

May 14 2015 Changes to distance transform.

1. It cannot segment objects of different sizes.
2. Objects can grow across boundaries causing poor segmentation.

After the distance seeds are created, the software now temporarily removes the pixels more than 50 gray levels below the background. The seeds then expand into this new mask. If there is a break and only one seed, this will leave an unprocessed area. The software will then check for any separated areas and add these to the object list. The removed pixels are restored and the list of objects are then expanded into the available pixels. This significantly improves the boundaries of newly segmented objects and also splits more nuclei. (See examples) without dropping areas.

Improvements to detecting inflections still include minor points. Routine will now find the 2 nearest points at least 1/3 of the chain apart with a separation of at most 50 pixels before segmenting. After segmenting, each object will have the separating line added to it boundary.

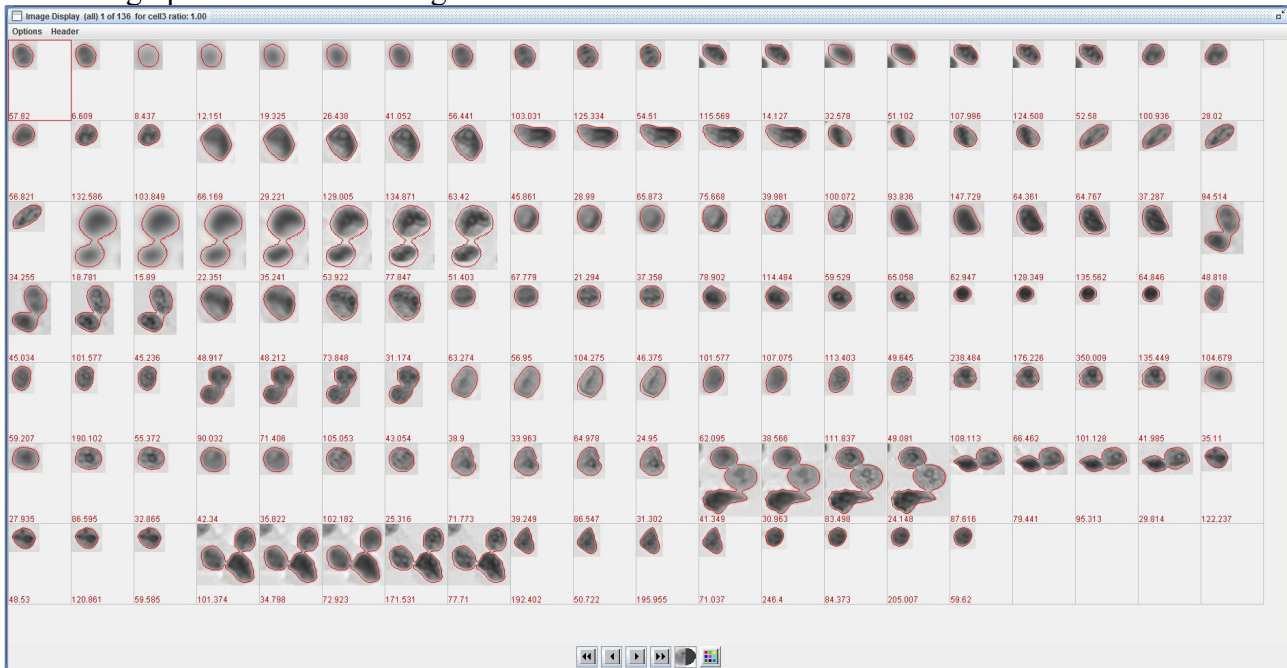
To remove excess white space around the nuclei, the routine will erode a given fraction of the lowest sobel values as a preprocess. Without this, the layers added to the boundary may not reach the dark edges of the nucleus.

Program was using the x,y centroids to track the shifting of nuclei during focus. This worked fine for single nuclei but the centroids drifted with multiple nuclei. Program now uses the centroids of the pixels under the chain code. The magnitudes of the shifts are divided by 2. Here is a graphic of nuclei without tracking.



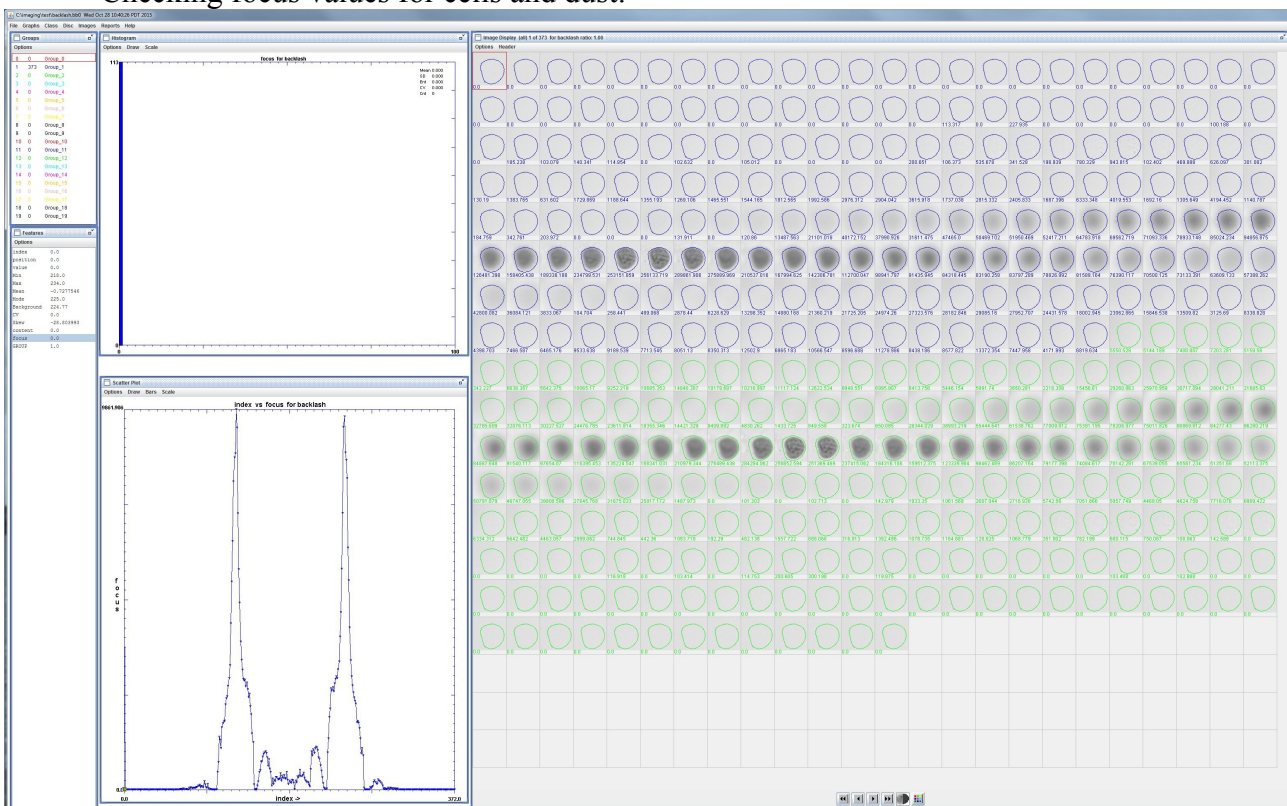
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Here is a graphic with the tracking enabled:



October 28

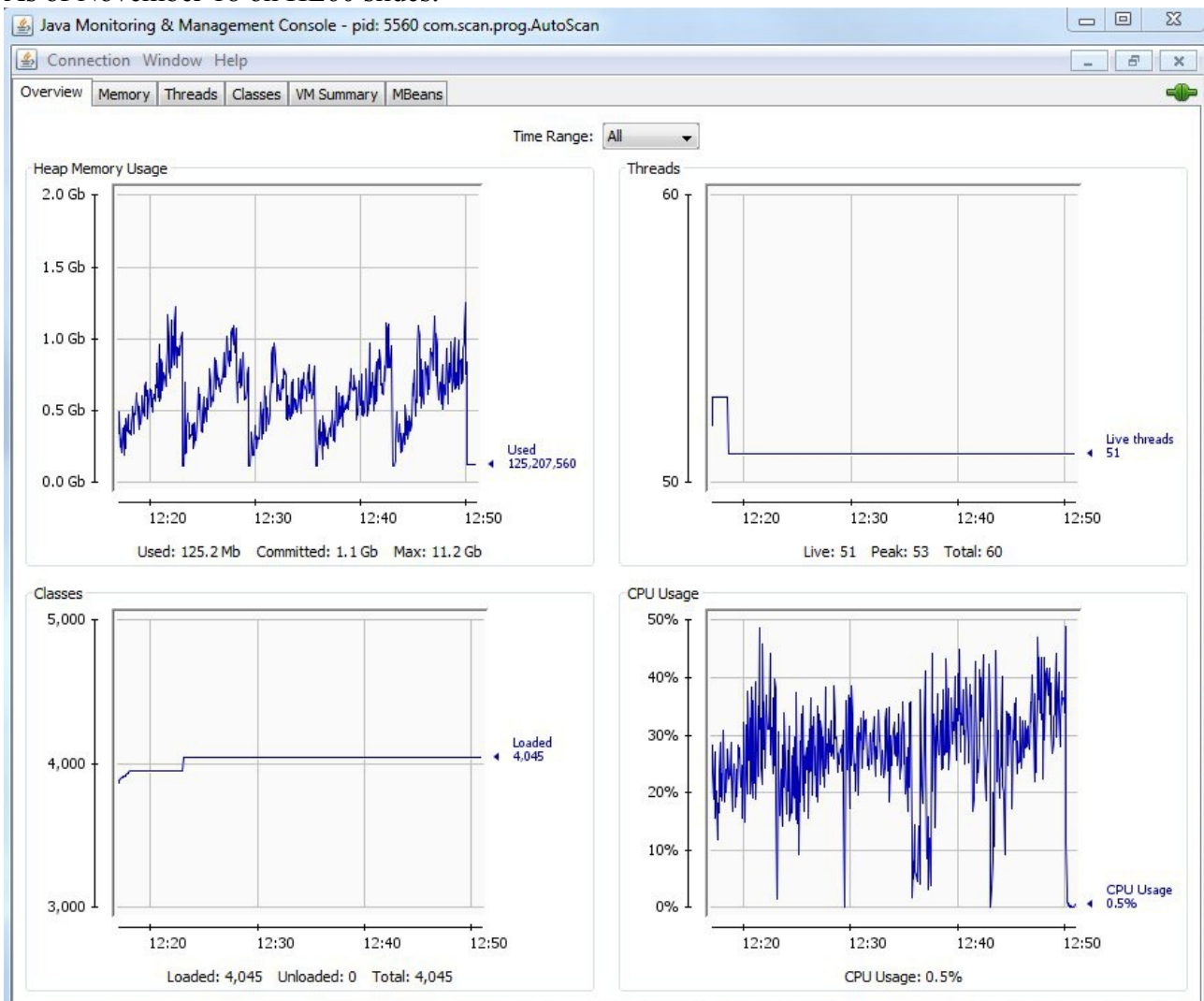
– Checking focus values for cells and dust:



Small peaks in the middle represent dust. Focus algorithms only include data for pixels a set distance below the background level. If the distance is less than 25, false peaks appear on both sides of the distributions. Scan program will now force a refocus if the mean area of the collected objects is less than 300 or if all objects are rejected as too small.

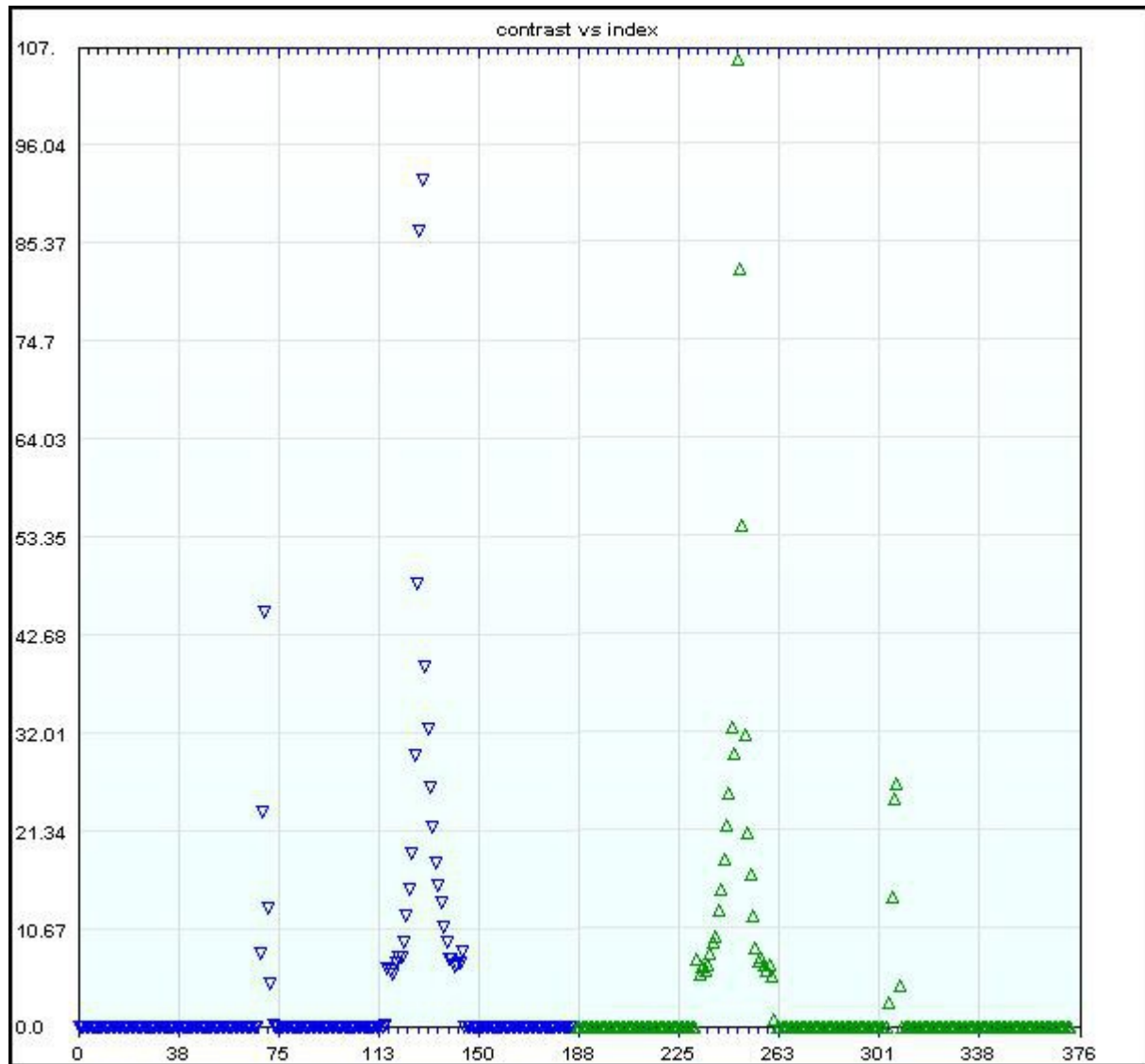
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As of November 18 on HL60 slides:



Cluster threads no longer being re-created. Program now waits long enough for threads to complete.

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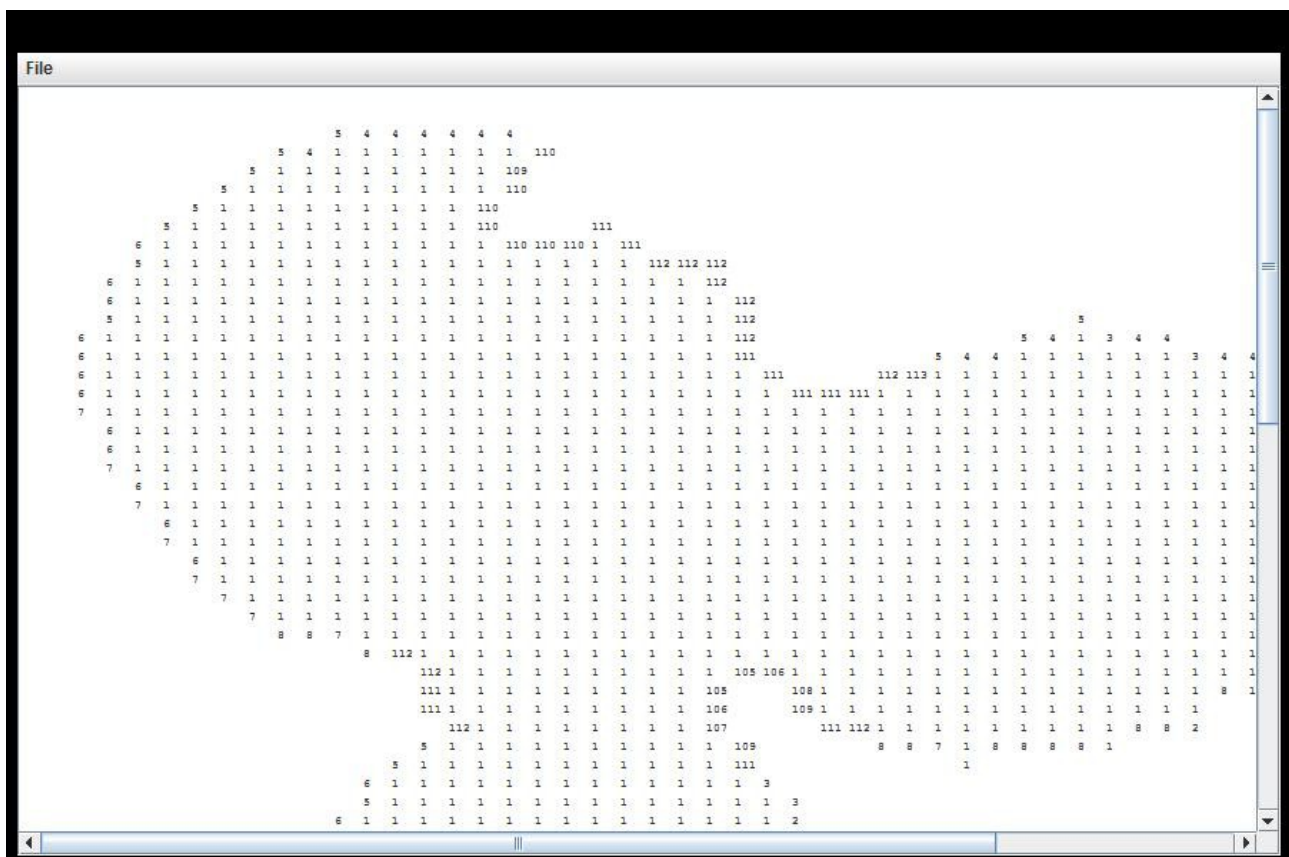
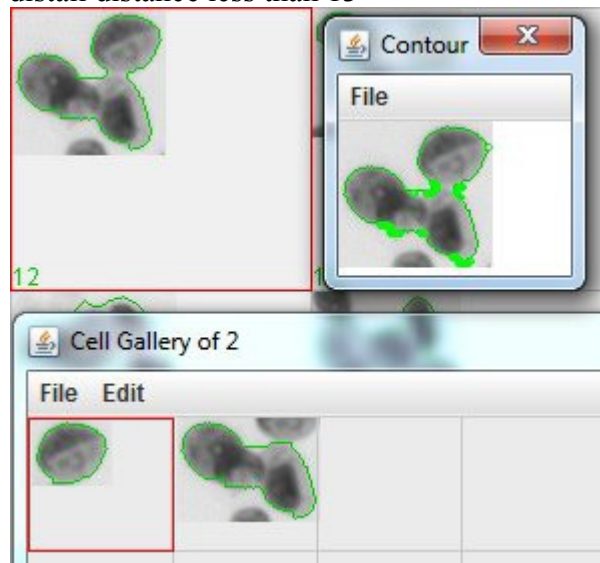


Cell focus using contrast (pixel to pixel variations) showing the effects of dust under the coverslip. This uses an offset below background to limit pixels used. This is with an offset of 5. With larger offsets > 20 , the dust peaks are reduced.

Background calculation appear unstable during focus so the program now uses variation of intensity. Cells with low values are too noisy so they will have to use the contrast function.

Aligning the light tube is quite difficult. The Backlash program now has a thread that moves the focus drive up, down and back continuously to facilitate the adjustment.

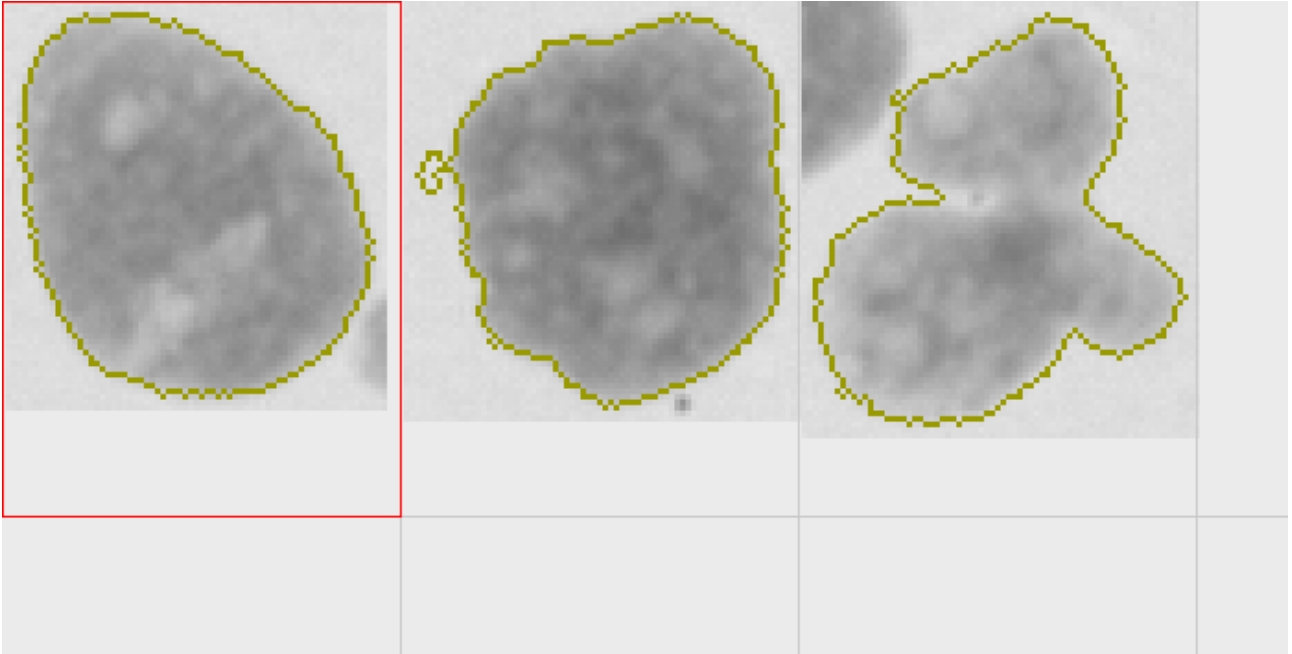
Inflection modified to use distall distance less than 13



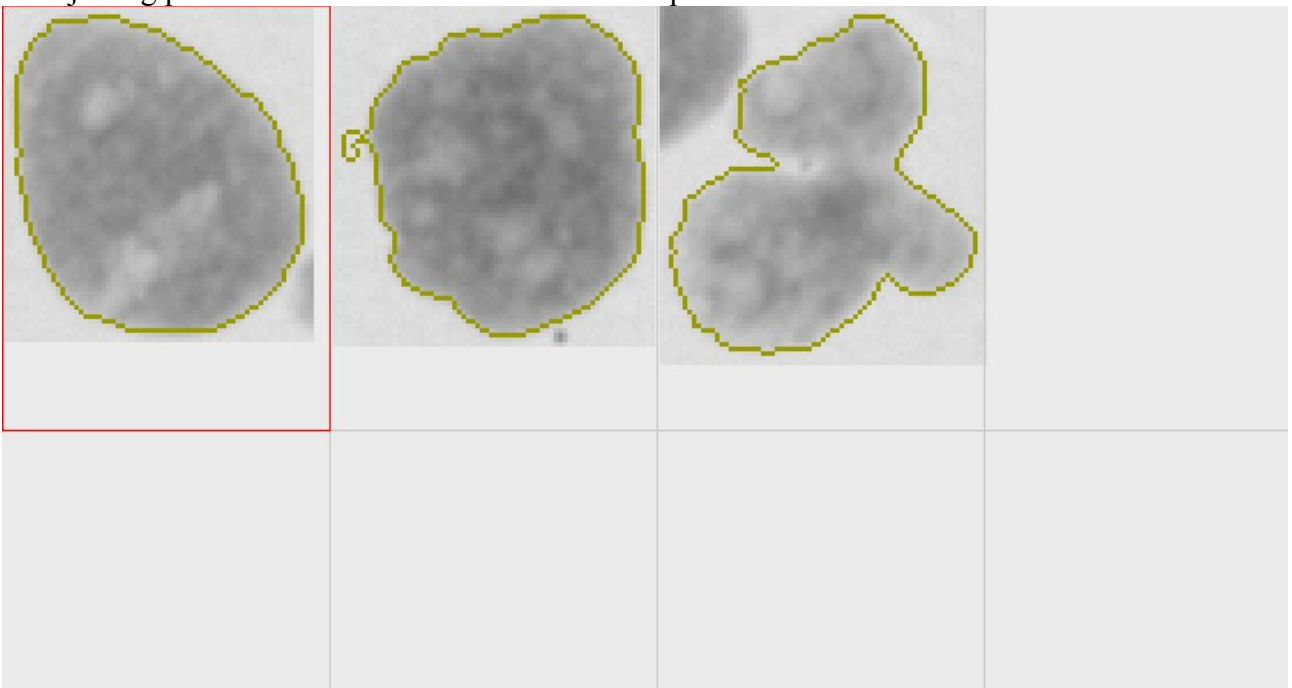
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Edge smoothing.

1. Rejecting pixels with no attachment to inside pixels

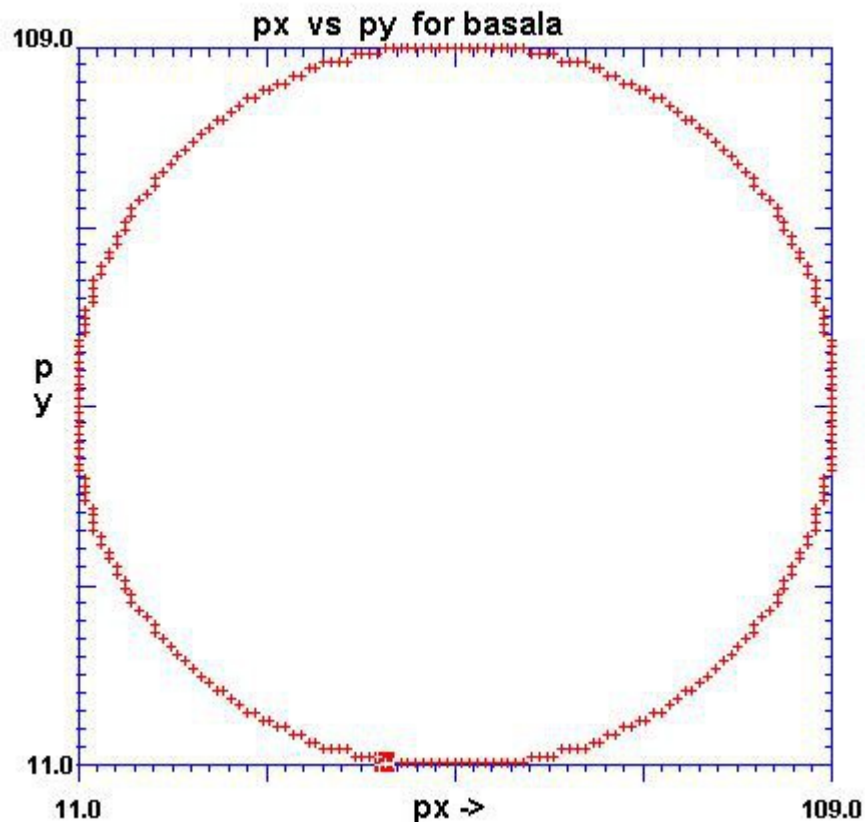


1. Rejecting pixels with no attachment to < 2 inside pixels



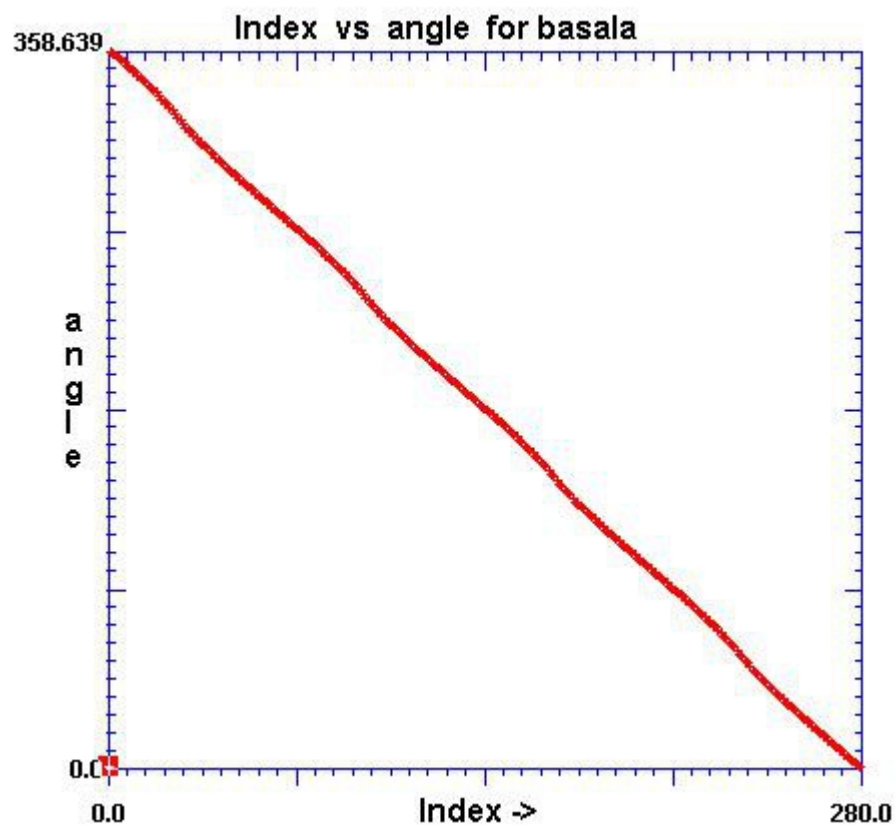
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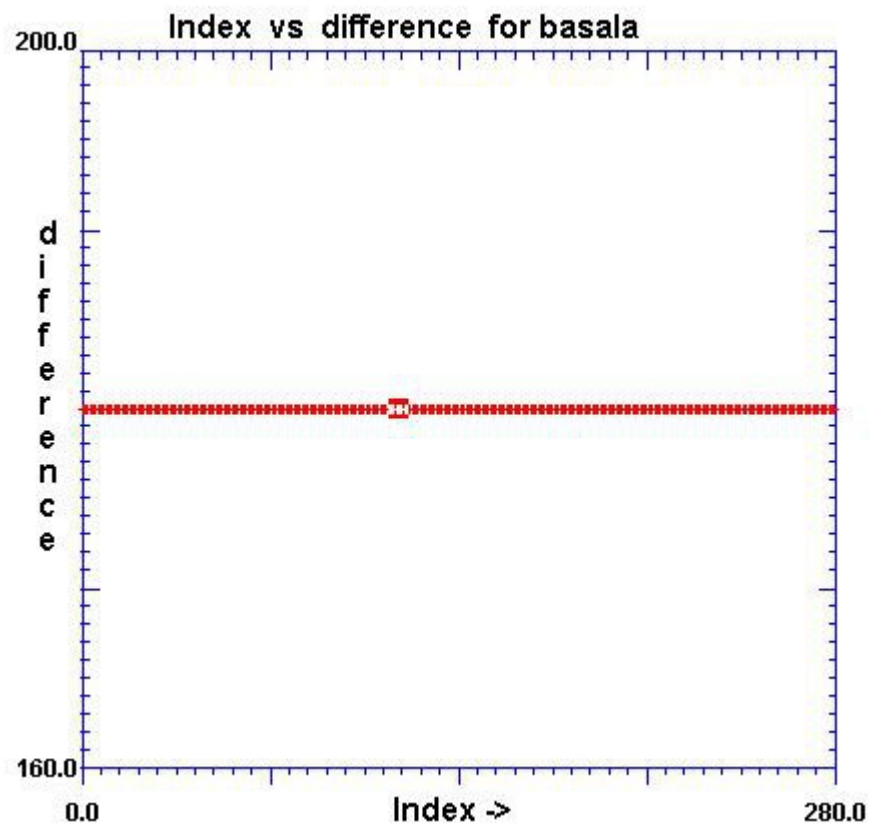
Basal angle testing.



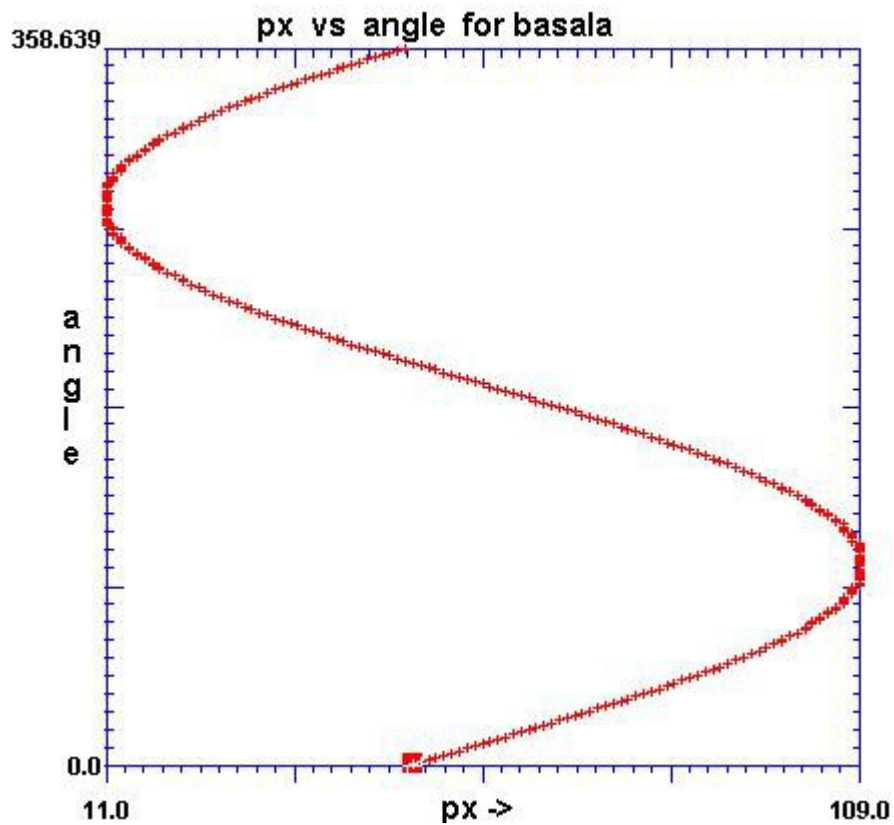
This displays the chain coordinates used for the testing.. The test uses a point at the centre, the start and consecutive points along the chain.

The following is the angle of the consecutive points relative to the centre.





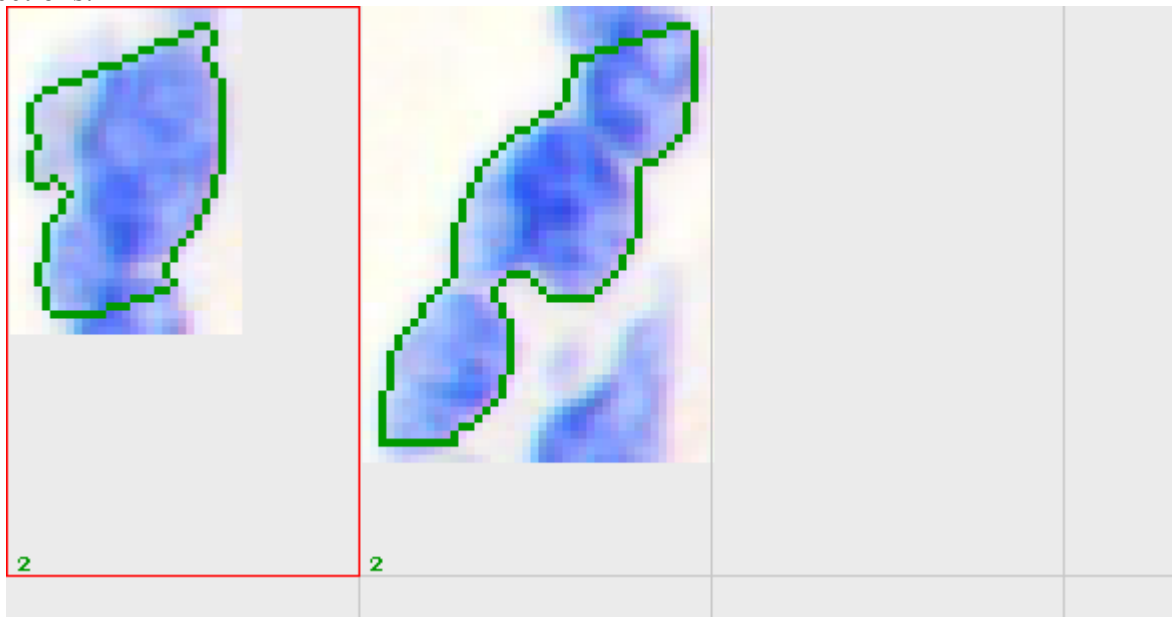
This figure represents the difference between the same points in both directions. As expected the values are 180 for the opposite directions.



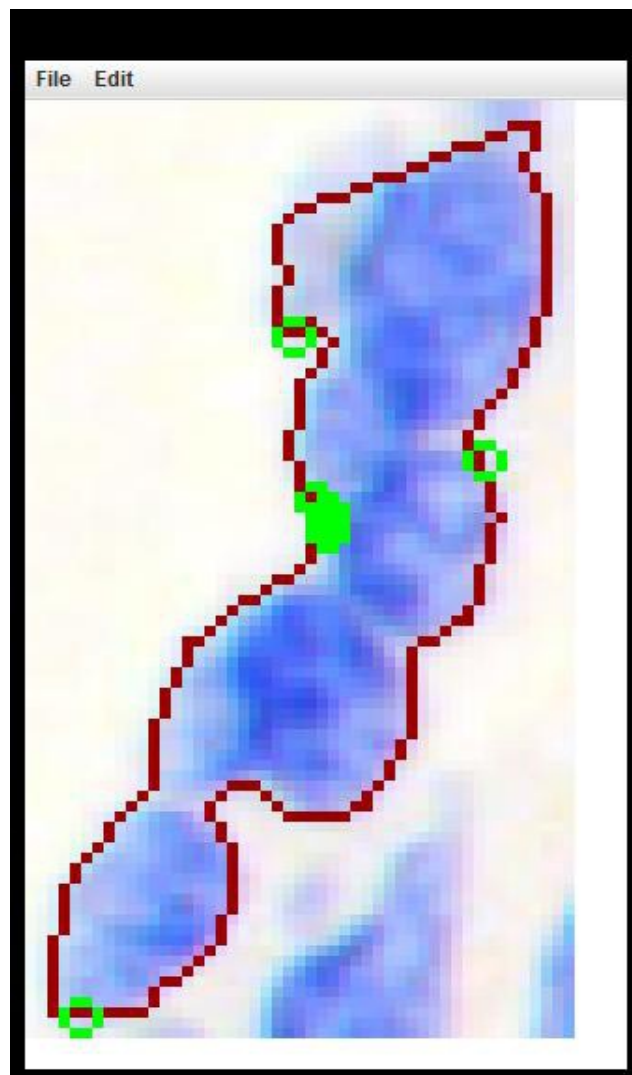
This figure represents the relative angle vs the X position of the chain.

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Inflections:



Inflection plot showing selected vertex points; Results of inflection splitting

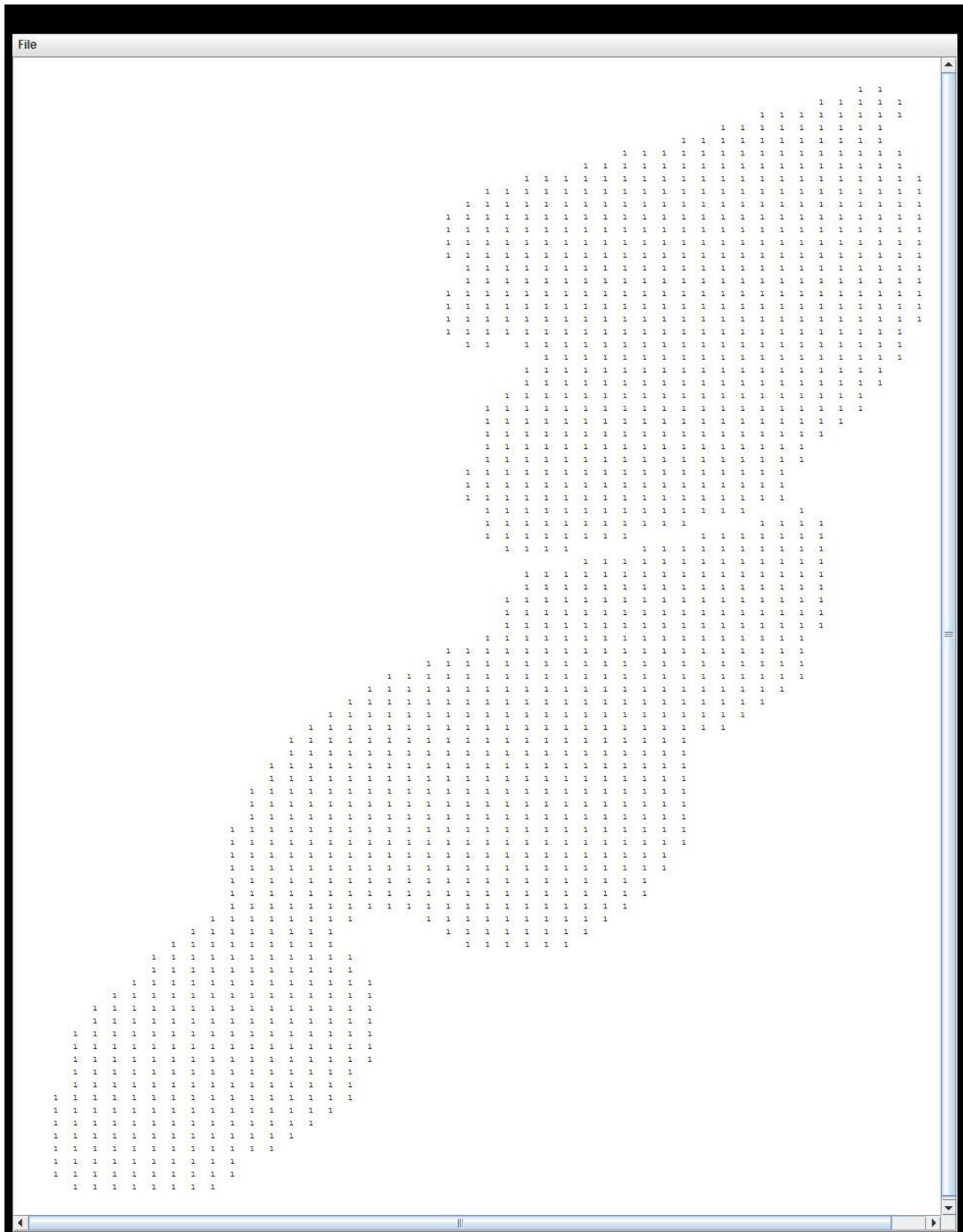


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Mask values showing angles at selected indents.

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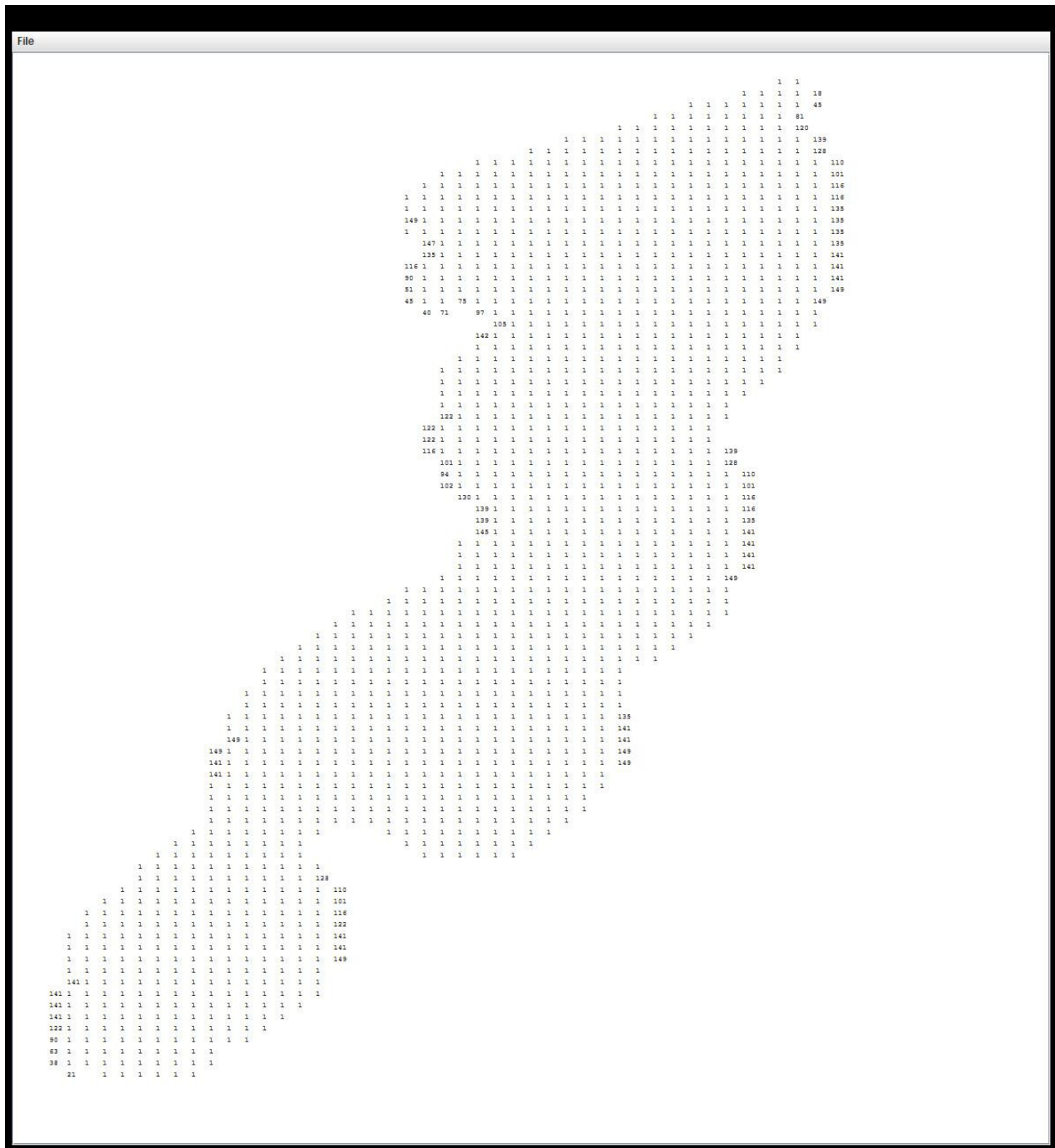
Mask displaying divided areas. Dividing line will be added back to each resulting area.

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This graphic shows the positions of the contour pixels

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This graphic shows the angles without the limitations of the indents.