Ex-Vivo Cardiac Fibre Imaging using Diffusion Tensor MRI and Optical Projection Tomography
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Introduction
Myofibre orientation in the heart strongly influences its ability to function efficiently. This is due to the importance of fibre co-ordination in both mechanical function and electric propagation. During myocardial infarction cardiomyocytes are starved of oxygen and a necrotic region forms that evolves into collagen based scar tissue, altering fibre structure. Knowledge of the fibre morphology in the myocardium would be a useful parameter for prognosis following myocardial infarction. By detecting the preferential movement of water molecules in the myocardium using diffusion tensor imaging (DTI) it is possible to investigate the orientation of muscle fibers in the tissue non-destructively. Optical Projection Tomography (OPT) is an emerging technique that enables imaging of fluorescent reporters within intact tissue by optically clearing the organ and provides complimentary information, such as gene expression, to DTI. This study aims to combine DTI and OPT in the adult heart to reveal structure/function relationships in the various components of the myocardium.

Methods
Myocardial infarction was induced in adult rats by occlusion using suture ligation of the left anterior descending coronary artery for 40mins followed by reperfusion. After seven days hearts were perfused with 150ml of heparinized phosphate buffered saline with high potassium followed by 150ml of 4% formaldehyde solution. The hearts were then stored in formaldehyde for 18hrs followed by PBS solution for 48hrs, changing the solution at 24hrs. Before scanning the hearts were embedded in 1% agarose to prevent motion. The hearts were first subjected to sonication for 48hrs, to lose much of their homogeneity (Fig 1 R insert), yet show a similar pattern in the unaffected myocardium. This is likely to be due to the remodeling of the tissue after cell death which is typical of infarcted tissue. Of disorder with surrounding pixels, i.e. bright regions show disorganization; which is typical of infarcted tissue.

Figure 1. Comparison between infarct (R) and control (L), voxel colour represents direction of primary eigenvector; pixel brightness shows the degree of disorder with surrounding pixels, i.e. bright regions show disorganization; which is typical of infarcted tissue.

Figure 2. Cardiac tractography showing macroscopic architecture of myofibres

Figure 3. OPT autofluorescence projection, showing high resolution (9μm) morphological detail

Discussion
This preliminary work shows that we are able to assess fiber orientation in control and infarcted rat heart, providing a platform for investigation of disease and novel therapies. Future work will combine and co-register DTI with optical projection tomography data together with vascular casts by computed tomography, to determine the relationship between myocyte orientation or vascular structure and fiber orientation.