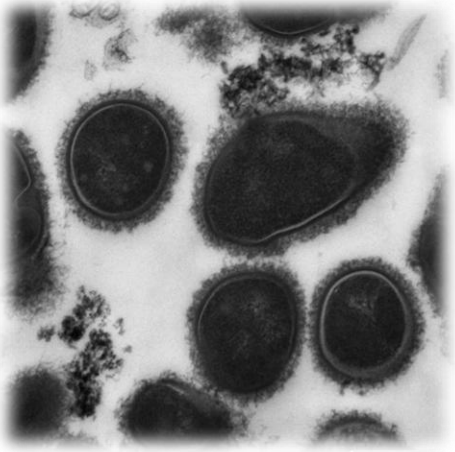
2. Immuno labelling „on grid“ of bacteria and viruses



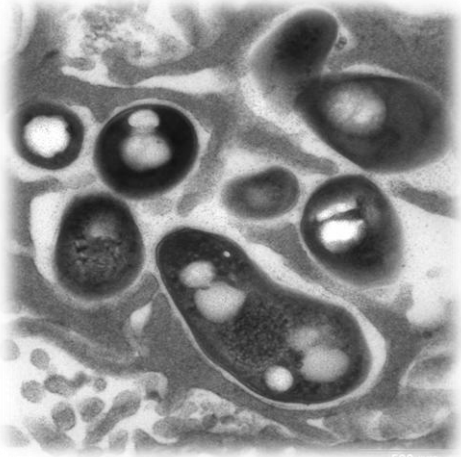




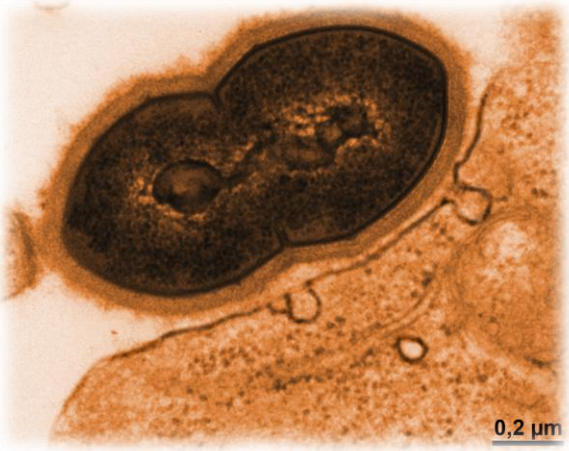
3. Resin embedding of bacteria and eucaryotic cells etc. at room temperature



S. pneumoniae



M. paratuberculosis

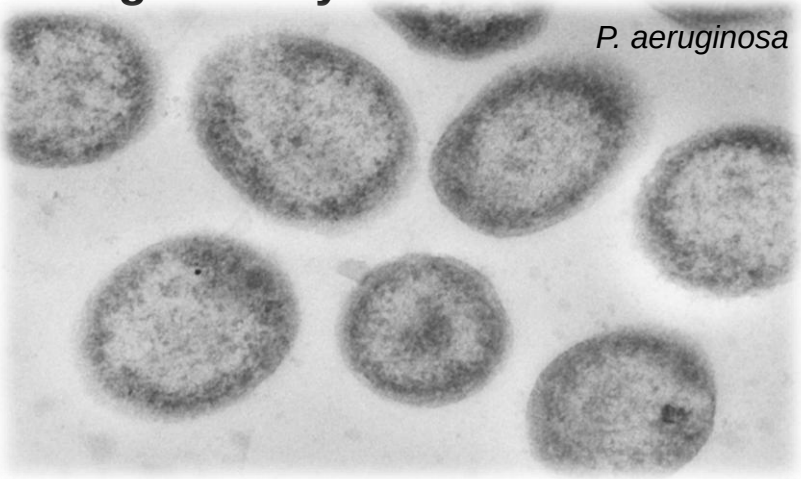


S. pyogenes A40

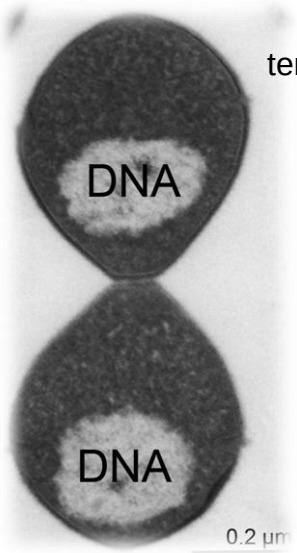
4. Resin embedding of bacteria, eucaryotic cells, tissues etc. at low temperature

5. Progressive lowering of temperature (PLT) embedding using Lowicryl resins

P. aeruginosa

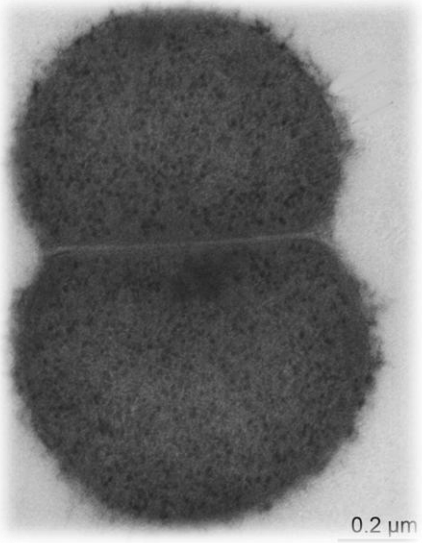


6. Freeze-substitution of cryo-fixed samples using epoxy or acrylate resins or Lowicryl resins



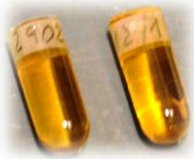
room temperature

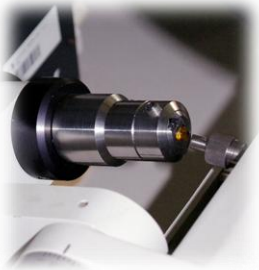
Group G streptococci

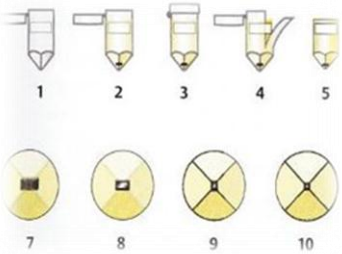


freeze-substitution

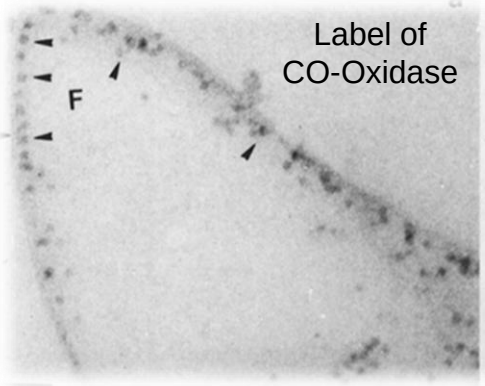
7. Preparation of ultrathin sections



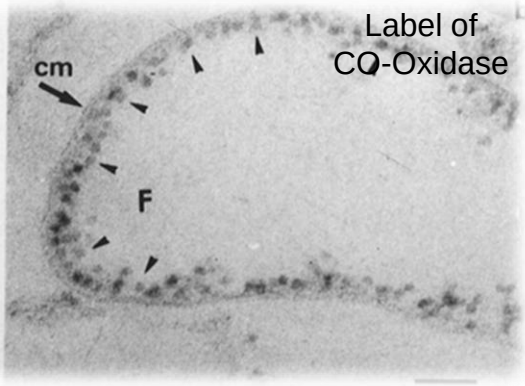




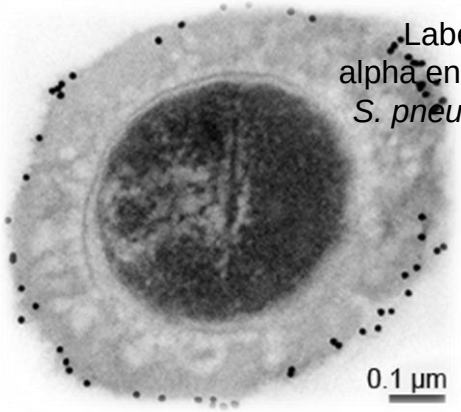
8. Pre-embedding immuno labelling of antigenes with antibodies and gold-nanoparticles exposed on surfaces



Label of CO-Oxidase



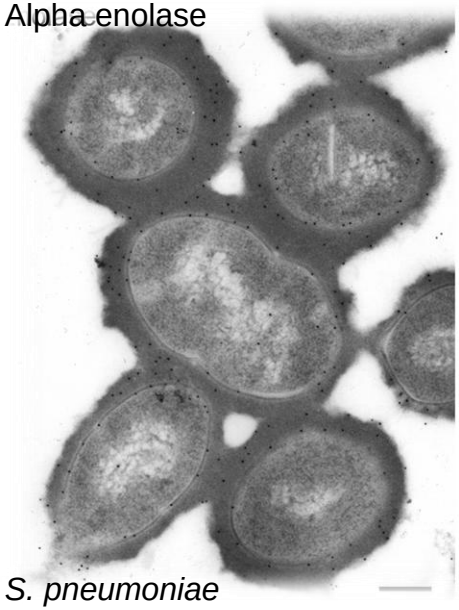
Label of CO-Oxidase



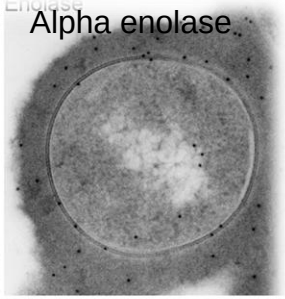
Label of alpha enolase on *S. pneumoniae*

9. Postembedding labelling of antigens with antibodies and gold-nanoparticles on ultrathin sections

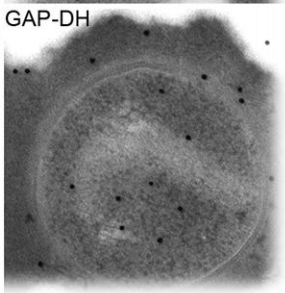
Alpha enolase

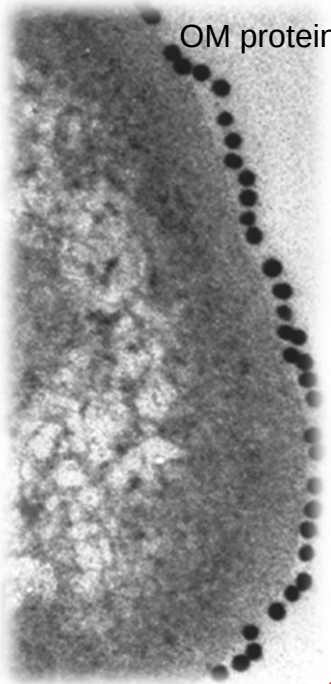


S. pneumoniae



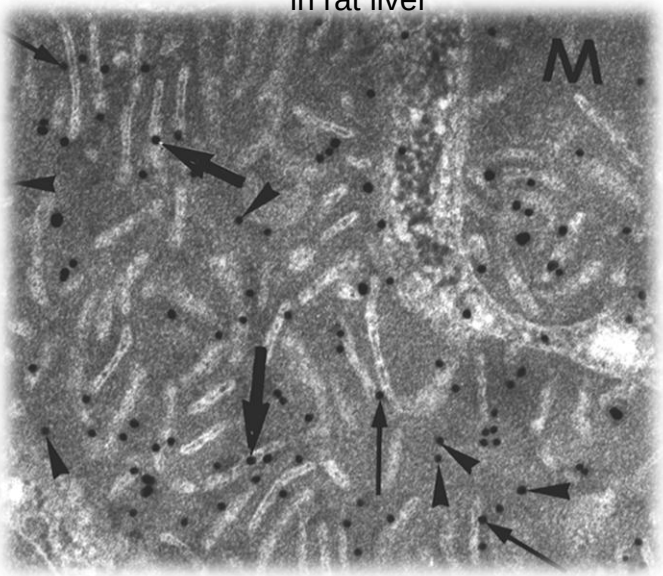
Alpha enolase



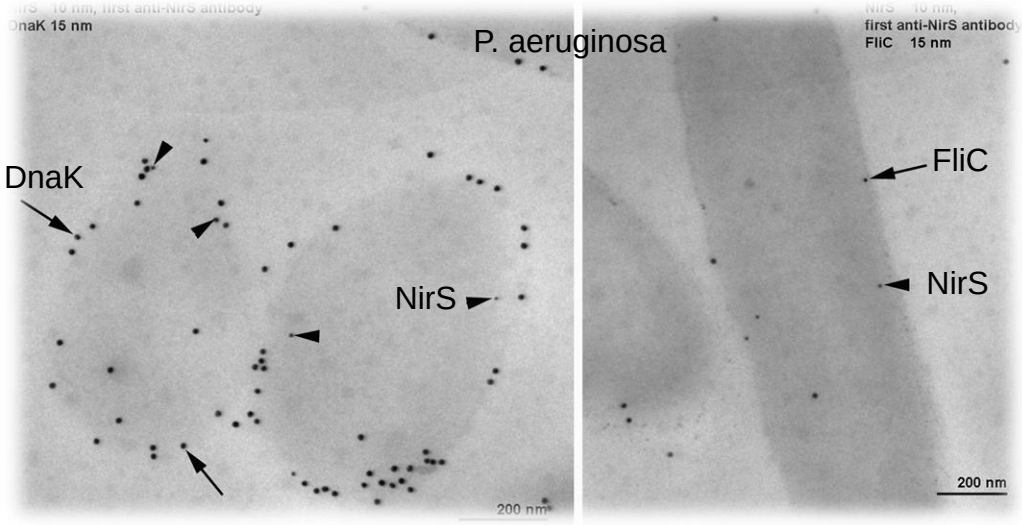


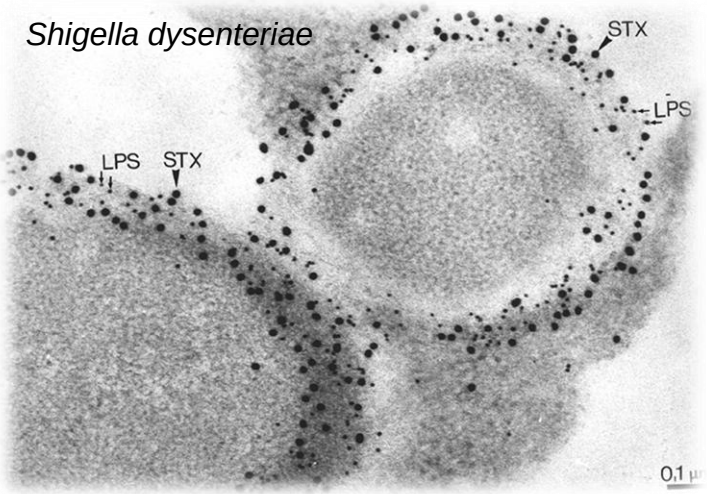
OM protein

Aminolevulinate synthase in rat liver

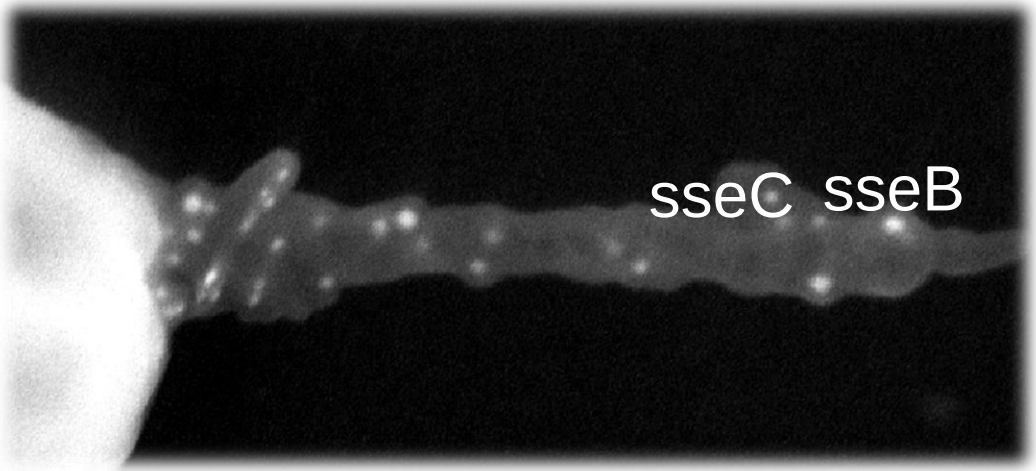


10. Double immuno labelling of two antigens on ultrathin section

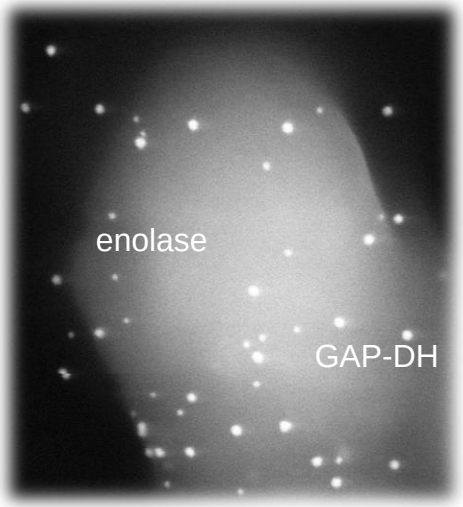




11. Double immuno labelling of two antigens on bacteria

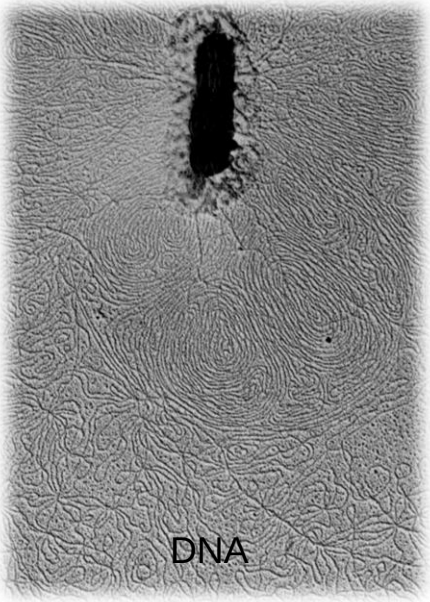


S. typhimurium, label sseB and sseC

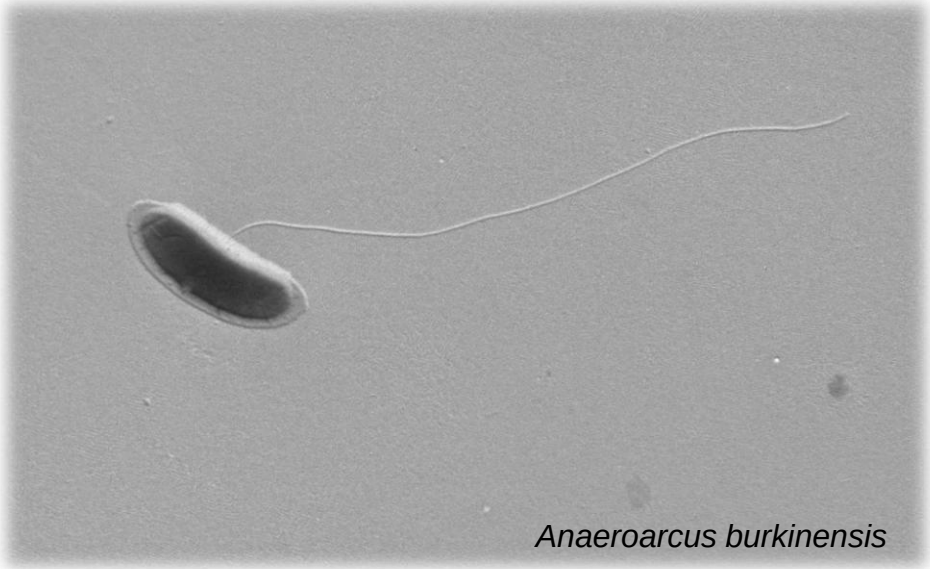


S. canis, label of alpha enolase and GAP-DH

12. Metal shadowing with platinum

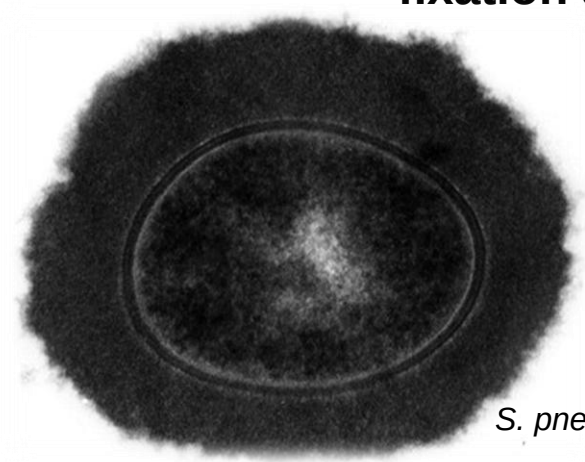


E. coli

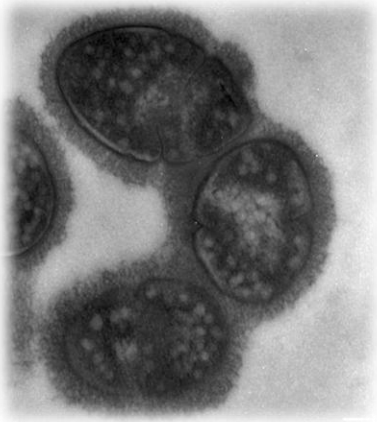


Anaeroarcus burkinensis

13. Detection of bacterial capsules applying LRR (lysine-ruthenium red-osmium) fixation and LRWhite resin embedding

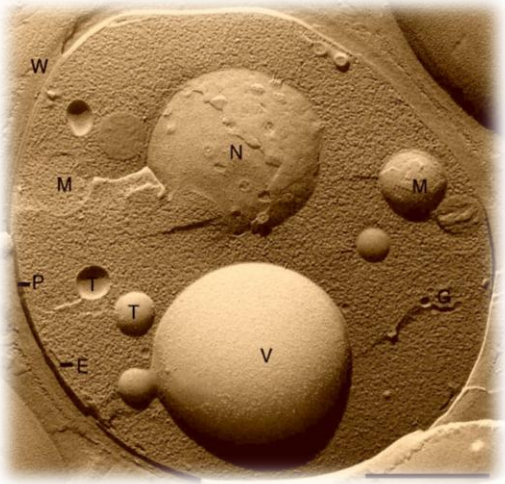


S. pneumoniae



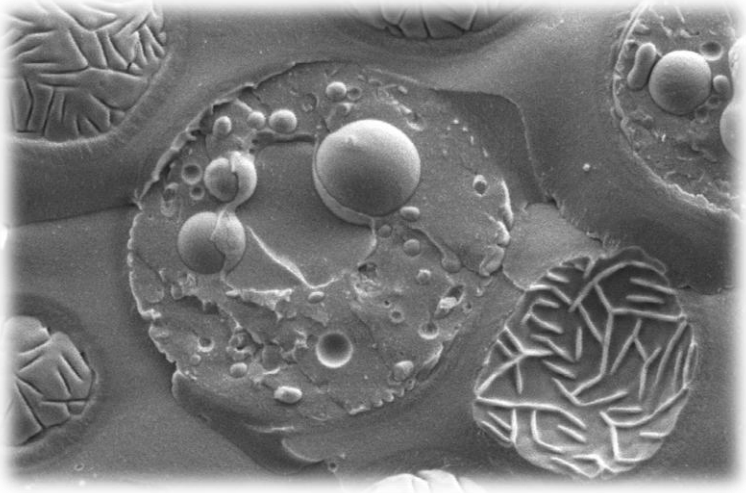
S. suis

14. Freeze-fracture replica preparation



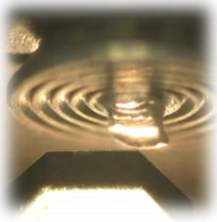
Yeast
S. cerevisiae

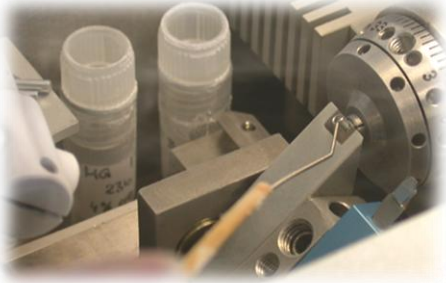
TEM

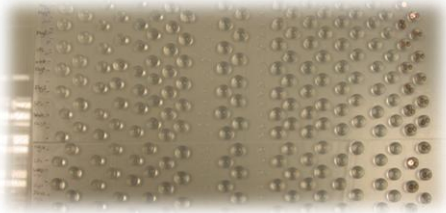


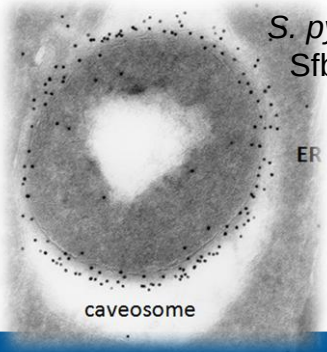
FESEM

15. Cryo sections with immuno labelling of antigens



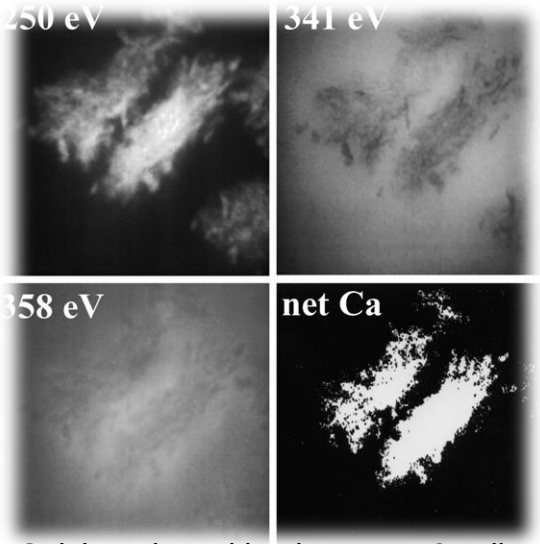






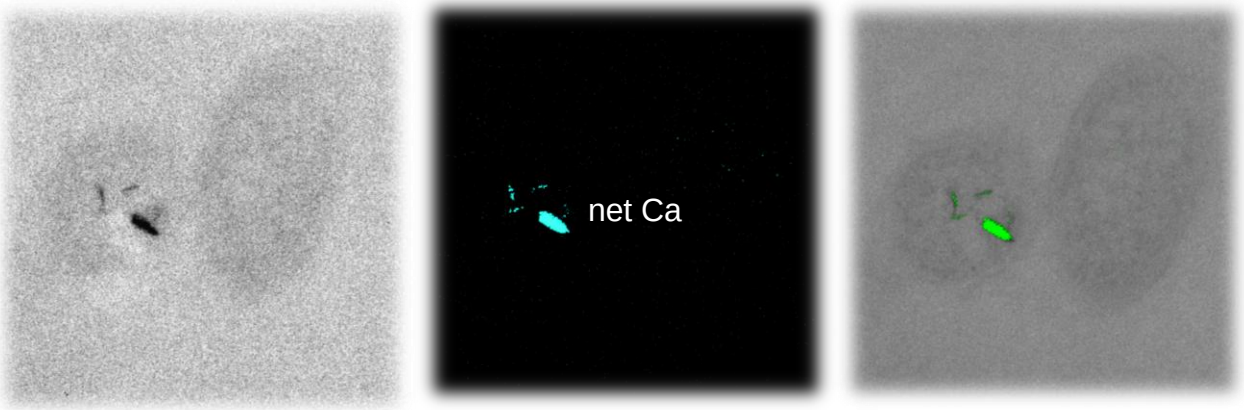
S. pyogenes
SfbI label

16. ESI imaging (electron spectroscopic imaging)

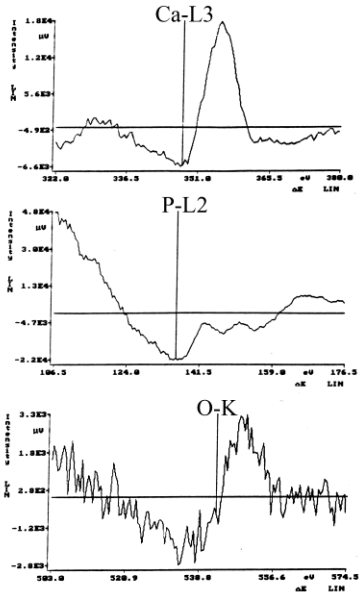


Calcium deposition in BBMP-2 cells

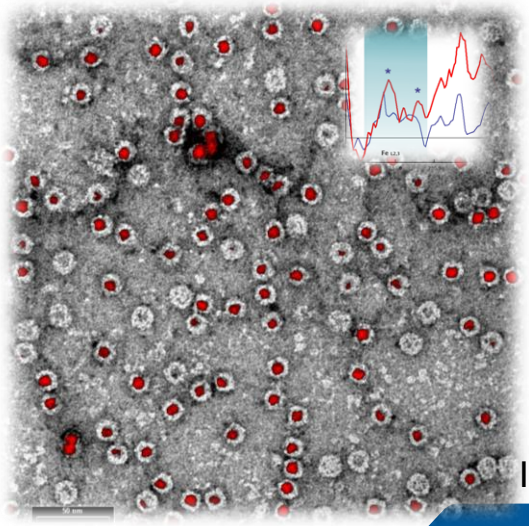
Calcium deposition in bacteria



17. Identification of elements applying EELS (electron energy loss spectroscopy)

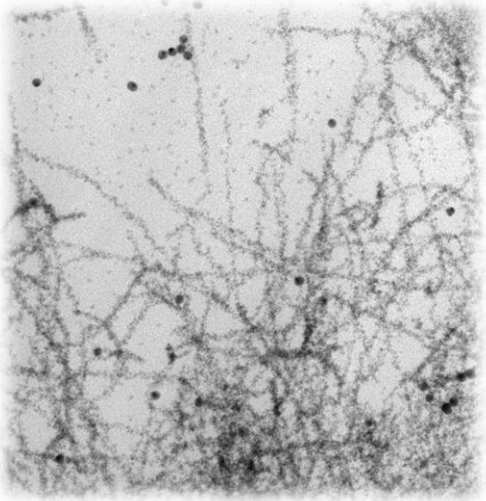


EELS of
image 16 above



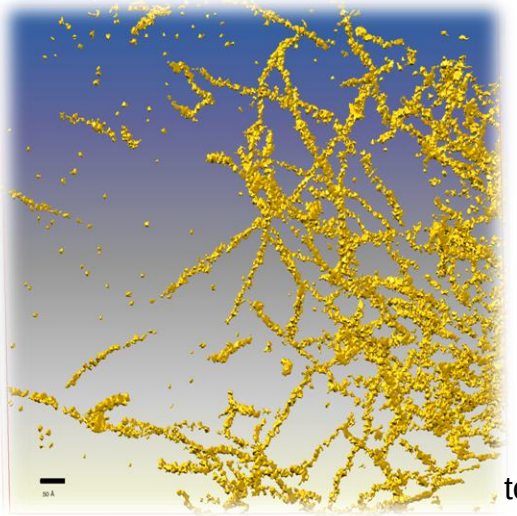
Iron core (red) in ferritin

18. Tomography of 500 nm ultrathin sections



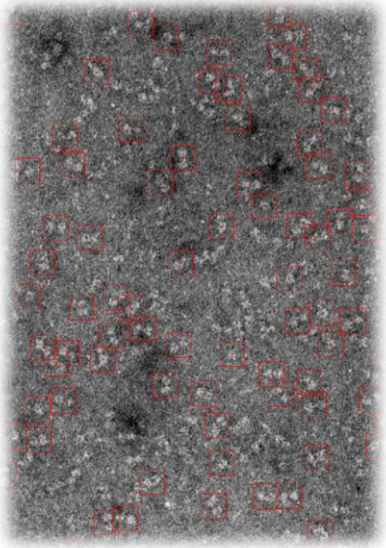
EPS matrix structures in biofilm

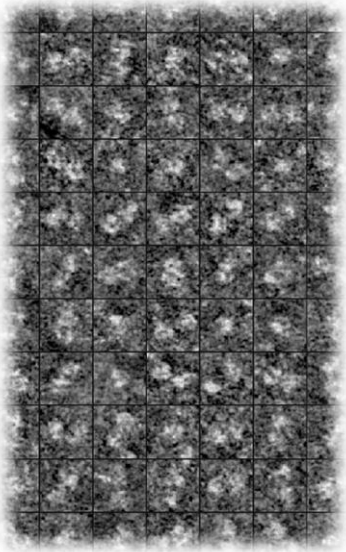
Ultrathin section

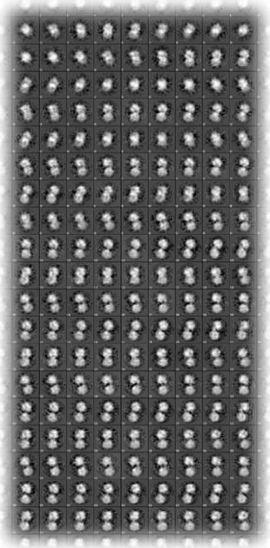


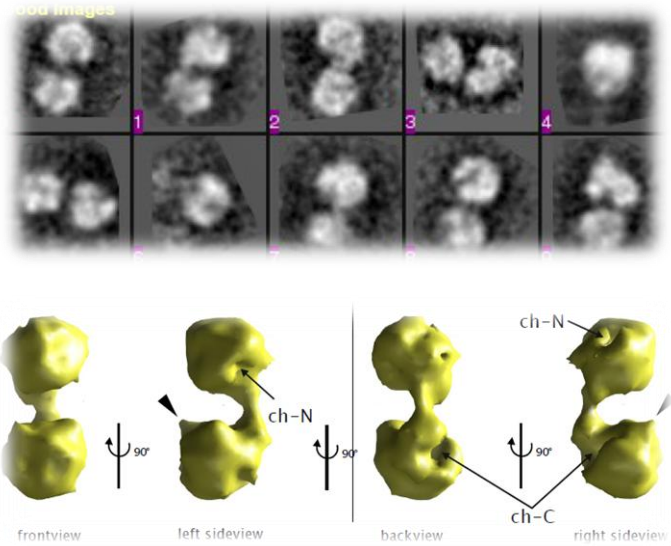
tomogram

19. Single particle analysis



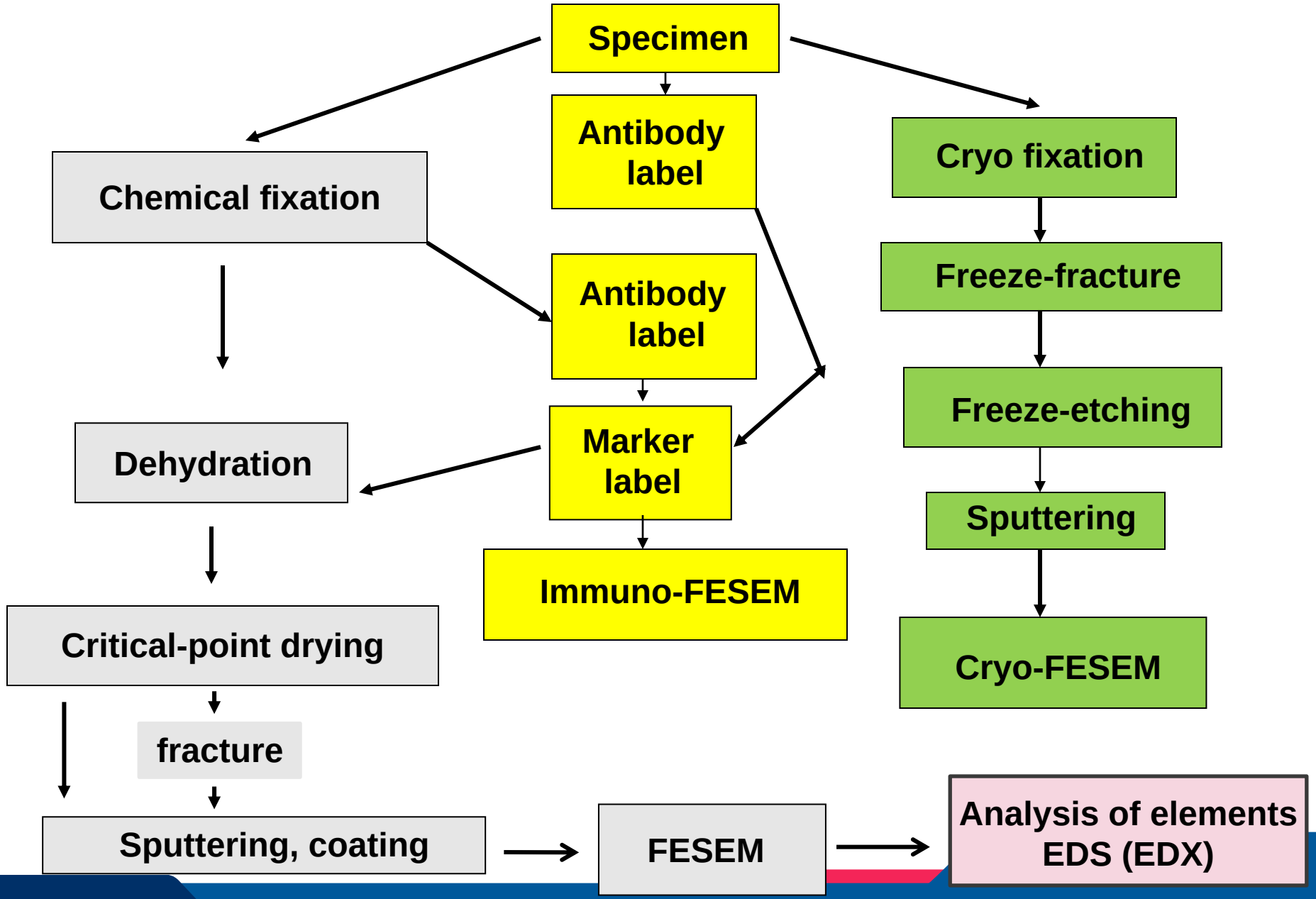






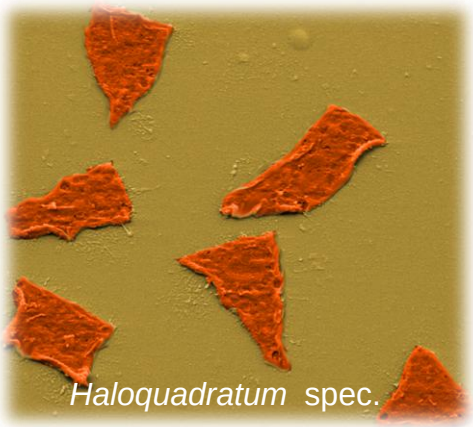
Angiotensin converting enzyme, ACE

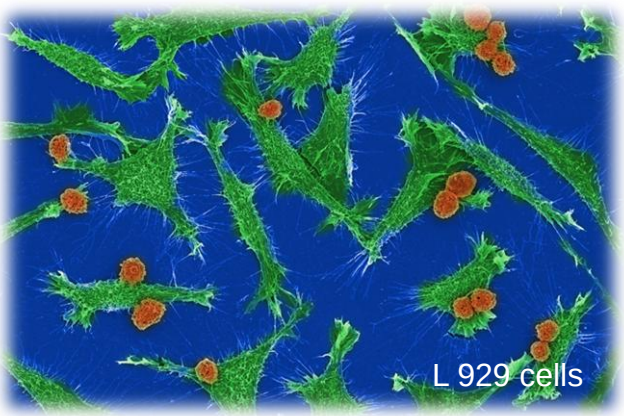
Overview methods Field Emission Scanning Electron Microscopy



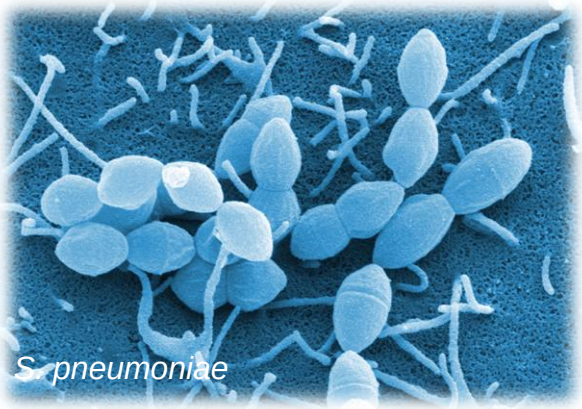
Methods for FESEM

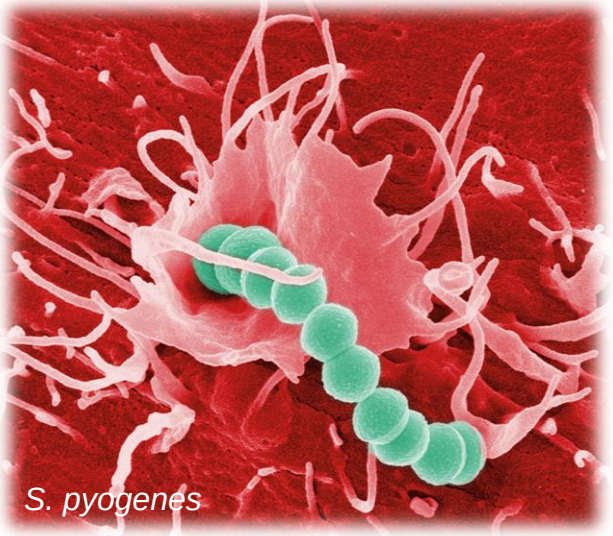
1. Morphological description of bacteria and cells



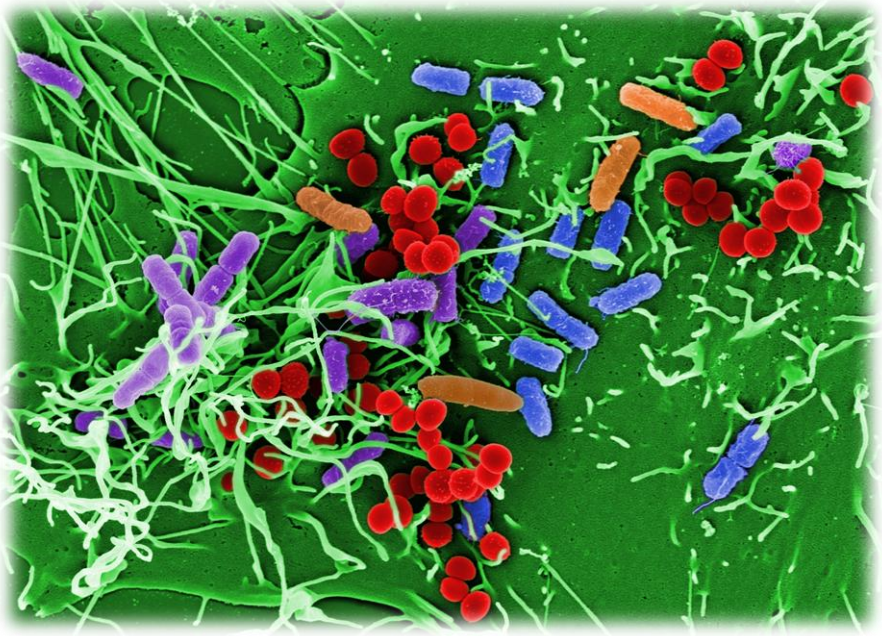


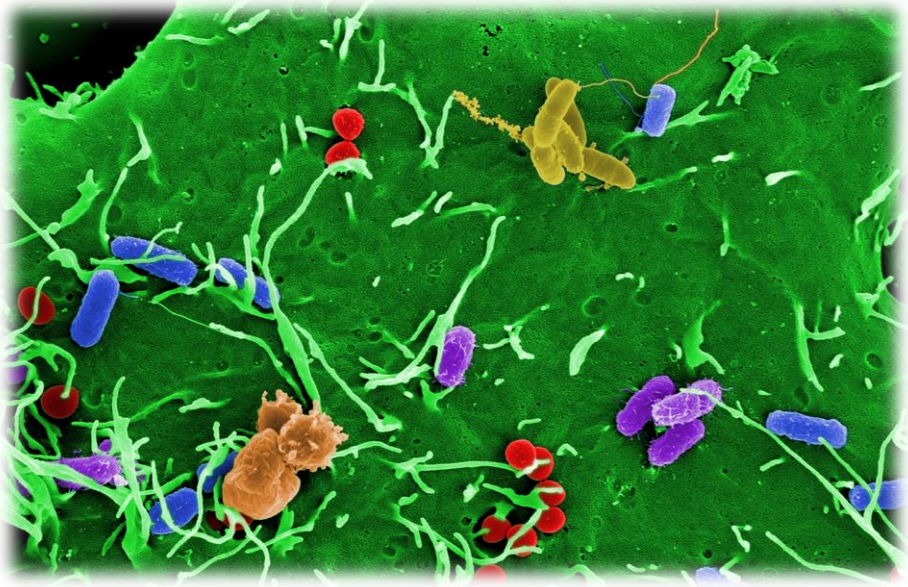
2. Morphological description of adhesion and invasion of pathogenic bacteria





FESEM allows to discriminate between 4 morphological different adhesive pathogenic bacteria





HEp-2 cells infected with

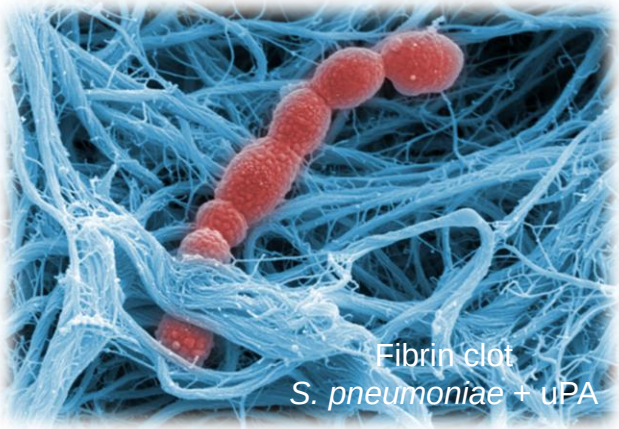
Staphylococcus aureus

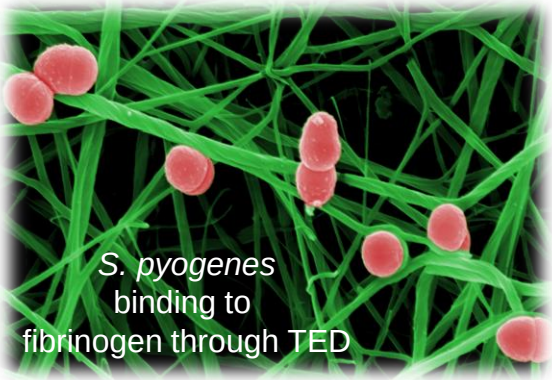
Pseudomonas aeruginosa

Escherichia coli

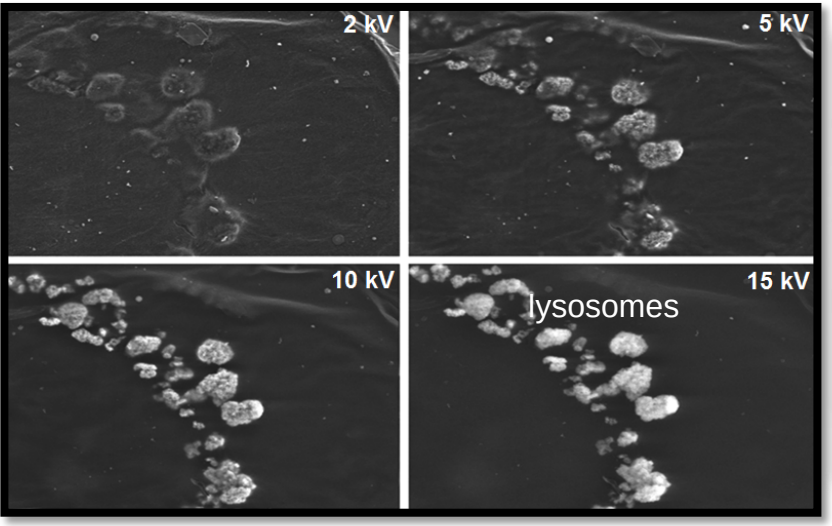
Salmonella typhimurium

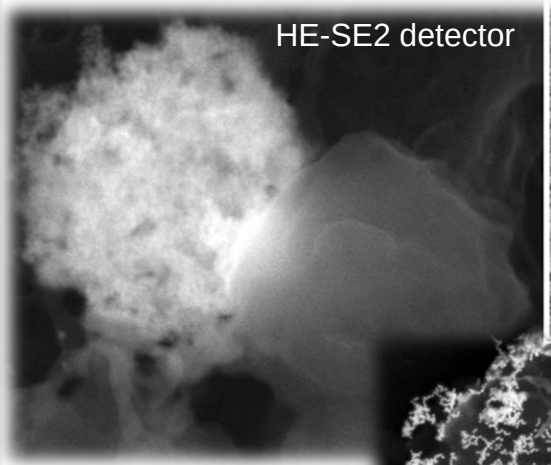
3. Interactions of matrix proteins with pathogenic bacteria



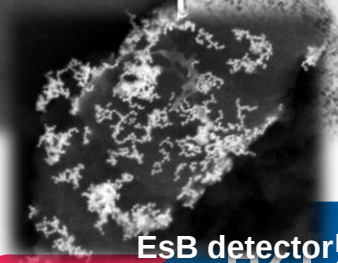


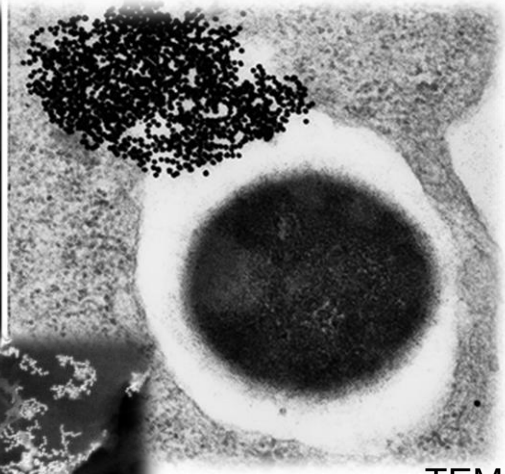
4. Intracellular trafficking, i.e., fusion with BSA-gold loaded lysosomes





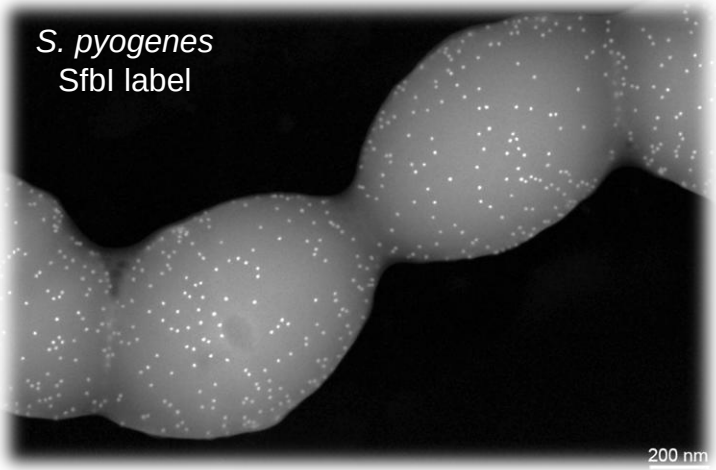
FESEM

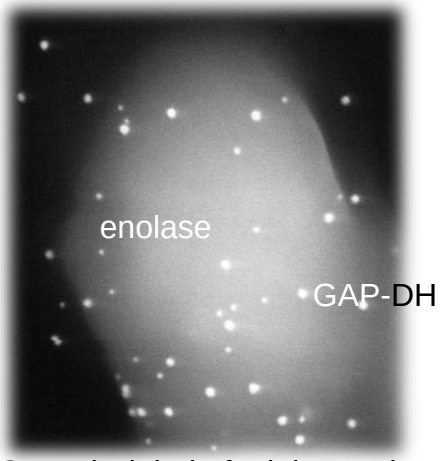




TEM

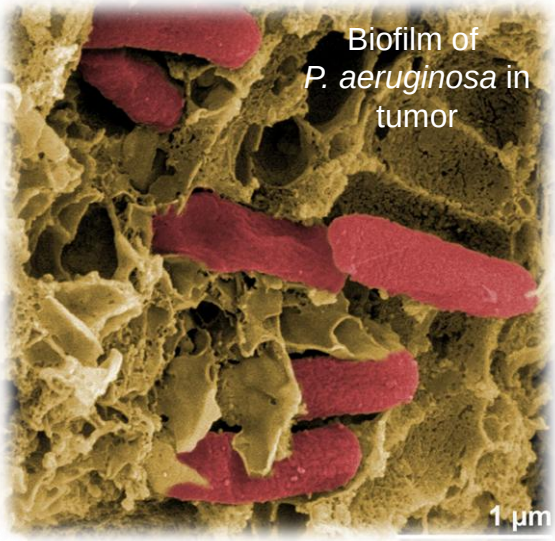
5. Identification of pathogenicity factors on bacterial surfaces with antibodies and gold-nanoparticles



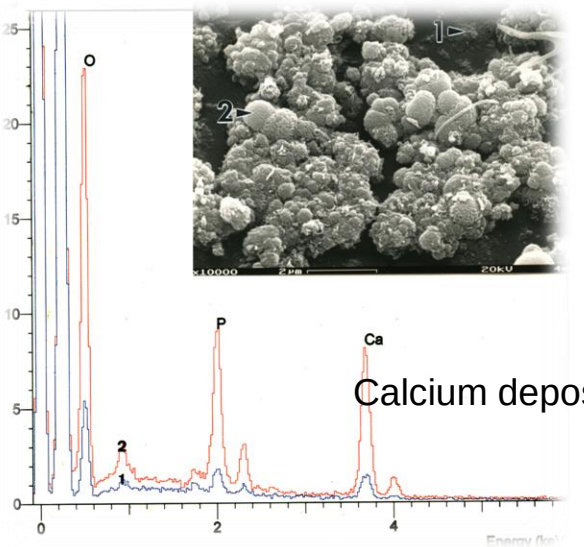


S. canis, label of alpha enolase and GAP-DH

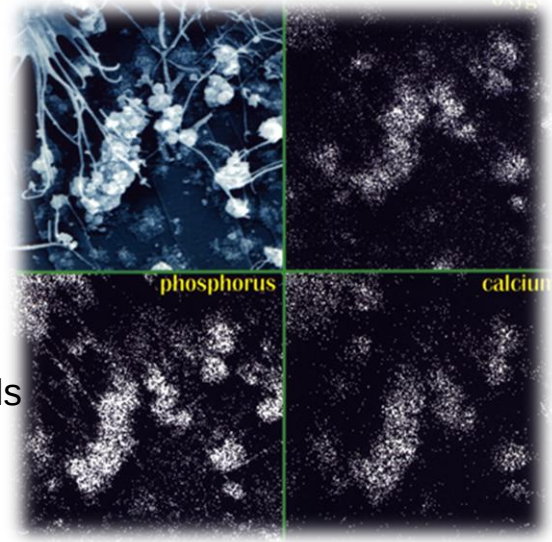
6. Fracture of critical-point dried samples for biofilm formation and dissemination of pathogenic bacteria in tissues

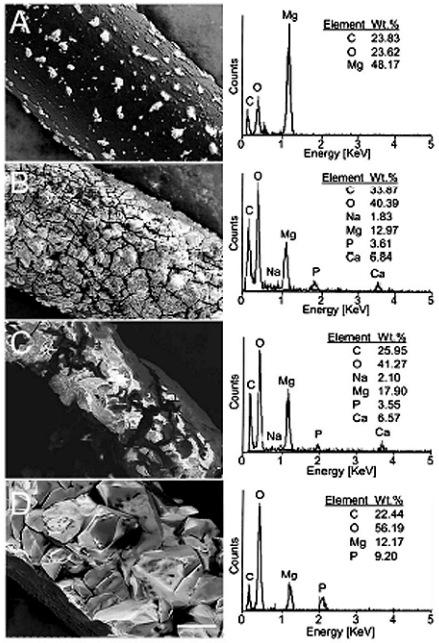


7. Identification of elements by EDS (energy dispersive analysis, EDX)



Calcium deposition in BMP-2 cells





Corrosion of magnesium implants

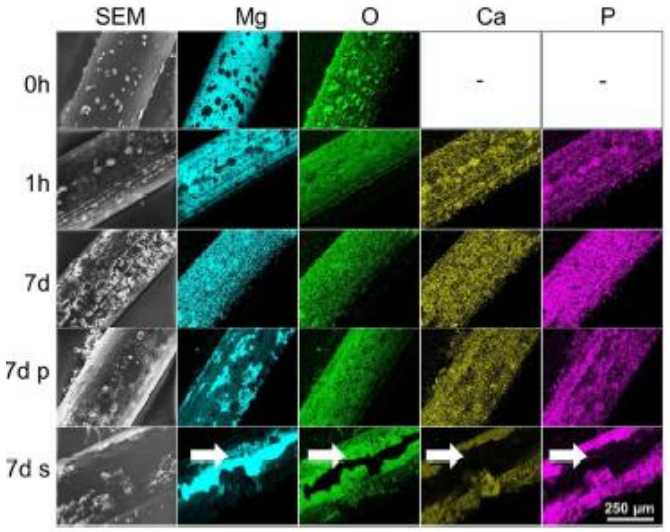


Image EDX of corroded magnesium implants

Overview Light Microscopy

Microscope configurations

- Inverted
- Upright

Transmitted Light Imaging

Phase contrast
DIC
Color brightfield

Tissue samples
Bacterial samples

Fluorescence microscopy

Live or fixed samples
Tissue samples, bacterial samples, nanoparticles
stained with fluorophores,
fluorophores bound to antibodies
(Immunofluorescence)
Fluorescent proteins

Fluorescence microscopes

- widefield images
- fluorescent images overlaid on the DIC image

Confocal Systems

Multi-dimensional acquisition
3D z-stacks
multi-channel
Live-cell imaging
Time-lapse at multiple locations

Specifications for light microscopes

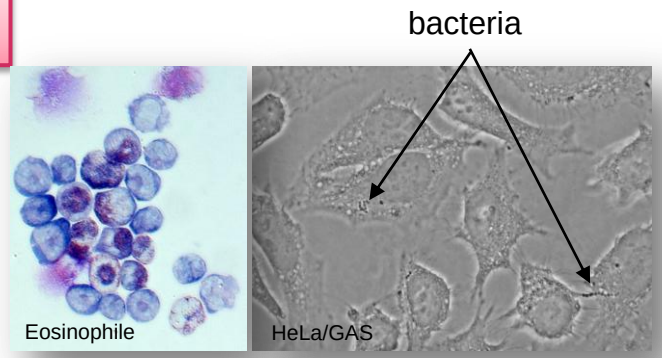
Mikroskop	Software, Kamera	Objektive	Filter set	Lampe	motori. Tisch	Computer
Zeiss Imager. A1	Zeiss Axiovision 4.6	**EC Plan-Neofluar**	DIC	HXP 120	nein	Fujitsu-Siemens
"ZEIM-Mikroskop 1"		10x/ 0,30 Ph1 420341-9911	DAPI	LED Lampe		Windows XP
	Zeiss	**EC Plan Neofluar**	FITC			Intel Xeon 2 GHz
	Axiocam MRm	40x/ 0,75 Ph2 420361-9910	CY3			2 GB RAM
	Axiocam Mrc	**Plan Apochromat**	CY5			
		63x/ 1.4 oil Ph3 420781-9910	FS24			
		Plan Apochromat				
		100x/ 1.4 oil Ph3 420791-9910				
Zeiss Imager.A2						
"Zeim-Mikroskop 2"	ZEN 2011 blue	**Plan Neofluar**	DAPI position 1	HXP 120C	nein	Fujitsu
		10x/ 0,30 440330	FITC	LED Lampe		Windows 7, 64 bit
	Zeiss	**Plan Neofluar**	CY3			Intel i5-2500
	Axiocam MRm	40x/ 0,75 Ph2 440351	FS24			3,3 GHz 8 GB RAM
		Plan Neofluar	CY5			
		40x/ 1.3 oil Ph2 440450	DIC			
		Plan Neofluar				
		63x/ 1,25 oil Ph3 440461				
		Plan Neofluar				
		100x/ 1,3 oil Ph3 440481				
Zeiss Imager.Z2	ZEN 2012 blue	**EC Plan Neofluar**	DAPI	HXP 120	ja	Fujitsu-Siemens
"ZEIM-Mikroskop 3"		10x/ 0,30 440330-9902	FITC	LED Lampe	großer Tisch mit	Windows 7, 64 bit
	Zeiss	**Plan Apochromat**	DsRed		Stitching	Intel Xeon
	Axiocam MRc5	40x/ 0,95 Korr 440654-9902	Cy5			2,66 GHz, 4 GB RAM
		EC Plan Neofluar	TL 1.6x			
		63x/ 1,3 DIC Imm Korr	free			
		440872-9970				
		Plan-Neofluar				
		2,5 x/ 0,075 440310				
		Plan Apochromat				
		20x/ 0,8 440640-9903				
		Plan-Apochromat				
		150x/ 1,35 DIC Gly Korr VIS-IR				
		420792-9970				
Zeiss Axiovert 100	Axiovision 4.8.2	**Plan Neofluar**	FITC	HBO 50 Watt	nein	Fujitsu
inverses Mikroskop		20x/ 0.30 Ph2	Rhodamin	Glühbirne	mit Inkubations-	Windows 7 Ultimate
"ZEIM-Mikroskop 4"	Zeiss	**LD Achroplan**	DAPI		kammer und	2 DUO E8400
	Axiocam HRc	40x/ 0.60 Korr Ph2			Flow chamber	3 GHz, 2 GB RAM
		Plan Neofluar			von IBIDI	
		63x/ 1,3 oil Ph3				
		Plan Neofluar				
		100x/ 1.30 oil				
Zeiss Axiovert 200 M	Axiovision 4.5.	**Plan Neofluar**	FITC	HBO 100 Watt	ja	Fujitsu
inverses Mikroskop		10x/0.30 Ph1	Rhodamin			Windows 2000
	Zeiss Axiocam HrC	**LD Achromat**	DAPI			1,7 Ghz, 500 MB RAM
		20x/0.40				
		LD Achromat				
		40x/0.60 Ph2				

Specifications for confocal microscopes

Mikroskop	Software, Detektoren	Objektive	Filter set	Lampe / Laser	motori. Tisch	Computer	am HZI Netz
Leica SP5 aufrecht							
"Leica SP5a"	LAS AF	HCX PL Fluotar 10x/0,30 (11506507)	A (UV)	Leica Kübler codix	ja	HP Pentium Workstation	nein
		HCX APO 20x/0,5 (11506147)	I3 (blue)		z-drive (piezo)	Windows XP	
DM6000B CS		HCX PL APO CS 63x/1,2W (11506281)	N21 (green)	405 (diode)		Intel Core 2 Duo	
	3x PMT	HCX PL APO 63x/1,4 Oil (11506206)		458/476/488/496/514 (Argon)		E8400 @3.00 Ghz	
	TL Detektor			561 (DPSS)		3,25 GB RAM	
		HCX PLAN APO 20x/0,7 (11506170)		633 /HeNe)			
Leica SP5 invers							
"Leica SP5i"	LAS AF	HCX PL Fluotar 10x/0,30 (11506507)	I3 (blue)	ebq 100	ja	HP Pentium Workstation	nein
		HCX PL APO 20x/0,7 (11506166)	N21 (green)		z-drive (piezo)	Windows XP	
DM6000B CS		HCX PL APO CS 40x/1,3 Oil UV (11506330)		405 (diode)		Intel Core 2 Duo	
	2x HyD	HCX PL APO lambda blue 63x/1,4 Oil UV (11506192)		458/476/488/496/514 (Argon)		E8400 @3.00 Ghz	
	2x PMT			561 (DPSS)		1,98 GB RAM	
	TL Detektor			633 /HeNe)			

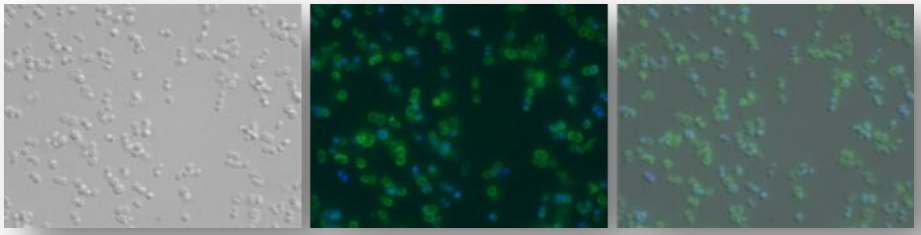
Light Microscopy

- ✓ Imaging of a huge range of bacterial and tissue samples
- ✓ Sample requiring from x10 to x100 objectives on a widefield
- ✓ Colour imaging

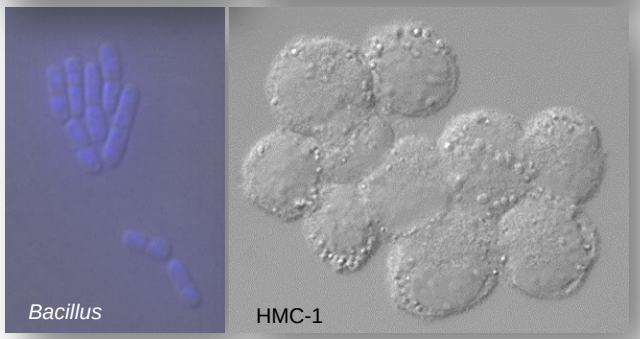


Fluorescence microscopy

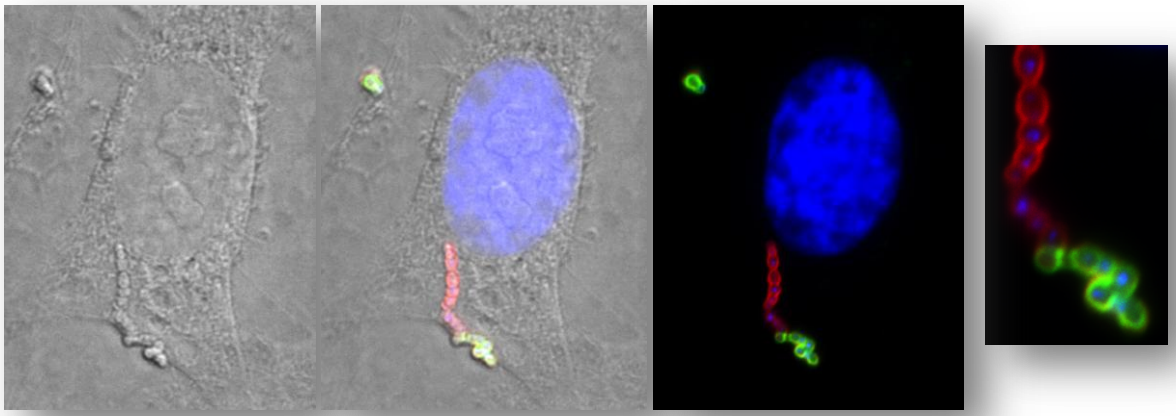
- ✓ Fluorescent images overlaid on the DIC image



antibody anti *Staphylococcus aureus* Sa02/DAPI



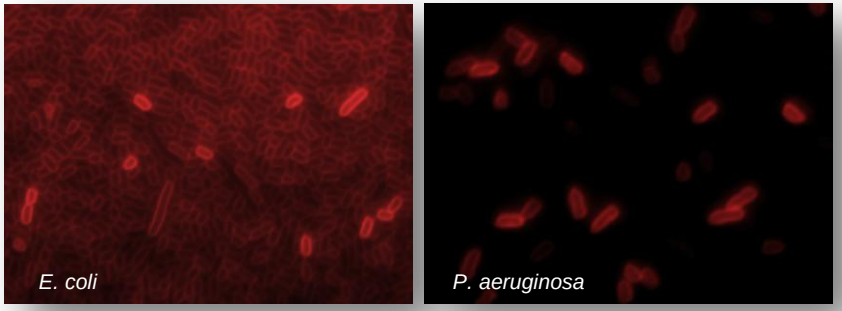
- ✓ Double immunofluorescence staining for extra and intra-cellular bacteria



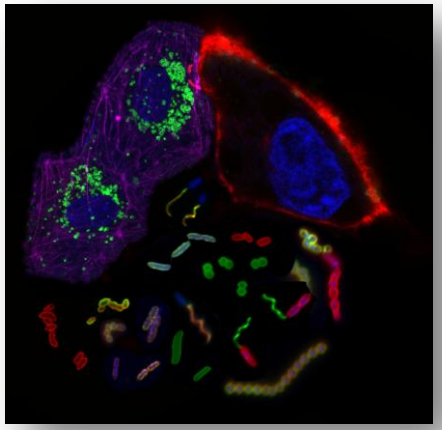
HeLa cells infected with
Streptococcus pyogenes (GAS)
Extra/Intra GAS
DNA
DIC

Fluorescence Microscopy

- Observation of life bacterial cells in agarose pads.
  Cells labeled or expressing a fluorescent proteins (e.g. GFP)



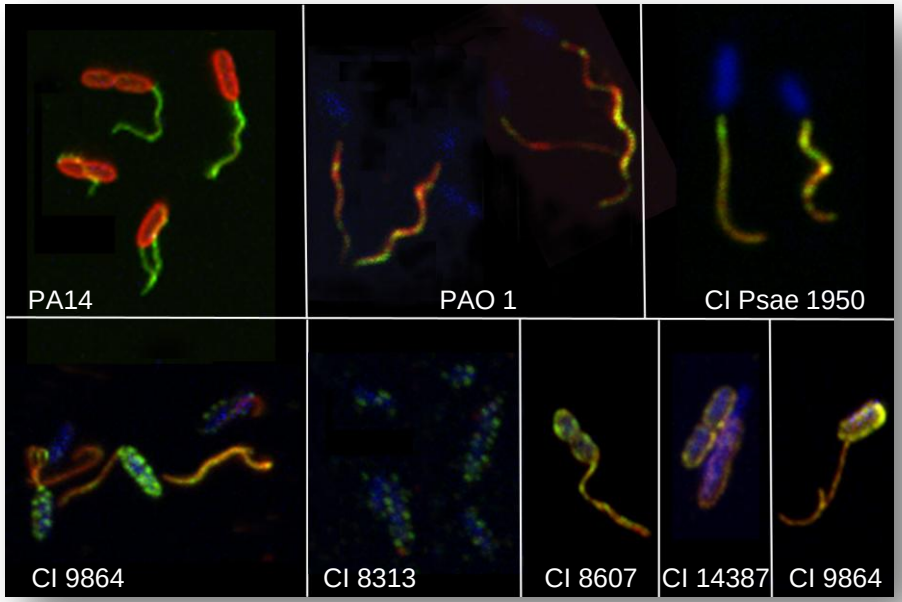
Live bacteria: labeling of membranes with the lipophilic dye FM4-64



Bacterial samples

Tissue samples

- Localization of proteins

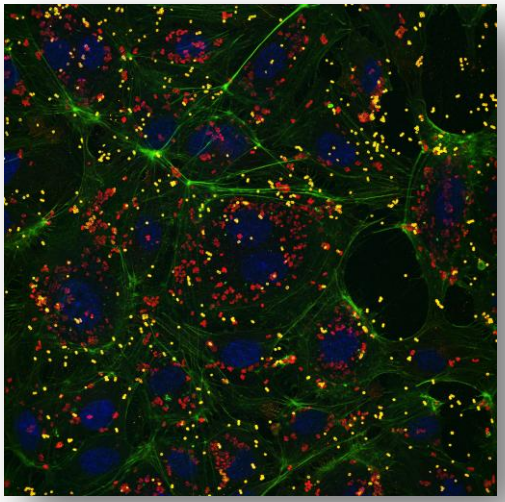


Pseudomonas aeruginosa: double immunofluorescence staining of **FliC**, the structural protein component of the flagellar filament and **DnaK**, a molecular chaperone. Strains PA14, PAO1 and different clinical isolates (CI).

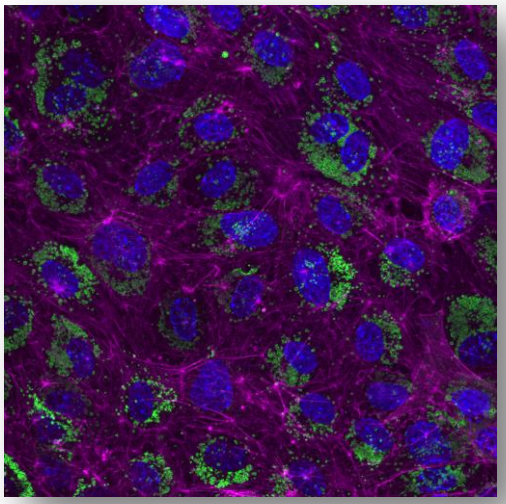
Confocal Microscopy

- **Multi-dimensional acquisition:**
- **Multi-channel and 3D z-stack**

- Protocol for adherent cells
- Host-pathogen interaction

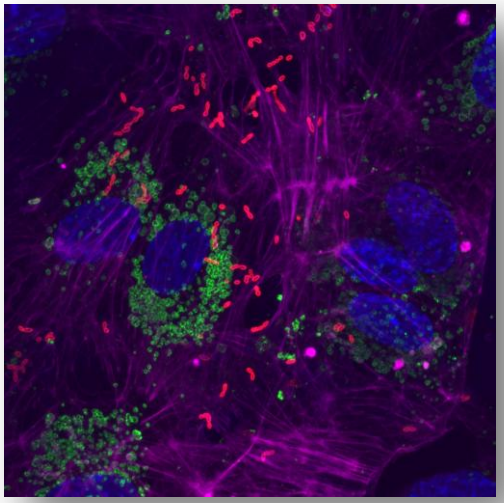


Intra and
extracellular GAS A60
Actin
HUVEC

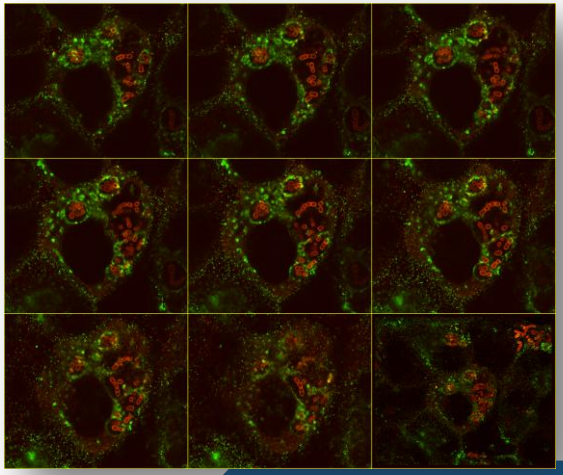


HUVEC
EEA1
Actin

- Intracellular compartments and trafficking pathways
- Co-localization studies



S. suis
EEA1
Actin
HUVEC

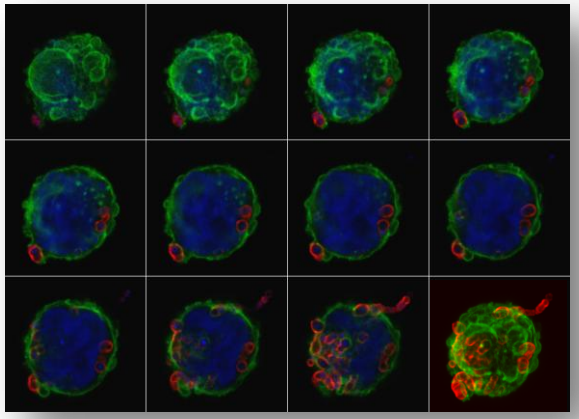


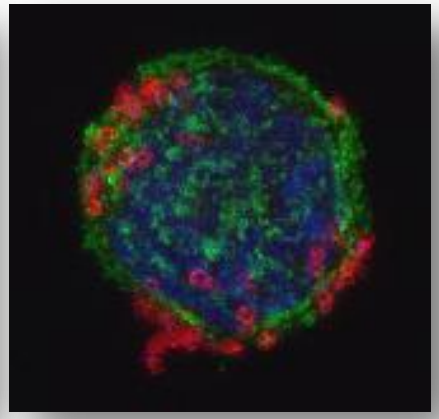
S. zooepidermicus
LAMP1
HeLa cells

Confocal Microscopy

- **Multi-dimensional acquisition:**
- **Multi-channel and 3D z-stack**

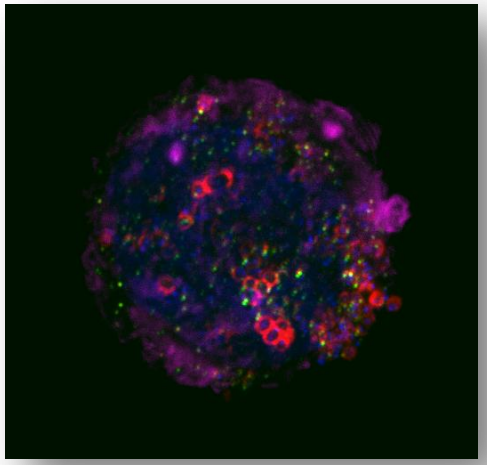
- Protocol for non-adherent cells: Immunofluorescence on cells in suspension
- Host-pathogen interaction





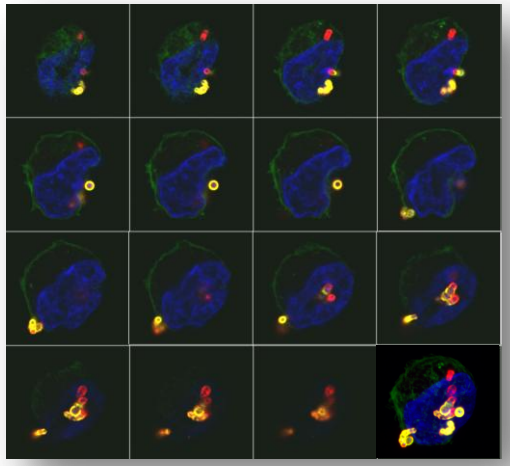
Staphylococcus aureus
Human Mast Cells HMC-1
Phalloidin
DAPI

- Intracellular compartments and trafficking pathways
- Co-localization studies

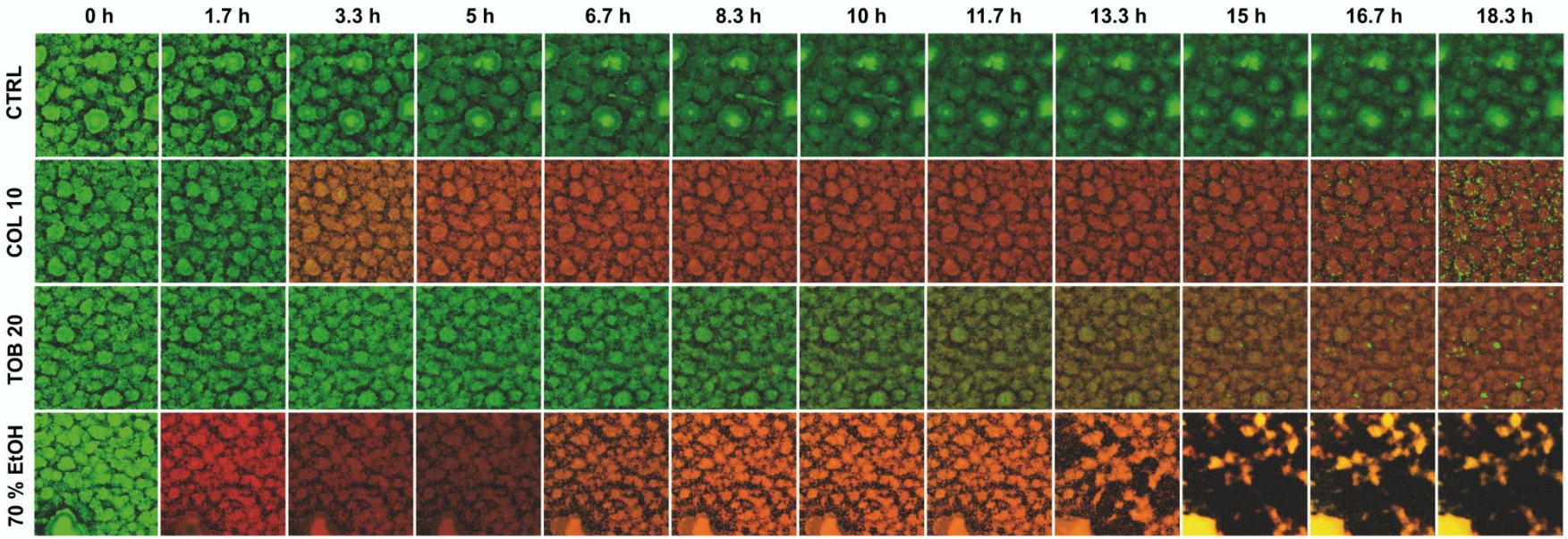


Staphylococcus aureus
Human Mast Cells HMC-1
Caveolin-1
Phalloidin
DAPI

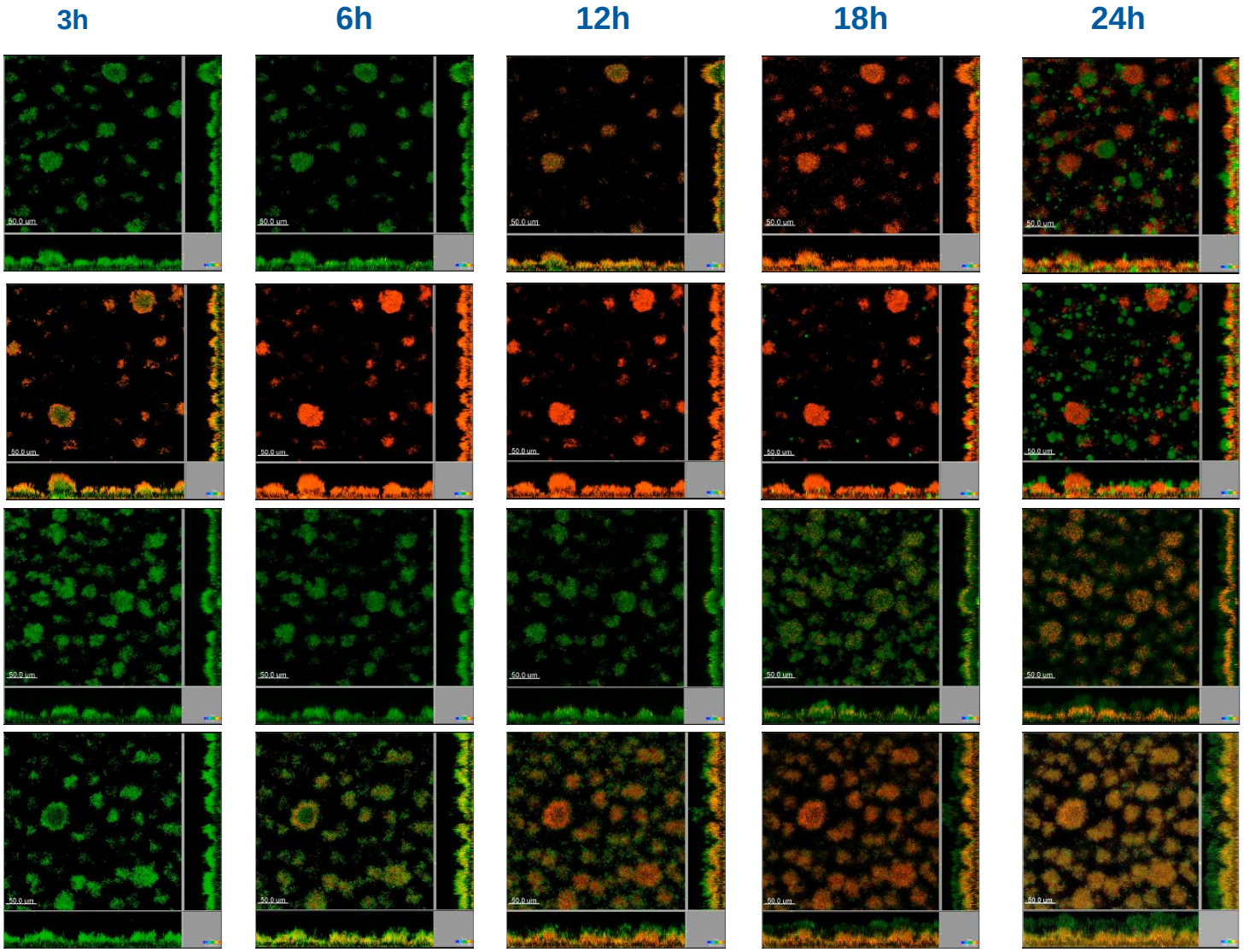
Intra and extracellular
***Streptococcus pyogenes* A8**
Human Mast Cells HMC-1
Phalloidin
DAPI



Live Cell Imaging:
P. aeruginosa biofilms treated with antibiotics



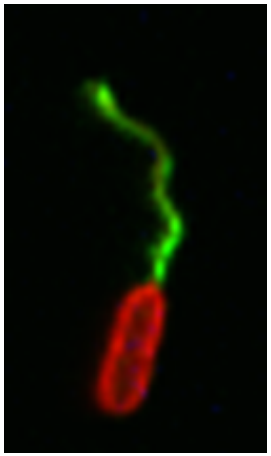
Live Cell Imaging:
P. aeruginosa biofilms treated with antibiotics

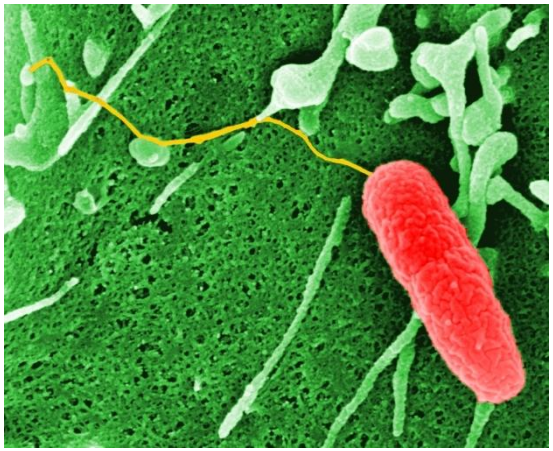


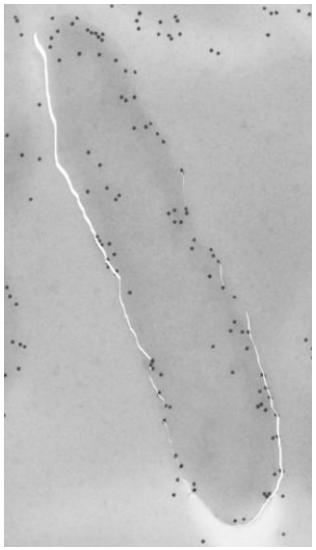
48 h old *P. aeruginosa* biofilms treated fror 4 hours with antibiotics

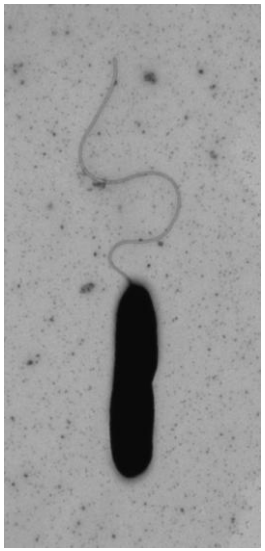
➢ A strength of ZEIM is applying a variety of approaches/techniques to investigate biological processes

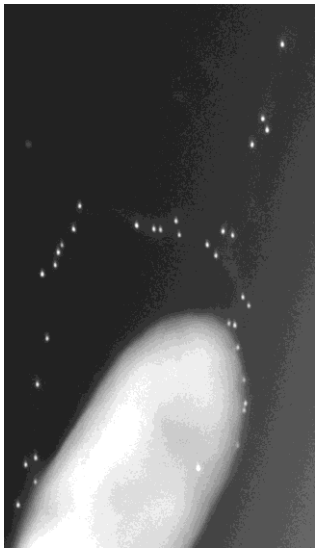
Pseudomonas aeruginosa: investigating the interaction between **FliC**, the structural protein component of the flagellar filament and the chaperone **DnaK**.

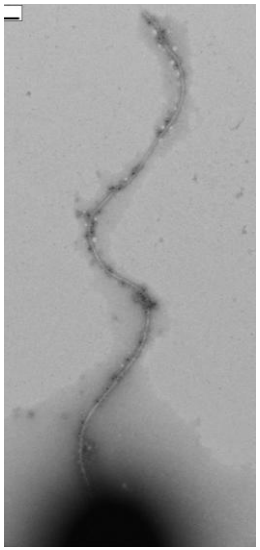


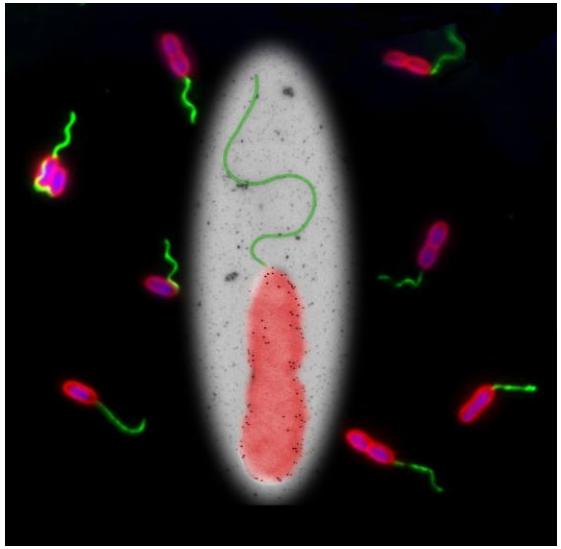






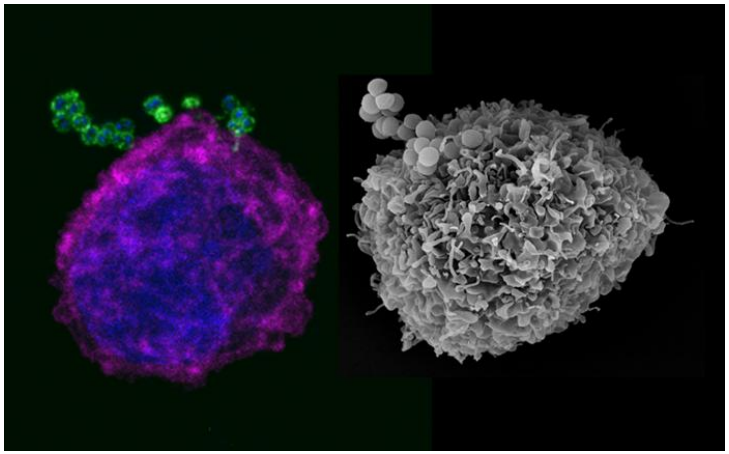




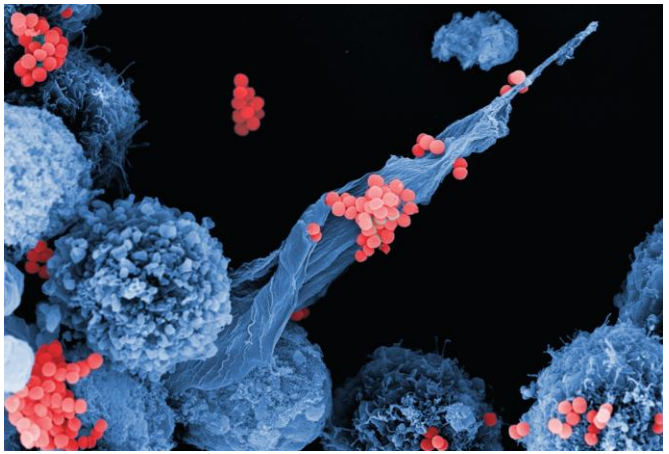


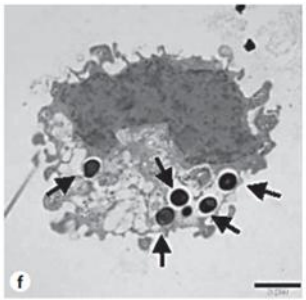
HZI (ZEIM) and TU-BS collaboration

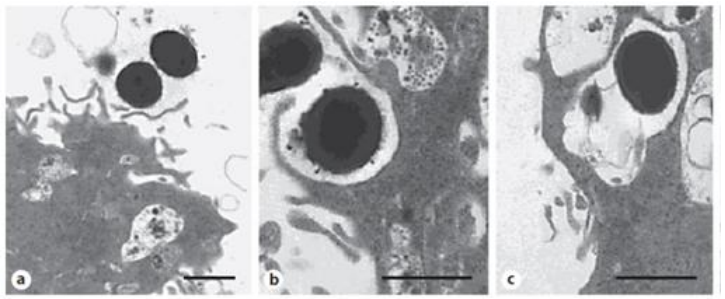
➢ A strength of ZEIM is applying a variety of approaches/techniques to investigate biological processes

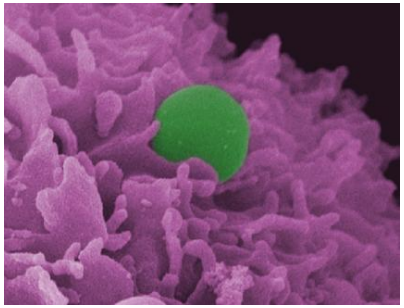


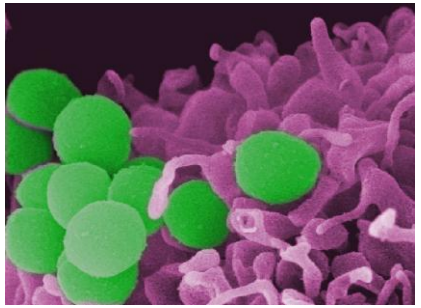
Unraveling the complex internalization process of *Staphylococcus aureus* in Human Mast Cells HMC-1

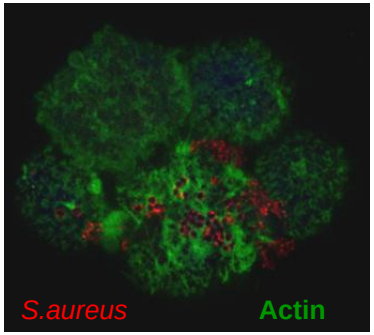


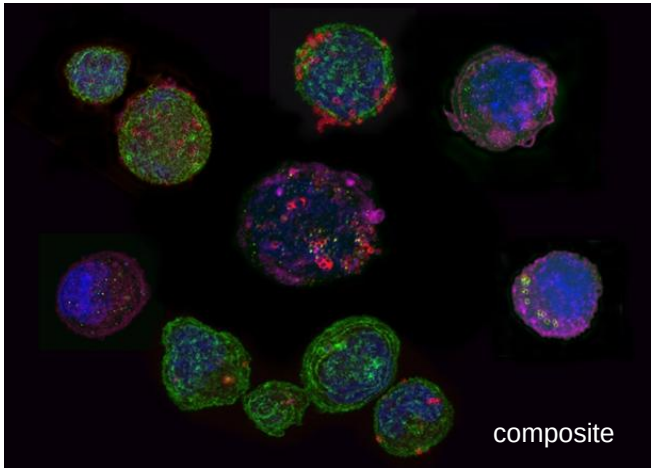


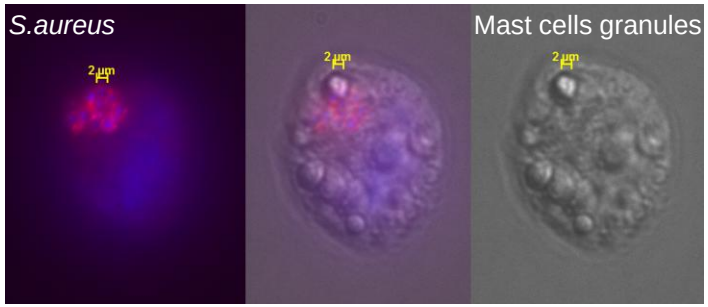






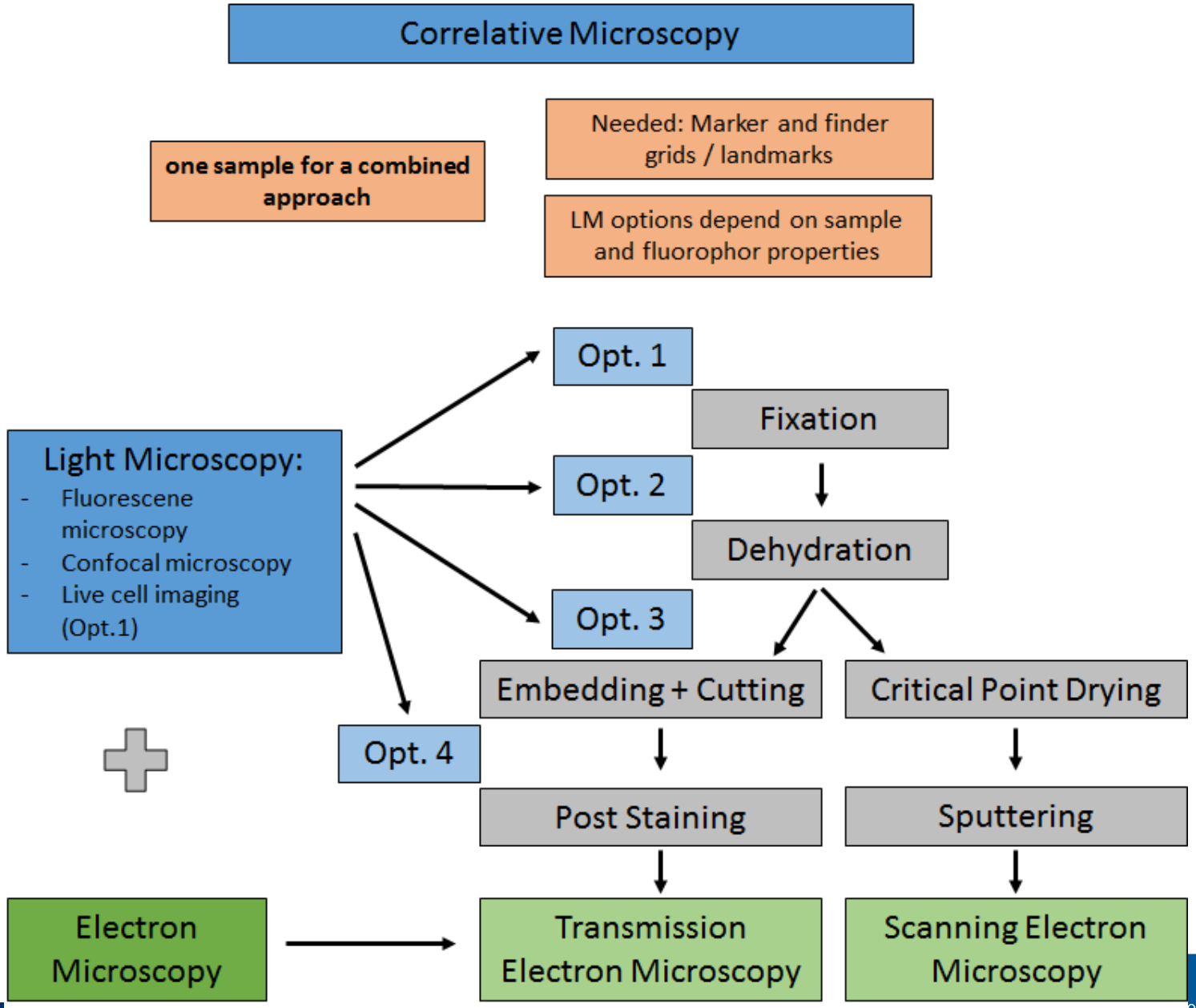




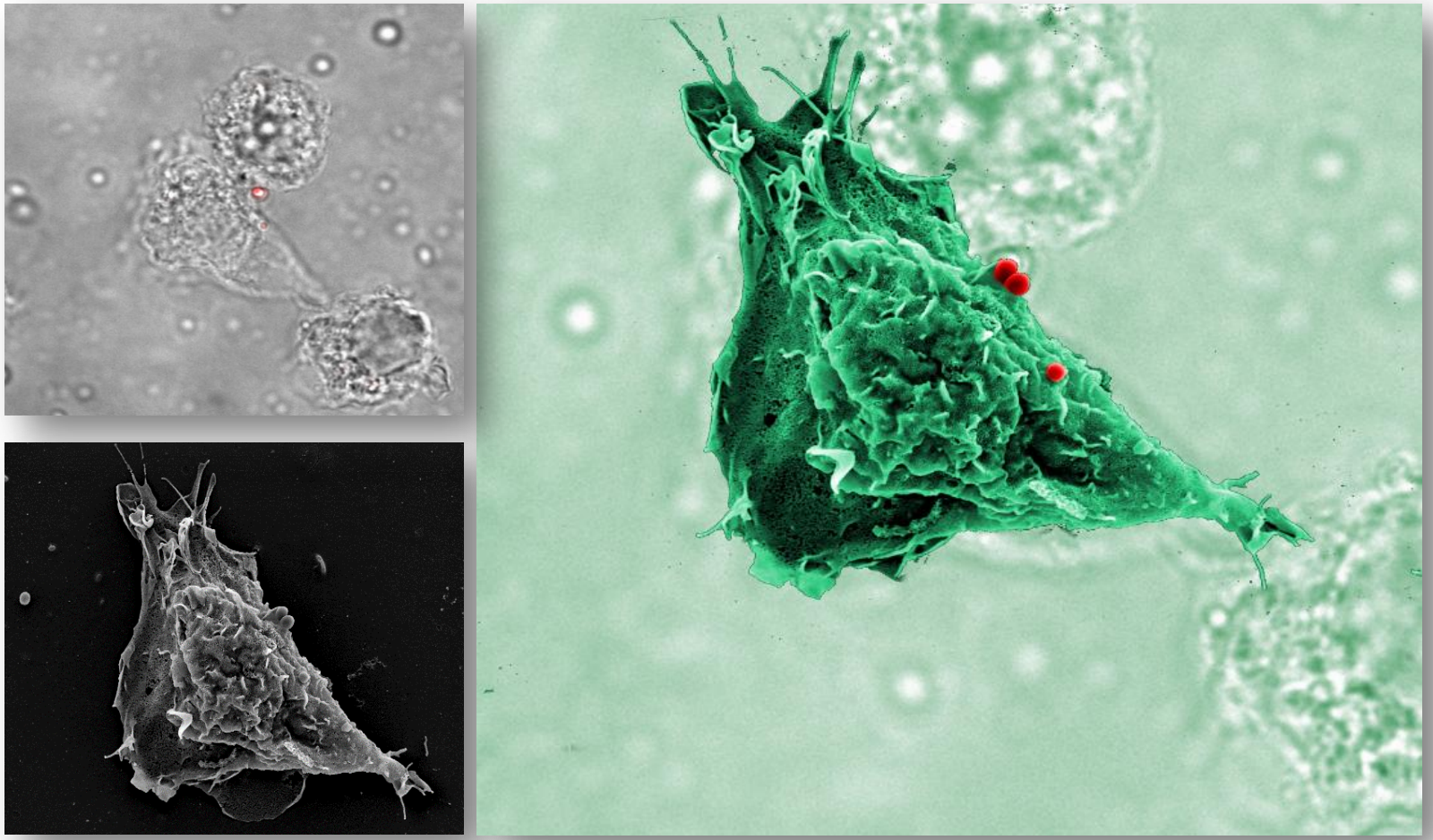


Co-operation INI and ZEIM

Correlative light and electron microscopy, CLEM

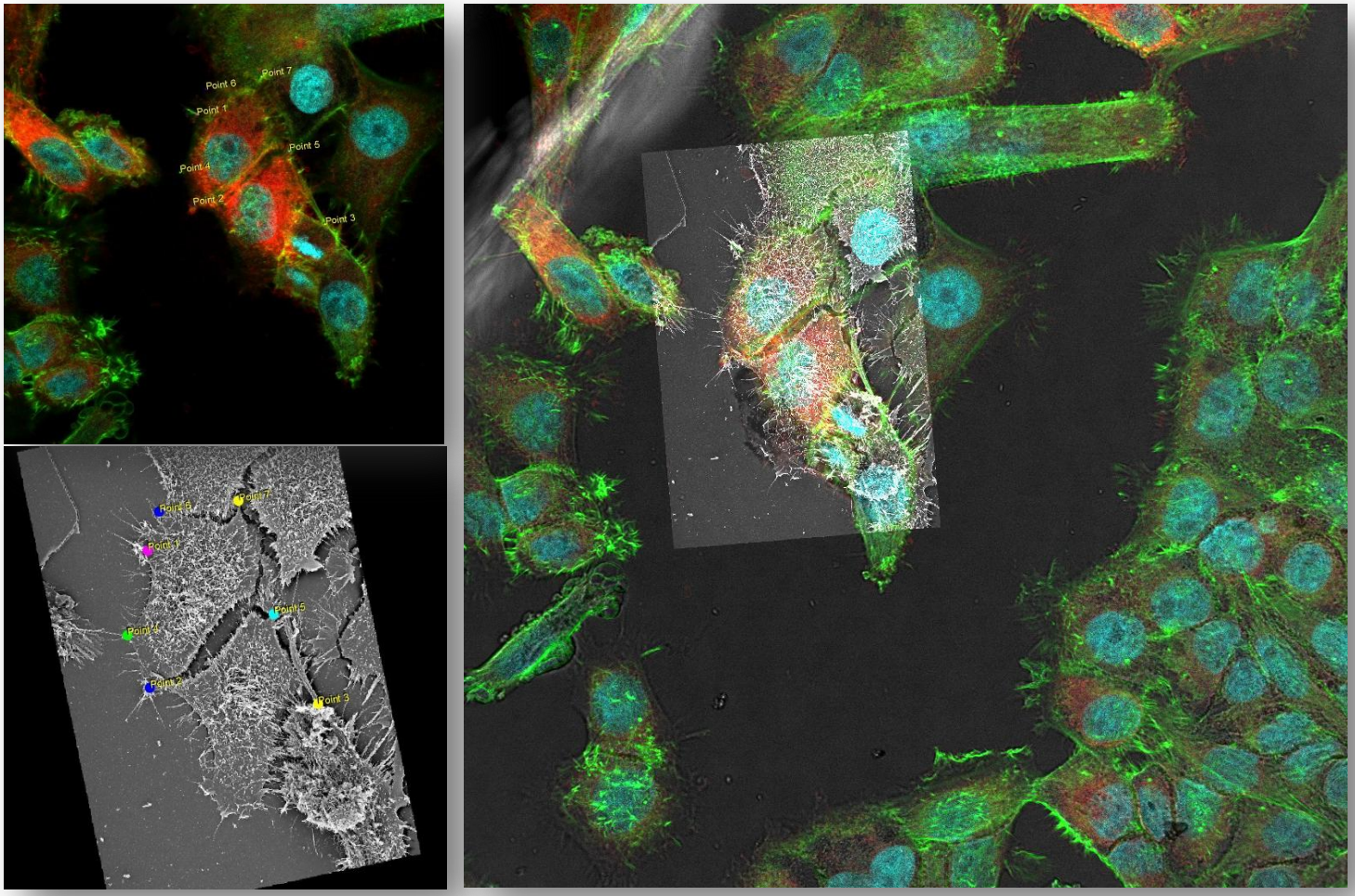


CLEM: Fluorescence + FESEM



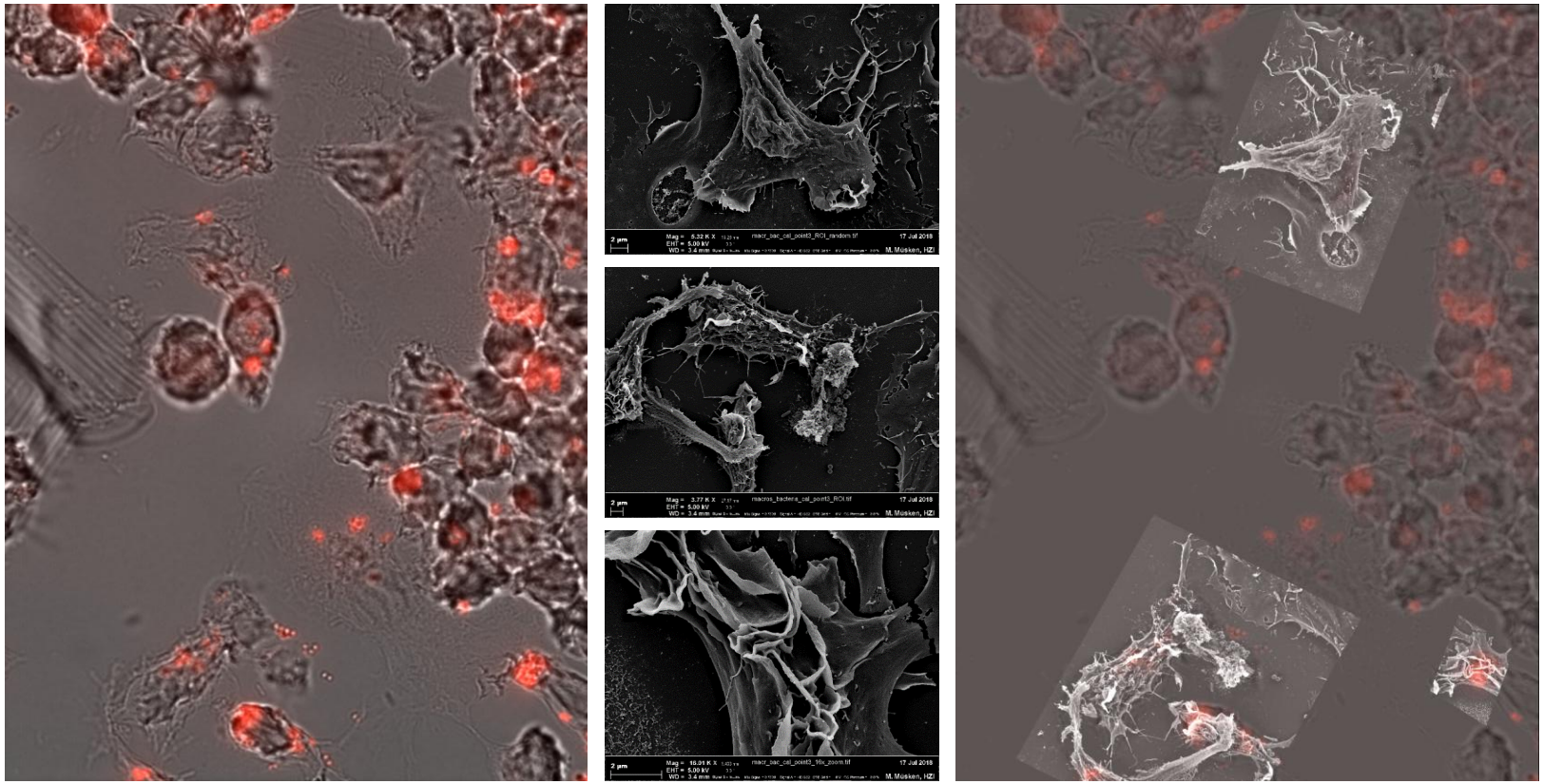
Macrophage with *S. aureus, red*

CLEM: Confocal + FESEM



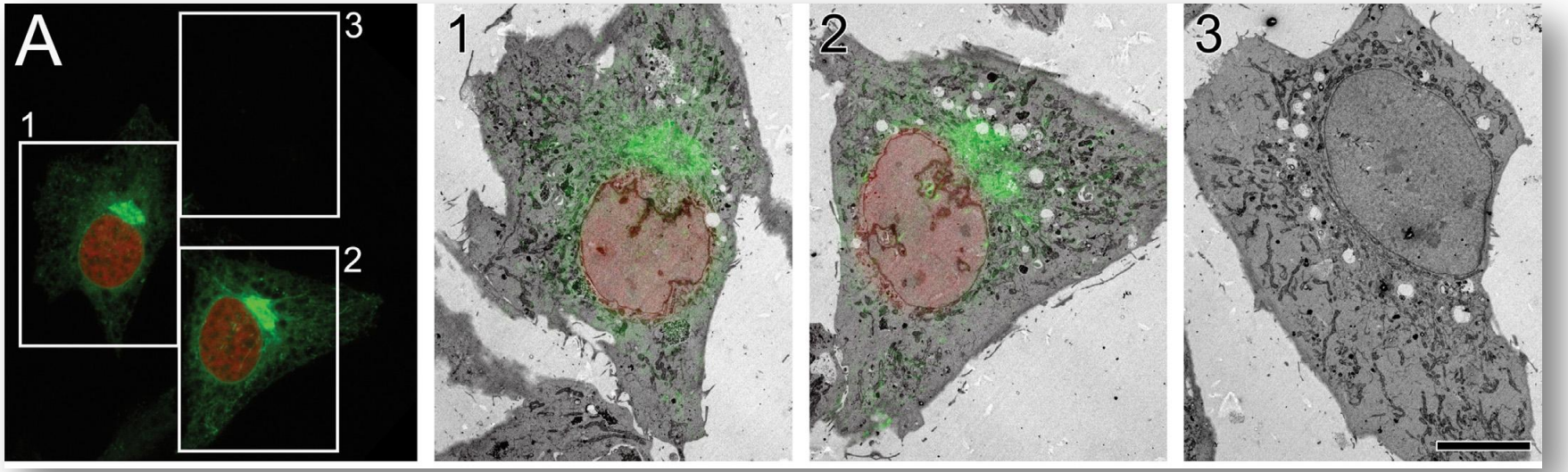
HEp-2 cells DAPI, Phalloidin Alexa 488nm for actin, cellmask deep red

CLEM: Fluorescence + 3x FESEM



HEp-2 cells with *S. aureus* (red)

CLEM: Fluorescence + TEM



Correlative and integrated light and electron microscopy of in-resin GFP fluorescence, used to localise diacylglycerol in mammalian cells, Peddie C. et al., Ultramicroscopy 143 (2014) 3–14

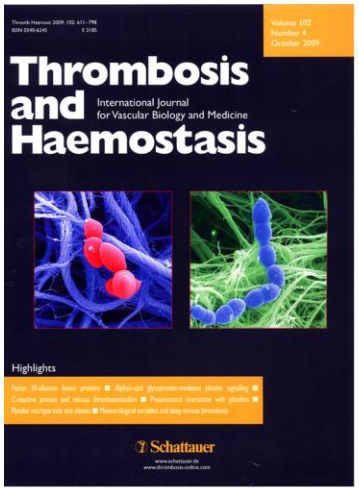
ZEIM Fees, internal use

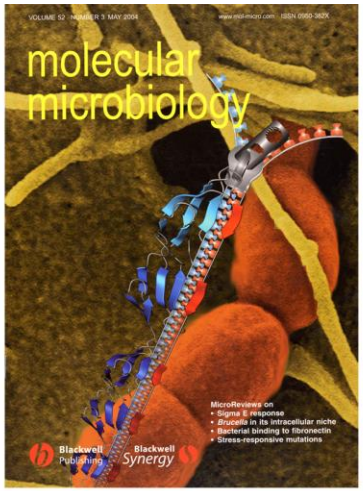
Equipment	Fees (1 h)
TEM EM 910	8,31 €
EM Libra 120 Plus	15,88 €
Field Emission SEM Merlin	16,48 €
Fluorescence Microscope Zeiss Imager Z2	1,47 €
Fluorescence Microscope Zeiss Axio Imager A2	0,30 €
Fluorescence Microscope Zeiss Axio Imager A1	1,02 €
Laser scanning Leica SP5 Inverted Confocal/Live Cell Station	7,16 €
Laser scanning Leica SP5 Upright Confocal	6,18 €

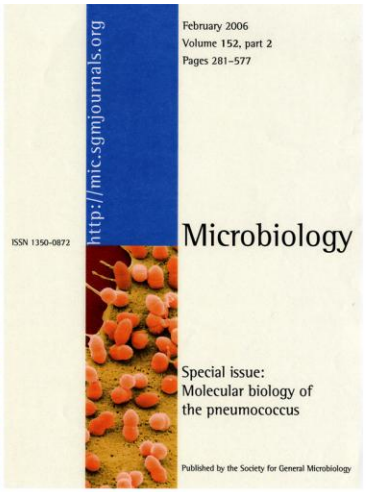
As 01.07.2018

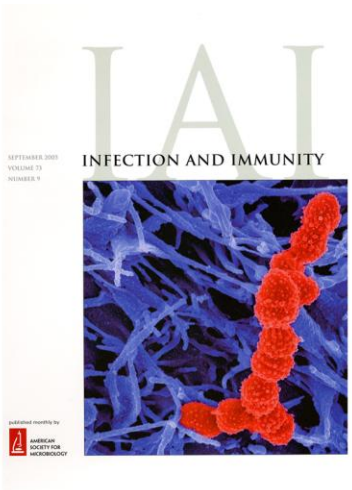
For internal invoicing of services delivered by the ZEIM platform and other fees see

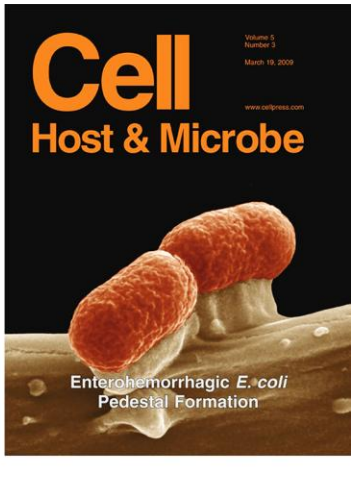
http://intranet-hzi/I/W/Seiten/Preisliste---Plattformleistungen.aspx

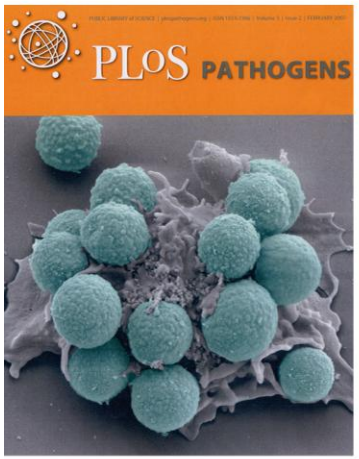

Thrombosis and Haemostasis
International Journal for Vascular Biology and Medicine
Volume 102
Number 4
October 2009
Highlights
Schattauer

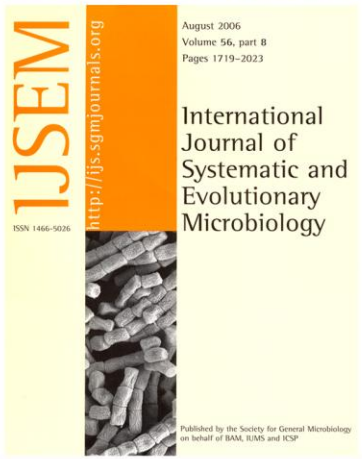

molecular microbiology
MicroReviews on
• Sigma E response
• Brucella in its intracellular niche
• Bacterial binding to fibronectin
• Stress-responsive mutations
Blackwell Publishing
Blackwell Synergy

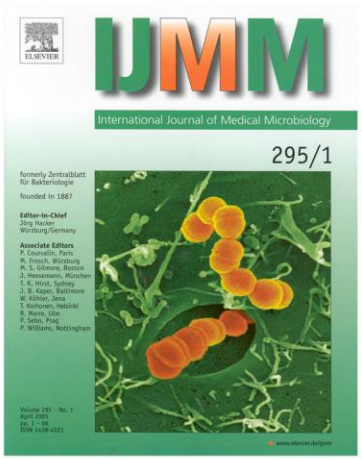

http://mic.sgmjournals.org
February 2006
Volume 152, part 2
Pages 281–577
ISSN 1350-0872
Microbiology
Special issue:
Molecular biology of the pneumococcus
Published by the Society for General Microbiology

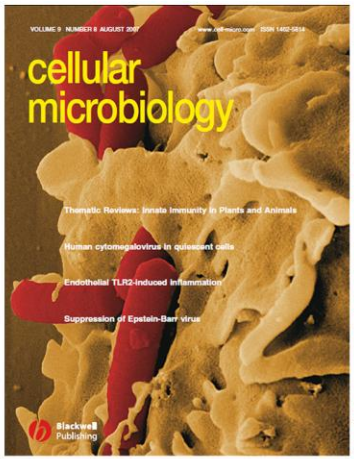

IAI
INFECTION AND IMMUNITY

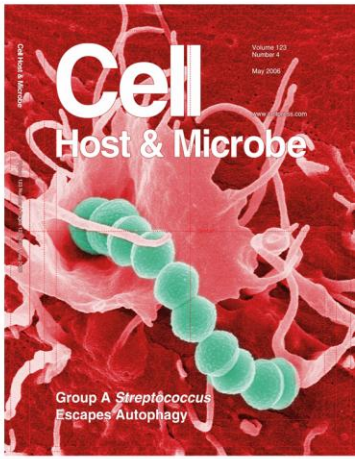

Cell
Host & Microbe
Volume 5
Number 3
March 19, 2009
Enterohemorrhagic E. coli
Pedestal Formation

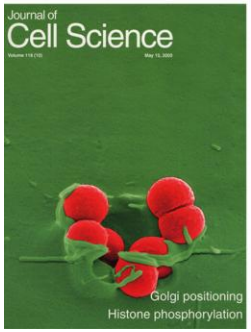

PLoS PATHOGENS


IJSEM
http://ijs.sgmjournals.org
ISSN 1466-5026
August 2006
Volume 56, part 8
Pages 1719–2023
International Journal of Systematic and Evolutionary Microbiology
Published by the Society for General Microbiology on behalf of BAM, IUMS and ICSP


IJMM
International Journal of Medical Microbiology
295/1

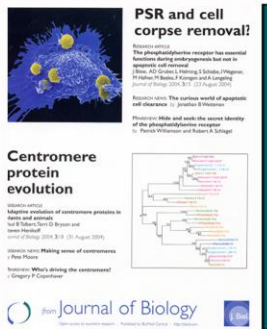

cellular microbiology
Thematic Reviews: Innate Immunity in Plants and Animals
Human cytomegalovirus in quiescent cells
Endothelial TLR2-Induced Inflammation
Suppression of Epstein-Barr virus
Blackwell Publishing


Cell
Host & Microbe
Volume 123
Number 4
May 2006
Group A Streptococcus
Escapes Autophagy

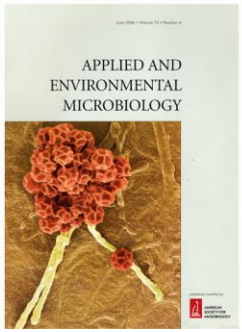

Journal of Cell Science
Golgi positioning
Histone phosphorylation


blood


molecular microbiology


PSR and cell corpse removal?
Centromere protein evolution
from Journal of Biology


CHEMBIOCHEM
11/2007


APPLIED AND ENVIRONMENTAL MICROBIOLOGY


JBC
JOURNAL OF BIOLOGICAL CHEMISTRY
ASBMB