doi:10.1038/nature22500

CELL IMAGING

An intracellular dance visualized

The development of a microscopy technique that enables observation of the interactions between six types of organelle, in 3D and over time, holds promise for improving our understanding of intracellular processes.

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Much as a human body contains many organs, its cells contain many organelles. These membrane-bounded compartments perform different functions but must interact physically, coordinating their activities to keep the cell alive and well. Cell biologists have long watched organelles dance inside living cells, either alone or in pairs. But technological limitations have made it hard to observe interactions between multiple organelles — little is known about the choreography of the dance troupe as a whole. In a paper online in *Nature*, Valm *et al.*[1] introduce an arsenal of tools with which to simultaneously visualize six types of organelle in a live cell, and use the video footage generated to define organellar structures, dynamics and interactions.

Fluorescence microscopy is the tool of choice for monitoring organelles in living cells. In this technique, different organelles are tagged with different fluorescent molecules called fluorophores; these are excited by illumination at certain wavelengths and themselves emit light at set wavelengths, producing different colours. However, routine fluorescence microscopes can distinguish only three or four such probes, owing to the broad range of wavelengths that each fluorophore is excited by and emits. More fluorophores can be distinguished using a method called linear unmixing, in which mathematical algorithms deconstruct the excitation or emission spectra from each pixel in an image to determine the combination of fluorophores excited in that region.

And there are other challenges to using fluorescence microscopy for live cells. First, the intensity of the fluorescence emitted by a fluorophore decreases continuously during light exposure — a phenomenon called photobleaching. Second, extended periods of light exposure can damage living cells. Together, these factors restrict the number of images that can be taken of any living cell. Limited snapshots can be obtained over time or in 3D stacks.

Valm *et al.* set out to simultaneously image six types of fluorescently labelled organelle — the endoplasmic reticulum (ER), mitochondria, Golgi, lysosomes, peroxisomes and lipid droplets — in live cells, both in 3D and over time. The authors first used a commercial fluorescence microscope and developed an information-processing system that could not only distinguish fluorophores through linear unmixing, but also outline objects from pixelated images, quantify organelle numbers, volumes and positions and, crucially, detect interactions between all organelles

The researchers used this tool kit to map the contacts between lipid droplets (which store and transport lipids) and other organelles. This analysis revealed reproducible interaction patterns. Lipid droplets made continuous contacts with the ER, in which most lipids are synthesized, but transient and less-frequent contacts with the other four organelles, to which droplets transport lipids for metabolic processing or degradation. However, these data were taken from a single, thick plane across the cell, because the microscope operated too slowly and caused too much damage to record 3D videos of the whole cell.

To overcome these challenges, Valm and colleagues developed a microscope based on a technique called lattice light-sheet microscopy, in which an ultrathin light sheet is placed perpendicular to the lens that connects to the camera and is projected onto the cell, illuminating only a thin section at a time (Fig. 1). This strategy enables high-resolution 3D imaging while limiting photobleaching and cell damage[2]. By quickly switching between six laser frequencies, the authors could rapidly

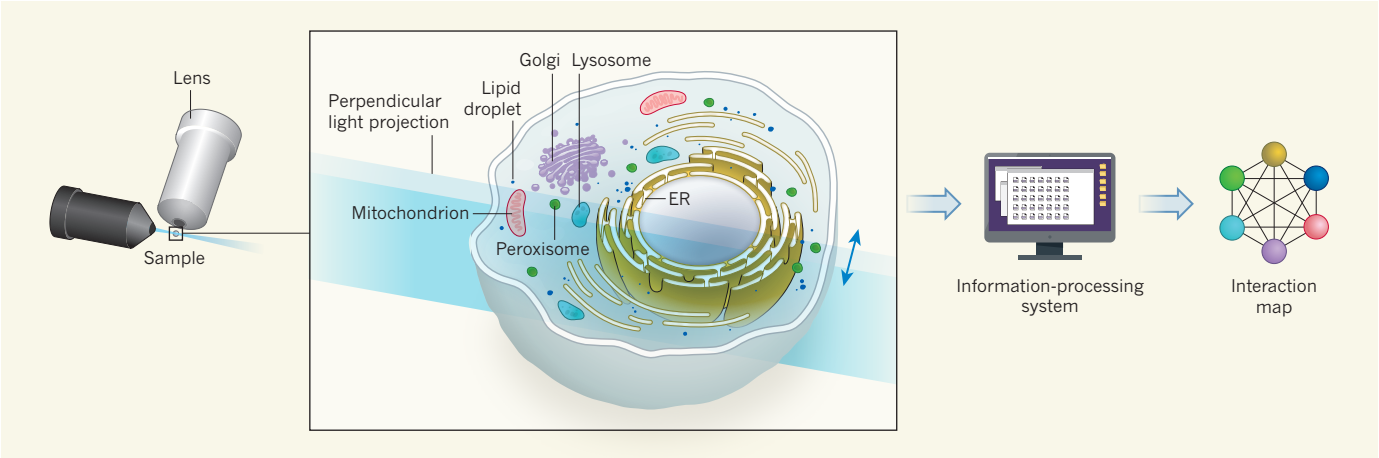

Figure 1 | Visualizing organelle dynamics in a living cell. Valm *et al.*[1] have developed a microscopy technique to analyse the interactions between six types of organelle: the endoplasmic reticulum (ER), mitochondria, Golgi, lysosomes, peroxisomes and lipid droplets. The authors projected lasers of six frequencies across the cell in a thin sheet perpendicular to the microscope lens. Each laser excites six fluorophores — molecules tagged to each organelle that emit fluorescent light — to varying degrees. Only the portion of the cell under observation is illuminated, preventing the damage to the cell or fluorophores that can result from prolonged light exposure. A complex information-processing system then computed organelle dynamics and interactions. Imaging at different levels across the cell and over time enabled the production of 3D videos from which organelle interactions could be mapped.

distinguish the different excitation spectra of each fluorophore.

The authors' 3D maps revealed that contacts between the ER and other organelles were uniformly distributed throughout the cell, whereas other organelle–organelle contacts were generally more frequent near the nucleus. The ER is mesh-like in shape, and the authors found that this mesh explored more than 90% of the cell within minutes. By contrast, other organelles moved much less. Taking all of their observations together, Valm and colleagues constructed a quantitative interaction network, which revealed that the ER acts as a central hub for the organelle network. The central role of the ER has been implicated in previous studies of organelle pairs[3], but the organelle network has never been quantitatively and systematically studied in individual cells. These observations provide a foundation from which to further investigate how the organelle network is coordinated during a variety of cellular processes.

Mitochondria, the cell's metabolic powerhouses, move continuously and stack in layers. To accurately map the contacts made by these organelles, the authors generated 3D time series of single cells. Like other organelles, mitochondria interacted most prominently with the ER. Finally, the authors defined contacts between ER–mitochondrion contact sites and the other four organelles over time. ER–mitochondrion contacts are of interest to biologists because they are well-established sites of calcium-ion signalling and lipid trafficking[3], and so are important for normal cell physiology.

Electron microscopy indicates that ER–mitochondrion junctions are 10–30 nanometres wide[3]. However, Valm and colleagues' microscope has a resolution of about 300 nm, so some of the contacts that the authors report could be false positives. Contact mapping could be made more precise either by increasing the resolution using spectral super-resolution fluorescence microscopy[4], or by using split fluorescent proteins[5] — two non-fluorescing protein fragments that fluoresce when they come into contact — attached to different organelles. In addition, the duration, recurrence or location of contacts could be monitored to form a score system to help identify functional contacts. Alternatively, perhaps such contacts could be pinpointed by measuring calcium-ion flux or labelling the molecular tethers that hold junctions together[3,6].

Valm and colleagues' breakthrough opens up wide-ranging opportunities for exploring the molecular mechanisms that underpin the organelle community's dance. If more dancers can be watched for longer, the potential for insight becomes even greater. Combinatorial labelling of organelles with more than one fluorescent protein, or the use of 'vibrational probes' that emit light over narrow bandwidths, could enable monitoring of several dozen cellular components[7,8], including signalling factors and metabolites that move between organelles. And techniques to amplify fluorescent signals could allow much longer time series to be taken[9]. In addition, the development of 'optogenetic' techniques[10] means that the positions of organelles can be controlled with light, enabling investigations of how the organelle network responds to altered behaviour of one or more chosen organelles. As the art of cell filmmaking matures, a new branch of systems biology based on images and video footage may emerge. ■

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