

# Spectrophotometry and Photoacoustic Imaging: A Comparative Study

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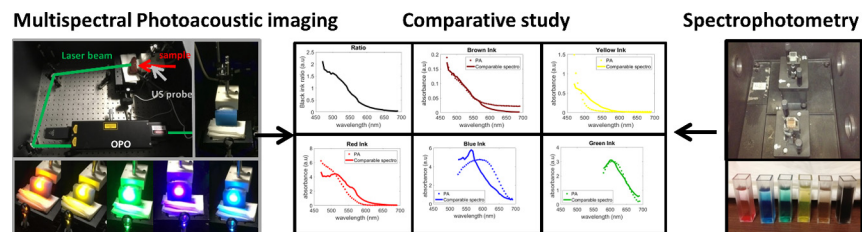
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## Highlights

- Spectrophotometry of six diluted inks.
- Multispectral photoacoustic imaging of agar phantoms coloured with the six inks.
- Similar multispectral evolutions obtained with both modalities.
- Spectrophotometry as reference to classify multispectral photoacoustic images.

## Graphical abstract



## Abstract

Photoacoustic imaging is a hybrid modality that is used to image biological tissues. Using multispectral optical excitation, a functional image is obtained due to the tissue specific optical absorption that depends on the wavelengths. To classify multispectral photoacoustic images, supervised methods are classically used. However, definition of the reference spectra is often difficult, and this choice can have a large impact on the classification results. A possible approach to build relevant reference spectra is to use spectrophotometry. This study aims at comparing absorbance measured by a spectrophotometer and multispectral photoacoustic signals of various coloured phantoms. We compare qualitatively the shape of the spectra obtained, using these two modalities for each sample. Our data suggest that spectrophotometry is a promising way to define reference spectra for classification of multispectral photoacoustic datasets.

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**Keywords:** Multispectral photoacoustic imaging; Spectrophotometry; Supervised classification method

## 1. Introduction

Photoacoustic imaging is a hybrid method that combines the advantages of acoustic and optical imaging [1]. This modality is based on the detection of acoustic waves that are produced by a medium under pulsed laser illuminations. The acoustic wave is created by the thermal expansion of the optical ab-

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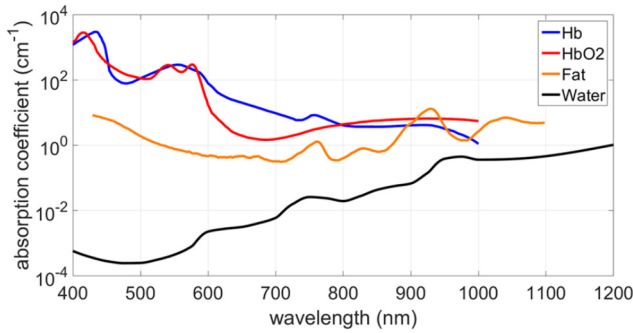


Fig. 1. Absorption coefficients of different media as a function of wavelength; adapted from [2,3].

sorbers, due to the local increase in temperature during the laser pulse. Each medium creates a specific acoustic wave [2, 3] that is related to its optical absorption at the wavelength used (Fig. 1). After propagation through the media to the surface, acoustic pressure waves can be detected using an ultrasound probe. A photoacoustic image of the region of interest can then be reconstructed.

The optical absorbance of tissue depends on the illumination wavelength, as does the photoacoustic signal. Scattering during acoustic propagation in tissues, which is also wavelength-dependent, has an impact also on the detection of the photoacoustic signals. Characterisation of media can thus be performed using multispectral photoacoustic signals obtained by acquisition of photoacoustic images of a region of interest at different wavelengths [4]. In this way, multispectral photoacoustic imaging allows a functional image to be obtained.

To classify multispectral photoacoustic images, supervised methods, such as the least-squares method, are typically used [5,6]. These methods require reference spectra for each medium to be classified. Most of the time, such references are calculated on selected pixels of the dataset, which does not accurately describe the point-to-point variation for the whole image. The quality of these reference spectra impacts a lot on the classification results, and they need to be as representative as possible. They have then to be independent of the calculation used in their construction, and their measurements with another modality might then be of interest.

Spectrophotometry has recently been suggested as a technique to construct such reference spectra [7]. To more deeply investigate this potential, in this study we aimed to compare the spectra provided by a spectrophotometer to the multispectral photoacoustic signal using a larger set of test media and a larger range of wavelength than seen in the literature [7]. In the future, such comparison should help in the development of complex multi-modal agar phantoms, to validate supervised multispectral classification methods. Here, we used agar phantoms coloured with six different inks for photoacoustic acquisitions. The spectrophotometry was conducted on the same six inks. The materials and methods used for the study are described first. Then, the results are presented. Finally, the data are discussed, and a conclusion to the study is provided.

## 2. Materials and methods

### 2.1. Spectrophotometry

Absorbances were measured from 400 to 1200 nm with 1 nm steps. These acquisitions were made on a Perkin Elmer Lambda900 spectrophotometer (Fig. 2, left) at the Institut Lumière Matière. We used a standard arrangement that measures the contributions of both the absorption and the scattering (i.e., the absorbance). The maximum absorbance that can be measured by the spectrophotometer made it impossible to perform the acquisitions directly on pure inks. The absorbance measurements were thus performed on diluted inks, with a dilution factor of 1:1601 (Fig. 2, right).

### 2.2. Multispectral photoacoustic imaging

Multispectral photoacoustic signals were acquired on the photoacoustic experimental set-up (Fig. 3a) in CREATIS. This is composed of a Nd:Yag laser coupled with an optical parametric oscillator (SpectraPhysics, USA) for the generation, and of an ultrasound scanner (ULA-OP) coupled with a CMUT probe for the detection [8]. The acquisitions were made from 410 to 690 nm, with 5 nm steps (Fig. 3c) on coloured 4% agar phantoms with a dilution factor of 1:1629 (Fig. 3b). For each medium, twenty images were acquired at the same location at each wavelength. The spectrum was averaged over the region of interest, corresponding to the laser beam illumination spot on the phantom. The spectra obtained in this way were then averaged over the 20 images, to remove any point-to-point variation.

### 2.3. Comparison method

The aim of the present study was to compare the absorbance measured by the spectrophotometer with the multispectral photoacoustic signal, as also done in [7] on a smaller selection of samples, and on a smaller range of wavelengths. This comparison will be useful for future photoacoustic dataset classification. However, data acquired with the two different modalities cannot be compared without previous cross-calibration. The absorbance measurements have first to be post-processed to make them comparable to the multispectral photoacoustic signals, as described below.

First, the photoacoustic laser energy has to be taken into account. As the absorbance spectra of black ink has a decreasing exponential shape, an optical signal is available for all of the wavelengths of the selected spectrum. This medium was used to calculate the ratio given in Equation (1) (Fig. 4a), to normalise the spectrophotometer spectra and compare them with the photoacoustic spectra:

$$Ratio = \frac{PA_{black}}{S_{black}}, \quad (1)$$

where  $PA_{black}$  is the multispectral photoacoustic signal, and  $S_{black}$  is the absorbance measurement of black ink. Then the

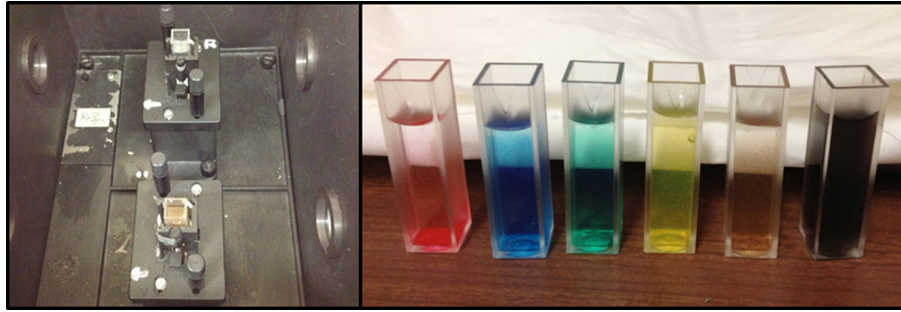


Fig. 2. Left – view of inside the spectrophotometer (brown ink and water as a reference in test tubes for the acquisition). Right – diluted inks for the optical spectroscopy measurements.

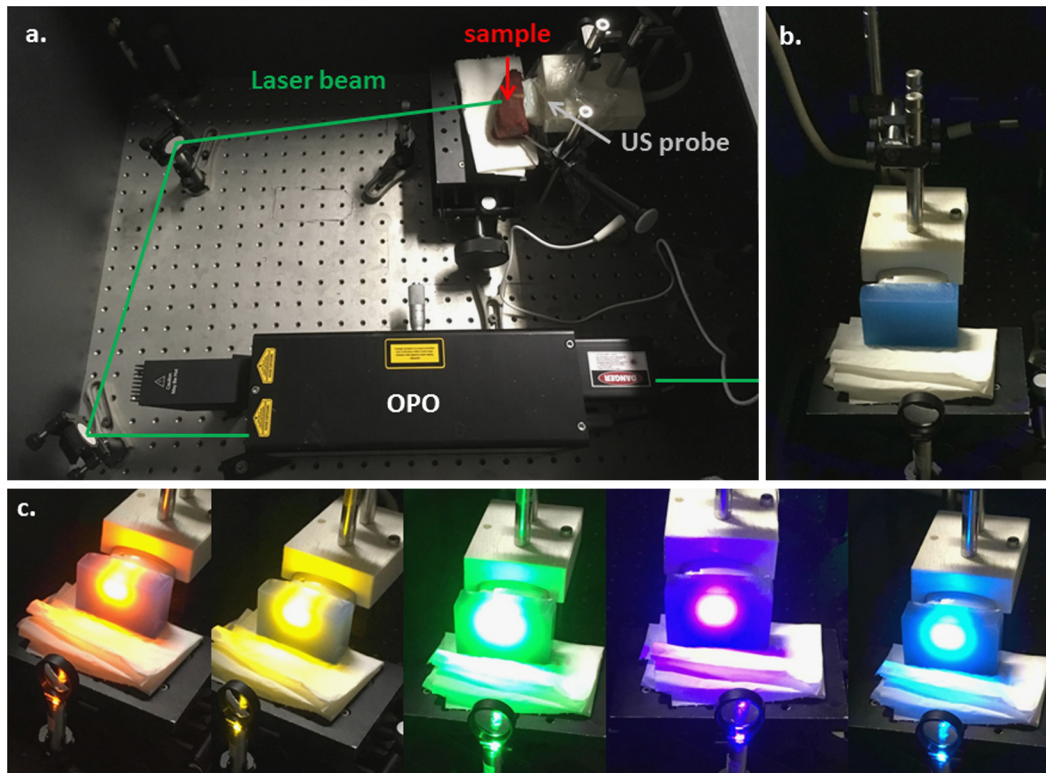


Fig. 3. (a) Photoacoustic set-up. (b) Blue phantom in the set-up. (c) Blue phantom during the acquisition of the multispectral photoacoustic signals.

ratio was applied to the five other absorbance spectra, as in Equation (2):

$$S_{PA} = S * Ratio \quad (2)$$

where  $S_{PA}$  is the normalised absorbance measurement, which can be compared to the multispectral photoacoustic signal, and  $S$  is the initial absorbance measurement. Using this ratio, the photoacoustic laser energy is then taken into account, assuming a constant energy distribution over all of the photoacoustic acquisitions.

For spectrophotometry and multispectral photoacoustic imaging, the concentration of the inks were slightly different. To avoid any discrepancies between the two types of measurements, we normalised them searching the best gain to minimize the root-mean square error (RMSE) between both measurements. RMSE was calculated as follow:

$$RMSE = \sqrt{\sum_{i=1}^{\lambda} \|S_{PA} - S\|^2} \quad (3)$$

where  $\lambda$  is the number of measurement wavelengths. This procedure provides access to the qualitative shape of the spectra, but does not allow quantification of the absorbance. As most classification methods are based on curve shapes rather than absolute values, this is not a limitation concerning the use of spectrophotometry as a reference for the classification of multispectral photoacoustic images.

### 3. Results

In this study, six inks were probed. One of these, the black ink, was used as the reference. Then, the comparison between spectrophotometry and multispectral photoacoustic signal was done on the five other phantoms (i.e., brown, yellow, red, blue,

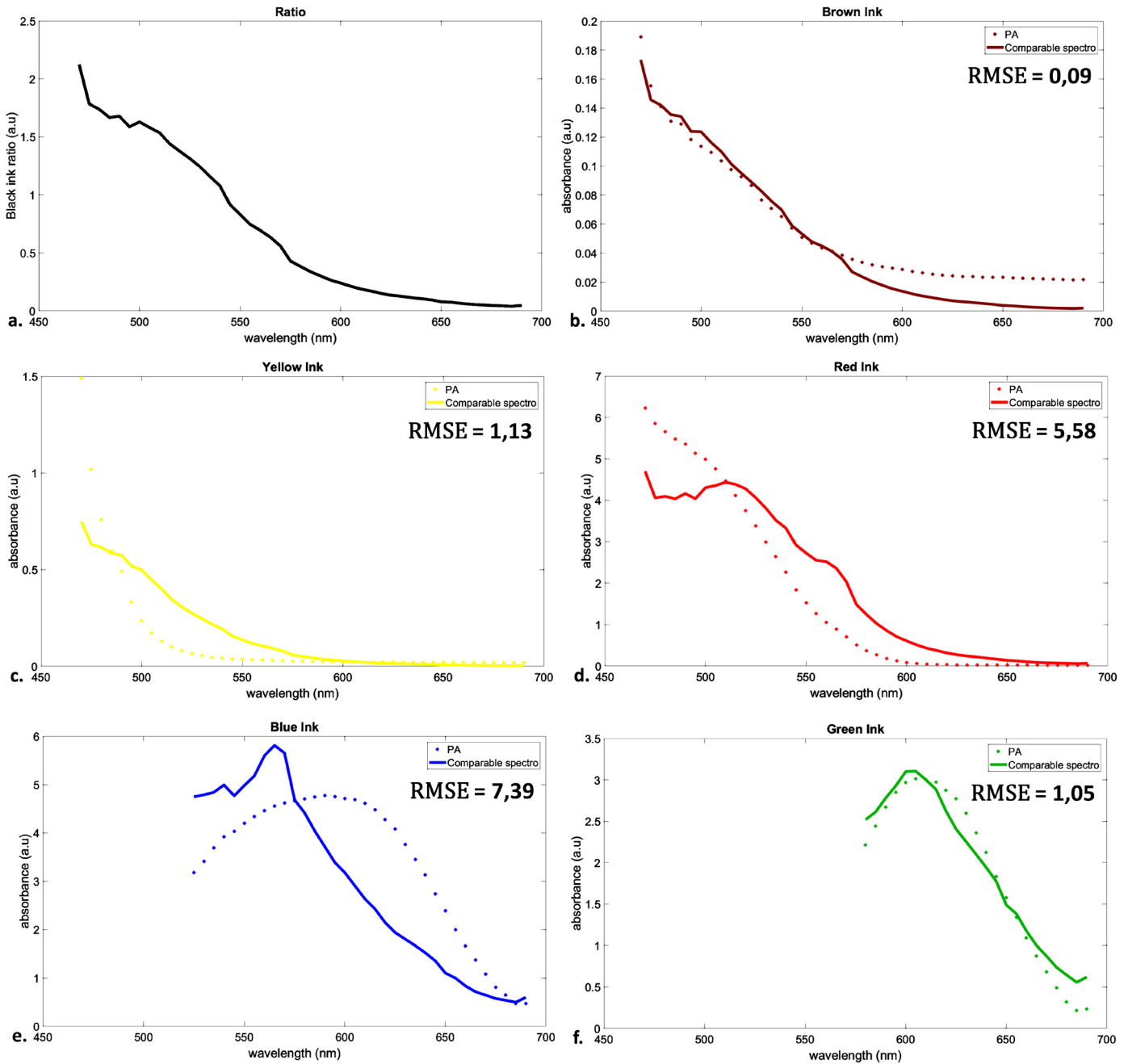


Fig. 4. Ratio used to modify the optical spectroscopies (a), and the comparisons for the brown (b), yellow (c), red (d), blue (e) and green (f) inks.

green inks). Note that the blue and green inks have a signal peak at around 600 nm, which means a very small signal amplitude for the smaller wavelengths of the study. The signal is below the noise level in these cases, so the measurements on these two media were restricted to a smaller range of wavelengths.

As shown in Fig. 4, the absorbances of the different inks follow the same tendency than the multispectral photoacoustic signals (Fig. 4, solid lines and \* markers, respectively). The blue ink, however, shows a larger discrepancy. To quantify this discrepancy, the RMSE was calculated for all inks. The error values are written on the different curves in Fig. 4. Then, the best fitting between both measurements is for brown ink (RMSE of 0.09) and the worst one is for blue ink (RMSE of

7.39). Indeed, the absorbance peak is much larger for the photoacoustic signal than for the spectrophotometry signal. With the yellow ink, there is also a difference in the decay rate of the absorbance between both of these modalities. However, for all of the other media, the agreement is largely comparable to that reported in literature [7], with both of these modalities giving similar trends in absorbance for the same medium.

#### 4. Discussion

For the five media used in this study, the normalised absorbance measurements and multispectral photoacoustic signal follow the same tendency. Some differences can still be ob-

served, which suggests the potential for future improvements to the method.

The role of scattering might represent an explanation for the differences between the two modalities used here. Indeed, as the size of the ink particles is comparable to the optical wavelengths used, scattering can occur [9,10]. However, spectrophotometry and photoacoustic measurements are both impacted upon by scattering and absorption in similar fashions [7]. Therefore, for qualitative studies where the respective contributions of absorption and scattering need not to be separated, the scattering by the inks can be ignored [7]. Moreover, it was demonstrated by [11] that it is possible to compare the signals measured on an agar–ink phantom and on a water–ink sample coloured with the same ink. Such comparisons suggest that the contribution of the agar to the total scattering is negligible. For these reasons, we believe scattering cannot explain the observed differences.

Contrary to what was proposed by [12], we did not consider the influence of light attenuation in the photoacoustic measurements, as the selected regions of interest were chosen close to the sample surface. Instead, the discrepancies between both measurements might come from the varying energy of the laser that was used. The data here were corrected assuming a constant distribution of optical energy over the different acquisition times. However, we have already observed that for a given wavelength, the energy can vary over time. Measuring laser energy at each acquisition might thus provide a strong improvement to our approach. Further study could also be done on molecular ink to validate the approach on other types of media.

This study is a part of a work on photoacoustic imaging classification. It is why the absorbance measurements was modified to be compared to photoacoustic signals. Nevertheless, if absorbance comparison was needed, multispectral photoacoustic signal could be modified and compared to absorbance measurements using only the inverse ratio.

## 5. Conclusion

The comparison described here suggests that absorbance measurements can be used to classify multispectral photoacoustic images with supervised methods like spectral fitting [4], and least-squares [5] or intra-class correlations [6]. As these methods have already given encouraging results for classification, the use of spectrophotometry spectra as a reference might increase the classification performance, as mentioned by [12]. However, to really be convinced about that, it appears that it is important to extend the present study to a larger range of media, and particularly biological media, to take into account in this case the role of light attenuation in the photoacoustic measurements [12].

Indeed, it would be interesting to see how usable the results would be if the study was to be conducted on media that have close multispectral signatures. The use of a larger range of wavelengths would also be of great interest.

## Conflict of interest statement

The authors declare that they have no conflicts of interest related to this study.

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