Advanced Microscopic Methods to Measure Dynamic Patterns of **Intracellular and Intranuclear Proteins in Living Mammalian Cells**

The Problem

The appropriate functioning of living cells depends on a variety of dynamic processes that necessitate delicate motion, transportation, association, and disassociation in time and space. For proteins, it is important to know:

1. Is there a mobile fraction and a fixed (bound) fraction?
2. What is the ratio between these two sub-populations?
3. What is the concentration of each one
4. Other relevant factors such as the number of binding sites.

In addition, both for proteins and other structures (such as DNA loci), it is important to know different dynamic patterns such as the type of their dynamics – is it a directed motion, is it normal diffusion or restricted diffusion. It is important to know the length scale of the dynamics, the diffusion coefficients and more. These parameters serves as a tool for exploring biochemical processes.

Although there are methods such as fluorescent recovery after photobleaching (FRAP) and fluorescence correlation spectroscopy (FCS), these methods can only give partial information from the list given above.

The Solution

We developed a method that combines intensity measurement with time-resolved measurement. It uses a confocal microscope setup and require labelling a tested protein (or structure) with a fluorescent dye (such as GFP) in live cells. Then, the sample (live cells) is placed under the microscope and using the confocal setup, the intensity is measured at a given point in the sample, normally for about 20-60 seconds. During the measurement, the life time of the emitted photons are also measured.

Then, the data is processed by combining few different methods. It gives many answers related to "the problem" described above. This includes 1. the ratio of bound and free sub-populations of the protein. 2. The diffusion coefficient of the mobile fraction. 3. The concentration of the mobile fraction and the bound fraction. 4. The parameters are confirmed by two different methods, both from the fluorescence intensity as well as from the fluorescence lifetime.

The Commercial Benefit

Our advanced disclosed methods and systems can:

- be implemented on existing confocal microscopy platforms.
- be implemented as a software interface for analyzing measured images.
- Provide information that cannot be found by other methods.
- Be used in different disciplines including biology, genetics, biophysics, neuroscience, bacteriology, signaling and more.
- be used for studying the character of many different proteins in cells, the nucleus, the membrane and many other species.
- Be used to study genetic abnormalities, diseases or disorders such as laminopathy, cancer, muscular dystrophy and others.

Market Potential

The global live cell imaging market is expected to reach USD 9.0 billion by 2025 (CAGR: 8.5%). Understanding the cellular behavior in its natural state is crucial in drug discovery and for targeted drug therapy. This, in turn, increases the need for live cell imaging, thus boosting the market.

Target Markets/Industries

- Manufactures of microscopy systems
- Manufacturers of imaging systems
- Manufactures of pharmaceutical drug discovery solutions
- Manufactures of biotherapeutic development
- Manufactures of Diagnostic tools and products

Intellectual Property

Patent pending

Team: Primary Inventor

Prof. Yuval Garini

- Prof. Yuval Garini is an associate professor at the Physics Department and the Nanotechnology Institute in Bar Ilan.
- Prof. Yuval Garni graduated the Technion Institute of Technology in Haifa, Israel (Physics Department).
- He was part of a startup company that developed unique spectral imaging systems and its genetics applications, including the development of spectral karyotyping (SKY).
- Before coming to Bar-Ilan, Prof. Garini was an assistant professor at the Applied Sciences department in Delft University of Technology, Delft, The Netherlands.

Future Research

Further research includes:

- Further development of methods to study live-cells and dynamic properties of proteins, DNA, organelles and other species in living systems.
- Studying the organization of the genome in the nucleus
- Developing spectral imaging systems for very large images and for applications such as pathology.
- Single molecule methods for studying DNA-protein interactions
- DNA origami and the use of other nano structures for biological applications.

The Opportunity

We are looking for investors that are willing to support the research and commercialize the developed IP.

Keywords

- Microscopy
- Fluorescence microscopy
- Optical imaging
- Live cell imaging
- Protein dynamics
- DNA protein interaction
- Continuous photobleaching
- Fluorescence correlation spectroscopy