

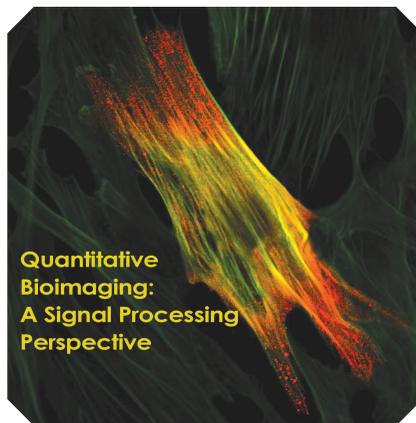
3-D Registration of Biological Images and Models

[Registration of microscopic images and its uses in segmentation and annotation]

The registration, segmentation, and annotation of microscopy images and respective biological objects (e.g., cells) are distinct challenges often encountered in bioimage informatics. Here we present several studies in widely used model systems of the fruit fly, zebrafish, and *C. elegans* to demonstrate how registration methods have been employed to align three-dimensional (3-D) brain images at a very large scale and to solve challenging segmentation and annotation problems for 3-D cellular images. Specifically, we consider two types of registration between images and models: image-to-image registration and model-to-image registration, where a *model* consists of a description of the geometrical shape or the spatial layout of biological objects in the respective images.

INTRODUCTION

The registration of objects or patterns (e.g., cells with a globular shape, gene expression patterns, and highly irregular arborization patterns of neurons) is a commonly used technique in biological and medical data analysis. Generally speaking, registration is a process to map one image, object, or pattern to another (often



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obtained from different sensors, times, subjects, etc.) so that they can be compared, analyzed, or visualized directly within the same coordinate system. A spatial coordinate system is often considered. Along with the development of time-lapse light microscopy, the registration of a time series of images is also common and deemed important for many developmental biology studies. As an enabling technique in many applications such as building digital atlases, assessing the invariance (stereotypy) of

patterns, profiling neuron connectivity, and studying the variation of cell populations, registration is essential in large-scale bioimage visualization, analysis, data mining, and informatics fields [1]–[3].

Segmentation and annotation of microscopy images and the respective biological objects are two challenging topics in bioimage analysis and informatics [1], [4], [5]. Segmentation refers to partitioning an image into multiple disjointed salient image regions, within each of which the image pixels share certain common characteristics. For 3-D cellular or brain images, the partitioned regions often represent interesting cells or compartments. In many cases, this partitioning process is realized by assigning a label to a group of pixels or by delineating the boundary of interesting objects and patterns. In contrast to segmentation, annotation is more closely related to the recognition of patterns or objects. Annotation often associates specific

semantic properties such as the identities or categories to objects or patterns. Segmentation and annotation are critical to address important biological questions (e.g., quantification of gene expression patterns, generation of the ontology databases, and digital atlases of model animals).

FROM IMAGE-TO-IMAGE REGISTRATION TO MODEL-TO-IMAGE REGISTRATION

Registration is often needed to compare, fuse, or quantify objects or patterns in images. In many cases, registration is also required to map images to models and vice versa. In these latter situations, a *model* often consists of geometric shape description of the anatomy or spatial layout of biological objects in the respective images.

IMAGE-TO-IMAGE REGISTRATION

Many system biology studies rely on aligning images of gene expressions in different cell populations [6]–[8] or specimens that correspond to different developmental times [9]. In several recent brain mapping projects of the *Drosophila* (fruit fly), it became critical to align a number of 3-D confocal images of the insect's brains. Each fly had been genetically engineered to express fluorescent proteins in a specific population of neurons, which were aligned to a standard space so that they could be compared with each other [Figure 1(a)]. The FlyCircuit project in Taiwan [10] and the FlyLight project at the Janelia Research Campus of the Howard Hughes Medical Institute [11] each generated tens of thousands of 3-D fruit fly brain image stacks represented some of the biggest neuroscience efforts to date to understand the brain's structure. In each of these brains, some neuron populations are labeled using genetic methods. In both projects, registration of brain images is crucial. Registering images that correspond to the same population is useful to quantify the intrapopulation variability of neurons, which can further help define the meaningful neuron types. Registering images that correspond to different populations is useful to quantify the spatial proximity of neurons and thus helps estimate the putative connectivity of neurons. Similarly interesting results for the zebrafish (*Danio rerio*) were also reported recently [3], [4], [12].

Sophisticated volumetric image registration methods have been developed in the biomedical imaging field. Many methods, such as mutual information registration [13], spline-based elastic registration [14], invariant moment feature-based registration [15], and congealing registration [16], [17], have been widely used and extended to align molecular and cellular images. However, since many of them were originally designed for magnetic resonance imaging and computer tomography data, in many cases it remains challenging to use them easily and effectively in aligning the microscopy images that have larger-scale and fuzzier contents.

Two major challenges in biological image registration are the scale (in terms of the number and size of images) and variation of data (morphology or shape of patterns, image intensity, and noise level). For the first challenge, when the number of 3-D image stacks of brains increases to the order of tens of thousands and each image stack normally has the dimensions of 1,024 voxels (X)

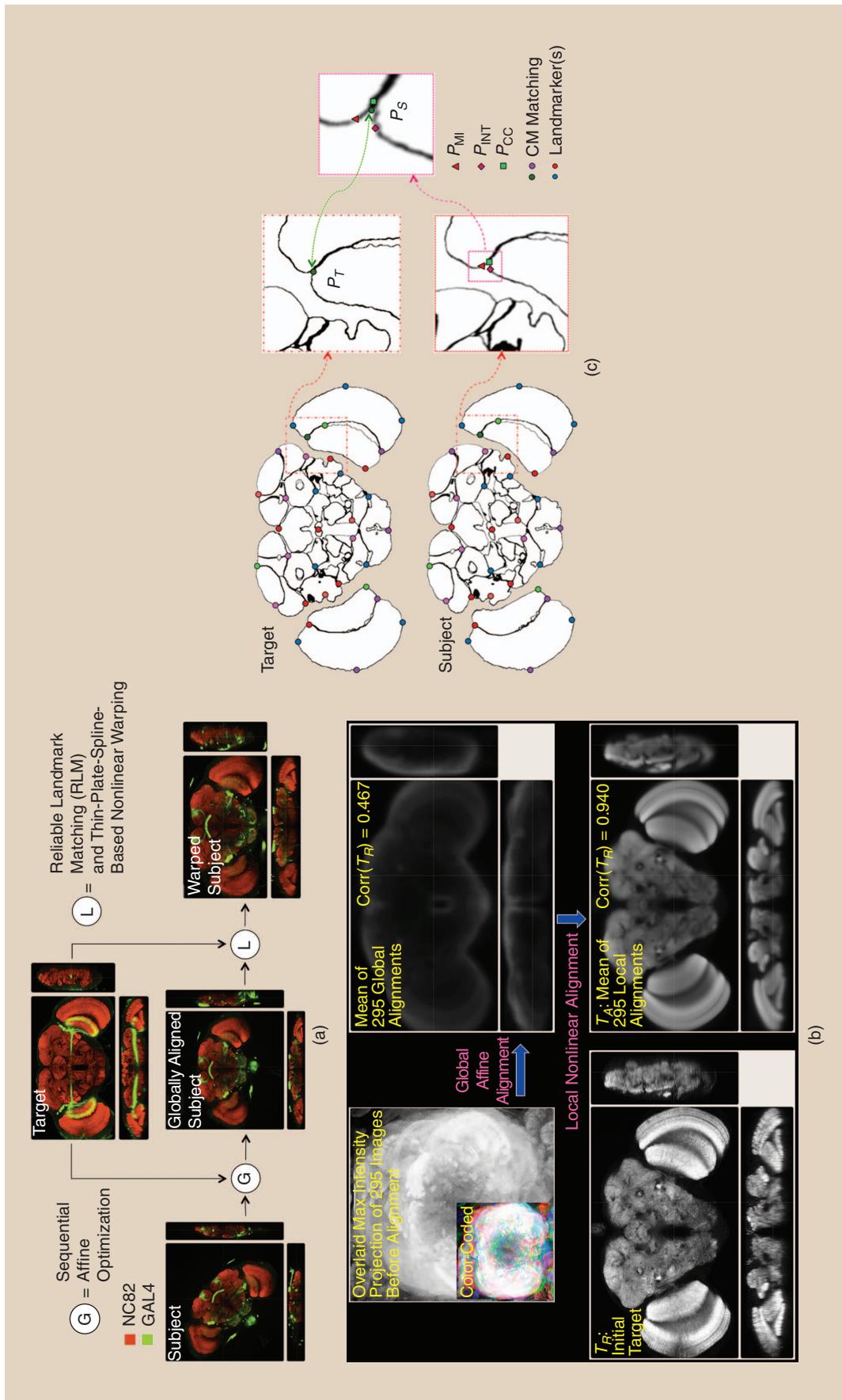
$\times 1,024$ voxels (Y) \times a few hundreds of voxels (Z), it will become exceedingly expensive to ask human annotators to supply even some simple prior knowledge of the data. The huge amount of image stacks requires that a successful registration scheme be highly automated, robust, and computationally efficient. These requirements limit the immediate applicability of many intensity-based registration methods in biomedical imaging field.

The second challenge is that the acquired microscopy image data not uncommonly display substantial variation of the appearance of the to-be-registered patterns. For instance, due to variable tissue labeling, light scattering, mismatching of reflective indexes of media along the light path, and many other issues in the automated image acquisition process, confocal microscopy data can exhibit a low signal-to-noise ratio. As in the fruit fly brain projects, an image normally comes with a neuropil staining that indicates the shape of the brain. Many times it is hard to threshold the neuropil image to segment the brain region from the image background. Therefore, it is often impractical to adopt boundary registration methods as used in the medical imaging field (see [15] for an example). In addition, complicated and varying shapes can arise from the flexible nature of specimens along with the sample preparation (e.g., tissue fixation). All these factors pose challenges to the image registration problem.

Many efforts were carried out to tackle these challenges. In an early effort of the FlyCircuit project, a simple affine transformation was used to align fruit fly brain images [10]. Unfortunately, the affine transformation is often not flexible enough to handle nonrigid deformations in images. In [18], 257 fruit fly brains are progressively registered using a method based on mutual information [19]. Such a method was also combined with multithreaded programming to accelerate the computation. However, nonsalient feature points used in registering different images can affect the accuracy of such a scheme.

BrainAligner [20] and ViBE-Z [3] are two programs developed recently to register sophisticated image patterns. ViBE-Z focuses on the registration of zebrafish brains. In such an application case, the image patterns consist of mainly line- and planelike structures [3]. ViBE-Z utilizes this feature by employing a trainable, rotation-invariant landmark detector. With 14 detected landmarks, a thin-plate spline transformation was used to perform a coarse but also elastic registration. Then, an intensity-based registration was used to realize a fine-scale elastic registration. In addition, a graph-based solver was used to determine the optimal deformation field in the fine elastic registration. This solver was shown to be efficient and less sensitive to local minima than commonly used gradient-descent methods.

We developed BrainAligner to detect the corresponding landmarks of any pair of images based on using a committee-machine algorithm [Figure 1(c)] to aggregate the feature matching results of a series of independent image feature analysis methods. In this way, the effect of pattern variation can be mitigated. The matched pairs of landmarks are further pruned using both the random sample consensus (RANSAC) algorithm [21] and tetrahedron pruning. RANSAC ensures all the corresponding landmark pairs form a globally consistent transform, which is the affine transform in our



[FIG1] The three-dimensional alignment of confocal image stacks of fruit fly brains. (a) The general scheme of image registration based on global alignment followed by nonlinear elastic local alignment. Red: reference channel (neuropil). Green: pattern channel (genetically labeled neurons or neuron-populations). (b) An example of aligning a number of fruit fly brain image stacks, each of which has a different orientation and size. Global alignment can only register these images to have approximately the similar size and orientation. Precise local alignment based on BrainAligner can produce sharply registered images. (c) An illustration of the first step in the reliable-landmark matching algorithm of the local alignment module in BrainAligner. Multiple independent matching criteria based on mutual information (MI), intensity (INT), and correlation (CC) are used to generate the initial candidates of matching locations (CM). Reliable-landmark matching will continue only when these candidate locations are close to each other. For more details regarding BrainAligner and reliable-landmark matching, see [20]. (Figure is adapted from [20] with permission.)

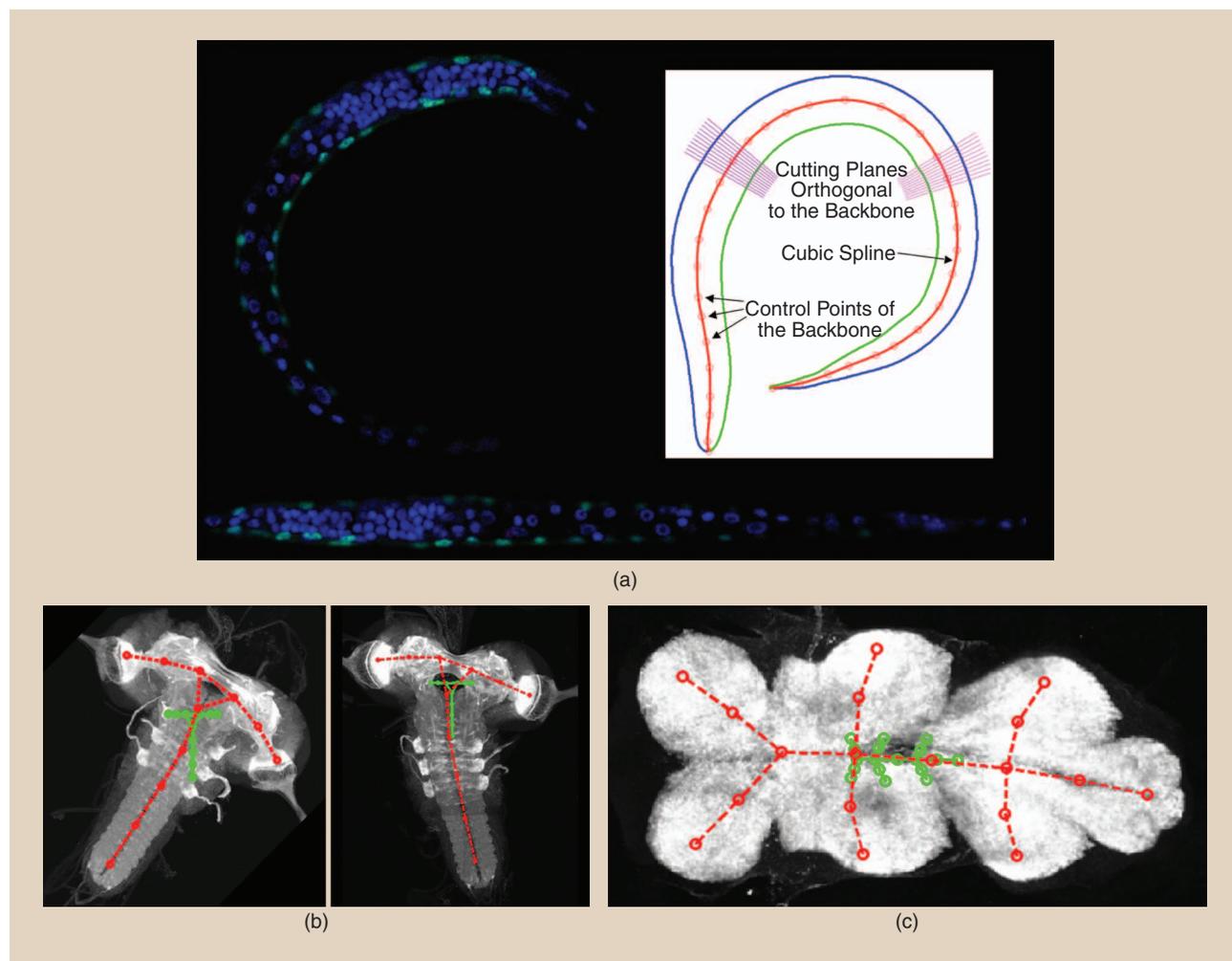
case. Tetrahedron pruning eliminates the cases of local self-intersection of corresponding landmark pairs and thus reduces the likelihood of occurrence of nonsmooth transform during registration. In addition, a hierarchical interpolation scheme for the 3-D thin-plate spline is employed in BrainAligner to quickly calculate the deformation field. Such an interpolation method considerably reduces both computation complexity and memory consumption of thin-plate spline warping. Together these components make BrainAligner robust to imperfect images (e.g., images of brains that have been partially damaged during sample preparation or images with fuzzy boundaries) and suitable for high-throughput processing. BrainAligner has aided a number of studies in fruit fly brain research by mapping neuron populations visualized using various genetic methods to a standard brain atlas model ([11], [20], [22]). This results in complete coverage of the fruit fly brain and a mesoscale connectome of the brain of the animal [23].

MODEL-TO-IMAGE REGISTRATION

PRINCIPAL SKELETON MODELS

Biological patterns often have highly curved, articulated, or branched structures. For instance, the bodies of *C. elegans* [Figure 2(a)] and zebrafish are usually curved. The fruit fly larval nervous system and ventral nerve cord of adult fruit fly have articulated shapes [Figure 2(b) and (c)]. The curved structure can be modeled as a lower-dimensional manifold pattern. A global affine transform is not suitable to globally register images of these patterns. Without being able to globally align these images, more detailed registration at local image regions will become impossible.

When the biological objects have an articulation or an embedded manifold, such patterns should be first globally standardized prior to the image-to-image registration (following the procedure discussed in the section “Image-to-Image Registration.”)



[FIG2] Model-to-image registration and its use in standardization of articulated shapes that are often seen in microscopy images of model animals. This process is done via detecting the principal skeletons of these shapes followed by unbending the structures using a smooth warp. (a) Detecting the center “backbone” curve of a *C. elegans* image stack (top left) and straightening this image by restacking resampled image data (bottom) of all cross-sectional planes orthogonal to the backbone curve (top right). (b) Registering an initial model (green) of a fruit fly larval nervous system to two different confocal images of this animal. The red color indicates the final detected principal skeletons (the control nodes are marked as small circles). Note that the same model was used in both examples to generate the correct results. (c) Registering an initial model (green) of a fruit fly adult ventral nerve cord to a confocal image of this animal. The red color indicates the final deformed principal skeleton (the control nodes are marked as small circles).

The *standardization* refers to unfolding the embedding-manifold structures or globally aligning the articulated components of objects so that they possess similar scales, positions, and directions.

To standardize a shape in the image, we first explicitly model the curved or articulated shape. A principal skeleton model [24] is suitable for this goal. The principal skeleton is defined by a set of connected polylines with intrinsic shape constraints embedded (Figure 2). For different shapes, different principal skeleton models should be created. The principal skeleton model of a shape should correspond to the simplest skeleton that is complicated enough to capture the major structure and major deformation of this shape. In the simplest case, a principal skeleton model consists of only a polyline without any branch, which is sufficient to capture the smoothly curved shapes in *C. elegans* [Figure 2(a)] or zebrafish. In a more complicated case, a connected multipolyline model is used to define the principal skeleton. This fits well the cases of fruit fly larval nervous system and adult ventral nerve cord [Figure 2(b) and (c)].

A principal skeleton model can be deformed to best register to the image content. This skeleton model, however, may not be easily produced using many approaches such as [25]–[30]. For instance, when the boundary of the animal's shape is not available [Figure 2(a)], a skeleton cannot be derived directly from the shape of the animal. Such cases are not uncommon in microscopy images. To solve this problem, we produced an optimized principal skeleton model for an image by iteratively mapping a predefined principal skeleton onto the image [24], [31]. Specifically, one can progressively update the control points in the principal skeleton while preserving the topology of the linkage between control points. To drive the deformation process, we defined a cost function to optimize two competing terms: one external force called *image force* and one internal force called *model force*. The image force is designed to push the principal skeleton to span as broadly as possible to cover the entire image pattern. This is realized by first generating the Voronoi partition using all control points and then minimizing the distance between each control point and the center of mass of its corresponding Voronoi region. The model force is designed based on the shape prior defined by the principal skeleton. Such a force is then minimized to attain the shortest overall length and the greatest smoothness of the principal skeleton. Figure 2 shows examples in which the initial model can deform to best register to images.

For multiple image patterns that have articulated structures, once their principal skeleton models have been generated, a thin-plate spline can be employed to warp these image patterns to a common coordinate system [24]. Such a method has been successfully applied to *C. elegans*, a fruit fly larval nervous system, and ventral nerve cord image data to perform more accurate global registration. Then local alignment methods such as BrainAligner can be used more effectively to generate high-resolution local registration.

SPATIAL LAYOUT ATLAS MODELS

In some cases, the model may need to be much more complicated than the aforementioned principal skeleton. One piece of

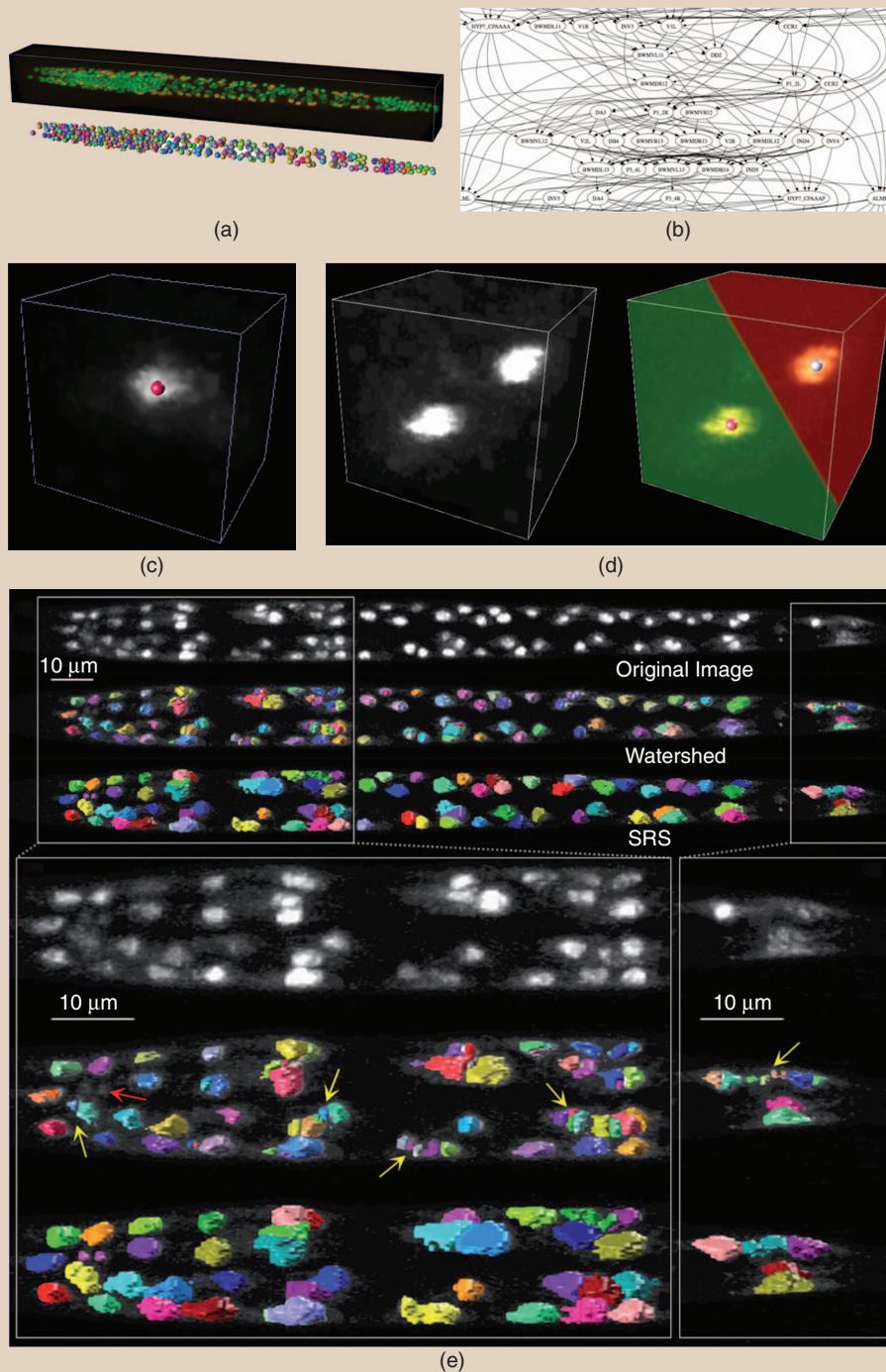
essential information is the complex 3-D spatial layout of objects. In addition, the model may also incorporate the objects' identities or some statistical information such as cell shape, size, and position variation, etc. [32]. With a complex version of the model, the model-to-image registration can be further extended to solve segmentation and annotation problems.

Here we restrict our discussion on *C. elegans* cell segmentation and annotation. For neuron- and whole-organism-level segmentation and annotation, we refer interested readers to [29], [33], and [34]. *C. elegans* is a model animal for a wide range of biological studies, from gene expression to brain function and even animal behavior [35]. This animal has an invariant number of cells, which also have invariant lineages during development. For the postembryonic *C. elegans*, a number of confocal images [Figure 3(a)] were segmented [32]. The results were further assembled as a 3-D digital atlas [Figure 3(b)] to describe the layout of cells at the single cell resolution [32]. This digital atlas can either be visualized in terms of a point-cloud [similar to Figure 3(a)] or a “wiring” graph of cells' relative locations [Figure 3(b)] in 3-D. The atlas was then used as a model to guide the recognition of cells in newly scanned 3-D image stacks of this animal.

Intuitively, recognition of these *C. elegans* cells could be achieved by first segmenting the cells in 3-D, followed by finding the correspondence between segmented cells in an image and the already standardized cells in the atlas model. Once cells have been segmented and recognized, useful information of cells, such as the expression level of specifically targeted cells, can be read out at these identified cellular locations. This routine was first developed in [36]. In the cell segmentation step, an optimized 3-D watershed algorithm was used. In the recognition step, since the relative locations of most cells are conserved from animal to animal, a graph-matching formulation of cell locations from the segmented cells to those recorded in the atlas was used. Both steps unavoidably had some errors. The biggest problem was that the information in the atlas (e.g., the number of cells, the variability of relative locations of cells) was not employed to help improve cell segmentation, which would also enhance the graph matching based recognition. In [37], the problem of over- and undersegmentation was alleviated by performing recognition on an abundant pool of segmentation hypotheses.

Instead of separating cell segmentation and recognition as two isolated processes, an alternative method is to perform segmentation and recognition in a simultaneous way with prior knowledge considered in both steps [38]. In short, this strategy was realized by “registering” the atlas to the image directly. The atlas itself in this case is a complex model that encodes both the identities and relative locations of all cells. The registration process is defined as deforming the 3-D locations of all cells in the model to best fit the cells in the image while keeping their relative locations. The cell segmentation in this case is implicitly realized via assigning a distinct group of image voxels to each cell.

To illustrate this idea, one may begin with a simplified case where there is only one cell in both the atlas and image. In this case, the best fit is apparently to move the cell's location to the center of mass of the image [Figure 3(c)]. In a slightly more complicated case where there are two cells (called u and v)



[FIG3] Three-dimensional segmentation and recognition of *C. elegans* cells. (a) Shown in the upper image is a 3-D confocal image stack of *C. elegans*, where different colors indicate different fluorescent labeling of cells; (a) (bottom) shows the point cloud representation of the 3-D segmentation result of this image stack, where different colors indicate different cells. The 3-D atlas is also often represented as a point cloud and visualized similar to the bottom of this picture. (b) A portion of the directed acyclic representation of the anterior-posterior location relationship in the 3-D atlas of *C. elegans*. The arrow from a cell U to a cell V means U 's location is always anterior of V in the atlas. Depicted in the middle of each circle (graph node) is the name of this cell. Similar left-right and dorsal-ventral graphs can be produced based on the atlas as well. (c) A schematic illustration of an image where there is only one cell and the optimal 3-D location of this cell should be the center of mass of image voxels. (d) A schematic illustration of an image where there are only two cells and the optimal 3-D locations of these two cells should be the centers of mass of the Voronoi regions. (e) Results of simultaneous-segmentation and recognition of *C. elegans* via deforming an atlas model of all cells to best register to the 3-D image, and a comparison with the 3-D watershed segmentation, which has both under- and oversegmentation at different regions. (Image taken from [38] and used with permission.) For more details on the *C. elegans* atlas and the algorithm, see [35] and [38], respectively.

convenience) in the atlas and image [Figure 3(d)] and assuming the cell u is always in the left of the cell v . In this case, we would partition the image into two portions, each of which would be assigned to one cell, and move the cell's location to the center of mass of the respective partition. Finally, the constraint for cells' relative positions can be guaranteed by switching u and v if such a constraint is violated.

Biologically, this approach is suitable for the *C. elegans* cell recognition problem because the number of cells of the worm is a constant and the relative spatial locations of individual cells are highly constrained [35]. We formulated this approach into an algorithm called *simultaneous recognition and segmentation of cells* [38]. Its optimization process consists of two iterative steps: 1) atlas-guided voxel classification and 2) voxel-classification-guided atlas deformation. A more detailed description is given in [38]. Interestingly, to make the algorithm more robust and efficient, several additional factors have also been considered [38]. First, because *C. elegans* is much more elongated along its anterior–posterior axis than the dorsal–ventral and left–right axes, the algorithm allows more flexible deformation of cells' locations along the anterior–posterior axis than the two other axes. Second, a “temperature”-modulated deterministic annealing optimization [39]–[41] was used to tackle the optimization problem by constraining the fuzziness of the classification probabilities. Thanks to this annealing method, simultaneous segmentation and recognition can even handle 180° flipped images [38]. Finally, to cope with the challenge of (usually) having an enormous amount of image voxels in a 3-D image, we downsampled the image before entering the iteration step. We also considered only sufficiently bright image pixels in the actual computation of likelihood and image partitioning (typically, only pixels with intensities greater than the average intensity of the image are included in the calculation). In the simultaneous segmentation and recognition result, the partition of the foreground image naturally translates to the segmented regions of cells.

Simultaneous segmentation and recognition has been applied to recognizing a number of cell types in *C. elegans*, including body wall muscle cells, intestinal cells, neurons, etc. It can recognize these cells reliably, even if the initial atlas of cells has a different orientation from the test image [38]. Simultaneous segmentation and recognition avoids many of the over- and undersegmentation problems [Figure 3(e)], compared to some widely used cell segmentation methods such as the watershed based [36], [42], graph-cut based [43], level-set based [44], and many other methods as mentioned in a recent review paper [5] and the many insight segmentation and registration toolkit methods wrapped up in the FARSIGHT project (see [45]). Such a feature indicates that this model-to-image registration-based approach can be used to solve challenging image segmentation in some situations.

DISCUSSION AND CONCLUSIONS

In this article, we introduced three cases of registration between 3-D images and models. We showed that registration-based approaches are useful for large-scale image alignment, as well as for the segmentation and annotation of 3-D cellular microscopy images. It is noteworthy that the generalization of registration-based approach

can be further applied to other bioimage analysis problems. These analyzed results could be further visualized or annotated by widely used manual tools such as Vaa3D (<http://vaa3d.org>) [46] and CATMAID [47].

The model-to-image registration can be combined with image-to-image registration in a pipeline, thus the articulated objects in a bioimage can be meaningfully aligned. Model-to-image registration can also be combined with image tracking, a whole field of methods not discussed in this article, to analyze two-dimensional or 3-D video-based animal motion or development (e.g., *C. elegans* or zebrafish kinetic motion analysis). Another promising direction is to integrate all the steps of animal tracking, shape standardization, cell segmentation, and recognition with microscope hardware control to build an “intelligent” system that can simultaneously perturb cells and screen corresponding behaviors in vivo.

Despite the several examples we showed, we also found several challenges in registration methods and applications. There is also a lot of room to improve the related algorithms. For example, cell recognition and segmentation, despite the exploration of relative spatial location information and position variation statistics, still lacks an efficient method to use the cell shape and size priors embedded in the atlas. Sophisticated machine-learning techniques, such as supervised learning, can play interesting roles in its further development. Not limited to registration, another key factor of consideration in many bioimage analysis applications is whether or not the prior knowledge can be effectively modeled and utilized. We hope this article can inspire more research into signal processing, pattern recognition, and machine learning for robust bioimage analysis.

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