

# OT\_Analysis: a software for rich analysis of force curves when probing living cells with optical tweezers

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## Software

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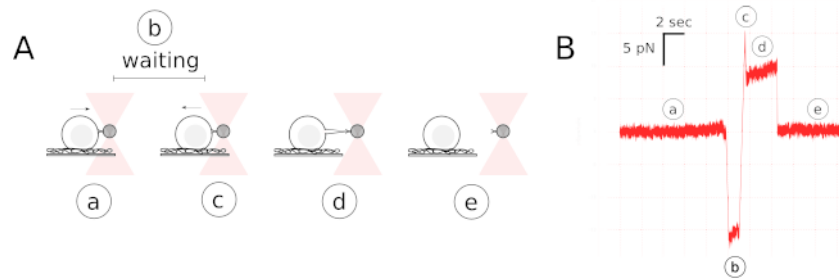
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## Summary

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 ([Gennerich, 2017](#)). 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 Puech & Bongrand ([2021](#))). In addition, cells can exert forces when performing their action, e.g. cytotoxic T cells are using forces to kill target infected cells ([Basu et al., 2016](#)).

Using optical tweezers and specifically decorated beads as handles, we pull membrane nanotubes from gently adhered living lymphocytes ([Sadoun et al., 2020](#)). Such nanotubes are usually used to probe the tension of adherent cells ([Diz-Muñoz et al., 2010](#)). 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, which is one of the key organizational structures that have profound implications in T cell recognition and action ([Baldari & Dustin, 2017](#)).

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 has been recently reported in a dedicated article ([Manca et al., 2023](#)). Furthermore, we will demonstrate the application of the software on full data.



**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 (Manca et al., 2023)

The experimentally obtained data consists of 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 detailed features that can relate to molecular and/or cellular mechanics that our model explores. It is therefore needed to standardize and semi-automate data analysis to help the experimentalist, often a biologist, to extract relevant features from the experimental data sets.

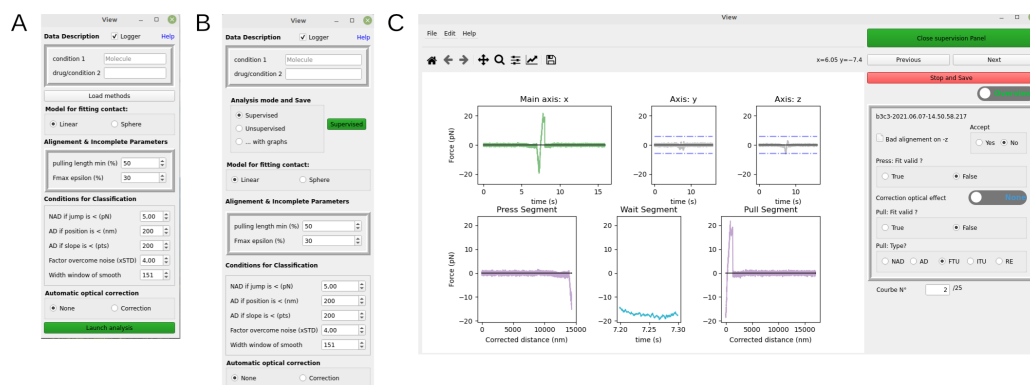
## Statement of need

The available 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, regarding the above described application, the user would have to interact a lot via mouse clicks and find an 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.

In addition, almost none, if any, open-source software has been proposed to the community e.g. via GitHub or GitLab for quantifying optical tweezers experiments with living cells, while some have been proposed for Atomic Force Microscopy force mode (Müller et al., 2019), 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](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 : The 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 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 to select curves where the force is detected mainly in one single direction corresponding to the one selected during the experiment. Furthermore, we introduced refined baseline corrections for forces that 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, 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.

## Example usage

The prototypical experiments consist in using optical tweezers to contact a cell with a trapped bead decorated with protein, in order to obtain some membrane tube pulling events upon separation. The first use of the present software is to ensure that such a pulling is specific to the protein decoration used. In this case, two sets of data curves (force vs. time) are obtained using an adhesive protein and a control protein (in our case an antibody specific for a surface protein of the cell and a second antibody, isotype to the first one, but not recognizing any surface protein a priori).

The software allows, after an automated pre-processing step, a inspection of the data by the user in order to amend the type selection and potentially refine, on a case per case basis, the data processing. The user can adjust several detection thresholds, e.g. for determining the duration and force amplitude that characterize adhesion events and potential tubes, and for tuning the detection and correction for optical artifacts.

The outcome will provide graphical representations to:

- evaluate the fraction of the positive events (adhesion + tubes) in the population of forces curves

- while rejecting the curves that are used for aligning the two substrates
  - while excluding the curves where optical artifacts can be detected
  - while removing from the analysis the curves that are “bad” (e.g. when the bead is lost or when a second bead enters the trap)
- measure the duration and force magnitude of the positive events
  - measure the amount of tubes in the overall population.

The output file can then be reloaded using ad-hoc procedures (e.g. in Python) to merge or sort the data, plot, perform statistical analysis on relevant biophysical parameters described in [https://github.com/phpuech/OT\\_Analysis](https://github.com/phpuech/OT_Analysis).

The results of the analysis can then be used to feedback on the experimental parameters (contact force, contact duration, molecular densities on the beads, bead size) and / or compare different molecules in order to examine a biological effect (for this last point, see (Manca et al., 2023)).

## Functionality documentation

We provide in the following figure the schematics of the structure of the code, with the main files.

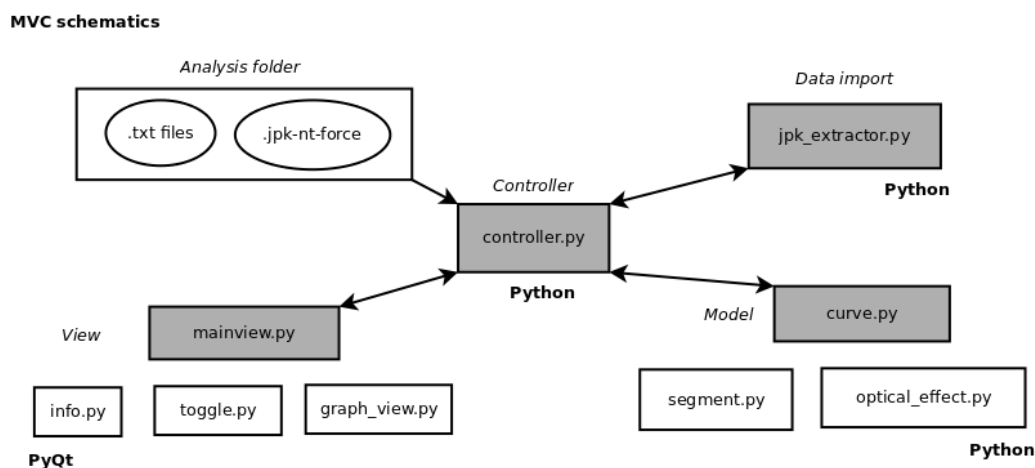


Figure 3: Schematics of the MVC conception of OT\_Analysis.

For a description of the functions and their arguments, please refer to the online documentation provided in the link below :

<https://otanalysis.readthedocs.io/en/latest/otanalysis.html?highlight=otanalysis>

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