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Quantification of astrocytic expression of a-syn in A53T mouse brain tissue

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We use this protocol and it's working

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Abstract

This protocol describes the imaging procedure used to quantify astrocyte and alpha-synuclein expression in multiple brain regions in 6 month aged transgenic A53T mice (M83) using the Leica THUNDER 3D Imager located in the Sydney Microscopy & Microanalysis facility in the Charles Perkins Centre at The University of Sydney

Attachments



[Leica THUNDER Quick ...](#)

8.2MB



[Sampling](#)

[Scheme_A53t...](#)

1.5MB

Guidelines

Slides should be mounted and coverslipped, ensuring enough time is left for the mounting medium to completely harden. Imaging should not be carried out if there is any indication the medium has not completely set.

****Note the microscope used in this protocol deploys an inverted stage.**



Materials

Imaging Equipment:

Leica THUNDER 3D Imager (Leica Microsystems)

Leica K8 sCMOS camera (Leica Microsystems)

All images used in this analysis were acquired on a widefield Leica Thunder (Leica Microsystems) with the following parameters

- Hoechst – 405 LED, acquisition time 75ms
- a-syn – 488 LED, acquisition time 250ms
- GFAP, ALDH1L1, S100B - 594 LED, acquisition time 250ms

Objectives:

- HC PL APO 40x/1.10 W CORR CS2 (Leica Microsystems)
- HC PL APO 63x/1.40 OIL PH3 CS2 (Leica Microsystems)

Consumables:

- 80% ethanol
- Kimwipes
- Lens wipes

Software:

- QuPath (Bankhead, P. et al., 2017)

Before start

This Imaging and Quantification protocol was used on fixed tissue that had been stained for immunofluorescence, mounted and coverslipped. Minimum 24 hours between coverslipping and imaging is required to ensure mounting medium has hardened. Startup details for this specific imaging setup are provided in the attachments. More information about the system used in this protocol is available at <https://www.sydney.edu.au/research/facilities/sydney-microscopy-and-microanalysis/our-facilities/light-and-laser-microscopy.html>

Slide Preparation

1. Ensure the mounting medium has completely dried and the coverslip is firmly fixed in place on the slide.
2. Clean both sides of the slide using a Kimwipe 70% ethanol, making sure to wipe the ethanol off to avoid leaving streaks.

Microscope Setup

1. Ensure appropriate objective lens' and filter cubes are equipped prior to imaging.
2. Turn on Leica Thunder 3D Imager as well as all light sources and cameras, as per specific setup requirements.
3. Clean each objective lens with lens paper ****ensure lens paper is used, paper towel/Kimwipes will scratch the lens**
4. Open Leica LAS X software on accompanying computer, open and save a new project in .lif form with specific identifiers, e.g., project ID, animal ID, brain region, antibodies used, etc.

Example: 9Mo_A53T_7616_SN_GFAP_aSYN (9 month A53T mouse project, animal ID-7616, region- SN, antibodies used - GFAP, a-syn)

Region Identification

1. Place slide(s) onto microscope stage, ensuring they are fixed firmly in place.
2. Use a low magnification objective (5X or 10X) and Brightfield light source to locate the sample and adjust until it is in focus.
3. Using the Leica THUNDER Acquisition function, take a low magnification scan of the entire slide area.
4. From the scans, identify regions of interest, and annotate as required.

Fluorescence Imaging



- 4
 1. Switch to fluorescent light source and select fluorescence filter cubes with appropriate excitation/emission ranges corresponding with fluorescent labels used.
 2. Beginning with the longest wavelength of light, set the exposure time for each antibody/channel, to optimise fluorescent signal clarity. Begin with a low intensity to avoid overexposure.
 3. Switch to a high magnification objective (40X or 63X) and adjust focus until sample is again in clear view.

Region Sampling Scheme

- 5
 1. Sampling scheme for each region as follows:

SN:
- One 40X field of view (FOV) per region
VTA:
- One 40X field of view (FOV) per region
STR:
- Four 40X fields of view (FOV) per region

**A schematic of the sampling scheme used in this protocol is provided in the attachments

Z stack capture

- 6
 1. Select the Z-stack acquisition button in the image acquisition panel of the LAS software, which will bring up a window to set the parameters for taking a Z stack series.
 2. Set the range of the Z stack as the focal planes by selecting the topmost and bottom-most planes where the sample is in view.
 3. The microscope sets default Z stack step size defined by Nyquist sampling theorem. Adjust if required.
 4. Once Z stack parameters set, click **Start** button for Z stack acquisition.
 5. Select Maximum Intensity Projection, and export Z stack image in .tiff form.

Astrocyte Quantification

- 7
 1. Using QuPath software, open an exported .tiff image and select fluorescence image type when prompted.
 2. Use Shift + C to open Brightness/Contrast panel.
 3. Isolate astrocyte channel, and count all astrocytes. Astrocytes were labelled with three astrocyte markers (GFAP, S100B and ALDH1L1) in the same fluorescent channel (594nm) to achieve the optimal profile.
 4. Once counted, add in the a-syn channel and count all astrocytes containing a-syn. A-syn expression within astrocyte soma or processes classifies an astrocyte coexpressing a-syn.
 5. Record total number of astrocytes, and total number of a-syn expressing astrocytes. Calculate the proportion of astrocytes that express a-syn.

Astrocyte Morphology Quantification

- 8
 1. Identify a sample astrocytes within a relevant field of view (min. 10 per sample).
 2. Trace the cell body using the QuPath polygon tool and record the size.
 3. Manually count the number of astrocyte processes, and record.
 4. Record the total number of astrocytes, the number of astrocytes positive for a-syn, the proportion of astrocytes expressing a-syn, astrocyte cell body size and the number of



astrocyte processes.

Statistical Analysis

- 9 After quantification, multivariate linear regression statistics is performed, with Tukey post hoc tests (IBM SPSS) to identify any differences in the above parameters between groups, using $p < 0.05$ as the level of significance.

Protocol references

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