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Abstract. With the use of longer near-infrared (NIR) wavelengths, image quality can be increased due to less scattering (described by the inverse wavelength power dependence $1/\lambda^n$ where $n \geq 1$) and minimal absorption from water molecules. Longer NIR windows, known as the second (1100 nm to 1350 nm) and third (1600 to 1870 nm) NIR windows are utilized to penetrate more deeply into tissue media and produce high-quality images. An NIR supercontinuum (SC) laser light source, with wavelengths in the second and third NIR optical windows to image tissue provides ballistic imaging of tissue. The SC ballistic beam can penetrate depths of up to 10 mm through tissue.

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Keywords: near-infrared light; scattering; tissues; supercontinuum laser; ballistic light; near-infrared imaging; imaging through turbid media; near-infrared therapeutic window; second and third optical windows.

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Images of turbid media, such as tissue, can have a reduction of image contrast from scattering (which causes blurring) and from absorption of light (which reduces the number of photons) by structures such as biomolecules (water, collagen and elastin, lipids, hemoglobin, and deoxyhemoglobin).[1,2] Scattering of light causes images to blur, while absorption of light by biomolecules will remove photons and cause fewer photons to reach the detector. At different regions along the electromagnetic spectrum, a reduction in the absorption of light by water, deoxyhemoglobin, and oxyhemoglobin, as well as in photon scattering can occur.[3] Near-infrared (NIR) light at wavelengths in the first NIR optical window (from 650 to 950 nm), known as the therapeutic window, is absorbed minimally by water molecules, and by oxygenated and deoxygenated hemoglobin. Thus, it can be used to reach greater penetration depths through tissue than in the visible range, resulting in higher-quality images.[4–7] This window is used mainly for phototherapy and can be imaged by silicon based detectors (for wavelengths <1000 nm). With the arrival of new photo imagers, such as indium gallium arsenide (InGaAs) (for wavelengths up to 1700 nm) and indium antimonide (for wavelengths longer than 1700 nm), novel imaging opportunities can be realized at longer NIR wavelengths. Smith et al. used advanced IR-CCD detectors and enhanced fluorescence from nanotubes, with longer NIR wavelengths from a second NIR window (1000 to 1350 nm), to image small animals *in vivo*.[8] Most recently, Sordillo et al used the IR-CCD InGaAs detector with a halogen lamp light source and showed that there are additional NIR windows suitable for imaging more deeply into tissue.[9,10] In this study, optical attenuation measurements from thin tissue slices of normal and malignant breast and prostate tissue, pig brain, and chicken tissue were obtained using the first, second, and third optical windows, in the spectral range from 650 to 2500 nm, and a fourth optical window centered at 2200 nm was also noted.[9] Total attenuation lengths (l_t) of light through these tissue samples were calculated. It was found that longer attenuation lengths occur with the second and third NIR optical windows than with the conventional first window. Table 1 shows the total attenuation lengths (l_t) from chicken tissue at wavelengths of 750, 1200, 1700, and 2200 nm (corresponding to the four NIR optical windows), using a halogen lamp light source and an IR-CCD InGaAs detector.[9]

Scattered light in tissue is wavelength dependent due to Rayleigh scattering (which varies as the inverse fourth power of the wavelength dependence) and Mie scattering ($1/\lambda^n$ with $n \geq 1$ dependence at longer wavelengths). Due to less scattering and minimal water absorption at longer NIR wavelengths, we concluded that the second and third NIR optical windows could be used to produce high-contrast images and to reach more deeply through layers of tissue.

In this study, the supercontinuum (SC) light source with wavelengths in the second and third NIR optical windows (from 1100 to 1350 nm and from 1600 to 1870 nm, respectively) was used with an NIR InGaAs CCD detector to reveal high-quality images with abnormalities (three black wires) hidden beneath thick layers of tissue. Optical images of tissue overlying black wires were also obtained using an InGaAs camera detector and light from a halogen lamp. The Leukos model STM-2000-IR SC laser light source, with optimized NIR emission and a total spectral range of 600 to 2500 nm, delivers 500 microwatt/nm of power at the second and third NIR windows and provides a greater number of outgoing photons compared to a conventional lamp light source. Transmission images were obtained using the optical setup shown in Fig. 1. This type of imaging technique (transmission imaging) has been applied to transillumination medical analysis and biological structural diagnosis. Transmission

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Table 1 Optical properties l_t (μm) from chicken tissue in the four (I, II, III, IV) optical windows.

Total attenuation lengths l_t (μm)			
I	II	III	IV
90	125	120	94

imaging utilizes one path to give information on the depth of penetration and is considered better at assessing depth than reflection imaging. Reflection imaging can also be used to show and locate abnormalities in the tissue. An IR-CCD InGaAs camera (Goodrich Sensors Inc. high-response camera SU320-1.7RT-D, Princeton, New Jersey) with spectral response from 900 to 1700 nm, and 1200 nm bandpass and 1500 nm longpass filters (representing the second and third windows) (Fig. 1) were also used. The detector has a fixed exposure time of 16.3 ms. Filter 2 was placed before the detector in a light blocking chamber to reduce noise and to isolate the desired wavelengths entering the detector.

Chicken tissue was sliced in increments of ~1 mm from ~2 to ~10 mm in thickness and placed (away from the detector) behind the three wires of thicknesses 1.56, 1.21, and 0.65 mm. Figure 2 shows the images of chicken tissue and the three wires using the Leukos SC laser and the conventional lamp source and wavelengths in the second (II) and third (III). The images were fitted with an ImageJ processing and analysis program. At a tissue thickness of ~10 mm, images acquired from the Leukos SC laser source with wavelengths in the second and third NIR optical windows show chicken tissue and the three wires. The ImageJ processing program was used to acquire a plot profile of the intensities (signal and background) over some distance along the image. From these results, the degree of contrast was calculated as the intensity of the signal minus the intensity of the background divided by the intensity of the signal plus the intensity of the background times 100. These results are shown in Figs. 3(a) and 3(b).

Optical attenuation measurements from a 200-micron chicken tissue were also obtained in the spectral range from 400 to 2500 nm. Figure 4 shows the total attenuation length (l_t), which was acquired from the total attenuation coefficient (μ_t) [defined by the absorption (μ_a) plus the scattering (μ_s) coefficients] and calculated using Eq. (1):

$$\mu_t = \frac{(2.303 \times \mathrm{OD})}{z}, \tag{1}$$

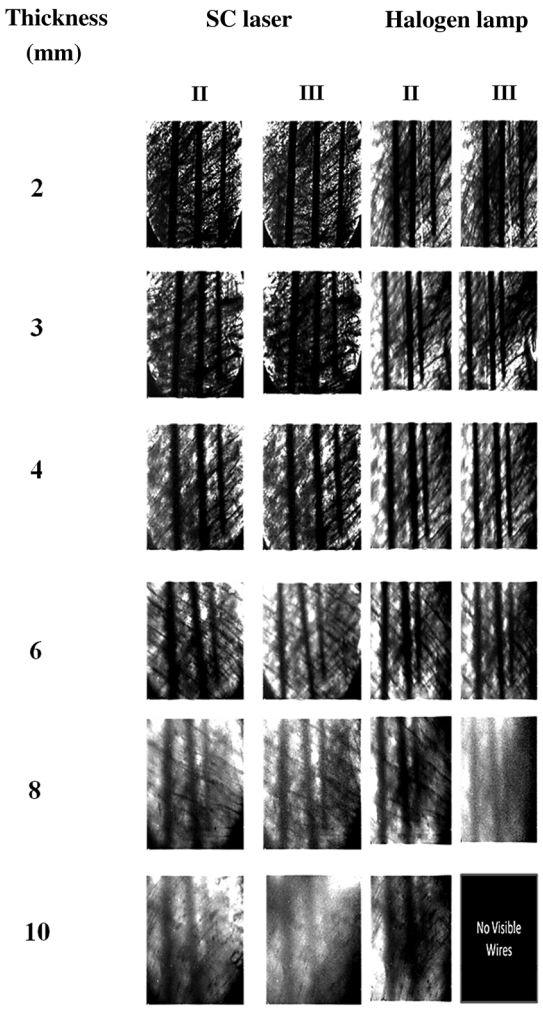

Fig. 2 Images of chicken tissue at various thicknesses (from ~2 to 10 mm) and three wires from the SC laser and halogen lamp light sources, using wavelengths from the second (II) and third (III) optical windows.

where OD corresponds to the optical density results from the tissue, z is the thickness of the tissue sample, and μ_t is derived from the Lambert-Beer's equation.

Unlike the halogen lamp source, the images of abnormalities acquired using the Leukos SC laser light source are visible at penetration depths of ~10 mm through tissue. As the thickness of the tissue increases, higher percent contrast results are also

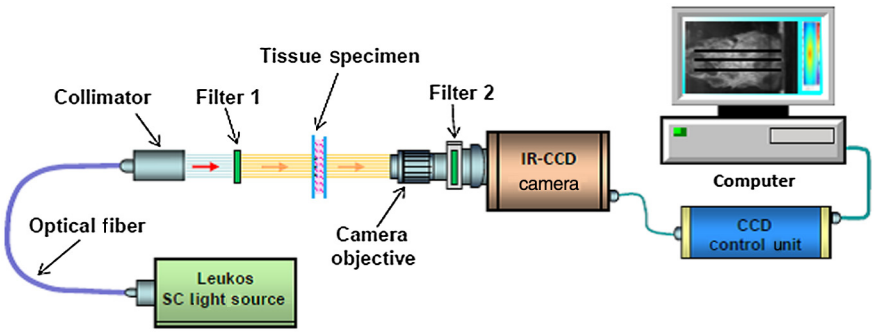

Fig. 1 Optical setup for optical imaging of tissue using selective filters 1 and 2 (1200 nm narrow-band and 1500 nm longpass) and wavelengths from the second and third windows from the Leukos supercontinuum (SC) light source.

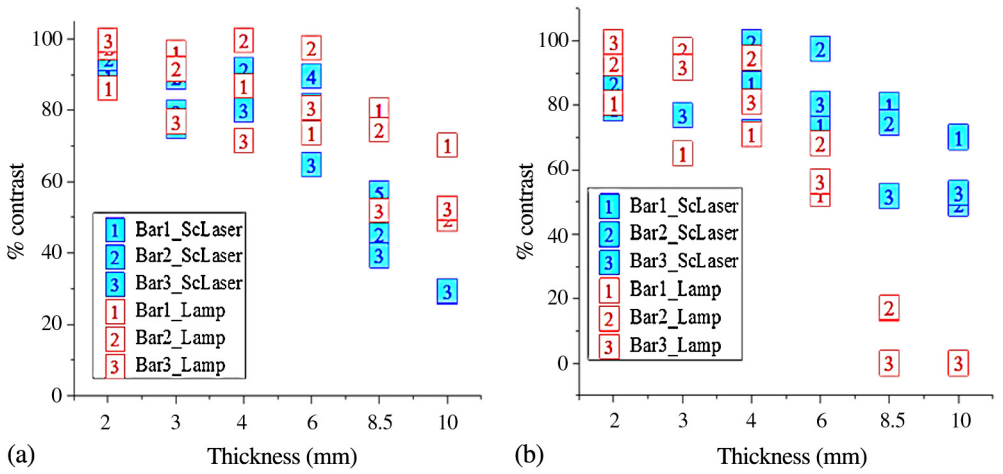

Fig. 3 Percent contrast results from images of chicken tissue and the three wires (marked as bars 1, 2, 3) using wavelengths from (a) the second and (b) third optical windows.

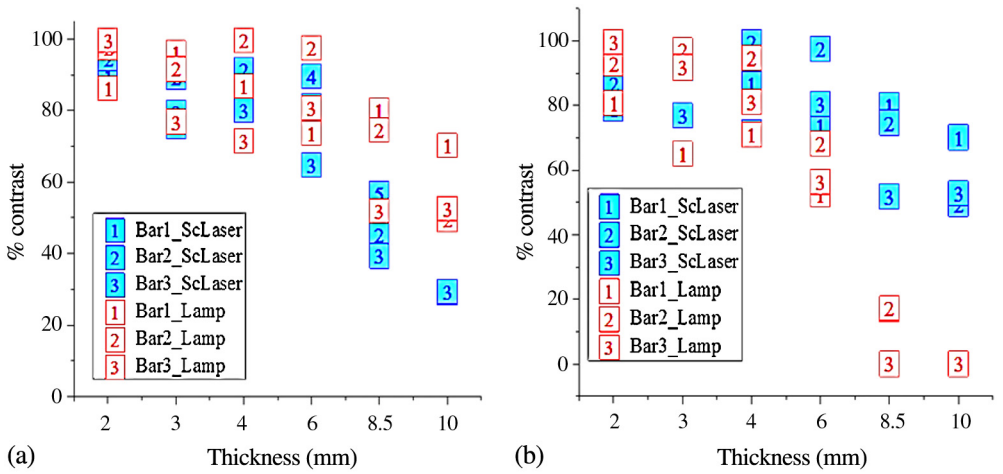

Fig. 4 Spectra of the total attenuation length (l_t) from chicken tissue in the I, II, III, and IV optical windows.

seen when using the SC laser in the third NIR window compared to the lamp source. The Leukos SC laser with wavelengths in the second and third NIR optical windows can be used to reach greater depths (>10 mm) through tissue and is an ideal light source for imaging through thick tissue. Overall, our ability to visualize structures is limited by the noise level. Signal-to-noise ratio (SNR) is an essential technique that can be used to statistically measure how much noise is present.[11] Increasing the exposure time will result in signals that are less corrupted by noise (higher SNR) and will improve image quality.

Acknowledgments

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