

Ultrahigh Resolution 3D Model of Murine Heart from Micro-CT and Serial Confocal Laser Scanning Microscopy Images

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Abstract — This study involves the reconstruction of a distortion-free ultrahigh-resolution 3D model of a whole murine heart. This is achieved by multimodal registration of serial images generated by confocal laser scanning microscopy (CLSM) with the aid of a micro-CT 3D image as a template. High-resolution information from CLSM is utilized for study of fine soft tissue structures in 3D, including fiber orientation and gap junctions. CLSM requires physical sectioning of the sample resulting in missing tissue and in various degrees of tissue distortion depending on thickness. The micro-CT data are distortion free and provide complete information on whole-object interfaces both external and internal. However, they do not provide information on the soft-tissue fine structure. In this project, we used a micro-CT image as template to spatially co-register all the individual CLSM images and to correct the resulting volume for distortion.

Index Terms — Confocal laser scanning microscopy, 3D large volume high-resolution reconstruction, micro-CT template for distortion correction

I. INTRODUCTION

THERE is increasing importance in analyzing high-resolution (micrometer-scale), large (millimeter-scale) biological samples in 3D for biomedical research. Confocal

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Laser Scanning Microscopy (CLSM) can generate images with high-spatial (sub-micrometer) and high-contrast resolution. However, light absorption and scatter limit the thickness of a section that can be scanned to 100–200 μm . This necessitates slicing a large specimen into a number of thin sections. We present here a robust, 3D image-reconstruction algorithm for generation of distortion-free, ultrahigh resolution 3D images of the whole murine heart. This has been accomplished by application of an affine transformation to the CLSM volume to register it with an undeformed template provided by a micro-CT image of the intact sample obtained prior to CLSM preparation.

II. MATERIALS AND METHODS

A murine heart (total size: $\sim 10\text{ mm} \times 5\text{ mm} \times 5\text{ mm}$) was imaged using micro-CT followed by CLSM techniques. The excised murine heart was arrested in diastole, and its chambers were filled with vegetable oil.

A. Micro-CT

The intact specimen was scanned using micro-CT at $\sim 15\text{ }\mu\text{m}$ resolution (Fig. 1). A SkyScan 1074 *in vitro* micro-CT scanner was used at 80kVp and 100 μA . Micro-CT data consisted of 400 projection (2D) images acquired at 0.45° steps through a 180° arc, with the x-ray signal integrated for approximately 1.8 s for each projection. Data pre-processing included subtraction of dark images, compensation for x-ray tube output fluctuation, reference detector normalization (via blank scan utilization), and logarithmic conversion of the signal. A modified Feldkamp [1] tomographic reconstruction algorithm was applied to pre-processed images, and a set of

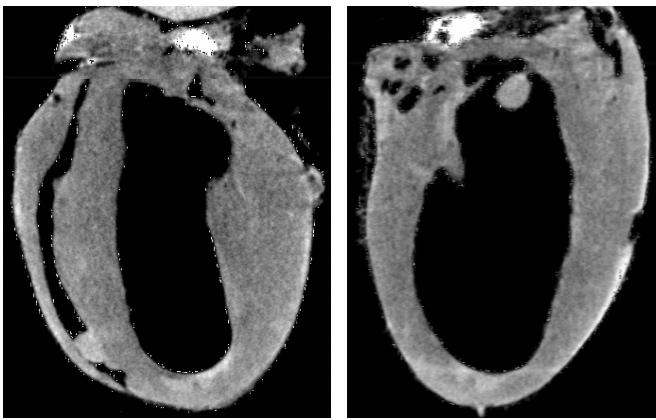


Fig. 1. Micro-CT images of the murine heart used in this study. *Left.* Vertical long axis. *Right.* Horizontal long axis.

transaxial (i.e. perpendicular to the axis of rotation) tomographic cross-section images was obtained.

B. Confocal Laser Scanning Microscopy (CLSM)

After completion of the micro-CT scan, the murine heart was immersion-fixed for 24 hours in 4% paraformaldehyde, and was sectioned (100–150 μm) on an Oxford vibratome. Sections were rinsed in PBS containing 0.1M glycine and 1% BSA, and stained with a 1:20 dilution (10 units/slide) of rhodamine phalloidin (Molecular Probes, Eugene OR) in PBS overnight at 4°C. Rhodamine phalloidin is a specific stain for f-actin, which is a primary component of the cardiac contractile apparatus. This was followed by vibratome- sectioning of the specimen into individual sections with thickness appropriate for CLSM ($\sim 100 \mu\text{m}$). For each physical slice, a series of images was acquired in parallel optical planes, defined by the varying axial focal depth (at $\sim 5 \mu\text{m}$ intervals). Imaging was performed with a 4 \times objective (NA 0.2, WD 15.7) on a BioRad MRC1024 ES confocal microscope, equipped with an Ar/Kr laser. Each image consisted of a 512 \times 512 pixel array (pixel size $\sim 4.83 \mu\text{m}^2$).

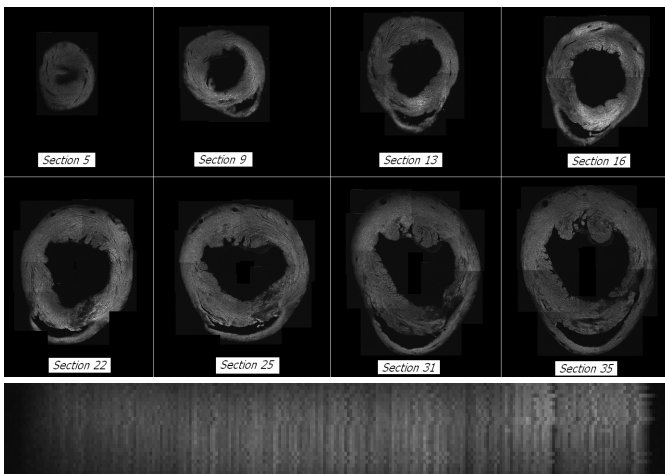


Fig. 2 *Top and middle row.* Short axis cross-section views of the murine heart imaged by CLSM and montaged. *Bottom panel.* 4 \times magnified septum wall in horizontal long-axis cross-section view of 33rd CLSM section.

C. Micro-CT vs. CLSM

CLSM images can be acquired with very high spatial (submicrometer) resolution and with excellent contrast resolution for soft-tissue objects. However, because of light absorption and scattering, the object to be scanned needs to be physically sectioned with an optimal section thickness depending on the type of tissue. In our case it was around 100 μm . Sectioning in turn causes loss of material ($\sim 5 \mu\text{m}$), and non-parallel cuts can occur. Thin sectioning and sample handling may cause soft tissue deformations, collapsed or ruptured walls (the relatively thin walls of the right ventricle are specially vulnerable). The relatively small field of view of CLSM, i.e. much smaller than the imaged heart cross section necessitates progressive scanning that yields multiple partially overlapping images that need to be montaged within a given optical plane. In turn, such synthesized optical planes need to be registered and stacked to obtain the 3D image of the whole imaged section of the sample.

By comparison, micro-CT data exhibit lower spatial resolution ($>5 \mu\text{m}$) and a very poor contrast resolution for soft tissue. However, they offer a large field-of-view and excellent contrast resolution for the interfaces between soft tissue and oil and thus allow for nondestructive imaging of the internal and external interfaces in an undeformed, intact murine heart suspended in oil, provided that the ventricles are filled with oil.

III. RECONSTRUCTION METHODS

A. Reconstruction of a physical section

The individual CLSM in-plane images were montaged using a phase-correlation maximization algorithm to generate 3D image data [2–3], resulting in images of individual optical planes (Fig. 2). The montaging process produces planes of different canvas sizes. These planes were brought to uniform dimensions. Individual optical planes were stacked together within each physical section, yielding a 3D (MHA image format, under ITK) section volume.

B. Volumetric registration of physical sections with micro-CT template

We have assumed that the total volume of a given section was preserved. Accordingly, we determined the location of every section along the long (z) axis of the heart. Micro-CT and CLSM volumes were transformed to common isotropic matrices with lower resolution (20 μm). An appropriate threshold was applied to CLSM data to segment the tissue from the background. Binary images were obtained for CLSM and micro-CT. In the next step, volume profiles of CLSM and micro-CT were matched with adjustable parameters, namely the average volume of material lost due to vibratome slicing and the CLSM optical plane thickness (Fig. 3). As a result, the appropriate location of each physical section along the long (z) axis of the heart was found.

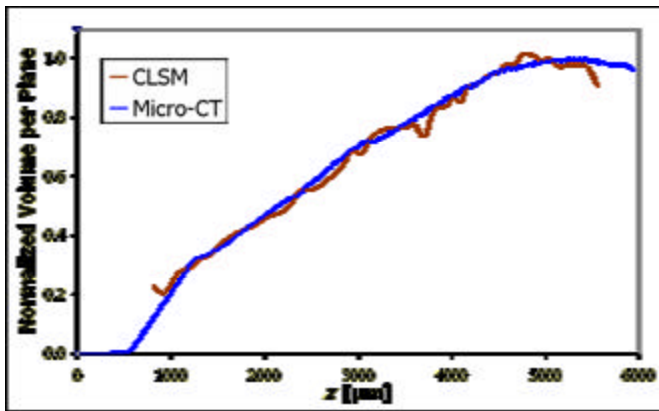


Fig. 3. Volume profiles along the long axis of the heart (z) obtained for micro-CT data (blue line) and CLSM data (brown line). Loss of material due to vibratome slicing and CLSM optical plane thickness was compensated to match the volume profiles.

C. Affine registration of individual physical sections to the micro-CT template

Registration, using an affine transformation (16 parameters) and a regular step, has been implemented as a pipeline (Fig. 4), built within the ITK Framework [4] in C++ using Visual Studio .Net and Windows XP. The initial center of rotation was estimated on the basis of the center of mass. The azimuthal angle of rotation of a given section about the z -axis was estimated by a search in the 0 – 360° range. The value of this parameter is “random” because it was defined by the CLSM technician who arbitrarily placed the vibratome section on microscopy slide. The registration loop shown in Fig. 4 was executed. At the end, the original high-resolution image was resampled.

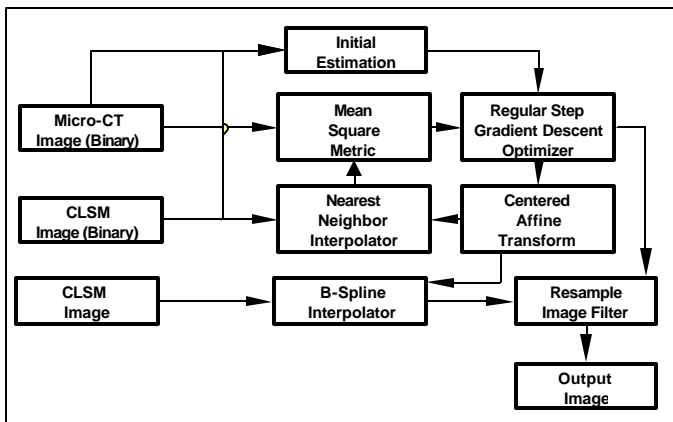


Fig. 4. Registration process implemented in ITK framework [3].

IV. RESULTS

Our test CLSM dataset (1.6 GB), obtained for a murine heart, consisted of 50 sections, each having 30–35 individual planes (totaling 1,418 planes). A 1.5 GHz computer with 1 GB of RAM was used to process all the sections. The elapsed times were a) 45 hours to find the registration parameters, and b) 33 hours to apply the transform parameters to the high-resolution image. The only cases where the registration failed were when the

input CLSM data had a missing “tile” in one of the montaged optical planes. However, even in those cases, after adjusting the parameters for the optimizer, a correct solution was obtained. As an example, Fig. 5 shows a few selected affine-registered sections superimposed on the micro-CT template.

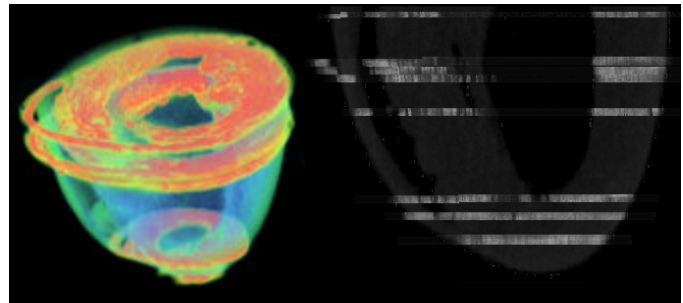


Fig. 5. *Left.* Selected CLSM sections affine-registered with micro-CT template. *Right.* Long vertical view of the same sections as shown in the left panel

V. CONCLUSIONS

We observe a monotonic increase in the value of the metric as we move from apex to base along the long axis of the heart. This indicates increasing deformation in the CLSM sections with increasing cross-sectional (short axis) size. These deformations are especially apparent along the wall of the right ventricle. This can be explained by the relatively small thickness of the right ventricle wall, compared with the left ventricle wall. The polar angle that the axis of rotation makes with the long axis of the heart was found to be constant within 0.2° for all CLSM sections.

Thus, micro-CT can serve as a useful template for global registration and reconstruction of a high-resolution 3D image of the murine heart from CLSM data. However, the affine transformation alone is insufficient for correcting the local deformations in the CLSM images.

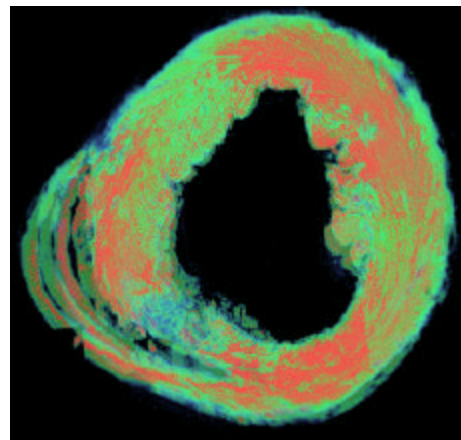


Fig. 6. Short axis view obtained for selected CLSM sections shown in green; affine registered with micro-CT template shown in blue.

VI. FUTURE WORK

We plan to implement local free-form transformation in addition to the global affine transformation to correct the

observed local deformation.

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