

### **Nassar and Shanklin Reticulin Stain**

Acid clean all glassware

1. Deparaffinize slides to ddH<sub>2</sub>O
2. Nuclear fast red – 3 minutes
3. Wash in ddH<sub>2</sub>O
4. Equal parts of .5% Potassium Permanganate and .5% sulfuric Acid – 2 minutes
5. Rinse in ddH<sub>2</sub>O and decolorize in 2% Oxalic Acid until slides are clear.
6. Give a good wash in ddH<sub>2</sub>O
7. In a beaker take 40 mL of 2% silver nitrate. Add 12 drops of pyridine. Place slides in silver for one hour in 65° C. Slides should be toasty brown.
8. Place 10 mL of 10% silver nitrate in a beaker. Add ammonium hydroxide drop by drop until clear. Add enough 10% silver to make solution slightly cloudy. Add H<sub>2</sub>O to make 50 mL (still cloudy). Add 15 drops of pyridine solution until it's clear.
9. Throw away the 2% silver, wash quickly in 100% alcohol. Place slides in 10% silver nitrate/ammonium hydroxide solution for 15 more minutes in the oven.
10. Make reducing agent: 2% formaldehyde (490 mL ddH<sub>2</sub>O and 10 mL Formaldehyde) and 100% alcohol – 50:50  
Wash slides in 100% alcohol. Place slides in reducing agent for 5 minutes (Never pour over on the slides)
11. Wash in ddH<sub>2</sub>O
12. 0.2% gold chloride solution – 3 minutes
13. Wash in ddH<sub>2</sub>O
14. 0.2% gold chloride solution – 3 minutes. Wash in ddH<sub>2</sub>O
15. 2% sodium thiosulfate – 2 minutes. Wash in ddH<sub>2</sub>O
16. Nuclear fast red for 3 minutes
17. Dehydrate and coverslip