Coherent Lensless X-ray Imaging

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Very high-resolution X-ray imaging has been the subject of considerable research in the last few decades. However the spatial resolution of these methods is limited by the quality of the X-ray optics that can be manufactured. More recently, lensless X-ray imaging has emerged as a powerful approach that is able to circumvent the limitations on X-ray optics. A number of classes of lensless X-ray imaging have been developed, many with origins in other forms of optics, and the key progress to date is here reviewed. We describe applications of the methods in imaging for biology and materials science, as well as the prospect for the imaging of single molecules using X-ray free electron lasers.

X-ray crystallography has a record of extraordinary achievement in science and its impact on the biological sciences has been immense. From its inception crystallography has numerically synthesized three-dimensional atomic-resolution images of molecules from the beams diffracted by their crystal forms. However, it was not until the influence of macromolecular crystallography was being fully realized that a prominent crystallographer, David Sayre, proposed[1] that the methods of crystallography might be

adapted to the imaging of general non-periodic objects to create a new form of lensless very high spatial-resolution imaging.

Sayre's idea became the subject of sporadic research in the ensuing period but a convincing experimental demonstration remained elusive for nearly twenty years. The first reported observation of X-ray diffraction from an isolated object was reported in 1987[2], however it was not until 1999 that an image was able to be recovered from X-ray data[3] and this result has stimulated a major international research effort aimed at developing coherent lensless X-ray imaging.

Solving the non-crystallographic phase problem

In 1952, David Sayre[4] applied Shannon's then recent work on sampling theory to crystal diffraction and noted that there may be sufficient information to uniquely solve for the diffracting object, if only one could measure diffraction intensities midway between Bragg peaks. It was not until 1980 that Sayre suggested[1] that a single isolated object permits such a higher density sampling scheme, since this non-periodic object produces a continuous X-ray diffraction pattern not restricted only to Bragg peaks. It was possible, therefore, to imagine that the formidable success of crystallography might be transferable to the study of finite but non-periodic objects and that one could create a lensless imaging methodology yielding images with extraordinary spatial resolution. This is the method now known as coherent diffractive imaging (CDI).

From a more modern perspective, Sayre's early work does not provide a compelling argument that a properly measured far-field diffraction pattern would necessarily yield sufficient information to fully determine an image of the diffracting object, primarily

because it does not deal with issues of the independence of the additional measurements. However independent and more or less contemporaneous theoretical work on the phase problem was being undertaken in the electron- and visible-optics communities. Fienup[5], working independently of Sayre in the area of visible optics, argued that it should be possible to recover an object distribution from its Fourier modulus and proposed some iterative approaches building on ideas from electron imaging[6]. Bates[7] argued that apart from some "trivial" ambiguities in the phase recovery problem (lateral translation of the object, complex conjugation, spatial inversion and absolute phase), the diffraction pattern from an isolated object would "almost certainly" lead to a unique solution. The residual ambiguity falls within the class of "homometric" structures in which different objects have identical diffraction patterns; these rather special objects do indeed exist[8] but the likelihood of them being of practical significance is vanishingly small. Miao and colleagues[9] showed that it is possible to recover images from data in which the total number of intensity measurements exceeds the number of unknown image points, though it is possible that this conclusion may depend on the nature of the other constraints available. In the case of illumination by a field that is coherent but contains a spherical phase curvature (ie. Fresnel diffraction), the methods of Bates have been used to show that the diffracted intensity has a truly unique relationship to the diffracting object[10].

The extension of crystallography, as envisaged by Sayre in 1980[1], is implemented by the simple process of measuring the far-field diffraction pattern from an object (Figure 1a). Numerical techniques are then employed to invert that diffraction pattern to recover an image of the object. In doing so, one constructs an imaging system that is devoid of image forming optical elements, promising spatial resolution limited in principle only by

the wavelength of the incident light. As Sayre pointed out[1] such a scheme loses a key benefit of crystallography, which is the coherent addition of intensities from the many molecules in the crystal. The dose to the object hence increases by many orders of magnitude, placing radiation damage as a practical limitation to the achievable resolution[11].

Data analysis

The fundamental CDI geometries are sketched in Figure 1. Figure 1a shows the original concept for CDI in which an isolated object is illuminated with a highly coherent beam of X-rays and the diffraction pattern produced by the sample is measured in the far-field. In practice the diffraction by an isolated object is very weak and so it is necessary to introduce a beam stop in order to prevent the direct beam damaging the detector and to facilitate the dynamic range required to properly measure the diffracted signal.

The algorithmic methods by which the image is recovered have their origins in electron microscopy through the iterative methods first proposed by Gerchberg and Saxton[6]. In the case of CDI, one iteratively seeks a solution that is consistent with both the measured diffraction pattern and any *a-priori* information held about the object, with the most commonly used information being the physical extent of the object, otherwise known as its support.[12] (See Box). The precise details of the manner in which the algorithm is implemented has been the subject of considerable recent research and, in practice, data is analysed using a cocktail of methods. The simple iterative replacement of the latest iterate with the *a-priori* information, such as the known support information, is known as error reduction; the use of a relaxation parameter is known as the hybrid input-output algorithm (HIO)[13]; and there are a range of other important variations on the theme[14].

Perhaps the most interesting innovation in this area is the "shrinkwrap" algorithm[15] in which the finite support of the object is estimated during the reconstruction process, a method that has found extensive use. These methods have been the subject of recent tutorial reviews by Quiney[16] and Marchesini[17] that also provide interesting and mathematically unified syntheses of the methods.

The algorithms assume a Fourier transform relationship between the field leaving the object and the field at the detector, an assumption that is only strictly correct in the case that the incident field is perfectly coherent. Williams et al[18] showed that even small deviations from perfect spatial coherence can prevent that data from being reliably analysed and this may have contributed to the long delay between the original Sayre proposal and its first experimental demonstration with X-rays, with the required coherence only becoming available with modern synchrotron facilities. However, Whitehead et al[19] have successfully modified the algorithms discussed here so as to account for the effects of partial spatial coherence providing potentially greater throughput and more reliable imaging.

A number of other geometries have also emerged, or been subject to a renaissance, in the last decade and one may now discern a number of distinct classes of coherent X-ray imaging methods. The first of these classes, denoted here as forward-scattering CDI, conforms to the initial vision by Sayre and involves the sample being illuminated by a coherent beam and its diffraction pattern is observed along the axis of the incident beam, which can be planar (Figure 1a) or curved (Figure 1b). A second class is here termed Bragg CDI and in this configuration a small crystal is illuminated and the structure of the Bragg spots is used to elicit information concerning the shape and internal structure of the

crystal, including its strain properties (Figure 1c). The third class we term here scanning CDI. This is the result of a resurgence of interest in the ptychographic method of electron microscopy[20] in which the image is reconstructed from a set of diffraction patterns obtained while the finite illumination is scanned across an extended sample (Figure 1d).

X-ray Fourier transform holography has also been subject to renewed attention and can rightly now also be considered under the rubric of coherent lensless imaging methods. However the development and applications of X-ray holography warrants considerably more discussion than can be covered in the space available here and so we are able to only lightly touch on the topic.

We discuss each of these approaches here before considering the role they might play with the X-ray free electron laser sources.

Forward-scattering CDI

The elementary geometry for forward-scattering CDI is shown in Figure 1(a). The requirement for a unique solution is that the beam immediately exiting the object must be finite in spatial extent and so, in the case of a planar incident beam, the sample must be completely isolated. In practice a very intense undiffracted beam mandates the use of a beam stop (or strong attenuator, which has not been realised) to prevent it from damaging the detector. The beam stop therefore prevents the measurement of the intensity of the small diffracted angles that scatter within its solid angle. However, as in crystallography, the fundamental experimental configuration is extremely simple.

Nugent et al[21] noted that modern X-ray optics are capable of producing such exquisitely small focused beams that it is conceivable to introduce significant phase curvature over

objects that might even be molecular in scale, leading to a Fresnel diffraction pattern at the detector plane, a pattern that uniquely defines the phase to within a constant and physically meaningless offset[10] (Figure 1b). Williams et al[22] experimentally demonstrated the use of Fresnel CDI. The advantage of the Fresnel approach lies in the reliability and consistency of the convergence of the iterative technique[23]. However the production of the incident curved wave adds some experimental complexity.

Miao and colleagues showed the first reconstruction of three-dimensional images[24] and Chapman et al[25] extended the method to use a full range of projections resulting in a full tomographic reconstruction . In the Chapman et al work, the iterative approach was applied directly to the three-dimensional set of diffraction data. The use of a focused beam has also been used to increase the illumination of the sample and very high spatial resolution has been reported for simple objects using refractive lenses[26] and X-ray mirror systems[27]. An important additional innovation was demonstrated by Abbey et al[28] in which it was shown that a finite expanding beam can be used to define a finite extent for the wavefield leaving the object, with the result that, in this configuration, CDI is not limited to objects of finite extent, thereby removing an important limitation on the applicability of coherent imaging methods.

A major part of the motivation for the development of the forward-scattering method is the development of improved high resolution imaging for biological samples, with the first example being from Miao et al[29] and a number of further studies have been published[30, 31] (Figure 2). The group working at the Advanced Light Source in California have compared their image of a yeast cell with that from a scanning transmission X-ray microscope (see article by Attwood) X-ray image of the same sample[32]. Subsequent work

has been demonstrated on the imaging of frozen hydrated samples, suggesting that real biological insights might be around the corner[33, 34]. The use of a curved incident beam has also led to the suggestion that it might be possible to amalgamate scanning X-ray fluorescence microscopy (and other imaging modalities) with high-resolution CDI[35] . Forward-scattering CDI has also been used to produce an image of a chromosome using 8keV X-rays[36] as well as a range of other biological targets.

As with other forms of X-ray microscopy, the problem of radiation damage is significant[11] and this radiation damage is indeed likely to limit the achievable resolution, especially for biological materials. These considerations are eased somewhat when materials samples are imaged and CDI has been used for demonstration experiments on simple materials. Barty and colleagues[37] obtained a three-dimensional high-resolution image of a ceramic nanofoam and showed that the image was consistent with the reduced statistical information of its structure obtained from incoherent small angle X-ray scattering methods, and Abbey et al[38] obtained images of buried structures in an integrated circuit.

Other forms of X-ray sources are also emerging, with the laser driven high-harmonic sources[39] showing consistent improvement. A major goal for these sources is to achieve substantial emission in the "water window" region of X-rays for which biological samples show a natural contrast due to the different absorption properties of carbon and oxygen (see article by Attwood). Forward scattering CDI has been demonstrated for these sources[40], as well as extensions that permit a number of harmonics to be utilised simultaneously and so increase the useable radiation flux [41].

Bragg CDI

CDI has its origins in the desire to extend ideas of crystallography to samples that are not periodic. However one of the most productive applications of CDI methods has been to the study of nanocrystals.

The diffraction pattern of an infinite crystal is the product of the reciprocal lattice with the molecular transform. Elementary Fourier analysis tells us that the diffracted field from a finite crystal differs in that the infinite reciprocal lattice is now convolved with the Fourier transform of the crystal shape – the resulting diffraction pattern is therefore the familiar crystalline diffraction pattern but with a distribution of intensity at the location of what would otherwise be a Bragg diffraction spot. Robinson and colleagues[42] recognized the importance of this information in the study of nanocrystals and showed that it is possible to deduce the detailed shape of a nanocrystal from the shape of the Bragg spots produced by that crystal.

A slight tilting of the crystal will have the effect of rapidly moving the diffraction spot through the diffraction condition[43] and so it is, in this case, quite easy to obtain a three-dimensional measurement of the Bragg spot distribution and therefore obtain information about the three-dimensional shape of the nanocrystal. Again, elementary Fourier analysis informs us that the imposition of simple shape information on a periodic sample will result in a centro-symmetric Bragg spot distribution. However it was observed that the spots rarely display the expected symmetry, implying that the shape function must be complex. The phase information arises from strains within the crystal and so Bragg CDI is able to yield very high resolution three-dimensional images of strain within a nanocrystal (Figure 3), in the direction of the reciprocal lattice vector[44]. The Bragg

diffraction used to form the pattern has the interesting aspect that the object does not need to be physically isolated since a non-periodic substrate, for example, will be invisible to the diffraction process. As a result, one can use the method to study the impact of an interface with the crystal under study. A long-term goal of Bragg CDI is to image the strain tensor by combining information from several Bragg spots, and to coherently combine perhaps many Bragg spots to obtain atomic-resolution images of the whole crystal. A useful overview of this field has been published by Robinson & Harder[45].

Scanning diffraction microscopy (ptychography)

The phase problem for imaging has been around for many years and was seriously addressed by the electron imaging community. Ptychography is a method that was proposed in the sixties for application in the transmission electron microscope[46]. The fundamental idea is to pass a probe beam (e.g. a focused or apertured beam) over a sample and to record the resulting two-dimensional diffraction pattern as a function of the position of that beam. In the typical case of a two-dimensional scan of the beam, the result is a four-dimensional set of data. The dataset is separable into the 4D Wigner functions of the probe and the sample[46], and knowledge of the probe allows the direct retrieval of the phased diffraction wavefield of the sample, as was experimentally demonstrated[47]. Iterative methods, fundamentally based on the ideas outlined in the present paper, have been developed which have eased the computational burden[48] and these methods have now been experimentally demonstrated[49]. This approach joins the work of Abbey et al[28] in opening up lensless X-ray imaging to extended samples (Figure 4). A further extension has been suggested in which an intermediate iterative step is

included that permits the probe beam distribution to be obtained[50] and which allows a further improvement in the resolution that can be obtained.

Ptychography, due to the large amount of highly-redundant data obtained, produces reliable and quantitative images of extended objects and can account for partial coherence[46], and so is showing promise of being a very valuable way forward for lensless imaging using synchrotron sources. Some interesting and potentially valuable bio-imaging results are now beginning to emerge[51].

Fourier transform holography

X-ray holography is the first coherent imaging technique identified for use with X-rays and the topic has a considerable history that is too extensive to be covered adequately by the present review. The key to holographic imaging is in the need for the wave scattered by the object to interfere with a known reference wave and allowing a direct and deterministic recovery of the image information. This contrasts with the methods that are the main subject of this review in which no reference wave is needed and the images are recovered using an iterative phase recovery method. Nonetheless, it can be argued that this topic should be covered, if only cursorily, in a review of coherent lensless imaging.

While X-ray Fourier transform holographic methods were successfully demonstrated[52] in the 1980s, the availability of X-ray sources with a high degree of coherence has enabled the method to find some important areas of application, the most important of which is to the study of magnetic samples beginning with the work of Eisebitt et al[53]. An example of the results obtained using this method is shown in Figure 5.

The science that has been enabled by the method is beyond the scope of the present paper, but the interaction with the coherent diffraction community has seen the method develop considerably. Of particular note is the use of multiple reference sources[54] and complex structured sources such as the uniformly redundant array[55], a structure that consist of a set of pinholes with a distribution such that the it has a perfectly flat power spectrum. It is hoped that these approaches will enable improved spatial resolution by creating a small characteristic scale in the reference source while retaining a large open area.

A particularly interesting hybrid approach that draws extensively from ideas of CDI has arisen from the work of Podorov et al[56] in which, instead of the pinhole reference source used in Fourier transform holography, diffraction from the edge of an aperture is used. In this way, the resolution of the reconstructed image is limited by, for example, the straightness with which an edge may be constructed and this opens an approach to very high spatial resolution holographic imaging. The method has been generalized by the work of Guizar-Sicairos and colleagues[57] and has been experimentally demonstrated for the imaging of magnetic samples[58].

X-ray free electron lasers

A possible solution to the problem, raised by Sayre[1], of radiation damage limiting the achievable resolution of lensless X-ray imaging, came with the proposal of imaging molecular scale structures using X-ray free electron lasers[59]. The goal is to subject a stream of single molecules to X-ray laser pulses and to observe the X-rays diffracted by the molecules. The intensity of the pulse will be so great that the molecule will

disintegrate and the hope is that the X-ray pulse is sufficiently short that the diffraction process will be complete before the molecule has undergone significant structural change.

Although simple in concept, the problems are substantial. The open questions surround the capacity to acquire adequate data, whether the molecule will disintegrate before the pulse is completed and the problem of assembling all of the data from the different random molecular orientations into a consistent single set of data.

Progress to date has been encouraging. The fundamental problem of recovering the electron distribution from a continuous diffraction pattern is, as is evidenced by the literature cited in the present paper, thoroughly demonstrated and, of course, the standard methods of crystallography, such as multi-wavelength anomalous dispersion, heavy atom replacement and so on, remain in the potential toolbox. Chapman et al[60], using the FLASH free electron laser in Hamburg, have experimentally demonstrated that it is possible to recover the structure of a sample before it disintegrates. CDI has been used to produce images of the disintegration of a sample after illumination from a free electron laser[61] and these studies have yielded results that are consistent with the motion predicted by dynamics codes. The nuclear motion of a disintegrating biomolecule have been studied computationally and it now seems safe to anticipate that motion is negligible on timescales shorter than about 5 fsec[62, 63]. It is to be anticipated that electron damage might happen on an even shorter timescale and the impact of this effect is the subject of ongoing study.

The classification of the very low signal diffraction patterns into their orientations is a further challenging problem and the methods of single-particle electron diffraction are unlikely to be suitable. However a recent proposal by Fung et al[64] suggests that the use of

sophisticated Bayesian statistics offers a path forward, and it might also be possible to align the molecules using, for example, laser methods[65].

It is the view of the authors that the problems standing in the way of obtaining the structure of biomolecules from free electron laser pulses are being steadily solved by the international research community and that there is every reason at the moment to anticipate success for this endeavour.

Summary

X-ray lensless imaging has been the subject of a very significant growth of activity in the last decade. CDI imaging is able to be applied to many important problems and we now know how the method may be applied to extended objects with very high spatial resolution, to the determination of strain distributions in crystalline samples and to the visualization on magnetic structures using holographic methods. These possibilities have arisen through the wealth of recent new ideas. We believe it is safe to assert that CDI and its related methods can now be regarded as an established form of high-resolution imaging using coherent X-ray sources.

The goal of imaging single, clusters or nanocrystals of biomolecules is still just out of reach. However the obstacles are yielding to concerted research and the barrage of new ideas. The authors believe that there is now an excellent chance that the goal can be achieved in the foreseeable future. It promises rewards in structural biology, condensed matter physics, magnetism and other correlated systems, as well as the detailed investigation of matter under extreme conditions.

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Iterative Phase retrieval

The far-field diffraction pattern of an object with a transmission $f(x)$ can be represented as its Fourier transform, with

$$F(u) = \mathfrak{F}\{f(x)\} = \int f(x) \exp(-2\pi i x \cdot u)\, dx.$$

The inverse Fourier transform synthesises the image of $f(x)$ from its complex Fourier components $F(u)$:

$$f(x) = \mathfrak{F}^{-1}\{F(u)\} = \int F(u) \exp(2\pi i x \cdot u)\, du.$$

However, the phase $\varphi(u)$ of the wavefield cannot be directly measured in the X-ray regime and must be recovered before the synthesis can be made. This inverse problem can be stated as determining $f(x)$ from samples of $|F(u)|$ by applying available *a priori* knowledge about $f(x)$. One of the strongest constraints that can be applied is that the object has a finite support, outside of which $f(x)$ should vanish. That is, the sum of all the frequencies $|F(u)|\exp\{i\varphi(u)\}\exp(2\pi i x \cdot u)$ is zero outside this region of x (the set S). The application of this constraint requires the diffraction pattern $|F(u)|$ to be measured finely enough to properly sample diffraction fringes that would occur from (the non-existent) scattering points in this region.

The simplest iterative scheme (Fienup's error reduction, or ER) starts from a random guess of $\varphi(u)$ to give $F_1'(u) = |F(u)|\exp\{i\varphi_1(u)\}$. Form an image $f_1'(x)$ by inverse transformation. This will not be zero outside the support, so update the estimate of the image by setting $f_2(x)$ equal to $f_1'(x)$ in the support and zero otherwise. Transform to a new diffraction pattern $F_2(u)$. This will not conform to the measured amplitudes, so set

$F_2'(u) = |F(u)| \exp\{i\varphi_2(u)\}$, which is transformed back to form $f_2'(x)$. This is repeated by alternately applying the "support constraint" and the "Fourier modulus constraint:"

$$f_n'(x) = \Im^{-1}\left\{|F(u)| \exp\left(i \arg\left\{\Im\left[f_n(x)\right]\right\}\right)\right\}$$

$$f_{n+1}(x) = f_n'(x) \text{ if } x \in S, \text{ 0 otherwise.}$$

This algorithm can be more compactly written in terms of projection operators, $f_{n+1}(x) = P_S P_M f_n(x)$ where P_M performs the first part of the iteration (the modulus constraint) and P_S the second. The solution occurs when both constraints are satisfied, or $P_S f_n(x) = P_M f_n(x)$, but ER often stagnates without reaching this solution. This is because the application of a projection operators always minimises the error between the iterate and constraint, which leaves no recourse to escape local minima of the solution space. A common problem is the stagnation at the sum of the correct image and its centrosymmetrical inversion (either of which is a valid solution, but not both). Zeroes in the diffraction intensities are problematic since the projection P_M is not well defined at those points. Often the incorrect locations of pairs of phase vortices (branch cuts of the complex-valued field) in the diffraction pattern cause stripes in the reconstructed image that form a deep local minimum. Modifications of the iterative schemes aim to avoid these stagnations, and Elser's difference map[14] formally avoids this case. Other constraints, such as positivity or atomicity, can be applied in a similar way.

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Figure Captions

Figure 1 A schematic summary of the experimental configurations for X-ray coherent diffractive imaging. (a) Plane-wave CDI in which a coherent planar beam of X-rays is incident on the sample. (b) Fresnel CDI in which a coherent phase-curved beam created by a zone plate is incident on the sample. An order sorting aperture (OSA) eliminates the unwanted diffracted orders. A beam stop prevents the undiffracted order from passing through the OSA. (c) Bragg-CDI in which a nano-crystal is illuminated and the detailed structure in the Bragg diffraction spots is used to recover information about the shape and strain distribution within the crystal. (d) Scanning diffraction microscopy in which a finite beam probe is scanned across the sample and the diffraction pattern observed at each beam position. The finite probe may be formed using a focusing optics such as a zone plate.

Figure 2: Biological imaging is an important area for applications of CDI. Figure 2(a) shows an optical image of a yeast cell using soft X-rays. The images show the reconstructed complex wave is represented using brightness for magnitude and hue for phase. The arrows indicate the location of immunogold labels. The details of this image may be found in ref [30], from which it has been adapted with permission.

Figure 3: Bragg CDI is able to recover the three-dimensional shape and strain structure from a nano-crystal. This figure shows the shape of a gold nano-crystal and the distribution of the strain field from within it. Reproduced with permission from ref[44]

Figure 4: Scanning diffraction microscopy is able to recover images of extended objects. Figure 4(a) shows that amplitude distribution of an integrated circuit sample used as a test object. Figure 4(b) shows the phase distribution. The form of the illuminating probe is also able to be recovered during the iterative image reconstruction scheme. Image provided courtesy of Dr Pierre Thibault of the Technical University of Munich.

Figure 5: Holographic reconstructions from a sample containing bit-patterned magnetic media. The bits consist of a substrate with 80 nm x 80 nm elevated squares in a 120 nm pitch array, coated with a magnetic multilayer film $[Co(5.5Å)/Pd(9Å)]_{24}$ plus seed and cap layers. The black/white contrast is based on x-ray circular dichroism and corresponds to the local magnetization in the magnetic film pointing up/down. Two magnetic states at different points within a magnetization cycle are shown and (a) and (b). Images courtesy of Prof. Dr. Stefan Eisebitt of the Technische Universität Berlin

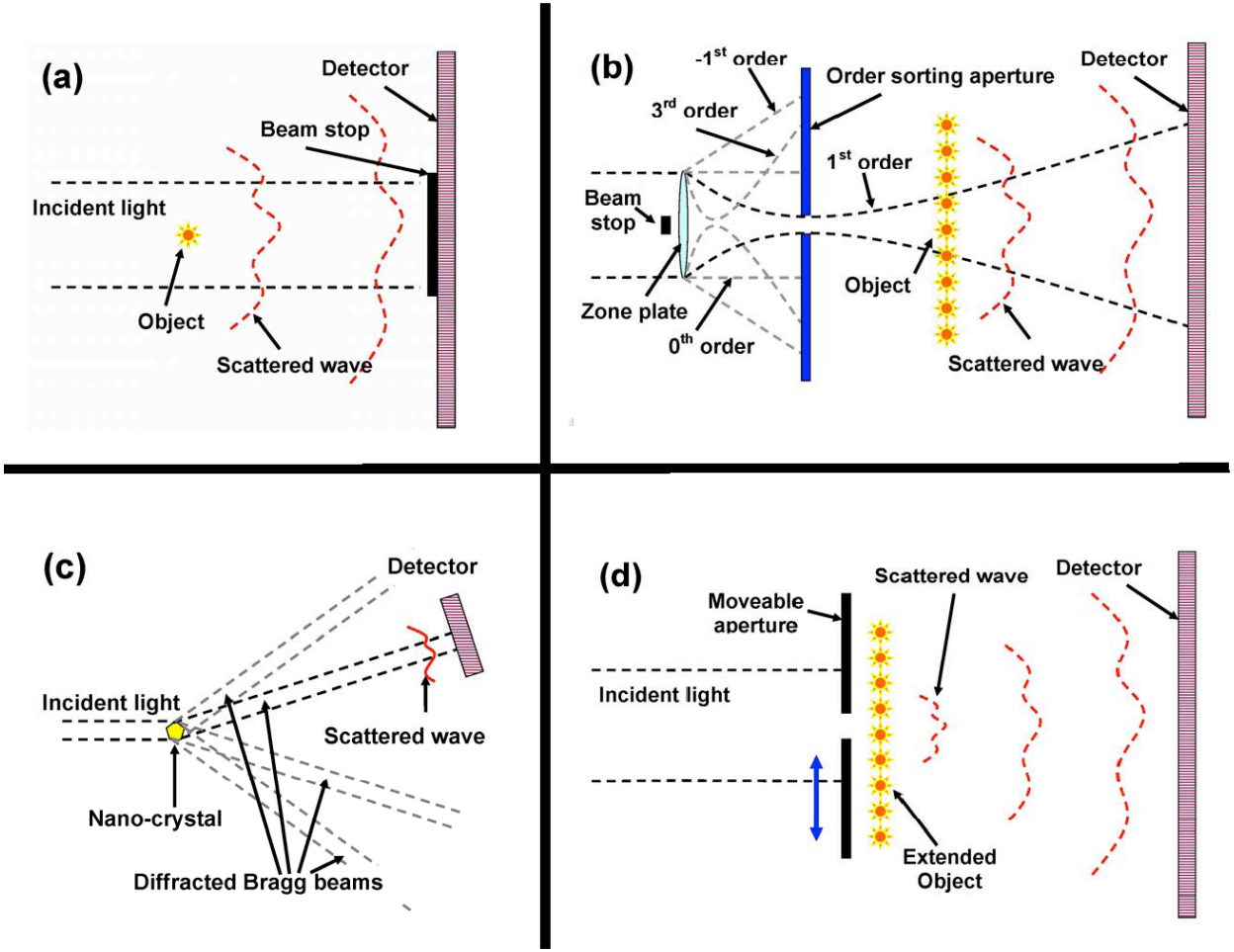
(a)
Detector
Beam stop
Incident light
Object
Scattered wave
(b)
-1st order
Order sorting aperture
Detector
3rd order
1st order
Beam stop
Object
Zone plate
0th order
Scattered wave
(c)
Detector
Incident light
Scattered wave
Nano-crystal
Diffracted Bragg beams
(d)
Scattered wave
Detector
Moveable aperture
Incident light
Extended Object

Figure 1

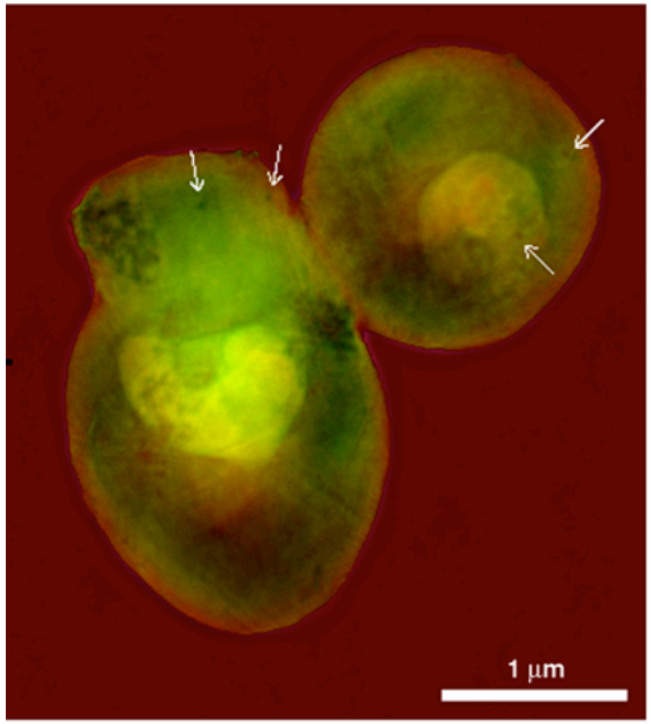

Figure 2

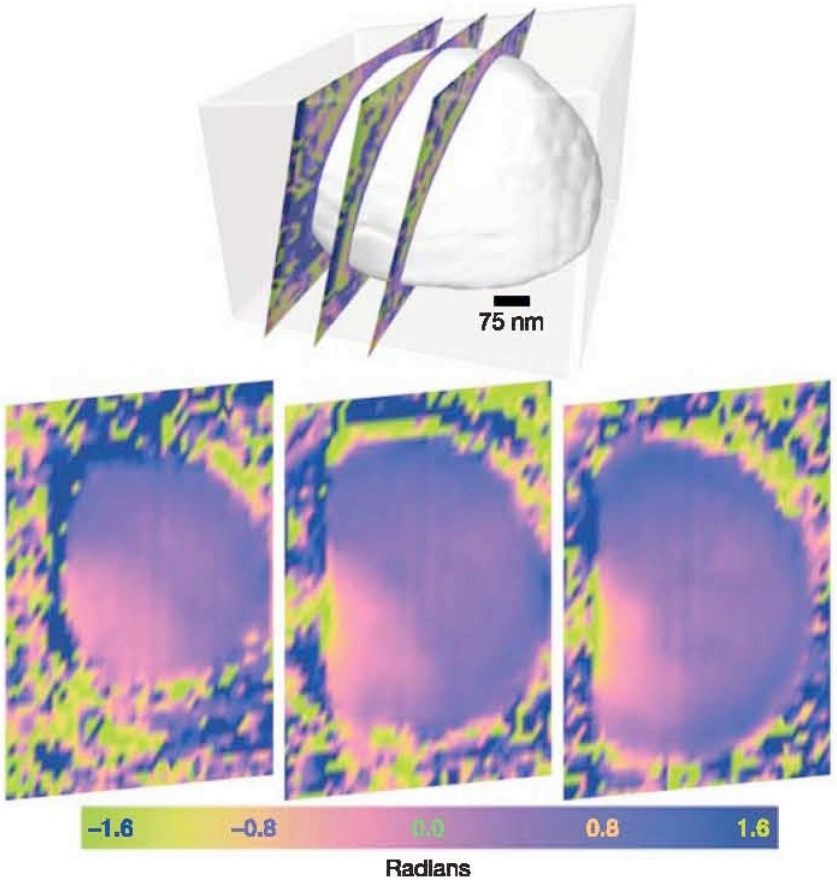

Figure 3

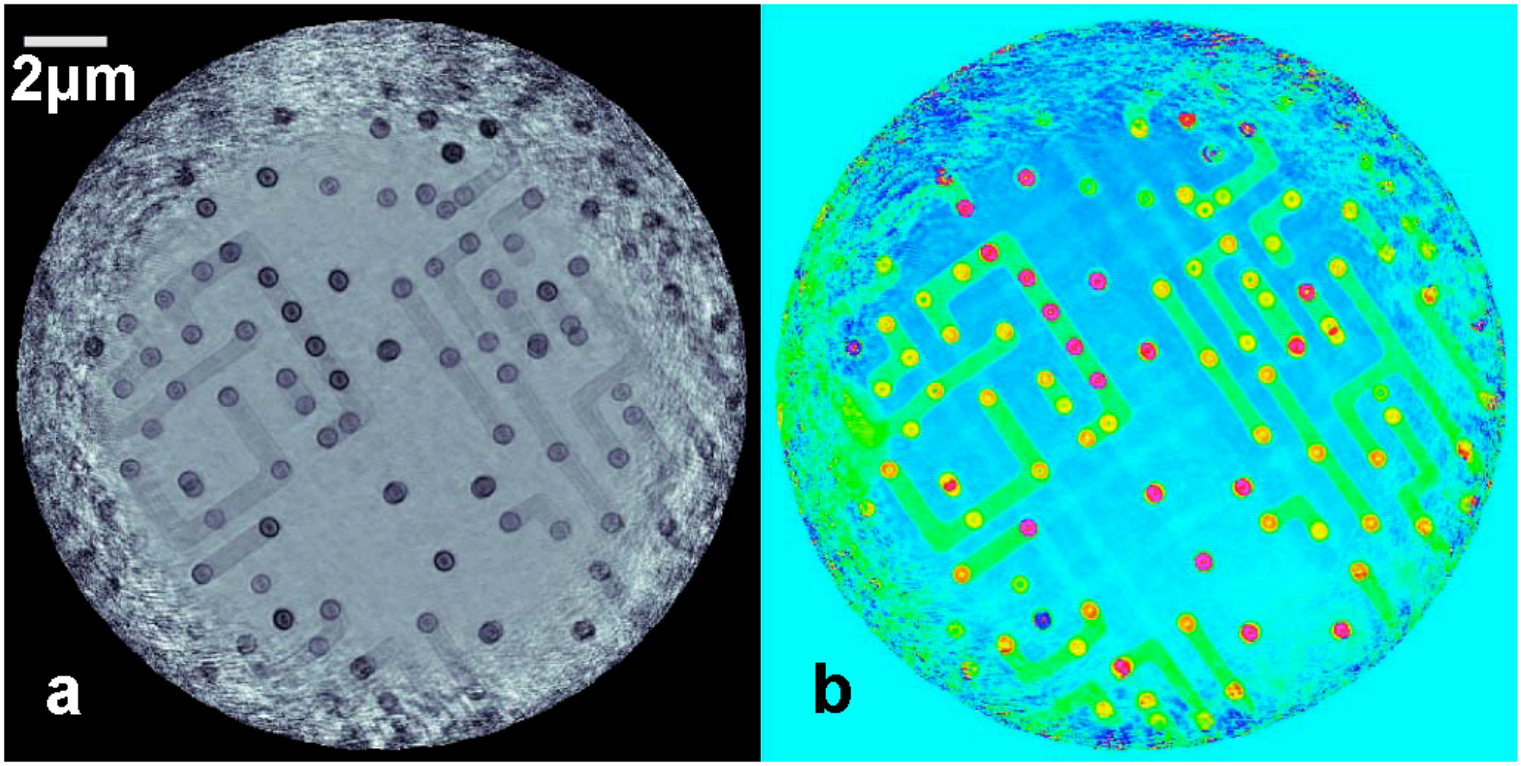

Figure 4

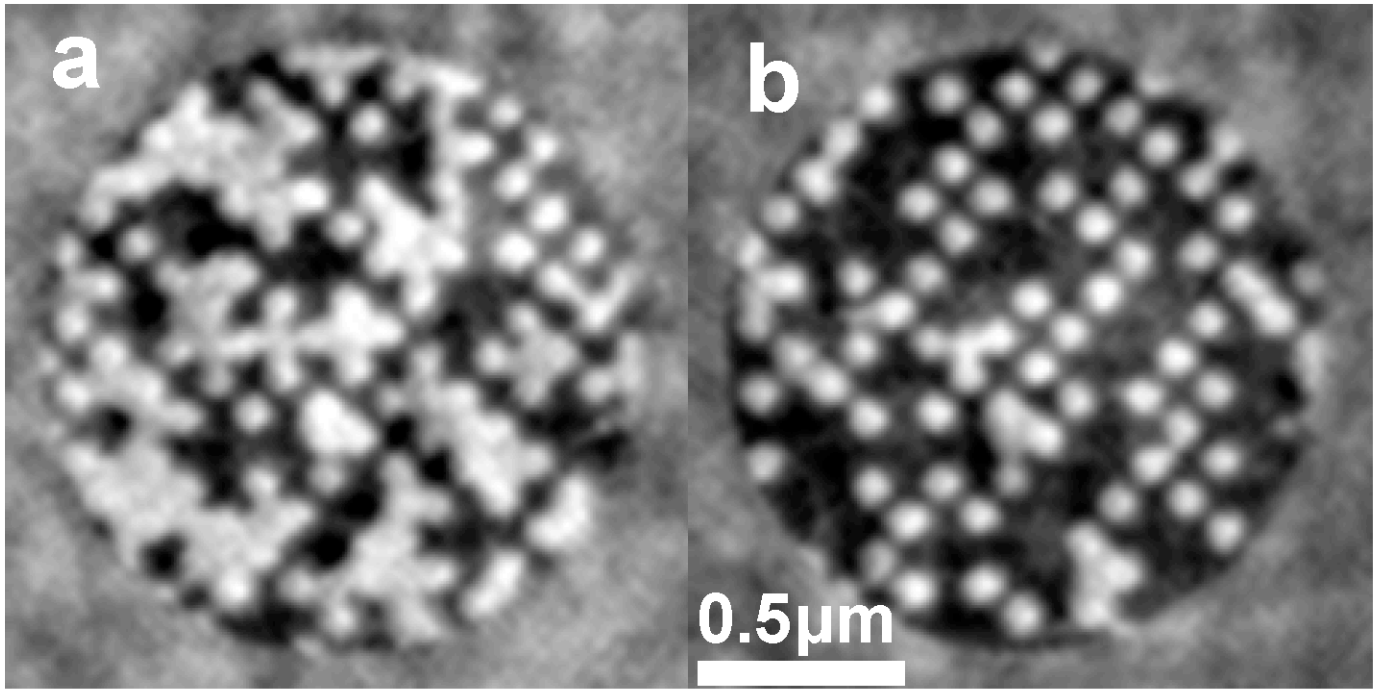

Figure 5