Single Molecule Detection - The Future of Life Science Imaging

Application Note

The behavior of individual molecules can have significant implications that affect biochemical processes and the properties of individual cells, ultimately influencing human behavior. Single molecule detection allows scientists to probe this molecular behavior with unprecedented detail.

Introduction: The Limitations of Conventional Fluorescence Microscopy

Microscopy has now become a regular part of most scientific investigations. It's a critical tool for understanding important biological mechanisms in life science, as well as advancing other high-tech fields such as nanotechnology, materials science and engineering.

In particular, fluorescence microscopy is now a staple of life science research. It’s a selective technique, in that it allows scientists to focus on specific biological features that are labeled with a fluorescent marker. This allows the imaging of specific regions rather than the entire sample (as is the case with standard bright-field microscopy).

But despite the benefits of fluorescence microscopy, the technique is at a disadvantage, due to the limit set by the diffraction of light. This limit is well above the resolution necessary to image, and discriminate between, individual molecules. In other words, it restricts the amount of detail that can be captured with standard objectives.

This means that imaging of individual cells, or even individual molecules is impossible with standard fluorescence microscopy. Since the behavior of an entire organism is determined by behavior and characteristics on the molecular level, techniques that facilitate this kind of imaging are critical to improving our understanding of human and animal behavior.

Single Molecule Detection may be the Answer

When higher resolution is needed, scientists will often turn to techniques such as confocal and total internal reflectance fluorescence (TIRF) microscopy. And for even more detail, super-resolution techniques such as structured illumination microscopy (SIM) and stimulated emission depletion (STED) microscopy are used. These are a relatively new group of techniques that push the resolution boundaries of conventional fluorescence microscopes.

However, even though these techniques provide better resolution, they still rely on stimulating multiple molecules simultaneously. It’s known that the information gathered from imaging a single molecule or fluorophore is superior to bulk measurements of many molecules. This is because it gives information on individual molecular properties and their environment. Imaging of single molecules has attracted much interest in several areas of research, because it allows us to study molecular properties normally disguised in large molecular groups.

As Professor David Walt of Tufts University says [1] “Single cells are the fundamental units of biology. Just as single cells have differences when you begin to look at the levels of proteins in them or the sequence of DNA in ostensibly identical cells, when you look at single molecules, you also see a distribution of behaviors.”

As a result, various techniques have emerged that make use of single molecule detection. For example, Morisaki et al. used nascent chain tracking (NCT) to study single mRNA protein synthesis dynamics, marking the first time this had ever been achieved in vivo.

Principle of Single Molecule Detection

Many techniques can take advantage of single molecule detection. These include laser scanning confocal microscopy, TIRF, wide-field microscopy, and near-field scanning optical microscopy (NSOM), as well as super-resolution techniques such as SIM and STED [3].

The general principle is as follows: using fluorescence microscopy for single molecule detection is fundamentally limited by the emitted photons from the fluorophore. Activated fluoroscent molecules must be separated by a distance larger than the Abbe diffraction limit. This allows parallel recording of many individual emitters, each having a distinct set of coordinates. A detector is used to collect image centroids of individual molecules, and the coordinates of these molecules are determined based on the number of photons emitted. A readout laser is used to collect images of the emitting molecules, until they spontaneously re-enter a dark state. Repeating this process for multiple cycles allows the positions of many fluorophores to be determined, and a summed image to be reconstructed at the end of the experiment [3, 4].

Pros and Cons

In contrast to observing several molecules simultaneously, single molecule detection provides certain benefits, as well as more information. For example:

- It allows highly accurate pinpointing of a molecule’s position within the labeled cell.
- Time traces of intensity, emission spectrum, or fluorescence lifetime provide information on local dynamics and diffusion.
- It can provide information on the proximity of specific labeled sites less than 10 nm apart, allowing for detailed probing of reaction mechanisms.
- Position sensitivity allows a scientist to locate a molecule and follow simultaneously the translational motion, re-orientational motion, and the internal dynamics of the individual molecules.

But single molecule detection does have some drawbacks:

- The ability to detect multiple fluorescent targets simultaneously can enable visualization of complex functional and molecular processes in-vivo. You can’t do this with the specificity provided by single molecule detection.
- By definition, you’re only looking at a tiny part of your sample at any one time. And the higher the resolution, the worse the sampling abilities. Before using single molecule detection techniques, you need to have studied your specimen with techniques that offer less detail, but better sampling.

Take the Next Step…
A defining technological development in supporting life science imaging was the introduction of low-light level electron multiplying charge-coupled device (EMCCD) camera systems.

Andor EMCCD cameras are considered the gold standard for single molecule detection. The iXon Life camera is designed exclusively for fluorescence microscopy, and drives the absolute best from EMCCD technology across all critical performance specs and parameters.

In particular, the accelerated readout rates - combined with ‘Optically Centered Crop Mode’ - means that dynamic single molecule processes can be better characterized. The 13m pixel of the 888 model provides superb single molecule resolving capability at the diffraction limit, while preserving optical photon collection efficiency.