

Quantitative Measurements of Scattering and Absorption by Low Coherence Spectroscopy

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Abstract: The accuracy of non-invasive spectroscopic absorption measurements in tissue is limited by lack of knowledge about the optical path length the light has travelled in the tissue. Low Coherence Spectroscopy (LCS) enables quantitative, path-length resolved measurements of absorption in scattering media, by combining reflection spectroscopy with low coherence interferometry.

This poster describes the *in vitro* proof of principle for an LCS system that operates in the 480 – 750 nm wavelength range. Absorption and scattering coefficients were measured quantitatively in media with varying absorption and scattering properties, comparable to tissue.

We conclude that LCS gives accurate, quantitative values of absorption and scattering coefficients in scattering media. LCS is a promising technique for the *in vivo* determination of tissue chromophores.

Background

Numerable physiological disorders are diagnosed by laboratory analysis of blood. This is an invasive and time consuming procedure, delaying time between probing and treatment. Complications as a result of invasive blood sampling (stress, infections) are often observed in preterm neonates, who require close monitoring of body function to prevent hyperbilirubinemia (an excess of blood bilirubin levels), anemia (a shortage of red blood cells) and hypoxia/hyperoxia (a shortage/access of tissue oxygen levels). If a non-invasive real-time analysis of blood could be developed, it would overcome these complications.

Optical spectroscopic techniques possibly offer a faster and non-invasive alternative to laboratory blood analyses, because of the ability to measure absorption and scattering coefficients of tissue which are, in turn, related to physiological states in biological systems. Although spectroscopic techniques are available for this purpose, most of them only measure the presence of tissue chromophores qualitatively. The accuracy of quantitative spectroscopic measurements of tissue chromophore concentrations is limited by lack of knowledge about the path length the light has travelled in tissue. To overcome this limitation, we developed a new technique called Low Coherence Spectroscopy (LCS). In LCS, low coherence interferometry and absorption spectroscopy are combined for quantitative, path-length resolved measurements of absorption properties in the visible wavelength region (480 – 750 nm).

Previous attempts to develop methods for quantitative spectroscopy involved spectroscopic optical coherence tomography (sOCT), which combines spectroscopy with high resolution imaging. To image at sufficiently high depths, sOCT requires wavelengths in the near-infrared (around 850 or 1300 nm). A disadvantage of this wavelength region is that it contains little spectral features of important tissue chromophores such as bilirubin and hemoglobin. In addition, spectral resolution is often sacrificed for high spatial resolution to produce high quality images. Therefore, the interest of most sOCT studies lies within contrast enhancement, instead of quantitative absorption spectroscopy.

Objectives

We developed an LCS system for the visible wavelength region with spectral and path length resolutions of 6 nm and 30 μm , respectively. The purpose of this study was to present *in vitro* proof of principle of our LCS system.

Absorption and scattering coefficients of media with varying absorbing and scattering properties were measured by LCS and verified with conventional transmission and reflection spectroscopy and Mie theory.

Materials & methods

LCS system

Our LCS system consists of a standard open air Michelson interferometer with an oscillating mirror in the reference arm. The single mode light from a supercontinuum light source (SC430-4, Fianium Ltd., UK) with a spectral bandwidth of 430 – 2500 nm is spectrally filtered and in combination with the detector response (2001, New Focus, USA) reduced to a bandwidth of 480 – 750 nm. A 50:50 beam splitter splits the source light into a sample and a reference arm and a graded index multimode fiber is used to guide the reflected light from both arms to a photodiode and lock-in amplifier.

The amplitude of the oscillating reference mirror produces a scanning window of 30 μm , which is used to obtain a back scattered spectrum from the sample. This results in path length and spectral resolutions of 30 μm and 6 nm, respectively. With a path length resolution of 30 μm , the sample is translated with respect to the sample focus to select the path length in the sample. Focus tracking is applied by adjusting the length of the reference arm in correspondence to the sample displacement.

Measurements

Back scattering was measured as a function of path length in varying concentrations of Intralipid[®] in water. The measurements were repeated for varying concentrations of absorbers (hemoglobin, ecoline green dye) in the Intralipid[®] solutions.

For each sample, a transmission and reflection spectrum were obtained by conventional transmission/reflection spectroscopy.

Data analysis

LCS power spectra of the back scattered light from the sample were obtained by Fourier transformation of the time signal on the detector. All LCS spectra were averaged 250 times.

The spectral area $I(\lambda)$ was calculated for wavelength regions λ of 6 nm in width between 480 and 750 nm. Beer's law was used to calculate the attenuation coefficient of the sample:

$$\mu_t(\lambda) = -\ln\left(\frac{I_t(\lambda)}{I_0(\lambda)}\right) \frac{1}{\ell} \quad (1)$$

Where $I_t(\lambda)$ is the spectral area as a function of wavelength (spectrum) at path lengths ℓ from 50 to 1000 μm in the sample, and $I_0(\lambda)$ is the spectrum for normalization. For $I_0(\lambda)$, the back scattered spectrum at a path length of 50 μm in the sample was used – assuming that little absorption and scattering events have occurred for this path length in the absorption and scattering range of our samples.

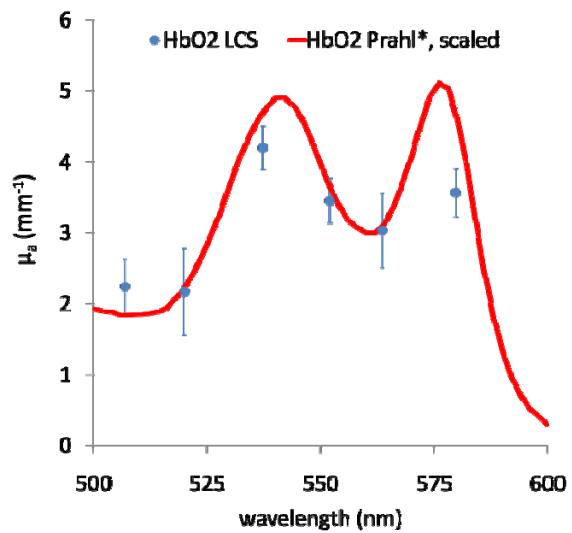
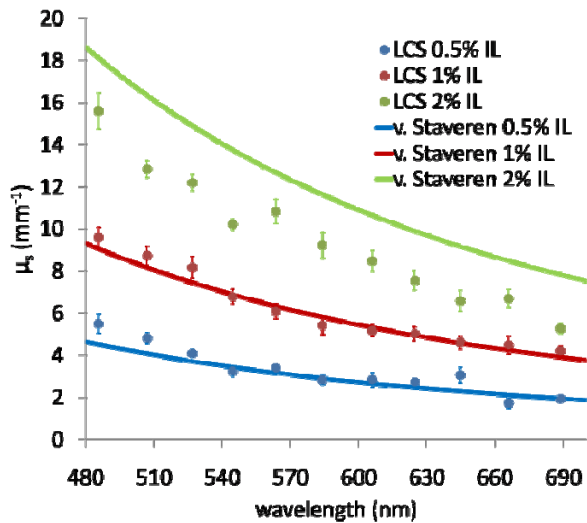
For the single scattering model in eq. 1, attenuation coefficients were averaged over the single scattering regime of the sample to obtain an average attenuation coefficient. For the scattering coefficients of the Intralipid[®] solutions without absorbers, we assume $\mu_t(\lambda) \approx \mu_s(\lambda)$. For the absorption coefficients of the Intralipid[®] solutions with absorbers, we assume $\mu_a(\lambda) \approx \mu_t(\lambda, \text{Intralipid}^{\text{®}} + \text{absorber}) - \mu_t(\lambda, \text{Intralipid}^{\text{®}})$.

Absorption and scattering coefficients were compared to those obtained by conventional transmission/reflection spectroscopy and Mie theory.

Results

- LEFT: scattering coefficient vs. wavelength between 480 and 700 nm for Intralipid[®] concentrations of 0.5%, 1% and 2%. Dots: LCS values, solid lines: Mie theory by Van Staveren (1991, Appl. Opt.). For the 2% Intralipid[®] solution, the LCS values for the scattering coefficient are lower than the predictions by Van Staveren. This may be caused by the detection of multiple scattered photons while we use a single scattering model for our data analysis.
- RIGHT: absorption coefficient vs. wavelength between 500 and 600 nm for oxyhemoglobin in 1% Intralipid[®]. Dots: LCS values, solid lines: scaled oxyhemoglobin spectrum from Prahl (Oregon Medical Laser Center). The absorption coefficient measured by LCS is slightly lower than the absorption coefficient expected from the concentration of oxyhemoglobin that was used.

(NB: wavelength regions were binned for the LCS data, resulting in wavelength resolutions lower than 6 nm.)



Discussion

The value of the attenuation coefficient is influenced by:

- errors in estimation of the refractive index used for focus tracking in the sample
- sample preparation; uncertainties in concentrations and possible interaction between Intralipid® and oxyhemoglobin
- use of a single scattering model in our data analysis

These factors may cause discrepancies between the measured value of the attenuation coefficient by LCS and the values expected from literature.

Conclusions

- We have shown quantitative measurements of scattering coefficients as a function of wavelength by LCS, corresponding to literature values.
- We have shown quantitative measurements of absorption coefficients as a function of wavelength by LCS.

Outlook

We will show results of the characterization of the system properties: signal to noise ratio and accuracy as a function of scattering and absorption coefficient. Also the use of a multiple scattering model for the data analysis will be presented.

We will extend our measurements to other scatterers, absorbers and to solid media.