# Microstructural Information from Single-Pulsed-Field-Gradient and Angular Double-Pulsed-Field-Gradient NMR: From Model Systems to Nerves

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**Introduction.** Water diffusion in neuronal tissues, at sufficient long diffusion times and high diffusion weighting, becomes non-Gaussian. Under such conditions the well-known Stejskal-Tanner equation used to analyze conventional DWI and DTI data does not accurately describe water diffusion in the CNS. However when complex biological specimens are investigated it is of value to test the new methodology on real, complex samples where the ground truth is known a priori. In the present work we acquired and modeled the signal from single (s-) and angular double-pulsed-field-gradient (d-PFG) diffusion NMR experiments performed on a series of well-defined phantoms of increasing complexity and on isolated pig optic nerves [1,2].

**Objectives.** To challenge the accuracy of the microstructural information that can be obtained by modeling the signal in s-PFG and angular d-PFG NMR experiments by using phantoms of increasing complexity, where the ground trough is known a priori, and subsequently to apply these methodologies to characterize both the averaged axon diameter (AAD) and the relative population of the different diffusing components in pig optic nerves.

**Methods.** Diffusion NMR experiments were performed on a Bruker 8.4 T spectrometer having gradient system able to produce pulsed gradients of up to 190 G/cm in each direction. Microcapillaries with known inner diameters (ID) of 5 ± 1, 9 ± 1, 15 ± 1 and 23 ± 1 and mixtures thereof were used both in the absence or presence of different amount of free diffusing $H_2O$. For a mixture of 23±1 and 15±1 μm (~1:1 volumetric ratio) microcapillaries the PGSTE experiments were performed with Δ/δ of 150/2 ms and $G_{max}$ of 160 G/cm, resulting in a maximal q-value of 1362 $cm^{-1}$. TE was set to14 ms. For the mixture of 9±1 and 5±1 μm (~1:1 volumetric ratio) microcapillaries the PGSTE experiments were collected with Δ/δ of 50/4 and $G_{max}$ of 160 G/cm, yielding a maximal q-value of 2724 $cm^{-1}$, respectively and the TE was set to 32 ms. The fixed pig optic nerves were studied when Δ was set to 30 or 90 ms, 40 q-values were collected with $G_{max}$ = 160 G/cm. The pulse gradient duration was set to 4 ms, resulting in maximal q-values of 2724 $cm^{-1}$. The angular double pulsed gradient spin echo (d-PGSE) and the bipolar (bp)-d-PGSE NMR experiments were performed when $G_1$ was fixed along the x-axis and the orientation of $G_2$ was varied in the x-y plane. The measurements were conducted for 25 different values of φ between 0° and 360°. For a mixtures of 23±1 and 15±1 μm microcapillaries the angular d-PGSE experiments were performed with $G_{max}$ of 80 G/cm and $\delta_1 = \delta_2 = \delta_3 = 2$ ms, resulting in a maximal q value of 681 $cm^{-1}$. $\Delta_1$ and $\Delta_2$ were set to 150 ms. For the mixture of 9±1 and 5±1 μm microcapillaries the angular bp-d-PGSE experiments were collected with $\Delta_1 = \Delta_2$ =50 ms and $\delta_1 = \delta_2 = \delta_3 = 4$ ms, $G_{max}$ of 80 G/cm, yielding a maximal q-value of 1362 $cm^{-1}$. In addition, pig optic nerves were studied with diffusion times of 30 ms, the pulse gradient durations were set to 4 ms, yielding a maximal q-value of 1362 $cm^{-1}$. Our model is trying to fit the signal decay by a superposition of free Gaussian diffusion and a series of restricted diffusion in cylindrical geometries [1,2]. No assumption is made on the number of compartments to be found and only $D_0$ and a size range has to be inserted a priori.

**Results and Discussion.** To challenge the modeling procedure of the s-PFG and angular d-PFG NMR experiments we first used these methods to study diffusion in model systems of increasing complexity, where the ground truth is known a priori. We started with single sized microcapillaries as a restricting compartment to which an increasing amount of free diffusing $H_2O$ was added. Then we studied mixtures of microcapillaries (23±1 and 15±1 μm and 9±1 and 5±1 μm) to which free $H_2O$ was added. Table 1 summarizes the results that were obtained by fitting the experimental data collected from s-PFG and angular d-PFG experiments performed on a mixtures of 23±1 and 15±1 μm and 5±1 and 9±1 μm microcapillaries, when the range of diameters that was explored was set to be between 10 to 40 μm or 2 to 12 μm, respectively. No assumption regarding the number of compartments that need to be identified was made. The fittings of the data were able to detect both the sizes and fractions of restricted compartments and to detect the increase in the fraction of free diffusing $H_2O$ in the sample. As expected, increasing the added volume of the free diffusing $H_2O$ results in an increase in the relative volume fraction of $H_2O$ exhibiting free diffusion with no effect on the sizes and relative fractions of the restricted components. In all phantoms the extracted sizes are in very good agreement with the nominal sizes. Figure 1A-B shows the results obtained from s-PFG and angular d-PFG NMR experiments performed on fixed pig optic nerves. Figure 1A shows the water signal decay for two optic nerves in PGSTE experiments as a function of q-value under different experimental conditions and the fittings of the experimental data. It can be seen that the signal decay is not monoexponential. Here the fitting was performed with 40 q-values assuming the diameters to be found are in the range 0.6–12 μm. Figure 1B shows $E(\varphi)$ profile of pig optic nerve in angular bp-d-PGSE experiments. It can be seen that $E(\varphi)$ is showing the expected bell-shaped dependency governed by microscopic anisotropy (μA) that arises from the boundaries of the axons. Here the fitting was performed with 6 q-values assuming diameters in the range 0.6–20 μm. Figure 1B shows that our model can fit the experimental data very well and the extracted AADs are summarized in Table 2. We found the AADs to be 2.3±0.2 μm very similar to the value obtained from histology. Tables 1 and 2 show the agreement between the single and angular double PFG MR results.

**Conclusions.** The modeling of both s- and angular d-PFG MR data enabled us to extract the microstructural features of the complex phantoms where the ground truth is known. In addition we were able to obtain reasonable estimation of the AADs of optic nerves. Importantly there was a good agreement between the results obtained from the s- and angular d-PFGMR experiments for both phantoms and nerves [3].

**Table 1.** Compartment sizes and volume fractions of free and restricted components of the phantoms comprising of a mixtures of 23 and 15 μm or 5 and 9 μm microcapillaries as a function of the addition of free diffusing water as obtained from the s-PFG and angular d-PFG.

| angular d-PFG | | Amount of free diffusing water | ID [μm] | restricted diffusion fraction | free diffusion fraction | SD of fit | s-PFG | Amount of free diffusing water | ID [μm] | restricted diffusion fraction | free diffusion fraction | SD of fit |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15:23 μm | 12 q-values | 1ml $D_2O$ | 14.9<br>22.7 | 0.47<br>0.47 | 0.06 | $3.06\cdot10^{-2}$ | 40 q-values | 1ml $D_2O$ | 15.2<br>23.6 | 0.45<br>0.51 | 0.04 | $1.04\cdot10^{-2}$ |
| | | 1 ml $D_2O$ +<br>1.5 μl $H_2O$ | 15.0<br>22.7 | 0.15<br>0.14 | 0.71 | $3.26\cdot10^{-2}$ | | 1 ml $D_2O$ +<br>1.5 μl $H_2O$ | 15.2<br>23.6 | 0.19<br>0.19 | 0.62 | $1.01\cdot10^{-2}$ |
| | | 1ml $D_2O$ +<br>3 μl $H_2O$ | 15.1<br>23.1 | 0.05<br>0.04 | 0.91 | $3.57\cdot10^{-2}$ | | 1ml $D_2O$ +<br>3 μl $H_2O$ | 15.2<br>23.1 | 0.06<br>0.05 | 0.89 | $5.56\cdot10^{-3}$ |
| 5:9 μm | 16 q-values | 1ml $D_2O$ | 4.8<br>8.3 | 0.35<br>0.45 | 0.20 | $3.42\cdot10^{-2}$ | 40 q-values | 1ml $D_2O$ | 5.2<br>9.0 | 0.43<br>0.41 | 0.16 | $5.34\cdot10^{-3}$ |
| | | 1 ml $D_2O$ +<br>0.5 μl $H_2O$ | 4.7<br>8.0 | 0.12<br>0.18 | 0.71 | $6.42\cdot10^{-2}$ | | 1 ml $D_2O$ +<br>0.5 μl $H_2O$ | 5.2<br>8.9 | 0.22<br>0.17 | 0.59 | $7.41\cdot10^{-3}$ |

**Table 2.** Compartment sizes and volume fractions of free and restricted compartments in optic nerves extracted from the s-PFG and angular bp-d-PFG NMR experiments

| q-value range | AAD [μm] | restricted diffusion fraction | free diffusion fraction | SD of fit |
|---|---|---|---|---|
| **angular d-PFG** | | | | |
| 851.6-1021.9 $cm^{-1}$ | 2.2 | 0.23 | 0.77 | $6.77\cdot10^{-2}$ |
| 1021.9-1192.2 $cm^{-1}$ | 2.3 | 0.21 | 0.79 | $5.31\cdot10^{-2}$ |
| 1192.2-1362.6 $cm^{-1}$ | 2.3 | 0.20 | 0.80 | $4.39\cdot10^{-2}$ |
| **s-PFG** | | | | |
| 40 q-values | 2.3 | 0.18 | 0.80 | $1.50\cdot10^{-2}$ |

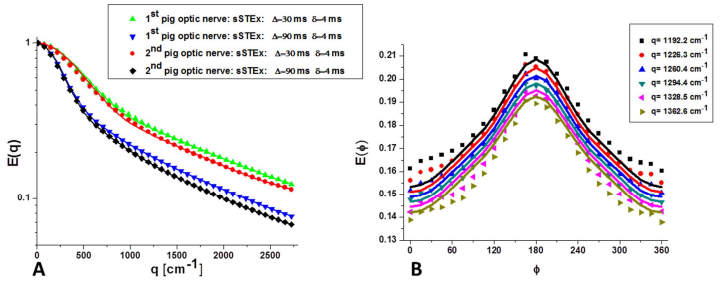


**Figure 1.** (A) Signal decay in s-PGSTE experiments in optic nerves. (B) $E(\varphi)$ profiles obtained from the angular bp-d-PGSE experiments performed on pig optic nerves. Symbols represent the experimental data while lines represent the fitting curves.

**References.** [1] D. Morozov el al., Magn. Reson. Med., DOI 10.1002/mrm25371. [2] D. Morozov el al., NMR Biomed. 2013; 26: 1787–1795. [3] S.N. Jespersen, NMR Biomed. 25 (2012) 813-818.