# Non-Gaussian diffusion in complex systems: results in bone marrow samples

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**Purpose.** Bone marrow is an heterogeneous complex system that fills pores of the cancellous bone (trabecular bone marrow, TBM), or it is free i.e. is not forced in pores (free bone marrow, FBM) in the central cavities of long bones. Both TBM and FBM are mainly characterized by different relative percentage of water and fat and comprised of several particles of size ranging from 6 μm to approximately 100 μm[1]. Because of the susceptibility mismatch between solid bone and bone marrow, the water component in TBM that diffuses in the interstitial space between solid bone and fat[2,3], is affected by strong internal magnetic field gradients[2,4], *Gi*. Moreover, *Gi* due to the magnetic susceptibility difference between fat and water is also present in both TBM and FBM and apparent diffusion coefficient (*ADC*) of fat component is approximately two orders of magnitude lower than that of water component[2]. Our aim was to investigate the potential of Gaussian and non-Gaussian diffusion methods to obtain microstructural information and water compartmentalization in TBM and FBM by investigating water and fat *ADC,* and γ stretched parameter of the Anomalous diffusion (AD) model, as a function of diffusion time Δ.

**Methods.** *Theory.* In pulse-field-gradient (PFG) diffusion experiments, the signal is recorded as a function of chosen b-values, where b includes both Δ, diffusion gradients strength *g* and diffusion gradient duration δ: $b=(\gamma G\delta)^2\Delta$, with γ the $^1$H gyromagnetic ratio. *ADC* can be extracted using Stejskal-Tanner equation which describes the signal *vs b*, as an exponential decay. However, the simple exponential decay of water signal is clearly an oversimplification when heterogeneous tissues are investigated[5]. Recently, AD stretched exponential model, in which γ arises from fitting the stretched function $S(b)=S(0)\exp(-(bADC)^{\gamma})$ to PFG data, has been proposed to investigate complex systems[5]. In particular it has been shown that γ quantifies both *Gi* and water compartimentalization[5,6]. In bone marrow we expected to find two distinct pools of diffusing water, fast and slow, plus the fat diffusion pool. As a consequence, the PFG signal attenuation follows a multiple exponential decay: $S(b)=f_{fast}\exp(-ADC_{fast}b)+f_{slow}\exp(-ADC_{slow}b)+f_{fat}\exp(-ADC_{fat}b)$ [1], where $ADC_{fat}$, $ADC_{fast}$ and $ADC_{slow}$ are the *ADC* of the fat, the fast and the slow diffusing water, respectively, and $f_{fat}$, $f_{fast}$ and $f_{slow}$ are the fraction of fat molecules, fast and slow water molecules, respectively. However, because of the presence of *Gi*, we hypothesized that the PFG signal attenuation due to the fast diffusing water may be better characterized by a stretched exponential decay and then we also tested $S(b)=f_{fast}\exp(-(ADC_{fast}b)^{\gamma})+f_{slow}\exp(-ADC_{slow}b)+f_{fat}\exp(-ADC_{fat}b)$ [2] relation. Moreover, diffusion motion propagators (MP) were obtained from q-space analysis[7]. *Experiments.* Four TBM and seven FBM samples extracted from calves, were investigated using a Bruker Avance-400 operating at 9.4T. A variable *g* Pulse Gradient STimulated Echo (PGSTE) sequence, with: *TE/TR* = 5/5000 ms, Δ = 64,128,256, 400,512,1024 and 2048ms, δ =4.4ms and 64 g steps from 1.2 to 120Gauss/cm (the corresponding *b* values run from 12 to $3.51\cdot10^6$ s/mm$^2$) was used to extract $ADC_{fat}$, $ADC_{fast}$ and $ADC_{slow}$ values and $f_{fat}$, $f_{fast}$ and $f_{slow}$ for all samples using relation [1]. Moreover, relation [2] was also used to extract γ values for all the samples.

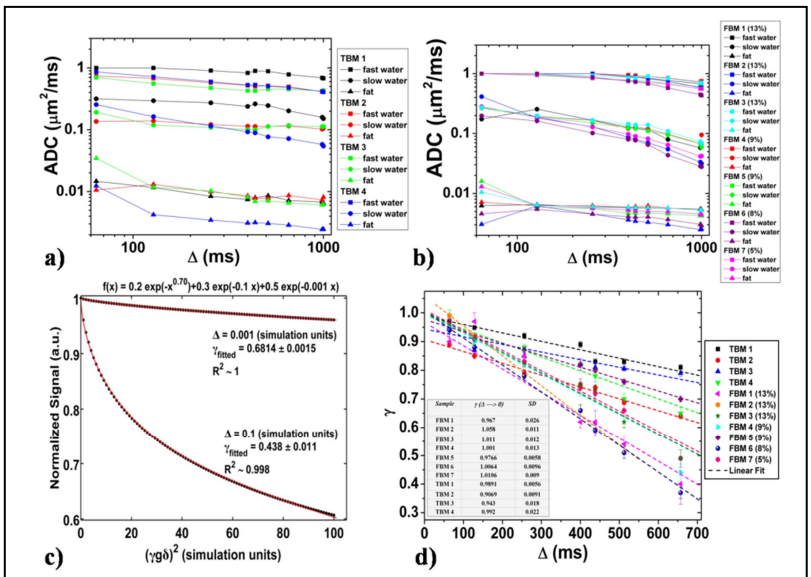


**Fig.1** : $ADC_{fast,\ slow,\ fat}$, *vs* Δ for TBM (a) and FBM (b) samples. c) Normalized signal decay *vs* $q^2=(\gamma g\delta)^2$ (in simulation units) simulated at different Δ, according to the function f(x) displayed on the top. Red lines represent the relation [2] fitted to the simulated data, with the corresponding evaluated γ. Fit goodness $R^2$ is also displayed. d) γ values as a function of Δ, for all samples. The table contains the γ values extrapolated at Δ→0 from the linear fit represented by straight lines.

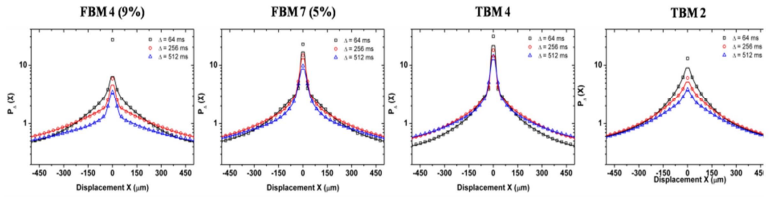


**Fig.2:** Diffusion motion propagators, obtained from q-space analysis, for four representative investigated samples at three different diffusion times, Δ= 64, 256 and 512 ms. Straight lines represent the corresponding expected pdf fitted to the data.

**Results and discussion.** $ADC_{fat}$, $ADC_{fast}$ and $ADC_{slow}$ values of TBM samples as a function of Δ are shown in **Fig.1 a**, while those of FBM samples are shown in **Fig.1b**. Specifically, the time behavior of the three ADCs associated to TBM1 and TBM4 are similar to that of FBM ones, while the time behavior of the three ADCs associated to TBM2 and TBM3 differs from that of all the others. These results, suggest that TBM1 and TBM4 samples show water and fat diffusion processes similar to those occurring in FBM. Indeed TBM1 and TBM4 are characterized by very large and interconnected pores in which water diffusion is poorly restricted. Conversely, results from TBM2 and TBM3 strongly suggest that water diffusion is altered and affected by the bone porous matrix. In order to give more insights into the differences between these samples, MPs were obtained (**Fig. 2**). The MP represents the molecular displacement probability density function (pdf) within the sample. To describe diffusion in FBM samples it is reasonable to use a sum of two Gaussian pdfs, associated to the fast water and the fat diffusion processes, and a decaying exponential function, associated to the slow diffusing water compartment[7]. Conversely, because of the presence of strong $G_i$ in TBM, the fast diffusing water term is no more well represented by a simple Gaussian, but it can be approximated by a stretched Gaussian function. As shown in **Fig.2**, molecular displacement pdf of TBM4 is different from that of TBM2. It is well fitted to a weighted sum of two Gaussians and a decaying exponential function like for the two FBM pdfs (FBM4 and FBM7). Conversely, diffusion propagators of the other TBM sample (TBM2) have a strong stretched Gaussian component, as suggested by the cusp behavior around 0 displacement in **Fig.2**. In order to quantify the non-Gaussianity of diffusion process in both FBM and TBM samples, the γ values associated to the water component as a function of Δ, are displayed in **Fig.1 d**. It is important to stress that in both FBM and TBM there are three diffusion regimes, depending on the Δ value used to investigate the dynamic process. At low Δ, i.e. $\Delta<(2R_p^2)/(3D_0)$, where $D_0$ is the bulk water diffusivity and $2R_p$ is the width of the wetting shell, the stretched exponential term in relation [2] dominates; conversely, at long Δ, i.e. $\Delta>>(2R_p^2)/(3D_0)$, the relation [2] is fully met. In the intermediate regime, the signal decay is not well defined. Therefore, the effective γ values should be evaluated for large Δ values (i.e. Δ>1000ms). Regrettably, due to $T_1$ relaxation effects, this limiting analysis cannot be done. An alternative solution is that to consider the limiting effective value of γ at Δ→0, as corroborated by simulation in **Fig.1c**. From this analysis we found that TBM3 and TBM2 have the lowest γ values (see table in **Fig. 1d**), in agreement with previous results[5,6] and structural considerations. Moreover, only the non-Gaussian diffusion analysis allows to discriminate between TBM2 and TBM3 (see table in Fig. 1d), because γ parameter is more sensitive to morphological and structural differences than conventional Gaussian diffusion parameters, due to its dependence on $G_i$.

**Conclusions.** In this work we highlighted the potential ability of Gaussian and non-Gaussian water diffusion to characterize and discriminate different bone marrow samples. Experimental results shown that γ is highly correlated to intrinsic local microstructural features at the interface between water and bone.

**References.** [1]Travlos GS. *Tox Pathol* 2006;**34**:548. [2]De Santis S, et al., *Phys Med Biol* 2010;**55(19)**:5767. [3]Capuani S, *Micropor Mesopor Mater* 2013;**178**:34. [4]Rebuzzi M et al. *BONE* 2013;**57**:155. [5]Palombo M, et al., *J Chem Phys* 2011; **135(3)**:034504. [6]Palombo M et al., *J Magn Reson* 2012;**216**:28.[7]Ong HH,Wehrli FW, *Neuroimage* 2010;**51**:1360.