Dose efficient Compton X-ray microscopy

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1. INTRODUCTION

X-ray imaging techniques have proven invaluable to study biological systems at high resolution due to the penetration power and short wavelength of this radiation. In practice, the resolution and sensitivity of current X-ray imaging techniques are not limited by the performance of optics or image-recovery methods but by radiation damage. We propose the use of Compton (inelastic) X-ray scattering for high-resolution cellular imaging and provide a study of a scanning microscope geometry that requires a dose to achieve a given resolution that is three orders of magnitude lower than for coherent (elastic) scattering. We find that the dose per imaging signal is minimized at a photon energy of 64 keV. This corresponds to a short enough wavelength (0.02 nm) to provide nanometer transverse resolution and micrometer depth of field for tomographic imaging of whole cells. The microscope could be implemented at future high-energy and high-brightness synchrotron-radiation facilities to provide images of unsectioned and unlabeled cells in their native conditions at enough detail to bridge the techniques of super-resolution optical microscopy and cryo-electron microscopy.

1. INTRODUCTION

The penetration power and short wavelengths of X-rays makes them a good candidate to address one of the widespread wishes of the biological community: to image soft tissue such as whole cells and organelles, at a few nanometers resolution in all three dimensions, in their natural state. At soft X-ray photon energies in the so-called “water window” between the carbon and oxygen absorption edges at 284 and 534 eV (wavelengths between 4.4 and 2.3 nm), the high X-ray absorption in carbon-rich protein relative to that of water produces high-contrast images of unstained biological samples in transmission microscopes [1–3]. As long as the thickness of the object does not exceed the depth of focus of the objective lens of the microscope, transmission images’ approximate projections through the object, which can be used to obtain three-dimensional reconstructions by the method of tomography. The high contrast enables images to be obtained at a tolerable dose at a resolution approaching 30 nm, but further improvements in resolution for this wavelength range require increasing the numerical aperture of the objective, which would reduce the depth of focus to substantially less than 1 μm, thinner than most cells [4].

Higher spatial resolution, depth of focus, and penetration can instead be reached by utilizing much shorter wavelengths or higher photon energies. The brilliance provided by third-generation synchrotron light sources [5] that enabled soft X-ray microscopy also facilitated the development of coherent X-ray imaging techniques at photon energies beyond 10 keV, such as Zernike phase-contrast microscopy [6], coherent diffractive imaging [7], ptychography [8,9], and projection microscopy [10]. Lensless techniques of ptychography and diffractive imaging are able to retrieve three-dimensional images with the desired resolutions of several nanometers by using spatially coherent hard X-ray beams with high penetration power and without limitations of depth of focus [11,12]. Additionally, recent developments in X-ray optics have demonstrated efficient focusing with large depth of focus to spot sizes of a few nanometers, using multilayer coated Kirkpatrick–Baez mirrors [13] and multilayer Laue lenses [14]. These, and new storage-ring designs that increase the brightness of synchrotron radiation by several orders of magnitude [15,16], open up new imaging modalities and opportunities for hard X-ray cellular microscopy that we explore here.

Although coherent scattering of X-rays transfers no energy, this process cannot occur without a proportional number of incident photons being absorbed by the object. Thus, independent of whether absorption or scattering is used to form the image, the signal strength of that image is proportional to the incident X-ray fluence and thus also to the amount of energy absorbed per unit mass of the object or dose [17]. The dose leads to structural changes that eventually impair the image that can be recorded. Biological and soft-matter samples are more easily damaged by radiation than hard materials. Keeping below the tolerable X-ray exposure limits the strength of the image obtained and thus...
the achievable imaging resolution [18–20]. Current X-ray microscopy techniques operate near this limit. Images of 5-μm-thick cryogenically cooled cells were obtained by water-window absorption-based imaging at about 30 nm resolution [2,4] at about 1 GGy dose. Cryogenically cooled cells of 18 μm thickness were imaged by ptychography at 6.2 keV photon energy, achieving 180-nm resolution at 670 kGy [21], and a 5-μm-wide alga cell was imaged in a similar fashion at 5.2 keV photon energy to achieve 18-nm resolution at 29 MGy [22]. (In the latter case, high-resolution fluorescence maps were obtained simultaneously.) These experimental results are consistent with imaging criteria, which assess the capabilities of coherent X-ray imaging techniques [23–25]. One criterion relates the number of scattered photons per voxel of a small feature compared with its surroundings [23]. This, and its extension to resolving small features embedded in larger objects [24], predicts that coherent diffraction imaging at energies between 1–10 keV cannot resolve features in biological samples finer than 10 nm without suffering radiation damage. Diffraction measurements must be sampled by detector pixels whose number increases with object size. Larger objects thus require greater exposures since the signal from a particular feature must be shared over more bins. Taking this into account gives a stricter criterion that predicts achievable resolutions of around 100 nm at 1–10 keV for realistically sized biological samples [25].

As the photon energy is increased beyond 10 keV, the photon absorption cross section decreases at a greater rate than that of coherent scattering processes [Fig. 1(a) shows the case for carbon]. An optimum ratio of the highest coherent image signal per degree of damage for soft tissues is expected at about 20–40 keV, depending on sample size [26]. However, at photon energies above 30 keV, Fig. 1(a) indicates the dominant interaction for low-Z materials is actually Compton scattering [27], which is usually considered as a noise-producing background in any coherent imaging arrangement. By comparing the cross sections of photoabsorption, coherent scattering, and Compton scattering as a function of photon energy for light elements [28], it is clear that an incoherent microscope based on the latter interaction has the potential for providing much higher imaging signal per dose than is possible with coherent scattering, for samples much thicker than can be imaged by absorption contrast in the water window.

This paper presents a dose-optimization study for hard X-ray imaging of cells, with realistic models of soft-tissue embedded in water and including all atomic cross sections for the energy range accessible at current high-energy synchrotron facilities. In view of this optimization a novel X-ray imaging technique is introduced, scanning Compton X-ray microscopy (SCXM), that exploits all the scattered photons from the sample. The performance of SCXM is then compared with hard X-ray coherent imaging techniques via simulations. From this comparison it is concluded that SCXM can achieve better resolution than coherent X-ray imaging techniques at a given dose. Finally, a SCXM design concept is described that utilizes an achromatic X-ray focusing lens.

2. SCATTERING-TO-DOSE OPTIMIZATION

The interactions of the X-rays with matter are photoabsorption, Thompson (coherent) scattering, and Compton (incoherent) scattering. Their cross sections as a function of the photon energy are depicted in Fig. 1(a) for carbon. Each of these interactions offers a different imaging modality, as well as a different dose penalty for a given achieved signal level or contrast. An image can be formed from the photoabsorption interaction by mapping the
transmission of the sample, a bright-field image whose contrast is provided by the removal of photons from the incident beam. Alternatively, the fluorescence or photoelectrons subsequently emitted by the sample can be mapped in a dark-field mode. Coherent scattering gives the opportunity for several modalities, including holography, coherent diffractive imaging, and phase-contrast imaging, whereas incoherent scattering allows a dark-field mapping of the scattered photons. Generally, imaging can be thought of as localizing particular interactions between X-rays and atoms in the sample. As such, before considering the actual contrast mechanism for a particular imaging modality, it is worth comparing the total photon count that would be available to an imaging process for a given dose. Figure 1(b) shows the number of total absorbed or scattered photons per atom for a given dose (here the energy deposited per atom), in the case of utilizing only the absorbed photons (black), coherently scattered photons (blue), or all scattered photons (red). The latter case includes detecting both the Thomson and Compton photons, but at photon energies above 10 keV this is mostly Compton scattering. It is clear from the graph that there are two regimes that offer the best performance. The first is at low photon energies below 1 keV where photoabsorption dominates, and the second is at about 50 keV utilizing Compton scattering.

The plot in Fig. 1(b) does not account for the achievable contrast in a particular imaging modality, but it does imply that at high photon energies of 50 keV (where the wavelength is short and penetration is high) a technique able to detect all the scattered photons from the sample has potential to obtain images with better resolutions and sensitivities than coherent X-ray imaging techniques and bright-field absorption imaging. The distribution of Compton scattered photons extends in almost all directions, as shown in Fig. 1(c). Directly forming an extended dark-field image of the sample with the Compton scattered photons is therefore inefficient, since only a fraction of the scattered photons will be collected by the numerical aperture of an objective lens, even when considering lenses with resolutions approaching 1 nm. Instead, an equivalent image can be formed in a scanning geometry, by mapping scattered photon counts as a function of the position of a focused X-ray beam that is scanned across the sample. A detector of coverage close to 4π steradians could be achieved in principle. As seen in Fig. 1(c) the Compton contribution is negligible in the forward direction (θ → 0), which is dominated instead by the coherent or elastic component as depicted in Fig. 1(d). Thus, it should be possible to discriminate coherent and incoherent photons using a suitable detector geometry.

To define the optimal imaging energy and capabilities for the proposed technique we employ a model of a cell consisting of water in which isolated voxels of a given size are embedded, consisting of biomolecular material 23,29. The composition of this material is taken to be the same as used in dose calculations by Howells et al. 23, H50C30N90O10S1 with a density ρ = 1.35 g/cm³. This allows direct comparison of our results with that work. To simulate the image, the total number of scattered photons in the detector solid angle, Ωdet, as contributed by illuminated voxels, must be computed. This quantity is given by

\[ N^i_S = \Phi \int_{\Omega_{det}} d\Omega \frac{d\sigma^i_S}{d\Omega} \, d\Omega, \]  

where \( \Phi \) is the incident flux (number of photons per unit area), \( d\sigma^i_S \) is the total scattering cross section, which includes the Compton and coherent components computed using Ref. 30, and the index \( i \) refers to the material composition: either the biomolecule (m) or water (w). As noted above, \( \sigma^i_S \) is dominated by Compton scattering. The detector solid angle excludes the incoming and outgoing (unscattered) focused beam given by the numerical aperture (NA) of the focusing optics, which itself is inversely related to the imaging resolution \( \Delta r \) as

\[ \Delta r = \frac{1.22 \lambda}{2NA}. \]  

In order to determine the optimal energy, a detectability criterion is used to establish if the biomolecule voxel embedded in water can be resolved. The chosen detectability criterion is the Rose criterion 31, i.e., the biomolecule can be discriminated if and only if the difference in signal between it and water is at least five times the noise level. Assuming that the noise is due solely to photon counting, this criterion requires

\[ 5 \leq \frac{|N^m_S - N^w_S|}{\sqrt{N^w_S + N^m_S}}. \]  

Equation (3) imposes a minimum required imaging fluence \( \Phi' \) to resolve features at the necessary contrast, which can be determined using the definitions of \( N^i_S \) in Eq. (1). We perform calculations only for two-dimensional images since the required fluence to achieve a particular contrast in a three-dimensional tomographic reconstruction is the same as for the two-dimensional image, according to the dose fractionation theorem 32. We find that the required fluence \( \Phi' \) depends on the width \( d \) of the feature and thickness \( L \) of the object in which it is embedded, as \( L/d^4 \). This dependence is deduced from Eq. (3) when comparing a thickness \( L \) of water with \( L - d \) water plus \( d \) protein. For details, see Supplement 1.

Figure 2(a) displays the required fluence in SCXM to discern a \( d = 10 \) nm voxel of biomolecular material from water \( (L = d) \), as a function of the photon energy. It is seen that the slope of the curve changes at photon energies greater than 30 keV as incoherent scattering becomes the dominant process for low-Z materials. Nevertheless, the plot also indicates that the required fluence to image a particular feature hardly changes as the photon energy is increased from 20 keV to beyond 80 keV. However, as the photon energy is increased, the probability for photoabsorption decreases strongly [see Fig. 1(a)], implying that the dose to the sample to image a particular feature also drops with photon energy.

At the photon energies considered here, energy is deposited into the sample mainly due to the photoelectric effect and the incoherent scattering. For simplicity, we assume that the entire volume of the sample is illuminated by a constant fluence (over the full exposure required to form the image), which is a good approximation for small biological samples (of few microns). The dose to the sample is given by

\[ D = \frac{E_{dep}}{\rho V}, \]  

where \( \rho \) is the density of the material contained in the illuminated volume \( V \) and \( E_{dep} \) is the deposited energy by photoabsorption and Compton scattering. A detailed explanation of the dose calculation is provided in the Supplement 1. Figure 2(b) displays the dose to the biomolecular component of the sample as a function of photon energy, at the fluence required to distinguish a 10 nm protein-containing voxel from a 10 nm water-containing voxel.
This figure shows that the optimal energy to image a radiosensitive material exploiting all the scattered photons is around 64 keV, although there is not a significant dependence on photon energy above 45 keV. This is a consequence of the similar energy dependence of the cross sections of water and biomolecules in this energy range. This optimal photon energy for Compton microscopy is independent of feature size since the dependence on $d$ can be factored in Eq. (3).

### 3. VALIDATION OF THE IMAGING CAPABILITIES OF SCXM

In order to check the calculations of Section 2, which were made assuming a simple sample geometry, we further simulated two-dimensional images of a 5-μm-thick cell-like object composed of water and with several submicrometer features. For this thickness of object, the dose to the protein material at the minimum required imaging fluence $\Phi^0$, as calculated from Eqs. (1), (3), and (4) is given by the red dashed curve in Fig. 3(a). This plot also displays the maximum tolerable dose with the black dotted line as determined by Howells et al. [23], which increases with the resolved feature size $d$. The intersection of these curves shows that the best achievable resolution that can be obtained with SCXM for this kind and thickness of sample is around 34 nm. We therefore simulated features of different materials of this size.

Images were simulated for 64 keV photon energy with a focal spot of 34 nm width that was scanned on a square lattice with steps of 7 nm. The sample was represented by the scattering cross sections of different materials and densities, discretized into a two-dimensional grid. The 3.5 nm × 3.5 nm grid was obtained after integrating the cross section of the different materials through the object thickness. At each point in the scan the total scattering signal in the detector was calculated by multiplying the focused beam fluence profile with the sample grid. Finally, Poisson noise was added to the simulated photon counts. One Compton micrograph is shown in Fig. 3(b), simulated at 64 keV photon energy and for the minimum required imaging fluence of $5.41 \times 10^{14}$ ph/μm$^2$ to discern 34 nm PMMA features in a 5 μm sample. In the zoomed region of interest shown in the figure, four different features can be seen, each of different contrast. Three of these correspond to Gaussian features of $2\sigma = 34$ nm width composed of: (i) PMMA with a density of 1.1 g/cm$^3$, positioned at the top-left corner; (ii) H$_{50}$C$_{30}$N$_9$O$_{10}$S$_1$ with a density of 1.35 g/cm$^3$, which mimics a typical protein, in the top-right corner; and (iii) H$_{35.5}$C$_{30.8}$N$_{1.7}$O$_{18.9}$ with a density of 1.7 g/cm$^3$ [29], mimicking DNA, in the bottom-right corner. Finally, (iv), an ellipsoidal DNA feature with semi-axes of 105 and 175 nm, is positioned at the bottom-left corner. Images at fluences of ten times that of Fig. 3(b) and of one tenth were simulated. For each fluence the contrast of the different features were evaluated, from which the minimum required fluence and dose to image the different 34-nm features were determined. More details of the study are provided in the Supplement 1. These results, summarized in Table 1, verify the calculations presented in the previous section, and predict that DNA and biomolecule features can be discerned at the tolerable dose below $4 \times 10^9$ Gy for this resolution.

![Fig. 3.](image)

(a) Plot of the dose required to discern a biomolecular voxel feature embedded in a 5-μm cell of water to a signal to noise level of 5 as a function of the width of the voxel in SCXM at 64 keV photon energy (red dashed line) and coherent diffractive imaging at 10 keV (continuous blue line). The black dotted line represents the maximum tolerable dose as a function of the resolution for a biological sample as reported in Ref. [23]. (b) Simulated images for SCXM and (c) coherent diffractive imaging of a cell-like object with objects of 34 nm width (Gaussian profiles with 1/e radius equal to 17 nm) at the predicted required fluxes and doses for that feature size. The dose for the coherent diffraction image is about $10^3$ higher than scanned image that makes use of all scattered photons.
4. COMPARISON BETWEEN COHERENT DIFFRACTION IMAGING AND SCXM

Current biological microscopies that use penetrating radiation of photon energy above about 1 keV primarily employ coherent techniques, such as coherent diffraction imaging, phase-contrast imaging, or ptychography. Such methods depend on the coherent scattering cross section (blue lines in Fig. 1) and provide imaging contrast based on phase effects and diffraction. These contrast mechanisms offer a significant dose advantage over absorption contrast, even though photoabsorption occurs more frequently than elastic scattering. We thus compare the performance of scanning Compton X-ray imaging to the method of coherent diffraction imaging.

The required fluence to form an image by coherent diffraction imaging has been previously estimated by considering the scattering power of a voxel of protein embedded in a surrounding matrix of water [23]. As mentioned above, we must also consider the sampling requirements of the diffraction pattern, whereby the scattered photons from that voxel must be partitioned to a greater number of detector pixels as the width of the entire diffracting object increases [25]. This introduces a reconstruction efficiency factor, which is dependent on the ratio of the size of the finest feature to be resolved and the larger object in which it is embedded. Based on this, as derived in Ref. [25], the dose at the minimum required imaging fluence for coherent diffraction imaging varies with the feature size \( d \) and object width \( w \) as \( \mu \frac{\Phi}{d^6} \). The blue line in Fig. 3(a) displays the minimum dose required to resolve biomolecular features in a 5-μm-wide water cell at 10 keV. The imaging dose is evaluated computing the surface dose [Eq. (2) Ref. [23]]. Comparing this to the maximum tolerable dose implies that the best achievable resolution for coherent diffraction imaging of this particular sample type and thickness would be about 100 nm.

To test the finding of the simple model that coherent diffraction imaging requires higher dose than Compton imaging, we simulated images of the same 5-μm-thick object used for the SCXM images in Section 3. As for the SCXM case, we invoked the dose fractionation theorem to obtain the signal (and hence required dose) from two-dimensional simulations rather than a full three-dimensional simulation of tomographic measurements. Using pixels of 3.5 nm width, the value of each pixel was taken as the projected (complex-valued) optical constants through the object for 10 keV photon energy. The width of the entire array was 10 μm, equal to twice the extent of the object itself. First, diffraction patterns of this coherently illuminated field were calculated directly from a Fourier transformation of this array, which was then discretized to photon counts for various X-ray fluences, and Poisson noise was added. The simulated noisy diffraction patterns at the different fluences were then phased to produce coherent images, via iterative phasing. In particular, iterations were performed several times in which 45 iterations of the hybrid-input-output algorithm [33] were followed by 5 iterations of the error reduction algorithm [34]. The operations of the block were repeated until the error converged to the Poissonian uncertainty simulated in the diffraction pattern.

One of the reconstructed images is shown in Fig. 3 (c) for a fluence of \( 5.24 \times 10^{15} \) ph/cm², which is that predicted to be required for discerning 34-nm PMMA features and corresponds to a dose of \( 2.68 \times 10^{12} \) Gy. The signal-to-noise ratio of the features in the image is very similar to those of the SCXM image of Fig. 3(b) simulated at \( 1.3 \times 10^{10} \) Gy dose. Further results are summarized in Table 2, which lists the minimum dose and fluence required to image the same features as described in Section 3 and can be directly compared with the SCXM case in Table 1.

Our simulations suggest that for radiosensitive materials embedded in water, imaging by SCXM at 64 keV requires a considerably lower dose than by coherent diffraction imaging at 10 keV, and thus better resolutions can be achieved within a tolerable dose to the sample by SCXM. It should also be remarked that the imaging dose for these different modalities has a different dependence on feature size \( d \), as depicted in Fig. 3(a). The dependence for SCXM is \( L/d^4 \), where \( L \) is the thickness of the object, whereas the dose for coherent diffraction imaging depends on \( w^2/d^6 \), where \( w \) is the width. Thus, as seen in Fig. 3(a), the advantage for SCXM increases quadratically as the feature size is reduced. This advantage also increases linearly with the sample size, assuming a roughly spherically shaped object. Specifically, the dose required to discern 34-nm biomolecular features with coherent diffraction imaging is up to three orders of magnitude higher than the dose required with SCXM. This difference in dose is due to the fact that SCXM optimizes the number and utility of the scattered photons available for a given dose to the sample.

5. CONCEPTUAL SETUP

As with all scanning X-ray microscopies, the spatial resolution in SCXM is determined by the spot size that an X-ray beam can be focused to. This requires a high-resolution lens that must be coherently illuminated by X-rays of a photon energy of about 60 keV. To achieve the coherence, and a high enough fluence to acquire images in a reasonable time, therefore requires a high-brightness source of this photon energy, such as will be produced by undulator radiation in upcoming diffraction-limited high-energy synchrotron light sources [15]. Meeting the damage-limited resolution in biological samples of a few tens of nanometers, as discussed in this paper, needs a lens capable of producing a focal spot of about 10-nm width. The most suitable devices for achieving such spot sizes at these photon energies and with high efficiency are multilayer Laue lenses (MLLs) [35]. These are volume zone plates, made by cutting a structure from

| Sample | \( \rho \) [cm\(^{-3}\)] | \( \Phi' \) [ph/μm²] | Dose (Gy) |
|--------|-----------------|------------------|----------|
| PMMA   | 1.18            | 5.41 × 10¹⁴      | 13.4 × 10⁹ |
| Biomolecule | 1.15            | 1.15 × 10¹⁴      | 3.63 × 10⁹ |
| DNA    | 1.70            | 2.83 × 10¹⁵      | 1.04 × 10⁹ |

| Sample | \( \rho \) [cm\(^{-3}\)] | \( \Phi' \) [ph/μm²] | Dose (Gy) |
|--------|-----------------|------------------|----------|
| PMMA   | 1.18            | 5.24 × 10¹⁵      | 2.68 × 10¹² |
| Biomolecule | 1.35            | 1.43 × 10¹⁵      | 1.16 × 10¹² |
| DNA    | 1.70            | 3.72 × 10¹⁴      | 4.24 × 10¹¹ |
a film made from many thousands of alternating layers of materials created by sputter deposition. The layer thickness and tilt is created to vary throughout the stack in such a way to ensure that diffracted rays meet at a common focus. A 10-nm-resolution lens at 60 keV (0.02 nm wavelength) has a numerical aperture of about 0.0012 [see Eq. (2)]. The required techniques to fabricate such lenses have recently been demonstrated [36,37].

Being diffractive lenses, the focal length of a MLL varies inversely with wavelength, as shown schematically in Fig. 4(a). The maximum relative bandwidth $\Delta \lambda/\lambda$ of the illumination to avoid chromatic aberration (that is, a spot size larger than achievable with monochromatic radiation) is equal to the inverse of the number of layers that make up the lens. Between $10^3$ and $10^5$ layers are needed to produce a 10-nm focal spot from a lens with a reasonable focal length and working distance of about 1 cm. This implies that the source illumination would be limited to a relative bandwidth between $10^{-3}$ and $10^{-5}$. To utilize the entire bandwidth of a particular harmonic of the undulator source ($\Delta \lambda/\lambda \sim 10^{-2}$), which would speed up image acquisition by several orders of magnitude, we therefore propose to use an X-ray achromat [38] to focus the beam.

An achromatic lens combines materials with differing dispersions to achieve a common focal length for two particular wavelengths. In the X-ray regime, Wang et al. proposed combining diffractive and refractive optics and relied in particular on the rapid variation of refractive index near an absorption edge [38]. More generally, it is possible to combine a compound refractive lens [39] with a diffractive optic. Given focal lengths $f_d$ and $f_r$ of the diffractive and refractive lenses that make up the pair, in the paraxial approximation, the achromatic condition is obtained for two lenses in contact when $f_d V_r = -f_r V_d$ and where $V_d$ and $V_r$ are the dispersive powers of the lenses, $V = (\Delta f/f)/(\Delta \lambda/\lambda)$. For a diffractive and refractive pair at high energies and away from absorption edges of the materials of both lenses, the ratio $V_r/V_d \approx 2$ [40]. Thus it is seen that an achromat can be achieved when the focal lengths of the lenses have opposite sign. It may be convenient to choose the compound refractive lens as having the negative (diverging) focal length, which would consist of a line of spheres, each centered on the optic axis. For example, a stack of 2000 negative compound refractive lenses of Be or Al at 64 keV, each with a radius of 55 μm, would compensate the chromatic aberration produced by a MLL with 10-nm resolution and 20-mm focal length, for $10^{-2}$ relative bandwidth. The proposed achromat, consisting of a set of negative refractive lenses and a positive MLL, is depicted in Fig. 4(b). Since there is little or no gap between the lenses and the focal length of the refractive lens is twice that of the MLL, the increase of the size of the beam illuminating the MLL is negligible.

We have assumed in our calculations of the achievable SCXM signals that all the scattered photons outside the main focused X-ray beam are efficiently detected over a 4π steradian solid angle. A suitable detector sensor material for the high photon energies needed for SCXM is CdTe, which gives efficiencies over 90% up to 100 keV [41]. With energy resolving elements, the detector could also be used to simultaneously map fluorescence from the sample [42]. A proposed design is shown in Fig. 4(c). We include in this design a means to reduce the background contribution of Compton scattering from air without having to use a vacuum environment, which would otherwise increase the complexity of the detector and sample preparation. Much of the scattering of the primary beam can be shielded from the detector with a small-diameter thin-walled beampipe that encloses the focused beam and which extends as close as possible to the sample [43].

With the conceptual design presented here, and the calculations in Section 3 we estimate image acquisition times for the expected coherent flux of PETRA IV of $10^{12}$ ph/s at 0.1% bandwidth, and the achromatic focusing system providing a $10 \times 10$ nm² spot with a 50% efficiency. Under this condition, images of 30-nm DNA and protein features embedded in 5-μm thickness of water can be acquired with dwell times of approximately 7 μs and 20 μs, respectively.

6. CONCLUSIONS

We have presented a concept for a scanning Compton X-ray microscopy (SCXM), which exploits all the scattered photons from the sample, including coherent and incoherent scattered photons, to form high-resolution images of radiosensitive samples at tolerably low doses. The study presented here shows that by detecting Compton scattering from the sample upon illumination by an incident focused beam of 64 keV photon energy, this technique can image features at higher resolution and lower dose than can coherent X-ray imaging techniques, which are optimized at about 10 keV. We find from a model and detailed simulations that 30-nm resolution images of biological objects can be obtained at $10^{-3}$ of the dose required for coherent imaging. Our proposed scanning microscopy technique could take advantage...
of new high-energy synchrotron light sources based on the multibend-achromat [15] that will provide large coherent fluxes at photon energies above 50 keV. This can be combined with new technologies for hard X-ray nanofocusing [13,14,36,37], to achieve dose-optimized and fast X-ray imaging. An advantage of the high photon energy used for SCXM is that the attenuation length of biological material is large, as is the depth of focus. For example, the attenuation length for cellular material at 64 keV is about 10 cm, and thus the transmission through an actual cell is essentially 100%. Furthermore, a lens with 10-nm resolution achieves dose-optimized and fast X-ray imaging. An advantage of energies above 50 keV. This can be combined with new bend-achromats [15] that will provide large coherent fluxes at photons.

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