Fixation: 10% Formalin (F-113)

Embedding: Cut paraffin sections at 4 to 5 um. (Thicker sections may stain better)

PROCEDURE:

1. Hydrate slides to distilled water.
2. Microwave in freshly prepared Rhodanine working solution for 60 seconds at 70 power. Allow slides to stand in solution for 30-60 minutes
   *To prepare Rhodanine Working Solution mix:
     Rhodanine Saturated Solution (J-602-1) 10 ml
     Distilled water 40 ml
   
   Note: Shake stock solution before measuring and mix solution well, filter through coarse filter paper. Shake working solution when pouring it on slides.

3. Wash well in several changes of distilled water.
4. Stain in Gill’s Hematoxylin #3 (J-602-2A) for 1-3 minutes.
5. Rinse with distilled water.
7. Rinse well with distilled water.
8. Dehydrate quickly through the alcohols, clear, and coverslip in PERMOUNT.

Stain Results:

<table>
<thead>
<tr>
<th>Material</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>Bright red to red yellow</td>
</tr>
<tr>
<td>Nuclei</td>
<td>Light blue</td>
</tr>
</tbody>
</table>

Note: With low copper concentrations in tissue, slight fading may occur after coverslipping and the golden precipitate may be difficult to distinguish from lipofuscin.

References: