**Counting rotifers with palmer slide**

1. Make sure it has the sticker facing up on the palmer slide.
2. Invert the 15 ml tube six times, making sure all of the liquid at the very tip of the tube has time to flow down to the cap during each inversion.
3. Pipette 0.1 ml of rotifer culture onto the palmer cell. When pulling up the rotifers from the 15 ml tube, dip the pipette tip until it’s ~75% submerged (there is a line on the tip that I aim for). Dispense the sample into the center of the sticker area. Cap the tube as quickly as possible, minimize the time it’s open. If anything goes wrong during this step, just go with it and do your best to still get a count, do not pull out another 0.1 ml from the tube, since that might alter our “common garden” condition among tubes.
4. Place a cover slip on the slide, trying your best to set it directly on top of the drop without squishing the sample to one side (this is almost impossible, but I try to do my best). If the sample doesn’t fully fill the sticker area, *gently* press on the center of the cover slip.
5. Take note of what sample you’re counting. I do this by clicking on the corresponding cell in the data sheet.
6. To count rotifers, there are two methods depending on concentration of the sample:

Method A (low concentration)

At 40X, start counting rotifers in a straight line from one notch to the other. Then, re-align your field of view to count above and below that center line. Try to avoid double-counting individuals, although that might be a challenge at high concentrations. If you feel like you can’t avoid double-counting, try method B.

Method B (high concentration)

At 100X, count rotifers in a straight line from one notch to the other. If that number is >10, multiply it by 6.8 and use that value as the count for that sample. If it’s <10, use method A.

Like all counting methods that involve taking a sample of a population, Palmer counting slides only give us an idea of the relative abundance of rotifers. This means we can compare the number of rotifers among tubes but can’t make any definitive assumptions about the actual number of rotifers in the tube. While we want to avoid double counting, by taking a small sample of the whole population we’re already introducing a ton of randomness into our count—that’s why we have replicates!