Validation of Hemoglobin and Water Molar Absorption Spectra in Near-Infrared Diffuse Optical Tomography

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ABSTRACT

Near Infrared diffuse optical tomography was used to measure the molar absorption spectra for de-oxyhemoglobin, oxyhemoglobin and water through phantom experiments. The measured spectra compensate for any systemic errors and errors arising from model-based estimation of concentrations of these chromophores, and show reasonable agreement with existing literature values. The spectra were validated by phantom experiments to obtain the oxygen dissociation curve. Variation of the partial pressure of oxygen in solution yielded results consistent with theory with mean error of 7.6% in estimation of oxygen saturation, and accurate within 5% in estimation of hemoglobin.

1. INTRODUCTION

The relatively good transparency of tissue in the red and near infra-red (NIR) spectrum (600-1000nm) allows light signals to penetrate up to a dozen centimeters into the tissue. The light interaction with tissue can be quantified in terms of optical parameters such as absorption and scattering coefficients, which are found by making suitable measurements (frequency domain or time-resolved techniques), and applying a suitable model-based reconstruction method [1]. The absorption of tissue is related to the concentration of the main chromophores present in the tissue, such as oxyhemoglobin, de-oxyhemoglobin and water. Hence, quantitative assessment of the hemoglobin and oxygen saturation in the tissue is made possible. The scattering is related to the scatterer size and number density [2] and this gives information about the structure of the tissue. The focus of this paper is on validating the ability to quantify regions of oxygen saturation.

This technique has been applied to study breast physiology in recent times [3-8]. The absorption of near infra red light by breast tissue is primarily due to de-oxyhemoglobin, oxyhemoglobin and water. Knowing the spectral features of these chromophores in the wavelength band of interest, and by obtaining the absorption coefficients through measurements on the breast and through image reconstruction, it is possible to quantitatively determine the hemoglobin, oxygen saturation and water maps of the breast. This gives valuable information about the breast in both its normal and diseased state. Pregnancy, age, and hormonal factors result in changes in the physiological state of the breast tissue which can be monitored noninvasively using NIR. During the menstrual cycle, blood flow can increase by 50% at time of ovulation(midcycle) and remains high until menstruation [9]. Shah et al[10] showed an increase in de-oxyhemoglobin of up to 48.3% and in water of up to 28.1% during the luteal phase. During pregnancy, by the time the baby is born, the glandular breast volume is more dominant than that of the compressed fibrous and fat stroma [9]. Using information from absorption and scattering, NIR may be able to track and quantify variations in the breast physiology occurring from these hormonal fluctuations.

Neovascularization in the tumor results in increased hemoglobin; and the source of contrast for diagnosing tumors in NIR imaging of breast tissue is believed to be the increase in absorption due to increased

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concentration of the total hemoglobin in tumor location. Several studies have shown this contrast of up to 2:1 in vivo breast cancer studies [4,11,12, 13].

On an average, the mean partial oxygen pressure (pO_2) values are lower in malignancies than in surrounding normal tissues, and there is a larger hypoxic fraction within tumor tissue [14]. In malignancies, the pO2 values are typically <= 20mm Hg [14]. Since the oxygen saturation of the tissue is related to the partial pressure of oxygen in the tissue by the Hill or oxygen dissociation curve, measurement of the Hill curve can give a good idea of the sensitivity of the imaging system to low pO2 values. In NIR tomography, quantitative oxygen saturation values used to provide information about the malignancy of a tumor is yet to be investigated in detail.

The spectral signatures of the main absorbers in breast tissue (which are assumed to be de-oxyhemoglobin, oxyhemoglobin and water) enable the calculation of the chromophore concentrations from the absorption coefficients. Several studies have been published on molar absorption spectra of hemoglobin, as will be reviewed here. Wray et al [15] found the near infrared hemoglobin spectra at various oxygenation levels from cuvette studies of lysed human red cells. This spectra has also been measured by Cope [16], in a similar way, in his Ph.D thesis. Zijlstra et al [17] have determined the millimolar absorptivities of the clinically relevant derivatives of fetal and adult human hemoglobin in the visible and near-infrared spectral range. The optical constants for water were found by Hale and Quarry.[18]. The extinction spectra for the chromophores of interest have been found in the past by different groups through chemical and clinical experiments, however, these literature values also differ from each other. For example, there is 11.7% difference between the values of Wray et al and Cope for the oxyhemoglobin spectra. The molar absorption coefficients would yield the chromophore concentrations without error, if the absorption coefficients are estimated without error, however, algorithms to convert light attenuation into corresponding concentrations, which seem to perform adequately in one system can apparently generate unrealistic results when transferred to another system as found by Matcher et al[19]. Most imaging systems introduce systemic errors in obtaining the absorption coefficients, which maybe either due to the measuring system or any reconstruction procedure. These errors have to be compensated for, and this can be done by using molar absorption spectra specific to the imaging system, which can be measured systematically by a simple procedure.

The aim of this study is to obtain the molar absorption measured within the imaging system and to obtain oxygen dissociation curve to establish the sensitivity of the imaging system to low oxygen pressures and validate the measured molar absorption spectra. In this study, all measurements have been done using a frequency domain diffuse tomography system with multiple source detectors in a circular configuration to image the whole uncompressed breast, and the optical parameters are obtained by carrying out a non-linear gradient based iterative reconstruction algorithm.

2. MATERIALS AND METHODS

2.1 Tomography System

The frequency domain data acquisition system is designed for cross sectional imaging of the pendant breast in three planes spaced 1cm apart. A 16 source-detector fiber system for each plane is set up in a radial configuration, and is automated so that both the height and the diameter of the ring can be changed. Light signals at six wavelengths between 660 and 850nm are intensity modulated at 100MHz and multiplexing of the source into each of the fibers is achieved by a circular translation stage. Light detection is done using high gain photo-multiplier tubes (PMTs) and heterodyning using mixers is carried out to obtain a low frequency signal (500Hz) which is read by the computer. Complete details of the instrumentation can be found elsewhere [20-22].

The measurements consisting of amplitude and phase undergo calibration[22] to account for any offsets or model-data mismatch. The calibrated data at each wavelength are reconstructed to give maps of absorption and scattering coefficients by using a finite element based calculation of the diffusion equation in frequency domain. Using least squares minimization of the difference between the measured and calculated fluence rate intensity and phase, absorption and scattering coefficients are obtained for the whole breast. The reconstruction procedure has been explained in detail in previous papers[23, 24, 31].

From the absorption coefficients and knowing the molar absorption spectra, the concentration of the chromophores (oxyhemoglobin-[HbO_2], de-oxyhemoglobin-[Hb] and water-[H_2O]) can be obtained by a linear least squares constrained fit to the equation

$$[\mu_a] = [\varepsilon\,][c] \qquad (1)$$

μ_a is a vector consisting of the absorption coefficients at six wavelengths for each point, and c is a vector of the concentrations of the three chromophores which is to be determined. ε is a 6X3 matrix containing the molar absorption spectra of the three chromophores at the six wavelengths. The total hemoglobin (Hb_T) is given by ($[Hb_T] = [HbO_2] + [Hb]$) and oxygen saturation (S_tO_2) is given by ($S_tO_2 = ([HbO_2] / [Hb_T]) \times 100$ %).

The relation between the reduced scattering coefficient μ_s', and wavelength (λ) is derived by an approximation to the Mie scattering theory given by [25 26],

$$\mu_s'(\lambda) = A\ \lambda^{-SP} \qquad (2)$$

where SP is the scatter power related to the scattering center size and number density, and A is the scatter amplitude, which also depends upon scatterer size and number density. These give further unique information about breast tissue structure

2.2 Phantom Experiments

To obtain accurate concentrations of the chromophores, it is necessary to have an accurate set of molar absorption spectra for the major absorbers, in this case, de-oxyhemoglobin, oxyhemoglobin and water. The molar absorption spectra have to be measured specifically within the system being used, to compensate for any systemic errors in the model-based estimation of concentrations. Phantom measurements are often performed on tissue-simulating objects, and a standard solution-based phantom is a mixture of Intralipid and blood [27]. The Intralipid provides Mie-like scatterer to simulate the light scattering from cells in the tissue [25]. Intralipid-based solutions were prepared with 1% lipid fraction, by volume, in sterile saline with whole blood added in varying concentrations. This solution was held in a plastic cup and positioned in the imaging array to mimic the scattering and absorption properties of the tissue. Measurements were carried out at each specific blood concentration level and then the blood concentration was increased successively between 0% and 1%, in steps of 0.2. By imaging the liquid phantom multiple times, we obtain the absorption coefficients at six wavelengths at each of the multiple blood concentrations, after reconstruction.

The same type of solution was prepared again and deoxygenated by addition of yeast, and the experiment was repeated for completely deoxygenated hemoglobin. The slopes from the graphs of μ_a versus blood concentration for oxygenated and deoxygenated solutions at each wavelength, yield the molar absorption coefficients for oxygenated and deoxygenated blood. These values are converted to the corresponding values for oxyhemoglobin and de-oxyhemoglobin by simply dividing by the hemoglobin concentration within the blood sample. This hemoglobin concentration was determined by measurement in a clinical co-oximeter system [28]. At each of the different wavelengths the molar absorption coefficient is then known. The molar absorption due to pure water is also given by extrapolation of the data to zero blood concentration at each wavelength. The experiments have been carried out four times for oxygenated and deoxygenated solution, to obtain repeatable data sets, and the mean has been used as the molar absorption spectra for the imaging system. Different sets of molar absorption spectra can also be obtained depending on the type of reconstruction used. Mainly, there are two types of reconstruction estimates used in this study.

1) Heterogeneous estimate: In this procedure, reconstruction is carried out iteratively as mentioned before to obtain maps of the absorption and scattering coefficients everywhere. The mean absorption coefficient of the whole image is used to obtain the molar absorption spectra. Ideally, the reconstruction should result in a homogeneous image for μ_a, However, because of the complex reconstruction problem, certain boundary artifacts are introduced in the image. Including these boundary nodal values in computing the mean for the absorption coefficient may introduce some error in its estimation. Hence a second way to obtain the homogeneous absorption coefficient is to calculate the absorption coefficient by specifying a region of interest in the image, which excludes the boundary artifacts, and taking the mean of the absorption coefficients in the region of interest. This yields a more accurate value for the absorption coefficient, which when used to calculate the molar absorption spectra, gives more accuracy.
2) Homogeneous estimate: In this procedure, a region based reconstruction is carried out where the phantom is considered as a single homogeneous region and reconstruction is carried out iteratively to calculate a global value for the absorption and scattering coefficients. The absorption coefficient obtained this way is quantitatively more accurate because it excludes any image artifacts or errors in region definition that may arise in the spatially-varying reconstruction and also makes use of the fact that the measurements have been done on a homogeneous phantom.

In this way, two different sets of absorption coefficients have been calculated, by using heterogeneous estimation with boundary artifacts correction, and homogeneous estimation. Using the processing explained, two sets of molar absorption spectra have been calculated, and are suitably used for studies depending on the type of reconstruction used for the study. The molar absorption spectra obtained this way vary slightly because each compensates for the error in the estimated parameters introduced by the particular reconstruction scheme.

Since the phantom under consideration is homogeneous, these types of reconstruction procedures can be used to generate the absorption coefficients.

2.3 Oxygen Dissociation curve

To test the sensitivity of the system to low pO_2 values, which are typically found in tumors, the next step was to find the oxygen dissociation curve (i.e. Hill curve) for our system. This involved obtaining the oxygen saturation values for the complete range of pO_2 values from 150mm to 0mm Hg. This was carried out by using a liquid phantom solution of 1% Intralipid and 1% whole blood in saline. The pO_2 was measured using a chemical microelectrode, after calibration of the electrode overnight in saline solution. By varying the pO_2 gradually, using a small amount of yeast and making measurements over the period of time, till the pO_2 reduced to zero, a complete set of data over the required range was obtained. Reconstruction of the data and use of the appropriate calculated molar extinction spectra depending on type of estimate used for the absorption coefficients, results in values of oxygen saturation, total hemoglobin and water concentration.

3. RESULTS

3.1 Molar Absorption Spectra

The molar absorption coefficients have been obtained by the slope-offset method described earlier. The absorption coefficients at 785nm, obtained from measurements on a completely oxygenated homogeneous phantom, are plotted against blood concentration in figure 1. The slope from the linear fit to the data points is divided by the hemoglobin content measured to give the molar absorption coefficient for oxy-hemoglobin at 785nm and the offset shown is the absorption due to water at 785nm. The absorption coefficients in figure 1 have been determined using the homogeneous estimate scheme. In this way, the values are obtained at all six wavelengths, and for both oxygenated and deoxygenated hemoglobin phantom solution.

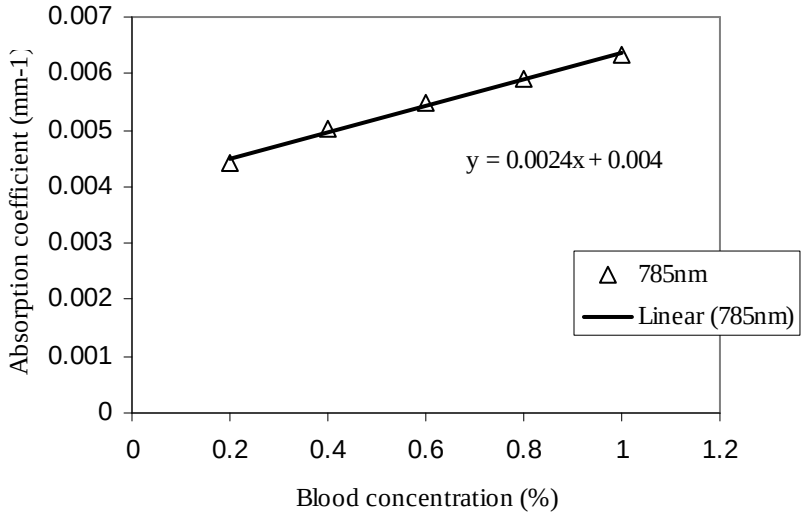
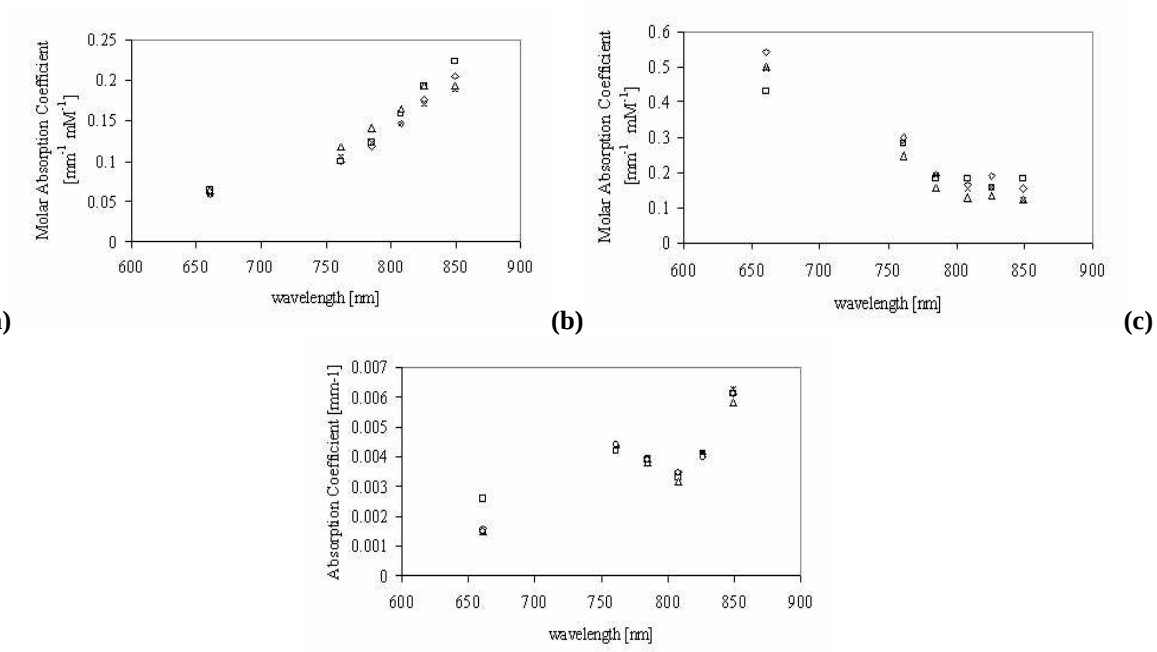


Figure 1. Absorption coefficients at 761nm obtained by measurements on fully oxygenated phantom solution with increasing amounts of whole blood.



(a) **(b)** **(c)**

Figure 2(a). Measured molar absorption spectra of oxyhemoglobin, calculated from tissue-simulating phantom experiments with increasing amounts of whole blood. **(b)** Molar absorption spectrum of de-oxyhemoglobin, calculated in the same manner, and **(c)** absorption spectra of 100% water, found from the offset in the phantom experiments.

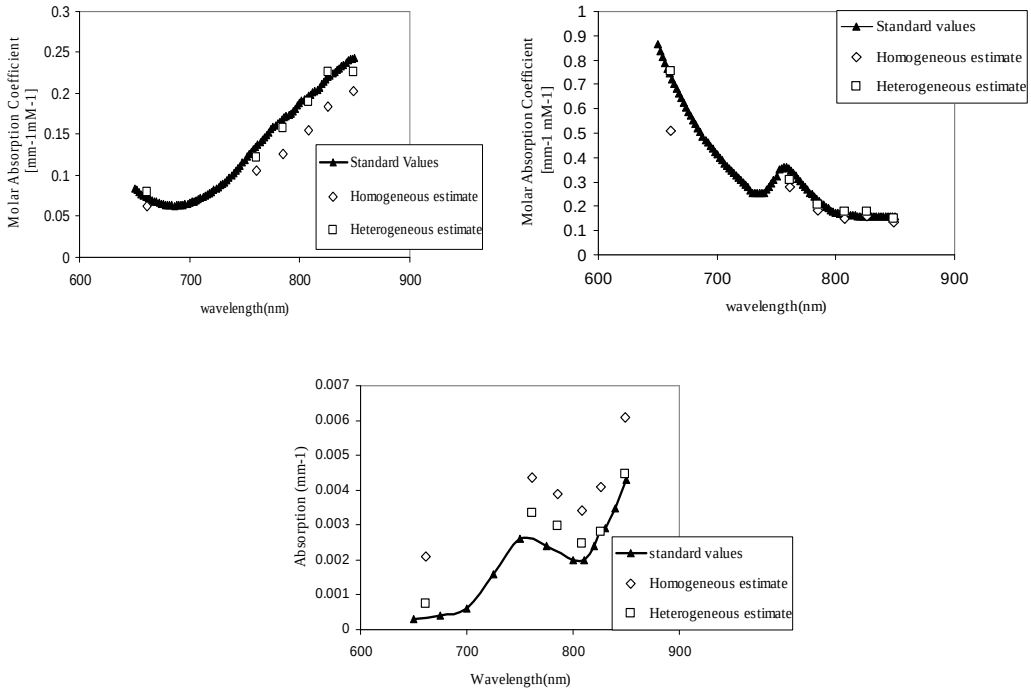


Figure 3(a). Mean molar absorption spectra of oxyhemoglobin, in comparison with standard literature spectra obtained from [29] **(b)** Mean molar absorption spectrum of de-oxyhemoglobin, in comparison with spectra obtained from Prahl **(c)** Mean absorption

spectra of 100% water, found from the offset in the phantom experiments, in comparison with standard values from Hale and Querry.

Figure 2 shows the measured extinction spectra for (a) oxy-hemoglobin (b) de-oxyhemoglobin and (c) water, obtained from our tomography system using homogeneous estimate. These data show the results from three repeated measures of these values, and the mean of set at a given wavelength was used as the standard value. It was estimated that these estimates have a standard deviation error of 6% for both homogeneous and heterogeneous estimates.

Figure 3 shows the molar absorption spectra for oxy-hemoglobin, hemoglobin and water obtained from both the homogeneous estimate and heterogeneous estimate, and the corresponding values from literature [18, 29]. Depending on the type of reconstruction being used, the corresponding molar absorption coefficients are used in other phantom and patient studies.

3.2 Oxygen Dissociation (Hill) Curve

In validation tests, these molar absorption coefficients were used to recover the properties of tissue-simulating phantoms of varying oxygen saturation. Phantoms were deoxygenated with the addition of yeast, and measurements of absorption and scattering versus solution pO_2 were recorded at multiple times during the deoxygenating process. The absorption coefficient data obtained from homogeneous or heterogeneous estimates was spectrally decomposed into concentrations of water, oxy-hemoglobin and de-oxyhemoglobin using the corresponding molar absorption spectra, and from these the oxygen saturation was calculated. The Hill curve characteristic of oxygen dissociation from hemoglobin is shown in Figure 4 (a), along with the total hemoglobin and water values with variation in pO_2, in Figure 4(b). The oxygen saturation values obtained using a homogeneous estimate and least squares constrained fit, is estimated to be accurate to within 7.6 % with the worst accuracy near zero pO_2 and higher accuracy above the 80% saturation value. For the values computed from heterogeneous estimate, the oxygen saturation values are accurate to within 4.8 %. Estimates of total hemoglobin only vary by 5% for both homogeneous reconstruction, and heterogeneous reconstruction, when oxygen saturation varies, and estimates of water concentration vary by less than 10% for both over the range of pO_2 values.

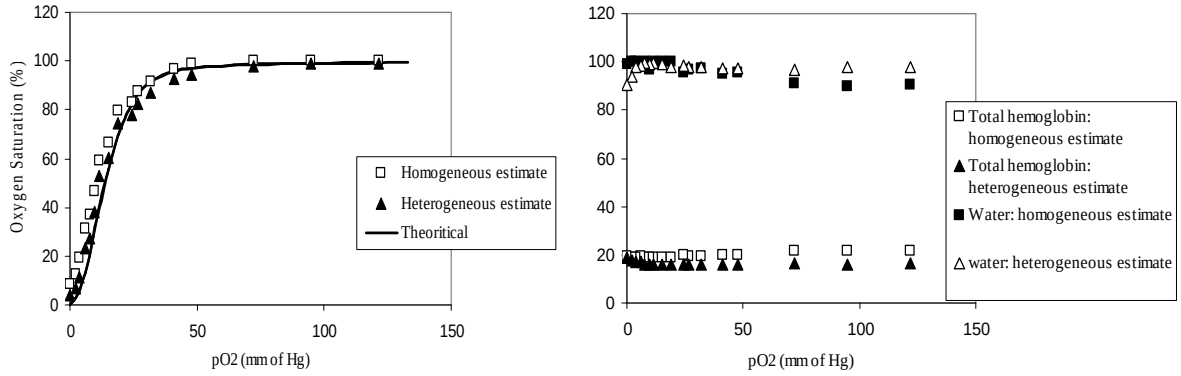


Figure 4(a)A plot of the calculated oxygen dissociation curve from both homogeneous and heterogeneous estimates as a function of solution oxygen partial pressure (pO_2) is shown, as measured in tissue simulating phantoms that were degassed of oxygen, and extinction spectra derived in Figure 3 were used. The theoretical curve was obtained from [30] **(b)** the hemoglobin and water variation with pO_2 in the same experiment are shown, which are nearly constant, since these were not varied in this experiment.

4. DISCUSSION

The goal of this study has been to ensure that quantitative accuracy is possible when imaging with our system, and the three absorption-derived measurements are separated well, without significant model-derived cross talk. The molar absorption coefficients have been derived experimentally and their accuracy has been investigated through phantom experiments. The absorption coefficients increase linearly with blood concentration and this is accurately depicted by the tomography system, and hence the procedure of obtaining the slope and offsets from a linear fit is well justified. The molar absorption coefficients for oxyhemoglobin and de-oxyhemoglobin calculated using the heterogeneous estimate, shows good agreement with the literature values with a mean error of 7.6%, and this fact shows the robustness of the nonlinear reconstruction used, when the boundary artifacts are removed. For the homogeneous estimate, this value is higher, at 23%, which may be because of the nature of the reconstruction in trying to find a single global value for the absorption and scattering coefficients which satisfies the measurements. The homogeneous estimate has been found useful in the past to study trends in optical properties in various studies where the global parameters are used instead of the average parameters, and in such cases, it is necessary to have molar absorption spectra which takes into account the difference between the global estimates and the heterogeneous estimates.

The Hill curve data plotted in Figures 4 confirms that we can image oxygen saturation while simultaneously imaging Hb_T and water accurately, and the data suggests that a level of less than 10% error is incurred in this situation, independent of type of reconstruction used.

Overall this study has shown that usage of the measured molar absorption spectra yields results in agreement with theory as shown by the Hill curve data. The spectra will be used in further studies to investigate the various parameters and information obtained from NIR tomography.

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