Introducing fluorescence-based, fast and accessible light intensity measurements

Two new complementary protocols are making accurate and versatile light intensity measurement possible across a wide range of wavelengths and intensities.

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International research supported in part by the EU-funded DREAM project has led to two new complementary methods that enable versatile but precise light intensity measurements in fluorescence imaging systems. The two protocols are described in a paper published in 'Nature Methods'.

Accurate quantification of light intensity is an important tool used by scientists in many different applications. Optical microscopists balance light intensity to optimise signals without phototoxicity. Biologists use photons to trigger physiological processes. Chemists use them to drive light-absorbing reactions. "Nowadays, a vast community of biologists, chemists, engineers and physicists are concerned with delivering precise numbers of photons," remark the authors in their study.

However, most current technologies are neither versatile nor accurate enough to meet current needs. They cannot measure light intensity and spatial distribution at the same time – at least, not over a wide range of wavelengths and intensities.

Speed, sensitivity and accessibility

Researchers led by DREAM project coordinator National Centre for Scientific Research, France, have now developed two rapid and straightforward complementary protocols that use organic dyes and fluorescent proteins as actinometers (systems that determine the number of photons in a beam). These fluorescence-based actinometers have proved to be faster and more sensitive and have also provided more accessible data for imaging systems.

The first protocol relies on five molecular actinometers that emit fluorescent signals when constant light is applied. The actinometers cover the entire spectrum of ultraviolet and visible light for measuring light intensity. Under certain conditions – where photoconversion happens so fast that there is minimal molecular motion – the protocol can also map the spatial distribution of light intensity.

As reported in the study, the team also sought "to make fluorescent actinometers accessible to different communities of end users." They therefore chose easily synthesised chemicals for chemists, and proteins and photosynthetic organisms for biologists.

The second protocol complements the first protocol's fluorescent actinometers, whose limited ranges of light absorption require several actinometers to cover the entire range of wavelengths. This protocol uses a photochemically inert fluorophore – a molecule with fluorescent properties that absorbs photons and emits photons of lower energy in return. The fluorophore transfers information on light intensity from one wavelength – measured with a fluorescent actinometer from the first protocol – to another.

Two are better than one

"Together, the two new protocols can be used in weak-light situations, shorter periods, and a wider range of wavelengths than conventional methods," the research team observes in a 'EurekAlert!' news release. The protocols have been used to accurately measure the spatial distribution of light in different fluorescence imaging systems and to calibrate illumination in commercially available instruments and light sources. The authors also expect their protocols to improve scientific insight into how light affects the health and viability of biological specimens. The research team offers online access to the actinometer properties and to codes and user-friendly data applications to facilitate the use of these actinometers across disciplines.

DREAM (Dynamic Regulation of photosynthEsis in light-Acclimated organisMs) is developing pioneering lighting, instrumentation and data acquisition protocols to promote precision agriculture in optimised and controlled environments such as greenhouses, vertical farms and indoor gardens. The project ends in 2026.

For more information, please see:
DREAM project website

Keywords

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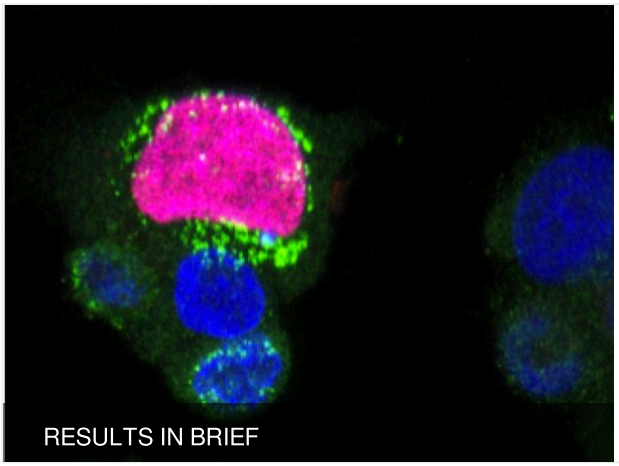

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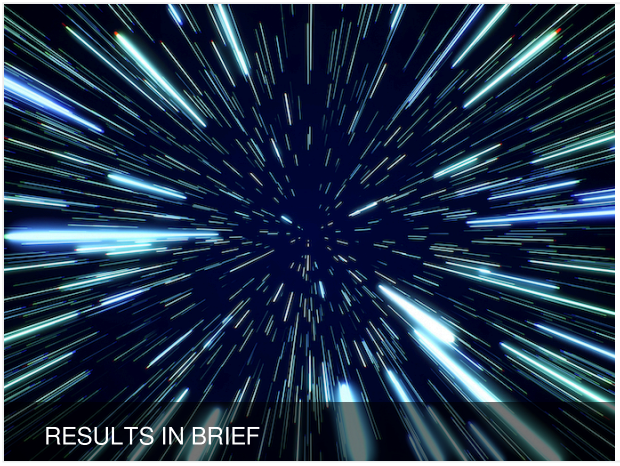

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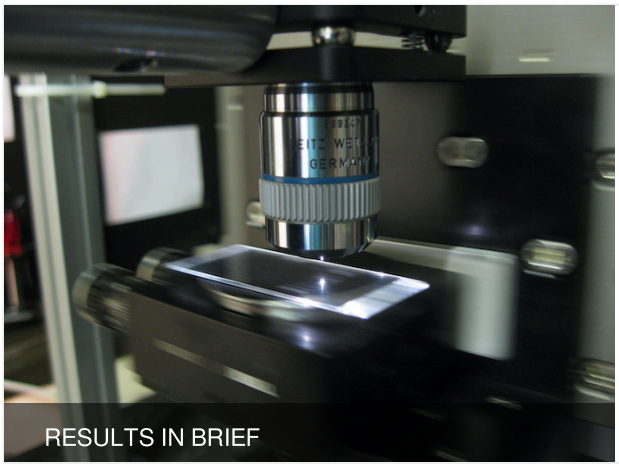

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