appropriate active phase. Unfortunately, however, overexpressing *Mc4r* in the ventrolateral VMH was not sufficient to restore the physical activity or body weight of ovariectomized mice to pre-ovariectomy levels. This perhaps hints at changed production of α-MSH in the absence of ovarian hormones.

The symptoms of menopause can be highly disruptive and can persist indefinitely. Reduced physical activity and changes in body composition coincident with menopause are also sometimes associated with social stigma. In this work, Krause and team elucidate an oestrogen-dependent neural mechanism that might well impose more-sedentary behaviour. Understanding the downstream brain regions and signalling molecules of the stepwise neurons in the ventrolateral VMH could provide insight into potential pharmacological targets to treat symptoms of menopause.

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1. Toth, M. J., Tchernof, A., Sites, C. K. & Poehlman, E. T. *Int. J. Obes. Relat.* **24**, 226–231 (2000).
2. Krause, W. C. *et al. Nature* **599**, 131–135 (2021).
3. Heine, P. A., Taylor, J. A., Iwamoto, G. A., Lubahn, D. B. & Cooke, P. S. *Proc. Natl Acad. Sci. USA* **97**, 12729–12734 (2000).
4. Quarta, C. *et al. Nature Metab.* **3**, 299–308 (2021).
5. Lee, H. *et al. Nature* **509**, 627–632 (2014).
6. Xu, Y. *et al. Cell Metab.* **14**, 453–465 (2011).
7. Musatov, S. *et al. Proc. Natl Acad. Sci. USA* **104**, 2501–2506 (2007).
8. Matharu, N. *et al. Science* **363**, aau0629 (2019).

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Imaging

A view to a cell

Jason R. Swedlow & Lucy Collinson

Efforts to generate nanoscale-resolution images of cell interiors have gained ground through the development and refinement of a microscopy method. The data sets are publicly available as resources for further discoveries. **See p.141 & p.147**

One long-sought goal of cell biology is the full and complete determination of the structure and composition of a single cell. If we could name every molecule that makes up a cell, know each molecule's location, map how the molecules interact, and understand how they come together to construct organelles and determine organelle function, we would have the building blocks needed to understand cellular physiology. Xu *et al.*[1] (page 147) and Heinrich *et al.*[2] (page 141) present combined advances in data acquisition and machine-learning (ML)-based analysis in microscopy that move us closer to achieving this objective.

The development of new technologies or the adaptation of existing tools from other domains have often driven crucial progress towards determining cellular architecture and composition. For example, an imaging technique called focused ion beam-scanning electron microscopy (FIB-SEM) was used for decades in materials science before it was repurposed for the biosciences[3].

In this approach, a series of consecutive images are acquired from biological samples embedded in resin. The method entails cycles of, first, imaging the sample's surface using an electron beam, and then milling away a thin layer of the sample to reveal a new surface for imaging. The resulting data reveal exquisite architectural detail of cells and tissues with a 3D resolution better than 10 nanometres. This is sufficient to clearly distinguish most of the organelles in cells from humans and other species. As a result, FIB-SEM (used alongside other techniques for volume electron microscopy such as serial block-face SEM and array tomography) has opened a window on the inner workings of organs and organelles alike, driving a new field called connectomics (the study of the connections between brain cells to build wiring diagrams of the brain)[4–7], and a surge in the number of studies reporting high-resolution 3D reconstructions of subcellular structures and organelles[8].

The two new papers present major advances in FIB-SEM image acquisition and data analysis that are tailored to the demands of biological research. Xu and colleagues report on the culmination of a multi-year effort to improve the spatial resolution and reliability of FIB-SEM systems, which they achieved by redesigning microscope hardware and monitoring systems to deliver fast, stable, high-resolution imaging over periods of months or years. The team further increased the possible speed of data collection by using a bank of microscopes running in parallel, echoing the use of banks of FIB-SEM systems for device analysis and characterization in the semiconductor industry. The result is spectacular imaging data that reveal the complex 3D constitution of whole cells, with individual organelles clearly visible (Fig. 1).

Xu *et al.* present data for different types of entire cell, including HeLa cells (a line of human cervical cancer cells), T cells of the immune system, and pancreatic β-cells (which secrete the hormone insulin needed to control blood glucose levels). These various cell types were imaged at a resolution of 4 nm in each dimension, meaning that the data can be 'resliced' and viewed from any angle, enabling the viewer to follow cellular membranes across a distance of many micrometres of cell space. Although other imaging methods can achieve higher resolution over smaller volumes, these 3D whole-cell nanoscale atlases will undoubtedly provide a treasure trove of buried information that should offer a deeper understanding of the fundamental processes of life.

With these data in hand, Heinrich and colleagues report the application of artificial intelligence, in the form of advanced ML-based tools, to automatically identify the subcellular organelles that these images reveal. But this is more than just another AI-driven application. The density of data in these new FIB-SEM reconstructions of cells is so great that it exceeds the capacity of manual annotation as a way to define the identity and boundaries of all the cellular constituents — a technique called segmentation. Heinrich *et al.* extended approaches used in several deep-learning-based organelle segmentation tools[9–12] to automatically identify the boundaries of some of the organelles contained in these huge data sets. Their methods are based on the detailed manual annotation of organelle boundaries by human observers in small subsections of the data. These human-annotated data are used as training sets for the ML-derived networks that are then applied to the whole-cell data. This analysis has produced some of the most-detailed models of the architecture of the interior of single cells ever generated.

Their data enabled the authors to measure the volume of individual organelles and the fraction of the total cell volume they inhabit, as well as to identify interesting organelle–organelle interactions; for example, the number of different organelles that make contact with a single microtubule and the various interactions between the endoplasmic reticulum and the Golgi complex. By training their models on annotated data sets from several types of cell, the authors show that generalizable models of cell organelles can be used for organelle segmentation in different cell types. We don't know exactly what features the trained models recognize, but their ability to recognize such features

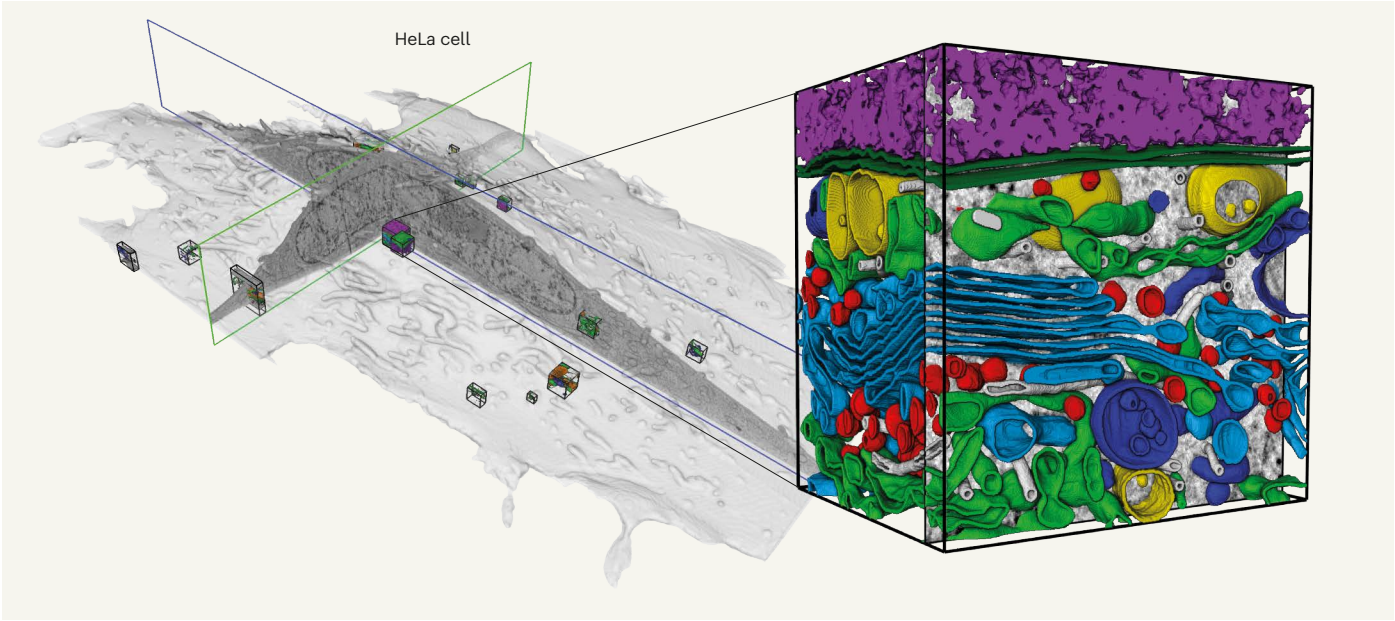

Figure 1 | Detailed picture of a human cell. Xu *et al.*[1] and Heinrich *et al.*[2] present work that has improved the options available for acquiring and analysing images using a microscopy technique called focused ion beam-scanning electron microscopy (FIB-SEM). This example of such an image is of a HeLa cell (a human cervical cancer cell). The authors manually identified organelle boundaries, and different types of organelle are shown in different colours. A machine-learning approach enabled Heinrich and colleagues to harness this manually produced information and develop a system that can automatically identify particular types of organelle in FIB-SEM images. (Image taken from Fig. 1 of ref. 2.)

suggests that subcellular organelles do not differ so much between cells as to prevent models derived from one cell type from being used for another. This is consistent with observations made in light microscopy, in which transferable models are becoming widely used for cell segmentation[13–15]. The authors exploited this ability to segment the interior of several types of cell grown in culture or in intact tissue, and have thus made a task that might seem impossible at least feasible, even if further improvements are needed.

These papers provide several notable steps forward, but it is nevertheless worth considering the limitations and the extra work that is still needed. For example, the reported ML methods reveal several components, such as the nucleus and plasma membrane, quite accurately, but are not as reliable in their definition of ribosomes and mitochondrial and lysosomal membranes, and in their identification of several other organelles. This underscores the difficulty of achieving the ultimate goal of defining all of the different components of a cell. Moreover, the authors' reported results are based on whole-cell reconstructions of just three HeLa cells (two at interphase and one at the mitotic stage of the cell cycle), one example of mouse pancreatic tissue and several T cells. One challenge will be to understand the level of variation naturally present at the nanoscale, and to reach the amount of sampling required to develop statistically valid measurements of different organelles as cells progress through the cell cycle, differentiate and respond to external stresses or drug treatments.

Another future challenge lies in data handling, sharing visualizations and analysis. The data sets are each several terabytes in size — far beyond the routine capabilities of typical individual laboratories or small imaging facilities. To make these data sets widely accessible, the authors built a dedicated web resource called OpenOrganelle (see go.nature.com/3bcowhl) that enables interactive visualization of the 3D data sets and their derived models, and that also makes the original data available for download. Browsing the site on a laptop screen gives a sense both of the extraordinary complexity of the human cell and of how little is currently known about its inner workings. Making data on this scale publicly available is no small feat, and required the development of a new data format and specifications for segmentation and visualization. The published data sets are an incredible resource and will undoubtedly be used by scientists globally.

In the future, it will be important to publish the models used to calculate the segmentations, so that the results from different such models can be compared. A far-off goal for the cell-biology community is to establish a library of deep-learning models that can be used to segment all components of all the different cell types. Indeed, the first library of such models, mostly for light microscopy, is now available (see go.nature.com/3lcr0gv), and it will be exciting to see how these efforts develop in the future.

This is part of a trend in cell biology in which the ultimate aspiration is to match achievements in genomics and in structural biology, by having the routine publication of complex and reusable multidimensional imaging data[16]. The data sets and visualizations provided by these two studies are a vital step in developing bioimaging into a field that fully connects and integrates experimental and computational sciences.

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1. Xu, C. S. *et al. Nature* **599**, 147–151 (2021).
2. Heinrich, L. *et al. Nature* **599**, 141–146 (2021).
3. Heymann, J. A. W. *et al. J. Struct. Biol.* **155**, 63–73 (2006).
4. Hildebrand, D. G. C. *et al. Nature* **545**, 345–349 (2017).
5. Helmstaedter, M. *et al. Nature* **500**, 168–174 (2013).
6. Zheng, Z. *et al. Cell* **174**, 730–743 (2018).
7. Scheffer, L. K. *et al. eLife* **9**, e57443 (2020).
8. Müller, A. *et al. J. Cell Biol.* **220**, e202010039 (2021).
9. Perez, A. J. *et al. Front. Neuroanat.* **8**, 126 (2014).
10. Liu, J. *et al. Front. Neurosci.* **14**, 599 (2020).
11. Guay, M. D. *et al. Sci. Rep.* **11**, 2561 (2021).
12. Spiers, H. *et al. Traffic* **22**, 240–253 (2021).
13. Hollandi, R. *et al. Cell Syst.* **10**, 453–458 (2020).
14. Stringer, C., Wang, T., Michaelos, M. & Pachitariu, M. *Nature Methods* **18**, 100–106 (2021).
15. Schmidt, U., Weigert, M., Broaddus, C. & Myers, G. in *Medical Image Computing and Computer Assisted Intervention – MICCAI 2018* (eds Frangi, A. F., Schnabel, J. A., Davatzikos, C., López Alberola, C. & Fichtinger, G.) 265–273 (Springer, 2018).
16. Ellenberg, J. *et al. Nature Methods* **15**, 849–854 (2018).

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