

F-390, BIELSKOWSKY'S METHOD

Fixation: Zenker's Stock Solution (F-155A)

Sections: Paraffin

Staining:

1. Deparaffinize and hydrate to distilled water.
2. Place sections in **Alcoholic Iodine, 0.5%** (F-390-6) for 5 minutes. Wash in water.
3. Place in **Sodium Thiosulfate, 0.5%** (F-390-7) for 5 minutes. Wash in water.
4. Treat sections for 5 minutes in **Potassium Permanganate, 0.25%** (F-390-8). Rinse in water.
5. Place sections in **Oxalic Acid, 5%** (F-390-9) for 15-20 minutes. Wash thoroughly in running water and rinse in distilled water.
6. Place sections in **Silver Nitrate, 2%** (F-390-10) for 48 hours, in dim light, but not dark. Wash briefly in distilled water.
7. Place in Ammoniacal Silver Solution * for 30 minutes. Wash quickly in distilled water.

*To prepare the Ammoniacal Silver Solution, add 20 drops of **Sodium Hydroxide, 40%** (F-390-2) to 20ml of **Silver Nitrate, 10%** (F-390-1) with stirring or continuous shaking. Add Ammonium Hydroxide (F-390-3) dropwise, with stirring until the brown precipitate is just dissolved. Do not add an excess of Ammonium Hydroxide. Bring the volume up to 80ml with distilled water, mix and filter before use. Prepare fresh as needed. Use chemically clean glassware throughout the preparation.

8. Reduce in **Neutral Formalin, 5%** (F-390-11) for 30 minutes, changing solution after the first 10 or 15 minutes. Rinse in water.
9. Tone in **Gold Chloride, 1%** (F-390-12) for 1 hour. Rinse in water.
10. Treat with **Sodium Thiosulfate, 5%** (F-390-13) for 2 minutes to remove excess silver. Wash thoroughly in running water for several hours.
11. Stain in **Delafield's Hematoxylin** (F-390-4A) or in **Harris' Hematoxylin** (F-390-4B) or in Weigert's working Iron Hematoxylin for 5-15 minutes. Wash in tap water until blue.
12. Counterstain in **Van Gieson's Solution** (F-390-5) for 30 seconds.
13. Dehydrate in 95% alcohol followed by absolute alcohol.
14. Clear in Xylene and mount in balsam.

Results:

Coarser collagenous fibrils	Red to rose
Finer collagenous fibrils (reticulum)	Black to dark violet
Nuclei	Black, blue or brownish
Cytoplasm	Grayish yellow
Muscle fibers	Brighter yellow
Elastic fibers	Pale yellow

References:

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Foot, N.C., & Menard, M.C., Arch. Path. & Lab Med. 4:211-4, 1927

Mallory, F.B. Pathological Technique, W.B. Saunders Co., Philadelphia, 1938

Clark, G. (ed), Staining Procedures, 3rd edit., The Williams & Wilkins Co., Baltimore, c 1973, p.67.