

Estimation of temperature rise at the focus of objective lens at the 1700 nm window

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Optical microscopy of biological tissues at the 1700 nm window has enabled deeper penetration, due to the combined advantage of relatively small water absorption and tissue scattering at this wavelength. Compared with excitation at other wavelengths, such as the commonly used 800 nm window for two-photon microscopy, water absorption at the 1700 nm window is more than one order of magnitude higher. As a result, more temperature rise can be expected and can be potentially detrimental to biological tissues. Here, we present theoretical estimation of temperature rise at the focus of objective lens at the 1700 nm window, purely due to water absorption. Our calculated result shows that under realistic experimental conditions, temperature rise due to water absorption is still below 1 K and may not cause tissue damage during imaging.

Keywords: Nonlinear microscopy; multiphoton processes; temperature.

1. Introduction

Optical microscopy enables us to uncover the structure of the microscopic world and even the dynamics lying within. After hundreds of years of development, many modalities of optical microscopy have flourished since their debut, catering to demands of imaging different kinds of objects or

samples. Excitation light is the prerequisite for optical microscopy. The wavelengths covering UV, visible and infrared have all been demonstrated for optical microscopy.

Recently, there has been rising interest in performing optical microscopy of biological tissues at the 1700 nm window. This is due to the fact that at this wavelength, tissue absorption due to water

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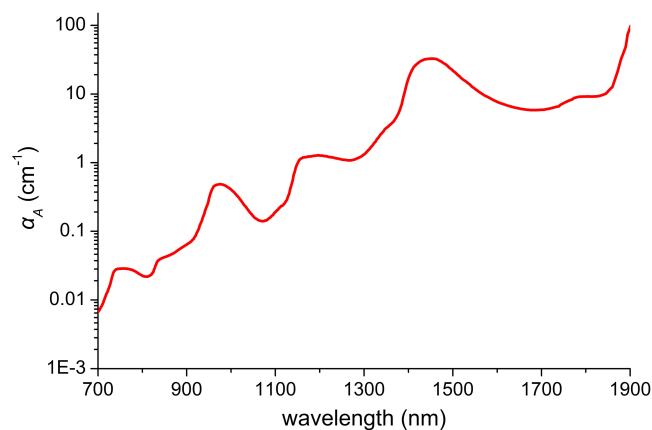


Fig. 1. Water absorption coefficient at different wavelengths.¹

absorption (most of the biological tissues comprise water) reaches a local minimum (Fig. 1),¹ and biological tissue scattering is also reduced due to the longer excitation wavelength compared with shorter wavelengths.² This combination leads to a reduction of the loss of excitation light during propagation in biological tissues. Consequently, this window is especially useful for deep-tissue optical microscopy and several modalities of deep-tissue optical microscopy have been demonstrated at this window. For example, long-wavelength optical coherence tomography (OCT) at 1700 nm enhanced imaging depth compared with widely used 1300 nm in various samples including human finger tips.³ Photoacoustic imaging at 1730 nm allows three-dimensional (3D) mapping of intramuscular fat with improved contrast and of lipid deposition inside an atherosclerotic artery wall in the presence of blood.⁴ 3-photon microscopy at 1700 nm enabled deep-tissue imaging into the hippocampus of living mouse,² revealing pyramidal neurons which could only be accessed through invasive techniques before.^{5,6} Furthermore, although not targeting deep-tissue microscopy, the low attenuation at this window even allows 4-photon fluorescence microscopy in mouse brain *in vivo*.⁷

Heating at the focus due to linear water absorption of excitation laser is always a concern when performing optical microscopy of biological tissue. Excessive temperature rise may lead to tissue damage. To estimate temperature rise at the focus for imaging, Schönle *et al.* performed theoretical investigation based on time-dependent heat equation.⁸ Their results indicate that irradiation with 100 mW of 850 nm light for 1 s leads to only 0.2 K

increase in temperature at the focus. As a result, heating under similar conditions in optical microscopy may not cause notable temperature rise.

Compared with excitation at shorter wavelengths, such as multiphoton microscopy at the 800 nm Ti: Sa mode-locked laser wavelength⁹ and 1040 nm Yb³⁺ fiber laser wavelength,¹⁰ OCT and photoacoustic imaging at 1300 nm wavelength,^{3,4} 1700 nm suffers from much larger water absorption. For example, water absorption coefficient $\alpha_A = 5.89 \text{ cm}^{-1}$ at 1700 nm, 268 times larger than that at 800 nm ($\alpha_A = 0.022 \text{ cm}^{-1}$) and 4.5 times larger than that at 1300 nm ($\alpha_A = 1.32 \text{ cm}^{-1}$). Consequently, it can be expected that more temperature rise will be induced for optical microscopy at this window. However, thus far a quantitative estimation of temperature rise at this window is lacking.

In this paper, based on the model proposed by Schönle *et al.*⁸ we performed numerical simulation of temperature rise due to water absorption at the focus of objective lens at the 1700 nm window. We obtained quantitative results for different irradiation time, power and numerical aperture (NA) of the objective lens. Temperature distribution, measured as the full-width-at-half-maximum (FWHM) values along both the axial and radial directions, was also calculated to estimate the spatial scales to which this temperature rise may influence the biological samples. Based on these results, we do not expect that tissue damage could be induced under practical experimental conditions, due to the $<1 \text{ K}$ temperature rise caused by water absorption.

2. Theoretical Model and Simulation Details

In accordance with (8), the governing equation for calculating time- and space-dependent temperature rise $\Delta T(r, t)$ is

$$(\partial_t - C\nabla^2)\Delta T(r, t) = f(r, t), \quad (1)$$

where $C = \Lambda/c_V$, and $f = p/c_V$. $\Lambda = 0.6 \text{ W/K/m}$ and $c_V = 4.17 \times 10^6 \text{ J/K/m}^3$ are heat conductivity and volume heat capacity of water, respectively, and p denotes the absorbed optical power per unit volume. In our simulation, absorption coefficients at different wavelengths are from (1). We followed the same simulation procedures in (8).

3. Simulation Results

First we quantitatively investigate the time-dependent heating at the focus of objective lens of different NAs at 1700 nm. Figure 2 shows that, as more power is deposited through focusing, the temperature at the focus keeps rising. Due to the relatively high water absorption, after only 10 ms for 20 mW irradiation, the temperature rises by 12.4 K with an NA = 1.05 objective lens. In contrast, for excitation at 850 nm, even after 1 s for 100 mW irradiation, the temperature rise was calculated to be only 0.23 K.⁸ This order of magnitude difference is purely due to the high water absorption at 1700 nm.

In (8), the authors introduced normalized form of Eq. (1), from which we learn that for a certain irradiation time, the calculated temperature rise is linear on power. This means for 10 ms of irradiation at 1700 nm, temperature rise will be 1.9 K for 3 mW irradiation, and 31 K for 50 mW irradiation.

Figure 2 also shows NA-dependent temperature rise. The overall trend is, when irradiation time is short, a tighter focusing (corresponding to a larger NA) induces a larger temperature rise. For example, after 1.28 μ s irradiation, temperature rise focused with NA = 0.65 objective lens is 1.09 K, 1.7 times smaller than that focused with NA = 1.05 objective lens ($T = 1.84$ K). As irradiation time increases, focusing with a higher NA objective lens will finally result in a slightly smaller temperature rise than with a smaller NA objective lens. For example, after 10 ms irradiation, temperature rise focused with NA = 0.65 objective lens is 12.8 K, 3% higher than

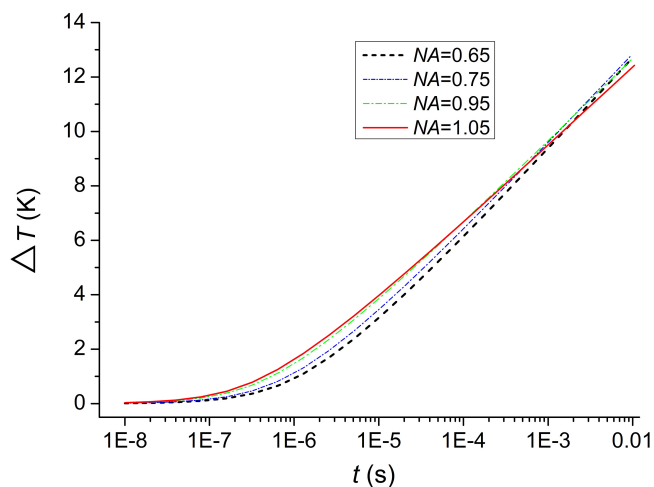


Fig. 2. Simulated temperature rise vs time for different NAs of the focusing objective lens. The excitation light is 20 mW at 1700 nm.

Estimation of temperature rise at the focus of the objective lens

that focused with NA = 1.05 objective lens ($T = 12.4$ K). We note the same complicated trend, although not plotted against time, was also partly shown by (8) for focusing at 850 nm (Table 1 in (8)).

According to Fig. 1, the water absorption at the 1700 nm window is relatively low. So previously two groups performing MPM² and photoacoustic imaging,⁴ respectively, both suggested 1600–1850 nm as the window for deep-tissue imaging. Next we investigate temperature rise for various wavelengths at this window. From Fig. 3 we can see that, at this window, 1700 nm excitation leads to minimum temperature rise at the focus due to water absorption. After 10 ms irradiation, temperature rises at 1600 nm (16.4 K) and 1850 nm (22.6 K) are 32% and 82% higher than that at 1700 nm, respectively. This suggests that at the 1700 nm window, if heating due to water absorption is to be minimized, 1700 nm excitation laser is preferred. Comparing temperature rise with water absorption coefficients at this window, we note that they agree in trend, i.e., the larger the absorption coefficient, the higher the temperature rise. So we can qualitatively infer relative temperature rise by simply comparing the magnitude of water absorption coefficients.

Heating by water absorption induces temperature rise not only at the focus, but also within the whole beam profile. Figure 4 shows that as irradiation time increases and more power is absorbed, temperature rise spreads to the peripheral of the focus, leading to non-negligible heating to the perifocal area. Since the biological structures to be imaged have finite size rather than an infinitesimal

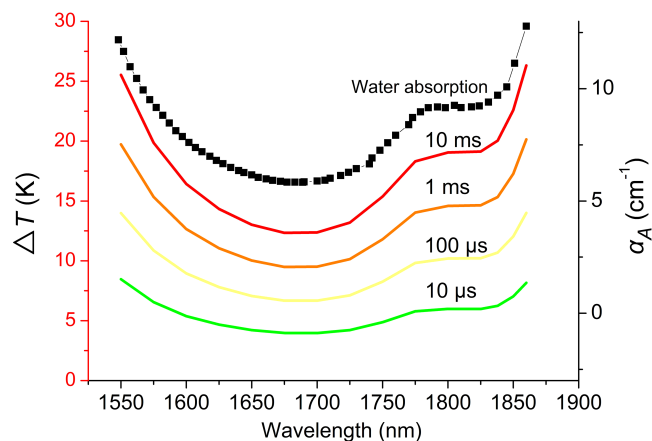


Fig. 3. Temperature rise vs wavelength for different irradiation time (left axis). Water absorption coefficient vs wavelength is also shown for comparison (right axis). The excitation light is 20 mW.

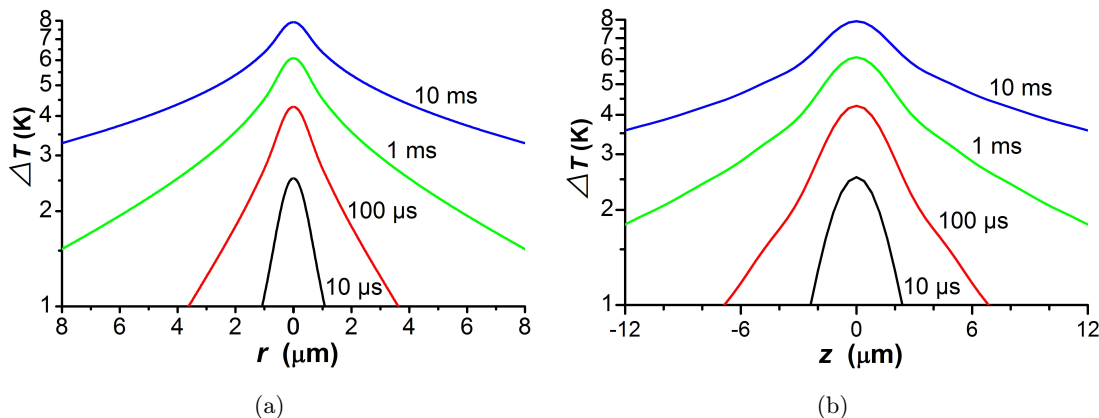


Fig. 4. Temperature rise after different irradiation time along the radial (a) and axial (b) axes. The excitation light is 20 mW at 1700 nm. NA = 1.05.

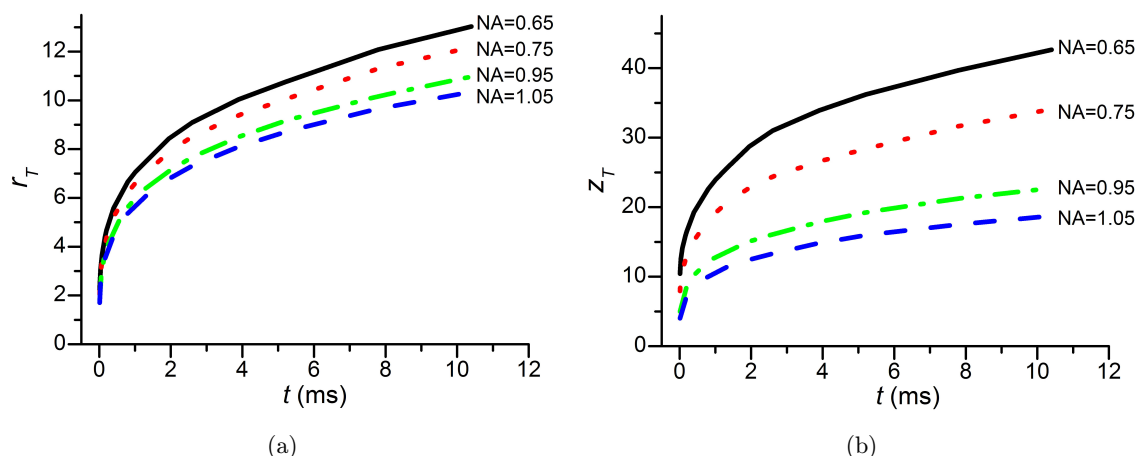


Fig. 5. Calculated FWHMs of temperature rise along both the radial (r_T) and axial (z_T) axes vs irradiation time, for various NAs as an estimation of temperature distribution. The excitation light is 20 mW at 1700 nm.

point, e.g., neurons are typically tens of microns in size and blood vessels are typically several microns in diameter, it is desirable that we know the extent to which this heating may affect the sample. To quantitatively estimate this temperature distribution, we calculated FWHMs of temperature rise along both the radial (r_T) and axial (z_T) axes. Figure 5 shows calculated results for different NAs of the objective lens. We can clearly see that as irradiation time increases, both r_T and z_T increase, affecting more and more perifocal area. Furthermore, the larger the NA, the smaller r_T and z_T . For example, after 1 ms irradiation, $r_T = 5.7 \mu\text{m}$ and $z_T = 10.5 \mu\text{m}$ for NA = 1.05, 81% and 44% of those for NA = 0.65, respectively. This means focusing with a higher NA objective lens induces less heating of the perifocal area. Physically, we attribute this smaller temperature distribution for higher NA

objective lenses to tighter focusing, i.e., both smaller focal size and smaller confocal length.

4. Conclusion and Discussion

In this paper, we have performed theoretical investigation of temperature rise at the focus of objective lens at the 1700 nm window due to water absorption. Compared with conventional irradiation at the Ti: Sa wavelength, excitation at the 1700 nm leads to order-of-magnitude temperature rise due to the larger absorption coefficients. If we compare different wavelengths at the 1700 nm window from 1600 to 1850 nm, temperature rise qualitatively follows the trend of the absorption curve. Furthermore, the smaller the NA of the objective lens, the more perifocal area will be affected by the heating.

However, this does not mean that 1700 nm is not suitable for tissue imaging due to heating by water absorption. We note that: (1) Experimentally, 3 mW on the sample was sufficient for acquiring a 512×512 image after 8 s exposure, corresponding to $30.5 \mu\text{s}$ irradiation time. The calculated temperature rise is only 0.79 K for $\text{NA} = 1.05$ objective lens. One concern is the transient heating due to each laser pulse especially for the low repetition rate (such as 1 MHz) laser system, as pulse energy is significantly higher than the high repetition rate ones. Based on the formula given in Ref. 8, the estimated temperature rise due to transient heating of a 1 MHz, 3 mW, 1700 nm pulse is 0.51 K. Another concern is the temperature accumulation effect due to the finite width of heating (Fig. 4), which leads to the heating of adjacent pixels and a further increase in temperature for the pixel to be imaged. An upper limit estimation can be given: The line dwelling time is ~ 15 ms. If we assume the whole line is dwelled on one pixel, then the temperature rise due to this 3 mW, 15 ms irradiation is 1.9 K. In reality, there are hundreds of pixel spanning hundreds of microns across the field of view, so the temperature rise in the presence of adjacent heating is < 1.9 K. (2) For live animal imaging, blood circulating the body will dissipate heat as heat sinks. This effect is not considered in our simulation. So our calculation here is an upper-limit estimation. Based on these results and the parameters pertinent to in real experiment, we expect a smaller temperature rise due to heating at the 1700 nm window and do not expect heating could cause harm to the tissue for imaging applications such as MPM.

Experimentally, to quantitatively measure temperature rise, a potential method is to use a tiny temperature probe such as a thermocouple. For example, there are commercial thermocouples with lateral dimensions on the order of $100 \mu\text{m}$. Although they may not be used to resolve the exact temperature distribution on the focal point and microsecond level, they may, however, give an estimation of the temperature rise on the entire field of view with second temporal resolution.

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