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NON-NEGATIVE MATRIX FACTORIZATION: A BLIND SOURCES SEPARATION METHOD TO UNMIX FLUORESCENCE SPECTRA

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ABSTRACT

Fluorescent imaging in diffusive media is an emerging modality for medical applications. Here, we use spectrally resolved measurements in order to separate several fluorescence sources. It is useful to filter parasite signal (such as the intrinsic biological tissues fluorescence, called autofluorescence) or enable the multiplexing of several fluorophores. A spectroscopic approach, based on the Non-negative Matrix Factorization (NMF) method, is explored to unmix overlapping spectra and thus isolate the specific fluorescence signals. This blind source separation method treats specific fluorescences (due to the various fluorophores) as different sources to separate; it only needs initial spectra, updated over iterations thanks to the classical gradient descent method. Fluorescence contributions of Alexa750 and ICG have been satisfactorily isolated on experimental data.

Index Terms— Signal processing, Fluorescence spectroscopy, Spectral unmixing, Multiplexing.

1. INTRODUCTION

Medical diagnostic systems based on fluorescent imaging are envisioned to be non-invasive, easy to use, and cost effective. Fluorescent markers are injected to a patient, and bind specifically to targeted compounds, like carcinoma. The region of interest is then illuminated with near infrared (NIR) light, at a wavelength optimized to propagate in the medium and to excite the fluorophores, and the fluorescence signal is measured. So far, NIR fluorescence imaging is mainly used on small animals where some fluorophores are available for injection.

Several fluorescent markers may be injected simultaneously in order to bind to different compounds or organs. The demultiplexing of these signals is obtained by using spectrally resolved measurements and a separation algorithm. Algorithms to unmix specific fluorescences or to filter autofluorescence have already been developed and tried on small animal examination equipments. Authors used methods such as non linear least squares [1], or spectra subtraction, principal component analysis (PCA) and singular value decomposition (SVD) [2, 3, 4]. (Those methods have been tested on our data but not selected: the principal inconvenient is that such

methods may return negative values though the initial data are non-negative). The algorithm used in this paper, is a blind source separation algorithm called Non-negative matrix factorization (NMF): it approximates a matrix with the product of two others matrices that carry information on the different spectra that compose the initial matrix. To our knowledge, the NMF method has only been used once on fluorescence spectroscopy data [5, 6]— but without any fluorophore injected to the subject — and represents a useful tool for spectral unmixing in fluorescence optical imaging.

In this paper, we introduce the NMF method and present ICG/Alexa750 separation results obtained on optical imaging experimental data.

2. THEORY

The NMF algorithm applied on spectroscopic data was first introduced by Gobinet [5, 6]. It approximates a non-negative matrix with the product of two others non-negative matrices; it thus differs from other factorization methods in that it enforces the constraint of non-negativity.

The mathematical translation of NMF is:

given a non-negative matrix $X \in \mathbb{R}^{N_x \times N_\lambda}$, find non-negative matrices $A \in \mathbb{R}^{N_x \times P}$ and $S \in \mathbb{R}^{P \times N_\lambda}$ such that:

$$X \simeq AS \quad (1)$$

Non-negative matrices are matrices whose all factors are non-negative and P is the number of specific fluorescence sources considered.

The decomposition is obtained by minimizing a cost function which may be the Euclidean distance between X and AS [7], written:

$$Q^{NMF} = \|X - AS\|^2 \quad (2)$$

or:

$$Q^{NMF} = \sum_{i=1}^{N_x} \sum_{j=1}^{N_\lambda} (x_{ij} - \sum_{p=1}^P a_{ip}s_{pj})^2 \quad (3)$$

The cost function Q^{NMF} is lower bounded by 0. The problem to solve is thus to minimize $\|X - AS\|^2$ with respect to A and S , subject to the constraints $A, S \geq 0$.

2.1. Regularization

Constraints may be added by using a more sophisticated cost function. Two constraints are considered here: one to smooth A and S and the other to limit the deviation between the initial spectra imposed and the results after the required number of iterations. The smoothing constraint (applied on A for example) can be written:

$$C_1 = \alpha_1 \|\nabla(A)\|^2 \quad (4)$$

while the constraint on the distance between the initial spectra and the final spectra (applied on S) can be written:

$$C_2 = \alpha_2 \|(S - S_0)\|^2 \quad (5)$$

Finally, the new cost function Q_2^{NMF} to minimize is a combination of the initial cost function and the constraints terms:

$$Q_2^{NMF} = Q^{NMF} + \sum_i \alpha_i \times C_i \quad i \in \{1, 2\} \quad (6)$$

We use the gradient descent method to minimize the cost function Q_2^{NMF} . Lee and Seung multiplicative updating rules [8] have also been tried on our data. Results appeared to be often less satisfying and will thus not be presented in this paper.

3. INSTRUMENTATION AND METHODS

3.1. Experimental set up

The experiment presented in this paper consists in illuminating an optical phantom which consists of water and intralipid (emulsion of soy bean oil and egg phospholipids for human use, used to simulate the scattering properties of biological tissues) (see Figure 1), with a planar laser along a line whose excitation wavelength is 785 nm. An emission filter that lets pass wavelengths over 820 nm is used to block the excitation. The phantom is translated after each acquisition in order to get data of the whole object. The fluorescence signal emitted back from the phantom is collected by an imaging spectrometer (along a line at the same position than the laser line) coupled with a charge-coupled device (CCD) camera above the object (as shown on Figure 2).

3.2. Acquired images

Using the set up described above, one can acquire the spectra of lines – 1mm separated from each other – of the phantom. Two capillary tubes containing respectively indocyanine green (ICG) and Alexa 750 fluorophores are immersed in

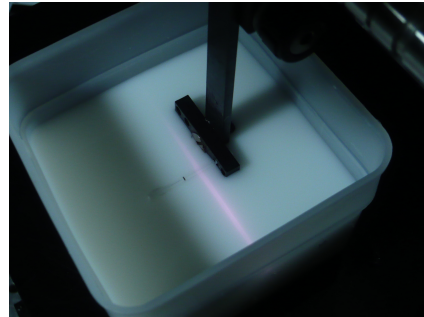


Fig. 1. Optical phantom: water and intralipid (5%)

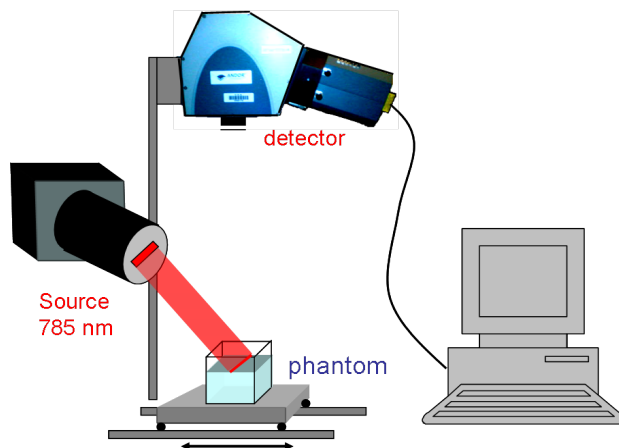


Fig. 2. Experimental set up

the liquid phantom presented before: the geometry of the experiment is presented on figure 3. The aim of the proposed algorithm is to get separate images of the fluorescence contributions of both features (ICG and Alexa 750). Both fluorophores are chosen to present different but very close fluorescence spectra to emphasize the method potentialities.

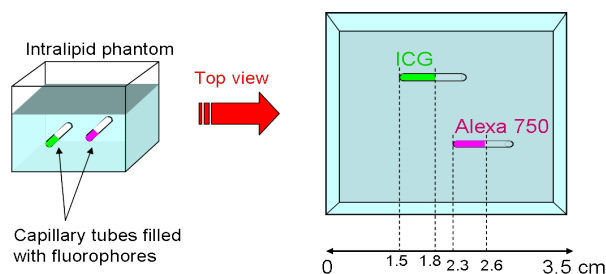


Fig. 3. Two capillary tubes containing fluorophores are immersed in the phantom

3.3. NMF applied on spectrometric data

As NMF separates a non-negative signal into a set of non-negative components, the method is suitable for spectroscopic

measurements. For our application, the NMF decomposition of an acquired signal X into $X \simeq AS$ can be interpreted with a physical sense.

Written more extensively:

$$X = \begin{pmatrix} x_{11} & \dots & x_{1,N_\lambda} \\ \vdots & & \vdots \\ x_{N_x,1} & \dots & x_{N_x,N_\lambda} \end{pmatrix} = \begin{pmatrix} a_{11} & a_{12} \\ \vdots & \vdots \\ a_{N_x,1} & a_{N_x,2} \end{pmatrix} \begin{pmatrix} s_{11} & \dots & s_{1,N_\lambda} \\ s_{21} & \dots & s_{2,N_\lambda} \end{pmatrix} \quad (7)$$

When the signal is supposed to be described by two sources, S contains the shapes of the two spectra and A contains the weights of these spectra for each spatial position as sketched by the following representation.

$$\begin{matrix} \text{line}_1 \\ \vdots \\ \text{line}_{N_x} \end{matrix} \begin{pmatrix} X \end{pmatrix} = \begin{pmatrix} a_{11} & a_{12} \\ \vdots & \vdots \\ a_{N_x,1} & a_{N_x,2} \end{pmatrix} \begin{matrix} S \\ \begin{matrix} s_1 \\ s_2 \end{matrix} \end{matrix}$$

Each line of the matrix X is a linear combination of the spectra s_1 and s_2 :

$$\text{line}_i = a_{i1} \times s_1 + a_{i2} \times s_2 \quad i \in (1, N_x) \quad (8)$$

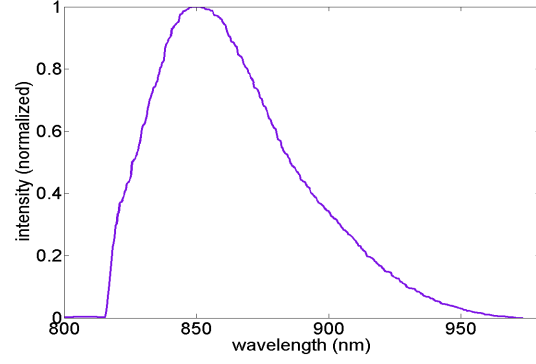
3.4. Algorithm

The NMF algorithm is an iterative scheme whose steps are the following ones: first, the two matrices A and S have to be chosen. For robustness reason, unlike Gobinet [5, 6] who utilized random matrices, we initialize S to be a matrix composed of a mean Alexa750 and a mean ICG fluorescence spectra calibrated on past experiments (Figure 4). Such initial spectra have been obtained by averaging spectra from past experiments *ex vivo* of samples of both fluorophores. Then, the cost function is minimized by using a gradient descent as described before, updating in turn both A and S .

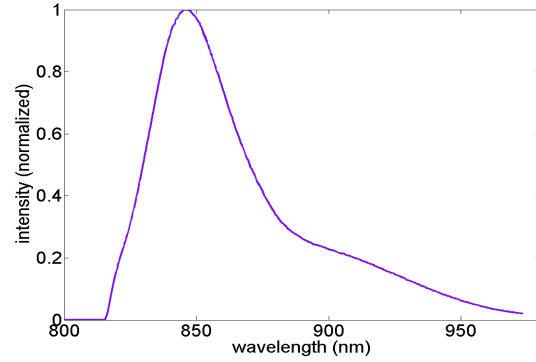
4. RESULTS AND DISCUSSIONS

We processed the NMF algorithm on the acquisitions of fluorescence obtained on the whole phantom 3 with the ICG and Alexa 750 initial spectra presented on figure 4: ICG and Alexa 750 spectra are largely overlapping. At convergence, the matrix S is composed of two spectra, presented on figure 5 (continuous lines) and compared with the initial spectra (dotted lines). Two top views of the phantom are returned, each respectively depicting the ICG contribution (Figure 6, (a)) and the Alexa 750 one (Figure 6, (b)).

We note that ICG and Alexa 750 spectra are correctly separated though the initial spectra were overlapping. The intensity of the ICG signal is larger than the alexa one. Two explanations may be given: first, the ICG concentration used here



(a) Alexa 750 spectrum model



(b) ICG spectrum model

Fig. 4. Models of fluorescence spectra chosen to initialize A and S

was larger than the Alexa 750 concentration, and second, the Alexa 750 position of the maximum fluorescence (around 750 nm) is located in the filtered part of the wavelength range (under 820 nm). The final spectra obtained are close to the models initially given, even if a few variations are noticed: the initial spectra are coarse spectra obtained from past experiments with different receiver media therefore spectral changes in the resulting spectra may be noticed.

Compared to other methods (singular value decomposition, least squares for example) tested on the same acquisitions of fluorescence, the NMF method turned out to be the most appropriate for our data (the spectra found were the closest to the ones expected) and is especially well adapted to the fluorescence spectra since all data are non-negative. It is also easy to implement and computationally fast. The cost function used here was the Euclidian distance since it is simple to implement and leads to acceptable results. We also imposed a smoothing constraint on A and a limitation of the deviation between the initial S and the resulted one. For now, the coefficients α_i have been empirically chosen after several tries, to give the best compromise between smoothing and limitation of the deviation, and to return final spectra as unmixed as possible.

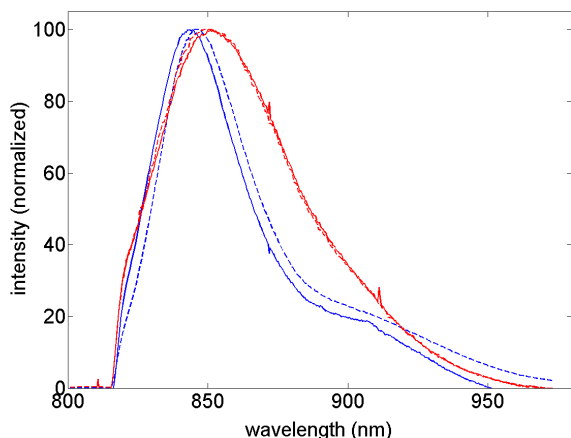
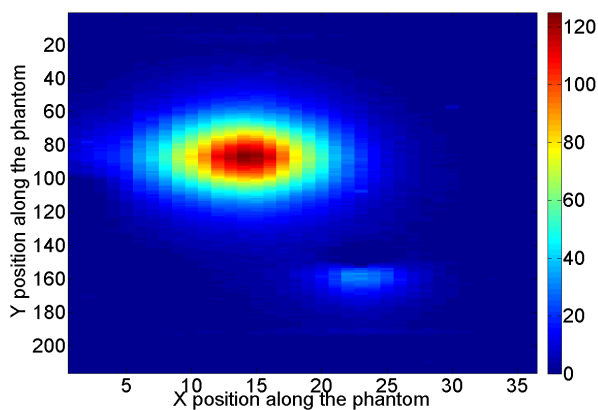
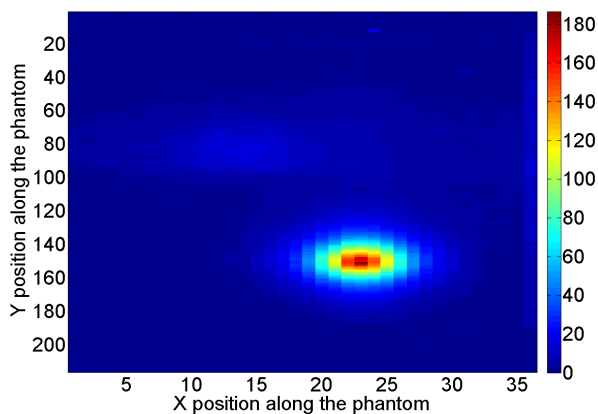


Fig. 5. ICG fluorescence (blue continuous line) and Alexa 750 fluorescence (red continuous line) spectra obtained after the NMF algorithm, and comparison with the initial spectra chosen (dotted lines).



(a) ICG part obtained



(b) Alexa 750 part obtained

Fig. 6. Spatial distribution of fluorophores estimated by the NMF algorithm

5. CONCLUSIONS

Experiments were performed to assess the capacity of NMF to unmix two different kind of fluorophores, ICG and Alexa 750. Two capillary tubes filled with those fluorophores were embedded in a diffusive medium; the aim was to get separated images of both specific fluorescence contributions.

NMF was found to be an efficient tool for spectra unmixing in fluorescence spectroscopy. It specifically allowed separating two different specific fluorescences, whose emission wavelength ranges were close, causing the fluorescence spectra to overlap and whose intensity values were comparable.

Separate and isolate specific fluorescence sources could be useful in the future for multiplexing *in vivo*. It could thus allow to analyze biological and chemical process of specific molecules, compounds or organs. An other application of this method would be to isolate a specific fluorescence signal such as one binded to carcinoma from the intrinsic fluorescence of biological tissues. It represents an improvement in fluorescence optical imaging for the detection of fluorescent probes embedded in a diffusive medium and may be useful for carcinoma detection in human bodies.

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