

Cilia Analysis with ProAnalyst®

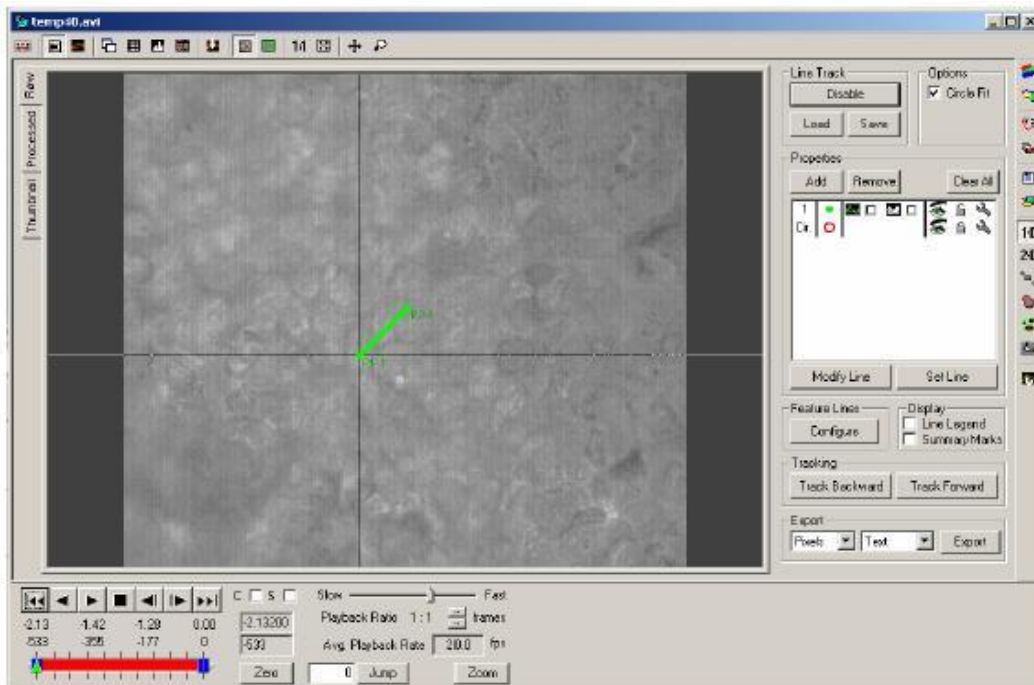
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Abstract

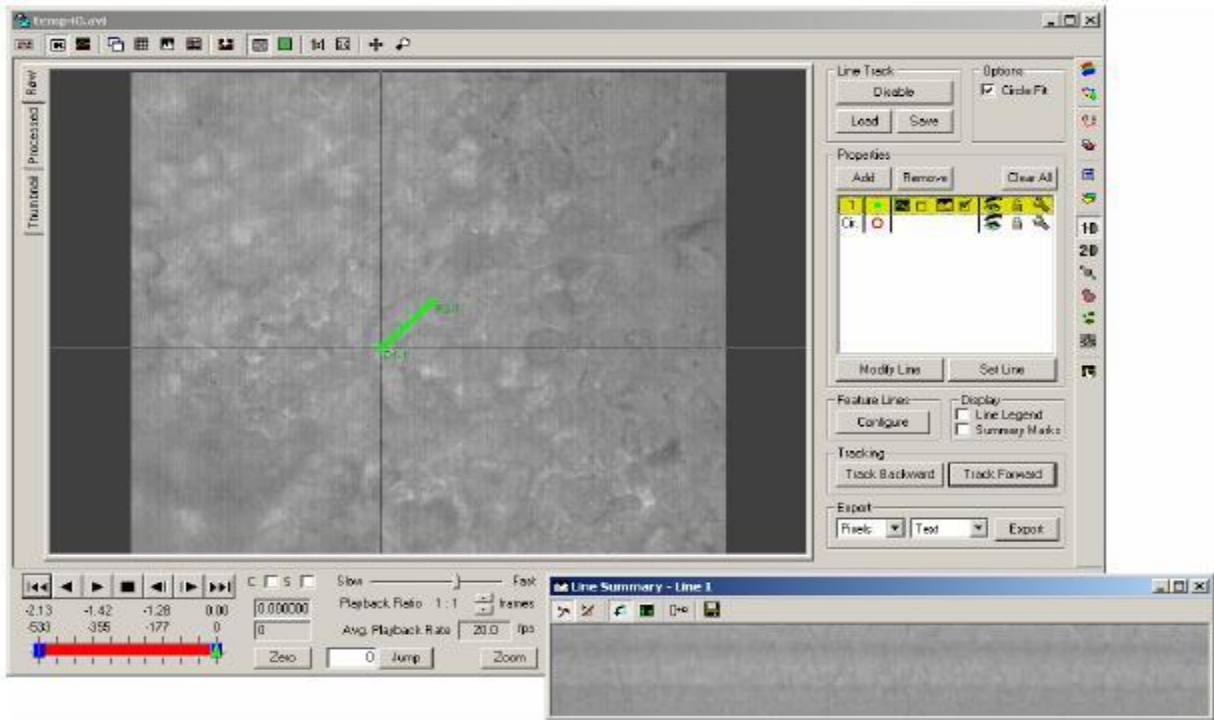
The analysis of motile cilia (small, numerous flagellae) is one example of how ProAnalyst can be adapted to applications in biological and medical science. This tutorial demonstrates how to use the 1-D Line Tracking and Line Summary features to track, record, view and analyze the motion of cilia. Although cilia analysis is a unique case, the principles illustrated in this tutorial can be applied to many other situations.

Instructions

1. Open the video file to be analyzed. Click the **1-D** button on the right side of the measurement window to open the Line Tracking control panel and click **Enable**. Place a line through the area of interest by using the **Modify Line** and **Set Line** buttons.

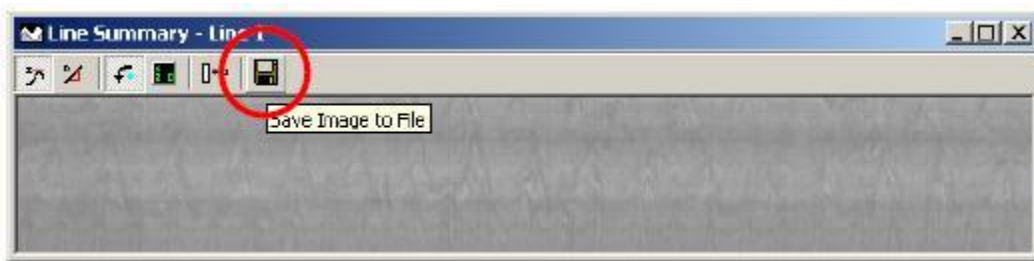


- Click the checkbox to the right of the Line Summary icon  in the Properties listing. The Line Summary window appears. Click **Track Forward**  to begin line tracking. You will see the Line Summary window update in real time.

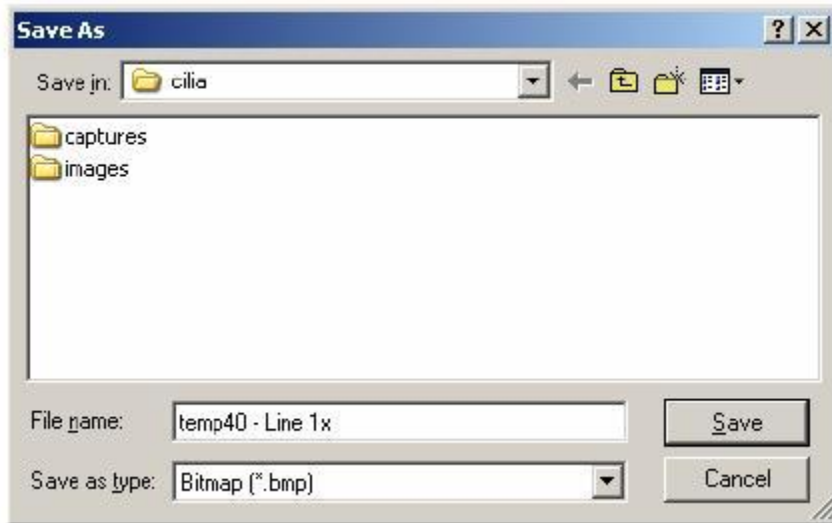


Note: The Line Summary window appears below the main window. The Line Summary window may be dynamically re-sized.

- Save the Line Summary image to file by using the **Save** button on the toolbar.

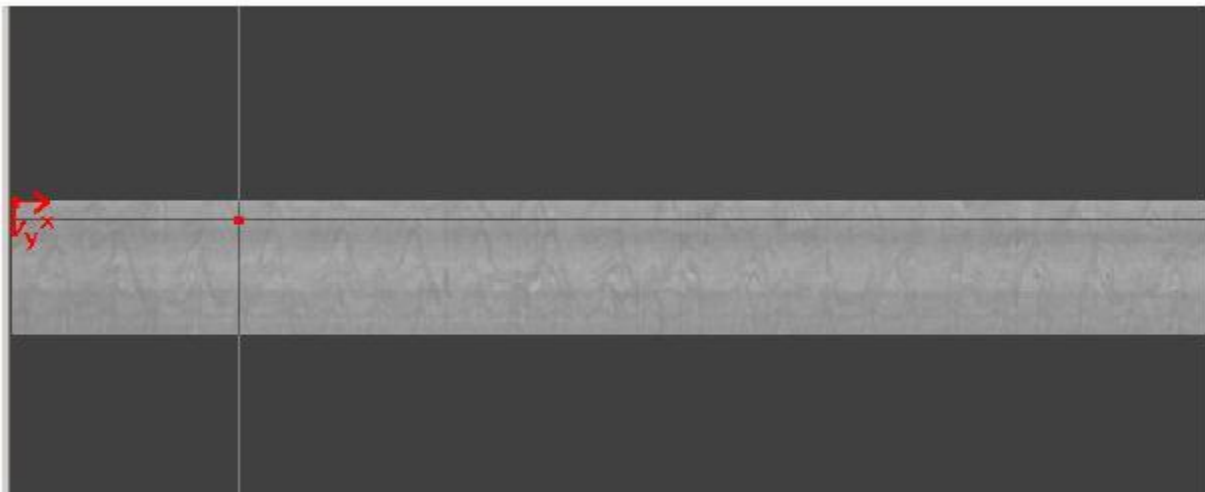


- Be sure to name the file so that the last character of the filename is not a number. In the following image, the default file name "Intensity" has been changed to "temp40" and an x has been added to the end of the file name. Appending the x or some other letter at the end of the filename is critical. This is because ProAnalyst interprets an image file that ends in a digit as one frame in a series of frames. This could cause confusion in the default loading of associated files. To avoid this problem, be sure to end the filename with a letter, not a number.

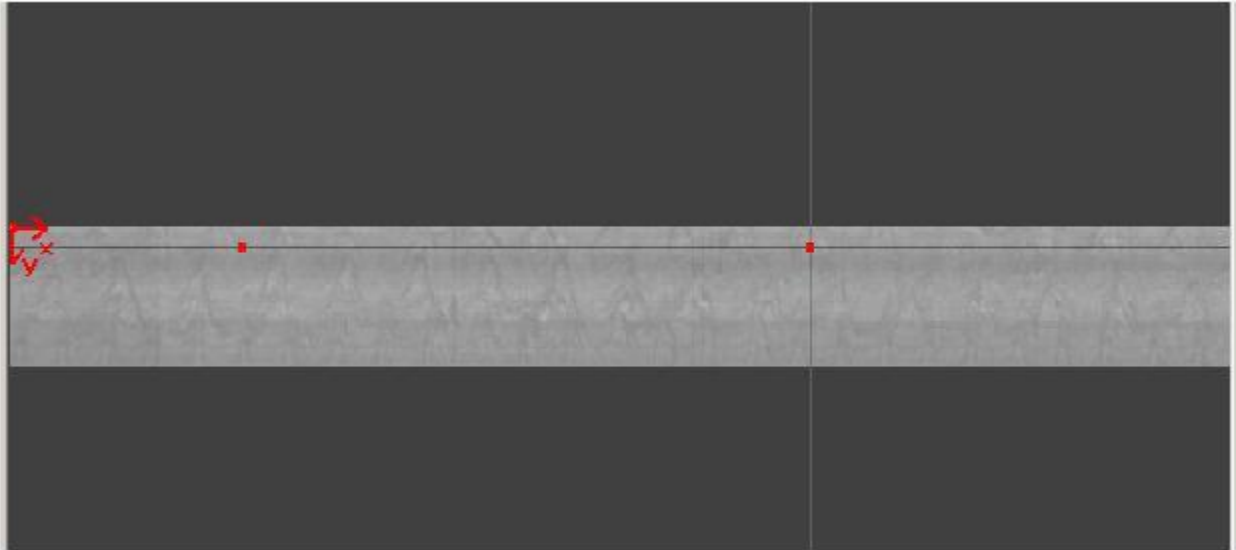


Note: Save your Line Summary image as a filename ending with a letter.

5. The Line Summary image that you have just saved will open automatically in a new window. Essentially, the Line Summary feature tracks the values of the pixels in a given line and “stacks” them in a horizontal sequence. The points along the vertical (y) axis are the pixels along the line that you drew in Step 1. As it stands, the points along the horizontal (x) axis are the individual frames of the video. Prior to analysis, we must calibrate the horizontal axis so that it represents time instead of frames. Place the first calibration point at any in the image and remember the coordinates. In the following image the reticle has been placed at coordinates (100, 8). The coordinates are displayed in the bottom status bar of the ProAnalyst window.

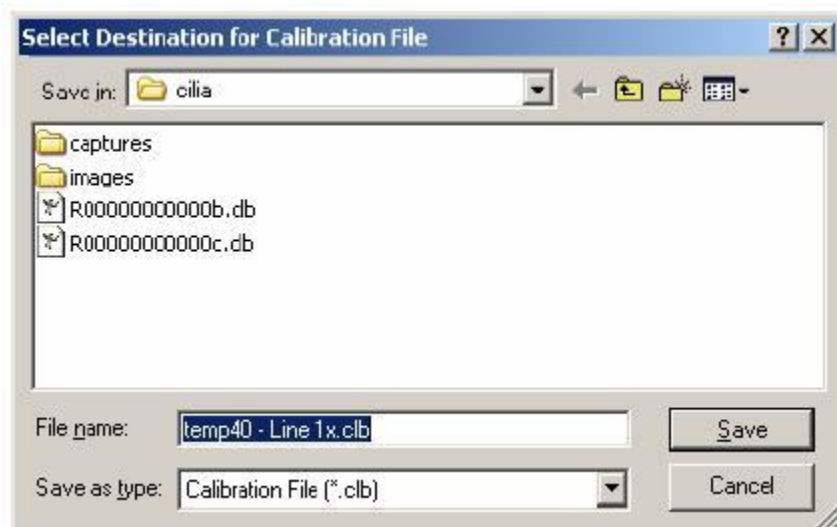


6. Add the frame rate to the x coordinate of your first point and place the second point at the new x coordinate and the same y coordinate. For instance, in this video, the frame rate was 250 frames per second. The first point was placed at (100, 8), so the second point should be placed at (350, 8). After both points have been placed, specify the scales as 1 of any calibration unit. When you click **Apply Scale** the result should show 250 pixel/unit.

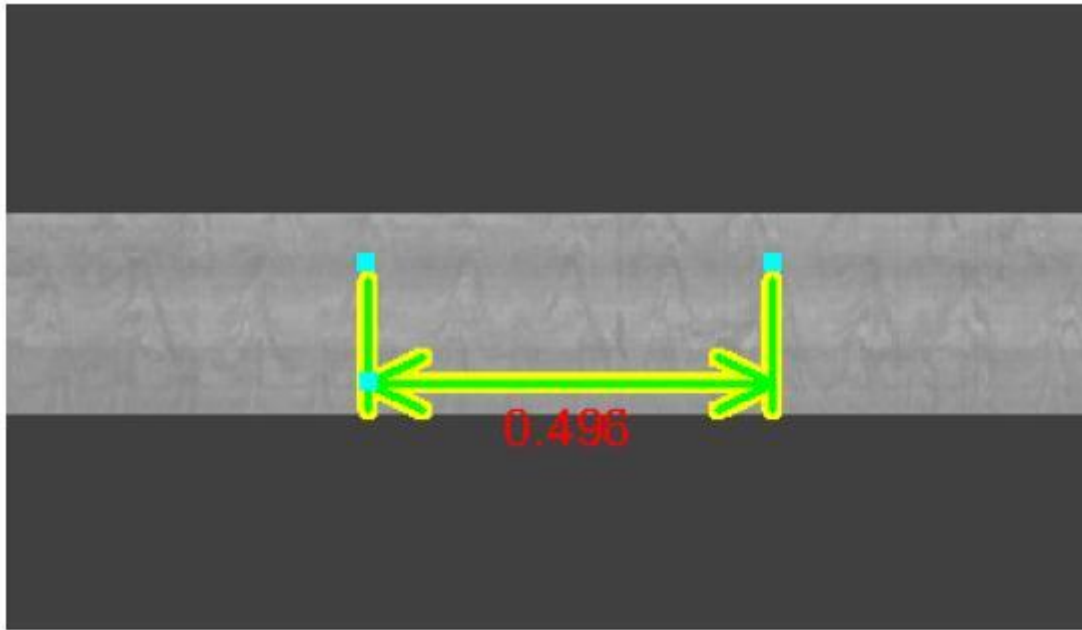


Note: The second calibration point should be placed at the original y coordinate and the original x coordinate plus the frame rate of the video. Apply Scale (in the upper right hand corner of the screen) displays 250 pixels/ut.

7. Save the calibration file using the **Save File** button.

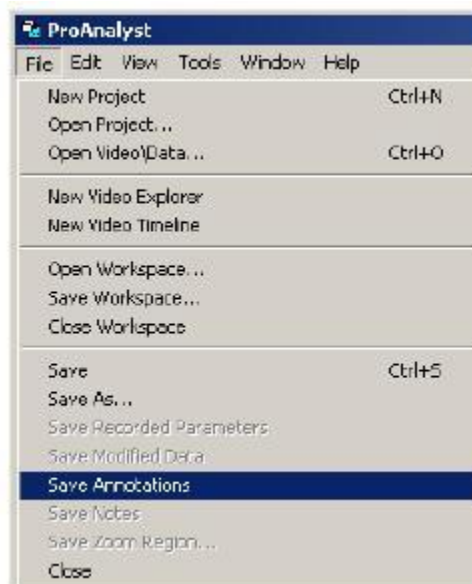


- Now that the image has been calibrated, you can use the Length Dimension annotation to easily measure the time between any two horizontal points. In the following image, the duration for four cycles of oscillation is shown to be 0.496 seconds.



Note: The arrows and lines indicate the duration of one oscillation.

- You can save the dimensions annotation you have just drawn by selecting **File ► Save Annotations**.



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