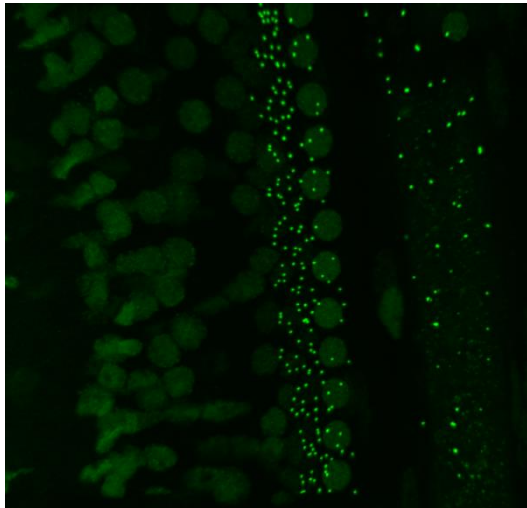**Published in:**

Pyott et al. 2004. *J. Neurosci.* Extrasynaptic localization of inactivating calcium-activated potassium channels in mouse inner hair cells. 24(43):9469-74.
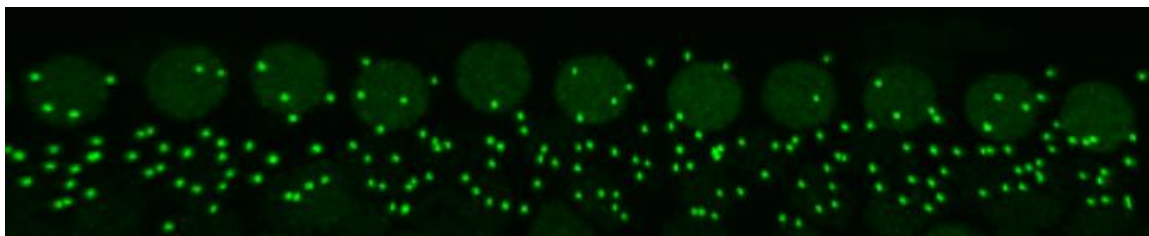
In Photoshop:

1. Open image (flattened z-projection)

(Raw image, reduced scale)

2. Rotate and crop to obtain region of interest
   - Right click on eyedropper tool to obtain ruler tool
   - Draw line of rotation
   - Go to image, image rotation arbitrary, OK
   - Crop image to exclude regions from analysis

(Rotated and cropped image)

3. Convert to grayscale image
   - Go to image, mode, grayscale

4. Adjust contrast if necessary
   - Image, auto contrast
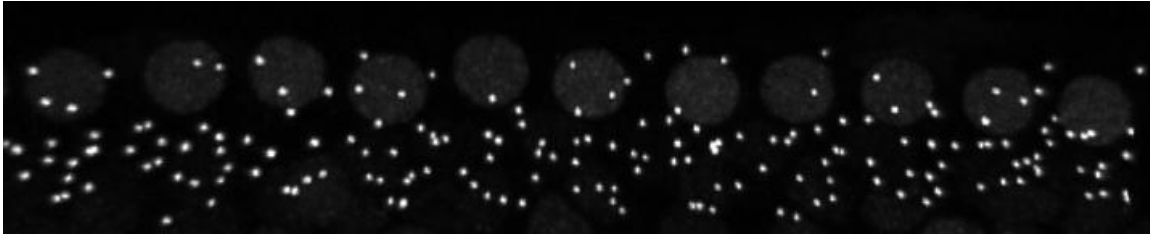
(Grayscale image)

5. Save as [filename]_ps.tif (or whatever name preferred)

In ImageJ:

6. Open processed image ([filename]_ps.tif)

7. Set scale
   • Go to analyze, set scale, and enter scale (pixels per microns)

8. Convert image to a binary (black and white file)
   • Go to process, binary, make binary

(Binary image)

9. Save as [filename]_ps_binary (or whatever name preferred)

10. Analyze particles
   • Set "size pixel^2": To be determined based on the approximate size of puncta
   • Adjust "circularity": To include/exclude only more/less spherical objects
   • Set "show" to "show outlines"

11. Set to "display results" and "clear results" (if you want to clear results between analyses)

12. The Results window displays measurements. You can set measurements displayed by clicking "Results" on the menu.
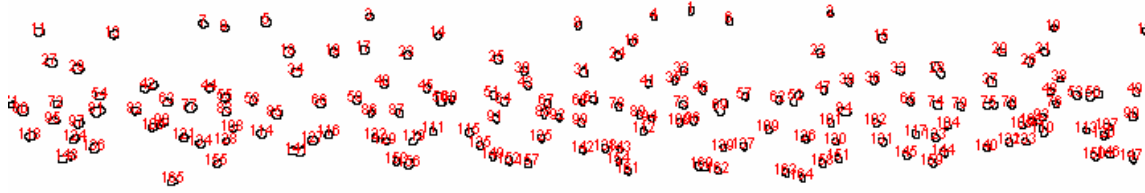
(Image with outlines in black and identified with red numbers)

(Results window)

13. Copy raw data to analysis software

14. Save as [filename]_ps_outlines (or whatever name preferred)

15. If needed, add a scale bar
    - Go to analyze, tools, scale bar