Estimation of Intra-Axonal Fraction in Spinal Cord White Matter by using Monte Carlo Simulation of Water Diffusion and High b-value Diffusion Sensitized MRI

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INTRODUCTION: In this project, we will estimate the intra- and extra-cellular water fractions (ICF, ECF) in white matter (WM) in cervical spinal cord (CSC) using diffusion sensitized imaging (DSI). Until recently, most of the observed DSI signals were fitted by a bi-exponential fitting function with two diffusivity components: slow and fast decaying components. Due to the complex nature of biologic tissues structure, it has not been straightforward to assign slow and fast decaying components to physiological intra- (IC) and extra-axonal (EC) space respectively. Several attempts have already been made to estimate ICF and ECF by assigning slow and fast decaying components to intra- and extra- axonal spaces, respectively; however, the estimated values did not agree with the expected values[1]. Based on the Monte Carlo Simulation (MCS) of water diffusion in CSC and DSI measurement of the pig CSC, we estimate ICF and ECF of water molecules in the WM in CSC and compare those fractions with fractions of the IC and EC areas measured from a section of light microscopy.

METHODS: **(a) Monte Carlo Simulation (MCS)**: Most axons in CNS are myelinated (g-ratio: axon diameter/ fiber diameter with myelin sheath= 0.66[2]) and randomly distributed with the size distribution given by γ-variate function (average diameter 1.2 μm[3]). Thus, WM in CSC is modeled as being mainly composed of axons (30 % volume fraction (VF) and water diffusivity D_{intra} =1×10^{-3} mm^2/s), myelin sheaths (39 % VF), and extracellular space (31% VF and water diffusivity D_{extra} =2.5×10^{-3} mm^2/s) as shown in Fig. 1a. Due to the very short T_2 value (10-20 ms)[4] of water molecules in myelin, the contribution of myelin water in DSI signal is negligible for relatively long TE (> 100 ms)[5]. Initially 10,000 molecules were randomly distributed over the IC and EC spaces (molecules belonging to the myelin space were discarded). The position of each molecule was updated every 100 ns using the random walk method[6]. When a molecule encounters a local boundary, such as membrane or myelin sheath, the last position of molecule is recalculated until it finds a new position belonging to the same space (no exchange is allowed). The position of each molecule was recorded in every 10-100 μs and post-DSI signal was calculated by computing phase accumulated by each molecule during course of diffusion and summing the contributions from all molecules. The effect of T_2 is neglected by assuming same T_2 values for both IC and EC waters. MCS software was written in Python and in Message Passing Interface (MPI) with C++ language using parallel processors in the High-Performance Computing Center, at Univ. of Utah.

(b) MRI Experiment: DSI measurements were performed on an excised pig CSC, which had been fixed for 3 days in buffered formalin and then stored at 4° C in PBS for 7 months, using a custom birdcage coil (0.5" ID, 1" length) and multi-shot diffusion-weighted EPI at 3T MRI system (Trio, Siemens Medical Solutions, Erlangen, Germany), equipped with maximum gradient strength 40 mT/m.

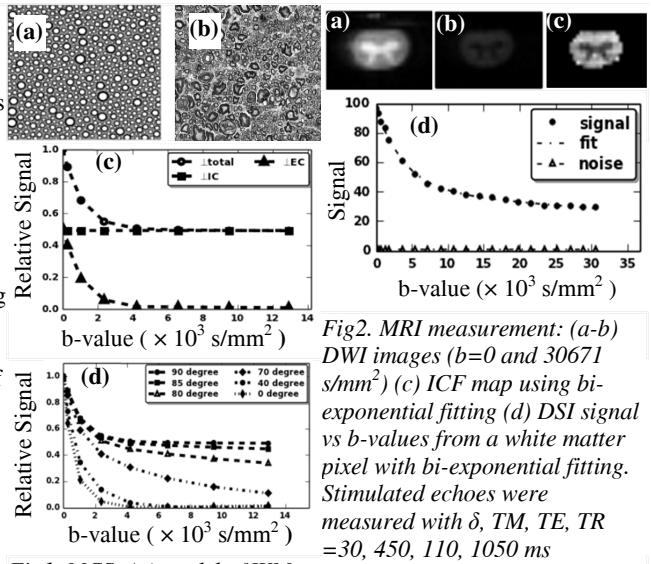

Fig1. MCS: (a) model of WM (b) light microscopy (c) total, IC, and EC signals vs b-values (d) total signal vs b-values. (δ=30 ms and Δ=465 ms)

Fig2. MRI measurement: (a-b) DWI images (b=0 and 30671 s/mm^2) (c) ICF map using bi-exponential fitting (d) DSI signal vs b-values from a white matter pixel with bi-exponential fitting. Stimulated echoes were measured with δ, TM, TE, TR =30, 450, 110, 1050 ms respectively. Diffusion gradient was applied perpendicular to the fiber direction.

(c) Light microscopy: Sections from the cord were post-fixed in osmium tetroxide, dehydrated in ascending concentrations of ethanol and embedded in epoxy resin. Cross sections of the cord were cut at about 1 μm in thickness and stained with toluidine blue prior to observation by light microscopy.

RESULTS AND DISCUSSIONS: The MCS of the water diffusion in WM model described in the METHOD section predicts that when the diffusion-sensitizing gradient is perpendicular to the fiber direction, the signal from the IC water does not decay with b values; however, the signal from EC water decays as a hindered diffusion and dies out to 1% with b = ~15,000 s/mm^2, assuming δ, Δ, and G are in the range of clinical scanner (see Fig. 1c). The total signal decay depends on the angle between diffusion gradient and fiber directions as in Fig. 1d. A bi-exponential fitting of the DSI signal calculated with various angles indicates that the estimated values of ICF (~50 %) and ECF (~50 %) do not change significantly with the angle; however, the diffusivities of the slow and fast decaying components increase from D_{slow} = 0.001 and D_{fast} = 0.96 to D_{slow} = 0.03 and D_{fast} = 1.00 in 10^{-3} mm^2/s unit, when the angle decreases from 90° to 80°. Thus, it is necessary to measure the angle and correct the effect. The MRI measurement of DSI signal in WM of the pig CSC decays bi-exponentially with b-values (0-30671 s/mm^2). D_{slow} and D_{fast} were estimated (0.013±0.004) ×10^{-3} mm^2/s) and (0.237±0.044) ×10^{-3} mm^2/s, respectively, with the corresponding fractions f_{slow} = 0.41 ± 0.06 and f_{fast} =0.59 ± 0.06 over all WM pixels present in a 2 mm thick slice as shown in Fig 2. In contrast to the MCS, the intra-axonal signal decays slowly and contributes 41 % to the total signal, and this contribution fairly agrees with the IC area measured in a section of light microscopy (Fig 1b).The discrepancy between the constant IC signal (50 %) estimated in MCS and slowly decaying IC signal (41 %) measured in DSI may be due to imperfect gradient direction, presence of some axons with larger size than estimated by the model, and very slow exchange. The significant effect of exchange has already been excluded by Niendorf et al.[1]

CONCLUSION: Based on the MCS and the DSI measurement, we can conclude that the DSI technique can be used to estimate ICF and ECF. However, the DSI signal measurement can be strongly affected by the various factors such as angle between the diffusion gradient and fiber directions, size of axons, and exchange between IC and EC spaces, which may lead to under or over estimation of the fraction of intra- and extra-axonal spaces.

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