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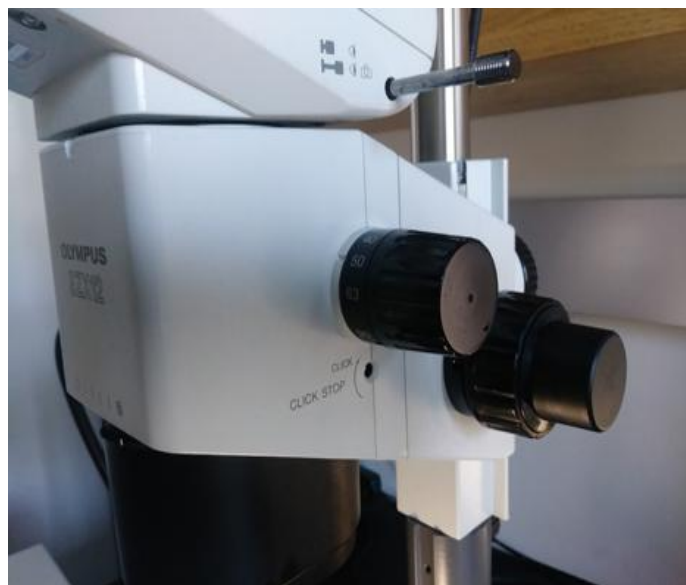
3. Select a specimen and, using the finger bowl filled with glass beads, orient the specimen with the curved edge of the valve pointing down so that it is secured by the beads. Orient the polished surface of the valve so that it is as perpendicular to the microscope lens as possible, with the hinge region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


4. Ensure that each hinge is clean, free of debris, and finger smudges.
5. Each hinge must be leveled, back to front and side to side, for the entire plane of the hinge. This ensures the hinge will be properly in focus. There is a level located next to the microscope for completion of the step.

6. 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.
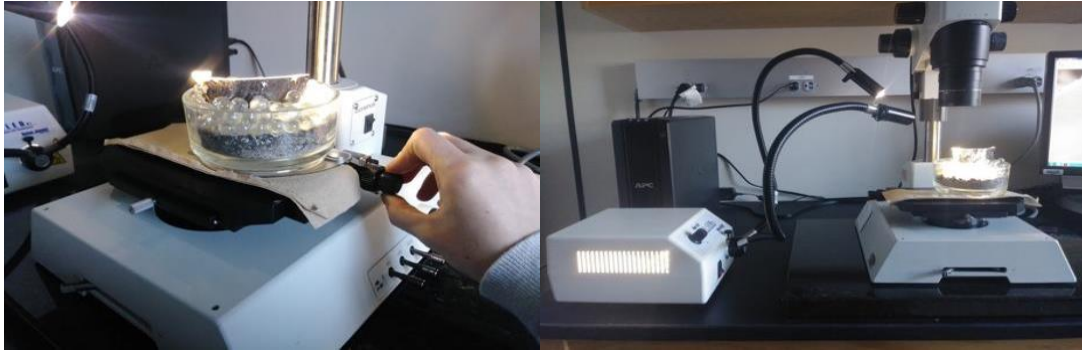
7. Back in CellSens, click on the leftmost tab (labeled "Camera Control") at the bottom of the column on the right side of the screen.
8. Toggle "RGB/Grayscale Mode" to "Grayscale" (if not already set).
   a. This setting appears as a small button below "Live" and looks like a few small blocks of grey to white rectangles when set to greyscale. It will appear as red, green, and blue rectangles with grey if RGB is enabled.
9. Under the "DP73: Custom Grey Scale" there are three buttons for contrast options; "low", "medium", and "high.'" Select "high."
10. 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."
11. 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.
12. Close the "Acquisition Settings" dialog box once appropriate settings have been made.
13. Click the "Live" button at the top of the column on the right side of the screen called "Camera Control."
14. 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.
15. Adjust the lighting as necessary to ensure even illumination of the specimen.
   a. A method that seems to prove effective is to set up the lighting such that the growth lines cup the incoming light direction. The lights are approximately in a 45 degree downward angle about 2-3 inches from the hinge.
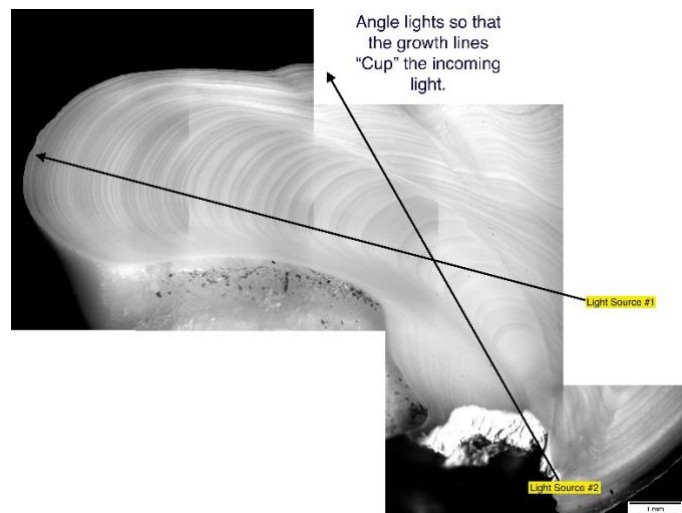
16. Adjust "Exposure" under the "Camera Control" tab. The exposure appears as a square dotted line box on the near the live image. This box can be moved around to sample lighting for auto-exposure. The exposure box size can be adjusted but is typically set at "spot 1%." Exposure can also be locked and adjusted incrementally by using the manual setting.

17. Once zoomed in and image focus has been optimized 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 18. It is important to remember to always update the magnification on the computer when magnification on scope changes. A fully stitched hinge photo is typically taken at minimum of 32 power. A single non stitched photo of the growth edge must also be included at a minimum of 90 power.

    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. 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 "undo last frame" button to retry.

        ● *Sometimes Helpful Tip* 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.

    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. Position the scale bar as close to the origin/growth edge as possible without covering the essential growth lines. To move the scale bar, click on the "Tools" menu, then "Options (Shift +F8)". Select "Scale Bar", "Display", "Select Position". There are four corner options to position the scale bar.

        i. If the scale bar is greyed out and not seen, move the "Info Stamp" and/or "Color Bar" options to free the corner spot. Even though the "info stamp" or "color bar" are not in use, their selected placement options will still block selecting a scale bar position.

    h. Click the stop button to finish the process and finalize the stitching of the image.

18. After the image is complete, you can adjust the image balance (if needed). Not all images need this however most do improve with it. Use the below options sparingly and consider levelness of the hinge, focus, light intensity, angle of light first before using this as a solution.
    a. Select "Adjust Display" (next to the Process Manager tab) and use "Display Enhancement" sliders; "Brightness", "Contrast", and "Gamma."
    b. Work in small increments to get the proper balance. Start with a small increase in contrast then lower the gamma. The brightness setting is adjustable but typically only when the contrast and gamma do not help. Look for the best settings that help define all the important lines throughout the growth series. Be aware that these image adjustments also exaggerate over and under exposed areas of the image that can hide important growth lines.
19. 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.
20. Click "File," "Save as," and then select the appropriate location for the file to be saved.
21. 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.
22. Save a version of the file as a .VSI, the proprietary format of the Olympus software, first (if stitching images only). Then save a version with the same name as a .JPG for ease of post-processing.
23. When finished, ensure all images generated are saved and shut down the computer. Turn off the fiber optic light source and cover the microscope.