F-375 MOLLIER’S QUADRUPLE STAIN

**Fixation:** Any suitable fixative for “Azan” stains.

**Sections:** Paraffin

**Staining:**

1. Transfer sections from 70% alcohol to **Orcin, 1%, in Acid Alcohol** (F-375-1) and stain for 12 hours. Wash in distilled water until free color is removed.
2. Stain in Weigert’s working Iron Hematoxylin for 12-30 minutes.

*To prepare Weigert’s Hematoxylin solution
  *Prepare fresh just before use.
  Mix equal parts of **Weigert’s Iron Hematoxylin A** (F-375--5), with **Weigert’s Iron Hematoxylin B** (F-375-4).

3. Rinse in distilled water.
4. Differentiate briefly in **Acid Alcohol, 1%** (F-375-9). Wash 6 minutes in running water.
5. Stain in **Azocarmine G Solution, 0.1%** (F-375-2) for 15-30 minutes. Check under microscope and do not over stain. Rinse in distilled water.
6. Place sections in **Phosphotungstic Acid, 5%** (F-375-10) for 3-6 hours, changing 2 or 3 times, until collagen fibers are completely decolorized. Rinse in distilled water.
7. Stain in **Naphthol Green B, 1%** (F-375-3) for 15-30 minutes.
8. Transfer directly to 95% alcohol and keep sections in motion for 30 seconds to avoid precipitation of Naphthol Green B.
9. Dehydrate, clear and mount in Balsam.

**Results:**

<table>
<thead>
<tr>
<th>Elastic fibers</th>
<th>Clear cut black</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen fibers</td>
<td>Green</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>Bright red</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>Pale red</td>
</tr>
<tr>
<td>Epithelium</td>
<td>Red</td>
</tr>
<tr>
<td>Chromatin of nuclei</td>
<td>Black</td>
</tr>
</tbody>
</table>

**References:**

Mollier, G., Z. Wissen, Mikr. 55:472, 1938
Clark, G. (ed) Staining Procedures, 3rd ed. Williams & Wilkins, c1973, p 64