Decoding Parkinson's Disease Pathology: Spatial Phenotyping through Ultrahigh-Plex Imaging

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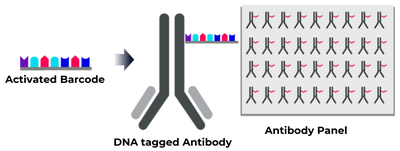
1. Parkinson's Disease

Background: Parkinson's disease (PD), a complex neurodegenerative disorder, poses a significant challenge in unraveling its complete pathology due to the intricate interplay of cellular and molecular manifestations. While neuropathological examinations of post-mortem brain samples have uncovered notable characteristics such as the **loss of nigral neurons** and the **presence of alpha-synuclein inclusions**, alongside markers of **inflammation and tau deposition**, the comprehensive understanding of this complex disease remains elusive. Techniques such as single-cell sequencing have the power to detect highly complex expression signatures from an individual cell but at the cost of spatial resolution at the cellular and subcellular levels. This warrants the need for protein level correlates that can capture both spatial and single-cell level measurements.

Objective: To provide a **detailed landscape of the pathology observed in PD**, we established a panel of oligo-labeled antibodies for ultrahigh-plex imaging of human post-mortem PD samples using Akoya's **PhenoCycler®-Fusion** technology.

2. Building a PhenoCycler Antibody Panel

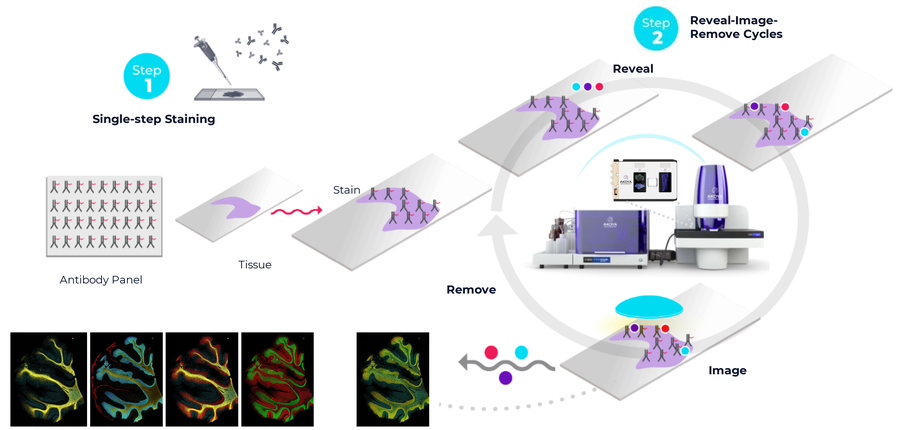


The PhenoCycler-Fusion 2.0 workflow is compatible with a wide range of commercially available antibodies. Antibodies can also be customized via tagged-to-activated oligonucleotide barcodes that are complementary to existing antibody panels. (e.g. Neuroscience core Module below). The antibodies are titrated and tested for appropriate target recognition and then added to a panel. We routinely deploy panels with 100+ antibodies. The table below shows a neuroscience antibody panel that is available and screened against **human FFPE** brain tissues).

Neuroscience Core	NeuN, MAP2, GFAP, Olig-2, IBA1, MBP, Vimentin, Neurofilament, Synaptophysin
Vasculature	a-SMA, Caludin-5, CD31, CD34, CollagenIV
Immune	CD45, CD68, CD163, TMEM119
Custom Module	ChAT, α-Synuclein, pRAb12, Total RAb12, Tau (pSer202), TH, Amyloid-β (Aβ), VGLUT1, pTDP-43, AQP4, GAD (65/67)

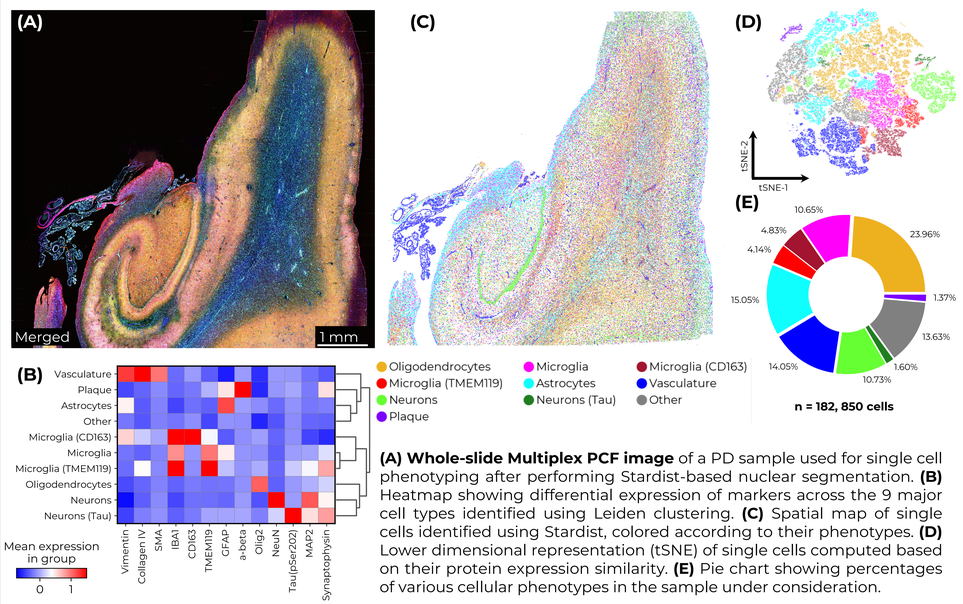
Our **panel of 30+ proteins** include markers of neuronal cells, immune cells, epithelial cells, pathological markers (Synuclein, TDP-43, Tau, and Amyloid beta) and a marker of LRRK2 signaling (Rab12, pRab12). We applied our antibody panel to a cohort of samples of substantia nigra, striatum and nucleus basalis from non-neurologically compromised controls, age matched PD and LRRK2- PD autopsy samples. For each sample, we performed cell segmentation and average intensity computation followed by unsupervised clustering to characterize major cell phenotypes and their spatial distribution.

3. PhenoCycler-Fusion Workflow (formerly CO-Detection by indexing CODEX)



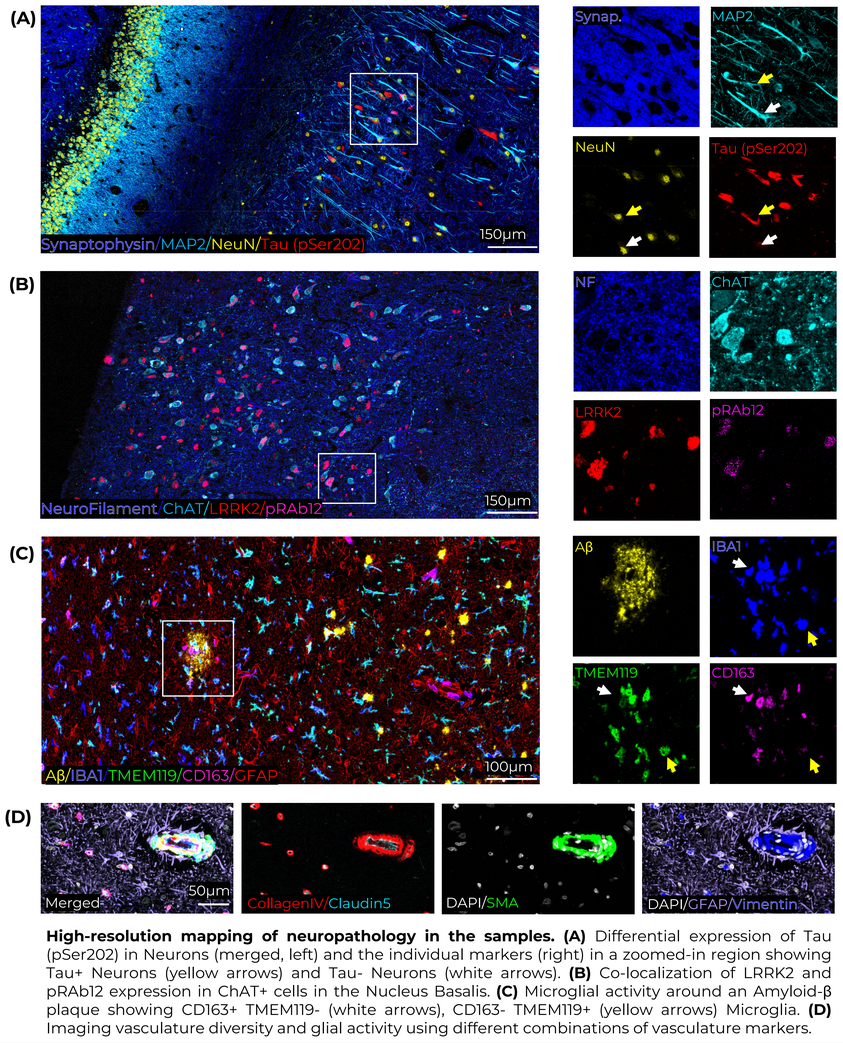
The PhenoCycler-Fusion (PCF) 2.0 workflow consists of iterative cycles of labelling, imaging and removing fluorescent reporters. In each imaging cycle, three fluorescent reporters are hybridized to their corresponding barcode-conjugated antibodies and imaged via standard fluorescent optics. Thereafter, the three reporters are gently removed, and a new cycle images additional reporters. The process is fully automated, and data are acquired across **whole slides at single-cell resolution.**

4. Spatial Phenotyping of Human Brain Tissue



(A) Whole-slide Multiplex PCF image of a PD sample used for single cell phenotyping after performing Stardist-based nuclear segmentation. **(B)** Heatmap showing differential expression of markers across the 9 major cell types identified using Leiden clustering. **(C)** Spatial map of single cells identified using Stardist, colored according to their phenotypes. **(D)** Lower dimensional representation (tSNE) of single cells computed based on their protein expression similarity. **(E)** Pie chart showing percentages of various cellular phenotypes in the sample under consideration.

5. Mapping PD Neuropathology in Human Brain Tissue



High-resolution mapping of neuropathology in the samples. (A) Differential expression of Tau (pSer202) in Neurons (merged, left) and the individual markers (right) in a zoomed-in region showing Tau+ Neurons (yellow arrows) and Tau- Neurons (white arrows). **(B)** Co-localization of LRRK2 and pRAb12 expression in ChAT+ cells in the Nucleus Basalis. **(C)** Microglial activity around an Amyloid-β plaque showing CD163+ TMEM119- (white arrows), CD163- TMEM119+ (yellow arrows) Microglia. **(D)** Imaging vasculature diversity and glial activity using different combinations of vasculature markers.

6. Ulrahigh-Plex Spatial Phenotyping Reveals PD Pathobiology

- We present a panel of markers for ultrahigh-plex characterization of PD phenotypes that quantitatively distinguishes between healthy control and PD autopsy samples.
- Phenotype neuronal and non-neuronal cell types such as microglia, astrocytes, and oligodendroctyes and their functional subtypes
- Detect key neuropathological markers in human brain: a-beta, tau, a-syn, TDP-43
- LRRK2 substrate pRAb12 is localized in Nucleus basalis of Meynert in ChAT-positive neurons

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