Yeast Showdown: Time-lapse Microscopy

NOTE: This exercise does not come with a separate hints document. For hints or guidance, refer to the solution file ***`YeastShowdownTLMicroscopySolution.m`***.

In yeast showdown I and II, you compared a wildtype yeast strain with a mutant strain by measuring their gene expression, via microscopy, and growth rate, via optical density. Sometimes, we may want to obtain growth rate directly from a microscopy experiment. This is possible, for example, if we take images over time, and use image processing to quantify the number of cells at each time point.

What are some advantages of using microscopy to quantify growth rates of yeast cells, compared to OD measurements of cultures? What are disadvantages of time-lapse microscopy?

In this exercise, you will analyze time-lapse images of yeast cells to address the question of whether there is a growth-rate cost to a yeast cell when it responds to galactose. Typically, in the presence of the sugar galactose, yeast induce the GAL pathway, which is a set of genes that metabolize galactose and allow the cell to grow. However, expressing the GAL genes requires considerable cellular resources. In the initial minutes after the pathway is activated, it is possible that the benefits of expressing GAL genes are outweighed by the costs of expression, and result in a temporary slowing of the cells' growth rate. After yeast have induced GAL genes, they should be able to grow rapidly, because galactose is a relatively efficient carbon source.

To detect possible induction costs in the GAL pathway, we will compare a wildtype strain of yeast that responds to galactose with a ΔGAL2 strain, which lacks the galactose transporter and therefore should not induce GAL genes in response to galactose. The wildtype strain is labeled with a constitutively expressed YFP and co-cultured with an mCherry-labeled ΔGAL2, and the cell mixture is imaged under the microscope in a flow chamber. For the first 5 hours of the time-lapse (frames 1-30), the flow chamber is perfused with raffinose, a poor carbon source. Starting at 5 hours (frame 31), galactose is added to the flow without changing the concentration of raffinose. The experiment lasts 16 hours, with images, or *frames*, taken at 10-minute intervals (97 frames total).

If there is a cost to responding to galactose, we should see differences in growth rate between the wildtype (galactose-responsive) and ΔGAL2 (nonresponsive) strains immediately after the media switch. To obtain the growth rates, you will analyze a sequence of images from one microscope field of this experiment.

All the data for this exercise is in a separate zip file that is around ~900MB in size. The time-lapse images are in the folder **`images`**. Different fluorescent *channels* were imaged separately, so there is a separate sequence of 97 images for both YFP and mCherry, or 194 image files total. The image files have complicated names, but all you need to know is that the end of each filename indicates the frame number—i.e. a file ending in **`_t15.tif`** is the 15th frame of a sequence of images.

Exercises:

1. View the time-lapse images. A pre-processed image stack **`overlay_sequence.mat`** containing YFP and mCherry images overlaid on bright-field DIC images has been provided for viewing purposes. Load the file into MATLAB. (This is a 300MB file and may take a minute.) What variable is loaded? How many dimensions does it have and what does each dimension represent? Play the image sequence as a movie using MATLAB's **`implay`** command (type **`doc implay`** or **`help implay`** to figure out how to use **`implay`**). What do you see? Can you tell by eye which strain is growing faster?

BONUS: Load the YFP and mCherry raw images into MATLAB as a sequence and play them back. Try overlaying the two sequences and play this back. How is this different from the movie stored in the file **`overlay_sequence.mat`**?

2. Segment the images. To assess whether one strain is growing faster than the other, we need to first computationally identify, or *segment*, the cells, and then use our segmentation results to quantify cell growth over time.

a. **Plan image analysis strategy.** Before we write any code, it's a good idea to come up with a general plan. In fact, it is often helpful to write down some "pseudocode", or a list of commands or tasks that your code will perform. For example, one approach for obtaining growth rates might involve the following steps:

```
For each channel:
    Loop through and load each frame
        Segment (i.e. identify) cells in frame
        Count number of cells and save to an array
        Keep track of which time point
    End loop
Plot number of cells versus time for both YFP, mCherry channels
```

One potential problem with this plan is that it requires that we write code to count exactly how many cells are in each frame. This can be difficult in crowded frames near the end of the experiment where there are many cells next to each other (see BONUS). Instead, we can start with a much simpler approach of simply segmenting cells from background, and use the number of pixels occupied by cells, instead of number of cells, as a proxy for cell mass. Sketch out a script in pseudocode for this strategy. What commands or matrix operations will you need to do the segmentation?

b. **Segment a single frame.** Now try to do the segmentation on a single frame. You may find it helpful to put the segmentation commands in a separate cell, script, or function. In other words, load up the first image in either channel, and threshold it to segment cells

from background. How does your segmentation compare to the original image? Where does thresholding tend to make mistakes? Finally, calculate the area of the cells in this frame, in pixels.

c. **Apply your segmentation to all frames.** Now apply the segmentation code you wrote in part b to all 97 frames in both YFP and mCherry channels. How should you load the files? (HINT: You need to make sure you are loading the frames in chronological order.) What results do you need to save?

   You will need loops for this part. Creating a function or two might also help. The processing may take quite a few minutes, so you may also find it helpful to have your code output feedback (i.e. at the beginning of each run of the loop) on its progress. You can do this using the **`fprintf`** command (**`num2str`** might also come in handy here), or simply by writing a string on a line by itself with no semicolon at the end (i.e. **`'this text will get outputted to the command line'`**). After you run your code, you should now have arrays containing the pixel area of cells in each frame of both channels.

3. Compare the growth of the two strains by plotting the pixel area of cells over time. Before reading further, plot the data yourself, adding labels, annotations, additional plots as you see fit. How should you scale your axes? What do you see? When is the media switch?

a. **Rescale and normalize the data.** Since the data represents areas of cells that are dividing exponentially, we can see more detail if we take the base-2 logarithm of the pixel areas before plotting. Also, since we are interested in growth rate and not absolute numbers of cells, it's helpful to normalize the pixel areas. How might you normalize the pixel areas? Normalize and log-transform your data, and replot it.

b. **Do some sanity checks on your data.** Do the plots look how you expected them to? What are possible caveats or limitations of this data? For example, before the media switch, we don't expect a difference in growth because ΔGAL2 shouldn't affect metabolism of raffinose. Are your plots consistent with this expectation? If not, what might be the reason?

c. **Consider the effects of galactose induction.** We are interested in whether the cells grow differently after galactose induction (t=5 hours, or frame 31). On your plots of log2(pixels) versus time, does it look like there a cost to GAL pathway induction? Is there a difference between the growth of cells in raffinose versus in raffinose and galactose? How long does it take before there is a difference in growth between the

wildtype and ΔGAL2 cells? To compare the two strains, you may want to normalize pixel areas to the time of media switch.

d. **Consider more data.** You might notice that the data from a single microscope field is a little noisy, since you are only considering a small number of cells. To get better statistics, you can image and analyze more fields. In fact, 20 microscope fields were acquired for this experiment. In the real world, this means that your next job will be to download and analyze 15GB of image files. To save you some time, the results of a threshold segmentation on all 20 fields have been provided in the file **`allfields.mat`**. This file contains a struct array called **`allfields`**, where **`allfields(i).YFP`** contains a list of the pixel areas of YFP-expressing cells in each frame of the i-th microscope field (and likewise for **`allfields(i).mCherry`**). Load this file and plot the data in it. How reproducible are the results between different microscope fields? The data that you analyzed is in field 11. How does your segmentation result compare to the result in field 11 of **`allfields`**? How does this affect your conclusion about the effect of galactose induction?

BONUS: Segment and count cells. For the above analysis we used the change over time of the total area of all cells as a proxy for cell growth. An alternative measure of cell growth is the number of cells over time. Revise your segmentation code to determine the number of cells. You will need the commands **`bwlabel`** and **`regionprops`** to count segmented regions—look these up in the MATLAB documentation (or in Google) to see details of how to use these functions.

NOTE: You may not be able to obtain perfect segmentation results for the bonus without some advanced image processing techniques such as filters and morphological operations, which will be covered on day 4. If you are curious you can read through the "MATLAB Image Analysis" file in the Day 4 packet to help you complete the bonus.

Created 5/29/2012 by JW.