A Review of Fluorescence Nanoscopy

In the last three decades a new microscopy technique, fluorescence nanoscopy, arose, offering scientists a new powerful tool to overcome the previous limitations and to explore the yet unveiled dynamics of cell biology.

Before its development, in fact, the usefulness of light-microscopy in biology seemed to have met the end, as Abbe's work had shown the intrinsic diffraction-limit in the resolution of light-focusing microscopy[1]: approximately half the wavelength of the light used, which led to a limit of 200nm for visible light. Even electron microscopy, although overcoming such limitation, due to several disadvantages[2], above all the impossibility of analysing live specimens, could provide limited benefits in the field[3,4].

The solution arose from a technique already in use in the 20th century: fluorescent labelling, which is the use of different fluorophores, i.e. molecules which re-emit light upon light absorption, to tag specific molecules they combine with. This phenomenon alone, however, is not enough, since fluorophores in the same diffracting region are excited together and emit together[1,5].

The key to nanoscopy, is, in fact, to prevent molecules within the same region from being detected together. It's realised by keeping fluorophores in a fraction of such region in a transiently dark state. The transition to the dark state is achieved by inducing stimulated emission, using multiple light sources[3].

In the coordinate-targeted methods such as STED[3,6] and RESOLFT[7], the result is achieved at defined spatial coordinates. Instead, the stochastic methods, such as PALM, STORM and PAINT, establish the "on" state at the single-molecule level, so that only a single fluorophore can emit. Each fluorophore is then separately localized by a centroid-calculation performed over the emitted light pattern[3,5].

Although such techniques haven't achieved the molecular scale (5nm) resolution they can theoretically reach, they have already proved their effectiveness, having accomplished multiple results in multiple fields. Among these there are mitochondrial biology[8], immunology[9], signalling[10], virology[11], bacteriology[12], cancer biology[13], and neurobiology[14], expanding our knowledge and potentially enabling the development of medical advancements.

Nevertheless, multiple challenges are still to be overcome for nanoscopy to achieve its maximum efficiency: above all, the ones concerning the labelling process itself[5], which, although fundamental, might affect the host molecule's functions, be incomplete or interfere with the localization. To avoid the last problem, small recombinant binders are likely to be developed. Additional challenges arise in living-cells nanoscopy, requiring high temporal resolution, which coordinate-targeted

methods are inherently well-suited for, as well as a minimization of phototoxic effects, for which longer wavelengths and lower intensities are desirable[15]. The intensities nanoscopy currently involves, which are the lowest in stochastic methods, might be too high for prolonged live-cell nanoscopy: live-cell imaging may ultimately require radically new concepts[5]. Finally, in thicker tissue slices, optical readout is complicated by stronger light absorption, scattering and aberrations[5]. Solutions implementing adaptive optics are currently being pioneered[16,17].

Although it's clear nanoscopy currently has its limitations, its benefits for biological and medical studies are undeniable. Given its potential, nanoscopy is rather likely to undergo future major upgrades and to further expand its frontiers.

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