Labeling of single, intact plant root cells using laser-mediated optoperforation

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Introduction

The interface between physics and biology has been the source of scientific advances in medical engineering and animal cell biology, but plant science has been slower to benefit from this synergy. Much of our knowledge of plants is derived from tissue-level studies; while easy to analyze they do not provide insight into the cellular-level responses that are central to the function of the whole organism. Thus, single-cell research is needed to discover how cells influence everything from their immediate neighbors to distant tissues. Current methods to modify individual cells are cumbersome (i.e. microinjection), or deleterious to the cell (i.e. biolistics). Recently, fluorescent markers and transgenes were introduced into single animal and cell wall-free cultured plant cells using a laser-based method known as optoperforation. However, the substantial structure and optical properties of the cell wall are particularly challenging for ablation. Here we demonstrate a successful system of transiently breaching the plant cell wall in order to allow passive uptake of fluorescent molecules and long-term survival of those individual cells.

Methods

Adapting optoperforation for plants

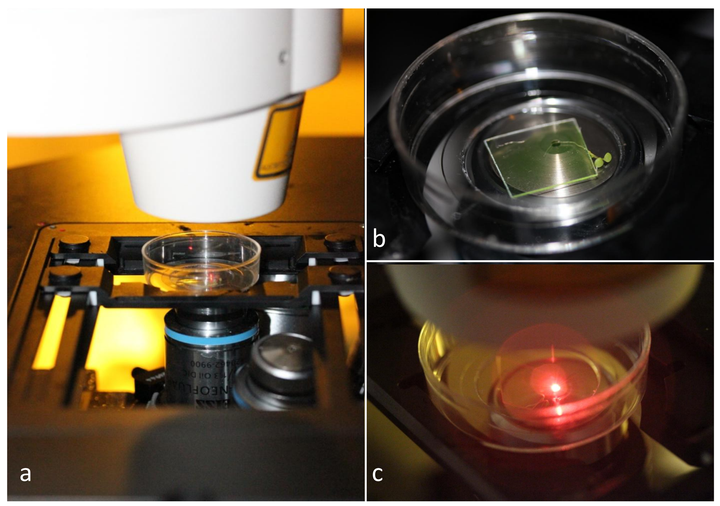
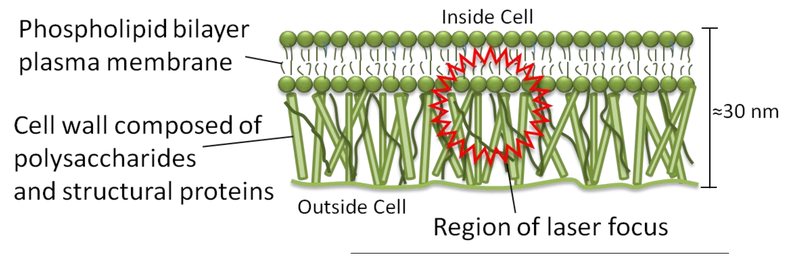
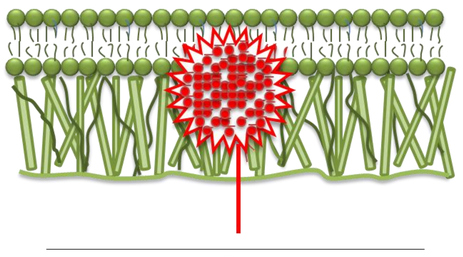


Figure 1. Overview of optoperforation setup optimized to minimize root damage and be consistently repeatable. (a)Confocal microscope stage with 15mm glass-bottom dish (MatTek®). (b) *Arabidopsis thaliana* orientation in the dish, immersed in a PBS-dextran solution, covered with plastic coverslip, mounted on 40X objective. (c) Focused femtosecond pulse of 700nm wavelength laser, aimed at the wall of a single root cell.

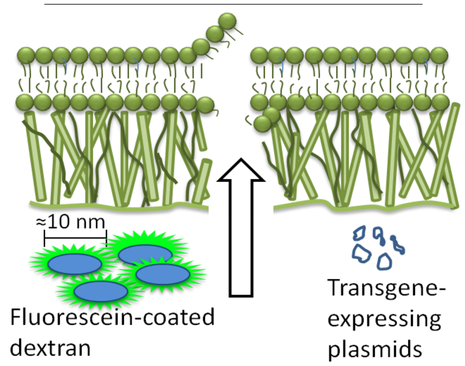
Laser-mediated cell infiltration



Femtosecond laser pulse forms a charged cloud of free electrons (plasma) at the focal volume through multiphoton excitation which breaks bonds in the cell wall and membrane.



A transient pore is formed, allowing an influx of external media – such as fluorescent markers or genetic material – via an osmotic gradient.



The plasma membrane and cell wall re-compose, trapping markers inside the cell.

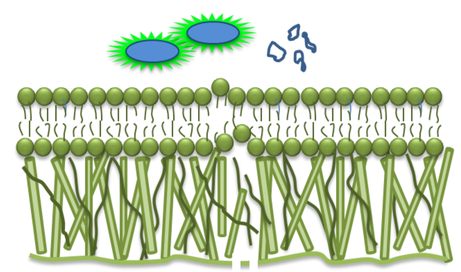


Figure 2. This injection method involves tightly focusing a pulsed low-energy, near-infrared laser onto a cell membrane. Within this small focal region multiphoton effects induce a cascade of electron excitations to form a low density electron cloud. As a result of this interaction, transient cell openings are created, allowing diffusion of surrounding particles into the cell. In this experiment we have had success with flourescein-tagged 70kDa dextran molecules. To aid in visualization, roots were stained with a red cell wall and nuclei stain (propidium iodide) and imaged on a Zeiss LSM 510 confocal microscope.

Results

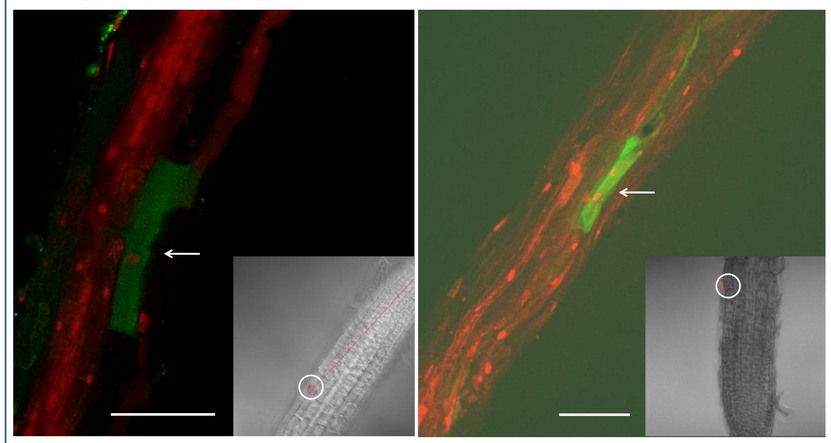


Figure 3. Single root cells (arrows) emitting green fluorescence from dextran-fluorescein introduced by optoinjection. Inset images taken prior to perforation and staining, white circles indicate site of laser-mediated perforation. Bars = 50µm

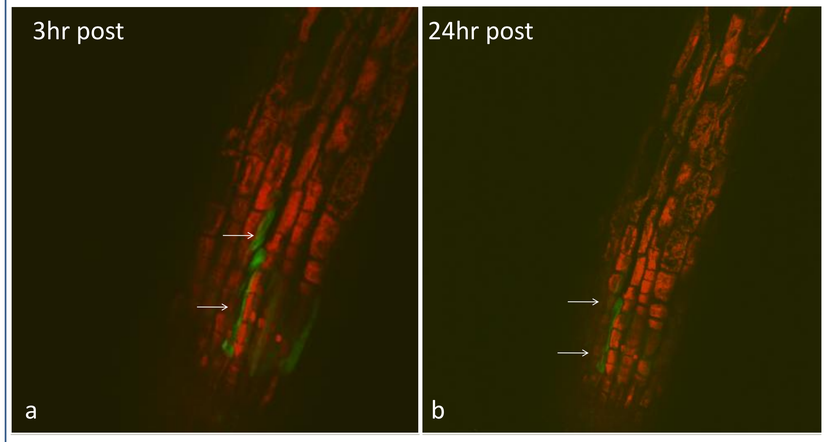


Figure 4. 3 hr (a) and 24 hr (b) post perforation roots showing persistence of fluorescein signal inside two adjacent laser injected cells (arrows) which indicates retention within an intact plasma membrane.

Conclusion

As a direct result of the collaboration between physicists and plant physiologists, we have developed a technique to show the first successful optoperforation of intact plant tissue via permeation with fluorescent 70 kDa dextran molecules. Cell survival was monitored for 24 hours and the dextrans remained distributed throughout the cytoplasm while the cell maintained integrity. This proof of principle paves the way for other exciting experiments, such as incorporating transgenes. This research has applications in studying the metabolism of single cells such as cell-to-cell movement of large molecules and may provide a new approach for generating transgenic plants.

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