Utilizing Fiji and Image J for Aging

Theresa Redmond, VIMS Molluscan Ecology

Manual 2019-3, April 2019

Counting Annuli

1. Open "Fiji". Must be sure "Fiji" has the "Object J" plug-in installed. Fiji (which is Image J) - https://fiji.sc/
2. If prompted to update the software, then click "Apply Changes" and allow the updates to install. Once complete, follow prompts to close the software and relaunch the software. If not prompted to update the software, then continue to the next step.
3. Click "Plugins" in the menu bar, scroll down, and click on "Object J." This will launch the plugin that is used for marking growth lines. Object J plugin for Fiji - https://sils.fnwi.uva.nl/bcb/objectj/2-Tutorial.html   (see step 1)
4. Before beginning you must create an ".ojj" file. We use the "TreeRing.ojj" file because it is already set up for line counting. The file can be downloaded from the VIMS Molluscan Ecology Website.
    https://www.vims.edu/research/units/labgroups/molluscan_ecology/_docs/lab_manuals/treering.ojj
5. Download the ".ojj" file, name, and save it based on your project needs. Save this file in an appropriate location, such as, the location you keep your images to be aged. You will need to open the ".ojj" every time you want to age as all your images from the 'project' will be linked to it. Due to the linking, it is advised to create a new ".ojj" file for each new 'project.' For example, if you are aging ocean quahogs and surf clams, create two separate ".ojj" files - one for each species.
6. Click on "Object J" in the menu bar and select "Project," then select "Open Project."
7. When opening project, navigate to the file location of the appropriate ".ojj" file. Select the ".ojj" file and open it.
8. Click on the "Link" button, then select the .jpg image file of which you would like to determine the age.
9. In the Object J window, double-click on the linked image that you would like to age. This should open the image.
10. On the Object J tools window, click on the upper left hand corner tool (set marker tool). Now, click on the location of the origin of hinge plate growth, between the light and dark regions. This will begin your object for marking lines. This marker should be electric purple.
11. Now, your mouse pointer should become blue, and you can add "Ring" markers at every annual growth line. Place the marker along an imaginary line that bisects the center of the hinge plate, so that each marker is in the same relative location of the growth line for each year. Markers should be placed at the edge of the dark line as it transitions to the light colored growth increment. Mark all annual lines in order as skipping annual lines will create errors in age and increment analysis. Be sure to include a mark on the growing edge when completed.
12. If the lines are difficult to see, then try adjusting contrast. Click on "Image" in the menu bar, scroll down to "Adjust" and click on "Brightness/Contrast." This will open a small window with sliders to adjust the appearance of the image. Use the contrast slider to adjust the image until you can discern the annual growth lines.
13. When done marking annual signatures, click "Object J" in the menu bar, and click "Enter Year." The year entered should be the year that the organism died. When entering make sure there is a "-" sign in front of the year. For example, enter "-2017" for the year. Click OK.
14. Click on "Object J" in the menu bar, and click on "Save Project."

Helpful Key Commands (MAC)

1. To zoom in/out of the image, simply press the "+/-" keys on the keyboard.
2. To drag the image around in the window when it is open, hold the spacebar and, with the pointer hovering over the image, drag three fingers across a trackpad to move the image within the window.
3. If you need to delete a "ring" marker, then select the gun tool from the Object J tools window. Hold the option key – the gun should turn blue instead of black. Then click on the point you would like to delete while continuing

to hold the option key. This will delete a single point. If you would like to delete an entire object, then simply click it with the gun without holding the option key.

    a. Deleting a single point can also be done by clicking the "backspace/delete" button on the key board. This is much easier than using the gun tool from the Object J tools window.

<u>Measuring Increments</u>

1. Open "Fiji". Allow the updater to run, and restart "Fiji" if files need to be updated. (See Step 2 in the Counting Annuli Section of this manual).
2. Once "Fiji" is open, click on "Plugins" in the menu bar, scroll down to "Object J" and click it. "Object J" should now appear in the menu bar at the top of the display.
3. Click on "Object J" in the menu bar, scroll to "Project" and click on "Open Project" in the sub-menu that appears.
4. Navigate to the project file that you would like to open, then open the project (.ojj file) within which you would like to measure increments.
5. You should now see the Object J project window and tool window on your display. "Images" is selected by default in the project window. Click on "Objects" to the right of "Images" in the project window. You should see three entries: "Begin," "Ring," and "Link."
6. Click on "Columns" to the right of "Objects" in the project window. Here you can define outputs from your markings within your Object J project. For example, you can output measurements between ring markers to a spreadsheet.
7. To define a new output, click the "new" button near the bottom left of the Object J project window. You can rename this column by double-clicking on the default name; in this case "Column1," and editing the name.
8. Select the output column title you just edited so that it is highlighted in blue, and then click on the menu for "Operations" under "Operands" in the right portion of the project window. In this case, click on "Distance" in the drop-down menu.
9. Under "1st Operand," leave the item type as "Begin," with clone# and point# as the default values of 1.
10. Under "2nd Operand," click on the drop-down menu and select "Ring," then leave Clone# and Point# as 1.
11. Click on the "Object J Results" button at the top of the project window. Your column should appear at the far right of the pop-up window. In this case, it is the value of the number of pixels between the "Begin" marker and the first "Ring" marker.
12. You can make another column that produces an output of the distance between the first ring marker and the second ring marker by defining the distance between "Ring" clone# 1 and "Ring" clone# 2. This can be done for as many increment widths as are necessary for your study.
13. Finally, within the Object J results window, you can click the button labeled "Copy/Export." A pop-up window titled "Export Object J Results" appears. Click "Export," and save the file to your project folder. This file can be opened, edited, and analyzed with Microsoft Excel as needed.