PhD Program in Physics and Nanoscience

Student: Lisa Cuneo
Cycle: XXXVI
Tutor: Alberto Diaspro

ANNUAL REPORT

1. RESEARCH ACTIVITY

My research activity deals with the design, implementation and application of machine learning algorithms to microscopy images. The aim is to extrapolate and structure the relevant information contained in the data acquired with the specific microscopy technique according to its peculiarities and drawbacks. In details, my work concerns the following different microscopy methods:

- Single-molecule localisation microscopy (SMLM): in this kind of experiments, the presence of background degrades the image quality and contrast, since it can compromise the localisation precision of the single molecules. In this contest, the aim is to develop an algorithm that accurately removes the background, improving the localisation accuracy without wasting photons.
- Fluorescence-Lifetime Imaging Microscopy (FLIM): the acquisition of the photon arrival time allows measuring the lifetime of fluorescence molecules point by point. Such temporal information, while it is a specific property of the fluorescence species, can also reflect local chemo-physical conditions. The goal here is to develop an algorithm that can spatially map the fluorescence molecules based on their specific temporal signature information, discarding the spectral one.
- Fluorescence Correlation Spectroscopy (FCS): provides estimations of dynamic properties, such as diffusion coefficients, through fitting methods applied to the auto/cross-correlation function. The idea is to consider the problem of estimating the diffusion coefficients from the autocorrelation function as in inverse problem and to solve it using a Bayesian method.
- Label-free methods generate contrast by exploiting specific physical properties of the light-matter interaction, therefore, imaging without the need for fluorescence labels. Label-free imaging can be obtained by means of Differential Interference Contrast (DIC), Phaco and Muller Matrix. The goal is to predict the corresponding fluorescence image from the label free one. Label free transformation in fluorescence imaging benefits of a training made using super resolved fluorescence images. The ambition is to offer the chance of obtaining molecular maps without the need of labelling.

A Neural Network is applied to all these kind of data in order to perform the required task. In details:

- For SMLM a Scattering Network followed by an up-sampling Convolution Neural Network (CNN) are applied in order to remove the background from the sequence of images by means of features extracted by a cascade of wavelet filters. To exploit not only the existing spatial information in the dataset but also the temporal one, another representation of the dataset was required. Therefore, the Singular Value Decomposition is applied and, by means of a threshold, it is possible to separate the meaningful data from the background.
- A more complex CNN architecture is required in the case of FLIM images. Convolution on time and on space are combined in order to extract the features needed to separate the contribution of two different fluorophores. According to the specific task, the architecture is also split to reconstruct two images. With the double aim of better explaining the network's behaviour and incorporating the physical model, a new CNN (Fig. 1) is trained to decompose the input sequence of images and reconstruct it respecting the photon flux conservation law.

- The first approach to the FCS problem was Bayesian. The algorithm solves the inverse problem related to the autocorrelation function calculated from the trace of photons detected on time. However, the aim is to find a good architecture that estimates the diffusion coefficient directly from the trace. Some Recurrent Neural Network as Long Short Term Memory was trained.
- The CNN applied to convert a label-free image into a fluorescence one is a U-net.

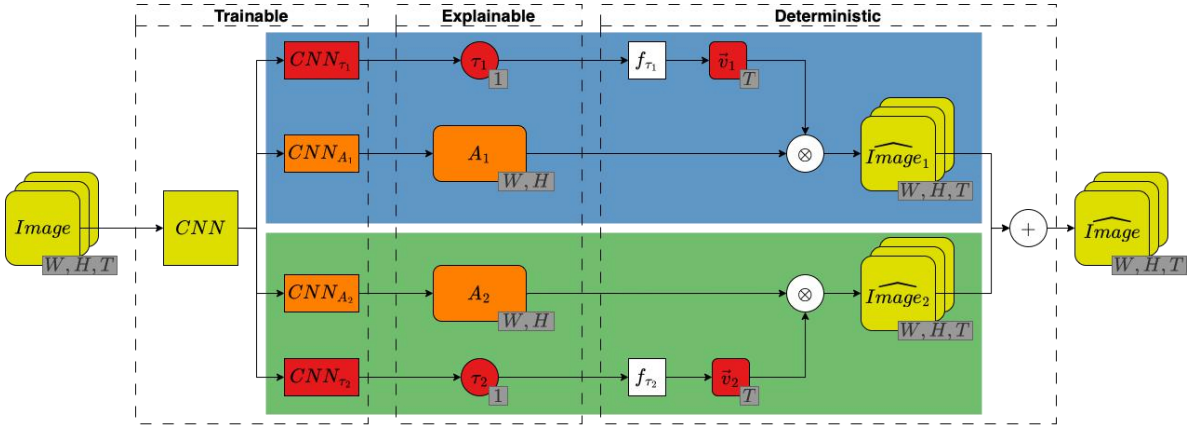

Figure 1: Design of the NN architecture. Each vector's dimensions are shown in grey. The parts estimating the spatial and temporal features are shown in orange and red, respectively. The two components are represented in blue and green. The network is divided into three sections. A trainable one, consisting of 5 CNNs, one to perform feature extraction and 4 for prediction. The second section provides an explanation of the network's output. A final deterministic one reconstructs the input image.

2. Pubblications

Under publication — IL NUOVO CIMENTO

A deep learning-based method to spectrally separate overlapping fluorophores based on their fluorescence lifetime

L. CUNEO, M. CASTELLO, S. PIAZZA, I. NEPITA, I. CAINERO, G. TORTAROLO, L. LANZANÒ, P. BIANCHINI, G. VICIDOMINI, A. DIASPRO

Conference proceedings — The Italian Physical Society, Volume 210, pp. 143–145, 2023

Scattering Networks: a tool to remove the background.

L. CUNEO, S. CIVITA, P. BIANCHINI, A. DIASPRO

Conference proceedings — Biophysical Journal 122 (3) pp. 462a, 2023

A deep learning method to separate fluorophores based on their fluorescence lifetime.

L. CUNEO, M. CASTELLO, S. PIAZZA, I. NEPITA, L. LANZANÒ, P. BIANCHINI, G. VICIDOMINI, A. DIASPRO

3. Other activities

27 Aug. – 1 Sep, 2023 — SIMAI 2023 – Matera, IT

Congress of the Italian Society of Applied and Industrial Mathematics.

I presented a talk with the title *'Bayesian approach to Fluorescence Correlation Spectroscopy'* inside the mini-simposium 'Bayesian and Monte Carlo methods in applied science and industry'.

Apr 10 – 13, 2022 — FOM 2023 – Porto, PT

Focus On Microscopy 2023.

I presented a talk with the title 'Scattering Networks and Singular Values Decomposition: different methods to remove background in *Single-Molecule Localization Microscopy images*'.

Feb. 18 – 22, 2022 — BPS 2023 – San Diego, USA

Biophysical Society Annual Meeting.

I presented a talk with the title *'A deep learning method to separate fluorophores based on their fluorescence lifetime.'* inside the section 'Computational Methods and Machine Learning, Artificial Intelligence and Bio-informatics'.