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Method to enhance the resolution of x-ray coherent diffraction imaging for non-crystalline bio-samples

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Abstract
To circumvent the problem of radiation damage when using an x-ray coherent diffraction imaging experiment to resolve the structure of biological samples, we propose a method to add objects made of heavy atoms with the bio-samples or we load the samples on a template made of heavy atoms. This template method is shown by a numerical simulation (including shot noise) to be able to resolve the structure of a virus better than without the template. A counter-intuitive result is obtained, where heavier templates have a better resolution, even if the diffraction intensity of the bio-sample is much smaller than the noise intensity. In addition, the method also helps to greatly increase the efficiency of phase retrieval. We also provide a way to estimate the error to be expected if a particular experimental setting were chosen once the charge ratio between the sample and the template is estimated. Hence, this method will also help experiments choose the optimal setting for the best resolution with minimal radiation damage.

Keywords: coherent diffractive imaging, lensless imaging, iterative phase-retrieval algorithms, oversampling, template method
1. Introduction

The advancement in coherent diffraction imaging microscopy has enabled the determination of structures of non-crystalline materials in the nanometer scale [1–4]. By utilizing the iterative algorithm, the diffraction patterns captured by the CCD camera can be phased and thus a real-space structure can be reconstructed [5–9]. Because of its longer penetration depth than electrons, an x-ray is able to probe the structure below the surface of thick samples. Moreover, without using optics, coherent diffraction imaging is free from the constraints of optical aberration and diffraction limits.

There are, however, several experimental and technical limitations preventing us from acquiring an exact and clear image. One of the main restrictions is the shot noise from the light beam. The fluctuation due to the shot noise can be, in principle, suppressed by increasing the exposure time or radiation intensity; however, the intense x-ray radiation [10] can also damage the vulnerable bio-samples [11, 12]. Through using single-shot schemes, such as the diffract-and-destroy experiments, Chapman and his coworkers [3, 10, 13–15] showed that a bio-sample can be imaged under the illumination of a high-intensity free electron laser (FEL) [16–18]. However, noise still serves as a major obstacle for high fidelity reconstruction of the structures.

The methodology for suppressing and dealing with noise during the experiment and in the post-experiment reconstruction has been widely discussed. The binning of pixels, which combines 2 × 2 or 3 × 3 pixels to wash out random noise, is considered to be an effective approach to enhance the signal-to-noise ratio (SNR), but at the cost of reduction of pixel number and resolution. To make the iterative algorithm more tolerant to the noisy diffraction intensities, Miao and his coworkers developed oversampling smoothness (OSS), which applies additional constraints to the region outside the support in the iterating process [19]. Martin et al proposed another numerical method, which is termed dark-field imaging, to ameliorate the phasing process at the presence of missing centres [20]. Although these approaches may help to enhance the image resolution at the post-experiment stage, it is desirable to explore possible new experimental approaches to systematically enhance the image quality under current x-ray coherent diffraction imaging (XCDI) set-up.

An early work by Szöke demonstrated holographic methods in x-ray crystallography, showing that a holographic reference helps to solve an unknown part of the structure [21]. In an experimental work by Marchesini et al, an array of gold scattering elements, termed uniformly redundant arrays, were placed next to the test sample and were shown to be able to enhance the image resolution [22]. These holography works have demonstrated that a point reference beam or a scattering reference might facilitate the imaging at a better SNR, but the holographic methods require prior information of the reference and, in particular, a comparable scattering intensity ratio between the sample and reference is usually desired because the interference contrast is critical for the hologram quality. In addition, these methods usually require the sample and reference object to be separated.

A method that adds heavy atoms, such as gold, has been employed in the study of microscopy and x-ray crystallography for various purposes. For example, a cryo-electron microscopy has used gold particles in the sample as markers for identifying and positioning the target [23]. In addition, the heavy-atom method widely utilized in the study of protein crystallography has demonstrated that heavy atoms added into the specimen can assist us to resolve the structure [24–26]. A rule-of-thumb of the heavy-atom method indicates that the best case is to have about similar intensity contributions from the heavy atoms and the original
sample is usually made of light atoms. If the intensity of heavy atoms dominates too strongly, then the errors in intensity measurement may become comparable or even larger than the contribution of the sample, making the structure either insoluble or less resolved than the case without using the heavy atoms. However, we will show below that, in the CDI microscopy for non-crystalline bio-samples, the concept of employing heavy atoms can enhance the images. The surprising result is that more or heavier added atoms will improve the resolution. In our approach, the bio-sample is placed on a specially engineered substrate with designed gold patterns, which is called a template. These gold patterns greatly enhance x-ray diffraction intensity. However, the shot noises are also enhanced. Even if the noise intensity is larger than the bio-sample intensity, the template will still help to resolve the structure of the bio-sample and can thus ameliorate the SNR under a low-dose condition. The mathematical analysis and simulation results will be given in the following sections.

2. Theory background

The specimen we consider has two parts, a metallic template and a bio-sample. For simplicity, we shall only consider a two-dimensional image reconstruction in this paper; the extension to three-dimensional reconstruction will be discussed in the last section. The projected electronic density of the template is \( f(x, y) \) with its Fourier transform \( F(k_x, k_y) \), where \((k_x, k_y)\) or \((k)\) represents the pixel number. The projected electronic density of the bio-sample is \( g(x, y) \) and its Fourier transform is \( G(k_x, k_y) \). In the absence of noise, the far-field diffraction intensity of this specimen measured by detectors is

\[
I(k) = \beta |A(k)|^2
\]

and \( \beta \), to be given explicitly later, is determined by the experimental parameters, including the photon flux and the beam time. We assume that our template covers most of the areas on the substrate and consists of heavy atoms such as gold, and its total charge density is much larger than the bio-sample; that is,

\[
\langle |F(k)| \rangle \gg \langle |G(k)| \rangle
\]

where \( \langle |F(k)| \rangle \) and \( \langle |G(k)| \rangle \) are defined as the averages among all pixels of \( |F(k)| \) and \( |G(k)| \), respectively. Then, for most pixels, we have

\[
|A(k)|^2 \approx |F(k)|^2 + F^*(k) \cdot G(k) + F(k) \cdot G^*(k) \gg |G(k)|^2.
\]

In general, measured experimental signals carry noises which can be attributed from the light beam or the detector. The measured intensity then becomes

\[
I'(k) = I(k) + \eta(k) \equiv \beta |A'(k)|^2,
\]

where \( \eta(k) \) denotes the noise intensity, which is assumed to be Poissonian and proportional to the square root of the intensity \( I(k) \). Thus, \( \eta(k) \beta^{-1/2} \) is of order \( |A(k)| \) and \( |F(k)| \), and, hence, it is generally much larger than \( |G(k)| \) as \( \langle |F(k)| \rangle \gg \langle |G(k)| \rangle \). Thus, using a template made of heavy atoms will bring up the noise level to much larger than the scattered intensities by the bio-sample. Naively, we will conclude that because the noise is larger than the information obtained from the sample, then the reconstruction of the structure of the sample is either
impossible or of much lower quality. However, a more careful examination below will show that the opposite is true.

In the presence of noise, $A'(k)$ defined in equation (3) can be defined as

$$A'(k) = A(k) + N(k), \tag{4}$$

where $N(k)$ denotes the contribution of noise to the Fourier transform of the projected electron density of the specimen. Using equations (3) and (4) we have

$$I(k) + \eta(k) = \beta |A'(k)|^2 = \beta [|A(k)|^2 + 2 |A(k)||N(k)| \cos \theta(k) + |N(k)|^2], \tag{5}$$

and the fluctuation term or noise

$$\eta(k) = \beta [2 |A(k)||N(k)| \cos \theta(k) + |N(k)|^2], \tag{6}$$

where $\theta(k)$ is the phase difference between $A(k)$ and $N(k)$.

If we now use the property of Poisson noise, $\eta(k) \approx \beta^{1/2}|A(k)|$, and the assumption that $\langle |A(k)| \rangle \gg \langle |N(k)| \rangle$, we obtain

$$\eta(k) \approx 2 \beta |A(k)||N(k)| \cos \theta(k). \tag{7}$$

and

$$|N(k)|^2 \cos \theta(k) \approx \beta^{-1/2}.$$ 

Taking the average with respect to all the pixels on the $k$-space, we have

$$\langle |N(k)| \rangle \approx \beta^{-1/2}, \tag{8}$$

which reveals that the contribution of Poisson noise to the diffraction amplitude is only dependent on the experimental setting and is independent of the sample property itself when the intensity contribution from the specimen is very large. Note that $N(k)$ is not the noise, but it is related to and caused by the noise, as shown by equations (6) and (7). Since $\eta(k)$ is proportional to $|A(k)|$ multiplied by a stochastic variable, equation (7) indicates that $|N(k)|$ is dominated by the stochastic variable. This is verified below in a numerical simulation. As far as we know, the last equation has never been discussed before. Besides being consistent with the fact that when SNR is increased the error gets reduced, the result makes the specific prediction of a square-root power law dependent on the experimental setting parameters. Most important of all is that the average $|N(k)|$ will not depend on the scattering amplitude of the whole sample and the template.

According to the last equation, to have a smaller error for a given specimen we must increase $\beta$ by increasing the exposure time or incoming x-ray fluxes in the experiments. Both of these methods would likely damage the bio-samples. However, we can go by another route to increase SNR. Instead of concentrating on $\beta$, we can change the specimen by adding the bio-samples with a heavy-atom template to enhance the total SNR ratio without directly increasing the radiation. An interesting result is that heavier templates have a better resolution of the bio-sample. This provides a means for us to control the error in the reconstructed image for the bio-samples. This is the central idea that underlies the theoretical basis of the template method. The numerical simulation to demonstrate the validity of this idea is given below. An added advantage of having a template, as shown below, is the increase of the rate of convergence in phase retrieval by using an iterative method. While the information of $G$ is immune from being
suppressed by noise induced by heavy atoms, the $F$ template helps to obtain the phases quickly during the reconstruction.

3. Simulation results

To demonstrate our template method, we performed a series of numerical experiments. In our simulations, a three-dimensional electron density data volume\(^1\) of a Mimivirus [14, 27] is used and projected on our designed metallic template. Figure 1(a) shows the 2D projection of electron charge distribution of the Mimivirus used in our simulations. Our gold template, shown in figure 1(b), consists of nine geometrical patterns, including triangles, squares and circles, on a single layer with a total area size of $2 \times 2 \mu m^2$. The height of the patterns will be varied to change the total electron charges of the template. The wavelength and photon flux of the coherent x-ray beam is set to $\lambda = 2.27 \AA$ and $\Phi_0 = 2.01 \times 10^{13}$ mm$^{-2}$ s$^{-1}$, respectively. The diffraction intensity is measured by a CCD camera with 1361 x 1361 pixels and each pixel has size $p$ of $20 \mu m$. The CCD camera is $z = 1.7$ meters away from the sample. This experimental configuration gives an oversampling ratio [5, 6, 28] of $\sigma \equiv \left( \frac{\lambda}{\pi p} \right)^2 \approx 93.1$ and a corresponding spatial resolution $\approx 14$ nm per pixel. The measured diffraction intensity can be quantified as

$$I'(k) \equiv \beta |A'(k)|^2 = \Phi_0 \rho_e^2 \Delta t \frac{\Delta s}{z^2} |A'(k)|^2,$$

where $\rho_e$ is the classical electron radius, the image acquired time $\Delta t$ is set to $1500$ s, and the area of a pixel is $\Delta s$. Hence, $\beta$ is about $3.3 \times 10^{-17}$. Standard Poisson noise is added to the diffraction intensities. In addition, a $21 \times 21$ pixel region at the centre is removed to emulate the beam stop which blocks the direct beam. Its size is not larger than the centro-speckle [8, 29], as shown in figure 2(a). The reconstruction was performed with the guided hybrid input and output (GHIO) [30] method, an improvement of the HIO [31] method by adding the concept of optimization and guided search. Instead of using one sample with a very large number of iterations, as in the

\(^1\) The 3D electron density data volume of Mimivirus is freely available from EM Navigator, PDBj.
usual HIO method, GHIO carries iterations simultaneously for a number of samples, 32 in this case. Here, we used 1000 iterations to generate the 32 reconstructed images in this first generation. Then, among these final 32 images we search for the best result with the lowest error in Fourier space, $E_F$, defined below. This best image is used as a reference to guide the search for the next generation. The method was shown to be much more effective and robust in finding a best solution than just HIO. A tight support of $145 \times 145$ pixels was employed. To monitor the reconstruction quality in GHIO, an error function $E_F$ is used and given by

$$E_F \equiv \frac{\left[ \sum_{k_x,k_y} \left( |A'(k_x, k_y)| - |A^{(c)}(k_x, k_y)| \right) \right]^{1/2}}{\left[ \sum_{k_x,k_y} |A'(k_x, k_y)|^2 \right]^{1/2}}$$

(10)

where $A'(k)$ and $A^{(c)}(k)$ are the measured and calculated Fourier modulus, respectively. To evaluate the image quality of the bio-sample obtained, we calculate

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{(a) Diffraction intensity of the Mimivirus with template (in logarithmic scale). A 21 $\times$ 21 pixel region in the centre has been set to zeros to emulate the beam stop. (b) A Mimivirus on the template without overlap. (c) A Mimivirus on the template overlapping with the square pattern. (d) The reconstruction of an isolated giant Mimivirus from noisy diffraction intensity. (e), (f) Reconstructed images of the virus from (b) and (c) after subtraction, respectively.}
\end{figure}
Figure 3. A selected ‘dip’ feature was correctly resolved by both cases: blue without overlap and red with overlap. The dashed black line is the model. Inset: the linear segment where we select the pixels.

\[
E_R \equiv \frac{\left[ \sum_{(x,y) \in U} \left( \rho_{(x,y)}^{(\text{recon})} - \rho_{(x,y)}^{(\text{model})} \right)^2 \right]^{1/2}}{\left[ \sum_{(x,y) \in U} \rho_{(x,y)}^{(\text{model})} \right]^{1/2}},
\]

where \( U \) is the support and \( \rho_{(x,y)}^{(\text{model})} \) is the model charge density of the Mimivirus.

The noisy diffraction intensity of an isolated Mimivirus without template was solved by GHIO, which gave the reconstructed image shown in figure 2(d) with corresponding \( E_R = 5.95\% \), which is arguably poor. Under the same configuration with noise included, we carefully put the virus over a 300 nm thick gold template (equivalent template to a Mimivirus total charge ratio of 52.1) without overlapping with the patterns, as shown in figure 2(b). In order to examine the reconstructed Mimivirus alone, we subtract the model template from the calculated image. The result is shown in figure 2(e). Comparing this result to that without the template, the \( E_R \) of the virus is greatly reduced by one order down to 0.74\%. Inevitably, there is a chance that the Mimivirus can overlap with the patterns on the template during the sample preparation. In figure 2(c), for example, the Mimivirus is partially covering the square pattern in the centre of the template. The simulation result shows that under this scenario the reconstruction shown in figure 2(f) can still achieve a relatively low \( E_R \) of 1.03\%.

To quantitatively evaluate the achieved resolution enhancement with the use of a template, we plotted the values of pixels along a selected segment indicated in the inset of figure 3. The plot shown in figure 3 exhibits the ‘dip’ for both the cases, both with and without overlap. Since our pixel is about 14 nm in size, which is about half of the range of the dip feature, this result demonstrates that the resolution has achieved about one pixel size. A comparison of figures 2(d) and (e) shows that the template method has greatly enhanced the resolution.

The theoretical analysis we made to reach equation (8) predicts that as the diffraction scattering intensity of the template becomes much larger than the sample, the noise contribution to the total signal will be proportional to \( \beta^{-1/2} \), where \( \beta \) is defined in equation (9). This prediction has two parts. The first part is that the contribution of noise, \( \ln |\mathcal{N}(k)| \), will not increase indefinitely as the total charge of the template increases. In fact, it will reach a constant value determined completely by the experimental parameters. This is contrary to the naive expectation.
that increasing the charge ratio will also enhance $|N(k)|$ as the noise $\eta(k)$ is proportional to the square root of the intensity $I(k)$. The second prediction is the very specific dependence of the power $-1/2$. It is simple to change the total charge of the template in our numerical simulation by just changing the thickness of the gold patterns shown in figure 1(b). In figure 4 the calculated error $E_R$ is plotted as a function of the charge ratio of the template and the bio-sample. As the ratio of the total charge of the template to the Mimivirus increases from 8.68 to 1736, the error approaches a constant of approximately 0.6% instead of increasing. Here we should note that the error $E_R$ in the reconstructed image is almost all from the noise because we obtain an error of $10^{-6}$ if there is no noise included in the simulation. Thus, the result confirms the prediction that the error will reach a constant value that is independent of the total charge of the template as long as it is much larger than that of the bio-sample.

To verify the second part of the prediction, we can vary the value of $\beta$ by changing the sample exposure time $\Delta t$. The theoretical prediction is that, as we shorten the exposure time $\Delta t$ by $\alpha$ times, the error will only increase by $\alpha^{1/2}$ times. The inset of figure 5(a) illustrates the trend of $E_R$ versus total charge ratios for several exposure time shortening $\alpha$ ratios, including $\alpha = 0.25$, 1, 4 and 25, which means that the exposure time varies from 6000 s to 60 s. It turns out that curves with different $\alpha$ or exposure time are actually of the same form when we rescale both the error and charge ratio by a factor $\alpha^{1/2}$, as shown in figure 5(a). The fluctuation for the result of $\alpha = 25$ is just a little bit larger than the others due to the fact that the average value of the amplitude $\langle |A(k)| \rangle$ is not much larger than $\beta^{-0.5}$, as we assumed in deriving equation (8). Figure 5(a) can help the experimental planning by providing an estimate of the error or resolution of the experimental outcome once the charge ratio and experimental parameters determining beta are known. Furthermore, in all cases, the error $E_R$ approaches a constant value, $E_{R0}$, as the charge ratio increases to infinity, which is shown in the inset of figure 5(a). The cyan curve in figure 5(b) shows the linear relation between $E_{R0}$ and $\alpha^{1/2}$ as predicted by equation (8).
It is also intriguing to quantitatively compare the strength of the bio-sample $G$ and the contribution of noise $N$, as defined in equation (4). The average amplitude of $N(k_x, k_y)$ can be approximated by

$$\langle |N(k_x, k_y)| \rangle \approx \langle |A'(k_x, k_y)| - |A(k_x, k_y)| \rangle$$

(12)

as we have assumed that $\langle |A(k)| \rangle \gg \langle |N(k)| \rangle$. We calculate the averaged $N(k_x, k_y)$ for the cases with maximum charge ratio for different $\alpha$ values, and the cyan curve in figure 5(b) shows the linear relation between noise contribution $\langle |N(k_x, k_y)| \rangle$ and $\alpha^{1/2}$, once again verifying our prediction in equation (8). In addition, figure 6 compares the Fourier spectra of measured intensity $|A'(k_x, 0)|^2$, the estimated noise contribution $|N(k_x, 0)|^2$, and the bio-sample ($a$ New J. Phys. 16 (2014) 033016 T-Y Lan et al

**Figure 5.** (a) Normalized $E_R$ versus total charge ratio under different exposure time shortening factor $\alpha$. Inset: $E_R$ versus total charge ratio without normalization. (b) Magenta: the average and corresponding error bars of $E_R$ versus the square root of $\alpha$. Cyan: the averaged $\langle |N(k_x, k_y)| \rangle$ normalized to $\langle |G(k_x, k_y)| \rangle$ versus the square root of $\alpha$.

**Figure 6.** The values of the power spectra of intensity $|A|^2$ (gray), noise contribution $|N|^2$ (magenta), and Mimivirus sample $|G|^2$ (cyan) along $k_y = 0$. All values are normalized to $|G(0, 0)|$. In this case, $\alpha = 4$ and the total charge ratio is 52.1 (300 nm template).
Mimivirus in our case) $|G(k_x, 0)|^2$. The spectral intensity due to the Mimivirus is almost completely buried under the stochastic part; however, even under this disadvantaged condition, the bio-sample can be reconstructed with good quality ($E_R = 2.0\%$).

We have further analyzed the process of convergence during the iterations of GHIO [30] by conducting another GHIO with 10 generations and 30 iterations per generation to solve the diffraction intensities with a 300 nm thick template (total charge ratio 52.1). We used the criterion of minimal $E_R$ to guide the search direction of GHIO and recorded the real-space images at each generation. As shown in Figure 7, the patterns of the template show up first. Then, while the image of the template is getting stable and fixed, the structure image of the

**Figure 7.** Reconstructed images by GHIO with 30 iterations per generation. The dashed circles indicate the location of the virus.

**Figure 8.** (a) Reconstructed Mimivirus images by GHIO with 30 iterations per generation. (b) The cosine of the phase difference $\Delta \phi$ between $\tilde{G}(k_x, k_y)$ and $A_G^{(0)}(k_x, k_y)$, where $\tilde{G}(k_x, k_y)$ is the Fourier transform of the Mimivirus image and $A_G^{(0)}(k_x, k_y)$ is the Fourier transform of the Mimivirus cropped from the solution with minimal $E_R$ at the end of the $i$th generation.
Mimivirus gradually appears and becomes clearer as the iteration continues. This demonstrates another advantage of the method in that we do not really need to know the structure of the template \textit{a priori} because it can be determined very easily. In figure 8(a) we only show the reconstructed Mimivirus in figure 7 by removing the template. In addition, it is interesting to examine the evolution of phases with the iterations. Here, we consider the cosine of the \(k\)-space phase difference between \(\tilde{G}(k_x, k_y)\) and \(A_{G}^{(i)}(k_x, k_y)\), as given by

\[
\cos(\Delta \phi) = \cos \left( \arg \left( \frac{\tilde{G}^{*}(k_x, k_y)A_{G}^{(i)}(k_x, k_y)}{|\tilde{G}(k_x, k_y)| |A_{G}^{(i)}(k_x, k_y)|} \right) \right)
\]

where \(\tilde{G}(k_x, k_y)\) and \(A_{G}^{(i)}(k_x, k_y)\) are the Fourier transforms of the Mimivirus image cropped from \(G(k)\) and from the on-flight data at the end of each \(i\)-th generation, respectively. Figure 8(b) illustrates the cosine of the phase difference between the \(G(k_x, k_y)\) and \(A_{G}^{(i)}(k_x, k_y)\) of each generation. The red pixel means that the phase of the model virus is obtained by the reconstructed image. The result shows a good agreement with our earlier assumption that the iterative algorithm retrieves the dominating \(F\) at the first few steps and then gradually yet stably resolves the structure of the Mimivirus, or \(G\).

4. Conclusion

In summary, we have proposed the template method to enhance the resolution of the reconstructed image of a bio-sample from noisy diffraction intensities of the XCDI. We have presented a theoretical analysis to show that, by placing the bio-sample on a template made of strong scattering heavy atoms, the bio-sample can be reconstructed much more accurately than without the template, even if the shot noises are considered. Unexpectedly, it was found that a larger average diffraction intensity of the template produced smaller errors of the reconstructed bio-sample. In fact the strength of the noise is only dependent on the experimental setting. The theory is verified in numerical simulations of a realistic XCDI experiment to resolve the structure of a Mimivirus. Furthermore, we also show that the convergence rate is greatly improved in the iterative method of image reconstruction, which greatly speeds up the calculation time required for the reconstruction.

Our template method can be extended to three-dimensional XCDI [9, 32] and applied to existing tomography methods [2, 33]. For each slice taken at a specific angle, the template can be removed because its profile is clear, leaving the projection of the biosample in the reconstructed image. Even at a tilted angle, the overlap of the template and biosample would not hinder the removal, since the template patterns can be accurately solved, as shown in figures 2(c), (f). On the other hand, the template can be engineered, particularly for the three-dimensional tomography. By carefully tailoring the template, the template can be completely measured in every single projection slice. The heavy-atom template not only enhances the image quality, but it also helps the total density normalization and alignment among all slices. The unboundedness problem mentioned in [33] should not be an issue.

There are several advantages of implementing this template method in XCDI experiments. The method can achieve high resolution for image reconstruction but it still significantly reduces the amount of exposure time for bio-samples. Since the patterns on the template can be
designed and fabricated in advance, it can be easily incorporated into the reconstruction algorithms. However, the present work has an intrinsic and important difference from the holographic method. Even if the template structure is not pre-determined, our method still works because the template can be easily determined from the strong scattering amplitude first. In addition, our method does not require any specific charge ratio, making the design of the template tolerant and not sensitive to any imperfection or mismatch during the fabrication. A template-assisting experiment is currently under investigation.

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