

1. Introduction : calculation of Stokes-Einstein law for free and cell water :

First, we verify that Stokes-Einstein law $D_{H_2O} = \frac{k_B T}{6\pi R_s \eta_{H_2O}}$ could be valid for free water itself (with H-O radius $R_s = 0.990 \cdot 10^{-10}$ m and the experimental free water dynamic viscosity η (Pa.s) : $\eta_{H_2O}^{37^\circ C} = 0.7606 \cdot 10^{-3} \text{ kg} \cdot \text{s}^{-1} \cdot \text{m}^{-1}$ [10]), by calculating the theoretical diffusion coefficient (SI units) : $D_{theo} = 3.016 \cdot 10^{-9} \text{ m}^2/\text{s} = 3.016 \text{ } \mu\text{m}^2/\text{ms}$ that we can compare to the experimental measure : $D_{exp} = 3.05 \text{ } \mu\text{m}^2/\text{ms}$ [11]. Considering the viscosity of cytoplasm as 3-4 fold that of the free water [12], the same calculation leads to a theoretical cytoplasmic diffusion coefficient of $0.754 \text{ } \mu\text{m}^2/\text{ms} < D_{cyto} < 1.00564 \text{ } \mu\text{m}^2/\text{ms}$. We can then calculate theoretical *RMSDs* (the real quantitative values measured by diffusion NMR) of water in tissue, if the decrease of the diffusion coefficient in brain would be *entirely* due to viscosity and would follow Einstein law : $RMSD = \sqrt{\langle r^2 \rangle} = \sqrt{(2n \cdot D \cdot t_d)}$ and with $t_d = 27.133$ ms we obtain a 3D displacement of $11.079 \text{ } \mu\text{m} < RMSD(\text{cell water}) < 12.795 \text{ } \mu\text{m}$ compared to $RMSD(\text{free water}) = 22.28 \text{ } \mu\text{m}$. In anomalous subdiffusion ($\alpha < 1$), the relation observed is : $\langle r^2(t) \rangle = 2n \cdot D \cdot t_d^\alpha$, where α , the anomalous exponent depends on the characteristic fractal walk dimension d_w of the tissue medium ($\alpha = 2/d_w$) [13]. A low α is associated with a more heterogeneous medium, with a higher number of components of different sizes (increase of medium's fractal dimension). Obstruction of free diffusion at different length scales (cell size, organelles size, tubulin and actin network size, hydrated proteins interaction size ...) would cause the anomalous behavior to appear by restriction of water displacement in large scale orders, by barriers of different sizes.

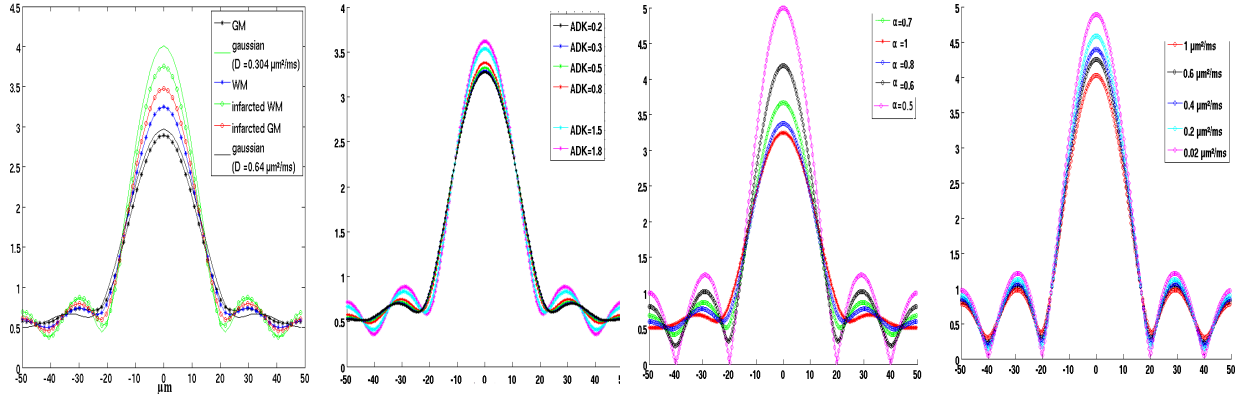
2. Material and Methods

DWI images of a patient were recorded 4 day after stroke in a cortical/subcortical area (Philips 3T Achieva DWI-EPI, $TR/TE = 3179.5/70.5$ ms, b -factors = 0,200,500,1000,2500 s/mm², $\Delta = 34.8$ ms, $\delta = 23$ ms, $t_d = 27.133$ ms) and registered. We calculated from segmented ROI an averaged S/S_0 signal fitted by ADI model : $S/S_0 = \exp[-(b \cdot DDC)^a]$ [5,13,14], DKI : $S/S_0 = \exp[-b \cdot ADCK + (b^2 \cdot ADCK^2 \cdot ADK)/6]$ [15], BEDI : $S/S_0 = F_{fast} \cdot \exp(-b \cdot ADC_{fast}) + F_{slow} \cdot \exp(-b \cdot ADC_{slow})$ or inverted to give q -space diffusion propagator ($b = q^2 \cdot t_d$) :

$$\bar{p}_s((\mathbf{r} - \mathbf{r}_0), t_d) = \int_{-\infty}^{+\infty} S(\mathbf{q}, \mathbf{r} - \mathbf{r}_0, t_d) / S_0 \exp(-i 2\pi \mathbf{q}(\mathbf{r} - \mathbf{r}_0)) d^3 \mathbf{q}$$

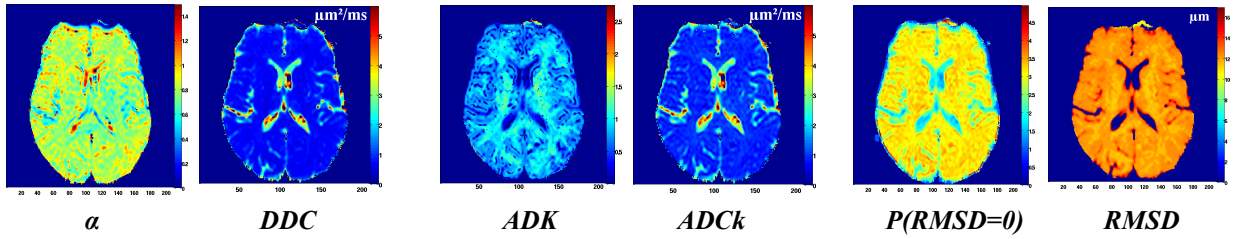
3. Results

For the diffusion time used here, experimental *RMSDs* measured (Fig.1, 2) are in the same order as those calculated for viscous cytoplasm. However, cell water diffusion propagator is not gaussian, probably because water diffuses in a less viscous, but structured media whose size is in the order of the length scale of brain cells diameter (glial cell, 2.5-9 μm ; neuron, ~5-20 μm [16]; dendritic tree arborescence, 13 μm diameter). Considering the imbrication and ramifications of cellular spaces, water mainly probes dense intracellular and extracellular matrix [17], and is able to diffuse towards the actin cytoskeleton [17] lattice mesh (0.1-1 μm), the tubulin/tau cytoskeleton network and to some packed axons (~0.5-2.5 μm diameter). A mean effect of the diffusion anisotropy at high b could decrease independently the *ADC* [17]. We made q -space signal simulations with variation of the anomalous exponent, kurtosis and of the diffusion coefficient. We found in healthy tissue the GM more anomalous than the WM. Simulated data compared to observed brain signals are presented in Fig.1. A limitation of qSI is that if the $P(RMSD=0)$ is an hypothese-free parameter, the deducted *RMSD* is under gaussian phase approximation. Decreasing D raises the $P(RMSD=0)$ but the propagator still remains gaussian (S3). Decreasing α (S2) or increasing kurtosis (S1) raise the peakedness of the initially gaussian propagator shape distribution and raise as well the $P(RMSD=0)$. This increase of the $P(RMSD=0)$ characterizes the differences between both healthy GM versus healthy WM and healthy versus ischemic tissue. Thus, to explain changes in healthy/ischemic tissue or in GM/WM contrast difference, we may invoke both a modulation of the water diffusion coefficient itself (linked in our interpretation to the water viscosity) and of a parameter representing a structural feature of the media, both acting on the *RMSD* determination. In ischemic GM, the rise in viscosity probably result from the associated loss of structural features because water diffusion becomes closer to gaussian when *DCC* and *ADCK* decrease (Fig. 2).



Experimental			Simulation (S1)			Simulation (S2)			Simulation (S3)		
Signal	RMSD (μm)	P(RMSD=0)	ADK	RMSD (μm)	P(RMSD=0)	α	RMSD (μm)	P(RMSD=0)	D (μm²/ms)	RMSD (μm)	P(RMSD=0)
WM	11.95315	3.25043	ADK=0.5	11.95310	3.32430	$\alpha=1$	12.28515	3.244405	1	10.62500	4.02410
Ischemic WM	10.95703	3.75542	ADK=0.8	11.62110	3.37940	$\alpha=0.8$	11.621093	3.377006	0.6	10.29296	4.24850
GM	12.61718	2.89380	ADK=1.5	11.28910	3.53520	$\alpha=0.7$	10.95703	3.66812462	0.4	10.29296	4.39420
Ischemic GM	11.28906	3.47904	ADK=1.8	10.95700	3.61630	$\alpha=0.6$	10.29296	4.1858259	0.2	10.29296	4.58610

Fig. 1 Diffusion propagators (p_s) resulting from averaging of localized magnetization DDI signal for WM, ischemic WM, GM, ischemic GM. $P(RMSD=0)$ is the peak maximum intensity. $RMSD$ is defined as the lateral gaussian width ($0.425 \times FWHM$). Simulations with increased kurtosis and α are realized with $D=0.7 \mu\text{m}^2/\text{ms}$. $\alpha=0.6$ for increased D (S3).



MEDI	$ADC \pm SD(ADC) \mu\text{m}^2/\text{ms}$	RMSD	DKI	$ADCK \pm SD(ADCK) \mu\text{m}^2/\text{ms}$	RMSD	$ADK \pm SD(ADK)$
WM	$0.58179 \pm (3.06024 \times 10^{-3})$	9.73210	WM	$0.91204 \pm (7.28192 \times 10^{-2})$	12.18520	0.82877 ± 0.0029386
Ischemic WM	$0.14488 \pm (1.6255 \times 10^{-4})$	4.85660	Ischemic WM	$0.24202 \pm (1.21010 \times 10^{-3})$	6.27700	0.76687 ± 0.0034765
GM	$0.73790 \pm (5.91233 \times 10^{-4})$	10.96030	GM	$1.28210 \pm (8.76839 \times 10^{-2})$	14.44730	0.72296 ± 0.0028751
Ischemic GM	$0.48258 \pm (5.91235 \times 10^{-3})$	8.86360	Ischemic GM	$0.71240 \pm (6.00953 \times 10^{-4})$	10.79630	0.98034 ± 0.003140

BEDI	$F_{\text{iso}} \pm SD(F_{\text{iso}})$	$ADC_{\text{iso}} \pm SD(ADC_{\text{iso}}) \mu\text{m}^2/\text{ms}$	RMSD	$F_{\text{iso}} \pm SD(F_{\text{iso}})$	$ADC_{\text{iso}} \pm SD(ADC_{\text{iso}}) \mu\text{m}^2/\text{ms}$	RMSD (μm)
WM	$0.64706 \pm (7.34166 \times 10^{-4})$	$1.54561 \pm (1.79224 \times 10^{-1})$	15.86260	$0.35293 \pm (7.34165 \times 10^{-4})$	$1.54561 \pm (1.79224 \times 10^{-1})$	6.37920
Ischemic WM	0.716373 ± 0.0015449	$0.97553 \pm (2.61919 \times 10^{-2})$	12.60220	$0.28372 \pm (2.95026 \times 10^{-4})$	$0.97553 \pm (2.61919 \times 10^{-2})$	3.51050
GM	0.58308 ± 0.0014755	$2.90698 \pm (4.42770 \times 10^{-2})$	21.75430	0.41691 ± 0.0014755	$2.90698 \pm (4.42770 \times 10^{-2})$	7.97370
Ischemic GM	0.45766 ± 0.0043817	$2.34798 \pm (1.14529 \times 10^{-1})$	19.55110	0.54233 ± 0.0043817	$2.34798 \pm (1.14529 \times 10^{-1})$	6.84610

DKI	$ADK \pm SD(ADK)$	$ADCK \pm SD(ADCK) \mu\text{m}^2/\text{ms}$	RMSD	ADI	$DDC \pm SD(DDC) \mu\text{m}^2/\text{ms}$	RMSD	$\alpha \pm SD(\alpha)$	d_i
WM	0.82877 ± 0.0029386	$0.91204 \pm (7.28192 \times 10^{-2})$	12.18520	WM	$0.65056 \pm (2.5639 \times 10^{-3})$	10.29130	0.83293 ± 0.0026382	2.40114
Ischemic WM	0.76687 ± 0.0034765	$0.24202 \pm (1.21010 \times 10^{-3})$	6.27700	Ischemic WM	$0.13538 \pm (1.47100 \times 10^{-4})$	4.69460	0.32889 ± 0.001187	6.08106
GM	0.72296 ± 0.0028751	$1.28210 \pm (8.76839 \times 10^{-2})$	14.44730	GM	$0.95598 \pm (1.78523 \times 10^{-3})$	12.47520	0.78473 ± 0.002778	2.54864
Ischemic GM	0.98034 ± 0.003140	$0.71240 \pm (6.00953 \times 10^{-4})$	10.76930	Ischemic GM	$0.518328 \pm (1.03380 \times 10^{-3})$	9.18600	0.81224 ± 0.0012935	2.4623264

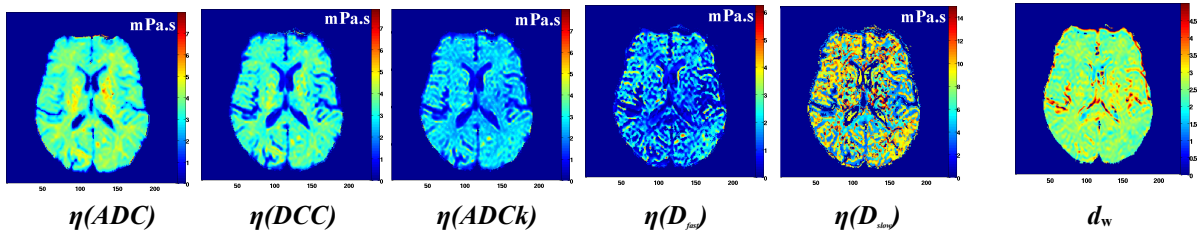


Fig. 2 On top, images showing the contrast between GM/WM results from pixel-by-pixels fitting of DDI, taken from the a slightly infarcted area. Table : spatial mean of fit parameters images and calculated RMSD for different DDI modelling (mean \pm standard deviation). On bottom, d_w and viscosity maps calculated with Stokes-Einstein. One can note that, contrary to $\eta(ADC)$ and to a little extent to $\eta(DCC)$ maps, $\eta(ADCK)$ do not present any WM/GM contrast.

4. Discussion

Recently, it has been hypothesized that cytoskeleton is the biological structure causing non-monoexponential water diffusion decay [17]. Cytoskeleton crowded network has been shown to be responsible of the anomalous diffusion properties of probes [4,6,7,8]. In a subcellular point of view, GM is mainly constituted of actin and associated proteins, and WM, of tubulin and neurofilaments (with a few perimembranar actin). Microtubules are empty cylinders, 50-100 μm length, 25 nm diameter. Tubulin polymerization is GTP-dependant and ATP depletion by ischemia induces a destructure of the microtubules with the loss of the axonal transport and the liberation of free tubulin [18], that could explain the *ADC* decrease (high water viscosity) in infarcted WM. Various chemotherapy drugs acting on microtubules have been shown to decrease WM *ADC* [19], as microtubule polymerisation inhibitors like Taxol, that causes an *ADC* decrease when injected in a tumor site [20] and vinblastine (preserving neurofilaments) that leads to *ADC* decrease in both perpendicular and parallel directions to the axon, together with conserved diffusion anisotropy [22]. If microtubules dissociation with nocodazole do not change the *ADC* in *Xenopus* oocytes [21], in nerves, it has been shown that the microtubule depolymerizer methylmercury rises the diffusion of water in axonal longitudinal direction [23]. Imidinodipropionitrile, causing the displacement of neurofilaments to the axon periphery, with microtubule moving to the center of the axon, induces in the spinal chord F_{slow} increase and D_{slow} decrease [24], the latter being responsible of the high *b*-value WM vs GM contrast [25] corresponding to an *ADC* decrease in WM. As diffusion probes a length scale of the cellular size order, water would be sensitive to the viscosity of the space traversed during the random walk, additionally affected by collision (and perhaps, binding) of water with local intra/extracellular microstructures [1,3], the latter causing a departure from gaussian diffusion. Disrupting cytoskeleton by ischemia (and with some chemicals) causes an α decrease representing the increase of the fractal dimension (higher order structure scale that water meets during diffusion), characterized by a peakedness of the gaussian distribution, together with a decreased diffusion coefficient that could be representative of increased viscosity in the stroke zone by a sol to a viscous gel transition. In conclusion, the use of the complete propagator informations, instead of *ADC* [1] would give valuable information about subcellular structures that have a functional role in cells, bridging the gap between the cell ultrastructure and energetic metabolism, both affecting the water diffusion signal. However, for qSI-based images extraction, some propagator informations are lost.

5. References

- [1] D.S Grebenkov, Concepts in Magnetic Resonance Part A 36A (2010) pp. 24-25.
- [2] A.S. Verkman, TRENDS in Biochemical Sciences 27(1) (2002) pp. 27-33.
- [3] D.S. Novikov, V.G. Kiselev, NMR in Biomedicine, 23 (2010) pp. 682-697.
- [4] M. Weiss, M. Elsnér, F. Kartberg, T. Nilsson, Biophysical Journal 87(5) (2004) pp. 3518-3524.
- [5] M. Köpf, C. Corinth, O. Haferkamp, T.F. Nonnenmachert, Biophysical Journal 70 (1996) pp. 2950-2958.
- [6] D.S. Banks, C. Fradin, Biophysical Journal 89(5) (2005) pp. 2960-297.
- [7] M. Tolic-Nørrelykke, E.-L. Munteanu, G. Thon, Physical Reviews Letters 93(7) (2004) pp. 078102-1 – 078102-4.
- [8] Y. Wong, M. L. Gardel, D.R. Reichman, Physical Reviews Letters 92(17) (2004) pp. 178101-1– 178101-4.
- [9] A. Schwarcz, P. Bogner, P. Meric, J-L Correze, Magnetic Resonance in Medicine 51(2) (2004) pp. 278-285.
- [10] L.Korson, W. Drost-Hansen, F.J. Millero, The Journal of Physical Chemistry 73(1) pp. 34-39.
- [11] C.A. Clark, D. Le Bihan, Magnetic Resonance in Medicine 44 (2000) pp. 852– 859.
- [12] K. Luby-Phelps, International Reviews of Cytology 192 (2000) pp. 189-221.
- [13] M. G. Hall, T.R. Barrick, Magnetic. Resonance in Medecine 59 (2008) pp. 447-455.
- [14] K. Bennett, K. Schmainda, R. Bennett, D. Rowe, Magnetic Resonance in Medecine 50 (2003) pp. 727-734.
- [15] J.H Jensen, JA Helpert, A Ramani, H. Lu, Magnetic Resonance in Medecine 53 (2005) pp.1432-1440.
- [16] G. Rajkowska, L.D. Selemon, P. S. Goldman-Rakic, Archives of General Psychiatry 55 (1998) pp. 215-224.
- [17] D. Le Bihan, Physics in Medicine and Biology 52(7) (2007) pp. R57-R90.
- [18] M.A. Petty, J.G. Wettstein, Brain Research Review 31(1) (1999) pp. 58-64.
- [19] C.S. Tam, J. Galanos, J.F. Seymour, A.G. Pitman, American Journal of Hematology 77 (2004) pp. 72–76.
- [20] Y. Mardor, Y. Roth, Z. Lidar, V. Jonas, R.Pfeffer, SE Maier, Cancer Research 61 (2001) pp. 4971-4973.
- [21] F.J.V. Sehy, L. Zhao, J. Xu, H.J. Rayala, Magnetic Resonance in Medecine 52(2) (2004) pp. 239-247.
- [22] C. Beaulieu, P.S. Allen, Magnetic Resonance in Medecine 31 (1994) pp. 394-400.
- [23] Y. Kinoshita, A. Ohnishi, K.Kohshi A.Yokota, Environmental Research Section A 80 (1999) pp. 348-354.
- [24] T.M. Shepherd, P.E. Thelval, E.D. Wirth, Proceeding of the ISMRM 2011 9 (2001) pp. 1624.
- [25] S.E. Maier, R.V. Mulkern, Magnetic Resonance Imaging 26 (2008) pp. 897-904.

Acknowledgments

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