Single-pulse enhanced coherent diffraction imaging of bacteria with an X-ray free-electron laser

Coherent diffraction imaging (CDI) is a very promising way for high-resolution imaging of non-periodic objects. This technique is based on the principle that the oversampled coherent diffraction patterns obtained from samples are recorded in the far field and then directly reconstructed real-space images using phase retrieval algorithms [1]. The achievable spatial resolution for CDI is principally limited by the wavelength of the incident X-rays. Recently, X-ray Free Electron Lasers (XFEL) with femtosecond and ultra-bright X-ray pulses combined with CDI overcome radiation damage limits and facilitate to achieve images by single-shot imaging based on a diffraction-before-destruction scheme [2]. However, presently, even with the ultra-bright XFEL, the achievable resolution of biomaterials is less than the required to study the cells and their internal structures because of the low scattering intensities. Hence, how to improve the low scattering ability of biomaterials is very important to the investigation of high-resolution single-shot imaging and its applications.

Theoretically, the scattering intensity at diffraction angle θ is proportional to the incident X-ray intensity and the form factors of the samples. Therefore, brighter X-ray source and increasing the form factors are two possible parameters. For single-shot CDI, the incident single pulse contains around 10^{12} photons at SACLA. Here, we demonstrated the promising single-shot CDI method [3] to enhance the scattering intensity and spatial resolution by labeling the low scattering biomaterials with heavy atoms. The bacterium *staphylococcus aureus* (*S. aureus*) was chosen as a model system and gold nanoclusters was selected as the labeling materials to investigate the scattering intensity and resolution enhancement. In order to compare the enhancement, two kinds of *S. aureus* samples were prepared: one, labeling of gold nanoclusters with a mean diameter of 9.8 nm; second, control samples with same culture conditions without gold labeling.

The experiment was performed at SACLA **BL3** [4]. Sketch of the experimental setup is shown in Fig. 1. To demonstrate the enhancement of signal and resolution by CDI, the labeled and control *S. aureus* were deposited onto 50-nm-thick Si_3N_4 membranes separately. By scanning the fixed membranes with focused XFEL single pulses, a multi-port charge-coupled device (MPCCD) detector was used to record the diffraction signals.

The diffraction patterns of the control and labeled *S. aureus* are shown in Figs. 2(a) and 2(b) respectively. Intuitively, the diffraction pattern of the labeled samples extended to the edge of the detectors, whereas the diffraction signals from the control sample are weak and mainly concentrated in the center of the detector. In order to quantitatively compare the signals intensity, the power spectrum density (PSD) of the two diffraction patterns were calculated and shown in Fig. 2(c) (blue curve for labeled and black curve for control). The highest spatial frequency of the labeled *S. aureus* is 81 μm^{-1} corresponding to a theoretical resolution of 12 nm, while the control sample is only about 45 μm^{-1} corresponding to a resolution of 22 nm. The spatial frequency of the labeled diffraction patterns is about twice of the control one. We also performed more diffraction patterns and the PSD curves showed the similar results. Since these diffraction patterns were recorded under the same experimental conditions, it confirms that diffraction signals has been significantly increased by labeling with gold nanoclusters.

Though the gold labeling increases scattering intensity, the achievable resolution depends on multiple factors, including the signal intensity, signal to noise ratio, missing low spatial frequency data and so on. To analyze the resolution enhancement, both the control and the labeled *S. aureus* patterns were reconstructed with HIO retrieval algorithm. Figures 3(a) and 3(b) show the reconstructed images from the diffraction patterns shown in Fig. 2. As there is few cellular structures could be identified with the achievable resolution, it is difficult to compare the image resolutions of the control and labeled *S. aureus* directly. To quantitatively estimate the resolution, the phase retrieval transfer function (PRTF) of reconstructed images were calculated. As shown in Fig. 3(c), the PRTF curves indicate that the achievable resolution is about 143.5 nm for control

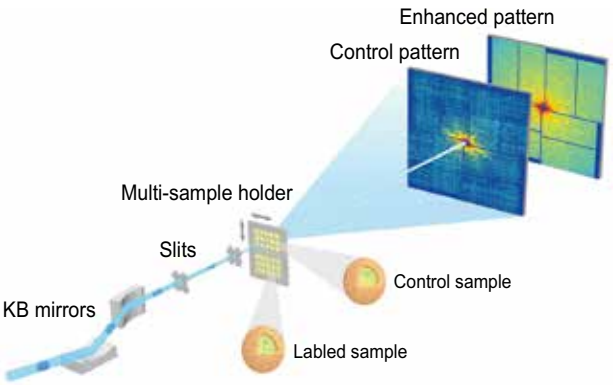

Fig. 1. Schematic layout of the enhanced single-pulse coherent diffraction imaging experiment.

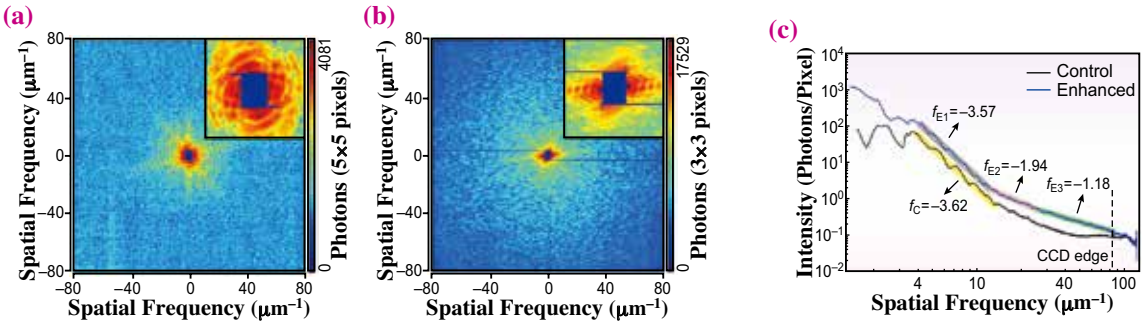

Fig. 2. **(a)** and **(b)** Representative diffraction patterns of control and labeled *S. aureus*. **(c)** Comparison of PSD curves of control and labeled diffraction patterns.

S. aureus (black curve) and 54 nm for labeled sample (blue curve) which is increased by a factor of 2.6.

During our experiment, it was found that by labeling with gold nanoclusters, both the diffraction signals and achievable resolution could be increased obviously. We analyzed and discussed the intrinsic causes for signal and resolution enhancement according to reference enhancement model. By introducing labeling, the recorded diffraction intensity includes three parts as presented in equation 1:

$$I_{sc} = I_{cell} + I_{Au} + 2\sqrt{I_{cell} I_{Au}} \cos(\phi_{cell} - \phi_{Au}) \qquad (1)$$

At high spatial frequency of the diffraction patterns, the signal mainly comes from the labeled gold nanoclusters and the interference between the cell and gold nanoclusters. Furthermore, the interference signal facilitates the phase retrieval and reconstruction. Since more such useful signals were used for reconstruction, the achievable resolution could be improved. Therefore, labeling biomaterials with appropriate heavy materials provides a promising way to enhance the scattering intensity and resolution using single-shot CDI without radiation damage.

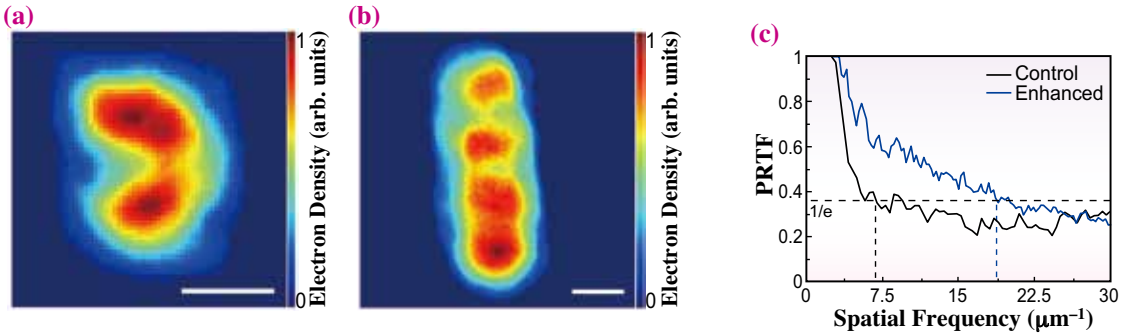

Fig. 3. **(a)** and **(b)** Reconstructed images of the control and labeled *S. aureus* from Figs. 2(a) and 2(b). **(c)** Phase retrieval transfer function curves calculated from the reconstruction results.

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References

[1] J. Miao *et al.*: Science **348** (2015) 530.
[2] R. Neutze *et al.*: Nature **406** (2000) 752.
[3] *J. Fan, Z. Sun, Y. Wang, J. Park, S. Kim, M. Gallagher-Jones, Y. Kim, C. Song, S. Yao, J. Zhang, J. Zhang, X. Duan, K. Tono, M. Yabashi, T. Ishikawa, C. Fan, Y. Zhao, Z. Chai, X. Gao, T. Earnest and H. Jiang: Sci. Rep.* ***6*** *(2016) 34008.*
[4] T. Ishikawa *et al.*: Nat. Photonics **6** (2012) 540.