

Science in the Classroom

Actin Network Architecture can determine Myosin Motor Activity Student Activity

Learning objective

In this activity, students will learn how to extract quantitative information from visual images. The objectives are for students to (1) learn how to make a kymograph using ImageJ, (2) extract numerical values from the kymograph, and (3) demonstrate the phenomena of actin filament formation with a hands-on activity.

Prior Knowledge and Skills Required

Students should be able to manipulate files on a computer (i.e. download, open). General experience in following instructions to perform functions in computer software is beneficial.

Materials Required

- Computer
- 3 pieces of yarn or thread (one 20" long, one 30" long, one 40" long)
- Various beads with centrally-extracted holes for stringing onto the yarn

General Introduction

An important visual representation of data used in this article is called a kymograph. The kymograph is a way to represent a single image or part of an image over time. Researchers make kymographs to show how a one-dimensional piece of an image (sometimes referred to as a "slice") changes in time. Constructing a kymograph essentially involves extracting a small linear region of an image at various time points, and stacking them on top of each other, in time order.

Kymographs are useful because they provide dynamic information about a single spatial location in a larger body (such as a living cell!). We used a kymograph in Figure 2C of the article to show how parallel-actin filament bundles elongate, while antiparallel filaments disassemble. Here, you will learn how to make a kymograph with the real images we included in the paper.

To get started, first download and install the freely available software, ImageJ, (<http://rsbweb.nih.gov/ij/download.html>). Open images by simply dragging the file in the lower band of the ImageJ window under the toolbox icons. If needed, modify the contrast by clicking on *Image > Adjust > Brightness / Contrast*.

Additionally, download the two images from Figures 2 and 4 to use for the activity.

Part 1

1. Open the Stars.tif file corresponding to the time series of the evolution of actin polymerization and dynamics on a star-shaped pattern without myosin (*left panel*) and in presence of myosin (*right*) in ImageJ.

2. The kymograph consists of plotting a profile for all frames along a desired line and encoding the intensity profile at a grayscale level. To do so, you will select the line along which we will measure the intensity profile as shown in Figure 2C. You will start by doing this on the experimental case in the presence of myosin (the *right panel* of the Stars.tif figure). Right click on the line tool (fifth tool in the ImageJ



window, shown in the screen shot below) in order to switch to “Segmented Line” and reproduce the blue dotted line shown in Figure 2B in the last image of the time series (Tip: You must click once at the edge of the array, then drag the cursor to the center, and double click, for a line to form).

3. Next, you will generate the kymograph. One way to do this is to use a plugin, which is a short computer program that performs a specific task. Rather than using a plugin for generating kymographs, we can use a built-in ImageJ function called “Reslice.” This function was built to process a collection of images in three dimensions, called a “Z-stack.” We will apply this function to create a kymograph. First, click on the *Image > Stacks > Reslice* button.
4. An option dialog appears; the default settings are fine for us, so just press “OK.” The kymograph similar to Figure 2C then appears in a new window.
5. You can now generate a similar kymograph for the other experimental case: in the absence of myosin. Draw another segmented line along the region of interest, in a similar location as the one you drew previously but this time on the *left panel*—the pattern in absence of myosin—of the Stars.tif image.
6. Then, click on *Image > Stacks > Reslice* once more.
7. Identify the main zones of actin dynamics in the presence of myosin (assembly, contraction, disassembly, elongation) as shown in Figure 2C and compare with what you obtained for the control case in the absence of myosin.

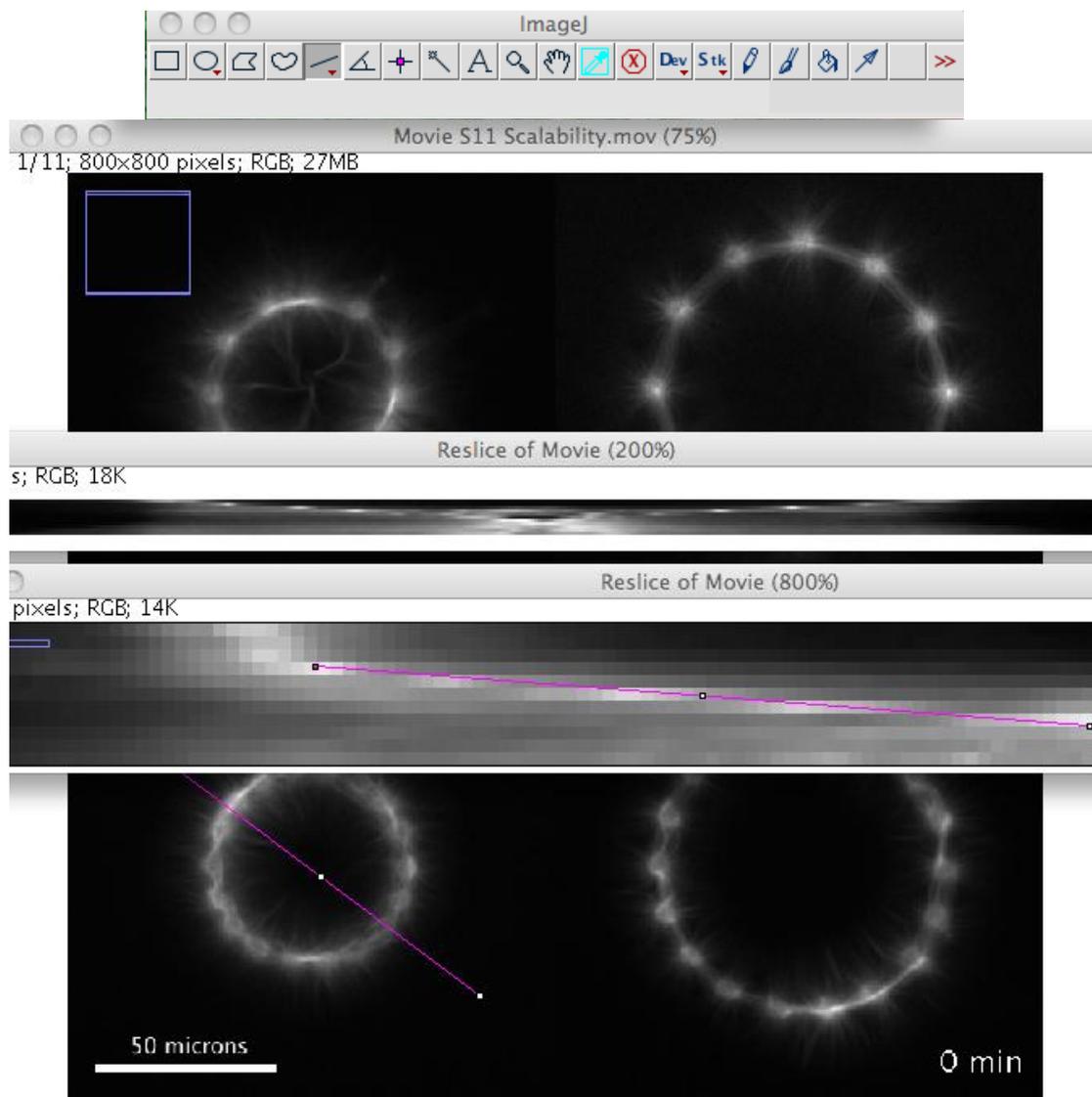
Part 2

Kymographs can also be used for more quantitative measurements, such as to calculate velocities. We will now use the time series corresponding to Figure 4 to compute the velocities of the contractile ring.

1. Open the Circle.tif file corresponding to the time series of the evolution of actin polymerization on circular dotted patterns in ImageJ.
2. Draw a line along the diameter of the circles (a straight line through the middle of the circle, extending slightly out of the edge of the circle).

3. Click on *Image > Stacks > Reslice* once more in order to generate the kymograph. (You may choose to do this four different times—once for each ring—if you would like to compare the different cases).
4. Next, you will draw a line along the border of the contractile actin network in order to extract its slope.
5. Click *Analyze > Set Measurement* and choose the Bounding Rectangle option only.
6. On the kymograph, draw a line on one of the sides of the border of the contractile pattern. You can zoom in using the magnifying glass before doing so. Once the line is drawn, click *Analyze > Measure*. You will then obtain the values of width, height or angle necessary for your quantification.

See the screen shot capture below for an example. The magenta-colored line represents the line drawn to measure the pattern.



7. Now, you know the scale of the image and the time interval between each frame, you should be able to estimate the velocities of constriction in nanometers per second (nm/s).
8. Compare your measurements with the values from Figure 4A. What is the drawback of using such a technique to obtain these velocities? Why do you think the authors chose to use another method as described in the article?

Part 3 – Hands-on Application Activity

1. Download and read the paper *Homeostatic Actin Cytoskeleton Networks Are Regulated by Assembly Factor Competition for Monomers*, and the summary below.
 - a. **Summary:** The main finding reported in this article is that the number of available competing actin monomers determines the length and formation of actin filaments. Authors show that disrupting patches of actin correlates with the growth of actin filaments in yeast cells *in vivo*. They demonstrate the importance of the Arp2/3 protein complex and a group of proteins called ‘formins’ in actin polymerization. When chemical agents that inhibit the Arp2/3 complex are introduced to the cells, the amount of actin assembled by formins increases. In the opposite case, when authors inhibit formins, the amount of Arp2/3-mediated actin increases, while the formin-mediated actin decreases. This demonstrates a “trade-off” between the actin assembled by the two different types of proteins. The paper also shows that the length of filamentous actin (F-actin) grows by incorporating free-actin monomers (G-actin) that are available after disassembly from their original location, within yeast cells. This is an *in vivo* application of the same finding discussed by authors in the *Science* article you have studied prior to this activity.
2. Read the paper and summary paragraph. What is the main finding of this paper? How is it similar to what was reported in the *Science* article with respect to actin filament growth and disassembly (Hint: It relates to the kymograph in Part 1 of this activity).
3. Next, you will demonstrate how actin monomers (G-actin) help form and define the length of actin filaments. Gather the beads and string listed in the materials above.
4. Arrange several “patches” of actin. These patches are made up of several beads sitting in small islands on a table. They also represent the “antiparallel branched meshworks” of actin from the original article.
5. Allow one of the patches to “disassemble.” This means the beads in one of the islands are no longer touching and have broken apart, separated randomly, and spread out on the table.
6. Now that the patches have disassembled, the individual beads that made up the patch are considered “monomers”. Use the recently-freed monomers to string onto the shortest string. Does this form a complete necklace?

***Note: A “complete necklace” is defined as a piece of string in which all beads are touching and there is no empty string space. Whether or not a necklace is formed will depend on the size and quantity of the beads used. This should be determined by an instructor ahead of time.*

7. Remove those beads from the shortest string and string those same monomers onto the medium, and longest size string. Does this form a complete necklace?
8. Remove the beads and disperse them around the table as free monomers once again.
9. Allow another actin patch to “disassemble.”
10. Use the newly disassembled beads (monomers) *in addition* to the dispersed monomers from the previously disassembled patch to string onto each string size. Does a complete necklace form on any of these strings? Which ones?
11. Remove the beads from the string and disperse them around the table as free monomers again.
12. Now disassemble ALL actin patches into free monomers.
13. Put beads onto each of the pieces of string. Does this form a complete necklace on any of the string sizes? Are there any beads left over? Compared to when you only disassembled one actin patch, now that there are more free monomers available, are you able to make more or fewer complete necklaces?
14. How does this exercise demonstrate the relationship between the disassembly of actin meshwork and the length of parallel actin bundles?