Macromolecular profiling of apoptosis via a multiplex biophotonic platform

Aliaksandr V. Kachynski and Paras N. Prasad

A nonlinear multiplex imaging technique reveals transformations in the cell during programmed cell death.

Traditional fluorescence microscopy is limited to visualizing labeled molecules and does not fully answer questions about molecular composition or cell/tissue organization. As all cells are composed of thousands of individual types of macromolecule, an understanding of the structural organization and specific mutual interactions between macromolecules is important for development in the biomedical sciences and applied medicine. Major vital cellular functions and substructures—such as cytoplasmic organelles—rely upon interactions between proteins, nucleic acids, saccharides, and lipids. Thus, for a comprehensive understanding of how the cell is organized and its function on a molecular level, knowledge of all types of macromolecular distribution, spatial dynamics, and transformations is required.

A multiplex biophotonic platform, recently developed by our group, allows for the quantitative characterization and real-time monitoring of local molecular environments in the structural domains of live cells. We combined coherent anti-Stokes Raman scattering (CARS) and two-photon excited fluorescence (TPEF) nonlinear optical imaging with fluorescence recovery after photobleaching (FRAP) and Raman spectroscopy to study the process of apoptosis, a programmed cell death essential for physiological regulation and elimination of genetic disorders. While the ability to combine nonlinear imaging has been demonstrated before in the pioneering CARS work of the Sunney Xie group at Harvard University, we applied the CARS/TPEF combination for quasi-synchronous multiplex imaging (multiple pictures in one scan) of proteins, lipids, DNA, and RNA during apoptosis. Proteins and lipids were distinguished by CARS, while acridine orange-labeled nucleic acids by TPEF responded in green and red spectral channels. CARS/TPEF imaging were augmented by FRAP measurements, which were employed for the characterization of the mobility of nuclear proteins during apoptosis.

Figure 1. The distribution of proteins, lipids, DNA, and RNA in HeLa cells during apoptotic development as visualized using multimodal coherent anti-Stokes Raman scattering (CARS) and two-photon excited-fluorescence (TPEF) imaging over 24h. Proteins (red) and lipids (grey) were observed in the CARS mode at the characteristic vibrations of 2928cm\(^{-1}\) and 2890cm\(^{-1}\), respectively. Nucleic acids, stained with acridine orange, were acquired in the red (RNA= green) and green (DNA= blue) fluorescence channels in TPEF mode.

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The apoptotic process was initiated in cultured cells and dynamic changes in the distribution of biomolecules were documented along its execution. We showed that, in the nuclei of proliferating cells, proteins are distributed uniformly with local accumulation in several nuclear substructures. This distribution is disrupted at the onset of apoptosis as it is transformed to a progressively irregular pattern. Additionally, FRAP studies indicated that pronounced aggregation of proteins in the nucleoplasm of apoptotic cells coincided with a gradual reduction in their mobility. This study demonstrated how modern biophotonic tools can reveal the molecular dynamics of fundamental intracellular processes (see Figure 1).

Our future research is focused on the study of biomolecular compositions of cellular structures, their transformations in cellular disorders and responses to specific therapeutic drug-cell interactions. We recently applied the basic concept of such biomolecular characterization of cellular dynamics throughout the cell cycle. The most exciting potential applications of our biophotonic platform embrace in vivo characterization and real-time monitoring of various pathogeneses, infectious diseases, nutrition imbalances, chemical contaminations, radiation-induced biomolecular damage, malignant transformations, and others factors causing abnormalities in cellular regulation. We anticipate that the macromolecular content of cytoplasmic organelles and nuclear compartments can serve as real-time indicators of the cellular physiological condition. Additionally, the ability to quantitatively characterize and monitor, in real-time, local molecular environments along the drug-cell interaction would be an invaluable tool for clinical analysis and therapeutic drug development.

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Author Information

Aliaksandr V. Kachynski and Paras N. Prasad

Institute for Lasers, Photonics and Biophotonics
University at Buffalo, The State University of New York
Buffalo, NY

Aliaksandr Kachynski is research associate professor at the Institute for Lasers, Photonics and Biophotonics. His research interests embrace lasers, laser spectroscopy, photonics, biomedical optics, and nonlinear optical imaging.

Paras Prasad is a distinguished professor of Chemistry, Physics, Medicine and Electrical Engineering and the executive director of the Institute for Lasers, Photonics and Biophotonics. He is a SPIE Fellow and has published over 620 scientific papers and three books: Introduction to Nonlinear Optical Effects in Molecules and Polymers, Introduction to Biophotonics and Nanophotonics.

References

