

# 24-hour monitoring of nsPEF induced morphological changes, in U-87 MG cells, using digital holographic microscopy

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## Introduction

Live cell imaging has been widely used to characterise changes in cellular dynamics, that occur in the seconds and minutes, following the application of nanosecond pulsed electric fields (nsPEF).

To study longer term effects, such as viability and cell death, a cuvette based exposure system is often used. This method requires cells to be in suspension, posing limitations for adherent cells.

Digital holographic microscopy is a real-time imaging technique that can be used to give quantitative measurements of cell morphology in unlabelled, adherent cells over extended time periods.

## Objective

To use a holographic microscope, developed for use within a standard cell culture incubator, to monitor both short term and long term changes in morphology of U-87 MG cells that have been exposed to nsPEF in their naturally adherent state.

## Material and Methods

**Digital holographic imaging:** U87 human glioblastoma cells were observed in real time using a M4 HoloMonitor (Phase Holographic Imaging AB, Sweden) placed on the workbench (30 minute experiments) or in an incubator (24 hour experiments) (fig 1).

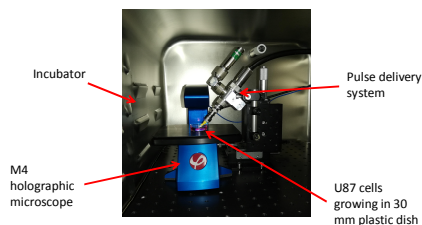


Figure 1. Holographic microscope setup

**Pulse exposure system:** composed of a nsPEF generator (50Ω output impedance, 10ns pulses), a 1GHz digital phosphor oscilloscope, a high-voltage measurement device (tap-off) and electrodes-based delivery system (fig 2). An electric field strength of 44 kV/cm was applied at 10 Hz repetition rate.

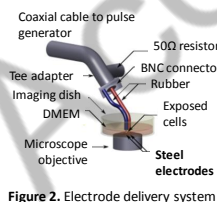


Figure 2. Electrode delivery system

## Results

### Short term imaging of morphological changes

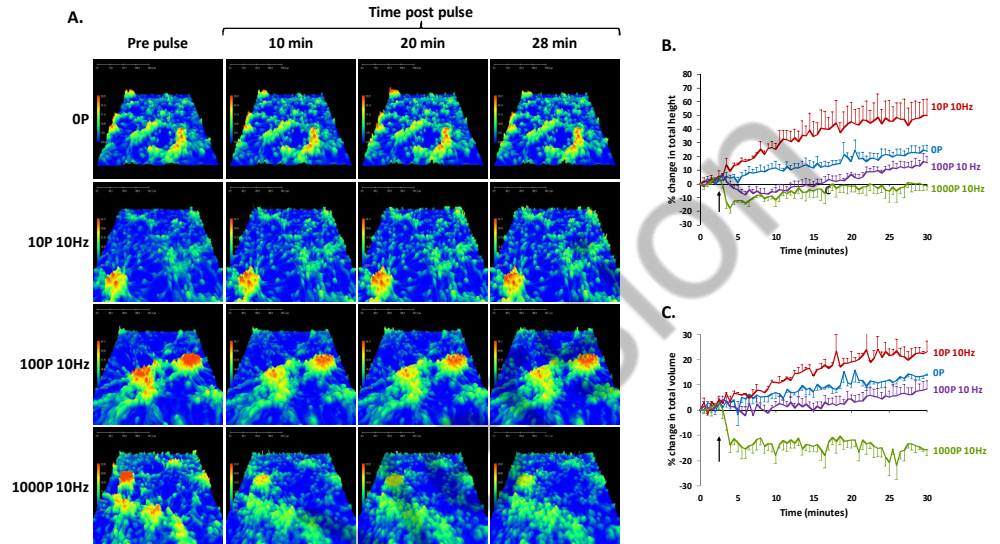


Figure 3. Representative holographic microscopy images of U-87 MG cells following application of 0, 10, 100 or 1000, 10 ns pulses at 10 Hz (A). Imaging was carried out at room temperature and images have been coloured to show differences in cellular height. Graphs show average % change in total cell height (B) and average % change in total cell volume (C) under the previously described pulse conditions. Arrows denote pulse application. In each case averages are from a minimum of two experiments and error bars show S.E.

- ~ 10 pulses caused a slight increase in total cell height, accompanied by a gradual increase in cell volume.
- ~ 100 and 1000 pulses caused an immediate decrease in cell height.
- ~ This decrease was more marked for 1000 pulses and corresponded with a 14% decrease in cellular volume.
- ~ No volume change was observed for 100 pulses.

### Longer term imaging of morphological changes

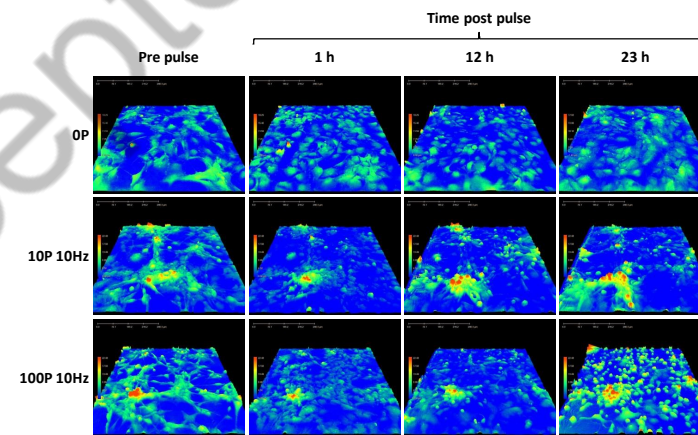
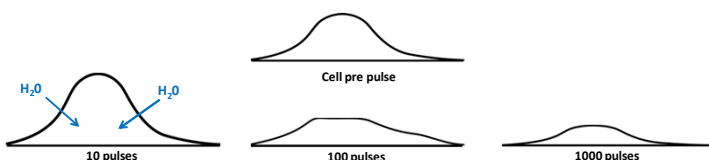


Figure 4. Representative holographic microscopy images of U-87 MG cells following application of 0, 10, 100, 10 ns pulses at 10 Hz (A). Imaging was carried out at 37°C in a CO<sub>2</sub> controlled incubator and the images have been coloured to show differences in cellular height.

- ~ Cells under control conditions maintained an elongated cell morphology throughout the experiment.
- ~ By 12 hours 10 and 100 pulses conditions have a loss of the elongate morphology, less cell-cell interactions and the presence of cells that are rounding up.
- ~ At 24 hours the number of rounded up cells has increased considerably following 100 pulses.

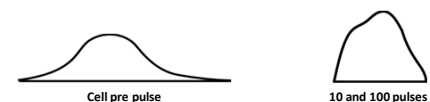
## Conclusions

### Short term imaging of morphological changes



**10 pulses:** Exposure causes plasma membrane poration → water enters via osmosis → cellular swelling [1]  
**100 and 1000 pulses:** Exposure causes plasma membrane poration [2] → cells resist osmotic swelling → PIP2/ IP3 pathway can be activated by nsPEF [3] and regulates cell volume in U87 cell → is this pathway activated by these higher exposure levels?

### Longer term imaging of morphological changes



Loss of the elongated morphology and reduction in cell-cell interactions could be due to disruption of the cells cytoskeleton [2,4].  
 Cell rounding occurs during mitosis → nsPEF disruption of microtubules could lead to malformed mitotic spindles → failure of cell division → increased number of rounded cells.