Diamond-II
Proposal for flagship project –
Large Volume Nanoscale BioImaging Beamline

Science Group: Life Sciences

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Large Volume Nanoscale BioImaging Beamline

1. Summary/Impact statement

Diamond has progressively strengthened its bioimaging capabilities since the first tranche of MX beamlines in 2007 came on-line allowing protein structures and large macromolecular complexes and viruses to be imaged at sub-nm resolutions. The portfolio now consists of a range of techniques that allows imaging across a range of resolution and length scales through dedicated beamlines for infrared imaging (B22), CD imaging (B23), BioSAXS (B21), a cryo soft x-ray transmission microscope (B24), a soft X-ray scanning microscope (I08) and hard X-ray imaging beamlines (I13, I14). In the last five years these SR-based beamlines have complemented Diamond's centre for electron bio-imaging, eBIC, which provides an exceptional user programme in cryo-electron microscopy and tomography.

The proposed beamline aims to extend these capabilities by providing the means to image thick/large volume biological samples at 20 nm resolution for sub mm specimens while allowing specimen sizes up to a few cm at somewhat lower resolution. This forms part of a phased approach, where first we propose to provide 3D cryoImaging capabilities for the user community through the I13L flagship project via an in vacuum cryo-endstation for imaging of biological samples. Whilst this current proposal forms the second phase through delivery of a dedicated cryo-biological imaging beamline that will realise the ambition of providing an integrated cellular and structural biology imaging approach. The flagship beamline is proposed to be delivered in the second tranche of new beamlines in the Diamond-II programme. The new beamline will provide a dedicated end-station, with an efficient detector and sample transfer system providing high throughput capability. The beamline will build on knowledge gained from I13L and have cryogenic capability to preserve the samples as close as possible to the native state and reduce radiation damage for nano-imaging. Data collection at room temperature will also be available. Examples of the scientific use of the beamline include study of biological tissue at sub-cellular level, comparison of organoids with natural tissue, imaging of neurons including connectomics, comparison of healthy and diseased tissue for the above and bioimaging of plant materials. The beamline will be optimised for phase contrast imaging using a variety of techniques such as near and far field ptychography. The beamline will exploit the increased coherent flux of Diamond-II in an energy range from 6 keV to 30keV. The main requirements are an undulator to provide bright radiation, beamline optics to provide a coherent and/or microfocus beam, a detector with an angular coverage and resolution matched to the properties of the sample and stable sample environments at cryo and room temperature. The proposal is very well aligned to the Diamond-II science case, in particular the Health and Well Being section.

2. Scientific Case

There is increasing demand to image biological tissue at high resolution to obtain information about the state of cellular and extracellular material. Ideally, the specimen should be examined in as natural as state as possible. Even bacteria can form colonies where the state of individual bacteria depend on their neighbours. For tissue samples, the cells grow within an extra-cellular matrix and complex intercellular signalling occurs. One cannot assume that an isolated cell will have the same state as a cell within the tissue and studies of large samples are therefore necessary for examining individual cells and the interactions between them. The ability to study thick samples is a key advantage of x-ray imaging compared with imaging by electrons (which generally can provide superior resolution). Du and Jacobsen [1] provide a comparison between the two methods for frozen hydrated samples. Radiation damage (resolution loss, specimen warping and swelling) can limit the possibilities for getting high resolution information from x-ray imaging and this can be mitigated by collecting data at cryogenic temperatures. Techniques such as freeze substitution and heavy metal staining can give reliable data and increase the contrast within the specimen compared with studying frozen hydrated specimens which are nearer the natural state. In many cases, the information can be correlated with other techniques such as various forms of fluorescence imaging. A key application is examining differences between normal and diseased tissue.

X-ray imaging of neural tissue is a specific example where the proposed beamline should make an impact by imaging neurons, glial cells and other components. Previous work includes imaging of mouse brain [2] and Drosophila [3]. Both were carried out at cryotemperature, with the sucrose cryoprotectant provided the contrast in the former case while the latter study used metal staining. A recent review [4] provides a comparison of the capabilities of electron and x-ray imaging methods for such studies. A specific feature of nerve tissue is the large distances covered by individual neurons. Due to the high penetration depth of multi keV x-rays, an x-ray imaging beamline therefore offers the capability of tracing these neurons over the whole organism and examining connections between them. The beamline would be ideally placed to play a role in connectomics, the mapping of all neural connections within an organism. Meinertzhagen reviews the strengths and limitations of connectomics [5] and further details of the requirements are summarised in the I13L proposal.

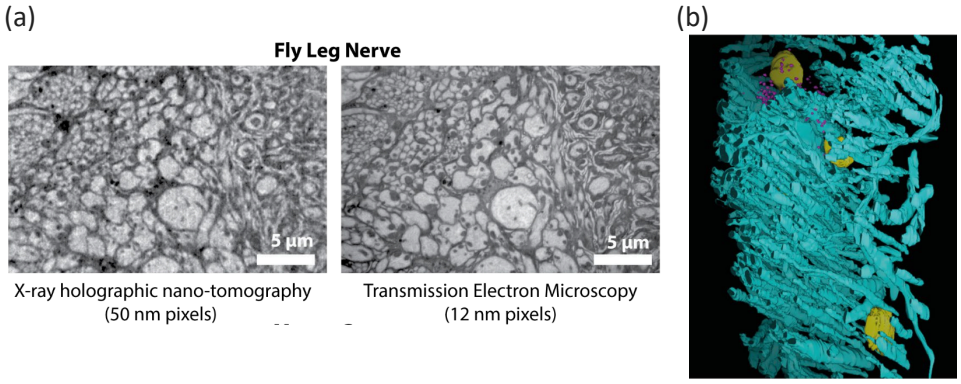

Figure 1 (a) Comparison between x-ray and electron imaging. Image taken from Pacureanu et al [3] (b) 3D Colour rendering of mouse brain tissue. Image taken from Shahmoradian et al [2]

As well as the requirement to study natural tissue samples, there is increasing interest in studying organoids. Organoid culture refers to growing cells in 3D to generate cellular units that resemble an organ in both structure and function. These organoids are then used for understanding normal development, modelling disease, testing therapies against disease and potentially for organ replacement [6,7]. Genome editing can be used to introduce specific changes (e.g. disease causing mutations) and the effects examined more easily in organoids than in whole organs. Imaging has a role to look at specific biological processes within and around the cells constituting an organoid. Organoids do not however mimic whole organs exactly both in terms of structure and function. Hence, imaging has an important role to play in characterising similarities and differences between organoids and tissue taken from whole organs.

The imaging of lignin in plants [8] is an example of how such a beamline could contribute to the area of biotechnology. Lignin represents a bottleneck to biomass degradation as it provides cell wall resistance to the release of sugars for fermentation or further processing. Using 3D-imaging to understand plant physiology can aid in assessment of strategies such as, modification of the lignin synthesis pathway to improve this aspect, while retaining the cellular rigidity necessary for plant growth and drought resistance. The study showed that the a selected mutant did not simply have lower values for the implosion parameter (dependent on the inter vessel wall thickness and vessel volume) but a narrower distribution for this parameter compared with wild type cells. The ability to monitor parameters such as in this case the implosion parameter provides information relevant to optimising the characteristics of the plant for biomass production.

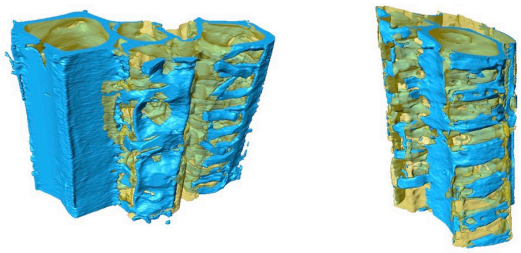

Figure 2 The morphological dissimilarities between wild type cells (left) and C4H mutant cells (right). Reproduced from Polo et al [8].

The beamline will interface to studies using microCT by examining selected parts or the whole specimen at higher resolution. Examples of the application of microCT include imaging of arteries and tendon within the tissue and applications to developmental biology [10]. For the above reasons, the need to examine biological material at different length scales (both object dimension and resolution) is a key driver for x-ray imaging. The proposal sits well within the scientific case for Diamond-II which includes "Coordinated access to a new cryo-biological imaging beamline, B24 and eBIC will provide a platform for imaging of cells and tissue in their near-native state across a range of length and resolution scales."

3. Benefit to the Diamond research community

The proposal complements existing facilities at Diamond for bioimaging by providing facilities for examining thicker biological specimens in 3D at both cryo and room temperatures. Such facilities are not presently available at Diamond. Some of the required developments, including the provision of an in vacuum cryo sample environment, will take place on I13 (see I13L proposal). It is envisaged that the present user community of existing bioimaging facilities will benefit significantly from these facilities. The ability to examine healthy and diseased tissue (e.g. from cancer biopsy, post mortem brain tissue) is expected to be of interest to a wider medical community and pharmaceutical firms with possible interest from biotechnology companies. The beamline will link in to the rest of the bioimaging facilities including cryo-electron microscopy, soft x-ray imaging, IR and CD beamlines and laser based facilities such as super resolution microscopy.

4. Outline Specification

In principle, the resolution obtained from coherent imaging is independent of the specimen size because the complex amplitudes from individual voxels are summed before squaring to get the intensity. At present, resolution is limited by focusing optics, mechanical stability and precision of stage movements, sample warping and performance of reconstruction algorithms [3]. Significant effort is going in to addressing these issues. Techniques available on the beamline will include near field holography, near and far field ptychography, structured illumination and fluorescence scanning. In some cases, the contrast within the specimen will need to be enhanced by specimen treatment such as freeze substitution or metal staining. The instrument will have the ability to select regions of interest for subsequent higher resolution examination. The instrument will allow full rotation of the specimen by, for example, milling specimens in to cylinders using FIB [8] In this case, the sample was resin embedded but the data was collected at cryotemperature. The possibility to examine samples milled at cryotemperature will also be included if this technique becomes available.

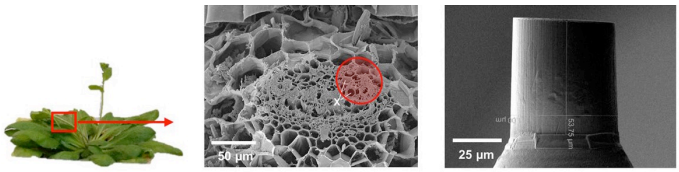

Figure 3 Sample preparation by GaFIB. Figure reproduced from Polo et al [8]

The instrument will require the maximum transverse coherent flux between 6 keV and 30 keV and will therefore exploit both the high brightness and increased flux at high energy on Diamond-II.

The beamline will require an undulator source to enable the production of both microfocus and coherent beams of sufficient intensity. It will need to select the coherent portion of the beam and produce focal spots down to 20 nm or less in size. A key issue for the design of the new instrument will be the necessity or otherwise of requiring a long beamline. I13 and I14 at Diamond or ID16A at the ESRF are approximately 200m long. Another possible model for the beamline is the cSAXS beamline at the SLS, which is only 40 m long. The decision will depend on factors such as the available focusing optics, range of sample sizes and detector characteristics. For some near field techniques, the smallest focal spot possible would be desirable. Options include KB mirrors, multilayer Laue lenses, refractive optics and waveguides. Scanning time scale linearly with volume and inversely with the fourth power of the resolution. Scanning times can be reduced both by exploiting the increased coherent flux of Diamond-II but also by a variety of other measures such as wider bandpass optics and more efficient focusing optics (see for example [9]).

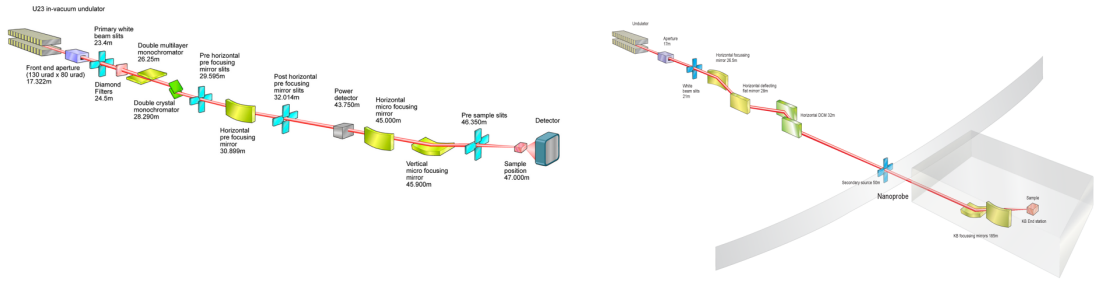

Figure 4 Schematic beamline layouts for a standard beamline (left, VMXi) and a longer beamline (right, I14)

5. State of the art benchmark

Within Europe, the cSAXS instrument at the Swiss Light Source operates in the energy range 4.4 – 17.9 keV and is equipped with two instruments for tomography, flOMNI [11] for room temperature studies and OMNY [12] for cryogenic temperature. Both use differential laser interferometry for an accurate positioning of the sample with respect to the beam.

Beamline ID16A at the ESRF operates in the range 17.0 - 33.6 keV with minimum beam sizes (H x V) of 30 x 30 nm^2 and maximum (H x V) : 400 x 400 μm^2. A 13 x 13 nm^2 focal spot has also been produced. The beamline is also equipped with accurate and stable specimen positioning and has cryogenic capability.

Both instruments are highly productive. The lower energy of the cSAXS beamline is suitable for minimising the exposure time as the coherent scattering varies inversely with the square of the energy. However, the attenuation length for soft biological material varies approximately as the energy cubed and is approximately 0.4 mm at 6 kev, 11mm at 18 keV and 30 mm at 30 keV. The proposed instrument on Diamond-II will therefore be positioned between the facilities at the ESRF and SLS. Both of these beamlines plan upgrades to

exploit gains from higher brightness sources along with other beamlines in Europe such as those at Petra IV and Max IV. Some details of the comparative performance of these facilities are given in the I13L document.

6. Community engagement

The existing bio-imaging community using facilities on the soft x-ray microscopy beamlines B24 and I08 at Diamond will benefit by extending the observations to biological cells grown within larger tissue samples. In addition, it is envisaged that the facilities will be of interest to a wider range of biomedical researchers including those investigating healthy and diseased tissue, organoids and connectomic studies. A workshop will be arranged to investigate the scientific requirements in these and related areas. The workshop could address the role of such an instrument in connectomic workflows, the need or otherwise for contrast enhancing techniques such as freeze substitution and the interface to higher resolution lower throughput techniques such as electron tomography and FIB SEM. A user working group would then be set up to assist in defining the requirements.

7. References

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