Glyoxylic Acid Fluorescence Histochemistry for Visualization of Catecholamine Containing Neurons

For sections:

Five steps: a) Perfusion with a glyoxylic acid (GA) solution 2% in Ringer buffer) (optional)

b) Sectioning on vibratome between 0-5 degrees Celsius between 30-35 μm thickness.

c) Total immersion of sections for no longer than 3-5 minutes in a ice-cold neutral 2% GA solution using a glass rod. Sections then transferred to gel-subbed slides.

d) Drying under a warm-air hair dryer for 15 minutes then optionally stored in a vacuator in darkness over fresh phosphorous pentoxide overnight.

e) GA vapour treatment (optional)

For whole-mount:
0.03-0.05 ml Xylazine @ 20 mg/ml + 0.27 ml Ketamine @100mg/ml IM to put animal to sleep.

Fresh unfixed PBS-washed arteries are open longitudinally, and immersed in 2% glyoxylic acid, 10% sucrose, 0.1M phosphate buffer at four degrees for 2 hours. They are mounted with lumen facing down on a gel-subbed glass slide and then dried for an hour with a hair dryer. They are then dipped in xylene (no alcohols) for a few minutes and coverslipped using fluorescent mounting medium.

The control and experimental tissue have to be treated a the same time.

Ref: Lindval et al. Histochem 39:97-127 (1974)

PS you could also look at Peptide Y, TH and dopamine hydroxylase (DH) immunocytochemistry.