Serial section Microscopy is an established method for volumetric anatomy reconstruction. Section series imaged with Electron Microscopy are currently vital for the reconstruction of the synaptic connectivity of entire animal brains such as that of Drosophila melanogaster. The process of removing ultrathin layers from a solid block containing the specimen, however, is a fragile procedure and has limited precision with respect to section thickness. We have developed a method to estimate the relative \( z \)-position of each individual section as a function of signal change across the section series. First experiments show promising results on both serial section Transmission Electron Microscopy (ssTEM) data and Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) series. We made our solution available as Open Source plugins for the TrakEM2 software and the ImageJ distribution Fiji.

Index Terms— Serial Section Microscopy, Section Thickness, Optimization, Fiji

1. INTRODUCTION

Serial section microscopy has been used to study the anatomy of biological specimens for over 130 years [1]. Today, neuroscientists use serial section microscopy to reconstruct the microcircuitry of animal nervous systems in their entirety. Electron microscopy (EM) offers the necessary resolution to resolve individual neuronal processes and synapses [2]. However, both serial section transmission electron microscopy (ssTEM), and block-face scanning electron microscopy (BF-SEM) generate section series with significant thickness variance. In a situation where the size of relevant structures is close to the resolution-limit imposed by section thickness, such variance can render accurate reconstruction impossible. Figure 1 top row shows an example of this thickness variance in a virtual \( xz \)-cross-section through a section series acquired with a focused ion beam milling scanning electron microscope (FIB-SEM).

In order to rectify these distortions, we have developed an image based method that estimates the position of each individual section along the \( z \)-axis of the volume. Using this information, a corrected volume can be rendered, greatly facilitating manual and automatic reconstruction efforts.

The underlying observation of our method is that the biological tissue is a signal whose changing speed varies slowly across the section series. Sections that are closer to each other generally look more similar than those farther apart. In addition, individual sections vary in image quality, however, this quality variance is uncorrelated across sections. We therefore simultaneously estimate the decay of simple pairwise similarity measures as a function of the distance between two sections, the quality of each individual section, and the optimal position of each section within the series such that the updated pairwise similarity measure is smooth. The result is a unique \( z \)-position for each section, compensating for variances in thickness (see Figure 1 bottom row). This estimate can be executed locally, accounting for varying section thickness across the section plane. We make our method available as plugins for the TrakEM2 software [3], and the ImageJ distribution Fiji [4], and have published the source code on GitHub.[5

[5]https://github.com/saalfeldlab/em-thickness-estimation
2. RELATED WORK

The problem of varying thickness in microscopy section series has been observed and addressed in previous work. The authors of [5] review four different methods to estimate section thickness: (i) measuring tissue folds that are twice as thick as the section, (ii) comparing electron scattering in the section with a standard test curve, (iii) estimating section thickness from interference patterns that occur when splitting a light beam and placing the transparent refractile Epon-section in one of the otherwise identical beam paths, and (iv) re-embedding the sections, re-sectioning them perpendicularly to the \(xy\)-plane, and then measuring the section thickness within these sections from subsequent EM. Method (i) depends on an artifact that compromises image quality, options (ii,iii) require specialized instruments, and option (iv) is laborious and requires that sections can be re-embedded and re-imaged which is not possible in destructive imaging modalities such as BF-SEM.

In [6], the authors manually annotate the top and bottom surface areas of small image stacks and describe these surfaces by polynomial functions. They then transform the image density (RGB-channel values) such that both surface areas are flat and perpendicular to \(z\). This method requires that individual sections are acquired as volumes correctly reproducing their physical thickness. It is not possible to compensate for thickness variances that are the result of physical compression that differs between adjacent sections.

In their recent work [7], the authors describe an augmentation of the FIB-SEM to correct for section thickness variation during image acquisition. Later, they ascertain structural isotropy of the sample and then transform \(z\)-coordinates such that the peak of the curve of autocorrelations of several \(xz\)-cross-sections of the data has the same half width in both dimensions. Finally, the \(z\)-coordinate of each section is determined by the average over all \(xz\)-cross-sections. This method enables automatic estimation of a scaling factor for sufficiently large section ranges that guarantees isotropy of the contained data. It is not possible to computationally estimate the thickness of individual sections in cases where this thickness varies rapidly.

Our method is unique among previous approaches in that it calculates an accurate \(z\)-position for each individual section directly from post-acquisition image data without further demands on instrument design and imaging modality. It has the potential to calculate section thickness varying in \(x\) and \(y\) in a straight forward manner and does not require manual annotations or processing of section images. As a result, our method readily scales to large EM data sets being routinely collected.

3. SECTION POSITION ESTIMATION

We start with the assumption that the spatial frequencies of tissue change slowly across the series of sections. As a result, the similarities between tissue sections shifted in space should decay with the magnitude of the shift. Since these tissue properties give rise to the image contrast in microscopy, we expect similarities between images of the tissue to reflect those properties. Our method corrects section spacing by estimating \(z\)-shifts that match a locally smooth tissue similarity decay curve. In our experiments, we have used normalized cross correlation (NCC) as a measure for similarity. In addition, we are experimenting with other metrics that are less sensitive to correct section alignment such as the inverse false positive rate of invariant feature matches or the average correlation coefficient of local blockmatches. However, regardless of which similarity metric is employed, the inherent similarity decay curve is generally unknown. It therefore needs to be estimated from the data while simultaneously correcting \(z\)-coordinates. Furthermore, each section is compromised by preparation artifacts (varying imaging conditions, differences in staining, etc.). Assuming that these quality differences apply to each section individually, the “quality” of a section affects all pairwise comparisons equally.

In Equation 1 we combine these assumptions into an optimization problem that jointly solves for

1. a “tissue inherent” similarity decay curve,
2. a quality measure for each section, and
3. \(z\) shifts for each section such that observed similarity curves are best explained by the tissue inherent curve

\[
\arg \min_{\rho, m, s} \sum_{i} \sum_{z} \left( w_{f}(z, \text{ref}) \sum_{c} \left( \rho_{s}^{(z)} - m_{s}(z) R_{c}(\rho_{s}^{(z)} - \rho_{c}(z)) \right)^{2} + \left( m_{s}(z) R_{c}(z - \text{ref}) - \rho_{s}(z) - \rho_{c}(z) \right)^{2} \right) + w_{a}(z, \text{ref}) \left( s(z) - \left( \rho_{s}(z) m_{s}(z) R_{c}(z - \text{ref}) - (c(z) - c(\text{ref})) \right) \right)^{2}.
\]
In our notation, $z$, $z_{\text{ref}}$ and $i$ are indices for sections, $\rho_{\text{z}}^{(i)}$ is the value of the fit of the similarity localized at $z$ and evaluated at $i - z$. $w_f$ and $w_s$ denote window functions that account for local smoothness, $m$ is a vector of quality measures for each section, $c$ holds the actual coordinates for each section, and $s$ holds the shifts with respect to the original (grid) positions. Finally, $R(\rho^{(z)}, \Delta z)$ is the measured value of the similarity for reference $z$ at a distance $\Delta z$.

This optimization problem has trivial solutions for all $m^{(z)} = 0$ and can arbitrarily stretch and compress the series, both potentially leading to further trivial solutions. We therefore apply two bounding conditions:

1. $\rho_{\text{z}}^{(z)} = 1$,
2. the minimum and maximum coordinates of the series remain unchanged, i.e. the series neither shifts nor scales globally.

As there is no closed-form solution to this non-convex optimization problem, we approximate Equation 1 by splitting it into three separate terms that we solve iteratively in alternating sequence until convergence is reached. Furthermore, an iterative approach gives us maximum flexibility to test a variety of further regularizing constraints. First, we estimate the similarity curve inherent to the tissue from the data (Figure 2a). Second, we estimate the quality of each section (Figure 2b) and, third, we calculate and apply the shift for each section (Figure 2c). We repeat these steps until a specified number of iterations is reached.

4. EXPERIMENTS

We applied our method to two different data sets: (i) FIB-SEM\(^2\) dimensions 2048×128×1000 px, voxel-size 8×8×2 nm, and (ii) ssTEM\(^3\) dimensions 2580×3244×63 px, voxel-size 4×4×40 nm. The voxel depth of 2 nm and 40 nm, respectively, is the reported nominal section thickness. As we will see later, the actual thickness of individual sections varies greatly.

4.1. Data Set 1: FIB-SEM

The section thickness variation in the FIB-SEM image data is apparent from the $xz$-cross-section shown in Figure 1. Tissue appearance changes abruptly from fast to slow at the indicated section. This indicates a sequence of thicker and thinner sections, respectively. Our method greatly reduces the severity of this artifact, as evidenced by the “squeezed” and “stretched” cross-sections through neuronal processes in the original image that appear round as expected after correction. We estimated a min and max section spacing of 0.14 and 10.2 px (0.28 nm and 20.4 nm) respectively.

4.2. Data Set 2: ssTEM

In the ssTEM data set, the variances in section thickness are not as dramatic as in the FIB-SEM data. Therefore, we corrected thickness in the original data set and used the result as the basis for two revealing simulated data sets: (i) we removed sections from the original series to artificially create gaps within the series, and (ii) we randomly reordered sections in the series. All results are visualized in Figure 3.

As a numeric measure of quality, we calculate mean and max absolute difference between estimated $z$-positions for the original stack and each modified stack. Any sections that have been removed during stack modification are ignored for this evaluation.

Original: The section thicknesses in the ssTEM data do not vary as much as in the FIB-SEM example. We estimated a min and max section spacing of 0.6 and 1.6 px (24 nm and 64 nm) respectively. As a result, the corrected ssTEM $xz$-cross-sections do not appear dramatically different than the originals as is the case for FIB-SEM.

Missing Sections: We removed sections 20, 21, 22, 46, 47. As a result, this stack contains five fewer sections than the original and structures are shifted compared to their original position (cf. Figure 3). Our method correctly estimates a large distance between those sections adjacent to those that were removed. Due to the applied floor interpolation in Figure 3, they are rendered as thick sections (see arrows). The estimated $z$-position of sections deviates from the corresponding $z$-position in the original reference series by 0.13 px (5.2 nm) on average, and 0.28 px (11.2 nm) at most.

\(^2\)FIB-SEM of *Drosophila melanogaster* CNS, courtesy of Ken Hayworth, Shan Xu, Harald Hess, HHMI Janelia

\(^3\)ssTEM of *Drosophila melanogaster* CNS, courtesy of Rick Fetter, Zhihao Zheng, Davi Bock, HHMI Janelia
Random Order: We randomly reposition each section within in a range of ±4 and then apply our algorithm, allowing for the order of the sections to be changed whenever indicated by the respective z-coordinates. Our method correctly recovers the initial order of the sections (Figure 3). The estimated z-position of sections deviates from the corresponding z-position in the original reference series by 0.044 px (1.76 nm) on average, and 0.13 px (5.2 nm) at most.

5. DISCUSSION & FUTURE WORK

We developed a method that estimates the accurate z-position of all images in image stacks with varying z-spacing. Our method can be used to estimate section thickness in complete section series. In addition, it can detect missing sections, however, it requires further inspection to distinguish gaps due to section loss from thick sections as the method reports only the position of individual sections. Finally, we demonstrated, that the method recovers the original section order in moderately randomized series which is particularly interesting for ssTEM where correct section order cannot be guaranteed.

In our experiments, we estimated a single z-position for each section, however, we are planning to extend the method to estimate thickness variation within sections, employing a hierarchical coarse-to-fine approach.

Our method and the source code are available as Open Source plugins for both TrakEM2 [3] and Fiji [4].

In this paper, we tested our method on cases with rather large variance in section spacing and demonstrated its efficacy. We believe that this correction will improve the performance of both manual and automated segmentation methods by providing more consistent data across sections in z, and will test this hypothesis in future work.

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