Structure Probing by Holographic Imaging at Nanometer scale with X-ray lasers (SPHINX)

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ABSTRACT

The SPHINX project aims building femtosecond X-ray holographic cameras for imaging microscopic samples and their internal parts with nanometer resolution. The proposal is based on an implementation of phase-contrast holography that overcomes limitations encountered in the absorption-contrast systems. A combination of polycapillary lenses, large X-Ray CCD arrays and XFEL sources will allow splitting the beam, focusing, magnification and phase-contrast imaging in the keV range. The advantage of diffusing optics comes from its divergence, driven by the single fiber. This allows overlapping on the detector area both object and reference beams, condition unreachable with a single Fresnel lens or a mirror.

Keywords: X-ray holography; nanometer resolution; capillary optics; in-vivo reconstruction; nano-robotics, phase-contrast imaging

1. INTRODUCTION

X-ray holography (XRH) has been proposed soon after the conventional holography introduction, being based on the same physical principle [1, 2].

- The advantage presented was straightforward: reconstructing not only the external but also the internal 3D structure of the samples, reaching, at the same time, a very high resolution allowed by the reduced diffraction limit. However, the first practical applications [3] were not immediately developed, due to the technical difficulties described in the next chapter.
- Hence, the objective of the current research is overcoming most current limitations and, at the same time, adding another important feature consisting in very short exposures so generating ultrafast X-ray holograms of microscopic samples and of their internal parts with nanometer resolution, by phase-contrast imaging. This would represent an improvement of the resolving power by one order of magnitude, so reaching the range accessed by Atomic Force Microscopy (AFM) and X-ray diffraction, without the altering treatments like polymer embedding, staining or freezing, required by the lasts. The significant parameters derive from a novel layout, using single-pulse XFEL sources, capillary focusing optics and large arrays of X-ray CCDs with small pixel size. Furthermore, the “flash” exposure (fs) grants structural stability, allowing reconstructing in-vivo cell elements, viruses and nanobots in their real, functional state, also during fast molecular processes, yet unexplored by imaging techniques.
- The current work will describe the proposed technique and the development of an experimental prototype of the apparatus.

2. STATE OF THE ART

To date, there are few XRH implementations, and we'll cite some of the most relevant:

In the “water window” (absorption contrast, O, C and N k-edges), a solution for the pinhole-limited flux consisted in replacing the pinhole with autocorrelated Hadamard arrays. The experiment was performed on the DESY-FEL (FLASH) at ~300 eV and the reconstruction done on a biologic cell reached 75 nm resolution, while for solid-state samples 43-50 nm was reported [4]. The method was extremely innovative, but is energy-limited by the apertures required, while the detector pitch adds further constraints.

The phase contrast holography, characterized by higher energy and smaller angles, had to face problems related to the long baselines required, over which the flux generated by small apertures is significantly reduced. A remarkable effort to exploit at best a synchrotron source was done in 2004-2005 at Spring-8 BL29XU beamline [5,6], where a beam traveling 987 m (granting an angle below the coherence limit), was used for illuminating a flat solid state sample, a 4 um thick

lithographic plate and produced holograms which presented phase contrast features. The resolutions were in the ~10^2 nm range, limited by the beam divergence in a Gabor-like configuration, by the Zone Plate resolving power and by the detector resolution.
A more recent experiment was carried out in 2012 at ESRF [7], where strong focusing was combined with ptychographic scanning for phase retrieval, allowing holographic reconstruction with 63 nm precision in the transverse plane. The obtained resolution was lower than the one in the absorption-contrast experiment due to the conventional optics and the synchrotron beam used, requiring a large number of scans and solid samples featuring structural stability and large variations in refractive index. The experiment presents a relevant impact on the current project, as it demonstrates the ptychographic wavefront reconstruction in holography.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

3.1. Premises

When passing from visible/UV light to shorter wavelengths, a series of experimental restrictions are to be considered, out of which the main ones are briefly recalled.

- Some optical elements do not exist (beam splitters) or are replaced with limited performance ones (low gain DO, mirrors working at very low angles, etc.); moreover, their efficiency drops with the energy.
- the most common X-ray sources exhibit a limited degree of spatial coherence which varies inversely with the flux and the energy, while good temporal coherence is achieved only with lasers (FELs).
- The investigated sample size is limited by the illumination.
- The typical elements for obtaining magnification by exposing the sample to a diverging beam, are the pinholes (Fresnel configuration). The technological limits (μm size) and the flux losses bound their use to the VUV/very soft X-ray range. For imaging at higher energies, the coherent sources are created by “virtual pinholes”, i.e. micron-size focus, far from the sample. This reduces both instantaneous flux and divergence, while the point-like focusing prevents the creation of a secondary, reference beam, overlapping the object one on the detector without supplementary mirrors, which further drop the illumination.

Therefore, long exposures are required, during which the samples might be altered, while vibrations induce additional smearing, making the nm scale unreachable.
For energies below 500 eV, the illumination problem was addressed in [4]. In this range, the diffraction is absorption-based and, for feature sizes of tens of nm, the corresponding angles are large, in the 10^{-2} rad range, so the exposure distance must be short. This conflicts with the low granularity of available detectors as compared to the photographic plates. Furthermore, structures behind a strong absorber will receive a reduced flux and might remain invisible.

3.2. Requirements for efficient phase-contrast imaging

From the above considerations, we can derive the working conditions needed to overcome the current limitations:

- Higher energy, for deeper probing and lower diffraction limit (in the keV range)
- Special beam focusing on the sample, allowing, at the same time, increased illumination and beam splitting, with controlled output divergence.
- Smaller diffraction angles, allowing far detector placement and therefore, lower "grain".

All the above can be addressed by a novel configuration, where classical optics will be replaced by polycapillary one and appropriate beam and detector configuration is employed. The capillary optics allows illumination gain, magnification, beam splitting and final overlap (interference) on the detector.

4. PROJECT RESULTS

The last requirement is just apparently in contradiction with the first; at higher energies the absorption on micron-thick samples becomes negligible so most of the photons will pass through. Each internal microstructure will have a specific refractive index; in the case of cells, the organelle, immersed in cellular fluid, behave like small “lenses”, hence the optical path and, consequently, the phase shift will be different when an X-ray crosses them. The phase variation in the small angle approximation is derived as

$$\Delta\phi(x,y) = -(2\pi/\lambda) * \int(1-\mathrm{Re}(n))dz, \qquad (1)$$

where n in the complex refractive index. Consequently, the corresponding diffraction angles are ~3 orders of magnitude smaller than absorption ones, in the range of μrad, for adjacent regions with n differing by few percent and photon energy of ~3 keV. The treatment was described in [8] while the complexity of the waveforms employed precludes analytic calculations. Taking into account the pixel geometry and the required number, the exposure baselines to be used are long, typically tens of meters.

4.1. Implementation

- Large detectors with small pitch (<30 μm), placed at large distances. The indicated value does not represent a technological limitation, pixel sizes of few μm are easily produced, but constitutes a good compromise between the fine granularity and two phenomena which contribute to image smearing, namely the charge sharing between pixels and the escape of deexcitation silicon photon (1.74 keV), which might end up in in the adjacent pixels
- Coherent, short time, high intensity X-ray pulses, to allow in-vivo imaging (XFELs). The necessity of a beam with high degree of spatial and temporal coherence is straightforward. However, at the aimed scale, the sample and setup stability during the exposure play a role of comparable significance and often were the main limitations in the past experiments. Despite the efforts in realizing ultra-stable mechanics, the structure of (unfrozen) biologic samples cannot be preserved at nm level during seconds or minutes of data taking. Apart from the internal dynamics, the beam itself constitutes a disturbing factor. By taking advantage of the currently available XFEL beams, with 10^{12}-10^{13} photons/bunch, the proposed apparatus will acquire the full image in a single shot with bunch duration (fs), before thermal effects develop inside the sample.

4.2 The Prototype

As a full setup realization involves a large investment in detectors and overcoming several technical difficulties related to the required ultra-high mechanical precision and stability, a lower resolution apparatus will be first tested with synchrotron light on a coherent optics beamline. This will allow verifying the working hypothesis and identifying the major points to be addressed in the final apparatus design. As mentioned above, a large-area X-Ray CCD array with the relative readout system, which should provide a low-noise image is one of the key elements. Therefore, the readout will be done in spectroscopic slow-scan mode, while the 16 X-ray CCD array will be operated under cryogenic regime.

The setup built for this purpose has an active area of 116 cm2 and a total of 22 MPixel with 22.5 μm pitch. This represents one of the largest X-ray spectroscopic cameras built to date and features a small pixel size (for X-ray), compatible with the grain required by holography. Most X-ray detectors exhibit a larger pixel, in the order of 100 um; the current configuration was chosen as best compromise between space resolution and setup scale. The silicon depletion is about 25 μm, granting, in ROI, a quantum efficiency near to unity. The layout of the detector is shown in Fig.1.

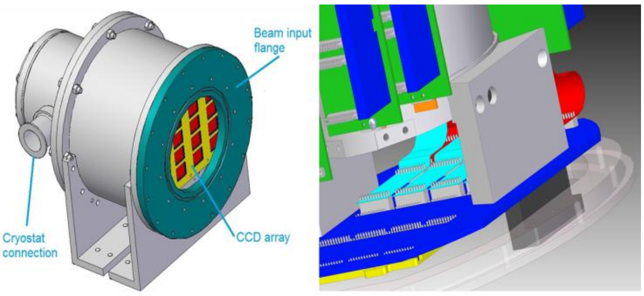

Fig. 1. Left: general layout of the 16 X-ray CCD detector. Right: detail of cryogenics and cabling.

Concerning the optics, the lenses must be done on custom design; here, the group can take advantage of the presence inside the INFN-LNF Lab of a full polycapillary lens factory. The moving stages contain six translations (lens and samples) and two rotations (lens). The translation actuators have a precision in the order of tens of nm and the connection between them is done with stable mechanics, both from thermal and vibrational point-of-view. A first dedicated optical element has been built (Fig. 2), using capillaries suitable for the design energy (2-5 keV). Other lenses will be produced after the first measurements with the current setup.

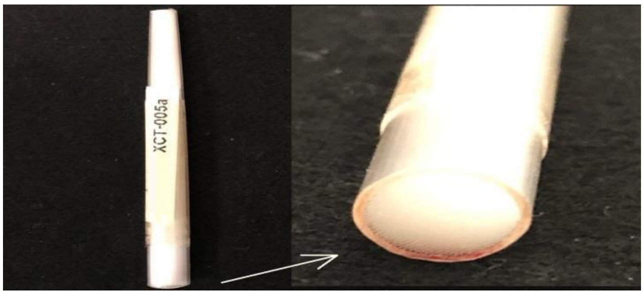

Fig. 2. A borosilicate polycapillary lens for soft X-rays.

5. FUTURE PROJECT VISION

The research is in an incipient phase, at the proof-of-concept level. To obtain the realistic response of a complex optical system, a full prototype has been built, while detailed simulation work is in progress. Unfortunately, a two-months measurement campaign, planned on a coherent optics beamline had to be postponed until international travel will be unlocked.

5.1. Technology Scaling

In case of successful validation, a large setup with more advanced features will be proposed. As a short list, an order-of-magnitude larger CCD detector, nanometer-precision movements, online calibration and long vacuum baselines are to be included. This will constitute a standard configuration, allowing industrial and medical XRH applications on any XFEL facility.

The key parameters are defined by the focusing elements. The advantage of diffusing optics is their divergence, driven mostly by the single fiber. This allows sending on the same detector area both object and reference beams, condition unreachable with a Fresnel lens or a crystal mirror. In practice, a set of capillaries will shine through the sample, placed off-axis, near the focus (object beam), while the others will direct their diverging rays unaltered, to the detector (reference beam).

After coherent propagation inside the capillaries, based on total external reflection [9-12], the photon paths will be different, transforming the initial plane wavefront in a very complex one. This last, scanned by ptychographic technique [7, 14], will offer a better reconstruction reference than a plane wave, referenced to the lab system. The holographic reconstruction up to 12 KeV with single, tapered and bent glass capillary waveguides was treated in detail in [10] as one of the most used layouts, while nm-resolution tomography by ptychographic phase-retrieval was demonstrated in [13].

The current project proposes the extension of this old idea, never implemented due to the low illumination carried out by a single waveguide, to a focusing optics containing many capillary fibers (tens to hundreds), where intensity gain, splitting and divergence control are all granted by a single optical element, a micro-polycapillary half-lens. Under special requirements, when large samples are to be examined or when the beam size is small, a second monocapillary beam-expander can be added. The magnification is that of a Fresnel system with off-axis reference and is given by:

$$M = (d1 + d2)/d1, \qquad (2)$$

where d1 is the distance between the object and the divergent source and d2 is the distance from the object to the detector. The "effective exposure distance", d_{eff}, will be optimized to match the detector size and the first absorption minimum, according to:

$$d_{eff} = (d1 * d2)/(d1 + d2) \cong N*P*\delta r/1.22*\lambda, \qquad (3)$$

with δr the minimal probe element, N the linear number of pixels, P the detector pitch and λ the wavelength. The formula refers to the whole area invested by the diffracted wavefront after having crossed the sample, so the expression is "Airy-like", independent of the refractive phenomena. The hologram interference pattern has much lower characteristic angles and is created by the refractive-diffraction associated to the phase-shifts between sample components.

The general layout of the setup and of the optical configuration, in an enhanced version of the Fresnel one, is presented below.

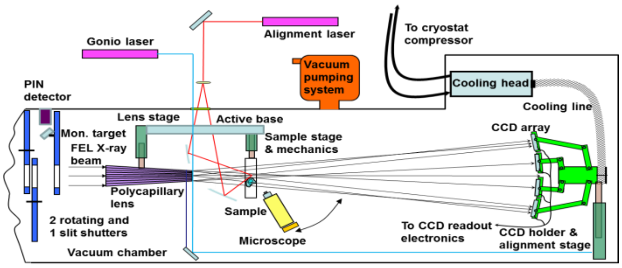

Fig. 3. Schematic layout of the X-ray holography apparatus

5.2. Project Synergies and Outreach

The investigated technology is conceived mainly for the recently built X-FELs. As a field of growing interest, big facilities are already operational, while many are under design. Nevertheless, an adaptation for wider scale application on synchrotron beams will be investigated. Therefore, the first collaborations (some already initiated) will be established with XFEL facilities and synchrotron laboratories.
After having defined a reliable reconstruction procedure, large interest is expected from cellular biology research and industry, as well as from nanorobotic industry.

All the results will be published on open access journals as well as on the ATTRACT and partners websites.

5.3. Technology application and demonstration cases

The potential applications are wide and straightforward; visualizing in-vivo 3D structures with resolutions comparable to the AF microscopes or coherent diffraction apparatus represents a step forward in understanding the cellular mechanisms, antibody-antigen interaction and specific mutation effects. In the nanotechnology field, the holograms will allow investigating the functioning of nano-robots. Moreover, the femtosecond timing will allow pinning up fast processes, difficult to describe in a chemical way and currently inferred from diffraction on frozen samples and from IR absorption spectroscopy. Therefore, a non-exhaustive list of possible application fields includes:

- *Health, especially microbiology;*
- *Nanorobotics;*
- *Microelectronics and quantum computing;*
- *Environement studies of nanocontaminats;*

One of the first structures on which the current research can enrich the investigation possibilities is the European X-Fel, the largest and most powerful to date.

5.4. Technology commercialization

Given the international scale of the involved structures the commercialization responsibility will be on the facilities management, who directly interact with the industries applying for accessing the investigation tools.

5.5. Envisioned risks

The project involves high-tech features not completely tested in the given environment. Through them, the nanometer stability of the optical system, the fast calibration procedures and the computing power required for digital reconstruction are some examples. The optics state under beam exposure is to be determined, too, since it will affect the costs of the single analysis.

5.6. Liaison with Student Teams and Socio-Economic Study

Given the reduced opportunity to obtain experimental data during the final part of Phase 1, collaboration with MSc./PhD. students was postponed for when experimentation will be possible. Still, the home Institute management expressed a high interest in the research and contacts with University will definitely help involving students in the project continuation.

6. ACKNOWLEDGEMENT

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