EASY-TO-USE TOOLS FOR CLEARED TISSUE LIGHT SHEET IMAGE ANALYSIS

TRANSLUCENCE BIOSYSTEMS

JACK G. ZEITOUN [1], ANTHONY REKSOATMODJO [1], CHASE REDD [1], RICARDO AZEVEDO [1,2], SUNIL P. GANDHI [1,2], DAMIAN G. WHEELER [1,3], PHILIP CHEUNG [1]

[1] Translucence Biosystems, Inc. (Irvine, CA, USA) [2] Univ. of California (Irvine, CA, USA) [3] Activity Signaling, LLC (San Diego, CA, USA)

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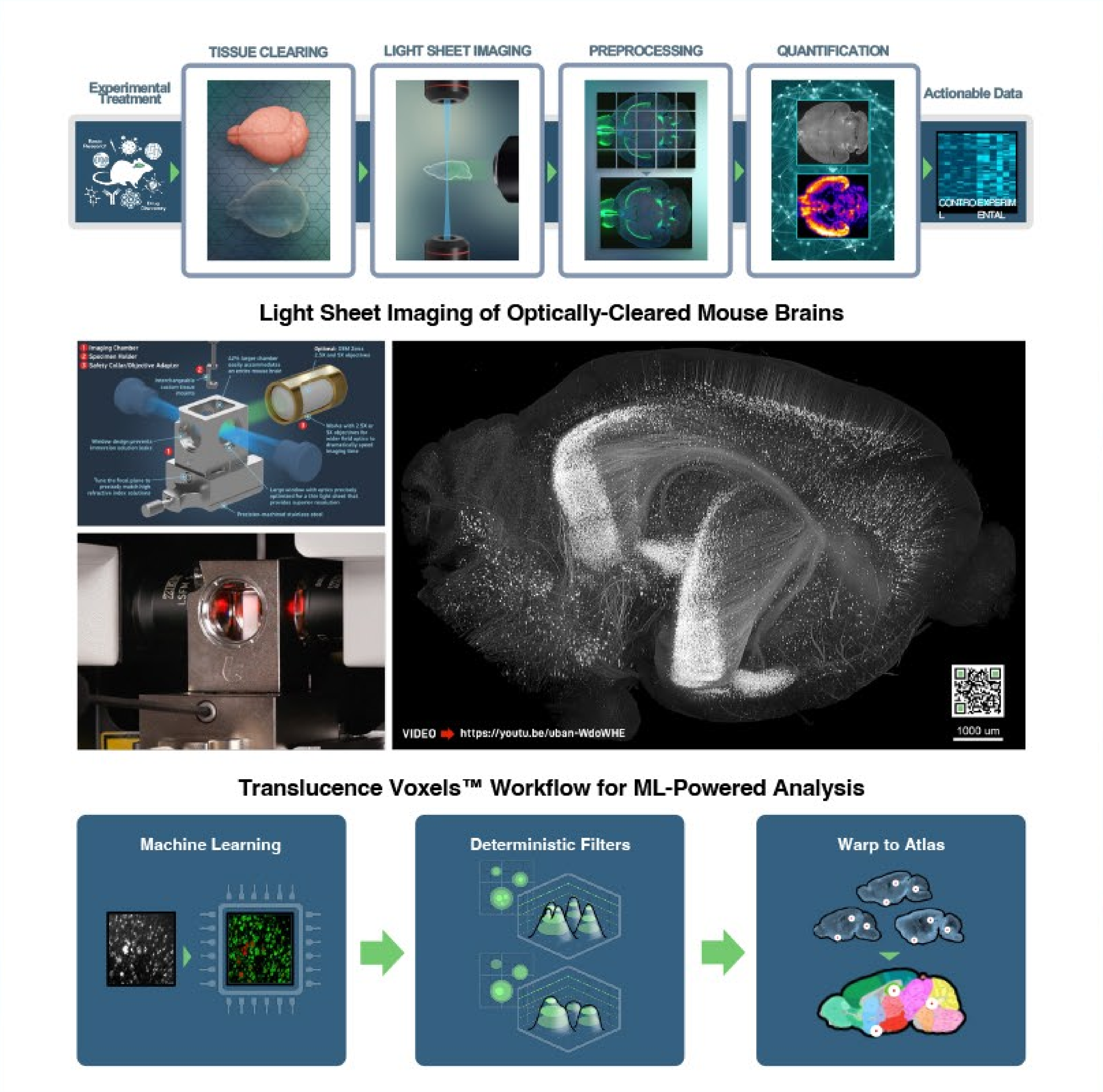
Introduction

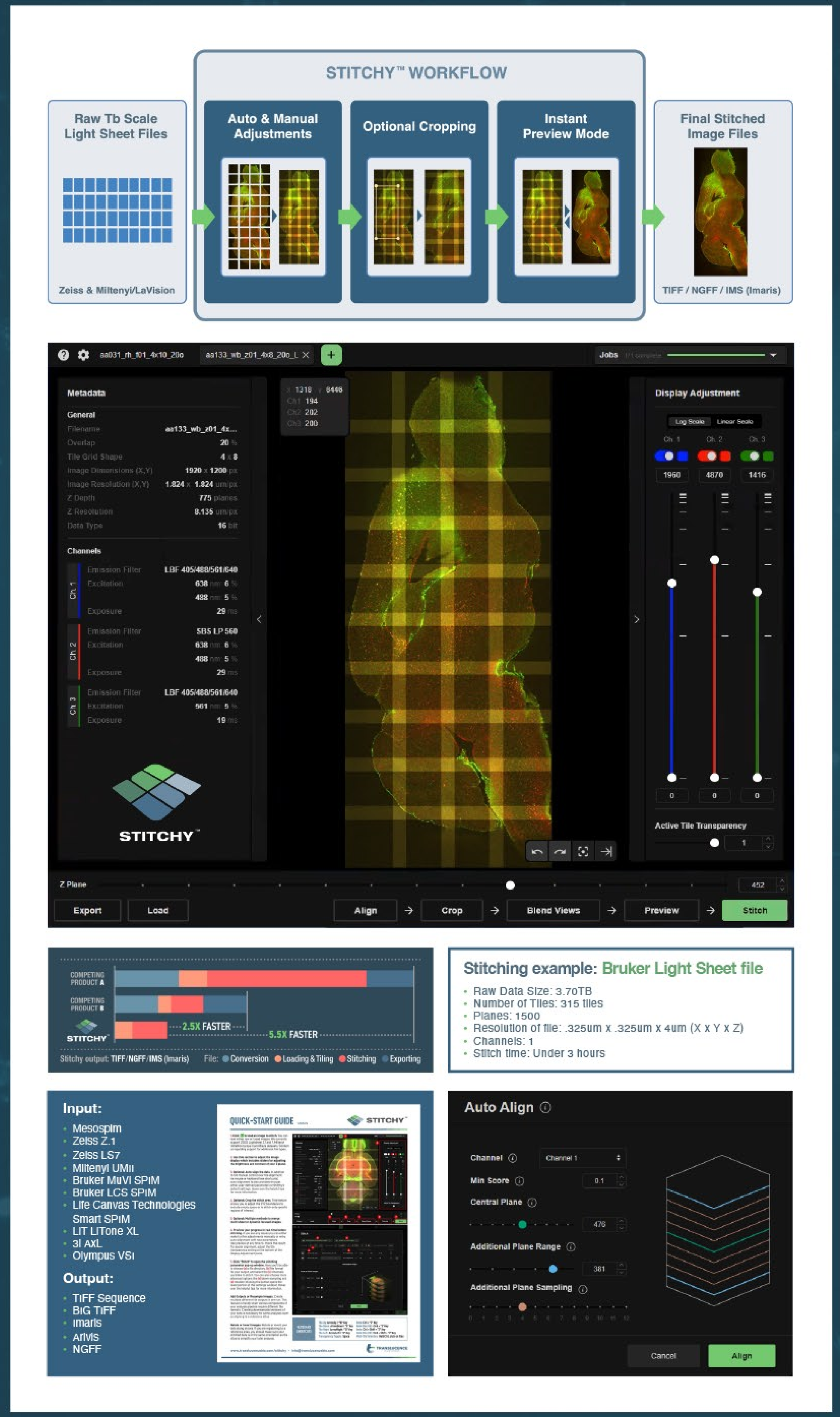
- Conventional 2D histology methods are limited to thin sections and regions of interest, restricting insight into brain-wide structure and function.
- Recent advances in tissue clearing and light sheet imaging have enabled the evaluation of whole-brains at cellular-resolution but have introduced massive data volumes that present a challenge for analysis and reproducibility.
- High-resolution 3D light sheet imaging requires efficient workflows for stitching, registration, and quantification across terabyte-scale datasets.
- Translucence Biosystems has developed a suite of AI-powered tools—Stitchy, BrainSeeker, Maverick, and Voxels—to streamline every stage of the 3D image analysis pipeline, making whole-brain analysis accessible, reliable, and reproducible.

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.

Stitchy



Stitchy enables scientists to stitch terabyte-scale light sheet data at high speeds, while running on a standard desktop computer. Stitchy supports reading and writing directly from a variety of popular file formats (listed above). A simple user interface allows for cropping of the stitch area, selection of auto-alignment parameters, hand-curated tiling, optional z-adjustment of tiles, instant previews, and selection of the output resolution and orientation. Stitchy avoids proprietary intermediate formats and provides significant stitching speed increases.

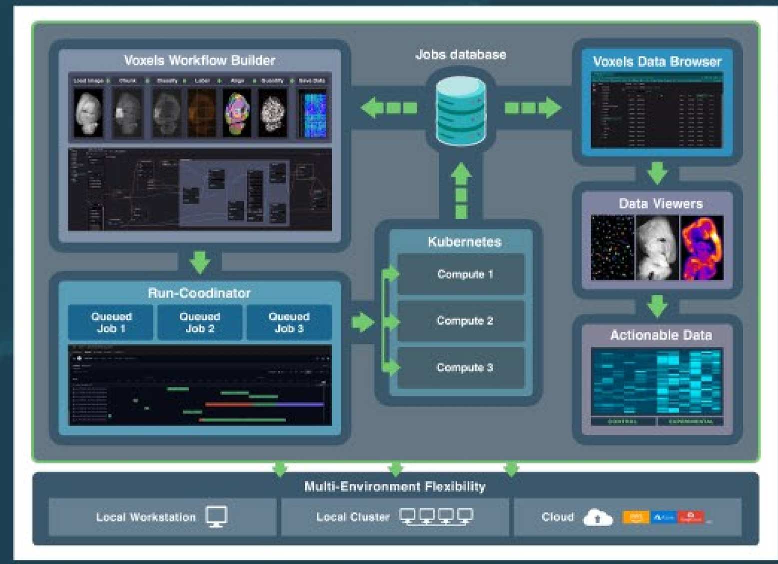
BrainSeeker

BrainSeeker is a tool that enables scientists to perform registration of brain images to reference atlases. BrainSeeker provides three primary features: 1) User friendly registration -- an automated interface that removes the complexity of the registration process; 2) Regional Image Array (RIA) -- allows users the ability to quickly explore and verify the quality of their staining and imaging without having to navigate large terabyte files; 3) Reverse atlas map overlay -- allows users the ability to overlay brain maps onto their light sheet images in raw image space.

Voxels

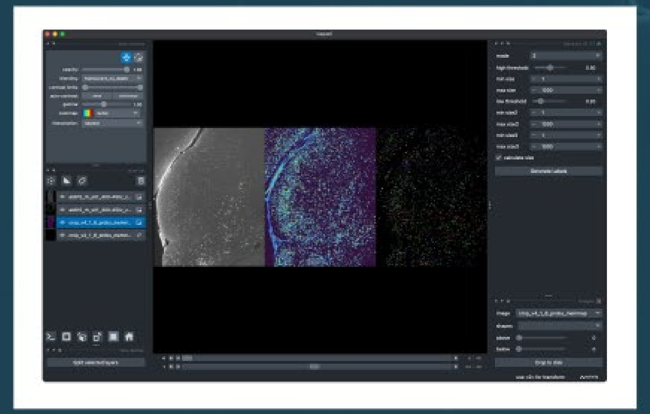
Voxels orchestrates end-to-end 3D Quantification at scale. Scientists compose multi-stage workflows, parameterize analyses, and operate on large datasets through a visual interface rather than code. The platform maintains audit trails, dataset and workflow versioning, and environment consistency to support reproducible results. Backed by Kubernetes, Voxels schedules containerized jobs, balances CPU/GPU workloads, and scales seamlessly from a laptop to an on-prem cluster or the public cloud.

VOXELS™

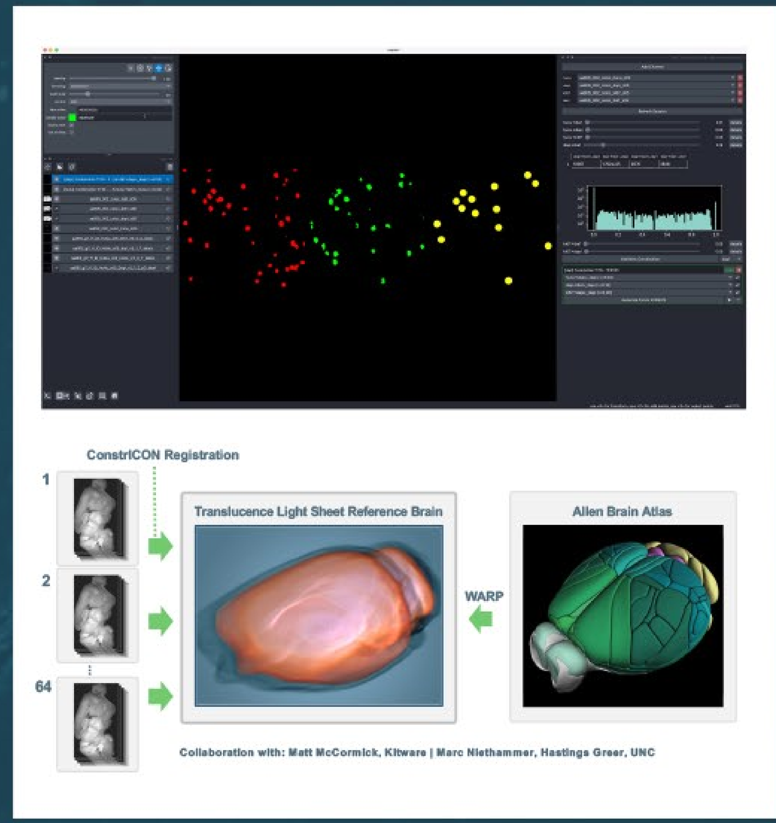


Maverick

One of our advanced labelling tools, Maverick, accelerates segmentation workflow development for cell-type identification. Maverick streams only the high-value regions of interest through our cloud platform, eliminating the need to transfer full multi-gigabyte volumes and enabling fast interactive parameter tuning with hands-on expert ground-truthing across multiple images. In 2D, teams can quickly develop reliable classifiers for IHC slides; in 3D, the same workflows scale to organ-level volumes without linear increases in compute or annotation time. Models are automatically versioned, validated on regional crops, and promoted to production for consistent, auditable quantification across studies.



Under Development Quantification Tools



This figure shows two tools under development: 1) object-based co-localization on multi-channel imaged data and 2) our Translucence reference brain. For our colocalization tool, segmented objects from two fluorescence channels: cFos(+) neurons are shown in Red and neurons expressing Npas4 are shown in green. Our workflow determined the closeness of the centroids of the segmented objects and the yellow dots indicate the location of neurons expressing both proteins. These calculation are done across the entire brain, but a simple cropped 2D plane is shown. The Translucence Reference Brain was generated in partnership with UNC and Kitware, using 64 light sheet autofluorescence image volumes registered together using a neural-network–based registration method called ConstrICON. Unlike the Allen Reference Brain, generated with serial two-photon tomography, the Translucence Reference Brain originates from light sheet data allowing for superior registration of light sheet images.

Summary

- The new Translucence software suite integrates image stitching, registration, and quantification into a unified, scalable workflow for 3D light sheet datasets.
- Stitchy enables automated tiling and z-plane correction across fluorescence channels, supporting all major LSFM file formats and large-batch processing.
- BrainSeeker provides rapid and accurate brain registration to common atlas spaces and advanced AI-based alignment tools.
- Maverick and Voxels empower users to design, visualize, and execute machine-learning–based analysis pipelines through a scientist-friendly, no-code user interface.
- Together, these tools accelerate data processing, improve reproducibility, and transform terabyte-scale 3D light sheet imaging datasets into actionable scientific insights.

INFO@TRANSLUCENCEBIO.COM

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