

## Custom microelectrodes delivery system for nsPEF cell electroporation

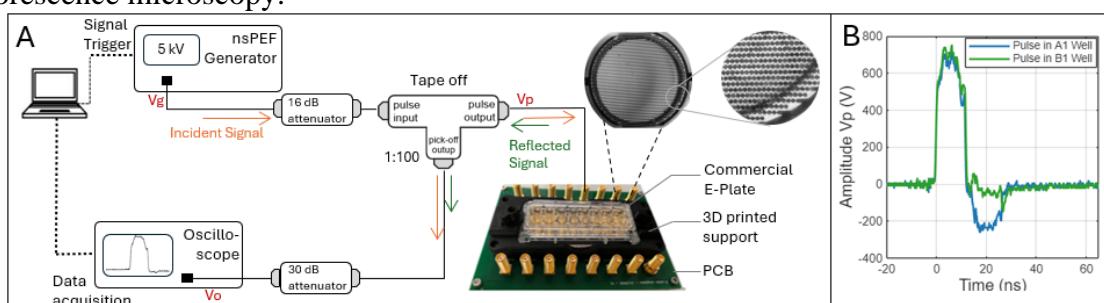
N. Van<sup>(1)</sup>, L. Michard<sup>(2)</sup>, N. Reitano Ferber<sup>(1)</sup>, G. N'kaoua<sup>(1)</sup>, F. Poultier de Gannes<sup>(1)</sup>, D. Arnaud-Cormos<sup>(2)</sup>, Y. Percherancier<sup>(1)</sup>, I. Lagroye<sup>(1,3)</sup>, P. Leveque<sup>(2)</sup>, N. Lewis<sup>(1)</sup>, R. Orlacchio<sup>(1,3)</sup>.

(1) Univ. Bordeaux, CNRS, IMS, UMR 5218, Talence, France (2) XLIM, Univ. Limoges, CNRS, Limoges, France (3) École Pratique des Hautes Études, PSL Research University, 75014 Paris, France

Nanosecond pulsed electric fields (nsPEF), characterized by high intensities (MV/m range) and ultrashort durations (<100 ns), have attracted increasing attention due to their ability to modulate a wide range of physiological responses and their potential therapeutic applications [1]. nsPEF delivery systems present engineering challenges including handling high-voltage and broadband signals while meeting bio-experimental geometric constraints. While cuvette-based systems ensure uniform exposure of up to millions of non-adherent cells, they are incompatible with real-time microscopy. In contrast, paired wire electrodes allow real-time imaging of adherent cells, yet expose only a limited region, leaving cells surrounding the electrodes unexposed. As a result, for 2D adherent cell cultures, a major constraint lies in the need to expose an entire monolayer to a spatially homogeneous electric field. In this context, microelectrode arrays or interdigitated microelectrodes represent well-suited solutions, enabling localized field application directly at the cell level [2].

This study characterizes a customized nsPEF delivery system based on commercial interdigitated microelectrode plates, i.e., XCELLigence E-Plate of 16 wells [3]. Each well contains two electrodes with a 40 µm gap integrating 25 gold fingers (40 µm-radius chained circular segments) at the bottom. A custom 4-layer printed circuit board (PCB) adapter was designed to connect the high-voltage generator and oscilloscope to the plate (Fig. 1A), using impedance-matched striplines buried between ground planes to minimize reflections and electromagnetic radiation.

The characterization of this setup relies on three complementary approaches: (1) Time domain pulse measurements to evaluate impedance matching and pulse integrity. (2) Numerical dosimetry via simulations (COMSOL Multiphysics) to quantify the electric field spatial distribution at the cell level. (3) Biological validation with nsPEF-induced membrane permeabilization in adherent cells monitored via fluorescence microscopy.



**Figure 1.** **A.** Representation of the experimental exposure system. **B.** 10 ns-pulse measured at the PCB input of two wells filled with 100 µL HBSS (Hank's Balanced Salt Solution).

1. A. R. Ruiz-Fernández, L. Campos, G. Núñez, F. Villanelo, and T. Perez-Acle, "Nanosecond pulsed electric field (nsPEF): Opening the biotechnological Pandora's box," *Int. J. Mol. Sci.*, vol. 21, p. 8882, Nov. 2020. doi: 10.3390/ijms23116158.
2. M. Schubert, J. Rasche, T. Vuorinen, M. Mäntysalo, and K. Bock, "Printed flexible microelectrode for application of nanosecond pulsed electric fields on cells," *Materials*, vol. 12, no. 17, Art. no. 2713, Aug. 2019, doi: 10.3390/ma12172713.
3. D. Arnaud-Cormos, R. O'Connor, Y. Percherancier, B. Veyret, and P. Leveque, "Delivery system setup and characterization for biological cells exposed to nanosecond pulsed electric fields," in Proc. IEEE Int. Conf. Pulsed Power Conf., CO, USA, 2015, doi: 10.1109/PPC.2015.7296952.