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Title: Digital microfluidic chip technology for water permeability measurements on single isolated plant protoplasts

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## ACCEPTED MANUSCRIPT

## Digital microfluidic chip technology for water permeability

## **measurements on single isolated plant protoplasts**

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### **Abstract**

- Dynamic follow up and analysis of isolated single cells that display non-adhering behaviour is hindered by the fact
- that they float in the suspension medium and thus requires the implementation of systems that physically entrap the
- 17 cells for successful analysis. We describe the potential of digital microfluidic (DMF) chip technology for conducting
- analysis of cells in suspension at the single cell resolution. More specifically, we demonstrate the use of DMF
- technology for the analysis of a group of single individual protoplasts from *Arabidopsis thaliana* plants that are
- 20 labelled with magnetic particles and are immobilized on the DMF chip by magnetic forces. By transporting droplets
- 21 with different osmotic conditions to the site where the cells are trapped, we challenge the cells and monitor their
- 22 responses dynamically with a camera. The use of DMF technology for performing water potential measurements has
- the following advantages: (i) solid particles such as cells and magnetic beads are manipulated on the chip without the
- 24 risk of clogging channels, (ii) low shear stress during droplet unit operations like mixing and transport that
- characterize DMF are particularly suited for analysis of delicate cells types that lack cell walls such as protoplasts,
- 26 (iii) the throughput of the analysis is strongly increased as multiple protoplasts are analysed simultaneously, in
- 27 contrast with the traditional methods that can handle and challenge only one cell at a time. We show that the DMF
- analysis platform is effective in creating the steep osmotic gradients required for calculation of water permeability
- coefficients (P) as the values found are comparable with previously reported ones thus validating the suitability of
- the technology for such studies. This work illustrates a proof of concept for the applicability of DMF as an
- 31 unparalleled and promising system for implementing single cells studies on non-adhering cells in an automated way.

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- **Keywords:** Digital microfluidic chip; Water permeability coefficient; Protoplasts; Magnetic cell immobilization;
- 34 Single cell analysis; Lab-on-a-chip

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#### 1. Introduction

37 Absorption of water molecules and solutes from the environment and their transport in plants represents one of the

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