# Paired-wavelength spectral approach to measuring the relative concentrations of two localized chromophores in turbid media: an experimental study

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**Abstract.** We present an experimental test of a new spectral approach that is aimed at quantifying the relative concentrations of two chromophores that are contained in a defect embedded in a turbid medium. The basic steps of our spectral approach are (a) perform a linear tandem scan of the source and detector across the defect; (b) measure the spectral dependence of the maximum change induced by the defect in the scanned intensity; (c) identify a set of appropriate pairs of wavelengths ($\lambda_1$, $\lambda_2$) at which such maximum intensity changes are the same; and (d) measure the reduced scattering coefficient spectrum of the background medium. For each wavelength pair ($\lambda_1$, $\lambda_2$), we obtain a measurement of the relative concentrations of the two chromophores, where the only required parameters are the extinction coefficients of the two chromophores and the ratio of the background scattering coefficients at $\lambda_1$ and $\lambda_2$. In a mixture of two test chromophores (blue food coloring dye and black India ink) contained in a 0.78-cm diameter cylinder, our spectral approach yielded relative concentrations values that were within 6% of their actual values. Although our paired-wavelength spectral approach is not generally applicable to any pair of chromophores, it is suitable for oxyhemoglobin and deoxyhemoglobin and is thus appropriate for oximetry of localized lesions in biological tissues.  [DOI: 10.1117/1.2779349]

Keywords: near-infrared spectroscopy; spectral image approach; turbid medium; breast imaging.

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## 1 Introduction

Optical measurements of the absorption coefficient of a sample can be used to quantify the concentrations of multiple chromophores in the sample, provided that the absorption coefficient is measured at a number of wavelengths that is at least equal to the number of chromophores. In the case of near-infrared spectroscopy of tissue, where oxyhemoglobin ($HbO_2$) and deoxyhemoglobin (Hb) are often the dominant chromophores, two wavelengths are sufficient, in principle, to separately measure their concentrations. A first complication of such measurement is the high scattering of light in tissues, which requires time-resolved measurement techniques for the separation of the absorption and scattering contributions to the optical attenuation.[1] A second complication arises from an inhomogeneous distribution of the chromophores, which requires the solution of the inverse imaging problem to map the spatial dependence of the chromophore concentrations.[2–6] The above two complications are mitigated in a measurement of the oxygen saturation of hemoglobin (which only requires the measurement of the relative concentrations of $HbO_2$ and Hb), and in the simplified inhomogeneous case of a single defect (containing the hemoglobin species) within an otherwise uniform medium. The simplified scenario of the measurement of the relative concentration of two chromophores that are confined within a single, localized defect inside a turbid medium is the topic of this paper. We propose that, under such conditions, it may be possible to obtain robust and quantitative measurement of the relative concentrations (i.e., oxygenation, in the case of hemoglobin) by means of a simple inversion procedure, without having to attempt a complete solution of the inverse imaging problem that aims to reconstruct the absolute values of the optical properties. Although we emphasize the measurement of hemoglobin saturation in a localized tissue inhomogeneity (e.g., a breast tumor or a well-defined activated brain region), the method proposed here is applicable to other chromophores as well, provided that they result in an overall absorbance spectrum that shows local maxima and/or local minima over the measured spectral region.

The spectral method proposed here to measure the relative concentration of two localized chromophores is based on the selection of a set of wavelength pairs such that the relative intensity change induced by the two localized chromophores

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is the same at the two wavelengths of each pair. The potential of our approach lies in its robustness, as we have previously indicated that it is relatively insensitive to the size, shape, and location of the inclusion containing the two chromophores.[7] Once the two wavelengths of a pair, denoted $\lambda_1$ and $\lambda_2$, have been identified, one only needs to input the extinction coefficients of the two chromophores at these two wavelengths, and the background reduced scattering ratio $\mu_{s0}'(\lambda_1)/\mu_{s0}'(\lambda_2)$ to obtain a measurement of the relative concentration associated to that wavelength pair. The wavelength pairs are identified by a broadband spectral measurement of continuous wave (CW) light attenuation, and the reduced scattering ratio may be measured with time-resolved methods or estimated from literature results. In fact it is critically important to observe that our method requires the spectral ratio of the reduced scattering coefficient at the wavelengths $\lambda_1$ and $\lambda_2$, not the absolute scattering spectrum.

We present an experimental test performed in the 520- to 800-nm spectral band on phantoms consisting of a liquid turbid medium (diluted milk), and a test tube filled with a mixture of black India ink and a blue food coloring dye. Although this experimental arrangement simulates the case of near-infrared tissue spectroscopy (where milk mimics the optical scattering in tissues and the two dyes mimic $HbO_2$ and Hb), it also points to the general applicability of the proposed method to chromophores besides $HbO_2$ and Hb.

## 2 Methods

### 2.1 *Basic Principles for the Paired-Wavelength Approach*

We have previously introduced our method to measure the oxygen saturation of hemoglobin in breast tumors (or the relative concentration of two chromophores in localized inclusions) by appropriately pairing wavelengths.[7] Briefly, we hypothesize that an inclusion characterized by an absorption contrast $\Delta\mu_a$ and a zero scattering contrast ($\Delta\mu_s'=0$) determines a maximal relative intensity change [$[\Delta I/I_0|_{max}(\lambda)$, where $I_0$ is the background intensity] that depends on the product $\mu_{s0}'(\lambda)\Delta\mu_a(\lambda)$ and on wavelength-independent parameters associated with geometrical factors and boundary conditions. Consequently, if two wavelengths $\lambda_1$ and $\lambda_2$ are such that $\Delta I/I_0|_{max}^{(\lambda_1)}=\Delta I/I_0|_{max}^{(\lambda_2)}$, then we can conclude that $\mu_{s0}'(\lambda_1)\Delta\mu_a(\lambda_1)=\mu_{s0}'(\lambda_2)\Delta\mu_a(\lambda_2)$. In other words, the ratio of the absorption perturbations at these two wavelengths is given by the inverse of the ratio of the background reduced scattering coefficients at the same two wavelengths. Because the oxygen saturation of hemoglobin is only a function of the ratio of the optical absorption at two wavelengths, one can use this relationship and translate a measurement of the background scattering ratio into a measurement of the absorption perturbation ratio associated with the embedded inclusion. In our experiment, we use a high-contrast defect, where the absorption perturbation ($\Delta\mu_a$) is much greater than the background absorption ($\mu_{a0}$), so that $\Delta\mu_a$ is representative of the defect absorption. If instead of using molar concentrations (as is typically done in the case of $HbO_2$ and Hb), one uses volume fractions (as we have done for the two chromophore solutions in our experimental test), the molar extinction coefficients of the two chromophores are replaced by the absorption coefficients of the two chromophore solutions. As a result, the expression for the relative concentration of ink ($RC_{ink}$), based on the expression for oxygen saturation reported previously,[7] is the following:

$$RC_{ink}=\frac{\mu_{adye}(\lambda_2)-\mu_{adye}(\lambda_1)\mu_{s0}'(\lambda_1)/\mu_{s0}'(\lambda_2)}{[\mu_{adye}(\lambda_2)-\mu_{aink}(\lambda_2)]+[\mu_{aink}(\lambda_1)-\mu_{adye}(\lambda_1)]\mu_{s0}'(\lambda_1)/\mu_{s0}'(\lambda_2)}, \quad (1)$$

where $\mu_{aink}$ and $\mu_{adye}$ represent the absorption coefficient of ink and blue dye, respectively, and $\mu_{s0}'(\lambda_1)/\mu_{s0}'(\lambda_2)$ substitutes $\Delta\mu_a(\lambda_2)/\Delta\mu_a(\lambda_1)$ based on our hypothesis.

### 2.2 *Experimental Procedures*

The experiments were performed in a highly scattering medium (2% milk-water mixture) that simulates the optical properties of breast tissue. The optical properties of the background medium were measured at two wavelengths (690 and 830 nm) using a multidistance frequency-domain method[8] with a commercial frequency-domain tissue spectrometer (OxyplexTS, ISS, Inc., Champaign, Illinois). The spectral extrapolation of the reduced scattering coefficient was performed by fitting the frequency-domain data at 690 and 830 nm with the power law $\mu_{s0}'=a\lambda^{-b}$.[9] The spectral measurements for our proposed method were performed with the source and detector fibers deeply embedded into the turbid medium and collinear with each other at a distance of 6 cm (simulating a typical breast thickness under mild compression). The optical inclusion is a transparent plastic tube (0.78 cm in diameter, 12 cm in length) containing a mixture of two test chromophores and background medium. The two test chromophores were black India ink and a blue food coloring dye. The absorption spectra of these chromophores ($\mu_{aink}$ and $\mu_{adye}$) were quantitatively measured in a nonscattering regime using a standard spectrophotometer (Lambda 35, PerkinElmer Instruments, Shelton, Connecticut).

A bandpass filtered (400 to 1000 nm) Xenon arc lamp (Model 66984, Oriel Instrument, Stratford, Connecticut) was coupled to the source optical fiber (internal diameter: 3 mm). The light collected with the detector optical fiber (internal diameter: 4 mm) was sent to a spectrograph (SpectraPro-150, Acton Research Corporation, Acton, Massachusetts) equipped with a 300-G/mm grating to achieve a spectral dispersion of

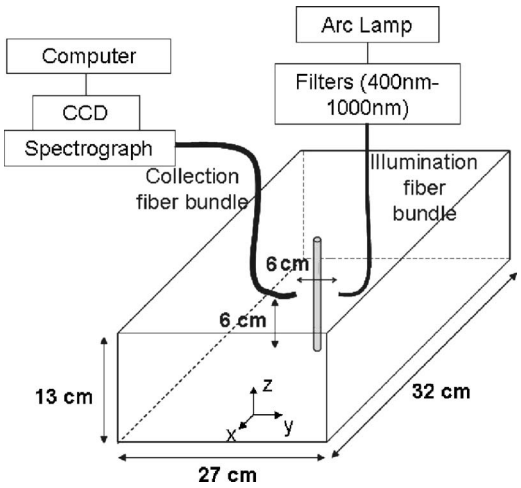


**Fig. 1** Experimental setup showing the container of the tissuelike liquid phantom and the cylindrical inclusion containing the two test chromophores. CCD: charge-coupled device.

20 nm/mm. A charge-coupled device (CCD) camera detector (DU420-BR-DD, Andor Tech., South Windsor, Connecticut) was placed at the exit port of the spectrograph for the simultaneous data collection of the intensity spectrum, with a spectral resolution of 0.5 nm. The experimental setup is shown in Fig. 1.

Eleven 0.78-cm diameter test tubes were prepared at the beginning of experiment, each containing various relative concentrations (by volume) of the two chromophores solutions, ranging from 0 to 100% by steps of 10% and a milk-water mixture with the same scattering coefficient as the background medium. Measurements were taken under three different conditions: (1) background medium with the tube containing the same medium as background to get the background intensity $I_0$; (2) tube center positioned on the midplane, [i.e., 3 cm away from both source and detector (centered case)]; (3) tube center positioned 1.5 cm away from the source fiber (off-centered case). Before taking the data for cases 2 and 3, we performed a tandem scan of source and detector along the transverse coordinate ($x$ in Fig. 1), to determine the position of maximum intensity change. Then the intensity data was recorded at this position with a CCD exposure time of 30 s.

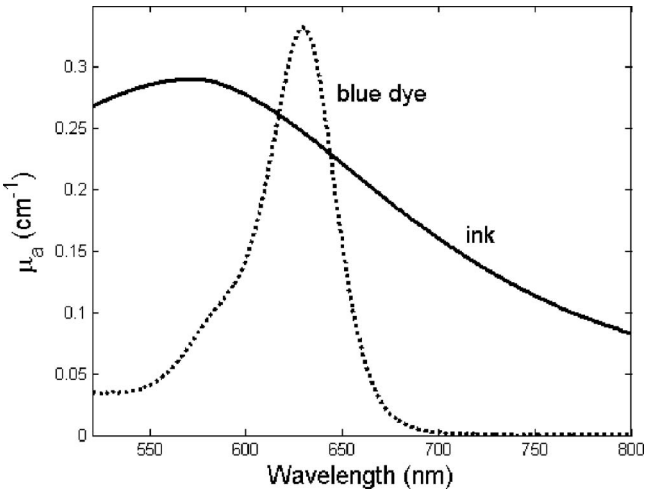


**Fig. 2** Absorption spectra of the aqueous solutions of ink and blue dye as measured by standard spectrophotometry in a nonscattering regime.

### 2.3 *Data Analysis*

We measured the spectral dependence of the maximum relative intensity change with respect to the background value, $\Delta I/I_0(\lambda)$. More precisely, $\Delta I(\lambda)=I(\lambda)-I_0(\lambda)$, where $I_0(\lambda)$ is the background intensity and $I(\lambda)$ the intensity measured when source and detector are collinear with the defect. These spectra of $\Delta I/I_0|_{max}$ were then used in our proposed method that first identifies paired wavelengths $\lambda_1$ and $\lambda_2$ such that $\Delta I/I_0|_{max}(\lambda_1)=\Delta I/I_0|_{max}(\lambda_2)$, then calculates the relative concentration of the chromophores ($RC_{ink}$) according to Eq. (1). In the case of spectral regions where the $\Delta I/I_0$ spectrum is relatively flat, the two wavelengths $\lambda_1$ and $\lambda_2$may be arbitrarily close to each other, in which case the numerator and denominator of Eq. (1) would both tend to 0. To prevent such situation, we adopted the criterion that the difference between the two paired wavelengths be greater than 15 nm ($|\lambda_1-\lambda_2|>15$ nm).

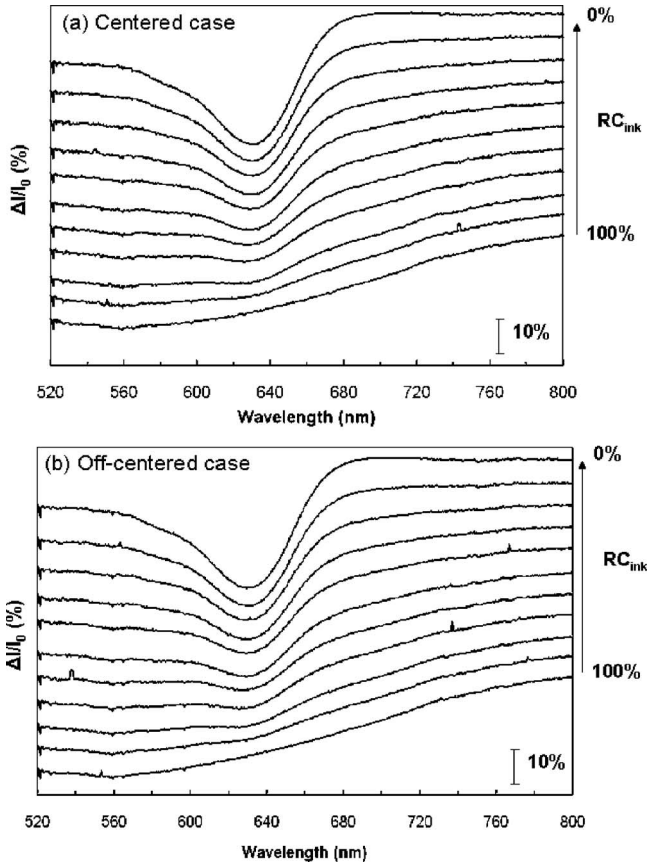


**Fig. 3** Intensity perturbation spectra induced by the cylindrical ink-dye inclusion for (a) the centered case (cylinder equidistant from source and detector), and (b) the off-center case (cylinder closer to the source).

**Table 1** Absorption coefficient ($\mu_{a0}$) and reduced scattering coefficient ($\mu'_{s0}$) of the background medium (diluted milk) at the two wavelengths measured with multidistance, frequency-domain spectroscopy.

| Parameter | 690 nm | 830 nm |
|---|---|---|
| $\mu_{a0}$ ($cm^{-1}$) | 0.010±0.003 | 0.040±0.003 |
| $\mu'_{s0}$ ($cm^{-1}$) | 9.55±0.06 | 7.4±0.1 |

Starting with the lowest wavelength (520 nm in this work) for $\lambda_1$, we look for its paired wavelength $\lambda_2 > \lambda_1$ according to the criteria already mentioned, namely $\Delta I/I_0|_{max}(\lambda_1) = \Delta I/I_0|_{max}(\lambda_2)$ and $(\lambda_2 - \lambda_1) > 15$ nm. For this wavelength pair, we calculate the relative concentration of ink according to Eq. (1), and we estimate its experimental error. The error in the relative concentration is induced by the experimental error on $\Delta I/I_0|_{max}$ (which in turn translates into errors in $\lambda_1$ and $\lambda_2$, which depend on the spectral shape of $\Delta I/I_0|_{max}$) and by the measurement error on the scattering spectral ratio $\mu'_{s0}(\lambda_1)/\mu'_{s0}(\lambda_2)$.We repeat the procedure for all measured wavelengths to find a set of wavelength pairs in this two-dimensional spectral space. We then set a 5% error threshold for $RC_{ink}$, so that any wavelength pair yielding an error greater than 5% in $RC_{ink}$ was discarded. The final measured value of $RC_{ink}$ for a specific ink and dye mixture is then given by the average of the $RC_{ink}$ values associated with all of the wavelength pairs that were not discarded, and the final error on $RC_{ink}$ was estimated by combining the average error in $RC_{ink}$ for the individual pairs and the standard error for the set of $RC_{ink}$ values.

We compared the results of our paired-wavelength method with a multiwavelength method that assumes that $\Delta I/I_0|_{max}$ is proportional to $\mu'_{s0}\Delta\mu_a$ (which is correct for diffusion theory within first-order perturbation when there is no scattering contrast). Both our paired-wavelength method and first-order perturbation for a pointlike defect do not require prior knowledge (and neither provide information) about the size, shape, and location of the defect. In our case, $\Delta\mu_a$ is given by the weighted sum of the absorption coefficients of the two chromophore solutions (where the weights are given by the corresponding relative volume fractions). This linear relationship makes it easy to determine the relative concentration of the chromophores by using the multiwavelength method[10] according to the following equation:

$$RC_{ink}^{pert} = \frac{\left(\sum_i \frac{\Delta I/I_0(\lambda_i)}{\mu'_{s0}(\lambda_i)}\mu_{ink}(\lambda_i)\right)\left(\sum_i \mu^2_{dye}(\lambda_i)\right) - \left(\sum_i \frac{\Delta I/I_0(\lambda_i)}{\mu'_{s0}(\lambda_i)}\mu_{dye}(\lambda_i)\right)\left(\sum_i \mu_{ink}(\lambda_i)\mu_{dye}(\lambda_i)\right)}{\left(\sum_i \frac{\Delta I/I_0(\lambda_i)}{\mu'_{s0}(\lambda_i)}\mu_{ink}(\lambda_i)\right)\left(\sum_i \mu^2_{dye}(\lambda_i)\right) - \left(\sum_i \frac{\Delta I/I_0(\lambda_i)}{\mu'_{s0}(\lambda_i)}\mu_{dye}(\lambda_i)\right)\left(\sum_i \mu_{ink}(\lambda_i)\mu_{dye}(\lambda_i)\right) + \left(\sum_i \frac{\Delta I/I_0(\lambda_i)}{\mu'_{s0}(\lambda_i)}\mu_{dye}(\lambda_i)\right)\left(\sum_i \mu^2_{ink}(\lambda_i)\right) - \left(\sum_i \frac{\Delta I/I_0(\lambda_i)}{\mu'_{s0}(\lambda_i)}\mu_{ink}(\lambda_i)\right)\left(\sum_i \mu_{ink}(\lambda_i)\mu_{dye}(\lambda_i)\right)}, \quad (2)$$

where the set of wavelengths $\lambda_i$ is within 520 to 800 nm to cover the whole measured spectral range.

## 3 Results and Discussion

### 3.1 *Experimental Tests*

Figure 2 shows the absorption spectra of the two test chromophores (black India ink and blue food dye) in water solution (no scattering) as measured by standard spectrophotometry. The absorption peaks of these dyes are at shorter wavelengths than those employed in near-infrared optical mammography, but our proof of principle does not necessarily need to be performed at the same wavelengths planned for the application to tumor oximetry for optical mammography. The absorption spectra of the two test dyes qualitatively mimic the absorption spectra of $HbO_2$ and Hb in the wavelength region of interest for optical mammography (680 to 880 nm). Namely, in this near-infrared spectral range, $HbO_2$ shows a relatively featureless absorption spectrum (similar to black India ink), and Hb shows local absorption maximum and stronger wavelength dependence (similar to the blue food coloring dye in the 520- to 800-nm region).

Table 1 shows the optical properties of the background medium at two wavelengths (690 and 830 nm) as measured with frequency-domain spectrometry. The absorption coefficient of the background medium is one order of magnitude smaller than the absorption coefficient of the chromophore solutions. The reduced scattering spectra is obtained by fitting the reduced scattering coefficients at 690 and 830 nm with the power function as mentioned in Sec. 2.2. We have found values of the fitting parameters $a = 6.25 \times 10^4$, $b = 1.344$ for $\mu_{s0}'$ ($cm^{-1}$) and $\lambda$ (nm). Because we have used the same scattering medium in the cylindrical tube as in the background, we assume no scattering mismatch between the defect and background medium.

The 11 curves in Figs. 3(a) and 3(b) correspond to 11 various relative concentrations of ink $[V_{ink}/(V_{ink}+V_{dye})]$, ranging from 0 to 100% by steps of 10%, for the centered cylinder case [Fig. 3(a)] and the off-centered cylinder case [Fig. 3(b)] as we have described in Sec. 2.2. Each curve has been shifted by an arbitrary offset for clarity. The top curve corresponds to the lowest ink concentration (0%), and the bottom curve corresponds to the highest ink concentration (100%). Our paired wavelength method requires that the relative intensity change caused by the defect show well-defined local maxima and/or local minima. This is not the case for the bottom three spectra in Figs. 3(a) and 3(b), which correspond to relative ink concentrations of 80, 90, and 100%. To quantify this situation, we define the useful contrast in each spec-

trum as the difference between the maximum and minimum values of $\Delta I/I_0|_{max}$ over the wavelength range where it is possible to find paired wavelengths that satisfy our criteria. The contrast-to-noise ratios (CNRs) for the highest three ink concentrations (80, 90, 100%) are 8.8, 9.2, and 8, respectively, but CNRs for all other spectra are higher than 12. As a result, we have applied our method only to the spectra corresponding to a relative ink concentration within the range 0 to 70%.

We have discussed (see Sec. 2.3) that our critical hypothesis is that the product $\mu_{s0'}\Delta\mu_a$ is the same at the two wavelengths $\lambda_1$ and $\lambda_2$ of each pair. Figure 4 shows the absolute values of $\mu_{s0'}\Delta\mu_a$ at the two wavelengths, their relative difference, the measured values of $RC_{ink}$, and $\lambda_2$ as a function of $\lambda_1$. Three representative cases are presented for actual $RC_{ink}$ values of 0% [panel (a)], 30% [panel (b)], and 70% [panel (c)]. One important point to observe in Fig. 4 is that the wavelength pairs for which the products $\mu'_{s0}\Delta\mu_a$ are equal, yield a measured value of $RC_{ink}$ that coincides with the corresponding actual value (shown as a horizontal dashed line in the $RC_{ink}$ graph). We observe that the collection of $RC_{ink}$values associated with the various wavelength pairs may be off by up to about 15%, but because measured values both overestimate and underestimate $RC_{ink}$, the average across all wavelength pairs tends to partially correct for these inaccuracies. Therefore, although our hypothesis on the functional dependence of $\Delta I/I_0|_{max}$ on $\mu'_{s0}\Delta\mu_a$ is not accurate over the whole spectrum, the deviation does not translate into dramatic inaccuracies on the measured relative concentration, and such inaccuracies tend to cancel out in the averaging process across wavelength pairs. We also notice highly accurate (to within 1 to 2%) measurements over extended spectral regions of $\lambda_1$ [e.g., 525 to 545 nm in Fig. 4(b) and 550 to 600 nm in Fig. 4(c)].

With regard to the sensitivity to the scattering spectral ratio, we point out that the spectrum of $\Delta I/I_0|_{max}$ for 70% $RC_{ink}$ (see Fig. 3) is relatively flat over the 550-to-600-nm wavelength region. This implies that $\lambda_1$ wavelength within this range (550 to 600 nm) corresponds to approximately the same $\lambda_2$ (638 nm). The ratio $\mu_{s0'}(\lambda_1)/\mu_{s0'}(\lambda_2)$ in this two-dimensional wavelength range is about 7%, but it translates into a variability for $RC_{ink}$ of only 1.3%. This result is due to the fact that the variation in the reduced scattering ratio is compensated by the different set of absorption coefficients for the ink and blue dye chromophores.

Figure 5 shows the measured relative concentration of ink $[RC_{ink}^{(measured)}]$ versus its actual value $[RC_{ink}^{(actual)}]$ for the centered [panel (a)] and off-centered [panel (b)] cases. The open circles are calculated by using the full spectrum and the assumption of linearity between $\Delta I/I_0|_{max}$ and $\mu_{s0'}\Delta\mu_a$ according to first-order perturbation theory, as discussed previously. The diamonds are calculated by using the new wavelength-paired method, which holds under any monotonic functional dependence of $\Delta I/I_0|_{max}$ on $\mu_{s0'}\Delta\mu_a$. The line indicates the ideal result for the measured values of relative concentration. These results show that the relative concentrations of ink measured with our wavelength-paired method deviate by no more than 6% from the actual values over the 0 to 70% range regardless of the location of the defect. By contrast, the $RC_{ink}$ values measured using the full spectrum and the first-order perturbation assumption of proportionality between $\Delta I/I_0|_{max}$ and $\mu_{s0'}\Delta\mu_a$have a much worse accuracy, with results that

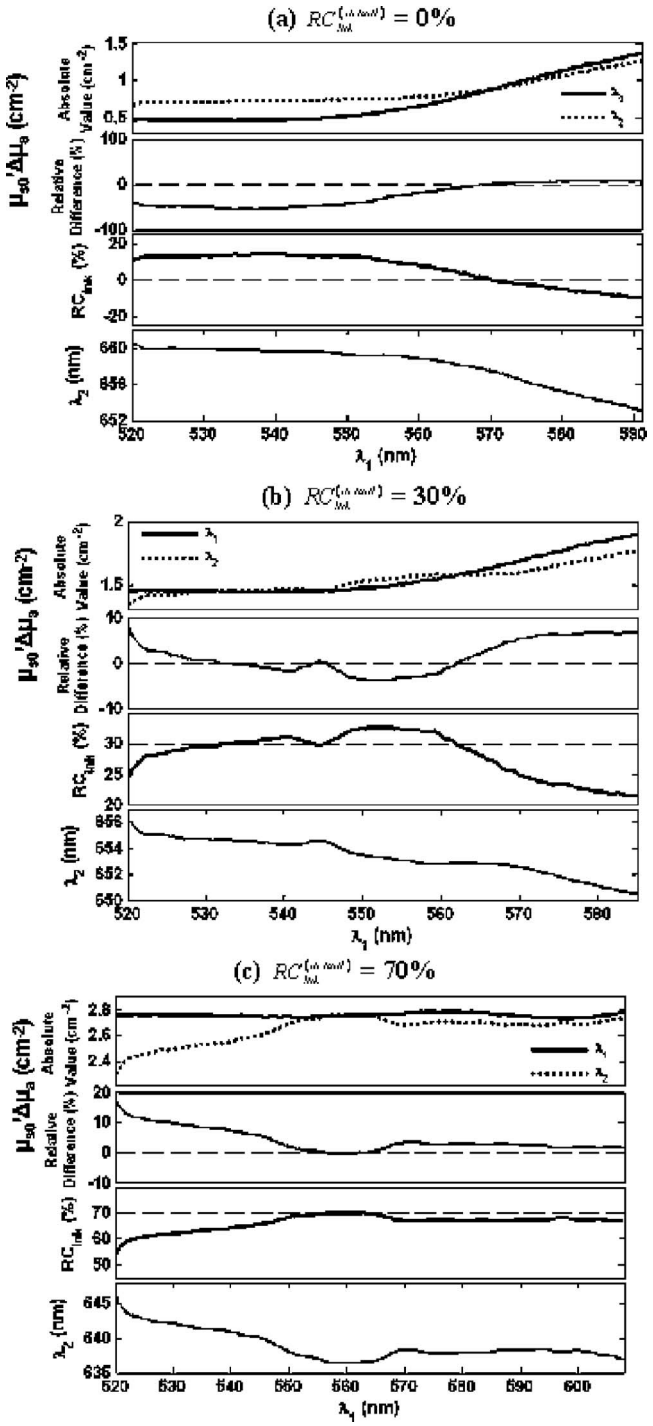


**Fig. 4** Absolute values of $\mu_{s0'}\Delta\mu_a$ as function of the paired wavelengths $\lambda_1$ and $\lambda_2$, their relative difference, and measured values of $RC_{ink}(\lambda_1,\lambda_2)$ as a function of $\lambda_1$ and $\lambda_2$ for relative ink concentrations of (a) 0%, (b) 30%, and (c) 70%.

are off by 15 to 30%. We assign the better performance of our method with respect to first-order perturbation theory to two reasons. First, our experimental conditions are beyond the limits of validity of first-order perturbation theory in quantifying the spectral dependence of the absorption contrast. Our paired-wavelength method, instead, is used to calculate ratios of absorption contrast, which is a more robust measurement.

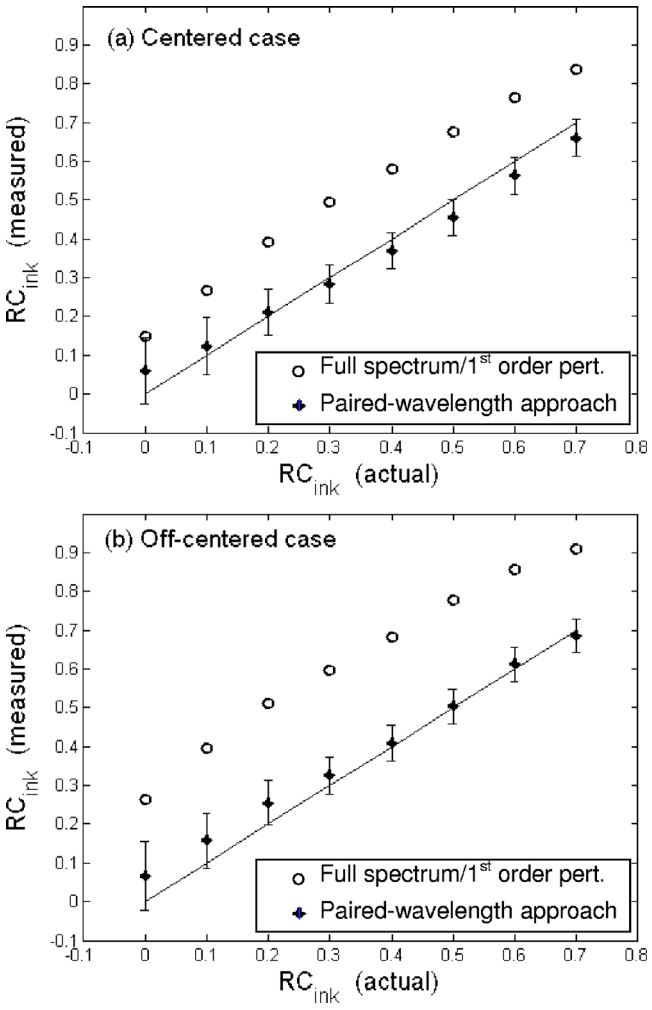


**Fig. 5** Measured versus actual values of relative concentrations of ink for the cases of (a) centered cylinder and (b) off-centered cylinder. Closed diamonds are obtained with our paired-wavelength approach; open circles are obtained from the fit of the full spectrum with first-order perturbation for the case of a pointlike object.

Second, the spectral information is used in a unique way by our method, where wavelength pairs are selected according to well-defined criteria. Therefore, the key point of the method is that it provides a correct estimate of the ratios of absorption contrast by choosing particular paired wavelengths. Also, we notice that because the paired wavelengths cannot be defined in advance, any multiwavelength approach that uses a discrete number of wavelengths instead of a broadband illumination is more limited in the application of our method.

Figure 5 allows us to quantify the sensitivity of our method (and the full spectral method based on first-order perturbation) to the location of the inclusion. We have found that for our paired-wavelength method, the relative concentration of ink measured in the centered case is about 4 to 5% lower than that measured in the off-centered case. In the case of full spectral analysis based on first-order perturbation, the relative concentration of ink measured in the centered case is about 9 to 13% lower than that measured in the off-centered case.

## Conclusion

We have presented an experimental test of a paired-wavelength spectral approach to measuring the relative concentration of two localized chromophores in turbid media, which is specifically aimed at identifying the oxygen saturation of hemoglobin in breast lesions. Only three parameters are needed to apply our method: (1) the extinction spectra (or absorption spectra) of the two chromophores; (2) the spectral dependence of the maximum relative intensity change caused by the chromophores as measured by a tandem scan of source and detector across the defect; (3) the reduced scattering coefficient spectrum of the background medium to within a multiplicative factor. Our method is computationally straightforward; it is indeed a partial inversion procedure aimed at reconstructing the ratios of two chromophores in a localized defect embedded in an otherwise homogeneous medium that uses "minimal" information, as discussed above, and, most importantly, no *a priori* knowledge about the geometry, location, and contrast of the defect. We stress that other reconstruction procedures based on the same set of minimal experimental data (no *a priori* information, linear tandem scan of a single source and single detector), most likely needs to make assumptions on the location and/or shape of the defect to solve the inverse problem. Another advantage of our approach is that it is relatively insensitive to the background optical properties as it only uses the spectral ratio of the reduced scattering coefficient, which is less affected than its absolute value by spatial heterogeneity.

The critical step of our proposed method is to identify two wavelengths $(\lambda_1,\lambda_2)$ such that $\Delta I/I_0|_{max}^{(\lambda_1)}=\Delta I/I_0|_{max}^{(\lambda_2)}$. This step requires that the spectral properties of the background and of the inclusion allow for the existence of such two wavelengths, in other words there has to be a local maximum and/or a local minimum of the intensity perturbation spectrum within the studied wavelength range. The hemoglobin spectrum features such local maxima and/or minima in the near-infrared wavelength range of 680 to 880 nm, with the exception of the fully oxygenated case ($HbO_2$). However, the extinction coefficient of ($HbO_2$) in the 650-to-750-nm spectral region is sufficiently low (it is 3 to 7 times smaller than the extinction coefficient of Hb) to approximately fulfill the first-order perturbation condition that $\Delta I/I_0|_{max}\propto\mu_{s0}'\Delta\mu_a$. Therefore, even if there are no two wavelengths $(\lambda_1,\lambda_2)$ for which the intensity perturbation is the same, substituting $\Delta\mu_a(\lambda_2)/\Delta\mu_a(\lambda_1)$ with $[\Delta I/I_0|_{max}(\lambda_2)\mu_{s0}'(\lambda_1)]/[\Delta I/I_0|_{max}(\lambda_1)\mu_{s0}'(\lambda_2)]$ may still achieve high accuracy in the measurement of high oxygenation values, as we have previously shown.[7] We observe that this is not the case in the experimental test reported here, where the high values of $RC_{ink}$ (for which the intensity perturbation spectra do not show local maxima or minima) correspond to the highest absorption for the mixture of the two dye solutions, rather than a minimum absorption as in the case of highly oxygenated hemoglobin.

By using black India ink and blue food dye to mimic ($HbO_2$) and Hb, we have experimentally demonstrated that our proposed paired-wavelength spectral approach is able to measure the relative concentrations of ink and blue dye solutions in a relatively large optical inclusion embedded in a highly scattering medium. We have found the accuracy of the

relative concentration measurement to be better than 6% over the measurable range 0 to 70%. This method shows promise in optical mammography as the oxygenation of individual optical inhomogeneities such as blood vessels and breast tumors may be robustly measured to complement spatial optical maps with physiologically relevant information.

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## References

1. P. Taroni, A. Pifferi, A. Torricelli, D. Comelli, and R. Cubeddu, "In vivo absorption and scattering spectroscopy of biological tissues", *Photochem. Photobiol. Sci*.," **2**, 124–129 (2003).
2. Z. M. Wang, G. Y. Panasyuk, V. A. Markel, and J. C. Schotland, "Experimental demonstration of an analytic method for image reconstruction in optical diffusion tomography with large data sets", *Opt. Lett.* **30**, 3338–3340 (2005).
3. S. Srinivasan, B. W. Pogue, B. Brooksby, S. Jiang, H. Dehghani, C. Kogel, W. A. Wells, S. Jiang, S. P. Poplack, and K. D. Paulsen, "Near-infrared characterization of breast tumors in vivo using spectrally constrained reconstruction," *Technol. Cancer Res. Treat.* **4**, 513–526 (2005).
4. X. Intes, V. Ntziachristos, J. P. Culver, A. Yodh, and B. Chance, "Projection access order in algebraic reconstruction technique for diffuse optical tomography", *Phys. Med. Biol.* **47**, N1–N10 (2002).
5. A. Zacharopoulos and S. Arridge, "3D shape reconstruction in optical tomography using spherical harmonics and BEM", *J. Electromagn. Waves Appl.* **20**, 1827–1836 (2006).
6. A. D. Klose and A. H. Hielscher, "Optical tomography using the time-independent equation of radiative transfer. Part 2: Inverse model", *J. Quant. Spectrosc. Radiat. Transf.* **72**, 715–732 (2002).
7. E. L. Heffer and S. Fantini, "Quantitative oximetry of breast tumors: A near-infrared method that identifies two optimal wavelengths for each tumor", *Appl. Opt.* **41**, 3827–3839 (2002).
8. S. Fantini, M. A. Franceschini, J. B. Fishkin, B. Barbieri, and E. Gratton, "Quantitative determination of the absorption spectra of chromophores in strongly scattering media: A light-emitting-diode based technique", *Appl. Opt.* **33**, 5204–5213 (1994)
9. F. Bevilacqua, P. Marquet, C. Depeursinge, and E. B. Dehaller, "Determination of reduced scattering and absorption-coefficients by a single charge-coupled-device array measurement. 2. Measurements on biological tissues", *Opt. Eng.* **34**, 2064–2069 (1995).
10. M. A. Franceschini, E. Gratton, D. Hueber, and S. Fantini, "Near-infrared absorption and scattering spectra of tissues *in vivo*", *Proc. SPIE* **3597**, 526–531 (1999).