Avian Egg Latebra as Tissue Water Diffusion Model

Stephan E. Maier¹ and Robert V. Mulkern²

¹Department of Radiology, Harvard Medical School, Brigham and Women's Hospital, Boston, MA, United States, ²Department of Radiology, Harvard Medical School, Children's Hospital, Boston, MA, United States

Target Audience: Researchers, who are interested in the investigation of non-monoexponential diffusion properties typically observed in tissues. **Introduction:** The presence of non-monoexponential water diffusion signal decay in tissues is well established [1]. A fully satisfying explanation for this deviation or a correct attribution of compartments in the case of the physically motivated biexponential model has to date not been achieved. This can probably largely be attributed to the complexity of tissue, not only at the level of imaging voxels but also at the level of individual cells. It is quite clear that the presence of cell and organell membranes and the consequential hindrance and compartmentalization of the diffusion process is fundamentally linked with the observed deviation from a basic monoexponential diffusion signal decay observed in fluids. It is thus of great interest to study simplified models, which also exhibit non-monoexponential diffusion. Here we present the avian egg latebra, a mass also known as white yolk in the center of the yellow yolk, as an easily accessible model for tissue water diffusion.

Method: Chicken eggs, known to be unfertilized, were purchased at local groceries. Imaging was performed on a clinical 3 Tesla system using a bird-cage wrist coil. T_2 -weighted images were acquired with a 16-echo Carr-Purcell-Meiboom-Gill sequence using the following parameters: 200 mm FOV; 3 mm slice thickness; 256x256x0.6 image matrix; +/–15.6 kHz bandwidth; 6000 ms TR; between 15 and 240 ms TE. Diffusion-weighted images were acquired with LSDI using the same FOV, a TR of 3000 ms and three orthogonal diffusion encoding directions at 16 evenly spaced b-factors. One data set was obtained with the diffusion weighting set between 5 and 5,000 s/mm² (3 mm slice thickness, 128x128x0.5 matrix, 85 ms TE) and a second data set with the diffusion weighting set between 5 and 50,000 s/mm² (6 mm slice thickness, 64x64x0.5 matrix, 144 ms TE). This shortest possible echo time was the result of using the maximum gradient strength of 40 mT/m and a combination of simultaneous gradient application along all three magnet main axes. A very low readout bandwidth of +/–2.4 kHz resulted in improved SNR with sufficient signal even at the highest b-factors and very reliable water/lipid separation based on spatial separation. Image data was processed off-line with dedicated software. For the low-b data exponential fitting was performed for each pixel followed by ROI analysis of the resulting maps. Whereas for the higher b-data set and the T_2 -data set, average values were assessed for each ROI and then used for mono-exponential fitting. Values that fell below a value equal to three times the average background signal were excluded from the analysis. To exclude any typically observed water diffusion in the high-b data, only values at b=20,000 s/mm² or above were considered. Ambient temperature was measured during each scan session and water diffusion values were corrected assuming a reference temperature of 20 C [2].

Results: The setup described above resulted in the reliable acquisition of excellent image data (see Fig 1). Findings of the quantitative T_2 and diffusion analysis are provided in the table below. In the latebra, but not in the other structures, there was clear evidence of non-monoexponential diffusion signal decay. A biexponential analysis of the latebra signal resulted in two diffusion components (ADC1 and ADC2) and respective volume fractions (VFR1 and VFR2). Moreover, the very high-b scan revealed a third very slow diffusing component ADC3. Olefins, which potentially could be associated with this very slow component, were excluded based on a much lower diffusion coefficient that was measured for the lipids.



Fig 1.: Subimages (100 mm FOV) of raw egg-pairs scanned with the LSDI sequence. The brightness of the individual images has been adjusted for optimal display. The latebra is clearly visible as a bright structure in the center of the yolk which evidently maintains signal above noise, even at a b-factor of 50,000 s/mm². In contrast, already at 5,000 s/mm² the water signal of the egg-white falls well below the noise threshold. On the diffusion-weighted images the predominantly lipid containing yolk is shifted upwards, permitting an unencumbered water diffusion analysis of the latebra.

Latebra (N=8)					
T ₂ [ms]	ADC1 [µm ² /ms]	ADC2 [µm ² /ms]	VFR1[]	ADC3 [µm ² /ms]	VFR3 []
120 (24)	1.33 (0.16)	0.25 (0.04)	0.80 (0.03)	0.013 (0.04)	0.06 (0.02)

Conclusion: The egg presents a simple but relevant object for exploring biophysical aspects of common tissue contrast parameters like diffusion and transverse relaxation. The biexponential characterization of the latebra diffusion signal decay results in diffusion coefficients and signal fractions that are very similar to those found in brain. However, a major difference between the present latebra data and in-vivo human data is the temperature. White yolk, which is found inside the latebra, is characterized by a lower lipid and higher water content than in yellow yolk. A relatively long T_2 relaxation time facilitates diffusion measurements with high b-factors. Yellow and white yolk contain spheres with membranes [3]. The size of the spheres observed in white yolk falls in the range of cell sizes typically encountered in tissues. Given the more recent yolk microstructural research results [4] that assume densely packed polyhedrons rather than spheres, there is no exterior or only a small exterior volume, which seems to discourage an interpretation of the biexponential diffusion as a consequence of separate intra- and extra-spherical compartments with different diffusion properties.

[3] Mulkern RV et al. NMR Biomed. 1999;12:51. [2] Le Bihan D. et al. Radiology. 1989;171:853. [3] Bellairs R. J Biophys Biochem Cytol. 1961;11:207. [4] Mineki M and Kobayashi M. J Food Sci, 1997, 62(4):757-761.

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