Using biophotonics to study programmed cell death

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Both light scattering and fluorescence of membrane glycoproteins can be used to detect programmed cell death (apoptosis) quickly and cheaply.

Our bodies consist of $10^{13} - 10^{14}$ cells, each having its own role and function. The life of a cell has a beginning (as a daughter cell during mitotic division) and an end (cell death). Every hour, approximately $10^9$ cells die in the body and the same number is produced by division.

Two types of cell death are known: pathological cell death, or necrosis, and physiological or programmed cell death, known as apoptosis. During apoptosis a cell performs suicide: it enzymatically slices the intercellular content, including DNA and proteins. As a result, the cell shrinks and is fragmented into membrane-coated vesicles, called apoptotic bodies, which are then engulfed by cells of the immune system.\(^1\)

Apoptosis removes elderly and impaired cells so they can be replaced with fresh cells. This huge turnover makes programmed cell death an important player in the game called “Life”: we will not notice anything when things are going right, but if a mismatch arises between cell production and death, we suffer from a range of diseases. For this reason, control and detection of apoptosis is of great importance for clinical and biomedical diagnostics.

There are many tests and specialized devices on the market for detecting apoptosis. Most of them exploit one of the numerous changes in biochemical content of dying cells. Since most of these changes occur inside the cell, however, detecting them requires violating the cell’s integrity. This type of analysis is costly and slow, and introduces additional errors.

We used two different approaches to making apoptosis detection fast and cheap (see Figure 1). First, we used the established method of light scattering to measure the distribution of cell sizes in the population.\(^2\) Changes in cell sizes indicate cell death and the accompanying cell fragmentation.\(^3\) In the second approach, we screened the cell surface for new biochemical markers of apoptosis that are readily accessible for analysis without the need for cell damage. We found that some glycoproteins in the plasma membrane undergo redistribution during apoptosis.\(^4, 5\) We used lectins—proteins able to bind specifically to the carbohydrate component of a glycoprotein molecule — to detect these changes by attaching a fluorescent label to them.\(^6, 7\)

The light-scattering approach successfully detected dying cells by their shrinkage and fragmentation into apoptotic bodies. We used a red laser to study side scattering of light by cells passing through the beam. We also implemented advanced analysis algorithms to obtain the particles’ size distribution. As a result, we detected quantitatively the fragmentation of cells to apoptotic bodies during programmed cell death, using the decrease in the particle size (see Figure 2). This is almost impossible with other methods. Since this technique deals only with physical parameters of the cells, such as size and refractive index, we avoided any need for labeling the cells and achieved a high speed of analysis.

The preceding method, however, had a relatively low specificity and could analyze only suspended cells. To overcome these difficulties we used an entirely different approach, looking at the surface of the cell: the plasma membrane. Previously, only one sur-

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**Figure 1.** When an intact cell (1) undergoes apoptosis (programmed cell death), it shrinks and often is fragmented to particles called apoptotic bodies (2). This phenomena is used in light-scattering analysis (4A). In addition, during programmed cell death, specific glycoproteins appear on the surface of the cell. Fluorescently labeled lectins—carbohydrate-binding proteins—are used to detect corresponding glycoproteins (3) by fluorescence detection (4B).
Figure 2. Physical changes in cells during programmed cell death. (A) Microscope images show dying cells fragmenting into apoptotic bodies. (B) Both fragmentation and shrinkage are evident in graphs of size distribution.

Surface change was known to accompany apoptosis, namely phosphatidyl serine externalization. Revealing this molecule requires a costly procedure using a jellyfish protein called Annexin V. Thus, we focused our attention on the other components of cell surface: glycoproteins, consisting of protein and carbohydrate parts. To study them, we used carbohydrate-binding proteins called lectins, discovered almost 100 years ago in plants and animals.

We screened changes in the carbohydrate moiety in glycoproteins during programmed cell death and found that some specific substitutions in glycoproteins’ structure were attributable to apoptosis. The lectin molecules that specifically recognize these changes in glycoprotein content were used as markers to detect programmed cell death. Finally, we attached fluorescent labels, such as fluorescein isothiocyanate (FITC) or Texas Red, to lectin molecules and obtained a powerful tool for the detection of apoptosis by means of fluorescence microscopy, flow cytometry, and other related methods. It relies on evaluation of programmed-cell-death markers on the cell surface, providing a high specificity and eliminating the need to compromise cell integrity (see Figure 3). We have tested that approach in different experimental cell models and currently are working on introducing it into clinical practice.

The science and technology underlying these results continually improve each other. In the future, we aim to combine the precision of light-scattering analysis with the specificity of plasma-membrane changes detected by fluorescence. We hope that the methods of biophotonics will help us to make the detection of the programmed cell death easier and more affordable, and our lives safer and more protected.

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Rostyslav Bilyy is a researcher at the Institute of Cell Biology, Lviv, Ukraine. Currently, he is finishing his PhD thesis on cell biology. Rostyslav is a co-author of 12 papers, three Ukrainian patents and one US patent application. In addition, he has been a member of SPIE since 2003 and has presented several papers. In 2006, Rostyslav was awarded a SPIE Scholarship.

**References**


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