Materials:

- 5% silver nitrate solution

- Distilled water

- 5% sodium thiosulfate solution

- Nuclear fast red solution (optional)

- Glass slides and coverslips

Procedure:

1. **Preparation of the tissue slides:** Begin by preparing 10 μm sections of your snap-frozen cardiac tissue. Mount the sections onto glass slides. Allow the slides to air-dry for 30 mins.
2. **Rinse** the slides in tap water for 1 minute before staining.
3. **Silver Nitrate incubation:** Incubate the slides in 5% silver nitrate solution under UV light for 30-60 minutes or until a brown/black precipitate forms. This step will help visualize the calcium deposits. UV light reduces the silver ions to elemental silver.
   1. Slide 1/2: 100 mins
   2. Slide 2/2: 120 mins
4. **Rinse** the slides in tap water for 3 minutes to remove excess silver nitrate.
5. **Sodium thiosulfate incubation:** Incubate the slides in 5% sodium thiosulfate solution for 5 minutes to remove unreacted silver. Sodium thiosulfate helps to "fix" the silver staining by reacting with unbound silver ions.
6. **Rinse** the slides in tap water for 1 minute to remove excess silver nitrate.
7. **Counterstaining (optional):** Rinse the slides again in distilled water and then counterstain with nuclear fast red for 3 minutes if you wish to visualize the cell nuclei.
8. **Rinse** the slides in distilled water for 1 minute again.
9. **Mounting:** Air dry the slides or dehydrate in graded alcohols, clear in xylene, and mount in a resinous mounting medium.
   1. Graded dehydration  Xylene (5 mins) x2  Air dry  Xylene (20s)  Mount