Aug 23, 2024

Image Capture and Neuropathological Quantification of post-mortem human brain tissue

DOI
dx.doi.org/10.17504/protocols.io.n92ld86qnv5b/v1

Felicia Suteja[1], Hongyun Li[1], YuHong Fu[1], Glenda Halliday[1]

[1]University of Sydney

ASAP Collaborative Rese… Team Kirik

courtney.wright Wright
University of Sydney

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.n92ld86qnv5b/v1

Protocol Citation: Felicia Suteja, Hongyun Li, YuHong Fu, Glenda Halliday 2024. Image Capture and Neuropathological Quantification of post-mortem human brain tissue. **protocols.io https://dx.doi.org/10.17504/protocols.io.n92ld86qnv5b/v1**

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

Created: August 23, 2024

Last Modified: August 23, 2024

Protocol Integer ID: 106311

Keywords: ASAPCRN, post-mortem , image analysis, human brain tissue, cell count, alpha synuclein, p62, tau

Funders Acknowledgement:
Michael J Fox Foundation
Grant ID: ASAP-000497

Abstract

This protocol details the fluorescent image analysis pipeline using QuPath to analyse pathological inclusions within human post-mortem brain tissue.

Materials

Software

- QuPath (version 0.5.1).

Safety warnings

It is recommended that you review the SDS (Safety Data Sheets) for all materials prior to commencement.

Ethics statement

Post-mortem brain tissue samples were obtained from Sydney Brain Bank (Sydney, Australia). Ethical approvals were acquired from the human research ethics committees of the University of Sydney (approval number: 2021/845).

Application of this protocol will need prior approval by the users' institutional review board (IRB) or equivalent ethics committee(s).

Before start

The protocol for labelling human tissue can be found here dx.doi.org/10.17504/protocols.io.n92ld8nxxv5b/v1.
This protocol can be modified to be applied for other combinations of markers and fluorophores.

Cell quantification

1 Post-mortem human immunofluorescent sections were scanned using an Olympus VS200 slide scanner at 20x for Quantification.

Cell count and area measurements were completed on digital images using QuPath (version 0.5.1). The QuPath quantification protocol followed the steps listed below.

Annotation

2
1. For the midbrain, Substantia Nigra pars compacta (SNC) was divided into non-overlapping subregions: SNC dorsal tier (SNCD), SNC ventral tier (SNCV), SNC lateral tier (SNL), SNC medial tier (SNCM), using tyrosine hydroxylase (TH) positive cells as an anatomical landmark.
2. For the cortex, the grey matter was divided into non-overlapping subregions: superficial grey matter (SGM) and deep grey matter (DGM), using HuD positive neurons as the guidelines. The white matter (WM) was categorized separately based on DAPI density and nuclear size.

Cell count: TH+ cell with/without aSyn+/Tau (AT8)+/p62+ signals

3
1. The point tool was used to identify TH+ neurons if cells contain visible TH+ signal and/or neuromelanin.
2. The point tool was used to identify aSyn+, AT8+, or p62+ signals based on cells recognised from step 1
3. Grid lines are then applied superimposed on the image to assist with cell counting and categorization using a counter.

Cell count: HuD+ cell with/without aSyn+/AT8+/p62+

4
1. Automated cell detection was used to recognize HuD+ cells. Parameters were optimized by comparing with manual counting until < 10% variance was achieved.
2. An object classifier was created to recognize HuD+ cells with/without aSyn+, AT8+, or p62+ signals. Parameters were optimized by comparing with manual counting until < 10% variance was achieved. These object classifiers were then applied to the step 1 recognised HuD cells.

Total aSyn+/Tau (AT8)+/p62+ particle count

5
1. Automated particle detection was used to recognise aSyn+, AT8+, or p62+ particles. The minimal size of aSyn+, AT8+, or p62+ particles was set at 10mm^2 to avoid of false counting. Parameters
were optimized by comparing with manual counting until < 10% variance was achieved.

2. The same object classifiers from the previous step 2 of the “HuD+ cell with/without aSyn+/AT8+/p62+” were applied to the aSyn+ and AT8+particles according to the order below:
    a. aSyn + particle detection -> AT8+ classifier and p62+ classifier applied sequentially
    b. AT8+ particle detection -> aSyn + classifier and p62+ classifier applied sequentially

Total cell count

6 Automated cell detection was used to recognize nuclei with DAPI. Parameters were optimized by comparing with manual counting until < 10% variance was achieved.