Fast Interactive Integration of Cross-Sectional Image Datasets and Surface Data for Morphometric Analysis

Oscar MERUVIA-PASTOR[1a], Jung SOH[a], Mei XIAO[a], Eric SCHMIDT[b], Cairine LOGAN[b], Julia C. BOUGHNER[b], Nicholas JONES[b], David OSBORN[a], Johanna SANTIAGO [a], Julian GITTLEMAN[b], Benedikt HALLGRÍMSSON[b], Christoph W. SENSEN[a]

[a]*Sun Center of Excellence for Visual Genomics, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Calgary*
[b]*Morphometrics Laboratory, Department of Cell Biology & Anatomy Faculty of Medicine, University of Calgary*

Abstract. To investigate external facial morphology and cell proliferation patterns and their relationship with cleft lip malformation in mice, we need to compare samples of mice tissue photographs and surface reconstructions from micro-CT scans obtained from mouse embryos. Tissue samples obtained through digital photography are typically misaligned with respect to each other, which prevents further analysis. We have developed a system for fast interactive alignment of these image stacks for volume reconstruction and data visualization and analysis in 3D. The system is designed to work in multiprocessor environments and can utilize an arbitrary number of processors, cutting down significantly the turnaround time and allowing users to quickly process sets of hundreds of high resolution images using a combination of automated and interactive tools. Additional modules are used to reconstruct the shape of the original subject. Our system is interactive, fully scalable and can be applied to any photographic sliced dataset, regardless of subject and reduces significantly the processing time for stack alignment.

Keywords. Image Registration, Histology, Parallel Processing, 3D Surface Reconstruction, Mouse Development

1. Background

Identifying cell proliferation changes within and across subjects at different stages of growth is important in morphometrics research. In particular, we want to model the relationship between cell proliferation patterns, external facial morphology and cleft lip malformation in mice. To investigate this relationship, various types of data sets are generated for each mouse embryo specimen. A 3D representation of the surface of a subject is obtained by processing MRI, CT or micro-CT scans (see Figure 1).

[1] Corresponding Author: Dr. Oscar Meruvia-Pastor (oscar.dot.meruvia@ucalgary.dot.ca)

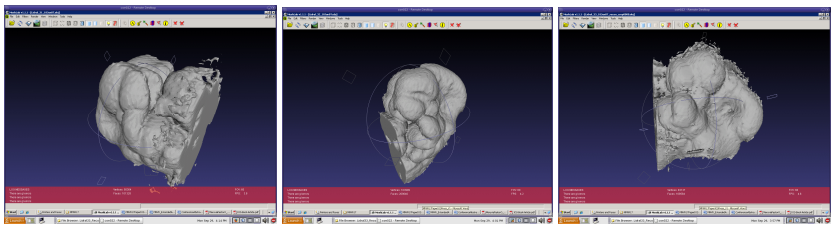

Figure 1: Surface models obtained from micro-CT scans of several mouse embryos before they were sectioned in thin slices. The surface models were cropped to show only the upper body, since we are interested exclusively in studying the region that contains the facial features.

Micro-CT scan data cannot be used to identify cell proliferation patterns, because the images resulting from CT scans do not reveal any differences between particular cell types. In order to identify cell proliferation patterns, particular cells are localized using antibody binding, and antibody-antigen interactions. These cells become visible only after slicing and digitally photographing samples from the subjects. In this process, technicians tend to spend several hours taking photographs using different illumination setups, mainly white- versus blue- or UV light, which highlights the different cell types. Allowing technicians to photograph each sample disregarding the alignment of the samples and to take batches of samples using different lighting conditions in separate batches would save several hours of work per specimen. Because each picture is taken individually, the resulting images are misaligned and can not be directly compared with each other before proper alignment. The disarray also prevents direct analysis of this data with respect to the surface model previously obtained through micro-CT scans. For this reason, a fast alignment technique (registration) is needed to convert data taken from photographs of tissue slices to an aligned volumetric dataset that can be then mapped to a 3D surface model.

2. Tools and Methods

We have created a parallelized, multiprocessor-oriented alignment system that uses the Insight segmentation and registration toolkit (ITK) [1]. Since registration algorithms do not guarantee successful alignment in all cases (e.g. [2,6,7]), we also provide users with an interactive alignment tool (AlignAStack & AlignAPair) that complements the automatic registration tool and is used to correct exceptional cases of misalignment. Finally, a 3D model alignment tool (AlignAModel) is used to find a mapping from the surface model generated with micro-CT scan data to the model originated through surface reconstruction from the stack of photographs.

2.1. Automatic Registration

Since each image in the stack is a digital photograph of a slice of tissue that has been manually placed in the microscope and photographed disregarding the orientation of previously photographed slices, the user ends up with a sequence of images which correspond to the original specimen, but which are all misaligned with respect to each other and cannot be directly mapped to the original 3D surface model obtained from the micro-CT.

Following a sequence of steps using our system, users can automatically manipulate high-resolution image sets, which in the case of a mouse embryos' upper body typically contain 70-150 images each. The first step is to do binary segmentation of the individual images. The second step is the automatic alignment of image pairs. The last step (consolidation) produces the aligned stack of images. Each step of the registration (segmentation, pair alignment and/or stack alignment) can de done in parallel. A process scheduler can spawn an arbitrary number of independent processes that act concurrently on the stack. Our solution therefore allows a multiprocessing machine or cluster of processors to act on the individual images of the stack concurrently, thus significantly reducing the time spent to align the whole stack.

2.1.1. Parallel Registration of a Stack of Images

When aligning a stack of N misaligned sequence of images (indexed I_1 to I_N), one safe approach would be to take the first two images in the stack, align them by having the first image fixed and the second image moved to coincide with the first, and then move on to take the second and third images, have the second image (already aligned with image I_1) be the fixed image, move the third image to be aligned with the second and move on to the next pair, and so on until the whole stack of images has been processed. This solution would have the stack aligned in linear time, depending on N. From a computational point of view, the process of aligning a pair of images $I_{i-1,}$ I_i using the sequential algorithm described before, is the equivalent of generating a 2D transformation T_i (represented as a 3×3 matrix in homogeneous coordinates), that could be applied on image I_i to bring it into alignment with image I_{i-1}.

2.1.1.1. Generating the Alignment Transformations

To align the stack of images in parallel, a scheduler provides each processor with a single pair of sequential images (e.g.: I_{i-1} and I_i), that the processor takes as input to generate the transformation Ti ($i \in [2..N]$). This transformation can clearly be computed by a processor with disregard of other processors working on other pairs of images that are also part of the same stack. Once all the transformations T_2 to T_N have been computed we can move to the consolidation stage. Note that at this stage, no single image has been actually aligned, *i.e.* we defer the application of the transformations on the images to the next stage.

2.1.1.2. Consolidating the Registration Process

In the consolidation stage, which is also executed in parallel, we apply a transformation T_{final} to each image I_i such that it is aligned with respect to the first image in the stack (which is the reference image):

$$I_{i\_aligned} = T_{final} \cdot I_i$$

For an arbitrary image in the stack I_i, the transformation that will bring it into alignment with the first image in the stack is the composition of the transformation that would have been necessary to bring image I_i in alignment with its predecessor I_{i-1} (T_i), with the transformations that would have aligned the predecessor with its own predecessor and so on, up to the first image I_1:

$$T_{final} = T_2 \cdot T_3 \cdot T_4 \cdot \ldots \cdot T_i$$

In the previous stage we computed each transformation from T_2 to T_N. In the next stage, each processor is provided with an image I_i and the set of transformations $T_{2..i}$; then, the processor computes the transformation T_{final} for each image and applies this transformation to the image.

A job scheduler provides the images and the transformations to a set of processors until all images in the stack have been processed. The key to this method is to defer the actual alignment of the images (application of the transformations) until all transformations T_2 to T_N are computed and T_{final} can be found for each image.

2.2. AlignAPair and AlignAStack

We have developed an interactive tool, AlignAPair/AlignAStack (see Figure 2), to assist the user in fixing the particular cases where the automatic alignment failed, which typically would happen when the automatic alignment's optimization algorithm gets stuck in a local minimum while searching for solutions. After a user performs the manual alignment, an additional pass of automatic registration can be applied to improve the alignment results, since the automatic registration tools are particularly effective for fine tuning.

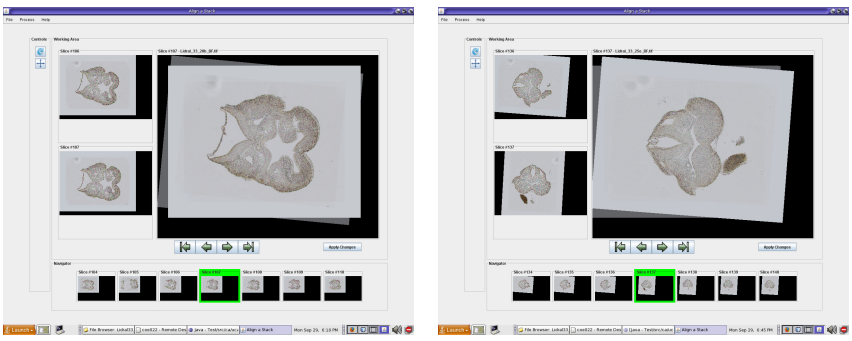

Figure 2: Screenshots of AlignAStack. Mouse embryos are sliced and photographed to identify cell proliferation regions close to the embryo's face. During this process, the images produced are misaligned with respect to each other. Parallel registration aligns most of the images in the stack, but for exceptional cases where parallel registration fails, we use AlignAStack.

AlignAStack handles images in the stack as if they were tightly coupled to each other. When a user performs a single alignment operation using AlignAStack, all images that are subsequent to the reoriented or moved image are affected by the user's operation as if they were stuck to the image originally displaced. This prevents individual user manipulations from creating misalignments in the images that are adjacent to the images being fixed. This approach is useful in our system because automatic alignment precedes manual alignment and several sections of the stack of images have already been aligned, so the user can focus on solving exclusively those cases where automatic alignment was not successful. AlignAPair is a simplified version of AlignAStack, where the user can align two images, for instance, one of which is the image of a tissue sample illuminated with white light while the other is the image of the same sample illuminated with UV light.

2.3. Surface Reconstruction

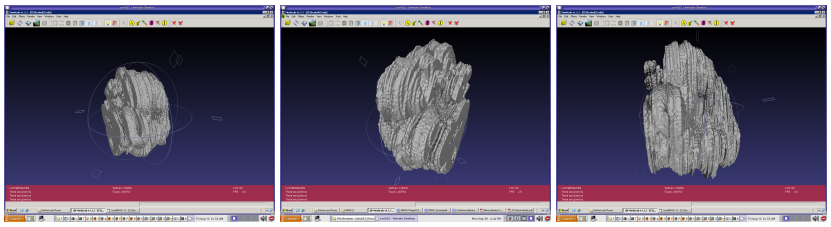

Figure 3: Once the stack of images has been aligned, we use a surface reconstruction algorithm (marching cubes) to recreate the surface of the model generated from the tissue slices taken from the subject.

To find the mapping between the information contained in the slice data and the morphometric data from the micro-CT scans, a 3D surface model is built from the stack of aligned images (See Figure 3). For this purpose, we have implemented a version of marching cubes [3] by Bourke[2], but this can also be done using other algorithms or libraries such as VTK [5]; an extensive survey of 3D surface reconstruction algorithms is available from Newman and Yia [4]. Figure 3 shows samples of the surfaces reconstructed from 3D models.

2.4. AlignAModel

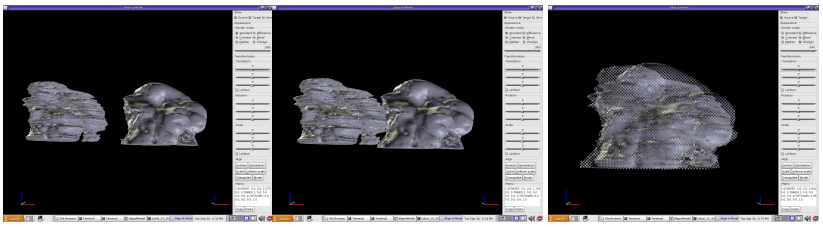

Figure 4: To integrate the histological data with the morphometric data in the same 3D space, the surface model from the slice data (Figure 3) is aligned with the model obtained from the micro-CT scan (Fig. 1) using AlignAModel.

Since the micro-CT model and the slice-based model were generated with different imaging procedures, we need to find a 3D affine transformation that binds both 3D models. To do this, we have created AlignAModel (Figure 4), an interactive program that receives the 3D models and lets the user change the translation, orientation and scale of one of the models to have it match with the other. AlignAModel provides automatic centering and automated rescaling and supports different rendering styles, using blended transparency, stencil buffers to highlight differences, and checkerboard overlays. The output of AlignAModel is a 3D transformation (represented as a 4×4 matrix in homogeneous coordinates) used to map data from the image space of the slice data to the 3D space of the micro-CT surface model.

[2] Available at: http://local.wasp.uwa.edu.au/~pbourke/geometry/polygonise/

3. Results

We have used the tools and methods presented to map the position of cells in the histological samples to the surface model obtained from the micro-CT scan, in an attempt to conduct morphometric analysis of the cleft lip malformation during mouse development. We have evaluated the efficiency of the parallel registration procedure with several datasets. With approximately 15 processes working concurrently in a multiuser environment, a stack of 73 high-resolution (2 Megapixels) images is automatically aligned in about 20 minutes, whereas the sequential process for this takes about 5 hours in the same setting. The fundamentals of our multiprocessor supported registration method are solid, generic, and can be used to speed-up existing registration systems, such as [6] and [7]. In addition, the system is fully scalable to an arbitrary number of processors: in a multiprocessor or cluster environment with a large number of processors, each processor could tackle one image pair from the stack and the whole set could be automatically aligned in about the same time taken to align a single pair.

4. Discussion

The methods described here are generic and can be applied to a variety of experimental setups where sectioning and 3D reconstruction are needed, (e.g. [2,6,7]). Additional modules and tools are being designed within the Cell Proliferation Visualization project to produce analysis tools for the integration of data from several specimens. In the future, we plan to enhance our system by implementing an algorithm that distributes the alignment process for a single pair of images over several processors. This work has been supported by Genome Canada through Genome Alberta; the NRC of Canada's IRA Program; Alberta Science and Research Authority; Western Economic Diversification; the Governments of Canada and of Alberta through the WE Partnership Agreement; the iCORE/Sun Microsystems IR Chair program; the Alberta Network for Proteomics Innovation; and the Canada Foundation for Innovation.

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