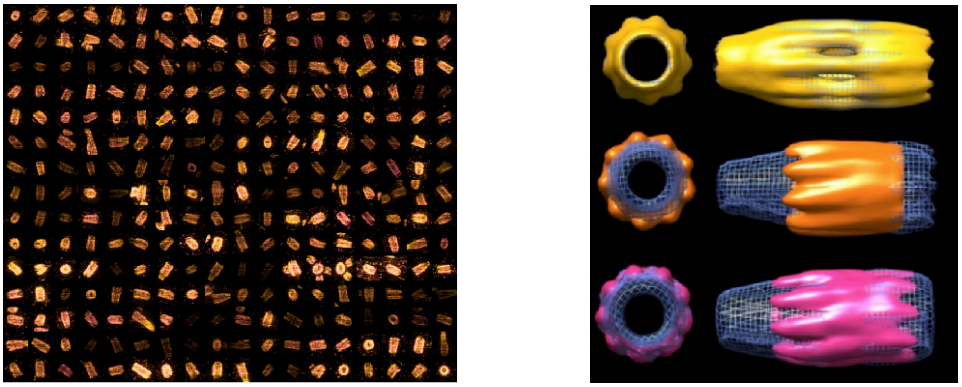
Master 2 Internship

Detection and segmentation of macromolecular assemblies with deep learning in fluorescence microscopy

Fluorescence microscopy is a fundamental observation tool to understand complex cellular mechanisms. The class of super-resolution techniques has been developing rapidly in recent years and it is now able to reach resolutions of a few nanometers in 2D. However, the 3D observation of small particles such as macromolecular assemblies is still hampered by a low resolution in the axial direction of the microscope. Moreover, the fluorescent proteins do not cover completely and uniformly the targeted particles, such that a single image yields only a partial view of the actual structure of cellular objects.

To overcome these limitations, this internship will take place in the context of a single particle reconstruction (SPR) project[1]. The principle of SPR is to acquire images that contain a large number of randomly oriented copies of a rigid particle, and to reconstruct a single model of the particle. The combination of multiple views highly improves the resolution and compensates for the partial fluorescent labelling in the input data. The IMAGeS team has developed reconstruction methods for SPR in fluorescence microscopy [3, 4], which led to promising results for biological applications [5, 2].



(a) (b)

Figure 1: Principle of single particle reconstruction [2]. (a): Examples of particles picked in the acquired images; (b): Examples of reconstructions of the particle model for different proteins.

The first step of the SPR pipeline is particle picking. It consists in detecting and segmenting the individual particles (typically several hundreds) in the acquired images. The accuracy of the picking has a crucial impact on all the subsequent steps of the pipeline, up to the final reconstruction. However, there exists

[1]Funded by the French National Research Agency (ANR), through the "SP-Fluo" project

no dedicated method for this task in fluorescence microscopy. In practice, the picking is often performed manually, which, in addition to be a tedious task, is prone to subjective bias and limits the number of picked particles.

The goal of this internship is to fill this gap and develop a particle picking method for SPR in fluorescence microscopy. Three main challenges have to be overcome: (i) the anisotropic resolution creates a large blur in the axial direction, such that particles with different orientations appear with different shapes; (ii) we do not have a prior model of the shape of the particle of interest; (iii) the images are corrupted with other undesirable cellular structures and with noise. We will rely on a semi-automatic approach, where a few particles are manually selected in a region of interest and are used to train a classifier that detects the particles in the rest of the images. A major difficulty will be to deal with the low number of training images resulting from manual picking. The starting point of the internship will be based on the literature on particle picking for another modality of microscopy called cryo-electron microscopy (cryo-EM), where the data is composed of 2D projection images of the 3D biological sample. In particular, we plan to investigate two main approaches:

- Extraction of rotationally invariant image features followed by particle detection with an ensemble classifier [6].
- Classification based on deep neural networks and positively unlabelled learning to deal with the low number of training images, following the approach described in [1].

These methods have been designed for 2D cryo-EM data, which fundamentally differs from our 3D fluorescence images with anisotropic blur. The first part of the internship will consist in adapting these ideas to the specificity of our data. Depending on the progress of the work, we will explore improvements such as refinement with iterative picking and reconstruction, or estimation of the orientations of the particles directly from the original images when a coarse template of the particle known.

The methods developed during the internship will be integrated to the existing SPR pipeline available in the IMAGeS team. The objective is to facilitate and improve the reconstruction process for the applications in biology developed by the group of Paul Guichard and Virginie Hamel in University of Geneva (`https://cellbio.unige.ch/research/paul-guichard/`). We already have at our disposal large datasets of images from various microscopy modalities that have been used in the past for biological applications [4, 5, 2].

Working environment

The intern will be a member of the IMAGeS team (`http://images.icube.unistra.fr/`) in the ICube laboratory in Illkirch. The internship will begin between January and May 2020, for a period of 6 months.
Supervisors: Denis Fortun (CNRS researcher, ICube, `dfortun@unistra.fr`), Etienne Baudrier (Assistant Professor, University of Strasbourg `baudrier@unistra.fr`).

Profile of the candidate

- Second year of Master studies in one of the following fields: computer science, applied mathematics, machine learning
- Good programming skills (the coding language will be Python)
- An interest for biomedical applications is welcome

Application

Send a CV and a short description of your motivation, as well as the transcript of marks for the past 2 years to Denis Fortun (`dfortun@unistra.fr`) and Etienne Baudrier (`baudrier@unistra.fr`).

References

[1] T. Bepler, A. Morin, M. Rapp, J. Brasch, L. Shapiro, A. J. Noble, and B. Berger. Positive-unlabeled convolutional neural networks for particle picking in cryo-electron micrographs. *Nature methods*, 16:1153–1160, 2019.

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