Seattle Alzheimer's Disease Brain Cell Atlas (SEA-AD)

Neuropathology Images and quantifications

For more information about the SEA-AD cohort of donors, please see the SEA-AD Documentation Page

Files Available:

File	Type	Description
Raw images	.svs	Full resolution raw brightfield images, stained for specific cells or pathological proteins
Quantified mask images	.tif	Processed images; segmented by the HALO image analysis software
Image annotations	.annotations	Bounding lines subdividing the quantified area into specific analysis regions (e.g., specific cortical layers)
Job ID mask and annotation crosswalk	.xlsx	Crosswalk file for specific image defines the analysis region that corresponds to a job ID number
Quantification Summary	.csv	Quantification summary file feature values corresponding to the segmentation done by the HALO image analysis software for each of the stains for each donor for a specific brain region.
Jupyter notebook	.ipynb	Jupyter notebook generates donor by quantitative neuropathology values csv files.

Methods Description:

1. **Tissue Processing**
   The non-frozen slices are fixed and blocks are sampled complementary to the standard blocks taken on the fixed hemibrain. The standard blocking of tissue samples is based on recommended consensus criteria for the evaluation of neurodegenerative diseases as well as function imaging connectivity patterns. Additional sections are taken based on the identification of grossly identified lesions as well as MRI-identified lesions.

MATERIALS:

- Camera
- Scalpel
- Tissue cassettes

PROTOCOL:

A. Photo documentation
   a. Starting with the frontal lobe, flip each slice and remove the surrounding agarose
   b. Lay the slices out from anterior to posterior and take pictures of the slices
      - Any lesions identified on the slabs should be marked with tape and photographed prior to sampling.

c. Perform all sampling (see part C) and take pictures of the boards again once sampling is complete

B. Perform modular sampling (Fig. 3)
   a. Block in the regions of interest for paraffin embedding in tissue cassettes.
      - Standard samples for NIA-AA diagnostics
      - Standard samples for neurotrauma
      - Standard samples for functional networks
      - Sampling of grossly-identified lesions
      - Sampling of MRI-identified lesions
   b. Store remaining tissue in formalin in air-tight containers so that it is available for additional sampling or research use as necessary.

NOTES:

- At the time of sampling the tissue slices should be thoroughly evaluated for pathology and assessments of atrophy.

1. Standard Neuropathological Diagnostic Workup

After sampling, all blocks are processed and embedded in paraffin according to standard techniques. Then, relevant blocks are sectioned and stained in accordance with the most up-to-date criteria for the assessment of various neuropathologic diseases. The National Institute of Aging – Alzheimer's Association (NIA-AA) have established criteria for the neuropathological assessment and diagnosis of Alzheimer's disease which dictate specific regions of the brain to be examined for that purpose(HYMAN; PHELPS; BEACH; BIGIO *et al.*, 2012). The histological characterization of the pathologic peptides in the relevant regions of brain is critical to properly diagnosis neurodegenerative disease and thus placing the utility of the research tissue in the proper context. As new consensus criteria emerge, the histologic workup evolves to keep pace. This has grown to include a more thorough evaluation for the histopathologic hallmarks of chronic repetitive neurotrauma, staging of limbic-predominant age-related TDP-43 (LATE) pathology, and assessments of age-related tau pathology in neurons and glia (ie. PART and ARTAG). A complete list of the brain regions and staining techniques used is provided in Table 3.

In addition to synthesizing the neuropathologic changes into a report with named neuropathologic entities for the study participants' family members and the clinical studies in which the subjects participated, the neuropathologic variables are entered into the database and this data is available to be shared as well as used for selection criteria when providing tissue for research requests.

The following protocol used in our laboratory covers the NIA-AA criteria for pathological diagnostics and achieves the high quality necessary to perform consistent digital image analyses.

MATERIALS:

- Standard histology lab equipment and reagents

PROTOCOL:

A. Section diagnostic tissue blocks and prepare unstained slides for staining
    a. Cut 5 micron thick sections of relevant blocks using a microtome
    b. Deparaffinize tissue sections
    c. Immerse in xylene for 3 minutes, 3 times.
    d. Rehydrate in graded ethanol (100%, 3x, 96%, 70% and 50%) for 3 min each
    e. Wash with TBST twice for 3 minutes
B. Perform immunohistochemical staining on subset of slides (list of common antibodies for neurodegenerative disease workup provided in table 2)
    a. Immerse the slides in Diva Decloaker 1x solution (Biocare Medical, DV2004) and heat-induced epitope retrieval (HIER) is performed using the Decloaking ChamberTM at 110℃ for 15 minutes (this works for the vast majority of antibodies).
    b. For some markers, enzymatic antigen retrieval is used.
    c. After the HIER is completed, the slides are cooled for 20 min at room temperature.
    d. The slides are then washed with TBST for 5 minutes, twice.
    e. Finally, the slides are placed in the automated stainer and protocols specific for the autostainer being used are followed.
    f. Once staining is complete, the slides are removed from the automated stainer and immersed in TBST, 3 min, then dehydrated in graded ethanol (70%, 96%, 100%, 2x) for 3 min and xylene 3 times each for 3 min.
C. Histochemical stains are generally performed manually using standard protocols
    a. Hematoxylin and eosin with Luxol fast blue (H&E/LFB)
    b. Modified Bielschowsky silver stain
D. Coverslip all stained slides, either manually or with an automated coverslipper
E. Perform miscroscopic examination of the stained slides
    a. All diagnostic slides are reviewed by a neuropathologist or neuropathology-trained individual following the latest published criteria.
    b. Generate a research diagnostic report that lists the neurodegenerative disease processes present along with any incidental findings.
    c. Record all neuropathologic data points in the database

Table 2. List of antibodies and antigen retrieval protocols used for each one.

PRIMARY ANTIBODY	CLONE/HOST	COMPANY	CATALOG NUMBER	DILUTION	PRETREATMENT
Alpha Synuclein	LB509/Mouse	Invitrogen	180215	1:200	Proteinase -K (10 min)
Beta Amyloid	6 E10/Mouse	Biolegend	803003	1:1500	Diva/Decloaker (110℃- 15min)
FUS/TLS	3A10B5/Mouse	Abcam		1:3000	Diva/Decloaker (110℃- 15min)

Anti-Huntington's Protein	mEM48/Mouse	Millipore	MAB5374	1:3000	Diva/Decloaker (110℃- 15min)
Notch 3/N3ECD	1 E4/Mouse	Millipore	MABC594		Proteinase -K (5 min)
SQSTM1/p62	2C11/Mouse	Abnova	H00008878 -M01	N/A	ER 1 (10 min)
PHF-Tau	AT8/Mouse	ThermoFisher	MN1020	1:1000	Diva/Decloaker (110℃- 15min) or ER 1 10 min
Anti-Prion Protein	3F4/Mouse	Millipore	MAB1562	1:50	ER1 (20 min)
TAU RD3	8E6/C11/Mouse	Millipore	05-803	1:2500	Diva/Decloaker (110℃- 15min)
TAU RD4	1E1/A6/Mouse	Millipore	05-804	1:400	Diva/Decloaker (110℃- 15min)
phosTDP-43 (Ser409/Ser410)	ID3/Rat	Biolegend	829901	1:2000	Diva/Decloaker (110℃- 15min)
TDP-43 (TARDBP)	Rabbit	Protein Tech Group	10782-2-AP	1000	ER 2 (20 min)
Ubiquitin	Ubi-1/Mouse	Millipore	MAB1510	500	ER 1 (20 min)

Table 3. Diagnostic stains by region

Brain Region	Stain	Laterality	Purpose
Middle frontal gyrus	H&E/LFB	Bilateral	Vascular pathology; other lesions
	6E10	Unilateral	Thal staging for amyloid plaques; CAA
	AT8	Bilateral	Braak staging of NFTs; CTE lesions; ARTAG; FTLD-tau
	pTDP-43	Unilateral	LATE staging; assessment for FTLD-TDP pathology
	a-syn	Unilateral	LBD staging; glial synuclein pathology (MSA)
	Bielschowsky	Unilateral	Neuritic plaque density (CERAD score)
Frontal white matter	H&E/LFB	Bilateral	Vascular pathology; other lesions
Inferior parietal lobule	H&E/LFB	Bilateral	Vascular pathology; other lesions
	6E10	Unilateral	Thal staging for amyloid plaques; CAA
	AT8	Bilateral	Braak staging of NFTs; CTE lesions; ARTAG; FTLD-tau
	Bielschowsky	Unilateral	Neuritic plaque density (CERAD score)
Superior/middle temporal gyri	H&E/LFB	Bilateral	Vascular pathology; other lesions
	6E10	Unilateral	Thal staging for amyloid plaques; CAA
	AT8	Bilateral	Braak staging of NFTs; CTE lesions; ARTAG; FTLD-tau

	pTDP-43	Unilateral	LATE staging; assessment for FTLD-TDP pathology
Occipital cortex with V1	H&E/LFB	Bilateral	Vascular pathology; other lesions
	6E10	Unilateral	Thal staging for amyloid plaques; CAA
	AT8	Bilateral	Braak staging of NFTs; CTE lesions; ARTAG; FTLD-tau
Anterior cingulate gyrus	H&E/LFB	Bilateral	Vascular pathology; other lesions
	AT8	Bilateral	CTE lesions; ARTAG; FTLD-tau
	a-syn	Unilateral	LBD staging; glial synuclein pathology (MSA)
Anterior hippocampus	H&E/LFB	Bilateral	Vascular pathology; other lesions
Mid-hippocampus	H&E/LFB	Bilateral	Vascular pathology; other lesions
	6E10	Unilateral	Thal staging for amyloid plaques
	AT8	Bilateral	Braak staging of NFTs; CTE lesions; ARTAG; FTLD-tau
	pTDP-43	Unilateral	LATE staging; assessment for FTLD-TDP pathology
Amygdala	H&E/LFB	Bilateral	Vascular pathology; other lesions
	AT8	Bilateral	ARTAG
	pTDP-43	Unilateral	LATE staging
	a-syn	Unilateral	LBD staging
Neostriatum	H&E/LFB	Bilateral	Vascular pathology; other lesions
	6E10	Unilateral	Thal staging for amyloid plaques
Thalamus	H&E/LFB	Bilateral	Vascular pathology; other lesions
Midbrain	H&E/LFB	Bilateral	Vascular pathology; neuron loss; other lesions
	6E10	Unilateral	Thal staging for amyloid plaques
	a-syn	Unilateral	LBD staging; glial synuclein pathology (MSA)
Pons	H&E/LFB	Bilateral	Vascular pathology; neuron loss; Lewy bodies; other lesions
Medulla	H&E/LFB	Bilateral	Vascular pathology; Lewy bodies; other lesions
Cerebellum	H&E/LFB	Bilateral	Vascular pathology; Purkinje cell loss; other lesions;
	6E10	Unilateral	Thal staging for amyloid plaques; CAA
	AT8	Bilateral	CTE lesions; ARTAG; FTLD-tau

NOTES:

- A thorough diagnosis may not be possible using the standard stains and slides. Additional stains on relevant sections may be considered.
- The methods described above are specific to single immunohistochemically-stained slides. However, immunohistochemistry for two protein markers (double-IHC) in a single tissue section can be used in some instances. Multiplexing protein markers in a single tissue section enables colocalization analyses of multiple antigens in a single tissue section, identification of different cell populations within a sample, and optimization of tissue, time, and histological reagents.

2. Digital Neuropathology

Beyond the traditional qualitative neuropathologic assessments, the stained slides are also used to generate quantitative measurements. The value of histologic stains can be increased by quantitative analysis of the pathologic peptide burden (LATIMER; BURKE; LIACHKO; CURREY *et al.*, 2019). Slide scanning analyses develop precise assessments of structural changes in

histologic sections across a wide range of brain regions, which can inform understanding of the basis of selective vulnerability in neurodegenerative disease. Such analysis can identify frequency and percent area involved by pathologic peptides, neuroinflammation, and neurodegeneration. Quantitative neuropathology is necessary to understand how pathologic burden differs across brain regions in association with cognitive function or biomarker studies, as similarly classified individuals can vary significantly. Additionally, double labeled slides can yield additional data on colocalization of these peptides in relation with their relative abundance.

The digital pathology concept refers to the use of computer work-stations to view the whole slide image (WSI) for sharing, teaching and primary reporting diagnostics(FRAGGETTA; YAGI; GARCIA-ROJO; EVANS *et al.*, 2018).

Image analysis has great potential to identify, extract, and quantify tissue features in greater detail than the standard pathologic assessment, which may produce improved prediction models or enhance task efficacy and speed beyond manual capability.

Optimal WSI quality and analyses are dependent upon high quality, reproducible histochemical and immunohistochemical staining in tissue.

MATERIALS:

- Slide scanner (Aperio AT2 scanner from Leica)
- Cloud based image storge system (Amazon Web Services)
- Image analysis software (HALO® software from Indica Labs)
- Computer

PROTOCOL:

A. Obtain a Whole Slide Image
    a. Clean slides with 70% ethanol and Kimwipes to remove any particles which might cast shadows on the underlying tissue sections
    b. Scan the majority of slides at 20x magnification, which is considered suitable for manual histochemical and IHC viewing and image analysis (Kumar, 2020), and is more cost effective compared to 40x due to the reduced storage space and scanning time required.
    c. Perform quality controls measures to assess for positivity in control slides, IHC artifacts (non-tissue or cell specific-DAB substrate stains, bubbles, unstained areas) and features which might impede digital analysis (dark shadows, folds, or tears).

B. Import images to image storage in cloud-based servers

a. These can be set up with individual user accounts to control access to groups of images and can be expanded as needed
b. Ensure HIPAA compliancy and compatibility with image analysis software
c. Sequential images using slide settings optimized for our IHC protocols are subsequently assembled or stitched into virtual slides (VS) and exact replicas of the glass slides.

C. Image visualization and analysis

a. Standardize immunohistochemical staining protocols for reproducible results
b. Annotate brain regions of interest and delineate objects of interest
c. Run image analysis algorithms to identify, extract and quantify neuropathologic features (image analysis modules used are described in Table 4)

Table 4: VS slide stains for HALO quantification

Slide name	Type of stain	Target-Molecule	Stain pattern
H&E-LFB	Hematoxylin and Eosin + Luxol Fast Blue		Nuclei (purple), Neuropil (pink) Myelin (blue)
NeuN	IHC- enzymatic reaction with DAB (diaminobenzidine)-brown precipitate for single molecule expression.	Neuronal Marker	Cytoplasmic and nuclear expression in neurons.
GFAP	IHC- enzymatic reaction with DAB (diaminobenzidine)-brown precipitate for single molecule expression.	GFAP (glial fibrillary acidic protein). Astrocytic marker	Cytoplasmic expression in astrocytes.
a-Syn	IHC- enzymatic reaction with DAB (diaminobenzidine)-brown precipitate for single molecule expression.	Alpha synuclein-Lewy bodies marker	Cytoplasmic inclusions and neurites expression in neurons.
AT8	IHC- enzymatic reaction with DAB (diaminobenzidine)-brown precipitate and AP (alkaline phosphatase)- blue precipitate for duplex molecule expression.	AT8 (Phosphorylated TAU (Ser202,Thr205) and p-TDP43 (phosphorylated-TAR DNA-binding protein 43)	AT8-Cytoplasmic inclusions and neurite expression in neurons (blue). pTDP43-Cytoplasmic and neurite expression in neurons (brown)
I6	IHC- enzymatic reaction with DAB (diaminobenzidine)-brown precipitate and AP (alkaline phosphatase)- blue precipitate for duplex molecule expression.	IBA1 (Ionized calcium binding adaptor molecule) and 6e10 (Amyloid beta clone 6e10)	IBA1-Cytoplasmic expression in microglia cells (blue. 6e10-Beta amyloid plaques expression (brown)

Table 5. HALO modules used and the type of data obtained by each one.

Module	Type of Data obtained by ROI	Unit
Area quantification (up to 3 markers)	Percentage of Area by marker	Percentage
Object Colocalization (up to 2 markers)	Total ROI Area	mm^2
	Total object 1 and 2 count	μm^2
	Total object 1 and 2 area	qty
	Object 1 and 2 percent area	Percentage
	Total object colocalized area	qty
	Percent of object 1 and 2 colocalized	Percentage
	Area of positive stain by object	μm^2
	Colocalized area by object	μm^2
	Percent of colocalized area of each marker by object	Percentage
Multiplex IHC (up to 5 markers)	Total cells by marker	qty
	Area of positive stain in nucleus by marker and by object	μm^2

	Area of positive stain in cytoplasm by marker and by object	μm^2
	Area of positive stain in membrane by marker and by object	μm^2

Tutorial on how to use the data:

While traditional histologic neuropathological assessment of neurodegenerative disease has tremendous diagnostic value, insights into neurodegeneration's pathophysiological underpinnings can be significantly enhanced with large-scale quantitative analyses. Using the whole slide imaging data described above, targeted analyses of each neuropathological feature, both within and across layers can be performed to understand these complex relationships.

For this tutorial, we will be analyzing the SEA-AD quantitative neuropathology dataset (SEA-AD_All_MTG_Quant_Neuropath_ByDonorID.csv). This dataset includes quantitative neuropathology data from 84 donors which was generated from 720 whole slide images across 7 stains, resulting in 15,440 individual data points. The dataframe will contain 84 unique rows (donors) and 392 unique columns (neuropathological features). Across the entire dataset, neuropathological features are quantified in both normalized and raw counts, so please consider whether the appropriate feature (column) is being used or a given analysis.

The following tutorial in R, walks the user through an example case of analyzing one feature of this dataset (Normalized AT8+ Cell Count), from reading in the data through making a heatmap.

Load required packages

```
library(tidyverse)
```

```
## ── Attaching packages ─────────────────────────────────────── tidyverse 1.3.1 ──
```

```
## ✓ ggplot2 3.3.5     ✓ purrr   0.3.4
## ✓ tibble  3.1.2     ✓ dplyr   1.0.6
## ✓ tidyr   1.1.3     ✓ stringr 1.4.0
## ✓ readr   1.4.0     ✓ forcats 0.5.1
```

```
## ── Conflicts ────────────────────────────────────────── tidyverse_conflicts() ──
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
```

```
library(ggplot2)
library(haven)
```

Read in data (.csv) as a data.frame

```
neuropath <- read.csv(file <- "~/all_quant_neuropath_by_donor_pivoted.2022-05-25.csv",
                      header = T,
                      sep = ",",
                      as.is = T)
```

Read in donor metadata (.sav)

```
metadata <- read_sav(file = "~/NeuropathMetadata_version103_20220415.sav")
```

Merge neuropath data.frame with donor metadata

```
neuropath_metadata <- merge.data.frame(x = neuropath,
                                       y = metadata,
                                       by.x = "case.number",
                                       by.y = "uwa")
```

Create list for features of interst

```
feature_list <- c('number.of.AT8.positive.cells.per.area_Layer1',
'number.of.AT8.positive.cells.per.area_Layer2',
'number.of.AT8.positive.cells.per.area_Layer3',
'number.of.AT8.positive.cells.per.area_Layer4',
'number.of.AT8.positive.cells.per.area_Layer5.6')
```

Create subsetted dataframe with features of interest

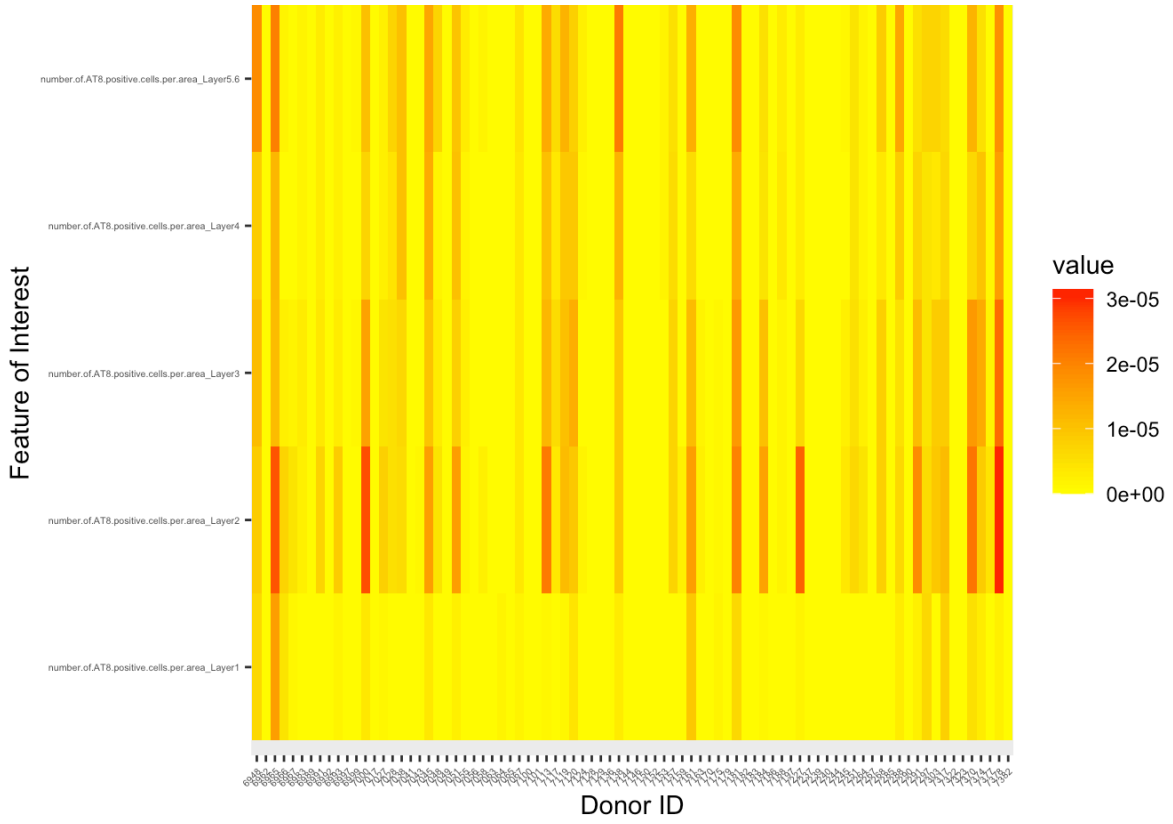
```
neuropath <- neuropath %>%
  column_to_rownames(var = "case.number")

neuropath_subset <- neuropath[,feature_list]
```

Restructure dataframe for heatmap

```
neuropath_subset_forheatmap <- neuropath_subset %>%
  rownames_to_column(var = "case.number") %>%
  pivot_longer(colnames(neuropath_subset),
               names_to = "neuropath_group",
               values_to = "value")
```

Plot heatmap

```
ggplot(neuropath_subset_forheatmap,
       aes(x = case.number, y = neuropath_group, fill = value)) +
  geom_tile(aes(fill = value)) +
  scale_fill_gradient(low = "yellow", high = "red") +
  xlab("Donor ID") +
  ylab("Feature of Interest") +
  theme(axis.text.x = element_text(size = 4, angle = 45, hjust=1),
        axis.text.y = element_text(size = 4),
        panel.grid = element_blank(),
        panel.border = element_blank())
```



Whole slide Image analysis:

The slides obtained from the SEA-AD IHC histological stains have two different analysis approaches.

The first one is performed by the Neuropathologist during the diagnostical assessment, where the slides stained with H&E-LFB, AT8 and 6e10, pTDP43 and a-Syn are used to determine Alzheimer's disease neuropathologic change and related disorders and produce the categorical analysis for Braak, Thal, CERAD, LATE and Lewy Bodies scores.

The second one is made on different VSs obtained from the whole slide images (WSI), not only to produce a numerical expression value for statistical analysis, but also to create an objective analysis of the massive amount information and expression patterns in a single slide, that goes beyond the standard neuropathological assessment.

According to Pantanowitz, et al. 2018, there are different approaches for quantification, characterization, and extraction of image features from WSIs using different image analysis algorithms.

1. Pixel-level features are lowest in the information hierarchy, and examples of pixel-level features include mathematical characterizations of color, texture, and spatial patterns. Gray-level intensity profiles, Haralick Gray-level co-occurrence matrix features, wavelets, Gabor filter responses, and statistics and frequencies of color histograms are a small subset of pixel-level features.
2. Object-level features are higher in the information hierarchy as they describe the characteristics of microanatomic objects such as nuclei, nucleoli, and cytoplasm.

3. Semantic-level features capture biological classification of microanatomic structures or regions. These features describe high-level concepts such as type of cell, or regions within the WSI.

4. Deep learning methods are rapidly making a major impact in digital pathology. These methods can be employed to identify and label objects WSI regions or to assign classifications to entire WSIs (2).

For Project 1, the WSIs obtained are analyzed using the HALO® v.3.4.2986 (Indica labs, Albuquerque, New Mexico, USA) software. As noted above, HALO uses different modules for the quantitative tissue analysis approach.

The WSI obtained from the NeuN slides is used to train the Deep Learning convolutional neural network (CNN) for the tissue classification into several cortical regions using the expression patterns of the different cortical layers in the MTG region, such as Layer1 (molecular layer), layer 2 (external granular layer), layer 3 (external pyramidal layer), layer 4 (Internal granular layer) and layers 5-6 (internal pyramidal and multiform layers).

Also, using the Serial Section registration tool, the different WSIs are registered, and the different cortical regions are annotated allowing comparable analysis on the same tissue regions in all the tissue sections obtained for every individual donor analyzed.

For the stains NeuN, alpha-Synuclein, GFAP and AT8 (blue)+pTDP43 (brown), analysis settings are based on the following algorithms: Color deconvolution v1.1, and Area Quantification v2.2 modules for analysis of optical density in IHC. Quantification of the single and duplex IHC is done with hematoxylin counter-stained slides on a pixel level basis. Quantification is done for the single and double immune-positive reacting cells and immune positive area thus: average optical density is measured for the DAB- stain and AP- stained slides, binned positivity based on optical density (light (yellow), medium (orange), strong (red)), as well as the co-expression area quantification is done for AT8 (red markup) and pTDP43 (green markup) double IHC.

Finally, the algorithm Object Colocalization v1.3, is used to count and measure the labeled objects in brightfield for the 6e10 (brown)+IBA1(blue) positive slides, counter stained with hematoxylin. The module outputs object 1 (red markup) and object 2 (green markup), object number, density, area (μm^2), perimeter, diameter, median, average, roundness, location in XY plane coordinates of the ROI, and area of colocalization (yellow markup).

The following table will describe the specific HALO modules, the level of analysis used, and the features extracted for the 5 slides of MTG tissue samples in 84 donors.

Table 6: HALO module quantification outputs

Slide name	Level of analysis	HALO module used	Features description
NeuN	Pixel, Object, Semantic, Deep learning	Color deconvolution, Area Quantification, Multiplex IHC, Dense Net (CNN).	Tissue Area Analyzed (µm²), Total of NeuN positive Area (µm²), Percent NeuN Positive Tissue area, Number of NeuN Positive Cells Avg NeuN positive Cell Area (µm²) Deep learning Cortical regions, classification, Layer1, Layer2, Layer3, Layer4, Layer5-6.
GFAP	Pixel, Object, Semantic	Color deconvolution, Area Quantification, Microglia module (adapted) to recognize the GFAP+ astrocytic branches.	Tissue Area Analyzed (µm²), Total of GFAP positive Area (µm²), Percent GFAP Positive Tissue area, Avg GFAP positive branch area, Avg GFAP positive Length.
a-Syn	Pixel, Object, Semantic	Color deconvolution, Area Quantification, Multiplex IHC.	Tissue Area Analyzed (µm²), Total of a-Syn positive Area (µm²), Percent a-Syn Positive Area Number of Positive a-Syn Cells, Avg a-Syn positive Cell Area (µm²).
AT	Pixel, Object, Semantic	Color deconvolution, Area Quantification, Multiplex IHC.	Tissue Area Analyzed (µm²), Total AT8 positive tissue Area (µm²), Total pTDP43 positive tissue Area (µm²),

			Total pTDP43 and AT8 positive co-expression Area (µm²), Percent AT8 Positive Tissue, Percent pTDP43 Positive Tissue, Percent pTDP43 and AT8 positive co-expression cells, Number of AT8 positive bearing Cells, Average AT8 positive bearing cell Area (µm²), Number of pTDP43 positive inclusion bearing Cells, Average pTDP43 positive bearing cell Area (µm²).
I6	Object, Semantic	Color deconvolution, Analysis Quantification, Object colocalization, Microglia module.	Tissue Area Analyzed (µm²), Total IBA1 positive tissue Area (µm²), Percent IBA1 positive Tissue Area, Number 6e10 positive objects, Number of 6e10 positive objects Per µm², Total 6e10 positive tissue Area (µm²), Total 6e10 positive tissue Area (µm²), Percent 6e10 positive tissue Area, Avg 6e10 positive Objects Area (µm²), Avg 6e10 positive Objects Median Diameter (µm), Number of IBA1 positive and 6e10 positive objects Colocalized, Percent of IBA1 and 6e10 positive Objects Colocalized, Total IBA1 positive cells, Total Activated IBA1 positive cells, Total Inactivated IBA1 positive cells, Avg IBA1 positive Process Area Per Cell,

			Avg IBA1 positive Process Length Per Cell Avg IBA1 positive Process Length Per Cell, Total IBA1 positive Process Area (μm²), Total IBA1 positive Process Length (μm).

References:

1. Kim So-Woon, Roh Jin, Park Chan-Sik. Immunohistochemistry for Pathologists: Protocols, Pitfalls, and Tips. Journal of Pathology and Translational Medicine 2016; 50: 411-418.
2. Pantanowitz L, Sharma A, Carter AB, Kurc T, Sussman A, Saltz J. Twenty years of digital pathology: An overview of the road travelled, what is on the horizon, and the emergence of vendor-neutral archives. J Pathol Inform 2018;9:40.