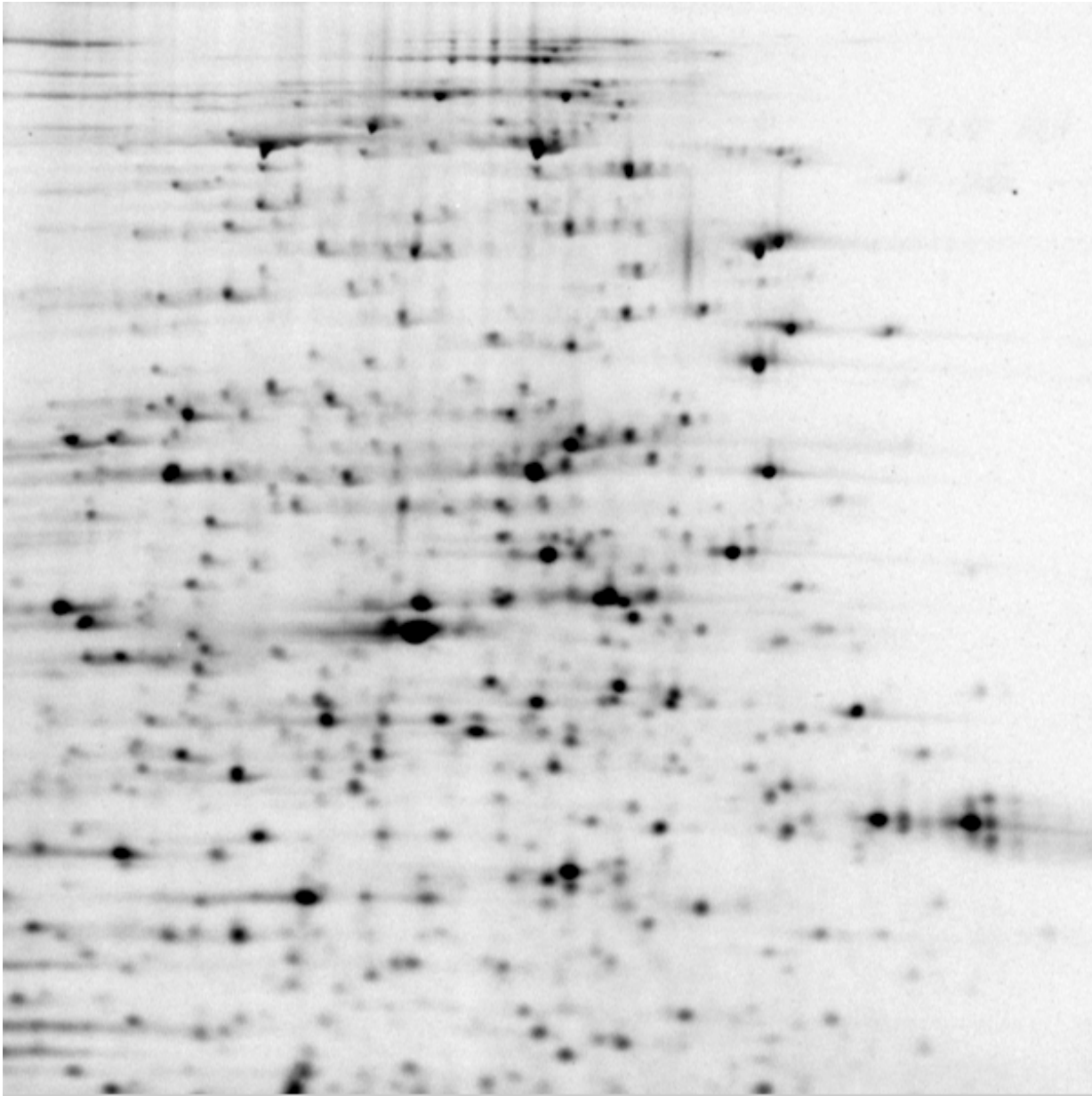
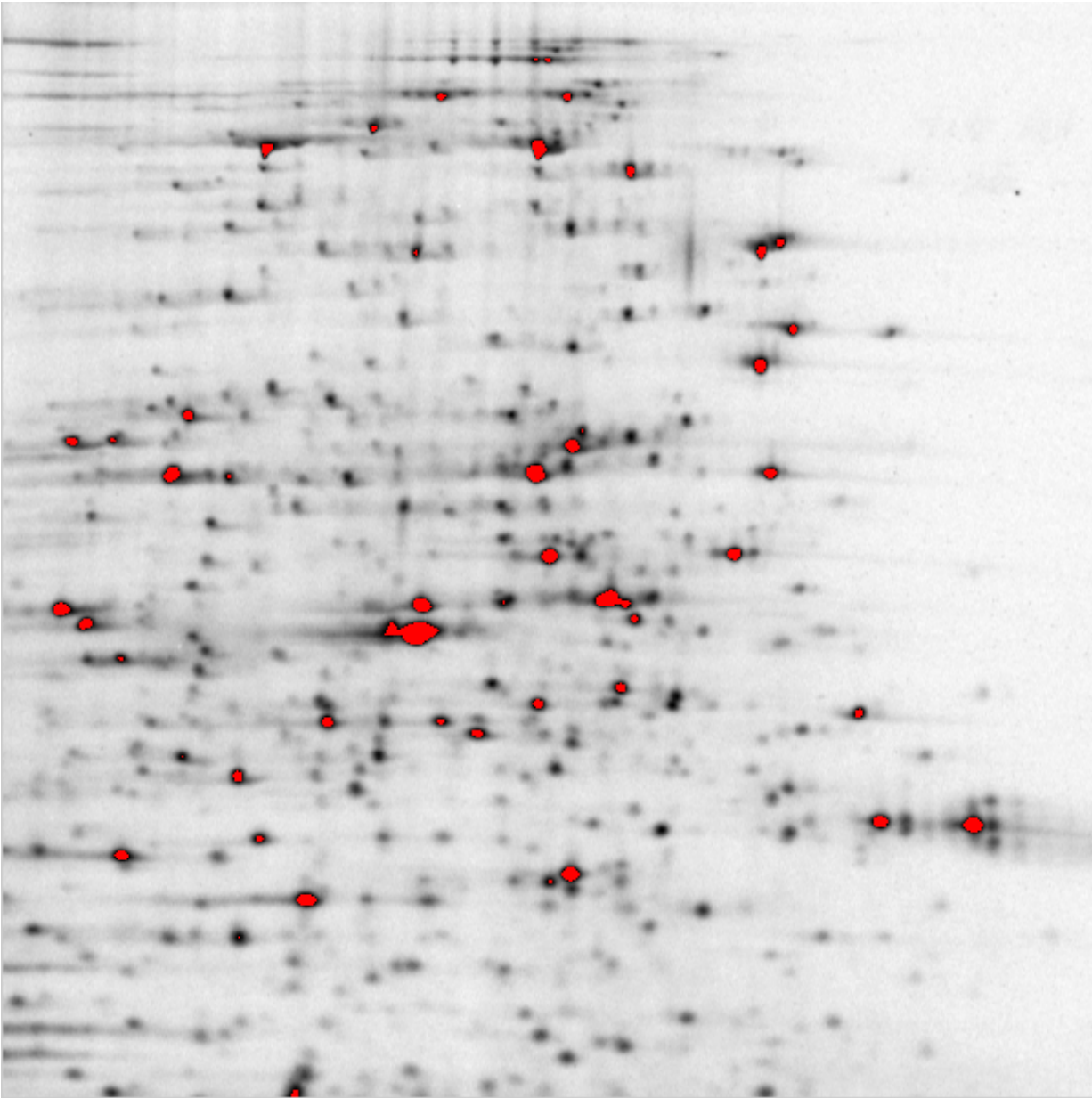


2D Gel Segmentation



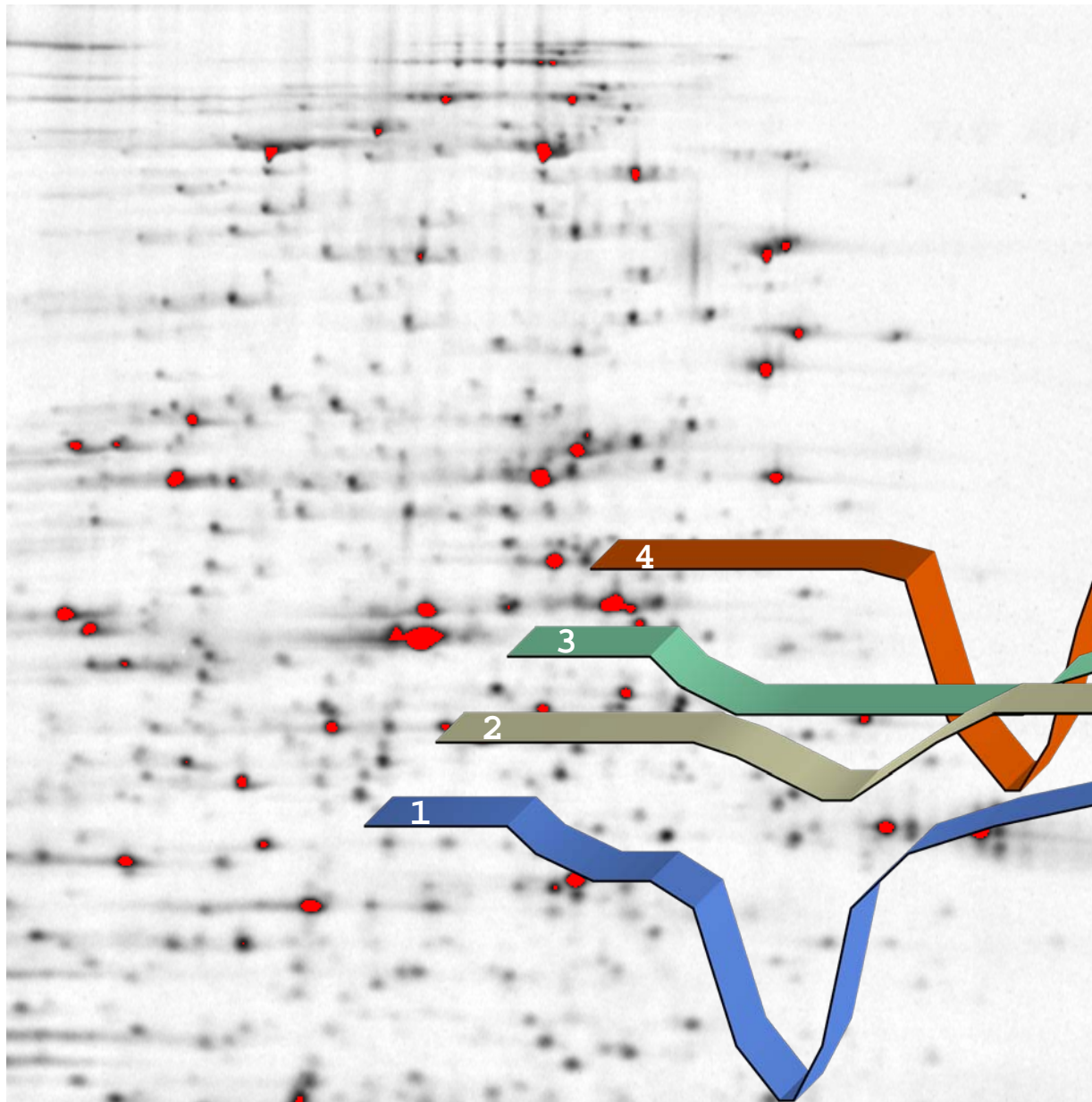
Areas of Saturation

Red areas have value 0. In these areas the real image value is unknown. They should be treated differently during analysis.



Background Removal by Top Hat Transformation

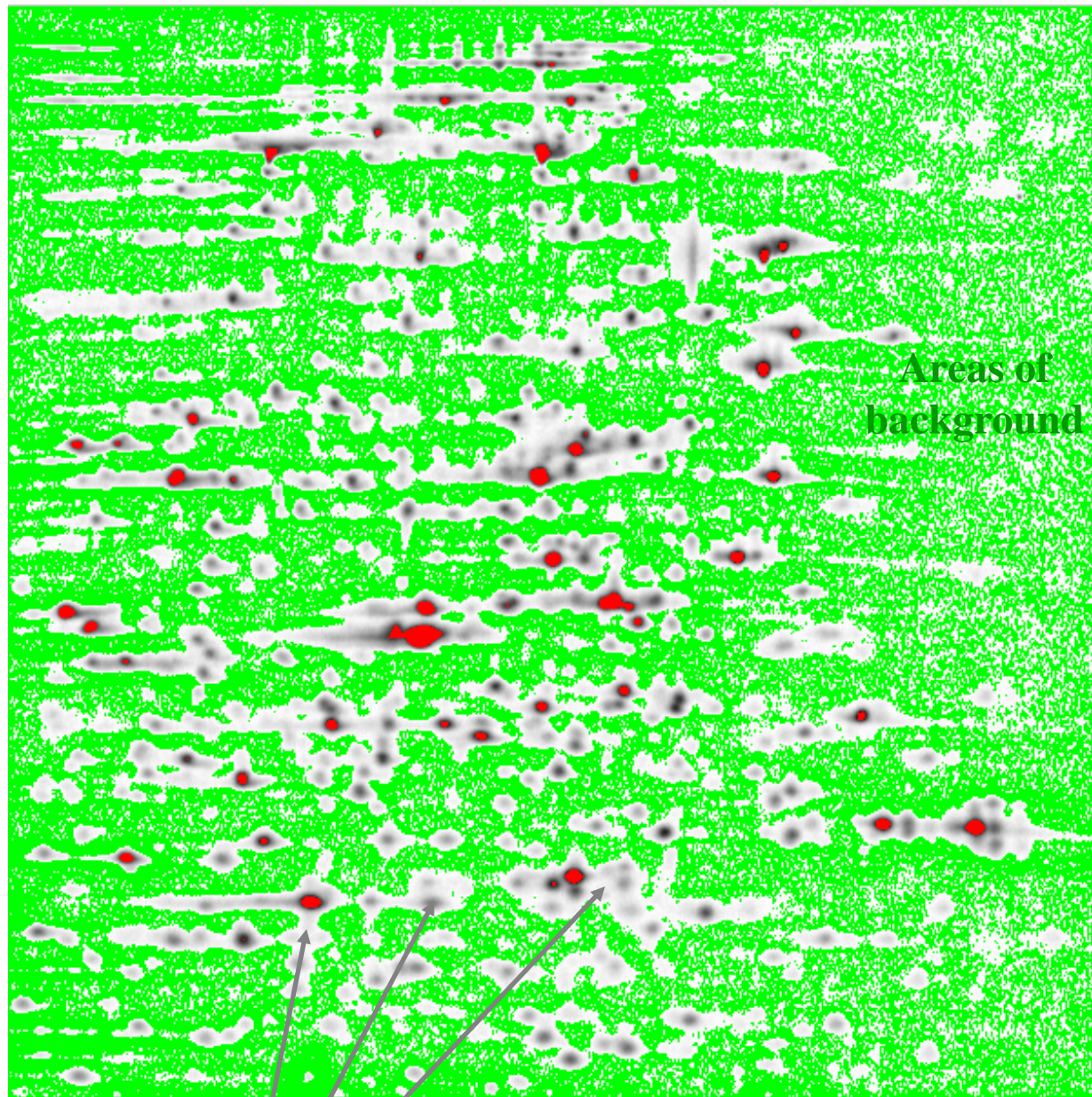
(But keep the saturation)



Density Profiles

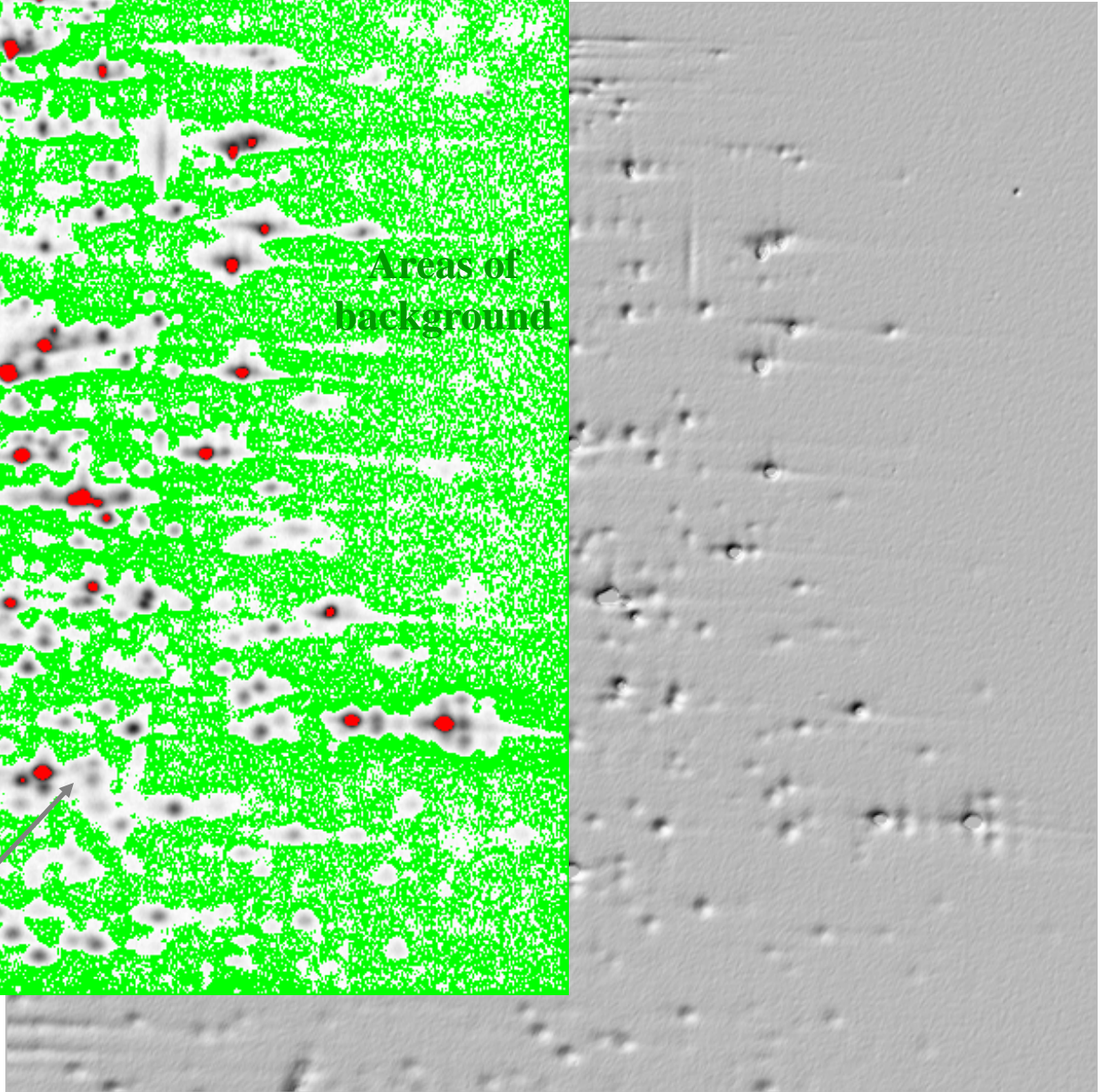
1. Original
2. Eroded
3. Dilated (this is the background)
4. Difference (background removed)

Fill Holes



Areas of
background

Areas of spots

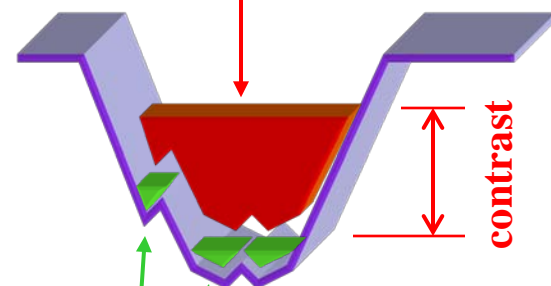


Spot Markers for Intensity Watershed



Background mask

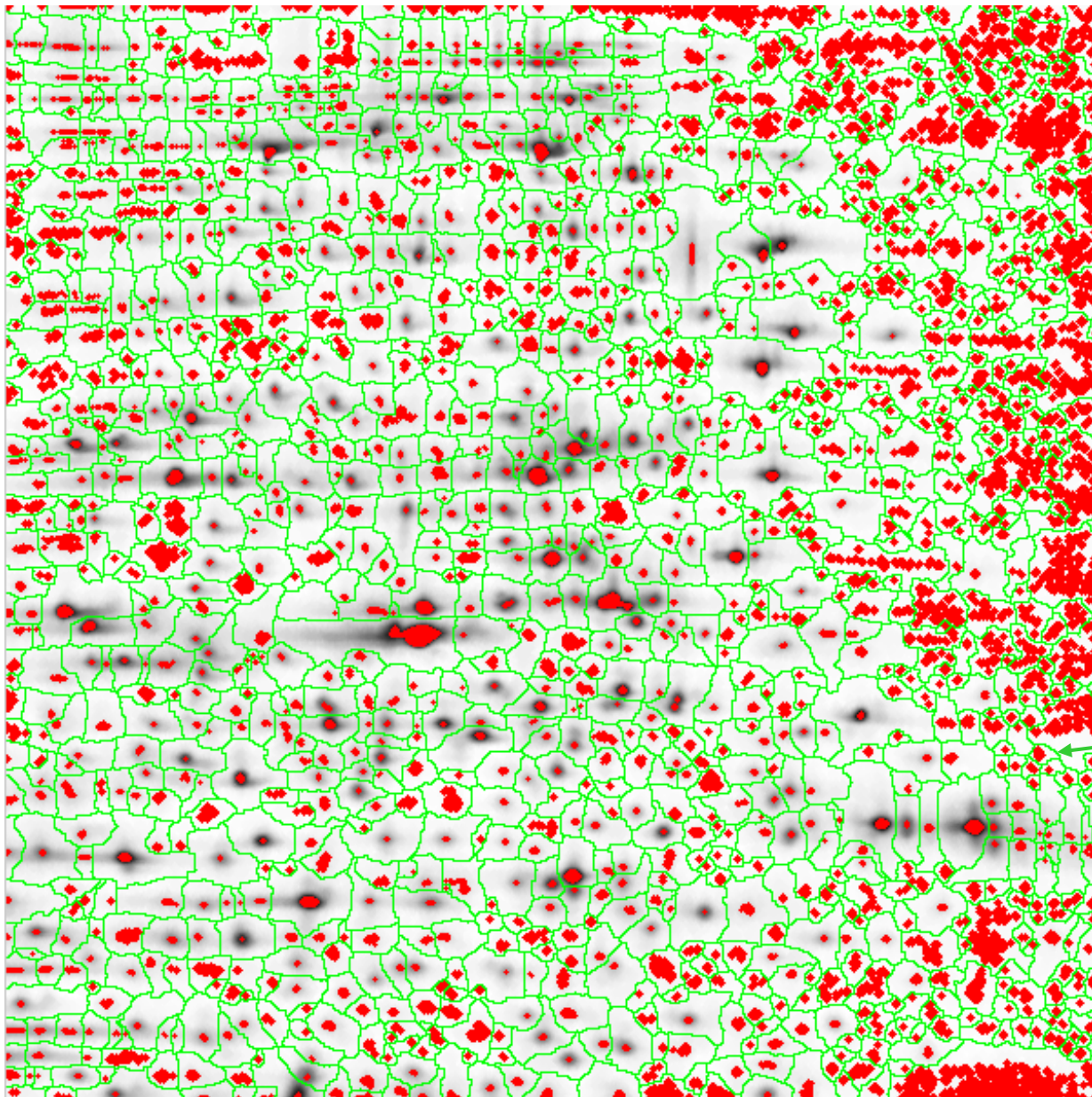
Regional minima with
given contrast avoid
detecting noise



Regional minima

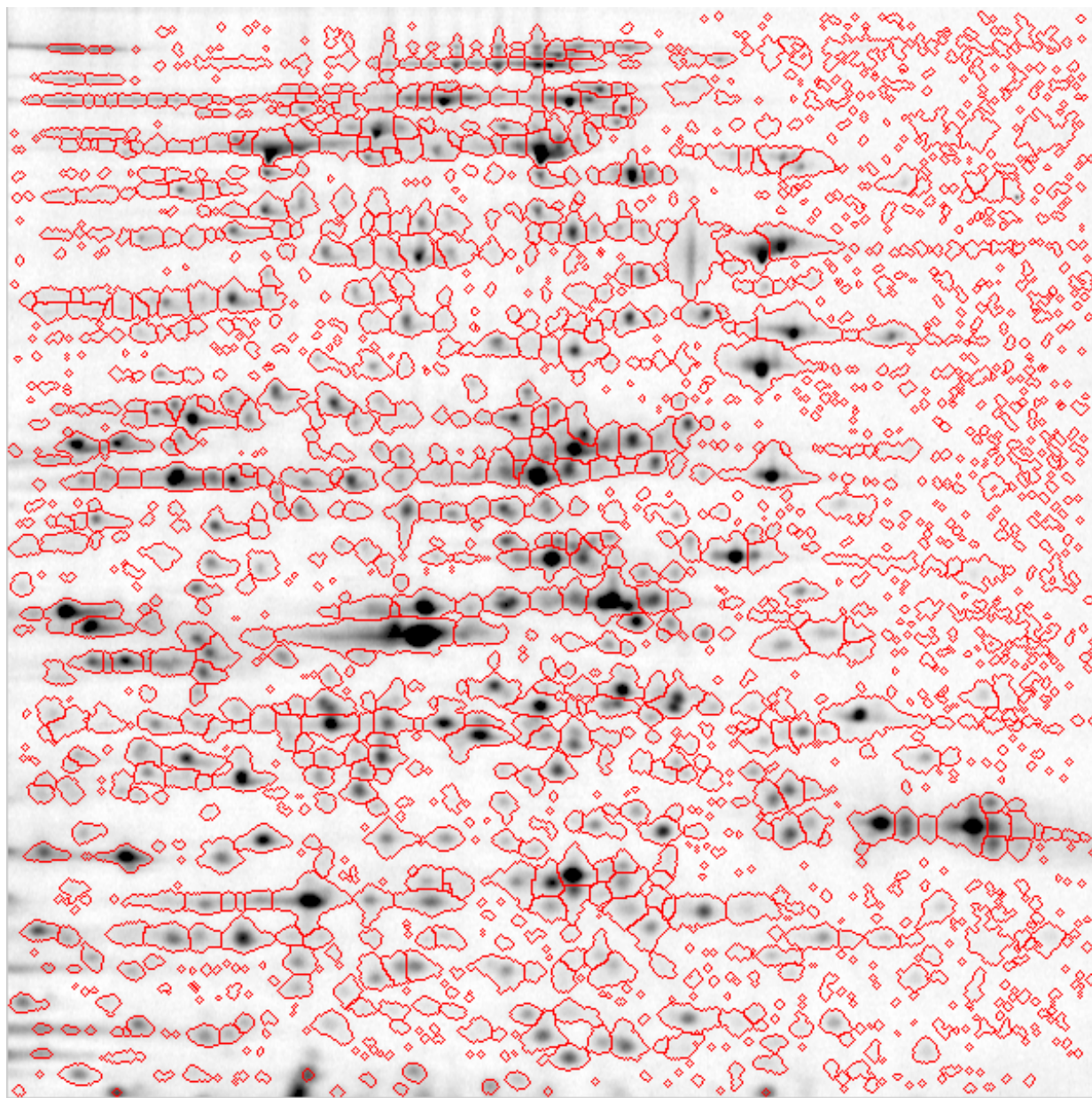
Watershed of Intensity Image

Watershed lines partition
the image into zones of
influence of spots



Watershed dividing
lines

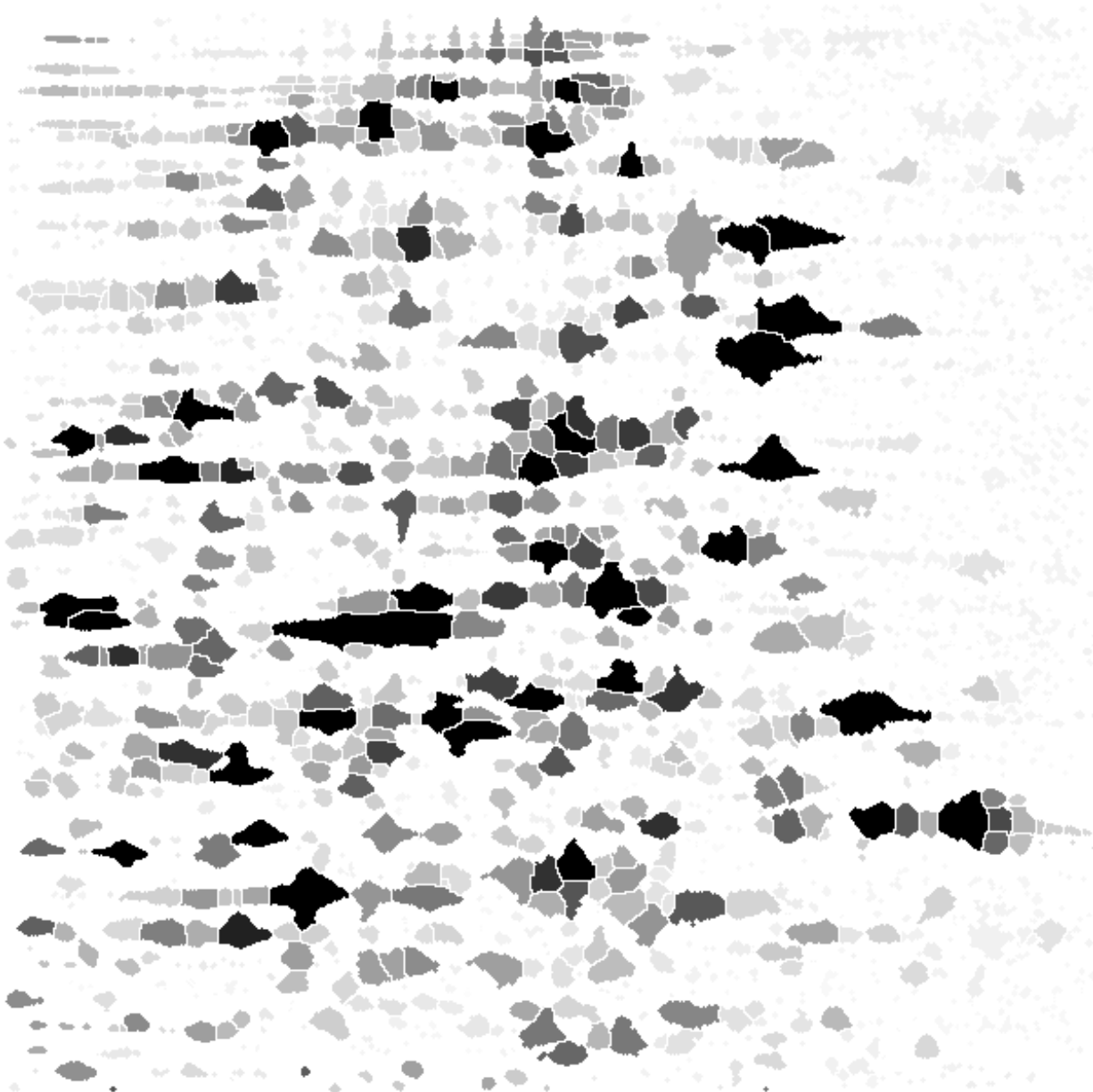
Markers



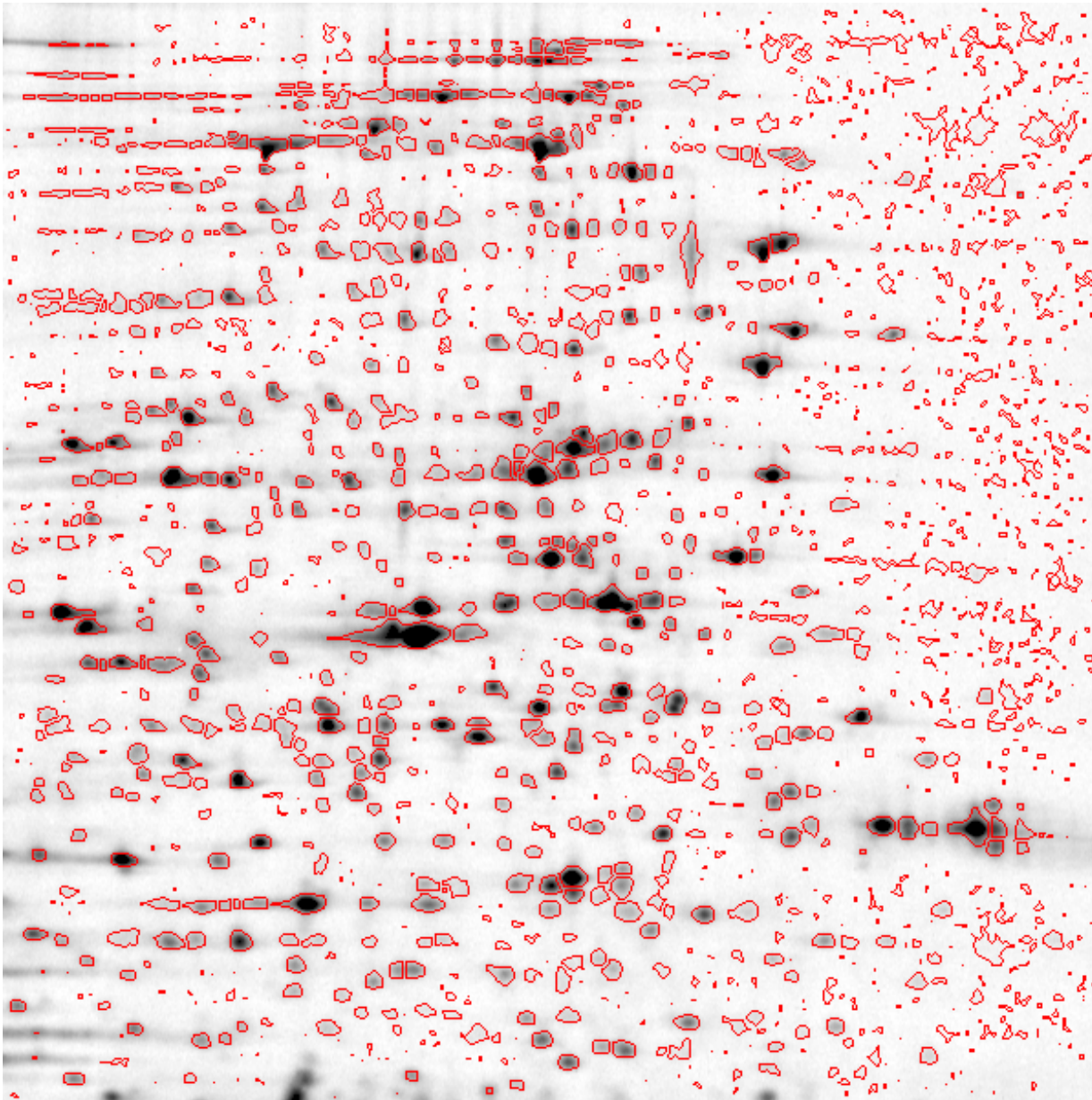
Spot Areas
Separated by
Intensity
Watershed

Adaptive Thresholding

1. Construct mosaic of maxima for each spot
2. Threshold each spot at a fraction of its maximum

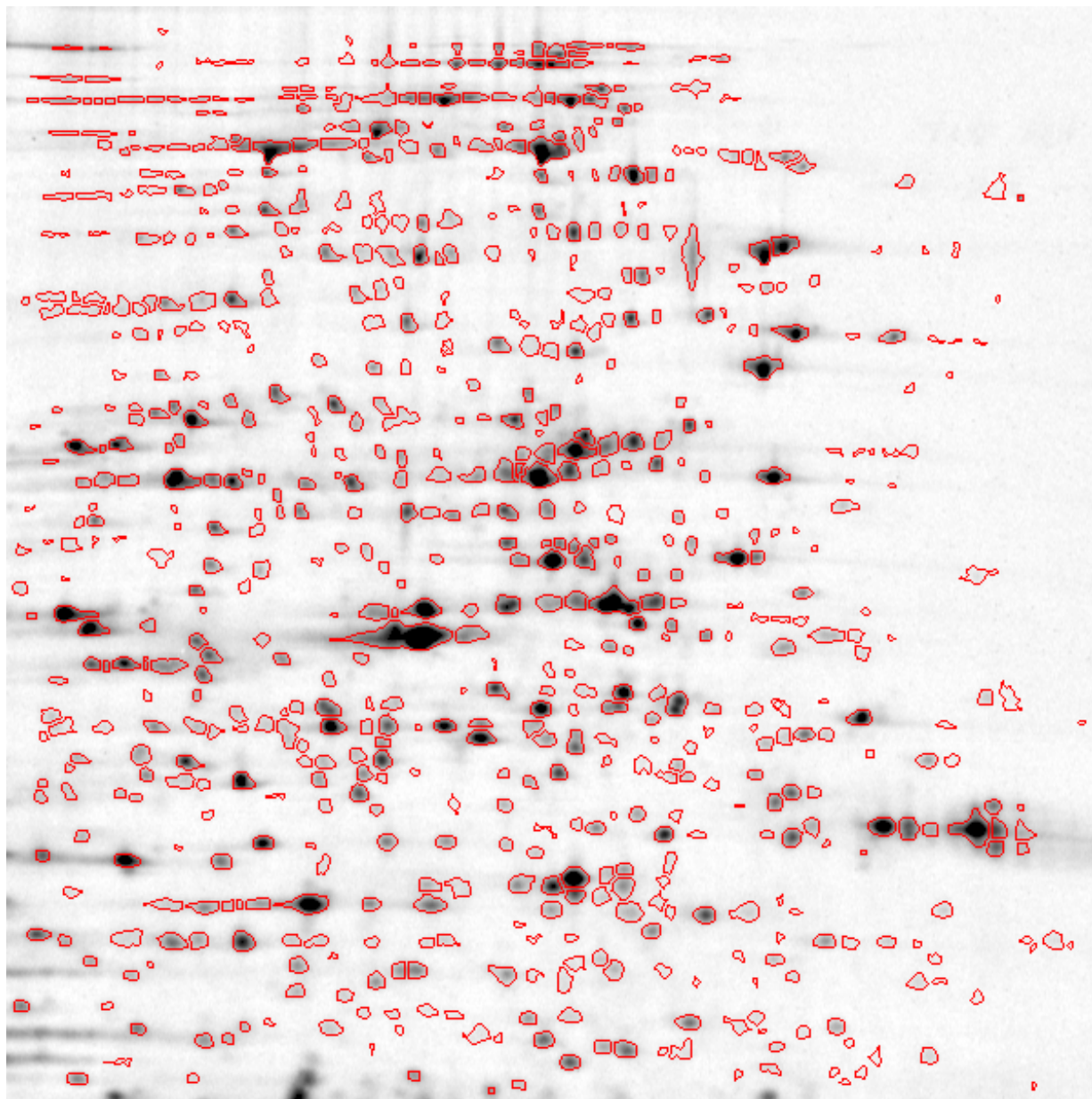


Spots
Individually
Thresholded

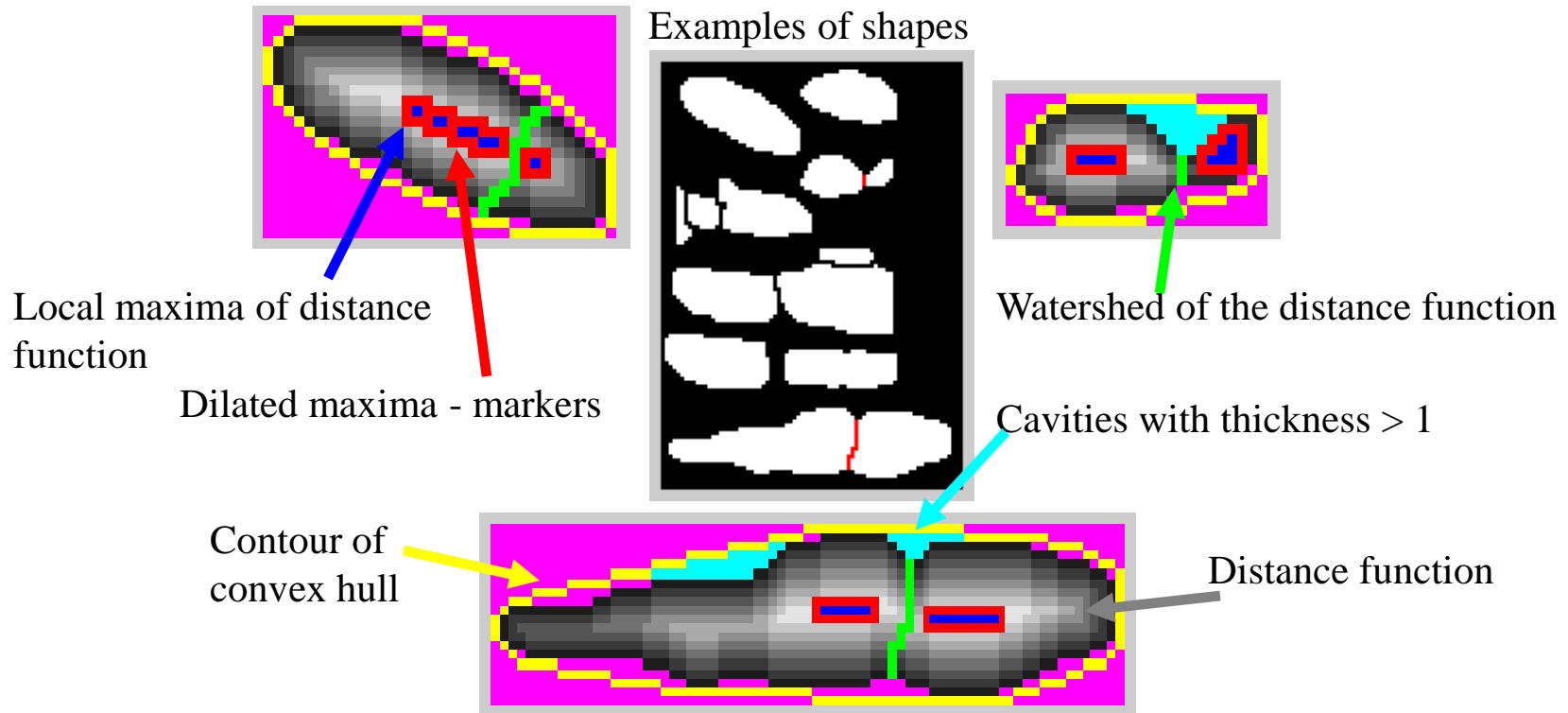


Filtering Spots

Spots with small area or low average intensity are removed



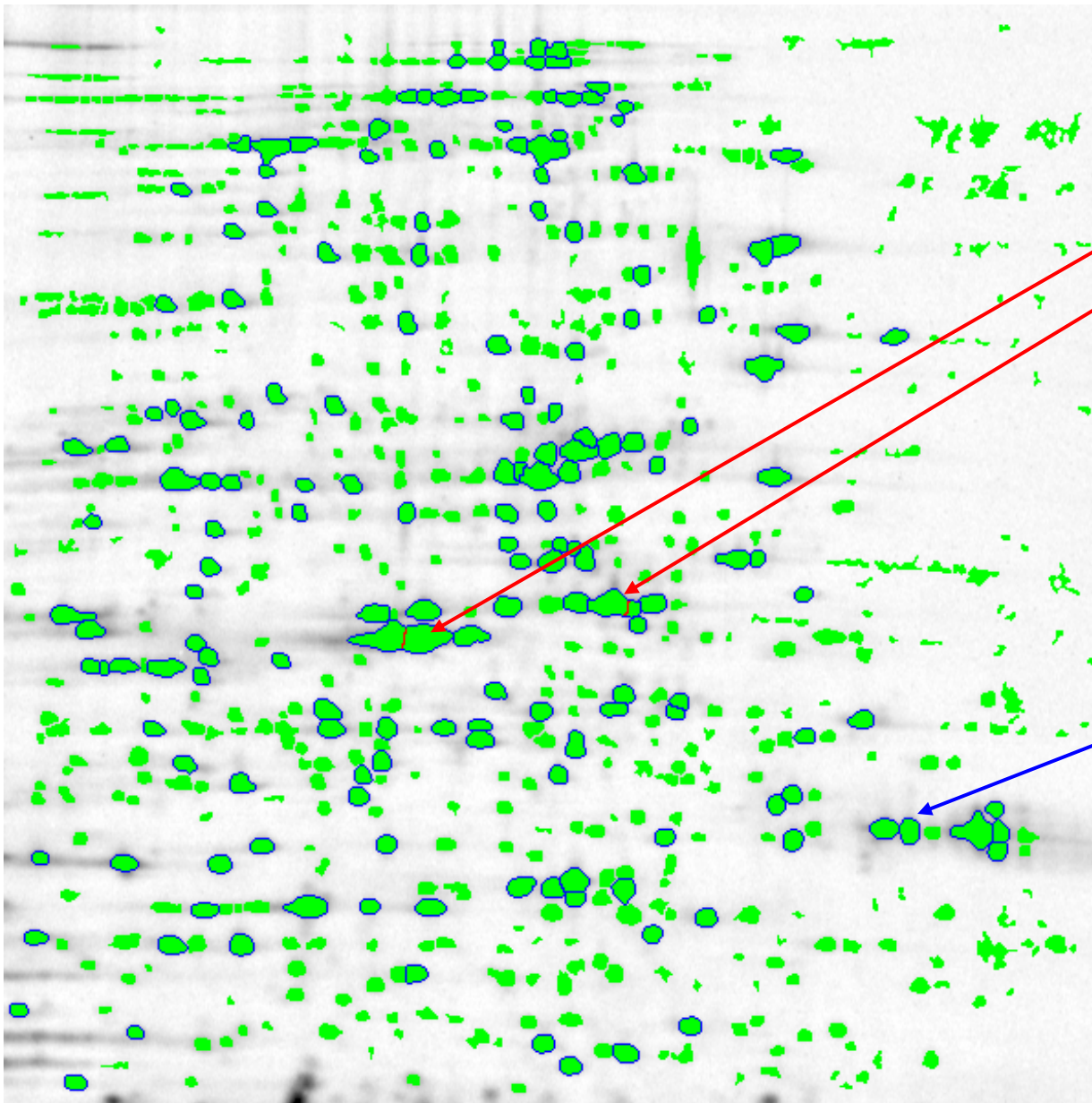
Shape Separation



Method:

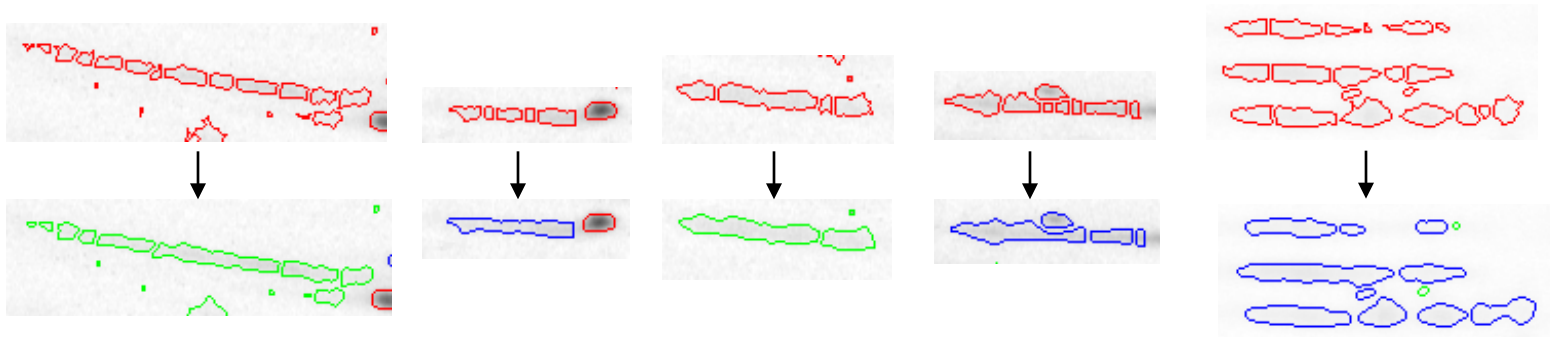
1. Distance function
2. Markers as dilated local maxima of the distance function
3. Watershed of distance function using markers
4. Convex hull of the shape
5. Cavities as difference between convex hull and the shape
6. Cavities with thickness > 1 .
7. Watershed lines touching filtered cavities

Spots Separated by Shape



Only spots more intense than the given threshold are considered for shape separation (shown here with blue outlines)

Merging of Low Intensity Spots into Streaks



Rows of low intensity adjacent spots are merged into streaks

1. Filter low intensity spots
2. Merge vertical lines by one step of thickening with

*	1	0	1	*
1	1	0	1	1
1	1	0	1	1
*	1	0	*	*
*	*	*	*	*

3. Add high intensity spots

Smoothing of Spot Contours



Original spots



Pruning endpoints with

0	*	1
0	1	*
0	0	0

and filling cavities with

1	1	*
1	0	0
1	1	*



Thickening towards convex hull with

1	*	0
1	0	0
1	1	*

5 steps

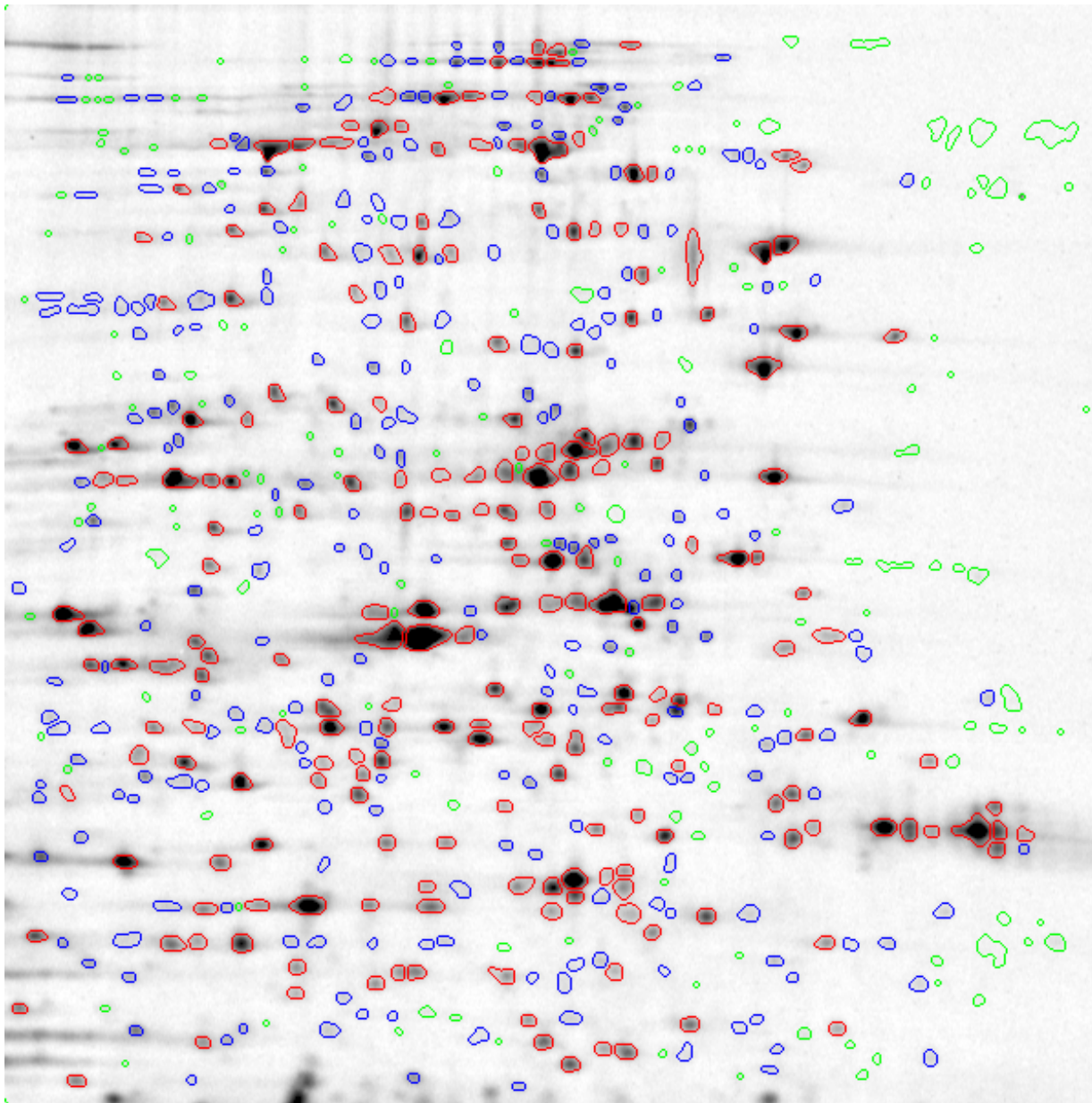
10 steps




20 steps

40 steps

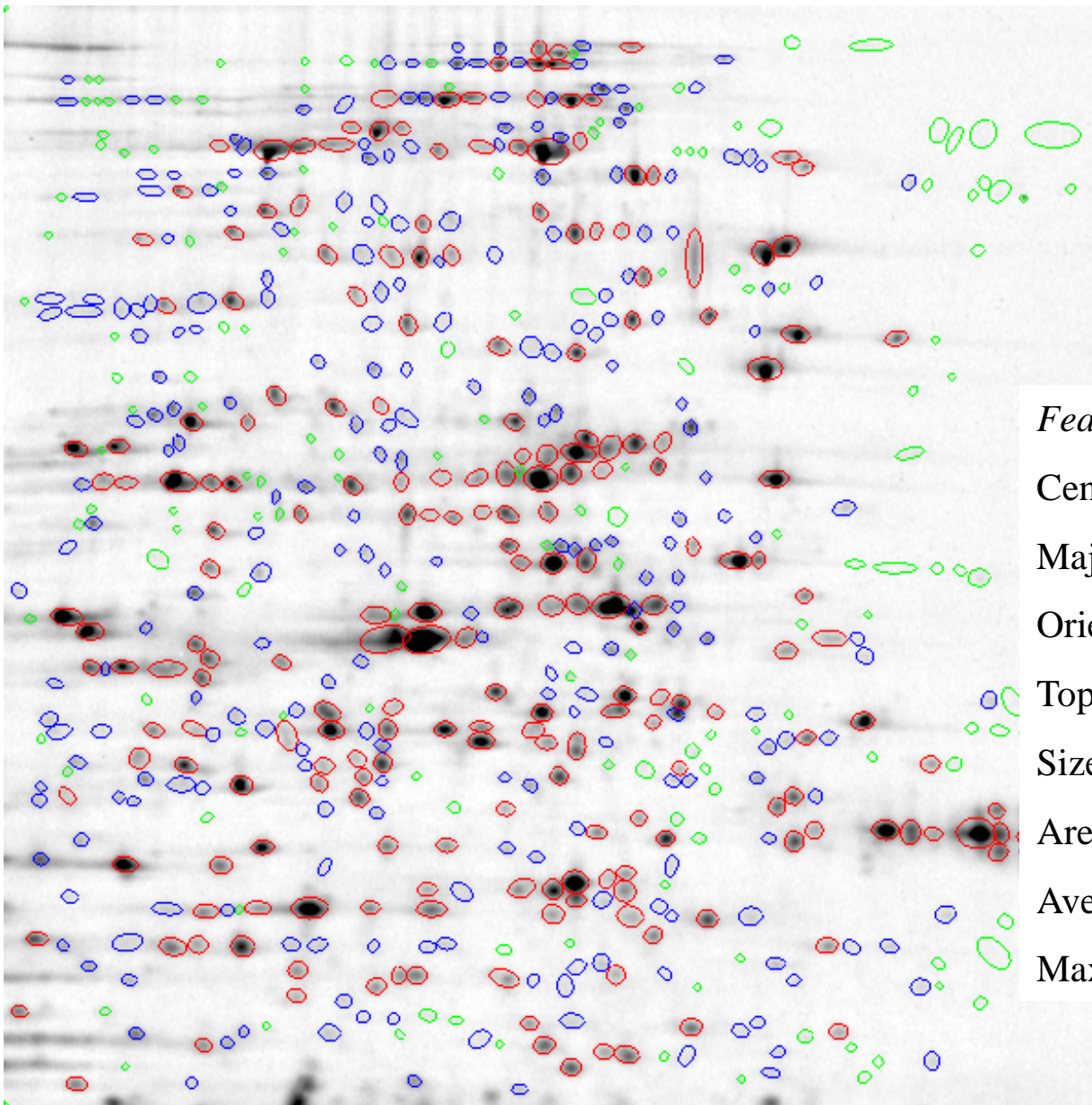
saturation

Filtration Into Classes of Spots by Intensity



-  Strong spots
-  Intermediate spots
-  Weak spots

Spot Table and Representation by Ovals



Features in the spot table:

Center of mass of the spot

Major and minor axes of the spot

Orientation of major axis of the spot

Top left corner of bounding box

Size of bounding box

Area of the spot

Average intensity of the spot

Maximum intensity of the spot