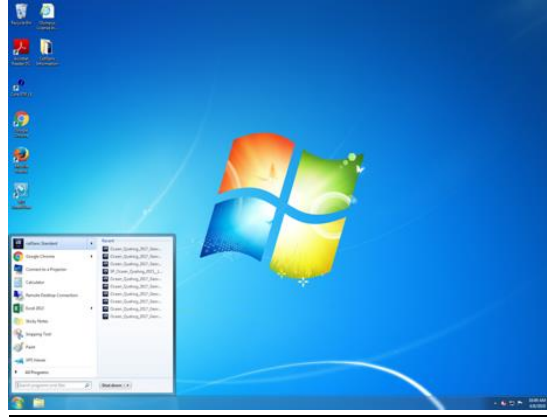


## Utilizing Olympus Microscope and Olympus CellSens for Aging

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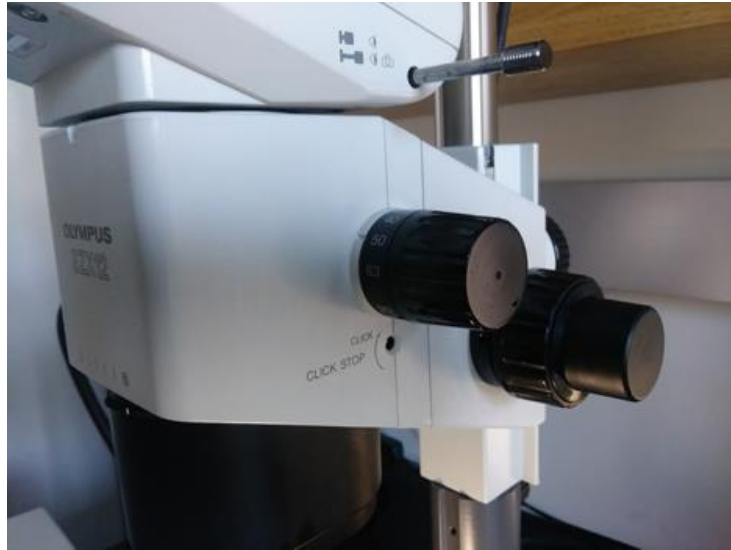
1. Open CellSens software.



2. Once the software is open, ensure that the microscope is ready for image capture:



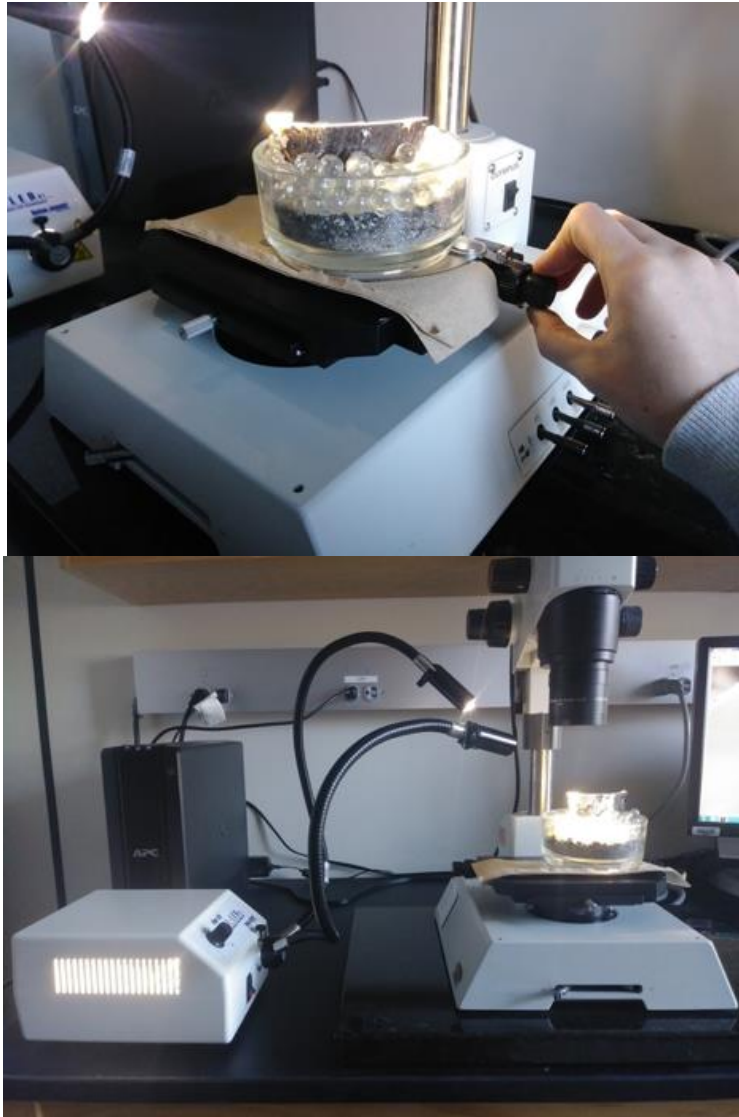
- a. Turn on the fiber optic light source, preferably to the “high” position.
- b. Ensure the microscope is allowing light to travel to the binoculars and to the camera mount.
- c. Set the magnification knob to the lowest setting.



3. Select a specimen and, using the finger bowl filled with glass beads, orient the specimen with the curved edge of the valve pointing down so that it is secured by the beads. Orient the polished surface of the valve so that it is as perpendicular to the microscope lens as possible, with the hinge region located as close to the center of the finger bowl as possible to provide for the most range of movement on the stage.

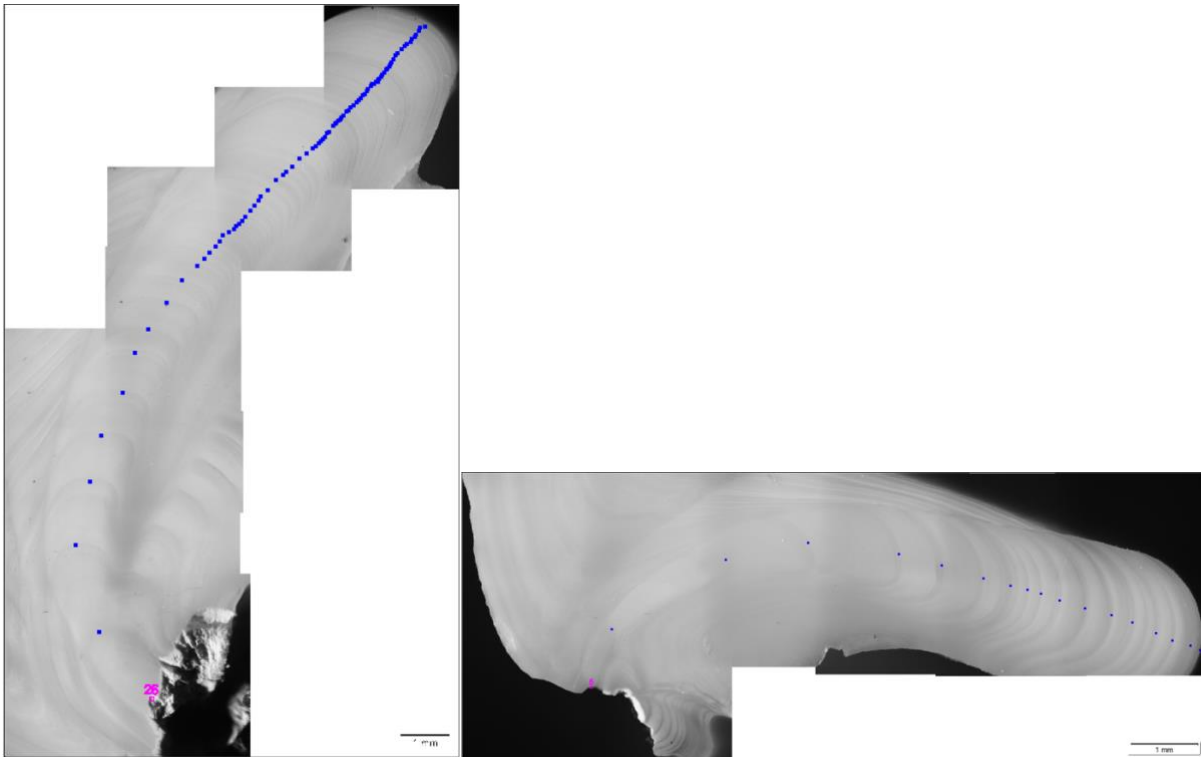


4. Once the specimen is aligned, use the fiber optic light source to illuminate the hinge region as evenly and brightly as possible from a steep angle so that reflections and glare are minimized.



5. Back in the program, click on the leftmost tab (labeled "Camera Control") at the bottom of the column on the right side of the screen.
6. At the top of the column "Camera Control," click the button with a picture of a hand atop a video camera. If you hover the pointer over this button, it will display "Acquisition Settings."
7. In the left column of the dialog box that appears is a setting tree labeled "Document Name." Click here, then click on "Snapshot" or "Process Manager" to access settings to customize the automatic naming format for files. This is where you can set automatic naming for still images and for "Manual Image Alignment" processes. Modify as necessary for the samples being processed.
8. Close the "Acquisition Settings" dialog box once appropriate settings have been made.
9. Click the "Live" button at the top of the column on the right side of the screen called "Camera Control."
10. A live image of the field of view of the microscope should appear on screen. Adjust the stage and the specimen until the polished section of the hinge appears within the field of view.
11. Adjust the lighting as necessary to ensure even illumination of the specimen.
12. Zoom in on the hinge and take images as necessary using the "Snapshot" icon. If multiple images are needed to be stitched together, see a (below). If only one image is necessary, proceed to step 13. It is important to remember to always update the magnification on computer when magnification on scope changes.
  - a. Zoom in on the growing edge of the hinge region so that the most recent growth lines are visible. Ensure that the magnification menu at the top of the screen is set to the magnification of the microscope being used to capture images (0.7x = 7, 1x = 1, etc.)

- b. Click on the tab at the bottom of the column on the right side of the screen labeled “Process Manager.”
- c. Ensure that the “Manual Processes” radio button is selected and that the button for “Manual MIA” is selected.
- d. Click the “Start” button at the top of the column to begin capturing images for a series of stitched images.
- e. Click the arrow buttons in the direction that you wish to capture and stitch additional images. You will need to move the stage so that the image on the screen moves in only the direction you want to take the next image. Try to move the image just past the position of best alignment so that the software can detect alignment. If the image is not moved far enough, or if the alignment is too perfect, then stitching will perform poorly. When ready to capture and stitch the image, click the arrow for the next image. Then review the quality of the previous stitch, and if it is not good then click the return arrow in the middle of the directional arrows to go back.
- f. Repeat step e until the entire hinge section from growing edge to origin is captured. A helpful trick to avoid a poor stitch of the final image is to click on a directional arrow after the last image of the origin so that the stitch can be examined before completion.



- g. Click the stop button to finish the process and finalize the image.
13. Select the image tab of the image you want to save near the top of the screen to the right of the “Start Page” tab.
  14. Click “File,” “Save as,” and then select the appropriate location for the file to be saved.
  15. When naming files, it is helpful to append the file name with the sample ID of the specimen, and include an “L” or “R” depending on if it is the left or right hand valve. Be sure to include any other pertinent information about the shell being imaged.
  16. Save a version of the file as a .VSI, the proprietary format of the Olympus software, first (if stitching images). Then save a version with the same name as a .JPG for ease of post-processing.
  17. When finished, ensure all images generated are saved and shut down the computer. Turn off the fiber optic light source and cover the microscope.