

FS-LASER TRANSFECTION OF LIVING CELLS – STRATEGIES FOR HIGH THROUGHPUT

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1. Abstract

Lately, several groups successfully used ultrashort laser pulses to selectively permeabilize the membrane of living cells to achieve transport of foreign molecules, like DNA, into the cells. For this, the high field intensities of tightly focused laser pulses are used to induce multiphoton absorption and the creation of a small scale optical breakdown at the membrane of the target cell. Afterwards, DNA or other foreign molecules are able to diffuse into the cell and achieve, for example, transfection of living cells. However, the cell throughput of this method is low, as, due to tight focusing. We present to possible techniques to achieve fs-laser transfection in living cells at higher throughput.

2. Methods and Experiments

To achieve high throughput transfection using ultrashort laser pulses, we will present two possible alternatives:

Microfluidic approach

Using microfluidics in combination with high numerical objectives, we successfully transfected non-adherent HL-60 cells within a laminar flow. For precise positioning of the cells with respect to the focus of the ultrashort laser pulses, a line trap (optical tweezers) at 1064nm was employed.

Nanoparticles approach

Using gold nanoparticles, we successfully used the plasmonic near-field enhancement of gold nanoparticles to perforate the membrane of living cells. As the ultrashort laser pulses need not to be focused at the single cells' membrane, a mildly focused laser beam with a spot size of 30µm was scanned across adherent cells.

Successful transfection was monitored 24-48 h after laser application by expression of several fluorescent proteins, as for example GFP-HMGB1.

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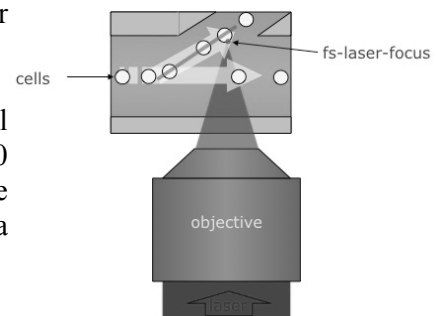


Figure 1: Microfluidic based optoporation of non-adherent cells