

4.1 Microscopy and tissue preparation

Name: _____ Class: _____ Date: _____

Total: 23 marks

KEY WORDS staining

Objective

Order the preparation sequence and reason about resolution and artefacts.

1 Key knowledge and worked examples

■ The pipeline

Fixation → dehydration → embedding → sectioning (3–5 μm) → staining. Light microscopy resolves ~200 nm; H = acidic blue (nuclei), E = basic pink.

■ Worked example: the empty magnification

Past ~1500 $\times$, magnification enlarges blur: 150 nm apart cannot be resolved at any light power ✓; 2 μm can.

2 Practice

2.1 Order the five preparation steps. [3]

2.2 What colour do nuclei stain with H&E, and why? [3]

2.3 Name one artefact of dehydration. [3]

3 Exam-style questions

3.1 A frozen section is requested mid-operation.

State which steps are skipped and why speed is gained. Name two quality costs. State the clinical trade-off being made. [7]

3.2 Two structures measure 180 nm apart; two others 1.5 μm apart.

State which pair is resolvable by light microscopy and why (the ~ 200 nm limit). Compute the advantage of EM (0.005 nm wavelength) and its cost. [7]

Solutions

2.1

- Fixation, dehydration, embedding, sectioning, staining ✓.

2.2

- Blue-purple: haematoxylin is basic and nuclei acidic.

2.3

- Shrinkage with artificial tissue spaces.

3.1

- Dehydration and embedding are skipped: the block is frozen and cut at once.
- Costs: ice-crystal artefact; poorer morphology and stain quality.
- Trade: a same-hour answer at lower certainty vs a perfect answer tomorrow.

3.2

- The 1.5 μm pair resolves; 180 nm < 200 nm does not ✓.
- EM wavelength 0.005 nm gives $\sim 40\,000\times$ the theoretical ratio – in practice $\sim 1000\times$ the usable resolution.
- Cost: vacuum, killed specimens, no living dynamics, no colour.