

MICROSCOPY

And then there was light

“non-fluorescent molecules can be imaged using a technique known as stimulated emission microscopy.”

Fluorescence microscopy is limited by the fact that many light-absorbing chromophores do not emit a detectable level of fluorescence and are not easily tagged with fluorophores in a physiological context. Min and colleagues now show how non-fluorescent molecules can be imaged using a technique known as stimulated emission microscopy.

Fluorophores absorb energy and drop back to a lower energy level (known as the ground state) by emitting a photon that is detected as fluorescence. However, the spontaneous emission of many excited chromophores is dominated by nonradiative decay — during which energy is released as heat — and their fluorescence is therefore too weak to be detected. The authors overcome this problem by using stimulated emission, which involves irradiating specimens with ultrashort pulses of light — electromagnetic pulses with a time duration in the hundred femtosecond order. A molecule (or

chromophore) that absorbs a photon from the first light pulse enters an excited state. A second pulse of light of a different colour (with a lower photon energy) de-excites the molecule (or chromophore) to the ground state, producing a duplicate photon of the same colour as the second pulse, the intensity increase of which is detected as the stimulated emission signal.

So, what can be imaged using this technique? The authors used stimulated emission microscopy to visualize proteins in several situations. They expressed genes encoding the *Goniopora tenuidens* and *Cnidopus japonicus* chromoproteins (gtCP and cjBlue, respectively), variants of green fluorescent protein that don't naturally fluoresce, in *Escherichia coli*, and visualized the proteins in the cytoplasm by stimulated emission. In another example, they used this label-free technique to image the microvascular structure in an *ex vivo* mouse ear by exciting haemoglobin — a light absorbing chromophore that usually emits an undetectable fluorescence.

The authors conclude that “Stimulated emission microscopy allows imaging of non-fluorescent chromophores with three-dimensional optical sectioning and high sensitivity, and extends the repertoire of reporters for biological imaging beyond fluorophores.”

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ORIGINAL RESEARCH PAPER Min, W. *et al.* Imaging chromophores with undetectable fluorescence by stimulated emission microscopy. *Nature* **461**, 1105–1109 (2009)

FURTHER READING Fernández-Suárez, M. & Ting, A. Y. Fluorescent probes for super-resolution imaging in living cells. *Nature Rev. Mol. Cell Biol.* **9**, 929–943 (2008)