Fixation: Any well-fixed tissue – 10% Buffered Neutral Formalin (F-113) 
or Zenker’s (F-155a) is preferred.

Sections: 5 microns.

Procedure:

1. Hydrate slides to distilled water.
2. Stain in Verhoffs solution for 1 hour. Tissue should be completely black.
   *To prepare working Verhoeff’s solution, add in order the following reagents – prepare fresh!
   - **Alcoholic Hematoxylin, 5%** (F-374-1) 20 ml
   - **Ferric Chloride, 10%** (F-374-2) 8 ml
   - **Weigert’s Iodine Solution** (F-374-3) 8 ml
   Mix well, solution should be jet black. Use immediately.
   At the conclusion of staining time, pour off the Verhoeff mixture into a container and 
   save it until after the differentiation process has been properly completed. If it should prove necessary, the 
saved solution may be used.
3. Rinse in tap water with 2 or 3 changes.
4. Differentiate in **Ferric Chloride, 2%** (F-374-4). Agitate slides gently during the process. Stop 
differentiation with several changes of tap water and check microscopically for black elastic fiber staining 
and gray background. Repeat Ferric Chloride, 2% treatment and tap water rinses as necessary for adequate 
demonstration. Kidney and myometrium are good controls. If the elastic fiber staining is too pale, restain in 
the saved Verhoeffs solution for 30 minutes and then proceed with the differentiation process.
Note: It is better to slightly underdifferentiate the tissue, since the subsequent Van Geison counterstain can 
extract the elastic stain somewhat.
5. Wash slides in tap water.
6. Treat with **Sodium Thiosulfate, 5%** (F-374-5) for 1 minute. Discard solution.
7. Wash in running tap water for 5 minutes.
8. Counterstain in **Van Giesons Solution** (F-374-6) for 3 to 5 minutes.
9. Dehydrate, clear in Xylene and coverslip, using a synthetic mounting medium.

Stain Results:

<table>
<thead>
<tr>
<th>Elastic Fibers</th>
<th>Blue-black to black</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fine elastic fibrils may not be stained with this method</td>
</tr>
<tr>
<td>Nuclei</td>
<td>Blue to black</td>
</tr>
<tr>
<td>Collagen</td>
<td>Red</td>
</tr>
<tr>
<td>Other tissue elements</td>
<td>yellow</td>
</tr>
</tbody>
</table>

References:
Sheehan, Dezna C., Hrapchack, Barbara B. Theory and Practice of Histotechnology, 2nd ed.
The C.V. Mosby Company, St. Louis, p. 196-197., c. 1980