Alignment of Large Image Series Using Cubic B-Splines Tessellation: Application to Transmission Electron Microscopy Data

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Abstract. 3D reconstruction from serial 2D microscopy images depends on non-linear alignment of serial sections. For some structures, such as the neuronal circuitry of the brain, very large images at very high resolution are necessary to permit reconstruction. These very large images prevent the direct use of classical registration methods. We propose in this work a method to deal with the non-linear alignment of arbitrarily large 2D images using the finite support properties of cubic B-splines. After initial affine alignment, each large image is split into a grid of smaller overlapping sub-images, which are individually registered using cubic B-splines transformations. Inside the overlapping regions between neighboring sub-images, the coefficients of the knots controlling the B-splines deformations are blended, to create a virtual large grid of knots for the whole image. The sub-images are resampled individually, using the new coefficients, and assembled together into a final large aligned image. We evaluated the method on a series of large transmission electron microscopy images and our results indicate significant improvements compared to both manual and affine alignment.

1 Introduction

Understanding the three-dimensional organization of complex structures and processes is a challenging topic in structural biology. The alignment of serial histological sections is a powerful way of performing such three-dimensional reconstruction of tissues and structures. Many linear and non-linear methods have already been proposed for alignment of histological images at the microscopical scale, including the alignment of images across medical image modalities (for example MRI, Magnetic Resonance Imaging). Works dedicated to the brain include [1], [2] for affine transformations, and [3], [4], [5] for elastic alignments.

The direct use of these methods is usually only possible on a series of images whose size is compatible with the processing capabilities of normal machines in reasonable amounts of time.

However, images with very large fields of view at very high resolution are becoming more and more common in structural biology imaging, in both light and electron microscopy. Creation of these images is facilitated by motorized

microscope stages and mosaicing softwares. The processing of series of large images is thus difficult because of the unusual size and resolution of the data. The degree of details contained in these images and the deformations induced by the distortion of the lens, the camera, the montage software (mosaicing), as well as the intrinsic deformation of the tissue from one section to another, necessitate the use of non-linear transformations.

Some methods have been specifically developed to process large microscopy images. Kremer [6] and Koshevoy [7] proposed a method to montage large multiple camera images into a single large field of view image. Burt [8] worked on the continuity of intensities when combining two or more images into a large mosaic, using multiresolution splines over transition zones. Thevenaz [9] proposed a mosaicing method to assemble microscopy images. Baronio [10] used parallel splines on sub-domains to restore large images and obtained continuity of the solution by adding an overlapping area. Mikula [11] proposed a framework to share large high resolution histological images using pyramid generation for Web-accessibility, whereas Kumar [12] proposed a framework to process very large images in a cluster environment. This list of dedicated methods to process very large images is not exhaustive.

However, for the specific problem of aligning large serial section images, very few solutions have been proposed: Kumar [12] proposed a section-to-atlas warping method, whereas Koshevoy [7] proposed a fiducial-point based approach to align electron microscopy sections, with no parallel implementation.

We propose in this work a solution to handle this problem based on the finite support properties of the cubic B-splines (see [13] for a description of spline transformations for image processing). We applied our method to achieve the 3D reconstruction of a series of Transmission Electron Microscopy (TEM) images. The method is well suited for parallel distributed computation on a networked cluster, permitting scalability to arbitrarily large images.

2 Method

General method: To align the series of images, we perform a slice-to-slice registration taking as reference the slice in the middle of the stack. Initially, each pair of 2D images is registered using first a rigid and then an affine transformation. These initial estimates are generated using the blockmatching technique as described in [2] (implemented in BrainVisa: <http://www.brainvisa.info>). The images are first downsampled (Gaussian smoothing plus bi-linear interpolation) so that they can be processed on a regular machine; typically the resulting downsampled image is less than 1,000 pixels large in both dimensions. The estimated affine transformation is then applied to the full resolution image.

After initial affine alignment, each large image is split into a grid of overlapping smaller sub-images whose size is suitable for the memory capacity of the machines used. The number of desired sub-images, as well as the number of control points in each sub-image (which will be used for the subsequent cubic B-splines registration) is set by the user. These parameters virtually define a grid of control points on each large image.

An array of sub-images is thus defined both on the target image and on the image to be registered (the “floating” image). The pairs of corresponding sub-images from the target and floating images are individually registered using cubic B-splines transformations estimated by optimization of the mutual information criterion as described in [14] (implemented using the Insight ToolKit: <http://www.itk.org/>). A pyramidal strategy is used both for the number of control points and for the image resolution.

Since the cubic B-splines transformations have a finite support, the deformation in a particular location is only influenced by the weights of a limited number of surrounding sub-images’ individual control point grids. For each control point of absolute coordinates (i_0, j_0) in the virtual global grid for the large image, the new pair of coefficients $c_{merged} = (c_{merged_x}, c_{merged_y})$ (one spline coefficient for each direction $\mathbf{x}$ and $\mathbf{y}$ in 2D) is estimated by blending the coefficients of all the sub-images whose individual grid contains location (i_0, j_0) as follows:

$$c_{merged}(i_0, j_0) = \sum_{P, Q \in \Omega(i_0, j_0)} \omega_{P, Q}(i_0, j_0) c_{P, Q}(i_0, j_0) \quad (1)$$

where $c_{i, j}(i_0, j_0)$ are the old coefficients estimated from the individual registration of sub-image (P, Q) in the global array of sub-images, $\omega_{P, Q}(i_0, j_0)$ are the weights controlling the contribution of the coefficient of the control point (i_0, j_0) from sub-image (P, Q) ; and $\Omega(i_0, j_0)$ is the set of indexes of sub-images (P, Q) which include the control point (i_0, j_0) in their own individual control points grid.

The parameters of the cubic B-spline transformation estimated for each sub-image are thus corrected to take into account the contribution of all the neighboring images and to guarantee the continuity of the elastic transformation at the border of each sub-image with its neighbors (see **Figure 1** just for two images).

All the sub-images are then resampled using their local corrected cubic B-spline transformation, and are assembled together into a final global large resampled image. The resampled floating image is then used as the target image for the next slice-to-slice registration of the series.

Application to Electron Microscopy image series: We tested the whole alignment method on a series of 57 TEM images of the lateral geniculate nucleus of a ferret. Each image was about $10,000 \times 10,000$ pixels large with a pixel resolution of $3nm$ and a slice thickness of $60nm$. We used *blendmont*, a utility that is part of the IMOD package ([6]), to reconstruct the big large field of view image from the 5×5 mosaic of smaller images coming from the camera¹.

The TEM images were downsampled by a factor of 10 for the initial rigid+affine estimation and were then resampled in full resolution. We chose to split the resulting large images into a $P \times Q$ grid, where $P = Q = 10$ of sub-images (100 in total) for the elastic registration. Each pair of sub-images (size about

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¹ The assembly of mosaic images from the camera is a pre-processing step out of the scope of this work.

1200 × 1300 pixels) was registered using two pyramid levels (level 1: 3 × 3 grid of control points and downsampling by 2 in each direction; level 2: 5 × 5 grid at full resolution) to estimate the B-spline elastic transformation. The weights from **Equation 1** were simply chosen as $\omega_{P,Q}(i_0, j_0) = \frac{1}{\text{card}(\Omega(i_0, j_0))}$, where card stands for the number of elements of a set.

Aside from the automated registrations, the sections were also manually aligned (affine transformations matching selected points, same reference slice as for the automated alignments) by a TEM expert, and several structures were manually segmented using *Reconstruct* ([15]). We performed a quantitative comparison between the manual, the affine and the elastic alignments described in this work by estimating the Dice score of superimposition ([16]: the Dice score ranges from 0 (no overlap) to 1 (total overlap)) between slices for three different segmented structures. Note that we used the original non-aligned slices as input data for both the affine and the elastic alignments.

Fig. 1. The merging of the 8 × 8 grids of control points for 2 neighboring sub-images. With more images, each control point can have up to 9 contributions from neighboring sub-images’ grids (for cubic B-spline functions).

3 Results

To qualitatively estimate the quality of the manual, affine and the elastic alignment, orthogonal views of the stack were created. Since the slices processed were very large, it was not possible to create and display an actual 3D volume. The orthogonal views were thus created by extracting the line of each large slice corresponding to the orthogonal view considered, and concatenating these lines together to create a 2D slice. Although the manual and affine alignment were already good, the smoothness of the reconstruction was improved using our elastic scheme (see **Figure 2**).

The slice-to-slice Dice score for the 3 alignment methods strongly varied across the series (see **Figure 3**). This score depends on the shape of the segmented structures and can drop dramatically at the end of a structure. The Dice score variations followed the same pattern for all the methods, which means no error propagation or particular bias was associated with our elastic method.

Fig. 2. Orthogonal views of the aligned stack using manual (a), affine (b) and elastic (c) transformations. The Arrow indicates structures which appear smoother on the elastic alignment.

The mean Dice score for the elastic alignment method was always better than the manual and affine methods. Since the Dice score of each method tested was already high (the data we processed did not suffer from very large non-linear deformations), the differences were relatively small. However, Student T-tests performed to compare the distribution of the slice-to-slice Dice scores between manual/elastic and affine/elastic alignment (paired values, two-tailed distribution) were statistically significant, except for segmentation C with manual alignment (see **Table 1**).

Table 1. Mean dice score and percentage of difference relatively to the elastic alignment over all the slices for the segmentations A, B and C for the three alignment methods. Significant differences ($p < 0.05$) are indicated in bold.

	Manual	Affine	Elastic
Segmentation A	0.773 -2.69% ($p < 10^{-7}$)	0.784 -1.24% ($p < 10^{-7}$)	0.794
Segmentation B	0.872 -0.60% ($p = 0.03$)	0.873 -0.47% ($p < 10^{-7}$)	0.877
Segmentation C	0.797 -0.94% ($p = 0.5$)	0.793 -1.50% ($p < 10^{-7}$)	0.805

4 Discussion

We chose B-splines transformations to model the deformations between corresponding sub-images because B-splines are flexible, easy to use and robust. Moreover, B-splines are a particularly powerful tool to control the continuity of the transformation between sub-images since just by operating on a limited

Fig. 3. Dice score for 3 different segmentations comparing manual, affine and elastic alignment for each pair of slices. The corresponding segmentations are displayed in red over an EM slice on the right.

number of discrete values (the control points' coefficients), the mathematical continuity of the whole image is guaranteed everywhere (see **Figure 4** for an example of continuity).

Because the global transformation estimate is obtained by merging independently computed transformations of each sub-image, our scheme is well suited for distributed computation. Provided enough disk space and compute nodes, our registration scheme permits registration of arbitrarily large images. It is thus particularly well adapted to the processing of very large field of view, high resolution microscopy images.

Fig. 4. Assemblage of the sub-images before (left) and after (right) merging the control points coefficients to guarantee continuity of the transformation (detail of a slice)

For very large images, libraries specialized for large image processing (in which the entire image is never completely loaded into memory) must be used to downsample the input image, apply the initial affine transformation and split it into sub-images prior to the actual elastic registration. The freely available package ImageMagick (<http://www.imagemagick.org/>) is one example of a library with these properties.

The tuning of parameters controlling the elastic deformation is particularly difficult when dealing with large data. The simple display of single result images, much less a stack of images, rapidly becomes impossible with classical tools. In our study, we performed not less than 5700 elastic registrations (and as many rigid and affine registrations). The parameters we chose allowed us to guarantee a robust behavior and to get significantly improved results compared to manual and affine alignment methods. However, automated parameter tuning on a cluster environment would probably result in transformations flexible enough to capture and correct the finest deformations.

The volume we reconstructed in this work was about $30 \times 30 \times 3.5 \mu\text{m}^3$, which is promising but still small for the purpose of understanding the three-dimensional organization of neuronal connectivity. Future efforts will include using our scheme on even larger images and longer series, and developing other processing methods, like automated segmentation, designed to perform on sub-domains individually with consistent global results.

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