The x-ray free electron laser (XFEL) marks a new epoch in the history of x-ray science with new lights of unprecedented characteristics: coherent, intense and short pulses, \(\sim 10^{11}\) photons/pulse in \(\sim 10\) fs. Single-shot diffraction imaging is born as a new paradigm in imaging science with the XFEL, and expects to open unique research areas. We introduce various single-shot coherent diffraction imaging (CDI) experiments at SACLA as well as perspective on emerging science applications and further upgrades.

**Keywords:** single-shot imaging, femtosecond x-ray laser

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1 Introduction

The ability to visualize features normally beyond the limit of the human eye has made microscopy an essential tool for investigating structures from newly introduced systems. As a result, one of the most practical issues with microscopy is in its image resolution, often bounded by the wave property of the light, the Abbe diffraction limit. One rational approach to achieve high-resolution would be then to use a short wavelength. Indeed electron microscopy, with a short wavelength from high-energy electrons, has been favorably employed to demonstrate atomic resolution 3D imaging, but limited to small specimens of less than several hundred nm. For samples of a micron or larger in size, however, destructive thin sectioning becomes indispensable.

X-rays have been attracting continued interest in this regard with wavelengths in the atomic scale. X-rays, compared to electrons, penetrate specimens deeper allowing non-invasive imaging of thick specimens. Synchrotrons expand their versatility further with the energy tunability to exploit characteristic absorption edges of atoms. This helps to gain enhanced sensitivity for specific ions, orbitals, spin-polarizations, chemical anisotropy, etc.

The focusing of x-rays is, however, much more elaborate when compared to the visible light; focusing lenses are readily available with large refraction of visible lights in general. Recent progress in x-ray focusing has achieved sub 10 nm using the K-B mirror, yet the state-of-the-art in resolution. This is in stark contrast with x-ray crystallography, which casually renders atomic scale structures insomuch as specimens are available in sizable crystals. Preparing a large crystal itself, however, often becomes a daunting task; frequently observed in membrane proteins, for instance. With technical challenges present in each different approach, x-ray imaging without using a lens or x-ray crystallography without using crystals would undoubtedly be the best approach, if possible.

2 Coherent diffraction imaging (CDI)

Coherent diffraction imaging, shown in Fig. 1, was introduced to this end. When coherent waves are diffracted by a sample, a characteristic diffraction pattern is formed, which in the far field has a one-to-one correspondence to the sample as a Fourier transform pair. The spatial dimension of the coherence length is required to be much larger than the sample to encompass the whole sample with a well-defined phase reference. It would then be tempting to directly invert the diffraction pattern to acquire a sample image. This is not feasible, however, as the measured data contain only amplitudes without phases, the ‘phase-problem’. D. Sayre proposed, in 1952, an idea to retrieve the phase of an x-ray diffraction pattern by fullfil-
Fig. 1 Coherent diffraction imaging. Schematics of coherent diffraction imaging are shown. The sample image is acquired from the measured coherent diffraction pattern via numerical iterative phase retrieval process\(^{28}\).
Coherent Diffraction Imaging and SACLA

Intense lights with ultrashort pulse width can provide a breakthrough, which has awaited the advent of x-ray free electron laser (XFEL)\(^{29}\).

3 CDI with the XFEL

SPring-8 Angstrom Compact free electron LAser (SACLA) was constructed as the second x-ray free electron laser facility in the world following the LCLS (Linac Coherent Light Source) in US\(^{30}\). SACLA produces femtosecond short (~10 fs) x-ray laser pulses containing more than 10\(^{11}\) photons per pulse. The femtosecond x-ray pulses enable us to acquire signals from samples before the radiation-induced damaged structures appear – “diffraction-before-destruction”. Intense x-rays strip off electrons from the atoms in specimens. With the loss of electrons, electric fields become unbalanced and ions experience strong repulsions between them to radically move out of original equilibrated positions rupturing specimens in a process known as Coulomb explosion. The “diffraction-before-destruction” scheme exploits ultrashort-pulsed lights to help the diffracted waves escape the sample before the destruction starts to set in. The sample gets damaged eventually, but the diffraction pattern conveys the undamaged structure.

At SACLA, several new types of experiments were introduced exploring the characteristics of the new light source. In this article, we will introduce applications by focusing our interest on single-shot diffraction imaging, which takes full advantage of the intense, short pulse, x-ray laser. Most of single-shot diffraction experiments, introduced here, unless otherwise mentioned, were carried out on the Multiple Application X-ray Imaging Chamber (MAXIC) installed at EH3 of BL3 at SACLA\(^{31}\). The MAXIC is a common platform established to carry out coherent diffraction imaging and scattering experiments with the emphasis on the femtosecond x-ray single-shot diffraction. The MAXIC was built, with high adaptability in mind, to conveniently accommodate various experiments of single-shot imaging, pump-probe imaging, and serial crystallography, etc.

In regard to coherent diffraction imaging, several essential features of the SACLA can be emphasized, distinguished from synchrotron radiation facilities. SACLA is a powerful x-ray source, and it is powerful enough to destroy samples irreversibly. This should not be misunderstood as that the total number of photons from SACLA overwhelms SPring-8. Counting for one second, the total number of photons from SPring-8 surpasses 10\(^{13}\), similar or even slightly higher than those SACLA produces. Instead SACLA delivers such a large number of photons within ~1/10\(^{14}\) second, uniquely enabling certain experiments. On the other hand, there are other experiments better suited for synchrotrons, of which specific examples will be provided later. Furthermore, SACLA is an x-ray laser facility producing spatially coherent x-rays. Less than 0.1\% of total photons are coherent at SPring-8, whilst it is near complete at SACLA. This implies that experiments contingent on coherent x-rays gain great advantages, well utilizing the unique characteristics of the XFEL. Single-shot coherent diffraction, with the reasons aforementioned, well suited to the XFEL, for instance. With the XFEL applied to single-shot imaging, almost radiation damage free images can be obtained. Also a great number of images can be obtained in a short time span, which opens a new paradigm in imaging science. Imaging of a single virus particle, which took several hours of exposure at SPring-8, was repeated at LCLS but using only a single XFEL pulse\(^{28,32}\).

4 Femtosecond x-ray single-shot imaging

4.1 Single-particle loading

Single-shot diffraction imaging contingently relies on an efficient single-particle loading scheme, as samples are destroyed by the full power exposure of micro-focus x-ray beam. Various single-particle loaders are introduced, which can be categorized largely into either a fixed or a flying target scheme as shown in Fig. 2. In the fixed target scheme of Fig. 2(a), samples are prepared on thin, x-ray transparent, solid membranes. Single-shot diffraction patterns are acquired by scanning the focused x-ray beam across the membrane. One single-shot exposure usually burns out the membrane by leaving a hole, and the next single-shot exposure is directed to a different region on the membrane controlled by motion stages. With the sample positions not controlled in relation to the x-ray beam position, the occurrence of a sample hit by the x-ray pulse is stochastic\(^{33}\). To increase the hit event (or ‘hit rate”), the sample number density is adjusted by controlling the concentration of samples dispersed in a solution. In the flying target scheme of Fig. 2(b), samples are aligned to the x-ray
focal spot via a focused stream. Using a liquid jet injector, for instance, samples are prepared dispersed in a solution, and that solution is injected into the diffraction chamber. The hit rate depends on a liquid beam diameter, focused x-ray beam size, sample concentrations, etc. An aerosol injector has also been introduced as a flying target loader to deliver the samples through a focused stream of sample flows. The aerosol injector has an advantage of removing any parasitic scattering resulting from the delivery media, but the hit rate is lower.

4.2 Applications of femtosecond single-shot diffraction

XFEL single-shot diffraction finds good applications in investigating nano-structures, biological cells, organelles and macromolecules. Intense x-rays make imaging of small specimens more feasible. Further equipped with femtosecond short pulses, XFEL single-shot diffraction has introduced serial femtosecond crystallography (SFX). The technique, as an intermediate step toward single molecule imaging, meets the significant demand from protein crystallography to analyze proteins available in small sized crystals such as in vivo protein crystals, membrane proteins, etc. The femtosecond x-ray pulses, greatly relaxing the concern for the radiation-damage, expands the application of crystallography to the room temperature for studying various redox reactions.

Dynamic reactions are prevalent in functional nano-structures, whose details influence device performance, such as electrochemical reactions in battery systems, for instance. In situ single-shot coherent diffractions make snapshot pictures of nano-scale structure. In a similar vein though more sophisticated, pump-probe single-shot diffraction well demonstrates the unique characteristics of XFELs. Ultrafast, intense x-ray pulses conveniently accommodate a few hundred femtosecond temporal and several nanometer spatial resolution pump-probe imaging when combined with femtosecond pump sources such as a femtosecond optical laser. This will open a route to study various transient phenomena under nonequilibrium conditions.

Imaging single biological specimens is of keen interest, especially with the potential to obtain native structures without radiation-induced alterations. As a great number of 2D images can be taken in a short time span, single-shot imaging can be applied to single-particle 3D imaging with individual samples of resembling structures, or statistical imaging to study nonidentical samples with common and varying features analyzed with good statistics from many images. The statistical imaging with XFEL single-shot diffraction is pertinent for cellular dynamic imaging, for instance, to track structural alterations in cells after a certain time lapse of controlled treatments.
5 Single-shot diffraction experiments at SACLA

Various single-shot imaging experiments have been performed at SACLA. Experiments were carried out on the MAXIC by fixing the photon energy at 5.5 keV. Femtosecond x-ray pulses were focused by a pair of K-B mirrors installed at the beamline (EH3 in BL3) to deliver 1 μm focused x-rays at the focal spot, 1.5 m downstream of the second K-B mirror as shown in Fig. 3. Two sets of four-way cross slits using bevel edged 700 μm thick Si slit blades were employed to filter parasitic scattering from the mirror and upstream optical components. These corner slits are a critical component in obtaining high-quality diffraction patterns with minimal background noises. The multi-port charge coupled device (MPCCD) detector records speckle patterns. Two MPCCD detectors were installed in tandem to increase the peak dynamic range effectively. Figure 4(a) displays a single-shot coherent diffraction pattern obtained from a colloidal nano-Au particle of 100 nm in diameter using the MAXIC. The sample image, in Fig. 4(b), was obtained by retrieving the phase of the diffraction pattern to display internal density distribution and sample morphology at a resolution better than 10 nm.31

Specimens studied at SACLA include bacteria, cellular organelles, biological macromolecules, etc. Single shot imaging of biological microsponges was realized as the first single-shot imaging of functional biological macromolecules. Imaging living cells is feasible with femtosecond single-shot exposures while specimens are prepared in a hydrated condition. The liquid jet injector provides a natural platform to carry out such experiments. Alternatively
samples can be prepared on fixed targets along with the buffer solution by encapsulating samples in solution using another membrane, whose feasibility was proved by imaging of fully hydrated yeast and plankton for the first time at SPring-8.\(^{36}\)

Rendering a 3D structure from single-shot imaging is feasible by acquiring multiple copies of 2D images. Resembling samples are imaged by single-shot exposures whose 2D images deliver samples at different orientations to accommodate a faithful ensemble of a 3D structure. Nano-particles with high symmetry have been imaged at SACLA to successfully render 3D structures at several nm resolution. Its general application to small biological specimens can be made, but still the weak scattering from small biological samples needs to be overcome.

The femtosecond x-ray pulse facilitates ultrafast single-shot pump-probe imaging. Using a femtosecond optical laser as a pumping source, x-ray 3D imaging of phonon excitations in Au particle has been demonstrated at LCLS recently.\(^{37}\) The experiment demonstrated advantages from short x-ray pulse and coherence in time-resolved imaging. Single-shot pump probe imaging can further unveil transient features during irreversible phase transitions by acquiring structures from single-shot exposures. We have studied nano-scale melting transition at SACLA at better than 10 ps temporal resolution via pump-probe imaging.

Serial femtosecond crystallography (SFX) using XFEL single-shot coherent diffraction attracts surging interest. Small protein crystals, with sizes ranging from several hundred nm to a few μm, are injected into the focused x-ray spot via single-particle loader such as liquid jet injector. A few hundred thousand copies of partial diffraction patterns from many small crystals in random orientation are recorded to unveil atomic structures of proteins. The technique is regularly employed at XFELs expecting to provide a breakthrough for studying proteins, which are not usually formed in sizable crystals.

6 Summary and discussion

Coherent diffraction imaging adapted to XFELs features single-shot diffraction imaging paving new routes to structure investigations. This article was aimed to introduce current research activities of single-shot diffraction at SACLA to address the capabilities available from the new light source. Whilst the XFEL realizes many experiments with intense and short x-ray laser pulse, many imaging projects could be more pertinent in synchrotrons such as 3D tomographic imaging with easier manipulations of samples and x-ray radiation dose. Experiments solely relying on the high photon flux would be more appropriate at advanced synchrotrons with more photon flux currently. The single-shot diffraction research starts to call for upgraded performance of the XFEL machine. Single-molecule 3D imaging would benefit from higher photon flux than currently achieved. A machine operating at a higher repetition rate would expedite data acquisition, of great benefit to the present analysis scheme that requires a few hundred thousand diffraction patterns for one 3D structure to be determined. However, it should be remarked also that this ‘wish list’ would be better accomplished by combined efforts in advancing analysis techniques, sample handling techniques, experimental schemes and XFELs, all yet in the beginning days of a long exciting journey.

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References


