UCSD Mousephenotyping Picro-Sirius Red Histo-chemistry

Reagents:

- Direct Red 80 (Sigma Cat#365548-5G)
- 1.3% saturated picric acid solution (Sigma Cat# P6744-1GAL)
- Weigert's Iron Hematoxylin Solution A (Harleco Cat# 15204-220)
- Weigert's Iron Hematoxylin Solution B (Harleco Cat#15204-222)
- Glacial Acetic Acid (Fisher Scientific Cat#A38-500)

Preparation of Reagents:

- Weigert's Iron Hematoxylin working solution: Mix 1:1 ratio of Solution A and B (this can be used for up to 2 weeks)
- 0.02% Picro-Sirius Red (0.1g Direct Red 80 in 500mL of 1.3% saturated picric acid solution): Can be used for 2-3 years
- 0.5% glacial acetic acid (50mL acid to 1L MQ water)

Staining Method:

- Deparaffinize paraffin sections and Place in slides Weigert's Iron Hematoxylin for *8 minutes*
- Move the sldies to Running tap water for *10 minutes*
- Immerse slides in 0.02% Picro-Sirius Red for *1 hour*
- Immerse slides twice (2x) in 0.5% Glacial Acetic Acid/MQ water -- *10 dips each*
- Physically shake slides to remove the remaining water
- Dehydrate (100% EtOH only) and clear in xylene in the fume hood.

Control Tissue:

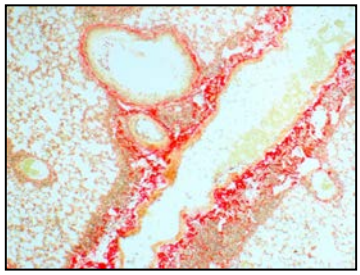
- Colon
- Lung

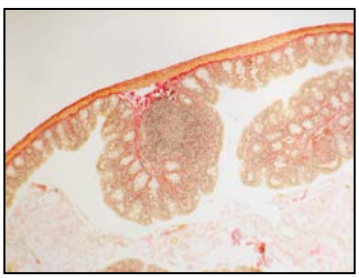
Expected Appearance:

- Red – Collagen
- Black – Nuclei
- Yellow – Everything Else

Notes:

- Works better on mouse tissue compared to Masson's Trichrome
- Can differentiate between collagen via polarizer
  - Larger fibers- yellow/orange
  - Thinner fibers- green





Example showing

Wild type mouse lung (left) and Wild type mouse proximal colon (right) with highlighted red collagen fibers, that can also be visualized using polarized light as well