

## **Microscopy on anesthetized worms**

### **Reagents needed**

- Vacuum grease syringe
- M9 + 5 mM Na Azide (3.3mg Na Azide in 10 ml of M9)
- Slides
- 22x22 mm coverslips
- watchglass slide

### **Protocol**

1. Place ~ 250 $\mu$ l of M9/NaAzide in a watchglass slide
2. Pick 5-10 worms off an NGM plate and into the azide solution - they should stop moving within 5 minutes. If they don't, make fresh azide solution)
3. Using the grease pen, draw a 10mm x 10mm square on a slide - make sure the square is closed on all ends.
4. Overlay a Kimwipe onto the square and give continuous pressure to even the grease pad
5. Pipet 5 $\mu$ l of M9 + Azide into the square.
6. Pick anesthetized worms onto the slide and gently overlay them with a 22 x 22 mm coverslip.
7. Image away...

### **Notes**

- If you don't want to use Na Azide, you can make M9 with levamisole (1mg / ml). However, it takes ~ 10 minutes to put worms to sleep with levamisole
- If worms explode when the coverslip is overlayed, your grease pad isn't thick enough. Try less pressure on the Kimwipe.
- Worms should only be imaged under these conditions for <30 minutes.