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Interrogation of living myocardium in multiple static deformation states with diffusion tensor and diffusion spectrum imaging

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ABSTRACT

Diffusion tensor magnetic resonance imaging (MRI) reveals valuable insights into tissue histo-anatomy and microstructure, and has steadily gained traction in the cardiac community. Its wider use in small animal cardiac imaging *in vivo* has been constrained by its extreme sensitivity to motion, exaggerated by the high heart rates usually seen in rodents. Imaging of the isolated heart eliminates respiratory motion and, if conducted on arrested hearts, cardiac pulsation. This serves as an important intermediate step for basic and translational studies. However, investigating the micro-structural basis of cardiac deformation in the same heart requires observations in different deformation states.

Here, we illustrate the imaging of isolated rat hearts in three mechanical states mimicking diastole (cardioplegic arrest), left-ventricular (LV) volume overload (cardioplegic arrest plus LV balloon inflation), and peak systole (lithium-induced contracture). An optimised MRI-compatible Langendorff perfusion setup with the radio-frequency (RF) coil integrated into the wet chamber was developed for use in a 9.4T horizontal bore scanner. Signal-to-noise ratio improved significantly, by 75% compared to a previous design with external RF coil, and stability tests showed no significant changes in mean T_1, T_2 or LV wall thickness over a 170 min period. In contracture, we observed a significant reduction in mean fractional anisotropy from 0.32 ± 0.02 to 0.28 ± 0.02, as well as a significant rightward shift in helix angles with a decrease in the proportion of left-handed fibres, as referring to the locally prevailing cell orientation in the heart, from 24.9% to 23.3%, and an increase in the proportion of right-handed fibres from 25.5% to 28.4%. LV overload, in contrast, gave rise to a decrease in the proportion of left-handed fibres from 24.9% to 21.4% and an increase in the proportion of right-handed fibres from 25.5% to 26.0%. The modified perfusion and coil setup offers better performance and control over cardiac contraction states.

We subsequently performed high-resolution diffusion spectrum imaging (DSI) and 3D whole heart fibre tracking in fixed *ex vivo* rat hearts in slack state and contracture. As a model-free method, DSI augmented the measurements of water diffusion by also informing on multiple intra-voxel diffusion orientations and non-Gaussian diffusion. This enabled us to identify the transition from right- to left-handed fibres from the subendocardium to the subepicardium, as well as voxels in apical regions that were traversed by multiple fibres. We observed that both the mean generalised fractional anisotropy and mean kurtosis were lower in hearts in contracture compared to the slack state, by 23% and 9.3%, respectively. While its heavy acquisition burden currently limits the application of DSI *in vivo*, ongoing work in acceleration techniques may enable its use in live animals and patients. This would provide access to the as yet unexplored dimension of non-Gaussian diffusion that could serve as a highly sensitive marker of cardiac micro-structural integrity.

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1. Introduction

Myocardial tissue structure is a key determinant of the mechanical and electrical properties of the heart in health and disease. Detailed characterisation of three-dimensional (3D) cardiac histoarchitecture, and its dynamic alteration during the contractile cycle, offers great potential for new insights into the mechanics of cardiac contraction. Most studies of myocardial microstructure have been conducted on fixed samples, using tissue-destructive techniques such as serial histology sectioning. In contrast, diffusion magnetic resonance imaging (MRI) is a non-invasive tool that enables determination of 3D morphological features in the intact organ (Plank et al., 2009; Scollan et al., 1998).

Diffusion MRI is based on the random diffusion or Brownian motion of water molecules in response to thermal energy, the mechanism of which was first described by Einstein (Einstein, 1905). MRI data acquisition is typically sensitized to diffusion by adding pairs of magnetic field gradients to an imaging sequence (Stejskal and Tanner, 1965). The first gradient labels protons with a given phase, while the second gradient reverses it. Any diffusion that occurs along the axis of the gradients during the time interval between the pair of gradients leads to measurable signal attenuation due to imperfect reversal of phase, which is dependent on the water diffusivity, D, and the degree of diffusion weighting, described by the b-value (Le Bihan et al., 1991). The experiment is typically repeated with diffusion gradients of different strengths and orientations. As water diffusion is influenced by the presence of micro-anatomical structures, its measurement provides information on cell and tissue micro-architecture at a scale beyond the imaging resolution.

Several diffusion MRI methods have been employed in cardiac imaging. These vary considerably in terms of the minimum number of images required, acquisition time, post-processing and information obtained. For instance, trace imaging is a rapid technique that enables calculation of the mean apparent diffusion coefficient (ADC), which may serve as a marker of tissue integrity after acute myocardial infarction (Rapacchi et al., 2011). Diffusion tensor imaging (DTI) is the workhorse of cardiac diffusion MRI, and models the diffusion displacement profile as a 3D tensor with a single Gaussian distribution (Basser et al., 1994). It provides information relating to the characterisation of histo-anatomy both in healthy hearts (Gilbert et al., 2012; Healy et al., 2011; Jiang et al., 2004; Poveda et al., 2012) and disease models such as myocardial infarction (Mekkaoui et al., 2012; Strijkers et al., 2009) or hypertrophy (McGill et al., 2012), by assessment of tissue anisotropy and locally prevailling cell orientations, referred to as “fibre orientation”. DTI provides additional indices such as fractional anisotropy (FA), helix angles and transverse angles of fibres. DTI also facilitates 3D fibre tracking and exquisite depictions of the whole-organ prevailing cell orientation, referred to as the cardiac fibre architecture (Sosnovik et al., 2009a), as validated with histology (Cooper et al., 2012; Hsu et al., 1998; Scollan et al., 1998). Importantly, cardiac DTI is well-suited for assessing myocardial microstructure throughout the cardiac cycle in the same heart (Chen et al., 2005; Hales et al., 2012; McGill et al., 2014).

While the single tensor model in DTI has been widely used as an efficient approach for investigating diffusion, Brownian motion of water in tissues is a more complicated process, in no small part due to the presence of multiple populations of cell types and orientations in each imaging voxel. A number of methods have been developed to better reflect multiple fibre populations within a voxel. These include multi-tensor fitting (Hosey et al., 2005), Q-ball imaging (Tuch, 2004), persistent angular structure MRI (Jansons and Alexander, 2003), diffusion orientation transform (Ozarslan et al., 2006), and spherical deconvolution (Tournier et al., 2004). These methods provide an estimate of the radial projections of the diffusion propagator, P (Wedeen et al., 2005) without the single tensor constraint. The development of these methods has been driven by brain imaging, with limited experience in cardiac applications. Therefore, beyond their higher acquisition overhead, the value of these imaging approaches in assessing cardiac microstructure remains to be established.

Another important consideration is that diffusion in tissue is impeded by membranes, organelles and other structures. This leads to diffusion that is hindered and restricted, and gives rise to a diffusion propagator, P with a non-Gaussian distribution. If for example, diffusing spins encounter an impermeable restriction during a finite diffusion time, Δ, then their measured or apparent diffusion will underestimate their true diffusivity. The cumulant expansion is a useful framework that expresses the magnitude of the diffusion-weighted signal as a power series of the b-value (Kiselev and Il'yasov, 2007). The first and second cumulants describe the Gaussian component of the distribution, while the fourth order cumulant describes the kurtosis, or sharpening, of the peak of P. This deviation from Gaussian distribution can be modelled using a number of methods such as diffusion kurtosis imaging (Jensen et al., 2005), hybrid diffusion imaging (Wu and Alexander, 2007), combined hindered and restricted model of diffusion (Assaf et al., 2004), and generalised DTI (Liu et al., 2004). Alternatively, the data can be fitted using gamma-distribution functions (Röding et al., 2012), stretched exponentials (Bennett et al., 2003) or bi-exponential functions (Maier et al., 2004). These methods are more demanding than DTI, requiring acquisition of data with multiple b-values, and they can require a relatively high maximum b-value to provide information about the shape of P. This remains a challenge in cardiac imaging, as addressed in a recent initial study (Liu et al., 2011).

Diffusion spectrum imaging (DSI) on the other hand, enables direct reconstruction of P, also referred to as the *probability density function* (PDF) (Wedeen et al., 2005). In contrast to other approaches, DSI facilitates quantification of non-Gaussian diffusion and resolution of multiple diffusion orientations in a model-free manner. The PDF can be calculated on a voxel-wise basis as follows:

$$\overline{p}_{\Delta}(\boldsymbol{r}) = S_0^{-1}(2\pi)^{-3}\int_{\mathbb{R}^3}\left|S_{\Delta}(\boldsymbol{q})\right|e^{-i\boldsymbol{q}\cdot\boldsymbol{r}}d^3\boldsymbol{q} \qquad [1]$$

where $\overline{p}_{\Delta}$ is the diffusion spectrum, S_0 is the signal without diffusion weighting, S_{Δ} is the diffusion-weighted signal, Δ is the diffusion separation time, $\boldsymbol{r}$ is the relative spin displacement, and $\boldsymbol{q}$ is the diffusion wave vector, where $\boldsymbol{q} = \gamma\mathbf{G}\delta/2\pi$, and γ is the gyromagnetic ratio, $\mathbf{G}$ is the diffusion gradient vector and δ is the diffusion pulse duration.

Correspondingly, the PDF can be obtained by the Fourier transform of the signal as measured in q-space. Consequently, diffusion-weighted data are sampled on a 3D Cartesian grid in q-space by acquiring data with different diffusion gradient strengths and orientations. For efficiency and isotropic resolution, the sampling scheme is usually restricted to a sphere and avoids sampling the corners of q-space. The PDF can then be integrated radially to yield the *orientation distribution function*, which describes its orientation with no prescribed limitation on the number of dominant directions. Likewise, the shape of the PDF can be described by parameters such as the probability at zero distribution, the full-width-half-maximum and its kurtosis (Lätt et al., 2008). Further fitting of the PDF to various non-Gaussian models is possible, providing access to yet more parameters. Due to the heavy acquisition burden, DSI in the heart is presently