Sample Preparation for X-ray Microscopy

Presented by
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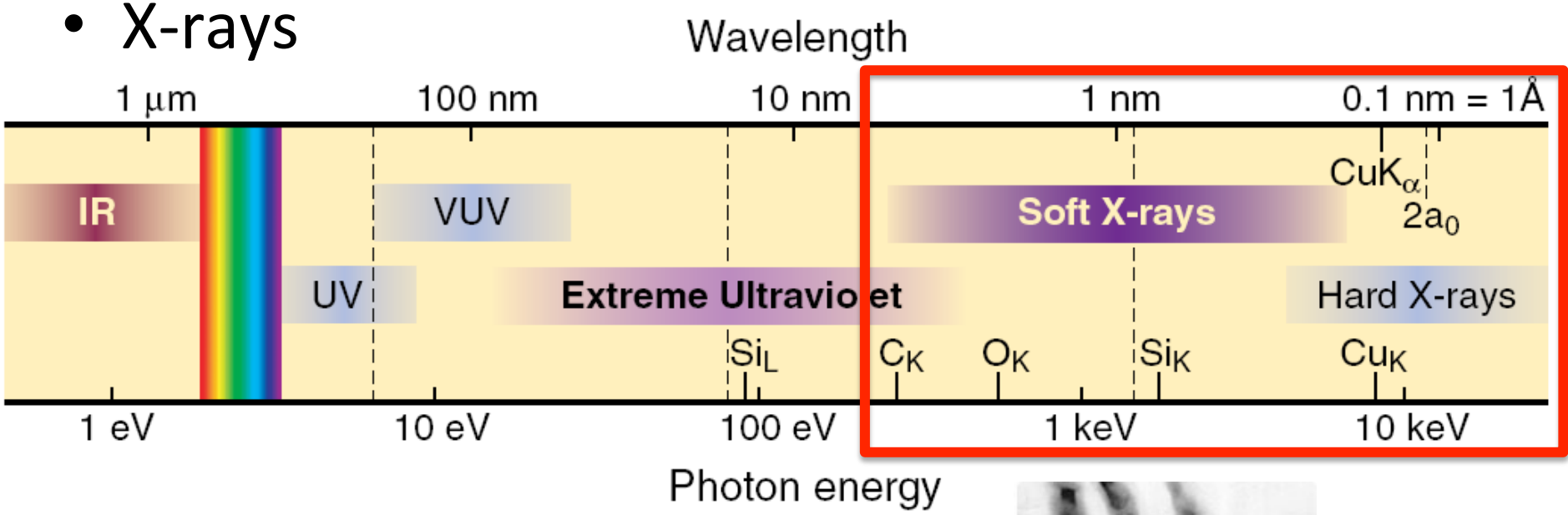
2014 DESY Summer Student Program
X-ray Crystallography and Bio-imaging Group

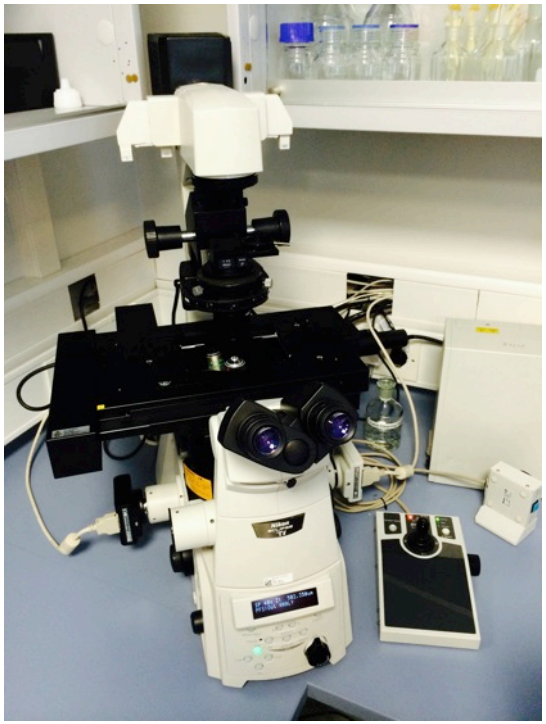
Introduction

- X-rays

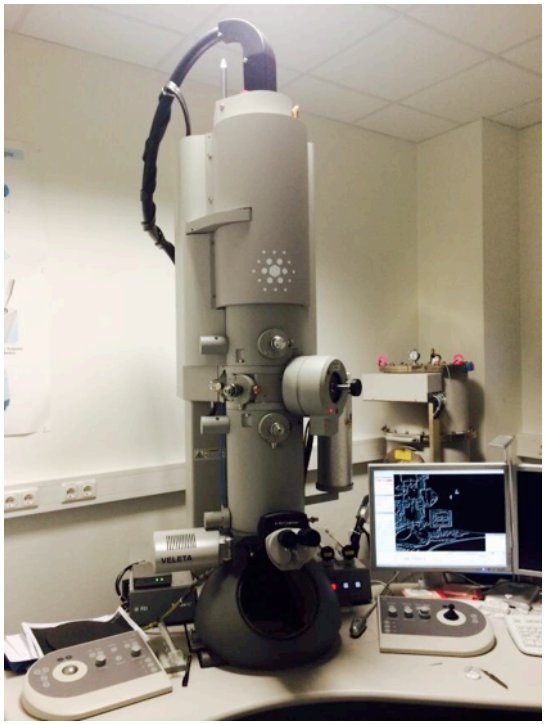


Introduction

- Currently common used microscopes



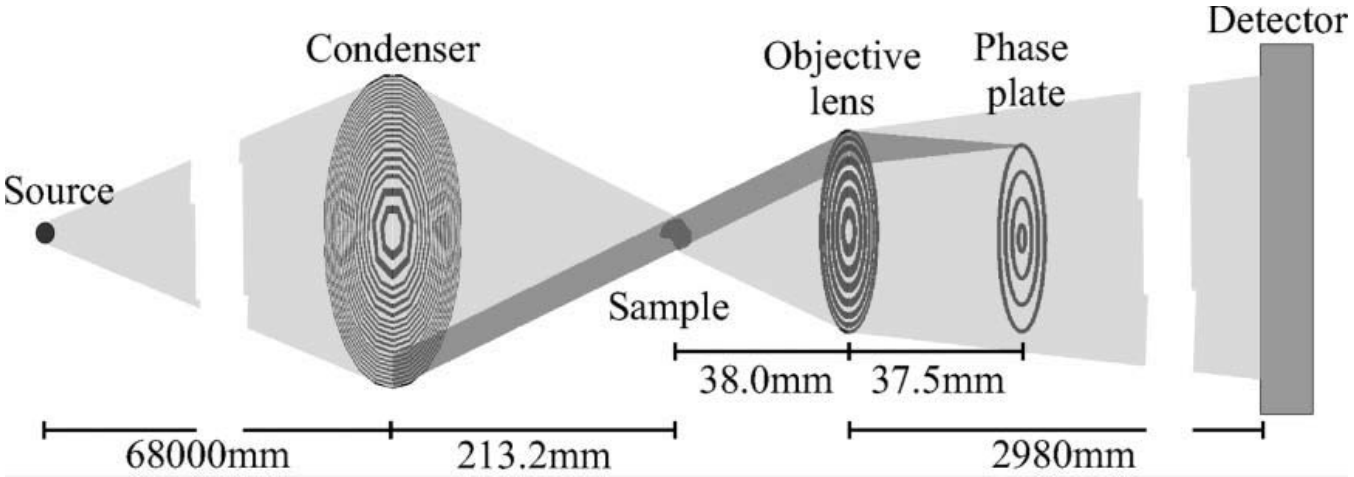
Light microscopy (LM)



Electron microscopy (EM)

Introduction

- X-ray microscopy at P11 beamline, PETRA III



Introduction

- Main ideas

Radiation sources
- Visible light (LM)
- Electron beam (EM)
- X-rays (XRM)

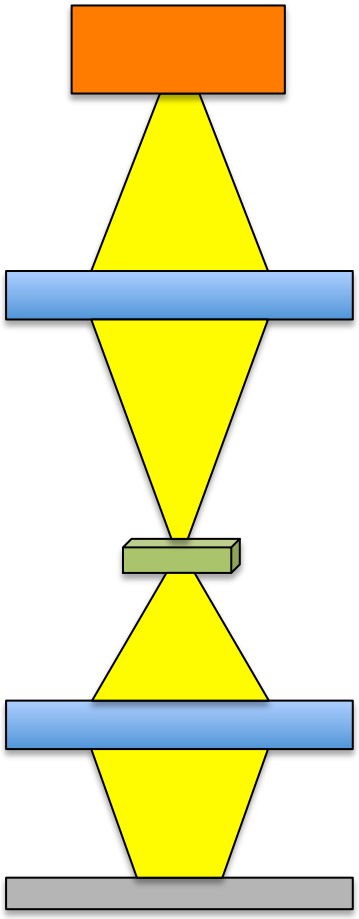
Condenser Lenses
- Optic lens (LM)
- Electromagnetic lens (EM)
- Zone plate lens (XRM)

Sample

Objective Lenses

Detector
- Charge coupled device (CCD)

Transmission mode



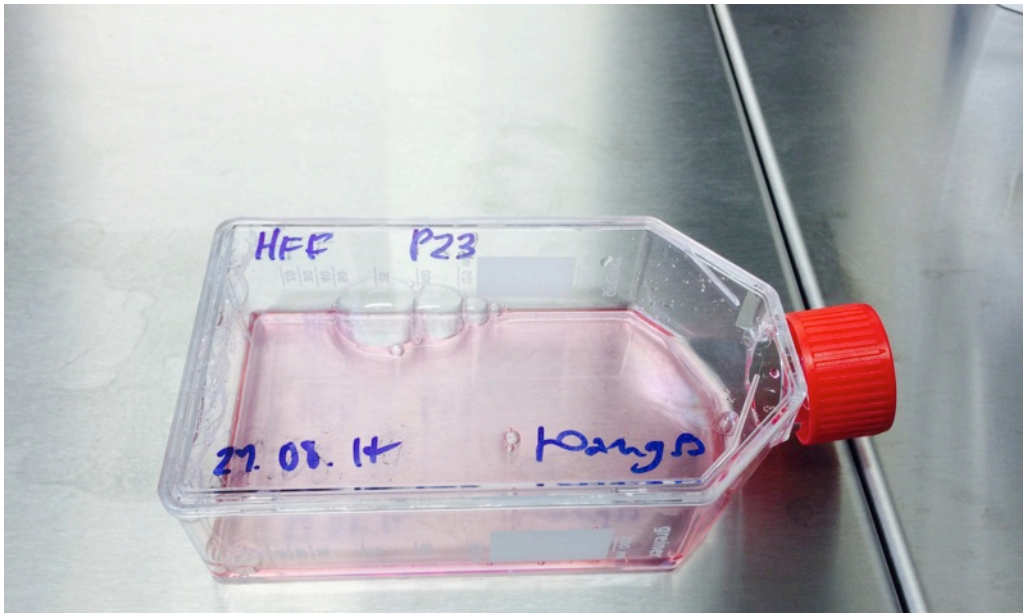
Radiation direction

Objectives

- Biological sample preparation for room temperature x-ray microscopy
  - The sample quality control without the need of an x-ray microscope
  - Correlation between x-ray and electron microscopy

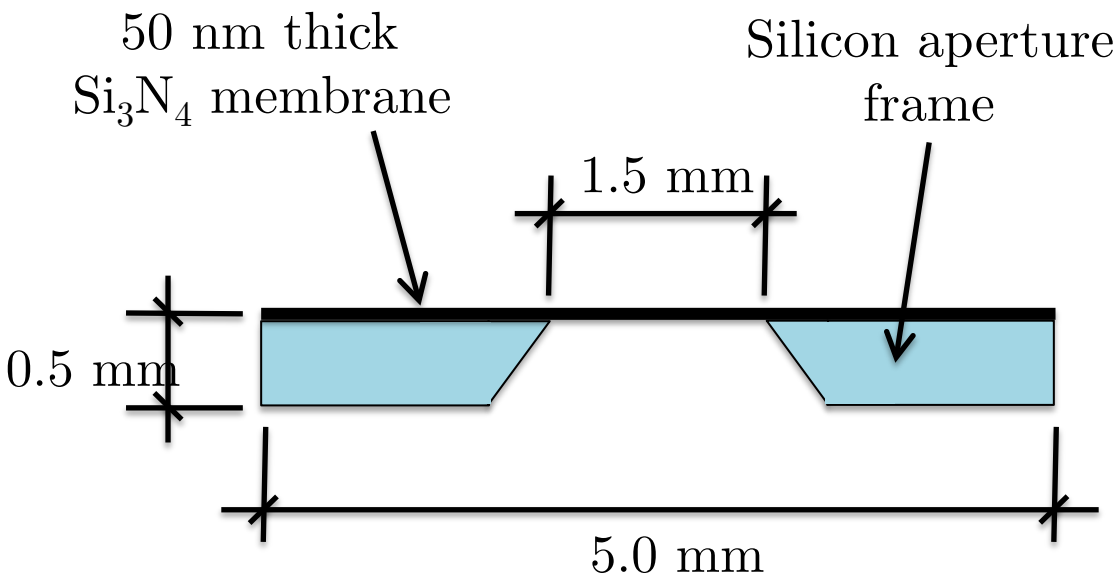
Sample Preparation

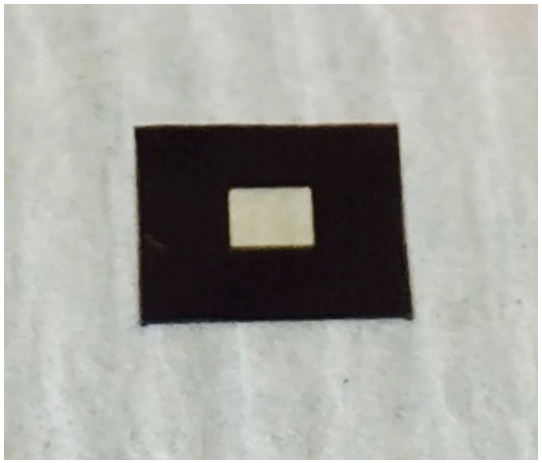
- Human foreskin fibroblast (HFF)
  - Cell culture in Dulbecco's Modified Eagles Medium at 37°C and 5% CO_2



Sample Preparation

- Human foreskin fibroblast (HFF)
  - Cell growth on silicon nitride (Si_3N_4) membrane





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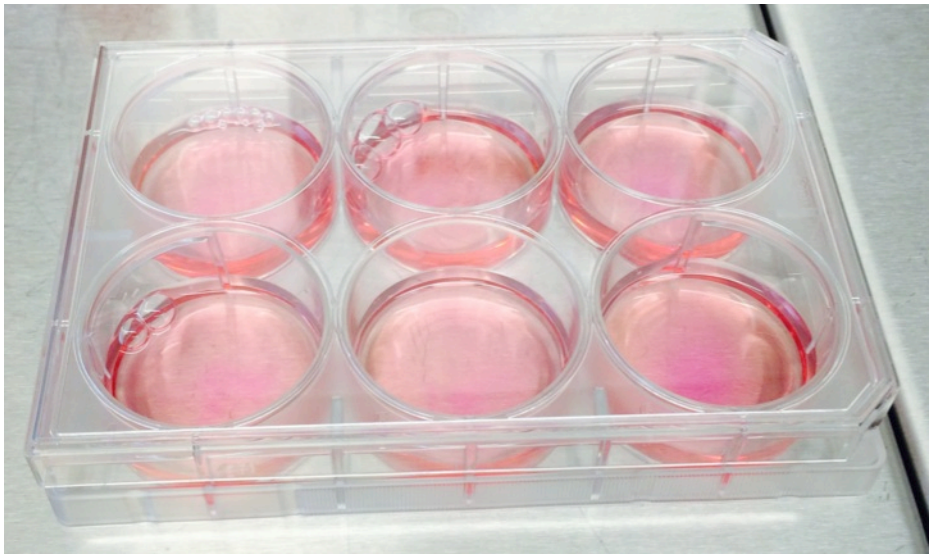
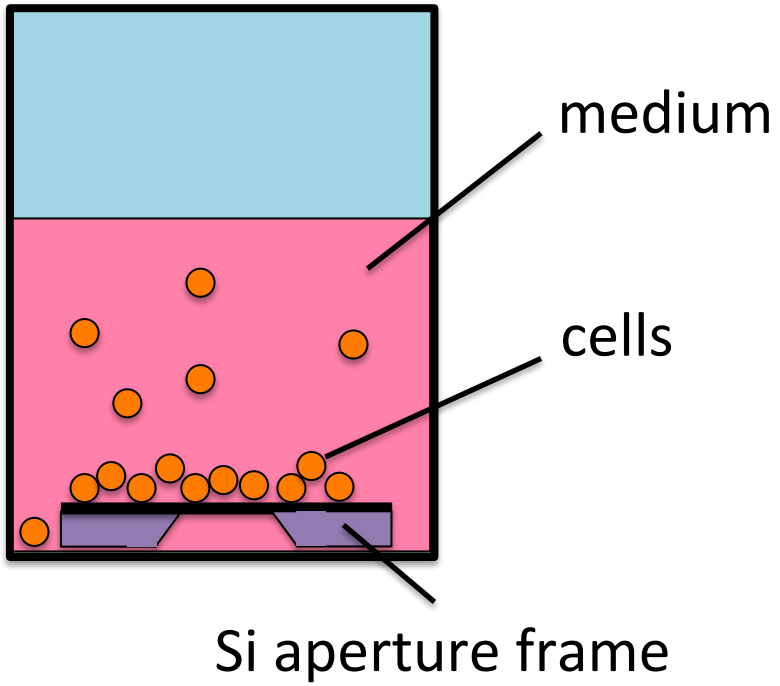




Sample Preparation

- XRM sample preparation based on EM methods

XRM Sample

Fixation

- To Inactivate all cellular activities with paraformaldehyde and glutaraldehyde solution

Staining with heavy metal salts

- To improve absorptivity and scattering for a good contrast image with osmium tetraoxide

Dehydration

- To remove all water in a cell with series of ethanol and freon 113 solution

----------check with SEM----------

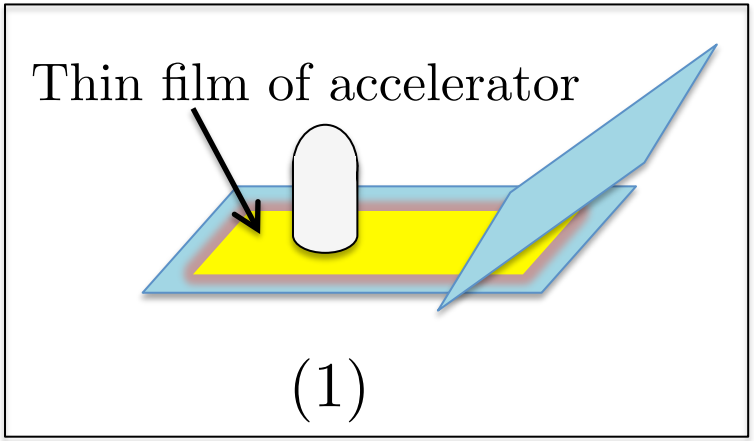
Evaluation in TEM

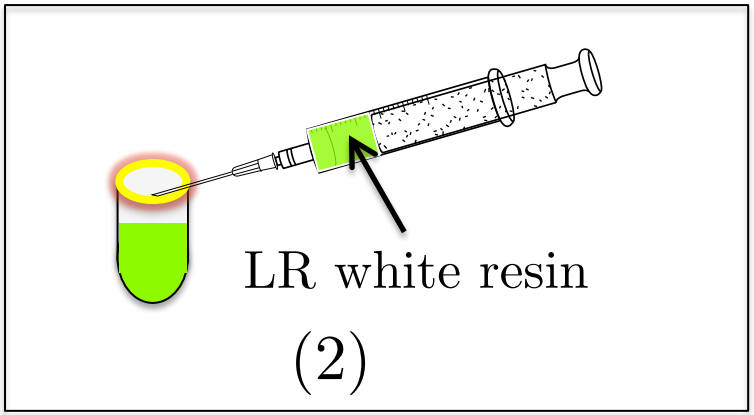
Resin embedding & sectioning (only in TEM)

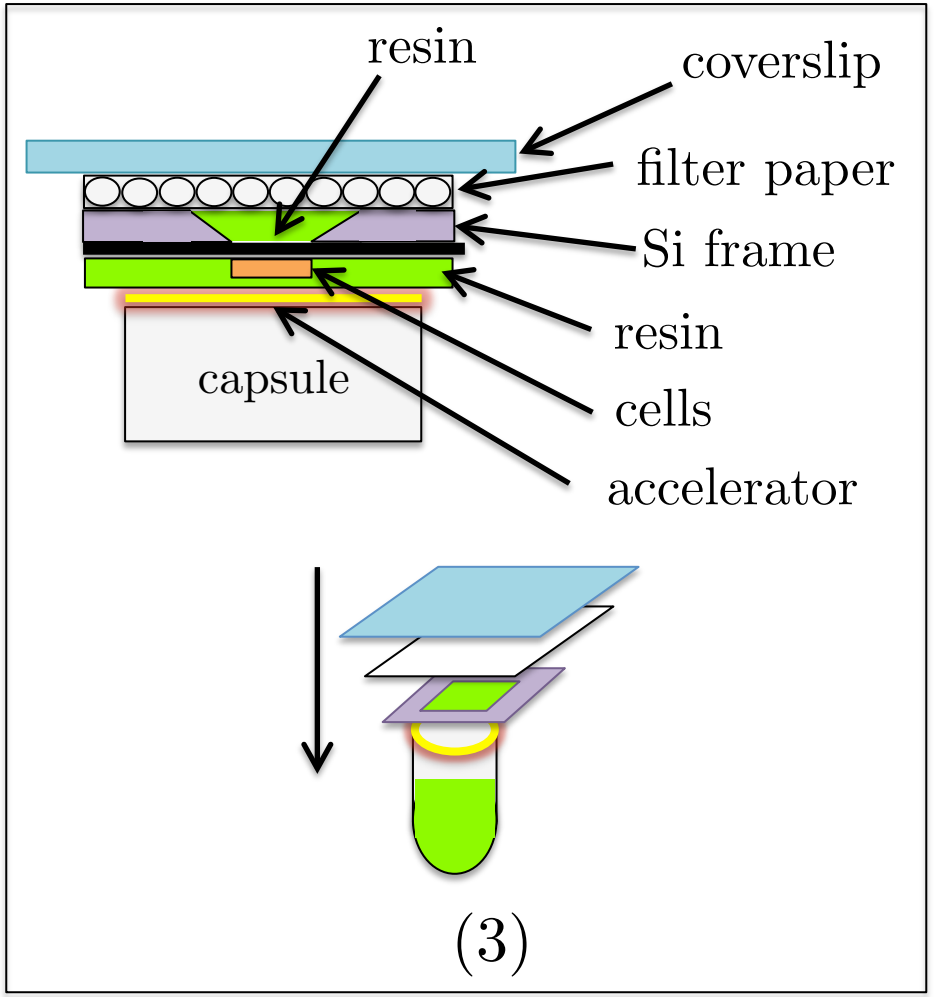
- To prepare corresponding TEM images to XRM samples

Sample Preparation

- Resin embedding

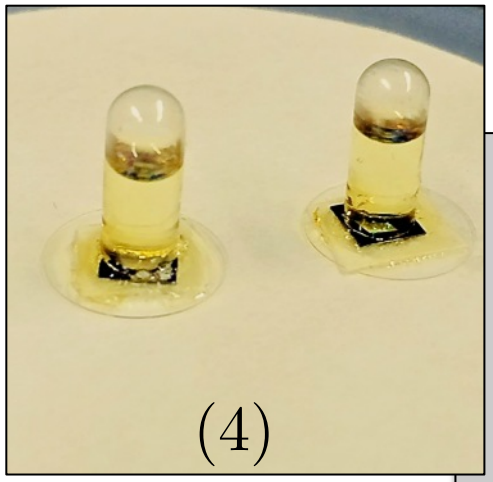






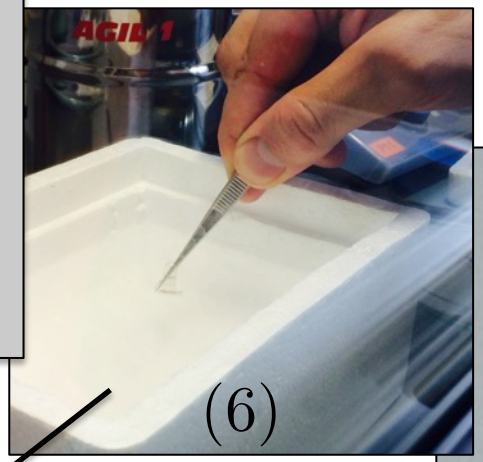
Sample Preparation

- Resin embedding

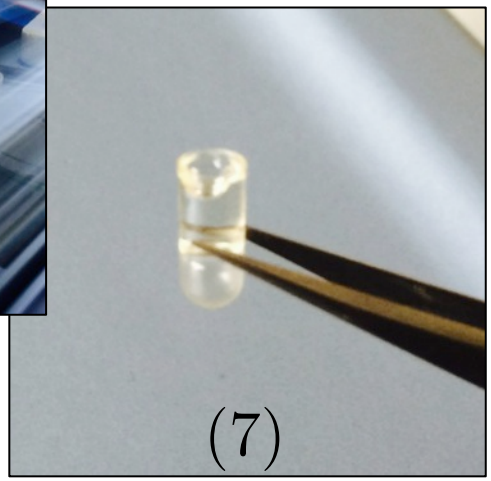


Incubate in oven at 50 °C at least 24 hours

(5)

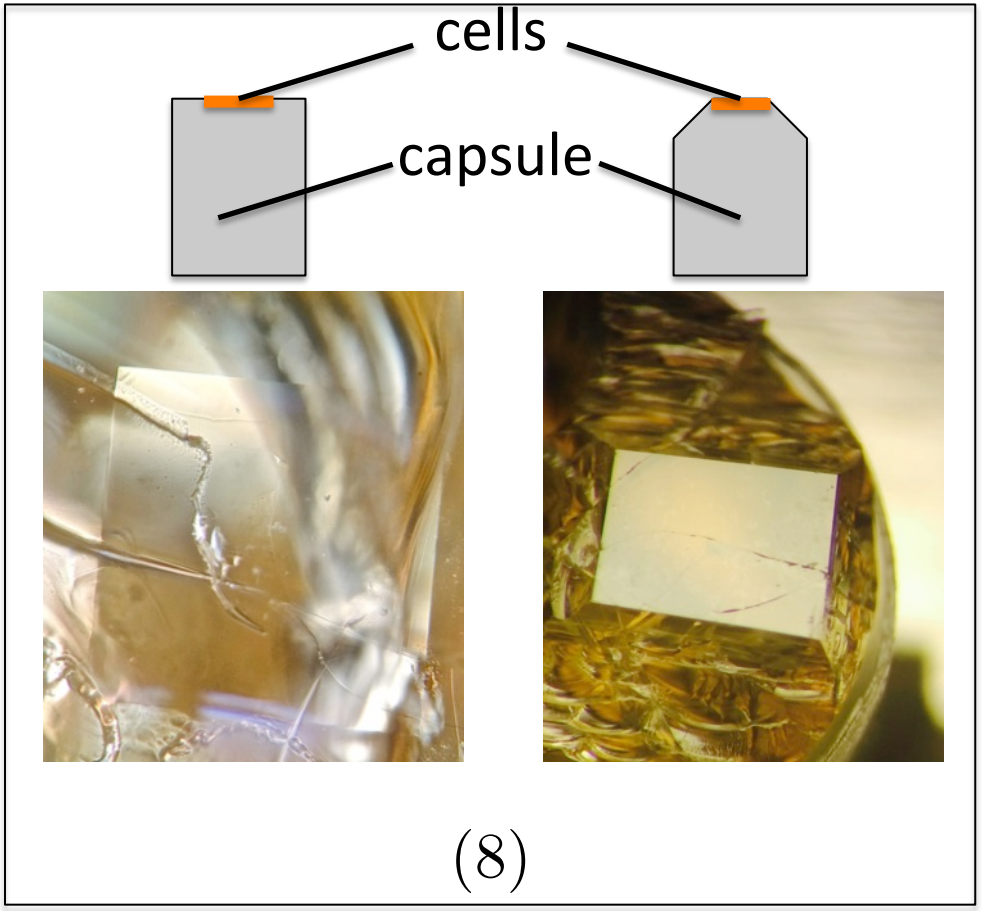


Liquid nitrogen

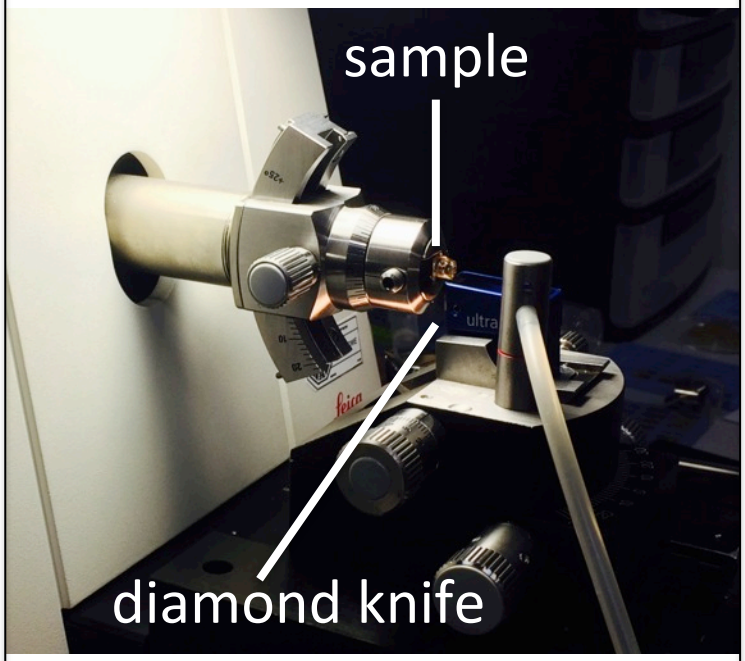


Sample Preparation

- Sectioning



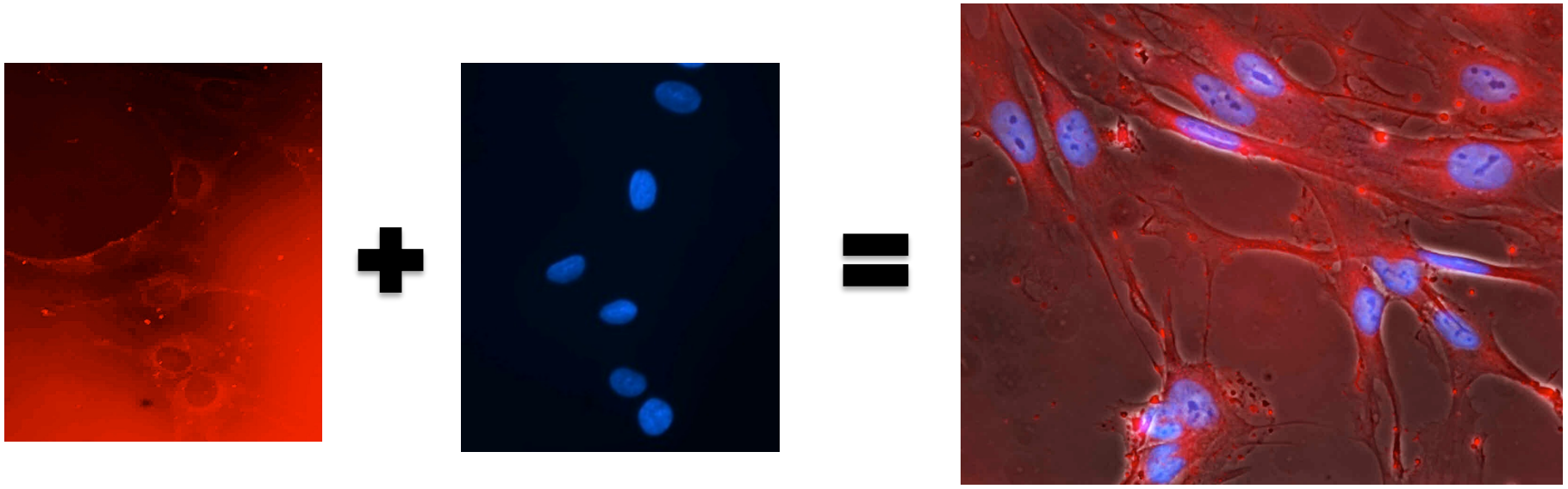
(8)



(9)

Results

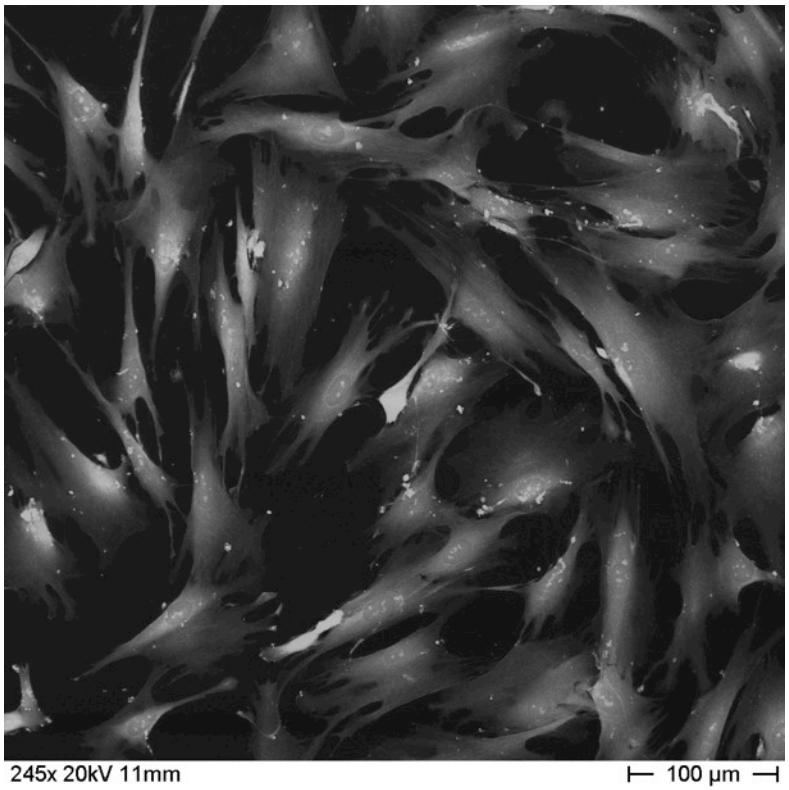
- Cell images
  - Fluorescence light microscopy



Nile Red channel DAPI channel

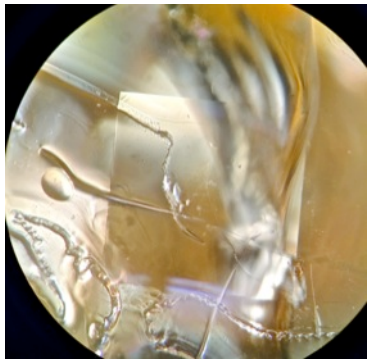
Results

- Cell images
  - Scanning electron microscope (SEM)

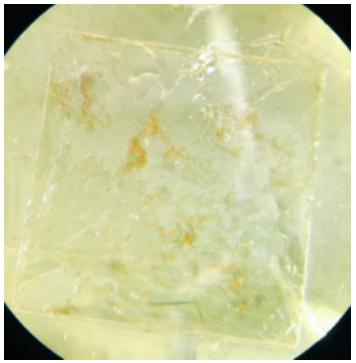


Results

- Resin embedding problems



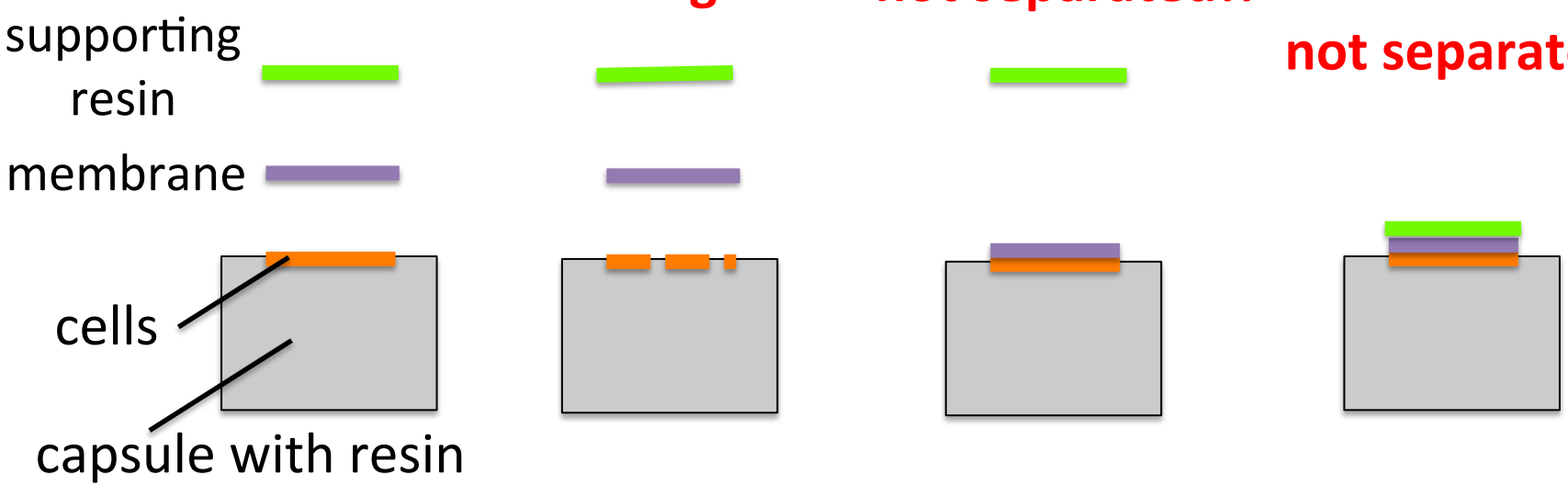
Good



Membrane damaged!!

Membrane not separated!!

Membrane & resin not separated!!



Conclusions

- The resin capsular embedding method has been adapted for Si_3N_4 membranes.
- The sample of XRM can be evaluated along with EM by developed procedure.

Outlooks

Possibility in …

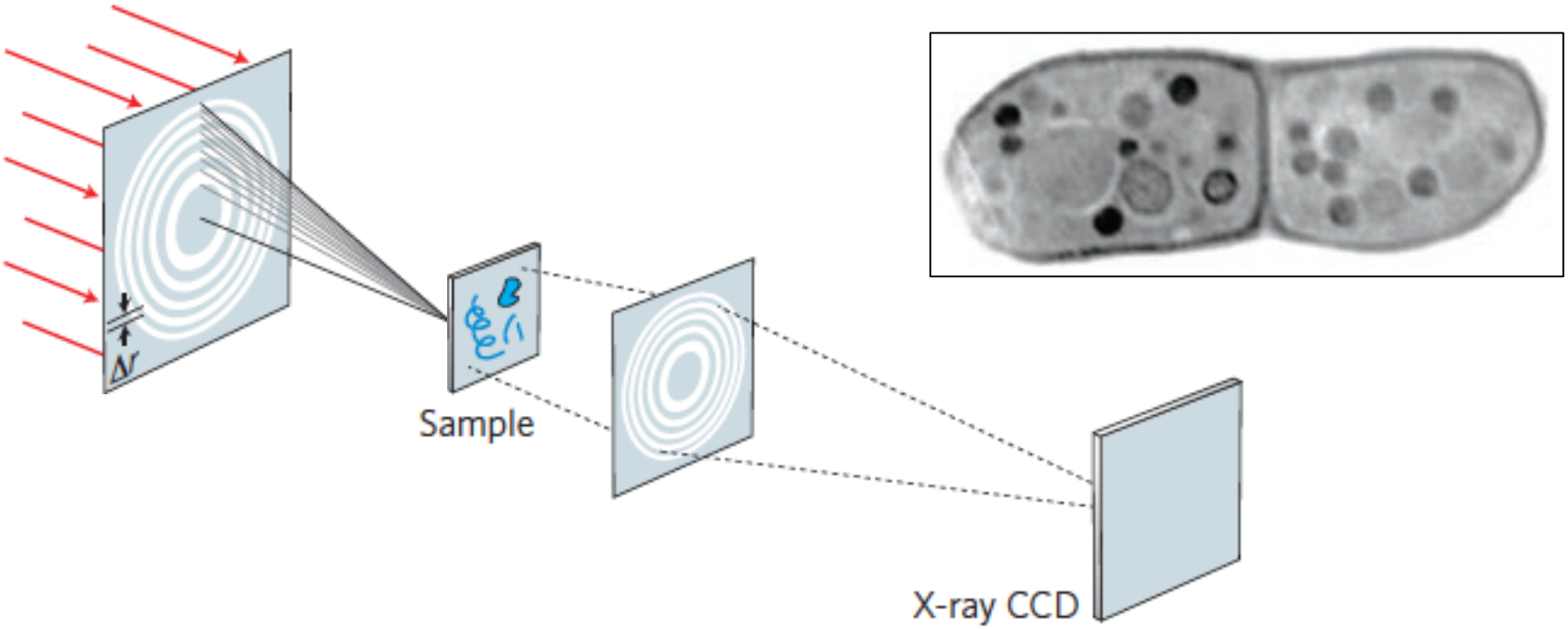
- Cryogenic fixation
  - Cell inactivation at liquid nitrogen temperature
- Freeze-drying dehydration*
  - Cellular water sublimation in cryogenic and vacuum conditions

* Must operate along with cryogenic fixation

Thank you for your kind attention

X-ray tomography

A not-yet-separated mother–daughter yeast cell pair is inspected in soft x-ray microscope.

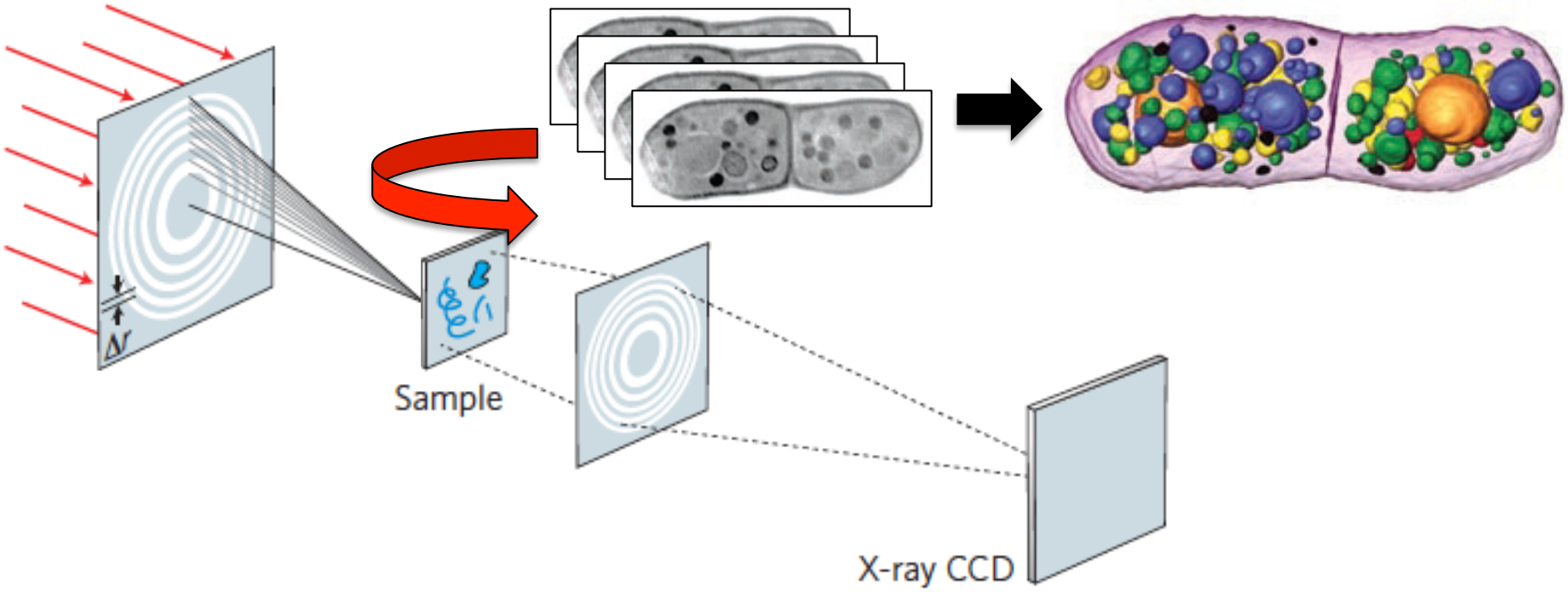


Sakdinawat, A.; Attwood, D. *Nature Photonics*. **2010**, *4*, 840-848.

X-ray tomography

- 3D structure study: x-ray tomography

A not-yet-separated mother–daughter yeast cell pair is inspected in soft x-ray microscope.



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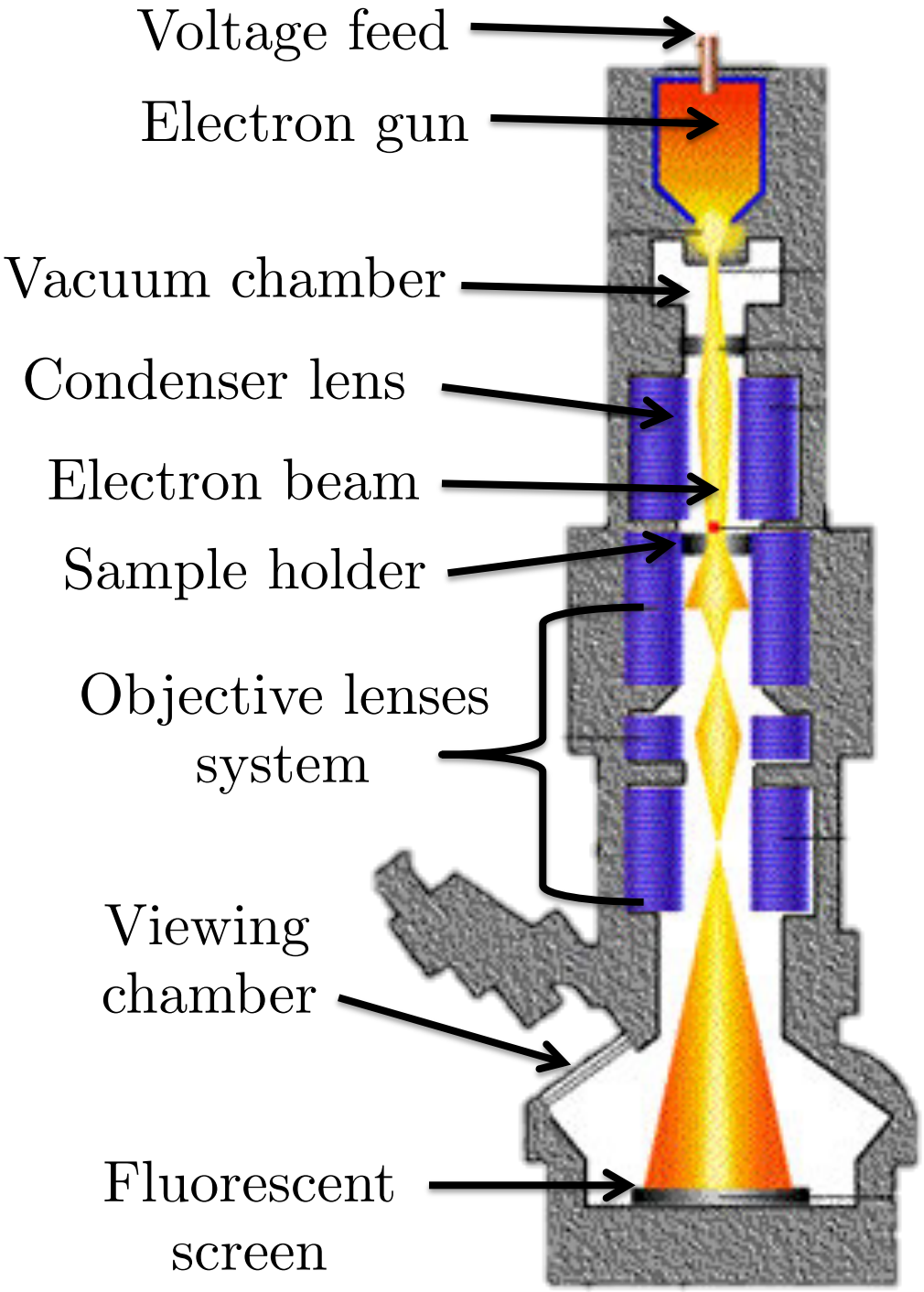
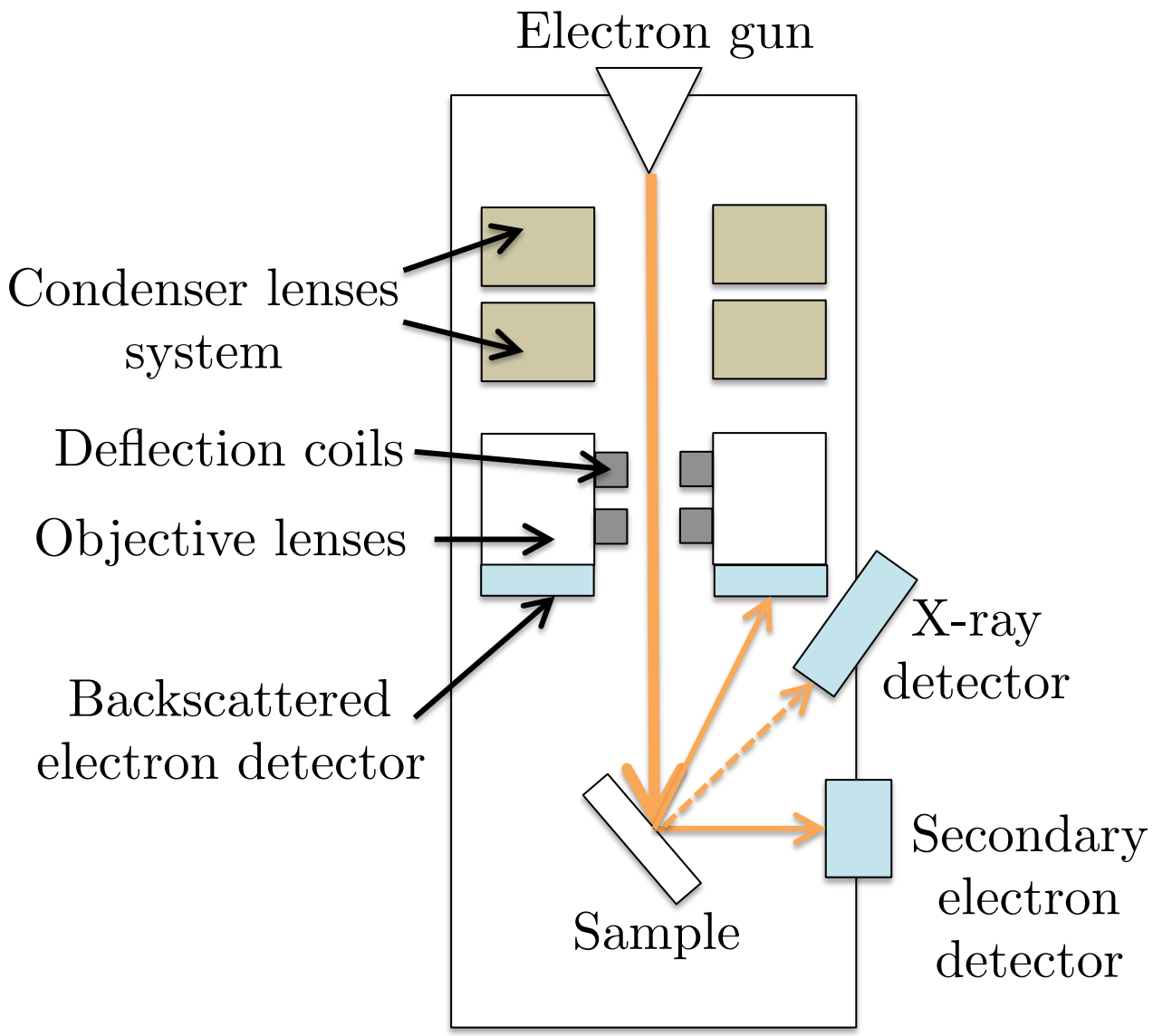
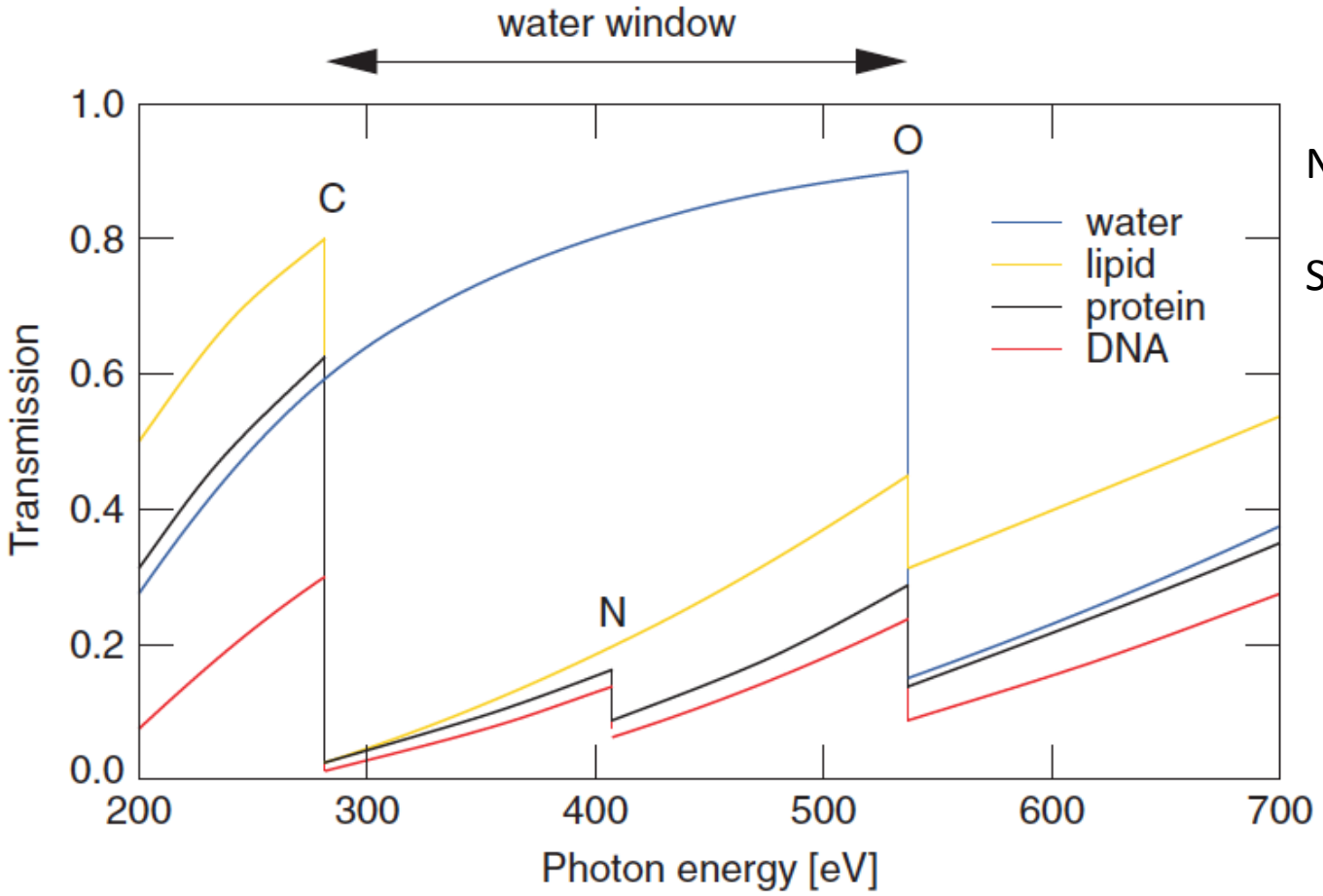

Voltage feed
Electron gun
Vacuum chamber
Condenser lens
Electron beam
Sample holder
Objective lenses system
Viewing chamber
Fluorescent screen


Electron gun
Condenser lenses system
Deflection coils
Objective lenses
Backscattered electron detector
X-ray detector
Secondary electron detector
Sample

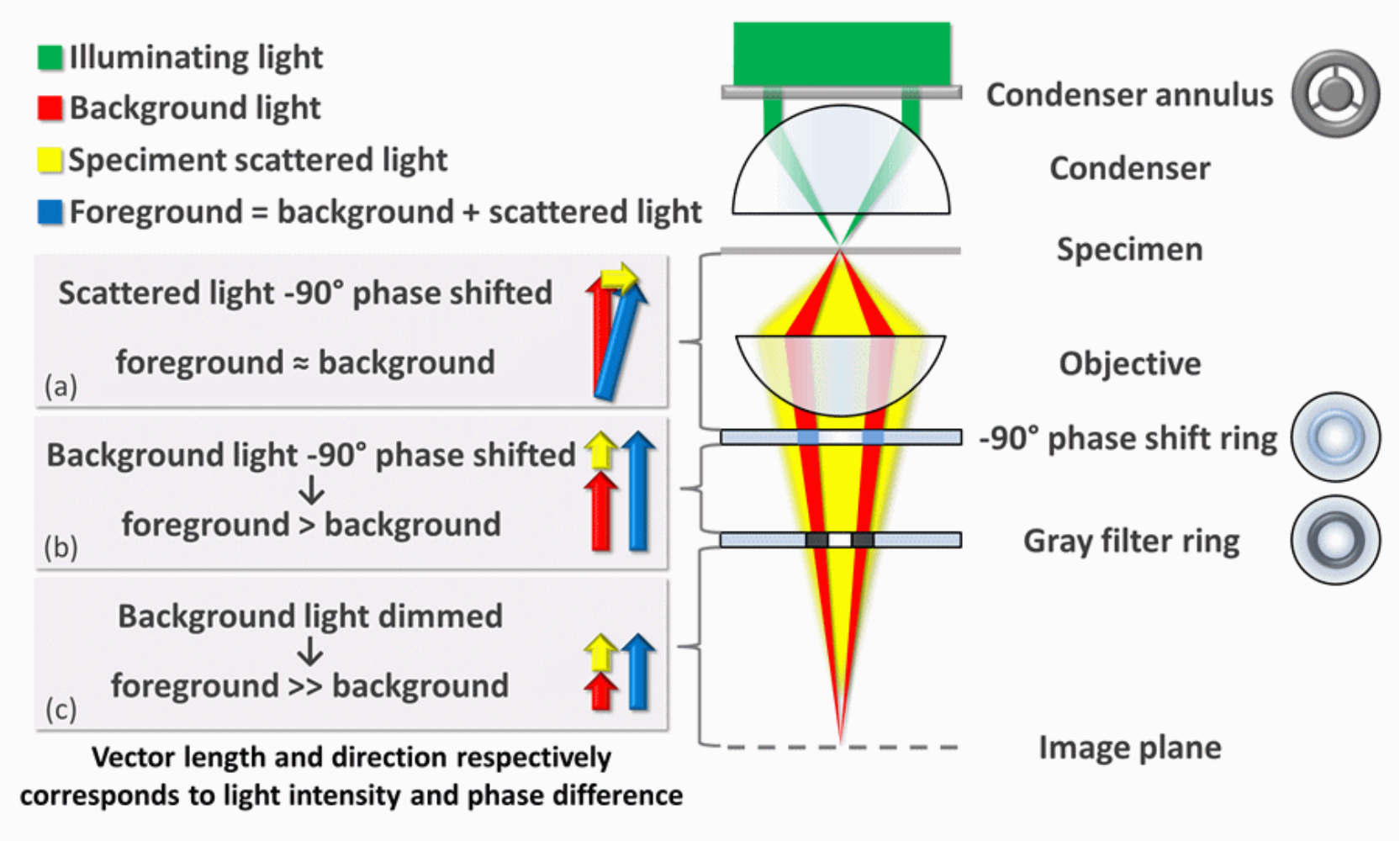
Water window



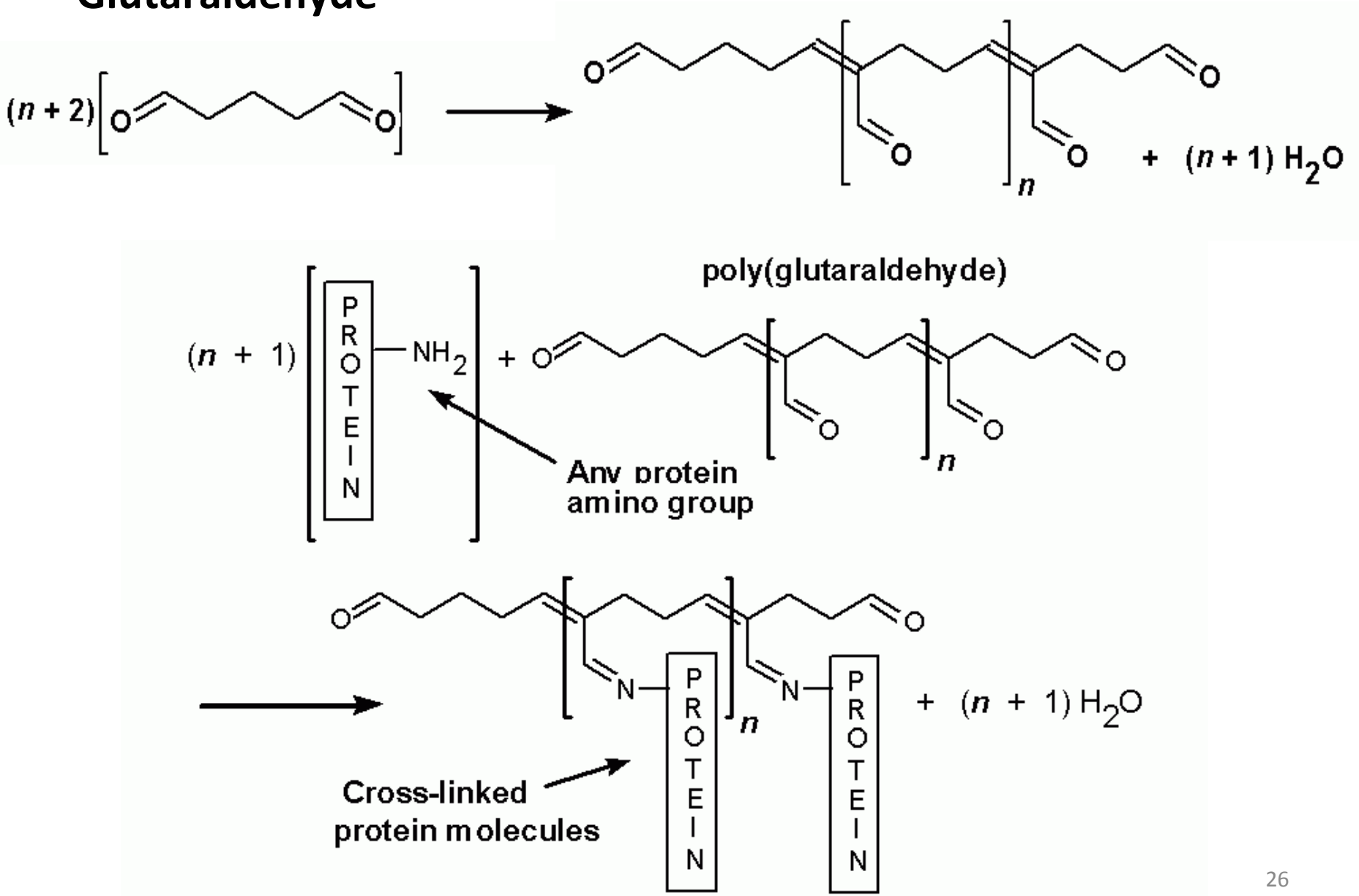
Note

Soft x-rays

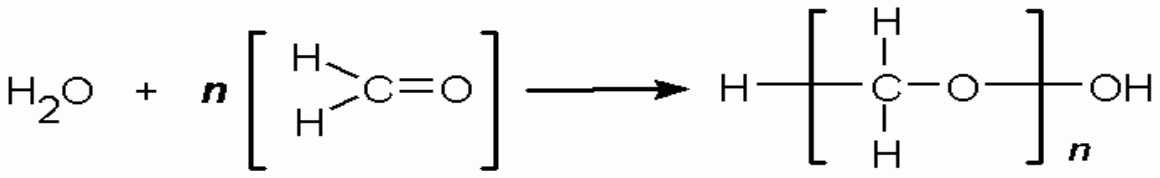
Phase contrast light microscope

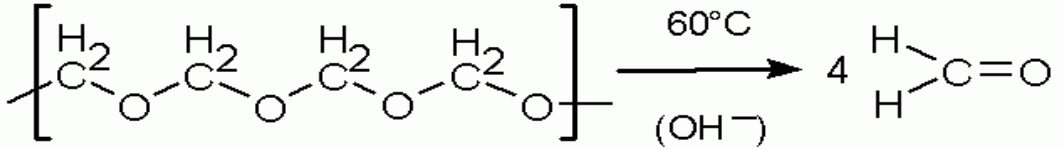


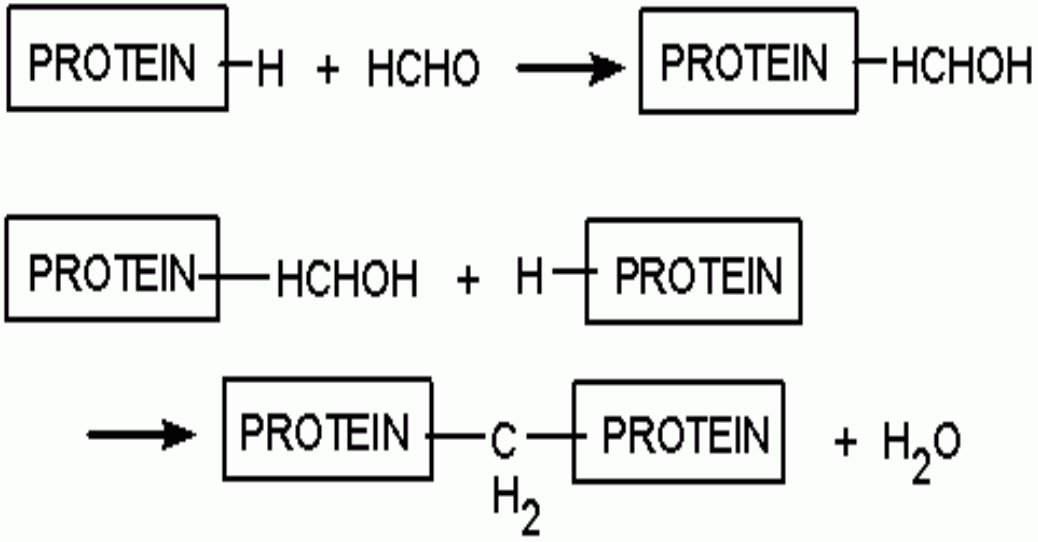
Glutaraldehyde



Paraformaldehyde







Comparative information

- Given by LM, TEM and XRM

	LM	TEM	XRM
Unstained samples	common	difficult	possible
Sample thickness	medium	low	high
Living sample	possible	impossible	possible
Resolution	150 nm	< 0.1 nm	Up to 10 nm
Radiation damage	less harmful	serious	violent
Focal depth	medium	low	high