Excellence in Electron Microscopy Award – Project Report

Photoacoustic and spectroscopy study of melanoma cancer cells. Linking absorption to signal strength

Francisco Cortalezzi [1], Grant Deana Grace [2], Luis Polo Parada [3*]

[1] Department of Chemistry, University of Missouri, Columbia, Missouri, United States
[2] School of medicine, University of Missouri, Columbia, Missouri, United States
[3] Electron Microscopy Core, University of Missouri, Columbia, Missouri United States
[*] Principal Investigators

Overall Hypothesis and Goals

In photoacoustic (PA) research, short and ultrashort laser pulses are used. An important issue in these investigations is to determine the cell photo-damage. It is generally assumed that the laser pulse creates no damage when cells are exposed to radiation times smaller than the thermal and stress relaxation time; however, this assumption hasn't been demostrated.

Here, we have evaluated nanosecond laser-induced cell damage by using UV-Vis spectroscopy, photoacoustics and transmission electron microscopy images. Fixed and fresh cultivated Hs936 melanoma skill cancer cells (dispersed in PBS solution) were transferred to a custom-made recording dish. Transmission spectra and PA signal from the center of the cell (5 um area) were obtained every time the laser was pulsed, and the complete trial was recorded in video at 30 fps. This trial was repeated twenty times.

Approach and methods

The experimental setup consisted of a tri-ocular upright microscope (BX51WI Olympus) with a 50/50 beam splitter dual top port (Figure 1). One port of the beam splitter directed the image from the microscope to a spectrophotometer through a100-micrometer optical fiber. The second port directed light to a digital camera to obtain images from the microscope. To produce the laser-induced ultrasound in one single cell, an 8-nanosecond laser pulse with a wave length of 532 nm and a fluence of 40 mJ/cm2 was directed toward it using a 1000-micrometers diameter optical fiber.

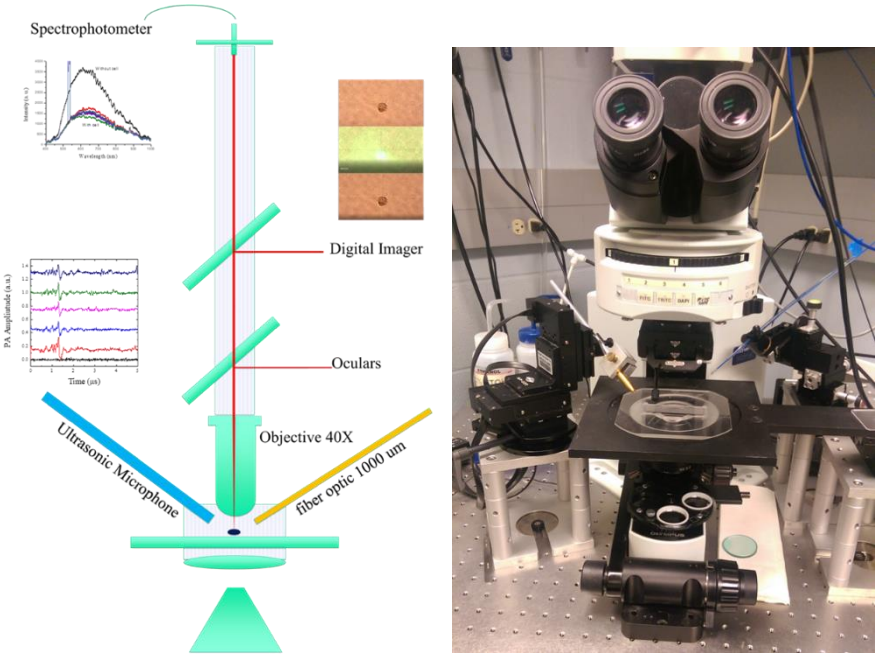

Figure 1: Microscope setup with light path and data collection.

Background

PA has gain momentum in the last 50 years with easier accessibility to small laser systems and ultra-rapid response ultrasonic sensors. In the past several years, several physical models have been proposed to relate cell absorption to its PA signal amplitude, but they fail to relate the theory to the experimental data. The complexity of this problem requires a better understanding of the relationship between the optical properties and the PA signal of individual cells.

Results and discussion

Our results showed that the energy per pulse used to damage and produce laser-induced ultrasound, does not change the optical properties of the studied cells and, in consequence, the amplitude of the PA signal. As it can be seen in figure 2, the number of observed vacuoles inside the cells increment and its size reduces with the number of pulses of energy given to the studied cells. For this particular subgroup of Hs936 cells, TEM images showed substantial cell damage to the intracellular components. In addition, figure 3 shows the absorption of light by a single cell as it is exposed to a laser pulse. From this figure we concluded that the absorption does not change much, with a trend of laser pulses at a macro level, although extensive damage is observed in the TEM images generated and also that the PA amplitude remains similar after several laser pulses.
Hs936 cells can present them self with different “coloration” due to presence of melanin, a strong absorber in the 532 nm wave length.
A more detail underway study of cell population will uncover details in the relation between optical properties and PA signal amplitude for strong absorbing cases (extremely dark cell) as well as for low absorbent cell that do not express a PA signal.

Selected Images

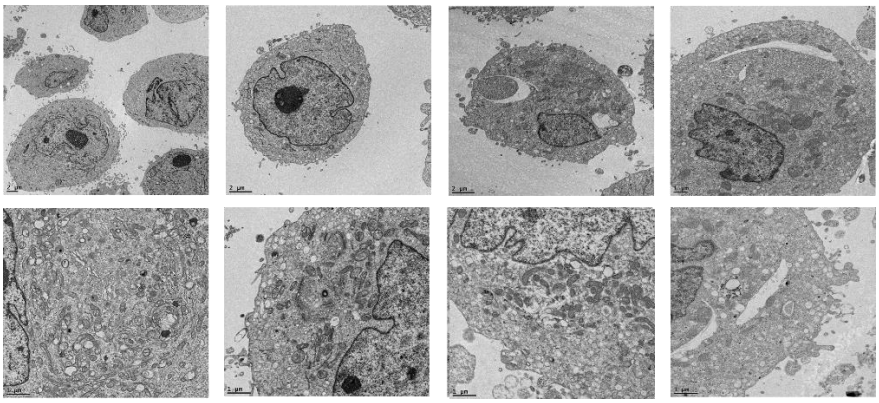

Figure 2: TEM analysis presented substantial damage to cells and internal organelles of Hs936 human skin melanoma cells when exposed to 8 nanosecond laser, fluence of 35-40 mJ/cm^2
From left to right, we show TEM images from a control sample, 1, 10 and 20 lasers pulses images.

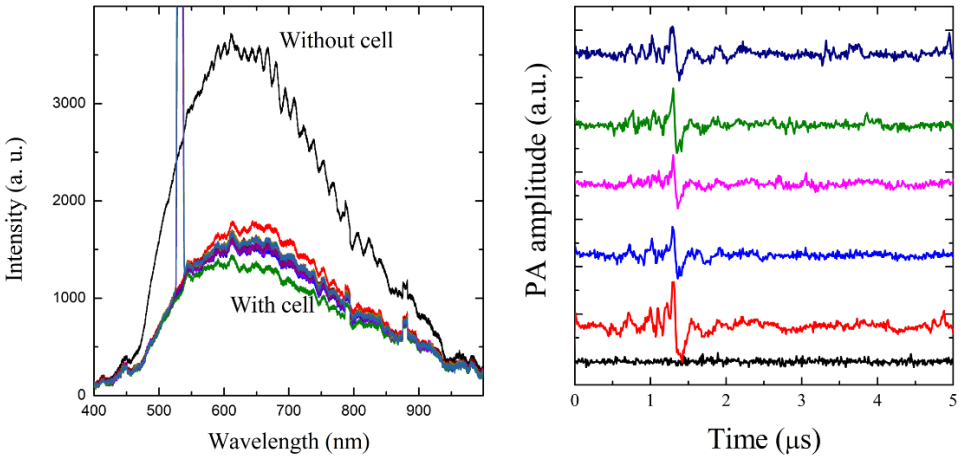

Figure 3: PA analysis of a trend of 5 short laser pulses (60 hrz) showed no variance in the amplitude of the signal of media to light colored Hs936 cells. Observation of cells through microscope ocular did not reveal apparent macroscopic cell damage in Hs936 cells.

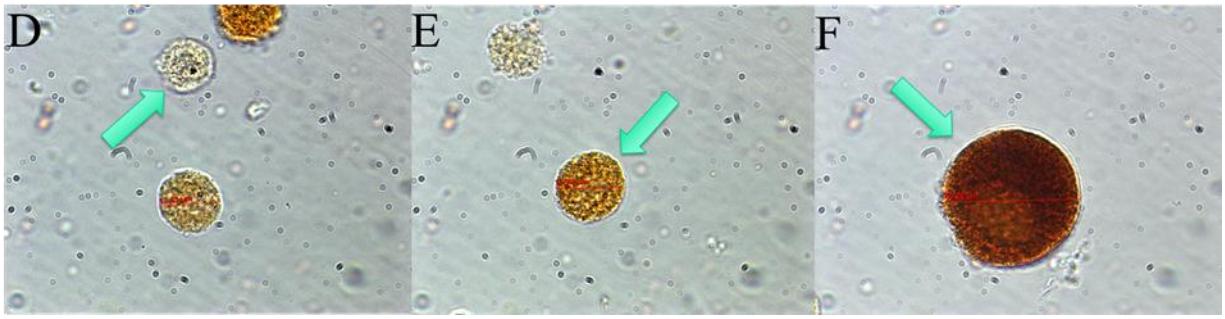

Figure 4: Images of Hs936 cells presenting different amounts and polymers of melanin D) light colored cell E) medium dark cell F) dark colored cell.

Future Research and Funding

Results obtained will be presented in a peer-reviewed publication as a continuation of "Spectrophotometric analysis at the single-cell level: elucidating dispersity within melanic immortalized cell populations", Analyst, 2017, 142, 1482. The newly obtained results can now relate in an experimental form the amplitude of the PA signal to the absorbance coefficient at the single cell level and relate this to cellular damage. The obtained results will help for the submittal of an NSF proposal in the near future.