OT-Analysis : a software for rich analysis of force curves when probing living cells with optical tweezers

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1 Summary

Optical tweezers are a light-based technique for micromanipulating objects. It allows to move objects such as microbeads and cells, and to record minute forces down to a few pN, which makes it a technique very well adapted to mechanical measurements on living cells [1]. We are interested in the mechanotransduction properties of lymphocytes. We seek to dissect the effect of forces and cell mechanics on the cellular response, in the context of the immune system. T cell mechanotransduction has been recently demonstrated to be instrumental in the finesse and accuracy of the response of the latter [2]. Aside, cells can exert forces when performing their action, eg. cytotoxic T cells are using forces to kill target infected cells [3].

Using optical tweezers and specifically decorated beads as handles, we pull membrane nano-tubes from gently adhered living lymphocytes [4]. Such nanotubes are usually used to probe the tension of adherent cells [5]. By varying the antibodies that are used to decorate the beads, we select the molecule type we specifically pull on, and we then explore the molecules which are characteristic of the immune synapse, that is one of the key organisational structures that has profound implications in T cell recognition and action [6].

Using this approach, we probe not only the forces of recognition of the given antibody to its target molecule, but also, by using strong extracellular bridges, we probe the cytosolic link of the probed molecule to the cytoskeleton. Such a link has been proposed to be instrumental in the way T cells can apply or feel forces through the molecule. A theoretical model has been built and will be reported in a dedicated article [7]. Aside, we will demonstrate the application of the software on full data.

The experimentally obtained data consists in force signal as a function of time (among other parameters), in the three directions of space, obtained in large quantities (at least 10 per cell / bead couple, and up to 20 couples tested per sample), containing rich and detailled features that can relate to molecular and/or cellular mechanics that our model explores. It is therefore needed to

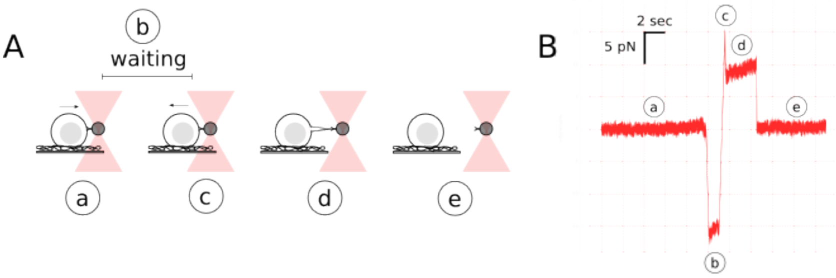

Figure 1: Schematics of the experiment. A : Sequence of events (a) antibody decorated bead, trapped by the focused infrared laser beam, and lymphocyte are far from each other, and approached ; (b) contact is formed under a controlled level of force and maintained for a given time ; (c) the cell is displaced away from the bead, eventually resulting in the pulling of a tube (d) and its rupture (e), back to the situation of (a). B : Resulting Force vs.Time curve with the indicated events. Adapted from [7]

standardize and semi-automatize data analysis to help the experimentalist, often a biologist, to extract relevant features from the experimental data sets.

2 Statement of need

The avalaible software that comes with the optical tweezers setup includes a data processing system, with a GUI, that allows the user to follow pre-defined data processing schemes. Even if it already allows the typical user to observe, manipulate, quantify and convert to plain text and images the data, it is closed source and, as such, cannot receive implementations of novel functions, depending on the experimentalist needs.

In particular, in regard to the above described application, the user would have to interact a lot via mouse-clicks and find alternative use of preexisting data processing functions to perform the (time) expensive analysis required. This may introduce bias in the data, which may impair user-to-user data comparison.

Aside, almost none, if any, open source software has been proposed to the community eg. via GitHub or GitLab for quantifying optical tweezers experiments with living cells, while some have been proposed for Atomic Force Microscopy force mode [8], with a modular capability. Among them, one can find, with the keywords ”optical tweezers”, on GitHub :

- https://github.com/ilent2/ott : an Optical Tweezers Toolbox for calculating the torques and forces on a bead in a simulated situation
- https://github.com/ilent2/tweezers-ml : an Interactive Optical Tweezers simulation using Machine Learning
- https://github.com/ghallsimpsons/optical_tweezers : set of macros for calibration
- https://github.com/softmatterlab/OpticalTweezersTutorial : a tutorial

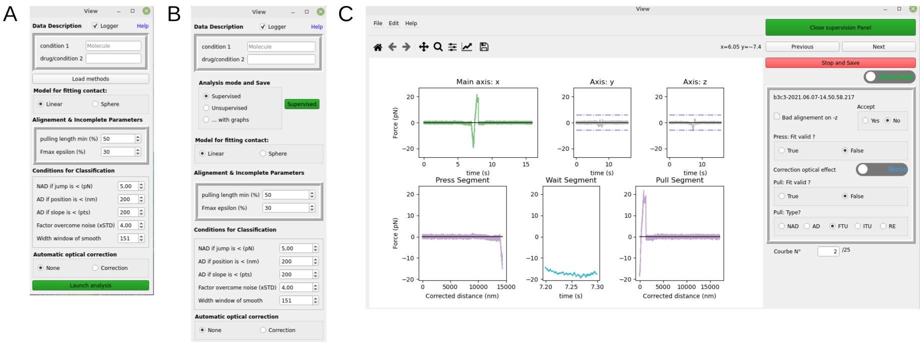

Figure 2: Snapshots of OT-Analysis software. A : Starting window where data can be selected and parameters for the analysis set. B : Setting the choice of analysis, from fully automated to user supervised. C : Main window of supervision showing the raw data in the three directions of space, and the results of the pre-analysis, allowing the user to amend the analysis if needed.

Of note, OT setups usually allow allows to quantify the forces in the three directions of space. Thus lateral forces resulting from small geometric misalignments between a given bead and the cell can be probed. As a consequence, our software has been designed to allow the direct comparison between these three directions in order to select curves where the force is detected mainly in one single direction corresponding to the one selected during the experiment. Aside, we introduced refined baseline corrections for forces which may be caused, for T cells, by the deformation of the trap close to contact. We quantify the cell mechanics, when pushing the bead on the cell, and also cell adhesion or tube pulling when separating them. Due to the large number of curves that are typically produced, we implemented data processing by subsets, in order to be able to use regular or old computers to be able to distribute it to our students.

We based our software on command line processing functions that we developed in the lab, and implemented a user friendly, modular, Qt based GUI which is more than needed when a non code-savy scientist wants to process complex biological data.

Our resulting software, as such, can serve as a basis for adding new features in the field of force measurements by optical tweezers.

3 Acknowledgements

We acknowledge data contributions from O. N'Dao, G. Eich, L. Normand. We also acknowledge the work on a previous Tk based version of the GUI from K. Himmet. We aknowledge the funding of Labex INFORM (ANR-11-LABX-0054 and AMIDEX project (ANR-11-IDEX-0001-02), funded by the "Investissements d'Avenir" French Government program managed by the French National Research Agency (ANR)) that allowed LAI to obtain the optical tweezers set-up, and the continuous help from JPK instruments/Bruker with it.

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