CELL BIOLOGY – FIRST YEAR

Tutorial 2: Methods of Studying the Cell

Chapter 2 – Course summary and exercises

Student name	
Group / Date	

Learning objectives

By the end of this tutorial, you should be able to:

- distinguish magnification from resolution and calculate both;
- compare light microscopy, transmission electron microscopy (TEM) and scanning electron microscopy (SEM);
- explain how histochemical, immunological and enzymological methods reveal the chemical composition and function of cells;
- interpret experimental results and choose the right method for a given question.

Plan: 2.1 Optical and electron microscopy · 2.2 Histochemical methods · 2.3 Immunological methods · 2.4 Enzymological methods.

Part 1 – Course summary

2.1 Optical and electron microscopy

Magnification is how many times the image is larger than the object. For a light microscope: total magnification = objective × eyepiece. **Resolution** (resolving power) is the smallest distance between two points that can still be seen as separate. It is limited by the wavelength (λ) and by the numerical aperture (NA) of the objective:

$$d = 0.61 \times \lambda / NA$$

Increasing magnification beyond the resolution limit only enlarges a blurred image (“empty magnification”). With visible light, the limit is about 0.2 µm, so useful magnification stops at roughly 1000–1500×.

Light microscopy. Several modes exist: bright field (stained fixed sections), phase contrast and differential interference contrast (living, unstained cells), dark field, fluorescence (labelled molecules emit light after excitation) and confocal (thin optical sections, 3-D reconstruction). Classical preparation of a tissue: fixation → dehydration → clearing → paraffin embedding → sectioning with a microtome (5–10 µm) → mounting → staining (e.g. hematoxylin–eosin).

Electron microscopy. A beam of electrons, whose wavelength is far shorter than that of light, is focused by electromagnetic lenses in a vacuum. In TEM, the beam crosses an ultrathin section (50–100 nm); structures appear dark where heavy metals (uranyl acetate, lead citrate, osmium tetroxide) are deposited. Preparation uses glutaraldehyde and osmium fixation, resin embedding and an ultramicrotome. In SEM, a beam scans the surface of a specimen coated with a thin metal layer (gold) and secondary electrons form a 3-D-like image of the surface. Other techniques: freeze-fracture and cryo-electron microscopy.

Table 1. Comparison of the three main microscopes (approximate values)

	Light microscope	TEM	SEM
Radiation	Visible light (400–700 nm)	Electron beam	Electron beam
Lenses	Glass	Electromagnetic	Electromagnetic
Resolution	≈ 0.2 µm	≈ 0.2 nm (1–2 nm for biological samples)	≈ 3–10 nm
Specimen	Sections 5–10 µm or living cells	Ultrathin sections (50–100 nm), vacuum	Whole specimen, metal-coated, vacuum
Image	Coloured, 2-D	Black and white, internal ultrastructure	Black and white, surface topography
Living material	Possible	Impossible	Impossible

2.2 Histochemical methods

Histochemistry detects and localizes chemical constituents (sugars, lipids, proteins, nucleic acids, ions) in tissue sections through specific colour reactions. Stains work through chemical affinity: basic dyes (e.g. hematoxylin) bind acidic, "basophilic" components such as DNA and RNA, whereas acid dyes (e.g. eosin) bind basic, "acidophilic" components such as most cytoplasmic proteins and collagen. In H&E staining, nuclei appear blue-purple and the cytoplasm pink. Note that lipids are dissolved by the solvents used for paraffin embedding, so they are studied on frozen sections.

Table 2. Common histochemical reactions

Method	Target	Result
PAS (periodic acid–Schiff)	Polysaccharides: glycogen, mucins, glycoproteins	Magenta
Feulgen reaction	DNA (after mild acid hydrolysis)	Magenta nuclei
Methyl green–pyronin	DNA / RNA	Green-blue / red
Sudan III, Oil Red O	Neutral lipids (frozen sections)	Orange / red
Perls' reaction	Ferric iron	Prussian blue
Alizarin red	Calcium deposits	Orange-red

2.3 Immunological methods

These methods use the very high specificity of an antibody for its antigen to locate a given protein in cells or tissues. In direct labelling, the primary antibody carries the label. In indirect labelling, an unlabelled primary antibody binds the antigen and a labelled secondary antibody, directed against the primary antibody, binds to it. The indirect method amplifies the signal because several secondary antibodies attach to each primary antibody.

- **Label:** a fluorochrome (e.g. FITC) → immunofluorescence; an enzyme (e.g. peroxidase, revealed by a coloured substrate) → immunohistochemistry; colloidal gold particles (dense, visible in TEM) → immunogold labelling.
- **Antibodies:** polyclonal antibodies recognize several epitopes of an antigen; monoclonal antibodies recognize a single epitope and give more specific results.

- **Controls:** omitting the primary antibody checks that the secondary antibody does not bind non-specifically. Related techniques include ELISA and Western blot.

2.4 Enzymological methods

Enzymological methods study the function of cell compartments through their enzymatic activity. Two approaches are used.

- **Enzyme histochemistry (in situ).** Fresh or frozen sections, lightly fixed to preserve activity, are incubated with a substrate. The reaction product is converted into a coloured or electron-dense precipitate at the site of activity. For example, succinate dehydrogenase (SDH) reduces a tetrazolium salt to a blue formazan deposit in mitochondria; acid phosphatase activity marks lysosomes; peroxidase/catalase marks peroxisomes.
- **Cell fractionation and enzyme assays.** Cells are homogenized and the homogenate is separated by differential centrifugation at increasing speeds: nuclei sediment first (≈ 1,000 g), then mitochondria, lysosomes and peroxisomes (≈ 10,000 g), then microsomes derived from the ER and Golgi (≈ 100,000 g), leaving the cytosol in the supernatant. Purity of each fraction is checked by measuring marker enzymes. Specific activity = enzyme activity / amount of protein; the purification factor = specific activity of the fraction / specific activity of the homogenate.

Table 3. Marker enzymes

Compartment	Marker enzyme(s)
Mitochondria	Succinate dehydrogenase, cytochrome c oxidase
Lysosomes	Acid phosphatase
Peroxisomes	Catalase
Endoplasmic reticulum	Glucose-6-phosphatase
Golgi apparatus	Galactosyltransferase
Plasma membrane	Na^+/K^+-ATPase, 5′-nucleotidase
Cytosol	Lactate dehydrogenase

Key terms: magnification, resolution, numerical aperture, fixation, embedding, microtome, ultramicrotome, contrast, basophilic / acidophilic, antigen, epitope, primary / secondary antibody, fluorochrome, marker enzyme, differential centrifugation, specific activity.

Part 2 – Exercises

A. Multiple-choice questions (one correct answer)

1. The main factor that limits the resolution of a light microscope is:

a) the magnification of the eyepiece
b) the wavelength of light
c) the thickness of the lens
d) the type of stain used

2. Which technique is best suited to study the three-dimensional surface of a cell?

a) Phase-contrast microscopy
b) Transmission electron microscopy
c) Scanning electron microscopy
d) Bright-field microscopy

3. Which reaction is specific for DNA?

a) PAS reaction
b) Feulgen reaction
c) Sudan III staining
d) Perls' reaction

4. Living, unstained cells are best observed with:

a) a phase-contrast microscope
b) a transmission electron microscope
c) a scanning electron microscope
d) an ultramicrotome

5. In indirect immunofluorescence, the fluorochrome is attached to:

a) the antigen
b) the primary antibody
c) the secondary antibody
d) the fixative

6. Which enzyme is a marker of lysosomes?

a) Succinate dehydrogenase
b) Catalase
c) Acid phosphatase
d) Glucose-6-phosphatase

7. Why are lipids usually studied on frozen sections rather than paraffin sections?

a) Paraffin is toxic to lipids
b) Organic solvents used in paraffin embedding dissolve lipids
c) Lipids are not stained by any dye
d) Frozen sections are thinner

8. Eosin is an acid dye. It therefore stains preferentially:

a) nuclei, which are acidic
b) cytoplasmic proteins, which are basic (acidophilic)
c) DNA only
d) lipid droplets

B. True or false? Correct the false statements

1. Magnification and resolution mean the same thing. ☐ True ☐ False

2. Electron microscopes have a better resolution because electrons have a much shorter wavelength than visible light. ☐ True ☐ False

3. Hematoxylin is a basic dye that stains nuclei blue-purple. ☐ True ☐ False

4. In indirect immunolabelling, the primary antibody carries the fluorochrome. ☐ True ☐ False

5. Enzyme activity is best preserved by prolonged fixation in strong fixatives. ☐ True ☐ False

6. SEM is used to observe the internal ultrastructure of organelles in ultrathin sections. ☐ True ☐ False

C. Matching

Match each technique (1–6) with its main application (A–F).

Technique	Application
1. Fluorescence microscopy	A. Locating a protein at the ultrastructural level
2. Confocal microscopy	B. Detecting mitochondrial activity (blue formazan deposit)
3. TEM	C. Revealing glycogen and mucins (magenta)
4. PAS reaction	D. Visualizing labelled structures that emit light after excitation
5. Immunogold labelling	E. Obtaining thin optical sections and 3-D reconstruction
6. SDH + tetrazolium salt	F. Observing organelle ultrastructure in ultrathin sections

D. Calculations

Exercise 1 – Magnification

A microscope has a ×10 eyepiece and a ×40 objective. (a) What is the total magnification? (b) A cell has a real diameter of 25 µm. What is the diameter of its image, in mm? (Recall: 1 mm = 1,000 µm = 10^6 nm.)

Exercise 2 – Electron micrograph

On a TEM micrograph, a scale bar of 2 cm represents 1 µm. A mitochondrion measures 3.5 cm in length on the picture. (a) What is the magnification of the micrograph? (b) What is the real length of the mitochondrion, in µm?

Exercise 3 – Resolution

Using $d = 0.61\ \lambda / NA$, calculate the resolution of a light microscope with an objective of NA = 1.4 under green light (λ = 550 nm). Then decide which of the following structures can be resolved: a bacterium (1 µm), a mitochondrion (0.5 µm wide), a ribosome (25 nm), a poliovirus (30 nm), the thickness of a plasma membrane (7 nm). What would change with blue light (λ = 450 nm)?

Exercise 4 – Specific activity

A liver homogenate contains 600 mg of protein and a total SDH activity of 3,000 µmol/min. The mitochondrial fraction obtained by centrifugation contains 12 mg of protein and 600 µmol/min of SDH activity. Calculate (a) the specific activity of the homogenate and of the fraction, (b) the purification factor, and (c) the percentage of the SDH activity recovered in the fraction.

E. Short-answer questions

Exercise 5

Define resolution and magnification. Why does increasing the magnification of a light microscope beyond 1500× not improve the image?

Exercise 6

Put the following steps of the preparation of a tissue for light microscopy in the correct order and give the purpose of each: staining – sectioning – paraffin embedding – fixation – dehydration – mounting.

Exercise 7

Explain why living cells cannot be observed with a conventional transmission electron microscope.

Exercise 8

Explain the difference between direct and indirect immunolabelling. Give one advantage of the indirect method.

Exercise 9

A tissue section stained with PAS shows strongly magenta goblet cells in the intestinal epithelium, whereas their nuclei are not coloured. What does this tell you about the content of these cells? Which reaction would you use to stain the nuclei specifically for DNA?

F. Experimental data analysis

Exercise 10 – Immunofluorescence

Cultured cells are fixed and incubated with an anti-tubulin primary antibody, then with a FITC-labelled secondary antibody. Green filaments radiate through the cytoplasm. In a control sample, the primary antibody was omitted.

- (a) Which cell structure is revealed, and with which type of microscope is it observed?
- (b) What result is expected in the control? What would a green signal in the control mean?
- (c) How could the same protein be localized with a TEM?

Exercise 11 – Cell fractionation

A liver homogenate is fractionated by differential centrifugation. The table gives the percentage of the total activity of four enzymes found in each fraction.

Enzyme	Nuclear (1,000 g)	Large granules (10,000 g)	Microsomal (100,000 g)	Soluble (supernatant)
Succinate dehydrogenase	10	80	5	5
Acid phosphatase	8	70	12	10
Glucose-6-phosphatase	5	10	80	5
Lactate dehydrogenase	3	5	2	90

- (a) Which compartment does each enzyme represent?
- (b) In which fraction are mitochondria mainly found? Why is acid phosphatase also abundant in this fraction?
- (c) Propose a method to separate mitochondria from lysosomes.

Exercise 12 – Choose the method

For each question, name the most suitable method and justify your choice: (a) Where is glycogen stored in a liver section? (b) What is the shape of the surface microvilli of an intestinal cell in 3-D? (c) Where exactly is a given receptor located on the plasma membrane at the nanometre scale? (d) How do mitochondria move in a living cell?