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# 🌐 Neuromelanin Processing and Image Acquisition

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**Protocol status:** Working
**We use this protocol and it's working**

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# Abstract

Protocol for preparing post-mortem tissue for intracellular NM quantification.

# Troubleshooting

## Tissue Processing

1. Formalin-fixed paraffin-embedded tissue blocks from the pontine and midbrain regions were sectioned at 6μm, collected onto adhesive microscope slides and allowed to dry in the oven at 37°C for 48 hours.

2. Slides were incubated in the oven at 60°C for 1 hour to melt the paraffin.

3. To remove the paraffin, sections were submerged in HistoChoice Clearing Agent (Sigma-Aldrich, H2779) for 2 × 7 minutes, followed by rehydration in decreasing ethanol concentrations (100% ethanol for 2 × 3 minutes, 95% ethanol for 3 minutes, 70% ethanol for 3 minutes) and distilled H2O for 3 minutes.

4. Slides were submerged in 1xPBS and coverslipped using Fluorescence Mounting Medium (DAKO, S302380-2).

## Image Acquisition

5. Sections were scanned using 20x objective (NA=0.8) with pre-set focusing and exposure parameters for optimal NM signal quality with an automated Slide Scanner (SLIDEVIEW VS200, Tokyo, Japan).

6. Identical parameters were applied to each scanned section to ensure consistency in capturing NM.

7. Once the scan was completed, sections were submerged in 1xPBS to remove coverslips and continue with IF processing.