Protocol: C. elegans DAPI Stain

DAPI is a fluorescent nucleic acid stain. In this protocol ethanol is used as a fixative. This protocol is adapted from Mair et al (2011).

What is needed:
2% agarose pads for microscopy
100% molecular-grade ethanol
M9
DAPI

1. Make 2% agarose pads on clean microscope slides.

2. Transfer worms to an empty NGM plate and let crawl around for ~30 minutes (longer is better).

3. Add 2 μl of M9 buffer to the agarose pad and transfer worms from the empty NGM plate to it with a pick. (This works best with two people: one to pick worms from the plate, and the other to transfer worms to the small drop of M9 on the agarose pad. The focus planes of the NGM plate and microscope slide are different, so each person operates a different scope.)

4. Remove remaining M9 with the corner of a KimWipe. Leave enough M9 behind so that the worms do not dehydrate completely.

5. Add 2 μl of stock DAPI (1mg/ml) to 2 ml of 100% ethanol. Keep the solution covered when not in use.

6. Slowly add 60 ul of the DAPI and ethanol mixture in 20 ul aliquots to the worms and cover until ethanol evaporates.

7. Once all the ethanol has evaporated, add 60 ul of M9 buffer and allow the slide to rehydrate overnight in 4℃ fridge. Put the slides into an empty freezer box so that way they are shielded from light.

8. The following day, add a drop of VectaShield mounting medium to the agarose pad. Add coverslip and seal with nail polish.