Meeting Report

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Future structural biology applications with a free-electron laser – more than wild dreams?†

Matthias Wilmanns

EMBL Hamburg Outstation c/o DESY, Notkestrasse 85, D-22603 Hamburg, Germany.
E-mail: wilmanns@embl-hamburg.de

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A workshop entitled *Potential Future Applications in Structural Biology of an X-ray Free-Electron Laser at DESY* was held at Hamburg, Germany, on 4–8 July 1999. The aim of the workshop was to identify and discuss potential applications in structural biology using the specific beam properties of the planned X-ray free-electron laser at DESY, Hamburg. The workshop focused on proposals in X-ray crystallography, spectroscopy, microscopy and holography. In the discussions during the workshop, the response of biological material to the expected high photon flux and its time dependence played a central role. Technological aspects in data recording and image interpretation were covered as well. There was general agreement that an X-ray free-electron laser source with its specific source parameters could offer unique opportunities for novel experiments in structural biology, complementing current synchrotron facilities. The realization of future applications will be challenged by the handling of large high-energy doses on the biological specimen and the development of equipment capable to respond to a pulse time structure in the femtosecond range.

Keywords: free-electron lasers; radiation damage; structural biology; peak brilliance; time resolution.

1. Introduction

The research centre DESY in Hamburg is planning to enter a new era of high-energy particle physics by building a linear collider, called TESLA (TeV superconducting linear accelerator), with a proposed centre-of-mass energy of 500 GeV. By making use of superconducting cavities, very high luminosity is expected to be reached with this 35 km-long linear collider. A central part of this project will be the incorporation of an integrated free-electron laser in the hard X-ray regime at ∼1 Å (X-FEL). Another X-FEL, known as the LCLS (linac coherent light source), is planned at SSRL, Stanford, and is expected to deliver X-rays at ∼1.5 Å. The planned X-FELs are anticipated to provide laser radiation with unprecedented characteristics in terms of average photon flux and brilliance, full transverse coherence, sub-ps pulse length and fast tunability. Therefore, they could offer radical technological advances in diffraction, scattering, spectroscopy, microscopy and quantum optics. Most of these techniques have important applications in biology. Diffraction methods, for instance, are now massively employed for structure determination of biological macromolecules and will play a central role in the emerging projects for functional and structural elucidation of entire genomes. Other important areas will be the elucidation of structures of above-molecular assemblies and complexes, complementing functional studies to analyse cellular processes (described by Iain Mattaj, EMBL, Heidelberg) as well as biophysical studies on protein folding and structures (described by Hans Frauenfelder, Los Alamos). To discuss potential future applications with an X-FEL in biology an International EMBO-funded workshop was organized by the EMBL Hamburg Outstation (Matthias Wilmanns, Paul Tucker, Victor Lamzin), jointly with DESY/HASYLAB (Jochen R. Schneider). The workshop was arranged in nine sessions (Table 1) including an introductional presentation of the TESLA project, putative applications in biology, processing of these expected data, and on the anticipated behaviour of biological material under X-FEL conditions. The workshop was attended by 95 scientists from 13 countries (Fig. 1).

A paperback book, covering the transparencies of most speakers at the workshop, will be available upon request by writing to the EMBL Hamburg Outstation, c/o DESY, Notkestrasse 85, 22603 Hamburg, Germany, or by e-mail to wilmanns@embl-hamburg.de.

2. The X-FEL project

Coherent stimulated emission from a free-electron laser (FEL) is generated by longitudinal density modulations of a bunched electron beam within an undulator. This process is known as self-amplified spontaneous emission (SASE) and occurs if the undulator resonance condition, determined by the photon wavelength, undulator parameters and by the electron beam, is met. The SASE principle does not require the optical cavity resonator, used in multipass longer-wavelength FELs, and can therefore generate radiation at high energies in the 10 keV (1 Å) regime. The technical feasibility of the SASE effect, however, still remains to be proven for the X-ray energy regime. The principal components needed for an X-FEL are: electrons from an RF photocathode gun; bunch compressors, reducing the electron bunch length; linear electron accelerators and appropriate undulators in SASE mode. The intensity (I_{ph}) of an SASE-generated FEL is approximately $I_{ph} \sim N_{el}^2$, where N_{el} is the number of electrons per bunch. Since the intensity of undulator-generated synchrotron radiation is $I_{ph} \sim N_{el}$, there is a gain in intensity, which is proportional to the number of electrons per bunch for the FEL, allowing peak intensities several orders of magnitudes higher than available from undulators at current synchrotron facilities.

In the DESY concept (reported by Jörg Rossbach, DESY, Hamburg), the X-FEL is integrated into the TESLA linear collider, where two electron beams are planned to branch off the main linear electron beam accelerator at 10–25 GeV and 20–50 GeV, feeding a number of FEL undulators (Table 2, Fig. 2). In the TESLA design the RF gun will operate at a low frequency of 10 Hz in an interleaved mode allowing alternating pulses for high-energy physics and for FEL operation. One FEL 'bunch train' is planned to consist of 11315 bunches, where each bunch is 80 fs (r.m.s.) long and separated by 93 ns, resulting in a bunch-train length of 1050 µs. The spectral photon flux of an FEL undulator has two components, with the FEL line generated in SASE mode,

† This article is *in memoriam* of Dr Werner Meyer-Ilse, who was an invited speaker at the workshop. He died in a tragic car accident in Göttingen, Germany, on 10 July 1999.

and a background of spontaneously emitted undulator radiation. It is anticipated that the time-average brilliance could be up to 10^{26} photons s^{-1} $mrad^{-2}$ mm^{-2} (0.1% bandwidth)$^{-1}$, exceeding current synchrotron radiation sources by about six orders of magnitude. The pulsed FEL peak brilliance, which has a very small energy bandwidth, could even be up to 10^{34} photons s^{-1} $mrad^{-2}$ mm^{-2} (0.1% bandwidth)$^{-1}$. The r.m.s. spot size at the undulator exit will be ~25 µm and the r.m.s. beam divergence will be of the order of 1 µrad. This very small emittance can be achieved in a LINAC because the emittance decreases with increasing electron energy. The timetable of the TESLA-XFEL depends on the approval of the TESLA concept by DESY, which is expected for 2003. Further details are described in a DESY report by Brinkmann *et al.* (1997). The other plan for an X-FEL, the LCLS project, was presented by Keith Hodgson (SSRL, Stanford). The main difference between the LCLS and the TESLA projects is in the LINAC technology and the time structure of the FEL radiation. The LCLS-FEL is considered as a first bid for an FEL in the true Ångström regime. Construction of the LCLS-FEL is expected for the years 2003–2005. For further information, see http://www-ssrl.slac.stanford.edu/lcls/.

Currently, DESY is building an FEL test facility, known as the TESLA test facility (TTF). Thomas Möller (DESY, Hamburg) reported that the TTF will be operated at 390 MeV in the first phase, generating radiation with wavelengths down to $\lambda = 42$ nm. During a subsequent second phase the desired wavelength will be $\lambda = 6$ nm at an acceleration energy of 1 GeV. User operation of this facility is planned to start in 2003. In addition, DESY is gathering experience with this facility on many components essential for the planned X-FEL. The development of novel superconducting cavities for the linear acceleration process with gradients up to 25 MV m^{-1} is critical for the planned TESLA project. The facilities, currently under construction, were visited by the workshop participants.

Gerd Materlik's (HASYLAB/DESY, Hamburg) contribution set up an ideal stimulus to discuss future applications of the X-FEL at the workshop by showing a series of impressive design slides. To restrict thinking to the current scientific activities might lead to what he called 'trivial extensions', but not to radically new ideas desired for the future X-FEL. Therefore 'wild dreams' (Jochen Schneider, HASYLAB/DESY) that could lead to 'unexpected surprises' were not only allowed but were actually desired during the workshop to stimulate further discussion.

Figure 1
Workshop participants flocking together in front of the DESY bistro.

Table 1
Sessions at the workshop.

Title	Chair
(1) The TESLA project with an integrated X-FEL	J. Schneider (Hamburg)
(2) Radiation damage	C. Kratky (Graz), P. Trucker (Hamburg)
(3) Image interpretation	M. v. Heel (London), V. Lamzin (Hamburg)
(4) Time-resolved crystallography in the femtosecond range	I. Schlichting (Dortmund), H. D. Bartunik (Hamburg)
(5) Spectroscopic applications	L. Tröger (Hamburg), W. Meyer-Klaucke (Hamburg)
(6) X-ray microscopy	G. Margaritondo (Lausanne)
(7) Biocrystallography	Z. Dauter (Brookhaven)
(8) Data recording	C. Brönnimann (Villigen)
(9) Holographic methods	A. Szöke (Livermore), G. Materlik (Hamburg)

3. Biological samples in the FEL

When discussing potential applications in biology with an X-FEL there is widespread concern by many scientists about the sample survival during such experiments, expressed as 'harsh reality' (Elspeth Garman). Indeed, the now available third-generation synchrotron facilities allow a detailed characterization of biological material when irradiated with a high-brilliance X-ray beam. Raimond Ravelli (ESRF, Grenoble) showed an interesting video displaying the breakdown of disulphide bridges in some test proteins during progressive doses of photons. Klaus Sokolowski-Tinten (Essen) went one step further by summarizing experimental experience on non-biological material exposed to laser-plasma X-ray sources with intensities of the order of 10^{17}–10^{19} W cm^{-2}. These intensities approach the expected experimental conditions of an X-FEL, however, in a different wavelength range. He illustrated the melting process of Ge and Si as a function of time under high-dose conditions, by analysing their (111) Bragg reflections.

There were three other speakers, two from the field of X-ray crystallography (Elspeth Garman, Oxford; Colin Nave, Daresbury) and one from X-ray imaging (Chris Jacobson, Stony Brook), who provided descriptions of the behaviour of biological material under strong radiation loads. Their discussion was based on the analysis of experimental observations, and on extrapolations from these data to higher doses. Elspeth Garman referred to the first systematic studies on radiation damage in proteins almost four decades ago (Blake & Phillips, 1962). In their calculation, for each 8 keV photon (Cu $K\alpha$ radiation) absorbed, disruption of ~70 molecules in the crystal occurs. This is sometimes referred to as the 'Henderson dose limit' for biological samples, which on a macroscopic scale is equivalent to 2×10^7 Gy or 1.6×10^{16} photons mm^{-2} (Henderson, 1990). This number is in qualitative agreement with experimental observations using high-brilliance beams (Elspeth Garman). Further steps in the sample response to irradiation, followed by 'classical' radiation damage, in the sense of partial or complete breakdown of the lattice in protein crystals, lead to a temperature rise within the sample, to thermal shocks in the sample, and ultimately to plasma generation. Protein crystals (Elspeth Garman) as well as cellular specimens for X-ray microscopy (Chris Jacobson) can be sufficiently robust to tolerate temperature jumps of a few degrees.

When discussing radiation-damage scenarios from an X-FEL, obviously one has to discriminate between the damage created from an integrated dose over some defined time and damage

resulting from fast pulses (Chris Jacobson, Stony Brook). The second scenario appears to be more relevant for an X-FEL, leading to the question: what is the time scale to destroy a sample under X-FEL conditions? Jacobson summarized his experience with scanning transmission X-ray microscopy (STXM) at extreme intensities providing insight into hydrodynamic shock and mass loss processes in the sample. In their experience immediate effects at the chosen specimens can be detected already beyond 10^4 Gy. He also demonstrated that in microscopy the observed or predicted damage scenarios are largely influenced by the sample preparation (experiment temperature, freezing procedure, fixed/unfixed specimens). Gerd Schneider (Göttingen) provided a list of techniques capable of stabilizing hydrated samples, indicating the importance of this aspect of sample preparation. In his calculations the dose limit for experiments with biological specimens is of the order of 10^8–10^9 Gy. He estimates a potential improvement of imaging techniques in spatial resolution below 10 nm to be achievable under appropriate X-FEL conditions.

Janos Hajdu (Uppsala) presented modelling experiments, using the unique parameters of the planned X-FEL. In his simulations a protein crystal is regarded more as a topographical entity than as a uniform macroscopic and periodic entity leading to Bragg diffraction (Neutze & Hajdu, 1997). He described his ideas on the 'cross-beam topography' where the radiation-damage barrier could be stretched to allow X-ray scattering without the requirement of amplifying the scattered radiation by Bragg reflections. Similar thoughts were expressed by Edgar Weckert (Karlsruhe) and Zbyszek Otwinowski (Dallas). Chris Jacobson mentioned recent results from Sayre and co-workers on diffraction patterns from non-periodic specimens using soft X-rays (Miao *et al.*, 1999). Jacobson thinks that the resolution obtainable with this method, in conjunction with an X-FEL, could be pushed down to 20 nm for non-disposable samples and to 200 nm for disposable samples.

Finally, Hermann Heumann (Munich) described an interesting application of hydroxyl-radical footprinting that takes advantage of progressing radiation damage in time. This technique can be used for probing the surface accessibility of nucleic acids in, for example, protein-DNA complexes or RNA molecules. This application was also, in part, described by Eric Renault (LURE, Orsay).

Table 2
X-FEL parameters in comparison.

The numbers for the TESLA-FEL have been adopted from the report by Brinkmann *et al.* (1997). The numbers for the LCLS-FEL have been updated (K. Hodgson, personal communication).

	LCLS-FEL	TESLA-FEL
Optimized gradient for X-FEL operation (MV m^{-1})	19	18
LINAC repetition rate f_{rep} for X-FEL (Hz)	120	5
Bunch length (r.m.s.) (fs)	67	83
Bunch spacing (ns)	8.7	93
Number of bunches per train	112	11 315
Bunch train length (μs)	0.35	1050
Bunch charge (nC)	1	1
Normalized emittance (π mrad mm)	1.5	1
Longitudinal emittance (keV mm)	60	27
Photon energy range (keV) (first harmonic)	0.8–8.3	0.1–12
Peak photon beam power range (GW)	11–9	280–60
Number of photons per bunch ($\times 10^{12}$)	22–2	100–1
Typical photon beam divergence (μrad)	3.2–0.38	1
Typical photon beam diameter (r.m.s.) (μm)	37–31	25

4. Potential applications in structural biology using an X-FEL

The main objective of the workshop was to identify and discuss potential applications in structural biology using the future X-FEL. Therefore, five sessions of the workshop were devoted to potential experiments, namely on novel opportunities in biocrystallography, possible applications in time-resolved crystallography, and applications in spectroscopy, microscopy and holography.

4.1. *Biocrystallography*

John Helliwell (Manchester) summarized the exciting developments in X-ray diffraction on biological macromolecules during the last decades where major advances were linked to the exploration of the possibilities of synchrotron radiation. As an example, he referred to the pioneering diffraction studies by Ken Holmes and collaborators at DESY about 30 years ago (Rosenbaum *et al.*, 1971), leading to a rapidly growing demand for synchrotron radiation beamlines for biological projects of increasing complexity. Later, structural biology, in particular

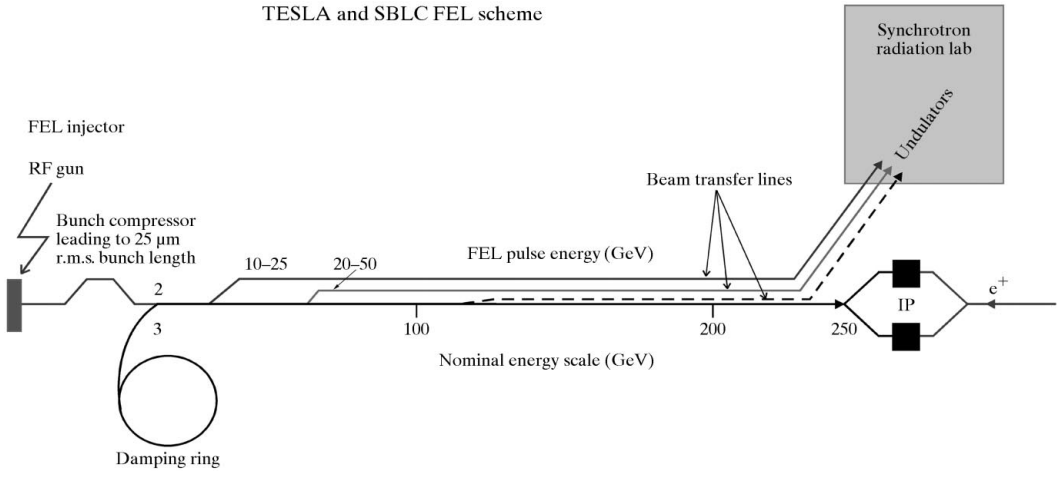

Figure 2
Sketch of a coherent X-ray source laboratory based on a linear collider installation. Adopted from Fig. 5.3.3. of the Brinkmann *et al.* (1997) report. (Courtesy of J. S. Schneider, DESY.)

biocrystallography, became a major factor for the construction of third-generation synchrotron facilities. Obviously, the funding of new state-of-the-art synchrotron facilities, mostly nationally (*e.g.* the DIAMOND project in the UK, or the Swiss Light Source in Switzerland), will continue. At the same time the scientific demand for novel scientific development will lead to some kind of fourth-generation synchrotron or synchrotron-like facilities where the most exciting and demanding option is offered by the emerging X-FEL technology.

The importance of biocrystallography in life sciences was underpinned in three other presentations by Martino Bolognesi (Genova), Martin Walsh (Pomezia, previously APS, Argonne) and Robert Sweet (BNL, Brookhaven). The latter two speakers demonstrated how state-of-the-art synchrotron facilities could accommodate current and future requirements for high-throughput structural determinations in the emerging structural genomics era. A number of speakers (Colin Nave, John Helliwell, Zbyszek Otwinowski) examined the experimental possibilities for very small crystals and crystals with very large unit cells, taking advantage of the expected small beam size and divergence of the X-FEL.

Starting a series of presentations on more practical aspects, Anastassis Perrakis described the design for an automatic centring goniostat on the microfocus beamline at EMBL/ESRF, Grenoble. Developments in this technology will be critical for sample mounting in an X-FEL. Single-shot experiments within extremely short time ranges will also require new techniques to monitor the sample status during the experiment (Zbyszek Otwinowski). Obviously, new techniques need to be developed to process X-FEL data (Zbyszek Otwinowski). Finally, Edgar Weckert (Karlsruhe) alerted the participants to demand an appropriate X-FEL time structure, to make it suitable for putative experiments in X-ray crystallography.

4.2. *Time-resolved crystallography*

Conformational changes in some proteins during biochemical reactions can be studied by Laue diffraction methods, where broad-bandpath 'white' radiation can allow rapid data collection with good data completeness within a few image frames (Ren *et al.*, 1999). A number of state-of-the-art examples were presented on myoglobin (Philip Anfinrud, NIH, Bethesda; Keith Moffat, Chicago; Ilme Schlichting, Dortmund; Hans Bartunik, Hamburg), PYP (Ulrich Genick, Scripps, La Jolla), bacteriorhodopsin (Richard Neutze, Uppsala), as well as ultrafast model studies on iodine gas (Richard Neutze). In particular, Philip Anfinrud demonstrated how time-resolved X-ray crystallography could be complemented by time-resolved spectroscopic methods. Gleb Bourenkov (MPG, Hamburg) showed how the efficiency of data collection could be maximized by using a limited bandwidth (∼2%) of white beam. His strategy could make time-resolved studies feasible for less ordered crystals than currently required when using a broad white-beam spectrum.

Some speakers further examined the possibilities for time-resolved crystallography offered by an X-FEL. By assuming that the required data content must be in one or a few images, the broad spectrum of the undulator radiation will be of most interest, whereas the bandwidth of the laser radiation will be too small. It will be critical that the amplitudes of the bunch-to-bunch fluctuations in the spectrum are small, to allow wavelength normalization which is essential for Laue data processing. The pulse length in the 100 fs range will be suited for achieving sub-ps time resolution in combination with rapid reaction initiation. Keith Moffat elucidated the possibilities of time-slicing ps pulses from existing sources, which could, in turn, be useful for the experiment design for an X-FEL source in the fs range. Philip Anfinrud presented a 'wish list' for a future X-FEL; on the X-ray pulse structure (minimum separation of pulses by 350 ns), the X-ray pulse spectrum (monochromatization to eliminate incoherent fraction of beam), X-ray pulse duration (less than 200 fs), X-ray pulse energy (1 mJ at 0.5–1.5 Å) and X-ray/laser synchronization (timing jitter shorter than X-ray pulse duration). He expects that some attenuation has to be employed in order to limit the temperature jump in the sample (10 K maximum). Alternatively, higher harmonics with reduced heat load could be used. There was a consensus that numerous challenges for further technical development remain. These include demands for ultrafast pixel detectors (see below); overcoming the gap of time and space resolution ('resolution wedge'; Ulrich Genick), which hampers many studies in time-resolved crystallography; expanding the field of feasible biological systems; methods for chemical reaction initiation and kinetic measurements; techniques for pump and probe synchronization at very short time intervals.

4.3. *Spectroscopy*

A number of important spectroscopic techniques, in particular in the field of nuclear resonance scattering, and their applications for biological systems were described by Fritz G. Parack (Munich) and Alfred X. Trautwein (Lübeck). Uwe Bergmann (Lawrence, Berkeley) summarized state-of-the-art applications of X-ray absorption spectroscopy in biology. These speakers emphasized the importance of high-brilliance synchrotron radiation beams and potential novel applications with X-FEL radiation for highly diluted samples (Uwe Bergmann). Unfortunately, spectroscopy applications utilizing the expected coherence of the X-FEL, like X-ray photon correlation spectroscopy, were not sufficiently covered during the workshop.

4.4. *Microscopy*

X-ray imaging techniques present a rapidly expanding field in structural biology. Although they do not reach the resolution of electron microscopy, they have the advantage of being applicable for rather thick samples (up to 30 nm). X-ray microscopy provides a variety of applications, ranging from morphological studies, mapping of elements, specific chemical bonds and proteins in cellular systems and microtomography studies. Gerd Schneider (Göttingen) summarized the experience of his group's X-ray microscope at the BESSY-II synchrotron facility. He emphasized, using observed and extrapolated data, the importance of sample stability at high dose rates (see above). Another aspect is the required stability of the Fresnel zone plates, which are one of the main factors determining the spatial resolution achieved. Werner Meyer-Ilse† and Chris Jacobson summarized their group's experiences in X-ray microscopy at ALS and Brookhaven, respectively. They demonstrated how this method can be utilized in a number of areas of biological research, in particular by specific protein labelling, and can complement confocal microscopy. It was noted that all three speakers of this session anticipate significant advances in their field from a future X-FEL. Harun Solak (Stoughton) summarized his experience in lithography, with implications for X-ray imaging. In his calculations the require-

† Deceased.

ments of spatial and time coherence are not very strong for phase contrast imaging and in some cases may be even counter-productive.

4.5. *Holography*

Holographic methods do not only provide the means to record an image of an object microscopically but also to receive phase information by splitting a wavefront in two parts, an object wave and a reference wave. This information is widely used for the holographic reconstruction of three-dimensional images. More relevant here is the potential of holography to extract phase information, which in turn can be used for the reconstruction of atomic three-dimensional structures. Abraham Szöke has pioneered one aspect of the field by using fluorescence as a source of coherent radiation in holography. These ideas were subsequently used for the experimental reconstruction of small molecules by Tegze & Faigel (1996) and Materlik and co-workers (Gog *et al.*, 1996). Dimitri Novikov (HASYLAB/DESY, Hamburg) and Stefano Marchesni (CEA, Grenoble) summarized their recent holography results by using synchrotron radiation at DESY and the ESRF, respectively. In Novikov's calculations the properties of the planned X-FEL could allow an expansion of holography into the field of protein crystals. Chris Jacobson showed some interesting applications on cellular systems. However, a hard X-ray FEL will not be suitable for these applications because of the mismatch of the desired atomic reference source (the X-FEL) and the objects to be studied. Abraham Szöke (Lawrence, Livermore) showed his attempts to make use of emission holography to solve the phase problem of 'complicated' structures (biological macromolecules), or part of these structures. The principle is to generate a continuous diffraction pattern of a crystal, which in turn has to be sampled on a very fine grid for reconstruction. In one of the simulation experiments, for instance, he was able to regain phase information for missing parts of a protein structure of moderate molecular weight.

5. Data recording and processing

The applications discussed in the previous sessions are not only demanding with respect to the desired beam properties of a future X-FEL but also require development of tools for data recording, processing and interpretation. Therefore, two sessions at the workshop were devoted to present and future possibilities for FEL-suitable detectors and to image interpretation.

5.1. *Data recording by pixel detectors*

One of the main reasons for the recent progress in biocrystallography is due to the advances in two-dimensional detectors, starting with multiwire and TV-based detectors, and succeeded by imaging-plate scanners and CCD detectors. However, there is a consensus that there must be further progress with the next generation of detectors, which will most likely be pixel array detectors. The objectives for the next detector generation are: (i) high angular resolution (in the μrad range) and intensity precision (less than 0.1%); (ii) high counting rates in the 10^{10} X-rays pixel^{-1} s^{-1} range; (iii) very short integration time per frame of the order of microseconds to seconds, and short dead times in the ns range; (iv) detector format in the $10^3 \times 10^3$ pixels range; (v) wide dynamic range of the order of 10^5–10^6; (vi) low electronic noise (less than 1 X-ray pixel^{-1}); (vii) high DQE from 5 to 25 keV, and (viii) robustness and cheapness! Eric Eikenberry (Cornell) described test experiments with their 100 × 92 pixel CMOS-chip detector, with 151 μm pixels and an active area of 13.9 × 15.1 mm. Initial results from a microsecond Laue experiment, using this detector at one of the CHESS beamlines, were of particular interest. The detector was also extensively tested for pixel radiation damage under high dose conditions, and technical solutions were discussed. Eikenberry pointed out that, despite the established pixel array detector technology, increasing the size of these detectors by about one order of magnitude in each dimension, which is required for biocrystallographic experiments for instance, remains a major challenge. Nikolai Pavel (Siegen) described the development and technical details of a prototype gas-filled MicroCAT two-dimensional-pixel detector. Its outstanding features are that it can be used both in single photon counting mode and in integrating mode with good timing resolution and a high dynamic range. This detector has been tested in a number of applications in X-ray crystallography and small-angle scattering. Finally, Carlo Fiorini (Milano) presented new developments on silicon drift detectors. These detectors are already used for high-performance X-ray spectroscopy with synchrotron radiation. Owing to its outstanding features, this type of detector is an obvious candidate for a large 4π multi-element detector for holographic data recording at an FEL. The three presentations have demonstrated that the development of new and fast X-ray detectors is progressing and that this is a key issue in instrumentation for modern synchrotron beamlines. For the X-FEL projects, the design of the FEL facilities should be very closely accompanied by dedicated detector development projects, which are optimized for the beamline parameters and the particular specific experimental needs.

5.2. *Image interpretation*

Processing and interpretation of synchrotron data in structural biology continues to undergo dynamic development, in particular for data/image pattern recognition and automation. Advances in this field in three major areas were presented by Anastassis Perrakis (EMBL, Grenoble) for X-ray crystallography, Dimitri Svergun (EMBL, Hamburg) for small-angle scattering of non-crystalline systems, and Marin Van Heel (London) for electron microscopy. Although most of the attention is currently focused on ideas for experiments at the future X-FELs and their technical feasibility, it is evident that, once data are measured at X-FELs, there will be a strong need for high throughput and automated data interpretation. Therefore, advances in the field of data processing and image interpretation are important for the success of potential X-FEL experiments.

6. Conclusions

Within a few years, free-electron lasers will provide sources with unprecedented characteristics in the hard X-ray energy regime, which is of particular interest for applications in structural biology. The planned X-FELs will be unique in terms of their peak (laser) and time-average photon flux, their time structure, and their spectral properties in terms of coherence, beam size and beam divergence. These, initially, 'test facilities' will offer unique opportunities for experiments, novel in their design and their potential to reach shorter time frames, better diffraction and scattering properties and higher complexity of samples to be studied.

The objective of this workshop was to gather new ideas for applications in structural biology. Naturally, most of these start from currently established technologies, and lead to a range of ideas from the realistic to the fantastic. When discussing structural biology the issue of sensitivity of biological material to high dose rates remains prominent. This debate is not new and has accompanied advances in the field over the years, sometimes leading to partly unexpected solutions, for instance the wide use of crystal sample handling at cryogenic temperatures. The current experience with high dose modes is mostly limited to the facilities that are widely used for structural biology experiments, like third-generation synchrotrons, and more experience on biological material with higher doses and dose rates is needed. Ultimately, it will be fascinating to participate in the first biology experiments using an X-FEL. It was repeatedly mentioned at the workshop (Jörg Rossbach, DESY; Keith Hodgson, SSRL) that there is still more flexibility in the design parameters for the future X-FELs than expected by many scientists. Rossbach requested, on behalf of other X-FEL designers, the input of biologists at the earliest possible stage for optimization of the X-FEL parameters to be suited for experimental applications.

In which field will the first applications be? X-ray diffraction experiments will probably be significant, most likely in the fields where the principal X-FEL parameters match the desired needs. These are expected to be time-resolved studies, crystallography of very small crystals and crystals with very large unit cells. The potential capabilities of an X-FEL to allow significant diffraction from small ensembles with fewer unit cells, and even topographical analysis (Hajdu), remains to be shown. At the workshop there was less enthusiasm to plan X-FEL experiments for tackling the crystallographic phase problem, largely because it is regarded as more or less solved, practically if not theoretically, by the establishment of the MAD technology. Another promising field is X-ray microscopy, which has a strong potential for bridging molecular and cellular structural biology. In this field there is hope that the X-FEL could improve the resolution of recorded images substantially. The X-FEL is also of particular interest for holographic methods because of its coherent properties of the source. To what extend holographic methods could be used for phasing in biocrystallography still remains a question.

As intended, the workshop mostly focused on the search for biological applications. Nevertheless, the challenges for designing the front ends and other beamline components are fully recognized. During the data-recording session of the workshop it became evident that there is already a community that is preparing detector devices for this next generation of beamline facilities. A promising field is the development of large pixel array detectors. No doubt many future applications will differ from the ideas and possibilities discussed at the workshop. After having experienced exciting, and sometimes exhausting, discussions at this workshop, the community in structural biology appears to be very eager to perform the first experiments using an X-FEL beam.

I thank the co-organizers of this workshop, the chairpersons and the speakers for their excellent scientific input, which is the basis of this report. Finally, all participants are appreciated for their presentations and their lively involvement in the discussions during the workshop. Paul Tucker, Christoph Hermes and Jochen R. Schneider are thanked for critical reading of the manuscript and comments. The workshop was, in part, funded by EMBO.

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