Registration and resampling of large-scale 3D mosaic images

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***Abstract* - Determining the detailed structure of intact organs such as the mammalian brain requires submicron (~0.1 µm^3 voxel size) imaging of large tissue volumes - often hundreds of mm^3. Due to limitations in imaging technologies, large numbers of smaller images, each representing a subset of the full sample, are acquired and assembled into a continuous whole using image registration techniques. The full mouse brain requires approximately 10 teravoxels for a complete representation at diffraction-limited resolution. However, relatively few tools exist for processing biological datasets of this magnitude. Here, we describe a procedure for the registration and resampling of large-scale imaging data that can be employed for assembling tens of thousands of individual image volumes tiling complete organs in three dimensions.**

***Index Terms*—Fluorescence microscopy, Neuroanatomy, Image registration.**

I. Introduction

In many biological applications, it is necessary to visualize complete organs at high resolution in order to investigate spatially extended, fine-scale structures. This is particularly true in mammalian neuroanatomy, as individual neuronal fibers as thin as 100 nm in diameter can traverse long distances before reaching their targets – e.g. many millimeters in the rodent brain. In order to image fluorescently-labeled structures in large tissue volumes at high resolution, serial two-photon tomography has been employed in the past [1], [2]. In this approach, a thin volume near the exposed surface of a tissue sample is imaged repeatedly while sections with a thickness of ~100 microns are progressively removed. While block-face imaging inherently produces images that are approximately aligned, the process of sectioning deforms soft tissue and creates severe discontinuities at the micron scale. For this reason, constructing complete, continuous image volumes from large tissue samples remains challenging. Furthermore, visualizing the resulting image volumes – typically ~20 TB per color channel for the mouse brain – is not possible using commonly available software packages for biological image processing. Here, we describe an approach for registering large collections (tens of thousands) of three-dimensional image stacks totaling 100 TB in size or larger and the resampling of such datasets into an efficiently navigable representation.

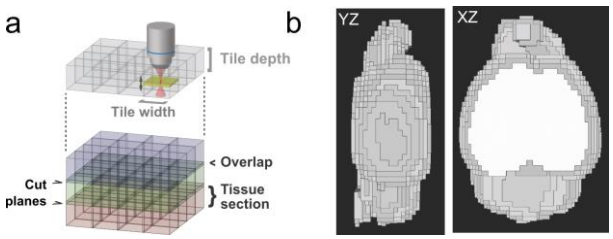

Fig. 1 – Schematic of 3D mosaic imaging. An image volume is acquired by serially imaging many smaller three-dimensional image tiles, which are arranged into layers. This approach can be used to image large structures, such as the full mouse brain.

II. Method

A. Image registration

We consider the case in which a large, three-dimensional tissue volume (up to 1 cm^3) is imaged using volumetric serial two-photon tomography. Here, individual three-dimensional sub-volumes (‘tiles’) covering the full tissue specimen are serially acquired. To facilitate post-hoc registration, tiles are acquired such that they overlap in all three dimensions (Figure 1). A high-fidelity stage system ensures that tiles bordering in the x- and y- directions can be aligned simply by translating adjacent image stacks over a distance specified by the corresponding movement of the stage system. However, physical sectioning of the tissue specimen, e.g. by an integrated vibrating microtome, introduces plastic deformation and subsequent misalignment between image tiles bordering in the z direction. This requires post-hoc registration of neighboring tiles.

In this case, image registration is first carried out pairwise between pairs of tiles that are adjacent in the z-direction. The adult mouse brain contains ubiquitous fluorescence puncta (‘lipofuscin’ [3]) that permit accurate registration using point-cloud registration algorithms. This procedure can be carried out in parallel on a high-performance computing cluster. In our approach, we first identified a set of matched descriptors contained within the overlap regions of all tile pairs across a pair of adjacent tile layers using a previously-described approach [4]. Next, using the coordinates of the stage system

recorded during acquisition of each tile and the microscope field of view dimensions, we projected the location of these descriptors into the coordinate system of the mechanical stages using an affine transformation. This procedure provided an estimate of the position of each descriptor before and after the tissue was sectioned. The displacement between these estimates represents the deformation due to sectioning. For each pair of descriptors, the displacement between them was halved and assigned to the position of each descriptor (with opposite polarity) producing two displacement fields – the first from the descriptors in the bottom of one section and the second for the corresponding descriptors in the overlap region at the top of the next section.

In order to construct a smoothly-varying displacement function across the full extent of the tissue, we used an existing surface-fitting procedure [5] parameterized from $\mathbf{R}^2 \rightarrow \mathbf{R}^3$ to find a surface corresponding to the displacement field in each dimension. This generated two sets of surfaces per tissue section - displacement in x, y and z for the top and bottom of adjacent tile layers.

For each tile, the surfaces corresponding to displacement of the top of the tile and displacement of the bottom of the tile were sampled at 9x9 grid of points equally spaced in the x and y directions (~50 um spacing between grid lines). The z position of these control points was set to the center of the overlapping region for each pair of tiles. The resulting displacement fields were used to adjust the three-dimensional position of the 162 control points for each tile (81 for the top of the tile and 81 for the bottom) in the coordinate system of the mechanical stages and then a new, final set of control points were produced on the top and bottom surface of each tile using linear extrapolation from the points of the initial control set.

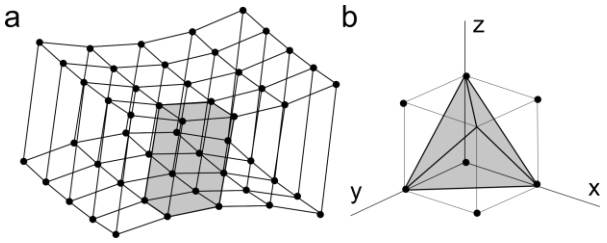

Fig. 2 – Location of control points for a single tile when partitioned using a 5x5 lattice (left). Deformation of tile exaggerated for illustration. Approximation of individual subvolume as the union of 5 tetrahedra (right).

B. Image resampling

To create a navigable representation of the full image volume, the collection of individual, overlapping tiles was resampled using the registration information into a new set of three-dimensional image stacks that tile the imaged volume in a non-overlapping manner.

Briefly, the 9x9 grid of points on the upper and lower surfaces partition each overlapping tile into 64 subvolumes which extend the full length of the z direction (Figure 2, *left*). Since the four corners of each subvolume face are displaced independently, they may not necessarily remain coplanar. Each face can be represented by two triangles, and thus the interior by five tetrahedral (Figure 2, *right*).

A barycentric transform was used to resample each interior tetrahedron into the coordinate system of the mechanical stages to produce the non-overlapping tile set. Trilinear interpolation was chosen to balance image quality and compute time. The resulting registration was markedly better than constraining the subvolumes to be parallelepipeds, i.e. using a unique affine transform for each subvolume.

Contiguous tiles were processed together on the same node of a computing cluster so that spatial locality could be leveraged to minimize file I/O. The entire brain volume was partitioned into contiguous chunks of tiles by iteratively dividing in half along the longest dimension until each partition contained a manageable number of tiles. Tiles in each partition were traversed in Morton order and the image stacks in the output set that overlapped multiple original tiles were merged in local memory before transferring to a file system shared across nodes. Care was taken to balance the load across multiple nodes so that no one job dominated the finishing time.

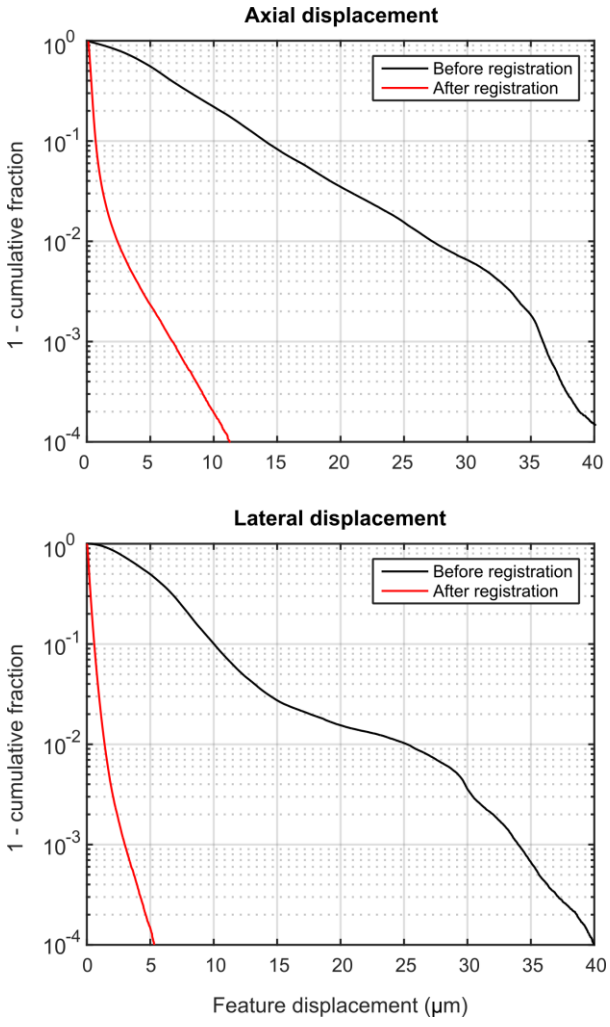

Fig. 3 – Reduction in feature displacement following registration in the axial (top) and lateral (bottom) directions.

Full-resolution non-overlapping output image stacks were downsampled by a factor of two in each dimension, six successive times to form a seven-level octree. The resulting 14% increase in required storage space facilitated exploration of the dataset a various levels of resolution in an efficient manner.

III. Results

We applied our registration procedure to a dataset wherein 32201 tiles represent the full mouse brain. This dataset contained 97 layers and the point-cloud matching algorithm identified an average of 13,756 descriptors per layer. Before registration, descriptors were displaced by 6.89 ± 5.61 microns axially and 5.72 ± 4.24 microns laterally on average, consistent with adjacent layers being approximately aligned after block-face imaging. Following our registration procedure, however, descriptor displacement was reduced substantially to 0.3 ± 0.51 and 0.27 ± 0.3 microns axially and laterally, respectively – values similar to the diffraction limit for fluorescence microscopy. In addition, the fraction of descriptors displaced by more than 1 micron was reduced dramatically in both the axial and lateral directions (axial: 0.92 to 0.04; lateral: 0.96 to 0.03. Figure 3 summarizes the displacement of all identified descriptors before and after our registration procedure.

Image resampling required 3-1/2 days of compute time on a high-performance computing cluster (13 nodes each with 32 2.3GHz Intel Haswell cores and 256 GB memory) using the Julia language to script custom compute-intensive C++ code, and generated 138,910 non-overlapping octree nodes precisely tiling the full mouse brain. These resulting registered and resampled image volumes could be effectively visualized and navigated using both custom [6] and existing applications [7-9].

IV. Conclusions and Future Work

The registration and resampling procedure described herein was found to be computationally efficient enough that it could be completed in substantially less time than that required for image acquisition and provided sufficient accuracy to make neuronal processes within individual tiles appear continuous across the vast majority of tile boundaries. Furthermore, the construction of a hierarchically-downsampled octree representation enabled efficient browsing of data at multiple spatial scales. The registration problem becomes somewhat more complex and computationally intensive when lateral registration between tiles cannot be assumed – e.g. when a high-fidelity mechanical stage system is not available to precisely translate the sample between acquisitions of subsequent tiles. This case remains an outstanding problem for future studies.

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