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