

1. Introduction

Immunofluorescence imaging is widely used in neuroscience, but few advanced multiplex immunofluorescence techniques have been implemented. **CO-Detection** by indEXing (**CODEX[®]**) is an ultra-high plex immunofluorescence imaging technology that enables imaging of 40+ biomarkers *in situ* and at single cell resolution. We demonstrate the successful implementation of CODEX[®] to image formalin-fixed paraffin embedded (FFPE) human brain slices. Our data confirm that CODEX[®] is suitable and immediately deployable for neuroscience research.

2. The CODEX System and Method

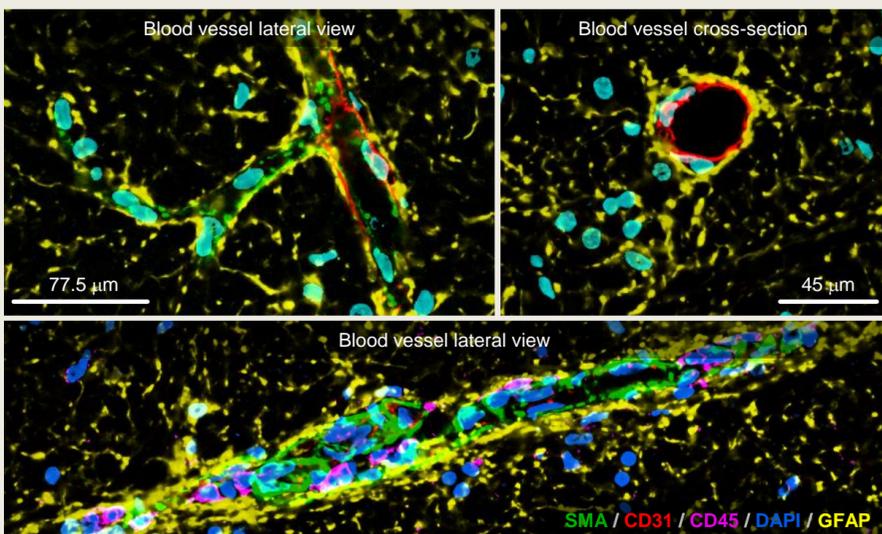
THE CODEX[®] SYSTEM

SPATIAL, SINGLE-CELL ANALYSIS OF 40+ MARKERS



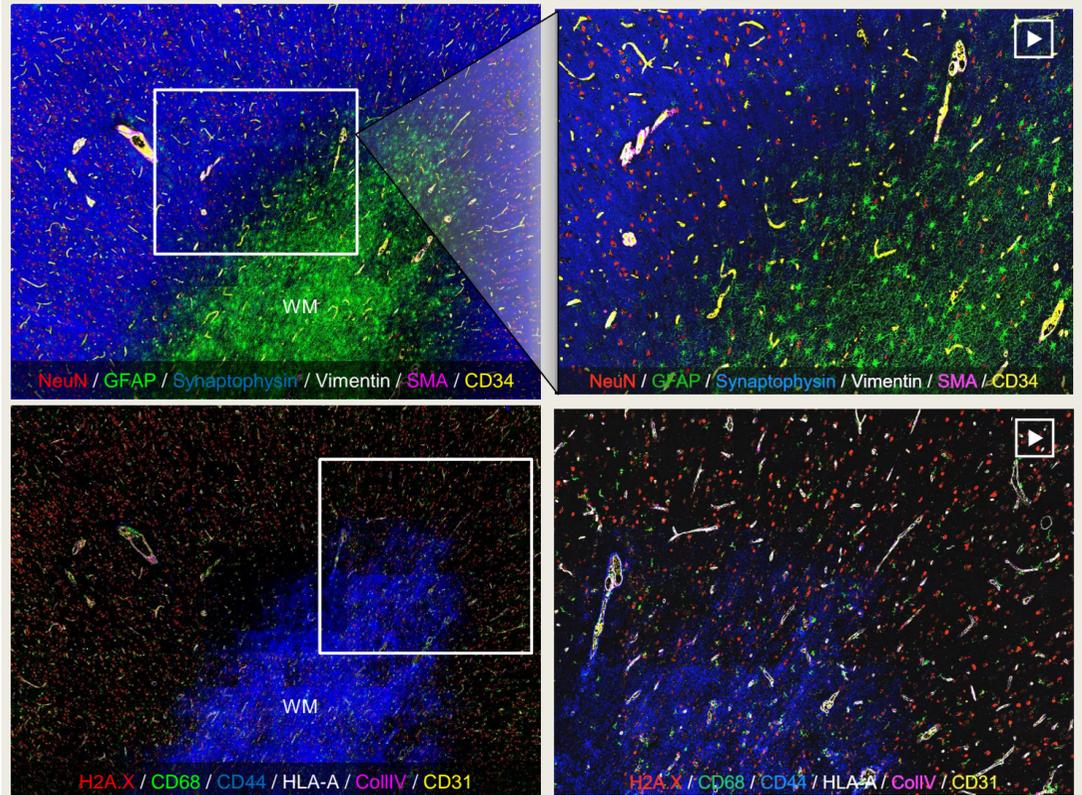
Post-mortem human brain tissue (8 μ m slices; FFPE; Seeley Lab UCSF) was stained with 22 oligo-barcoded CODEX antibodies (**STAIN**). Antibodies were visualized with fluorescent CODEX reporters via iterative reveal-image-remove cycles (**IMAGE**). Tissues were imaged with a Keyence BZ-X series epifluorescence imaging system and data were analyzed with the CODEX Multiplex Analysis Viewer MAV (**ANALYZE**).

3. Neurovascular Labeling



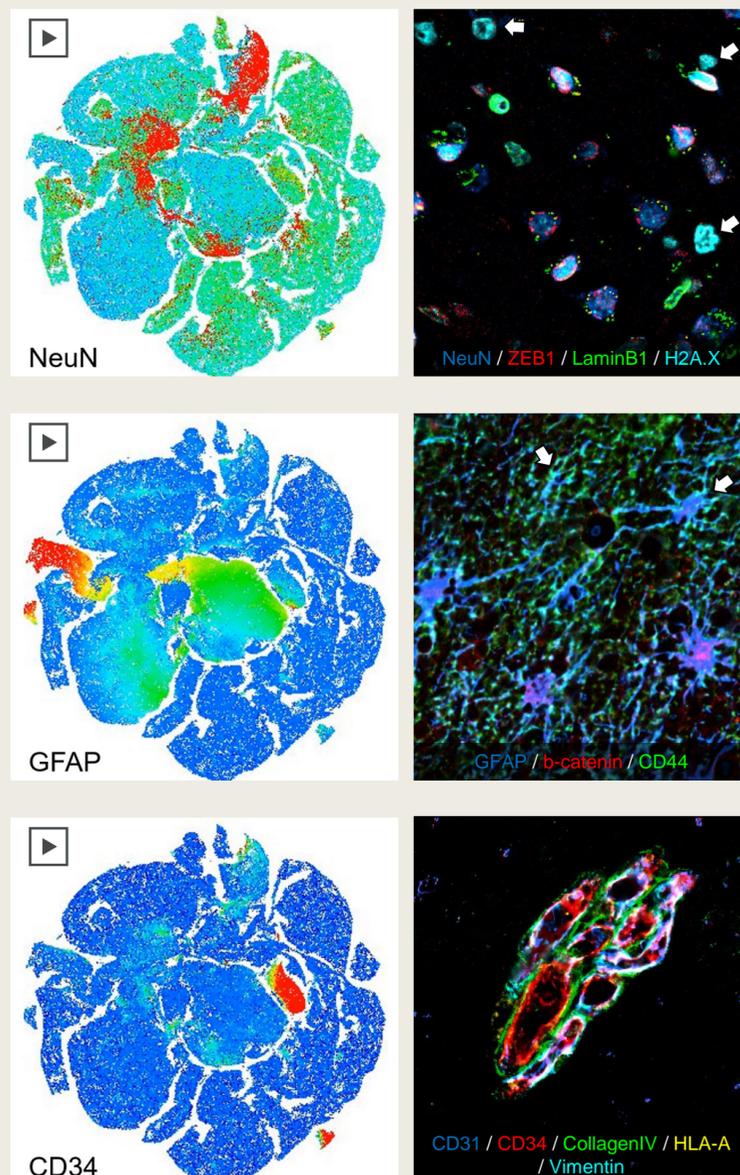
Neurovascular details illustrate the sensitivity & utility of CODEX for spatial analyses. Systemic CD45 immune cells (magenta) interact primarily with vascular SMA (heatmap: log-odds ratio of cells neighboring within 20 μ m for 4555 SMA and 1799 CD45 cells). This result confirms that CD45 cells are trapped in the blood vessel and that the neurovascular unit in this brain is intact.

4. Regionally Distinct Labeling



Overlaid imaging data from 12 antibodies from four imaging cycles. All images are from the same sample at the same imaging position; cortical white matter is indicated as WM. The right-hand column contains animated data, zoomed in, and separately shown for each antibody.

5. Cell-type Specific Biomarker Clustering



T-SNE plots showing the expression of distinct biomarkers in neurons (top row), glia (middle) and the vasculature (bottom). Images on the right show biomarkers that are in the T-SNE. Note that H2A.X is also expressed in non-neuronal cells (arrows top). CD44 localizes specifically to glial processes (arrows middle). The bottom row shows multiple layers of the neurovascular unit, starting with CD34 and HLA-A in the lumen vs. CollagenIV and Vimentin in the outer layers. All data were clustered with CODEX MAV software and are consistent with prior reports.