High throughput imaging of extended tissue volumes

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In many fields, three-dimensional (3D) information is necessary to understand the organisation and spatial relationships within biological samples. Our group has previously used a confocal microscope and microtome to assemble large, high-resolution volume images from resin-embedded heart tissue (Young *et al*., 1998). Digital reslicing, segmentation and volume rendering methods can be applied to the resulting volumes to provide quantitative structural data about the 3D organisation of myocytes, extracellular collagen matrix and blood vessel network of the heart not previously available. Information such as this is necessary to quantify the heart wall remodelling associated with various types of cardiac disease. It is also required for computer models, which are necessary to examine the effects of myocardial structure on the function of the heart. For example, we have used structural data extracted from an extended volume image of rat left ventricular myocardium (3.8mm × 0.8mm × 0.8mm at 1.5μm pixel size, 0.72×10^9 voxels) to model the influence of structural discontinuities on the propagation of electrical activation in the heart (Hooks *et al*., 2002). However, acquisition of volume images of this scale requires weeks of painstaking work using conventional techniques.

We have developed a novel high throughput imaging system that enables extended volume images to be collected flexibly and efficiently. The system consists of a confocal microscope (Leica TCS 4D) with a Kr/Ar laser, a variable speed Ultramill (Leica) which cuts to 1μm over a 75mm path using diamond or tungsten carbide tips, and a three-axis translation stage (Aerotech) with XYZ movement of 1000, 200 and 75mm, respectively at 100nm step size. This stage controls the positioning of specimens for imaging and milling. The microscope and mill are supported above the translation stage using rigid mounting systems designed to facilitate alignment of imaging and cutting planes. The system is mounted on an anti-vibration table. Z-stack volume images are acquired for overlapping x-y areas that cover the region of interest. The imaged volume is then milled off and the process is repeated. The images acquired may then be combined to reconstruct the volume in 3D. A major advantage of this method is that alignment of the sample elements is maintained throughout the imaging and milling operations, thereby preserving spatial registration and making reconstruction of the complete volume image easier and faster.

The system is controlled using a dedicated computer (Dell P4, 1.8GHz, 1GB RAM, Windows 2000) using custom software written using the LabVIEW™ programming language. A single user interface has been developed that enables image acquisition and milling to be controlled interactively or automatically and allows the operator to process, reconstruct and visualise the image volumes. The flexible user interface provides the ability to image chosen sub-volumes at high resolution, but placing them within the context of a large volume imaged at lower resolution.

Preliminary studies carried out with cardiac tissue specimens demonstrates that the system has the capacity to acquire a 62.5 million voxels per hour, each averaged over 8 scans. This translates to acquisition of a fully registered image volume $1mm^3$ at 1μm pixel size (10^9 voxels), with 8× averaging, in 16 hours, representing greater than an order of magnitude speedup from the manual technique. The volumes imaged to date have been limited to heart tissue perfusion stained with picrosirius red. We are currently working on techniques to extend the range of tissues and fluorescent markers suitable for imaging with the system.

Hooks, D.A., Tomlinson, K.A., Marsden, S.G., LeGrice, I.J., Smaill, B.H, Pullan, A.J. & Hunter, P.J. (2002) Cardiac microstructure: implications for electrical propagation and defibrillation. *Circulation Research,* 91: 331-338.

Young, A.A., LeGrice, I.J., Young, M.A. & Smaill, B.H. (1998) Extended confocal microscopy of myocardial laminae and collagen network. *Journal of Microscopy,* 192, 139-150.