

Microimaging

Imaging bees,
pollen and
atoms



thomas wendler
saint petersburg, april 2006

Outline

- Definition
- Classification
 - Modality
 - Dimension
 - Magnification
- Examples

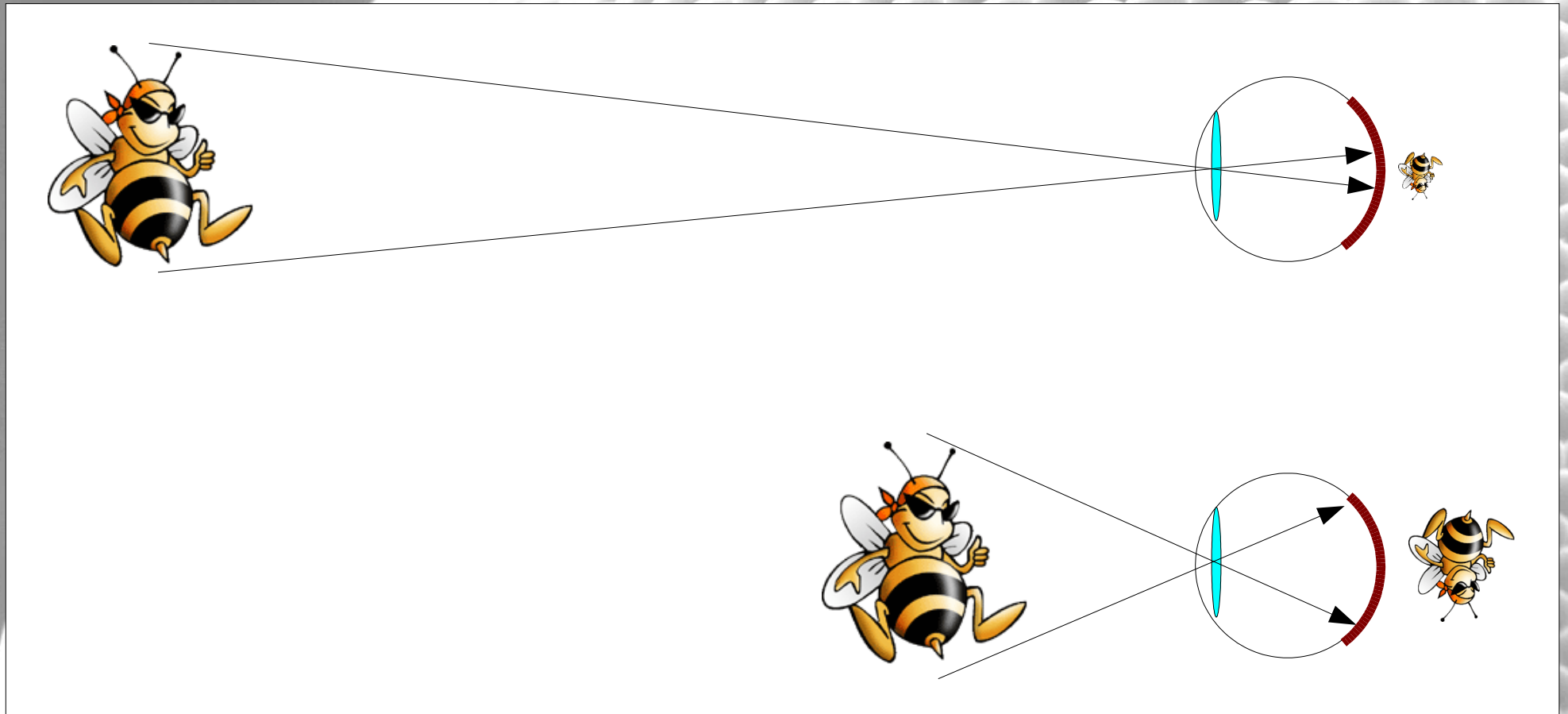
Microimaging goes beyond the human eye

- The smallest structure a human eye can see is about 25 [μm] at 10 [cm].
- There is but far more to see beyond that limit!
- Microimaging is imaging structures smaller than that.
- Microimaging is not restricted to visible imaging.

Microimaging implies “coloring” signals and increasing angles

- Images are representations of the light information acquired by our eyes.
- Imaging requires converting the signal into light that our eyes can see (“visible range” from 400 to 700 [nm]).
- Furthermore it can differentiate points with an angle of 3×10^4 [rad].
- The size of the object seen depends on the size of its projection on the retina.

Closer objects look bigger, because they ARE bigger



Imaging can be divided according to the physical signal “imaged”

- Common imaging modalities are:
 - Visible light: photography, microscopy, ...
 - X-rays, γ -rays: X-ray images, X-ray fluoroscopy, ...
 - IR-rays: IR-imaging, thermal imaging, ...
- However, almost *everything* can be imaged!
 - Conductivity, hardness/stiffness, sound, temperature, ...

Images can be divided according to their spatial dimension

- 2D images
 - Projection images (X-rays, 2D ultrasound, ...)
 - Surface images (β -camera, strain images, ...)
- 3D images
 - Tomographic reconstructions (CT, PET, ...)
 - General 3D images (MRI, 3D ultrasound, ...)
- Virtual 3D images
 - Stereoscopic images, 3D surface imaging, ...

Microimaging can be also divided by the magnification method

- The common magnification used is based on the concept of magnifying the input signal.
- Nonetheless there is a second approach: miniaturizing the sensor.

There are many flavours of microimaging

	Optics	Scanning	Combined
Visible light	Loupe, microscopy	NSOM, OCT	
Fluorescence	Fluorescence microscopy		CLSM
X-rays	X-ray microscopy	EPMA (EDX)	
Electrons	TEM, electron tomography	SEM	STEM
Ions		FIB	
Conductivity		STM	

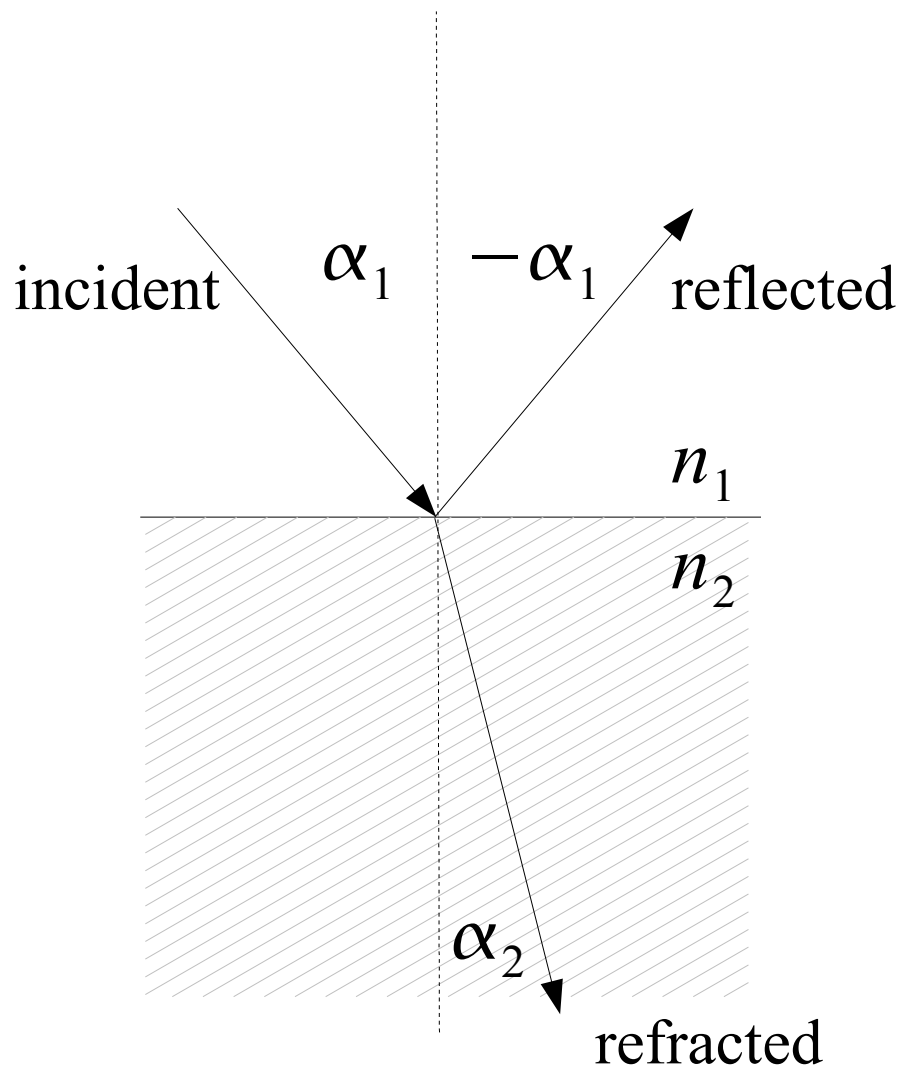
A scanning electron micrograph (SEM) showing a dense, regular array of rounded, lens-like structures. The structures are arranged in a hexagonal or similar lattice pattern, with each lens having a slightly darker, central region. The overall appearance is that of a highly organized biological surface, possibly a lens or a specialized epithelial layer. The text "Rays and lenses" is overlaid in the center of the image.

Rays and lenses

The signal magnification is based on the concept of lenses

- Lenses are devices for deflecting rays.
- This restricts their use to... rays, of course..., i.e. electromagnetic, acoustic and particle “rays”.

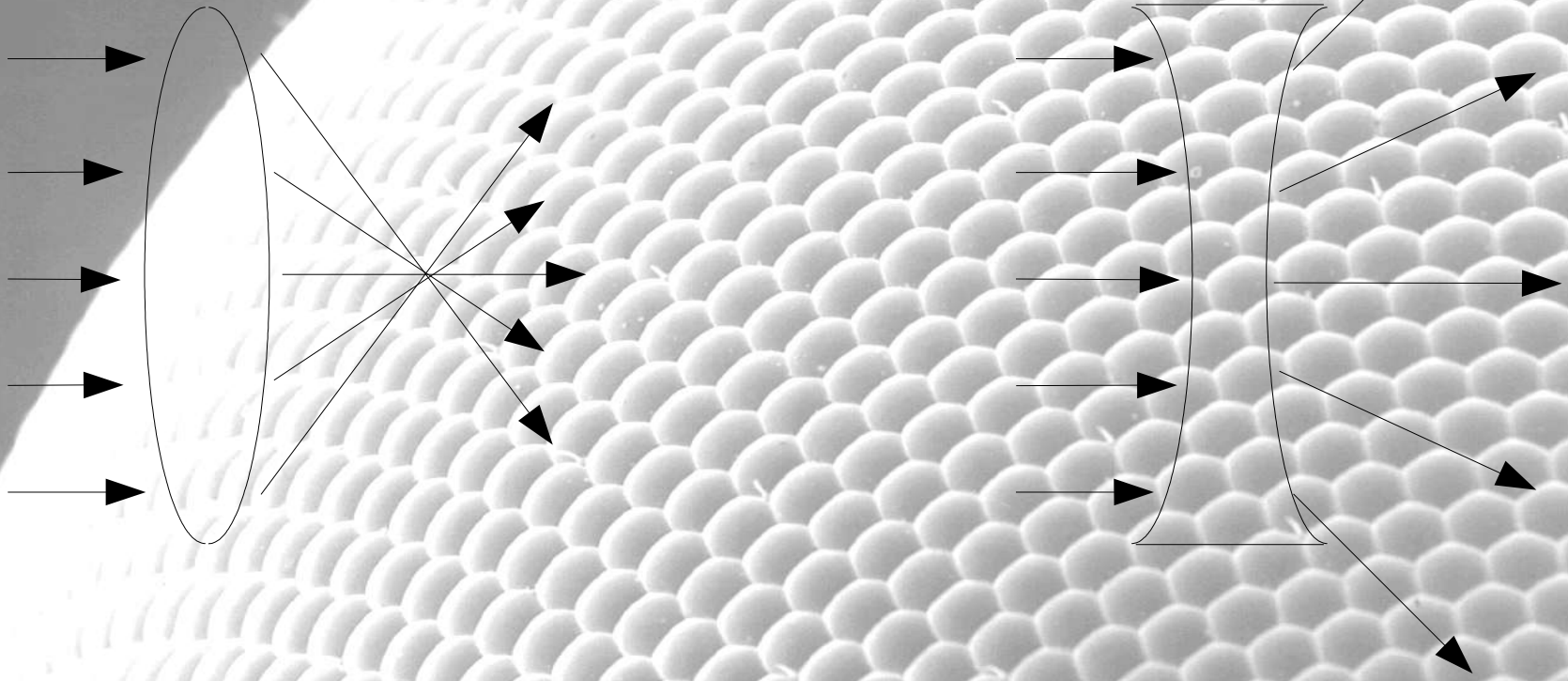
Light deflects when reaching interfaces



$$\frac{\sin \alpha_2}{\sin \alpha_1} = \frac{n_1}{n_2}$$

$$\frac{\alpha_2}{\alpha_1} \approx \frac{n_1}{n_2}$$

Converging and diverging depends on the geometry



Electromagnetic and acoustic lenses are based on refraction

- Changes in propagation velocity of the rays in the medium imply changes in the direction of propagation.
- The relationship of incident and refracted rays is given by the law of Snell.
- The rest is geometry.

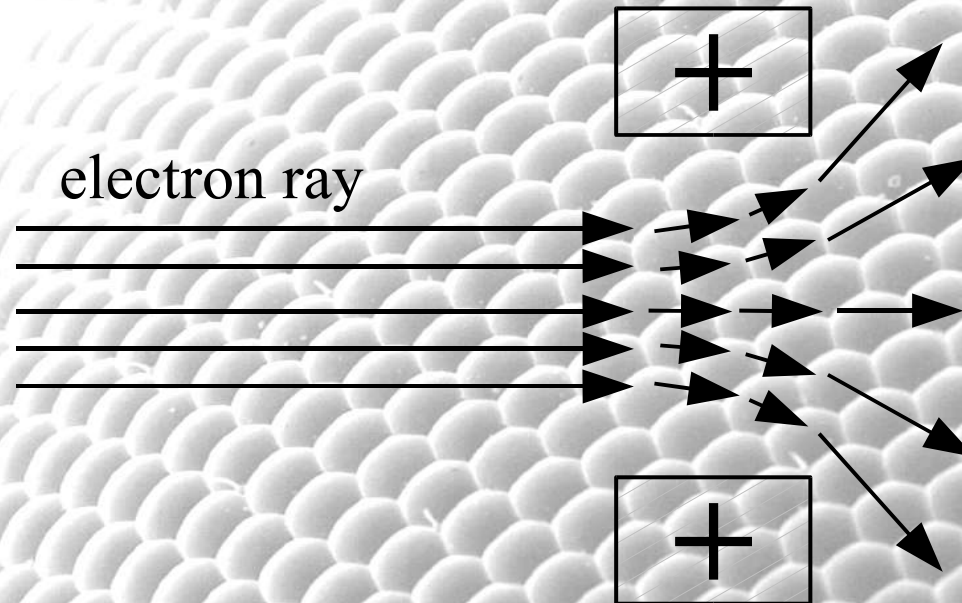
X-rays lenses exist

- X-rays are electromagnetic waves, thus can be focused or spread.
- The refraction index is though slightly less than one.
- First approaches use several cylinders to gradually bend X-rays.

Charged particles can also diverge and converge

- Electrostatic and magnetic fields are used to change the trajectory of charged particles.
- A proper geometric construction can result in lens equivalent devices.
- All common lens properties can also be calculated for these lens system.

Charged particles can be focused and spread with fields

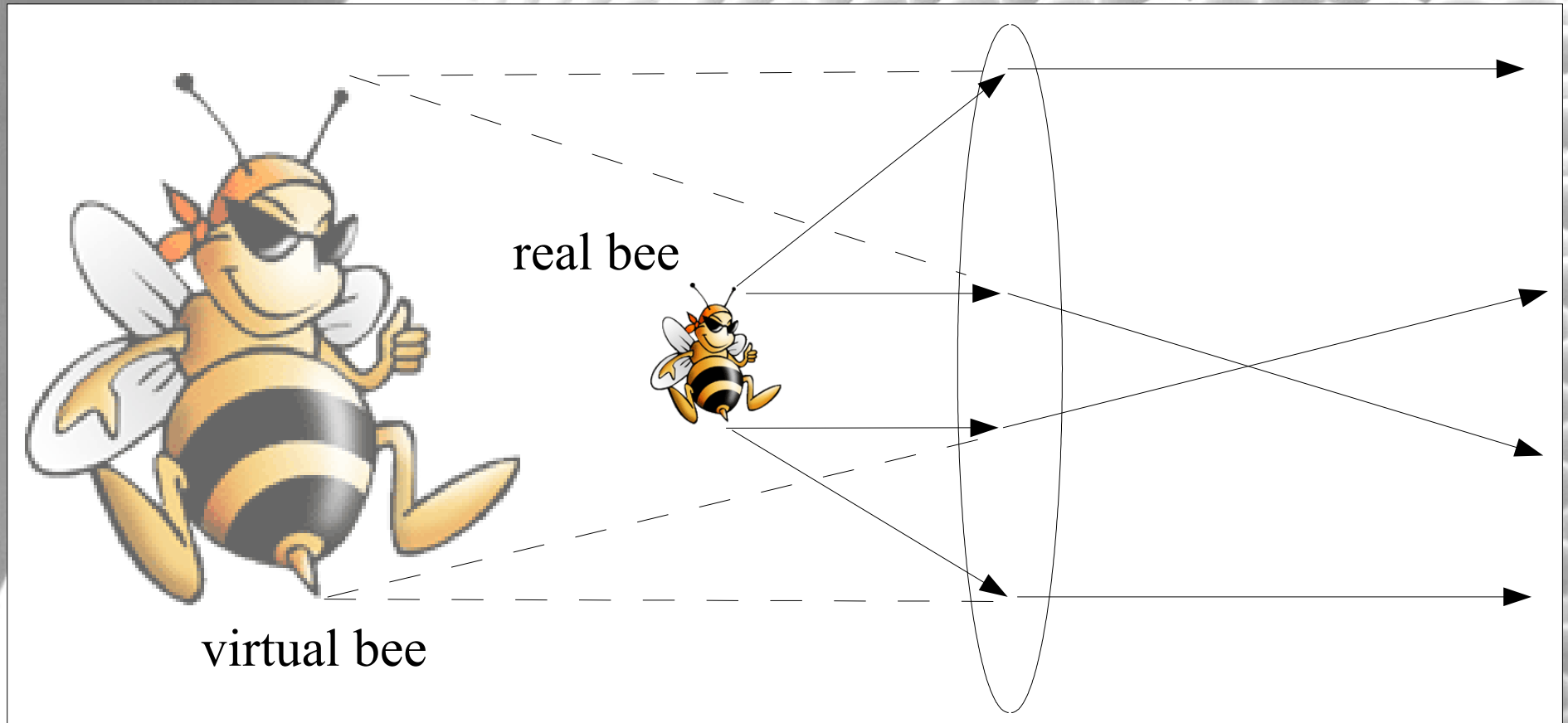


Traditional microscopy is based on visible light

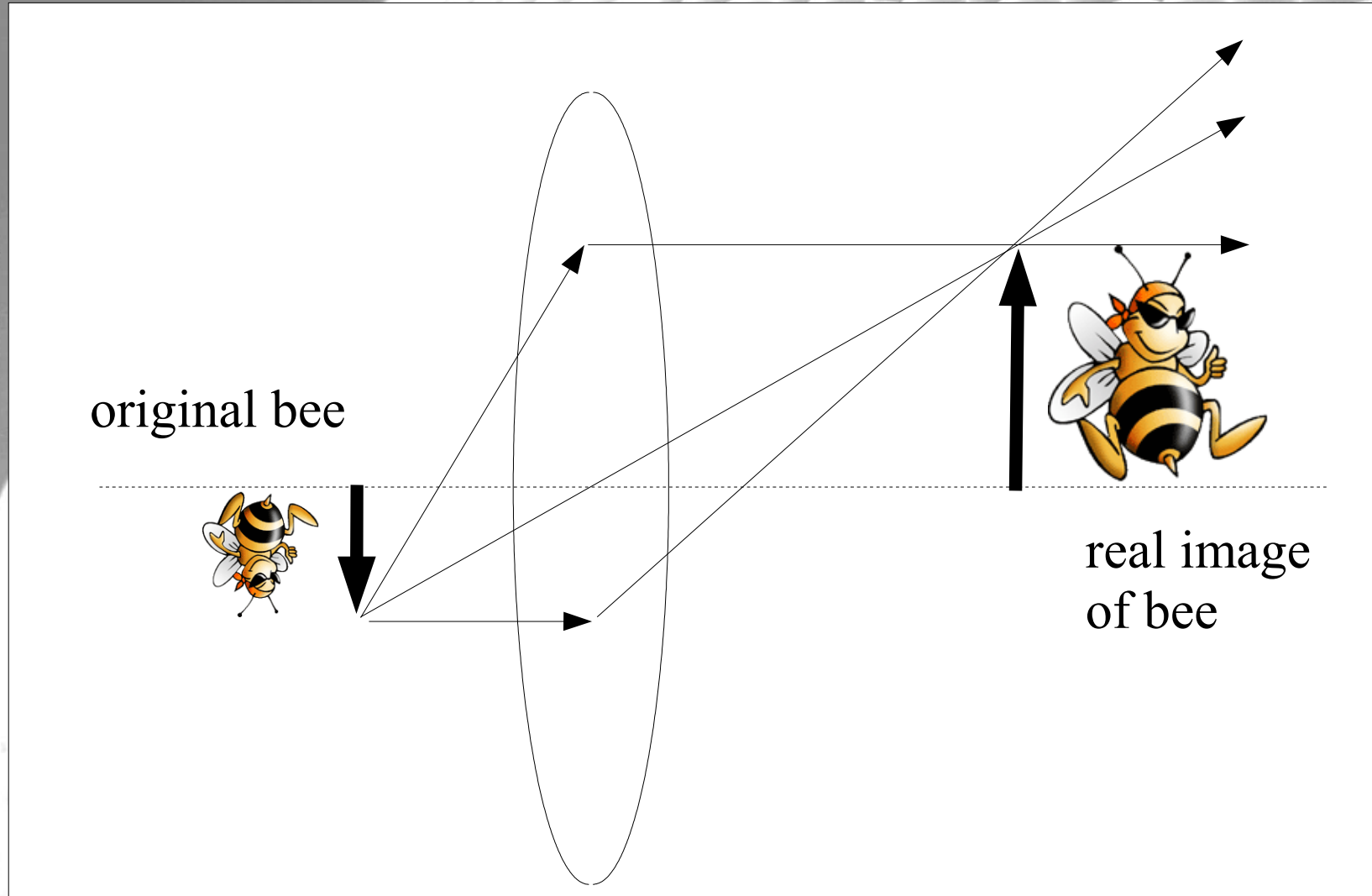
- Thin semitransparent objects can be projected using lens systems to bigger sizes.
- There are many modalities:
 - Bright field microscopy
 - Dark field microscopy
 - Phase contrast microscopy

A loupe is a one lens magnification system

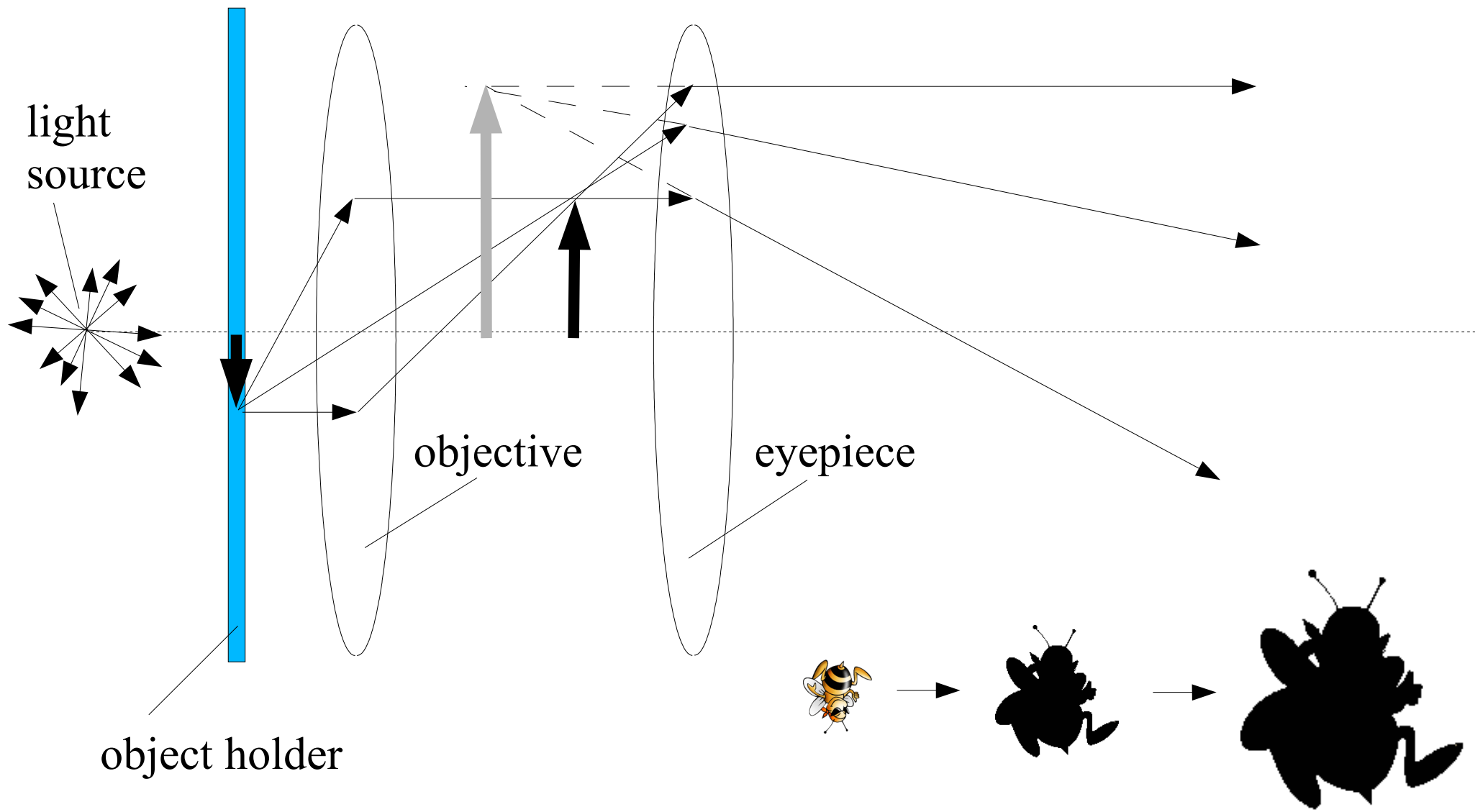
- An object close to a lens can be magnified by generating a bigger virtual image of it.



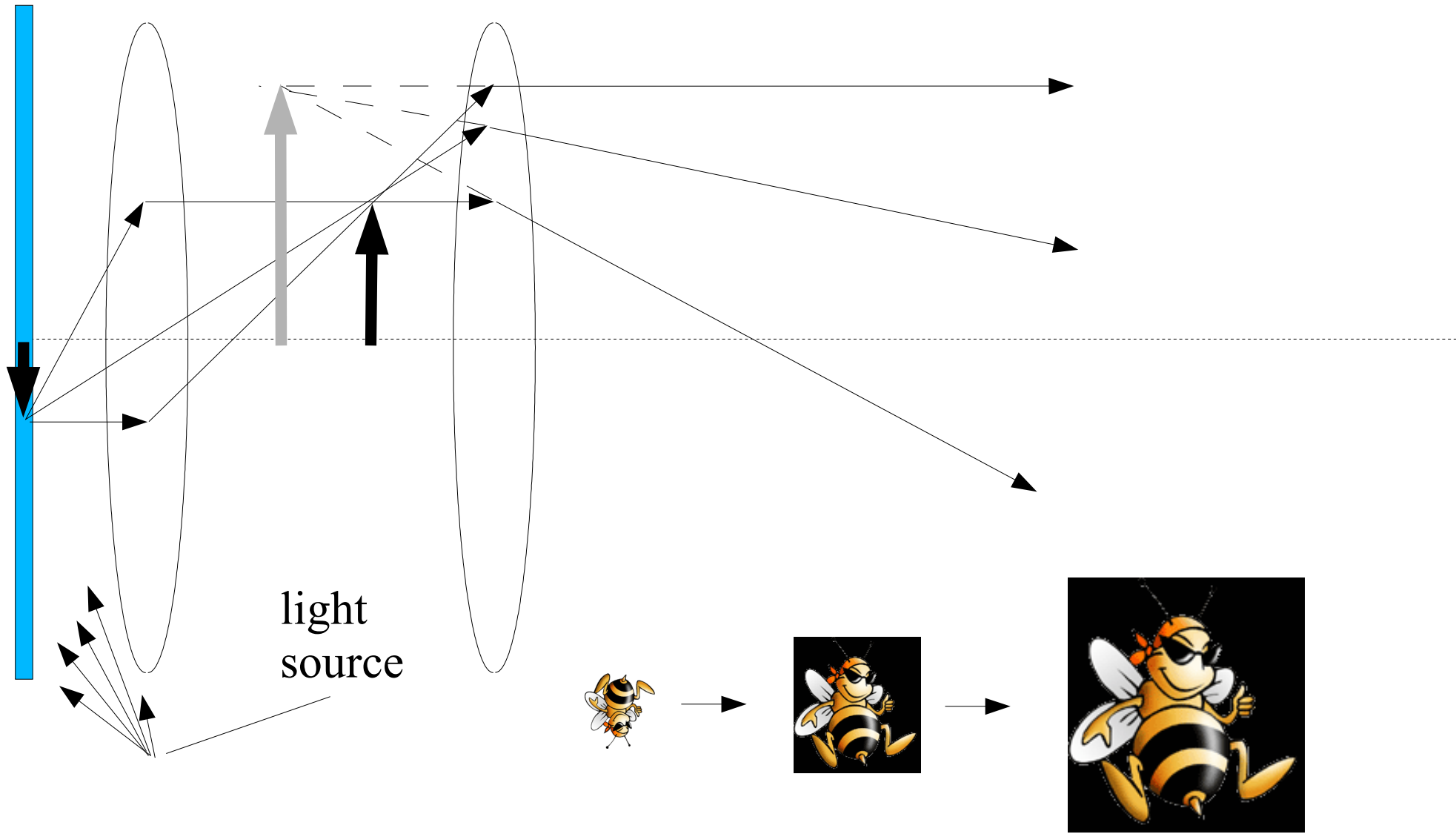
Loupes can also be used to generate real images



Bright field microscopes show objects dark



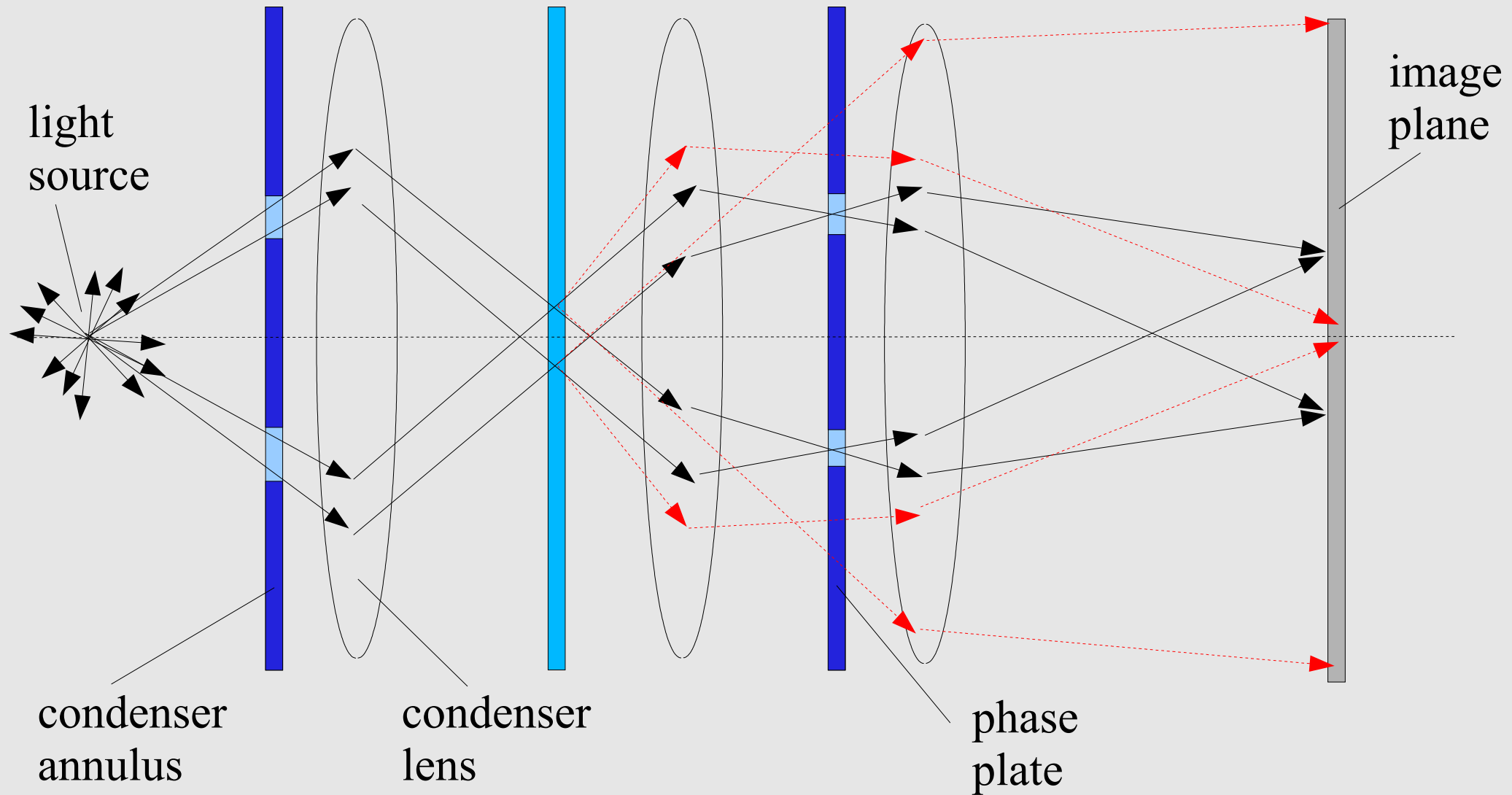
Dark field microscopes show objects bright



Phase contrast microscopes allow to see invisible things

- The main idea is to separate diffracted light from light that did not interact with the object.
- Phase differences are enhanced to generate desired interferences.
- Transparent objects that affect phases can thus be imaged.

Spatial separation and interference are the essence of phase contrast



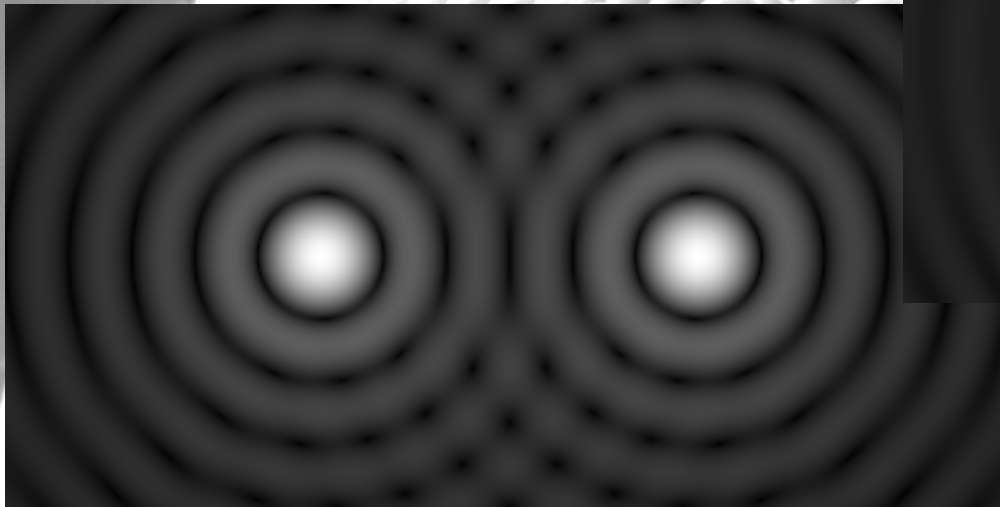
Ray (wave) systems are constrained.

- Points are imaged as discs given to diffraction.
- This fact shows a limit for resolution.
- This can be minimized with corrected lenses, bigger “numerical aperture” and shorter wavelength.

$$d_{min} = \frac{0,61 \lambda}{N.A.} = \frac{0,61 \lambda}{n \sin \alpha}$$

The main parameter in microimaging is resolution

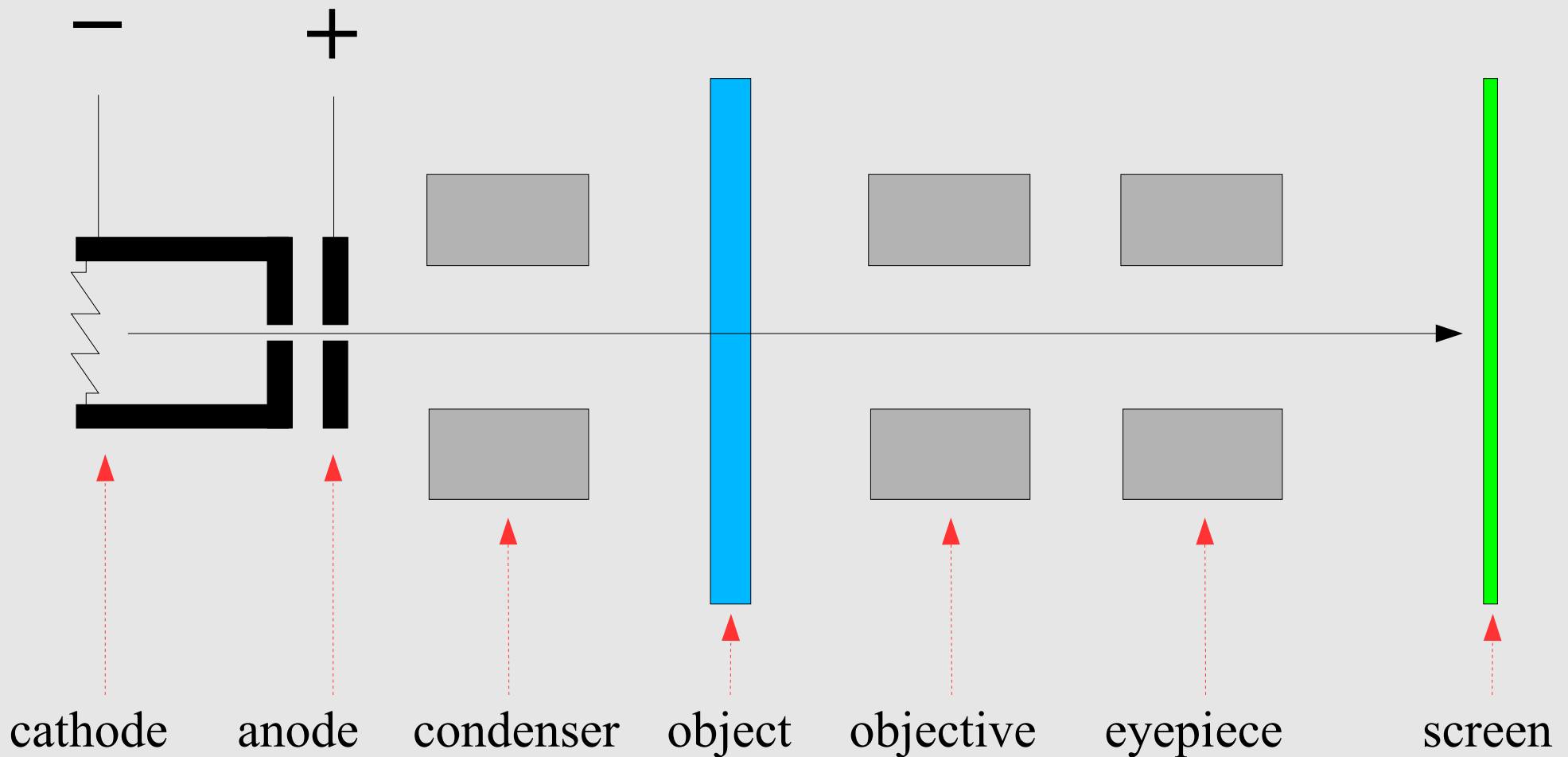
- Resolution is calculated from the minimal distance between to points to consider them different entities.



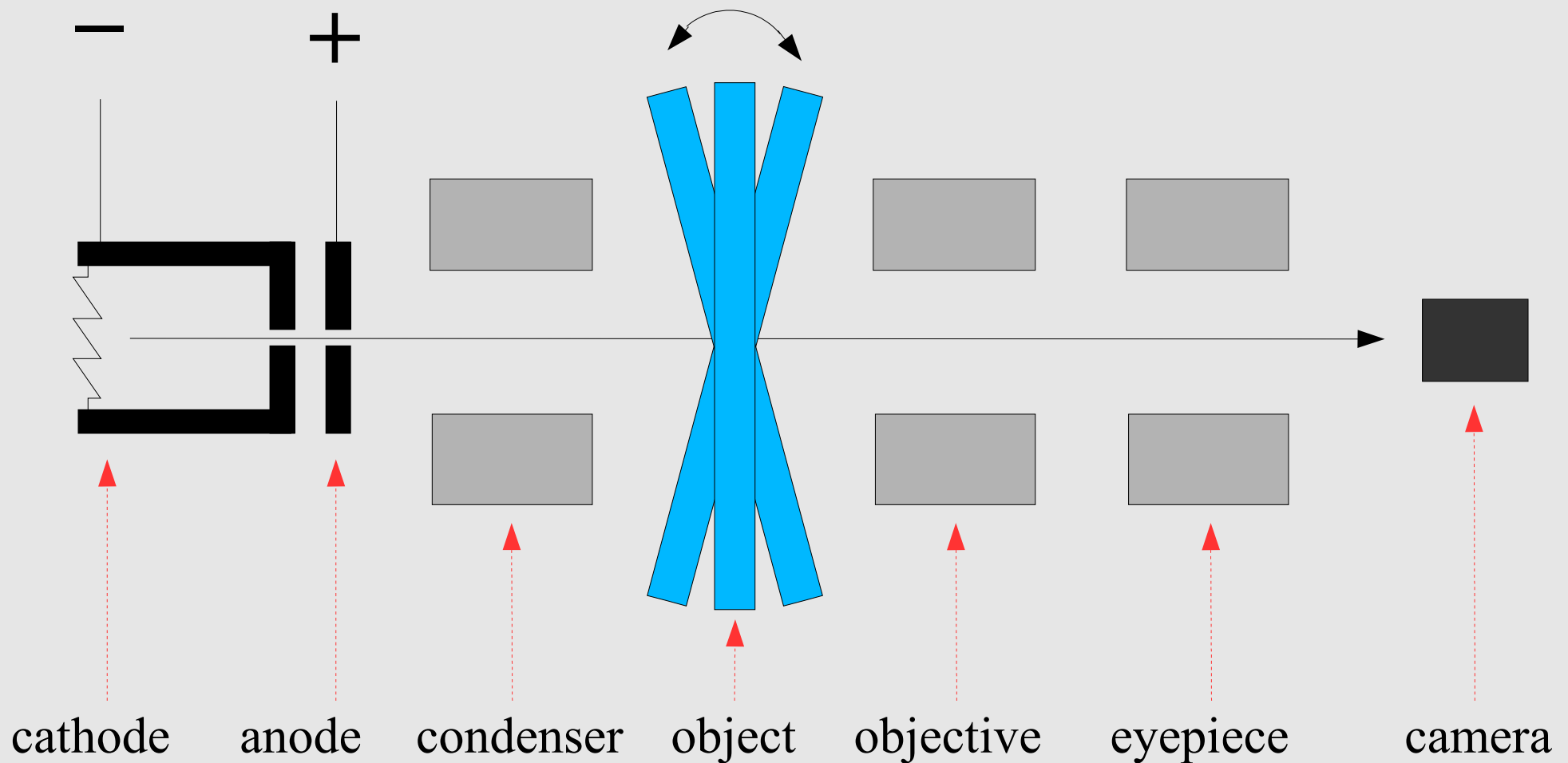
Transmission electron microscopy uses electrons as light

- According to the relation of De Broglie matter is also a wave.
- Electrons can be seen as high frequency, i.e. small wavelength rays.
- Diffraction also exists!

TEM requires high voltage, lenses and deflecting structures



Capturing TEM images at different angles allows tomography





Miniaturization and atoms

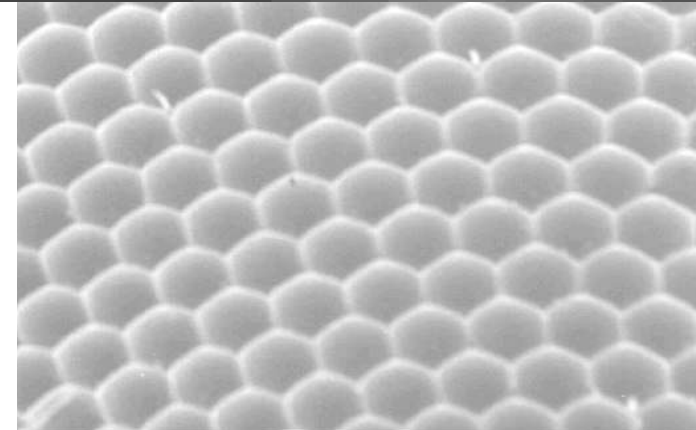
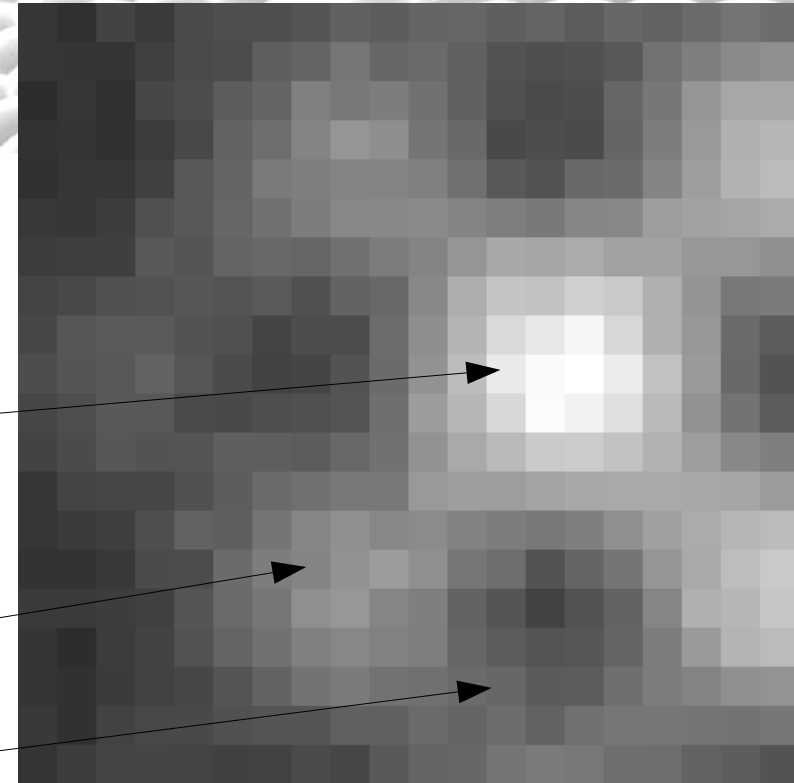
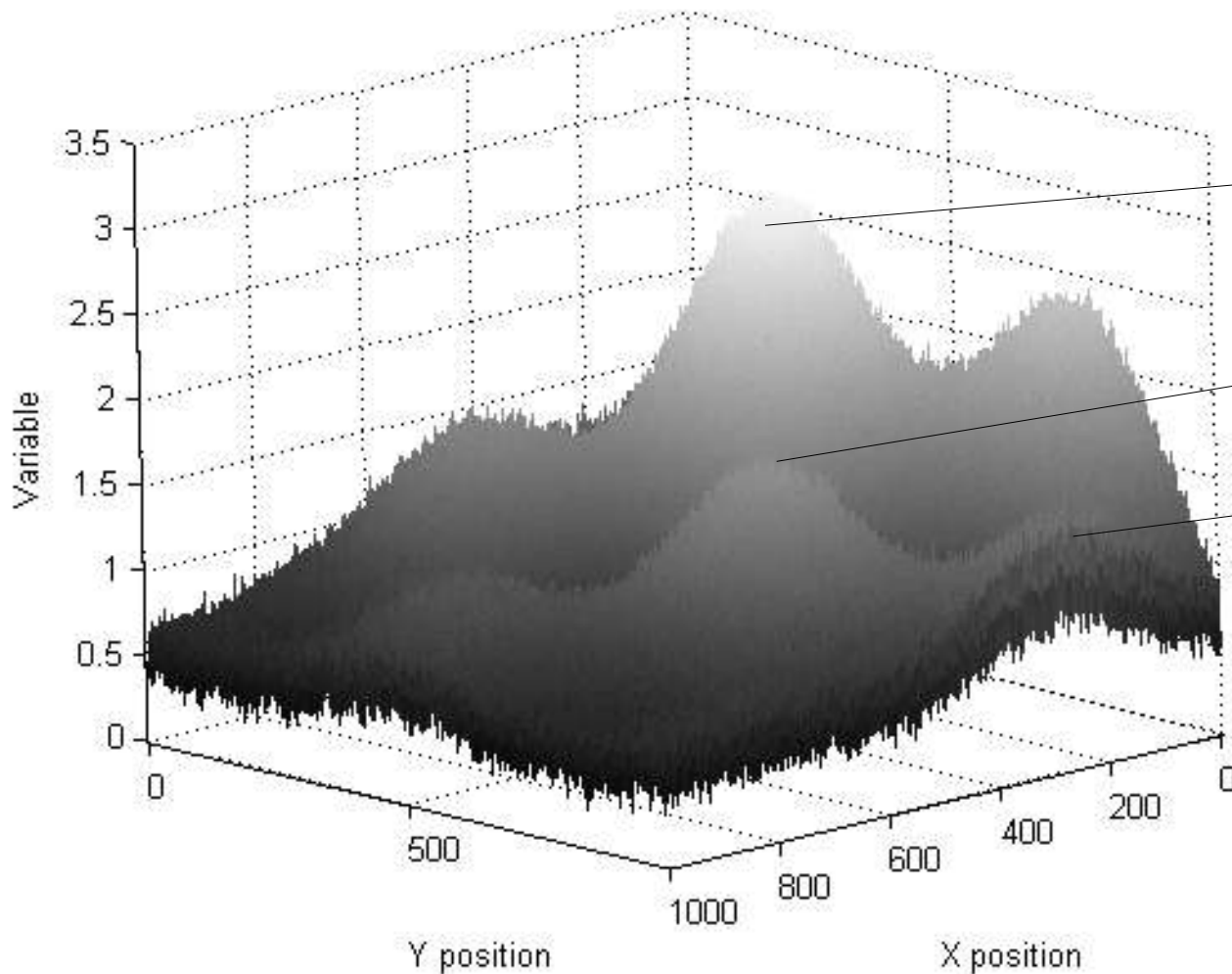
Miniaturization of sensor arrays allows also magnification

- The independent readings of a small pattern sensor array can be reproduced in a larger pattern.
- To surpass the human eye really small sensors are needed.
- An option for that is “scanning”.

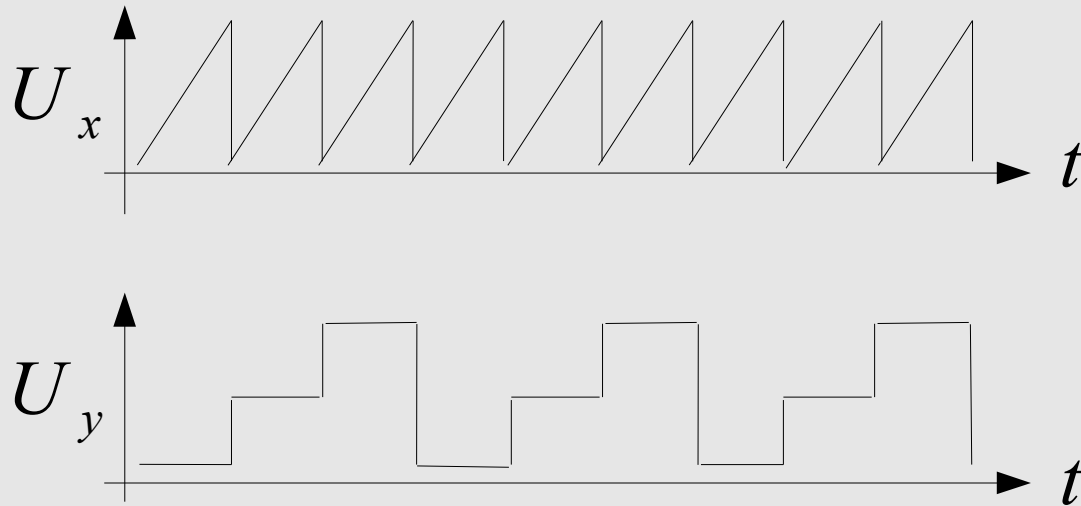
“Scanning” means measurement and position recording

- Assuming a constant measurement field singular points can be acquired simultaneously acquiring the position.
- The collection of measurement and positions can be rendered virtually into images.

Scanning on a grid allows imaging



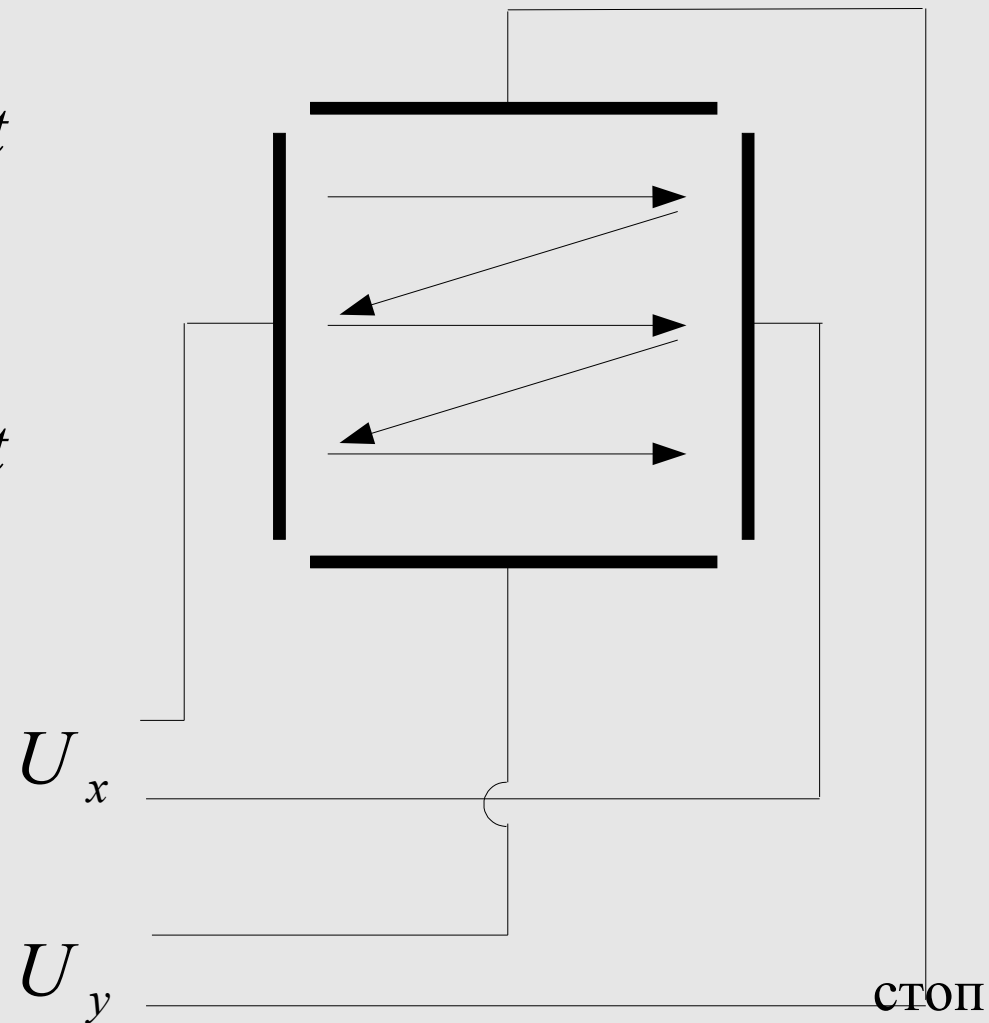
Scanning requires synchronization



U_x Voltage in x deflector

U_y Voltage in y deflector

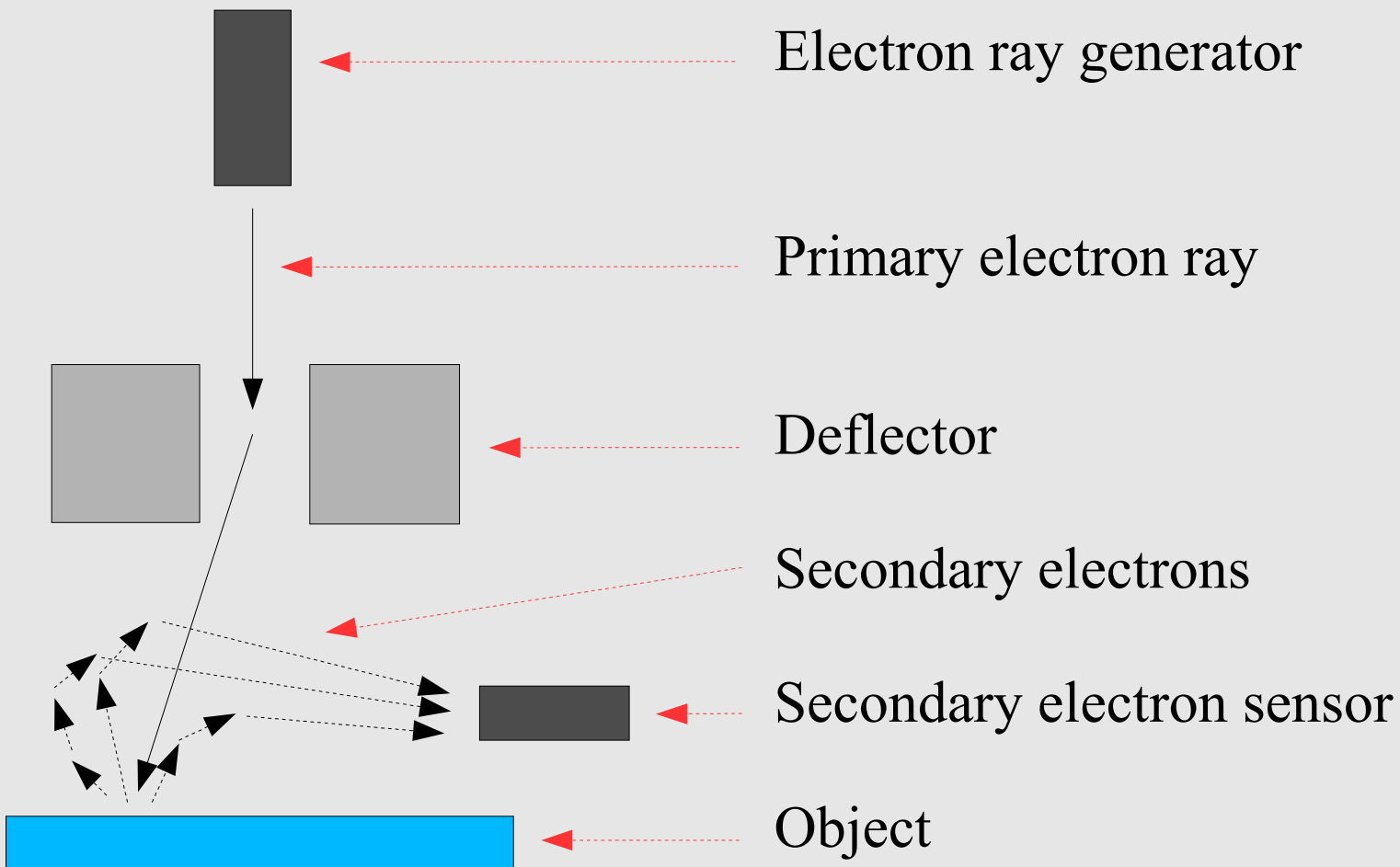
t Time



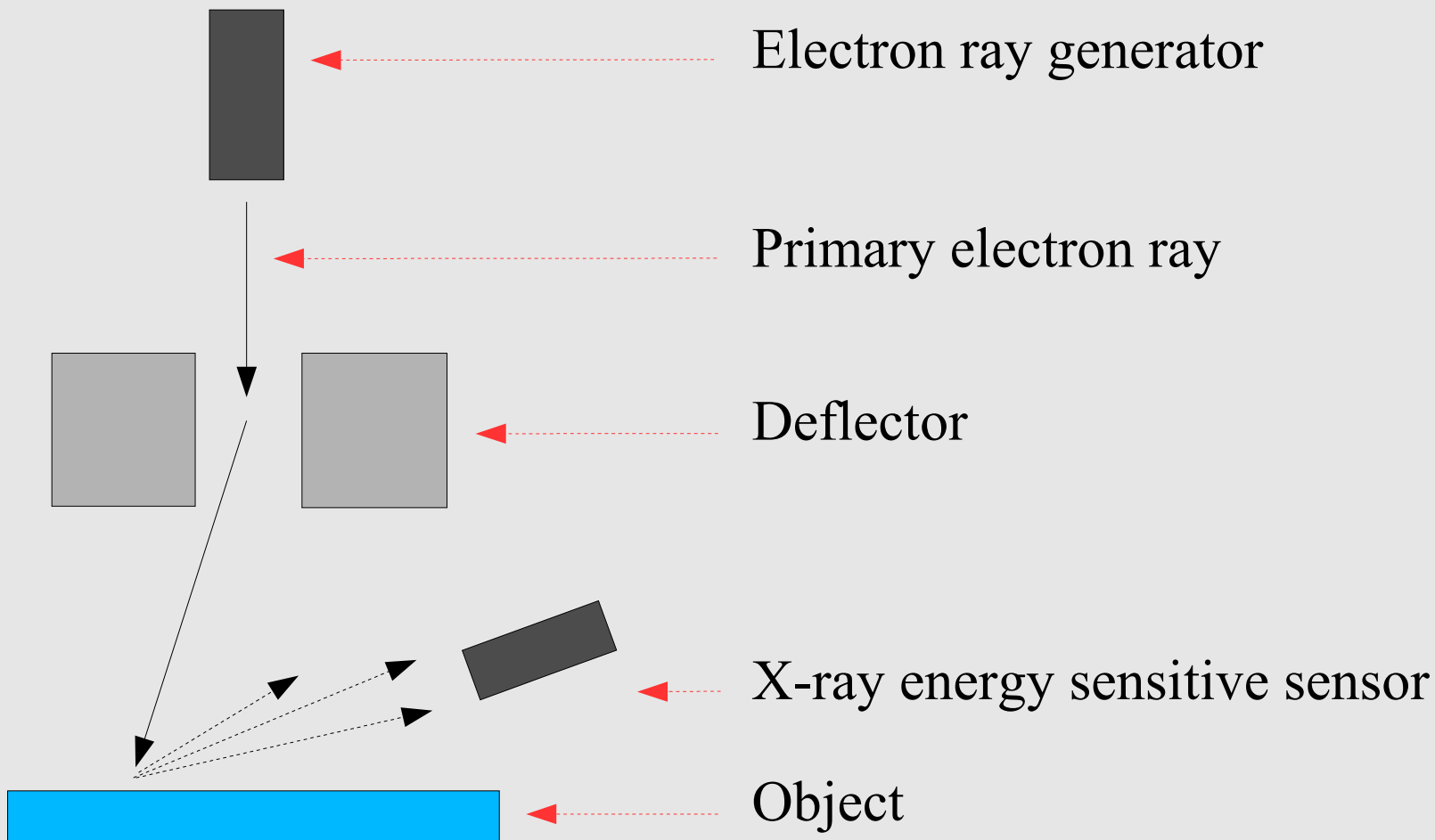
Scanning electron microscopy acquires secondary electrons

- Secondary electrons are electrons that are drawn out of atoms, ions and molecules by high energetic interactions.
- By scanning the secondary electrons produced on a surface information on it can be obtained and put together in an image.
- The current limit for SEM is 0,2 [nm].

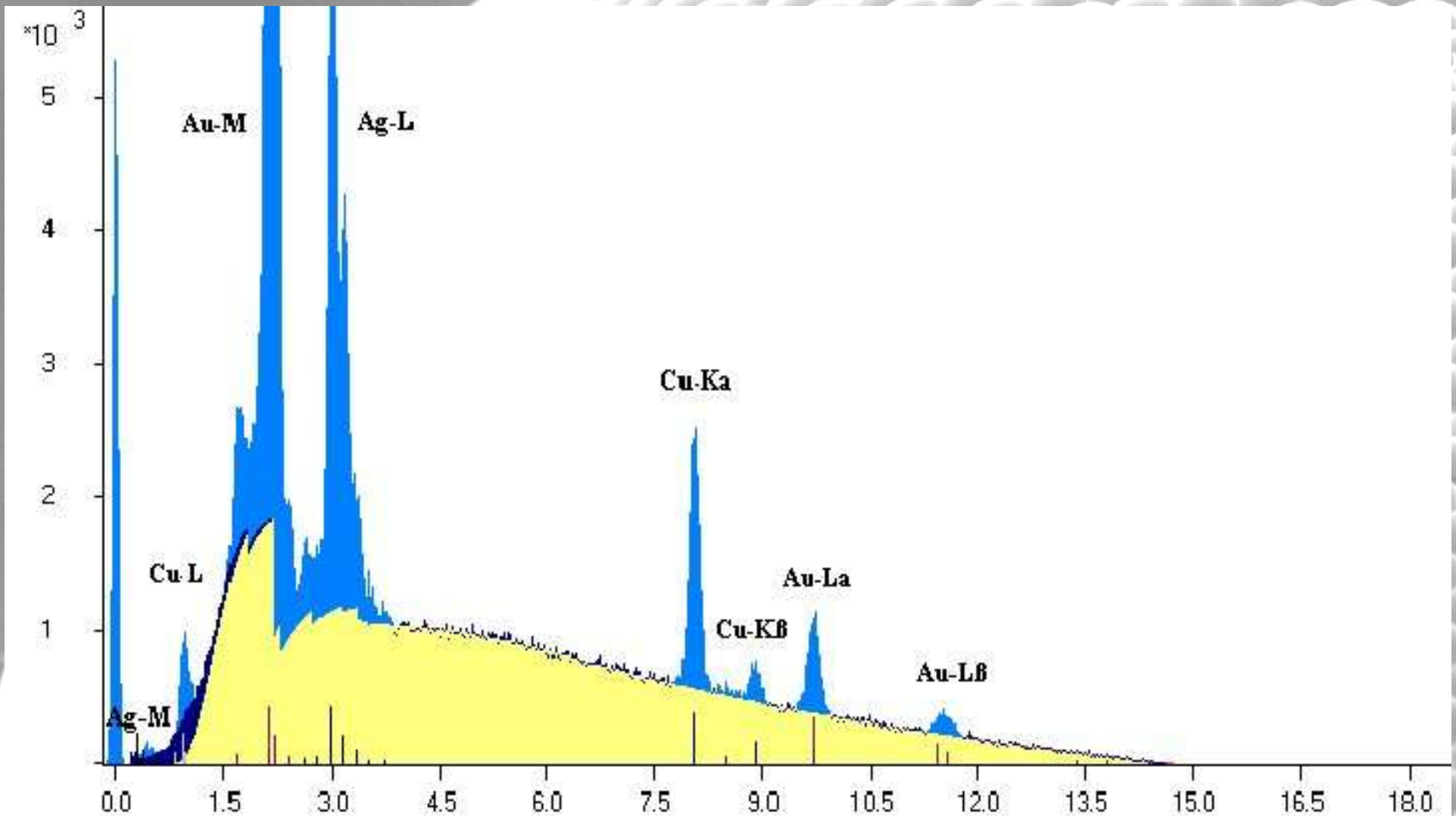
The scanning electron microscope looks at the secondary electrons



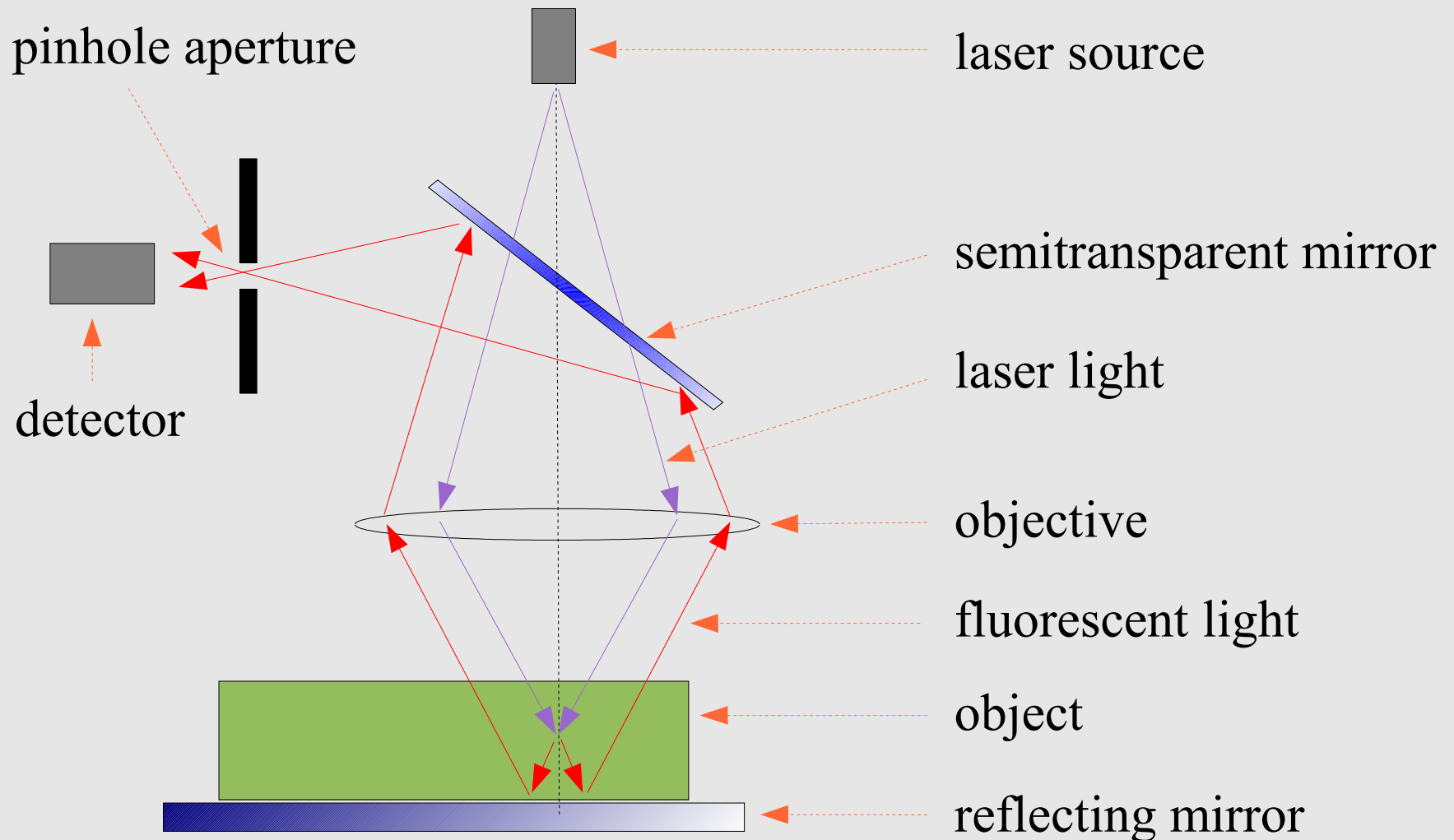
The electron probe microanalysis looks at the X-ray radiation



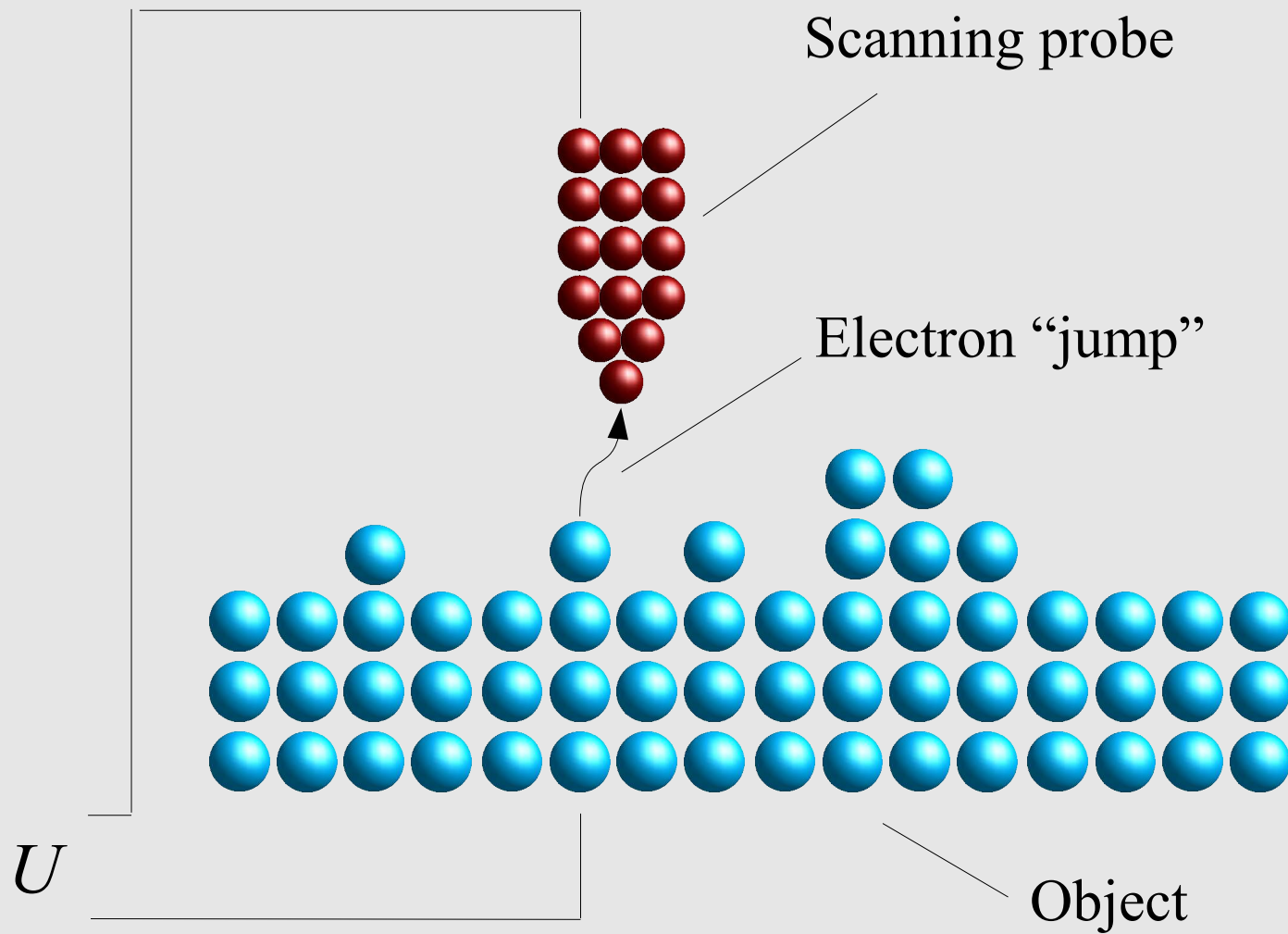
Peaks in the spectrum EDX allow seeing atoms



Confocal laser scanning microscopy

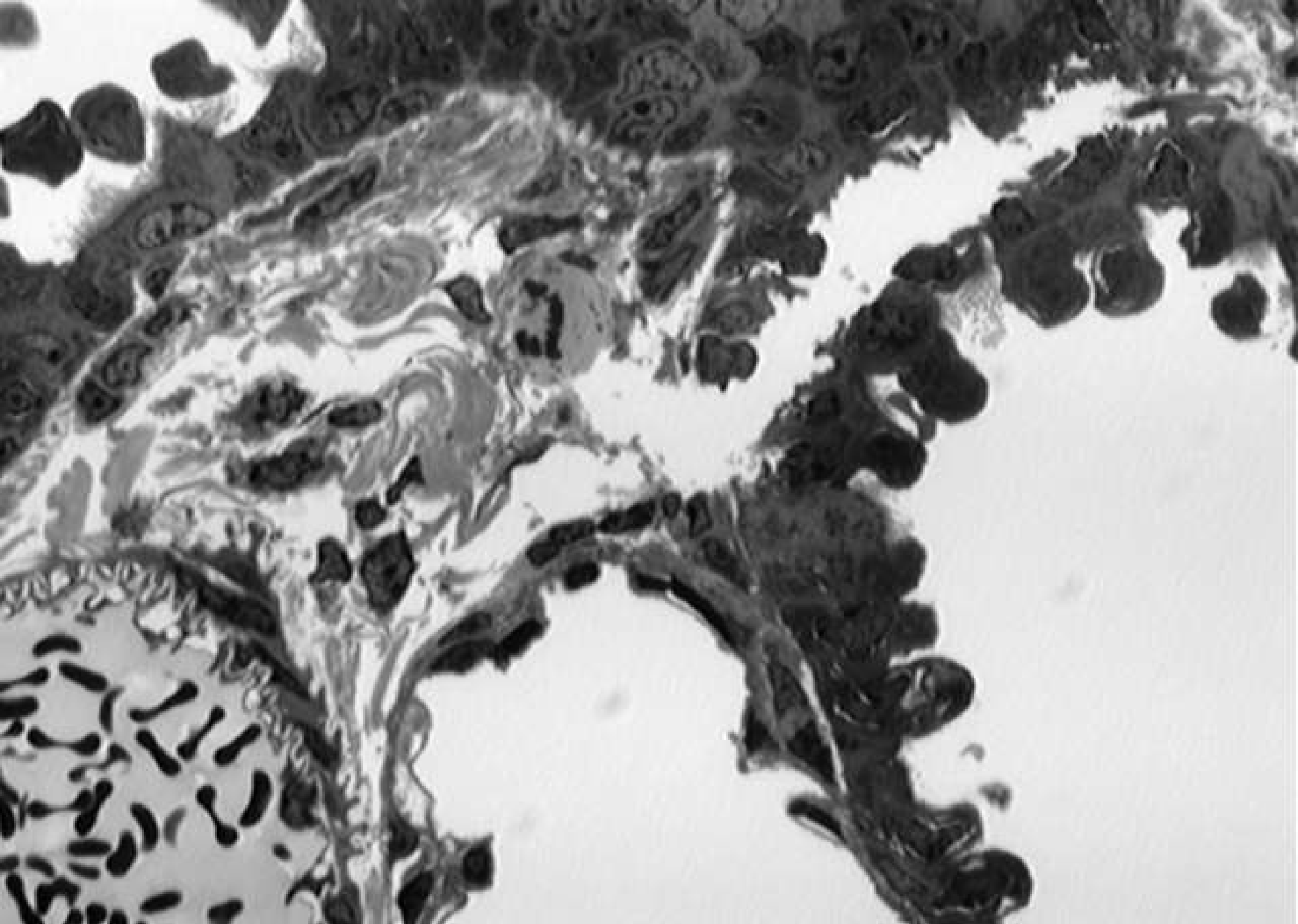


Scanning tunneling microscope



Bees, pollen...

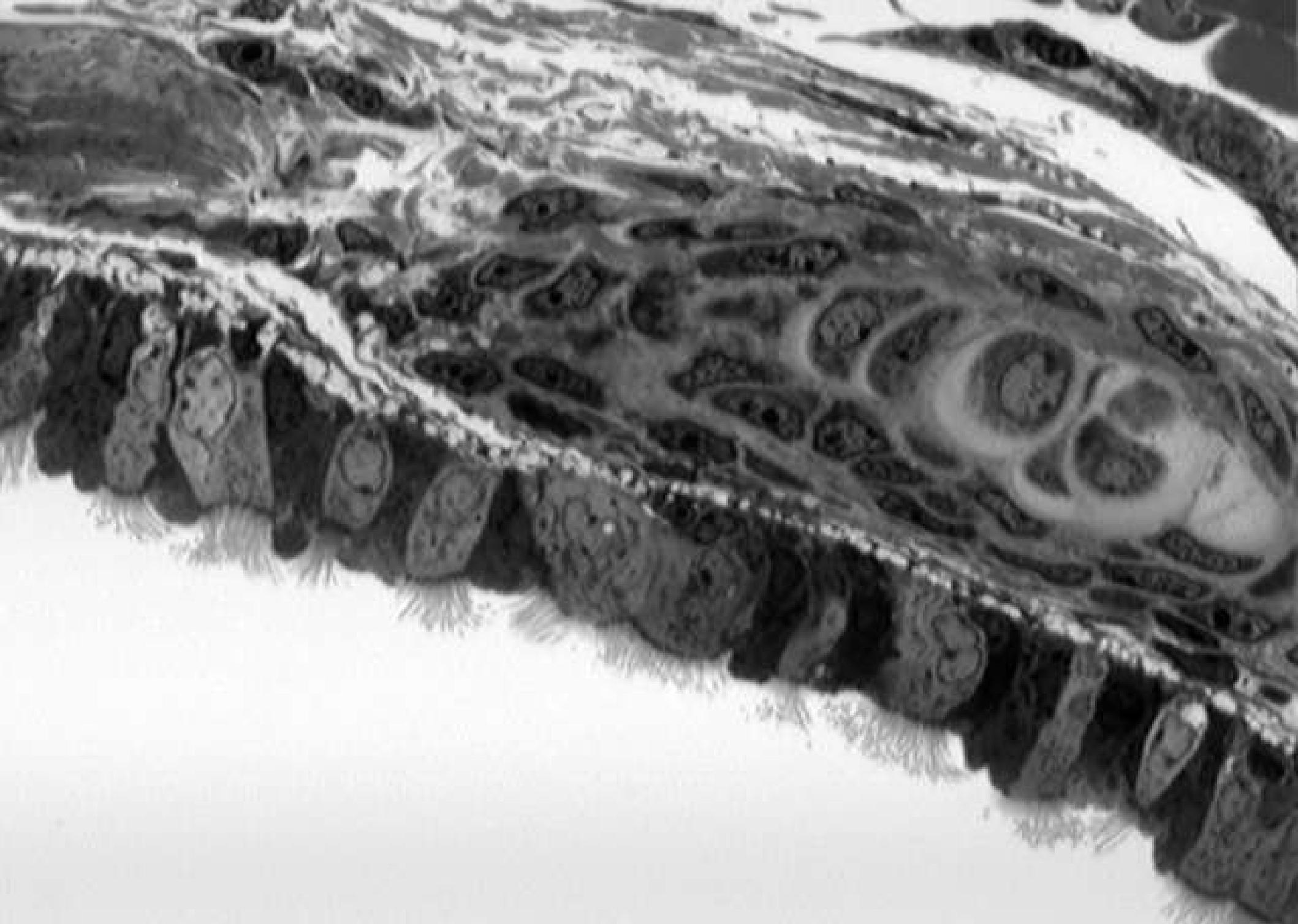
... and atoms



24 μm 40.0X obj.

Lung Sample #8(40x-1.25). 2

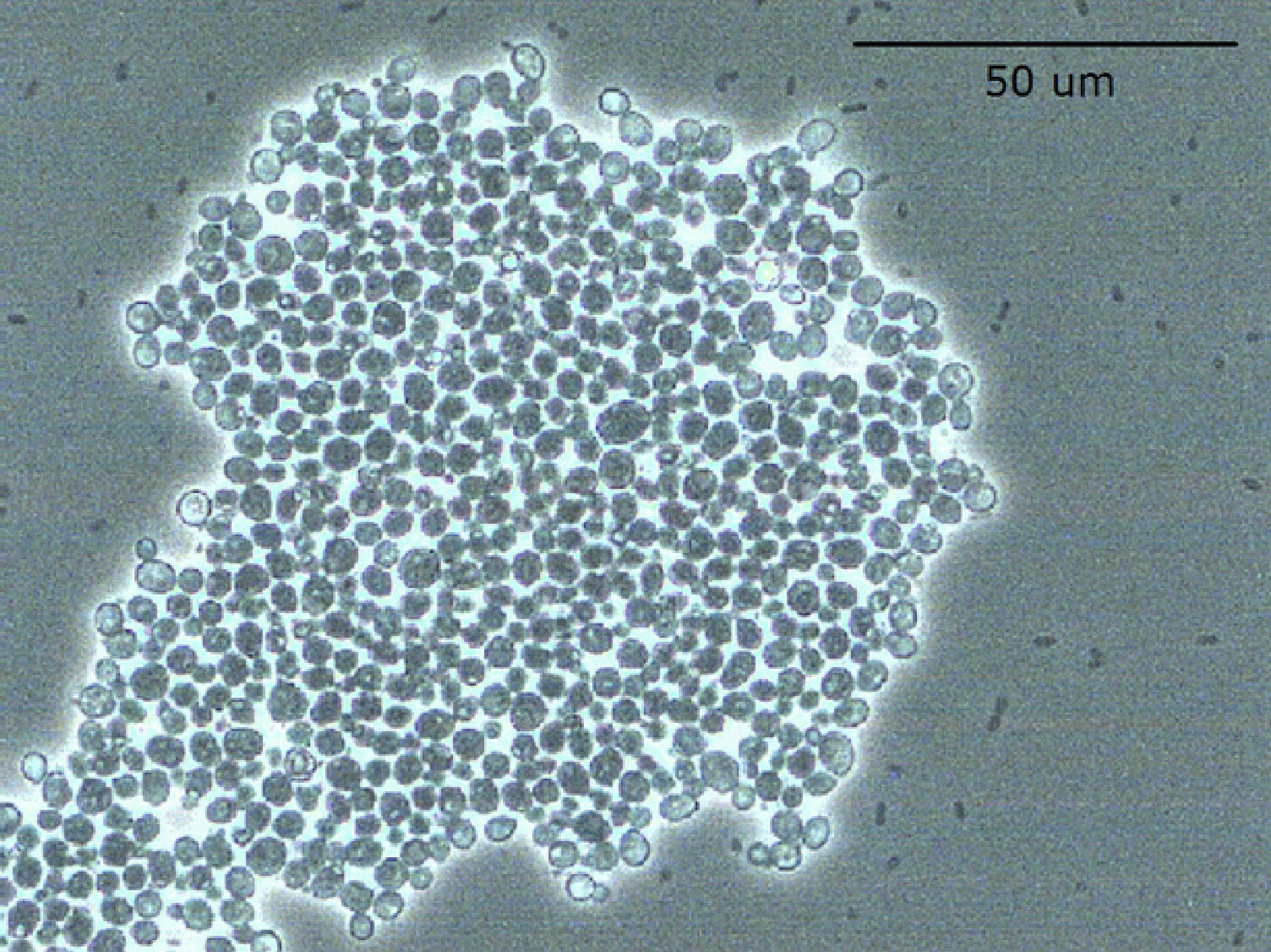
11/ 2/99 12:46:18



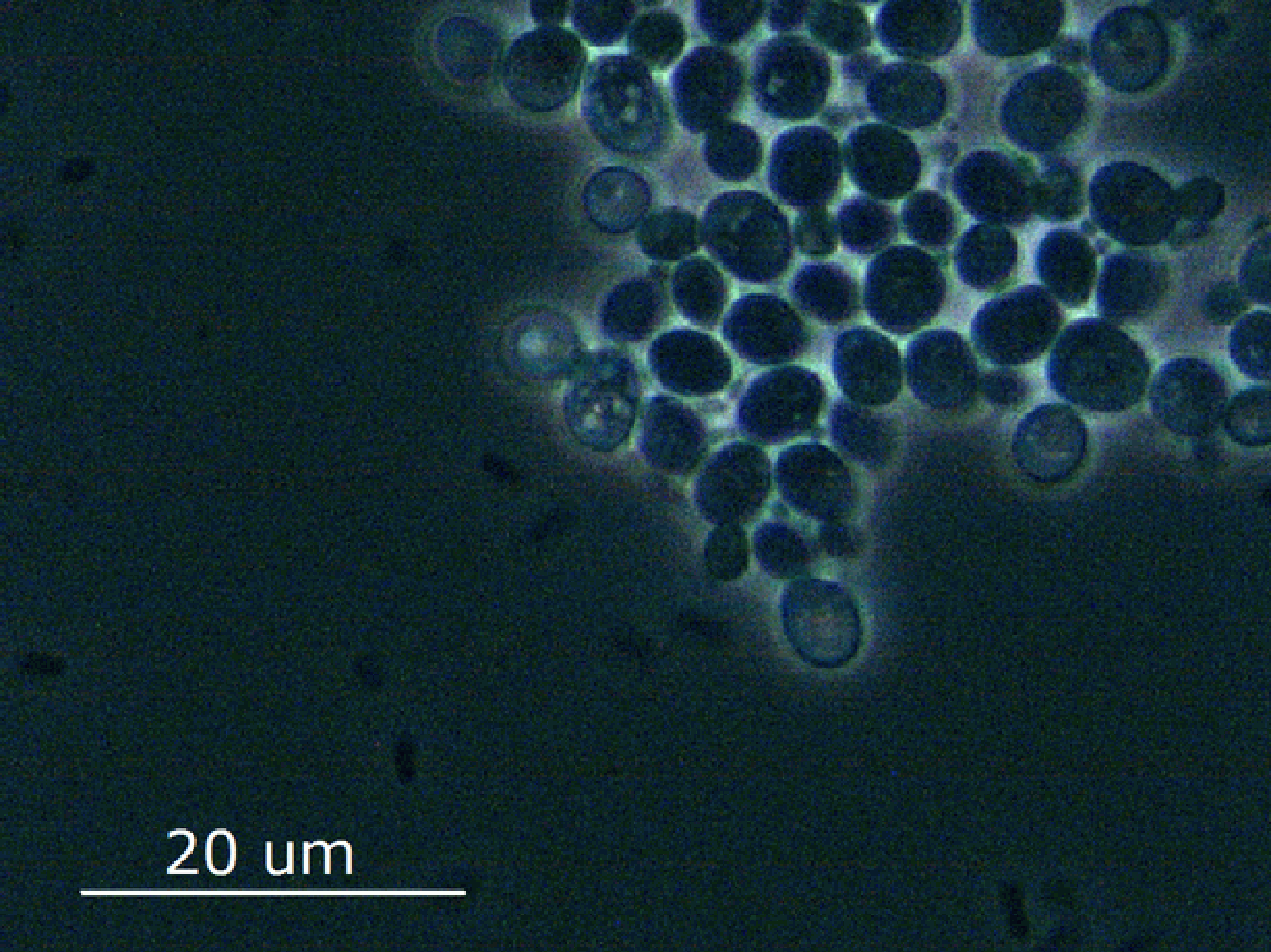
24 μm 40.0X obj.

Lung Sample #8(40x-1.25) 2.1

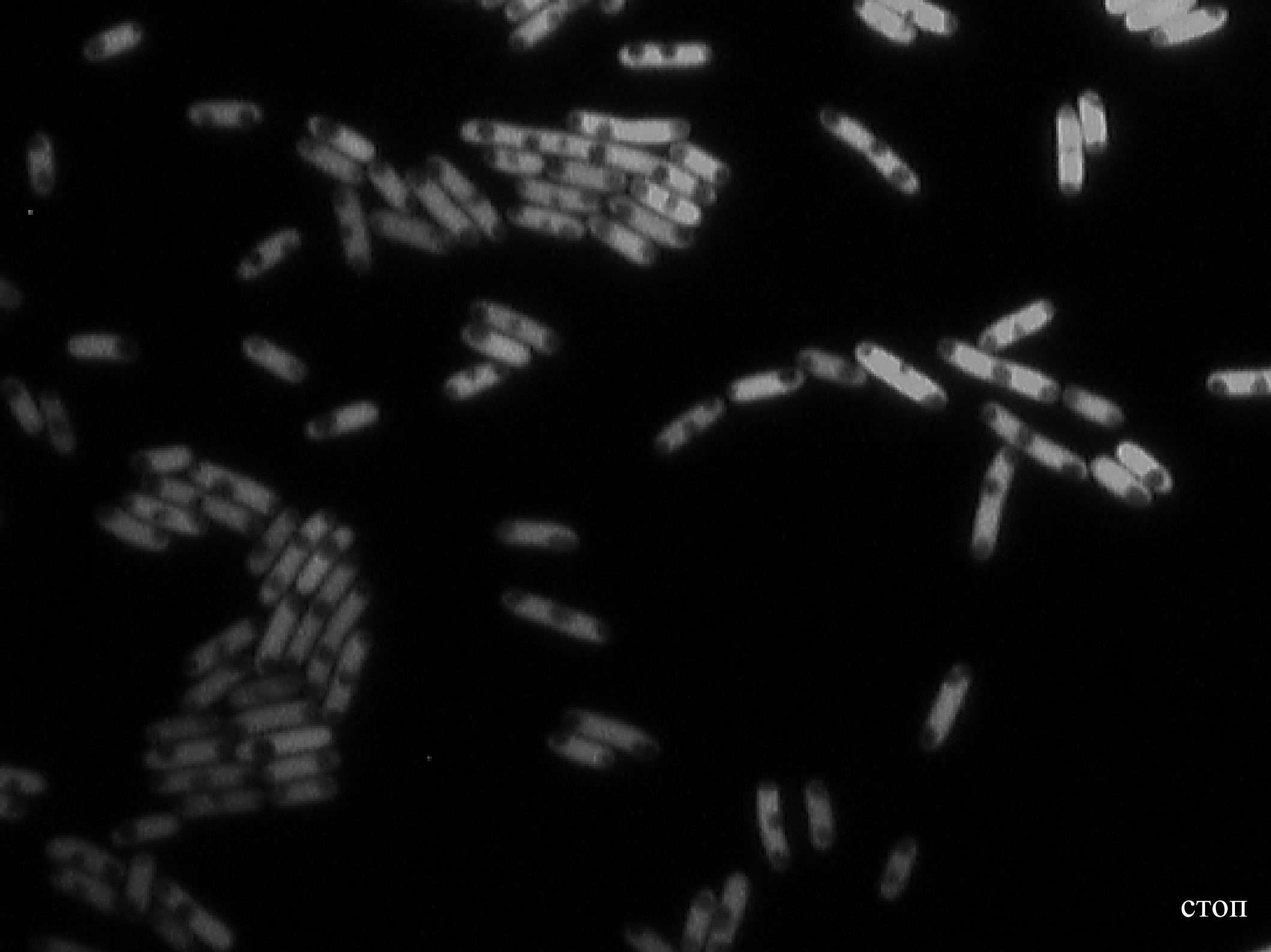
11/2/99 13:0:3



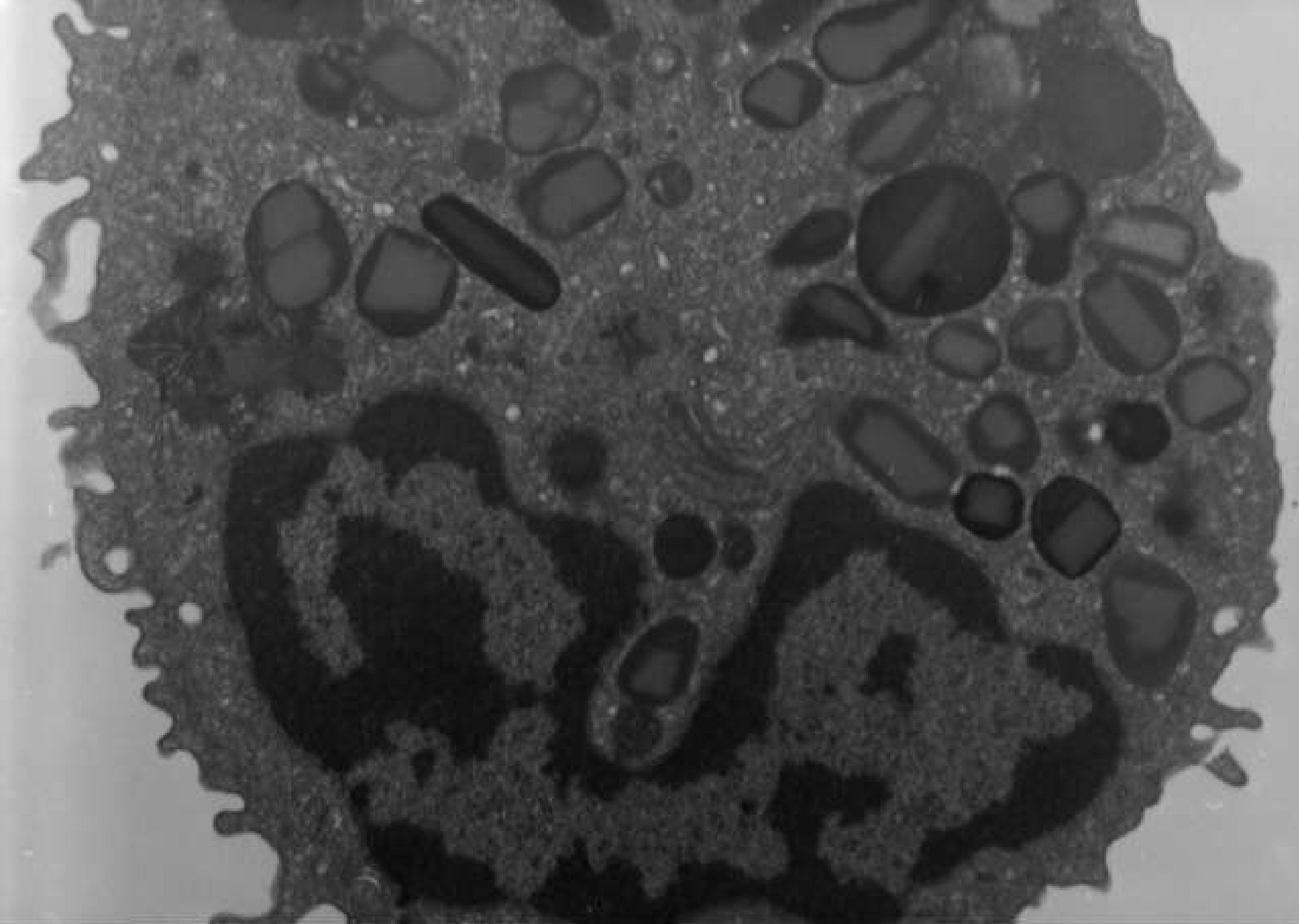
50 μm



20 um



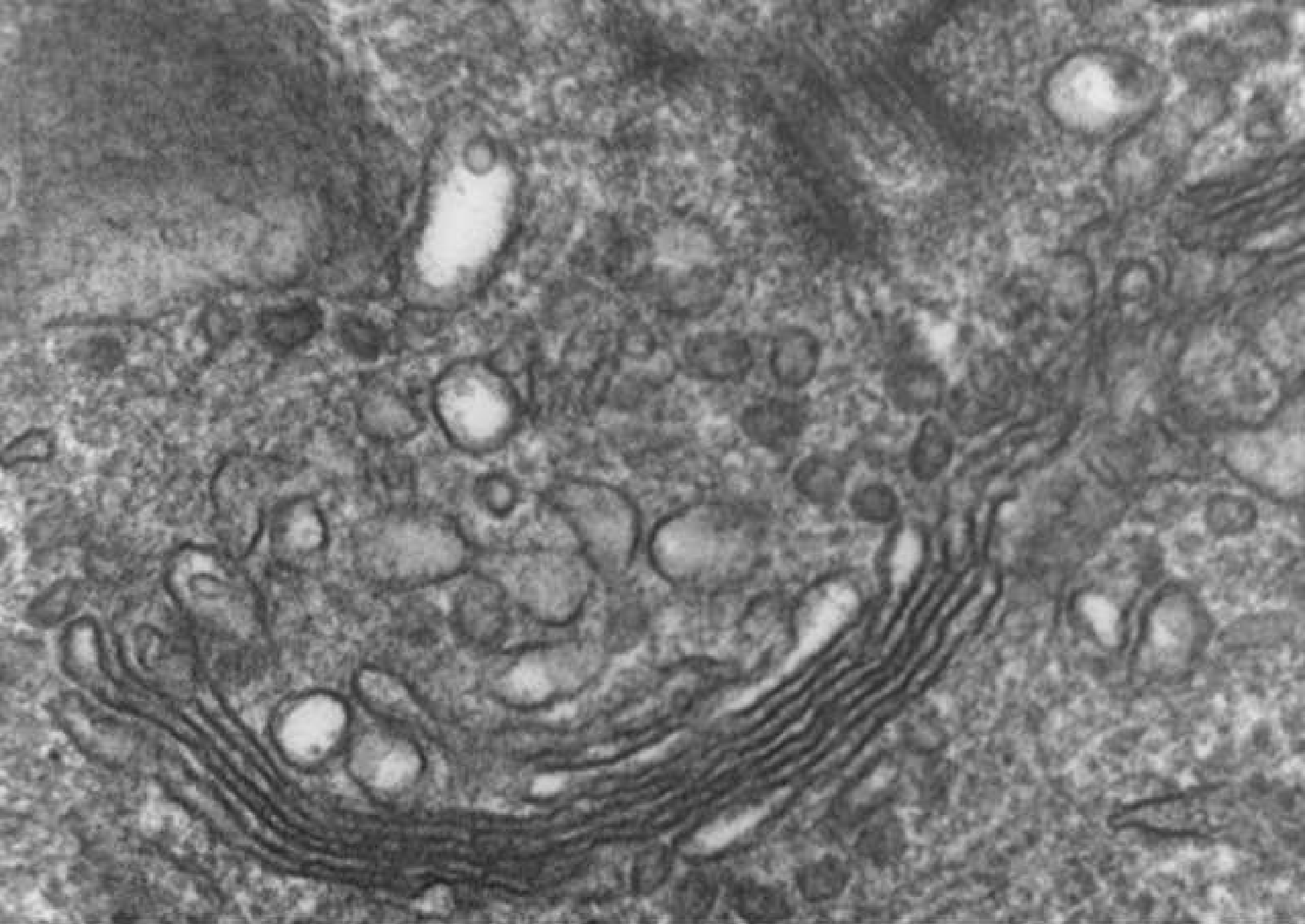
СТОП



500 nm

1Blood Cells

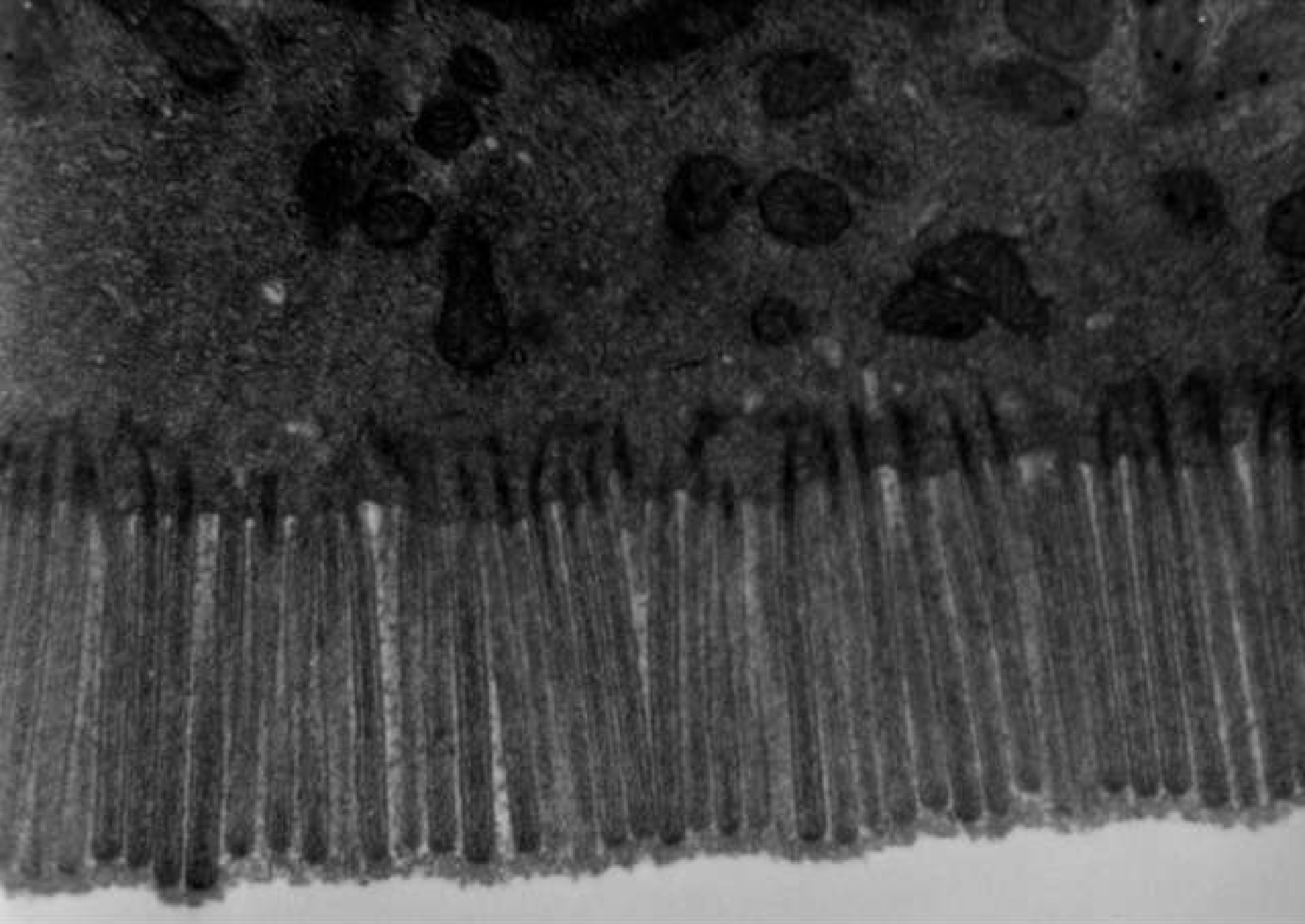
1/7/0 REMF



50 nm

3Blood Cells

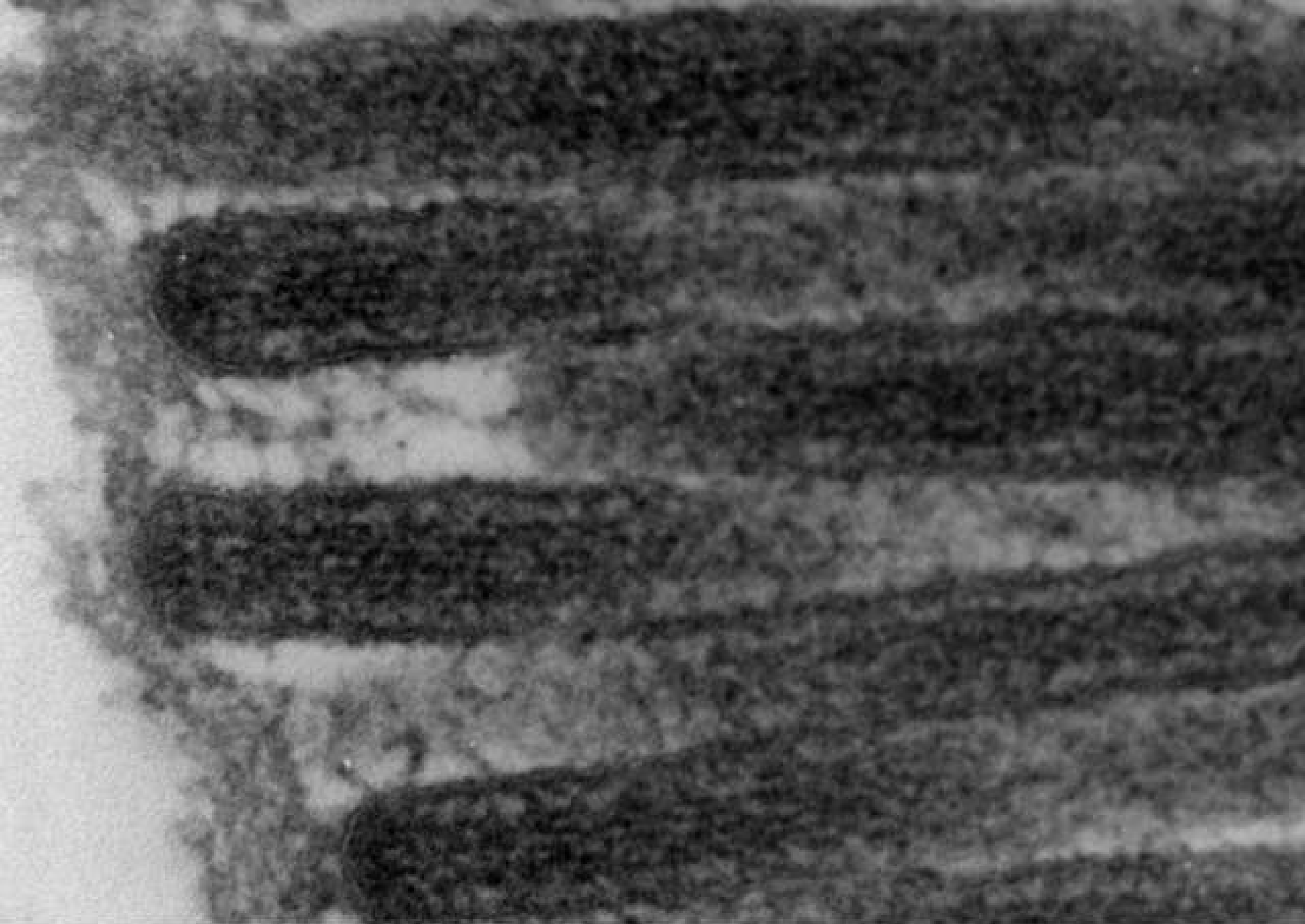
1/7/0 REMF



—
100 nm

1Microvilli

1/7/0 REMF



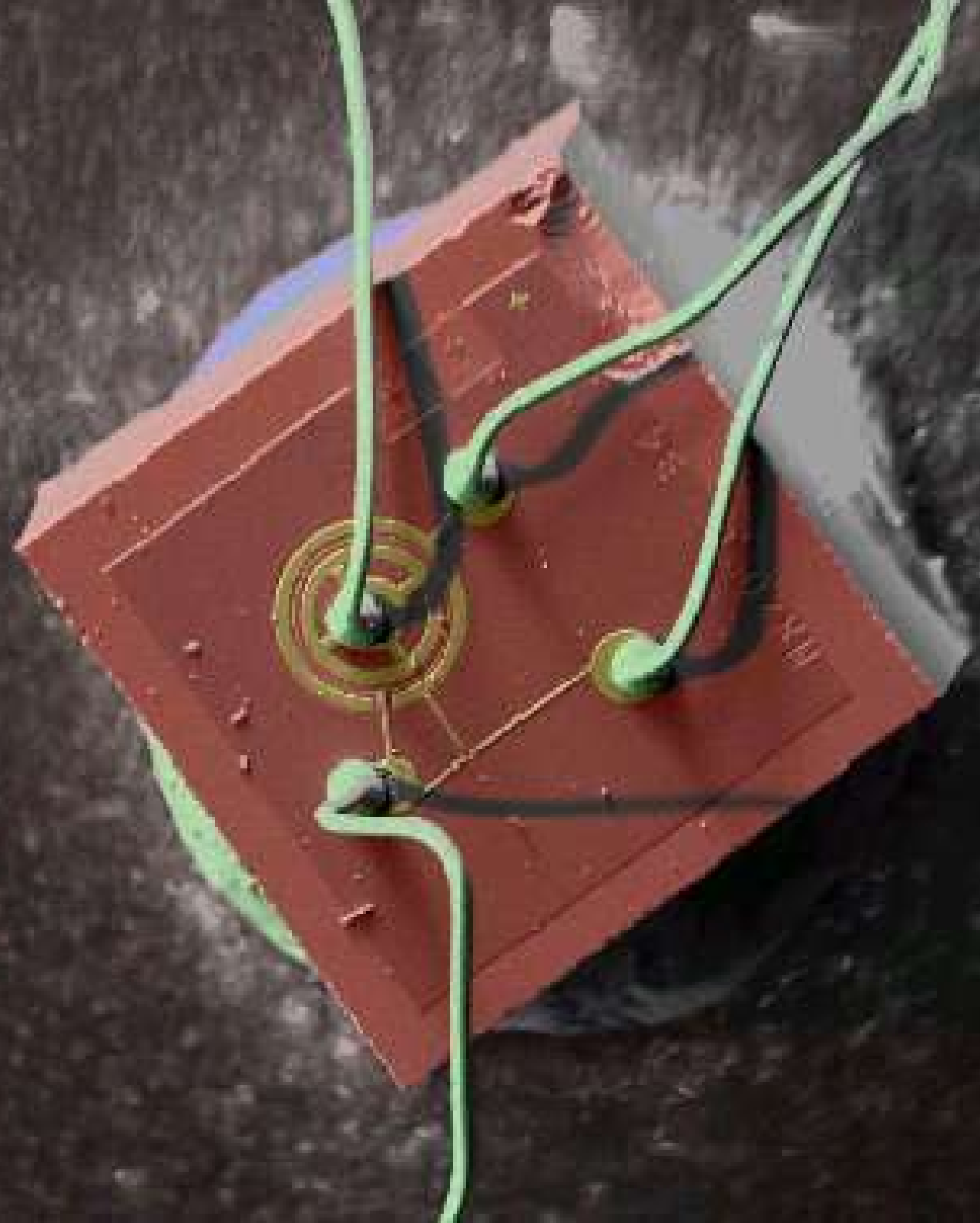
100 nm

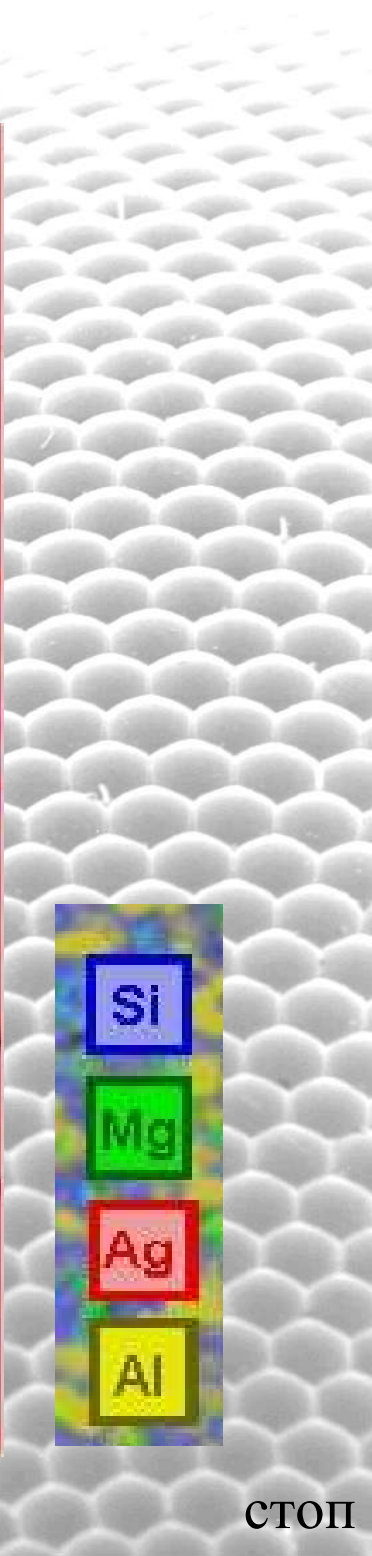
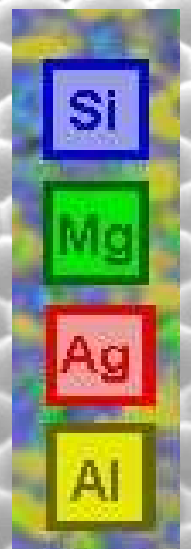
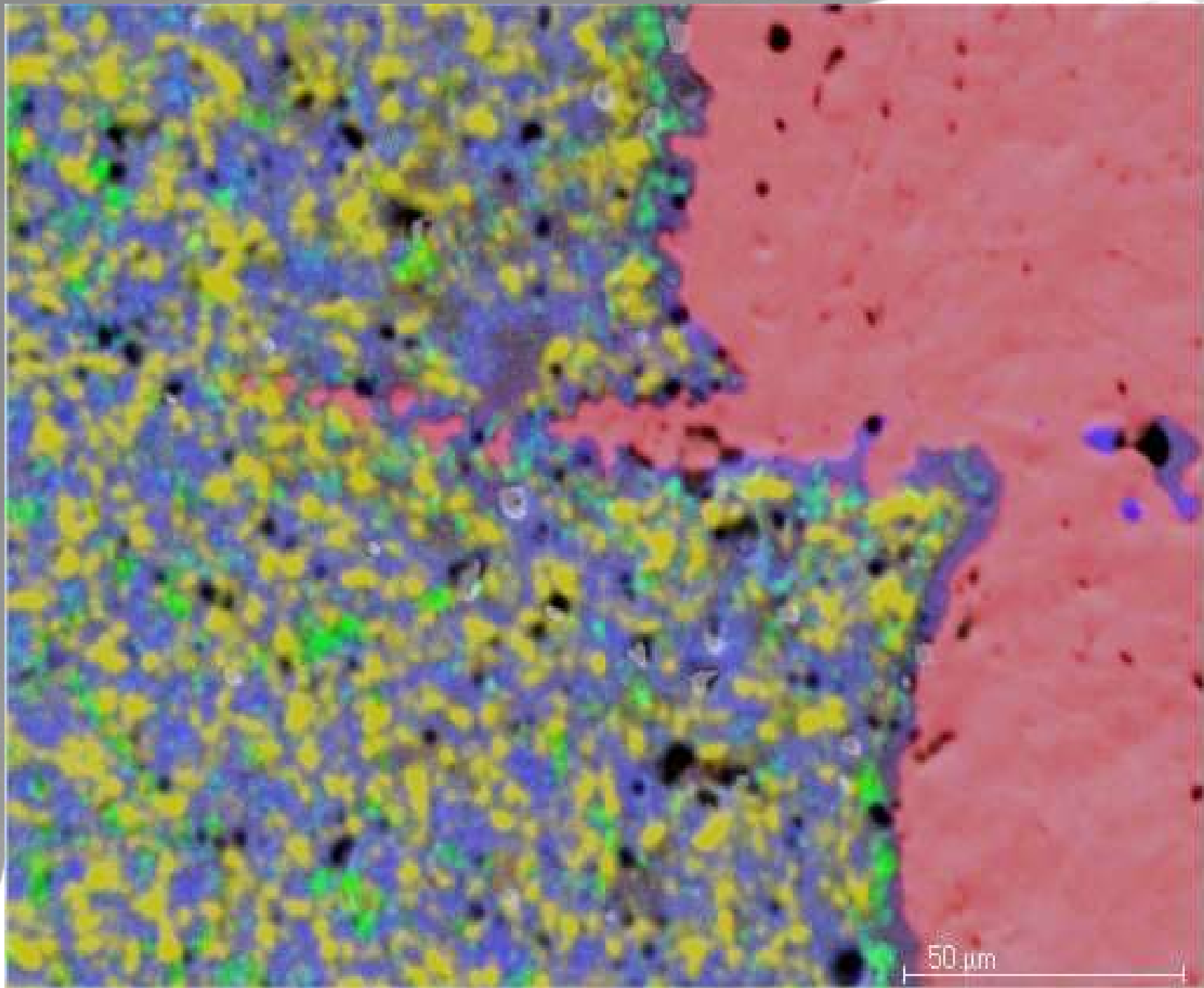
5Microvilli

1/7/0 REMF

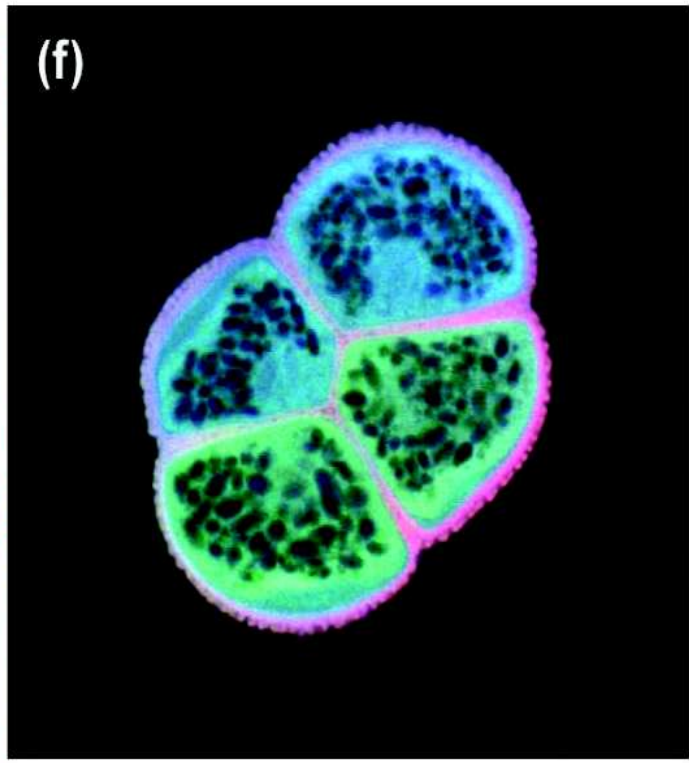
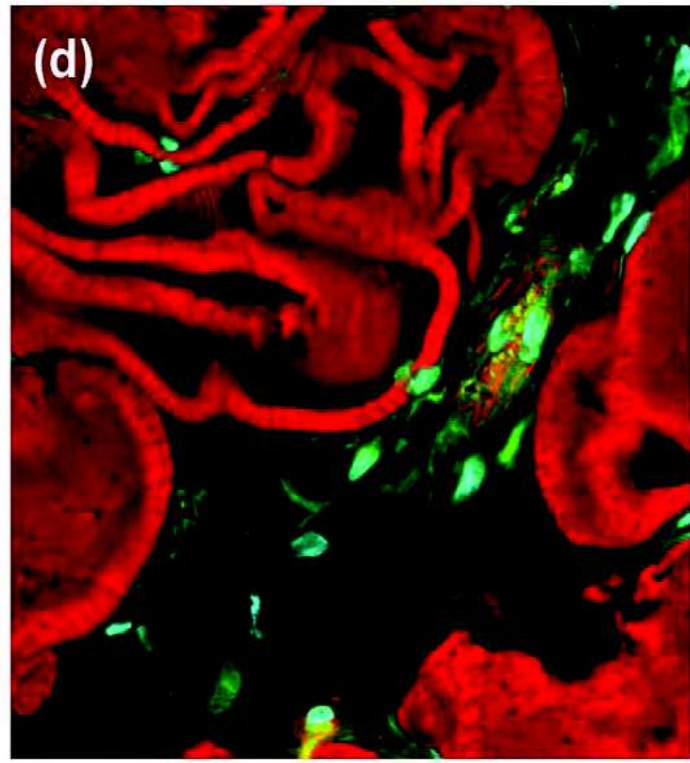
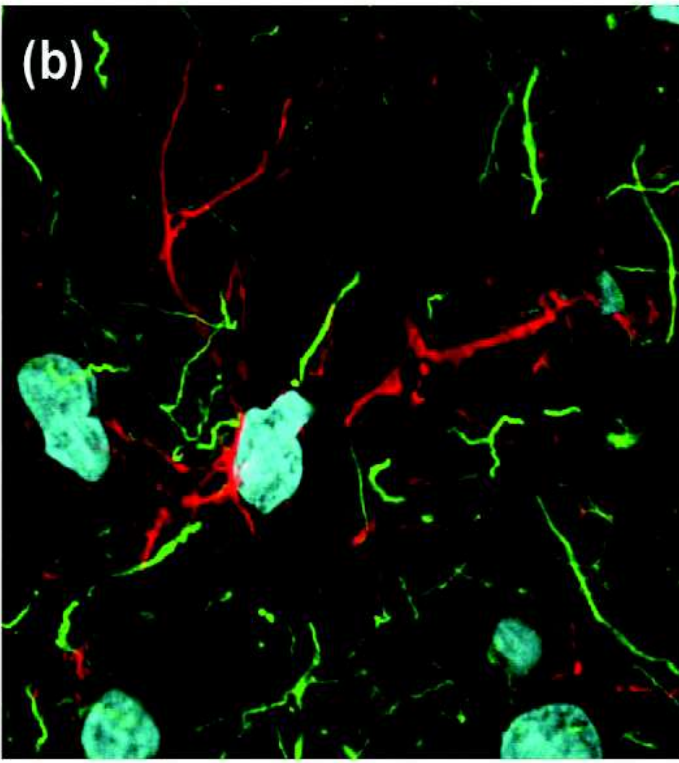
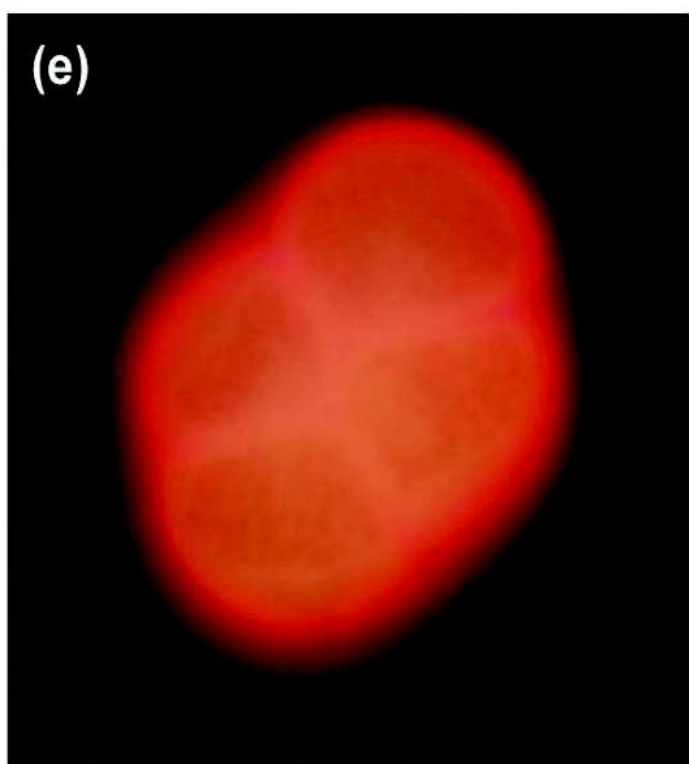
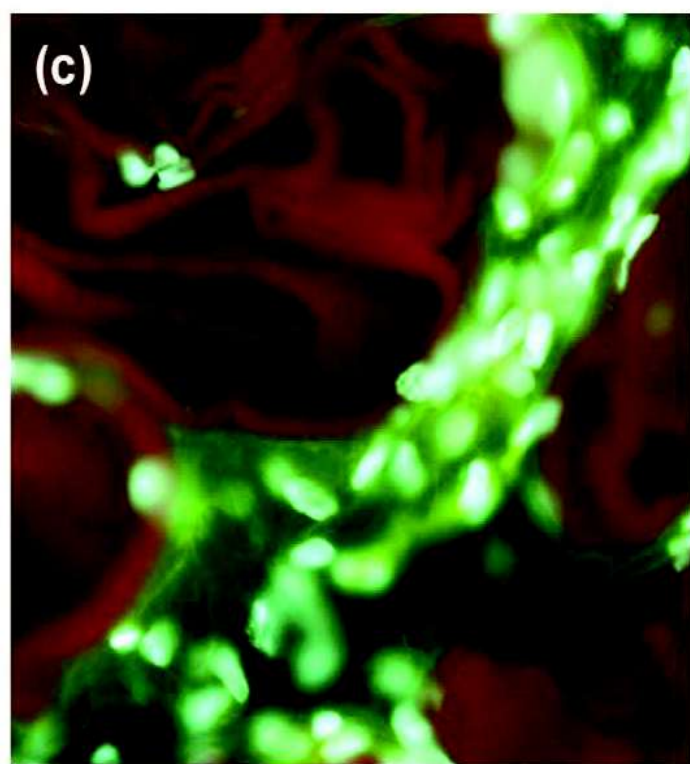
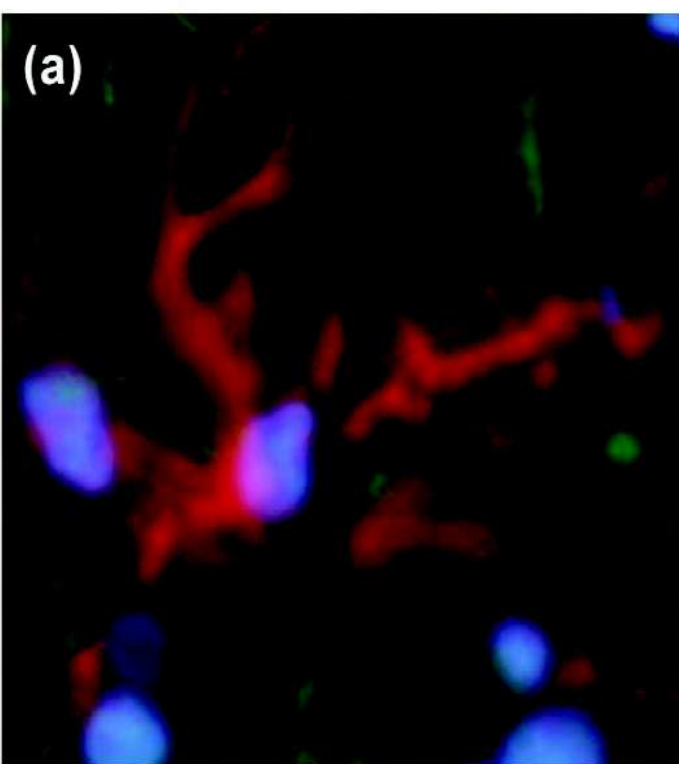
CTOII

- Si
- Al
- Sn
- Au

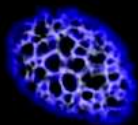




CTOII



1



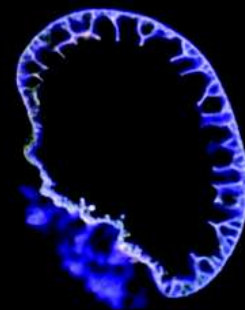
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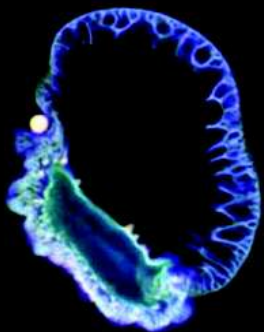
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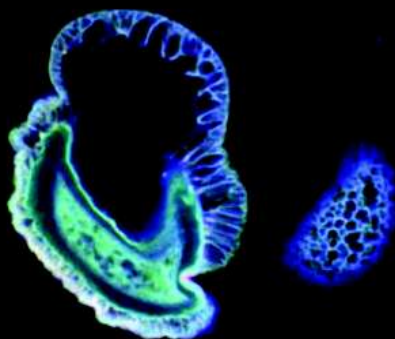
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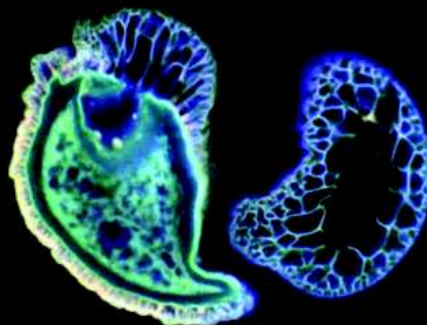
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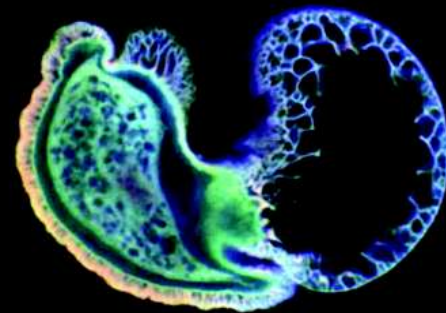
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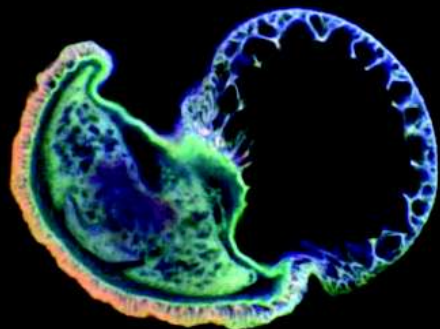
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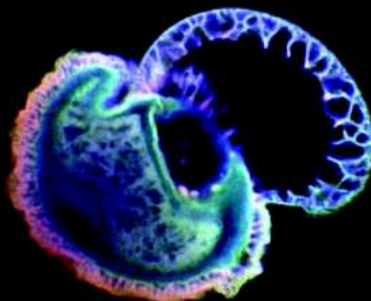
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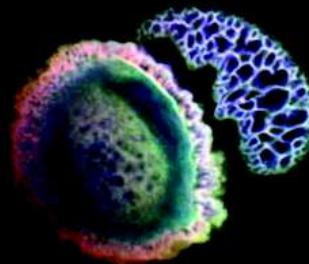
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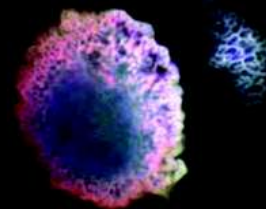
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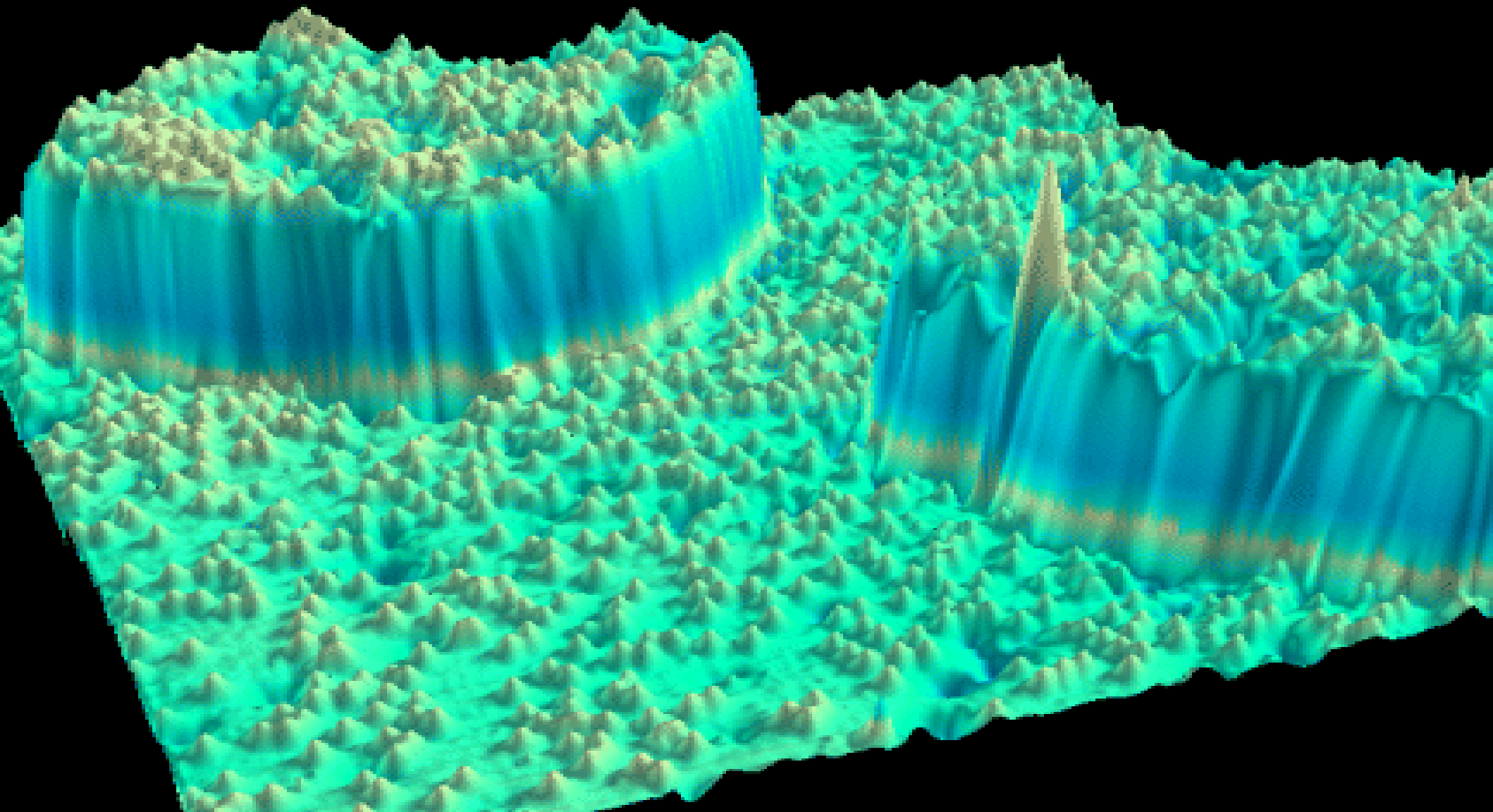
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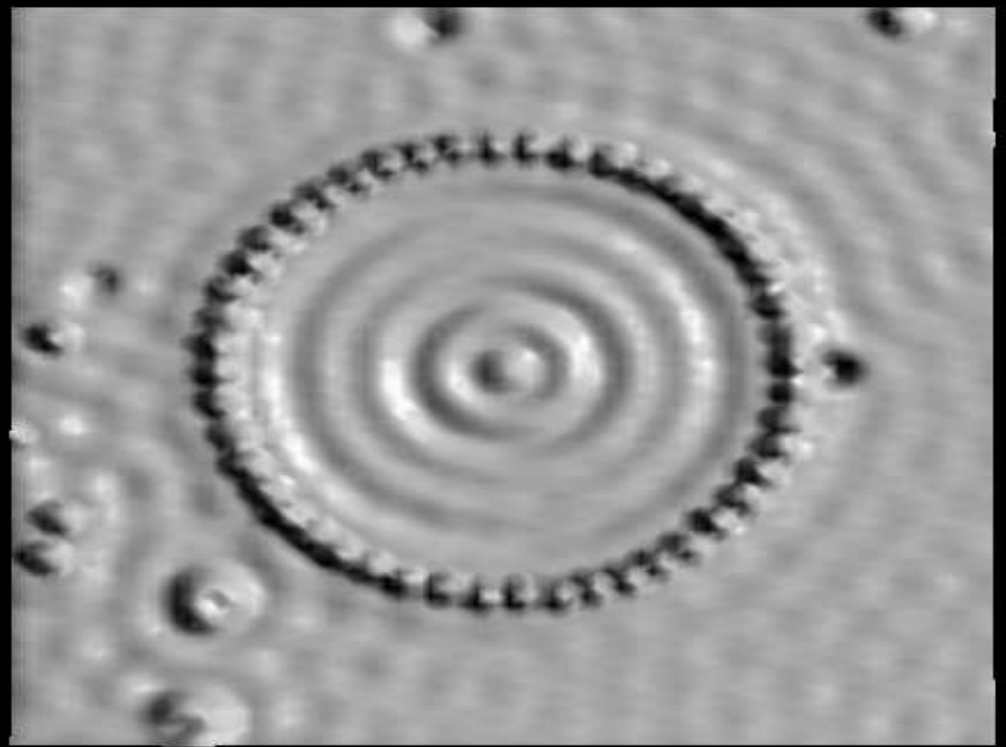
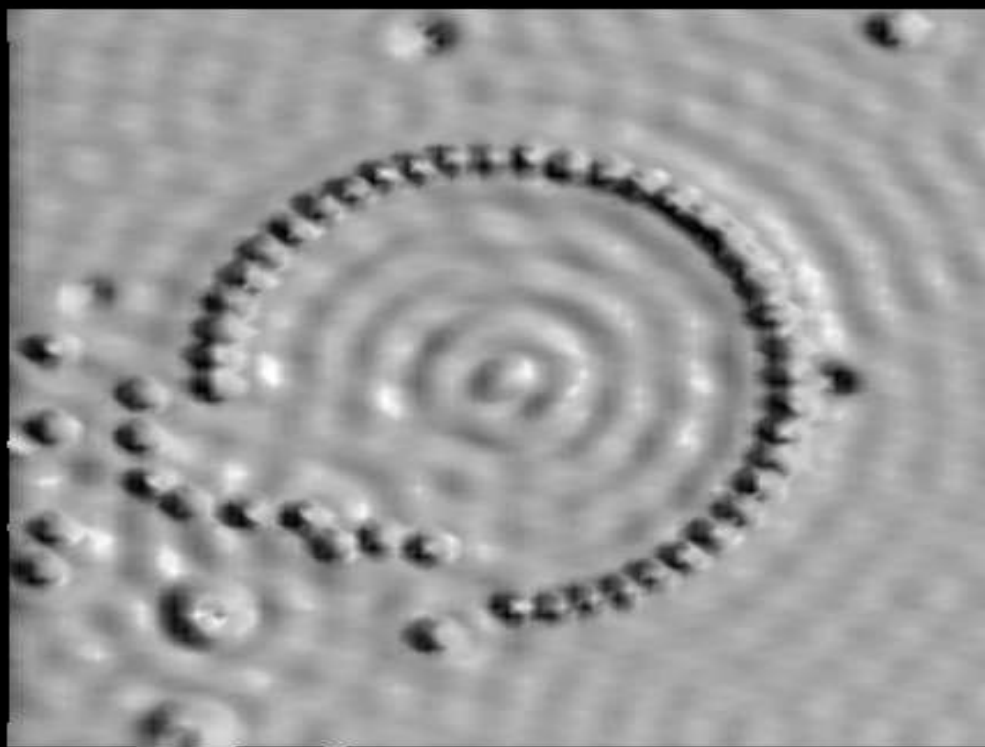
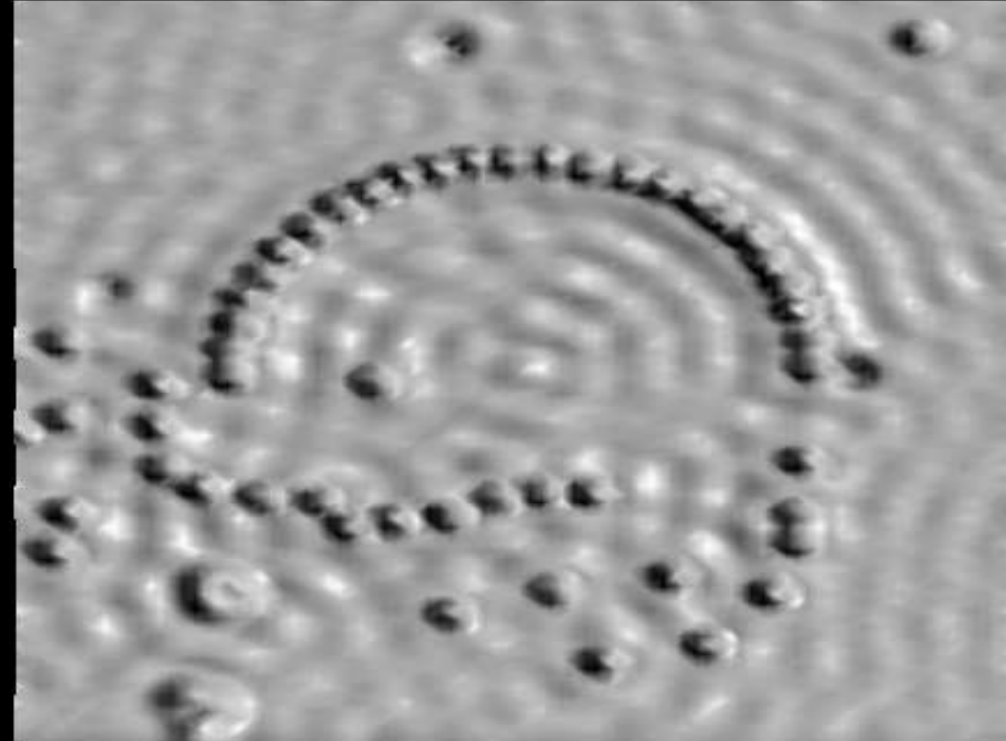
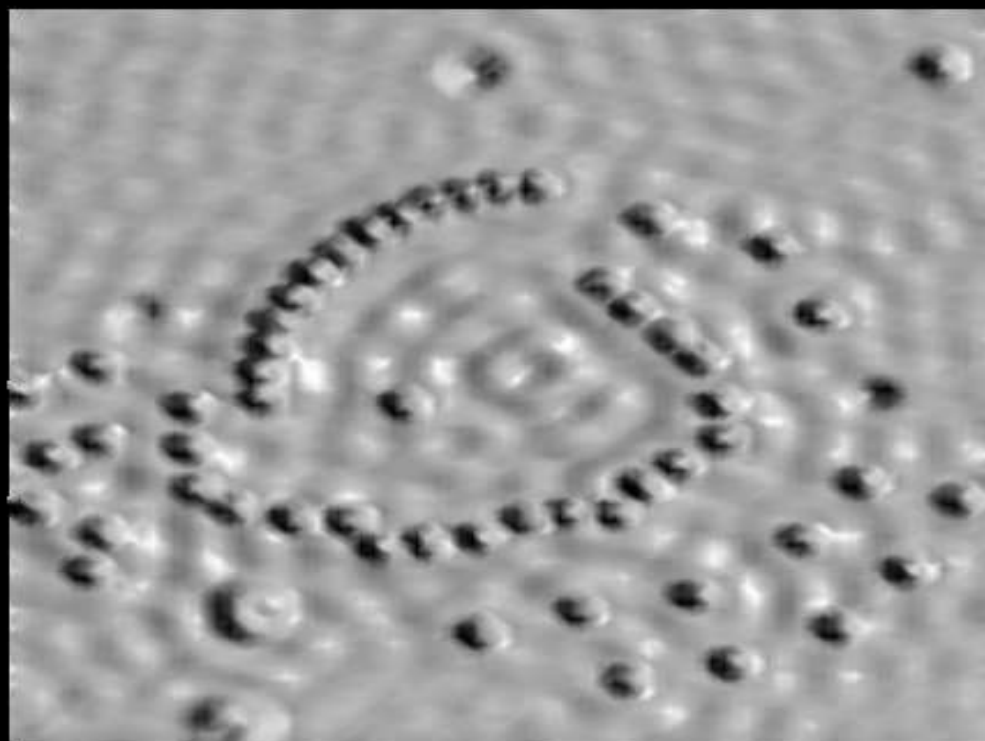


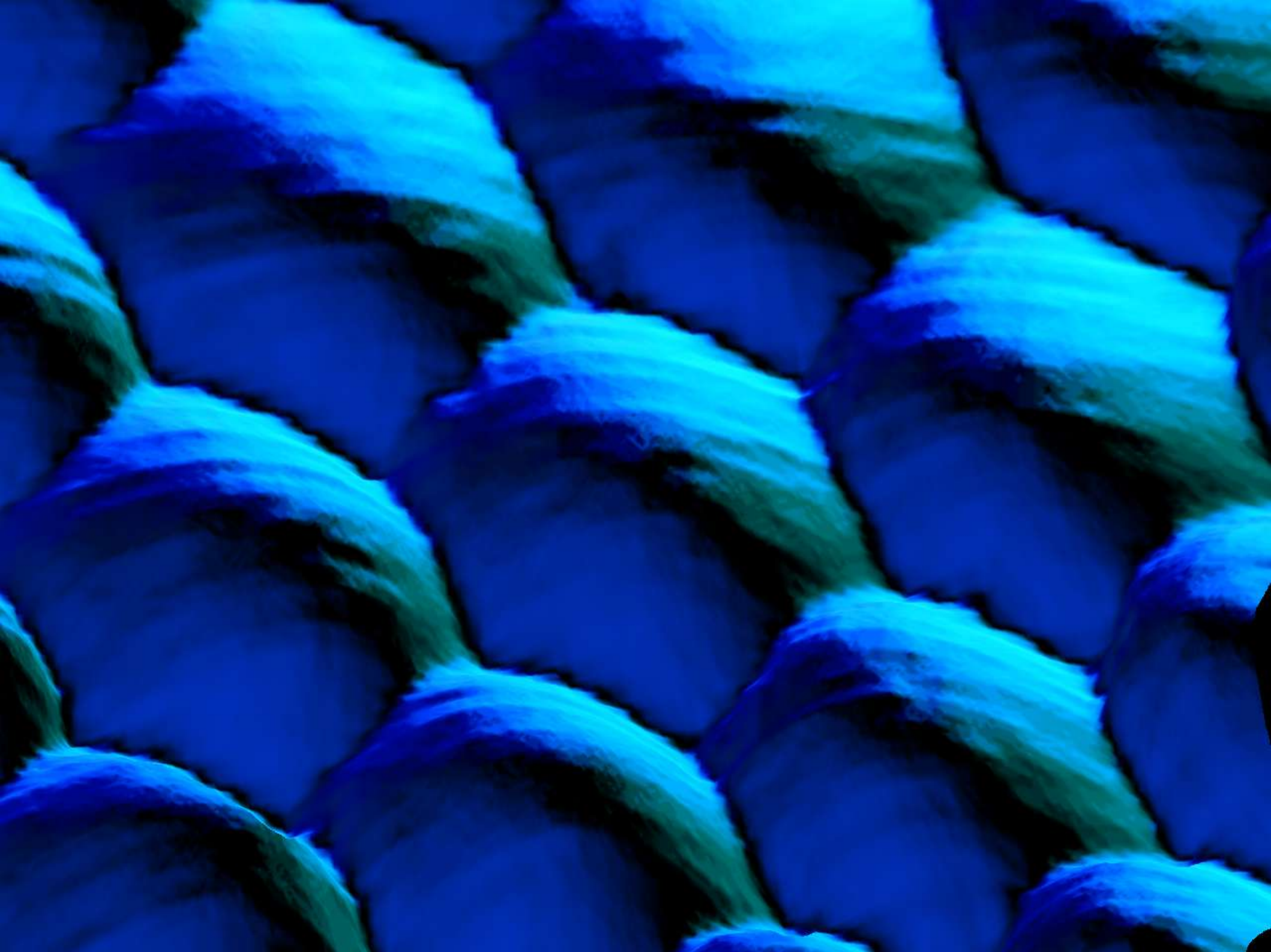
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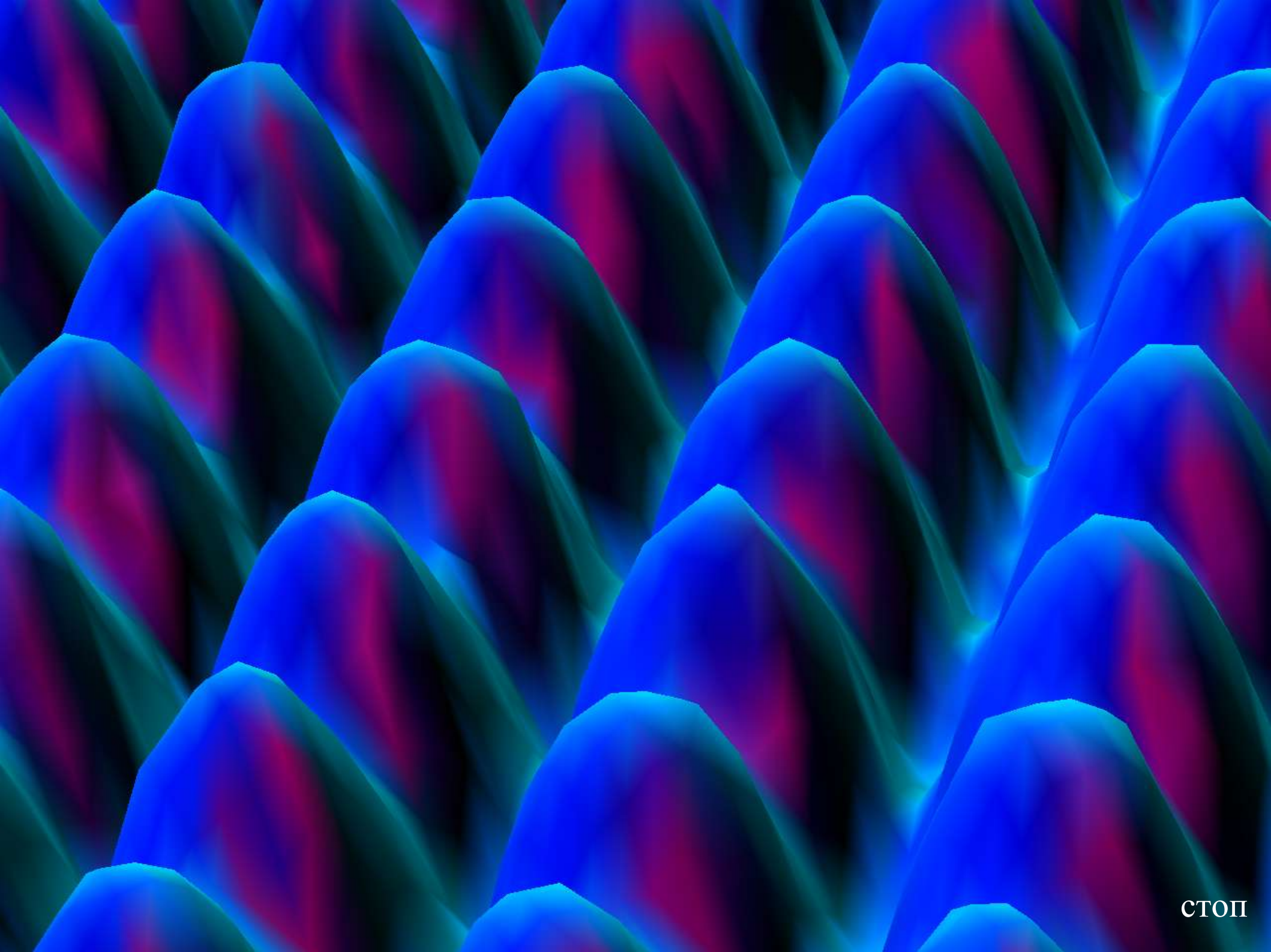


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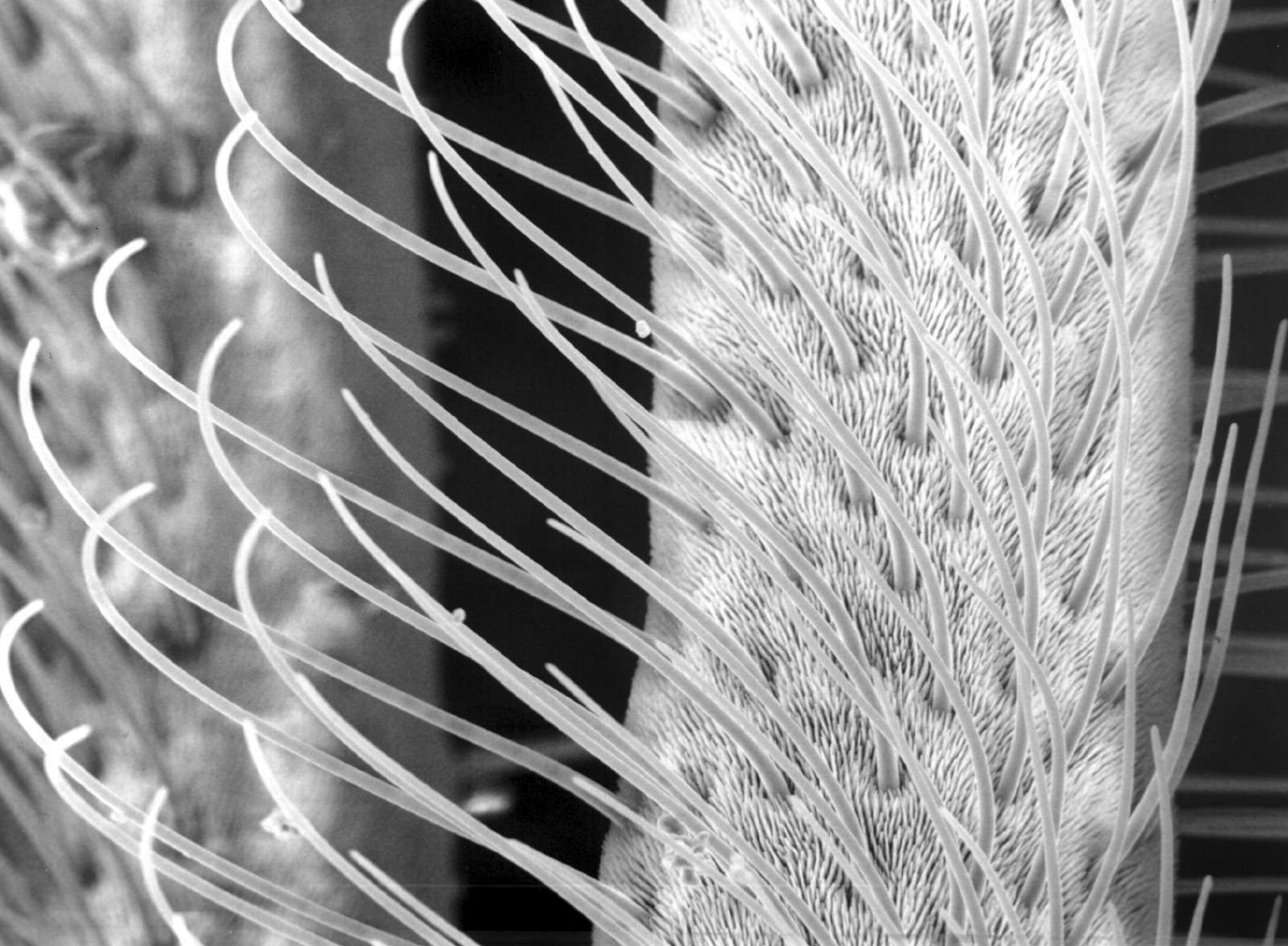




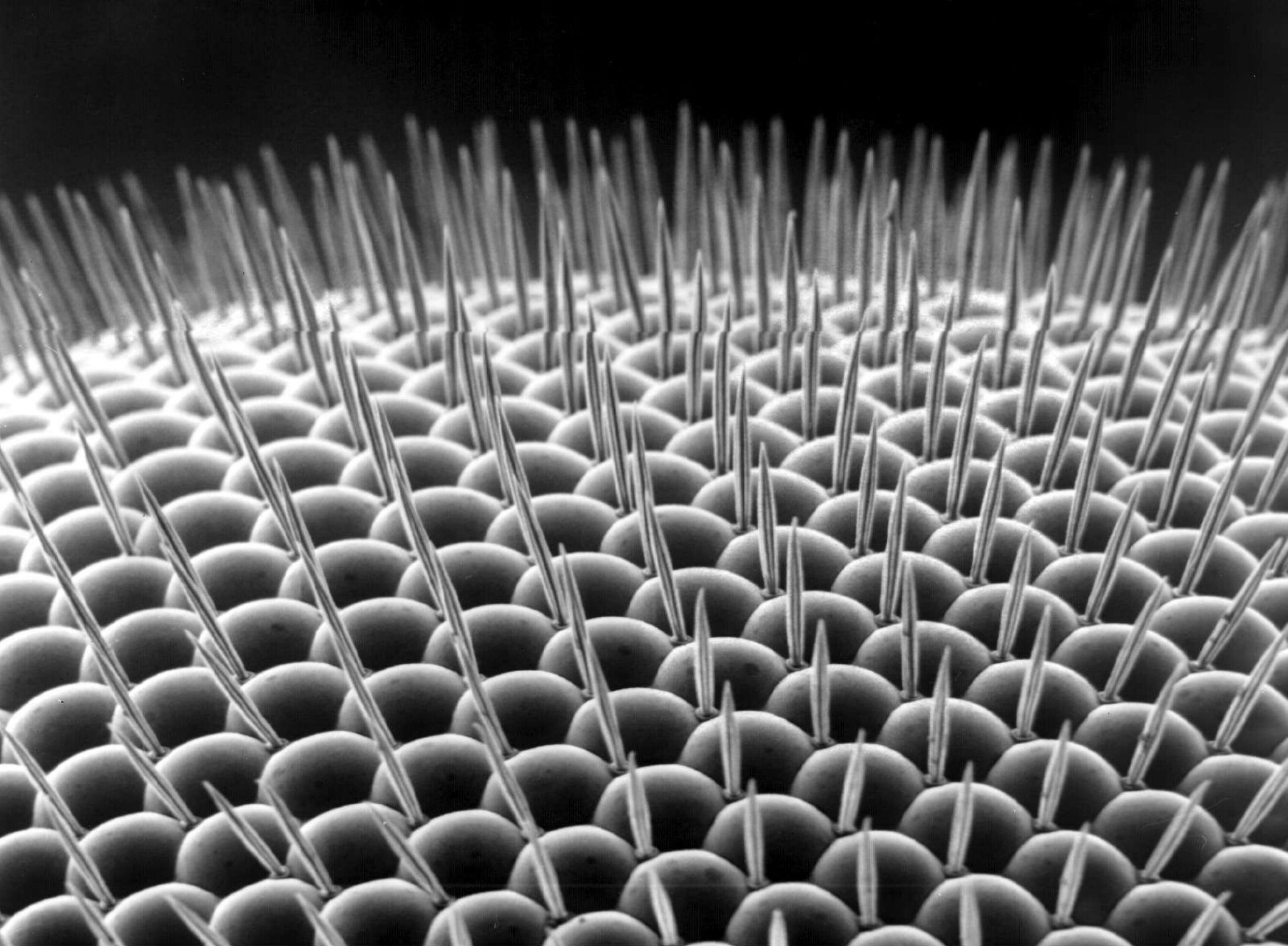


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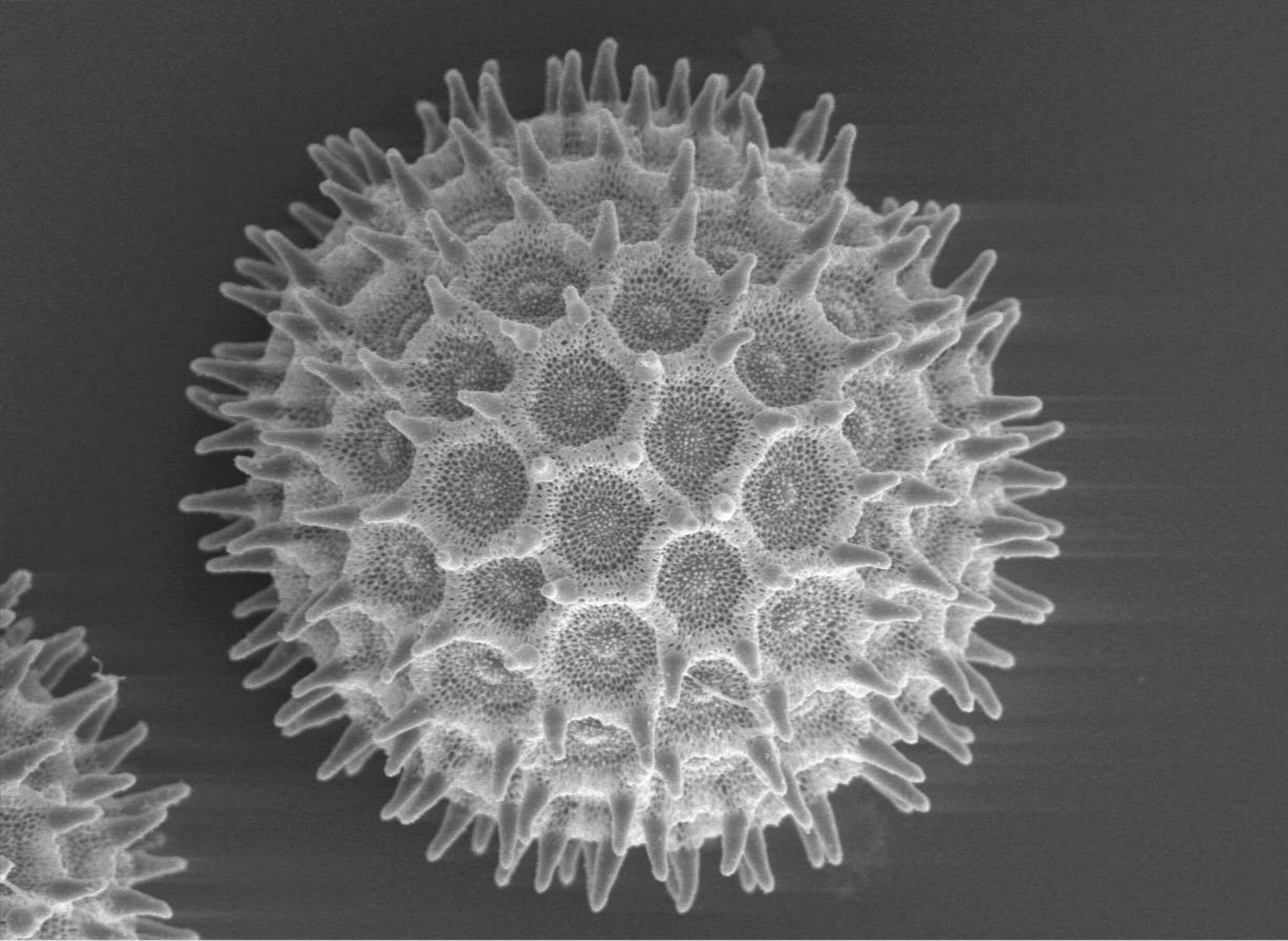








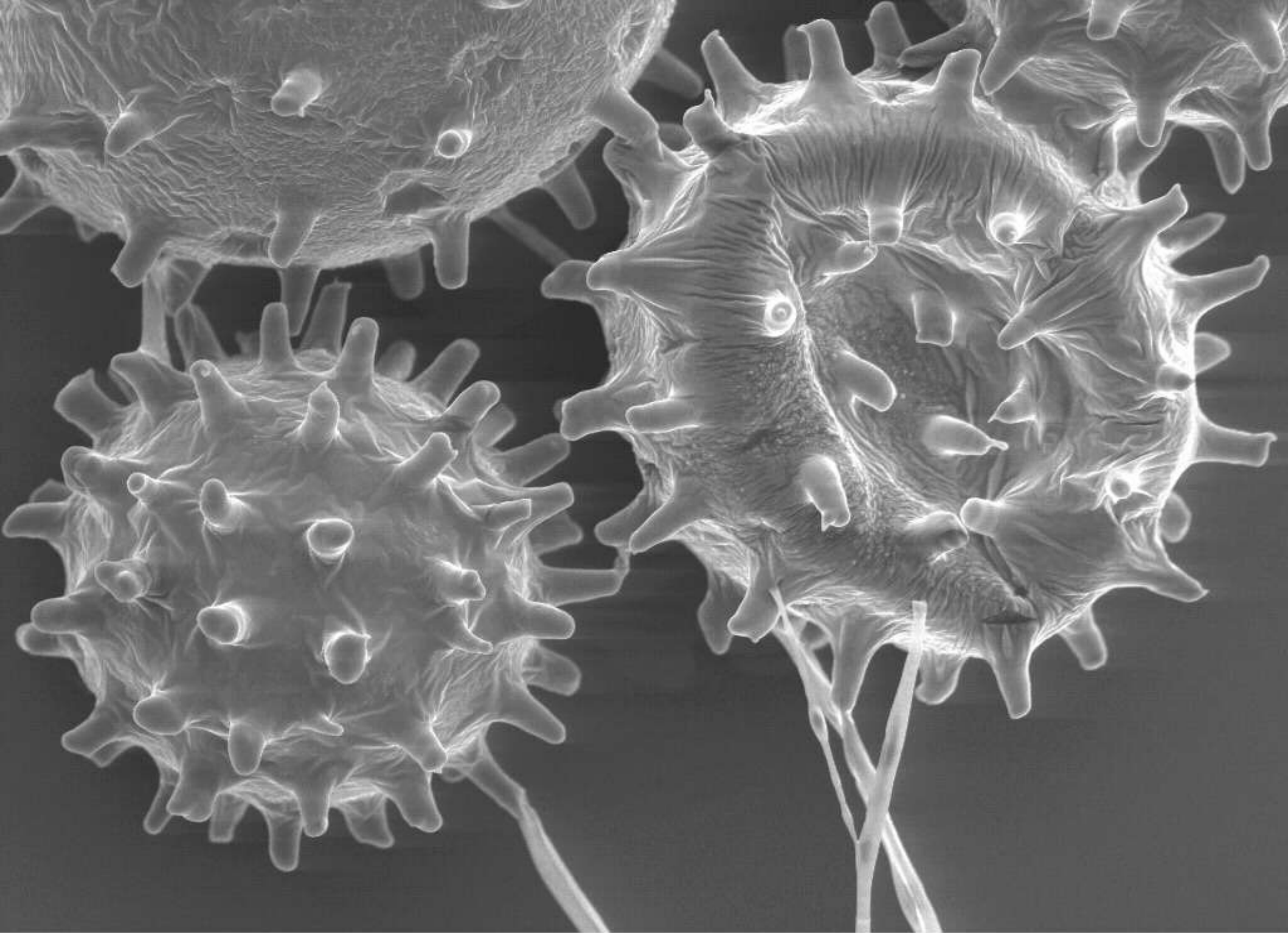




20 μm

04ConvolvulaceaePollen

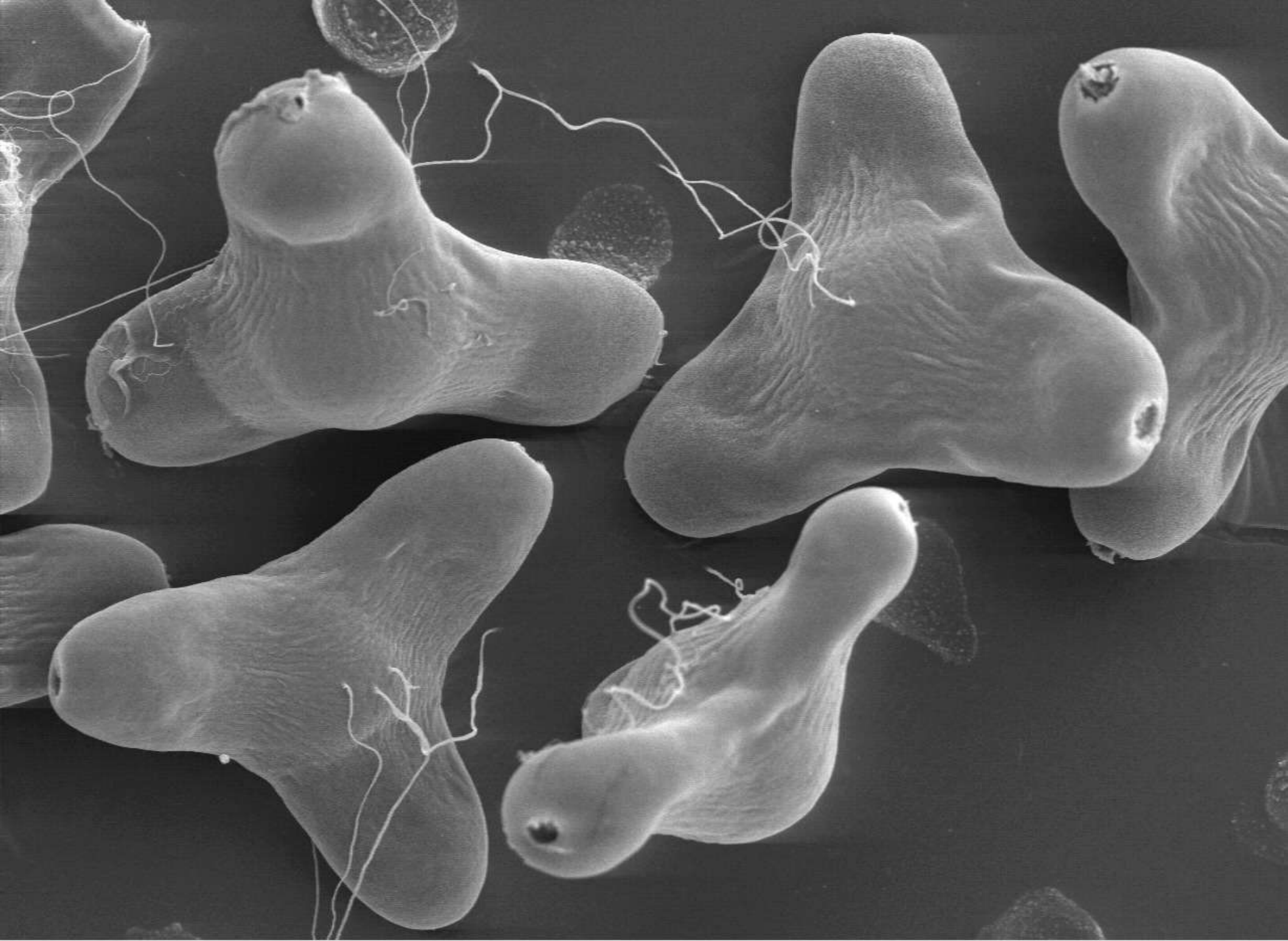
1/11/0 REMF



50 μm

Pollen1

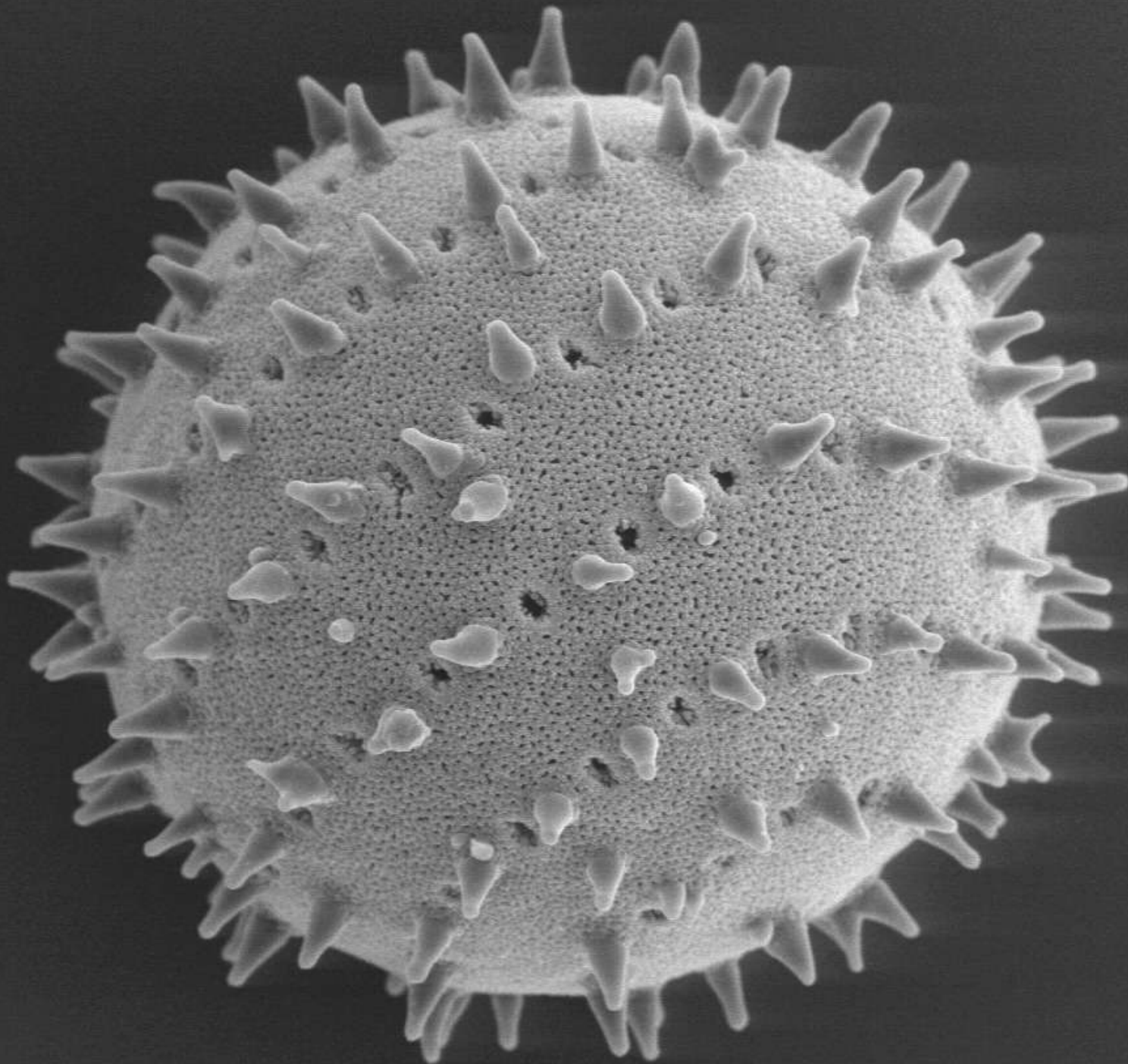
1/6/0 REMF



30 μ m

05OnagraceaePollen

1/6/0 REMF



Summary

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