Unstained Targeting: X-ray Phase-Contrast micro-CT as a bridging imaging modality for intravital CLEM (UTXμCT)

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ABSTRACT

An important trend in life sciences is to integrate imaging modalities, on the same sample, that inform on function brought by fluorescence microscopy with sub-cellular ultrastructure by electron microscopy. For multicellular specimens, X-ray imaging provides a solution for a current gap in multi-scale biological imaging: the precise targeting of key features within soft tissues. While absorption contrast relies on heavy-metal staining of the specimen, phase-contrast instead offers the possibility to visualise and differentiate soft tissues under close-to-native preparation conditions. We have achieved synchrotron-based X-ray computed tomography images of unstained samples, and experimentally demonstrated sub-micron resolution phase-contrast imaging with a laboratory-scale system, paving the way for the development of a system prototype.

Keywords: Intravital CLEM, X-ray phase-contrast, X-ray micro CT

1. INTRODUCTION

The recent technological breakthrough in cryoelectron tomography opens to imaging, at close to atomic resolution, of macromolecular complexes in their native cellular context. This technique is seminal for a new field in Life Sciences referred to as cellular structural biology which is starting to shed light on how fine conformational states of protein complexes are linked to biological functions. In parallel, molecular cell biology is evolving towards integrated systems, where fundamental cellular functions are studied at multiple dimensions of space and time in model organisms. Whilst cellular structural biology is now routinely performed on simplified single cell models, it is still extremely challenging on organisms, mostly due to technical hurdles linked to the huge scale range that one has to cover for switching from millimetre-scale organisms or tissues to the nanometre-range objects imaged by electron tomography. For doing so, objects of interest have to be selected and targeted in three dimensions, then physically extracted for high resolution imaging.

The capability of targeting by exploiting phase-contrast effects, thereby removing the need for heavy metal staining, is a breakthrough for the field of X-ray imaging because it opens the possibility to study multicellular organisms preserved in close-to-native conditions, that is in vitrified specimens. We envisaged the removal of the need for heavy sample preparation to be a crucial advancement with great potential also for other areas such as materials science and biomedicine.

This project produced the experimental proof-of-concept results for sub-micron phase-contrast X-ray imaging within a laboratory compact setting. This result underpins the development of a solution to a current practical problem in multi-scale imaging workflows: the precise targeting, within a relatively large volume, of micron-scale structures. Knowing the location of these structures of interest with high accuracy enables targeting for the subsequent multi-scale imaging steps, spanning across a huge range of scales, without losing the contextual information. This is the stepping stone for developing a full prototype and demonstrate its operation in a research environment by seamless integration of this 3D non-destructive imaging technique within the entire experimental research workflow.

2. STATE OF THE ART

X-ray phase-contrast imaging offers a unique opportunity to visualise soft tissue with high resolution, especially when the conventional absorption contrast does not provide a good enough signal to differentiate the structures of interest[1, 2]. We propose the application of

this imaging technique to solve a problem that is currently holding back the multi-scale imaging of biological soft tissue: high-resolution three-dimensional targeting. This procedure requires the inspection of cubic-millimetre samples micron-scale resolution. Moreover, the imaging technique must be able to differentiate between tissues with very similar attenuation properties. This can currently be achieved by using contrast agents and conventional micro-CT, however staining the sample is a step that would be much more desirable to avoid. Combining conventional micro-CT with intravital correlative light and electron microscopy (CLEM) [3] with micro CT uniquely reveals anatomical features of the tissues as seen in the resin embedded material, ready to be observed by EM. Providing enhanced precision (few microns), the targeting - by EM - of the region of interest was dramatically accelerated, enabling multiple observations. Because it is based on absorption contrast, this method requires chemically fixing and heavy metal post-staining the samples, preparation methods that come with artefacts and that limit the field of application. High-resolution phase-contrast X-ray tomography is currently feasible by means of synchrotron radiation [4], nonetheless, the need for such a high specialised facility severely constrains its portability and accessibility. Our aim is to be able to offer unstained targeting within a compact scanner, which can be easily deployed in laboratories across the entire research community, maximising its outreach and impact of this technology.

3. BREAKTHROUGH CHARCTER OF THE PROJECT

Non-destructive imaging at isotropic resolutions better than one-micron is the first step towards the development of unstained targeting in millimetre-scale biological samples. As such it will constitute a revolutionary tool for multi-scale imaging in the field of cellular structural biology [5-8] in the Life Science, and with a vast discovery potential for biomedical and materials science investigations. X-ray phase-contrast imaging offers a unique opportunity to visualise soft tissue with high resolution and enable the navigation of the anatomy. Targeting, the ability to precisely locate a given structure within a large volume in three dimensions, is key to the development of multi-scale imaging workflows that can reach close to atomic resolution without loss of the contextual information which is essential for the study of organisms and for the extension of cellular structural biology from simplified single-cell models to multi-cellular organisms. Oher research areas where we envisage that multiscale navigation without loss of contextual information will play a crucial are Additive and Composite manufacturing and batteries. Additive and Composite manufacturing are critical to future lighter weight design and to enable more sustainable components for a more agile and smarter manufacturing industry. The ability to measure size and location of defects at multiple scales, and by maintaining contextual information, will be key for assessing components safety and determine the life of parts manufactured in these innovative ways. Battery manufacturing is in need for the development of designs that mitigate against failure, as highlighted by high profile failures in automotive, aerospace and consumer electronics. X-ray phase-contrast imaging will underpin these studies by providing the highest possible spatial resolution as well as by enabling sensitivity for materials buried deep inside cells and the study of next generation materials.

4. PROJECT RESULTS

Core results of UTXμCT are summarised in Figure 1 and Figure 2.

Figure 1 reports qualitative results measured by means of a custom-built phantom. Panels (a) and (b) show the X-ray images of the phantom which is composed by polydisperse borosilicate microspheres on a membrane, as seen in absorption contrast and phase contrast, respectively. The sphere diameters range from few to twenty microns, with most of the distribution between 5-15 μm. The dramatic increase in visibility brought by the phase contrast is apparent, especially of the smallest details is apparent. Red arrows point at places where the detection of such small features would be very difficult if not impossible by using the image in panel (a) whilst it is clear by using the image in panel (b). The two images were obtained by using the same exposure time and the same number of X-ray photons, therefore offering a fair base for comparison of image quality.

Figure 2 reports quantitative results, measured by means of a calibration standard, fabricated ad hoc for this purpose. Panels (a) and (b) report the real and reciprocal space characterisation of the system spatial resolution. The Line Spread Function (LSF) (real space case, panel (a)) was measured through differentiation of the edge response function. Data were fitted with a Gaussian distribution of Full Width Half Maximum (FWHM) of 719 nm. The Modulation Transfer Function (MTF) (reciprocal space case, panel (b)) was directly sampled by measuring the modulation of bar patterns of progressively decreasing pitch. Both these measurements indicate that the system achieved sub-micron resolution. Together with the image in Figure 1, panel (b), they demonstrate that our compact laboratory system is capable of producing X-ray phase contrast images at sub-micron resolution.

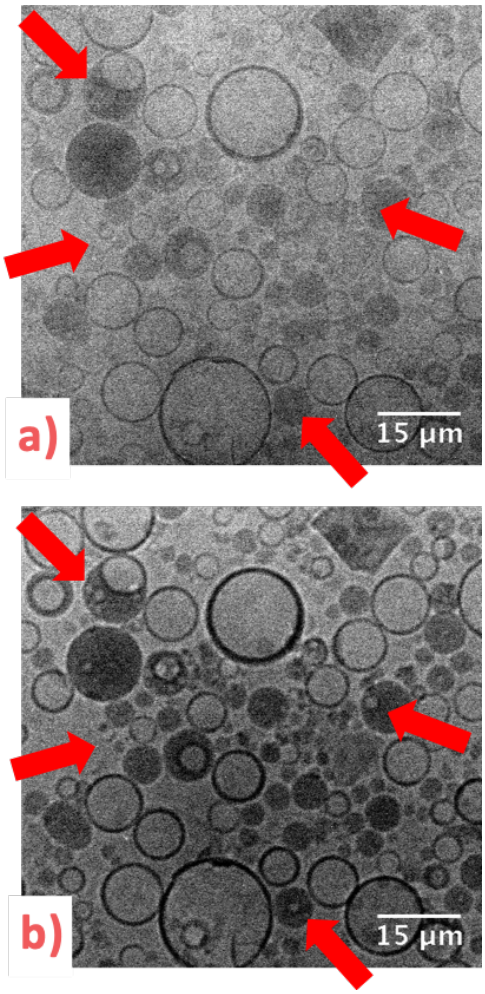

Fig. 1. Qualitative experimental results demonstrating X-ray phase contrast imaging with our compact laboratory system. Panel (a) and (b) compare a custom-built phantom (polydisperse borosilicate microspheres on film) as seen in absorption contrast (a) and phase contrast (b). The dramatic increase in visibility, especially of the smaller details is apparent. Red arrows are pointing at features undetectable with absorption contrast which are instead well visible with phase contrast.

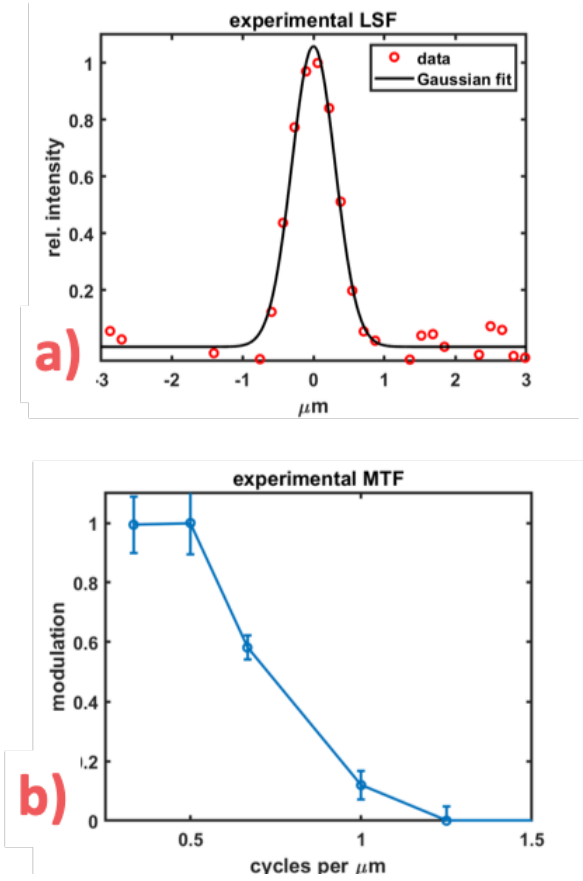

Fig. 2. Quantitative experimental results characterising the spatial resolution properties of the imaging system. Panels (a) and (b) report real and reciprocal space characterisation of the spatial resolution properties of the imaging system, respectively. Panel (a) show the Line Spread Function (LSF) and its Gaussian Fit with 719 nm Full With Half Maximum. Panel (b) reports the Modulation Transfer Function (MTF) measurement in panel (d). They demonstrate sub-micron resolution and, together with Fig. 1b, the sub-micron X-ray phase-contrast imaging capability of our laboratory scanner.

5. FUTURE PROJECT VISION

5.1. Technology Scaling

The roadmap we have envisaged to scale our proof-of-principle experiment up to a prototype capable of demonstrating operations in a research environment encompasses a bottom-up approach where we engineer all system components and optimise them towards the development of the prototype. The current proof-of-principle was obtained with mostly already-available instrumentation and large room for improvement exists on each of the system's components. Scaling-up is envisaged as broadly divided in two steps: first one dedicated to the validation of our technology in progressively more complex environments, and the second one dedicated to the demonstration of its viability by operating a first prototype directly in its field of application. The first step will target validation with the existing yet sub-optimal instrumentation that will inform the final design of the bespoke hardware that will be assembled in the first prototype, in such a way we can run the validation steps and the prototype design in parallel, for a faster turnaround. Once the bespoke hardware is available, the prototype will be built and tested in order to demonstrate our approach in the field. Early adopters will conduct a series of demonstration experiments that will be unlocked by the availability of our new technology and these will be the first points of dissemination of our technology in the research community.

5.2. Project Synergies and Outreach

The Consortium will be extended to involve SMEs (3), Academic Institutions (2) and Research Laboratories (3). The SMEs are world-leaders in their field and can offer a degree of customisation hardly available elsewhere. We will work in tandem with synchrotron facilities and manufactures during the validation phase to inform the design and optimise performance as much as possible. The validation phase will entail experiments at the partner Research Laboratories as well as data analysis and modelling performed at the coordinating Academic institution (UCL, AXIm group [9]). Once the first prototype will be assembled, it will be hosted in an environment dedicated to new technology incubation at a partner Research Laboratory and directly tested within its target workflow. In this way we will be able to demonstrate the validity of our approach directly in-operation. Synergies exist with our other ATTRACT projects ML-CYCLO-CT and DM-MX; discussions will be held with consortia on detectors (X-COL, where we are partners, and FASTPIX / ESSENCE) and CT reconstruction (QuIT).
Public dissemination of the results will be integral to the project and the main route for dissemination will be scientific publication and events such as conferences and workshops. We will reach out to the diverse user communities by showcasing selected case studies lead by opinion leaders in their fields, who will demonstrate the potential unlocked by our technology. All our partners have extended networks spanning across diverse fields of application, with links with world-leader who will be able to act as champions through their involvement in the early experiments on selected case studies. Public engagement (PE) will be key. Our entire team received training from the PE unit created when UCL was selected as a centrally funded "Beacon of PE", and we regularly engage with non-specialist audiences; this will be scaled up to guarantee appropriate dissemination of our ATTRACT results.

5.3. Technology application and demonstration cases

Developing an unstained targeting workflow for multi-scale imaging is foundational for the Life Sciences, and has great potential to generate impact across diverse fields. Examples are materials science and energy research where solid-liquid interfaces, albeit representing an important range of physical, chemical and biological processes, are often difficult to understand due to the lack of high-resolution methods that are compatible with both solid and liquid components. Enabling new research on the soft and liquid phases will greatly benefit the design and development of new materials, tools and devices that will used by our community in daily life. We will target at least three demonstrations in collaboration with end users:

- *Health, through ultra-low dose dynamic lung imaging.* Excellence exist at UCL (Prof. Rachel Chambers / EMINENT consortium) plus we have links with EU leaders (Sam Bayat, CHU).
- *Secure, clean and efficient energy,* through studies in batteries with our collaborator Prof. Paul Shearing who runs the world-leading Electrochemical Innovation Lab.
- *Smart transport/resource efficiency* by expanding our activity in additive manufacturing and composite materials.

The EU Research Infrastructure will benefit through deployment of the technology at synchrotron facilities and in flagship research laboratories such as the ESRF and EMBL. We have established collaborations with these and other research institutions, thus enabling installation and thorough testing of the technology and facilitating ensuing its deployment in other facilities.

5.4. Technology commercialization

The steps envisaged for commercialisation are the dissemination of the new technology in the scientific

community to identify its early adopters. Researchers will be able to access the prototype for research on a free-at-point-of-access basis. This will provide visibility of the techniques across diverse areas of scientific research and already during ATTRACT Phase 2 project, shall our proposal be successful. With an established early adopter's base, the most promising commercialisation route will be identified with the support from the Technology Transfer Division at UCL (UCLB, with which we already have long-standing collaboration in place). We currently collaborate with several X-ray instrumentation manufacturers [9], which puts us in the ideal position to target the exploitation of our research, be it through agreements with a single company, or creation of a consortium. Established mechanisms exist for both, as do collaboration agreements which both de-risk and reduce the bureaucratical burden of any step in this direction.

5.5. Envisioned risks

The main technical risk is the difficulty in the full integration of the technology within an extremely complex and delicate workflow. Mitigation against this will be achieved by the design of the programme itself, which will be structured in levels of gradually increasing complexity. This guarantees that even if the most ambitious goals cannot be fully achieved, there will be still significant benefit brought by the less ambitious ones. The key non-technical risk is the current pandemic which limits the ability to travel and therefore to perform experiments at synchrotrons and in the collaborators' laboratories. This will be mitigated by designing work-packages that can be carried out by local teams through remote communication with the co-investigators.

5.6. Liaison with Student Teams and Socio-Economic Study

All our Departments run popular MSc programmes. Shall our Phase 2 be successful, we will offer about 10 projects per year, both to our cohorts and to our partner universities [10]. We are setting up a Doctoral Training Programme [11], and we will leverage on this to promote activities across MSc and PhD students to build and reinforce their links and networking. Students will be involved in the design of teaching and training material on our technology which will facilitate public dissemination activities, ranging from scientific, to demonstrations to public engagement. We also have a track record in hosting and training EU students through the Erasmus+ traineeship programme (2 per year). We will be constantly available for interviews and technology impact references and we will also actively look for opportunities to include and showcase our technology in such activities.

6. ACKNOWLEDGEMENT

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