Overcoming challenges in neuroscience: Multiplex immunofluorescence and autofluorescence isolation in brain tissue using multispectral imaging

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BACKGROUND

Disorders that affect the central nervous system are highly complex and often the result of many players interacting with one another. The ability to visualize and quantify multiple antigens and complex phenotypes within the same tissue section could lead to better treatment development and more accurate predictive outcomes.

Brain tissue (FFPE) is known to have an inherent autofluorescence signal that has made traditional multiplexing a challenge. Using Akoya's OPAL-TSA staining technology and spectral unmixing with autofluorescence isolation, we have been able to overcome some of these challenges in order to enhance the signal-to-noise ratio and to visualize the tissue environment more completely.

METHODS

Tissue Preparation and Staining

Tissue was fixed in PLP and sectioned, blocked, and embedded in paraffin. 10 micron slides were cut using a microtome and dehydrated. After de-paraffinizing, the slides were stained manually, using the following steps: 1) 15 minute antigen retrieval using a microwave protocol; 2) 30 minute 3% DKS block; 3) 1 hour primary antibody incubation; 4) 30 minute secondary antibody incubation; 5) 10 minute Opal dye incubation. All steps were performed at room temperature. After staining was finished, the slides were coverslipped and sealed, and then stored at 4°C.

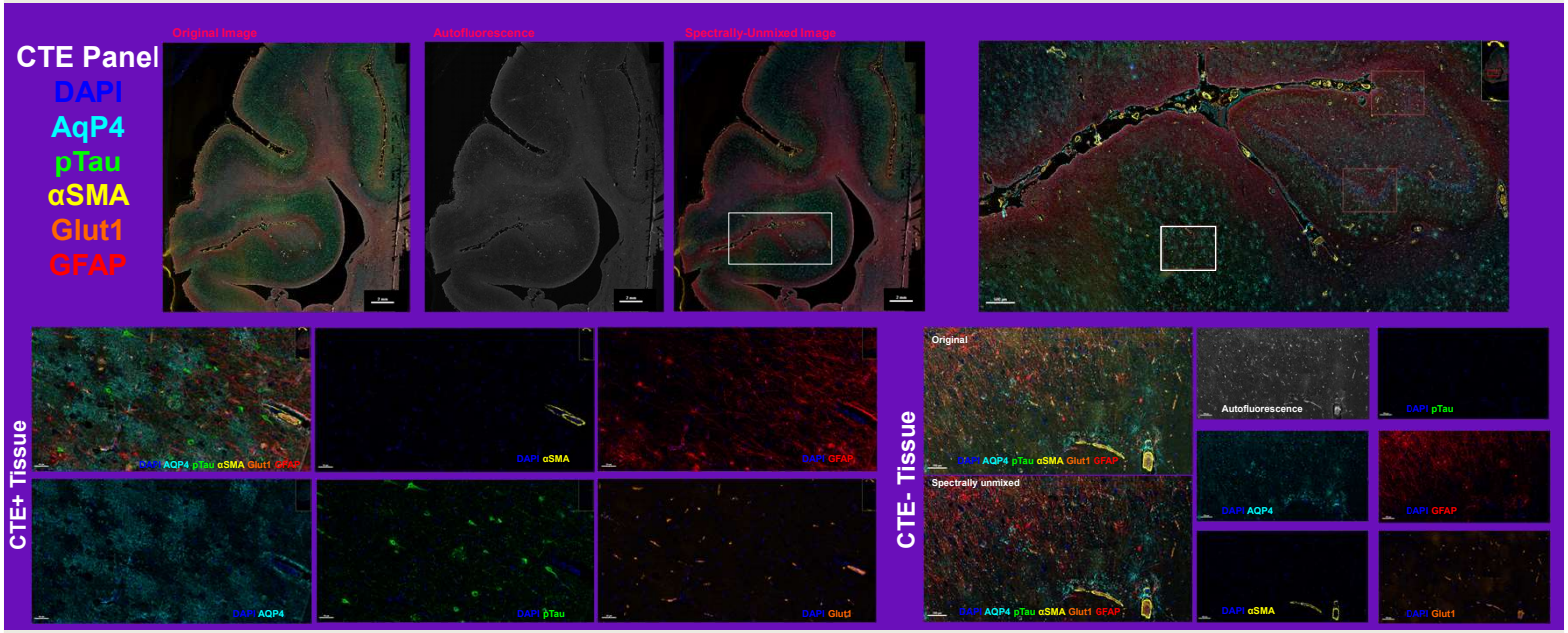
Vectra Polaris Scanning

The Vectra Polaris was set up using the MOTiF whole slide scan workflow with each channel autoexposed. At the same time and under the same conditions as the multiplex images, an unstained but rehydrated slide was scanned and used to determine and separate the inherent autofluorescence of the tissue without the use of Sudan Black or other harsh chemical treatments.

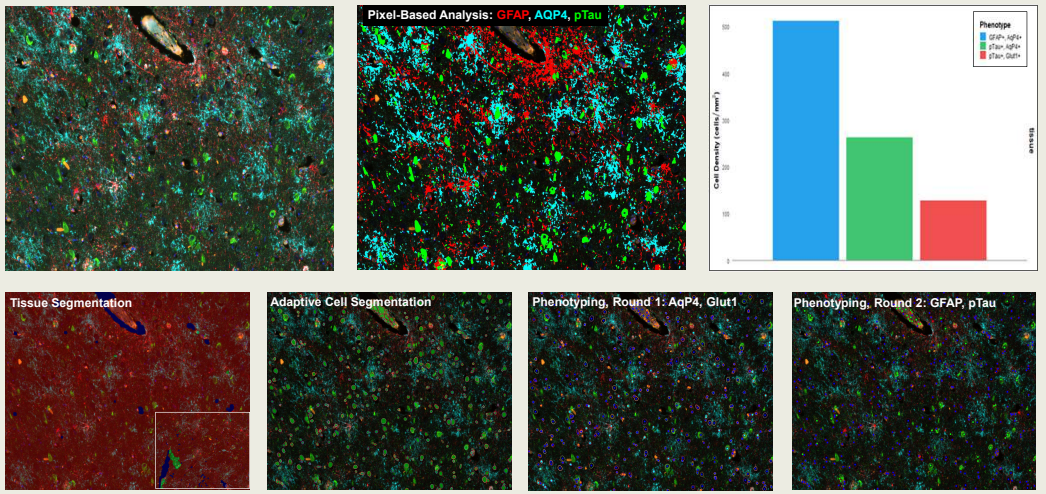
inForm Software Analysis

inForm software was used to create an image analysis algorithm to spectrally unmix Opal dyes and autofluorescence on tissue samples annotated in the Phenochart software. Next, an analysis project was created to analyze pixel-based signal through colocalization and thresholding. Finally, a project was created to evaluate cell-based signal using tissue segmentation, adaptive cell segmentation, and two rounds of phenotyping. Complex phenotypes were identified and analyzed with phenoptrReports.

RESULTS – MULTISPECTRAL WHOLE-SLIDE IMAGING – HUMAN BRAIN TISSUE



RESULTS – INFORM IMAGE ANALYSIS



Average Signal to Noise Ratio			
	Without AF removal	With AF removal	Fold Increase
DAPI	26:1	999:1	38.42
Opal 480	14:1	867:1	61.93
Opal 520	41:1	651:1	15.88
Opal 570	229:1	999:1	4.36
Opal 620	41:1	194:1	4.73
Opal 690	43:1	53:1	1.23

CONCLUSIONS

- The addition of two markers over traditional FL-IHC allowed for more complete and complex phenotyping of cells.
- The isolation of autofluorescence signal allows for an increased signal-to-noise ratio without the need for chemical treatment (up to **60x higher SNR** in the Opal 480 channel).
- The use of inForm software allows for both pixel- and cell-based analysis.

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