Instructions for PointsFromKymograph ImageJ Plugin:

1. Open a kymograph image in ImageJ. The time axis should be vertical. If it is not, rotate the image. The plugin is designed for 32-bit images. If your kymograph is not a 32-bit image, convert it (in the menu, select Image -> Type -> 32-bit). Here is a sample kymograph:

2. Run the plugin (in the menu, select Plugins -> Compile and Run…, and then locate the PointsFromKymograph.class or PointsFromKymograph.java file). Two windows should appear. One is a duplicate of the image file with the heading "select your point's region," and the other is an empty table titled "Results."

3. Select the points that define your search type and region. The plugin will trace a maxima, minima, or point of maximum slope through time from the center of the two points you choose.

The search begins at the midpoint of the line defined by the two points, and the horizontal distance between the two points determines how far the plugin will search in a given step. If this distance is too small, the plugin may lose a feature that moves too far in one time step. If this distance is too large, the plugin may skip to a nearby feature.

If the first point you choose is vertically lower than the second, the plugin sea rches for a maximum or minimum. Whether it searches for a maximum or minimum is determined by the initial region: If the value most different from the average of the endpoints is bigger than that average, it follows a maximum. Otherwise it looks for a minimum. A red line traces the feature, allowing for visual confirmation.
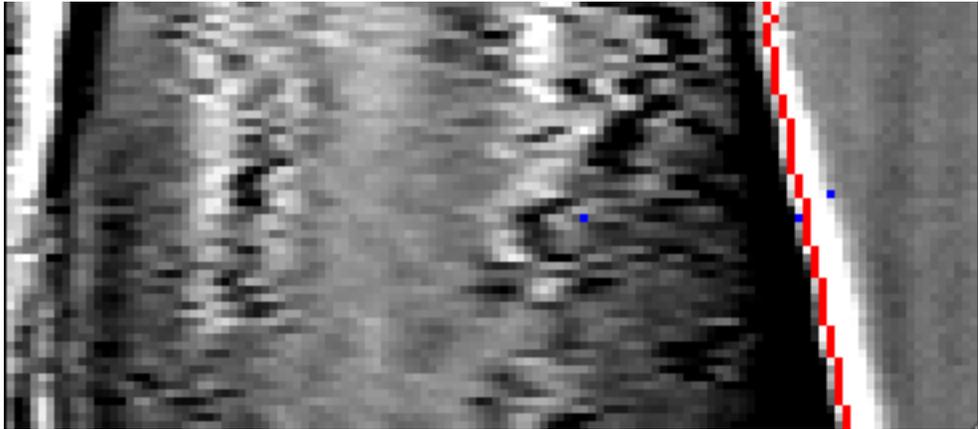
If the first point you choose is higher than the second, the plugin instead follows the highest-magnitude derivative. A green line traces this feature.
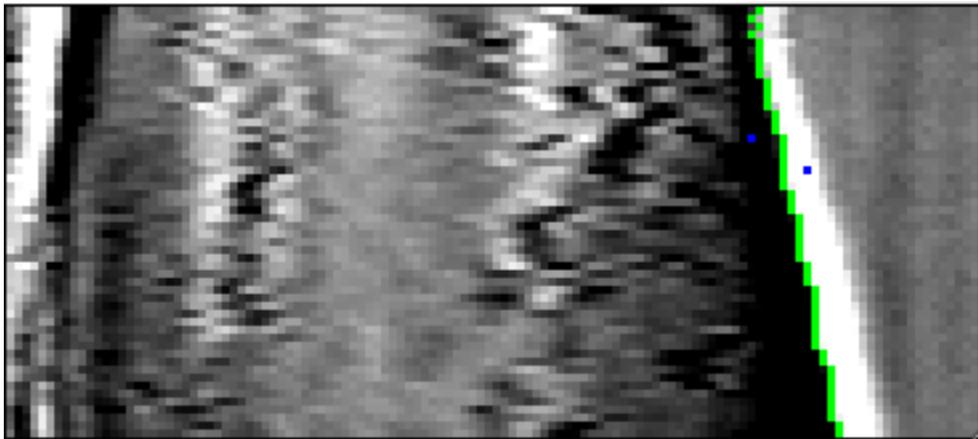
Multiple features can be traced on the same image consecutively:
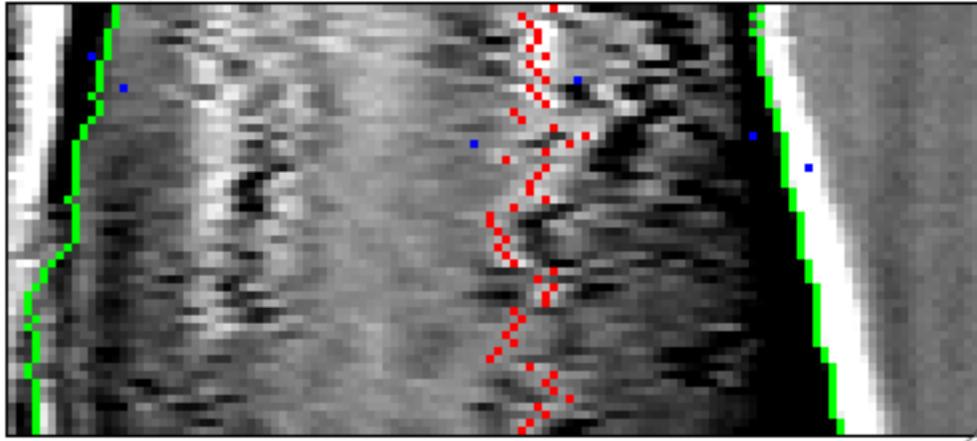
4. Get the data. A successful trace will populate the results table. This can be copied into a spreadsheet program like Excel or saved directly from the menu (File -> Save As…). The accuracy of the plugin in following the desired feature should always be confirmed visually.

Results

time	point
0	94
1	93
2	94
3	93
4	94
5	94
6	94
7	94