Volumetric Reconstruction of Cellular Organelles using Z-splitter Microscopy

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Abstract

Capturing the three-dimensional dynamics of cellular organelles is essential for understanding rapid biological processes like dielectrophoresis. Using images of mitochondria and lysosomes gathered with z-splitter microscopy, they are deconvolved and reconstructed using deep learning-based interpolations. This study aims to assess the performance of these methods in generating precise volumetric images, and implement an integrated visualization program.

1. Introduction

In the modern field of biological sciences, precise observation and visualization at the cellular level is essential not only for deriving reasonable interpretation to the research in itself, but also plays an important role in providing new inspiration to scientists. Although two-dimensional imaging using optical microscopy has been a standard method to observe cells in the early days, there are limitations to practical interpretation with only flat, single-plane information about the three-dimensional structures and dynamics of cells.

The simplest and slowest way to obtain volumetric cellular images is to capture multiple 2D images while translating the camera sensor. Image stacks can be acquired more quickly with a fast z-scanner [4] or light-sheet geometries [2], but they are still slow compared to biological processes, such as dielectrophoresis, which can occur on millisecond time scales.

One of the great scientific proposals for obtaining instantaneous volumetric data is z-splitter imaging [10], which is being cited and developed by various medical and biological studies [7, 12]. It uses a z-splitter prism to split the detection path of a microscope into multiple paths of increasing path length, so that each path results in images of different depth of the sample. Additional clarity and image quality can be acquired through some appropriate deconvolution methods for confocal microscopy here [5].

Since z-splitter imaging takes just several z planes from the sample, the full volumetric shape would be reconstructed with interpolation along the z axis. It could be achieved by traditional B-spline interpolation [11], but thanks to neural networks, now there are lots of more elaborate deep learning based interpolation methods for z-geometric upsampling, including 3D U-Net [1] and deep-Z [8].

In this research, the images of cellular organelles (mitochondria and lysosomes) under the effect of dielectrophoresis are obtained by high-speed z-splitter microscopy. They will be subjected to deconvolution and reconstructed using deep learning-based interpolations to obtain a complete volumetric visualization of them. We will verify the efficiency and precision of the above process and compare the performance of the aforementioned interpolation methods. We will also explore whether there are additional heuristics for the given samples.

2. Method

2.1. Z-Splitter Imaging

The raw 6-plane image stacks of cellular organelles are gratefully gathered from the advisory graduate of this research. The structure of z-splitter microscope is based on the schematics proposed by xiao et al. [10]

(Additional information of how the images are obtained will be added in the final report)

2.2. Extended-Volume 3D Deconvolution

Since z-splitter imaging inevitably contains out-of-focus background data, background suppression and contrast enhancement is essential for image clarity. We use EV-3D deconvolution algorithm shown in Fig. 1 proposed by xiao et al [10], which even considers possible far-out-of-focus

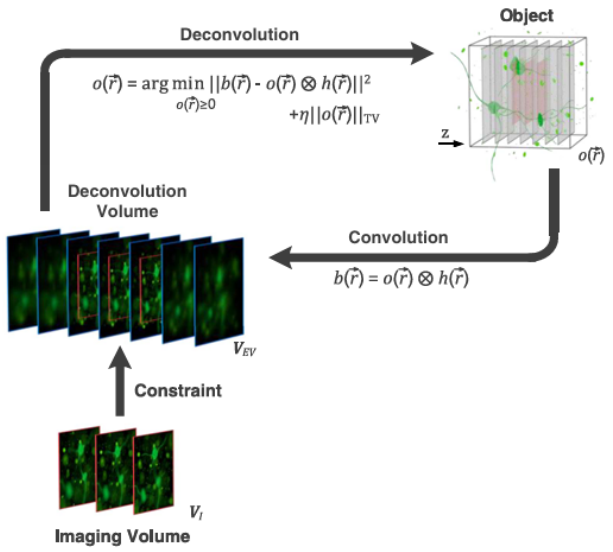

Figure 1. Schematic of EV-3D deconvolution algorithm proposed by xiao et al [10].

fluorescence outside the imaging volume using deconvolution. The algorithm is about a minimization problem for estimated image $o(\vec{r}) \geq 0$ and raw image $b_0(\vec{r}) \geq 0$

$$\min_{o,b} f(o,b) = \min_{o(\vec{r}),b(\vec{r})} ||(o \otimes h)(\vec{r}) - b(\vec{r})||_2^2 + \eta||o(\vec{r})||_{\mathrm{TV}} \tag{1}$$

$$\text{s.t. } b(\vec{r}) = b_0(\vec{r}) \text{ for } \vec{r} \in \mathbf{V}_I \tag{2}$$

in cartesian coordinate $\vec{r} = (x, y, z)$ where $h(\vec{r})$ is the point spread function (PSF) of the system, $\mathbf{V}_I$ is the imaging volume before deconvolution, and $||o(\vec{r})||_{\mathrm{TV}} = \sum_{\vec{r}\in\mathbf{V}_{\mathrm{EV}}} \sqrt{(\partial o/\partial x)^2 + (\partial o/\partial y)^2}$ is the isotropic total variation norm in the extended volume $\mathbf{V}_{\mathrm{EV}}$ when the regularization parameter η is small but greater than zero.

Starting from $b^1(\vec{r} \notin \mathbf{V}_I) = 0$, this deconvolution procedure takes nested iterations for optimization as following Algorithm 1 and 2.

Algorithm 1: EV-3D Deconvolution

Initialize: $b^1 = b^0$
for $k = 1, 2, \cdots, K$ **do**
  $o^{k+1} = \mathrm{argmin}_{o\geq 0}\, f(o, b^k)$
  $b^{k+1} = \mathrm{argmin}_{b\geq 0}\, f(o^{k+1}, b),\ b(\vec{r} \in \mathbf{V}_I) = b_0$

Thanks to the author of z-splitter imaging research, we take the provided MatLab code in GitHub repository [9] as reference for implementation. However, we migrated the code to Python with *cupy* package (the GPU version of well-known *numpy* package) to integrate the project with the visualization tool.

Algorithm 2: Update of o at Iteration k

Initialize: $o_1 = b^k$
for $n = 1, 2, \cdots, N$ **do**
  $o_{n+1} = (\frac{b^k}{o_n \otimes h} \otimes h^*) \cdot \frac{o_n}{1-\eta \mathrm{div}(\nabla o_n/|\nabla o_n|}$
  $o_{n+1} = \max(o_{n+1}, 0)$
return $o^{k+1} = o_{N+1}$

The computation time is tested with a deconvolution volume of size $580 \times 580 \times 25$ pixels, outer iteration $K = 20$ and inner iteration $N = 80$, using a desktop equipped with i9-10900K CPU, 64GB RAM and GeForce RTX 3090 GPU. It takes about 50 seconds per image, which means 20 hours of operation is required to process deconvolution for 24fps one-minute-length video.

This EV-3D deconvolution algorithm is presented to be much faster with 'warm start', by initializing $b^1(\vec{r} \notin \mathbf{V}_I)$ optimally (i.e. using projection onto the imaging volume). It is stated to result in the reduction of outer iteration to $K = 1$ with small degradation of image quality. (This 'warm start' strategy will be applied to organelles image and discussed with its precision and speed in the final report)

2.3. Volumetric Reconstruction

Although EV-3D deconvolution algorithm contains depth extension during computation, it is on purpose of dealing with far-out-of-focus background and not for reconstruction. Full volumetric reconstruction can be roughly done with spline interpolations using Python *scipy* package, but deep-learning based reconstruction would be more elastic way for interpolation from such sparse z-stack images.

3D U-Net [1], first proposed in 2016, is still a good option for volumetric reconstruction. It is a dimensionally extended version of U-Net [6], which has an U-shape network with contracting path for downsampling and expanding path for upsampling, as shown in Figure 2. U-Net is based on fully convolutional network [3] thus generally designed for segmentation tasks. However, it is also usable for reconstruction by expanding the output shape of the network, which is possible with the existence of upsampling process transforming coarse feature map into dense map of an arbitrary shape.

Since there on the Internet is no proper dataset of volumetric organelle images to train networks, we have generated randomly bended cylinders and dented spheres to get 'fake' three-dimensional images of mitochondria and lysosomes. They are arbitrarily sliced to produce z-stacks as training input, and used intact as expected output of the network.

(The last paragraph would be either omitted or fulfilled in the final report after tests) Alternatively, there is an U-Net based pretrained network of z-stack fluorescence microscopy images called deep-Z [8].

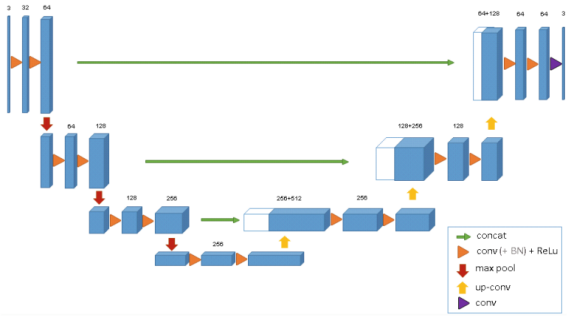

Figure 2. The 3D U-Net architecture proposed by ronneberger et al [6]. Blue boxes represent feature maps. The number of channels is denoted above each feature map.

2.4. Visualization

After the deconvolution and reconstruction, the data is expected to be a three-dimensional array that represents a single alpha-channel volumetric image. Three-dimensional visualization can be done with Python *vedo* package, which is easy-use and supports basic interaction such as rotation and zooms. Here we additionally use Unity3D, a well-known game engine, to enhance interaction with the sample. The reconstructed data is transformed its type from 3D numpy array to json (serialized text data), loaded in the Unity executable, and applied as an alpha-channel texture of transparent raymarching shader of Unity to generate highly-interactive visualization with some helping user interface.

3. Experiments

(The result will be presented in the final report. In this midterm report, here are some figures 3 being tested before the main implementation)

4. Conclusion

(Conclusion will be presented in the final)

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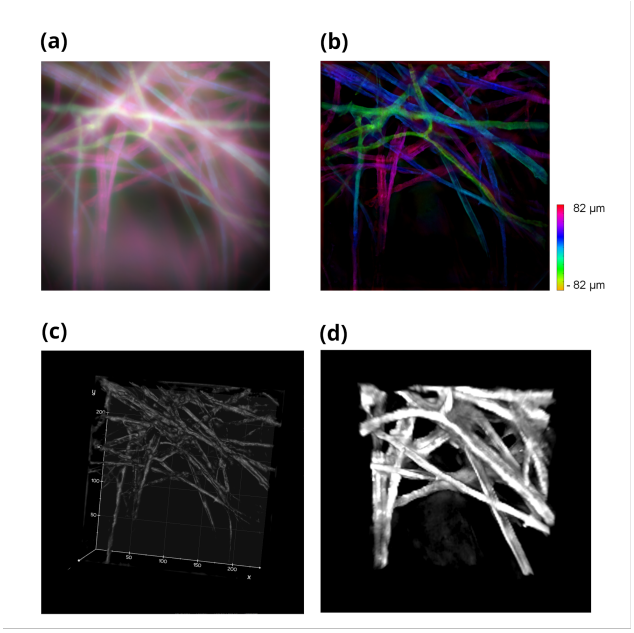

Figure 3. (a) Color-coded extended-depth-of-focus (EDOF) microtubules image of the 9-plane image stack, whose shape is $480 \times 480 \times 9$. It is provided from GitHub repository of xiao et al [9]. (b) Color-coded EDOF image stack computed by EV-3D deconvolution algorithm with outer iteration $K = 20$ and inner iteration $N = 80$. (c) three-dimensional *vedo* visualization of reconstructed microtubules structure of shape $480 \times 480 \times 175$. The reconstruction is roughly done with b-spline interpolation. Alpha threshold 0.2 and some visual adjustment is applied for clarity (each voxel with alpha-channel value lower than 0.2 becomes transparent in the visualization) (d) three-dimensional *unity* visualization of half-compressed structure of shape $240 \times 240 \times 88$. Alpha threshold 0.1 and some visual adjustment is applied for clarity. Each z plane is independently drawn here.

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