Cellular Size Distributions Revealed by Non-Uniform Oscillating-Gradient Spin-Echo (NOGSE) MRI

Noam Shemesh[1], Gonzalo A Alvarez[1], and Lucio Frydman[1]
[1]*Chemical Physics, Faculty of Chemistry, Weizmann Institute of Science, Rehovot, Israel*

Target Audience. Researchers and clinicians interested in CNS microstructure.

Introduction. Shape and size distributions have a direct impact on the underlying functional and biological aspects of cells. In the Central-Nervous-System, the axonal size distribution determines conduction velocity[1], and disease-induced aberrations in cellular dimensions often lead to detrimental consequences[2]. Noninvasive characterization of the salient features of cellular distributions via existing methods[3-5] requires very strong gradient amplitudes and multidimensional experiments, as well as extensive tissue modeling. Here, we harness Non-uniform Oscillating-Gradient Spin-Echo Magnetic-Resonance-Imaging (NOGSE MRI, Fig. 1A) – a methodology probing diffusion dynamics[6] recently shown to exhibit extraordinary sensitivity towards compartmental dimensions[6,7] – for reporting on size distributions noninvasively and in a simple, one-dimensional fashion. Theoretical considerations show the possibility of using NOGSE to accurately extract size distributions; the methodology was successfully tested in yeast cells and validated against light microscopy. With it, size distribution contrasts in both white matter and gray matter are revealed.

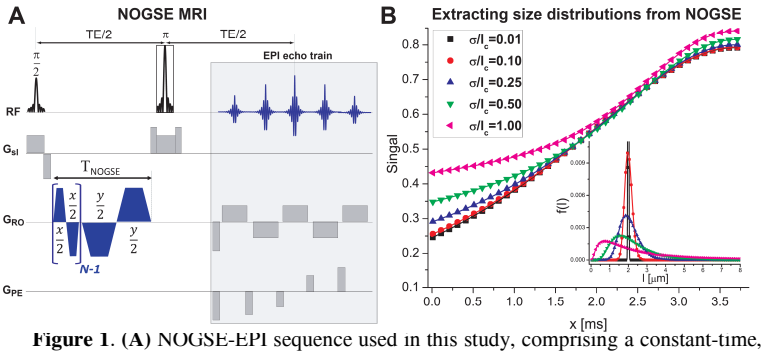

Figure 1. **(A)** NOGSE-EPI sequence used in this study, comprising a constant-time, constant *N*, variable-delay probe of diffusion played out at a constant time (T_{NOGSE}), and constant gradient (**G**). **(B)** Simulations showing the marked effects of size distributions on NOGSE signals. The inset shows the extracted distributions from the simulated curves (symbols) with the ground truth (solid curves).

Purpose. To map size distributions in the mouse brain using NOGSE – a new experiment with an exquisite length[6] sensitivity.

Methods. All experiments were performed on a 9.4 T Bruker Avance scanner operating at a ^{1}H frequency of 400.17 MHz and equipped with a micro5 imaging probe. NOGSE MRI experiments were performed using the sequence shown in Figure 1. NOGSE is a diffusion-based experiment, comprising an oscillating gradient train of *(N-1)* bipolar pairs of duration *x*, followed by another bipolar pair of duration *y*; *x* and *y* are chosen such that the overall evolution time $T_{NOGSE} = (N-1)*x + y$, remains constant. Simulations for the NOGSE signal in lognormal size distributions incorporating H_2O subject to diffusion in impermeable pores were written in Matlab®. Validation NMR experiments were performed in yeast cells with T_{NOGSE}=30 ms, *N*=8, G = 87 G/cm, and *x* was varied between 1 and 3.75 ms in 29 steps. Cellular dimensions were quantified under a light microscope for several thousands of cells. NOGSE-EPI experiments in the brain were performed with T_{NOGSE}=30 ms, *N*=8, *G* = 57.6 G/cm, and *x* was varied between 0.8 and 3.75 ms in 31 steps. The ensuing data were analyzed pixel-by-pixel and fitted to a lognormal distribution, and the distribution's mean, peak, and width were mapped.

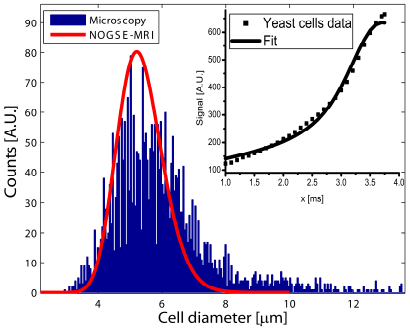

Figure 2. Size distribution obtained from light microscopy (blue bars) and from an analysis of the NOGSE signal shown in the inset (symbols represent experimental points, solid lines represent fitted curves). Excellent agreement is observed, validating NOGSE's capability of reporting on cellular size distributions.

Results and Discussion. In the presence of restricted diffusion, NOGSE's sensitivity towards compartment size, which varies with length to the power of six[6,7], can be highly sensitive and informative. The simulations shown in Figure 1B corroborate this hypothesis, and demonstrate that structural distributions can be faithfully reconstructed from the NOGSE responses (Fig. 1B, inset). To experimentally corroborate these important trends, NOGSE experiments were performed on yeast cells where the ground truth cellular sizes were obtained from ancillary light microscopy. The results shown in Figure 2 evidence an excellent agreement between the noninvasive, one-dimensional NOGSE experiments and the intracellular sizes quantified from microscopy, validating the NOGSE methodology for extracting size distributions. Perfused mouse brains were subsequently targeted for size distribution evaluation by NOGSE-MRI. The results shown in Figures 3A-C represent size distribution maps in the white matter (corpus callosum). Notably, NOGSE clearly segments this tissue into its well-known[4] five anatomical regions; furthermore, all of the corpus-callosum's size distribution features well-known from histology[8], such as the increase in distribution width from genu to splenium[8], are reproduced by the NOGSE maps These striking contrasts are not confined to white matter: when experiments were preformed on a coronal section, the NOGSE appears to parcellate cortical gray matter into distinct layers, faithfully depicting this major GM structural hallmark.

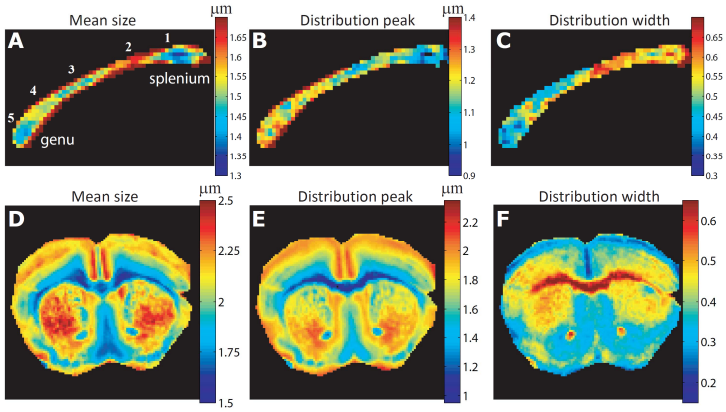

Figure 3. NOGSE EPI experiments in the *ex-vivo* mouse brain. (**A-C**) Maps of distribution mean, peak, and width, respectively, in images masked for the corpus callosum. (**D-F**) Maps of distribution mean, peak, and width, respectively, in a coronal image. Notice the strong contrast in the white matter, encompassing a segmentation of the corpus-callosum into its anatomical fiber composition, that recapitulates all the trends well-known from histology[8]. Notice the strong grey matter contrast in the coronal images, clearly revealing the cortical layers.

The sensitivity of NOGSE towards size distributions is likely arising from its previously reported[6,7] sensitivity towards the restricting length to the power of six, coupled to its multi-frequency probe of diffusion-driven dynamics governing transitions between free and restricted diffusion[6]. This enables one to direct image cellular size distributions, with minimal tissue modeling and with robustness. The experiments can be modified to accommodate relatively weak gradients, especially at lower clinical fields where T_{NOGSE} can be made longer; however, a limitation of this method is that it will inherently be biased towards longer T_2 species. Nevertheless, the detailed maps derived from NOGSE-MRI are highly promising for studying cellular-scale aberrations in white matter tissues upon disease, as well as modifications in the tissue's ultrastructure upon normal CNS processes, such as plasticity.

Conclusions. Cellular size distributions were unraveled by a simple, 1D NOGSE experiment. Simulations and experimental corroboration in biological cells validate the methodology, and the ensuing contrasts in the mouse brain segment white and gray matter into hallmark underlying structures. These features augur well for NOGSE as an ultrasensitive probe for microstructure *in-vivo*.

Acknowledgements. This work was supported by an ERC Advanced Grant #246754, a Helen and Martin Kimmel Award for Innovative Investigation; Israel Science Foundation grant ISF 1142/13; the Perlman Family Foundation; and a European Commission under the Marie Curie Intra-European Fellowship for Career Development Grant No. PIEF-GA-2012-328605.

References. [1] R. Caminiti et al., Proc. Nat. Acad. Sci. U.S.A. 106 **(2009)** 19551-19556. [2] G. Lovas et al., Brain 123 **(2000)** 308-317. [3] Y. Assaf et al., Magn. Reson. Med. 59 **(2008)** 1347-1354. [4] D. Barazany et al., Brain 132 **(2009)** 1210-1220. [5] D.C. Alexander, Magn. Reson. Med. 60 **(2008)** 439-448. [6] G.A. Álvarez, et al., Phys. Rev. Lett. 111 **(2013)** 080404. [7] N. Shemesh et al., J. Magn. Reson. 237 **(2013)** 49-62. [8] F. Aboitiz et al., Brain Res. 598 **(1992)** 143-153.