FIJI Particle Analysis Procedure

- Load NucleiDAPIconofcal.png
 - You may need to convert to 8-bit.
 - Image from:

http://imagej.net/Nuclei Watershed Separation



- 1. Process -> Subtract Background...
 - Use Preview
 - $\circ~$ Try sliding paraboloid
 - $\circ~$ Adjust rolling ball radius



- 2. Process -> Filters -> Gaussian Blur
 - \circ Use Preview
 - Adjust Sigma (blur effect)
 - o This smooths the nuclei



- 3. Image -> Adjust -> Threshold
 - Use Dark Background
 - \circ Apply
 - $\circ~$ This creates an image mask







- 5. Analyze -> Analyze Particles
 - Adjust Size Range
 - Adjust Centricity
 - \circ 0 is a line, 1 is a circle



- 6. Reopen NucleiDAPIconofcal.png
 - o Select overlays from the ROI manger individual overlays or crtl+A to select all
 - Measure
 - Copy results to excel



- 7. Analyze -> Set Measurements
 - Select properties to measure

| Set Measurements | | |
|------------------|------------------------|---------------------|
| | I ✓ Area | 🔽 Mean gray value |
| | Standard deviation | 🗖 Modal gray value |
| | 🔽 Min & max gray value | Centroid |
| | Center of mass | Perimeter |
| | Bounding rectangle | 🗖 Fit ellipse |
| | Shape descriptors | Feret's diameter |
| | Integrated density | 🥅 Median |
| | Skewness | 🗆 Kurtosis |
| | Area fraction | Stack position |
| | | |
| | Limit to threshold | Display label |
| | Invert Y coordinates | Scientific notation |
| | Add to overlay | NaN empty cells |
| | | |
| | Redirect to: | None 💌 |
| | Decimal places (0-9): | 3 |
| | | |
| | _ | OK Cancel Help |

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