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# Fourth-generation X-ray sources: some possible applications to biology

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The term 'fourth generation X-ray sources' has come to mean X-ray free-electron lasers which use multi-GeV electron beams from linear accelerators to generate X-rays by self-amplified stimulated emission when fired into long undulators. Properties of the radiation expected from such sources are reviewed briefly and two possible applications of the resulting pulsed highly collimated X-radiation to problems in biology are discussed: use of X-ray photon correlation spectroscopy to measure time correlations of atoms in protein crystals, and use of Mössbauer radiation extracted from the photon beams by resonant Bragg diffraction from $^{57}$Fe-containing crystals, for MAD phasing of very large unit-cell biomolecular crystals and possibly for photon echo measurements.

**Keywords: X-ray lasers; X-ray photon correlation spectroscopy; thermal diffuse X-ray scattering; protein crystallography; Mössbauer radiation; MAD phasing; X-ray photon echo measurements.**

## 1. Introduction

The term 'fourth-generation X-ray sources' has come to mean the application of X-ray free-electron lasers (XFELs) to the production of extremely highly collimated pulsed X-ray beams which would exceed the brilliance of currently available X-rays from third-generation sources, *i.e.* high-brightness storage rings such as those at ESRF (Grenoble), APS (Argonne) and SPring-8 (Kyoto), by several orders of magnitude. Unlike infrared FELs, where the resonant cavity can be pumped by the train of electron pulses, an X-ray FEL has to operate on a new principle – that of self-amplified spontaneous emission (SASE) in which the spontaneously emitted X-ray radiation from an electron bunch in an undulator becomes strong enough to modulate the electron density in the bunch in such a way as to further amplify the spontaneous emission in phase with the originating X-ray radiation field. Currently this concept is being tested on multi-hundred MeV electron linacs (see Table 1) and there are plans to build an X-ray FEL using multi-GeV electron bunches from the Stanford Linear Accelerator (the Linear Coherent Light Source; LCLS) and from a proposed multi-GeV superconducting linac being planned by the DESY Laboratory in Hamburg in connection with their plans for a particle physics linear collider.

How can such fourth-generation sources be applied to produce new X-ray science? The scientific case for such sources has been the subject of much work in the last two or three years and is summarized on the DESY web site (http://www.desy.de/~wroblewt/scifel/). Given the vast scope of current X-ray science based on second- and third-generation sources, it is clear that there are a very wide number of scientific fields to which fourth-generation sources will bring new capabilities. In this paper, after summarizing some of the key properties of the new sources, I focus on two possible applications of biological interest: the application of X-ray photon correlated spectroscopy (XPCS) to the study of Brownian motion of biomolecules in crystals and the application of Mössbauer radiation to protein crystallography.

## 2. Key properties of X-ray FEL radiation

The plans for X-ray FELs at SLAC and DESY call for electron bunches in the 10–25 GeV energy range (up to 15 GeV at SLAC) to be sent through a 100 m undulator in order to generate SASE radiation. The resulting radiation will have extremely high lateral coherence based on the fact that the undulator radiation opening angle varies as $n^{-1/2}$, where $n$ is the number of undulator periods. This will result in a 1 μrad opening angle to be compared with the $1/\gamma$ = 36 μrad divergence from an equivalent wiggler. In contrast to this extreme lateral coherence the temporal (or longitudinal) coherence will be relatively poor since the time structure of the beam will be broken up into a number of spontaneous micropulses (Bonifacio *et al.*, 1994), each of duration less than 1 fs. The total pulse duration will be of the order of 280 fs (composed of many such micropulses) with a total X-ray energy per pulse of 2 mJ, equivalent to $\sim 2 \times 10^{12}$ photons per pulse in a bandwidth of ~30 eV at 8 keV. This peak power will correspond to an electric field in the unfocused X-ray beam of around 3 V Å$^{-1}$. This is getting close to the ionization threshold for atoms and, when focused, the beam can very well vaporize a sample in a matter of femtoseconds (see Doniach, 1996). The plans

**Table 1**
Timelines for SASE experimental facilities to reach saturation at shorter wavelengths.

| Laboratory | λ | Target dates |
|---|---|---|
| VISA (visible to infrared SASE amplifier): BNL–LANL–LLNL–SLAC–UCLA collaboration undulator built and installed in the accelerator test facility at NSLS (4 m long, strong focusing) | | |
| SASE saturation experiment | 8–0.6 μm | Early 2000 |
| Source Development Laboratory (SDL) at NSLS: 210 MeV linac, SASE demonstration | 300 nm | 2000 |
| LEUTL at ANL: uses part of the APS linac | | |
| Some gain seen | 0.53 μm (218 MeV) | 2000 |
| Eventually down to | 0.12 μm (440 MeV) | |
| Mid-infrared saturated amplifier (MISA) at LANL: capitalizes on availability of existing hardware at AFEL | 19 μm | Early 2000 |
| TTF-FEL at DESY | | |
| Phase 1 | 390 MeV, 420 Å | First operation 2000 (some gain seen already at 109 nm) |
| Phase 2 | 1000 MeV, 60 Å | Start commissioning in March 2002 |
| TESLA (DESY linear collider) | 1 Å | 2010 (?) (proposed) |
| SLAC (LCLS) | 1.5 Å | 1999–2002 R&D (funded) |
| SLAC (LCLS) | 1.5 Å | 2003–2005 construction (proposed) |

are to generate the X-radiation in the form of pulse trains. The SLAC FEL plans call for 112 pulses per train while the DESY plans call for $10^4$ pulses per train, which is feasible for them because of the superconducting nature of the RF cavities in the proposed DESY linac. The repetition rate for pulse trains will be 120 Hz at SLAC. The transverse size of the laterally coherent radiation emerging from the undulator will be of the order of 70 μm. These properties are summarized in Table 2.

## 3. Use of XPCS for time-dependent correlation measurements in protein crystals

The idea of XPCS is to extend to the X-ray regime the techniques used in dynamic light scattering. However, in contrast to the light-scattering case in which optical laser beams with very high longitudinal coherence may be used to perform the heterodyne spectroscopy, the short longitudinal coherence of the X-ray beam necessitates the use of post-detection interferometry, following the principles established in astronomy by Hanbury-Brown and Twiss. Here one simply measures the deviation of the scattered intensity, $I(t)$, from the time average value, $\langle I\rangle$, by counting individual photons and then electronically multiplying together the photon counts separated by time interval $t$ to generate the time correlation function of X-ray intensity deviations, $\delta I(\vec{q}) = I(\vec{q}, t) - \langle I(\vec{q})\rangle$,

$$c(\vec{q}, t) = \left\langle \delta I(\vec{q}, 0)\, \delta I(\vec{q}, t)\right\rangle. \quad (1)$$

The advantage of XPCS in contrast with visible-light correlation spectroscopy is that the correlations may be generated in a given scattering wavevector channel so that one can work back to directly measure spatially resolved correlations in addition to the temporal resolution coming from the time dependence of the correlation functions.

This technique is, of course, not dependent on the existence of a fourth-generation source and has been applied at third-generation sources to a number of problems in materials science and soft condensed matter physics (Mochrie *et al.*, 1997; Price *et al.*, 1999). However, a major advantage will be obtained at fourth-generation sources since the correlation function, $c$, depends on the square of the scattered intensities so that there will be a big advantage in having sources in which the average X-ray power is concentrated into very short pulses as opposed to storage-ring sources where the equivalent power is spread out into many more weaker pulses on the microsecond time scale for circulation of the electron bunches in the storage ring (see Doniach, 1996).

However, there will be an additional problem with the X-ray FEL sources as presently conceived because of the stochastic nature of SASE, in which micropulse initiation is a stochastic event so that the total power per pulse is expected to be quite noisy. One way around this, which will benefit from the very high lateral coherence of the beam, will be to use X-ray delay lines based on Bragg scattering in which the X-rays from a given pulse are split into two beams, one of which is delayed and the other direct, so that the resulting scattered intensity is a direct measure of the correlations as a function of the delay time. A microsecond delay line would be about 30 m in length, which is perfectly feasible given the microradian divergence of the X-ray beam.

How would the resulting time correlations be useful in protein crystallography? Basically, application of this technique will simply be a generalization of thermal diffuse scattering in which scattering intensity is measured away from the Bragg peaks. In the XPCS measurement the time correlations of this thermal diffuse scattering would be measured directly, thus enabling a measurement of time correlations of the Brownian motion for individual atoms in the protein molecule. Note that this is a very different measurement from the time-dependent measurements currently performed in protein crystallography in which a

**Table 2**
Comparison of projected performance of the LCLS and DESY XFELs.

| | LCLS | DESY |
|---|---|---|
| FEL wavelength (Å) | 1.5 | 1 |
| Bandwidth ($\delta E/E$) | 0.003 | 0.002 |
| Pulse duration (fs) | 280 | 200 |
| Peak coherent photons per pulse | $\sim 2 \times 10^{12}$ | |
| Peak brightness [flux mm$^{-2}$ (0.1% bandwidth)$^{-1}$] | $10^{33}$ | $3.5 \times 10^{33}$ |
| Peak EM field (unfocussed) (V Å$^{-1}$) | 3 | |
| Repetition rate (Hz) | 120 | 50 |
| Transverse size of coherent beam at exit (μm) | 70 | 28 |
| Divergence of coherent radiation (μrad) | 1 | 1.4 |
| Number of bunches per train | 112 | $10^4$ |

conformational change of the protein is triggered by a photo event, either directly acting on a chromophore in the protein itself (Genick *et al.*, 1997), or a photodissociation event such as in CO-myoglobin experiments (Teng *et al.*, 1997), or photorelease of a chemical agent leading to subsequent change of protein conformation (Duke *et al.*, 1994; Perman *et al.*, 1998). In all these events a change in the molecular conformation is then measured by time-resolved crystallography of the Bragg peaks themselves as a function of time elapsed from the triggering event. Use of XPCS for thermal diffuse scattering, on the other hand, simply looks at the natural Brownian motion of the atoms in the protein molecule without any external triggering. Of course, some combination of these two could be thought of for future experiments, such as monitoring changes in Brownian motion during a chemical reaction, for instance.

One of the advantages of the thermal diffuse scattering experiment is that, in principle, the background is zero (although in practice crystal defects will lead to some static disorder which will appear as a background) so that most of the photons scattered are directly measuring the thermal motions in the crystal. Then, if the Brownian motions are considered as purely harmonic so that the resulting correlation functions are purely Gaussian in character, it may be shown that the intensity–intensity correlation function can be rewritten as the square of the density–density correlation function, just as in the case for Brownian motion in liquids as studied by dynamic light scattering,

$$c(\vec{q}, t) \cong \left|\left\langle \rho_{\vec{q}}(t), \rho_{-\vec{q}}(0)\right\rangle\right|^2, \tag{2}$$

where we have used $I(\vec{q}, t) \equiv |\rho_{\vec{q}}(t)|^2$.

Finally, by expanding to lowest order in the atomic displacement it may be then shown that one can measure directly the pair correlation functions between individual atoms in the protein molecule (including the autocorrelation functions) as in

$$\begin{aligned}\left\langle \rho_{\vec{q}}(t), \rho_{-\vec{q}}(0)\right\rangle &= \left\langle \sum_i \exp\left[i\vec{q}\cdot\vec{r}_i(t)\right] \sum_j \exp\left[-i\vec{q}\cdot\vec{r}_j(0)\right]\right\rangle \\ &\cong \delta(\vec{q}, \vec{G}) + \sum_{ij} \exp\left[i\vec{q}\cdot(\vec{X}_i - \vec{X}_j)\right] \\ &\quad \times \left\langle u_i(t), u_j(0)\right\rangle, \end{aligned} \tag{3}$$

where $\vec{G}$ are the Bragg vectors, $\vec{r}_i(t)$ are the atomic positions at time $t$, $\vec{X}_i$ are their average positions, $\vec{u}_i$ their displacements, and we have set the atomic form factors to unity for simplicity.

To illustrate how this information could be useful in practice, we consider a couple of recent examples, one from static measurements of Brownian motion in crystals of calmodulin and a second one from $^{15}$N NMR measurements of dynamic motions in the HIV protease molecule.

A recent study by Wall *et al.* (1997) illustrates the usefulness of thermal diffuse scattering for understanding intramolecular Brownian motion in proteins. They report results of X-ray diffraction from crystals of a calmodulin-peptide complex. Uncomplexed calmodulin has the form of two calcium binding domains joined by a rather long linker helix. In the peptide complex the two domains are pulled around to bind to the peptide, and the linker helix is bent into an elbow shape. The crystallography data show that there is quite a bit of flexibility in the complex and the authors fit the Bragg peak intensities with a multiple copy refinement method which shows strong thermal variations, not only in the flexible linker but also in other non-helical domains, including three of the four calcium binding sites.

They then go on to look at the diffuse scattering pattern and analyze this in terms of a liquid-like model in which real-space correlations decay exponentially. The important result from this analysis is that the motions of the two domains at the end of the linker are rather weakly correlated with a correlation length of $\sim$50 Å along the linker direction. Furthermore, motions are primarily along the head-to-tail packing directions of the unit cell and motions in one molecule strongly influence the movements of neighbouring molecules along these same directions.

Clearly, since the measurements reported by these authors are static measurements averaged over long time periods, they do not contain any information about the time dependence of the various motions seen in this study. One way to obtain the time scale of intramolecular motions in proteins is by the use of $^{15}$N NMR. NMR measurements are normally made on a time scale of a few nanoseconds, so that time-dependent correlations in proteins which are extracted using the 'model free' approach generally provide information about Brownian motions on these nanosecond time scales. However, the effect of 'chemical exchange' can lead to splitting of the $^{15}$N NMR lines as a function of conformational change in a protein. Thus the NMR can sample two different conformations and, provided the measurements are made on a time scale slower than the time for the conformational transition, two separate $^{15}$N lines will show up corresponding to changes in chemical shifts between the two conformational states. (This assumes that the chemical shifts in the two conformational states are sufficiently different to show up on the time scale of the conformational changes.) A recent study on HIV protease (Freedberg *et al.*, 1998) has highlighted this conformational change effect. The wings of this protein which are involved in the substrate binding site of the

protease are quite mobile and NMR studies show that they move on the microsecond-to-millisecond time scale.

Since XPCS has the capacity to measure atomic correlations certainly out to the microsecond time scale (see above), and possibly beyond, this will add a significant tool to understanding motions in proteins and their importance in protein functions such as substrate binding, enzyme action *etc*.

## 4. The X-FEL as a source of Mössbauer radiation

The possibility of collimated X-ray beams with the extraordinarily fine energy resolution of the decay of a Mössbauer nucleus, of the order of $10^{-8}$ eV, presents some very attractive features for biophysical studies. In particular, the change of the real part of the scattering amplitude $f'$ on going through the Mössbauer resonance of $^{57}$Fe is of the order of $500\,e^-$, to be compared with $\sim 20\,e^-$ for the lanthanide $L$-lines. This offers an extremely attractive potential for MAD phasing of crystals of large macromolecules, particularly those which do not possess pseudo-rotational symmetry such as viruses.

Another interesting possibility is that of extending photon echo experiments, such as those currently being performed using infrared FELs (Rector *et al.*, 2000), to X-ray wavelengths.

Because of the poor longitudinal coherence of the XFEL source, only a very small fraction, *i.e.* $10^{-8}$ eV in the 30 eV line width of the source, around one part in $10^9$, of the X-rays fall into the Mössbauer wave band. In terms of the average X-ray flux delivered by the XFEL, this amounts to about $10^7$ Mössbauer photons per second from the LCLS (which would have to run in the third undulator harmonic to reach this X-ray energy) and, because of the superconducting functioning of the proposed DESY X-ray FEL, about $10^9$ photons per second from the latter source.

How would the Mössbauer radiation be filtered out from the background, which is made up of about $10^9$ times more non-Mössbauer X-rays? One method would be by diffraction from a $^{57}$Fe crystal such as yttrium iron garnet, which has been studied by several groups (Ruffer *et al.*, 1987; Baron *et al.*, 1994; Chumakov *et al.*, 1993). The interesting physics which occurs here is that when the scattering angle is adjusted so that a Bragg peak falls exactly on the Mössbauer frequency of 14.4 keV, then a standing wave is set up in the crystal which oscillates at the Mössbauer frequency and is in phase at every $^{57}$Fe nucleus in the crystal. [The theory of this has been worked out in detail by Trammell & Hannon (1978).] Because of the existence of the standing wave, the incoming X-rays can induce a superradiant state in the Mössbauer nuclei when all the effective radiating nuclear dipoles are in phase with each other. This has the effect of a flywheel which would convert the incoming very short pulses of radiation in the XFEL into highly coherent wave trains on the scale of 100 ns or so. It is interesting to think about the coherence properties of such a source. Since each micropulse produced by the SASE mode will be coherent over the 1 fs or so time scale of the micropulse (*i.e.* about 1000 oscillations at the X-ray frequency), the superposition of a series of such micropulses will still retain some coherence properties since the coherent amplitude of the superposition of random wave trains will scale as the square root of the number. Thus a train of 100 or so micropulses making up a given X-ray pulse will have a coherent amplitude corresponding to 10% of the total X-ray flux. In principle, this will allow for modulation of the Mössbauer wave trains emitted by the super-radiant state of the YIG crystal, for instance, by applying time-varying magnetic fields which will shift the nuclei in and out of resonance with the coherent wave train on a nanosecond time scale. This could then allow for experiments where Mössbauer nuclei in a target sample can be hit with a series of pulses at the X-ray emission frequency of the nuclear dipoles, which could conceivably lead to phenomena such as photon echo (Mukamel, 1995; Rector *et al.*, 2000). For instance, one could imagine looking at time-dependent conformational changes in a macromolecule by measuring the resonant energy transfer of Mössbauer radiation between two Fe atoms in the molecule.

The use of a $^{57}$Fe YIG crystal also raises another interesting possibility: that of using non-linear nuclear scattering to perform frequency doubling of a 7.2 keV X-ray beam to the Mössbauer frequency of 14.4 keV. The strength of such a frequency doubling may be estimated by considering the effect of a non-linear term in the Hamiltonian interaction between the Mössbauer oscillators and the radiation field of the form

$$\mathcal{H}_{\rm int} = (e^2/\delta\varepsilon) \sum_i \vec{A}(r_i)^2. \qquad (4)$$

Here, $\delta\varepsilon$ has been inserted as a parameter to represent the order of magnitude of the energy needed for an internal transition which will act as an intermediate in a two-photon summation to the Mössbauer radiating level. Under the Mössbauer–Bragg condition discussed above where a standing wave is produced in which all Mössbauer nuclei radiate in phase, the (electromagnetic field)$^2$ term will combine two photons of wavevector $\vec{k}_1$ and $\vec{k}_2$ satisfying the condition $|c\vec{k}_1 + c\vec{k}_2| = \omega_{\rm M}$, where $\omega_{\rm M}$ denotes the Mössbauer frequency. Because of the very high transverse coherence of the XFEL beam, the effects of deviation from the forward direction, $\delta\vec{k}_\perp$, which could lead to deviations from fulfilling this condition, will be very small. Thus the effect of this interaction term may be rewritten in terms of the interaction of the single resultant Mössbauer photon with the radiating dipoles multiplied by the strength of the electric field at half the Mössbauer frequency,

$$\mathcal{H}_{\rm int} \simeq \frac{|eE_{7.2}|}{\delta\varepsilon/\lambda} \sum_i \exp\left(i\vec{k}_M \cdot \vec{X}_i\right) e\dot{\vec{u}}_i \cdot \vec{A}(X_i), \qquad (5)$$

plus a similar term for the Bragg-reflected wave in the standing-wave combination. Here we have used $\vec{u}_i$ to

denote coordinates of the nuclear dipoles in a very simplified representation.

Since the field strength emerging from the XFEL is of the order of 1 V Å$^{-1}$, then, provided the non-linear energy parameter $\delta\varepsilon$ is of the order of 10 keV, *i.e.* close to the Mössbauer energy itself, the factor $|eE_{7.2}|/(\delta\varepsilon/\lambda)$ will be of the order of $10^{-4}$, and this will lead to a photon beam of roughly the same intensity as an equivalent Mössbauer photon beam at 14.4 keV (since the beam intensity varies as the square of the interaction strength). The point here is that *all* the photons in the incoming X-ray pulse can contribute to the outgoing Mössbauer frequency since the condition $|c\vec{k}_1 + c\vec{k}_2| = \omega_M$ can be satisfied for any given photon $\vec{k}_1$ in the 7.2 keV X-ray pulse. Thus this frequency-doubling mechanism is a kind of flywheel for compressing a fraction {of the order of $[|eE_{7.2}|/(\delta\varepsilon/\lambda)]^2$} of essentially all the longitudinally incoherent photons within the bandwidth of the 7.2 keV X-ray pulse into the highly coherent $10^{-8}$ eV-wide coherent Mössbauer beam at 14.4 keV. Thus even though the non-linear frequency addition amplitude is very low, of the order of $10^{-4}$, under the above assumptions about $\delta\varepsilon$ for the field amplitude, *i.e.* $10^{-8}$ for the intensity, it can still be potentially competitive with the direct Mössbauer scattering process. Clearly this suggestion is highly speculative since at the present time the conversion efficiency represented by the parameter $\delta\varepsilon$ is not known. Nevertheless, it seems like a worthwhile potential to follow up, particularly since there are a variety of Mössbauer nuclei available and it may be that some of them are more efficient at frequency doubling than others.

## 5. Concluding remarks

As may be seen from the above two examples, the fourth-generation X-ray sources have the potential to produce new and significant information in structural molecular biology and can potentially complement neutron scattering sources by extending the time range for obtaining information about the dynamics of internal motions of biomolecules.

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