HIGH-THROUGHPUT IMMUNOHISTOCHEMISTRY IMAGING AND QUANTIFICATION USING PMT-GENERATED IMAGES AND CLOUD-BASED ML-POWERED SOFTWARE

198.22

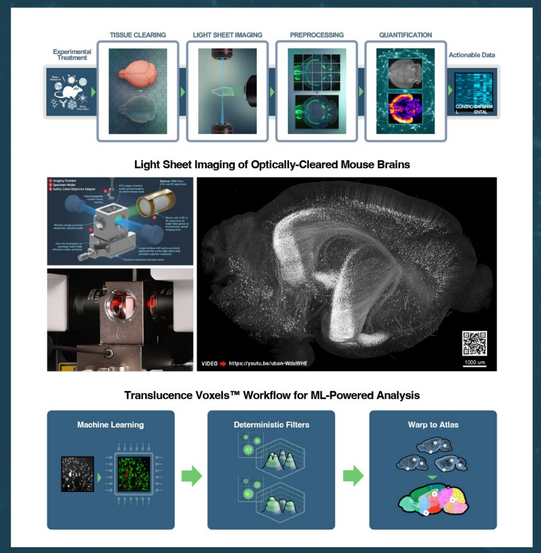
ERIK CASTILLO [1], NATHANIEL GUANZON [1], ALEJANDRO CERVANTES [1], C. CHARLINE BARABAN [2], PERRINE BOREL [2], DAMIAN G. WHEELER [1,3]

[1] Translucence Biosystems, Inc. (Irvine, CA, USA) [2] Innopsys (Carbonne, France) [3] Activity Signaling, LLC (San Diego, CA, USA)

Introduction

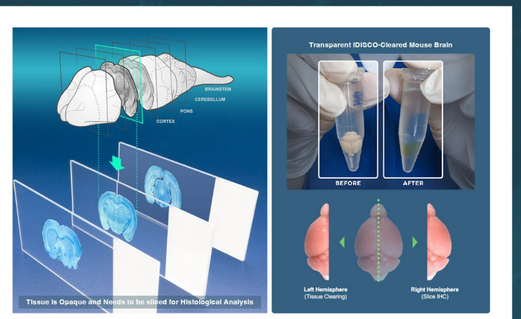
- Traditional 2D immunohistochemistry relies on wide-field microscopes that illuminate slides with LED sources and capture tiled mosaics using CMOS cameras. Each slide must be imaged manually, stitched, and quality-checked under continuous supervision. This workflow is time-intensive and difficult to scale for large studies.
- The InnoQuant™ slide scanner automates large-area imaging, capturing full slide ROI at uniform illumination without mosaics, avoiding tiling or stitching artifacts.
- By integrating Translucence Biosystems' AI-powered Voxels™ platform, 2D datasets can now be quantified at slice-wide and regional scales with reduced background and improved signal fidelity.
- In this poster, we outline how this collaboration provides researchers with an end-to-end solution—from imaging to analysis—enabling reproducible, high-throughput quantification of immunostained tissue sections.

Pipeline For the Generation of 3D Anatomics Data



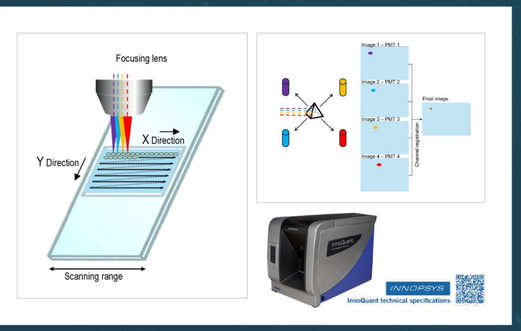
Mice are experimentally manipulated, including treatment with small molecules, antibody therapeutics, cellular therapeutics and gene therapies. Fixed and perfused brains are cleared and immunostained intact and then imaged on the ZEISS Lightsheet Z.1 microscope with our Mesoscale Imaging System™, which adapts the ZEISS Lightsheet Z.1 and 7 microscopes for imaging large cleared tissues in high refractive index solutions with mesoscale optics. The Thy1-GFP brain above was imaged in ~25 minutes using this setup. After tiling and stitching in our Stitchy™ software, our machine learning-enabled Voxels™ software produces actionable anatomics data. Voxels begins with machine learning–based voxel classification, generating probability maps of objects of interest across whole-brain images. These probability maps are then refined using deterministic filters to produce segmented objects. In parallel, each brain is aligned to a common reference atlas using linear and nonlinear transformations. Segmented objects are then warped into this common atlas space, enabling regional quantification across hundreds of brain regions. Metrics are extracted hierarchically, supporting both region-specific and whole-brain analysis.

Traditional Slice IHC Complements 3D Histology



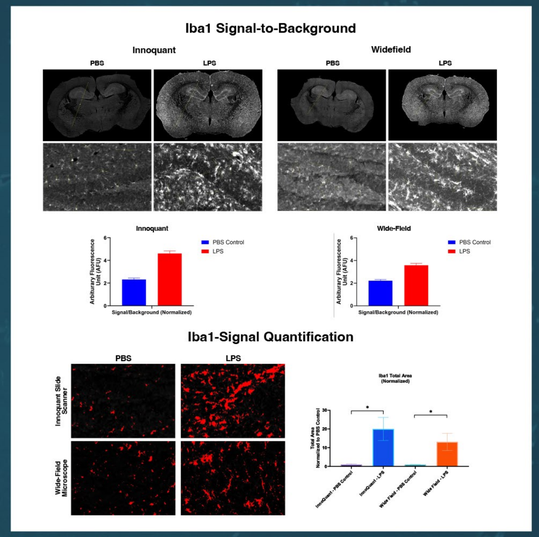
Traditional 2D immunohistochemistry and 3D whole-brain clearing offer complementary insights. 2D histology enables finer assessment of biological details through high-resolution coronal sectioning and imaging, while tissue clearing and 3D imaging provide a broader, brain-wide view of spatial patterns and network-level changes. In addition, 2D histology remains the gold standard for regulatory review and quantitative validation.

InnoQuant Scanner Multiplexed Acquisition Principle



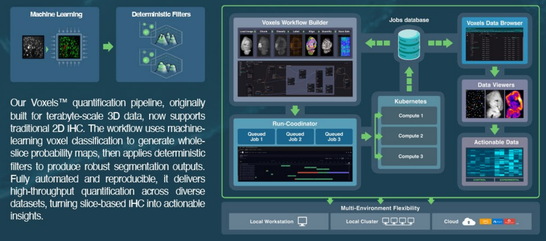
The InnoQuant slide scanner operates on a unique optical design that enables simultaneous acquisition across four fluorescence channels. Four excitation lasers (375, 488, 561, and 640 nm) are co-focused onto the sample through a single focusing lens and spatially separated on the slide by a few micrometers. The lens scans across the full width of the slide to generate a line, while the sample is translated along the Y-axis to scan the next line and complete the acquisition. Emission signals are separated by a pyramidal beam splitter and detected by four dedicated PMTs. The four channel images are subsequently merged to produce a composite raw image. The resulting data are free from mosaic stitching, illumination artifacts, and post-acquisition corrections, ensuring high quantitative accuracy and reproducibility.

Innoquant Benchmarking



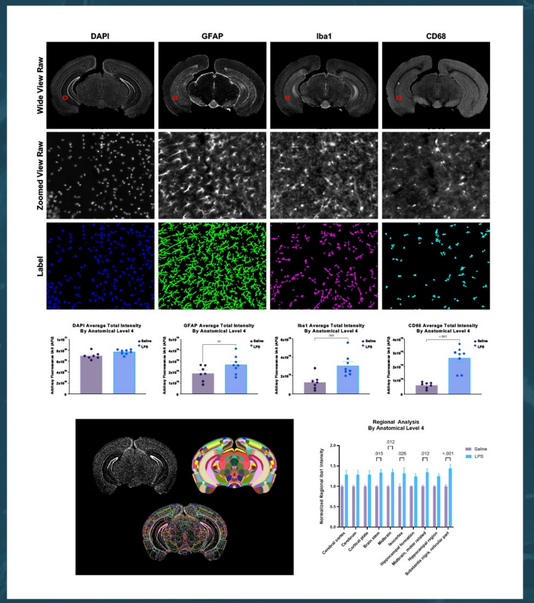
To validate the InnoQuant system for use in larger high-throughput 2D histology projects, brain sections from PBS- and LPS-treated mice were stained for the microglial marker iba1 and imaged using both the InnoQuant PMT slide scanner and a previously validated research-grade wide-field microscope. Quantitative analysis showed comparable Iba1 intensity profiles across platforms. Images acquired with the InnoQuant scanner exhibited higher signal-to-background ratios, improving assay sensitivity and reproducibility. Both systems detected a significant LPS-induced increase in microglial activation, confirming that the InnoQuant PMT scanner provides equivalent results while enabling scalable, time-efficient imaging.

Translucence Voxels Workflow for ML-Enabled Analysis



Our Voxels™ quantification pipeline, originally built for terabyte-scale 3D data, now supports traditional 2D IHC. The workflow uses machine-learning voxel classification to generate whole-slice probability maps, then applies deterministic filters to produce robust segmentation outputs. Fully automated and reproducible, it delivers high-throughput quantification across diverse datasets, turning slice-based IHC into actionable insights.

Slice-wide and Regional Quantification: PBS vs LPS



The InnoQuant scanner, combined with our Voxels machine learning workflows, enables quantification across four epitopes (DAPI, GFAP, Iba1, CD68) using three sections spanning the hippocampal region (anatomical Level 4). Slice-wide metrics capture broad effects, and regional analysis retooled from our 3D pipeline maps each section to a reference atlas. The integrated approach rapidly delivers actionable data, revealing both section-wide trends and anatomical specificity.

Summary

- Validation showed that InnoQuant™ imaging produced higher signal-to-noise data and quantitative outcomes equivalent to those from research-grade wide-field microscopes.
- Combined with Voxels™ analysis, the workflow yielded statistically robust quantification of Iba1 immunostaining across multiple treatment conditions with high signal-to-background ratios.
- Regional metrics and cell-level data were automatically extracted across all slides, enabling reproducible assessment of biological variability and treatment-induced effects.
- Together, the InnoQuant™ + Voxels™ pipeline delivers a fully automated imaging-to-analysis platform that accelerates quantitative histology, reduces user variability, and saves hundreds of hours compared to conventional workflows.

INFO@TRANSLUCENCEBIO.COM

WWW.TRANSLUCENCEBIO.COM