

Bi 1X, Spring 2011

Week 6

- **Session 2: Single-cell movies of dividing bacteria**

As a way to complement the bulk measurements you did we'll be measuring cell division at the single cell level under the microscope today. The idea is to not only compute the cell doubling time based on the movies you'll obtain but to introduce the single cell measurements that can be done using an automated microscope.

Preparing the agar pads:

1. Stretch a piece of parafilm on a glass surface (or the bench). Place a 24x50mm or 24x60mm coverslip on the parafilm.
2. Pipette 2 – 3ml of LB with 1.5% low melting temperature agarose.
3. Drop another coverslip on top in order to “sandwich” the agarose. This step might be easier to do with your gloves off.
4. Let it dry for 30 minutes. Note that in the interest of time we'll be giving you some premade pads.

Setting up the pads:

1. Cut two 3 – 4mm squares of agarose and put them on the slide. Cover the pads while they are drying (without touching them!). The idea is to make sure the pad is not too wet – your cells would float in the drop of water, and not too dry at the same time – cells need moisture to divide. We're making two pads in case something goes wrong with one!
2. Spot 2ul of cells on each pad. Remember that you should NOT touch the pad with the pipette tip!
3. Cover the pads with a tip box lid and let them dry for a few minutes until the water spot is no longer visible. Depending on how dry the pad is, we might put it at 37°C to speed up drying.
4. Gently transfer the pads to a Wilco petri dish as demoed by your TA. Remember that the side where you spotted the cells should face the bottom of the Wilco petri dish.
5. Place the dish on the microscope stage leaving the lid open. Make sure that the environmental chambers are properly closed. The idea behind this step is to let the pad equilibrate with the temperature of the scope. If you close the lid and see condensation forming then open it again and wait some more time.
6. After around 15 minutes seal the dish using parafilm. Make sure the parafilm doesn't interfere with the placement of the dish on the scope. This step is meant to reduce evaporation from the pad which would result in drying.
7. Put a drop of oil on the 100X objective and on the bottom of the dish where the pads are located. Load the dish onto the stage with a petri dish adapter. Make sure the phase ring corresponds to Ph3.

Setting up the movie:

1. Load “MicroManager 1.3.39”. **NOTE:** It’s very important to load this version of MicroManager. If not your movie might not work.
2. Look for cells on the pad and setup Köhler Illumination in bright-field live.
3. Go to the “Multi-D Acquisition” window and open the XY list (next to the “Use XY list” option).
5. Move around the pad to mark 5 – 10 different positions on the XY list where you see interesting things. For example, you might want to include some areas with only one or two cells in the middle and some other areas with a lot more cells.
6. Set up the channels to be used. You should use the brightfield setting without binning. Make sure that you have a reasonable exposure!
7. Set up the autofocus by selecting “Autofocus” and open its option dialogue. Let’s choose the following options:

1st number of steps: 6

1st step size: 1

2nd number of steps: 6

2nd step size: 0.3

Threshold: 1

Crop ratio: 0.25

Channel: Brightfield

What do these options mean? Check out the [MicroManager manual](#) you were given in Week 1. Explain how the search for the optimal focus will go.

Note for the Nikon scopes: The Nikon scope does not need any software autofocus. You’ll have to set up the Perfect Focus. Also, make sure that the “Hardware Autofocus” options “Switch off for XY move” and “Switch off for Z move” are selected. Ask your TA how to work with Perfect Focus.

8. Choose “Save files to acquisition directory” and create a subfolder in “Bi1X2011” folder on the desktop to save your images. Also, choose “Single Window” in the “Display” option. **NOTE:** Always save your files in the local drive first!
9. Before you start taking your movie let’s make sure that everything works. Let’s take only one frame. If one of the frames fails to focus go back to the position and make sure it hasn’t drifted out of the range of the autofocus search. If you’re having issues, ask your TA! **Be patient!**
10. Now you’re ready to take your movie. Think carefully about how often you want to take frames.

What’s the maximum time resolution you could get? What’s the limiting factor (the bottleneck) in the acquisition at each position? You’ll probably want to take a frame every 5 minutes.