Fluorescence polarization measurements of GFP expressed in individual live cells, under various functional conditions

The study introduces the following two achievements: the examination of the primary defense mechanism of plant cells and the identification of lymphocytes that underwent mitogenic activation. Thus, this multi-disciplinary work concerns two subjects, which dictated the study design.

The first part of this study considers the physical backgrounds, common to both research subjects, including a brief survey regarding fluorescence and fluorescence polarization that served as the basis of the present work. The first part also includes a survey of the materials used during this research, the general methods, the measurement systems and their calibration.

The second part is dedicated to the plant cell investigation and includes 3 chapters:
The introductory chapter describes the biological backgrounds and motivation for the present work: While the cytoplasm occupies only 10% of the cell volume, the vacuole occupies 90%. According to many researchers, the vacuole serves as a water buffer for the cell, which allows for the continuation of normal biochemical activity under stress conditions. However, as far as I am aware, presently there is no scientific method that enables the monitoring of this defense mechanism.

The Methods chapter describes the unique methodology developed for this part of the study, specifying, among other topics, the process of deriving protoplasts (plant cells without the wall) and sample preparations for measurement.

The third chapter presents the results and achievements: to the best of my knowledge, the first work to suggest a scientific method for monitoring and quantification of the protoplast responses to osmotic stress. I demonstrate that by using fluorescence polarization measurements of specific organic markers hosted in the vacuole and in the cytoplasm, it becomes possible to differentially monitor their interrelations under osmotic

stress. The entire study was performed on protoplasts and it is also the first study to present this type of measurements in isolated, live plant cells.

The protoplasts were labeled with cytoplasm-specific and vacuole-specific markers, and were suspended in isotonic, hypotonic and hypertonic solutions, and the fluorescence polarization emitted from each specific labeled compartment was measured, as shown in Figure A.

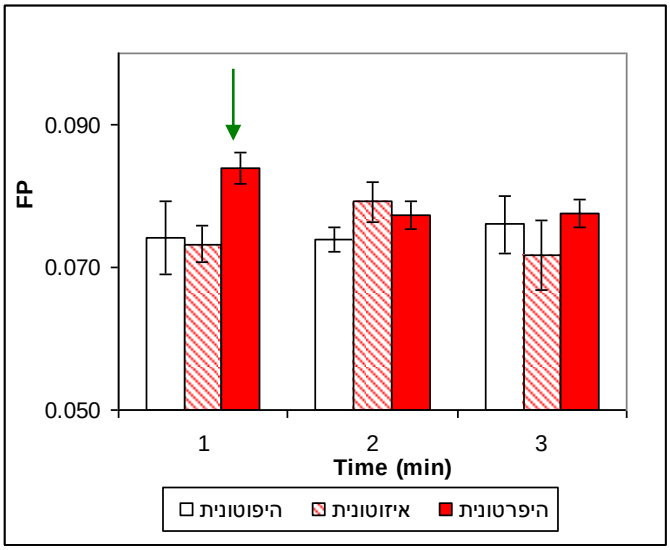

Fig. A2. Fluorescence polarization of Acridine Orange in the vacuoles of protoplasts suspended in different osmotic solutions: Hypotonic (white), isotonic (striated), hypertonic (red)

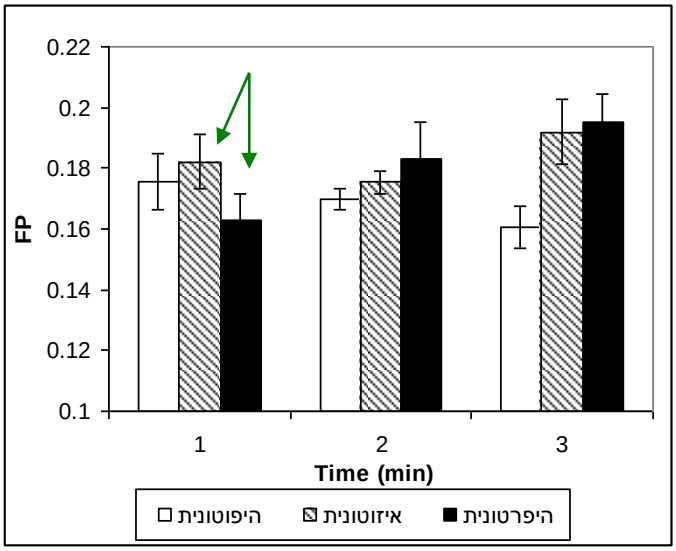

Fig. A1. Fluorescence polarization of Fluorescein in the cytoplasm of protoplasts suspended in different osmotic solutions: Hypotonic (white), isotonic (striated), hypertonic (black)

When suspending the cells in the hypertonic environment, the fluorescence polarization of the cytoplasmic marker remained constant and even slightly decreased (Fig. A1, columns indicated by green arrows). In contrast, the fluorescence polarization of the vacuolar marker increased (Fig. A2, the column indicated by the green arrow).

The present work is, to the best of my knowledge, the first to confirm that the vacuole indeed serves as a water buffer for the plant cell. The study findings demonstrate that it is possible to monitor the water status in different cell compartments and their mutual influences.

Beside the scientific achievement of this work, it appears that it opens a new way for the establishing of physical measurements/variables in plant physiology research that can have great importance for developing new tools to evaluate plant resistance to stress.

The Third Part of this work considers the research of lymphocytes and includes 3 chapters, similarly to the second part.
The first chapter describes the biological background and motivation for this research: namely, the identification of lymphocytes that underwent activation, a very important process in basic as well as applied biomedical research. This part relies on the fact that, using fluorescence polarization measurements of exogenous intracellular markers, it is possible to evaluate lymphocyte activation. In this work, I used a fluorescent protein, which is synthesized by the cell and does not interfere with cell physiology, in order to identify activated lymphocytes.

The second chapter describes the methods unique for this part of research, and includes, among other subjects, the method of transfection used to obtain a stable cell line expressing the fluorescent protein: This work utilized the Jurkat cell line (human lymphocyte cell line of T type) that express free fluorescent protein in the cytoplasm.

The Results chapter first describes the experiments performed to identify special characteristics of the fluorescent protein that make it suitable for the present study. The main part of this chapter presents the major achievements of this work. This work is, for the best of my knowledge, the first to utilize the fluorescent protein as an independent marker, and not as a reporter of another protein, in order to examine lymphocyte activation. For the activation, Jurkat T cells were treated with the mitogen PHA and the lymphocyte fluorescence polarization was measured immediately upon the mitogen activation (time zero) and after 24 hours, as presented in Figure B.

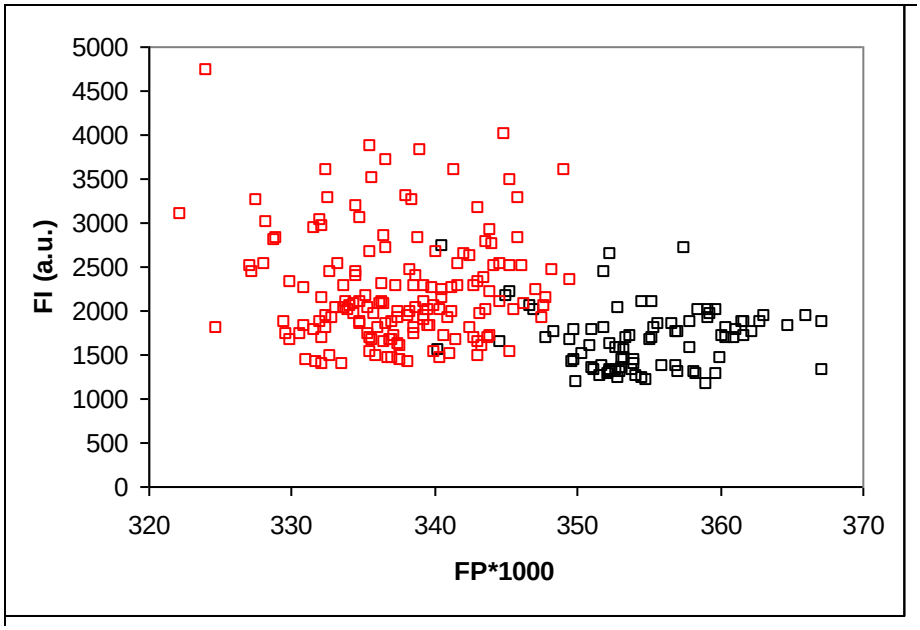

Fig. B. The relation between fluorescence intensity and fluorescence polarization for cells treated at time zero (black squares) and after 24 hours (red squares)

Approximately 20 hours after the mitogenic activation, RNA synthesis commences, which leads to the general protein synthesis, including the fluorescent protein expression in the lymphocytes. As the protein quantity increases, there increases the probability for energy transfer between the fluorescent proteins, which leads to a decrease in fluorescence polarization emitted from the proteins as a result of HomoRet. The present work demonstrates that, through the measurements of fluorescence polarization emitted from the protein expressed in the cytoplasm, and through the complementary measurements of fluorescence intensity, it becomes possible to clearly distinguish between lymphocytes at the beginning of the mitogen activity and lymphocytes under mitogenic activation for 24 hours.

The results obtained in this study for the lymphocytic cell line open a new way for the detection of activated lymphocytes via fluorescence measurements of intracellular fluorescent proteins.