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Brain microscopy point spread function in a photon diffusion limit

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In fluorescence microscopy of the brain, the image can be significantly degraded by light scattering. The individual scattering events in the brain tissue are very anisotropic, preserving to a large degree the photon direction. When the scattering coefficient is low or when the fluorophore light source is close to the tissue surface, the photons may enter the microscope without having scattered more than a few times, with only a limited detrimental effect on the point spread function (PSF). On the other hand, in highly scattering tissue or when imaging deeper sources, photons will scatter many times. Ultimately, they will lose their directional memory and enter the photon diffusion regime.

We simulated microscopy imaging of a point source located at a fixed depth of 100 μm inside a brain tissue slice 400 μm thick and varied the tissue scattering coefficient μs until the photon diffusion limit was reached. The full width at half maximum (FWHM) of the PSF was measured in each case. The simulation parameters corresponded to a typical experimental setup used in integrative optical imaging [1], with immersion objective magnification of 40, numerical aperture 0.8, and indexes of refraction 1.37 and 1.33 in the tissue and the surrounding solution, respectively. The scattering anisotropy factor g was kept constant at 0.9 and light absorption was assumed negligible [2]. The simulations employed a modified MCML program [3] to model scattering of 10,000,000 photons in each of the 22 runs.

The results are shown in Figure 1. Note that without scattering, the ballistic PSF in a typical experimental setup can be close to the pixel size of a CCD detector (in our experimental setup, less than 0.5 μm in the object space). In the other extreme, at the photon diffusion limit, no ballistic photons survive their tissue journey and the PSF is akin to a steady state diffusion pattern with a Newton boundary condition at the tissue surface. This PSF is over 100 times wider than that of the ballistic PSF, effectively destroying the image resolution. A similar effect would be observed with a constant scattering coefficient but increasing depth of the source. In conclusion, light scattering can profoundly affect the brain microscopy PSF, possibly reducing resolution by two orders of magnitude.

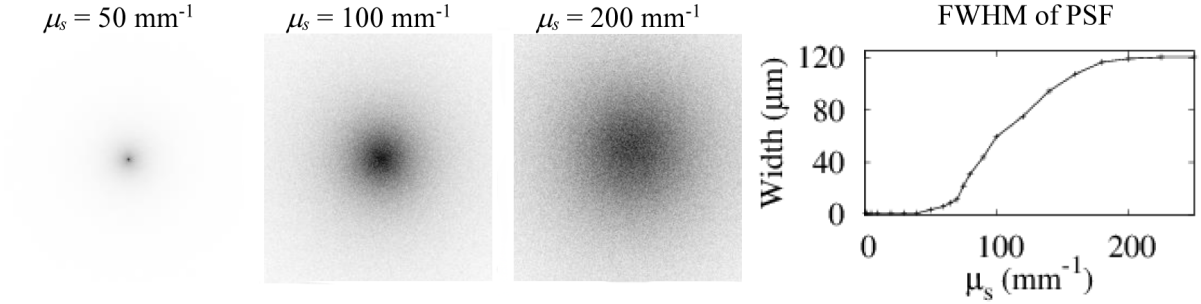

Figure 1: Simulated microscopy images of a point source for various values of μ_s (ballistic signal is omitted), and graph of the full width at half maximum of the point spread function as a function of μ_s.

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