# Ex-Vivo Arterial Collagen Fibre Tractography Using Micro-diffusion Tensor Imaging (μ-DTI)

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## INTRODUCTION

Collagen is the most significant load-bearing constituent in arterial tissue and its arrangement within arteries can therefore provide critical information on the health of the tissue. Among various non-invasive imaging techniques, Diffusion Weighted-MRI (DW-MRI) can provide sub-millimetre level details of collagen fibre architecture in tissues [1], however, to-date spin-echo based sequences with no more than 10 diffusion gradient directions have been used [1–4]. Whilst six non-collinear diffusion directions should be sufficient to trace local morphological variations in collagen fibre architecture, poor SNR, fast T2-decay of arterial tissue and long acquisition time limits the application of such DTI acquisitions in arterial imaging. In this study, a segmented spin-echo Echo-Planar Imaging (EPI) sequence has been used to acquire DWI with 256 diffusion directions in less than 2 hours. The aim of this study is to assess the potential of micro-DTI to robustly characterise the collagen fibre architecture in intact arterial walls.
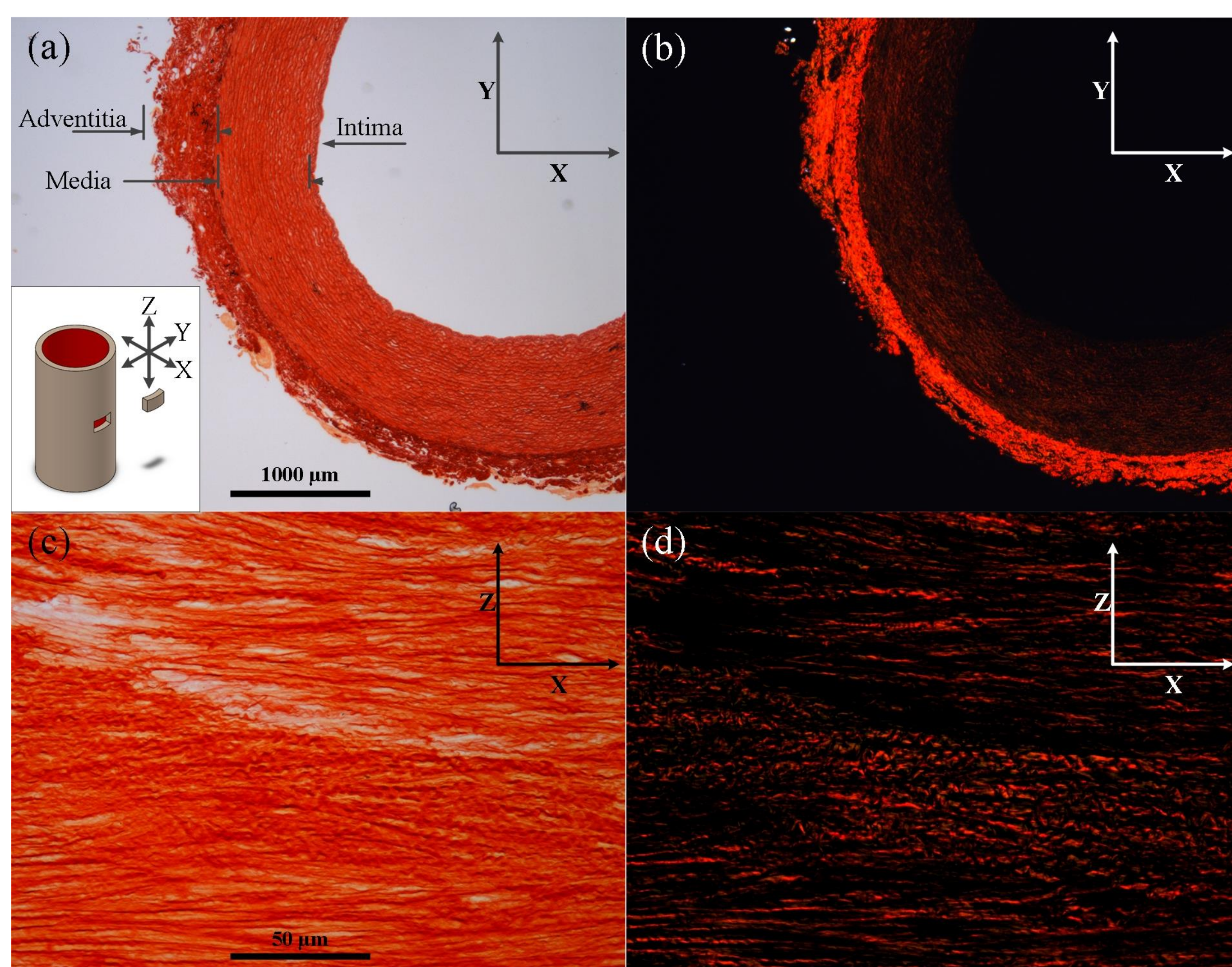


Figure 1: Ring section of a common carotid artery stained with picrosirius red (a) under visible light (b) under polarised light to isolate collagen from the rest of the constituents (magnification 2X). The layers of the arterial wall: tunica intima, tunica media and tunica adventitia are also marked in (a). Small flat section of the carotid artery under 40X magnification (c) under visible light (d) under polarised light.

## METHOD

Two carotid arteries (2 samples from each artery) were harvested from pigs aged five months. Samples were cryopreserved at -80°C and thawed prior to scanning. DT-MRI was performed on a Bruker BioSpin 7T-MR scanner equipped with a cryo-coil. A parameter-selective (T2-decay and b-value) multishell diffusion-encoding scheme based on a spin-echo EPI sequence was optimised for optimal SNR. DW-images were acquired with anisotropic voxel resolution ($0.07 \times 0.07 \times 0.25$ mm$^3$) and isotropic voxel resolution (0.117 mm$^3$). For each sample, field-maps were also acquired to compensate for non-zero off-resonance fields. The datasets were post-processed in FSL (Oxford, UK) to remove artefacts associated with B0 field inhomogeneity and eddy currents. Local Tensor information was obtained using the DtiFit (FSL) along with fractional anisotropy (FA), mean diffusivity (MD), eigenvector and eigenvalue maps (Figure 2).
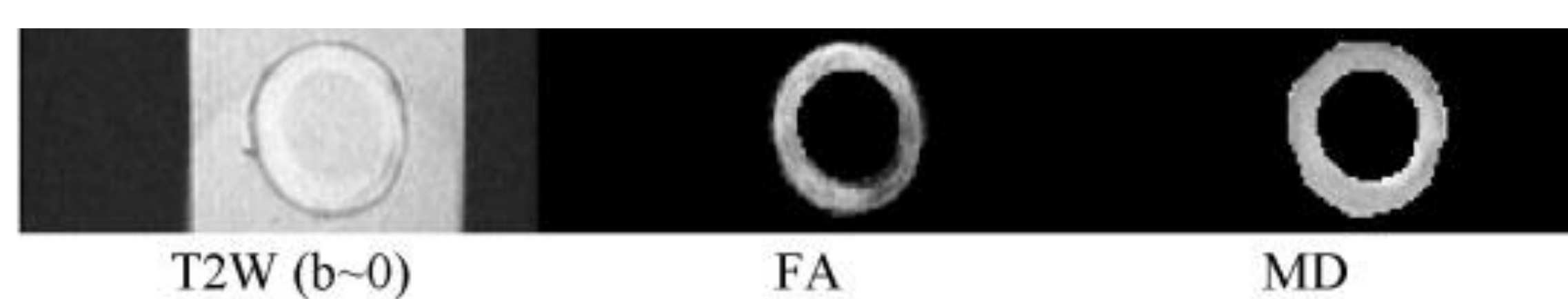


Figure 2: T2-weighted image, fractional anisotropy and mean diffusivity maps of the isotropic voxel resolution DTI dataset.

Under the assumption that water self-diffusion is characterised by a multivariate Gaussian distribution, the diffusion tensor in each voxel was represented by an ellipsoid, highlighting the probabilistic behaviour of molecular diffusion at a voxel scale. Diffusion tensors in each voxel were also classified in terms of basic geometric measures ($C_L$, $C_P$, $C_S$ and $C_a$) (Figure 3) [5]. Each geometric measure represents either a linear ($C_L$), planar ($C_P$), spherical ($C_S$) or non-spherical ($C_a$) case.

Weighted least square DTI fitting (DtiFit) provided a quality check of the DWI data. The Ball and Stick model (B & S) was then used to estimate the local fibre orientation distribution (FOD) in each voxel (BedpostX) [6]. Parameters such as mean fibre orientation distribution, dispersion (spread of the distribution), azimuthal angle and elevation angle were calculated for each voxel to generate FODs. These indices were then used to generate 5000 fibre tracts using the RK4 tracking algorithm with an angular threshold of 20°, a step size of 0.01mm and a minimum length of 0.4mm.
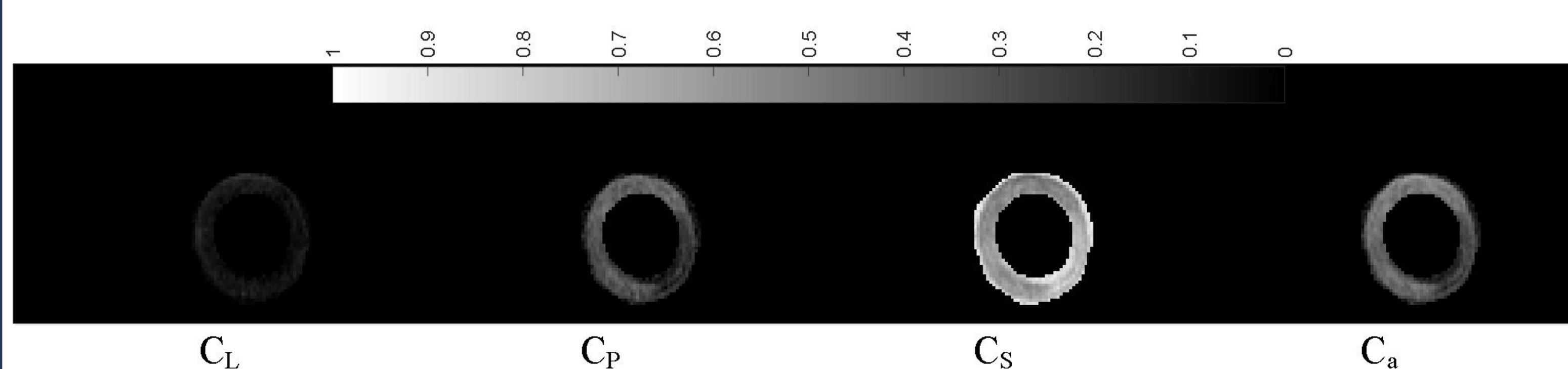


Figure 3: Quantitative maps from the isotropic voxel resolution DTI dataset.

## RESULTS

For isotropic/anisotropic voxel resolution datasets the mean FA was 0.32/0.35 (stdev 0.07/0.055). The helical angle (HA) as described in [3] was in the range of ±15° for both the isotropic and anisotropic (resampled) datasets. Figures 4 and 5 illustrate the indices that were used to assess collagen fibre structure in an intact arterial sample. The FOD per voxel, which was calculated from B & S model, shows the off-plane non-rotationally symmetric nature of fibre dispersion. Kappa ($\kappa$) which is a rotationally invariant index was used to estimate the in-plane degree of dispersion. Kappa values were in agreement with the B & S model based assessment of in-plane fibre dispersion.
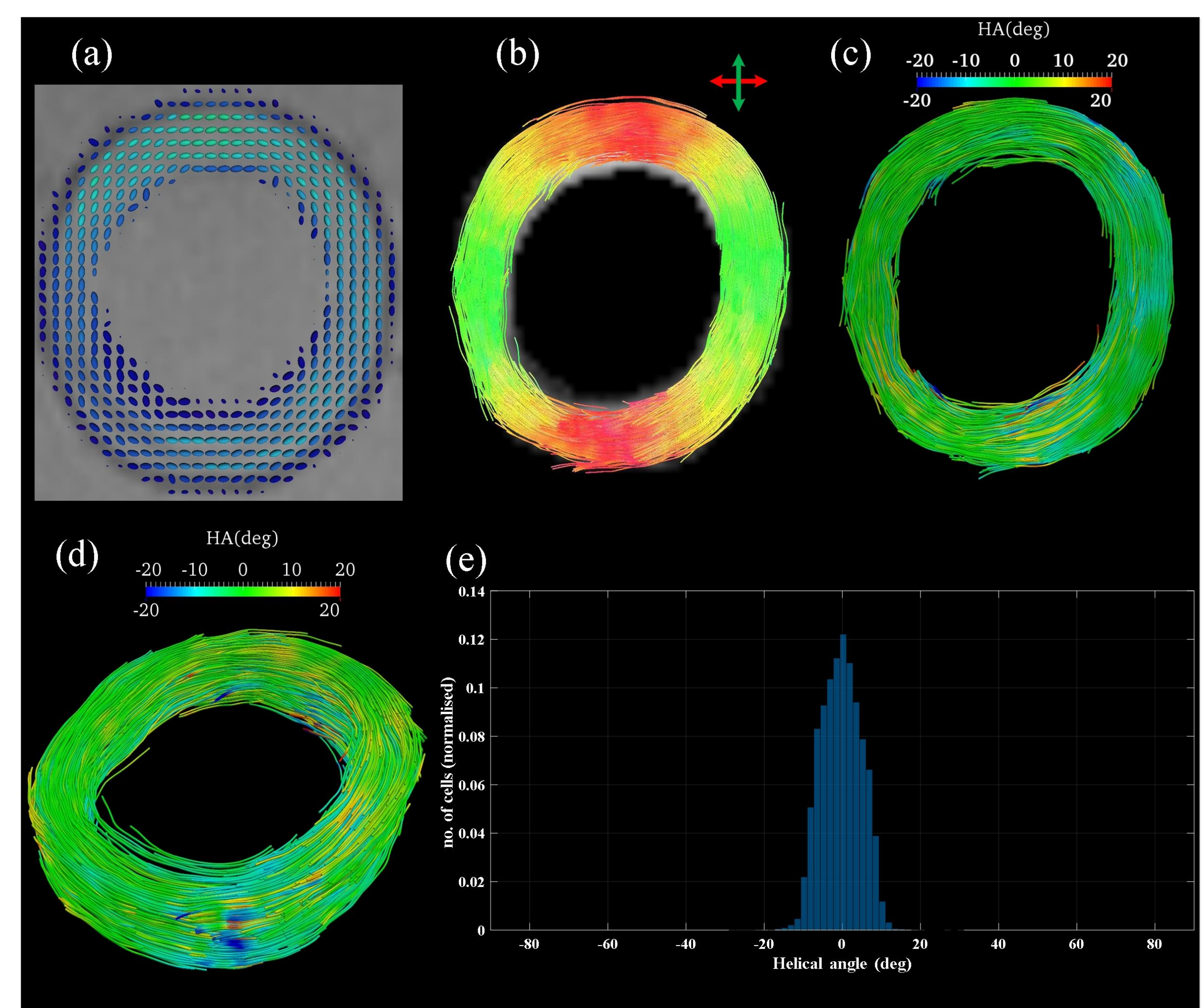


Figure 4: Visualization of DTI (isotropic voxel resolution) dataset in terms of (a) ellipsoidal representation, where the principal orientation of collagen fibres is defined by the long axis of the ellipsoid (b) fibre tracts with directionally encoded colour (c) projection of helical angle along the fibre tracts (d) perspective view of fibre tracts with the projection of helical angles along the tracts (e) distribution of helical angles.
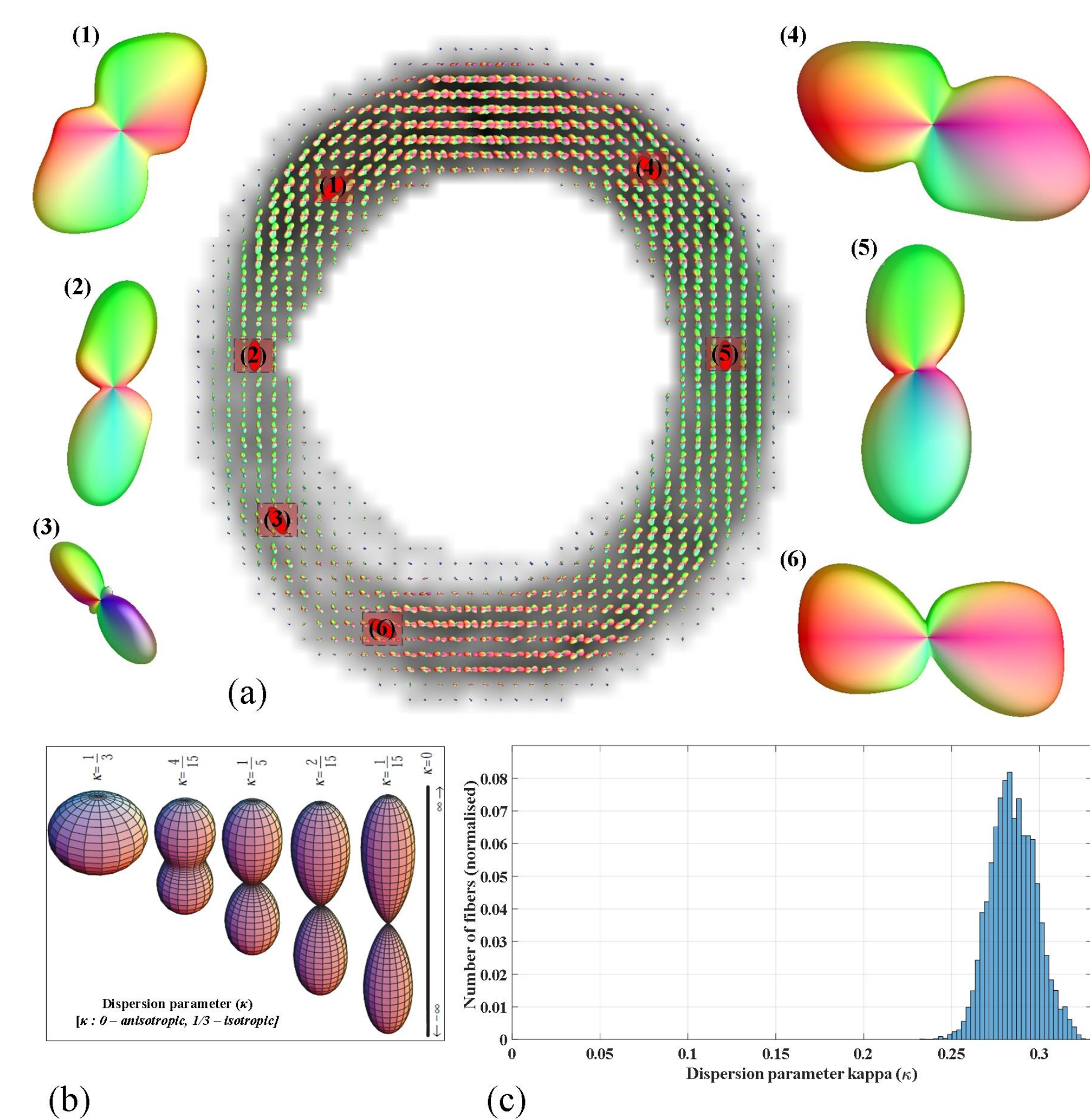


Figure 5: (a) Fibre dispersion in terms of fibre orientation distribution (FOD) in XY-plane (axial slice). Insets 1 − 6 show the FODs from six regions of interest (ROI). (b) graphical representation of fibre dispersion (taken from [7]) (c) dispersion parameter kappa ($\kappa$ : 0 − anisotropic 1/3 − isotropic) calculated from the secondary and tertiary eigenvalues.

## CONCLUSION

This study demonstrates the merits of an EPI based multishell diffusion encoding scheme for generating reproducible collagen fibre tracts in arterial samples. Our results confirm the near circumferential alignment of fibres in intact arteries and for the first time identified the presence of non-rotationally symmetric dispersion of collagen fibres using diffusion tensor imaging. Future work aims to use these protocols to explore load induced fibre reorientation and fibre remodelling in arterial tissues and tissue engineered blood vessels.

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