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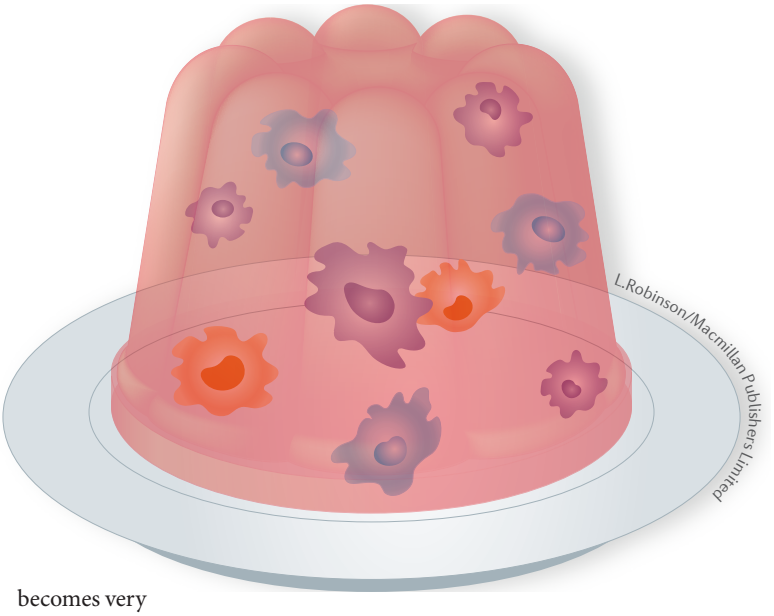
3D BIOIMAGING

Cells in gels



A new computational framework that utilizes confocal Raman spectroscopy allows 3D cell imaging with quantitative mapping of intracellular biomolecules. As Molly Stevens and co-workers report in *Nature Communications*, this framework provides unprecedented insight into cell biology as well as cell–material interactions in advanced biomaterials.

Understanding the behaviour of cells in culture systems is essential for developing biomaterials for tissue engineering that more closely mimic the *in vivo* microenvironment. The most common tool used to study 3D cell cultures is confocal fluorescence microscopy; this requires a fluorescent label and can only provide semi-quantitative information. Addressing the need to develop a complementary method that is both label-free and quantitative, the researchers turned to Raman spectroscopy. "In principle, every molecule has a unique Raman fingerprint that reflects how the atoms are vibrating," explains Mads Bergholt, co-author of the study. "Therefore, we can use this technique to quantify molecules without labelling them. This is a huge advantage because labelling, in general, disrupts cellular processes."

Raman imaging has previously been used to study the distribution of biomolecules and cell morphology. Stevens' team has previously developed methods to improve the quantification and visualization of molecules in 2D, and in the present work, has extended this to the third dimension. "This was not a trivial task" says Charalambos Kallepitis, first author of the study. "Raman signals are inherently very weak when using high-resolution Raman microscopy and the amount of data becomes very large; conventional computational methods therefore become inefficient." In a collaborative effort involving physicists, engineers and cell biologists, the team succeeded in overcoming these challenges by developing a new computational framework to process the Raman spectra. "We were able to identify very tiny components, such as lipid droplets, DNA and protein clusters. Hence, by using this technique we can better measure, visualize and quantify the individual cellular structures at the molecular level," notes Kallepitis.

> "Using this technique we can better measure, visualize and quantify the individual cellular structures at the molecular level"

The researchers used this method to image different cell types in both conventional cell cultures and 3D hydrogel biomaterials. In conventional cell culture, they demonstrated differences in the morphology and biochemical components of adult heart cells and heart cells derived from human-induced pluripotent stem cells — a level of detail that has not previously been accessible with label-free methods. They also demonstrated volumetric quantification of the different lipid subtypes that characterize the differentiation of monocytes to macrophages; this could be valuable, for example, in the study of diseases such as atherosclerosis, which is characterized by the transition of macrophages to foam cells.

In a polyethylene glycol hydrogel — a common artificial biomaterial — they used the signals from phospholipids (in the cell membrane) and from the polymer backbone of the hydrogel to discriminate stem cells from the matrix. In the 'inert' hydrogel, the stem cells retained a spherical shape and did not interact with the material. By contrast, when the hydrogel was made bioactive through peptide functionalization, cells became elongated and interacted with the material. Hence, this demonstrates the applicability of the method in probing the effect of biomaterials on cell behaviour.

The new quantitative volumetric Raman imaging technique has the potential to be used to investigate cell response and behaviour in stem cell research, cancer biology and drug discovery. "In the future, this will allow us to better characterize cell systems at the molecular level. Further, we are planning to correlate the imaging results that we find with various other complementary techniques used in cell biology," concludes Stevens.

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ORIGINAL ARTICLE Kallepitis, C. *et al.* Quantitative volumetric Raman imaging of three dimensional cell cultures. *Nat. Commun.* http://dx.doi.org/10.1038/ncomms14843 (2017)