Automated Identification of Beta-Amyloid Plaques by Image Analysis

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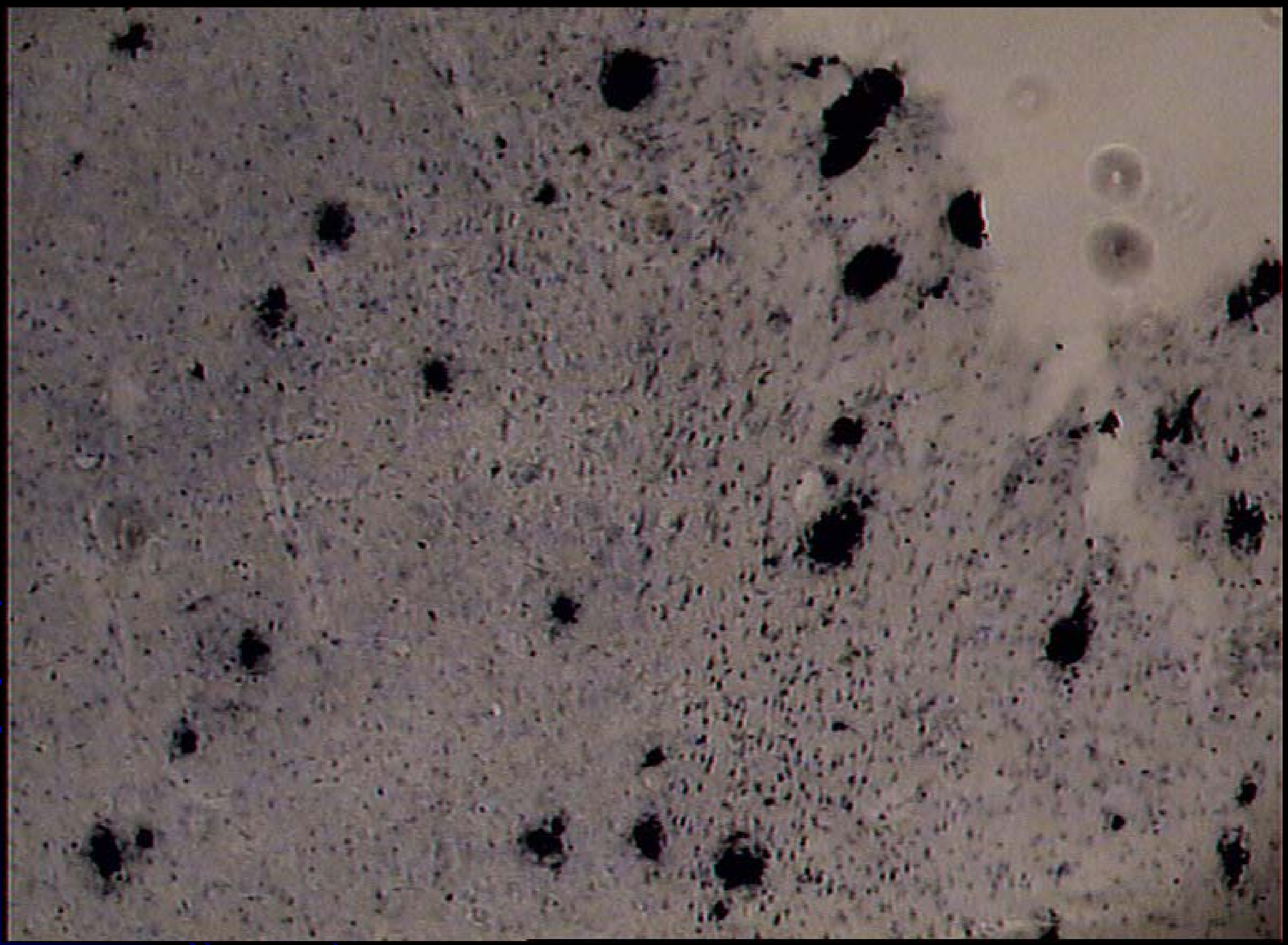
Beta-Amyloid (Aβ) Plaques



Aβ Plaques – Key Points

- Implicated in Alzheimer's Disease –
  inflammation + permanent neurodegeneration
- Increase in size + number as disease progresses

The ImageJ Image Editor

- Written in Java
- Open Source
- Widely used in various scientific fields
- Highly customisable through use of plug-ins and macros
- Plug-ins/macros are often made publicly available from labs that use them

The Task

- To develop an algorithm which will accurately identify Aβ plaques
- Implement this algorithm as a ImageJ plug-in or macro, ideally requiring a minimum of human interaction

Stages

- Image Preparation
- Image Segmentation
- Feature Extraction
- Validation

Image Preparation

- Due to variation in microscope magnification, CCD FOV/crop factor and resolution, the images must be calibrated in terms of pixels/µm
- ImageJ's Set Scale plug-in does this

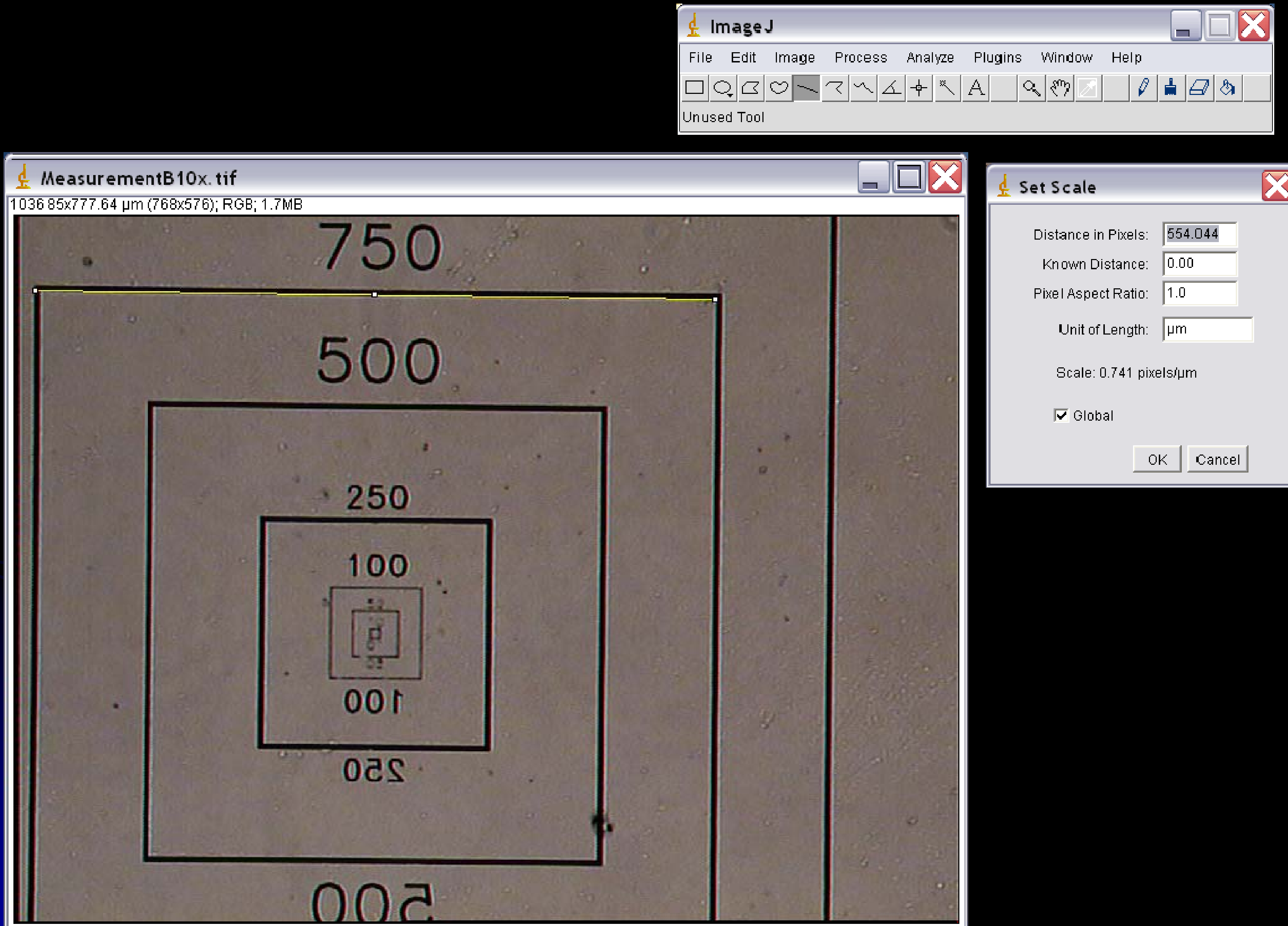
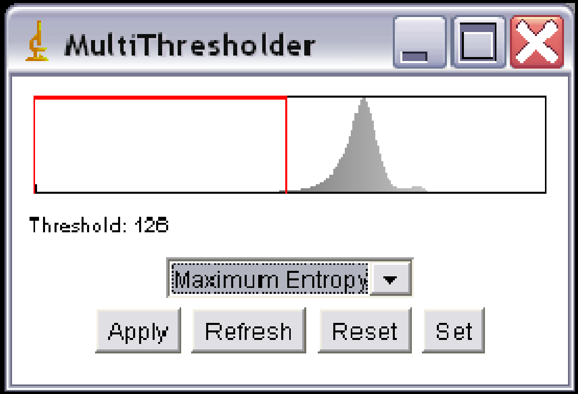

ImageJ
File Edit Image Process Analyze Plugins Window Help
Unused Tool
MeasurementB10x.tif
1036.85x777.64 µm (768x576); RGB; 1.7MB
750
500
250
100
Set Scale
Distance in Pixels: 554.044
Known Distance: 0.00
Pixel Aspect Ratio: 1.0
Unit of Length: µm
Scale: 0.741 pixels/µm
Global
OK
Cancel

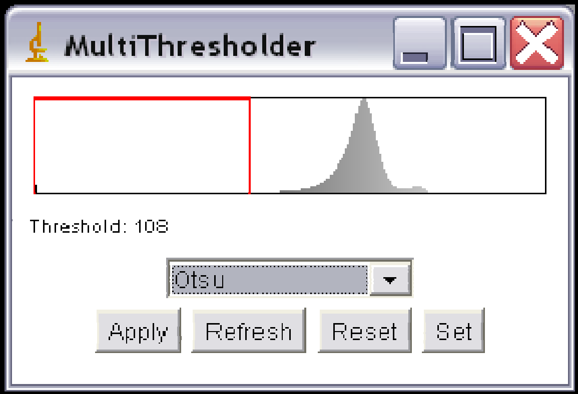
Image Segmentation

- This step is perhaps the most critical
- Divides the image space into two parts – i.e. plaques v everything else
- Requires a greyscale image, therefore convert directly from colour, or perform RGB split
- Segmentation requires thresholding

Thresholding

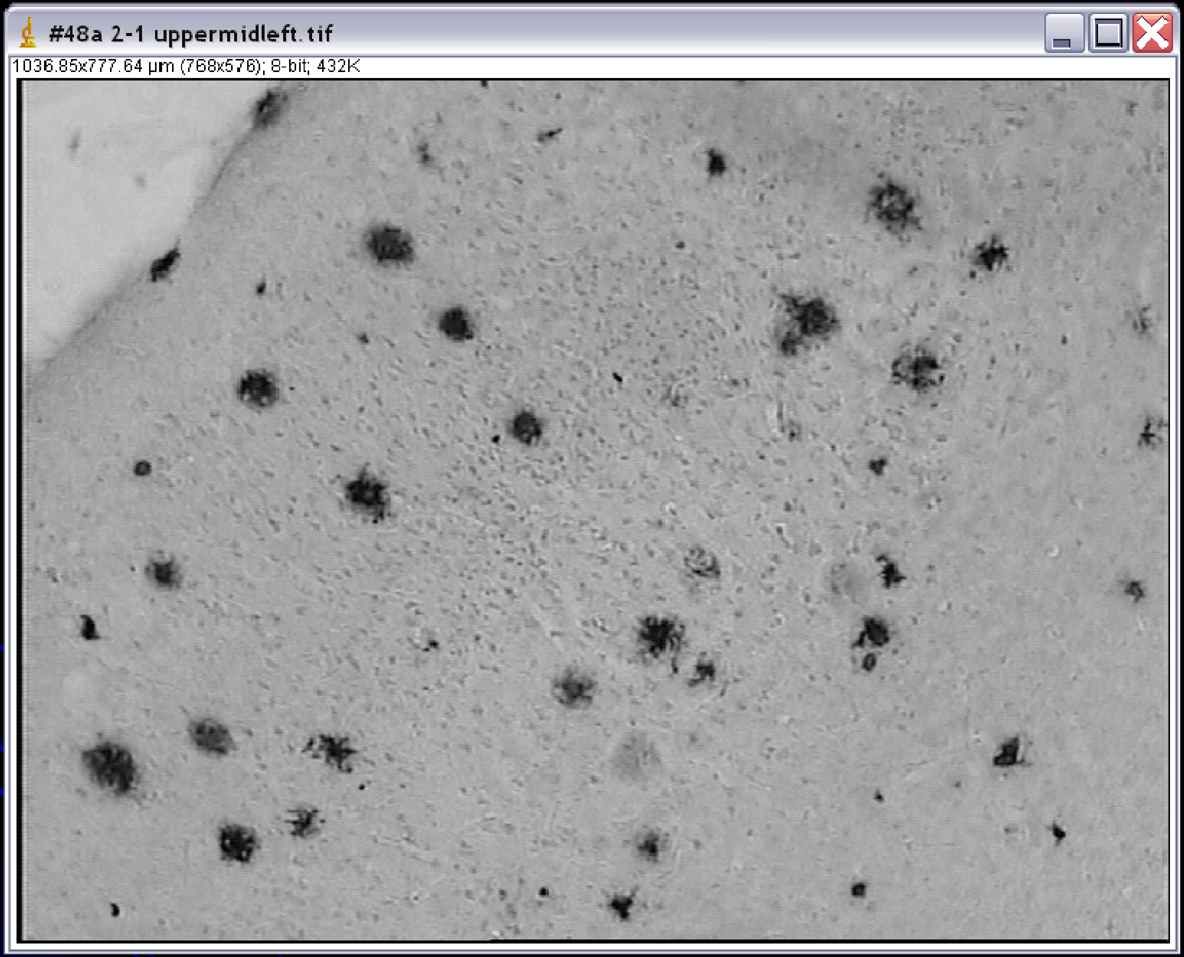
- Two methods were discovered to be effective
  - Maximum Entropy Threshold
  - OTSU



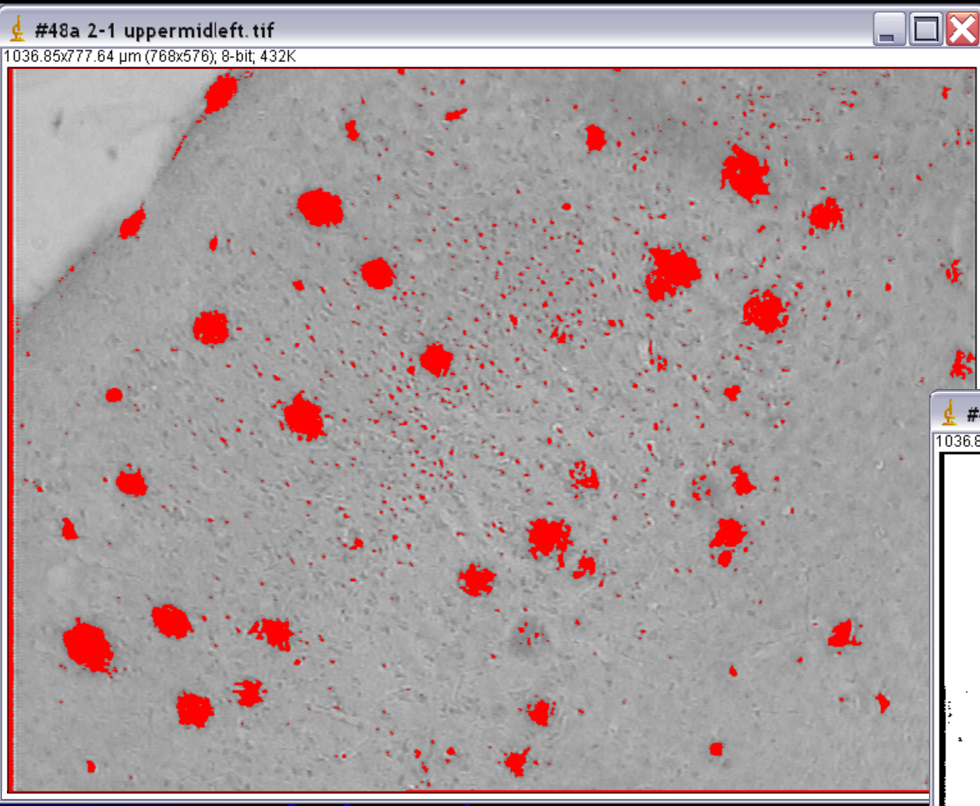


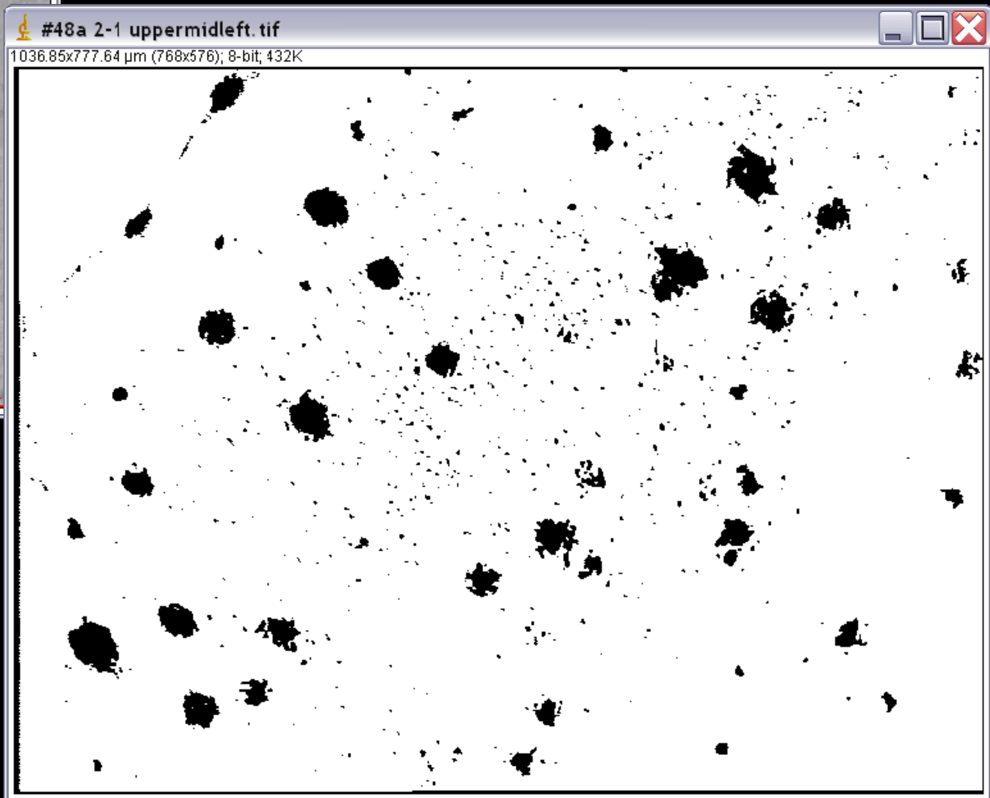
- Both found on the same plug-in - MultiThresholder

Grayscale – before thresholding

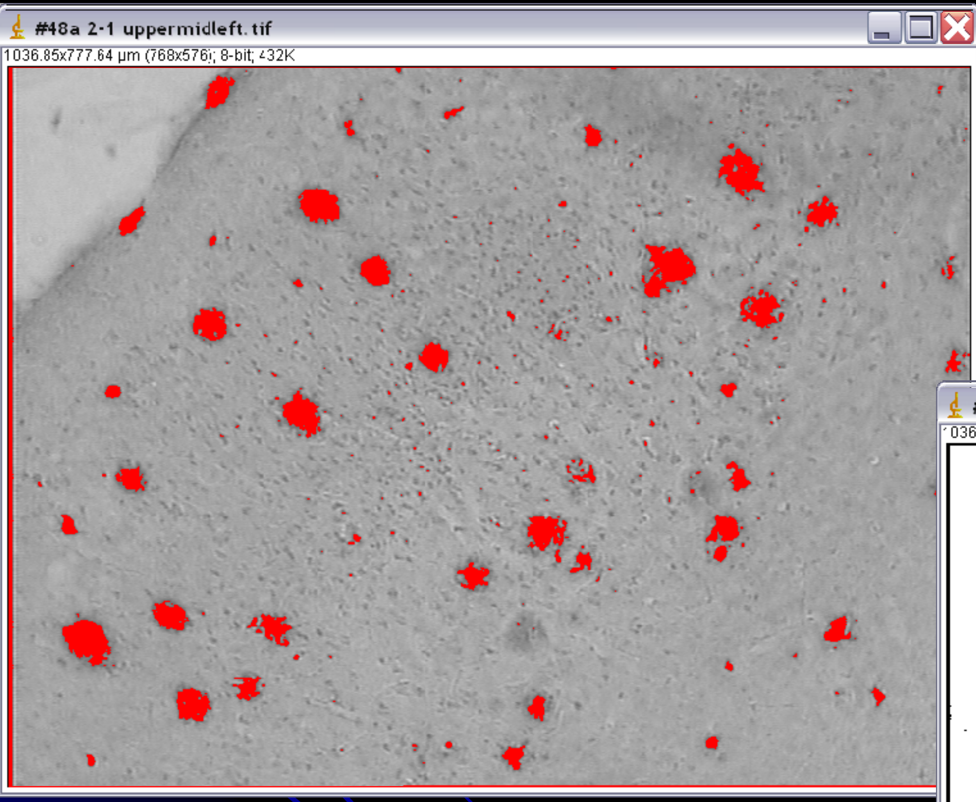


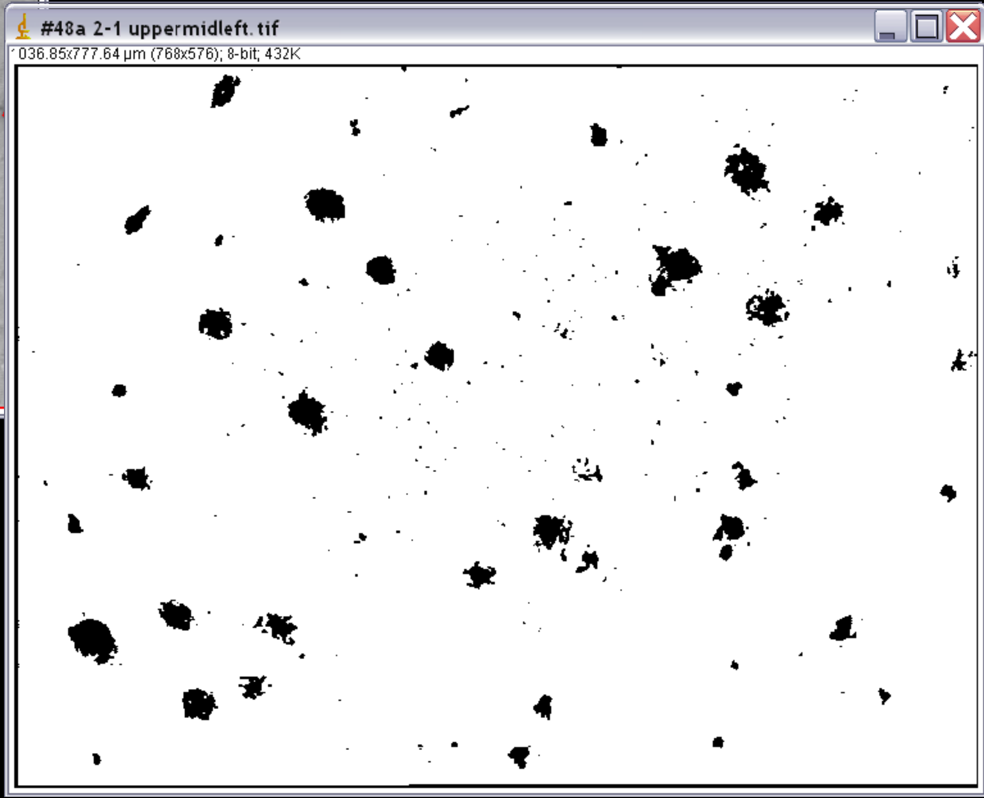
Maximum Entropy Threshold





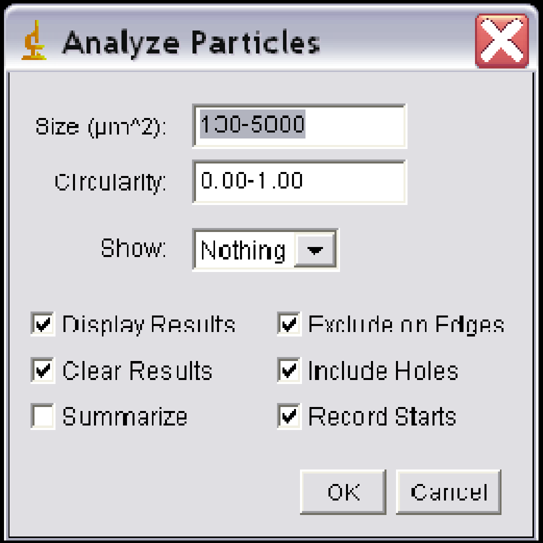
OTSU Threshold

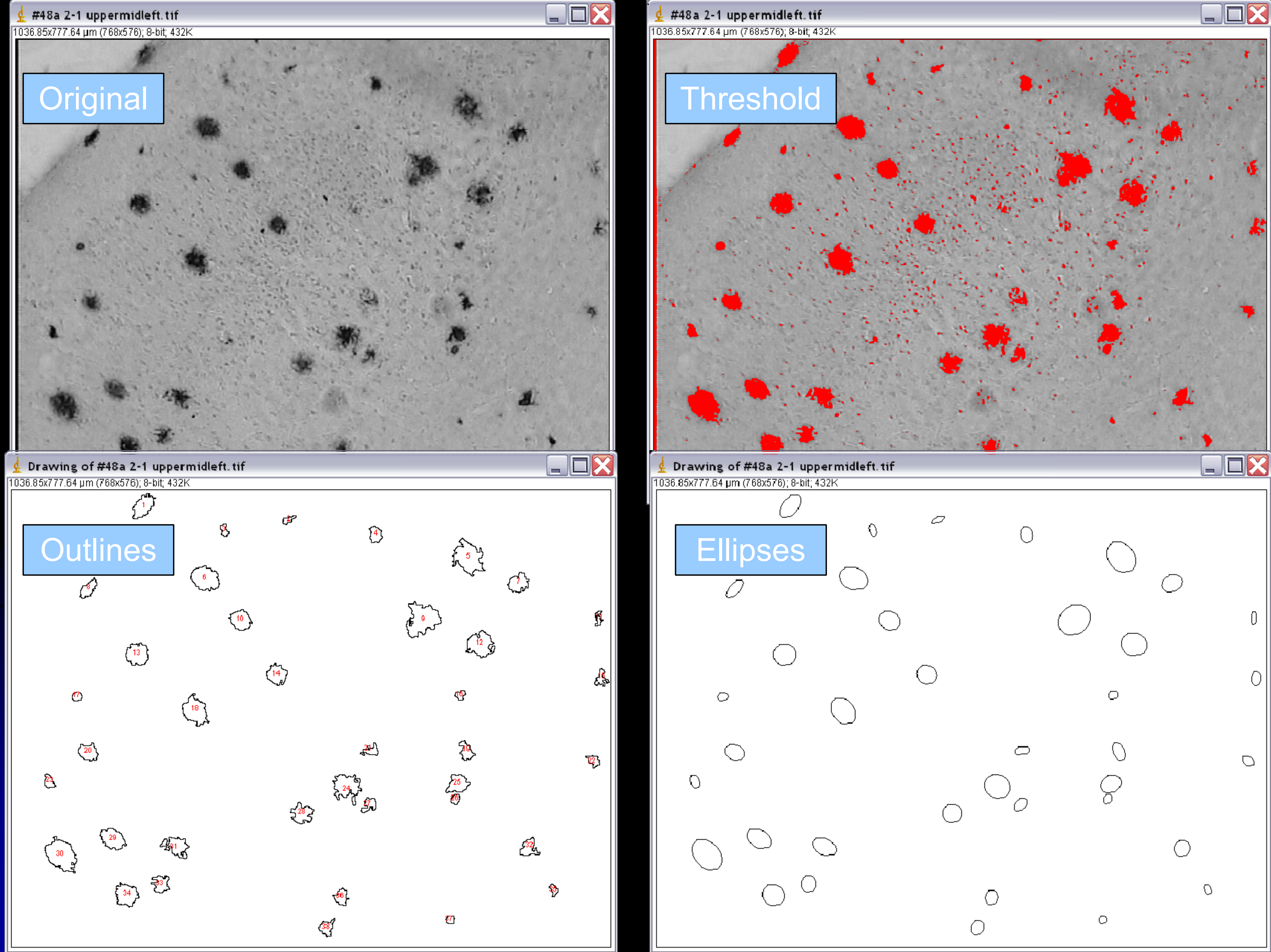




Feature Extraction

- Image data is collected as numerical output
- In ImageJ, the Particle Analyzer (sic) is used
- Allows selection of particles by min and max area, and circularity




#48a 2-1 uppermidleft.tif
1036.85x777.64 µm (768x576); 8-bit; 432K
Original
#48a 2-1 uppermidleft.tif
1036.85x777.64 µm (768x576); 8-bit; 432K
Threshold
Drawing of #48a 2-1 uppermidleft.tif
1036.85x777.64 µm (768x576); 8-bit; 432K
Outlines
Drawing of #48a 2-1 uppermidleft.tif
1036.85x777.64 µm (768x576); 8-bit; 432K
Ellipses

Results

File Edit Font

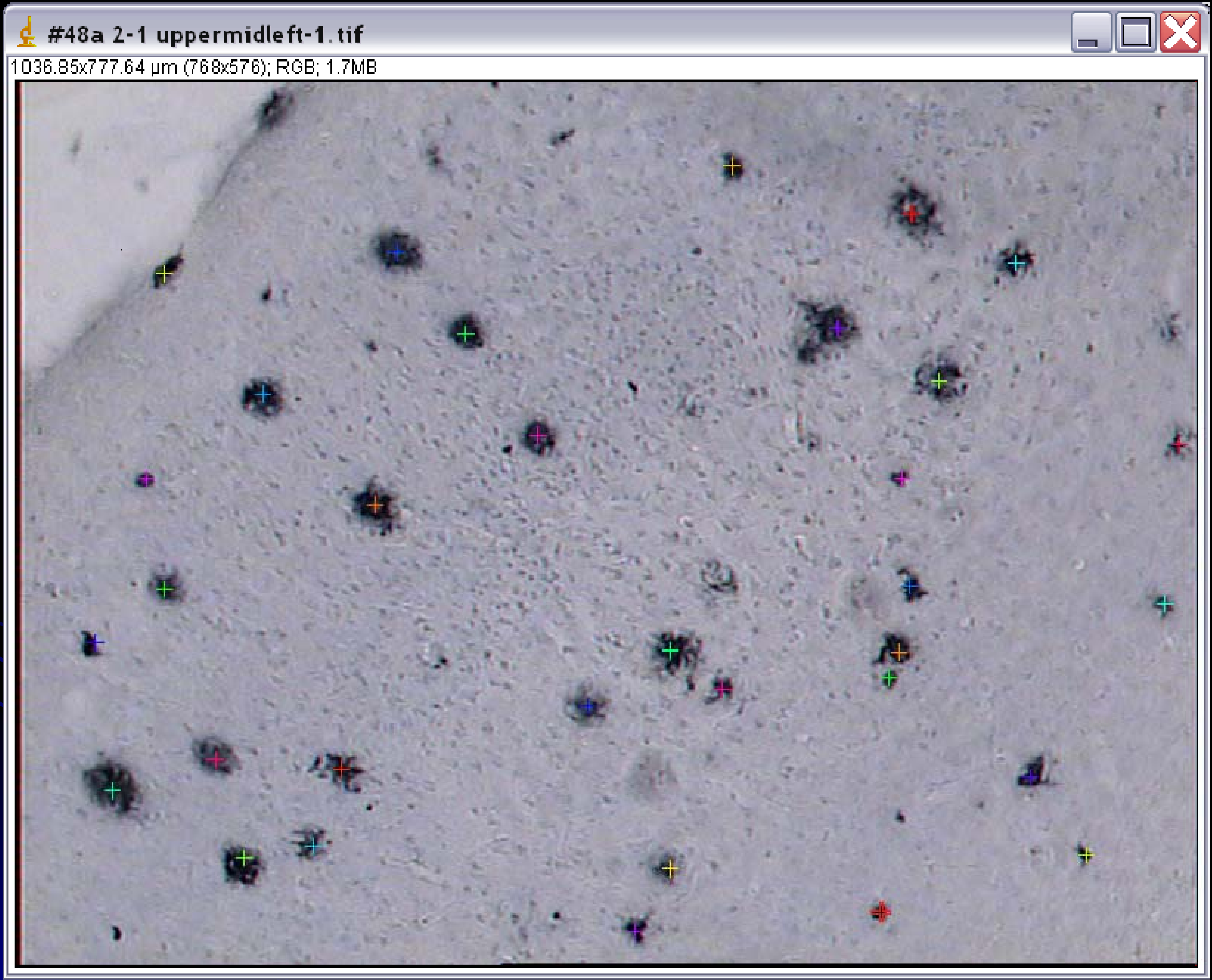
	Area	X	Y	Perim.	Major	Minor	Angle	Circ.	Feret
1	953.255	227.088	27.235	156.313	46.606	26.042	51.853	0.490	52.583
2	162.217	478.415	50.081	65.514	23.497	8.790	24.459	0.475	25.969
3	198.671	367.421	67.608	66.305	22.125	11.433	103.853	0.568	22.631
4	439.263	629.429	75.567	89.680	27.961	20.002	99.656	0.686	29.885
5	1944.786	789.676	114.006	275.422	56.812	43.586	128.183	0.322	67.799
6	1480.006	334.460	150.005	169.022	49.612	37.983	156.624	0.651	52.583
7	794.683	876.689	157.514	143.738	35.616	28.409	30.544	0.483	38.965

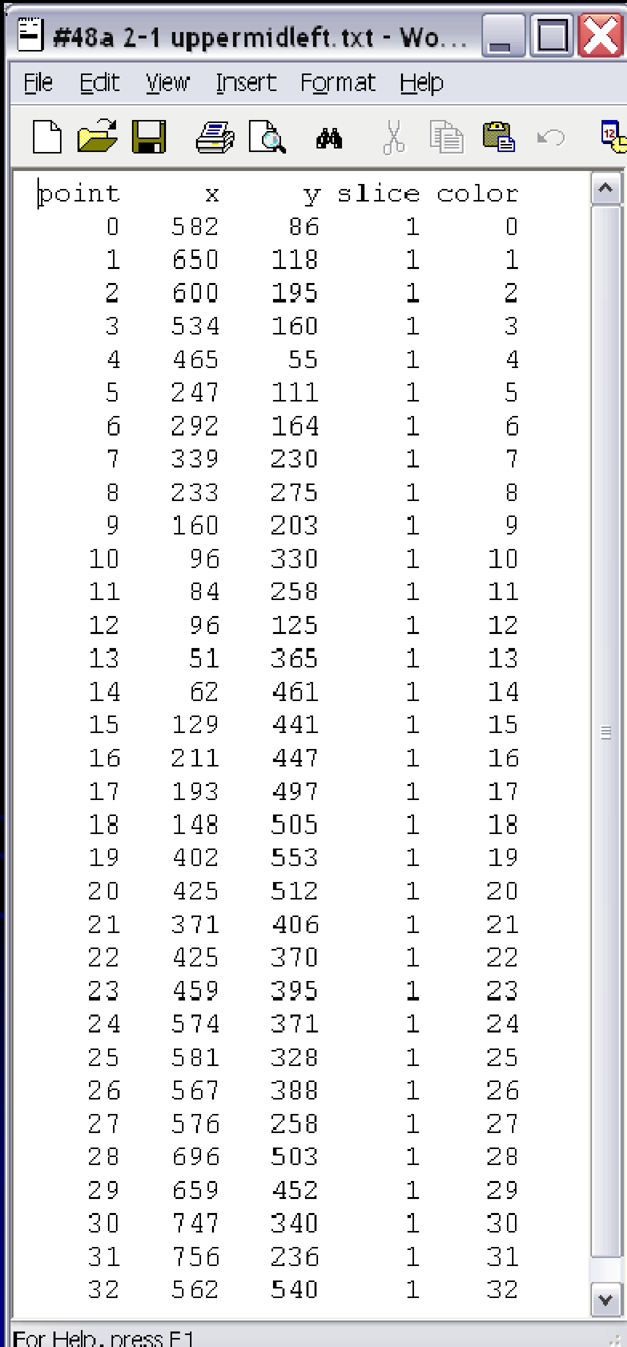
- X and Y represents centroid coordinates
    - Simply put, it is the average of all points of the particle.
- Circ. represents circularity of the particle:
    - circularity = 4pi(area/perimeter^2)
- Feret diameter is the maximum distance between any two points on the particle
- Major, Minor and Angle relate to ellipses…

Validation

- To check the accuracy of the algorithm
- Requires a ‘ground truth’ data set

- An expert identified plaques on a random selection of images
- The ImageJ plugin PointPicker_ was used


#48a 2-1 uppermidleft-1.tif
1036.85x777.64 µm (768x576); RGB; 1.7MB



point	x	y	slice	color
0	582	86	1	0
1	650	118	1	1
2	600	195	1	2
3	534	160	1	3
4	465	55	1	4
5	247	111	1	5
6	292	164	1	6
7	339	230	1	7
8	233	275	1	8
9	160	203	1	9
10	96	330	1	10
11	84	258	1	11
12	96	125	1	12
13	51	365	1	13
14	62	461	1	14
15	129	441	1	15
16	211	447	1	16
17	193	497	1	17
18	148	505	1	18
19	402	553	1	19
20	425	512	1	20
21	371	406	1	21
22	425	370	1	22
23	459	395	1	23
24	574	371	1	24
25	581	328	1	25
26	567	388	1	26
27	576	258	1	27
28	696	503	1	28
29	659	452	1	29
30	747	340	1	30
31	756	236	1	31
32	562	540	1	32

- X and Y represent coordinates (in this case by pixel, not by µm)
- So how to compare these to the coordinates of the Particle Analyzer?

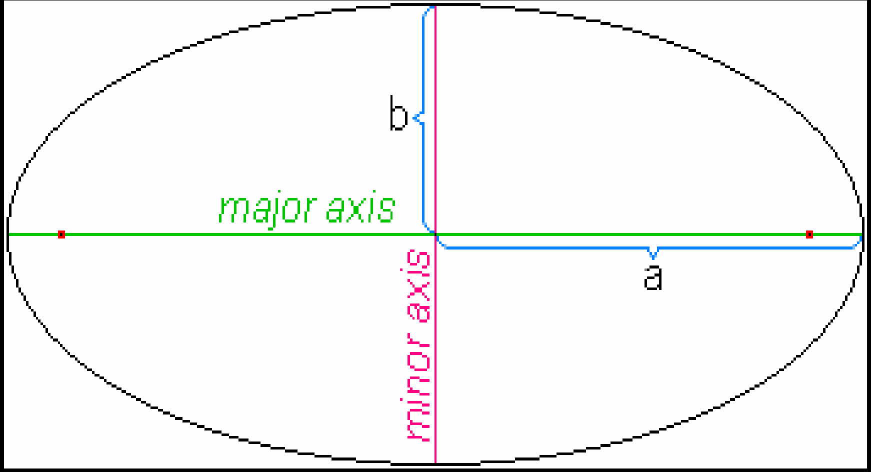
Validation – Feret's diameter

- The easy way – for every possible match, calculate the distance between the expert's and the Particle Analyzer's centroid coordinates
- Check if this distance is less than half the Feret's diameter of that particle

Validation - ellipse

- The hard way – for every possible match, check if the expert's coordinates lie within the Particle Analyzer's elliptical approximation of the particle
- As the ellipses have an angle, the expert coordinates must be transformed (rotated about the centroid by the same angle as the ellipse)

Ellipses



$$\frac{x^2}{a^2} + \frac{y^2}{b^2} = 1$$

		X																													
	0	**-15**	**-14**	**-13**	**-12**	**-11**	**-10**	**-9**	**-8**	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
	-15	5.1	4.9	4.7	4.5	4.4	4.2	4.1	4.0	3.9	3.8	3.7	3.6	3.6	3.5	3.5	3.5	3.5	3.5	3.6	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.4	4.5	4.7	4.9	5.1
	-14	4.6	4.4	4.2	4.1	3.9	3.8	3.6	3.5	3.4	3.3	3.2	3.2	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.2	3.2	3.3	3.4	3.5	3.6	3.8	3.9	4.1	4.2	4.4	4.6
	-13	4.2	4.0	3.8	3.6	3.5	3.3	3.2	3.1	3.0	2.9	2.8	2.8	2.7	2.7	2.6	2.6	2.6	2.7	2.7	2.8	2.8	2.9	3.0	3.1	3.2	3.3	3.5	3.6	3.8	4.0	4.2
	-12	3.8	3.6	3.4	3.3	3.1	2.9	2.8	2.7	2.6	2.5	2.4	2.4	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.4	2.4	2.5	2.6	2.7	2.8	2.9	3.1	3.3	3.4	3.6	3.8
Y	**-11**	3.5	3.3	3.1	2.9	2.7	2.6	2.5	2.3	2.2	2.1	2.1	2.0	2.0	1.9	1.9	1.9	1.9	1.9	2.0	2.0	2.1	2.1	2.2	2.3	2.5	2.6	2.7	2.9	3.1	3.3	3.5
	-10	3.1	2.9	2.7	2.6	2.4	2.3	2.1	2.0	1.9	1.8	1.7	1.7	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.7	1.7	1.8	1.9	2.0	2.1	2.3	2.4	2.6	2.7	2.9	3.1
	-9	2.8	2.6	2.4	2.3	2.1	2.0	1.8	1.7	1.6	1.5	1.4	1.4	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.4	1.4	1.5	1.6	1.7	1.8	2.0	2.1	2.3	2.4	2.6	2.8
	-8	2.6	2.4	2.2	2.0	1.8	1.7	1.6	1.4	1.3	1.3	1.2	1.1	1.1	1.0	1.0	1.0	1.0	1.0	1.1	1.1	1.2	1.3	1.3	1.4	1.6	1.7	1.8	2.0	2.2	2.4	2.6
	-7	2.3	2.1	1.9	1.8	1.6	1.5	1.3	1.2	1.1	1.0	0.9	0.9	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.9	0.9	1.0	1.1	1.2	1.3	1.5	1.6	1.8	1.9	2.1	2.3
	-6	2.1	1.9	1.7	1.6	1.4	1.3	1.1	1.0	0.9	0.8	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.7	0.7	0.8	0.9	1.0	1.1	1.3	1.4	1.6	1.7	1.9	2.1
	-5	2.0	1.8	1.6	1.4	1.2	1.1	1.0	0.8	0.7	0.6	0.6	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.5	0.5	0.6	0.6	0.7	0.8	1.0	1.1	1.2	1.4	1.6	1.8	2.0
	-4	1.8	1.6	1.4	1.3	1.1	0.9	0.8	0.7	0.6	0.5	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.5	0.6	0.7	0.8	0.9	1.1	1.3	1.4	1.6	1.8
	-3	1.7	1.5	1.3	1.1	1.0	0.8	0.7	0.6	0.5	0.4	0.3	0.3	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.3	0.3	0.4	0.5	0.6	0.7	0.8	1.0	1.1	1.3	1.5	1.7
	-2	1.6	1.4	1.2	1.1	0.9	0.8	0.6	0.5	0.4	0.3	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.3	0.4	0.5	0.6	0.8	0.9	1.1	1.2	1.4	1.6
	-1	1.6	1.4	1.2	1.0	0.9	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.9	1.0	1.2	1.4	1.6
	0	1.6	1.4	1.2	1.0	0.8	0.7	0.6	0.4	0.3	0.3	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.3	0.3	0.4	0.6	0.7	0.8	1.0	1.2	1.4	1.6
	1	1.6	1.4	1.2	1.0	0.9	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.9	1.0	1.2	1.4	1.6
	2	1.6	1.4	1.2	1.1	0.9	0.8	0.6	0.5	0.4	0.3	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.3	0.4	0.5	0.6	0.8	0.9	1.1	1.2	1.4	1.6
	3	1.7	1.5	1.3	1.1	1.0	0.8	0.7	0.6	0.5	0.4	0.3	0.3	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.3	0.3	0.4	0.5	0.6	0.7	0.8	1.0	1.1	1.3	1.5	1.7
	4	1.8	1.6	1.4	1.3	1.1	0.9	0.8	0.7	0.6	0.5	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.5	0.6	0.7	0.8	0.9	1.1	1.3	1.4	1.6	1.8
	5	2.0	1.8	1.6	1.4	1.2	1.1	1.0	0.8	0.7	0.6	0.6	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.5	0.5	0.6	0.6	0.7	0.8	1.0	1.1	1.2	1.4	1.6	1.8	2.0
	6	2.1	1.9	1.7	1.6	1.4	1.3	1.1	1.0	0.9	0.8	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.7	0.7	0.8	0.9	1.0	1.1	1.3	1.4	1.6	1.7	1.9	2.1
	7	2.3	2.1	1.9	1.8	1.6	1.5	1.3	1.2	1.1	1.0	0.9	0.9	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.9	0.9	1.0	1.1	1.2	1.3	1.5	1.6	1.8	1.9	2.1	2.3
	8	2.6	2.4	2.2	2.0	1.8	1.7	1.6	1.4	1.3	1.3	1.2	1.1	1.1	1.0	1.0	1.0	1.0	1.0	1.1	1.1	1.2	1.3	1.3	1.4	1.6	1.7	1.8	2.0	2.2	2.4	2.6
	9	2.8	2.6	2.4	2.3	2.1	2.0	1.8	1.7	1.6	1.5	1.4	1.4	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.4	1.4	1.5	1.6	1.7	1.8	2.0	2.1	2.3	2.4	2.6	2.8
	10	3.1	2.9	2.7	2.6	2.4	2.3	2.1	2.0	1.9	1.8	1.7	1.7	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.7	1.7	1.8	1.9	2.0	2.1	2.3	2.4	2.6	2.7	2.9	3.1
	11	3.5	3.3	3.1	2.9	2.7	2.6	2.5	2.3	2.2	2.1	2.1	2.0	2.0	1.9	1.9	1.9	1.9	1.9	2.0	2.0	2.1	2.1	2.2	2.3	2.5	2.6	2.7	2.9	3.1	3.3	3.5
	12	3.8	3.6	3.4	3.3	3.1	2.9	2.8	2.7	2.6	2.5	2.4	2.4	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.4	2.4	2.5	2.6	2.7	2.8	2.9	3.1	3.3	3.4	3.6	3.8
	13	4.2	4.0	3.8	3.6	3.5	3.3	3.2	3.1	3.0	2.9	2.8	2.8	2.7	2.7	2.6	2.6	2.6	2.7	2.7	2.8	2.8	2.9	3.0	3.1	3.2	3.3	3.5	3.6	3.8	4.0	4.2
	14	4.6	4.4	4.2	4.1	3.9	3.8	3.6	3.5	3.4	3.3	3.2	3.2	3.1	3.1	3.1	3.1	3.1	3.1	3.1	3.2	3.2	3.3	3.4	3.5	3.6	3.8	3.9	4.1	4.2	4.4	4.6
	15	5.1	4.9	4.7	4.5	4.4	4.2	4.1	4.0	3.9	3.8	3.7	3.6	3.6	3.5	3.5	3.5	3.5	3.5	3.6	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.4	4.5	4.7	4.9	5.1

- Points inside the ellipse are < 1
- Points outside the ellipse are >1

What’s next?

- The comparison of expert and Particle Analyzer data will be performed by a function written in R
- This will allow easy determination of information from the ‘expert sample’ such as:
  - the average min and max area of particles
  - min circularity of particles
  - comparison of results for each thresholding method
- A macro can then run the Particle Analyzer with these values implemented
- From this macro, a plug-in can be developed with a simple dialog allowing the user to choose key characteristics, along with batch processing options – this may be operated via GUI or command line

Example macro:

MaxEntropyMin130Max5000.txt - WordPad

```
run("Set Scale...", "distance=555.53 known=750 pixel=1 unit=µm global");

open("D:\\Documents and Settings\\Colin Morrison\\My Documents\\Bioinformatics Degree\\Final Project\\Points&Images\\#48a 1-1 lowleft.tif");
run("8-bit");
run("MultiThresholder", "Maximum Entropy");
run("MultiThresholder", "Maximum Entropy apply");
run("Analyze Particles...", "size=130-5000 circularity=0.00-1.00 show=Nothing display exclude clear include record");
saveAs("Measurements", "D:\\Documents and Settings\\Colin Morrison\\My Documents\\Bioinformatics Degree\\Final Project\\Points&Images\\#48a 1-1 lowleft(ME130-5000).xls");

close();
```

For Help, press F1

Notes

- Fluorescently labelled samples captured through a confocal microscope may yield better results using this algorithm
- This algorithm cannot detect overlapping particles. It may be possible to use the Watershed algorithm to solve this, but may require significant alteration of the image (e.g. smoothing plaque edges) which may adversely effect accuracy

References:

- ImageJ:
  - Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2006.
  - Abramoff, M.D., Magelhaes, P.J., Ram, S.J. "Image Processing with ImageJ". Biophotonics International, volume 11, issue 7, pp. 36-42, 2004.
- MultiThresholder:
  - Kevin (Gali) Baler - http://rsb.info.nih.gov/ij/plugins/multi-thresholder.html
- Maximum Entropy:
  - P.K. Sahoo, S. Soltani, K.C. Wong and, Y.C. Chen "A Survey of Thresholding Techniques", Computer Vision, Graphics, and Image Processing, Vol. 41, pp.233-260, 1988.
- OTSU:
  - N. Otsu "A threshold selection method from gray level histograms", IEEE Trans. Systems, Man and Cybernetics, Vol. 9, pp.62-66, 1979
- PointPicker_:
  - Philippe Thévenaz - http://bigwww.epfl.ch/thevenaz/pointpicker/