Spot Image Analysis V5R8

info.txt in the images directory (example), new for R7 and R8 parameters in red

gridy 6
gridx 8
refx 1 (use knowledge of reference spots in X)
ngroups 16
group 1 2 3 4 5 6 7 8 1 2 3 4 5 6 7 8 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 9 10 11 12 13 14 15 16 9 10 11 12 13 14 15 16
groupnames GRP1 GRP2 GRP3 GRP4 GRP5 GRP6 GRP7 GRP8 GRP9 GRP10 GRP11 GRP12 GRP13 GRP14 GRP15 GRP16
skippoints 1
normpoints 3 (normalization of plots is done to the average of normpoints following skippoints; default = 1)
trendpoints 5 (Removal of linear trend if trendpoints>=2. Trendpoints after skippoints are used to estimate the trend.)
basepoints 0 (use as base points for curve analysis. If 0 – finds automatically)
plotgrp 2 3 4 5 6 7 8 9 10 11 12 13 14 15
~~date DD-MON-YYYY~~ (removed)
copy_number COPY NUMBER (used in titles)
target TARGET (used in titles)
chipbottom CHIPBOTTOM (used in titles)
~~assay ASSAY 1~~ (removed)
gtolerance 50
ringw 8
ringg 4
minspotarea 500
maxspotarea 2500
nominalspotdiam 60
asnominallim 0.60
cocentrlim 0.25
solidlim 0.80
bgrmedian 1 (median or mean as estimate of background: 0-mean, 1-median, default – mean)
quantile 0.85 (quantile for first thresholding after background removal)
times_sd 10
gamma 0.7 (grayscale remapping after background removal, lower for dim spots)
votingmasks 0.8 (controls calculation of the union of masks from individual images: 0.0 – spot is where at least one mask is present, 0.99 – spot is where all masks are present; default 0.8)
figs 1
cfigs 0
histfigs 0
ppt 1

launchcompiled.bat

C:\Users\ilya\Documents\MATLAB\NVS\NVS_V5\distrib\NVS_V5R1.exe
C:\Users\ilya\Saphicon\NVS\Images
pause

Root

Algorithm at a Glance - 1

For each directory of the directory tree under Root:

Create subdirectory for results of the current analysis run

First pass through all files – spot finding {

Read image

Adjust contrast to full range

Remove background

Remap with gamma

Find adaptive threshold as quantile of histogram and make spot mask

Modify spot masks:

fill by majority, remove small components, fill holes,

for large components repeatedly raise threshold for possible acceptance,

smooth spot contours

Filter spots by shape

Find grid from projections of intensity image masked by spots and remapped with gamma for the second time

Analyze by spots and leave only if they satisfy given limits: gtolerance, asnominallim, cocentrlim, solidlim

Write results table

}

Find the best image and extract its results

Find union of all spots in all images (with shifts)

for best image and each current image find the best pair of spots to determine shift, record shifts for the second pass

add current mask with shift to accumulator

Find final mask where >= 80% of existing masks agree

Find grid from projections of accumulated masks

Renumber spots by rows and columns

Form label markers: reconstruct labels for spots and form labels for rings

Algorithm at a Glance - 2

Second pass through all files – spot measurement {

Read image

Retrieve shifts and shift the label images for spots and rings

Measure:

integrated intensity in spots, integrated intensity above background in spots,

average intensity above background in spots, average intensity in rings (negative values are forced to zero)

}

Write results for spots

Average by groups, write and plot normalized (to the first non-skipped point) results

Analyze cycle curves for individual spots by groups (skipping a given number of points)

Align by regression analysis all curves from different spots within one group

Remove linear trend using trendpoints points for the estimate

Find by ANOVA the first run of non-significantly-different points

For these points find average, SD, and threshold level = average + SD * times_sd

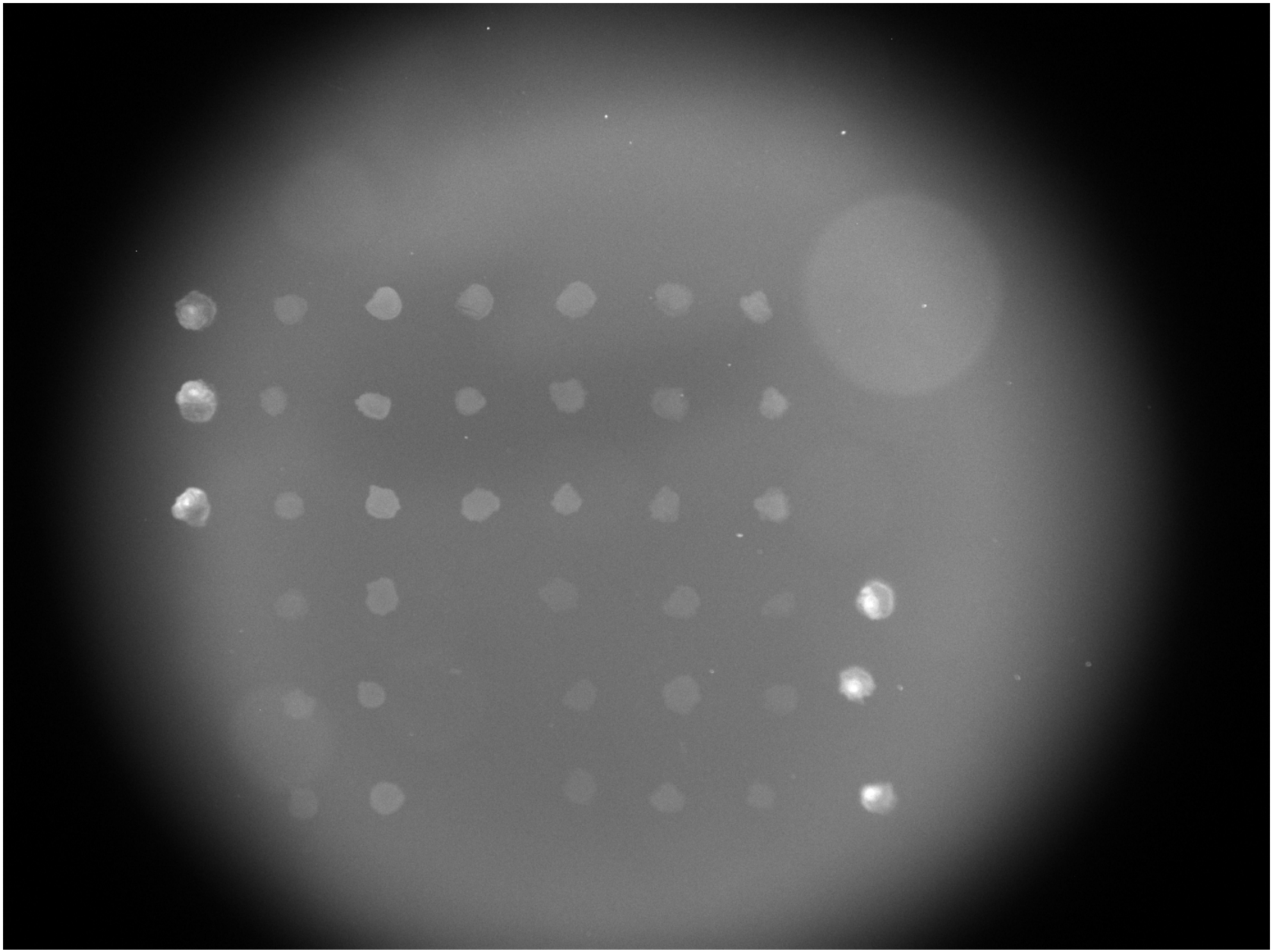
Find cycle threshold where threshold level intersects the curve using polynomial approximation (power=4)

Write threshold results

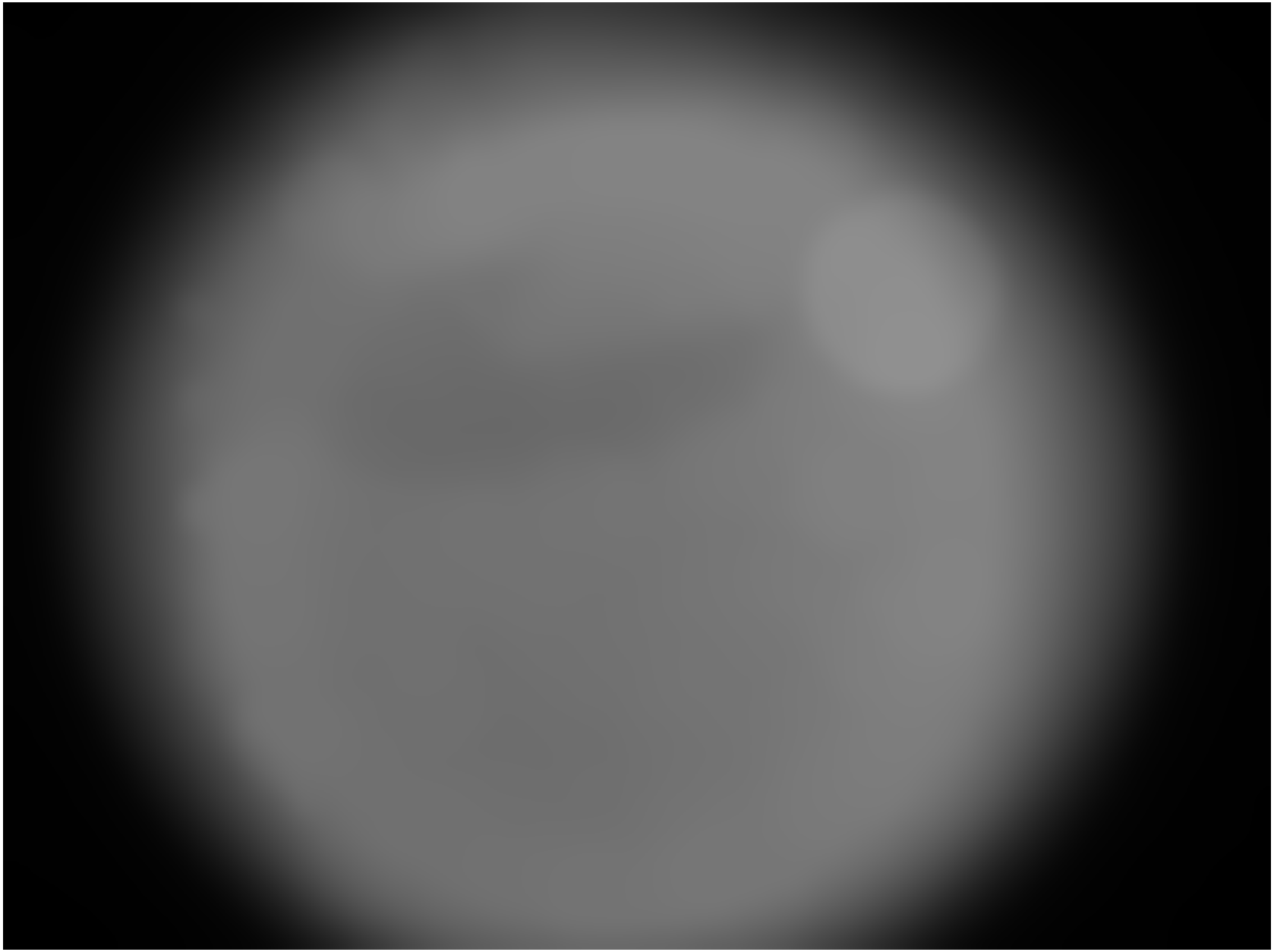
Automate PowerPoint to create a presentation with figures and data

Original

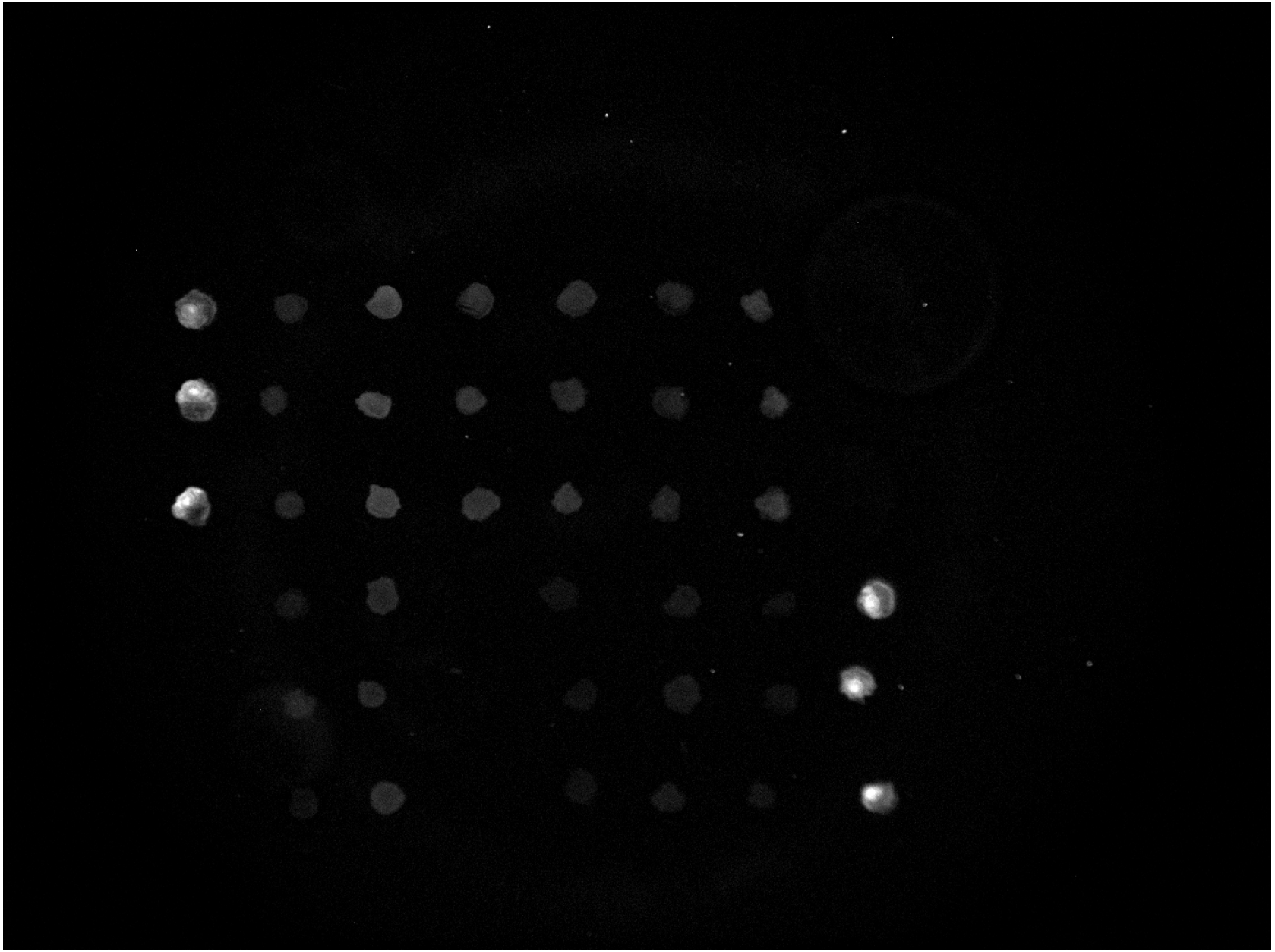
Contrasted



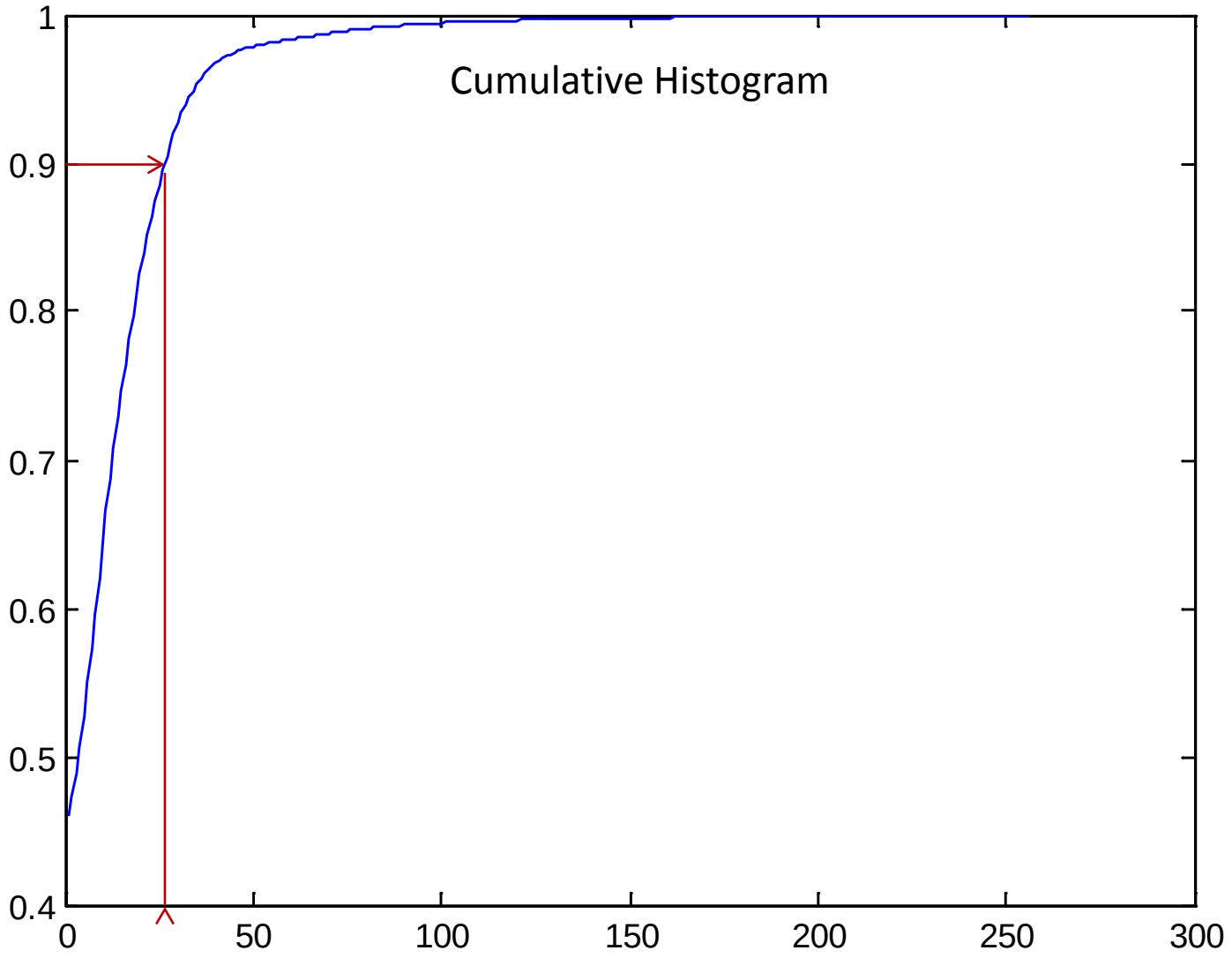
Background



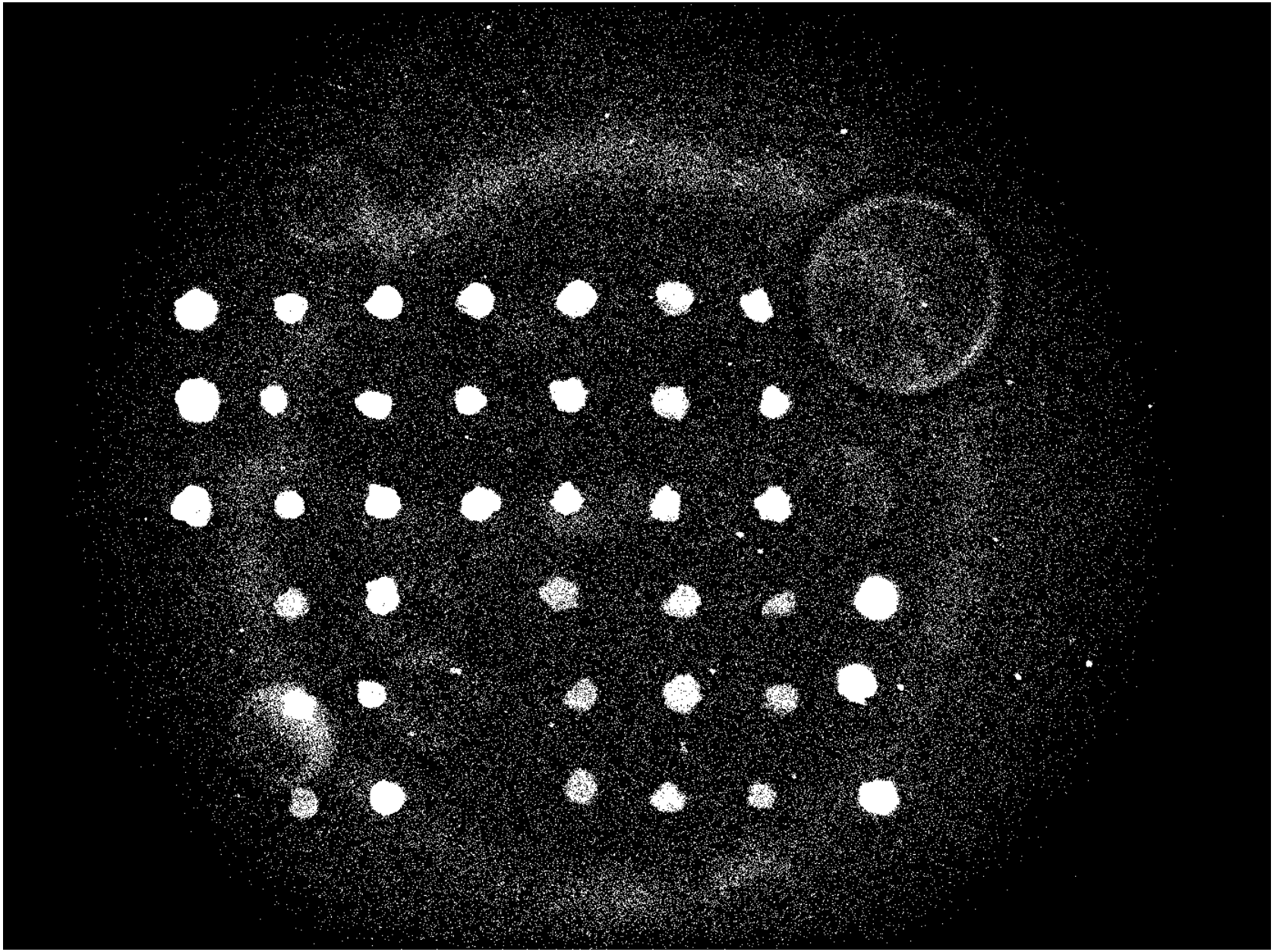
After Background Removal and Second Contrasting



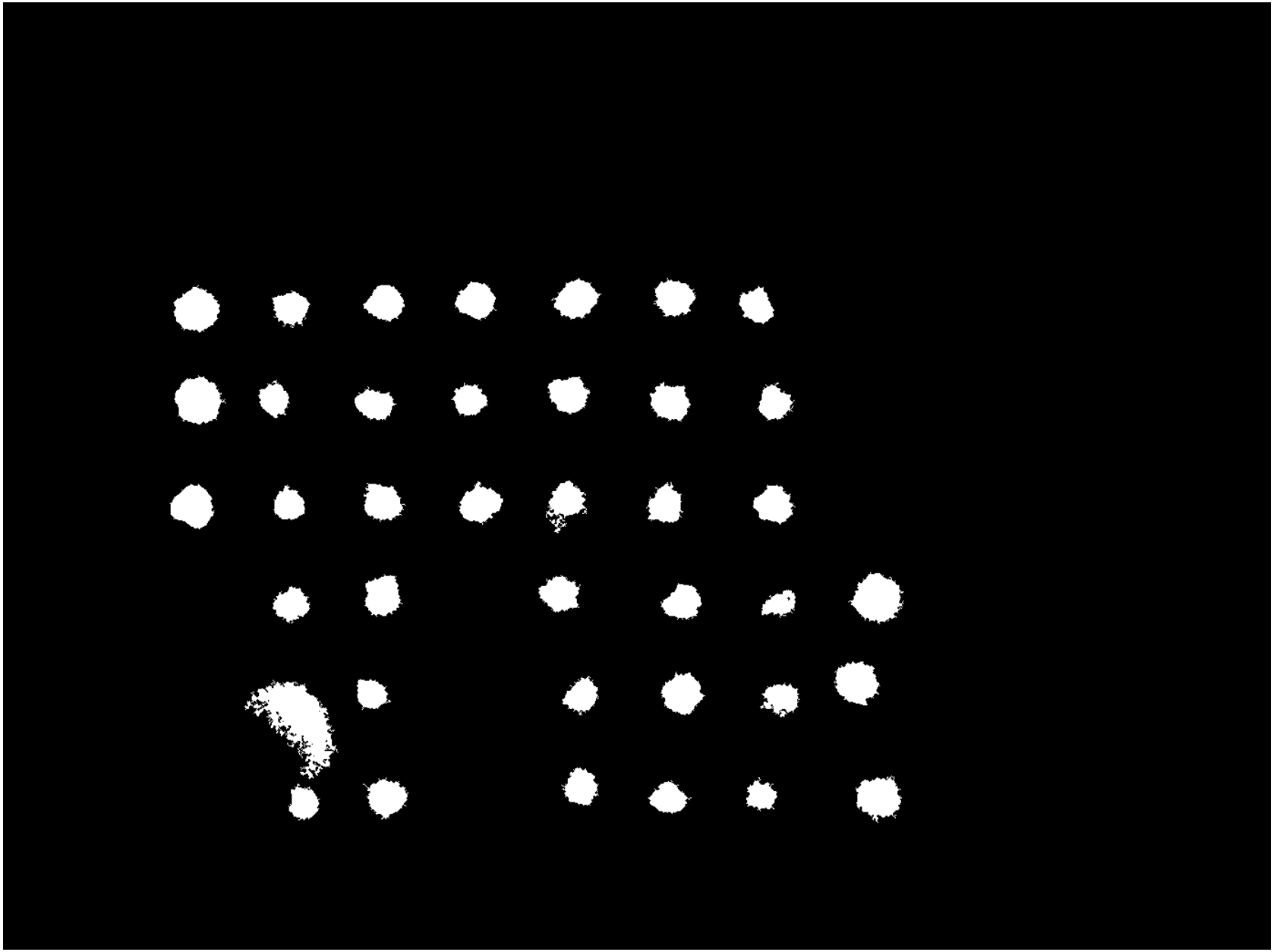
Adaptive Threshold as Quantile of Histogram



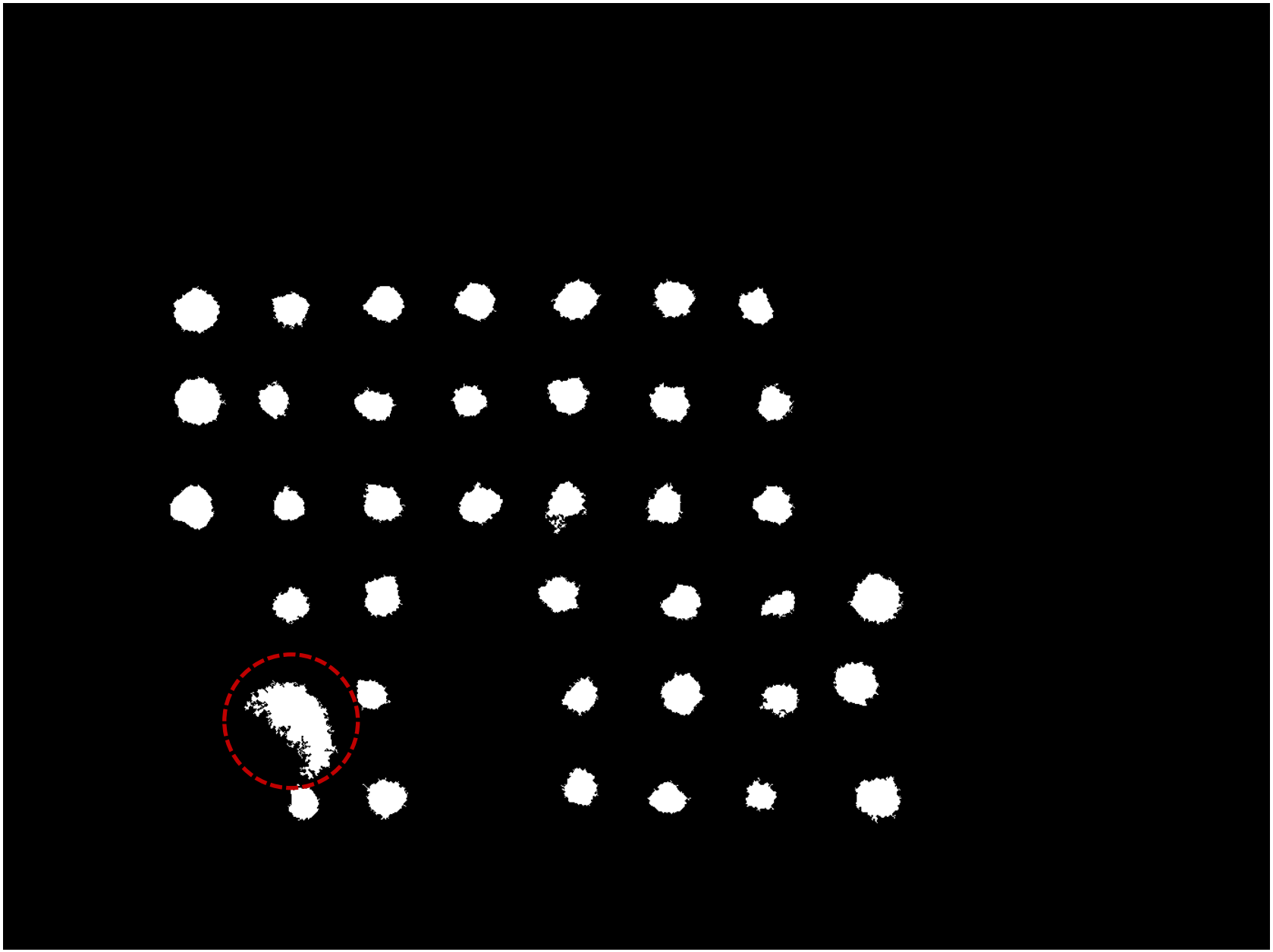
Raw Spot Mask after Thresholding



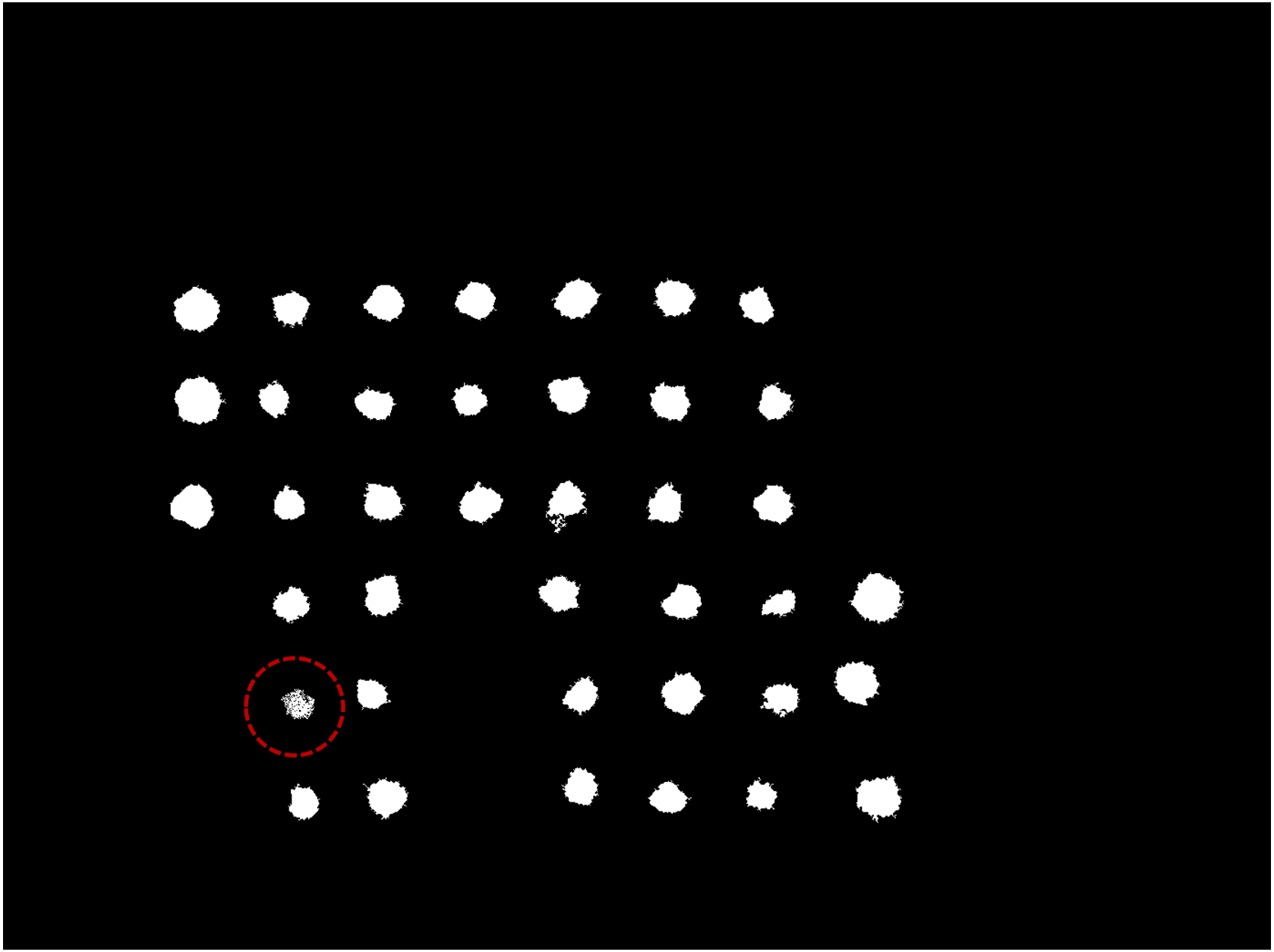
Mask after Removing Small Components



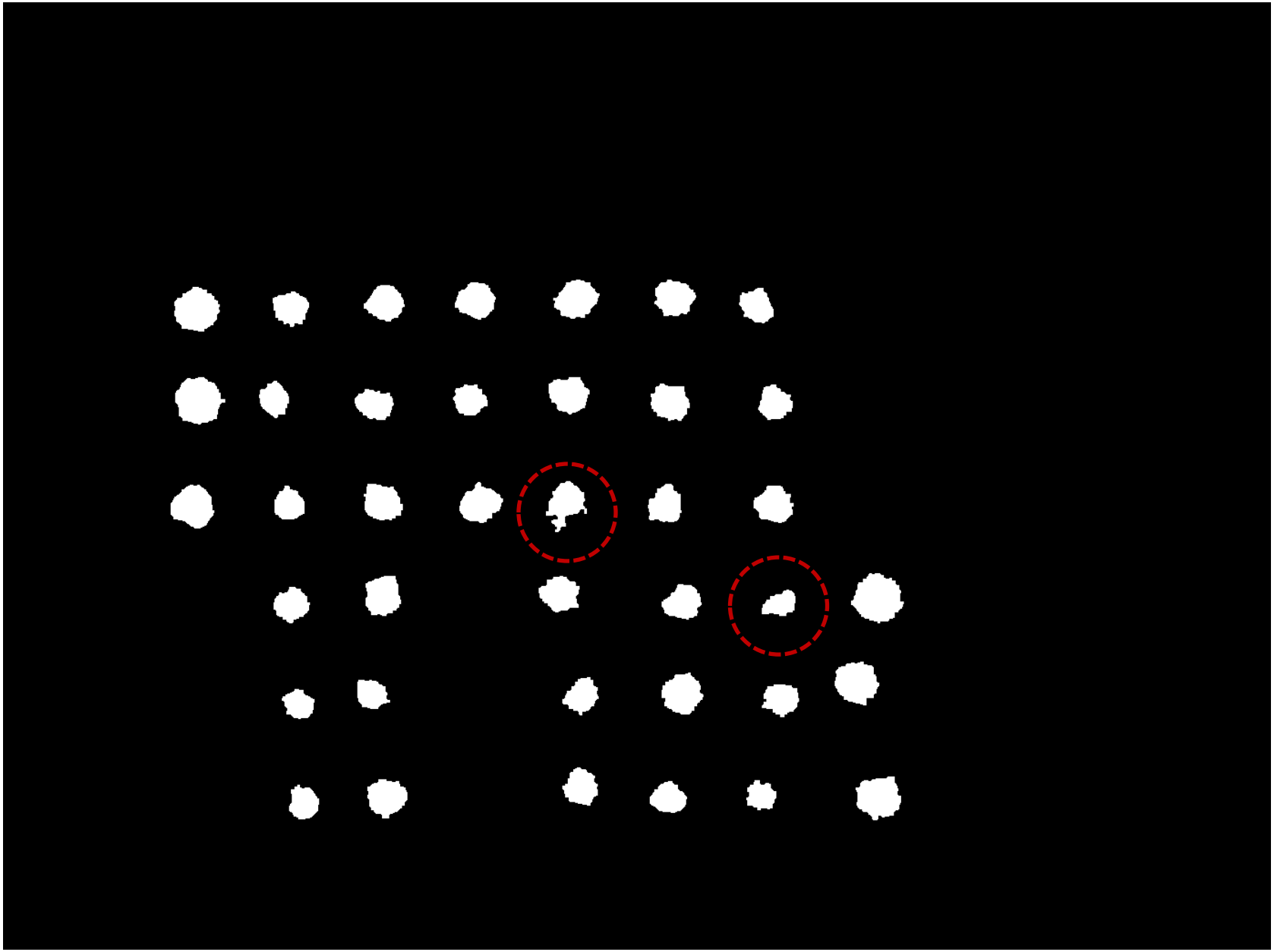
Mask after Filling Holes



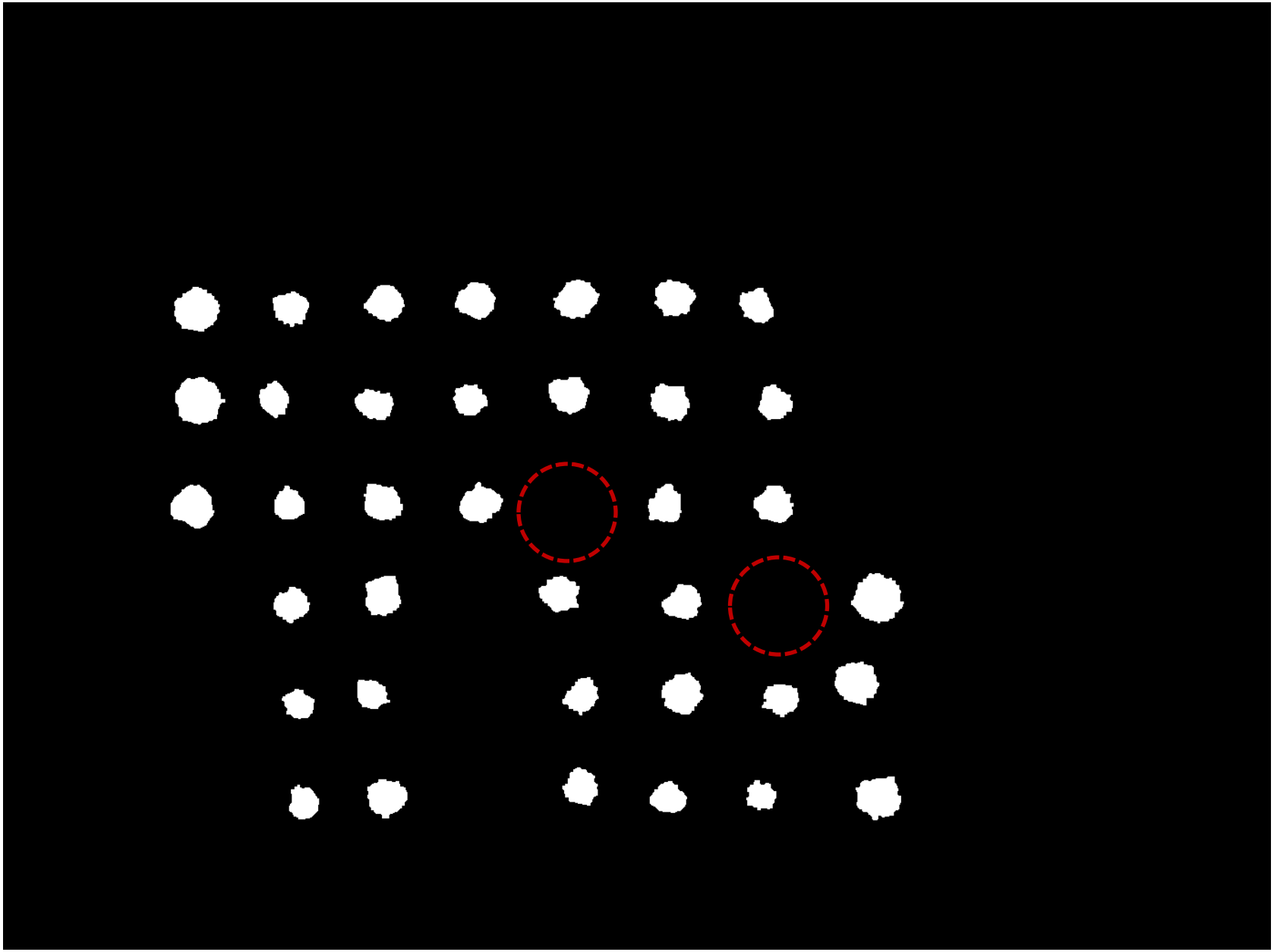
Mask after Raising Threshold in Large Components



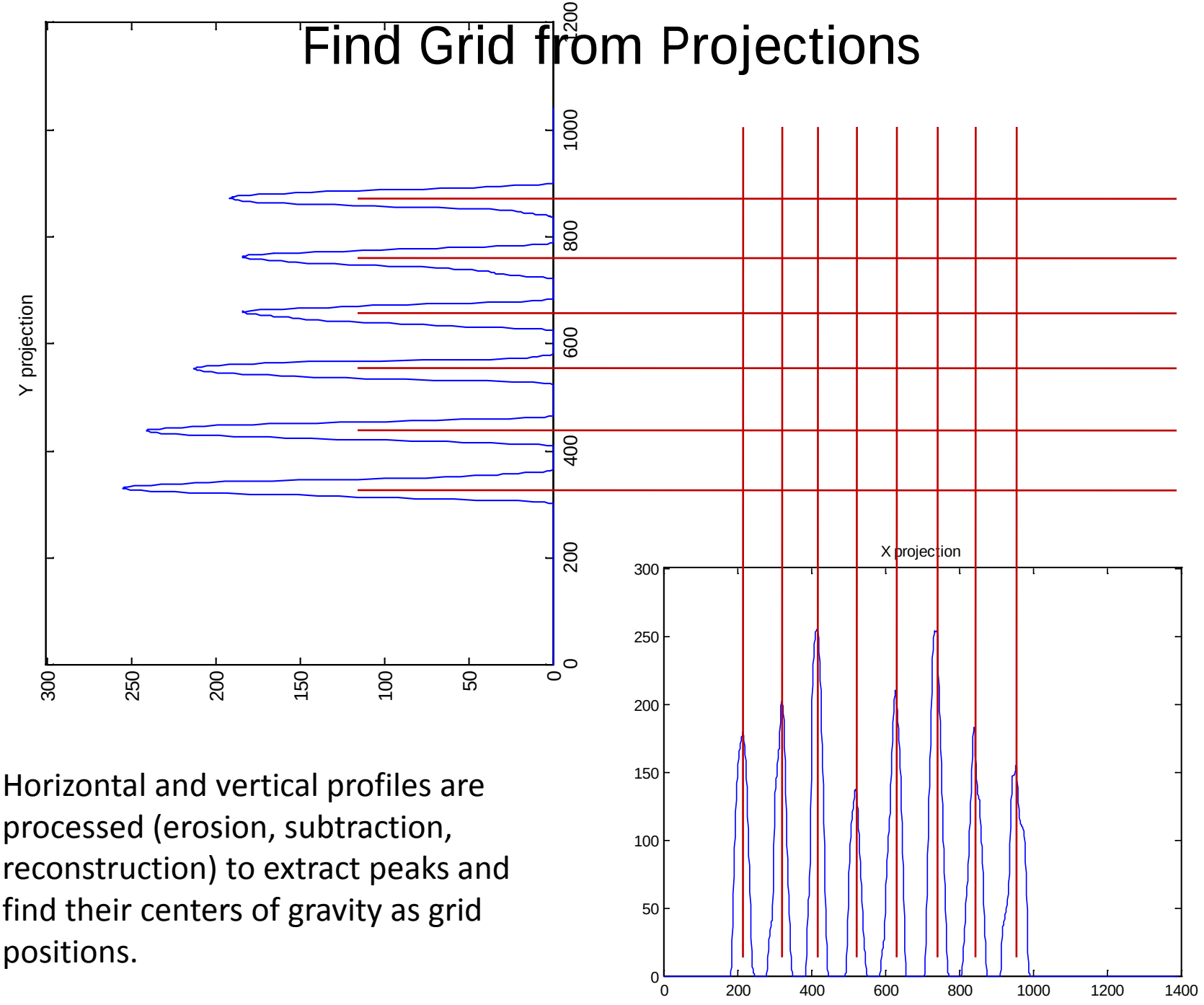
Mask After Smoothing Spot Outlines



Mask After Filtering Spots by Shape

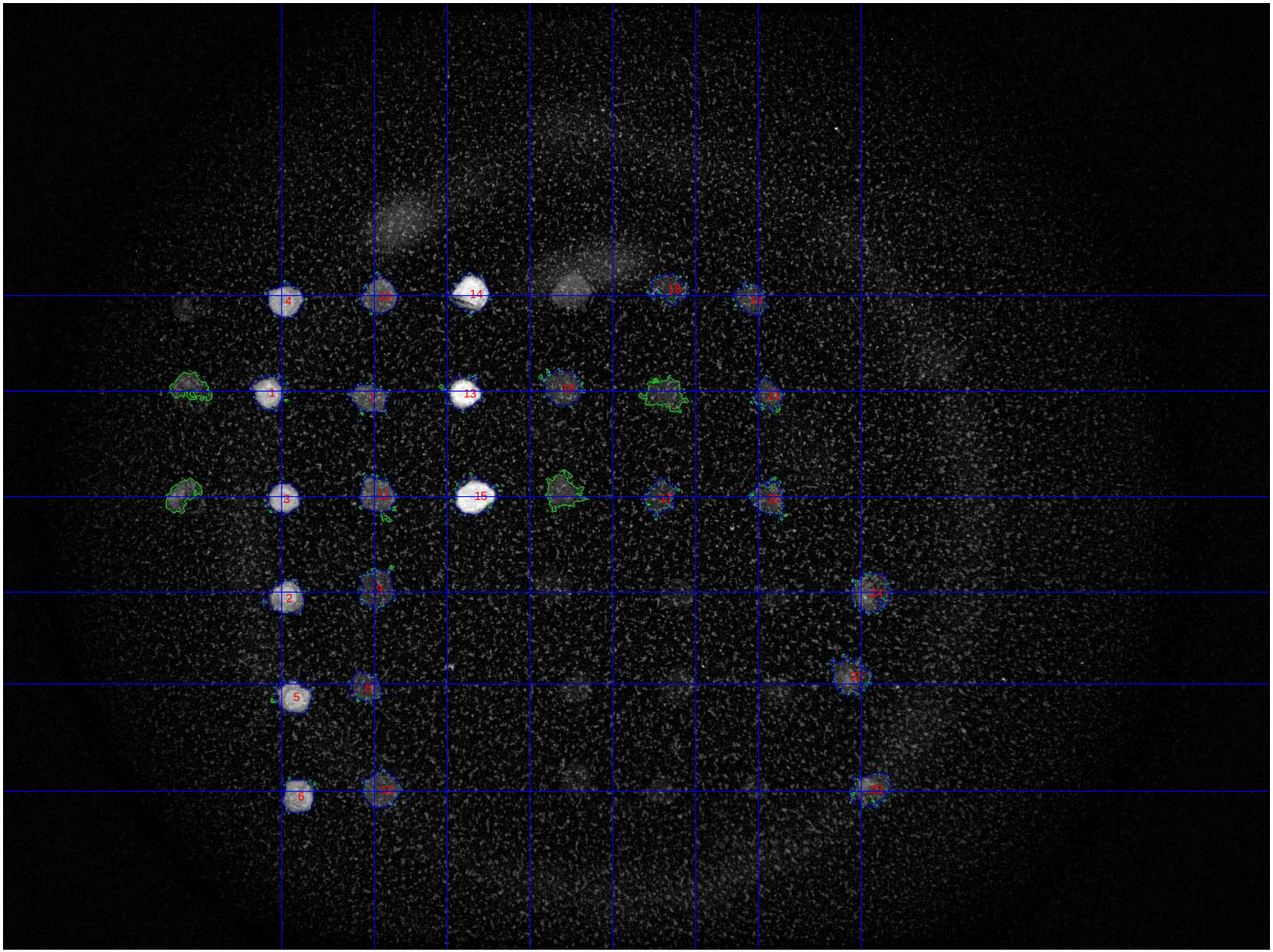


Find Grid from Projections



Horizontal and vertical profiles are processed (erosion, subtraction, reconstruction) to extract peaks and find their centers of gravity as grid positions.

Formation of Grid if Not Enough Spots



Best Pair of Spots to Determine Shifts

Algorithm of finding shifts between the best image and current image:

1. For each spot of the best image find minimal shift distance to spots of the current image
2. Take a few spots giving low distance
3. Choose among them the spot with the best quality measure (brightness and roundness)
4. Record shift from this spot

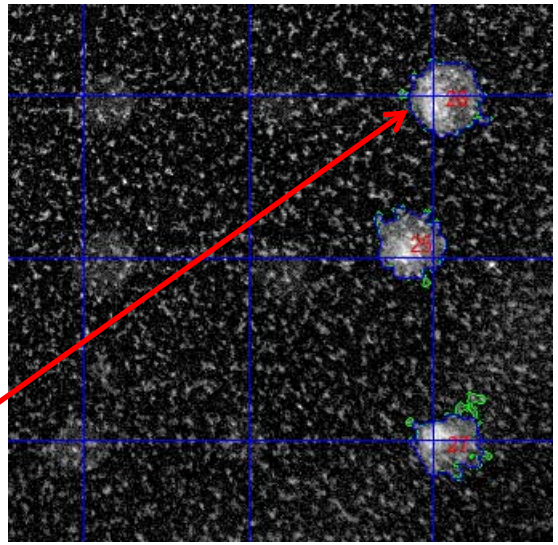
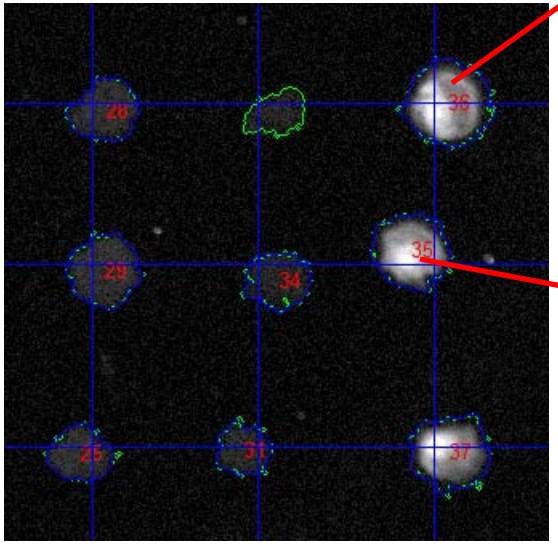


Image #6



Best image - #1

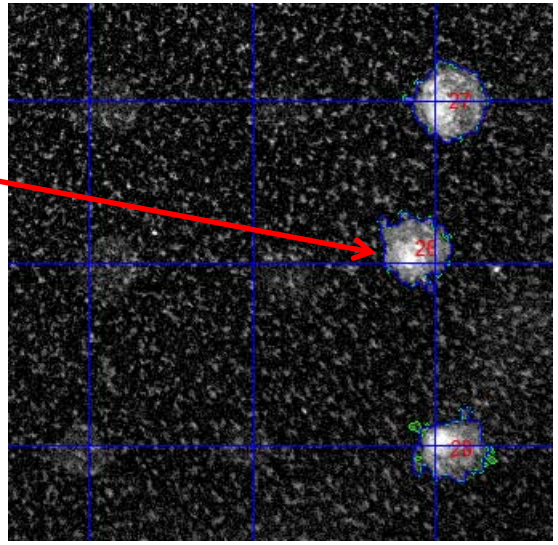
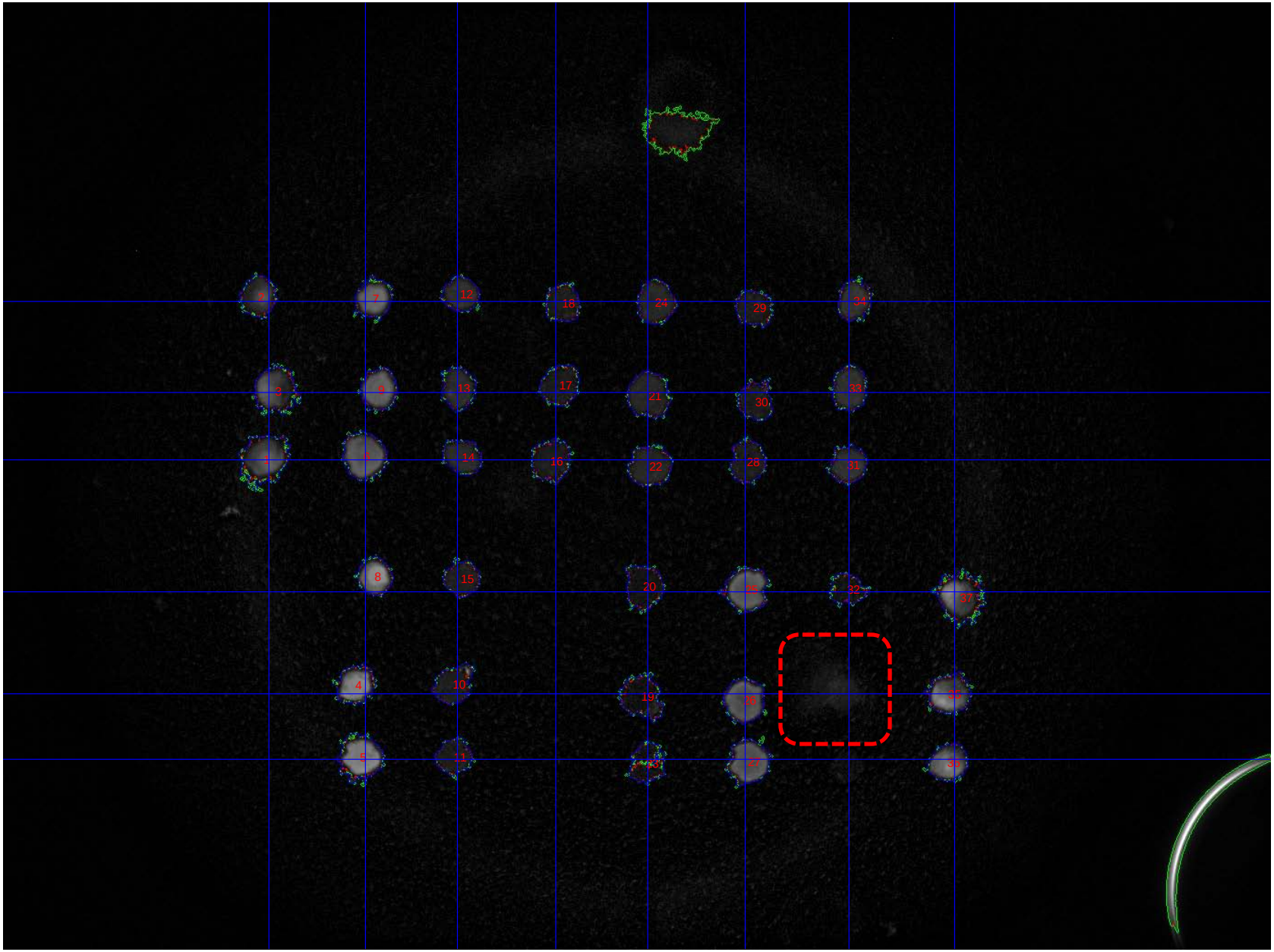


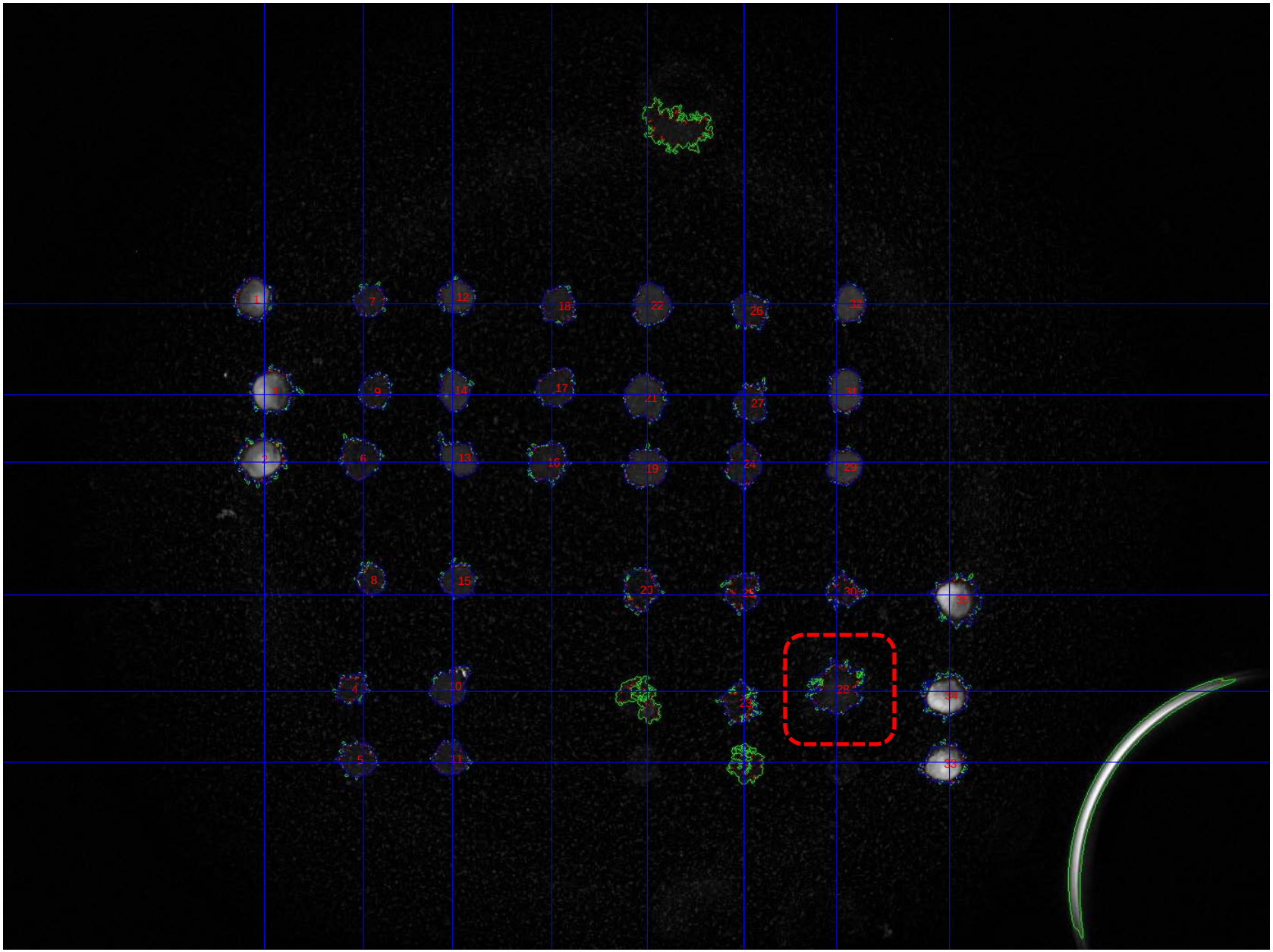
Image #2

All Masks - Voting

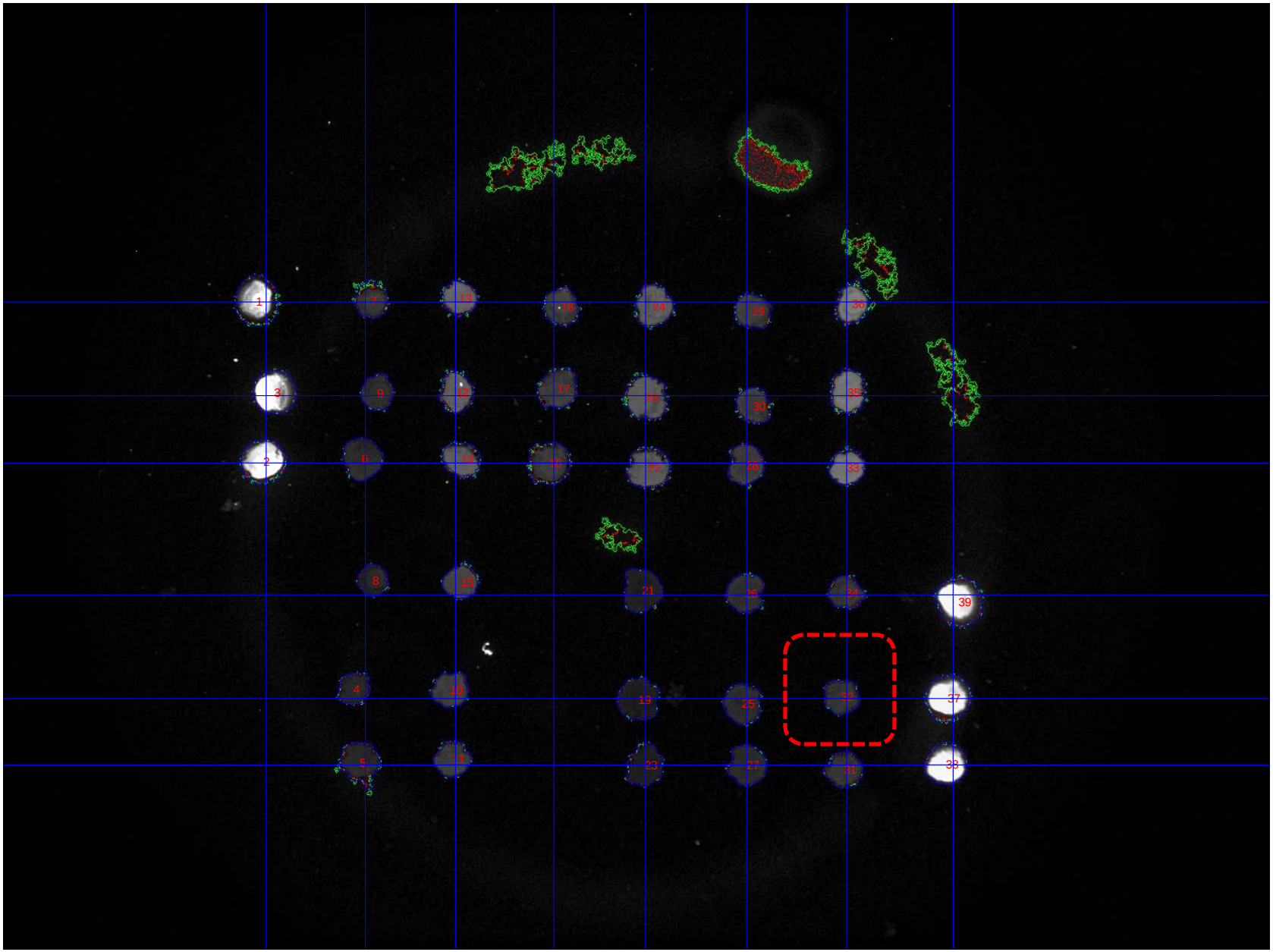
Mask Voting 1: Spot Not Detected



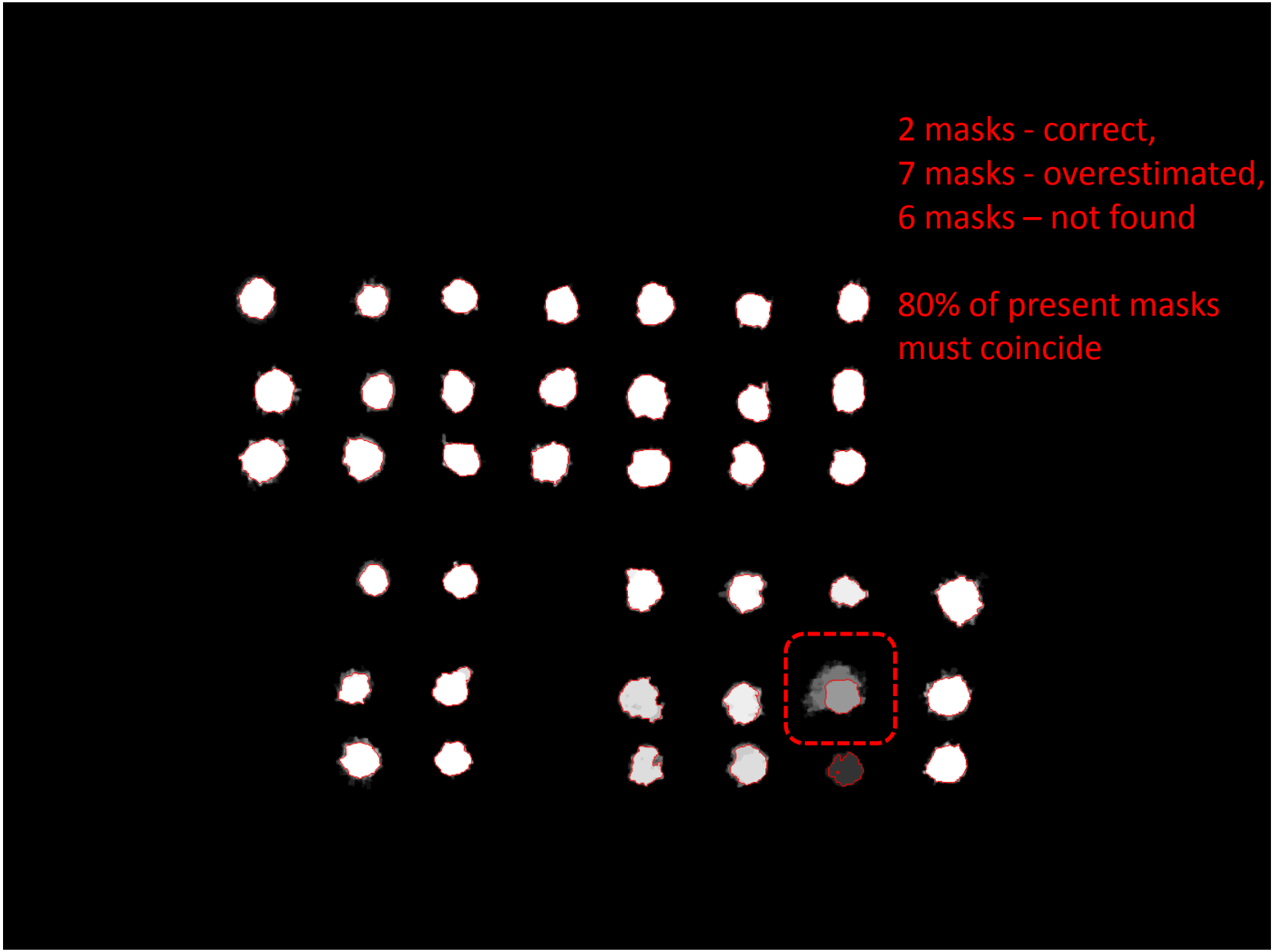
Mask Voting 2: Spot Overestimated



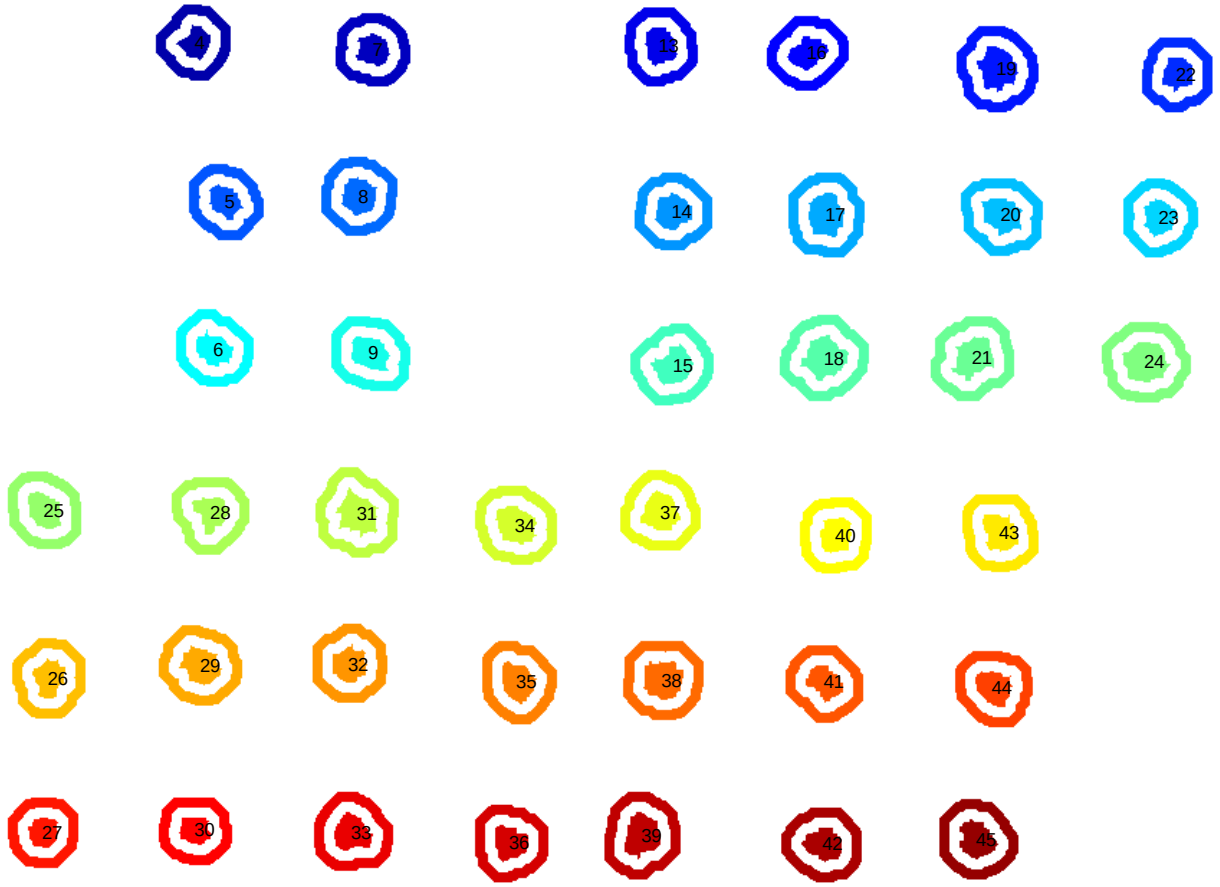
Mask Voting 3: Spot Estimated Correctly



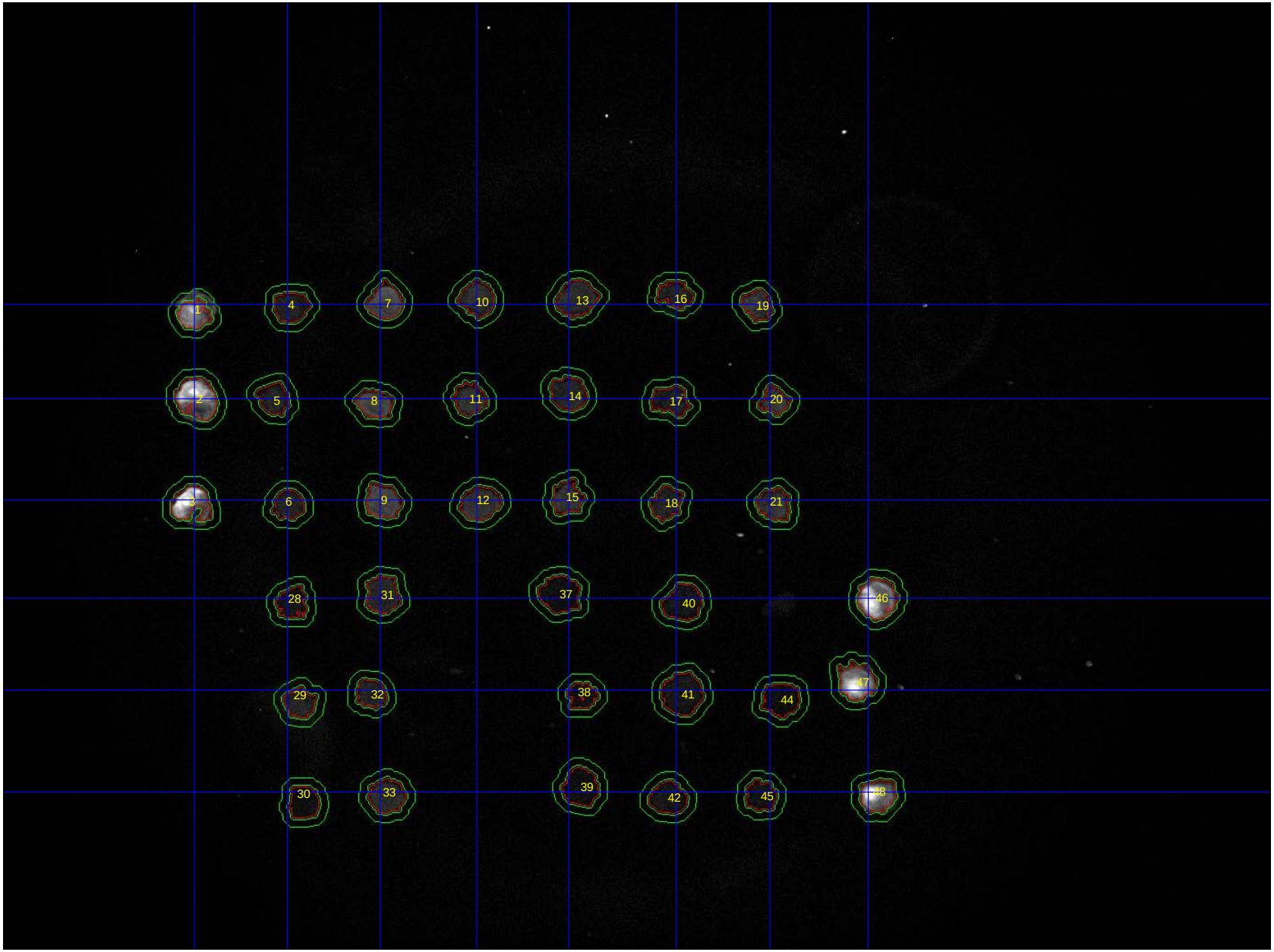
Mask Voting 4: Result



Measurement Masks: Spots & Rings



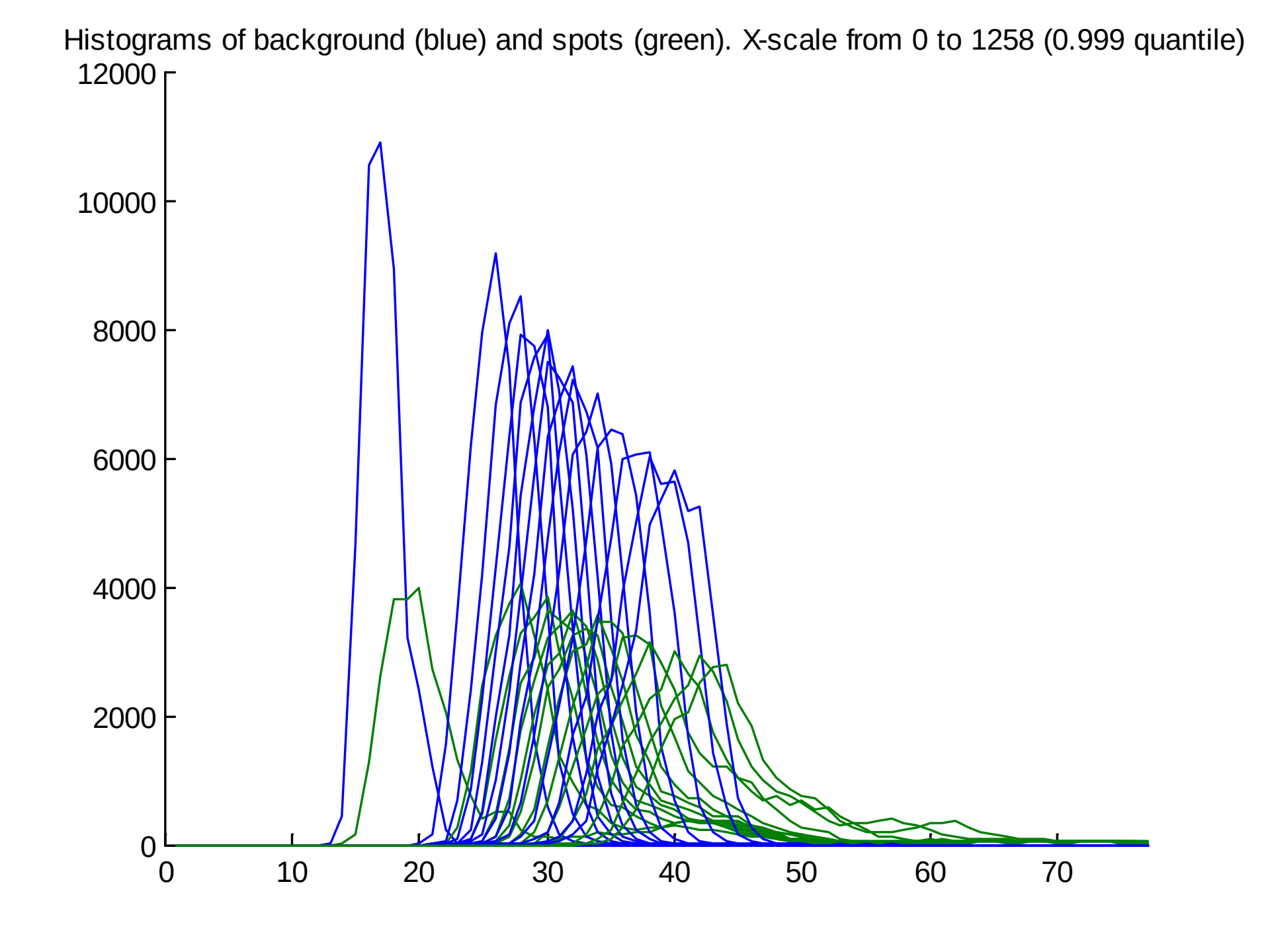
Summary of Spot Finding



Shifts
(between best image and each image)

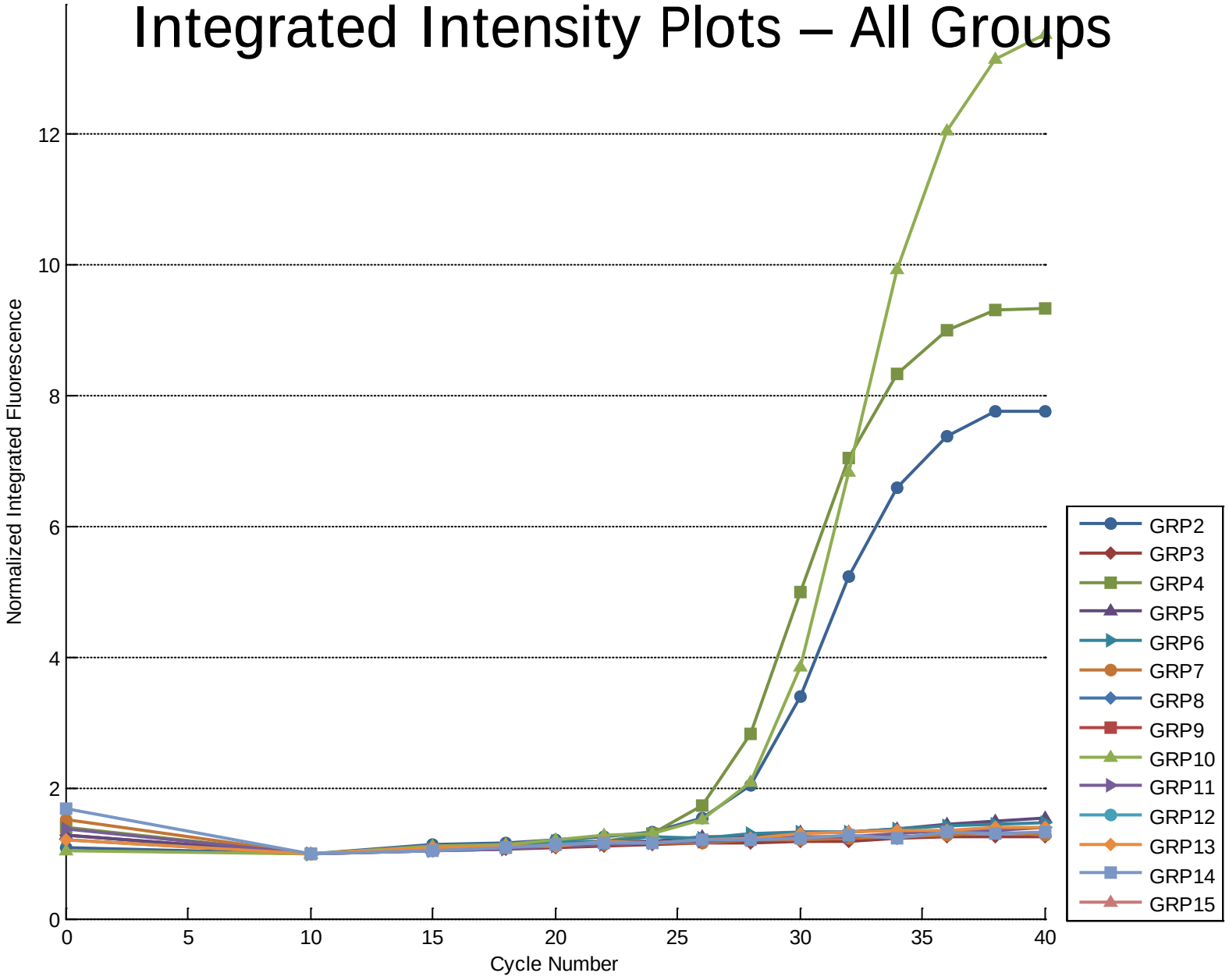
Best Image →

Y-shift	X-shift	Spot Y	Spot X
0	0	382	306
-23	-1	717	1046
-26	-2	376	720
-28	-2	717	1046
-30	-3	382	306
-32	-3	378	954
-31	-5	717	1046
-34	-4	382	306
-34	-5	382	306
-35	-7	382	306
-36	-7	382	306
-36	-8	382	306
-36	-8	717	1046
-38	-7	476	314


Histograms of background (blue) and spots (green). X-scale from 0 to 1258 (0.999 quantile)
12000
10000
8000
6000
4000
2000
0
0
10
20
30
40
50
60
70

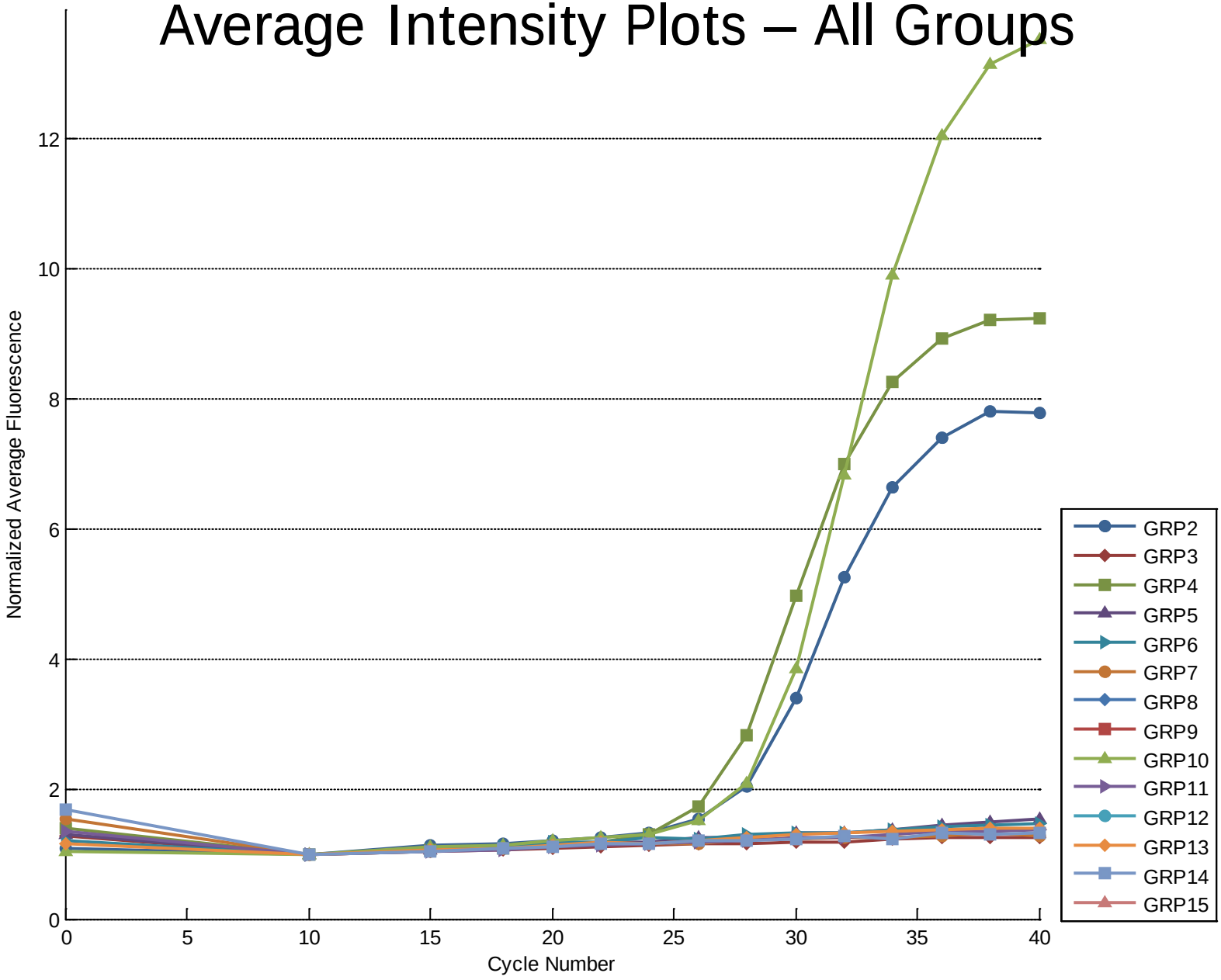
DD-MON-YYYY, COPY NUMBER, ASSAY 1

Integrated Intensity Plots – All Groups

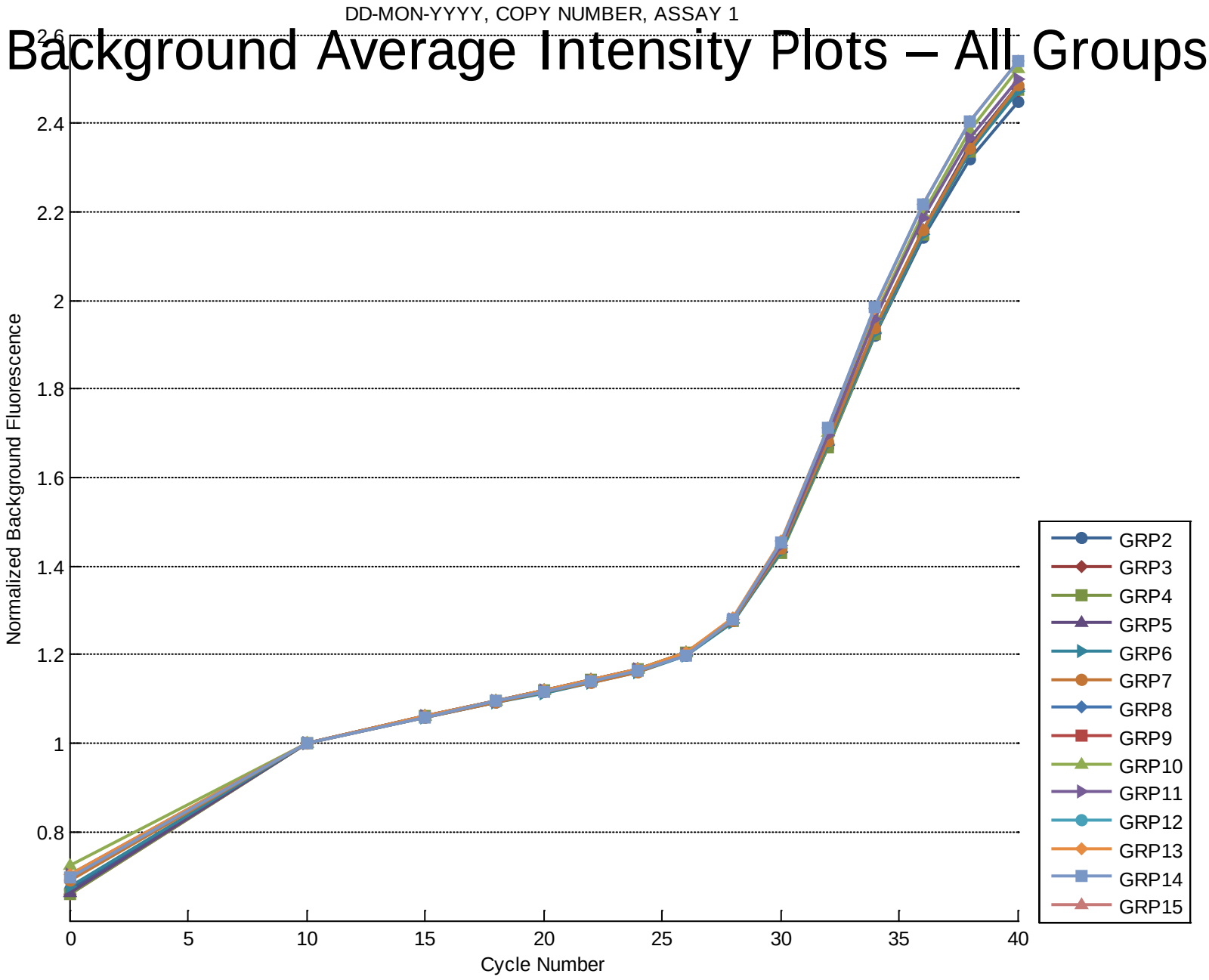


DD-MON-YYYY, COPY NUMBER, ASSAY 1

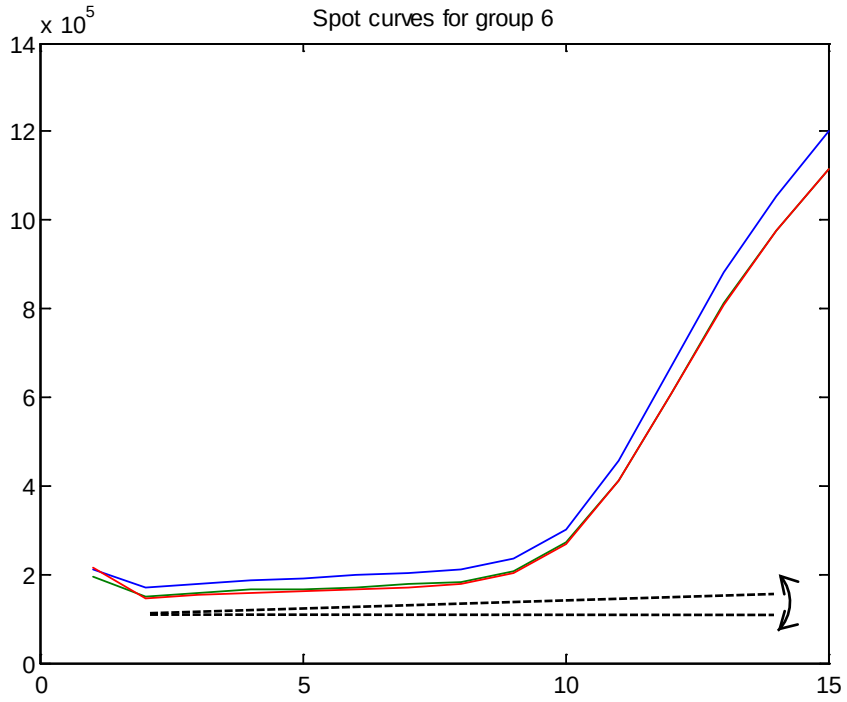
Average Intensity Plots – All Groups

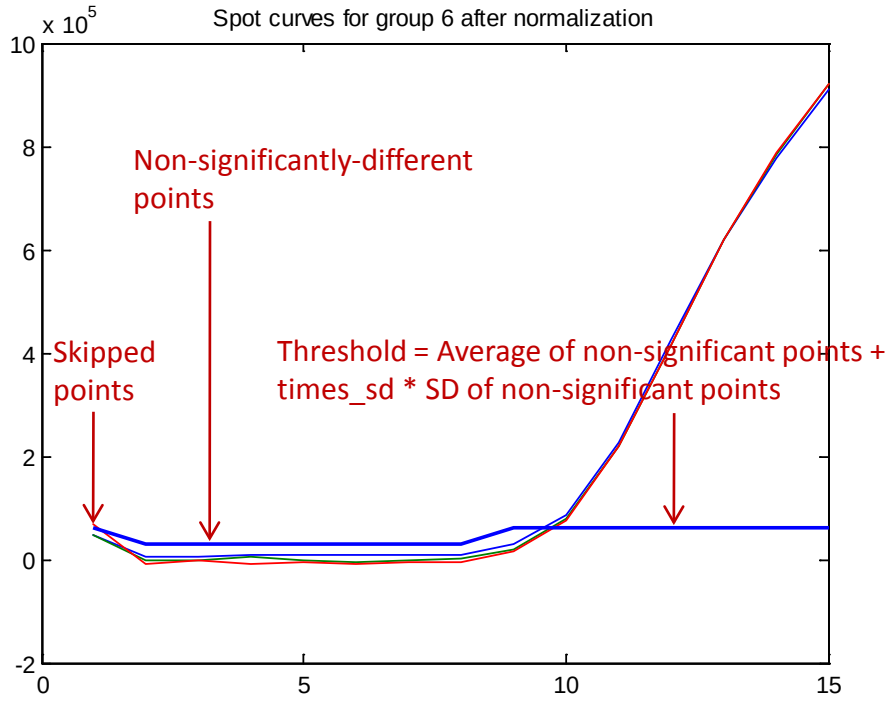


Background Average Intensity Plots – All Groups



Intensity Curve Normalization and Analysis

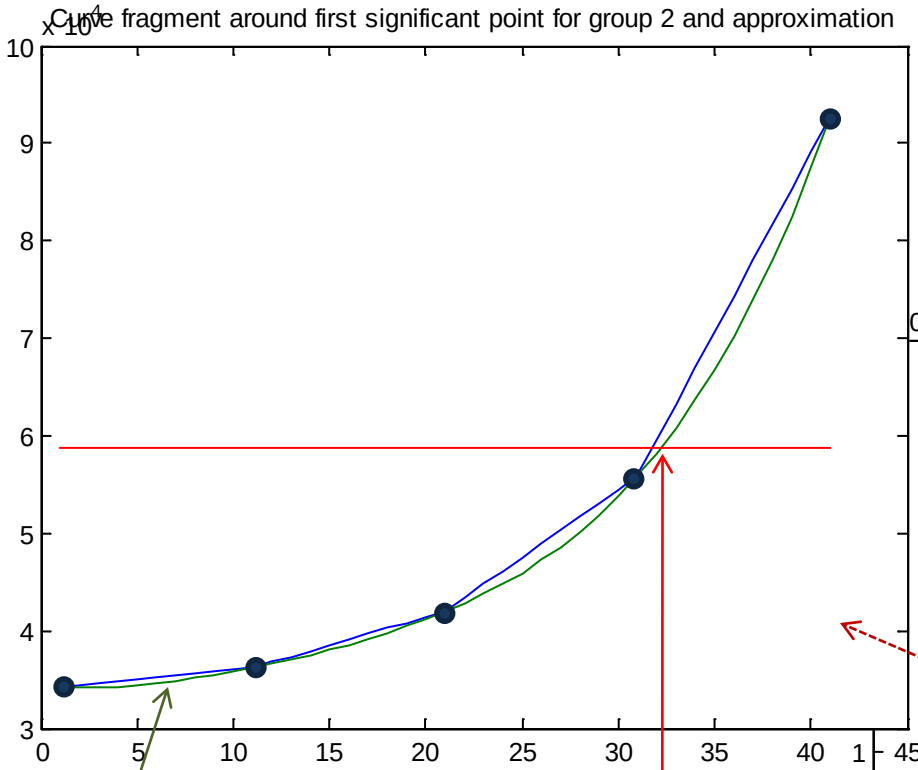


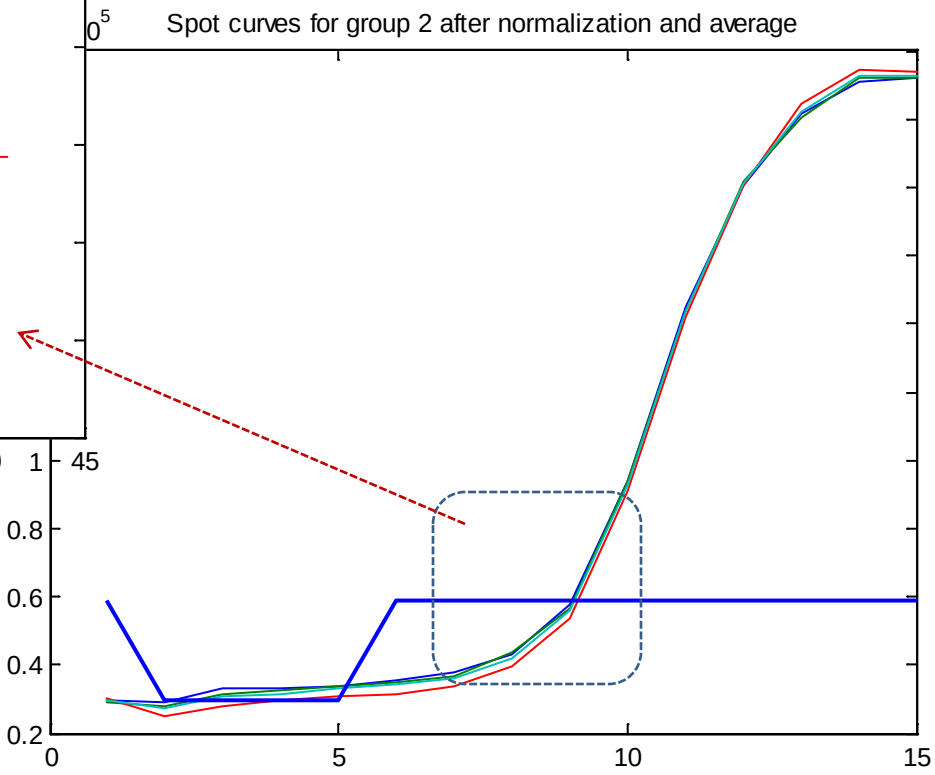


Curves show different scale for different spots in the same group, probably due to illumination non-uniformity, or spotting variability, or both.

1. Linear regression of curves for spots 2, … on the curve for spot 1.
2. Normalize to keep the same average.
3. Remove linear trend.
4. Perform 1-D ANOVA of increasing curve fragments until first significant difference among points.
5. Calculate threshold.

Intensity Curve Approximation





Cycle Threshold found by solving equation with the polynomial

Approximation of 5-point curve with 4-d degree polynomial

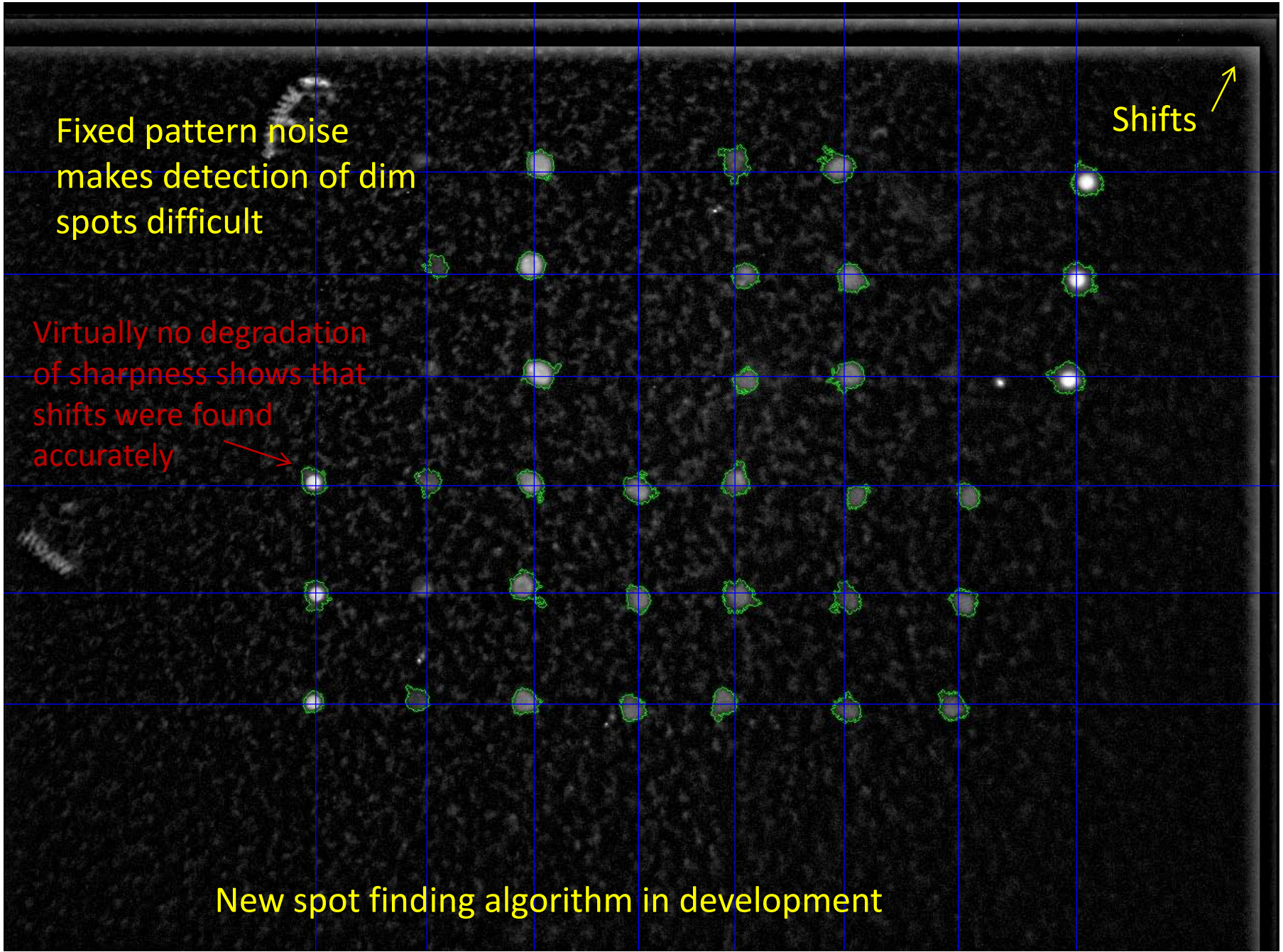
Sequence Threshold

#	GRP	Thresh	BasePts	BaseVal	ThrVal
1	GRP1	0.00	2	0.97	1.35
2	GRP2	0.00	2	1.05	1.67
3	GRP3	10.73	2	1.02	1.26
4	GRP4	9.62	2	1.02	1.25
5	GRP5	12.85	2	1.03	1.36
6	GRP6	0.00	2	1.04	1.46
7	GRP7	0.00	2	1.02	1.27
8	GRP8	0.00	0	0.00	0.00
9	GRP9	0.00	0	0.00	0.00
10	GRP10	0.00	2	1.04	1.56
11	GRP11	8.43	4	1.04	1.47
12	GRP12	0.00	0	0.00	0.00
13	GRP13	8.43	2	1.01	1.19
14	GRP14	13.28	2	1.03	1.40
15	GRP15	0.00	2	1.03	1.44
16	GRP16	0.00	2	0.97	1.33

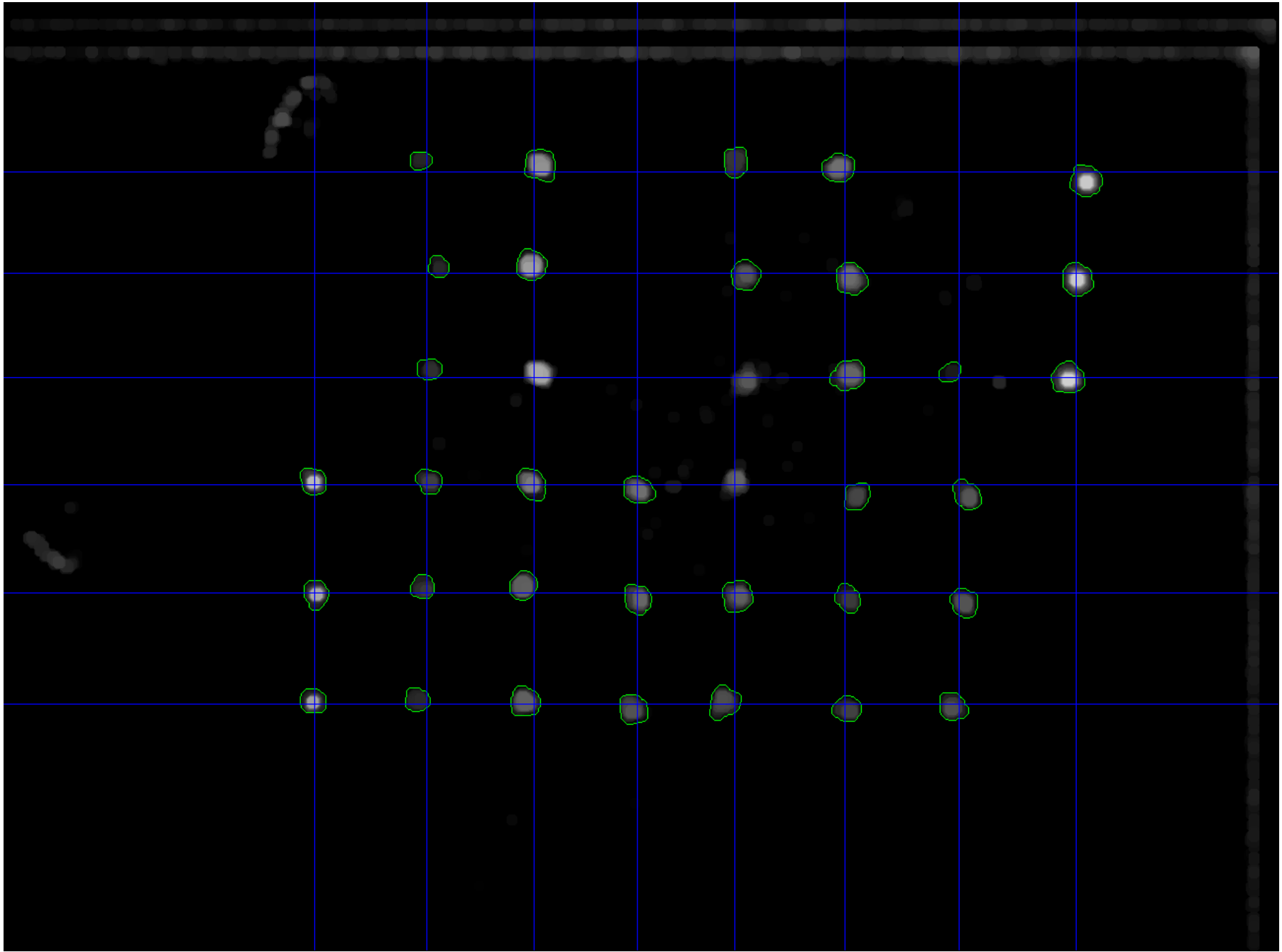
Cycle Threshold

#	GRP	Thresh	BasePts	BaseVal	ThrVal
1	GRP1	0.00	2	0.97	1.35
2	GRP2	0.00	2	1.05	1.67
3	GRP3	31.46	2	1.02	1.26
4	GRP4	29.24	2	1.02	1.25
5	GRP5	35.71	2	1.03	1.36
6	GRP6	0.00	2	1.04	1.46
7	GRP7	0.00	2	1.02	1.27
8	GRP8	0.00	0	0.00	0.00
9	GRP9	0.00	0	0.00	0.00
10	GRP10	0.00	2	1.04	1.56
11	GRP11	26.87	4	1.04	1.47
12	GRP12	0.00	0	0.00	0.00
13	GRP13	26.86	2	1.01	1.19
14	GRP14	36.56	2	1.03	1.40
15	GRP15	0.00	2	1.03	1.44
16	GRP16	0.00	2	0.97	1.33

Accumulated Image with Shifts and Found Spots



Same, After opening



Explanation of Figures in Debug Mode. 1

figs = 1:

i - sequential image number
200 + i - Result of finding spots on each image, grids, spot numbers are in order of finding (not by rows and columns)
17 - X-projection for grid finding (all masks with shifts)
18 - Y-projection for grid finding (all masks with shifts)
21 - Addition of all masks with shifts and final mask from voting
22 - Spot and ring masks for measurement
23 - All histograms of background and spots
24 - Best image with final mask, grids, spot numbers by rows and columns (remapped through groups)
25 - Plots of integrated intensity in spots by groups
251 - Plots of integrated intensity in spots by groups (subtraction instead of division)
26 - Plots of average intensity in spots by groups
261 - Plots of average intensity in spots by groups (subtraction instead of division)
27 - Plots of background intensity by groups
28 - Accumulated image with shifts after background removal, contrasting, gamma; spots and spots filtering by grid
29 - Same, with opening (to remove fixed patter noise)

figs = 2:

1 - Original image
2 - Contrasted image
3 - Background image
4 - Background-removed and contrasted image
5 - Cumulative histogram of background-removed and contrasted image
6 - Thresholded (with quantile) image
7 - Filtered by area
8 - With filled holes
13 - With smoothed edges
14 - With smoothed edges - iteration 2
15 - Spots filtered by shape
19 - Only good spots
300 + i - Original image with final mask

Explanation of Figures in Debug Mode. 2

cfigs = 1:

i - sequential image number
30 + i - Spot curves for group i
50 + i - Spot curves for group i after normalization and average
70 + i - Curve fragment around first significant point for group i and polynomial approximation

histfigs = 1:

90 + i – Histograms of background and spots