MOVING TOWARD FASTER MEASUREMENTS OF MICRON-SIZED AXON DIAMETERS *IN VIVO*

SUMMARY: Methods to determine micron-sized axon radii using oscillating gradients were too time consuming for clinical use. With fewer gradient frequencies, imaging is 4.16 times faster.

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INTRODUCTION

Autopsy studies indicate that the distribution of axons in brains with schizophrenia could differ from brains without schizophrenia [1]. Magnetic resonance imaging (MRI) has inferred axon diameters and distribution in the brain [2], typically for axons larger than 5 µm in diameter [3]. The goal of this work is to modify these methods to adapt oscillating gradients (OG) to target small axons (1 to 2 µm range) which constitute the majority of cortical connections and shorten the data acquisition time so that the method can be used to measure axon diameters *in vivo*.

Conventional MR inferences of axon diameters use the pulsed gradient spin echo (PGSE) sequence [4,5]. The time over which water diffusion can be studied, or diffusion time, using PGSE cannot be reduced enough to study diffusion over a micron scale because of the presence of the π pulse, and the need for large gradient strengths to measure a sizable effect. Thus, PGSE can be used to study larger axon diameters, but another pulse sequence is needed for smaller axons.

The oscillating gradient spin echo (OGSE) pulse sequences use high frequency sinusoidal gradient pulses in order to target smaller diffusion distances [6]. The signal from diffusion experiments measured using different gradient frequencies and strengths can be fitted to different models which

describe the geometry of the sample to find the distance between barriers causing restrictions to the diffusion. Previous measurements required long imaging times which make them unsuitable for clinical and preclinical imaging. The goal of this work was to reduce imaging times so that the methods could be more suitable for clinical and preclinical imaging and to determine more optimal frequency ranges for a more flexible measurement routine depending on the sizes of axons in the sample.

METHODS

Data from previous studies using phantoms, vegetables and brain tissue and those from Monte Carlo simulations were used in this study [7-10]. A portion of human corpus callosum tissue was collected from an autopsy specimen, under the protocol approved by the institutional health REB along with the consent obtained from the family members. OGSEs are used to probe diffusion in smaller time regimes (higher frequency) to target smaller axons. Diffusion of water within the brain can be categorized into three regimes: restricted (intra-axonal), hindered (extra-axonal) and free (cerebrospinal fluid) [11]. Free water diffusion signal is usually from ventricles and can possibly be from intracellular and extracellular space with very high frequency gradients and makes insignificant contributions. The main contribution to the MR signal changes measured in our experiments comes from restricted water. Axons are modelled as long cylinders and fibers are modelled as groups of parallel cylinders, possibly of varying diameters. The signals are then fitted to a model called AxCaliber [12],

$$S = f_{axon} S_r(N_{OG}, G) + (1 - f_{axon}) S_h(N_{OG}, G) \quad (1)$$

where *S* is the MRI signal, f_{axon} is the volume fraction of axons within the voxel, S_r and S_h are the signals from the water within the restricted and hindered compartments respectively. The AxCaliber model, with data collected using OGSE sequences, requires images collected using N_{OG} different OGSE gradient frequencies and at least two gradient strengths *G*. Previous experiments used as many as N_{OG} = 20 gradient frequencies and as many 6 gradients strengths per gradient frequency [7,8]. Using OGSE and AxCaliber smaller radii have been measured in phantoms, such as celery [7], and human corpus callosum [8]. MR data [7,8] was collected from a 7 T Bruker Avance III NMR system with Paravision 5.0 and BGA6 gradient set with a maximum gradient strength of 1.01 T/m. For experimental and simulated data, the OGSE pulse sequences had two 20 ms sinusoidal gradient pulses separated by 24.52 ms with a maximum range of frequencies from 50 to 1000 Hz along with 5 or 6 gradient strengths depending on the experiment. The geometrical model reflects the average axon widths and axon packing fraction.

With the higher gradient frequencies, the diffusion of water in the extra-axonal space became less hindered and freer, so we studied the effect of abandoning the hindered part of the AxCaliber model when fitting the data due to its negligible contribution in short-time diffusion regime. Rather than 20 different oscillation frequencies, data collected from fewer frequencies were studied to determine if fewer frequencies could be used to reduce imaging time without compromising the precision of the results. With a narrower range of frequencies being recommended for use, the next step was to determine which frequency would be optimal for the center of the range. Theoretically, the expected displacement of water diffusing in a certain effective diffusion time, Δ_{eff} , can be calculated using Einstein's relation [13]

$$\langle x^2 \rangle = 2N_d D \Delta_{eff(cos)}, \left(\Delta_{eff(cos)} = \frac{T}{4N_{OG}} \right) \quad (2)$$

where $\langle x^2 \rangle$ is the expectation value for displacement squared, N_d is the dimensional freedom of movement, *D* is the diffusion coefficient, *T* is the gradient pulse duration, and $\Delta_{eff(cos)}$ is the effective diffusion time for cosine wave oscillation gradient [14]. We expected that the optimal range of effective diffusion time, or gradient frequency would be sufficient to characterize the expected displacement of water diffusing approximately the distance of an axon radius. This assumption was tested with our data.

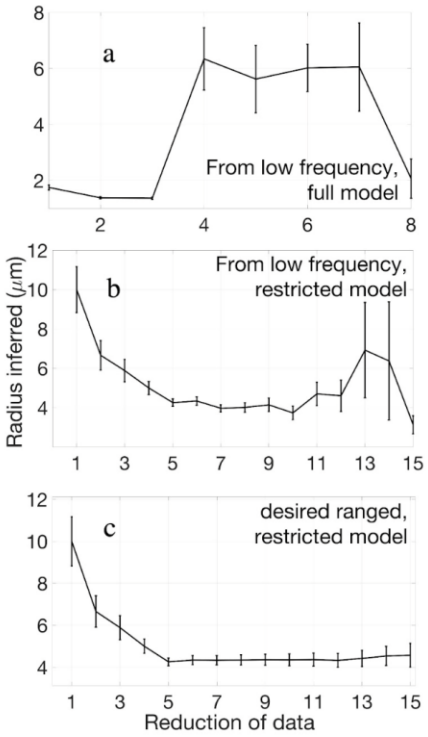

Figure 1. Inferred Radius vs trial number, which is one more than the number of excluded oscillating gradient frequencies from Monte Carlo simulation data. The geometry was hexagonally packed parallel cylinders with radii of 4.5μm and 60% packing fraction, this Figure serves as an example of how different models respond to less data.

a. The full model is used to fit to infer the radius. While data are dropping from N_{OG} = 1 in step of +1. In the first iteration, no data have been dropped. The inferred radius and the error bar both show an inconsistent trend.
b. Restricted compartment purely from the AxCaliber model was used in the fit to infer the radius. Data are dropped similar to A. Visually it can be seen that for these data, a reduction of 5-1 = 4 frequencies resulted in smaller error bars or lower limit of N_{OG} = 5.
c. Continuing with B, starting from N_{OG} = 5, Data are dropping from N_{OG} in step of -1 the inferred parameter(radius) loses its consistency and error bar width at n = 12. Visually it can be seen that for these data, a reduction of (12-1) - 5 = 6 frequencies additionally resulted in consistent error bars and inferred parameter or upper limit is found as N_{OG} = 15.

Overall, it can be seen that the model that excludes the hindered water diffusion contribution appears to provide more consistent and accurate results than the full model with a smaller data set and that approximately 12 frequencies can provide optimal inferences of axon sizes.

RESULTS

Plotted in Figure 1 is an example comparing different inference models using Monte Carlo simulation data, similar to those published previously, from 4.5 µm hexagonally packed cylinders or axons with an axonal packing density of 60% [15]. All data were initially fitted assuming uniform-sized axons with either the full(a) or only restricted(b,c) AxCaliber model in the first trial. The data set was reduced by removing images from one frequency at a time as described below and the inferred radius was recorded at the end of each trial. The inferences from the fit to the full model always under- or over-estimate the axon size, whereas the inferences from the restricted model converge close to the correct size. The restricted model inferences have the lowest uncertainty when the 4 lowest frequencies were removed from the data, suggesting that using the 16 highest frequencies resulted in the optimal inference of axon radius. Continuing with the reduced data set, that is the one using the 16 highest frequencies, the upper frequency limit was determined to be at the point (i.e., 12th trial, upper limit is found to be $N_{OG} = 15$.) where the uncertainties lose their consistency as shown in Figure 1c. For all the data, it was found that on average, 12 frequencies optimized the uncertainties in the inferences of axon radius. A moderate correlation (R = 0.6487) was found between the expected diffusion displacement based on Equation (2) and the inferred radius based on the reduced data sets as shown in Figure 2. *A priori* knowledge of the expected range of axons desired to be targeted in the tissue, and the expected intra-axonal apparent diffusion coefficient of the water in the sample under the conditions being imaged could be used to calculate the optimal middle frequency choice for the range of diffusion frequencies used in the experiments according to the fit equation shown in Figure 2.

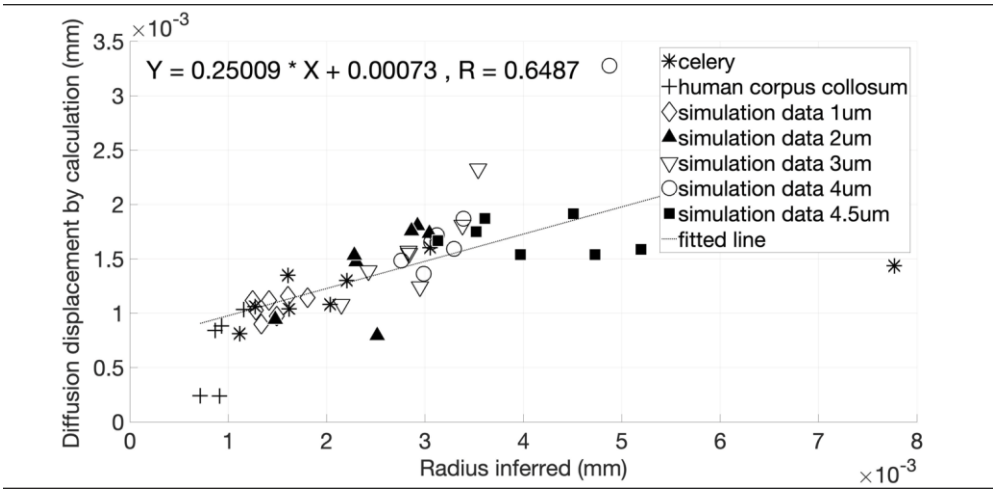

Figure 2. Diffusion displacement calculated using Equation (2) vs inferred radius plotted for all data available during the pandemic. The correlation coefficient is found to be R = 0.6487 (moderate correlation). The least squared fitted line on the plot is $y = 0.25009x + 0.00073$. Knowing the expected size of the axon, *x*, we can calculate the optimal diffusion displacement, *y*, based on this fitted equation. Using the intra-axonal apparent diffusion coefficient and Equation (2) we can then calculated the optimal effective diffusion time Δ_{eff} and thus optimal central frequency for OGSE measurements.

DISCUSSION

Using only 12 gradient frequencies rather than 20 gradient frequencies reduced imaging time by a factor of 1.6 without a significant effect on the inferred radius, based on the plots in Figure 1. The hindered part of the model was abandoned because it appeared that water within the extracellular space produced a relatively weak signal for the selected range of oscillation frequencies. Thus, the hindered extracellular water diffusion portion of the model could be neglected in an analogous way to free water diffusion signal being excluded from the full AxCaliber model. In addition to the reduction of the number of gradient frequencies, it has been proposed to reduce the number of gradient strengths used per frequency to two [9] which would cause the imaging time to be 4.16 times faster than using the full AxCaliber model with all gradient frequencies and amplitudes that were used before. Our results found an intermediate correlation between the inferred axon radius and the expected axon radius, based on Equation 2, and suggest that the hindered portion of the AxCaliber model can be dropped for this range of frequencies for OGSE. Our dataset is heavily biased by simulation data thus more brain tissue data are needed to confirm that the central frequency is optimal and the hindered portion can be dropped. Rather than not studying the extra-axonal water diffusion, the discovery of an equation that can fully describe different diffusion regimes in a wide variety of diffusion times or frequency could possibly lead to more accurate inferences. Other modifications to the model can be made, for instance, including a variety of axon radius rather than one, and tested to determine if they help improve the accuracy. Each modification has to be evaluated for the improvement in accuracy versus the increase in imaging time as well as for the bias the modifications could bring to the results. For instance, including a spectrum of radii for fitting could reduce the uncertainty in the inferences but it could also bias the result depending on the chosen spectrum.

CONCLUSION

In conclusion, reducing the number of gradient frequencies and gradient strengths used to collect images with the OGSE sequence and analyzed with AxCaliber appears to be possible, in theory. It will reduce the imaging time by a factor of 4.16 without a significant change in the precision of the inferred axon radii and consideration of any noise contaminations. The method proposed here requires *a priori* knowledge of the desired cell sizes. Imaging data needs to be collected post-Covid-19-pandemic along with electron microscopy to verify theoretical predictions. More work is needed to obtain a better model of the packing fraction. This work is the first step to reducing the imaging time so that an OGSE sequence can be used with the AxCaliber model to infer 1-2 µm axon sizes in live mouse brains.

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