Haematoxylin & Eosin

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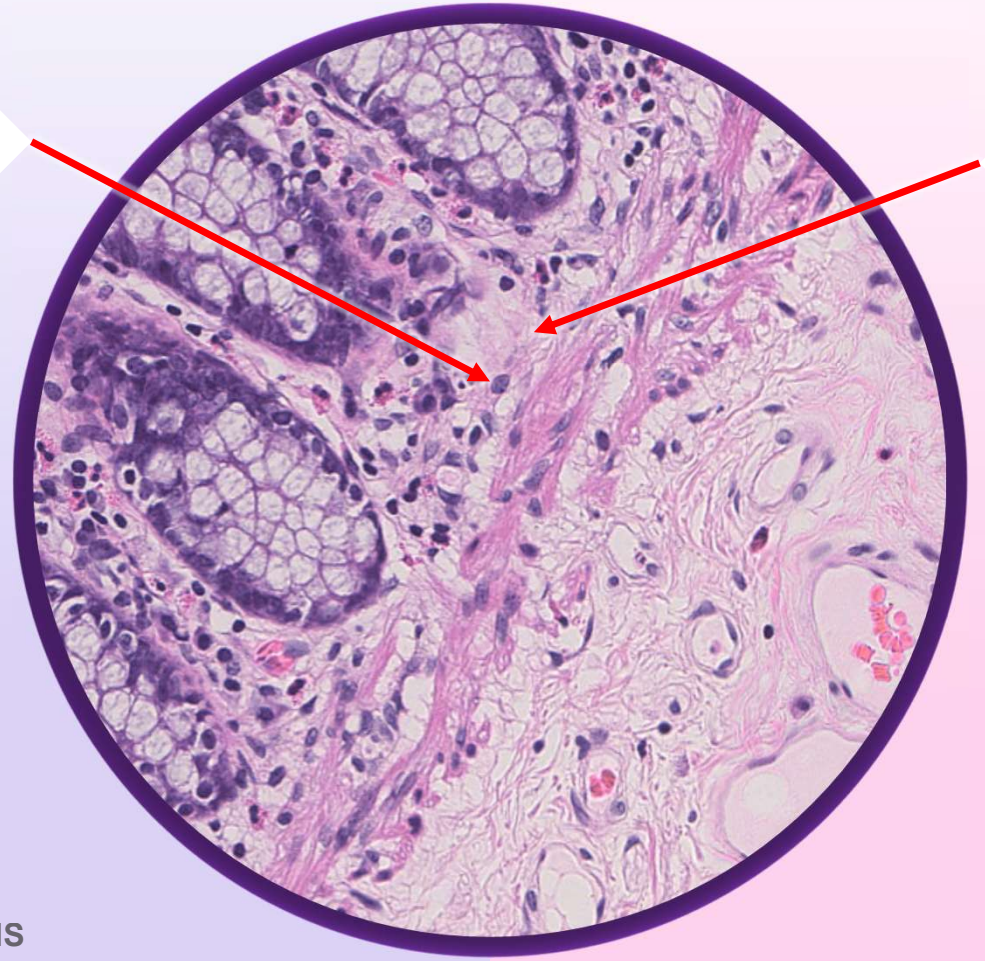
Learning objectives:

- Understand the **principles** of H&E staining and dye–tissue interactions
- Identify **nuclear** and **cytoplasmic components** based on staining characteristics
- Recognise common **technical issues** affecting contrast and morphology

Haematoxylin and eosin (H&E) staining is a fundamental technique used in histopathology to visualize the microscopic structure of biological tissues.

Haematoxylin

- a **basic dye**
- stains acidic structures of tissues and cell structures (basophilic)
- primarily **cell nuclei,** which contains mucin, chromatin & ribosomes in purplish blue



Eosin

- an **acidic dye**
- stains the basic structures of a cell (acidophils)
- **RBCs, cytoplasm, muscle and collagen** in varying intensities of pink

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Leica Biosystems – H&E staining overview

The combination of **haematoxylin and eosin** in tissue staining shows the general distribution of cells and provides a general overview of a tissue sample's structure.

It provides essential information on:

Cell morphology

The shape, size, and organization of cells

Pathological changes

Abnormalities such as tumors, necrosis, or fibrosis

Leica Biosystems – H&E in diagnostic histopathology
Histology Guide – teaching H&E slides

TYPE OF STAINS

REGRESSIVE		PROGRESSIVE
The tissue is intentionally over-stained and then differentiated until the it reaches the desired endpoint	DEFINITION	The tissue is left in the staining solution just long enough to reach the desired endpoint
YES	DIFFERENTIATION STEP	NO
Slow	SPEED OF PROCESS	Fast
• Nuclear detail stands brighter and crisper • Does not produce background staining • Over differentiation will cause pale nuclei • Under differentiation will obscure fine details	PROS & CONS	• Nuclear staining is more consistent and not prone to errors • Produce background staining • Nuclei may not stained brighter and crisper

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Leica Biosystems – standard H&E interpretation guidance

PROCEDURE

Harris Haematoxylin & Eosin Y with Phloxine Stain, Regressive

1 min — **Xylene**

↓

2 min — **Xylene**

↓

Deparaffinization

Since tissue sections are embedded in paraffin, the wax must be removed before staining

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IHC World – Harris H&E regressive staining protocol

University of Rochester Medical Center – H&E SOP (PDF)

1 min	Absolute Alcohol
1 min	Absolute Alcohol
1 min	95% Alcohol
1 min	75% Alcohol
15 sec	Wash

Hydration

This is done by passing the slides slowly through a **series of decreasing concentrations** of alcohols and eventually introduce water into the cells

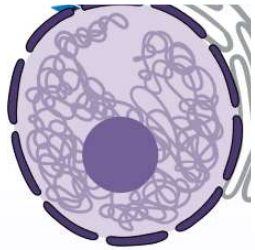
This step ensures that tissues are **properly prepared** to absorb aqueous staining solutions

IHC World – Harris H&E regressive staining protocol

Mycetoma Research Centre – SOP for H&E staining (PDF)

9 min **Haematoxylin**

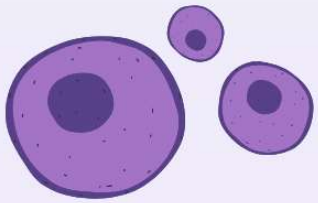
15 sec **Wash**



Nuclear Staining

Haematoxylin stains the nucleus of the cell,
(chromatin, ribosomes & mucin)

It allows the chromatin activity
to be seen easily



Leica Biosystems – nuclear staining principles

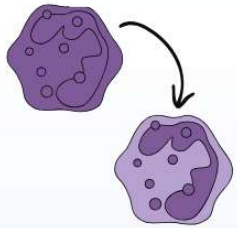
1 sec — **0.5% Acid Alcohol**

15 sec — **Wash**

10 sec — **Ammonia Water**

30 sec — **Wash**

Differentiation



removal of excess dye from the section

Ensures precise nuclear staining (proper coloration) by reducing background coloration

Bluing

changes the reddish-purple haematoxylin to a blue or purplish-blue colour

This step enhances the contrast and clarity of the nuclear staining

IHC World – acid alcohol differentiation and bluing

Newcomer Supply – Harris hematoxylin technical memo (PDF)

5 sec **70% Alcohol**

1 min **Eosin**

LL1

Counterstain

stains the RBCs, cytoplasm, muscle fibers, collagen and extracellular matrix **in shades of pink**, providing contrast to the blue-stained nuclei

Leica Biosystems – eosin staining characteristics

5 sec — 75% Alcohol

5 sec — 95% Alcohol

10 sec — Absolute Alcohol

20 sec — Absolute Alcohol

1 min — Absolute Alcohol

Dehydration

Dehydrate in increasing concentration of alcohols

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Mycetoma Research Centre – SOP for H&E staining (PDF)

30 sec — Xylene

30 sec — Xylene

Exit drawer — Xylene

Clearing

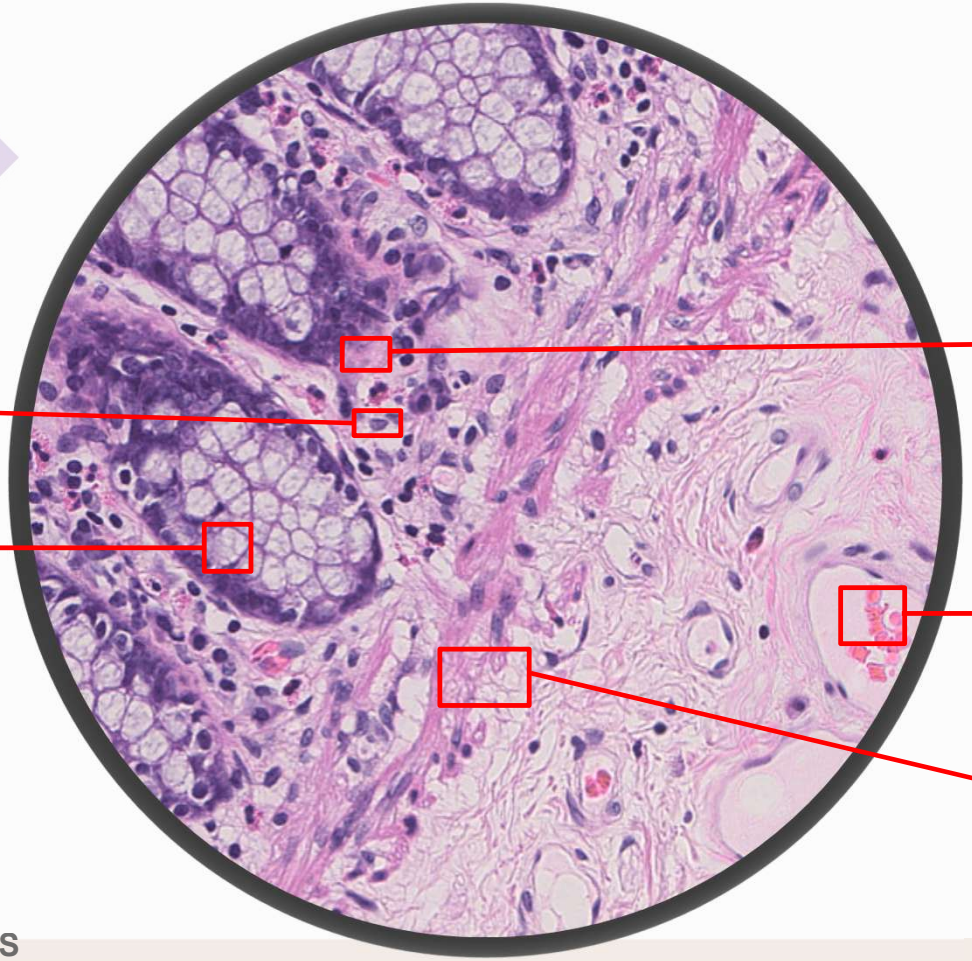
Removing of alcohol from the tissue sections by the solvent to assure miscibility when cover-slipping with xylene

Pathweb, Department of Pathology, NUHS

University of Rochester Medical Center – H&E SOP (PDF)

Result

Haematoxylin



Nucleus
purplish blue

Mucin
grey blue

Eosin

stains **in varying intensities of pink**

cytoplasm
pink/purplish pink

RBCs
orange red

Connective tissue
pink

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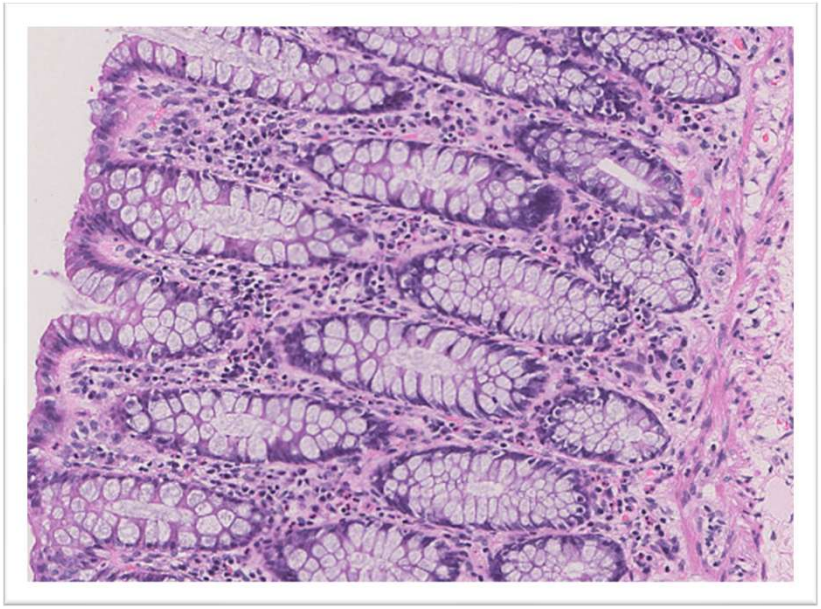
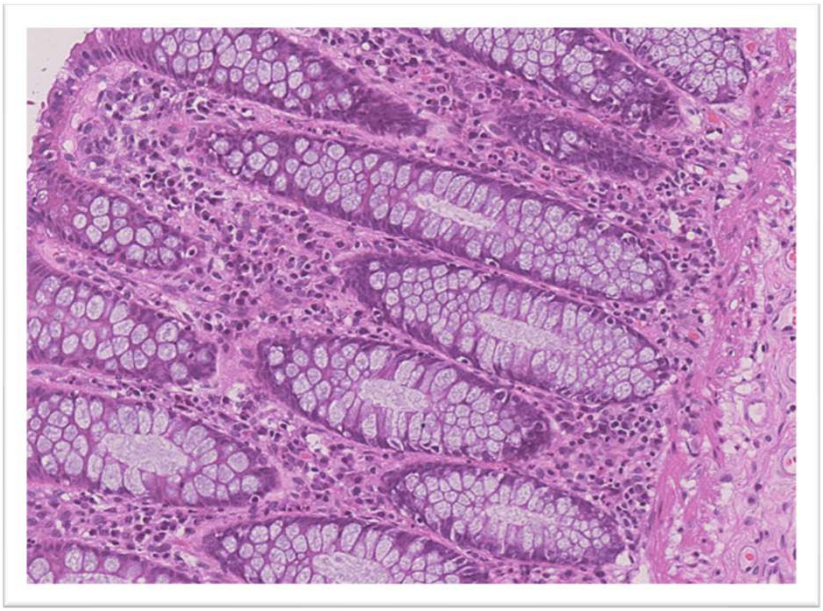
Troubleshooting

Bluing Issue

Control



Without Ammonia



[Nuclei may remain red/purple]
[X contrast]

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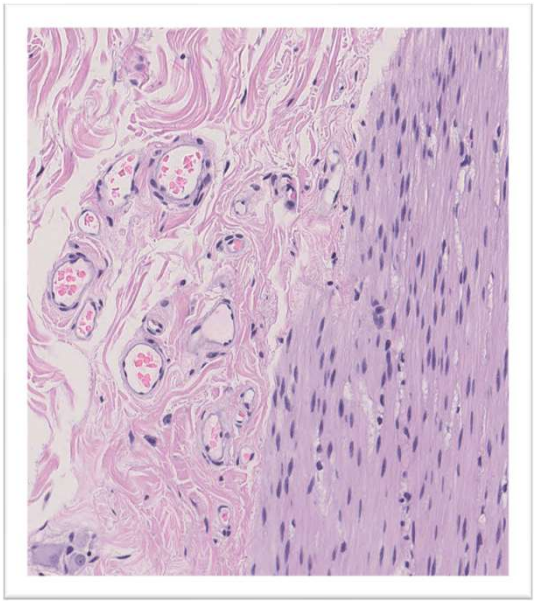
Leica Biosystems – H&E troubleshooting

Troubleshooting

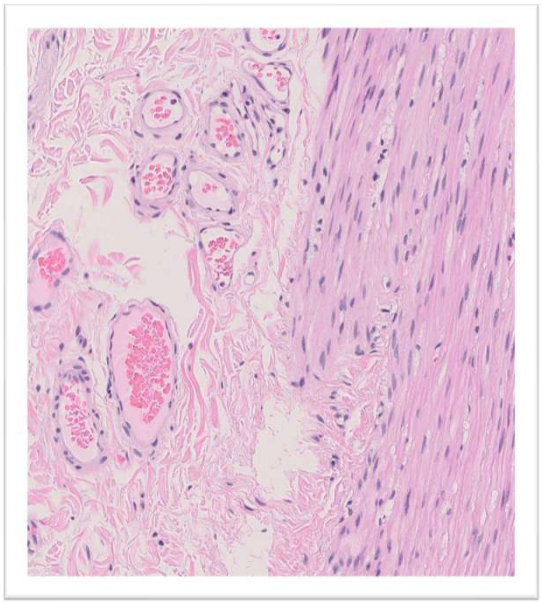
Eosin intensity

Under-staining

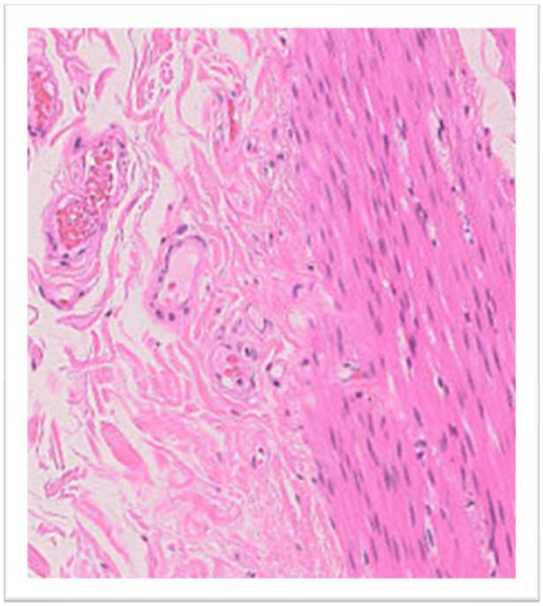


[X contrast]

Control



Over-staining



[X different shades]

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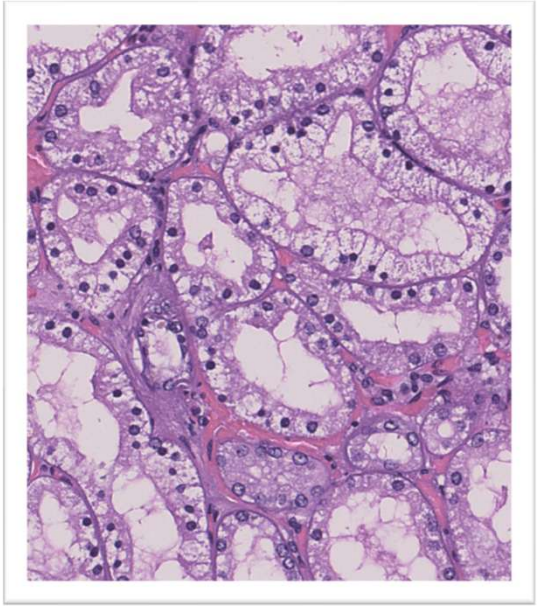
Leica Biosystems – eosin under- and over-staining

Troubleshooting

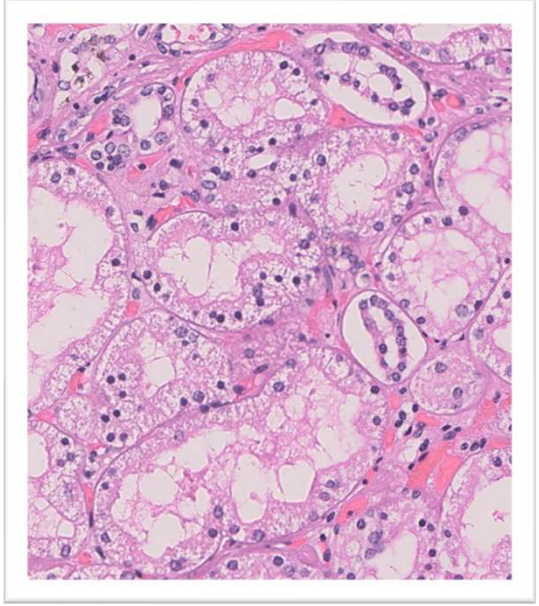
Different Strength of Acid Alcohol

Under-differentiated

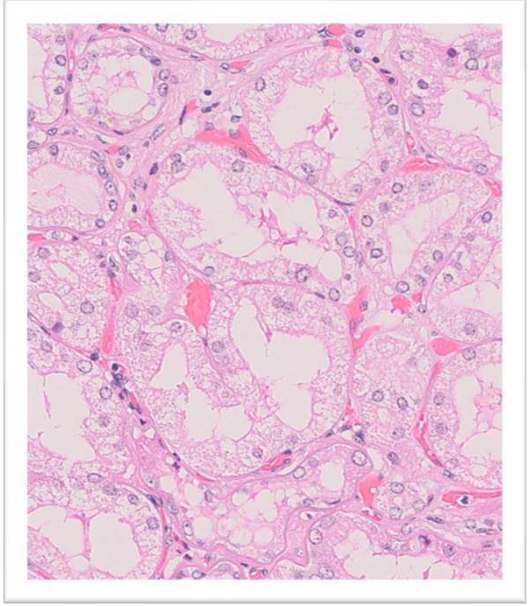


[Nuclei are too dark]
[X Chromatin activity]

Control



Over-differentiated



[Nuclei appear faint or poorly defined]

IHC World – differentiation step

Newcomer Supply – Harris hematoxylin

Take Home Points:

- H&E is the **foundation stain** for routine diagnosis and slide orientation
- Haematoxylin is a **basic dye** that stains basophilic structures (e.g. nuclei) blue–purple
- Eosin is an **acidic dye** that stains acidophilic components (cytoplasm, muscle, collagen, RBCs) pink to red
- Proper **differentiation and bluing** are critical for sharp nuclear detail and contrast