Unsupervised analysis of FLIM-FRET data

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***Abstract*— We have developed an unsupervised method to analyze fluorescence lifetime imaging microscopy data based on non-negative matrix factorization. Additionally, by modelling the Förster resonant energy transfer process between donor and acceptor fluorophores, we retrieve the dynamics and the distribution of donor-acceptor pairs.**

Keywords—Fluorescence lifetime imaging, Förster resonant energy transfer, Non-negative matrix factorization

I. Introduction

In fluorescence microscopy, the biomolecule of interest is labelled with an efficient emitter, for example by immunostaining or genetically encoding of fluorescent proteins. An image of the emitted intensity reveals the spatial distribution of the labelled biomolecules in cells and tissues, with high contrast, and spatial resolution. Beyond the emission intensity and colour, the decay dynamics of the fluorescence intensity following pulsed excitation can be used to distinguish spectrally overlapping fluorophores [1] and reveal information on the local environment of the emitting fluorophore, such as temperature and pH. This concept is used in fluorescence lifetime imaging microscopy (FLIM).

Förster resonant energy transfer (FRET) is a non-radiative energy transfer between two fluorophores, called donor and acceptor, where the emission of the donor spectrally overlaps with the absorption of the acceptor. The transfer rate is significant for short distances the nanometer range, enabling to use the process to study protein-protein interaction by tagging two proteins of interest, one with the donor and the other with the acceptor. The energy transfer can be measured in FLIM by observing an increase in the donor emission decay rate and a delayed maximum of the acceptor emission. Different methodologies have been proposed to analyse FLIM-FRET data such as least-square fitting [2], phasor analysis [3] or deep learning [4]. In these cases, some prior knowledge on the decay dynamics needs to be assumed.

We have developed an unsupervised FLIM analysis method (uFLIM) based on non-negative matrix-factorization (NMF), which decomposes the data into a linear combination of few components defined by their spatial distribution and does not require prior knowledge of spectra or dynamics [5]. By extracting the donor and acceptor dynamics from control samples using uFLIM, we introduce a FRET analysis method which retrieves the modified dynamics due to FRET and the distribution of the donor acceptor pairs.

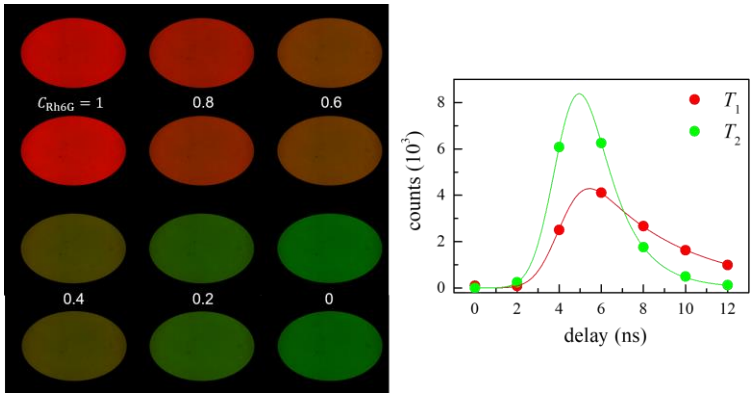


Fig. 1. uFLIM results on time gated FLIM data on mixtures of Rhodamine 6G and Rhodamine B. Left: Spatial concentrations **S** displayed as colour overlay with the red (green) channel given by the first (second) component. Right: Temporal dynamics , with red (green) symbols representing the first (second) component. The solid lines are fit to the data.

This work was supported by the Cardiff University Data Innovation URI Seedcorn Fund, the Ser Cymru II programme (Case ID 80762-CU-148) of the Welsh Government and the Royal Society Wolfson award (Grant WM140077)

II. Method

The FLIM data are reshaped as a matrix **D** of dimension ($N_s \times N_t$), where N_s and N_t indicate the number of spatial and temporal points, respectively. A NMF is then performed on **D**, which is factorised as a product of two matrices **S** and **T** of ($N_s \times N_c$) and ($N_c \times N_t$) elements, respectively, where N_c represents the number of components. We utilise a fast NMF algorithm, which minimises the residual Frobenius norm, i.e. $\|\mathbf{D}-\mathbf{ST}\|$.

The dynamics of interacting donor and acceptor molecules, e.g. undergoing FRET, can be calculated from the dynamics of the non-interacting species by considering at a given time point the transfer up to that time point. The FRET process can be modelled an additional decay channel with rate γ. To consider the variation of distance between donor and acceptor, a distribution of rates is used described by mean and standard deviation. If the acceptor emission is detected, a relative efficiency of detecting donor and acceptor emission is introduced. The most likely values for these parameters are determined as those minimizing the residuals of the NMF.

III. ResultS

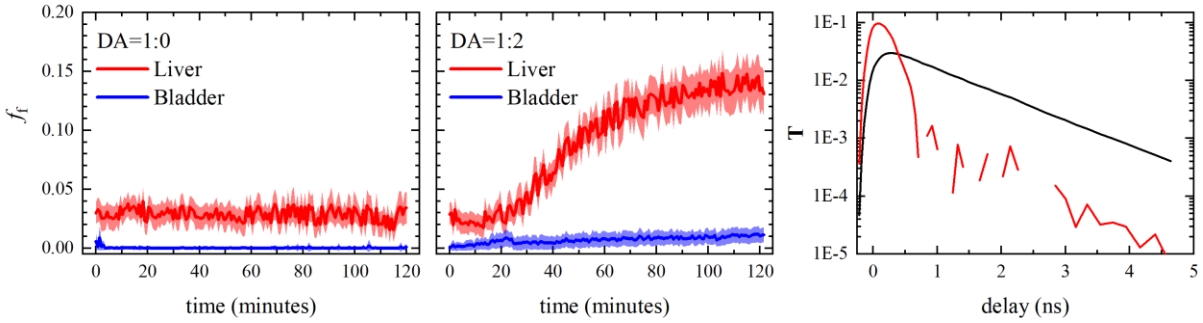
A. *FLIM data from controlled mixtures*

To demonstrate that uFLIM can unravel FLIM without prior knowledge, we analyzed FLIM data from mixtures of Rhodamine 6G and Rhodamine B at different relative concentrations [2]. Figure 1 shows the results of the NMF using two components and random initial guess for **T** and **S**. The two components of **S** are encoded as red and green channels in Fig. 1, showing the first component decreasing as the concentration of Rhodamine 6G increases. The resulting dynamics (symbols) show the first component slower than the second. An exponential fit (solid lines) results in lifetimes of 4.2ns and 1.5ns for the first and second component, respectively, confirming that the first (second) component represents the Rhodamine 6G (Rhodamine B).

B. *In-vivo FLIM-FRET data*

We have analyzed FLIM-FRET data, where donor and acceptor-labelled transferrin (Tf) were injected into mice to monitor engagement of Tf with its receptor and non-specific accumulation of Tf in the liver and its elimination via the bladder [4]. A series of images of the liver and bladder regions were acquired every 45 seconds to study the accumulation of Tf in the liver. The non-interacting donor dynamics was extracted via a uFLIM analysis of the bladder regions before injection of the acceptor-labelled Tf. The uFLIM-FRET analysis retrieves a FRET component with an average γ=6.8GHz and negligible distribution width (Fig. 2). The fraction of donor molecules undergoing FRET is close to 0 in the bladder region, indicating that only non-interacting donor are accumulating there. In the liver this fraction increases to about 14% after acceptor injection.

Fig. 2. Analysis of FLIM-FRET data on Tf-TfR engagement. Left: evolution of fraction of donor molecules undergoing FRET in a control sample



(Donor:Acceptor=1:0) in liver (red) and bladder (blue). Center: same as left for the sample with acceptor-labelled Tf (Donor:Acceptor=1:2). Right: dynamics of the non-interacting donor (black) and donor molecules undergoing FRET (red)

IV. Conclusions

We have developed an unsupervised method to analyze FLIM data using non-negative matrix factorization to infer the emission dynamics and the spatial distribution of emitting molecules. Modelling the FRET process in the dynamics, distributions of FRET rates and donor-acceptor pair densities can be extracted.

Acknowledgment

This work was supported by the Cardiff University Data Innovation URI Seedcorn Fund .F.M. acknowledges the Ser Cymru II programme (Case ID 80762-CU-148) which is part-funded by Cardiff University and the European Regional Development Fund through the Welsh Government. P.B. acknowledges the Royal Society for her Wolfson research merit award (Grant WM140077).

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