Diffusion anisotropy in breast cancer tissue corresponds to spatial patterns of collagen alignment from structure tensor analysis of histology

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Synopsis

Directional and anisotropy measures from a diffusion model composed of VERDICT compartments were compared with directional and anisotropy measures from structure tensor analysis of registered histology images. A significant positive correlation was found between the direction of the Zeppelin component of the diffusion model (assumed to represent the extracellular space) and the predominant direction of the structure tensor from the stroma, where the primary feature is aligned collagen. The correlation of anisotropy measures was weak, which may be due to difficulties in detecting alignment in regions with densely-packed collagen, which have nearly uniform intensity on H&E staining.

Purpose

Diffusion tensor imaging results in breast have been conflicting[1–3] and the source of potential anisotropy is unknown. Some[4] suggest a relationship to the breast ducts, while preclinical and *ex vivo* studies suggest an association with the stroma[5] and collagen/hypoxia[6]. We hypothesize that the diffusion orientations will reflect those derived from structure tensor (ST) analysis of histology and clarify the origin of diffusion anisotropy in breast. This study examined *ex vivo* breast tissue using a model of tissue microstructure based on VERDICT MRI[9] and quantitatively compared diffusion orientations with ST from histology.

Methods

MR acquisition and fitting

Seven formalin-fixed tissue samples containing invasive breast cancers were rehydrated with saline and scanned at 9.4 T (Varian Inc). The protocol[10] consisted of 42 diffusion-weighted images (0.25 x 0.25 x 0.5 mm^3, 3 gradient directions + 1 unweighted), b_{max}=21 960 s/mm^2 and gradient separations from 10-80 ms. Two DTIs (42 directions + 6 unweighted), b=1000 and 1500 s/mm^2 were also acquired. A fast spin echo (fSE, 0.125 x 0.125 x 0.5 mm^3) was acquired for registration. Data were fitted voxelwise to a Zeppelin-Sphere model (cylindrically symmetric tensor + restricted isotropic diffusion)[9].

Histology and structure tensor analysis

Three micron slices were cut every 100 μm and stained with H&E. Slides were digitised (Hamamatsu Nanozoomer) at 20x magnification.

ST analysis was conducted at 5x magnification using the freely available Structure Tensor Toolbox[8]. The ST describes the local image-texture orientation by convolving the image with a 2D Gaussian weighting function over a neighbourhood[7,8]. The Gaussian full width at half maximum used was 15 μm, approximately the distance a water molecule is expected to diffuse over a 30 ms experiment. Analysis was restricted to the extracellular space by a mask of the stroma obtained via k-means clustering.

An anisotropy index was calculated using the eigenvalues $\lambda_1 \geq \lambda_2$ of the structure tensor: $AI = \frac{\lambda_1 - \lambda_2}{\lambda_1 + \lambda_2}$. The eigenvector of λ_2 gives the dominant direction.

Image registration and correlation

Adjacent slices were stacked at 100 μm intervals into a 3D volume using 2D pairwise rigid registration with a block-matching strategy[11,12] and correlation coefficient as similarity measure. The 3D stack was registered to the fSE volume using an intensity-based affine registration from ITK[13] with normalized mutual information as similarity metric. The resulting transformation was applied to the diffusion parametric maps and directional vectors.

ST results were downsampled to the MRI resolution, normalizing AI by stromal area to reflect extracellular anisotropy. The Zeppelin component of the diffusion model was assumed to represent the extracellular space. The 3D MRI vectors were projected into the 2D histological plane (from registration) for comparison.

Direction of the Zeppelin projection (φ) and fractional anisotropy (FA) were compared to ST direction and AI, averaging over a 5x5 window around each pixel. Pearson correlation coefficients were calculated, weighting the direction regression by the FA from MRI to limit the influence of nearly isotropic pixels with uncertain directions.

Results and Discussion

Figure 1 shows the registration.

Figure 2 demonstrates the results of the structure tensor analysis.

Registered sections of the ST and diffusion directional analysis are shown in Figure 3 for one sample.

Figure 4 depicts the correlations between MRI and histology for both the direction and anisotropy measures. There is a significant positive correlation between the dominant directions directions of MRI and ST. The correlation plots demonstrate quite large variance. This is likely due to errors in the registration, which affect both the pixel locations for comparison and the pixel value φ, calculated when the transformation is applied to the diffusion vectors.

The anisotropy correlation is weaker. This may be due to difficulties capturing orientation of dense collagen (Figure 5). Tightly packed collagen demonstrates less intensity variation in the histology images, producing AI estimates that may be low even when collagen anisotropy is high (visible as the spread in AI as FA increases in the bottom left plot of Figure 4). Additionally, AI measures the gradient in the stroma, where the dominant feature is collagen orientation, while FA measures the hindrance of water, which may result from structures other than collagen.

Conclusion

Structure tensor analysis on histological images was compared to directional and anisotropy parameters from a VERDICT model in *ex vivo* breast tissue. The primary diffusion direction of the anisotropic MRI compartment has a significant positive correlation with the primary direction from ST in the stroma. This is evidence of diffusion anisotropy even in the absence of ductal structures. If this is confirmed in more samples, high-resolution VERDICT measurements may provide a method of detecting collagen realignment, a valuable biomarker for hypoxia[5] and tumour progression[14].

Acknowledgements

This work was supported by funding from MIMIC (EP/K020439/1), the VPH-PRISM EU project (FP7-ICT-2011-9, 601040), the CRUK-EPSRC Cancer Imaging Centre at KCL/UCL, Horizon2020-EU.3.1 CDS-QUAMRI (ref: 634541) and by EPSRC fellowship EP/N021967/1.

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Figures

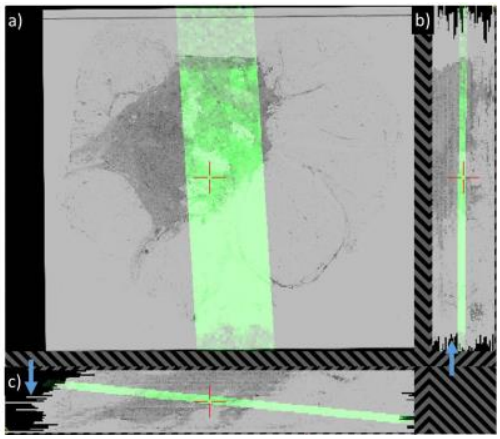

Figure 1 (a) A single histological slice and (b, c) orthogonal views of the stacked slides. The jagged black/white edges (blue arrows) demonstrate the slide translation needed to achieve a stacked histological volume. Overlaid in green is the aligned MRI diffusion-weighted image. This slice does not correspond exactly to the histological slicing plane, meaning that only a portion of each histological image corresponds with the MRI data.

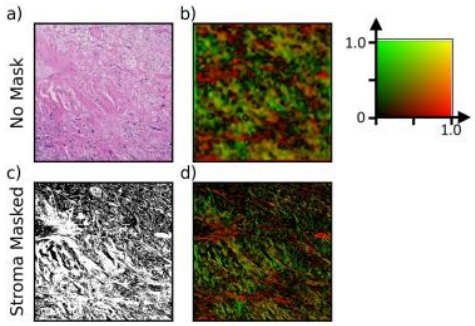

Figure 2 (a) H&E-stained image and (b) the Structure Tensor analysis for all voxels in the image. (c) Mask of the stroma obtained by k-means clustering and (d) the structure tensor analysis when restricted to only stromal voxels. Colours indicate direction and brightness indicates magnitude of anisotropy, AI, as depicted in the legend at the top right.

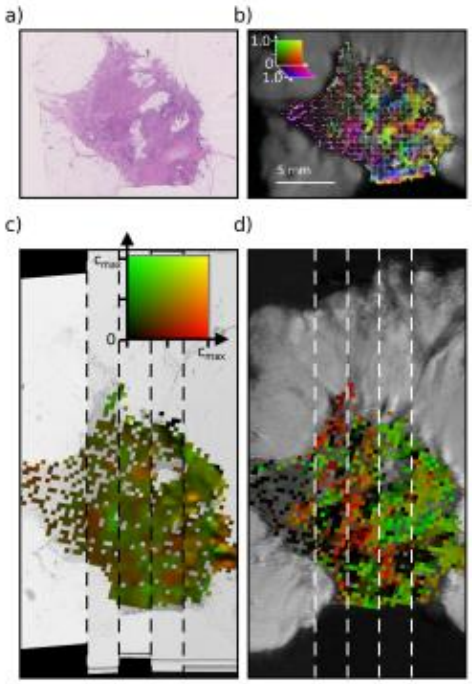

Figure 3 (a) A histological slice and (b) the (unregistered) colour FA map for the Zeppelin portion of the model. Registered analyses are shown underneath: (c) directional map of the ST, downsampled to MRI resolution and normalized to the stromal fraction; (d) projection of the primary diffusion direction vector into the histological plane. Colours and brightness correspond to direction and anisotropy, as depicted in the legend, where c_{max} = 1 for FA from MRI and 0.8 for AI from histology. MRI and histology slices are not co-planar and maps have been stitched together, indicated by the dashed lines.

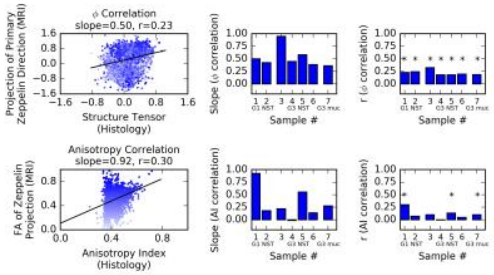

Figure 4 Correlation between MRI and histology for (top) directional information and (bottom) anisotropy. The correlation plot for the sample shown in Figure 3 is on the left, with the best fit line in black. Whiter points have lower anisotropy and are weighted less in the regression for φ (the anisotropy fit is not weighted). The slopes and correlation coefficients for all samples are shown in the bar graphs to the right, grouped by grade and type (G1/3=grade 1/3, NST=no special type, muc=mucinous). * indicates r significantly different from 0 at p<0.01 significance level.

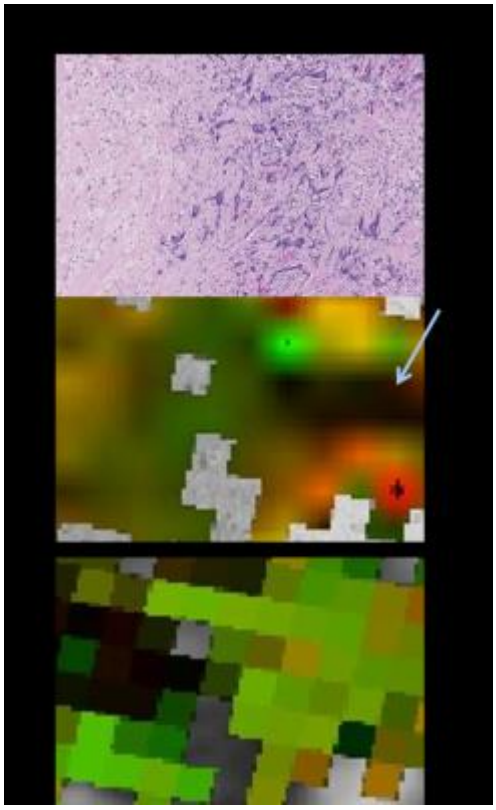

Figure 5 A region with less dense collagen on the left and more dense collagen on the right. The structure tensor shows a dark region (blue arrow) even in the aligned collagen because there is less intensity variation when aligned collagen is tightly packed. The MRI appears bright in this region and has lower FA toward the left, where loose collagen would not hinder diffusion as much.