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Mengran Wang, Chunyan Wu, Bo Li, Fei Xia, David Sinefeld, Chris Xu, "Comparison of excitation wavelengths for in vivo deep imaging of mouse brain," Proc. SPIE 10498, Multiphoton Microscopy in the Biomedical Sciences XVIII, 104982A (23 February 2018); doi: 10.1117/12.2289065

SPIE.

Event: SPIE BiOS, 2018, San Francisco, California, United States

Comparison of excitation wavelengths for *in vivo* deep imaging of mouse brain

Mengran Wang*[a], Chunyan Wu[a], Bo Li[a], Fei Xia[a], David Sinefeld[a], and Chris Xu[a]
[a]School of Applied and Engineering Physics, Cornell University, Ithaca, NY 14853

ABSTRACT

The attenuation of excitation power reaching the focus is the main issue that limits the depth penetration of high-resolution imaging of biological tissue. The attenuation is caused by a combination of tissue scattering and absorption. Theoretical model of the effective attenuation length for *in vivo* mouse brain imaging has been built based on the data of the absorption of water and blood and the Mie scattering of a tissue-like phantom. Such a theoretical model has been corroborated at a number of excitation wavelengths, such as 800 nm, 1300 nm[1], and 1700 nm[2]; however, the attenuation caused by absorption is negligible when compared to tissue scattering at all these wavelength windows. Here we performed *in vivo* three-photon imaging of Texas Red-stained vasculature in the same mouse brain with different excitation wavelengths, 1700 nm, 1550 nm, 1500 nm and 1450 nm. In particular, our studies include the wavelength regime where strong water absorption is present (i.e., 1450 nm), and the attenuation by water absorption is predicted to be the dominant contribution in the excitation attenuation. Based on the experimental results, we found that the effective attenuation length at 1450 nm is significantly shorter than those at 1700 nm and 1300 nm. Our results confirm that the theoretical model based on tissue scattering and water absorption is accurate in predicting the effective attenuation lengths for in vivo imaging. The optimum excitation wavelength windows for *in vivo* mouse brain imaging are at 1300 nm and 1700 nm.

Keywords: absorption, scattering, effective attenuation length, excitation wavelength

1. INTRODUCTION

Biological tissue is composed of a variety of cells and organelles. The inhomogeneity and refractive index variation cause tissue scattering. In addition, biological tissues absorb photons before they reach the focal point, thus reducing the number of photons contributing to multiphoton excitation. Attenuation length is used to describe the distance that an optical beam could propagate before being attenuated. Scattering length (ls) and absorption length (la) are terms used to characterize scattering and absorption events, respectively. Both scattering and absorption reduce the power reaching the focal point.

For Nth-order excitation, the signal scales with Nth order of the excitation power at the focus:

$$S^N(z) = P^N(z) = P^N(0)e^{-\left(\frac{Nz}{l_s}+\frac{Nz}{l_a}\right)}, \quad (1)$$

where z is the imaging depth below the brain surface, P(0) is the power at the surface, P(z) is the power at the depth z. Both scattering and absorption lengths are strongly dependent on wavelength. Various models have been developed to fit the scattering properties in biological sample[3–5]. Here, we used glass beads embedded in agarose gel[6,7] to characterize the wavelength dependence of scattering coefficient ($\mu_s=\frac{1}{l_s}$) with calculations based on Mie scattering theory. Such a model has been used successfully in the past to model the mouse brain. For the absorption contribution, specifically in brain tissue in vivo, blood and water are the most important factors. Therefore, many studies have been carried out to determine the absorption coefficient ($\mu_a=\frac{1}{l_a}$) of water[9] and hemoglobin [10]. For shorter wavelengths <1.1 μm, absorption in blood dominates but when wavelengths exceed 1.2 μm, water absorption takes over.

Taking account of both scattering and absorption effect, the effective attenuation coefficient is defined as μ_e ($\mu_e=\mu_s+\mu_a$). Figure 1 shows the calculated wavelength dependence of water absorption, blood absorption (oxy and deoxy-hemoglobin), scattering, and effective attenuation coefficients. Figure 1 is consistent with measured data in the mouse brain *in vivo* [1,2,11].

Multiphoton Microscopy in the Biomedical Sciences XVIII, edited by Ammasi Periasamy, Peter T. C. So, Karsten König, Xiaoliang S. Xie, Proc. of SPIE Vol. 10498, 104982A

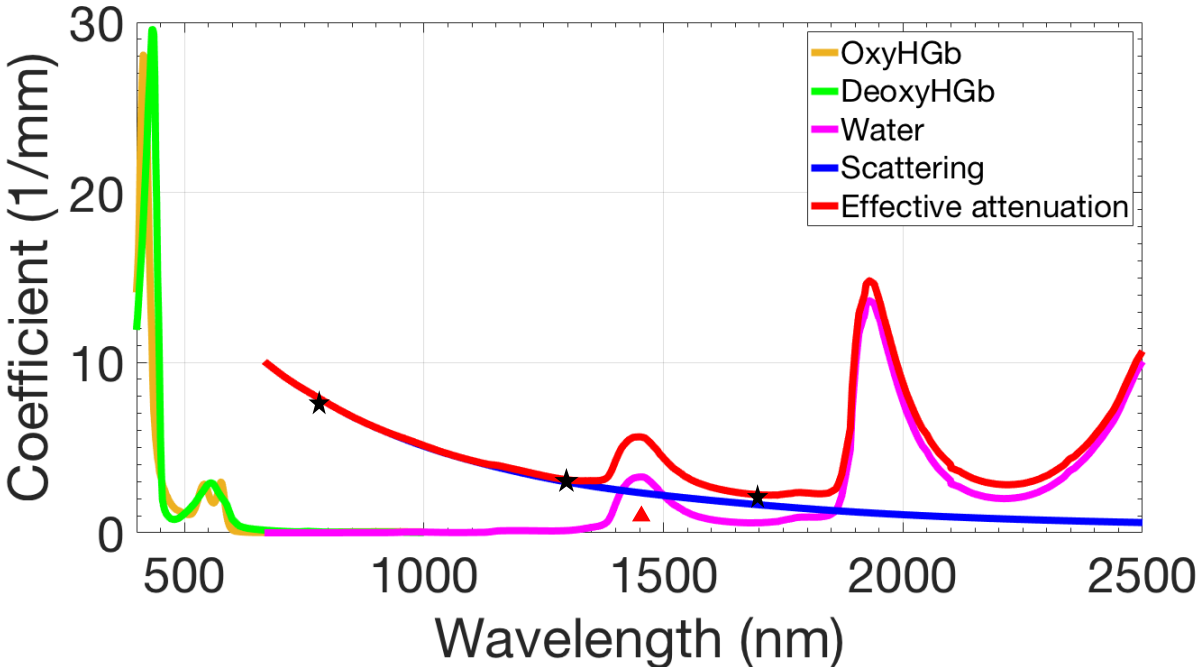

Figure 1. Theoretical calculation of the effective attenuation coefficients based on water, blood (at approximately 10% of the physiological value) absorption data and Mie scattering data. Scattering coefficient, calculated using Mie-theory for a tissue-like colloidal solution containing 1-μm diameter glass beads at a concentration of 5.4×10^9 /mL. The black stars indicate the reported effective attenuation coefficients (131 μm at 775 nm, 285 μm at 1280 nm and 383 μm at 1680 nm). Red triangle indicates the wavelength where water absorption dominates (i.e., absorption by water is stronger than tissue scattering).

2. METHODS

We use three-photon microscopy (3PM) to image Texas Red-labeled blood vessels in the same mouse brain with different excitation wavelengths, in the sequence of 1700 nm, 1550 nm, 1500 nm and 1450 nm. The excitation source is a wavelength-tunable optical parametric amplifier (OPA, Opera-F, Coherent) pumped by a Monaco amplifier (Coherent). The excitation wavelengths are measured by the Optical Spectrum Analyzer (OSA, Thorlabs). To enure the effective attenuation length measurement is not biased by the beam size of different excitation wavelengths, we measured the beam size of each excitation beam by an InGaAs camera (Axiom Optics, WiDy SWIR 640) (Figure 2). The measurement is taken before the scanner. The scan lens and the tube lens provide a magnification of 6. The measurement shows that the beams of different wavelengths are similar to size, and the impact of the beam-size variation on the decay-length measurement will be small. The maximum power used for all the excitation wavelengths are limited at 40mW to avoid potential heating of the brain tissue.

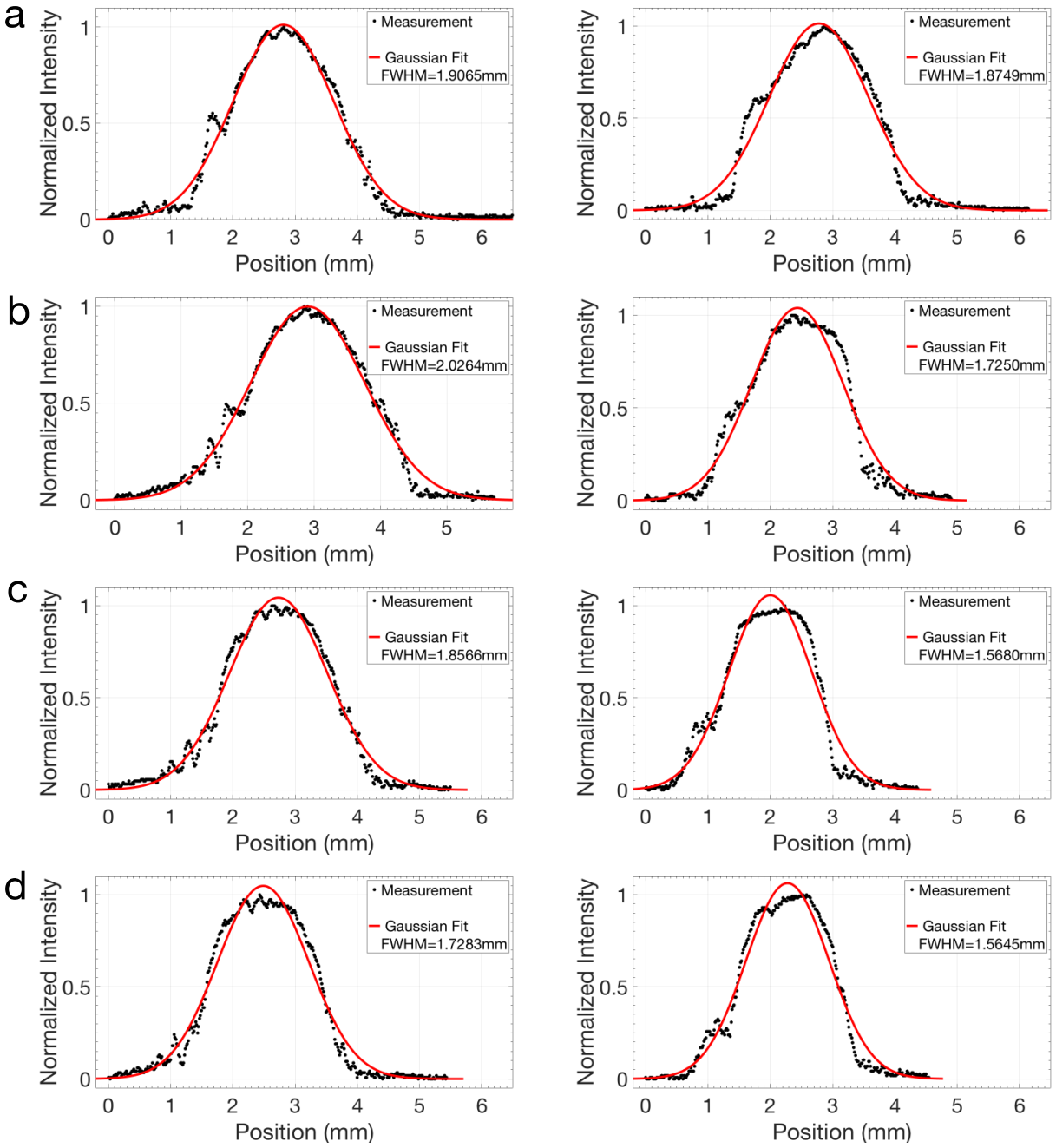

Figure 2. Beam size measurements. a. 1680 nm excitation beam. b. 1550 nm excitation beam. c. 1500 nm excitation beam. d. 1450 nm excitation beam. There is some ellipticity in the excitation beam. Left figures are measurements along long axis; right figures are measurements along short axis. Full-width-at-half-maximum (FWHM) is labeled in each figure.

3. RESULTS

We carry out several imaging sessions to compare the effective attenuation lengths at different wavelengths, two of them are presented in Figure 3. To confirm that the imaging sequence has no impact on our results, we repeated the imaging with 1700 nm excitation wavelength (labeled as "1700 nm repeat") at the end of the imaging session. The 1700 nm results at the beginning and the end are similar, confirming that the tissue properties remained stable throughout the

imaging session. The results of the effective attenuation lengths are summarized in Table 1. For 1700 nm excitation, the effective attenuation length is 370 μm and 405 μm, which is similar to previous results. For 1450 nm excitation, which is at the water absorption peak, the effective attenuation length is the shortest, around 188 μm and 212 μm, which is much shorter than those reported previously at 1300 nm (~ 300 μm). This wavelength dependence matches the theoretical prediction based on the absorption data and scattering data.

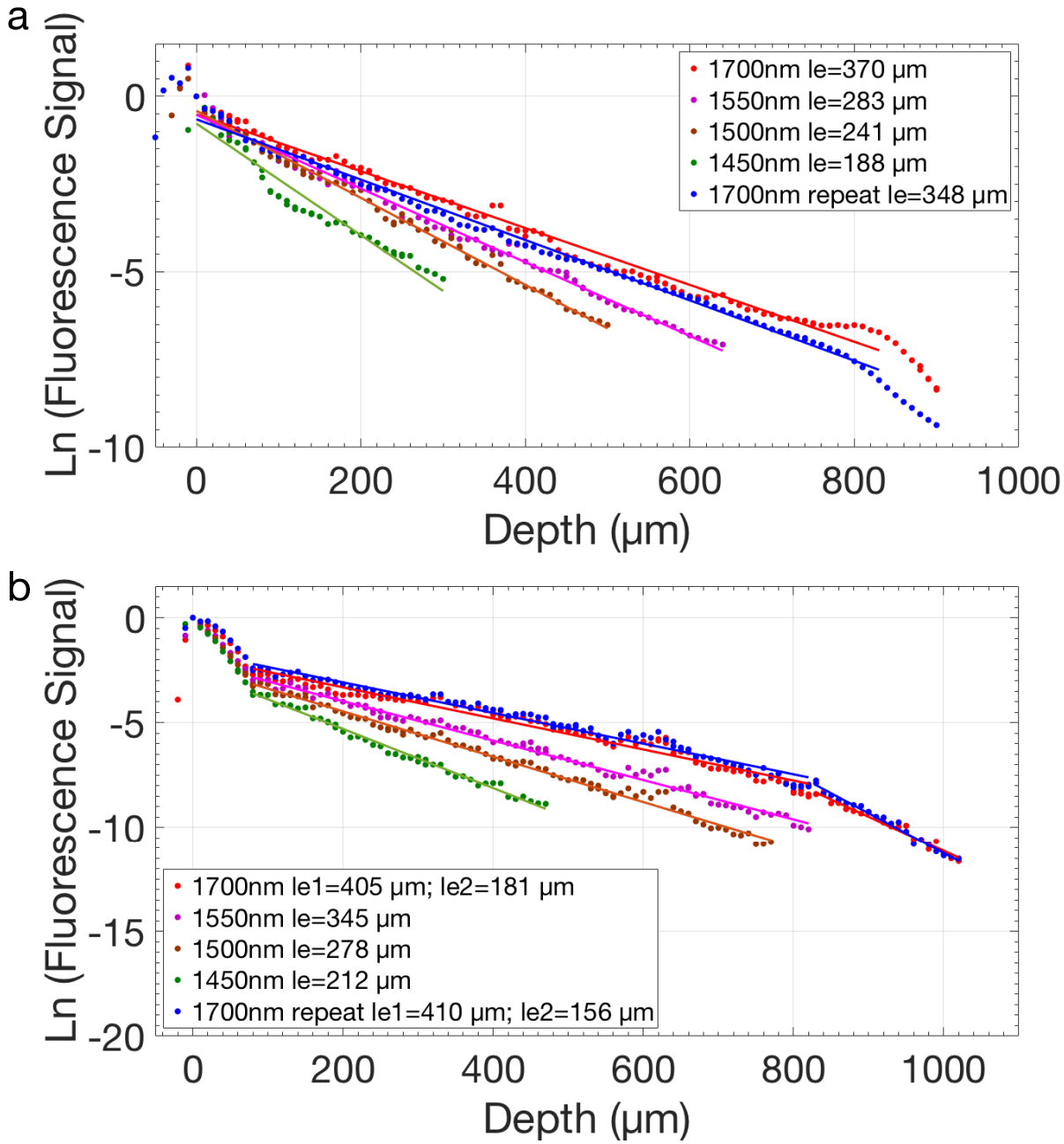

Figure 3. Comparison of decay length at 1680 nm, 1550 nm, 1500 nm, and 1450 nm excitation. Effective attenuation lengths (l_e) are indicated for each wavelength. le_2 indicates the decay length in the external capsule (i.e., the white matter).

Table 1. Effective attenuation lengths (l_e) for 1680 nm, 1550 nm, 1500 nm, 1450 nm excitation in two imaging sessions in Figure 3.

	1700 nm	**1550 nm**	**1500 nm**	**1450nm**	**1700 nm repeat**
Imaging 1 Cortex (μm)	370	283	241	188	348
Imaging 2 Cortex (μm)	405	319	278	212	410
Imaging 2 White matter (μm)	181	N/A	N/A	N/A	156

4. CONCLUSION

We performed a systematic comparison of the effective attenuation lengths at excitation wavelengths of 1700 nm, 1550 nm, 1500 nm and 1450 nm through three-photon imaging of mouse brain vasculature *in vivo,* and confirmed that water absorption at 1450 nm strongly attenuates the excitation light.

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