Jul 12, 2024

Cluster Counting

DOI
https://dx.doi.org/10.17504/protocols.io.5jyl8224rl2w/v1

daniel.dautan daniel[1,2], Per Svenningsson[1,2]

[1]Department of Clinical Neuroscience, Karolinska Institutet, 171 76 Stockholm, Sweden;
[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815, USA

Jacquelyn Haytayan
Weill Cornell Medicine

DOI: https://dx.doi.org/10.17504/protocols.io.5jyl8224rl2w/v1

Protocol Citation: daniel.dautan daniel, Per Svenningsson 2024. Cluster Counting. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl8224rl2w/v1

Protocol status: Working
We use this protocol and it's working

Created: June 06, 2024

Last Modified: September 23, 2024

Protocol Integer ID: 101328

Keywords: ASAPCRN, alpha-synuclein, imaging, immunofluorescence, synuclein in mouse, synuclein, counting cluster, cluster counting, scanning, upper gastrointestinal tract

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: 020608

Abstract

Used for counting clusters labeled for pSer129 alpha-synuclein in mouse upper gastrointestinal tract (intestine). Sections should be stained, mounted, and imaged with high resolution (2048 × 2048 scanning).

1 Stain structures for pSer129 and scan with high-resolution (pixel size of 6.25µm).

2 Import the images into ImageJ.

3 Convert to grayscale.

4 Adjust signal intensity to a standardized threshold of 95%. (All thresholds, exposure settings, and laser intensities were applied consistently across all scans).

5 Employ the "Process - Binary - Convert to Mask" function.
The mask function can be determined using the default method against a black background.

6 Apply a watershed filter to select clusters with similar distribution.

7 Execute the "Analyze Particles" function.
Size range: 0 to infinity
Display and summarize results.
This will extract all clusters including their coordinates and areas.

8 To extract clusters with a size equal to or smaller than 1 pixel (6.25µm²) and clusters identified as artifacts (>15000 µm²), use the Excel COUNTIF function, count the number of small clusters (6.25 to 50 pixels), medium-sized clusters (50 to 200 pixels), and large clusters (>200 pixels).

9 Determine relative density of clusters for the amount of tissue area analyzed.