Integrative optical imaging of molecular diffusion in strong light scattering brain tissue

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Integrative optical imaging (IOI) is a method for measuring the effective diffusion coefficient (D^*) of fluorescent molecules in brain extracellular space (ECS) [1]. Over the last two decades the IOI method has been applied, both in brain slices and *in vivo*, to a wide variety of substances. These include epidermal growth factor, quantum dots and the synthetic drug-carrier PHPMA [2, 3]. Here we discuss further development of IOI to compensate for light scattering in brain tissue.

IOI uses epi-fluorescence microscopy combined with a CCD camera to determine D^* by fitting a theoretical expression to the image intensity of a cloud of fluorescent molecules emitted from a point source and subsequently diffusing in ECS [1, 4]. The original theoretical expression does not take into account the light scattering (LS) in tissue. Therefore in tissues that exhibit strong scattering the images may be significantly distorted and the original theoretical expression will not provide satisfactory curve fits, leading to an inaccurate determination of D^*.

In order to extend the application of the IOI method to strong light scattering tissues, we derived a new expression for image intensity. The approach is to separate the signal contributions of un-scattered and scattered photons. The un-scattered light is treated as before and therefore has an intensity profile similar to the original expression but with a reduced amplitude. The scattered photons are treated with a diffusion approximation [5] and their signal contribution is calculated by convolution of the diffusing cloud of fluorescent molecules with an approximate Green's function. As a result, the new expression involves a multiple integral that has to be calculated numerically.

The new expression has been implemented in a curve-fitting program to analyze experimental data and results obtained with 3,000 MW dextran molecules have shown that:

- In low LS media, like agarose gel, the new expression continues to fit experimental data very well, and generates essentially the same D^*-values as the original expression.

- In high LS media, the new expression fits experimental data much better and generates reduced D^*-values compared to the original expression. In more opaque brain slices, the correction to the D^*-values could be significant.

References

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