Microstructural models for diffusion MRI in breast cancer and surrounding stroma: an ex vivo study

Colleen Bailey1 | Bernard Siow2 | Eleftheria Panagiotaki1 | John H. Hipwell1 | Thomy Mertzanidou1 | Julie Owen3 | Patrycja Gazinska3 | Sarah E. Pinder3 | Daniel C. Alexander1 | David J. Hawkes1

1 University College London, Centre for Medical Image Computing, London, UK
2 University College London, Centre for Advanced Biomedical Imaging, London, UK
3 King’s College London, Guy’s Hospital, Breast Research, Pathology, London, UK

Correspondence
Colleen Bailey, University College London, Medical Physics, University College London, Eng Front Bldg., Gower St., London WC1E 6BT, UK.
Email: colleen.bailey@ucl.ac.uk

INTRODUCTION

Breast cancer screening allows the early detection of cancerous lesions, but improved technology increases the likelihood of detecting small, slow-growing cancers that do not require aggressive treatment. It is estimated that 10% of women who have mammographically detected cancers would not have required treatment in their lifetime (‘overdiagnosis’),1,2 and that post-surgical radiation treatment may not improve 5-year overall survival in some groups of women (a form of ‘overtreatment’).3

There is therefore a need for further tumour characterisation, i.e. beyond cancer detection, to identify patients in whom overdiagnosis and overtreatment are likely; magnetic resonance imaging (MRI) is particularly appealing because of its non-invasive nature and sensitivity to microstructure. Breast cancers show great variation in microstructure: higher grades tend to have increased cell density and a more disorganised structure;4 immune cell infiltration and cell differentiation affect the distribution of cell types and sizes; and there are changes in the extracellular matrix related to invasion.5,6

Diffusion MRI is sensitive to many microstructural features. Diffusion tensor imaging (DTI)7 and neurite orientation dispersion and density imaging,8 for example, have produced maps of brain architecture. In the context of cancer, methods such as VERDICT9,10 and restriction spectrum imaging11 estimate tissue features such as those related to cell density. However, the exploration of breast microstructure has been relatively limited in comparison.
Clinical work has focused on data acquisition at a small number of low (≤1000 s/mm²) b values and mono-exponential fitting to give an apparent diffusion coefficient (ADC). The ADC shows a difference between benign and malignant lesions,12–15 but there is a large overlap in the ADC values of the two groups in many studies, with an area under the receiver operating curve ranging from 0.72 to 0.97 for ADC alone.16,17 Attempts to use ADC to distinguish histological grades have produced mixed results, even with large numbers of patients.18–20 Some variation in ADC with molecular subtype has been observed,21 although results may be affected by the inclusion of necrotic regions common in triple-negative cancers.

However, ADC assumes that all of the water in a particular voxel can be represented by a single ADC. In reality, intracellular water is at least partly restricted by the cell membrane, extracellular diffusion depends on the extracellular space and organisation of cells, and anisotropic structures in the tissue produce diffusion orientation dependence. The use of an inappropriate model yields ADC values that are dependent not just on physiology, but on the choice of b value itself,23 making comparisons between different scan protocols and between centres with different scanners difficult. The results from a given patient might also be difficult to interpret: although ADC correlates with cellularity in breast tumours,24,25 immune responses and changes can be represented by a single ADC. In reality, intracellular water is although results may be affected by the inclusion of necrotic regions common in triple-negative cancers.

The nature of the ADC values and mono-exponential fitting to give an apparent diffusion coefficient (ADC). The ADC shows a difference between benign and malignant lesions,12–15 but there is a large overlap in the ADC values of the two groups in many studies, with an area under the receiver operating curve ranging from 0.72 to 0.97 for ADC alone.16,17 Attempts to use ADC to distinguish histological grades have produced mixed results, even with large numbers of patients.18–20 Some variation in ADC with molecular subtype has been observed,21 although results may be affected by the inclusion of necrotic regions common in triple-negative cancers.

However, ADC assumes that all of the water in a particular voxel can be represented by a single ADC. In reality, intracellular water is at least partly restricted by the cell membrane, extracellular diffusion depends on the extracellular space and organisation of cells, and anisotropic structures in the tissue produce diffusion orientation dependence. The use of an inappropriate model yields ADC values that are dependent not just on physiology, but on the choice of b value itself,23 making comparisons between different scan protocols and between centres with different scanners difficult. The results from a given patient might also be difficult to interpret: although ADC correlates with cellularity in breast tumours,24,25 immune responses and changes can be represented by a single ADC. In reality, intracellular water is although results may be affected by the inclusion of necrotic regions common in triple-negative cancers.

However, ADC assumes that all of the water in a particular voxel can be represented by a single ADC. In reality, intracellular water is at least partly restricted by the cell membrane, extracellular diffusion depends on the extracellular space and organisation of cells, and anisotropic structures in the tissue produce diffusion orientation dependence. The use of an inappropriate model yields ADC values that are dependent not just on physiology, but on the choice of b value itself,23 making comparisons between different scan protocols and between centres with different scanners difficult. The results from a given patient might also be difficult to interpret: although ADC correlates with cellularity in breast tumours,24,25 immune responses and changes can be represented by a single ADC. In reality, intracellular water is although results may be affected by the inclusion of necrotic regions common in triple-negative cancers.

However, ADC assumes that all of the water in a particular voxel can be represented by a single ADC. In reality, intracellular water is at least partly restricted by the cell membrane, extracellular diffusion depends on the extracellular space and organisation of cells, and anisotropic structures in the tissue produce diffusion orientation dependence. The use of an inappropriate model yields ADC values that are dependent not just on physiology, but on the choice of b value itself,23 making comparisons between different scan protocols and between centres with different scanners difficult. The results from a given patient might also be difficult to interpret: although ADC correlates with cellularity in breast tumours,24,25 immune responses and changes can be represented by a single ADC. In reality, intracellular water is although results may be affected by the inclusion of necrotic regions common in triple-negative cancers.

However, ADC assumes that all of the water in a particular voxel can be represented by a single ADC. In reality, intracellular water is at least partly restricted by the cell membrane, extracellular diffusion depends on the extracellular space and organisation of cells, and anisotropic structures in the tissue produce diffusion orientation dependence. The use of an inappropriate model yields ADC values that are dependent not just on physiology, but on the choice of b value itself,23 making comparisons between different scan protocols and between centres with different scanners difficult. The results from a given patient might also be difficult to interpret: although ADC correlates with cellularity in breast tumours,24,25 immune responses and changes can be represented by a single ADC. In reality, intracellular water is although results may be affected by the inclusion of necrotic regions common in triple-negative cancers.

However, ADC assumes that all of the water in a particular voxel can be represented by a single ADC. In reality, intracellular water is at least partly restricted by the cell membrane, extracellular diffusion depends on the extracellular space and organisation of cells, and anisotropic structures in the tissue produce diffusion orientation dependence. The use of an inappropriate model yields ADC values that are dependent not just on physiology, but on the choice of b value itself,23 making comparisons between different scan protocols and between centres with different scanners difficult. The results from a given patient might also be difficult to interpret: although ADC correlates with cellularity in breast tumours,24,25 immune responses and changes can be represented by a single ADC. In reality, intracellular water is although results may be affected by the inclusion of necrotic regions common in triple-negative cancers.

However, ADC assumes that all of the water in a particular voxel can be represented by a single ADC. In reality, intracellular water is at least partly restricted by the cell membrane, extracellular diffusion depends on the extracellular space and organisation of cells, and anisotropic structures in the tissue produce diffusion orientation dependence. The use of an inappropriate model yields ADC values that are dependent not just on physiology, but on the choice of b value itself,23 making comparisons between different scan protocols and between centres with different scanners difficult. The results from a given patient might also be difficult to interpret: although ADC correlates with cellularity in breast tumours,24,25 immune responses and changes can be represented by a single ADC. In reality, intracellular water is although results may be affected by the inclusion of necrotic regions common in triple-negative cancers.

However, ADC assumes that all of the water in a particular voxel can be represented by a single ADC. In reality, intracellular water is at least partly restricted by the cell membrane, extracellular diffusion depends on the extracellular space and organisation of cells, and anisotropic structures in the tissue produce diffusion orientation dependence. The use of an inappropriate model yields ADC values that are dependent not just on physiology, but on the choice of b value itself,23 making comparisons between different scan protocols and between centres with different scanners difficult. The results from a given patient might also be difficult to interpret: although ADC correlates with cellularity in breast tumours,24,25 immune responses and changes can be represented by a single ADC. In reality, intracellular water is although results may be affected by the inclusion of necrotic regions common in triple-negative cancers.

However, ADC assumes that all of the water in a particular voxel can be represented by a single ADC. In reality, intracellular water is at least partly restricted by the cell membrane, extracellular diffusion depends on the extracellular space and organisation of cells, and anisotropic structures in the tissue produce diffusion orientation dependence. The use of an inappropriate model yields ADC values that are dependent not just on physiology, but on the choice of b value itself,23 making comparisons between different scan protocols and between centres with different scanners difficult. The results from a given patient might also be difficult to interpret: although ADC correlates with cellularity in breast tumours,24,25 immune responses and changes can be represented by a single ADC. In reality, intracellular water is although results may be affected by the inclusion of necrotic regions common in triple-negative cancers.

However, ADC assumes that all of the water in a particular voxel can be represented by a single ADC. In reality, intracellular water is at least partly restricted by the cell membrane, extracellular diffusion depends on the extracellular space and organisation of cells, and anisotropic structures in the tissue produce diffusion orientation dependence. The use of an inappropriate model yields ADC values that are dependent not just on physiology, but on the choice of b value itself,23 making comparisons between different scan protocols and between centres with different scanners difficult. The results from a given patient might also be difficult to interpret: although ADC correlates with cellularity in breast tumours,24,25 immune responses and changes can be represented by a single ADC. In reality, intracellular water is although results may be affected by the inclusion of necrotic regions common in triple-negative cancers.
TABLE 2 Models tested (with fitting parameters in parentheses). Compartment shapes are described in the text and Appendix.

| Models tested | Extracellular compartment | Intracellular compartment | No. of fitting parameters |
|---------------|---------------------------|---------------------------|--------------------------|
| Ball (ADC)    | Ball (D₁)                 | Ball (D₁)                 | 3                        |
| Zeppelin      | Zeppelin (D₁, D₂, θ, ϕ)   | Ball (D₁)                 | 6                        |
| Tensor (DT)   | Tensor (D₁, D₂, D₃, θ, ϕ, α) | Ball (D₁, R)             | 8                        |
| Ball–Ball     | Ball (D₁)                 | Ball (D₁)                 | 5                        |
| Zeppelin–Ball | Zeppelin (D₁, D₂, θ, ϕ)   | Ball (D₁)                 | 8                        |
| Tensor–Ball   | Tensor (D₁, D₂, D₃, θ, ϕ, α) | Ball (D₁)             | 10                       |
| Ball–Sphere   | Ball (D₁)                 | Sphere (D₁, R)            | 6                        |
| Zeppelin–Sphere | Zeppelin (D₁, D₂, θ, ϕ) | Sphere (D₁, R)            | 9                        |
| Tensor–Sphere | Tensor (D₁, D₂, D₃, θ, ϕ, α) | Sphere (D₁, R)          | 11                       |

spectral area in the T₂ spectrum from non-negative least-squares analysis. Compartment shapes are described in detail in Panagiotaki et al. and are summarised in the Appendix: a Ball describes unrestricted (free or hindered) isotropic diffusion; a Tensor describes anisotropic free diffusion (with diffusion coefficients D₁, D₂, and D₃ in three orthogonal directions characterised by angles θ and ϕ for the primary diffusion direction and α describing the angle of the secondary diffusion direction in the perpendicular plane); a Zeppelin is a cylindrically symmetrical tensor; and a Sphere describes diffusion restricted isotropically by an impermeable membrane with radius R. In this nomenclature, the conventional ADC model is represented by a Ball and a bi-exponential fit by Ball–Ball. In addition to the diffusion, shape and orientation parameters for each compartment shown in parentheses in Table 2, two-compartment models have extracellular and intracellular volume fractions fₑ and fᵢ, respectively, and all models include the equilibrium signal S₀ and the T₂ relaxation time constant as fitting parameters.

Data were fitted using an iterative maximum likelihood procedure that accounts for local minima and Rician noise. The noise was derived from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region. The noise was derived from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region from correction of the standard deviation of signal in an empty region.

2.4 Histology and registration

After imaging, the samples were processed and embedded in paraffin wax, and sections approximately 3 μm thick were cut at every 100 μm through the block. The slides were stained with haematoxylin and eosin (H&E), and digitised using a C9600–01 NanoZoomer Digital Slide Scanner (Hamamatsu, Hamamatsu City, Japan) at 20× magnification (21 708 pixels/cm).

Histological images were stacked into a volume using two-dimensional pairwise registrations between adjacent slices based on a block-matching strategy. The transformation model used was rigid body and the similarity measure was the correlation coefficient. This pairwise registration aligns each slice with the subsequent slice and then concatenates transformations to generate a volume consisting of stacked slices registered with respect to a reference slice in the middle of the image stack. The slice separation was taken as 100 μm.

To register the stacked histology volume to the T₂-weighted MRI, 9–15 manually selected corresponding landmarks were identified in each MRI/histology volume pair. This provided an approximate initial alignment as a result of the differing volume orientations. For the final registration, the volumes were resampled to isotropic voxels (0.5 mm for MRI and 0.1 mm for histology) to reduce orientational bias and the relevant regions (bright foreground voxels in MRI and non-zero voxels in histology) were selected to restrict the region over which the similarity measure was calculated to the internal tissue contrast. An intensity-based affine registration from ITK was then performed using normalised mutual information as the similarity measure and a regular step, single-scale optimisation with MRI as the target volume. The shear
component of the affine registration will, to first order, correct for any residual cumulative stacking error of the histology slices. A sample of the three-dimensional histology stack in each of three orthogonal views is shown in Figure S1 with the diffusion MRI slice overlaid. As a result of the orientation of the MRI slice with respect to the histological slicing plane, only a portion of the histology slice corresponds to the diffusion image, which is indicated by outlines of tumour regions in subsequent figures. The transformation was also applied to the primary diffusion vectors to keep alignment consistent with the image orientation.

3 | RESULTS

Fitting quality, parameter reproducibility and posterior parameter distributions were first examined as part of the model selection process. In a second section, model parameters were compared with histology, first examining parameters associated with compartment size and restriction, and then those associated with orientational structure.

3.1 | Fitting results and model selection

Figure 1a shows a sample diffusion-weighted image \( (b = 1076 \text{ s/mm}^2, \Delta = 30 \text{ ms}, \delta = 3 \text{ ms}) \); the yellow point indicates the voxel for which the data (points) are plotted in Figure 1b–d. Fits (full lines) are shown for the standard DT model (b) and the Tensor-Sphere model (d) (fits for other models can be seen in Figure S2). The plotted fitted lines are calculated assuming Rician noise (mean noise/\( S_0 \) = 0.01 for this voxel), so that any remaining systematic bias should be the result of model choice. Values are normalised using the fitted \( S_0 \) value. The residuals (Figure 1c, e) emphasise that even the most complex single-compartment model, the Tensor, overestimates the signal at low diffusion times and \( b \) values, and underestimates the signal at high diffusion times and \( b \) values. This voxel is typical of those that have higher fitted \( f_I \) values in two-compartment models (\( f_I = 0.44 \) for the Tensor-Sphere in this voxel); fits for voxels with lower \( f_I \) deviate less from the data, but have a stronger orientational dependence.

\( T_2 \)-weighted and diffusion-weighted images for each sample are shown in the two left-most columns of Figure 2 with the tumour focus outlined in cyan. Most of the remaining tissue is fat; some fibroglandular voxels outside the main tumour focus are also present. The best model for each voxel in each sample is shown in the third column of Figure 2 for AIC and in the fourth column for BIC (regions with no colour were excluded either as fat or for having non-mono-exponential \( T_2 \)). The distributions of relative AICs and BICs across each sample are shown in the boxplots, with the line at the median.
the box extending to the quartile values and the whiskers showing the range. The four larger samples (Figure 2a, c, e and f) are best explained by models with anisotropy; the remaining three cases (Figure 2b, d and g) include large amounts of fat, with a smaller tumour focus near the edge of the field of view, where the Ball–Sphere model is selected in some voxels. This could be a result of signal-to-noise ratio (SNR) issues at the image edge, contamination from interspersed fat, or may be a true biological difference (the case with the
largest number of voxels best explained by the Ball–Sphere model is an invasive carcinoma of histological grade 1 and NST. Many voxels are best explained by a model with a restricted Sphere component, but some regions, particularly in the grade 3 mucinous carcinoma (Figure 2c), are better explained by an unrestricted Zeppelin–Ball or Tensor–Ball model. There are no voxels in any of the samples in which a conventional ADC or DT is the best choice. Subsequent results focus on the four samples with large central sections of invasive cancer (Figure 2a, c, e, f), but the remaining cases can be seen in the Supporting Information.

Figure 3 demonstrates the parameter variance using histograms from the MCMC procedure for data from a single voxel with moderately high \( f_i \). The width of the diffusion coefficient distributions is larger for the two-compartment models, but the distributions of the angular parameters are similar. The mean values are similar across two-compartment models with restriction (Ball–Sphere, Zeppelin–Sphere, Tensor–Sphere) for \( f_i, D_1, R, \theta \) and \( \phi \), but differ for related diffusion coefficients (e.g. \( D_1 \) from Ball–Sphere is between \( D_1 \) and \( D_2 \) for Zeppelin–Sphere). Histograms from voxels with lower \( f_i \) (see Figure S3) showed similar patterns, but with narrower \( \theta \) and \( \phi \) distributions, probably because of the larger extracellular signal.

There was generally good agreement between the posterior distributions and parameter maps (see Figure S4) for the Zeppelin–Sphere and Tensor–Sphere models. Reproducibility (Figure S5) was also similar, and so subsequent results are presented for the simpler Zeppelin–Sphere model.

3.2 Model parameters – \( f_i \) and \( R \)

Figure 4 shows H&E-stained histology in the top row, parametric maps from ADC (second row) and selected parameters from the Zeppelin–
There was variation in all parameters across samples, including the characteristic high ADC in the mucinous carcinoma in the last column (mean ± standard deviation across fitted tumour voxels \(\times 10^{-3} \text{ mm}^2/\text{s}\): 1.3 ± 0.3 for grade 3 mucinous; 0.67 ± 0.18 and 0.50 ± 0.17 for grade 1 NST; 0.9 ± 0.6, 0.55 ± 0.09, 0.68 ± 0.14 and 0.48 ± 0.22 for grade 3 NST).

Regions of low cellularity on histology tend to correspond to regions of high ADC and low \(f_a\) (red outlines). However, some regions (e.g., cyan outline) have high cellularity relative to their surroundings, but higher ADC, which may be explained by a larger cell radius, \(R\). Additional samples are shown in Figure S6. Regions without colour were excluded either as fat (orange outline in first column) or as non-mono-exponential \(T_2\) (orange outline in second column), which often corresponded to necrotic regions on histology.

For the mucinous carcinoma (last column), the fitted \(R\) parameter hits the 20 \(\mu\)m maximum allowed by the fitting procedure in most voxels. This large \(R\) value is equivalent to unrestricted diffusion given amongst cancerous epithelial cells. The ADC in this region is relatively uniform \([0.77 \pm 0.16] \times 10^{-3} \text{ mm}^2/\text{s}\), but \(R\) increases from 6.4 ± 0.4 \(\mu\)m on the left side of the image to 8.2 ± 3.1 \(\mu\)m on the right side of the image, where the proportion of larger epithelial cells increases. The boxes in Figure 5b show a region of low cellularity (near the cyan outline from Figure 4) where ADC is lower than in the surroundings \([0.51 \pm 0.07] \times 10^{-3} \text{ mm}^2/\text{s}\) versus \((0.68 \pm 0.08) \times 10^{-3} \text{ mm}^2/\text{s}\) in the same-sized region above], contrary to conventional thinking about ADC, but the \(R\) map and high magnification histology suggest that small cells in this region restrict diffusion, limiting diffusion decay in spite of the lower cellular volume fraction.

For the mucinous carcinoma (last column), the fitted \(R\) parameter hits the 20 \(\mu\)m maximum allowed by the fitting procedure in most voxels. This large \(R\) value is equivalent to unrestricted diffusion given
the diffusion lengths probed in this experiment; thus, this finding is consistent with Figure 2 data that the Zeppelin–Ball model is a better choice in this sample.

3.3 | Model parameters – orientation

Colour FA maps from the Zeppelin portion of the Zeppelin–Sphere model (i.e. removing the isotropic spherical component from the FA calculation) are shown alongside the H&E-stained histology in Figure 6. Small regions of coherent direction (approximately 4 voxels = 1 mm) were observed and are highlighted for regions from two samples in Figures 7 and 8.

Figure 9 displays the colour FA maps for the original data (a) and data downsampled in-plane (b, c), demonstrating that anisotropy becomes weaker (colours less bright) at lower resolution, particularly at 2 mm resolution.

4 | DISCUSSION

4.1 | Model selection

This article presents detailed diffusion data of breast tissue samples acquired using a rich imaging protocol, testing a variety of one- and two-compartment models with different shapes, with and without restriction. The model that best explains the data varies in different tumours and regions, which is not unexpected given the diversity of breast cancer microstructure. This variation has also been reported in ex vivo prostate studies. A small fraction of voxels were excluded from fitting as a result of non-mono-exponential $T_2$ decay, and histology revealed that many such voxels were in necrotic regions. In most voxels, the data were best explained by the anisotropic two-compartment models: Zeppelin–Sphere and Tensor–Sphere in regions of higher cellularity, indicating that a restricted diffusion component is present in these areas, or Zeppelin–Ball and Tensor–Ball in regions of...
low cellularity. There was no clear trend in parameters with grade, although the number of samples is small and the samples demonstrated heterogeneity. There were no regions in which conventional ADC or DTI best explained the data. These models are by far the most commonly used clinically, albeit with more limited single-shell protocols consisting of lower $b$ values.

Although the constraints of clinical scans limit the diffusion data that can be obtained, the results of the rich protocol suggest that there is valuable information that is not captured by most clinical protocols and diffusion models. For example, a clinical protocol with scan parameters producing signal sensitive to the observed $R$ range of 6–9 μm could be designed. This approach has been successfully applied in prostate cancer to distinguish tumour from benign regions, and may improve tumour characterisation in breast.

4.2 Parameter values

Parameter values varied both across and within samples. The MCMC parameter distributions suggested that the fitted parameters were relatively stable, and comparison with histology further supported the hypothesis that parameter variations reflected true microstructural differences. This heterogeneity makes a simple summary of parameter
values challenging. The mucinous carcinoma examined had the highest ADC (Figure 4, third column), as has been reported previously, and is attributed to low cellularity.\textsuperscript{13,15}

In other samples, the ADC in regions of low cellularity was approximately $1.3 \times 10^{-3}$ mm$^2$/s, which is in agreement with the value of $1.23 \times 10^{-3}$ mm$^2$/s for interlobular stroma found by Norddin et al.\textsuperscript{38} at $22^\circ$C. Regions of higher cellularity tended towards lower ADC (approximately $0.6 \times 10^{-3}$ mm$^2$/s), in agreement with the mean diffusivity in breast lobules ($0.59 \times 10^{-3}$ mm$^2$/s) and regions of invasive ductal carcinoma ($0.45 \times 10^{-3}$ mm$^2$/s), despite differences in scan parameters and resolution.\textsuperscript{38} However, ADC does not fully characterise the histological features: some regions with little variation in ADC (box in Figure 5a) showed variations in cellularity and cell size that more closely reflected variations in the $i$, and $R$ maps from the Zeppelin-Sphere model; other regions with low ADC actually had low cellularity relative to their surroundings (Figure 5b). The map of $R$ suggested that this was a result of smaller cell size, and a qualitative estimate of cell size based on nuclear size in H&E supported this. This is the first study using two-compartment restricted diffusion models to examine \textit{ex vivo} breast tissue. However, Lasić et al.\textsuperscript{50} examined MCF-7 cells that had been grown \textit{in vitro} and found a median size of 13.2 µm assuming a log-normal distribution and a width of 0.6 µm. This is larger than the radius range observed in most sample regions in the present study (6–9 µm). This may reflect true biological differences − the \textit{ex vivo} samples include stromal regions containing smaller cells such as lymphoid cells − or may indicate cell shrinkage as a result of fixation or the use of a single cell radius parameter rather than a cell size distribution. Models incorporating cell size distributions were beyond the scope of this study, but should be examined in the future.

The diffusion coefficients themselves are affected by the room temperature scan, e.g. they are lower than the intracellular ($1.5 \times 10^{-3}$ mm$^2$/s) and extracellular ($2.8 \times 10^{-3}$ mm$^2$/s) values observed for \textit{in vitro} breast cell samples at $37^\circ$C.\textsuperscript{50} Fixation may also affect diffusion through cross-linking and decreased water content, but work in prostate suggests that the relative signal fractions in compartments are similar before and after fixation and that changes are unlikely to affect model ranking.\textsuperscript{31,52} Studies in brain\textsuperscript{53} and optic nerve\textsuperscript{54} also demonstrate that the microstructure and anisotropy are largely unaffected by fixation. Thus, cell size and organisational information should reflect the \textit{in vivo} situation reasonably well, but further experiments are needed to verify this.

\section*{4.3 Anisotropy}

Previous work\textsuperscript{31} has suggested that anisotropy in breast DTI might be a result of breast ductal structures. We were unable to examine this hypothesis in this study because of the limited number of normal duct structures in the samples, but anisotropy was observed in regions in which no breast ducts were present on histology. The regions with strongest anisotropy correspond to regions of lower cellularity in which H&E staining demonstrates a coherent collagen pattern. The possibility that structures not visible on H&E staining may contribute to diffusion anisotropy cannot be eliminated, but work in gels and tumour xenografts suggests that collagen results in the anisotropic diffusion of large molecules\textsuperscript{55} and may affect the smaller water molecules that provide the signals measured here. These findings are consistent with the higher FA observed in fibrous stroma relative to breast lobules,\textsuperscript{38} and with the higher FA in regions of hypoxia with increased collagen fibre density.\textsuperscript{36}

The regions of coherence on the FA maps (Figure 6) are relatively small, approximately 1 mm, and are likely to be averaged out at resolutions approaching 2 mm (Figure 9), which may account for the inconsistency in previous clinical findings: in healthy breast tissue, the ducts and/or surrounding structures may produce large regions of anterior–posterior anisotropy;\textsuperscript{35} tumours disrupt this structure and lower the large-scale anisotropy, but smaller regions of coherence with varying direction exist in the stroma, and may produce observable anisotropy depending on the image resolution, SNR and how disruptive the tumour is. For example, higher order diffusion tensor methods have been successfully applied in patients, and demonstrate the presence of multiple diffusion fibre directions in some voxels of malignant tumours, but anisotropy is not present in benign tumours.\textsuperscript{17} There is also evidence that collagen reorients in invasive tumours,\textsuperscript{6} and is more strongly aligned in malignant samples relative to hyperplastic samples.\textsuperscript{56} Of particular note in this study is that, although the mucinous carcinoma had lower anisotropy, small regions of coherence were still observable and corresponded to stromal orientation patterns, whereas mucinous carcinomas have proven difficult to distinguish from normal and benign tissue using conventional ADC methods.\textsuperscript{13} Thus, the ability to detect small regions of anisotropy within and around tumours is a potentially valuable biomarker, and may become achievable in the near future with the use of reduced field-of-view sequences, double diffusion encoding sequences\textsuperscript{57} or higher order diffusion tensor methods.\textsuperscript{17}
4.4 | Limitations

In addition to the use of a single average cell size parameter, and the fixation and temperature issues already discussed, this study has several limitations. The number of samples was small, but variation in the preferred microstructural model and parameters was observed even within this range of grades and histological subtypes of breast cancer. Samples were examined voxel-wise to maximise the information obtained about different microstructural environments, but additional samples are needed to determine whether the findings are generalisable across all breast cancers.

The gradient strengths used were larger than those commonly available clinically, but Figure 1 demonstrates that single-compartment models, such as DTI, diverge from the data even at low b values (e.g. red circles). More limited gradient strengths and diffusion times may result in more uncertainty in model parameters, but a priori information, such as that obtained from ex vivo studies and validated in vivo, may be useful in constraining models applied to more limited clinical data.

All models assumed no exchange of water between compartments during the measurement, although there may be some additional signal decay, particularly at long diffusion times and high b values, arising from exchange effects. \( T_2 \) was assumed to be mono-exponential, and a separate sequence ascertained where this assumption failed and excluded these voxels from fitting. The method could potentially be extended to include regions with multi-exponential \( T_2 \), given sufficient data. We assumed spherical cells of uniform size, which is a simplification of the real biological system. In cases in which there is some eccentricity in the cell shape, the radius estimate will represent a volume average of this parameter. Future work could extend the model selection to include compartments with anisotropic restriction; however, fitting both a cell size and compartment eccentricity using a basic pulsed gradient spin echo sequence biases both the radius and eccentricity parameters.58

5 | CONCLUSIONS

This is the first study to examine such a broad range of diffusion data in human breast tissue samples and to model the data using both anisotropy and restriction. The data from most cellular cancer regions and the adjacent fibro glandular tissue were best explained using a Tensor–Sphere or Zeppelin–Sphere model, indicating that both restriction and anisotropy are present in breast cancer tissues. There were no voxels in which ADC or DTI were the best models. Although variations in ADC often corresponded with variations in cellularity on histology, there were exceptions in which additional information was provided by the radius parameter \( R \) and intracellular volume fraction \( f_i \) from the Zeppelin–Sphere model. Regions of anisotropy corresponded to extracellular regions with aligned collagen on histology, but directions were only coherent over areas of approximately 1 mm and require high spatial resolution or diffusion techniques sensitive to sub-voxel anisotropy17,37 for their detection.

ACKNOWLEDGEMENTS

This work was supported by funding from Engineering and Physical Sciences Research Council (EPSRC) grant ‘MIMIC’ (EP/K020439/1) and EU FP7 Virtual Physiological Human grant ‘VPH-PRISM’ (FP7-ICT-2011-9, 601040).

REFERENCES

1. Zackrisson S, Andersson I, Janson L, Manjer J, Garne JP. Rate of over-diagnosis of breast cancer 15 years after end of Malmö mammographic screening trial: follow-up study. Br Med J. 2006;332:689–692.
2. Independent UK Panel on Breast Cancer Screening. The benefits and harms of breast cancer screening: an independent review. Lancet. 2012;380:1778–1786.
3. Kunker IH, Williams LJ, Jack WJL, Cameron D, Dixon JM. Breast-conserving surgery with or without irradiation in women aged 65 years or older with early breast cancer (PRIME II): a randomised controlled trial. Lancet Oncol. 2015;16:266–273.
4. Galbán CJ, Hoff BA, Chenevert TL, Ross BD. Diffusion MRI in early cancer therapeutic response assessment. NMR Biomed. 2016; DOI: 10.1002/nbm.3458
5. Witkiewicz AK, Dasgupta A, Nguyen K, et al. Stromal caveolin-1 levels predict early DCIS progression to invasive breast cancer. Cancer Biol Ther. 2014;8:1071–1079.
6. Conklin MW, Keely PJ. Why the stroma matters in breast cancer: insights into breast cancer patient outcomes through the examination of stromal biomarkers. Cell Adh Migr. 2012;6:249–260.
7. Basser PJ, Mattiello J, LeBihan D. MR diffusion tensor spectroscopy and imaging. Biophys J. 1994;66:259–267.
8. Zhang H, Hubbard PL, Parker GJM, Alexander DC. Axon diameter mapping in the presence of orientation dispersion with diffusion MRI. Neuroimage. 2011;56:1301–1315.
9. Panagiotaki E, Chan RW, Dikaos N, et al. Microstructural characterization of normal and malignant human prostate tissue with vascular, extracellular, and restricted diffusion for cytometry in tumours magnetic resonance imaging. Invest Radiol. 2015;50:218–227.
10. Panagiotaki E, Walker-Samuel S, Siow B, et al. Noninvasive quantification of solid tumor microstructure using VERDICT MRI. Cancer Res. 2014;74:1902–1912.
11. McCamack KC, Schenker-Naor NM, White NS, et al. Restriction spectrum imaging improves MRI-based prostate cancer detection. Abdom Radiol. 2016;41:946–953.
12. Partridge SC, Rahbar H, Murthy R, et al. Improved diagnostic accuracy of breast MRI through combined apparent diffusion coefficients and dynamic contrast-enhanced kinetics. Magn Reson Med. 2011;67:1759–1767.
13. Woodhams R, Kakita S, Hata H, et al. Diffusion-weighted imaging of mucinous carcinoma of the breast: evaluation of apparent diffusion coefficient and signal intensity in correlation with histologic findings. Am J Roentgenol. 2009;193:260–266.
14. Kul S, Eyuboglu I, Cansu A, Alhan E. Diagnostic efficacy of the diffusion weighted imaging in the characterization of different types of breast lesions. J Magn Reson Imaging. 2014;40:1158–1164.
15. Cakir O, Arslan A, Inan N, et al. Comparison of the diagnostic performances of diffusion parameters in diffusion weighted imaging and diffusion tensor imaging of breast lesions. Eur J Radiol. 2013;82:e801–e806.
16. Bokacheva L, Kaplan JB, Giri DD, et al. Intravoxel incoherent motion diffusion-weighted MRI at 3.0 T differentiates malignant breast lesions from benign lesions and breast parenchyma. J Magn Reson Imaging. 2014;40:813–823.
17. Teruel JR, Goa PE, Sjebakk TE, Østlie A, Flåsne HE, Bathen TF. Diffusion weighted imaging for the differentiation of breast tumors: from apparent diffusion coefficient to high order diffusion tensor imaging. J Magn Reson Imaging. 2016;43:1111–1121.
18. Cipolla V, Santucci D, Guerrieri D, Drudi FM, Miggiorini ML, de Felice C. Correlation between T2 apparent diffusion coefficient values and grading of invasive breast carcinoma. Eur J Radiol. 2014;83:2144–2150.
19. Belli P, Costantini M, Bufi E, et al. Diffusion magnetic resonance imaging in breast cancer characterisation: correlations between the apparent diffusion coefficient and major prognostic factors. *Radiol Med*. 2015;120:268–276.

20. Martincich L, Deantoni V, Bertotto I, et al. Correlations between diffusion-weighted imaging and breast cancer biomarkers. *Eur Radiol*. 2012;22:1519–1528.

21. Youk JH, Son EJ, Chung J, Kim J-A, Kim E-K. Triple-negative breast cancer on dynamic contrast-enhanced and diffusion-weighted MR imaging: comparison with other breast cancer subtypes. *Eur Radiol*. 2012;22:1724–1734.

22. Kim EJ, Kim SH, Park GE, et al. Histogram analysis of apparent diffusion coefficient at 3.0 T: correlation with prognostic factors and subtypes of invasive ductal carcinoma. *J Magn Reson Imaging*. 2015;42:1666–1678.

23. Nilsson LF, Fangberget A, Geier O, Seierstad T. Quantitative analysis of diffusion-weighted magnetic resonance imaging in malignant breast lesions using different b value combinations. *Eur Radiol*. 2013;23:1027–1033.

24. Guo Y, Cai Y-Q, Cai Z-L, et al. Differentiation of clinically benign and malignant breast lesions using diffusion-weighted imaging. *J Magn Reson Imaging*. 2002;16:172–178.

25. Hatakenaka M, Soeda H, Yabuuchi H, et al. Apparent diffusion coefficient and major prognostic factors. *Eur Radiol*. 2002;12:4748–4755.

26. Sigmund EE, Cho GY, Kim S, et al. Intravoxel incoherent motion imaging in tumor microenvironment in locally advanced breast cancer. *Magn Reson Med*. 2011;65:1437–1447.

27. Liu C, Liang C, Liu Z, Zhang S, Huang B, Intravoxel incoherent motion (IVIM) in evaluation of breast lesions: comparison with conventional DWI. *Eur J Radiol*. 2013;82:e782–e789.

28. Sun K, Chen X, Chai W, et al. Breast cancer: diffusion kurtosis MR imaging–diagnostic accuracy and correlation with clinical-pathologic factors. *Radiology*. 2015;277:46–55.

29. Wu D, Li G, Zhang J, Chang S, Hu J, Dai Y. Characterization of breast tumors using diffusion kurtosis imaging (DKI). *PLoS One*. 2014;9:e113240.

30. Lima M, Yano K, Kataoka M, et al. Quantitative non-Gaussian diffusion and intravoxel incoherent motion magnetic resonance imaging: differentiation of malignant and benign breast lesions. *Invest Radiol*. 2015;50:205–211.

31. Eyal E, Shapiro-Feinberg M, Furman-Haran E, et al. Parametric diffusion tensor imaging of the breast. *Invest Radiol*. 2012;47:284–291.

32. Tsougos I, Svolos P, Kougi E, et al. The contribution of diffusion tensor imaging and magnetic resonance spectroscopy for the differentiation of breast lesions at 3 T. *Acta Radiol*. 2014;55:14–23.

33. Partridge SC, Ziadlo A, Murthy R, et al. Diffusion tensor MRI: preliminary anisotropy measures and mapping of breast tumors. *J Magn Reson Imaging*. 2010;31:339–347.

34. Jiang R, Zeng X, Sun S, Ma Z, Wang X. Assessing detection, discrimination, and risk of breast cancer according to anisotropy parameters of diffusion tensor imaging. *Med Sci Monit*. 2016;22:1318–1328.

35. Furman-Haran E, Grobgeld D, Nissan N, Shapiro-Feinberg M, Degani H, Can diffusion tensor anisotropy indices assist in breast cancer detection? *J Magn Reson Imaging*. 2016;44:1624–1632.

36. Kakkad SM, Zhang J, Akbardeh A, et al. In vivo and ex vivo diffusion tensor imaging parameters follow Collagen 1 fiber distribution in breast cancer xenograft model. *Proc Int Soc Magn Reson Med*. 2015;22:222.

37. Mayr NA, Staples JJ, Robinson RA, VanMetre JE. Intracutal breast carcinoma: initial results of a morphometric study using computerized digital image analysis. *Clin Oncol (R Coll Radiol)*. 1990;2:66–70.

38. Norddin N, Power C, Watson G, et al. Microscopic diffusion properties of fixed breast tissue: preliminary findings. *Magn Reson Med*. 2015;74:1733–1739.

39. Alexander DC. A general framework for experiment design in diffusion MRI and its application in measuring direct tissue-microstructure features. *Magn Reson Med*. 2008;60:439–448.

40. Modat M, Ridgway GR, Taylor ZA, et al. Fast free-form deformation using graphics processing units. *Comput Methods Programs Biomed*. 2010;98:278–284.

41. Whittall KP, MacKay AL. Quantitative interpretation of NMR relaxation data. *J Magn Reson*. 1989;84:134–152.

42. Panagiotaki E, Schneider T, Siow B, Hall MG, Lythgoe MF, Alexander DC. Compartment models of the diffusion MR signal in brain white matter: a taxonomy and comparison. *Neuroimage*. 2012;59:2241–2254.

43. Henkelman RM, Measurement of signal intensities in the presence of noise in MR images. *Med Phys*. 1985;12:232–233.

44. Akaike H. A new look at the statistical model identification. *IEEE Trans Automat Contr*. 1974;19:716–723.

45. Schwarz G. Estimating the dimension of a model. *Ann Stat*. 1978;6:461–464.

46. Mertzaniotou T, Hipwell J, Dalmis M, et al. Towards spatial correspondence between specimen and in-vivo breast imaging. In: Fujita H, Hara T, Muramatsu C, eds. *Breast Imaging: 12th International Workshop, IWDM 2014, Gifu City, Japan, June 29 – July 2, 2014. Proceedings*. Cham: Springer International Publishing; 2014:674–680.

47. Ourselin S, Roche A, Subsol G, Pennec X, Ayache N. Reconstructing a 3D structure from serial histological sections. *Image Vis Comput*. 2001;19:25–31.

48. Yoo TS, Ackerman MJ, Lorensen WE, et al. Engineering and algorithm design for an image processing API: a technical report on ITK–the Insight Toolkit. *Stud Health Technol Inform*. 2002;85:586–592.

49. Liang S, Panagiotaki E, Bongers A, et al. Diffusion kurtosis imaging: differentiation of malignant and benign breast lesions using different b value combinations. *Eur Radiol*. 2013;23:1027–1033.

50. Lasić S, Oredsson S, Partridge SC, et al. Apparent exchange rate for breast cancer characterization. *NMR Biomed*. 2016;29:631–639.

51. Bourne RM, Bongers A, Charles N, Power C, Sved P, Watson G. Effect of formalin fixation on biexponential modeling of diffusion decay in prostate tissue. *Magn Reson Med*. 2013;70:1160–1166.

52. Bourne RM, Panagiotaki E, Bongers A, Sved P, Watson G, Alexander DC. Information theoretic ranking of four models of diffusion weighted signal attenuation in fixed prostate tissue. *NMR Biomed*. 2015;28:660–671.

53. Dyrbyr TB, Baeré WFC, Alexander DC, Jelsing J, Garde E, Søgaard LV. An ex vivo imaging pipeline for producing high-quality and high-resolution diffusion-weighted imaging datasets. *Hum Brain Mapp*. 2011;32:544–563.

54. Richardson S, Siow B, Batchelor AM, Lythgoe MF, Alexander DC. A viable isolated tissue system: a tool for detailed MR measurements and controlled perturbation in physiologically stable tissue. *Magn Reson Med*. 2013;69:1603–1610.

55. Stylianopoulos T, Diop-Frimpong B, Munn LL, Jain RK. Diffusion anisotropy in collagen gels and tumors: the effect of fiber network orientation. *Biophys J*. 2010;99:3119–3128.

56. Ambekar R, Lau T, Walsh M, Bhargava R, Toussaint KC. Quantifying collagen structure in breast biopsies using second-harmonic generation imaging. *Biomed Opt Express*. 2012;3:2021–2035.

57. Shemesh N, Ozarslan E, Komlish ME, Basscr PJ, Cohen Y. From single-pulsed field gradient to double-pulsed field gradient MR: cleaning new microstructural information and developing new forms of contrast in MRI. *NMR Biomed*. 2010;23:757–780.

58. Ivanu A, Drobnjak I, Alexander DC. Model-based estimation of microscopic anisotropy using diffusion MRI: a simulation study. *NMR Biomed*. 2016;29:672–685.

59. Stepnišk J. Time-dependent self-diffusion by NMR spin-echo. *Phys B Condens Matter*. 1993;183:343–350.
SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Bailey C, Siow B, Panagiotaki E, Hipwell JH, Mertzianidou T, Owen J, Gazinska P, Pinder SE, Alexander DC, Hawkes DJ. Microstructural models for diffusion MRI in breast cancer and surrounding stroma: an ex vivo study. NMR in Biomedicine. 2017;30:e3679. doi: 10.1002/nbm.3679

APPENDIX

Mathematical descriptions of the model components are presented here for the pulsed gradient spin echo (PGSE) sequence. Compartments are assumed to have slow exchange of water between them, such that the total signal in a voxel \( S \) is the sum of all signal compartments \( S_i \) weighted by their respective fractions \( f_i \): \( S = S_0 e^{-\frac{B_0}{2} \sum f_i S_i} \), where \( S_0 \) is the equilibrium signal intensity, TE is the echo time and \( T_2 \) is the relaxation time constant (\( S_0 \) and \( T_2 \) are fitted parameters in all models).

The Ball compartment is equivalent to the signal for Gaussian diffusion: \( S_{\text{Ball}} = e^{-b \Delta^2} \), with \( b = (g\delta)^2(\Delta - \delta/3) \), where \( g \) is the gyromagnetic ratio, \( \delta \) is the gradient strength, \( \Delta \) is the gradient duration and \( \delta \) is the gradient separation.

The Zeppelin is the product of signal along the primary diffusion direction and the direction perpendicular to this: \( S_{\text{Zeppelin}} = e^{-b \cos^2 \psi \delta_1 D_1} e^{-b \left(1+\cos^2 \psi \right) \delta_2 D_2} \), where \( \psi \) is the angle between the normalised gradient direction \( \hat{g} \) and the parallel diffusion direction \( \hat{n} \) defined in spherical co-ordinates with \( \theta \) and \( \phi \): \( \cos \psi = \hat{g} \cdot \hat{n} \), \( \hat{n} = (\sin \theta \cos \phi, \sin \theta \sin \phi, \cos \theta) \).

The Tensor signal is given by \( S_{\text{Tensor}} = e^{-b \cos^2 \psi \delta_1 D_1} e^{-b \cos^2 \psi \delta_2 D_2} e^{-b \cos^2 \psi \delta_3 D_3} \), where \( \cos \psi_1 \) is the angle between the gradient direction and the \( i \)th eigenvector from the diagonalisation of the diffusion matrix, such that \( \hat{n}_1 = (\sin \theta \cos \phi, \sin \theta \sin \phi, \cos \theta) \), \( \hat{n}_2 = \hat{k} \cos \alpha + (\hat{k} \times \hat{n}_1) \sin \alpha + \hat{n}_1 (\hat{k} \times \hat{n}_1) (1 - \cos \alpha) \), \( \hat{k} = (\sin \theta + \frac{1}{2} \cos \phi, \sin \theta - \frac{1}{2} \sin \phi, \cos \phi + \frac{1}{2}) \) is a vector orthogonal to \( \hat{n}_1 \) rotated by an angle \( \alpha \) in that plane and therefore \( \hat{n}_3 = \hat{n}_1 \times \hat{n}_2 \).

The Sphere signal is calculated using the Gaussian phase distribution approximation: \( S_{\text{Sphere}} = \exp \left(-2Y^2 \Delta^2 \sum_{m=0}^{\infty} \frac{2(\Delta - \delta/3)^2}{\delta} \beta_m (\beta_m R)^2 J_{2m}(\beta_m R) \right) \). Here, \( Y(x) = e^{\beta_m \Delta x} \), \( \beta_m \) is the \( m \)th root of \( J_2(\beta_m R) - \beta_m R J_1(\beta_m R) = 0 \) and \( J_v \) is the Bessel function of the first kind, order \( v \). The summation was carried out over the first 31 roots of the equation.