

Exercise walk-through

4.1

Microscopy and tissue preparation

University Medicine

Preparation: making the picture

- 1 Sequence** Fix (formalin cross-links) → dehydrate → embed (paraffin) → section (microtome, 3–5 μm) → stain (H&E).
- 2 Resolution** Light microscopy caps at $\sim 200\text{ nm}$; EM at 0.005 nm buys $\sim 1000\times$ at the cost of a dead specimen.
- 3 Logic** Basic haematoxylin stains acidic nuclei BLUE; acidic eosin stains basic cytoplasm PINK - charge pairing.

Preparation - five steps and their risks



H = acidic structures blue (nuclei) · E = basic pink (cytoplasm)

Fix → dehydrate → embed → section → stain: five steps, five failure modes.

Exercises 2.1-2.3

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]

Exercises 2.1-2.3

2.1 Order the five preparation steps. [3]

2.1

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

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

2.2

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

2.3 Name one artefact of dehydration. [3]

2.3

- Shrinkage with artificial tissue spaces.

Exercises 3.1-3.2

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]

Exercises 3.1-3.2

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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.
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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]

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.