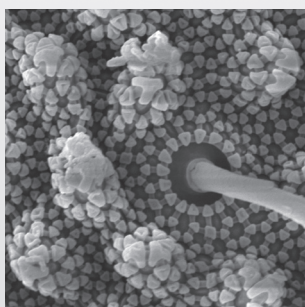


Living up to Life

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MICROSYSTEMS

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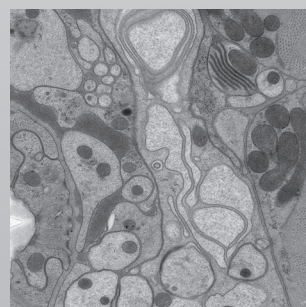
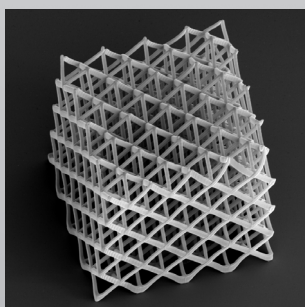
Life Science
Research

Application Note

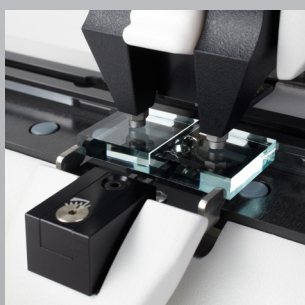
Epoxy resin embedding of animal and human tissues for pathological diagnosis and research

related instrument Leica EM AMW

Medical
Research



Industrial
Manufacturing



Natural
Resources



Epoxy resin embedding of animal and human tissues for pathological diagnosis and research

*Courtesy of: Dr. Josef A. Schröder, Universitätsklinikum Regensburg, Institut für Pathologie
Zentrales EM Labor, F-J-Strauss Allee 11, 93053 Regensburg, Germany*

Ultrastructural analysis

Epoxy resin embedding of different tissues
e.g. animal tissues, human tumours and non-neoplastic lesions

i. Reagents

Cacodylate buffer (0.1M)

Fixatives: modified Karnovsky fixative (2% paraformaldehyde + 2.5% glutaraldehyde in 0.1M cacodylate buffer); Osmium-tetroxide OsO₄ (1% in 0.1M cacodylate buffer)

Ethanol (50%, 70%, 95%, abs.)

Acetone abs.

Epoxy resin (all EPON components from Electron Microscopy Sciences, Munich/Germany):

EMbed 812	90g	#14960
DDSA	60g	#13710
NMA	40g	#19000
DMP-303g	#13600	

ii. Fixation protocol

The tissues were fixed in the modified Karnovsky fixative generally by immersion overnight (at minimum 4h at room temperature). Then pieces of approx. 1mm³ were cut with a sharp razor blade and processed for embedding in the AMW.

iii AMW programs

Reagent list

Name	Type	Max. Temp. (°C)	Max. Power (watt)	Drain pause
Cacodylate buffer	Rinse	60	30	0
1% OsO ₄ (in buffer)	Fixative	50	30	0
dH ₂ O	Rinse	90	30	0
50% Ethanol	Solvent	66	30	0
70% Ethanol	Solvent	66	30	0
95% Ethanol	Solvent	66	30	0
Ethanol abs.	Solvent	66	30	0
Acetone abs.	Solvent	46	30	0
Acetone:EPON 1+1	Resin	50	30	10
Acetone:EPON 1+3	Resin	50	30	20
EPON	Resin	95	30	50

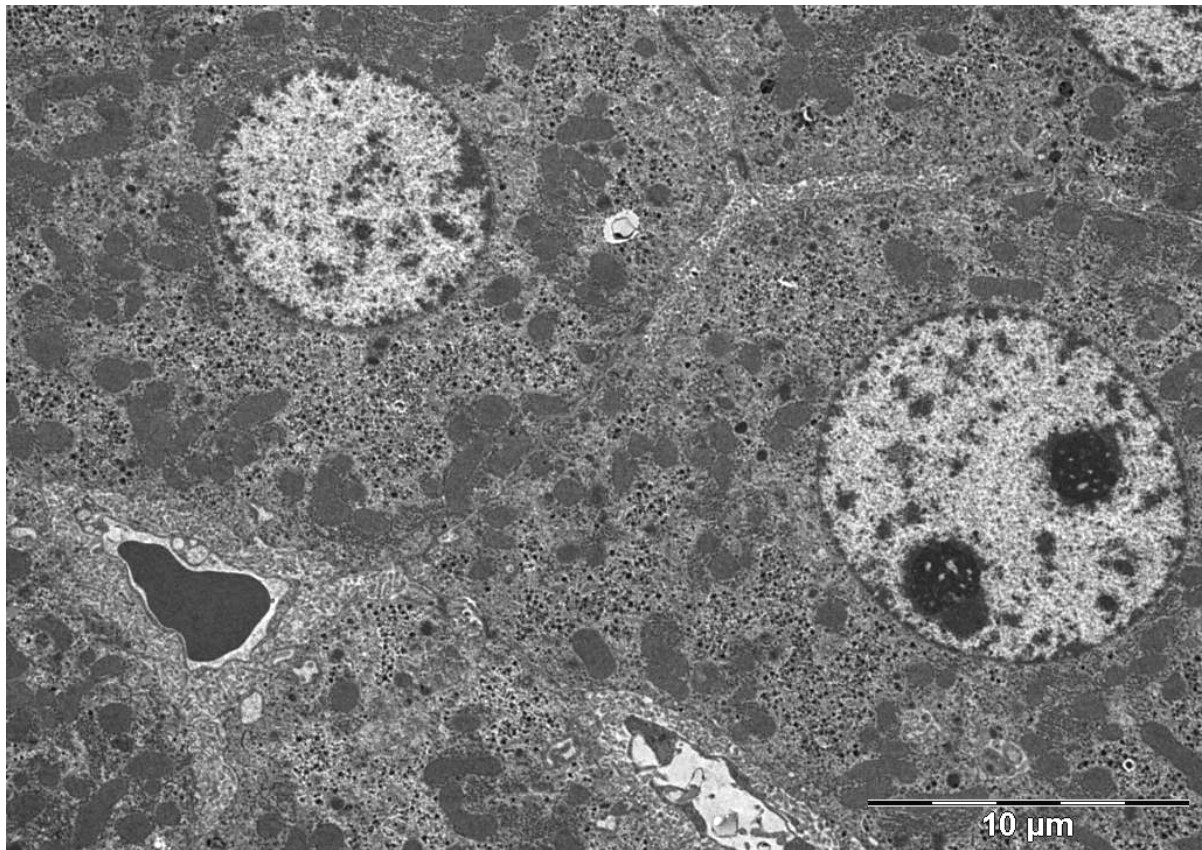
Epon embedding

Vial	Reagent	Step #	Time (hh:mm:ss)	Temp. (°C)	Power (watt)	Mode	Pause
1	Cacodylate buffer	1	00:05:00	35	15	Slope	
2	Cacodylate buffer	1	00:05:00	35	15	Slope	
3	Cacodylate buffer	1	00:05:00	35	15	Slope	
4	1% OsO ₄ (in buffer)	1	01:00:00	37	25	Pulse	
5	Cacodylate buffer	1	00:05:00	37	15	Slope	
6	dH ₂ O	1	00:05:00	37	17	Slope	
7	dH ₂ O	1	00:05:00	37	17	Slope	
8	50% Ethanol	1	00:05:00	37	15	Continuous	
9	70% Ethanol	1	00:05:00	37	15	Continuous	
10	95% Ethanol	1	00:05:00	37	15	Continuous	
11	Ethanol abs.	1	00:05:00	37	14	Continuous	
12	Acetone abs.	1	00:05:00	37	13	Continuous	
13	Acetone abs.	1	00:05:00	37	13	Continuous	
14	Acetone:EPON 1+1	1	00:15:00	40	11	Continuous	
15	Acetone:EPON 1+3	1	00:20:00	40	11	Continuous	
16	EPON	1	00:20:00	50	11	Continuous	
17	EPON	1	00:20:00	50	11	Continuous	

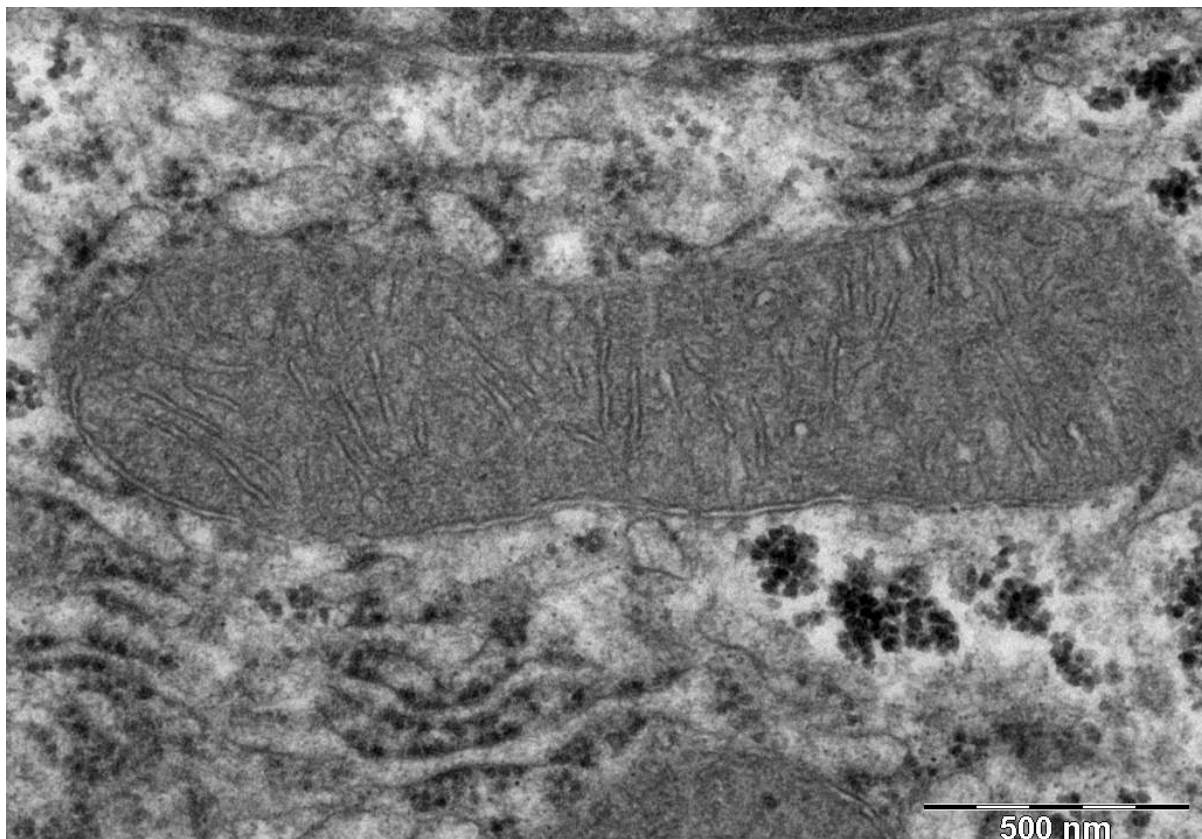
Epon polymerization

Vial	Reagent	Step #	Time (hh:mm:ss)	Temp. (°C)	Power (watt)	Mode	Pause
n	EPON	1	00:05:00	63	30	Slope	
		2	00:05:00	75	30	Slope	
		3	00:15:00	83	30	Slope	
		4	01:45:00	83	30	Continuous	

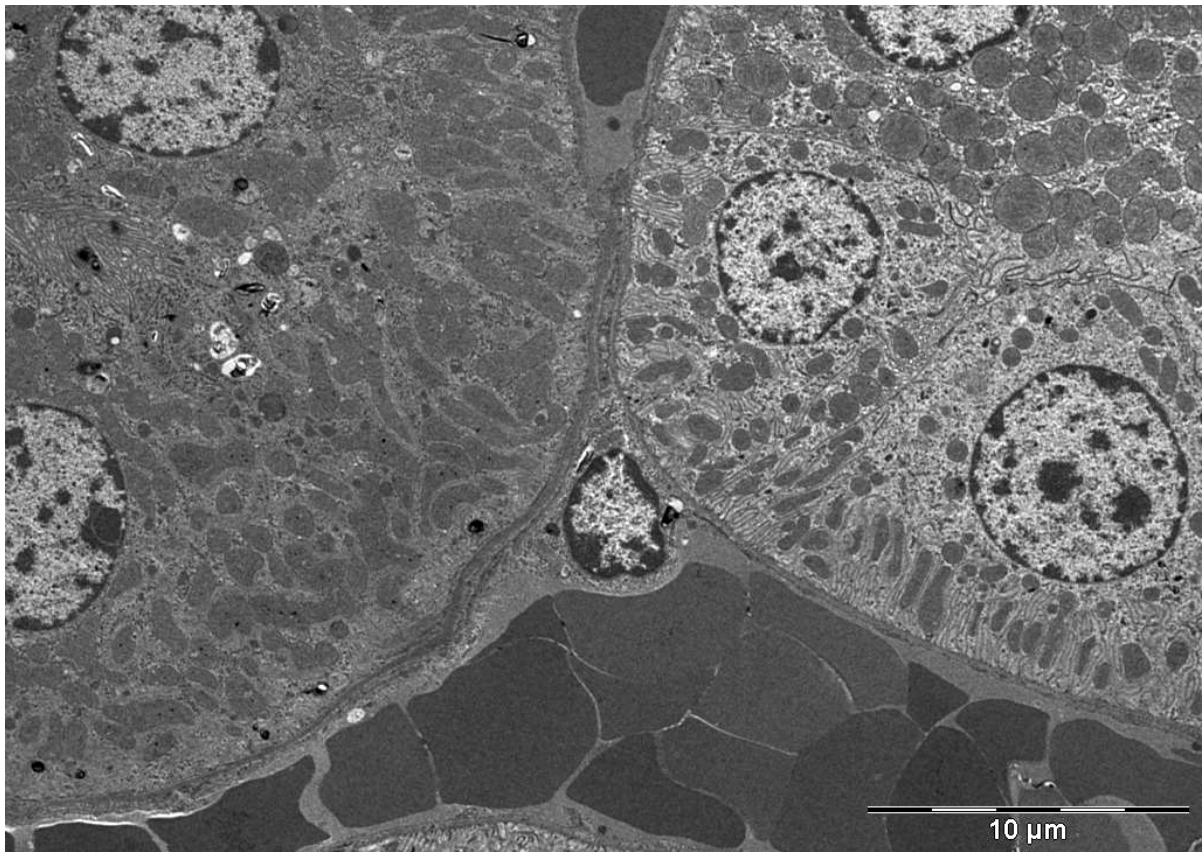
iv. Images



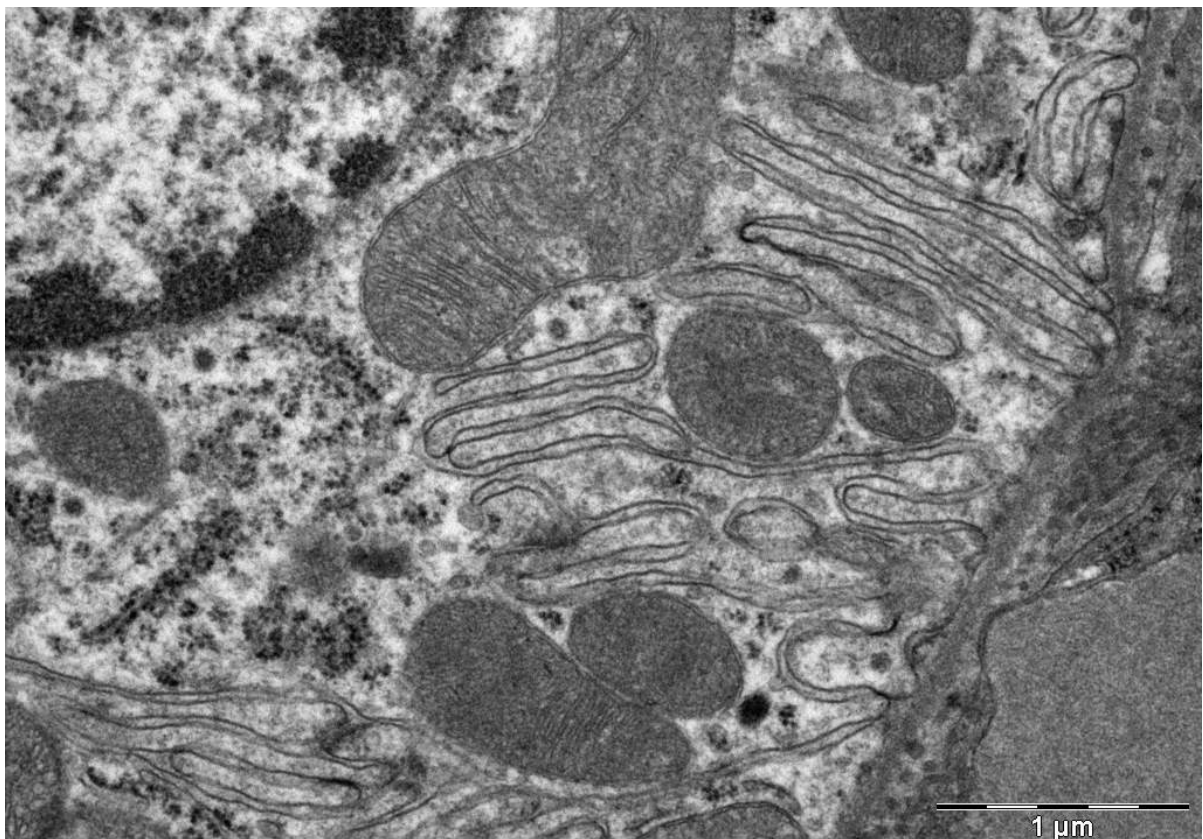
Mouse liver (immersion fixed). Hepatocytes with organelle-rich cytoplasm and round cell nuclei containing prominent nucleoli; in between a blood sinusoid with preserved space of Dissé and bile canaliculi with numerous microvilli. Original magnification: 1,250x.



Mouse liver, cell details. Mitochondrion, nuclear membrane, RER, and glycogen. Original magnification: 20,000x.



Mouse kidney, cortex (immersion fixed). Parts of a proximal and of a distal renal tubule with blood capillaries (filled with erythrocytes) in between. Original magnification: 1,250x.



Mouse kidney, detail of a cell of the distal renal tubule. The cytoplasm displays convolutions of the cell membrane and numerous mitochondria. Original magnification: 10,000x.

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Leica EM AMW

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