

Metadata

General information

Title of the dataset/output	Phototoxicity_induced_in_living_HeLa_cells_by_focused_femtosecond_laser_pulses/ <ul style="list-style-type: none">- Data_T0_laser_fixed.xlsx- Data_T0_scansione.xlsx- Data_T18_scansione.xlsx
Dataset Identifier (using the naming convention)	
Responsible Partner	PoliMI/3rdPlace
Work Package	
Author	Benedetta Talone (PoliMI)
Date of data collection/production	November-December 2018 June-July 2019

Access information

Access restrictions placed on the data	Open access
Data repository	Zenodo - CRIMSON community
Links to other publicly accessible locations of the data	-
Links to publications that cite or use the data	Phototoxicity induced in living HeLa cells by focused femtosecond laser pulses: a data driven approach (WIP)

Dataset/Document overview

Status of the documented data (draft, final)	Final
Date of submission/publication	18-11-2021
Keywords that describe the content	HeLa cells, optical microscopy
Version number	1.0
Format	Zip folder containing 3 .xlsx
Size in kBs	42.6 kB + 26.9kB + 52.4kB

Methodological Information

Materials & Methods	<p>For our experiments we used HeLa cells, a human cell line derived from cervical cancer cells, which are extensively employed in in-vitro phototoxicity experiments, and in particular in the field of laser microscopy, thanks to their availability, ease of cell manipulation and reproducibility of the results. HeLa cells are cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1% L-glutamine (2 mM), penicillin (10 units/ml), and streptomycin (10 μg/ml) at 37 ° C and in 5% CO₂ atmosphere (Euroclone, Italy). The culture medium is changed every 2-3 days. Cells are seeded in gridded cell-culture Petri dishes (IBIDI, 35mm μ-Dish Grid-500). The effective area available for the cell adhesion is 40 mm². The cells are stored in the incubator until the 100 % confluence condition is reached, then they are ready to be used for our experiments. Just before laser exposure, the cell medium is replaced with a DMEM solution containing a live/dead cell double staining kit consisting of calcein-acetomethoxy (AM) 0,2 μL/mL (excitation at 473 nm, emission at 490-590 nm) and ethidium homodimer-1 0,5 μL/mL (excitation at 559 nm, emission at 570-670 nm) (ThermoFisher Scientific, Italy), which are fluorescent dyes able to detect live and dead cells. In particular, the AM derivate of calcein is transported through the cellular membrane into live cells, thus colouring alive cells in green. On the other hand, the ethidium homodimer-1 is a membrane-impermeable fluorescent dye which binds to DNA, so that it enters only into dead cells, colouring them in red. We decided to insert the fluorescent probes before laser irradiation experiments and not after, as their addition requires performing some washing steps to remove the culture medium (DMEM), which often caused the detachment from the Petri dish of many cells that were damaged and stressed by the NIR pulsed illumination, thus inhibiting their count and evaluation. We note that we have not found any evidence in the literature that calcein-AM and ethidium homodimer probes may contribute to phototoxic damage. We have also experimentally verified this result, observing that, for those samples in which we managed to avoid cell detachment from the Petri dish, the damage extent in the two conditions (i.e. adding the fluorescent probes before or after irradiation) was the same. The cells, stored in the incubator at 37° C and in 5% CO₂ atmosphere, were quickly picked up and brought to the optics laboratory to be exposed to laser light. In the optics laboratory the temperature was kept at 27° C to minimize thermal gradients and reduce cell stress. Irradiation experiments were performed employing an ytterbium-doped fiber laser which uses a master oscillator power amplifier (MOPA) architecture (Fidelity HP, Coherent, USA). This laser source emits pulses at 1040 nm wavelength, with repetition rate of 80 MHz, transform-limited duration 130 fs, a negative group delay dispersion variable from 0 to -120,000 fs² and up to 18 W of output average power. Samples were placed in a home-built inverted microscope setup equipped with white-light Köhler illumination and a CCD camera with 1024x768 pixels (Thorcam, Thorlabs, Germany), with a field of view of 200x266 μm², employed to inspect the sample before the measurement and to put it in focus (by adjusting a linear translation stage along the z axis). To focus the laser beam on the sample, we employed two different optical systems: a lens with focal length $f = 30$ mm for the experiments presented in Section 3, using a fixed and rather large (15μm radius, intended as half width at half maximum (HWHM)) illumination spot, and an objective (Melles Griot 10x, 0.25 NA) for the experiments presented in Section 4, based on raster scanning a smaller (6 μm radius, intended as HWHM) illumination spot. We chose these two illumination configurations with large focal spots on the sample on purpose to avoid the problem related to the vertical position of the laser beam waist with respect to the cell plane. The thin (170 μm) glass bottom of the Petri dish often presents variations in thickness. Furthermore, due to strains or bending due to gravity, it is not perfectly flat. As a result, it is rather difficult to keep the laser focus on the cells while scanning the sample over large fields of view using a high-numerical aperture objective. Using a low-NA one and a lens with long depth of focus we avoided the problem, obtaining a very good reproducibility. For the latter experiments, in particular, we combined a fast galvanometric mirror (GVS311/M, Thorlabs, Germany) with a slower motorized translation stage (8MFT-75LS05 Standa, Lithuania), moving along two orthogonal directions</p>
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	<p>In order to prevent distortion phenomena like vignetting, a 4f-relay system was implemented between the galvanometric scanner and the focusing objective. After laser exposure, samples were imaged using a confocal microscope (Olympus Fluoview FV10i) equipped with four diode lasers (emission wavelengths 405, 473, 559, 635 nm), an integrated incubator, and with a 10x phase-contrast objective, 0.4 NA (image size 1.2x1.2 mm²). The pinhole was set to 2.5 Airy Unit. Two-colour images were acquired to detect live cells and dead cells. Images were analyzed with the open-source software ImageJ, counting live and damaged cells and measuring the extent of the damaged area.</p>
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