Image Registration Based Navigation of Region of Interests in Volume Correlative Light and Electron Microscopy

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Background and Purpose:
Volume correlative light and electron microscopy (vCLEM) has the potential to provide both function and ultrastructure of biological specimens by combining light and electron microscopy. One attractive approach for vCLEM is to prepare samples for electron microscopy (EM) in a way that preserves fluorescence even after they've been embedded in resin (in-resin fluorescence preservation). This allows for navigation to a ROI using fluorescence and EM images acquired at various points through the imaging workflow, including the final ultrathin sections used for transmission electron microscopy (TEM) imaging.

Image registration of light microscopy (LM) and EM images at various scales is required throughout this process. Current trends in image registration rely on methods that warp one image based on user-defined corresponding landmarks. While these methods yield high accuracy, they require significant user time and effort in selecting corresponding landmarks. Recent advancements have introduced automated methods that leverage Laplacian-of-Gaussian filtering or point cloud mapping. While these methods enhance both efficiency and reproducibility, their applications have primarily been limited to cell CLEM, leaving their efficacy in the realm of tissue CLEM unverified. Moreover, achieving ROI navigation through image registration across multiple scales remains a challenge, as existing tools lack the capability to visualize this process effectively.

Here, we aim to bridge this gap by proposing an efficient ROI navigation workflow that leverages new image registration techniques.

Methods:
We use image datasets encompassing mouse brain tissues. The mice are labelled with intrabodies specifically targeting inhibitory synapses, alongside cytoplasmic markers that comprehensively labelled the entire neurons. They are anesthetized, perfused, and brain vibratome sections are collected. Then, cell nuclei are labelled with DAPI and blood vessels with tomato lectin LEL. Next, the tissues are high-pressure frozen, freeze-substituted, and resin embedded. Following the acquisition of a confocal image of the resin block, it is sectioned into ultrathin slices (200 nm) using an ultramicrotome, and

fluorescence images collected of some of those sections. Finally, TEM images of these ultrathin sections are obtained.
Our workflow facilitates the acquisition of EM images corresponding to ROIs identified using LM images. It encompasses two key components:

1. Semi-automated image registration algorithm: This algorithm aims to achieve the registration of images acquired through diverse microscopy techniques. Its core functionality consists of two primary processes: (a) object segmentation and (b) image registration. In (a) object segmentation, the objects that appear commonly in both images are segmented either automatically or with a degree of user interaction. During (b) image registration, images are warped to match objects in (a) across imaging modalities. Image registration metrics such as mutual information are calculated to assess the quality of the warping. Arranged in descending order of value, a user is presented with the overlayed images corresponding to the warping yielding the highest metric value. With the help of the user, the successful registration parameters are determined. In scenarios where one image has greater dimensions than the other, the larger image undergoes a raster scan. During this process, the image value registration metric is computed at each scan position. Notably, if the input consists of an image stack, additional scanning additionally occurs along the depth axis.

2. ROI navigation viewer: This viewer facilitates the visualization of image stack and section image registration results, ultimately guiding navigation towards ROI acquisition via high-resolution EM. The viewer leverages the Napari framework for its implementation and has multiple advantages.

(a) Three-dimensional visualization
This function accepts the image stack and warping parameters to display the three-dimensional positioning of the ROI and ultrathin section fluorescence (and TEM) images relative to the entire low-magnification confocal microscopy image stack. This comprehensive view allows for at-a-glance comprehension of the spatial relationships between multiple images and datasets. Additionally, the depth information from the ROI surface serves as a guide for selecting ultrathin sections.

(b) Overlaying an ROI on an ultramicrotome stereomicroscope image
This function accepts an ultramicrotome stereomicroscope image, a transmitted DIC image, and a confocal microscope image of a tissue block and overlays the ROI location onto the ultramicrotome image to facilitate the precise trimming of the tissue block containing the ROI.
Notably, the transmitted DIC image serves as an intermediary to achieve accurate superposition onto the ultramicrotome image.

(c) Overlaying ROI on low-magnification EM image

This function accepts block confocal microscopy image of a tissue block and its ultrathin section EM image, and displays the location of the ROI overlayed on the low-magnification ultrathin section EM image to facilitate acquisition of high-magnification EM images of the ROI.

Results:
We assessed the efficacy of the proposed workflow using image datasets encompassing brain tissues from two mice. The image acquisition procedure is detailed within the background section. The key distinction between the two datasets is the presence or absence of blood vessel staining (Datasets 1 and 2, respectively).

For the Dataset 1, we succeeded in navigation of the entire block image (2 mm x 2 mm x 0.1 mm, pixel size 0.57 µm in the X and Y, 1 µm in Z) to the single nuclei (roughly size of 5 x 5 µm2, pixel size: 7 nm x 7 nm). For the Dataset 2, we applied Functions 1 through 3 sequentially and succeeded in navigation of the entire block image (600 x 600 x 35 μm^3, pixel size: 0.63 µm in X and Y, 1 µm in Z) to the single axon (roughly size of 1 x 20 µm2, pixel size: 3.4 nm x 3.4 nm).

Conclusion:
While vCLEM offers unparalleled access to three-dimensional information regarding both cellular function and ultrastructure, navigating the ROI for EM image acquisition has been laborious. Our segmentation-based image registration algorithm facilitates seamless navigation across modalities and scales. Coupled with our interactive viewer capable of visualizing registration results and guiding ROI navigation from LM to EM imaging, this integrated approach enables efficient ROI navigation.

Keywords:

Image registration, Automation, Visualization

Reference:

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