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Neuroinformatics of the *Allen Mouse Brain Connectivity Atlas*

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ABSTRACT

The *Allen Mouse Brain Connectivity Atlas* is a mesoscale whole brain axonal projection atlas of the C57Bl/6J mouse brain. Anatomical trajectories throughout the brain were mapped into a common 3D space using a standardized platform to generate a comprehensive and quantitative database of inter-areal and cell-type-specific projections. This connectivity atlas has several desirable features, including brain-wide coverage, validated and versatile experimental techniques, a single standardized data format, a quantifiable and integrated neuroinformatics resource, and an open-access public online database (http://connectivity.brain-map.org/). Meaningful informatics data quantification and comparison is key to effective use and interpretation of connectome data. This relies on successful definition of a high fidelity atlas template and framework, mapping precision of raw data sets into the 3D reference framework, accurate signal detection and quantitative connection strength algorithms, and effective presentation in an integrated online application. Here we describe key informatics pipeline steps in the creation of the *Allen Mouse Brain Connectivity Atlas* and include basic application use cases.

1. Introduction

Modern neuroscience is increasingly exploiting three-dimensional digital brain atlases as a means for understanding complex brain anatomy, localizing experimental data, and planning experiments. Such atlases are critical to integrating diverse information in a topographically meaningful fashion [1]. Several recent studies and atlases of brain connectivity have become available, spanning scales from micro through meso to macro, that pose new challenges for data mapping and organization [2–5]. Beyond conventional structure-based atlases, large scale atlases of connectivity of the brain pose special demands on informatics and quantitative processing of the data. In addition to issues of data preprocessing, image registration, and signal quantification, there are substantial challenges in tracing neuronal connections and proper interpretation of the data.

The *Allen Mouse Brain Connectivity Atlas* is a comprehensive database of high-resolution images of axonal projections originated from distinct anatomical regions of wild-type mouse brains or from various genetically labeled cell populations in individual brain regions of Cre-driver mice [5]. Briefly, to create the atlas, each mouse brain was injected with an enhanced green fluorescent protein (EGFP)-expressing adeno-associated virus (AAV) as an anterograde viral tracer into a particular brain region. EGFP labeled axonal projections throughout the brain were systematically imaged using a customized TissueCyte 1000 serial two-photon (STP) tomography system, which couples high-speed two-photon microscopy with automated vibratome sectioning [6]. By imaging serially at 75 μm below the tissue surface of the brain, STP tomography reduces tissue distortions that occur in conventional section-based histological methods, yielding a series of high resolution, inherently pre-aligned images amenable to more precise 3D spatial mapping.

The three dimensional geometry of projections is obtained by sequentially sampling high resolution 2D imagery. For each brain specimen, 140 coronal plane images with high resolution (0.35 μm) images were obtained at a *z*-sampling interval of 100 μm. This procedure spans the entire brain during a continuous 18.5 h scanning period, resulting in an approximately 750 gigabyte data set per brain. The GFP signals from the injection site and

Abbreviations: 2D, two dimensional; 3D, three dimensional; EGFP, enhanced green fluorescent protein; IDP, informatics data pipeline; INCF, International Informatics Coordinating Facility; MIP, maximum intensity projection; MRI, magnetic resonance imaging; R,G,B, red,green,blue; STP, serial two-photon; WHS, Waxholm Space; ZAP, zoom-and-pan.

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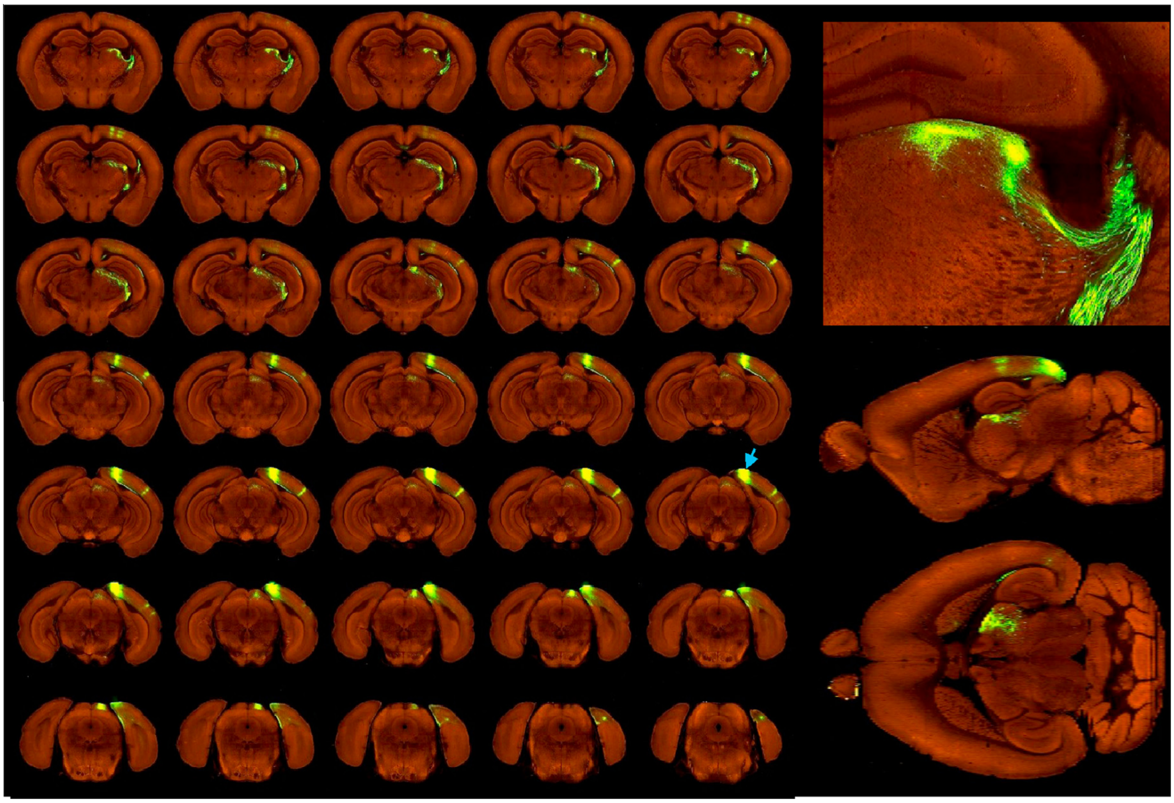

Fig. 1. High-resolution image series of a coronally sectioned brain specimen from the *Allen Mouse Brain Connectivity Atlas* with EGFP viral tracer injected into the primary visual area (blue arrow). The injection site and major projection targets are visible in green. Sequential imaging at 100 µm intervals generates inherently pre-aligned images amenable to precise 3D reconstruction and mapping. The coronal images on the middle and left side of this figure are displayed from a medial to a caudal plane of section through an adult mouse brain. The inset at the upper right shows a detail of EGFP-labeled axonal projections, terminating in the lateral geniculate nucleus. The sagittal and horizontal planes are virtual extrapolations derived from reconstruction of the coronal image series.

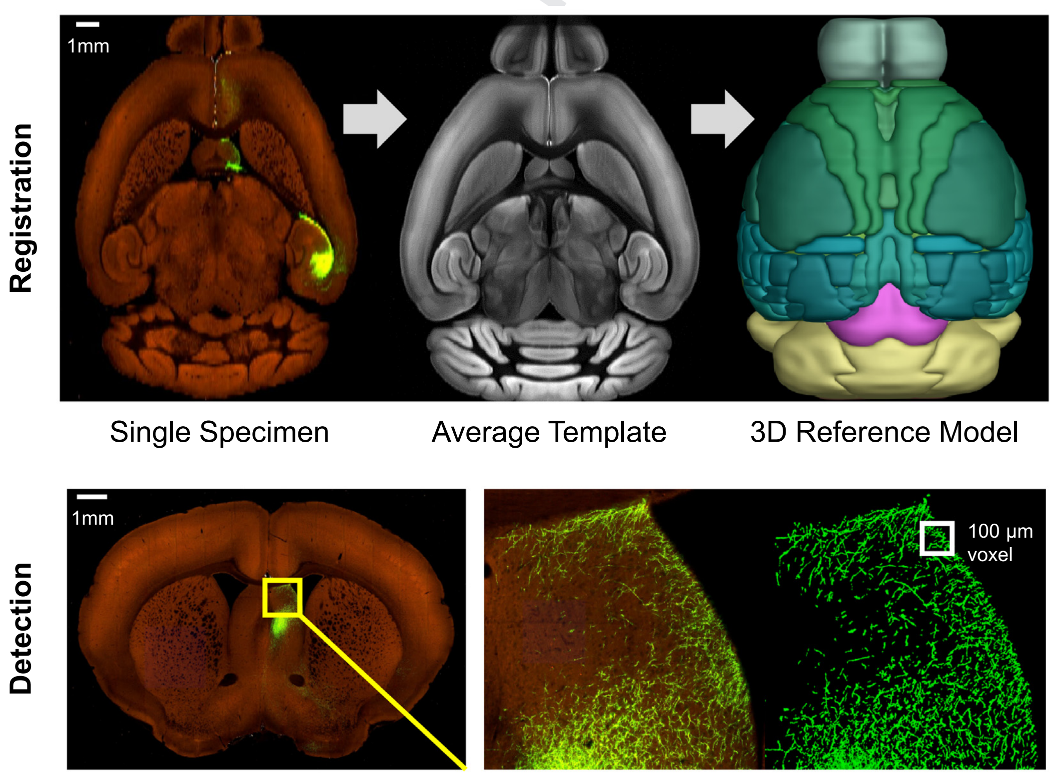

Fig. 2. Major informatics pipeline algorithms of the *Allen Mouse Brain Connectivity Atlas*. Pipeline algorithms implemented include registration and averaging methods to bring all data into the common coordinate framework of a 3D reference space (top panels), and methods to segment and extract labeled axons (detection) from 2D digitized images (bottom panels).