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# **Breast Tumor Microstructure Imaging with Diffusion MRI**

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### Abstract

Despite the notable scientific progress in screening procedures and treatment strategies, breast cancer remains a significant cause of mortality, suffering and financial burden. Breast tumor detection, classification and treatment response monitoring currently rely on combinations of invasive biopsies and imaging procedures that use contrast agents, which increase the patient risks and discomfort. Diffusion MRI (dMRI), as a non-invasive and contrast agent-free imaging method, could be considered a viable alternative approach. Conventional dMRI methods already possess a role in breast cancer protocols, due to their contribution to the sensitivity and specificity of tumor diagnosis and assessment. This role however has remained purely a supplementary one, due to their low capability to resolve sub-voxel tissue heterogeneities that yield low specificity. Novel diffusion imaging techniques that claim microstructural imaging capabilities, could only recently be clinically investigated, after advancements in MRI software and hardware. By providing microstructural information and measuring biochemical properties on cellular scales, the hypothesized higher specificity of the obtained diffusion-based parameters could expand the clinical role of dMRI in breast cancer. This hypothesis is investigated in this review article, by presenting a compilation of findings from studies that report on breast tumor microstructure obtained by either conventional or microstructural dMRI methods. In an attempt to explore the feasibility of dMRI becoming a stand-alone breast cancer approach, the clinical value of the microstructural findings reported in each method is highlighted.

Keywords: Diffusion MRI, Breast Cancer, Microstructure

# 1. Introduction

Diffusion Magnetic Resonance Imaging (dMRI) is an imaging method that generates image contrast based on the microscopic diffusion motion of water molecules contained within biological tissues. Its ability to capture diffusion properties at the micrometer scale [1] and to produce a plethora of parameter maps based on them, renders dMRI a uniquely versatile method with successful applications in both preclinical and clinical settings. Changes in local diffusion properties are indicative of alterations in the microstructure and the physiology of certain tissues that are linked to specific clinical and even behavioral conditions [2]. One major category of such conditions notorious for their capacity to disrupt tissue physiology is cancer.

Initially, the successful applications of dMRI were targeting mainly neurological conditions of the brain. The high sensitivity of the diffusion signal to microstructural alterations eventually has driven the use of dMRI in oncological cases of various organs [3]. In fact, over the past two decades, dMRI has been proven to provide valid imaging biomarkers for the detection and characterization of tumors, the assessment of treatment response, as well as survival prognostic markers [4].

Breast cancer can be considered a representative example of the significant contribution of diffusion MRI on a very relevant oncological disease. Despite occurring in women almost exclusively (99% of the cases), breast cancer was estimated in 2020 to be the most prevalently diagnosed form of cancer with 2.26 million new cases, and responsible for 685.000 deaths, rendering it the 5th deadliest cancer (WHO - Global Health Estimates 2020.) Overall, the global cumulated burden on women due to breast cancer is estimated at 19.8 million Disability-Adjusted Life Years (DALYs) [5]. The upwards trend of the global average of the Human Development Index (HDI) over the last three decades, mainly due to the westernization of low- and medium-income countries, has been strongly correlated with an increase in incidence and mortality rates due to cancer [6]. These, along with the improved awareness and screening processes, lead to projections that indicate an increase in new annual incidences to 2.7 million in 2030 (Cancer Tomorrow).

Current breast cancer protocols are primarily based on Dynamic Contrast-Enhanced (DCE) MRI and histological analyses of either tissue samples acquired by biopsy, or resected tumors. While the sensitivity of DCE-MRI to malignant breast lesions has been reportedly found in a metaanalysis [8] at 90%, its low specificity at 76% can lead to multiple unnecessary biopsies to be performed. In addition, evidence suggesting the toxicity of gadolinium used in the contrast-agents has been gathered [9]. Therefore, an accurate and reliable dMRI-based method could provide a non-invasive and agent-free approach to breast lesion screening and tumor evaluation. In pursuit of this goal, the diagnostic capabilities of diffusion MRI in breast lesion screening have been acknowledged, which has led to its incorporation into the standard breast lesion screening protocols (BI-RADS) [4].

However, only the simplest dMRI techniques are clinically applied, due to the limited timeframes of medical examinations, and the limited availability of the required specialized hardware. In principle, all dMRI methods indirectly capture characteristics of tissue microstructures through the properties of the water diffusion within tissues. Conventional dMRI methods are generally regarded to yield parameters of low specificity since their estimation is based on voxel averaging of the diffusion signal. With typical voxel sizes of a few millimeters, the morphological details of tissues that are on the micrometer scales are challenging to be resolved. A representative example of this limitation can be the case of white matter structures in the brain, which can be seen in Figure 1.

Novel dMRI techniques claimed to be capable of resolving sub-voxel heterogeneities and providing microstructural information and properties on a cellular scale, have been developed. Often referred to as Microstructure Imaging, these dMRI methods rely on diffusion encoding sequences and diffusion signal representation frameworks of high complexity. Only recently these methods could be investigated in the clinic, after the advancements in the MR scanner hardware, which are capable of stronger and faster gradients, as well as higher signal sensitivities to the diffusion.

Only recently these methods could be investigated in clinical settings, mainly due to advancements in MRI scanner hardware design and image reconstruction methods. In the first, stronger and faster magnetic field gradients are now possible, achieving higher signal sensitivities to diffusion. The latter, based on signal under-sampling techniques and the novel simultaneous multi-slice acquisitions, led to acquisition times that fall within the clinically-acceptable limits. Despite this progress, the vast majority of studies that develop and validate these dMRI methods specifically target brain pathologies, with only a few focusing on different body sites.

Following the successes in brain applications, interest in the translation of advanced microstructural dMRI techniques to breast cancer applications is rising. Essentially, harnessing the unique microstructural information obtained by such techniques could lead to superior performance over standard protocols. An increased specificity, coupled with the noninvasive and contrast agent-free nature of dMRI, could ultimately allow for dMRI-based approaches to replace DCE-MRI in breast imaging. The additional advantages offered by microstructural imaging are potentially numerous [11]. The possibility of replacing the invasive histological assessments can be hypothesized, provided that sufficient microstructural information can reliably be obtained through them. In such a scenario, the relative convenience and repeatability of dMRI imaging methods would allow the safe use of larger healthy control groups, enabling greater progress in breast cancer research to be made.

In this article, the feasibility and the potential of microstructural dMRI methods in breast cancer are explored by compiling results from studies reporting on breast tumor microstructure. To highlight the clinical value of the obtained microstructural information, the reported associations between diffusion parameters and clinical statuses are listed. Before these, a brief overview of the theory of both conventional and recently-developed microstructural imaging methods is additionally presented.



Figure 1. An illustrative example of the root cause of the low of specificity in dMRI-derived metrics. (A) Histological images of typical glioma (left) and meningioma (right) structures at micrometer scales. The shapes of the water diffusion within them are approximately annotated by the blue glyphs. Gliomas are characterized by isotropic diffusion of various magnitudes, and meningiomas by anisotropic diffusion. (B) Representations of voxels containing diffusion patterns of various shapes that can be found in brain, at millimeter-scales as typically acquired in dMRI. Despite the obvious differences in the underlying microstructure that cause these diverse diffusion patterns, conventional dMRI measurements would yield similar results. Adapted from (Reymbaut & Descoteaux 2019, [10]).

#### 2. Breast Tumor Histopathology and Microstructures

As breast cancer is described the malignant neoplasia, or else the abnormal and excessive growth, of any mammary tissue. Breast cancer subtypes are formed based on several classifications [12], each one of which has distinct risk profiles and treatment strategies [13]. Accurate breast lesion screening and tumor classification, along with treatment response monitoring and prognostic biomarker extraction, are therefore of utmost clinical importance. The contribution of dMRI methods to these tasks is based on the associations of their estimated parameters to the histological and microstructural features and, by extension, to their clinical indications.

Whether the cells of the neoplasm are derived from the epithelium that lines the ducts or the lobules, which is the most prevalent scenario, leads to the categorization into ductal carcinomas (DC) and lobular carcinomas (LC) [12], corresponding to 77% and 13% of the breast cancer cases, respectively [14]. An additional classification describing the localization of the cancerous cells is used. As in-situ (IS) carcinoma is described as the confinement of cancerous cells within the lobular-duct system, while invasive carcinoma (I) their expansion to different tissue compartments, such as the lymphatic system [15].

A molecular classification is based on the presence of three antigens in the tissue: estrogen (ER), progesterone (PR), and human epidermal growth factor 2 (HER2) hormonal receptors. Hormones bind to these receptors, causing changes in the cancer cells [16]. Depending on their presence, a further division of breast tumors into four major classes is made:



Table 1. Breast Cancer Characteristics.

Figure 2. Graphical representations of microstructural and microenvironmental characteristics of healthy and cancerous breast tissues. Adapted from (Moccia et al. 2021, [24]).

luminal-A, luminal-B, and HER2<sup>+</sup> carcinomas and triplenegative breast cancer (TNBC) [17].

Depending on the tumor subtype characterization, anatomic cancer stage and patient preferences, different treatment strategies of neoadjuvant and adjuvant chemotherapies (NACT and ACT) are followed [13]. Currently, for the characterization of a lesion that shows up in imaging as malignant or as benign dense fibroglandular tissue (FBGT), tissue samples or the entirety of the lesion are excised in an operating room. After the sample preparation process of formalin fixation and embedding in paraffin blocks, sections of 3-5 µm are generated using microtomes to be examined microscopically [18]. Immunohistochemistry (IHC) tests are then used as a staining technique to highlight the cell organization, extra- and intracellular structures, such as cell membranes and nuclei. Ultimately, quantitative histological features such as the ones found are then estimated based on which inferences about the tumor are made.

Breast lesions have distinct morphological features on milliand micro-meter scales, that influence the diffusion properties that dMRI is sensitive to. A synoptic list of the most notable of these features as found in the literature is listed in Table 2. Tumors inherently disrupt normal cell organization and differentiation due to their rapid cell division [16], and present increased cellularity compared to healthy FBGT. Water diffusion tends to be restricted and anisotropic within the healthy mammary ducts and lobules, with the degree of restriction being increased in case of malignancy due to blockages by malignant cells [19]. On the microscopic scale, cellular properties such as size, nuclei uniformity membrane permeability to water are also affected [16]. Water contained in tissues is of particular interest for dMRI, and it approximately accounts for 60% of the human body contents. Physiologically, body water can be broken down into two major compartments [20]. Intracellular fluid (ICF) is contained within cells and accounts for 70% of the total body water [21], and the rest is Extracellular fluid (ECF), which is contained in areas outside of cells. One of the sub-compartments of the ECF is the interstitial fluid by 80% [22] that is located between blood vessels and cells, and the rest is plasma located in the blood.

Neoplasms have been found [23] to distort the physiological size and the shapes of these compartments on a microscopic level. A prevalent example is the increased presence of blood perfusion in tumors, which is related to the angiogenesis that comes with their growth [24]. Inspired by these findings, multiple dMRI techniques make biophysical assumptions using compartments to achieve improved interpretations of the dMRI signal and specificity of their derived estimates. Within each voxel, the sizes and the shapes of these tissue compartments contained are therefore considered microstructural features of major importance. The dMRI techniques with that aim, such as compartmental models and microstructure imaging methods, will be covered in Chapters 4.2 and 5. of this review, respectively.

# 3. Diffusion Encoding Methods

Signal acquired in conventional MRI methods is mainly generated by the spin precession of hydrogen nuclei, primarily the ones forming water molecules. For the signal to contain sufficient localization information for image reconstruction, magnetic field gradients are used to encode the 3D position of the molecules. A parallel could be drawn with the case of dMRI. To harness information based on the characteristics of

Microstructural Features	Description	Reasons	
Cellularity	Cell or packing density	Increased cell density or heterogeneities can indicate malignancy [25]. Necrotic tissue has decreased density.	
Cell Size	Cell diameter	Cellular size is one of the most quickly affected parameters in chemotherapies. Tumor status and its response to treatments can be observed by size changes of tumor cells [26].	
Cell Shape	Eccentricity and directionality	It can be used to distinguish the cell of origin and tumor aggressiveness.	
Cell Membrane Permeability	Permeability of cell membrane that allows the molecular water exchange between the intra- and extra-cellular compartments	Cell membrane permeability depends on cancer subtypes, tumor microenvironment, proliferation, drug delivery [27] and treatment-induced apoptosis [26].	
Transcytolemmal Water Exchange	The water exchange between the cellular-interstitial water compartments	It is predicted associated with intracellular water lifetime, which reflects the status of the cellular metabolism, which is a characteristic of cancer aggressiveness [28].	
Tumor Vascularity	Presence of micro capillaries	Hypervascularity and angiogenesis are observed in or around malignant tumors. They can influence cancer development and can act as a prognostic factor [24].	
Stroma Types	Prevalence of collagen, fibroblasts, and lymphocytes stroma types	Stroma type ratios and the dominant stroma type can be independent prognostic factors in mammary cancer [29], [30].	
Subcellular Architecture	Sizes of nuclei and organelles, [Jiang 2021] and nuclei uniformity [Iima 2019]	Cell nuclei become less uniform [16].	

Table 2. Microstructural features and their relevance to breast tumors.

the water diffusion in a tissue, an additional 3D encoding is needed for each voxel [31], which we describe as diffusion encoding.

Diffusion encoding sequences are composed of timevarying magnetic field gradients. These are applied before the signal readout, for which Echo-Planar-Imaging (EPI) -based sequences are most commonly used [32]. With these acquisition schemes, the acquired signal is sensitized to the diffusion motion of the water molecules within tissues, unveiling characteristics of its underlying structure. Therefore, a brief overview of such encodings is necessary before any attempt to estimate any microstructural or compartmental parameters based on the diffusion signal. As such, the encoding schemes used in dMRI studies of breast tumors are briefly covered in this section.

### 3.1 Linear Diffusion Encoding (SDE)

The majority of dMRI acquisitions rely on the Pulsed-Gradient Spin-Echo (PGSE) sequence as introduced by [33] in 1965 for diffusion encoding. Diffusion encodings using nonspin-echo sequences of pulsed gradients also exist, such as the pulsed-gradient stimulated-echo [34] sequences. Therefore, we will adopt the nomenclature introduced by [35] and refer to this family of diffusion encoding sequences as Single Diffusion Encodings (SDE).

The most commonly used form of SDEs is the one depicted in Figure 3. They are composed of pairs of diffusion weighting gradient pulses with identical magnitudes, G, and time durations,  $\delta$ , that are applied before and after a refocusing pulse. With the ramp times of the pulses neglected, the time interval between the onset of the two gradient pulses is  $\Delta$ . The sensitivity of the acquisition to the diffusion motion is referred to as *b*-value and is defined by these scalar parameters according to the equation:

$$b = (\gamma G \delta)^2 \tau_d$$

where  $\gamma$  the proton gyromagnetic ratio, and  $\tau_d$  the diffusion time is defined as  $\tau_d = (\Delta - \delta/3)$ . The spatial orientation of the applied gradient pulses is determining the axis along which the signal is sensitive to diffusion, which renders SDEs a *linear* diffusion encoding method. In dMRI experiments, measurements of multiple orientations are always performed, to probe diffusion characteristics along different directions.

By varying the encoding parameters and, by extent, the amount of diffusion weighting in an acquisition as described by the *b* value, one attempts to obtain morphological features of tissues [11]. In most of the SDE-based acquisitions, this is achieved by varying *G* while keeping  $\delta$  and  $\Delta$  constant [36]. The capabilities of SDEs however are limited, since different diffusion characteristics, such as anisotropy, orientation dispersion and variance can not be resolved [37].



Figure 3. Sequence diagrams of the conventional PGSE (top) and the oscillating gradients (bottom) diffusion encodings. Adapted from (Afzali et al. 2021, [11]).

# 3.2 Variations of Linear Diffusion Encoding

The potential of varying the gradient durations and the diffusion times of the linear diffusion encodings has been explored in the quest for higher tumor conspicuity and specificity in the inferences about histological features of the micrometer scale. In the context of breast tumor imaging, most of the novel imaging methods aiming at microstructures rely on the SDE-based encodings of Oscillating Gradient Spin-Echo (OGSE) [38] and Time-Dependent Diffusion (TDD) [39].

# Oscillating Diffusion Encoding (ODE)

By replacing the pulse gradient pair of an SDE with a series of oscillating pulsed gradients as seen in Figure 3, we obtain OGSE diffusion encodings. Adopting [35] once again, these are also referred to as Oscillating Diffusion Encodings (ODE). The number of oscillations N, and their frequency  $\omega$ , are characteristic parameters of the ODE encodings, with optimal values being under investigations [40]. In practice, values of 30 repetitions and 1 *KHz* are typical [40], however since the *b*value is proportional to  $G/\omega^2$ , strong gradients are required to compensate for increased frequencies [40], which limits the use of ODE to advanced MR-scanners.

In general, achieving high b-values with ODEs is challenging, due to the limited duration of the applied gradients in each oscillation. In contrast, [11] claims that ODEs are capable of maintaining such high b-values with short diffusion times. This capability allows for increased diffusion sensitivity to small pores, which ultimately renders ODEs a suitable encoding scheme for cell size estimation [41]. Microscopic variations in tumors can be revealed by methods using ODEs [42], and multiple microstructural methods applied in breast cancer rely to a certain extent on them.

# Time-Dependent Diffusion (TDD)

Diffusion time  $\tau_d$  indicates the amount of time during which water molecules diffuse and sense the microstructure before taking a measurement [43]. Based on this principle, a variation of SDEs is increasingly in use by varying the diffusion time  $\tau_d$ to increase the specificity of dMRI to specific microstructural features. Different diffusion times are acquired by using combinations of the  $\delta$  and  $\Delta$  time parameters, while maintaining the same gradient strength *G*. These methods, which are named Time-Dependent Diffusion (TDD) encodings [41], have been described by [39] as "a strong candidate" for characterizing tumors with high specificity, after implementing and testing several TDD-dependent models that estimate microstructural features on various tumors, with breast ones among them.

A dependency on diffusion time is added to all diffusionderived metrics, which often leads to the use of the term "temporal spectroscopy" to describe TDD in the literature. This dependency is characterized by the presence of restricted structures, the permeability of barriers, and the length scale of barriers compared to diffusion length range [44], and multiple signal representations and models exist to leverage it, some of which will be covered in the following chapters. The timedependent scheme of TDD can also be applied to other diffusion encodings such as the ODE ones. Information acquired with the time-dependent extension of any encoding requires the generalization of the traditional *b*-value of the SDEs with a more versatile *b*-tensor to describe the complexity of the acquired signal [1].



Figure 4. Sequence diagrams of different MDEs and their shapes of b-tensors they generate. The linear (G), planar (H), and spherical (I) b-tensors can be generated by applying the simplistic single (A), double (B) and triple (C) diffusion gradients, or their free gradient waveform equivalent ones (D-F). Adapted from (Afzali et al. 2022, [37]).

#### 3.3 Multidimensional Diffusion Encoding (MDE)

Alternative approaches that encode diffusion along multiple orientations simultaneously are increasingly being applied in dMRI research. By assigning characteristic shapes to the sensitivity of the diffusion motion, increased signal specificity is achieved, which enables the resolution of signal ambiguities [45]-[47] that result in entangled measurements of orientation coherence, microscopic anisotropy and isotropic heterogeneity. These approaches are described as Multidimensional Diffusion Encodings (MDE), and rely on pulse sequences that involve more than one diffusion orientation encoding before the readout. According to such schemes, encoding of diffusion with *b*-tensors that resemble planes or spheres is possible by using combinations of two or three pairs of orthogonal gradients. These gradient forms are named Double Diffusion Encoding (DDE) and Triple Diffusion Encoding (TDE), respectively, and can be seen in Figure 4.

Optimizations towards more efficient MDE sequences have led to very powerful encodings whose gradient waveforms deviate from the conventional pulsed gradient designs. These encodings use multiple complex waveforms of magnetic gradient intensity that give complex shapes to the diffusion sensitivity. Relying on the use of *b*-tensors, combinations of optimized free gradient waveform encoding acquisitions were found to unveil microstructural information [48]. In Figure 4, the free gradient waveform variants of the MDE encodings along with the b-tensors they generate can be seen. The signal interpretation of such encodings relies on the mathematical framework of Q-space, the details of which are beyond the scope of this article, however, an exhaustive review of the formulation can be found in [10]. In a nutshell, the Q-space framework allows for diffusion measurements and interpretations using arbitrary trajectories of gradient waveforms. The use of the framework is free of assumptions and restrictions [31], which provides alternative diffusion interpretations non-parametrically.

# 4. Conventional Diffusion Representation and Biophysical Tissue Models based on Pulsed-Gradient SDEs

Once sufficient diffusion data are collected using the appropriate encodings, their interpretation takes place using diffusion signal representation frameworks and biophysical tissue models [49]. These methods, based on various physical and histological assumptions, provide the diffusion-based metrics used for inferences of morphological and functional features of tissues.

As described in [50], representations and models are being used in conjunction to increase sensitivity and specificity to microstructural features, respectively. Several representations and models pose additional requirements, such as limited ranges of *b*-values, a minimal number of gradient orientations, as well as specific diffusion encodings. These aspects are briefly covered in this chapter, while many publications and their findings relevant to BC and their conclusions about tumor microstructures are presented.

# 4.1 Representations

# Trace Diffusion Weighted Imaging (DWI)

Post diffusion encoding, the acquired MRI signal exhibits a decaying intensity with an increase in diffusion sensitivity. The simplest approximation can be then made by fitting the diffusion-weighted signal  $S_{DWI}$  with a monoexponential decay function using the following equation:

$$S_{DWI} = S_0 \exp(-b ADC) \quad (1)$$

where  $S_0$  is the diffusion-unweighted signal (b=0) acquired with no encoding gradients, and *ADC* is the apparent diffusion coefficient of the tissue that describes the amount of diffusivity per voxel. This technique is referred to as Diffusion Weighted Imaging (DWI), and it is most commonly used with SDE encodings. Therefore, in practice, a minimum of 3 orientations of measurements are required to capture the spatial distribution of diffusion. A minimum of one non-zero b-value measurement is necessary, with two often being chosen within the range 500-1000 s/mm2.

In a perfectly homogeneous medium, water diffusion is random and isotropic, and can be described by a Gaussian distribution. In this case, an accurate fitting of the above representation to the diffusion data can be achieved. Human tissues, however, have complex cellular configurations and present a large heterogeneity in the sub-millimeter scale of voxels in MRI. For this reason, signal deviations from the equation (1) are observed in high b-values, which limit the use of this method to b-values in the range of 0-1000  $s/mm^2$ . In general, even within the optimal b-value ranges, ADC is generally considered a low-specificity metric, since multiple distinct structures can lead to the same ADC values. Despite these limitations, the relative simplicity of DWI has led to numerous extensive performance investigations of ADC over the decades, concerning its ability to distinguish breast lesions, characterize tumors, provide prognostic factors and monitor the effects of treatments.

The malignancy detection capabilities of ADC in breast lesions have been showcased. The improved specificity achieved by acquiring DWI along with DCE MRI has led to the inclusion of DWI in breast screening protocols. Tumors generally presented a lower ADC value than lesions [51]. In response to treatment, the increase in tumor ADC has been found to be detectable earlier than changes in size and vascularity as measured by DCE MRI [52], offering indications about the treatment efficiency earlier. In the opposite direction, an extensive metanalysis [53] comprising 2990 breast tumors that studied the associations of ADC and the classification into molecular subtypes, concluded that ADC is unable to discriminate between different molecular subtypes. Moreover, the association of ADC values with tumor proliferative markers, such as Ki-67 expression and response to neoadjuvant chemotherapy is controversial [54].

Microstructurally, ADC has been inversely correlated to tissue cellularity of breast tumors in many studies [3], [51]. This is commonly attributed to the hindrance and restrictions experienced by diffusing molecules in high cellularity tissues, such as dense breast tumors [55]. However, in [55] skepticism is expressed about the increased cellularity being the sole contributor to a decrease in ADC, after citing many studies with weak to moderate ADC correlations with cellular density. The integrity of cell membranes is also reported to have an association with ADC in a study using the DWI method with reduced Field-of-View that enabled a sub-millimeter resolution [56]. Overall, the ADC hypo-intensity remains informative as it is considered to be represented by voxels with high water content and low cell density [57]. Concerns however about the lack of specificity due to the signal contribution from blood flow and tissue heterogeneities on a sub-voxel scale, repeatability, and validation of the findings are raised [58]. These concerns are often regarded as indicative of the limited potential of ADC to reliably capture microstructural features.

# Diffusion Tensor Imaging (DTI)

A method named Diffusion Tensor Imaging (DTI) extends the representation of DWI by replacing ADC with a tensor, providing a mathematically more versatile framework to describe diffusivity along the orientation of the used gradients. The signal then follows the equation:

$$S_{\text{DTI}} = S_o \exp(-b\boldsymbol{g}^T \, \boldsymbol{D}_{\boldsymbol{DTI}} \, \boldsymbol{g}) \quad (2)$$

where  $D_{DTI}$  is a rank-2 tensor that contains 6 independent parameters, and  $\boldsymbol{g} = (g_1, g_2, g_3)$  the gradient direction vector. Thus, for a DTI application, we estimate 7 parameters (including  $S_0$ ), at least 2 b-values, and 6 gradient directions. In practice, multiple measurements are acquired, especially since it has been shown that the angular precision of the signal is increased [59]. The choice of one b = 0 with over 15 measurements at  $b = 1000 s/mm^2$  is commonly used. Using singular value decomposition methods, diffusion signal is decomposed to three mutually orthogonal eigenvectors that describe the spatial orientation of the ADC. Based on their eigenvalues, the diffusion measures of Mean Diffusivity (MD) and Fractional Anisotropy (FA) are usually acquired. With MD, the average amount of diffusivity along all directions is calculated per voxel, while FA reflects the degree of diffusion anisotropy. Due to the directional versatility of the diffusion tensor introduced, MD and FA are considered more reliable diffusion metrics over the conventional ADC.

Leveraging the increased specificity of DTI for breast screening has been the goal of multiple studies. Based on the principal eigenvectors obtained by DTI, a study using tractography on breast tumors obtained comparable sensitivity, specificity, and accuracy to DCE MRI [60]. A characteristic lack of organization in the tractography paths of tumors was also identified in the same study. Further evidence showing the lesion characterization abilities of FA, led the authors to support the use of DTI-Tractography as a breast screening method. The presence of studies finding significantly lower FA and MD in breast tumors than in healthy FBGT [60], [61], combined with studies claiming the opposite [44], has led to the dispute of the added value of DTI to the existing screening protocols [19].

Investigating FA and MD for tumor classifications, it has been reported that MD can discriminate between in-situ and invasive ductal carcinomas, but also that MD and FA are unable to distinguish their molecular subtypes [61]. Features that indicate a poor prognosis, such as tumor size, histological grade, and lymph node metastasis, were also associated in the same study with lower MD values.

The microstructural interpretations of DTI metrics were not extensively reported. The lower FA in tumors found in [60] was attributed to the structural organization disruption of the normal breast tissue. Similarly, the associations of the lower diffusion magnitude, as expressed by MD, with tumor aggression that was reported in [61], were attributed to blockages of the ductallobular network.

#### Diffusion Kurtosis Imaging (DKI)

In the high b-value regimes of  $2000 - 3000 s/mm^2$ , the potential of DTI for higher specificity to sub-voxel microstructures is limited due to the imposed monoexponential signal behavior. To address this deviation from the Gaussian behavior, Diffusion Kurtosis Imaging (DKI) was introduced. DKI is an extension of DTI to higher cumulants of the Taylor expansion of the exponential signal equation, leading to a signal equation with the following form:

$$\left(-b\boldsymbol{g}^T\boldsymbol{D}\boldsymbol{g}+\frac{1}{6}b^2\left(\frac{1}{3}\sum^3 D_{ii}\right)^2\right)$$

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$$S_{DKI} = S_0 \exp \left\{ \begin{array}{c} -b \boldsymbol{g}^T \boldsymbol{D} \boldsymbol{g} + \frac{1}{6} b^2 \left( \frac{1}{3} \sum_{i=1}^{3} D_{ii} \right) \cdot \\ \sum_{i=1}^{3} \sum_{j=1}^{3} \sum_{k=1}^{3} \sum_{l=1}^{3} g_i g_j g_k g_l W_{ijkl} \right\}$$
(3)

The added terms to the DTI equation (2) capture the deviation of the DTI representation from the Gaussian diffusion. An additional rank-4 kurtosis tensor W is introduced, which adds another set of 15 independent parameters to the 6 pre-existing ones of DTI. This translates to a total of at least 21 diffusion encoding orientations to be acquired, with two different non-zero b-values. In practice, approximately 50 orientations are typical for a DKI analysis. A parametric form of equation (3) is often used, that uses the kurtosis coefficient K and the diffusivity,  $D_{DKI}$  as follows:

$$S_{DKI} = S_o \exp(-b D_{DKI} + b^2 D_{DKI}^2 K^2/6) \quad (4)$$

Through the DKI framework, a set of metrics is estimated in addition to the ones of DTI, with mean Kurtosis (MK) being the most notable among them. DKI is characterized as highly sensitive to microstructural properties, at the same time sensitive to noise and image artifacts, and the interpretation of its metrics is not intuitive [43].

The lesion malignancy detection was claimed to be possible based on MK and DKI-estimated MD, with superior diagnostic performance to ADC [62]. Agreeing results were reported in another study [63]. In particular, MK and MD have been



Figure 5. Acquired signal dependence to diffusion sensitivity as characterized by the b-value, and signal modeling. In (A), the decay of the diffusion signal is observed with increasing b-values. Approximations of this decay are the purpose of signal representations and models found in (B-D). DWI, as seen in (B) models the signal with a mono-exponential decay, which is acceptable only in the ranges of (0-1000 s/mm^2). In (C), DKI models the deviation from the Gaussian assumption made by DWI and DTI, allowing measurements at higher b-values. In the shorter b-value rangers, IVIM offers more accurate modeling that accounts for the contribution of the microperfusion to the signal. Adapted from (Nilsson et al. 2018, [84]).

consistently found significantly higher and lower in malignant lesions, respectively [63], [62], [64]. Two of the studies obtained an improved specificity when DCE-MRI is used in conjunction with DKI, however, only one of them [64] proceeded to suggest the use of DKI as a potential replacement for DCE-MRI, after the necessary validations. On the opposite side, a study [44] comparing conventional DWI, DTI, and DKI for lesion differentiation, found that ADC to had a superior discriminative ability to the ones added by DTI and DKI. The differentiation between IDC and DCIS based on the significantly higher value of MK in the first has been reported [62]. In addition, the ability to distinguish molecular subtypes. histological grade, and lymph node status based on MK and DKI-estimated MD has been reported by the same study. Similarly, histologic grade and the expression of the Ki-67 protein were found negatively correlated to  $D_{DKI}$  [62], [63].

Only general microstructural interpretations of the above results could be found, with concrete correspondence of microstructural features with specific DKI metrics to be absent. The deviation from the Gaussian assumption in breast tumors at high b-values has been interpreted in [62] and attributed to cellular and microenvironmental changes due to the malignant status. Generally, DKI is claimed to be sensitive to cellular membranes and intracellular organelles [57].

#### 4.2 Models

As discussed in chapter 2, the water content is physiologically categorized into compartments. The shapes and the sizes of them, which are on a micrometer scale, are influencing the dMRI signal from which the voxels are on a millimeter scale. Therefore, adapting the signal representations to models that a-priori include such compartmentalization, allows for the ability to resolve further sub-voxel heterogeneities. However, biophysical assumptions about the tissues are often made for the application of these models, which may introduce ambiguous and biased results.

# Intra-Voxel Incoherent Motion (IVIM)

Blood perfusion contributes to the diffusion signal, introducing further deviation from the Gaussian assumption. This contribution is significant in the small b-value regimes  $(0 - 200 \ s/mm^2)$ , at which the characteristic diffusion times acquired become comparable to slow perfusion. A popular biophysical tissue model that can discern the contribution of the microcapillary perfusion to the diffusion signal, without the use of contrast agents is Intra-Voxel Incoherent Motion (IVIM) [65]. IVIM extends the mono-exponential diffusion signal to the following form:

$$S_{IVIM} = S_o[f \exp(-bD^*) + (1-f)\exp(-bD)]$$
 (5)

where the pseudo-diffusion coefficient  $D^*$  reflects the contribution of slow perfusion effects to the acquired signal, weighted by the volume fraction of the vasculature in a voxel f. IVIM parameters provide an indirect measure of tissue microvasculature and perfusion effects, and they have also been associated with tumor angiogenesis [58].

Since sensitivity in perfusion effects is claimed by both IVIM and DCE-MRI, the first is being increasingly

investigated for malignancy detection in breast lesions [66] and often compared to DCE-MRI. A summary of 15 studies that include a total of 1089 women regarding lesion discrimination has been reported in [23]. Among the studies, 8 and 7 showed a significant decrease in D and an increase in f in malignant lesions, respectivelly. Comparing D and f against ADC in the same study, IVIM parameters were claimed to be diagnostically superior. Lastly, a study using IVIM along with DKI, observed a pattern of tumors being surrounded by a combination of increased f, K and D, with the opposite was observed within necrotic tissue and fibroadenoma [57]. Despite the development of IVIM dating for a few decades, the characteristic perfusion information provided by IVIM was claimed to be increasingly acknowledged recently [23].

Studies with findings based on IVIM-estimated parameters to microstructural features could be found, that could potentially be used in tumor classification, monitoring, and treatment prognosis. D and f were found in a study [30] to be significantly associated with the tumor-stroma ratio and capable to distinguish collagen-dominant tumors among fibroblast or lymphocyte ones. An additional comparison of D and f with DCE-MRI biomarkers showed a moderate correlation [67]. Using a combination of DKI and IVIM, the area ratios of interstitium and cancer cell nuclei were found in [68] significantly correlated with ADC and K. The prediction of tumor response to NACT was also claimed in [69]. The dependence of the overall quantification accuracy of IVIM on the chosen echo time, was showcased in a recent study [70]. A significant overestimation of pseudo-diffusion fraction using the conventional IVIM was reported, by comparing it with an echo-time-corrected variant of IVIM. However, the improvement in accuracy was believed not to justify the increased scan-time required.

# Multiple-Compartment Gaussian Models

Multiple alternative biophysical tissue models exist that attempt to unveil intra-voxel heterogeneities toward increasing the specificity of dMRI. In general, they share similar principles with IVIM, with compartmental assumptions and multi-exponential components.

A combination of bi-exponential and tri-exponential models in b-values up to 4000 s/mm2 was used in a study within the Restricted Spectrum Imaging (RSI) framework. Comparisons between the two showed an improvement in the fitting of breast dMRI signal using the tri- over the bi-exponential model. In the same study, it was concluded that the slower diffusion component of the models could be used for tumor diagnosis since they were found larger in malignant lesions. The discrimination of breast tumors from healthy FBGT using a triexponential model has been also investigated in [71]. After comparisons with conventional DWI and DKI, its superior screening accuracy led the authors to claim the method as a potential screening alternative to DCE-MRI.

A bi-exponential model of diffusion that regards the signal as originating from fast and slow water diffusion compartments has been used to investigate the influence of collagen content at high b-values [55]. Higher ADC values and associations with the fast signal fraction were reported to be indicative of the stromal collagen content in the tumor, which is of high clinical value. The response of breast tumors to NACT, as assessed by DWI, IVIM, and Stretched-Exponential models, was compared in a study [72]. The findings indicated that only a small number of the model-estimated parameters could predict any pathological response.

#### 5. Novel Diffusion Methods for Microstructure Imaging

# 5.1 Filter Exchange Imaging

Cell permeability can be characterized using Filter Exchange Imaging (FEXI) [73] that relies on DDE with a specific mixing time between the pair of diffusion blocks. Sensitizing the signal to the water exchange between intra- and extracellular compartments, and subsequently fitting it using a mono-exponential decay of diffusion, the Apparent Exchange Rate (AXR) is estimated, which is claimed to quantify the molecular water exchange across cell membranes [74].

Studying the use of FEXI for tumor classification on 4 patients with IDC, [27] was able to estimate AXR only on the breast tumor ROI, with AXR in normal tissue being outside the experimental range. In parallel, an in vitro study using breast tumor cell suspensions of various subtypes was conducted, in which a low AXR was associated with less invasive cancer subtypes. They concluded that since relatively low AXR values for tumor cells were reported in both in vivo and cell suspension experiments, AXR value can potentially distinguish different cancer subtypes. However, the low SNR of the in vivo protocol poses limitations on the resolution capabilities of the method.

# 5.2 Diffusion-Time Dependence

# **IMPULSED**

A microstructural imaging method that aims to estimate the mean cell size, which can be indicative of biological tissue alterations, is IMPULSED (Imaging Microstructural Parameters Using Limited Spectrally Edited Diffusion) [75]. IMPULSED combines the use of conventional SDEs with varying diffusion times  $\tau_d$  that allow measurements at long diffusion times, with ODEs of different frequencies that are capable to measure shorter diffusions times. Since each of their acquired signals, S(SDE) or S(ODE), naturally generates a unique set of diffusion estimates, their differences are claimed [26] to be experimentally associated with the length scales of major restrictions to diffusion through which inferences of the cell sizes can be made [41]. Based on this principle and on the compartmental modeling, in which the diffusion-encoded signal is modeled as the sum of signals originating from an intracellular and an extracellular compartment,  $S_{in}$  and  $S_{ex}$ , IMPULSED estimates their corresponding diffusion coefficients  $D_{in}$  and  $D_{ex}$  respectively. Additional parameters obtained by this modelling are the intracellular volume fraction  $f_{in}$  and the volume-weighted mean cell size d, which relies on the chosen model of cellular shape. The cell density is also estimated based on the  $f_{in}$  and d.



Figure 6. Dependence of the acquired signal to the b-value, to the diffusion time,  $\tau_d$ . In diffusion-time dependent microstructural methods, acquisitions with different diffusion times are combined to resolve microstructural features such as the mean cell size (A,B) or the permeability of the cellular membranes (C). Adapted from (Nilsson et al. 2018, [84]).

Only one study was found in the literature applying IMPULSED to breast imaging by [26], that claims the feasibility of mean cell size and density estimations. Using the diffusion data acquired and analyzed from 7 patients of various tumor types and grades, along with in vitro breast cancer cells and breast cancer xenografts in mice, the robustness of the method was showcased. Investigations suggested that the extracellular diffusion coefficient remains unaffected by diffusion times and incited the authors to model it by a constant  $D_{ex}$ . All validations suggested that IMPULSED is capable to provide accurate and reliable measurements of mean cell size, alterations of which are valuable for tumor progression during treatment.

Caution was advised in the interpretation of cell density acquired from IMPULSED, since  $f_{in}$  might be compromised by the increased cell-membrane permeability due to therapyinduced apoptosis. A bias in the estimation of the cell density was speculated to be introduced since water exchange between the compartments was ignored [26]. The article concludes that further investigation towards optimization of the experimental parameters should be performed with a stricter patient selection protocol. Additional concerns stated in IMPULSE-based studies are the increased scan time that limits its clinical use [76], and the need for stronger gradients and higher slew rates to achieve greater diffusion weighting [26]. Despite these, the ability of IMPULSED to estimate mean cell size variations could lead the way for accurate and reliable monitoring of the tumor response to therapies, once properly validated.

#### MRI-Cytometry

Addressing the inability of IMPULSE to characterize cell size heterogeneity, which may itself be a diagnostic biomarker, a follow-up study based on the data acquired [26] was conducted [77]. They introduced a framework termed MRI-Cytometry that is claimed to estimate cell size distributions, without assumptions based on parametric distributions. It is a

2-step fitting method that is based on the 2 compartment intraand extra-cellular models and assuming the free water is included in the extra-cellular one, from which their size distributions are being estimated  $P(D_{in})$ ,  $P(D_{ex})$ , respectively. In addition, the cell density distribution P(d) is estimated, the diffusion dispersion rate  $\beta_{ex}$  is introduced with its distribution  $P(\beta_{ex})$ .

Validation of the method was claimed after correlating cell size distributions obtained by computer simulations, in vitro cell cultures, and mice xenografts with histological data. An additional significant correlation between the mean of the MRI-Cytometry-estimated cell size distributions and the IMPULSED-estimated mean cell sizes were reported. It was concluded that the ability of the method to map and distinguish different cell populations, can potentially assist in the monitoring of anticancer treatments.

# 5.3 Conventional dMRI Methods with Diffusion-Time Dependence

As hinted in chapter 3.2, the microstructural imaging potential of conventional dMRI methods, when coupled with diffusion time  $\tau_d$  investigations have been showcased in several studies. In a nutshell, these used acquisition schemes with multiple diffusion times; i.e., mapping conventional measures such as ADC as a function of diffusion time rather than relying on an overarching time-dependent model. The rate of change in their values as a function of diffusion time is then estimated, with a subsequent search of correlations with histopathological features. With the majority of them being applied in the brain and the prostate, only a handful of applications on breast imaging could be found that were based on TDD-variants of DWI [78], [79], DKI [28], [79] and IVIM [79] which are being synopsized.

# Diffusion-Time Dependent DWI

A recent study that investigated the diffusion-time dependency of *ADC* as a potential biomarker for breast cancer screening, subtype classification, and prognostic value was found in the literature [78]. A combination of SDE and ODE was used for a single b-value at a 3T scanner. The diffusion-time dependency was investigated by using one short and one long diffusion time, based on which an  $ADC_{short}$  and an  $ADC_{long}$  were obtained respectively. Their difference,  $\Delta ADC$  that indicates the rate of change in ADC values due to diffusion time, was also estimated.

The characterization of breast lesions using TDD-DWI was performed on a cohort of 86 malignant and 46 benign tumors, and its performance was compared to both conventional DWI and DCE-MRI. The specificity of the TDD-DWI derived metrics of  $ADC_{long/short}$  and  $\Delta$ ADC was superior to those obtained by conventional methods, with 87.0%, 95.7%, and 73.9% respectively. However, a lower sensitivity was reported with 87.2%, 90,7%, and 100%, again, respectively. The potential of the TDD-DWI-derived metrics for distinguishing the molecular subtype of breast tumors was investigated by estimating their correlation with hormone receptor statuses present in the malignant tumors. The presence of the ER was correlated with lower  $ADC_{short}$  and ADC values. Similarly, the presence of the PR showed a lower  $ADC_{short}$ ,  $ADC_{long}$  and ADC values. Lastly, the prognostic value of the metrics was assessed by comparing them to the presence of the Ki-67 protein, which is related to the tumor proliferation status.  $\Delta ADC$  values were found higher in Ki-67-positive tumors, which was considered a notable finding towards improved breast cancer management.

# Diffusion-Time Dependent DKI

The monitoring potential in breast tumors based on estimations of the transcytolemmal water exchange time was investigated in a time-dependent DKI (TDD-DKI) [28]. Acquisitions from 2 biopsy-proven IDC patients and using a 3T scanner were used, whose scan time was approximately 5 mins.

The protocol in more detail included SDEs of only one orientation, five diffusion times in the ranges of 120-650 ms, and 3 different b-values (200, 1000, and 2000  $s/mm^2$ ). By fitting the diffusion-time-encoded data to the DKI representation and a two-compartment model, the dependence of diffusivity  $D(\tau_d)$  and kurtosis  $K(\tau_d)$  on diffusion time could be leveraged to estimate the cellular-interstitial water exchange time  $t_{ex}$ , with an average ROI-median value of 86 ms being reported. Additionally, a 30% decrease in K(t) was observed with increasing diffusion time while D(t) remained constant. This behavior was consistent with in vivo experiments on mice that were injected with brain and breast cancer cells that were conducted in parallel by the authors. Observations of the estimated exchange time being much longer than the characteristic times in intracellular diffusion of  $\tau_c = 10 ms$ , led the authors to the validation of the Kaerger model that considers cell membranes to be permeable to water molecules. Since  $\tau_{ex}$  is related to intracellular water lifetime that in turn is reportedly correlated to the metabolic activity of cells, the findings of the study suggest that TDD-DKI could potentially provide accurate prognostic biomarkers of tumor aggressiveness and response to therapies.

Despite the study eventually claiming that transcytolemmal water exchange can be estimated in both clinical and preclinical settings, a lack of validation of the  $\tau_{ex}$  measured by the TDD-DKI method should be noted. The assumption that the diffusion is isotropic in a tumor that led to the application of diffusion weighting gradient in only one direction can raise questions. An additional assumption was made regarding the differences in the  $T_1$  values between the two compartments and their resulting influences on the measurements concerned the authors.

#### Diffusion-Time Dependent DKI with IVIM

The associations of several conventional dMRI parameters acquired by TDD with histological biomarkers in breast xenograft mice models were investigated in a recent study [79]. For tumor differentiation, 7 xenograft mice with the estrogendependent tumor line MCF-7 and 15 with the aggressive TN breast tumor MDA-MB-231, were used with a 7T scanner.

Both SDEs and ODEs were used with 4 values of  $\tau_d$  in the range of 2.5 – 27.6 ms. From the data, a plethora of time-

dependent diffusion parameters ADC, K, f and  $D^*$ , from DWI, non-Gaussian DWI, and IVIM were estimated, respectively. Previous studies [80] showing that a "shifted ADC" (sADC) based on two key b-values showing increased sensitivity to diffusion, non-Gaussian diffusion and perfusion effects, led the authors to include it in their analyses. Differences between ADC and sADC of different diffusion times, and specifically the ones  $ADC_{2.5ms} - sADC_{27.6ms}$  and  $sADC_{9ms} - sADC_{27.6ms}$ in the b-value range of  $200 - 1500 \text{ s/mm}^2$ , showed statistically significant differentiation between the two xenograft groups. Similar differentiation abilities were found in  $K_{9ms}$ . It was also observed that sADC and ADC, in line with findings reporterd in the literature, decreased with diffusiontime in breast tumors. Notable is the authors' observation that despite the MCF-7 and the MDA-MB-231 cell lines having very distinct histopathological features, their distinction was not feasible by using quantitative parameters obtained by any diffusion measurement or model using a single diffusion time.

#### 5.4 Multidimensional Encoding Methods

Microstructural imaging approaches that rely on advanced gradient waveforms and b-tensor diffusion encoding schemes, such as MDE and free gradient waveforms, have only recently been feasible at clinical MRI scanners [48]. These methods combine diffusion information acquired by multiple b-tensors of different but complementary shapes, and in multiple directions. This increases the sensitivity of the diffusion signal to very specific microstructural properties and disentangles sub-voxel structures [1], [48]. The capabilities of b-tensor encodings to resolve complex breast tumor structures have only recently been investigated using Diffusion Tensor Distribution (DTD) [46] and Q-Space Trajectory Imaging (QTI) [45], [47], [81].

# Diffusion Tensor Distribution (DTD)

An interpretation of the MDE-acquired signals was performed in [1] by using a representation-model hybrid of DTD. This technique is claimed to estimate intra-voxel distributions of the diffusion tensors non-parametrically [1]. This is achieved by employing a quasi-genetic algorithm to computationally estimate the likelihood of measured signals being explained by a set of hundreds of different b-tensors. As a result, the means and the variances of shapes and sizes based on the tensor distributions are estimated using the Isotropic Diffusivity  $D_{iso}$ , and the Diffusion Anisotropy  $D_A^2$ , which are associated with cellular shapes and sizes respectively. Signal fractions based on  $D_{iso}$  and  $D_{\Delta}^2$  are used to created distinct sizeshape bins of diffusion distributions, each of which can correspond to specific microstructural properties. Distinguishing the signals coming from elongated cells, isotropic cells and free water is considered feasible using these bins. The microstructural fractional anisotropy  $\mu FA$  is introduced, which is claimed to be the FA variant that excludes the the effects of orientation dispersion [48]. Based on the  $FA/\mu$ FA ratio, the orientational order parameter (OP), is



Figure 7. The generated signal from tissue model simulations of voxels containing variable levels of diffusion anisotropy and isotropic heterogeneity can be resolved using Multidimensional Diffusion Encodings. Acquisitions using multiple different b-tensors, such as LTEs and STEs, can obtain diffusion metrics with higher specificity, such as  $MK_I$  and  $MK_A$ . Adapted from (Szczepankiewicz et al. 2016, [81]).

is defined as the ratio  $(FA/\mu FA)$  that is believed to reflect the degree of alignment of elongated cells within a voxel.

In the breast study published by [1], multiple uses of the above method were investigated. To distinguish between healthy FGT and tumor tissue, as well as characterization of a tumor as invasive or as in-situ, a group of 16 patients with these types of breast carcinomas was imaged using a 3T scanner. The MDE data were acquired using free gradient waveforms of 37 isotopically-distributed LTEs and 73 STEs were used for a set of five b-values in the 0-2000s/mm2 range. The bin-based analysis of DTD was showcased to be able to distinguish between malignant tumors and healthy FBGT, as well as significantly higher values of FA and mean  $D_{\Delta}^2$  in the former. The mean  $D_{iso}$ , which corresponds to the conventional *ADC*, and a number of binned-signal fractions were found as capable to distinguish invasive carcinomas with in-situ ones. In conclusion, the MDE-based DTD method was

Author - Date	Methods	Encoding	Estimated Parameters	Related Microstructural Features	Study Group	Potential in
Lasič et al. 2016 [27]	FEXI	DDE	AXR	Cellular Membrane Permeability	Patients: 8 IDC	Classification (Cell Types)
Xu et al. 2020 [26]	IMPULSED	TDD (SDE, ODE)	$d \ v_{in} \ D_{ex}$	Mean Cell Size	Patients: 7 Tumors>1cm	Monitoring
Xu et al. 2021 [77]	MRI- Cytometry	TDD (SDE, ODE)	P(d) $P(v_{in})$ $P(D_{ex})$ $\beta_{ex}$	Cell (and Compartment) Size Distributions	Patients: 7 Tumors>1cm	Monitoring
Iima et al. 2021 [78]	TDD(DWI)	TDD (SDE)	ADC <sub>short</sub> ADC <sub>long</sub> ΔADC	-	Patients: 132 (86 mal., 46 ben.) IDC	Screening Classification (molecular subtype) Prognosis (Ki-67)
Zhang et al. 2021 [28]	TDD(DKI)	TDD (SDE)	$D(t) \ K(t) \  au_{ex}$	Transcytolemmal Water Exchange Cellular Membrane Permeability	Patients: 2 IDC	Monitoring Prognosis
Naranjo et al. 2021 [1]	DTD	Free Gradient Waveforms (LTE,STE)	$Mean[D_{iso}] \ Var[D_{iso}] \ Mean[D_{\Delta}^{2}] \ FA, OP \ f_{bin1}$ , $f_{bin2}$ , $f_{bin3}$	Cell Shape (Elongation/Eccentricity) Cell Orientational Order	Patients: 16, various subtypes	Screening Classification (Invasive - In-Situ)
Cho et al. 2022 [48]	QTI	SDE + Free Gradient Waveforms (LTE,STE)	MD,FA MK <sub>T</sub> MK <sub>A</sub> MK <sub>I</sub> μFA	Cellularity Tumor Growth Patterns	Patients: 29, IDC, various mol. subtypes	Screening Monitoring

Screening: Characterization of a breast lesion as benign (e.g. FBGT) or malignant (tumor)

Classification: Categorization of a malignant tumor into the multiple tumor histological types, subtypes, molecular classes, and stages

Monitoring: Tumor response to Neoadjuvant (NACT) or Adjuvant (ACT) Chemotherapies. Prognosis: Provide prognostic biomarkers of tumor aggressiveness (e.g. stage, Ki-67).

Table 3. Microstructural Methods in In-Vivo Breast Imaging Studies

able to provide qualitative and quantitative maps of the composition and the orientational order of the breast tissues with high clinical value. The authors conclude that *FA* and mean  $D_{\Delta}^2$  could be appropriate for breast cancer diagnosis. However, low in-plane resolutions and a few methodological limitations were reported that need to be addressed in a validation study of the method.

# Q-Space Trajectory Imaging (QTI)

A very extensive breast tumor study using b-tensor encoding was published recently by [48] by applying a Q-Space-based acquisition strategy with efficient free gradient waveform MDEs. In QTI, the total diffusional variance described by the mean kurtosis  $MK_T$  is decomposed into isotropic,  $MK_I$ , and anisotropic,  $MK_A$ . The first one has been linked to the heterogeneous isotropic diffusivity present in voxels containing multiple cell densities and tissue mixtures [48]. The second one is claimed to be correlated to diffusion anisotropy on the microscopic level such as in eccentric cells and tissue structures [48]. In addition to MD and FA, microscopic fractional anisotropy  $\mu FA$  is also estimated.

These parameters were estimated in a cohort of 29 IDC cases of various molecular subtypes. Analyses including parameter comparisons between IDC and healthy FGBT, and their association with histopathologic features were performed. For the diffusion acquisition, a combination of SDE data of 2 bvalues and 29 directions, with LTE and STE gradient waveforms of 3 b-values and 26 directions was used. The comparisons showed a significant increase of  $MK_T$ ,  $MK_A$ , FA and  $\mu FA$  in IDC when compared to FGBT, while MD showed a significant decrease in the same comparisons. For the cases of FA and  $\mu$ FA, the results are interpreted to be caused by the disorganized growth and cell density of IDC. It was noted that  $\mu FA$  was the dominant cause of restriction in IDC that captures the high cellularity and prominent nucleoli present in them. Only  $MK_I$  was found to be positively correlated to the size of the tumor, without any conclusions being drawn about its interpretation. In addition,  $MK_T$ ,  $MK_A$  and  $\mu FA$  were significantly higher in a group with lymph node metastasis. The findings of the study show the potential of QTI for breast tumor screening, as well as monitoring based on  $\mu FA$  could be considered an imaging biomarker for evaluating the status of breast tumors before surgery or chemotherapy. It is also noted that the lack of postprocessing corrections applied to the data might have undermined the accuracy of the method. However, a significant increase in scan time to counter the low SNR was also necessary.

# 6. Discussion

A synopsis of diffusion MRI methods found in the literature that yield microstructural information about breast tumors was compiled. Due to the large versatility of dMRI with numerous techniques and with different nomenclature used, it was proven challenging to construct a concise narrative and to highlight the modularity of the methods and their interdependencies. To address this and for completeness, an introductory tone was adopted that covers dMRI prerequisites such as diffusion encodings and conventional dMRI representations and models. The current review was focused on in-vivo breast microstructural imaging, excluding articles and findings that purely rely on in-vitro cell suspensions, xenograft mice, and computer simulations. Despite this choice potentially limiting the range of novel dMRI methods included, it was deemed important to cover applications that studied tumors within their native environment, whose importance is highlighted in [82]. In addition, novel dMRI methods are often developed in NMR in-vitro samples, whose transfer to clinical applications can be hindered by hardware limitations.

# Microstructural Methods

The potential of novel microstructural methods in breast tumors could be explored only in a very small subset of publications. With the majority of development and validation of these methods being performed in the brain, the estimation of distinct microstructural features in breast tumors is increasingly proven possible. However, further validation of the methods and evidence of the added clinical value is required. The potential ability of these methods to resolve the lower specificity shortcomings that conventional dMRI is accused of is evident. Microstructural imaging could assist dMRI to assume a leading role in breast cancer imaging.

In microstructural imaging studies investigating the diffusion time dependence of conventional methods, such as [79], the degree of parameter correlation to microstructural features depended on the chosen diffusion time. The significance of this finding should be highlighted and further investigated. In the majority of older studies using conventional dMRI methods, the values of diffusion times applied were not

reported. Since different diffusion times yield distinct contrasts, contradictory findings found in the literature could be attributed to different unreported diffusion times used. To address this, [78] and [79] emphasize the importance of reporting diffusion time in future studies. An additional recommendation would be to retrospectively analyze previous studies and attempt to associate the diffusion times used with their findings.

Microstructural techniques rely on complex diffusion encoding schemes and frameworks to interpret the acquired signal. The diffusion encodings require medium to high-end MRI systems of usually 3T, at least. With the faster and stronger gradients that such scanners are capable of, higher bvalues, wider ranges of diffusion times, and more complex diffusion encoding waveforms can be obtained. However, these requirements currently confine these methods within the domain of research, and could potentially hinder the possibility of their clinical adoption.

# Breast Tumor Screening

The potential of dMRI methods to substitute DCE-MRI and histological replacement in breast screening protocols by providing sufficient microstructural information could be of interest. Especially in light of increasing evidence of the harmful effects of contrast agents and the invasiveness of biopsies, an agent-free non-invasive dMRI-based approach could be a potential alternative approach.

A plethora of studies has reported on the diagnostic performance of DWI, which are synopsized in [52]. Despite studies that reach up to 89% of sensitivity and excellent repeatability [57], DWI has been suggested [52], [57] to be



Figure 8. A set of parameter maps acquired with different conventional (B-E, I) and microstructural (F-H, J) dMRI techniques in an example case of a stage I IDC. The corresponding DCE-MRI (A) and histological assessment (K,L) images are also included. Adapted from (Cho et al. 2022, [48])

used only as a supplement to the current DCE-based protocols, maintaining the status quo. To date, any valuable contribution to the sensitivity and specificity of DWI using DTI has not been found. An exception could be the improvement found using fiber tractography in a study [60], however, DTI has been claimed to have reproducibility issues [57]. DKI-estimated ADC has been reported to have a comparable diagnostic performance to DCE-MRI [62], but it was only suggested as a complementary approach to it. A similar conclusion had another DKI-based study [64]. Parameters based on IVIM were found to be superior to ADC, especially when coupled with DKI as in [23]. Tumors could be distinguished from normal breast tissue based on the parameters of DKI and IVIM. Unfortunately, a large parameter overlap between malignant and benign lesions was also found, raising skepticism about the method's reliability [23]. On the opposite side, the screening advantages of tri-exponential models were claimed in two studies [71], [83], following comparisons to DWI, DKI, and a bi-exponential model.

An absence of studies directly comparing DCE and diffusion MRI diagnostic performances was surprisingly observed, especially using more advanced microstructural dMRI techniques. The publications found are either focused on conventional DWI and DTI methods for diagnostic purposes or only on microstructural features obtained by the advanced methods without detailed suggestions on their clinical relevance in breast tumor screening. Two articles using microstructural techniques were found that mentioned their potential use for breast malignancy detection. The first one [1] using DTD features such as the transcytolemmal water exchange and cellular membrane permeability are claimed to be quantified. In the latter [48], measurements of cellularity and the directionality and eccentricity of tumor growth are claimed. With the degree to which these features could be clinically valuable still being hypothesized, the translation of these microstructural techniques to clinical applications is currently unattainable. Obstacles such as the low acquired SNR, longer scan times, and the need for stronger gradients for higher bvalues [57] are adding to the validation challenges, which eventually could render these methods too complex to be clinically adopted.

# General Concerns

Several practical considerations should be taken into consideration for any microstructural information obtained by the methods covered. Most of these are inherited from shortcomings present in most dMRI methods, such as the low SNR, the dependency on b-value selection, post-processing and corrections applied [58]. The determination of threshold values of diffusion-based parameters is considered important for multiple tasks such as lesion screening and tumor classification. A method can be claimed as repeatable and reliable after sufficient cross-study agreement in threshold values. The repeatability and reliability of the novel breast tumor microstructural methods are unvalidated, due to the insufficient number of studies reporting on threshold values. The methodological dependency of the results on ROI selection has been also mentioned in multiple studies and highlighted for its ability to undermine the quantitative accuracy and repeatability of a method. In [58], the whole tumor volume method of slice selection for ROI analysis is recommended for optimal interobserver consistency and repeatability. An exhaustive and highly informative review was found [50] that covers multiple topics regarding dMRI modeling for microstructure imaging. The dMRI validation concerns are addressed, leading to suggestions for methodological approaches for future studies. Additional suggestions are presented in [49] and [11], however, they focus on brain imaging.

The methodological approaches found in the existing literature could be classified into two groups, the clinical and the technical. In the clinical approaches, dMRI-based parameters were associated with clinical statuses and outcomes, and rarely included any interpretations about the underlying microstructure. In the technical ones, the validation of the dMRI techniques was sought by using simulations, invitro cell cultures, xenograft mice, in-vivo and ex-vivo tumors, for specific microstructural features. The link between the breast microstructural features acquired by dMRI and their clinical relevance involves medical expertise. Despite that link being beyond the scope of this present review, a sparsity of such information was observed. As a result, it is suspected that a reader with an imaging background struggles to assess the status of breast microstructural imaging and the clinical relevance of its findings. To address this and to assist the transfer of novel dMRI techniques into clinical tasks, further systematic and targeted research that involves both medical and diffusion experts is needed. Highly recommended is the use of more standardized nomenclature, such as the one introduced by [35], detailed acquisition characteristics, and findings reporting. By offering more clarity to the complex and interdependent methods used in dMRI, gaps in the current literature could be more easily identified, guiding future dMRI research.

# 7. Conclusions

Through the broad and non-technical overview of the existing literature, microstructural features of breast tumors were found obtainable using both conventional and novel diffusion MRI techniques. Although conventional techniques could provide only a limited number of such features, mostly due to their lack of specificity, they remain promising alternative approaches for multiple clinical tasks. Microstructural imaging techniques were found capable to obtain previously unattainable microstructural information. Due to their novelty, the reliability of the techniques and the clinical value of the obtained microstructural information is yet to be proven. Further evidence of the associations of the diffusion-estimated parameters to histopathological features is needed.

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