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Volume EM: a quiet revolution takes shape

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Volume electron microscopy (vEM) is a group of techniques that reveal the 3D ultrastructure of cells and tissues through continuous depths of at least 1 micrometer. A burgeoning grassroots community effort is fast building the profile and revealing the impact of vEM technology in the life sciences and clinical research.

The awarding of three Nobel Prizes for imaging technologies between 2008 and 2017 has highlighted the key importance of imaging in present-day life science research. The expression of proteins tagged with green fluorescent protein in living cells and organisms transformed the way in which life science research was conducted. The ability to visualize the dynamic nature of proteins in cells and tissues was recognized with the Nobel Prize in Chemistry in 2008. This was followed by another Nobel Prize in Chemistry in 2014 for the development of super-resolution light microscopy technologies, in which Abbe's resolution limit was finally broken to allow localization of fluorescently tagged molecules with a precision of tens of nanometers. Most recently, the 'resolution revolution' in cryogenic electron microscopy (cryo-EM) has enabled the determination of the molecular structure of isolated proteins and protein complexes, and was recognized with the 2017 Nobel Prize in Chemistry. There is now another imaging revolution underway that reveals the exquisite complexity of cells and tissues at the membrane and organelle scales in three dimensions — volume electron microscopy (vEM), which was recently highlighted as one of the 'seven technologies to watch in 2023' by *Nature*.

Volume electron microscopy

vEM is a group of techniques that are used to image the structure of cells and tissues through continuous depths of at least 1 micrometer at nanometer resolution[1] (Fig. 1). vEM encompasses imaging technologies based on both transmission electron microscopy (TEM) and scanning electron microscopy (SEM). In all cases, vEM generates a series of images of the specimen that, when combined, form a digital representation of the specimen volume. The individual images are acquired by repeatedly slicing the specimen into thin sections, and then imaging either the sections (from different angles in the case of tomography) or the exposed face of the sample. Without using the exact term, various kinds of vEM have been in use for decades and have contributed substantially to our understanding of the complexity of cells, tissues and organisms.

What vEM can reveal about life

vEM reveals the structural beauty and complexity of life, from the membranes that make up organelles, through the arrangement of organelles within cells, to the communities of cells that make up tissues and the architecture of tissues that make up organisms. This makes vEM a critical tool for understanding biological complexity across scales. Indeed, the development of vEM was originally driven by the quest to understand the connections in the brain, from individual vesicles that release neurotransmitters at synapses to entire neurons that make connections across different brain regions. Since the 1980s, vEM has delivered connectomes from model organisms including *Caenorhabditis elegans*[2], *Drosophila melanogaster*[3–5] and *Danio rerio*[6]. However, complexity across scales is present in every organism and vEM is now being used throughout the life sciences, revealing the structural complexity of fertilization[7] (Fig. 2), blood vessels[8,9], muscles[10], sensory organs[11,12], tumors[13,14], pathogen-infected cells and tissues[15,16], plants[17,18] and marine organisms[19,20], to name but a few.

vEM workflows

There are three main components that are common to all vEM workflows: (1) sample preparation, (2) imaging and (3) data.

In the first step, the sample is prepared by chemical or cryogenic fixation, followed by staining using heavy metal salts of osmium, lead and uranium to add electron contrast to the membranes and make the sample more conductive. The sample is then dehydrated using a solvent, infiltrated with a liquid resin and the resin is polymerized using heat or UV light. This encases the cells and tissues like a mosquito in amber, resulting in a hard block that can be sliced using a diamond knife or an ion beam. Slicing is an essential part of the vEM workflow because of the poor penetration of the electron beam into the samples. Slicing or sectioning may be performed manually on an ultramicrotome using a diamond knife, and the ultrathin sections collected onto metal grids, tape or wafers for imaging. Alternatively, the sections may be removed and discarded, and the block surface imaged after each cut. In both cases, the result is a set of sequential images that represent the volume of the original sample.

In the second step, vEM imaging is performed using TEM or SEM (Fig. 1). TEM methods include imaging of serial sections on grids (ssTEM) and serial section electron tomography, which improves axial resolution. SEM-based vEM techniques became viable following the switch from analog to digital electronics and improvements in sources and detectors, resulting in TEM-like imaging in the SEM. Serial blockface SEM (SBF-SEM) incorporates an entire ultramicrotome device within the SEM chamber, enabling some automation of the slicing and imaging cycle[21]. Focused ion beam SEM (FIB-SEM) adds a second column to the chamber, using gallium ions to mill away thin layers of the specimen surface before imaging the blockface[22]. Array tomography uses large silicon or conductive glass substrates to hold hundreds or thousands of ultrathin sections for serial imaging in an array format[23]. A recent review[1] provides further details of the different vEM techniques.

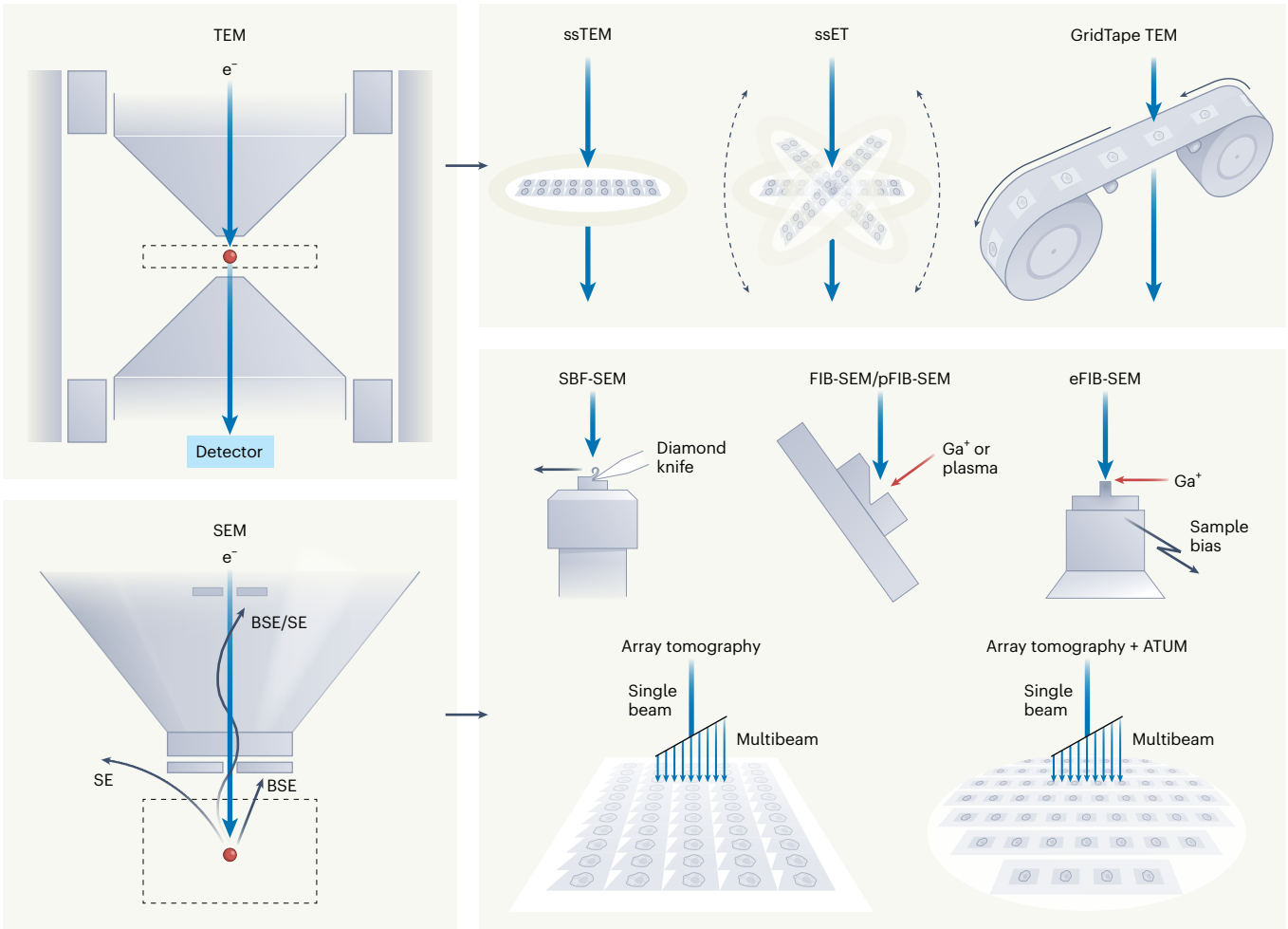

Fig. 1 | vEM technologies. vEM encompasses a range of technologies based on transmission and scanning electron microscopes that enable the collection of a series of images through the volume of resin-embedded cells and tissues[1]. The source (e^-) generates a beam of electrons (blue arrows), which passes through (in TEM) or interacts with (in SEM) the sample (red dot) and forms an image on the detector. The image is generated by the scattering of the primary electron beam in TEM, and by secondary electrons (SE) or backscattered electrons (BSE) in SEM. TEM (top row) can be used for vEM by sequential imaging of serial sections on grids (ssTEM and serial section electron tomography (ssET)) or on tape (GridTape TEM). SEM (bottom row) can be used for vEM by sequential imaging of an exposed sample surface that is cut with a diamond knife (SBF-SEM) or ion beam (FIB-SEM, plasma FIB-SEM (pFIB-SEM) and enhanced FIB-SEM (eFIB-SEM)), or by sequential imaging of serial sections on a substrate such as a silicon wafer (array tomography). Ultramicrotomy may be partially automated by collecting sections onto tape. ATUM, automated tape ultramicrotomy.

In the third step, the vEM data are processed and analyzed. vEM now joins light-sheet microscopy and cryo-EM in catapulting the life sciences into the 'big image data generator' regime, approaching data output levels that are more familiar in astrophysics and particle physics. A single vEM instrument can easily generate 250 GB of image data per day, with new high-speed imaging systems promising rates of 5 or more TB per hour. In addition, considering that many vEM experiments involve correlative and multimodal workflows, the complexity of the data is multiplied. This gives rise to heterogeneity in data types, file formats and branched data flows within a single experiment, requiring the development of a coherent global data model. Much of the data generated by vEM workflows thus remain unanalyzed owing to the rich content of the images and the lack of automated image analysis methods available, although this is changing quickly with the explosion of artificial intelligence methods. Public archiving of data and user-friendly implementations of advanced analysis algorithms will be critical to ensure that vEM data are fully mined. The Electron Microscopy Public Image Archive (EMPIAR)[24], although originally developed to host raw cryo-EM data sets, now also supports the archiving of vEM data (currently about 10% of new depositions). EMPIAR is working actively with the community to further accommodate the requirements of vEM data producers as well as users from communities as diverse as cell biology and machine learning. The connectomics community have developed their own solutions for sharing vEM data, including the Open Connectome Project and the Registry of Open Data on Amazon Web Services (Open NeuroData)), and the 3D viewing tools neuroglancer, webknossos and the Collaborative Annotation Toolkit for Massive Amounts of Image Data (CATMAID).

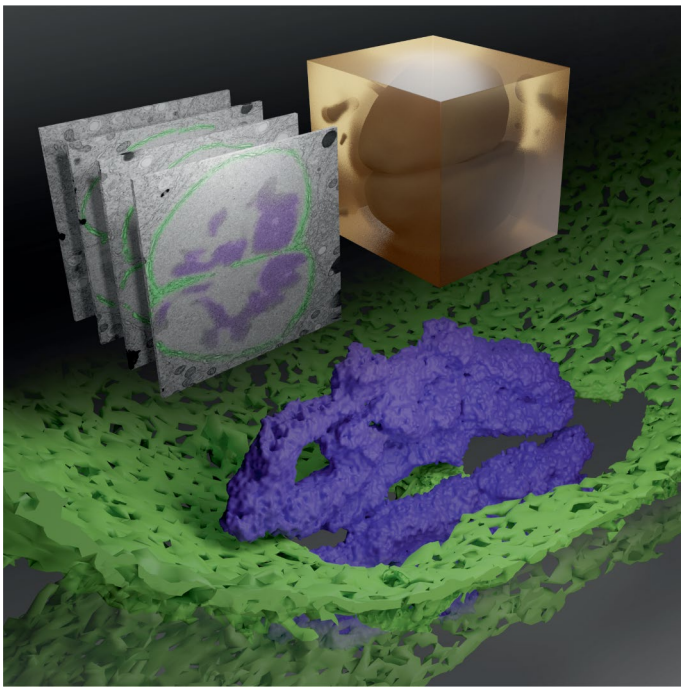

Fig. 2 | First contact. Rendering of a vEM workflow to capture in 3D and at high resolution the coming together of haploid parental genomes following fertilization[7]. A high-pressure frozen, stained and resin-embedded *C. elegans* zygote (amber block) was imaged by FIB-SEM to generate a stack of grayscale images of the targeted volume. Features of interest were reconstructed in 3D, revealing the fenestrations in the breaking-down nuclear membrane (false-colored green in 3D and highlighted in green in grayscale images), through which the condensed parental genomes (purple) make contact before full mixing. Image credit: Joseph Meyer, Kedar Narayan.

The cutting edge in vEM technology

The vEM field is currently expanding, and technologists and engineers are delivering improvements in speed, throughput and usability at pace (reviewed in ref. 1). Sample slicing is becoming faster with the automation of ultramicrotomes and adoption of fast milling technologies from the materials sciences (plasma FIBs and gas cluster ion beams). Imaging throughput is increasing with the development of tape feed-through systems (GridTape TEM) and multibeam SEMs that image the sample with multiple parallel electron beams (the Zeiss multiSEM and the Delmic FAST-EM). Automation of pipelines in image-acquisition software and monitoring systems aid in both usability and error-free data collection over days, months and even years[25]. So, although the technical capabilities are increasing quickly, other issues such as accessibility and data handling and analysis are now major bottlenecks.

vEM-associated technologies

Although vEM does not directly reveal the functional state of the cellular structures that are captured in the images, the integration of other spatial analysis technologies into correlative and multimodal workflows allows additional information to be mined from the sample. For example, X-ray microscopy of the entire sample volume adds context to smaller regions imaged by vEM; light microscopy (for example, fluorescence microscopy) adds dynamic molecular localizations; electron-dense probes (for example, APEX2 and nanoparticles) add static molecular localizations; nanoscale mass cytometry (for example, nanoscale secondary ion mass spectrometry (nanoSIMS), OrbiSIMS and X-ray fluorescence) adds spatial chemical signatures; and spatial transcriptomics adds gene expression signatures.

In situ structural cell biology, in which molecules are imaged in their cellular environment at near-atomic-resolution, is a related field that also depends on electron imaging. Although there are overlaps in some of the sample preparation techniques and microscopes, in situ structural cell biology is distinct from vEM because it is performed entirely at cryogenic temperatures and is limited to a continuous sample thickness of about 200 nm (ref. 26), which is too thin to be considered vEM by the current definition proposed by the community[1]. However, there are some nascent areas of crosstalk between the cryo-EM and vEM worlds: for example, the combination of cryo-fixation with vEM to improve sample preservation[27] and the use of cryo-FIB-SEM as a vEM imaging tool[28,29] (although both are limited to samples smaller than 200 micrometers in at least one dimension owing to the physical limits of vitrification).

Volume electron microscopists

The demands of sample preparation and the expense and complexity of many of the vEM instruments and workflows have generally limited access to vEM to a small number of experts. There are many and varied paths to becoming a volume electron microscopist. For instance, to become a vEM expert in a core facility one may have studied biosciences, used light microscopy to image a sample, run out of resolution or needed to see where the fluorescent protein was localized within the context of cell structure, acquired first electron microscopy images of a sample with the help of an electron microscopy expert, been captivated by the complexity and beauty of cell structure, started training in the full vEM workflow, joined a specialist electron microscopy laboratory or facility, and eventually become a vEM expert. In other settings (for example, in specialist connectomics and vEM technology development laboratories), multidisciplinary teams that efficiently integrate varied individual expertise are more common. Here, the early experiences and career paths of vEM experts are varied, and involve fields such as engineering, physics, chemistry, data science and the life sciences. A profile of a vEM expert may thus include the following traits: multidisciplinary (for example, cell biology, light microscopy, X-ray microscopy, electron microscopy, big data and engineering), collaborative (tackling complex projects as part of a larger team), and patient (vEM projects take months or years, not days or weeks).

Access to vEM

Considering the limited number of vEM experts (currently perhaps a few hundred worldwide), the complex multimodal workflows, intricate sample preparation with toxic chemicals and the expensive and sensitive instrumentation, vEM is well suited to the central-facility access model. vEM microscopes have been gradually incorporated into existing electron microscopy facilities, but the diversity of vEM workflows means that there are few facilities that are large enough to offer all vEM options.

The choice of a particular vEM modality is driven by the scientific question and incorporates considerations of sample size, target structure size, field of view and resolution required to capture the target structure, and availability of probes to highlight and confirm the identity of the target structure. vEM modalities overlap in their capability and therefore one biological question might be addressed by (for example) ssTEM, SBF-SEM or array tomography, whereas another

Table 1 | vEM community working groups

Working group	Remit	Co-chairs in 2020–2022
Community	Support all those who are practically involved in vEM techniques, across sample preparation and ancillary techniques (for example, X-ray imaging and correlative light and electron microscopy); including microscopists and data scientists.	J.J.B. (University College London) C.J.P. (Francis Crick Institute)
Outreach	Reach out to the wider international biomedical science community in the form of: (1) education about the various vEM techniques and (2) engagement of stakeholders, including end users, engineers, software developers, funders and the public.	G.F. (BioImagingUK) A.B. (University College London)
Sample preparation	Curation of sample preparation resources for the vEM community, including a protocol library that encompasses preparation steps through to imaging, with a focus on discussion and dissemination of knowledge around why each step is done, health and safety information, and annotations to indicate options and when they might be useful.	C.B. (Francis Crick Institute) M.C.D. (Rosalind Franklin Institute)
Data	(1) Investigate community requirements for data storage, transfer and archiving. (2) Investigate community requirements for software and data analysis platforms. (3) Promote the development and use of metadata ontologies. (4) Democratize access to data and tools for the whole community.	K.N. (Frederick National Laboratory, National Cancer Institute, National Institutes of Health) M.W. (Science and Technology Facilities Council, UK Research and Innovation)
Training	Provide resources and training on both the technical and managerial skills relating to all aspects of vEM.	E.J. (Oxford University) R.C. (Zeiss Research Microscopy Solutions)
Infrastructure	Define, seek funding for, build and run a coordinated accessible infrastructure for vEM to meet the needs of the UK bioscience research community and to facilitate similar applications elsewhere.	C.C. (ConnectomX) C.W. (Portsmouth University) P.V. (Bristol University) L.M.C. (Francis Crick Institute)

can only be answered by (for example) FIB-SEM. Thus, some biological questions may be answered by local electron microscopy facilities with access to only one or two vEM modalities, whereas others will require a large facility with the ability to flexibly combine multiple vEM (and perhaps other imaging) modalities into a custom workflow to produce a multiscale, multimodal dataset that will answer the question.

The piecemeal incorporation of vEM into existing electron microscopy facilities and the low throughput of multiscale, multimodal projects means that global vEM capacity is still low. When considering projected future vEM needs, the gap between supply and demand becomes more worrying — especially as the life sciences community becomes increasingly aware of the potential of vEM to reveal complex cell and tissue interactions across scales. We propose an urgent review of international supply and demand in vEM, and construction of open-access local, national and/or international facilities to fill any gaps, alongside training programs to ensure the availability of a sufficiently large skilled vEM workforce.

The international vEM community

The vEM community consists of imaging scientists who develop and deliver vEM and end users who apply vEM to answer their research questions. The goal of the grassroots vEM community initiative is to build a coherent international community of vEM experts and users to facilitate science delivery, and to build capacity to meet the vEM needs of the bioscience research community.

In the 2000s, the 'volume revolution' gathered pace and so too did the growth of the vEM expert community. Despite local collaboration and networking by vEM experts, it was initially difficult to communicate the exciting advances being made in vEM to the wider life sciences community in the shadow of the burgeoning 'resolution revolutions' in both light microscopy and single-particle cryo-EM. Taking inspiration from the UK cryo-EM community, a vEM town hall meeting was organized by some of authors of this Comment (L.M.C., G.J.K., A.P. and P.V.), supported by and held at the Wellcome Trust in London, UK in October 2019. Sixty members of the expert vEM community attended, representing biologists, microscopists, technologists, facility managers, hardware and software engineers, image analysts, public image archivists and commercial suppliers. Although there were international attendees, the meeting was driven by and focused on the UK vEM community, and from this meeting came the first formal proposal for a vEM infrastructure capable of meeting UK bioscience demand. The proposal suggested the formation of multiple centers of excellence for established vEM techniques (SBF-SEM, FIB-SEM and array tomography) housed close to key end-user communities in universities or research institutes, and a national facility that would host newly developed vEM techniques that were not yet user-friendly enough for broader rollout (for example, plasma FIB, multibeam SEM and GridTape TEM). All facilities would have the supporting hardware and expertise for sample preparation, correlative multimodal workflows (light microscopy, SEM, TEM and X-ray microscopy) and advanced data handling and analysis.

The proposal was submitted to UK funders who provided positive feedback, but requested further development of the concept. Before further progress could be made, the COVID-19 pandemic swept across the world. As with much of science, the vEM community continued their work online and held town hall meetings in October 2020 (60 attendees) and October 2021 (200 attendees). In August 2020, at a vEM microlaboratory workshop organized by another author of this Comment (K.N.), around 50 domain experts identified current issues in vEM, which were articulated as key 'how can we?' questions. These questions were:

- How can we retrieve, preserve and prepare large-volume biological samples to obtain appropriate information?
- How can we accelerate end-to-end sample throughput and interpretation?
- How can we improve software to transform vEM from a qualitative to a quantitative method, enabling user-friendly extraction of insights from biological samples?
- How can we share vEM data quickly and efficiently for viewing and analysis without overburdening local storage and computing capacity?
- How can we set up an efficient vEM facility?

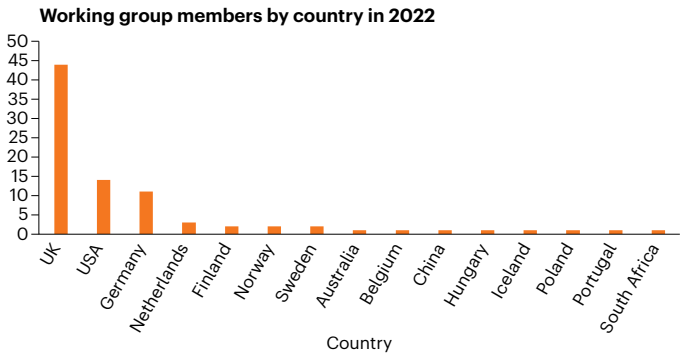

Fig. 3 | Working group membership. vEM community working group membership by country in 2022.

- How can we improve communication and information exchange within the vEM community and across disciplines?

From these meetings emerged two goals: to build (1) a coherent international community of vEM experts and users to facilitate science delivery and (2) a vEM infrastructure that can serve the needs of the international bioscience research community. To achieve these aims, six working groups (focusing on infrastructure, community, outreach, sample preparation, data and training) were formed, each of which was co-chaired by two volunteers from the vEM community (Table 1). As of early 2023, there are almost 80 international imaging scientists who are volunteering their time and enthusiasm across the working groups (Fig. 3). As well as increasing the global representation of the working groups and networking colleagues across disciplines, the groups have delivered resources that include:

- A website to link the community and share resources
- A mailing list for the vEM community
- A vEM Twitter account and hashtag (#volumeEM)
- A vEM community logo
- Blog posts from the working group co-chairs on the community site FocalPlane
- An edition of *Methods in Cell Biology* focused on vEM methods
- A special issue of *Frontiers in Cell and Developmental Biology*, focused on methods and applications in correlative light microscopy and vEM
- A *Nature Reviews Methods Primer* article on vEM for those new to the field[1]
- Case studies showing the impact of vEM in the biosciences
- A new Gordon research conference focused on vEM, starting in 2023
- A list of volunteer vEM expert reviewers for journal editors and funders (available from the corresponding authors on request)
- A database of vEM-related resources set up in collaboration with Microlist
- A vEM-focused virtual seminar series in collaboration with Euro-BioImaging
- A sample preparation widget in collaboration with the European Molecular Biology Laboratory European Bioinformatics Institute (EMBL-EBI)
- Training videos to explain complex vEM workflows
- Explainers of the toolkit required to get started with vEM workflows.

Next steps for vEM

The impressive outputs of the grassroots vEM community have thus far been supported by the efforts of volunteers, all of whom have day jobs running imaging facilities and conducting bioscience research and technology development. The resources delivered to date demonstrate the power and the enthusiasm of the community for realizing the full potential of vEM to drive discovery and translational research. However, delivering the next phase of community resources will require funding for dedicated people to coordinate and conduct larger-scale activities. As of January 2023, we are happy to announce that the Chan Zuckerberg Initiative has awarded funding to the vEM community for this purpose through their 'Advancing Imaging Through Collaborative Projects' scheme.

Future resources planned by the community include:

- An annual vEM meeting for technologists to coordinate global efforts to further develop vEM into a quantitative imaging science (community working group).
- A global map of vEM facilities to help bioscience researchers to access expertise and instruments (infrastructure and community working groups).
- Development of a website for sharing expertise and spare capacity on instruments to make optimal use of existing vEM resources (infrastructure working group).
- A series of training videos and animations explaining complex vEM workflows (training working group).

Activities with a more technical focus that will support the development of community standards and FAIR (meeting findability, accessibility, interoperability and reusability criteria) data practices include:

- A project to gather and analyze sample preparation protocols from across the community to help to map the diversity of methods and identify critical steps, with an eye to improving reproducibility (sample preparation working group).
- A project to create a vEM ontology to help to standardize the metadata that describe vEM datasets (data working group).
- A project that harmonizes protocols and ontologies into a sample preparation widget implemented at EMBL-EBI, which will associate complex protocol metadata with every vEM image set stored in public archives to support FAIR practices and metastudies of deposited data (sample preparation and data working groups).
- The creation of a collaborative computational project for vEM to support users and developers of vEM software. The vEM community is keen to follow community standards such as Recommended Metadata for Biological Images (REMBI)[30], and the EMPIAR team at EMBL-EBI is working with the data working group to devise a REMBI-compliant data model that captures metadata from vEM experiments (data working group).

In addition to supporting the community to self-organize and establish key standards and practices in vEM, the critical issue of capacity for conducting vEM experiments remains. Since the initial proposal was submitted to the UK funding bodies, we have gathered further supporting evidence of the demand for a vEM infrastructure. On the basis of discussions within and beyond the community, the proposal has evolved to incorporate in situ structural biology, encompassing

correlative and multimodal imaging across scales (from proteins in cells to tissues and organisms). Building on a foundation of best-possible structural and molecular preservation required for electron microscopy, this infrastructure would be unique in allowing researchers to understand the high-resolution spatial organization of complex biological systems. Further development of the proposal will leverage the expertise of the growing community and incorporate globally relevant insights, as vEM working group members are based in six continents (North America, South America, Africa, Asia, Oceania and Europe). In building vEM capacity, we will critically consider equity, diversity and inclusion to ensure that the technology is open to all regardless of local conditions. As a relatively new community, we also have an opportunity to design and adopt new strategies for minimizing the environmental impact of large-scale technical and data infrastructures into our programs of work. By embedding these principles in our community and infrastructure, we will ensure that we are consistent in working toward improving the health of the population and the health of the planet.

If you have enjoyed this Comment and are interested in learning more (whether as a technical specialist or as a potential end user), we encourage you to make contact through our website and to join this exciting, open and welcoming community at the forefront of imaging science.

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Author contributions

L.M.C., G.J.K. and P.V. wrote the first draft of the paper. L.M.C., A.P., G.J.K. and P.V. led the original vEM community initiative. C.B., A.B., J.J.B., R.C., C.C., M.C.D., G.F., E.J., K.N., C.J.P., M.W. and C.W. wrote sections and/or reviewed the manuscript and co-chaired the six vEM working groups. C.J. P., K.N., L.M.C. and G.J.K. prepared figures.

Competing interests

C.C. is a co-founder and shareholder of Connectomx Ltd. M.C.D. is an employee of SPT Labtech Ltd. R.C. is an employee of Zeiss Group. The remaining authors declare no competing interests.

Additional information

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