Quantifying biological forces involved in the process of cell-mediated cytolysis

Experimentation based on responses of living cells (Cell-based assays) represents an important stratum in biological and clinical investigations, in diagnosis and therapy. The vast majority of tests in living cells are based on invasive techniques which, almost all of them require cell labeling by specific markers (usually fluorescent markers). In the majority of the cases, such measurements are binary, i.e. enabling measurements at a single time point. The marker in the cell/membrane can influence the cell response and thus affect the measurements' validity.

In addition, inasmuch as cell populations are heterogeneous, measurements at the single cell level within a given population have a clear advantage, particularly for discovering sub-populations that vary in their responses.

In contrast to the above, the present study utilized a non-invasive method, based on optical force. The present work includes tow parts: The first part concerns the development of a new measurement technique and its application to examine cellular changes induced by hydrogen peroxide and by the mitogen phytohemagglutinin (PHA). The second part examines the possibility of multi-channel measurements (i.e. the simultaneous measurements on a large number of cells), also based on optical force.

The basis for the proposed technique is the following: the optical force acting on a captured object depends on the properties of the capturing ray and the optical system, the electrical properties of the host medium and the properties of the examined object (its composition, refraction index, size, shape). Due to its thermal energy, the captured object's localization is not constant, but vibrates around the trapping point. The extent of the object vibration depends on several factors: its structure, properties of its suspending liquid, temperature and the arrangement of the optical force. Hence, the physiological changes taking place in the cell can influence the active optical force and thus change vibration characteristics of the object.

The first part: Initially, I examined the influence of the diameter and refraction index of a captured object on its vibration. Since, in the present work, the object dimensions are much larger than the wave length (830 nm) of the capturing light

source (diode laser), it was possible to apply Mie approximation in estimating the efficiency of optical force and its dependence on the object refraction index and dimensions.

In the second stage, I revealed the measurement limitations and consequently defined the subsequent protocols both with regard to measurement performance and result analysis. In order to present the differences in the protocols that I have developed, I have reviewed the majority of presently accepted techniques for the calculation of the force constant and measurement parameter definition. After having established the measurement protocols, I have characterized the signal to noise ratio and assessed the intensity of each noise component.

Finally, I applied the conclusions reached and the protocols developed for the actual examination of three lymphocyte populations: the Jurkat T cell line, the Molt4 cell line, and peripheral blood lymphocytes (PBL). These cell populations have different characteristic diameters. And indeed, I have found that this is reflected in the varianceof their vibrations. An example is given in Figure A, which shows cell scattergram in the vibration variance – cell radius domain. The results demonstrate that, in the living cells, the variance increases with cell diameter. However, within a specific population, I have not found a similar pattern.

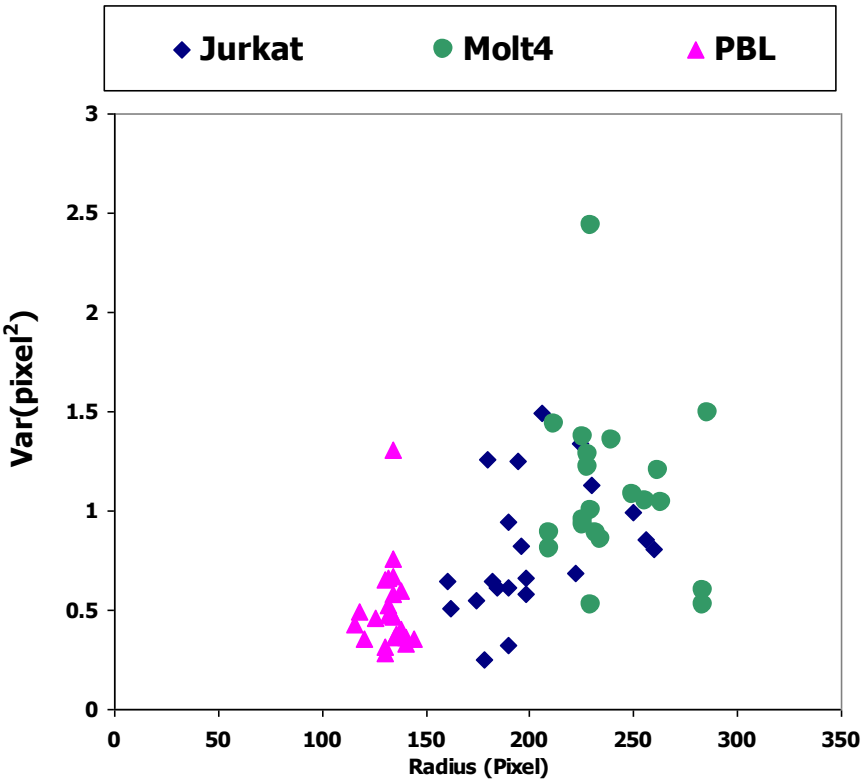

Figure A. The relation between cell radius (in pixels) and the variance of cell vibration.

I have tested the technique performance in revealing induced cellular physiological changes on cell populations that underwent two types of treatment. The results are summarized in Figure B. It demonstrates the frequency of vibration variance in cells incubated in the absence or presence of hydrogen peroxide (for apoptosis induction) and in the absence or presence of the mitogen PHA (for cell division induction). The results indicate that, at the cell population level, the incubation with hydrogen peroxide reduces the value of variance, whereas the incubation with PHA increases the variance.

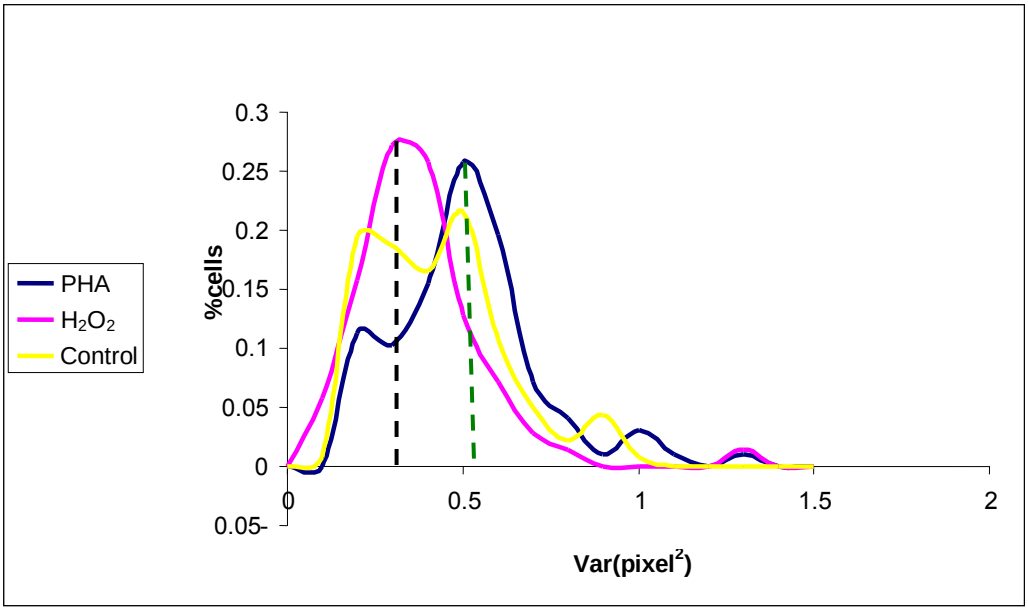

Figure B. Histogram of the vibration variance of cells incubated without the reagents (yellow curve) or with H_2O_2 (purple curve) or with PHA (blue curve).

In Figure B, the dashed perpendiculars (green and black) respectively emphasize the change in the distribution of vibration variance of cells treated by the mitogen versus those treated by hydrogen peroxide. After the treatment with PHA, the percentage of cells with high vibration variance increased. On the other hand, since it has been found that the average radius of cells did not change, I concluded that, following the mitogenic PHA treatment, the refraction index decreases. An opposite result was obtained after treatment by hydrogen peroxide.

The second part: The cell measurements, as presented in the first part of this work, are performed consecutively, cell after cell, and therefore are not suited for the simultaneous measurement of a number of cells in a population. In this part of the work, I focused on the development of a novel technique that would allow multi-channel measurements of vibration variance on a large number of cells.

For this purpose, I have tested the application of the optical cell platform (live cell array – LCA). I found that, in addition to the mechanical capacity of the optical platform to maintain individual cells in their place during the treatments, it also made possible, by using an appropriate optical arrangement, to develop temporally constant optical traps for each cell in its specific locale. This carries several significant benefits:

- The mechanical capture shortens, by orders of magnitude, the duration of optical trapping operation (of the laser).
- The trap operation is limited to the measurement of vibration variance only, as the mechanical entrapment is sufficient to maintain the identity of the cell during its exposure to different biological treatments. Therefore,
- The duration of interaction of laser radiation with the cell decreases, by orders of magnitude, as compared to any existing system.
- The optical trap array is not based on time-sharing, but is constant in time.
- The control over the force of optical trapping can be mechanical.

Initially, I used simulation to test the properties of the multi-channel array of optical traps, using the Beam Propagation method. The results of simulation were confirmed by trap image acquisition experiments. Figure C shows the presence of secondary rays formed from the interference pattern of the original ray.

Figure C. Interference pattern of converging rays passing through the micro-lens array.

In an additional experiment, I tested the effect of the multi-channel trap on micro-bead suspension. Figure D shows aggregations of beads (each black dot in the image represents a single bead). In the present work, I found that the distribution of aggregates precisely follows (in fact, is determined by) the distribution of traps in the multi-channel array.

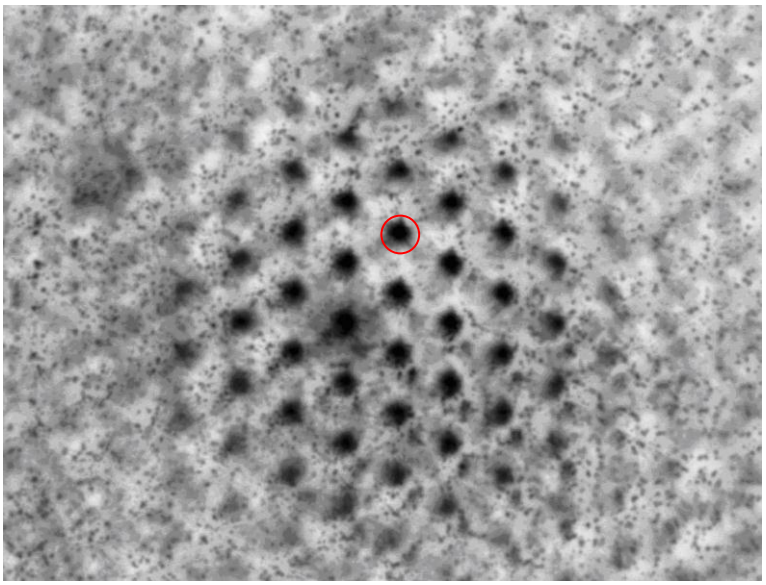

Figure D. The formation of beads' aggregates. The micro-beads in suspension (black dots) form aggregates (large black spots – see the example indicated by the red circle) in the spatial order that is determined by the arrangement of optical traps in the multi-channel, honeycomb-like array.

In conclusion, the outcomes of this research may enable the investigation of living cells, using early, non-invasive markers. In addition, it may make it possible to conduct such investigations simultaneously on a large number of cells.