

MASSON TRICHRROME

**PRINCIPLES
TROUBLESHOOTING
APPLICATION**

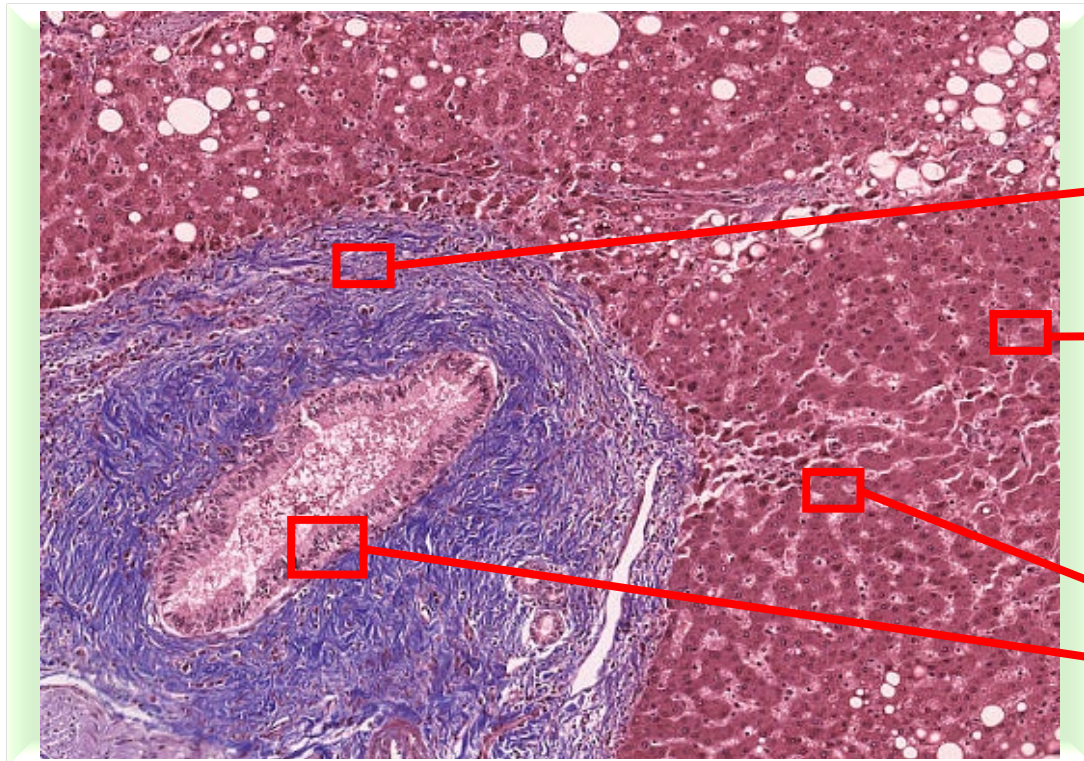
WONG XUE YI

LEARNING OBJECTIVES

- Describe how Masson Trichrome differentiates **collagen from muscle and cytoplasm**
- Interpret colour patterns in **normal and pathological connective tissue**
- Identify factors affecting **staining intensity and contrast**

- To distinguish collagen from other tissue components such as muscle and cytoplasm
- To study the diseases that involve changes of connective tissue such as hepatic disease, cardiac pathology, renal disease, muscle disorder etc

Masson Trichrome



Collagen

Blue or green

Nuclei

Black or brown

Fibrin and erythrocytes

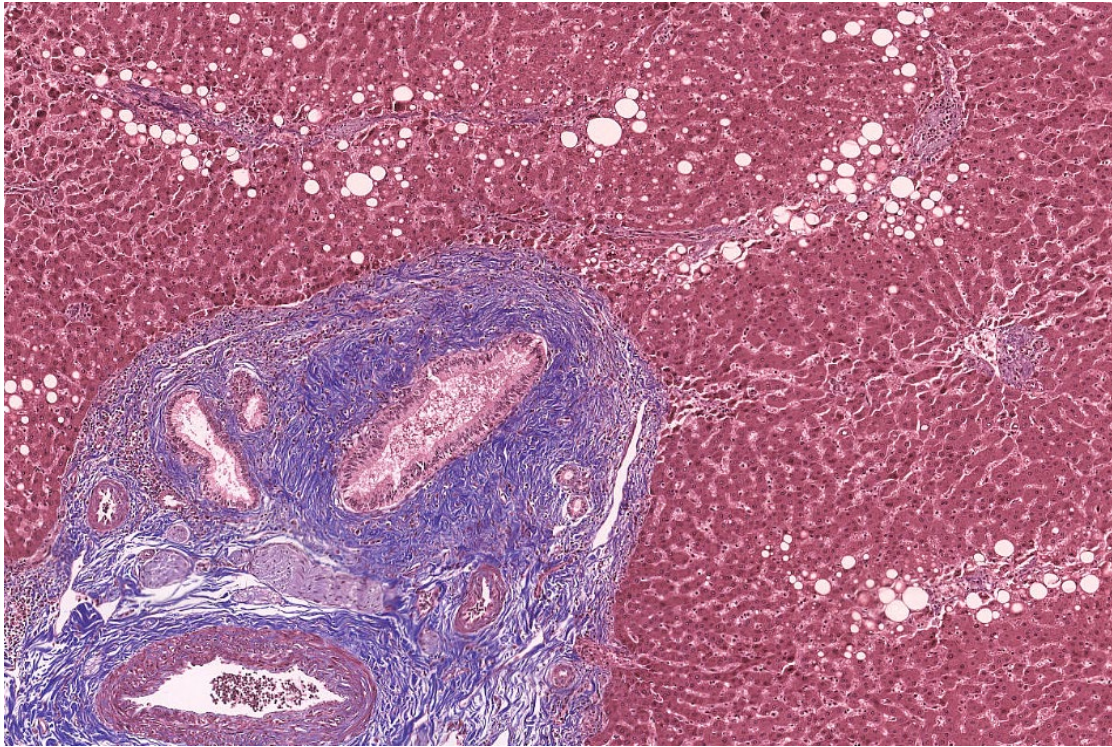
Also typically stained red

Muscle and cytoplasm

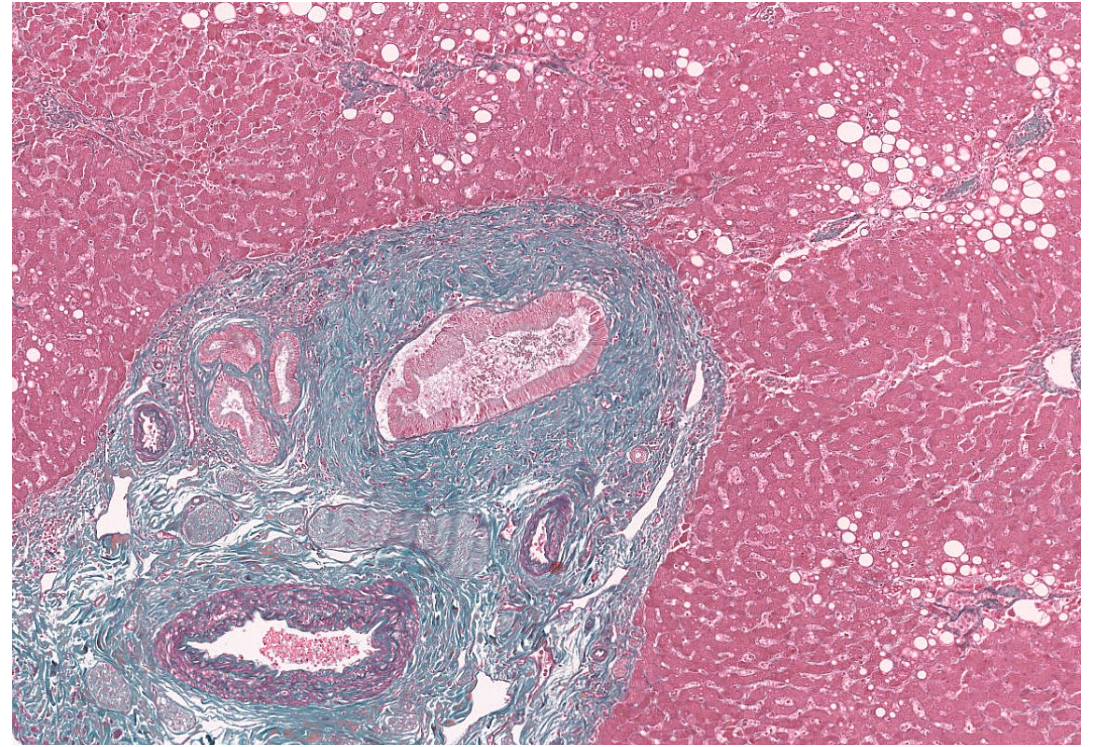
Red

MT (control)

Machine-stained MT



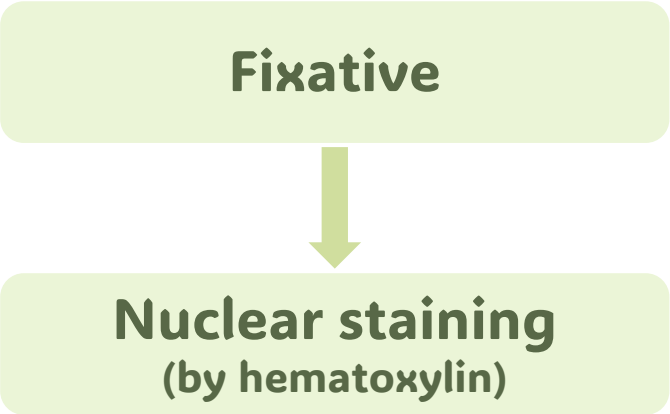
Manual-stained MT



Staining Principle

- Based on the size of dye molecules and permeability of the tissue structures
- Works by replacing the smaller dye molecules with larger ones in the more permeable structures

Fixative



**Nuclear staining
(by hematoxylin)**

Bouin's solution

- Picric acid
 - Protein precipitate
 - Enhancing staining
 - Nuclei acid interaction
 - Tissue shrinkage
- Acetic acid
 - Coagulate nuclei acid
 - Swollen effect
- Formaldehyde
 - Stabilizes protein structure

Staining Principle

Ponceau Fuschin



Phosphotungstic Acid



**Aniline Blue or
Light Green**

Initial stain

Binds to the acidophilic tissue components
Cytoplasm, muscle and collagen

Differentiation

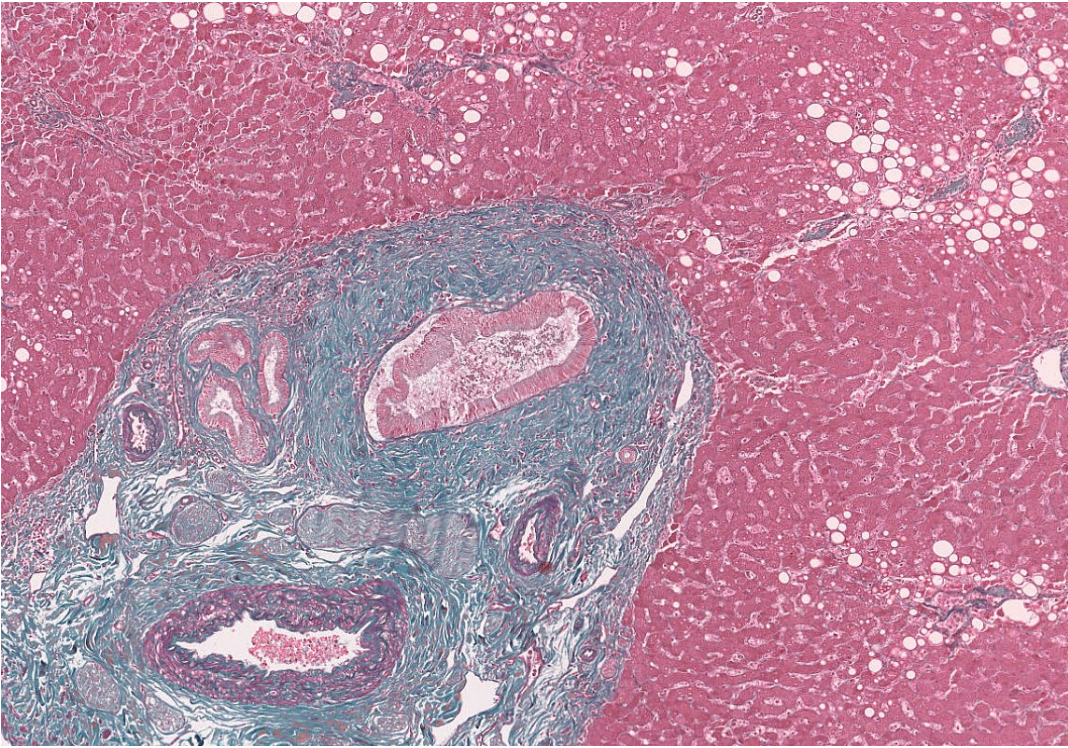
Removes the red stain from the relatively porous collagen
fibers

Collagen staining

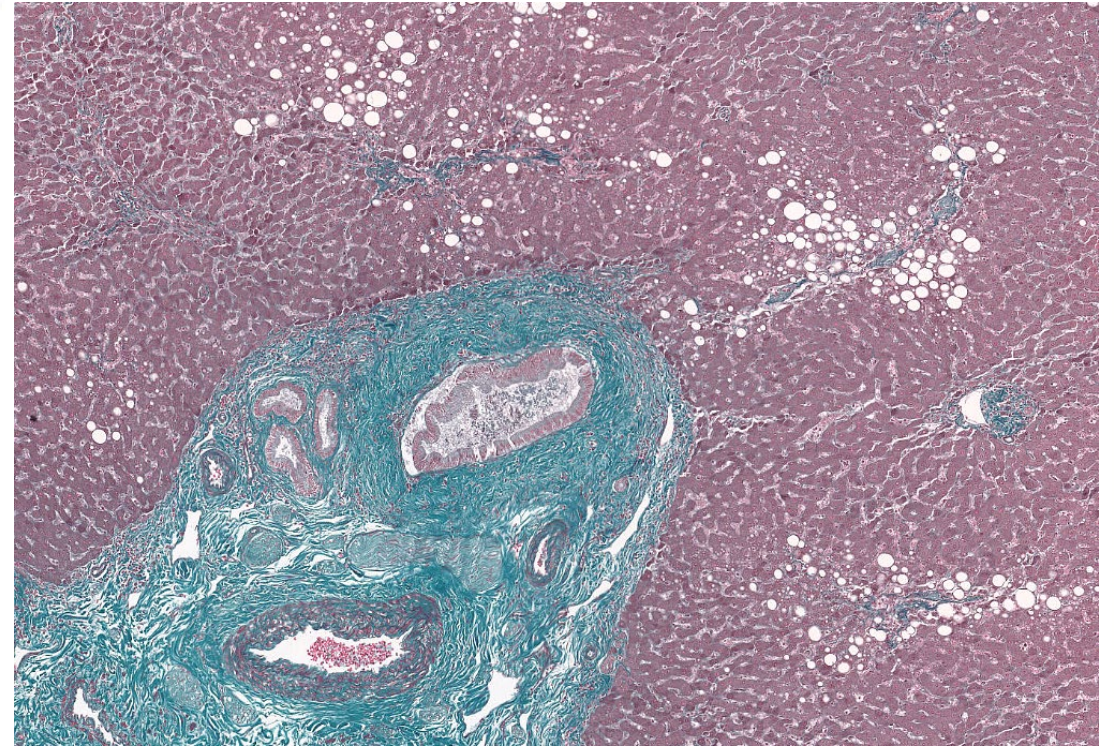
Penetrate the spaces in the collagen and bind, replacing
the polyacid and stain the collagen blue or green

Troubleshooting

Control



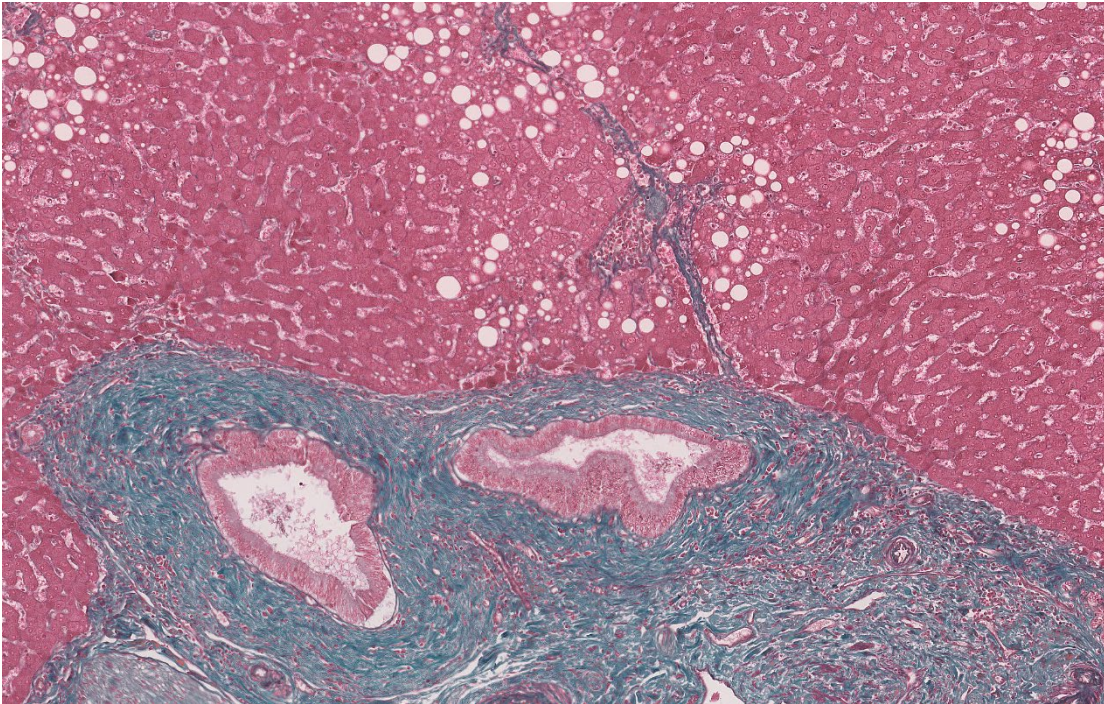
X Bouin's solution



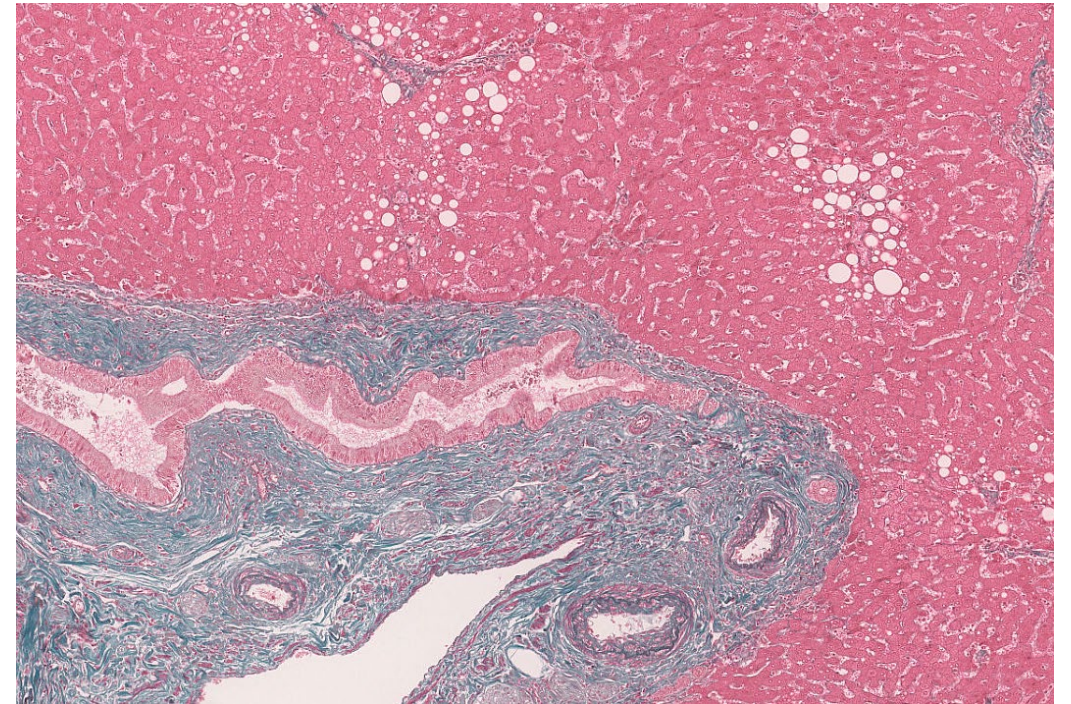
[x bright, x sharpness]

Troubleshooting

Control



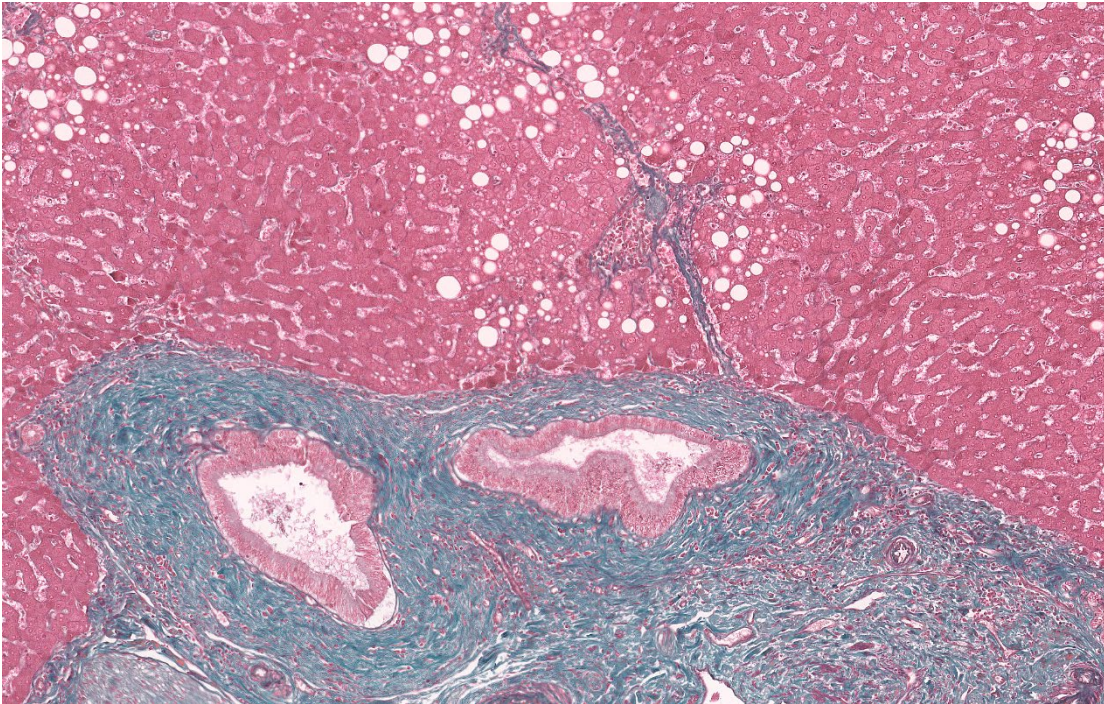
DH₂O after Ponceau



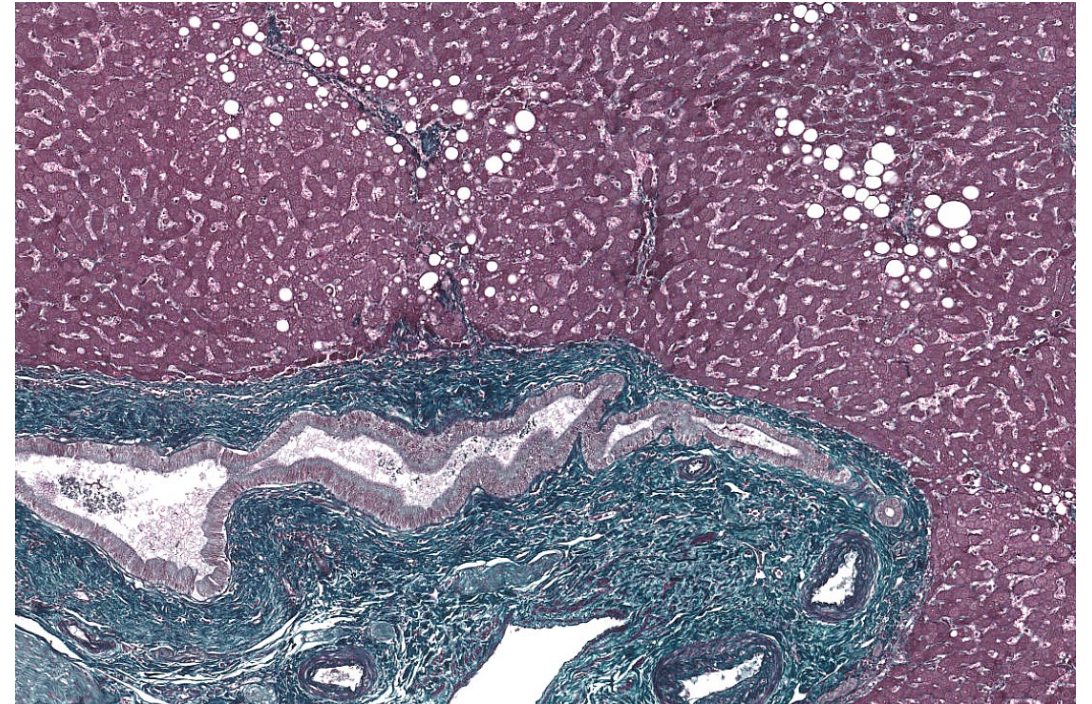
[x cytoplasm, x muscle]
↓ staining intensity

Troubleshooting

Control

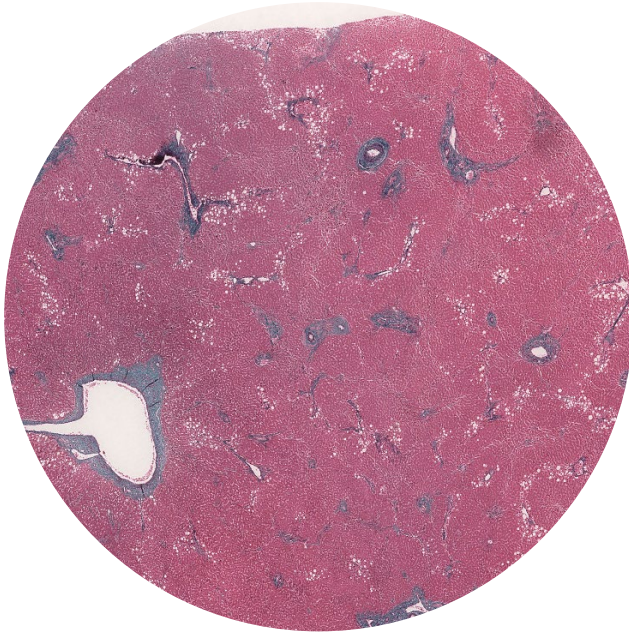


X Phosphotungstic Acid



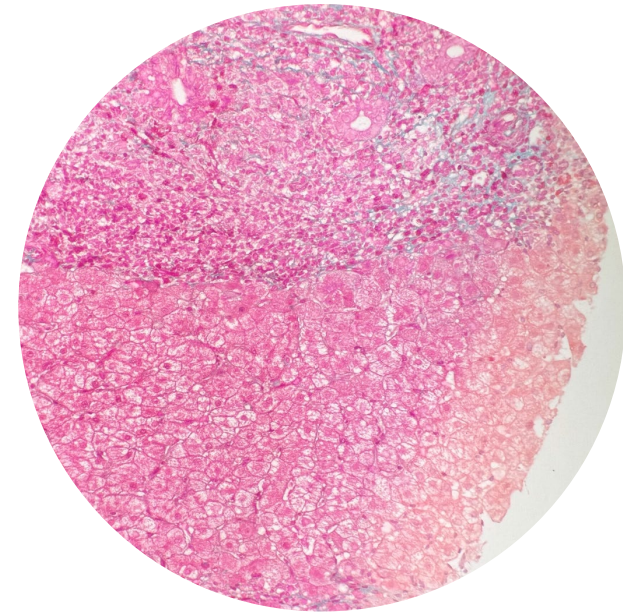
x contrast

Troubleshooting



Uneven

- Possible causes:**
- 1. Uneven Bouin's fixative**
 - 2. Reagent level**
 - 3. Uneven staining**



Sunset effect

TAKE HOME POINTS

- Masson Trichrome is based on **differences in dye molecule size and tissue permeability**
- Collagen appears **blue or green**, while muscle and cytoplasm appear **red**
- Nuclei appear **black or dark brown** (hematoxylin)
- Useful in assessing **fibrosis**, scarring, and connective-tissue-related diseases