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SPEAR3 Workshop: Making the Scientific Case

Report from the Workshop held at Stanford Synchrotron Radiation Laboratory (SSRL), May 29-30, 1997

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Executive Summary

Preface

As part of the planning process for the proposed upgrade to the SPEAR electron storage ring, SSRL, the SSRL Users Organization and the SSRL faculty sponsored a 1 1/2 day workshop on May 29-30 1997. The goal was to assess and document the impact of SPEAR3 on current and future science and technology research programs of the users of SSRL.

The hard and soft x-ray beams produced at SSRL are used in a number of different scientific and technological disciplines. The workshop was organized by defining a set of areas of science and technology covering the basic activities at SSRL and inviting key people from outside Stanford to work with the SSRL faculty and staff in a set of topical groups on estimating the impact of SPEAR3 on their respective fields and developing a vision of the future opportunities.

This report documents those scientific and technological opportunities and provides written summaries of the discussions. The report is organized with a brief technical description of SPEAR3 and planned beam line upgrades (which summarizes material presented to the workshop participants prior to the breakout sessions) following this executive summary. More detailed information from the topical working groups then follows. Finally, an appendix provides a list of workshop participants and a copy of the workshop agenda as well as some more detailed information on the SPEAR3 lattice and machine.

Overall Impact of SPEAR3 on Hard and Soft X-ray Measurements

The most immediate impact would be improvements in capabilities on all of the operational experimental stations on SPEAR. By decreasing the emittance of SPEAR by a factor of 7 and increasing the beam current to 200 mA, existing insertion device end stations would experience an increase of focused photon flux density at the sample of approximately a factor of 10. As the radius of curvature of the bending magnets would be reduced with the new lattice, the critical energy of those beam lines would increase from the present 4.8 keV to 7.1 keV. Bending magnet beam lines equipped with focusing mirrors would see an increase in focused flux ranging from ~30 at low energies up to a factor of 200 at 20 keV. Therefore, one consequence of the upgrade is that the focused flux density of focused bending magnet lines would nearly equal that of the

existing beam line 10-2. This would have a major impact on the usefulness of these bending magnet lines which would then become workhorses in a variety of disciplines including protein crystallography, thin film diffraction studies and applications of x-ray absorption spectroscopy.

The improved brightness would also have significant impact on the potential of SSRL for future developments in undulator beam lines. Although not explicitly included in the SPEAR3 storage ring upgrade, an undulator line optimized for the photon energy region 1-4 keV would provide the potential for spectroscopy experiments which require very small focal spots in this energy region. The potential impact (which is discussed in detail below) for experiments in environmental science (Mg, Al, Si, K, and Ca K-edges) and biologically important elements (P, S, Cl, K, and Ca) is very exciting. Separate funding will be sought for such a line that could take advantage of one of the new long straight sections, or an existing interaction region straight section, in SPEAR3.

Finally, the potential for producing microfocus beams with dimensions in the few micron range would open up SSRL for a range of element specific imaging and local probe spectroscopies which would have a major impact on a wide range of applications, particularly in the environmental and biological sciences.

Highlights of SPEAR3 Working Group Reports

I: Biological Macromolecular Crystallography

The goal of understanding life has evolved into a large interdisciplinary effort which integrates information that extends from experimental results at the atomic and molecular level to studies of organelle, cellular and tissue organization and function. There is a strong interplay among disciplines such as chemistry, structural biology, biochemistry, genomics, informatics and computational sciences. Atomic level information will increasingly provide the window through which biological function, and malfunction that leads to disease, will be understood. Macromolecular crystallography has provided the vast majority of information on three-dimensional molecular biological structure and will play an even greater role in the future. Information relating structure to function has also led to the development and successes of new approaches to drug discovery.

The high intensity of synchrotron radiation (SR) plays a seminal role in enabling these advances as first demonstrated by two important classes of protein crystallography studies at SSRL more than two decades ago; 1) the tunability of SR provides a completely new approach based upon multiple wavelength phasing (MAD) to solve the phase problem and 2) the high intensity and collimation enables the study of much smaller crystals and to a significantly higher level of atomic resolution, all in a much shorter time. Beam lines at SSRL for crystallographic studies have enabled scientists to understand structural and functional aspects of processes like muscle contraction, respiration and other cellular processes, cellular control, bacterial infection, nitrogen fixation and photosynthesis. Such studies have contributed to fueling an explosive growth in demand and scientific productivity in the protein crystallographic field at SSRL (and other SR facilities as well) during the past decade.

SPEAR3 will build upon the already formidable capabilities at SSRL for protein crystallography to provide world class capabilities that will enable new protein crystallography experiments. The enhanced brightness and flux density will enable structure determination from crystals of even larger molecular assemblies (such as larger viruses and multiprotein complexes) to higher resolution and in shorter times. Such studies are intrinsically difficult because the systems have large unit cells and often will not grow into large crystals. SPEAR3 will also enable many more applications of ultra-high resolution (below 1 Å) protein structure determination providing the means to unravel the structure-function relationship truly at the atomic level, including direct visualization of hydrogen atoms that are so crucial in both structure and catalysis. The possibility for very rapid data collection of short-lived reaction intermediates will become feasible, providing even more direct probes of biological reaction cycles. MAD phasing can be applied to new classes of proteins, especially the larger and more complex systems, therefore greatly expediting structure solution and thus being directly relevant to the need for high throughput structure determination as we seek to understand the structure and hence function of the proteins derived from the thousands of genes that will be elucidated from the human and other genome efforts. Finally, the opportunity will exist for the development of a very intense undulator source which would produce extremely bright lower energy x-rays that would extend the MAD phasing technique to new systems and would provide the means to collect data from microcrystals. SPEAR3 would have an immense impact by enabling the study of new problems,

by dramatically improving quality of all studies and by significantly increasing the number of samples studied.

II: Biological Small Angle X-ray Scattering and Diffraction

Studies of structure-function relationships have become a central part of modern biology. There are a number of important biological molecular assemblies whose characteristic lengths are in the range of nanometers to micrometers, such as virus particles and muscle fibers. Many of those complex molecular assemblies are in a partially ordered (*e.g.* fibers) or randomly oriented state (*e.g.* protein complex in solution) that is not suitable for high-resolution crystallographic analysis. Studies of dynamical structural changes of such large molecular assemblies that are linked to specific biological functions are required to expand our knowledge of atomic resolution protein structures to more complex biological systems which have remarkably diverse and important roles in enabling life processes.

Small angle x-ray scattering/diffraction (SAXS/D) is a technique very suited for studying such non-crystalline biological structures but its use becomes feasible only through the application of synchrotron radiation which provides the needed high flux density, small natural beam divergence and energy tunability. The small angle scattering instrument on SSRL BL 4-2 makes use of these properties and accommodates a diverse spectrum of SAXS/D experiments (solution scattering, fiber and low angle crystal diffraction). It serves the structural biology community exceptionally well as the primary synchrotron SAXS/D instrument for structural biology research currently available in the United States.

The characteristics of the proposed SPEAR3 would play a major role in making SAXS/D experiments even more effective for structural biology research and in addition making new experiments possible. The reduced emittance of SPEAR3 would significantly increase the focused beam flux to the experiments by as much as 10-fold (providing $>10^{12}$ photons/sec with Si(111), $>10^{13}$ with multilayers). Direct benefits in SAXS/D experiments would be: *i*) accessibility to shorter time scales by a factor of at least 10, reaching the submillisecond regime where important biological dynamics is found; *ii*) enhanced ability to examine smaller and hard-to-obtain biological samples due to the smaller beam size; *iii*) improved accessibility to smaller angles by a factor of 5 in the horizontal diffraction plane and 2 in the vertical plane, covering

larger structural periodicities because the direct beam can be separated more easily from scattering at very small angles; and *iv*) increased flux by a factor of approximately 5 for anomalous scattering experiments with biological specimens. The existing wiggler on BL 4 should be replaced with a new permanent magnet wiggler in order to take full advantage of the low emittance beam. The significant upgrade of the beam line optics planned in the next few years, which is important to be able to manage high heat loads with SPEAR3 and the implementation of a new, fast detector system to handle the high counting rates are both very highly desirable complementary upgrades. Taken together, these would establish the BL 4-2 SAXS/D instrument as world class for a large range of important biological studies.

III: Biological X-ray Absorption Spectroscopy

X-ray absorption spectroscopy (XAS) enables the study in solution of the electronic and structural aspects of metal sites which play seminal roles in many biological processes ranging from respiration to photosynthesis and nitrogen fixation. The SPEAR3 increase in flux, when coupled with anticipated improvements in detectors, would lead to a factor 5 increase in sensitivity of the technique, making measurements of ~0.1 mM absorber concentrations in solution feasible. This would allow studies of metalloproteins that are presently too insoluble to be studied in solution (for example, many DNA binding proteins) or that cannot easily be concentrated (for example, membrane-proteins). The higher brightness of SPEAR3 would also allow studies of smaller sample volumes which is especially important in cases where only limited amounts of biological samples are available.

The SSRL wiggler beam lines remain oversubscribed by a factor of ~2. An increase of even a factor of 2 in productivity (*i.e.*, time required to measure the present samples with data of the present quality) would have an important impact (equivalent, effectively, to constructing several more beam lines). In addition, in SPEAR3 the bending magnet beam lines would have sufficient flux for most protein extended x-ray absorption fine structure (EXAFS) spectroscopy experiments that currently can only be done on wiggler beam lines. Thus their usefulness would be enormously enhanced in SPEAR3.

The application of XAS to single crystals can yield bond-specific metrical information and the means to determine electronic structure, including properties such as oxidation state, spin state,

and covalency, thus providing a complementary technique to protein crystallography for zeroing in on structure-function relationships in metal ion based active sites. Single crystal XAS experiments would benefit dramatically from the proposed gain in flux density through the SPEAR3 upgrade because of the small volume of the samples compared to normal solution XAS. It is estimated that high quality XAS data could be collected on crystals of ~150 microns^3, and single crystal diffraction data could easily be measured on the same samples. Such combined studies would provide definitive characterization of all aspects of metal ion active sites.

The increased flux of SPEAR3 would greatly enable new classes of experiments where samples are also more dilute. These include kinetic studies involving rapid mixing and freezing and continuous-flow techniques which can provide information on intermediates of catalytic turnover, with potential time resolution significantly faster than rapid freezing (*e.g.* <1 msec, compared to >5 msec). The high flux densities from SPEAR3 would make these experiments practical for a range of biologically interesting systems. Examples of possible applications include the study of catalytic intermediates in enzymes such as sulfite oxidase, a key enzyme in mammalian sulfur metabolism, and nitrate reductase, a key enzyme in the global nitrogen cycle.

It is in the 1-4 keV energy range that the greatest benefits of brightness from a new undulator source on SPEAR3 can be realized. This energy range includes the K-edges of Na through Ca, the L-edges of Cu through Cd, and the M-edges of I through Pu. Despite the wealth of information available from this energy range, experimental access has to date been very limited at SSRL and other synchrotron facilities worldwide. The SPEAR3 upgrade with existing beam lines would provide significantly enhanced capabilities in the 2-4 keV region (BL 6-2) and the 1-2 keV region (BL 3-3 with YB_{66} or InSb monochromator crystals). To date, the greatest potential has been realized at the sulfur K-edge, with important progress in understanding the electronic structure of sulfur ligands in metalloproteins, and the speciation of sulfur in complex samples such as microbial cells, oils, coals, soils and sediments. The SPEAR3 upgrade would also facilitate similar experiments with phosphorus, aluminum, *etc.* In the longer term a new undulator beam line operating in this photon energy range would make SSRL the premier facility worldwide for spectroscopy in this hitherto little-explored spectral region.

IV: Molecular Environmental Science

The application of synchrotron radiation techniques, particularly XAS, to problems in environmental sciences has grown dramatically at SSRL in the last seven years. This growth has come in response to the ability to directly probe the speciation of metal ions and organic species that are contaminants, or regulate the mobility of contaminants, in natural samples such as groundwater, soils, microbes, and plant matter. The speciation of contaminant ions (*i.e.*, the elemental identities of the contaminants, their physical states, oxidation states, host-phase identities, molecular structures and compositions) controls their toxicities, bioavailabilities, and transportabilities in soils and aquifers. Synchrotron-based methods can be used under environmentally relevant conditions, namely, in the presence of water at ambient pressures and temperatures and at dilute metal ion concentrations (*i.e.*, >1 ppm). The success of these techniques in solving environmental problems has led to the development of a new multidisciplinary field, molecular environmental sciences (MES) which has attracted a large MES user community at SSRL. Synchrotron-based methods, particularly XAS and micro-XAS, will continue to play important roles in solving environmental problems for the foreseeable future.

The SPEAR3 upgrade will positively impact the capabilities of all types of MES research at SSRL. Natural samples are often heterogeneous, containing colloidal- to sand grain-sized solids that are microfractured and/or coated by secondary and tertiary phases, all of which have different reactivities with dissolved metal ions and organics. The ten-fold increase in brightness and flux density provided by SPEAR3 will make it possible to probe the speciation and distribution of metal ions in such complex natural samples on length scales of approximately 10 μm. The increase in flux density will also substantially improve the ability to characterize environmentally important chemical reactions and transformations at the surfaces of natural materials and at mineral-water interfaces. For example, it will be possible to perform XAS investigations of metal ions adsorbed on single-crystal metal oxide surfaces at defect-level concentrations, which will permit studies of environmentally important chemical reactions at defect sites on mineral surfaces. The two-fold improvement in absolute beam line flux will facilitate the analysis of more dilute, smaller, and less hazardous samples than is currently possible. Furthermore, the stability of x-ray beams, which is currently a limiting factor in many

MES experiments and will be cruicial to microspectroscopy investigations, will be substantially improved by the SPEAR3 upgrade.

V: Materials X-ray Absorption Spectroscopy

All of the materials problems that are currently being studied at SSRL using x-ray absorption spectroscopy will be enhanced by the increased flux density of the proposed SPEAR3 upgrade. Examples of these are systems involving the structure of surfaces or interfaces, such as catalytic materials, or those which study the structure of materials at high pressure where the aperture into the sample cell is quite small. In both cases the increased flux density will enable researchers to study either smaller amounts of sample or more realistic concentrations than was previously possible. There are a variety of materials problems for which radically new information would be gained about the systems, due to the increased spatial resolution of the EXAFS or edge structure techniques enabled by the increased flux density of SPEAR3.

VI: Condensed Matter, Materials Science and Technology

The structural details of thin films, surfaces and interfaces at atomic resolution are of fundamental importance for applications in microelectronics and related applications. The increased flux density would enable increased signal rates from scattering experiments that probe the structure at the surface of a growing film. The effect of this increased flux density on applications such as the study of islands forming during the homoepitaxial growth process of GaAs by chemical vapor deposition would be to enable researchers to more accurately understand the size and asymmetry of these islands.

The high performance of the SPEAR3 bending magnet beam lines would be extraordinarily useful for x-ray diffraction and reflectivity measurements of the thin films that are used in the semiconductor, display and magnetic storage industries. An example would be read heads in magnetic storage devices, which are made up of multilayers of alloys of third row transition metals. Diffraction studies would help in understanding the structure of the interfaces in these multilayers on which the functioning of these heads depends critically, and which is currently largely unknown. Routine access to these more effective beam lines would aid the reliability for

researchers to more rapidly characterize the films structurally and compare those results to the magnetic measurements that are available in their laboratories.

Powder diffraction is a powerful analytic tool for understanding crystallite structures in complex materials. By raising the critical energy in bending magnet beam lines, SPEAR3 would extend the range of elements for which anomalous (resonance) diffraction effects can be exploited. With an accessible energy range of 5 to 30 keV, powder diffraction measurements would have easy access to the absorption edges of most elements. Another advantage of scattering at high energies is accessibility to a much larger reciprocal (Q) space making it possible to determine structural details at sub-Ångström resolution and obtain increasingly accurate and robust measurements of thermal displacement parameters, leading to improved knowledge of lattice dynamics in complex materials.

Feature sizes in microelectronics circuits are currently in the sub-micron regime and the need for diagnostic probes that match these features becomes compelling. The increased brightness of SPEAR3 would make possible the development of high intensity x-ray microprobes. Their primary use would be element-specific analysis using fluorescence (preferably in the scanning mode), diffraction or structural analysis on the micron scale or less and grazing incidence defect characterization in thin films or diffused surface layers.

Trace element analysis on wafer surfaces ("microcontamination") using total reflection x-ray fluorescence (TXRF) would benefit from the SPEAR3 upgrade by taking advantage of both the reduced source size as well as the increase in ring current. A quantification of this improvement is the minimum detectability of the trace metals. Microcontamination analysis efforts are being developed at the 3rd generation synchrotron radiation sources in Europe and in Japan. The continued improvement of this expertise and capability in the US, with the momentum presently being generated by the work between SSRL and the Sematech member companies should be a tremendous advantage for the semiconductor industry. The proximity of SSRL to many research laboratories of the major semiconductor manufacturing companies makes the continued leadership of SSRL in the field of TXRF an important goal.

VII: Imaging Techniques in Materials Engineering and Medicine

X-ray topography allows imaging of the defects and long-range strains present in semi-perfect bulk crystals and thin films. This is a very useful, non-destructive technique that requires large beam size and high geometrical resolution. With SPEAR3 one would be able to image with energies up to 60 keV (bending magnet) or 100 keV (wiggler lines) with a vertical field of view of 3-4 mm; this combination would be unique and would be extremely valuable for crystal characterization of highly absorbing materials, particularly in the transmission setting.

For SPEAR3 the usable intensity available for x-ray microbeam diffraction would be extended to 45-50 keV for bending magnet radiation. This roughly doubled maximum usable energy makes diffraction studies possible for interior volumes of bulk samples of important engineering materials, including Ti, Fe, Ni, Cu and Pb. An example of this type of application is in the reliability of new generations of turbine blades. Single-crystal blades are in service commercially. Damage accumulation mechanisms and rates within these blade crystals are poorly understood. Transmission polychromatic topography would reveal all of the accumulated damage within a blade and this type of nondestructive interrogation would allow the same blade to be examined periodically throughout its life.

SPEAR3 would bring qualitative improvements to the biomedical applications of X-ray Tomographic Microscopy (XTM) by which three–dimensional images of bone and tissue (or other objects) can provide a quantitative map of the tissue composition and density with a spatial resolution of a few cubic micrometers. Increase of spatial resolution by two- to three-fold can be expected enabling new types of biological imaging applications that are impractical with the present source. In addition, *in-vivo* biomedical applications are currently limited by the x–ray optics (the lack of good cooling on the crystal monochromators). Planned improvements to the beam line infrastructure would, therefore, be a great enhancement to SSRL capabilities.

An example of this application is in research in the use of magnetic fields, in combination with low dose parathyroid hormone treatment, to reverse osteoporosis in an experimental animal model. Electric and magnetic fields have been demonstrated to affect bone metabolism in several animal model systems and have been successfully applied clinically for therapeutic benefit in fracture healing and bone fusion. The changes in bone accompanying these experimental

procedures is being studied using single photon absorptiometry and three–dimensional histomorphometry by XTM; these studies would be much enhanced by SPEAR3.

VIII: Vacuum Ultraviolet and Soft X-ray Applications

The VUV community feels a strong need for continuing efforts both at SSRL and at the ALS. While certain experiments (mostly micro-spectroscopy types) would always be better performed at the ALS, SSRL with the SPEAR3 upgrade would be strongly competitive for the majority of experiments and preferable to the ALS for some (particularly those that need long beam lifetimes and exceptional beam stability, such as with dichroism experiments).

It should be noted that the flavor of experiments in the low energy regime is different from those in the higher energy regimes. Due to the difficulty of working in the UHV environment, the necessity for *in-situ* sample preparation and the necessity to repeat the experiments many times due to surface degradation, it often takes months rather than days to complete a careful experiment and the VUV community is facing a clear deficiency in high performance (high resolution/high flux) beam time.

In the VUV/soft x-ray regime SPEAR3 would give competitive photon fluxes on the sample which would allow for improved spectral resolution giving much greater information content to all experiments, allow the study of smaller or short-lived samples, and allow for new types of experiments, *e.g.* spin-resolved photoemission or x-ray absorption on very dilute samples. SPEAR3 would also provide long lifetimes and state-of-the-art beam stability for stable uninterrupted experiments. This is especially important for long scan experiments, experiments that need a very large number of spectra from the same sample surface, and experiments looking for very small effects, such as magnetic circular dichroism studies.

These improvements are critically important both for science and for technology in five currently active areas - condensed matter physics, surface and interface science, materials science, chemical science, and biological science - and also in a sixth area, environmental science, which is rapidly increasing in importance. SPEAR3 would bring significant benefits and enable new science in all these areas.

IX: Conclusions and Recommendations

- The important new opportunities for Science and Technology afforded by SPEAR3, which are detailed in this report, provide compelling reasons to proceed in the most rapid way possible with the SPEAR3 upgrade. This will be a cost effective means to preserve and enhance the significant public and private investment at SSRL and will provide world class science and technological capabilities that will become increasingly important into the next century. Such capabilities will also be of increasing importance to the research and technological infrastructure of the western United States, which is served in the x-ray region by SSRL. The SPEAR3 project receives the strongest possible endorsement from this workshop.

- In parallel to the machine upgrade, the program to improve the beam line optics is of crucial importance and efforts should be made to accelerate this effort as much as possible so that all the beam lines can take full advantage of the new capabilities. Efforts should also be directed towards adding new capabilities to existing (and future) beam lines through focus on research and development in areas such as advanced detectors.

- The use of undulators on SPEAR3 will provide unparalleled capabilities in the soft (1-4 keV) spectral region. This spectral region has broad implications for new scientific advances in many fields including molecular environmental science, structural molecular biology, and condensed matter physics, surface and interface science. SSRL should work closely with the user community to aggressively develop a specific proposal to seek funds to exploit this new opportunity.

- SPEAR3 will also provide extremely valuable long straight sections (4 meters and two even longer ones) and very potent radiation from the bending magnets. Plans should be evolved to develop these capabilities and should consider both state-of-the-art applications (such as microprobe and microcrystal applications) and the class of industrial analytical applications that are so critical to the regional semiconductor, biotechnology and petrochemical industries.

Technical Description of SPEAR3 Upgrade Proposal

Although designed as a high-energy physics colliding beam storage ring, the Stanford Positron-Electron Asymmetric Ring (SPEAR) has been an effective source of synchrotron radiation for most of its 25 year history. Dedicated to synchrotron radiation since 1990, it has run at 3 GeV, 100 mA since that time. While prior studies had been made to reduce the SPEAR emittance in the 1970s and 80s[1,2], the addition of a third injection kicker enabled a practical alteration of the lattice magnet settings in 1991 that reduced the emittance from ~500 nm-rad to 130 nm-rad[3]. Alternative lower emittance lattices, which require new magnets and vacuum chamber, have since been considered and proposed[3,4]. These studies have evolved into a specific proposal for a 3 GeV, 200 mA low emittance SPEAR3[5]. The highlights of this proposal are the reduction of the natural electron emittance to 18 nm-rad, the increase of the stored beam current to 200 mA, achievement of both high beam stability and long lifetime (~50 hrs), maintenance of the existing beam line alignment, the addition of several long (~4 m) straight sections and at-energy injection at 3 GeV. As part of the SPEAR3 upgrade plan, the electron vacuum chamber would be replaced with a chamber designed to handle up to 500 mA at 3 GeV. Since several of our existing beam lines are not designed for this higher current, the initial goal of the project would be to increase the current to 200 mA, but by having the higher current vacuum chamber in place it would allow the ring current to be increased as beam line optical components and masks are upgraded. Further details on SPEAR3 can be found in the preprint included in the Appendix[5].

From the standpoint of the users, one of the most important aspects of the SPEAR3 upgrade is the reduction of the electron source size. For the existing SPEAR, the insertion device source size is roughly 2000 μm horizontal by 53 μm vertical. These are the 1-σ values of a Gaussian electron density profile. The proposed SPEAR3 lattice would have insertion device source sizes of 511 μm by 38 μm, a reduction in source size of ~5.5. This, combined with a factor of 2 increase in current, results in an increase in focused flux density of an order of magnitude. This increase will be seen on all beam lines which have insertion devices with relatively small electron excursions, which means all but beam lines 4 and 7, the two electro-magnet wiggler beam lines. The focused flux density is presented in Figure 1, comparing the flux density from both a bending magnet and beam line 10-2 for both the present SPEAR source and the proposed

SPEAR3 source. For comparison, certain other beam lines are also presented, including an NSLS bending magnet, the NSLS X-25 wiggler, and an APS Undulator A.

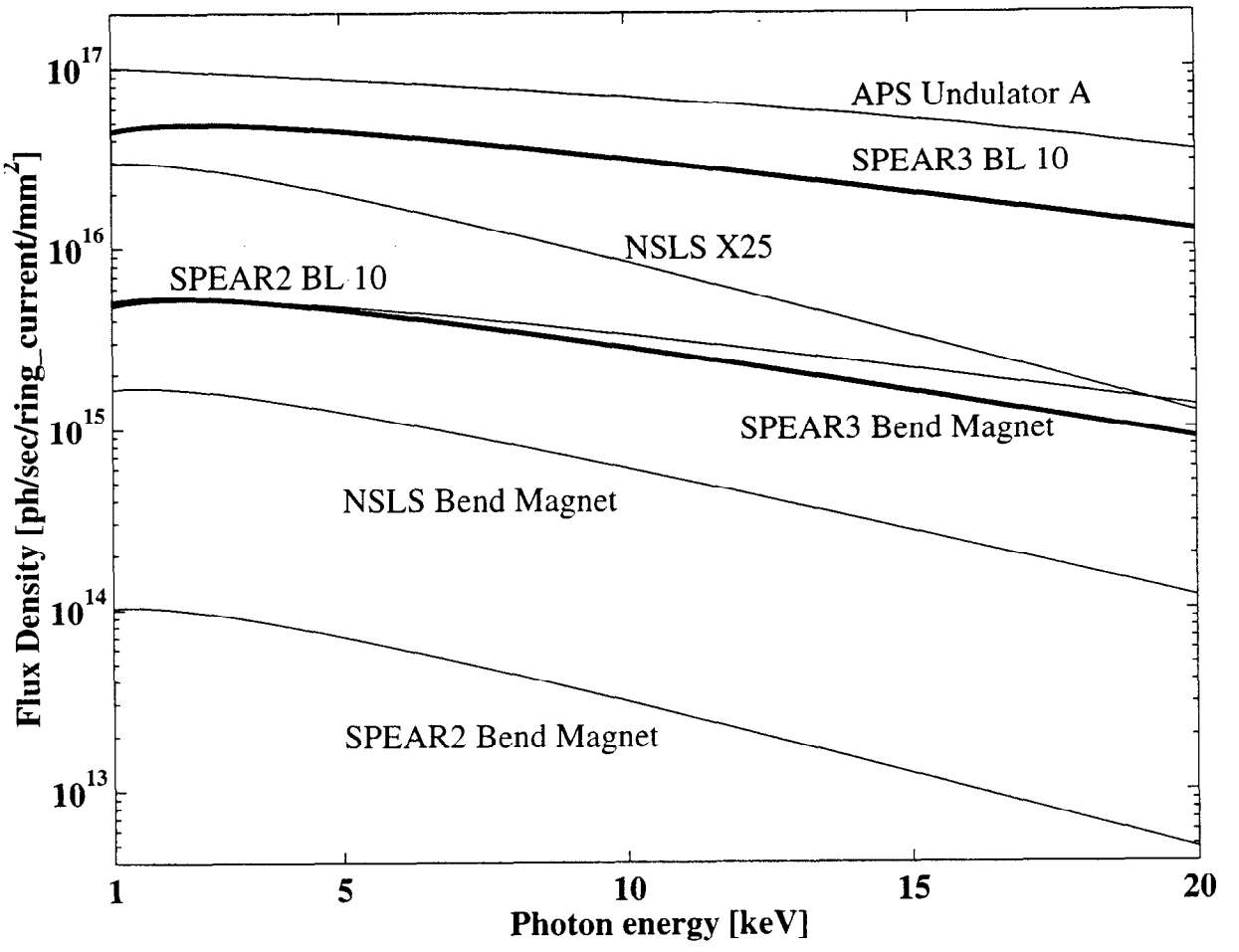

Figure 1. Focused flux density for several beam lines on the existing SPEAR source, the proposed SPEAR3 and beam lines from other existing synchrotrons.

The bending magnet beam lines will see several improvements with the SPEAR3 upgrade plan. First, the radius of the bending magnets is being reduced such that the critical energy of the photons radiating from these sources increases from 4.7 keV to 7.1 keV. This harder spectrum means that the improvement in flux of SPEAR3 over the existing bending magnet sources is even more marked at higher photon energies. The SPEAR bending magnet source size is 767 μm horizontal by 200 μm vertical. This would be reduced with the proposed SPEAR3 lattice to

162 μm by 36 μm. This is a factor of 26 reduction in source size, which is again augmented by the doubling of the current. Thus focused beam lines using light below 1 keV would see roughly this factor of 50 increase in focused flux, and experiments at higher energies, which are also affected by the hardening of the spectrum, would see a factor of 100 increase at 12 keV, increasing to nearly 200 at 20 keV. The focused flux from these beam lines is also presented in Figure 1.

For those beam lines without focusing optics, the increases are more modest. Wiggler side stations see the projected length of the wiggler as their effective source rather than the actual electron source, so those will not see the reduction in horizontal source size, but will see the reduction in vertical source size (~40% smaller) if equipped with vertically focusing optics. All wiggler side stations will see the doubling of the current. Unfocused bending magnet beam lines will see the doubling of the current and the hardening of the spectrum, so they will see increases of between 2 and 8 in the photon energy regime from 2-20 keV.

Without significant upgrades, however, the existing beam lines can not take full advantage of the improvements to the source. In particular, higher current operations will produce power loading on many masks, slits, and windows that exceed their thermal design limit. Thus in phase one of the SPEAR3 upgrade, all masks, slits, and windows will be upgraded for 200 mA operations. Few changes are anticipated on the newer beam lines (*e.g.*, beam lines 5-4, 9, 10, and 11) which were designed for 200 mA operations. Most of the older beam lines, however, require significant mask and window improvements. Beam lines 4 and 7, designed in the late 70's, will need significant modification to run at 200 mA. The existing electro-magnet wigglers on BLs 4 and 7 are poorly matched to take advantage of the proposed reduction in horizontal electron source size of SPEAR3. Thus there is a proposal (to be separately developed and submitted) to replace the existing 4-period electromagnet wigglers with ~10 period hybrid wigglers which would increase the flux density for the end stations by a factor of ~25, and a factor of 6 increase in flux for the wiggler side stations. For beam lines 4 and 7, all front end masks and windows require replacement. In so far as practical, the replacement components will utilize concepts developed for SSRL's newest beam line, BL 11.

While many optical elements can safely handle the power increase of SPEAR3, they will experience increased thermal distortion and the associated degradation in optical performance. Consequently, delivering photon beams that take full advantage of the source emittance improvement requires replacement of the monochromators and mirrors on most of the older insertion device beam lines. We anticipate using monochromator and mirror cooling technologies developed for third generation sources and adapted to SSRL's needs as part of recent beam line construction and improvement projects. These technologies include pinpost Si monochromator crystals (BL 9), liquid nitrogen cooled Si monochromator crystals (BL 11), internally cooled Si mirrors (BL 9), and side-clamp cooled Si mirrors (BL 7). Where possible, the optics upgrades will be designed to expand existing beam line capabilities by, for example, changing mirror cutoff energies or optics acceptances.

The emittance reduction of SPEAR3 opens possibilities for new undulator beam lines as well as improving the performance of existing beam lines. SPEAR has always been unique in the US in that it has two long straight sections (the former high-energy interaction regions) which have the potential to be used for very long undulator devices. The SPEAR3 upgrade plan would shorten those to 13 m, (still longer than any other insertion device straight sections in the US), but would as a result gain 4 straight sections of 4 m length, one on either side of the long straight sections. These 4 m long straight sections offer the possibility of undulator beam lines emanating from those sources. Given the 3 GeV source energy, the brightness of undulator beam lines is optimal in the 1-2 keV energy region, one which is not as well covered by the existing 3rd generation sources. As will be discussed in the panel reports, a 1-4 keV undulator offers many exciting opportunities for new science at SSRL. Although not specifically optimized for the SPEAR3 source, it is informative to plot the brightness from a 4 m version of the APS undulator A source using the SPEAR3 source parameters. This is shown in Figure 2, plotting the tuning curves for the first 5 harmonics[6].

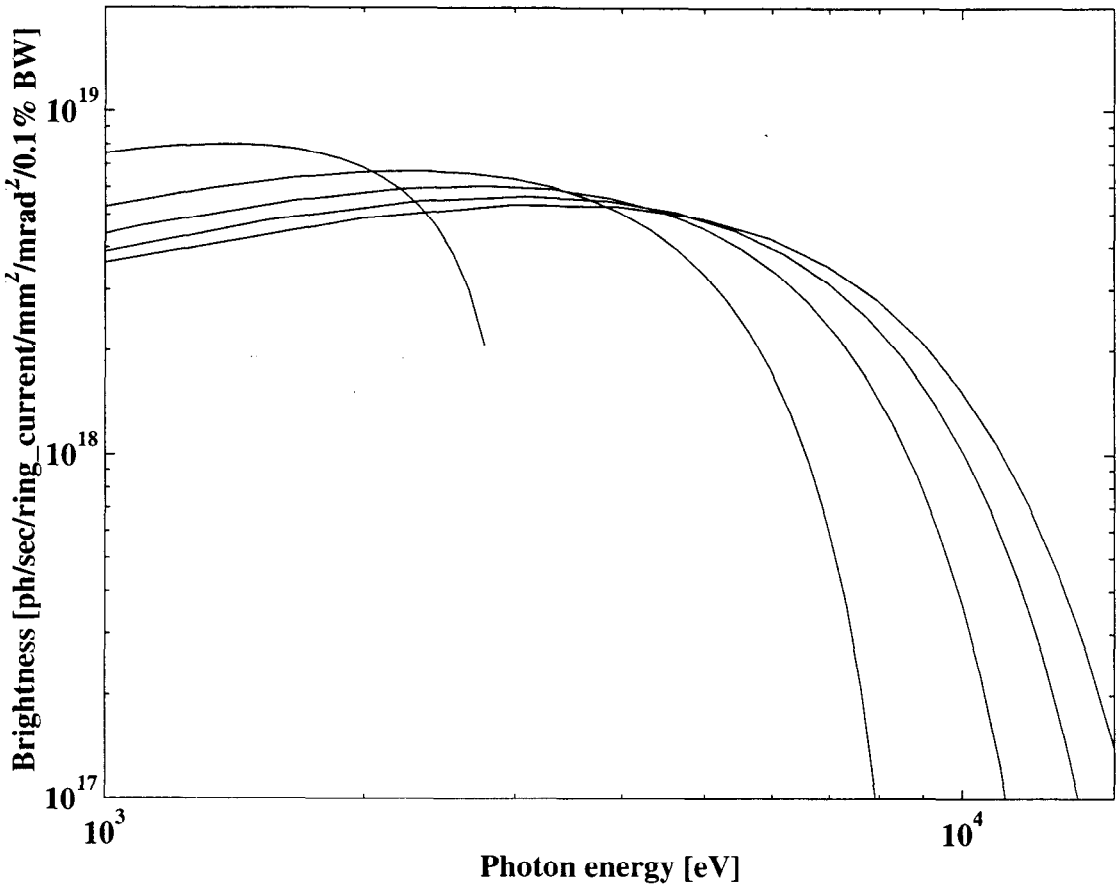

Figure 2. Brightness curves for a 4 m version of an APS Undulator A installed on SPEAR3.

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6. Calculations courtesy of Roger Dejus, Argonne National Laboratory.

Biological Macromolecular Crystallography

Introduction

Synchrotron radiation has been an essential component of the explosive growth of structural biology over the last 10-15 years. Bright synchrotron sources allow measurement of high-resolution data from crystals that diffract poorly on laboratory rotating anode sources, and those with large unit cells. The tunability of synchrotron radiation has made multiwavelength anomalous dispersion (MAD) phasing an expeditious method for structure determination.

Improvements in synchrotron radiation sources open new areas of investigation in structural biology. As the recent structure determinations of such proteins as nitrogenase[1], myosin[2] and the F1 ATPase[3] demonstrate, asymmetric structures with molecular weights of several hundred thousand daltons can be presently solved. Nonetheless, many fundamental problems require structural analysis of much larger macromolecular complexes, with molecular weights approaching one million for systems with little internal symmetry. The SPEAR3 upgrade will have profound consequences for these future advances in structural biology. In particular, by overcoming limitations of small crystal size and large unit cell parameters, it will be technically feasible to solve the structures of multimolecular assemblies central to the processing of information and energy in the cell. Structures of assemblies in the 100 to 1000 Å size range bridge the size scales between the atomic resolution methodologies currently available and the range accessible to optical microscopy, providing a complete molecular description of cellular processes.

Brighter sources will enable analysis of protein structures at extremely high resolution. Precise molecular models are required for detailed understanding of molecular mechanisms and are critical for rational drug design and other efforts in computational chemistry. *De novo* macromolecular structure determination by direct methods, which require very high resolution data, will also benefit from the proposed upgrade.

The design goals of SPEAR3 with respect to beam stability and lifetime continue the tradition of SPEAR that make it one of the most attractive synchrotron sources for data collection, especially for MAD phasing. In this regard, application of MAD phasing to larger problems than currently possible at SSRL will have a major impact on biological crystallography.

The breadth of research carried out at SSRL promises to open new avenues of investigation once a brighter source is available. For example, the development at SSRL of high pressure Xe as a heavy atom derivative, combined with brighter tunable beam lines, makes possible a new class of MAD experiments. Likewise, expertise in both biological XAS and single crystal diffraction at SSRL can be combined to study detailed chemistry of metalloproteins by a combination of crystallography and single-crystal XAS.

As molecular structure is critical for understanding cell biology in terms of chemistry and physics, rapid determination of crystal structures is essential for keeping pace with the advances in molecular biology and databases generated by large-scale sequencing efforts. Thus, in addition to new applications, the upgrade to SPEAR3 will move biology forward simply by enabling more structures to be solved. This is particularly true for MAD experiments, which are still difficult for weakly diffracting crystals. Also, with only a few sources in the United States, travel costs, both time and money, are significant issues for most laboratories. SSRL has a critical role in meeting ever-increasing demands for synchrotron beam time, especially in the western United States.

As detailed in the following subsections, the proposed SPEAR3 upgrade will be critical for advances in biological crystallography. First, the enhanced brightness of the source will allow data collection from smaller crystals and larger unit cells than is currently possible, and make ultra-high resolution data collection a practical method. Second, it will allow MAD phasing to be applied to larger systems, expediting their solution. Third, time-resolved studies on shorter time scales will be possible. Fourth, the new source opens the possibility for the use of undulator radiation for very small crystals and large unit cells requiring very bright, low-divergence beams.

Impact of Enhanced Brightness on Monochromatic Data Collection

The SPEAR3 upgrade will significantly impact data collection from poorly diffracting crystals and those with very large unit cells that are not optimally served by SPEAR:

- Exposures even on the newest and brightest beam line, 9-1, can be several minutes for such crystals. Even though most of the samples are flash-cooled and maintained in a cold nitrogen gas stream throughout the data collection, many show a time-dependent decay that causes the sample

to lose diffraction power. Shorter exposure times will reduce this effect significantly, and will be essential for small crystals. In cases where complete high resolution data can be measured only by translating the crystal with respect to the beam, the enhanced brightness will allow use of more finely collimated beams, making translation of the crystal for illuminating fresh volumes more precise.

- For non-mosaic crystals with large unit cells, horizontal beam divergence limits the spatial resolution of diffraction spots on the detector. The reduced emittance of SPEAR3 will improve spatial resolution without loss of intensity.

- High resolution data collection requires several passes at different exposures to stay within the dynamic range of the detector. This slows total data collection time, and with limitations of available beam time resolution is often sacrificed so that a larger number of data sets can be collected using shorter exposure times.

- The total time required to collect complete data from weakly diffracting crystals goes well beyond one ring fill period. This necessitates scaling the data over the full range of regular beam decay and more significantly over beam dumps, which can introduce scaling errors and decreased data quality. Shorter exposure times will reduce or eliminate this problem.

- On CCD detectors, longer exposure times lead to a significant increase in the number of zingers from background radiation, which reduces data quality.

Poorly Diffracting and/or Small Crystals

Measurement of accurate data from poorly diffracting crystals is a critical application of synchrotron radiation, and is often the decisive factor in being able to determine a structure. Many important problems have proven extremely challenging in terms of production of well-diffracting crystals, including ribozymes[4,5], molecular chaperones[6], membrane proteins[7], and cell-surface receptors[8].

The increased brightness will permit structure determination from smaller crystals than possible with SPEAR. Macromolecular crystals studied with a laboratory rotating anode source are

typically ~0.2 mm on a side with unit cell dimensions of ~100 Å, and hence contains ~10^{13} unit cells. In contrast, at synchrotron sources it is possible to collect data from crystals ~0.01 mm on a side that contain ~10^{9} unit cells using cryocrystallographic methods. Extension of diffraction data collection to smaller and smaller samples is critical for the analysis of ion channels and other complex systems; progress on these projects has been frustratingly slow in large part due to the inability to prepare large, well-ordered samples. More generally, use of small crystals can reduce significantly the total time for structure determination, and is especially cost-effective in terms of eliminating material and manpower expense for sample preparation and crystallization trials required to produce larger crystals. This is especially important in cases such as production of proteins in mammalian cell culture, where material is frequently limited by the expression levels and the cost of large-scale culture.

Large Unit Cells

One of the most important needs for brighter synchrotron radiation is for structure determination of macromolecular complexes, ranging from studies of binary protein-protein complexes to large assemblies such as viruses, proteosomes, and ribosomes. For crystals with large unit cells, the beam can be focused or collimated to a small size to optimize spatial resolution of diffraction spots without sacrificing intensity. For example, Roger Kornberg's group at Stanford is determining the structure of RNA polymerase II, the large multiprotein enzyme complex responsible for transcription. Exposure times for these crystals are many minutes even on the present beam line 9-1 (P. David, personal communication).

Studies of viruses will greatly benefit from the SPEAR3 upgrade. Whole virus particles with radial dimension between 150 and 300 Å crystallize with unit cell dimensions often in excess of 1000 Å. Studies aimed at mapping the structural basis of the virus life cycle and cellular responses to viral infection, such as those by Jack Johnson at The Scripps Research Institute, employ crystallizing virus mutants[9], particles in complex with monoclonal antibodies[10] and fragments of cellular receptors, which produce crystals with very large unit cells. The primary technical problem in these studies is in the measurement of relatively high resolution data (~3.5 Å) from unit cells with axes in excess of 800 Å. The problem becomes acute above 6 Å resolution, where the mosaic spread of the crystal and beam divergence lead to serious problems of reflection overlap even at crystal-to-detector distances of 800 mm. Surprisingly, the mosaic

spread of many virus crystals is less than 0.1°, so the major contribution to reflection overlap is the horizontal beam divergence. The primary need for these experiments is therefore extremely low beam divergence and very high intensity such as that provided by SPEAR3.

Although cryopreservation has largely eliminated radiation damage as a problem in data collection, the success rate of freezing virus crystals is significantly lower than for protein crystals generally. In such cases, rapid data collection allows measurements to be obtained before diffusion of free radicals generated by radiolysis of water destroy the illuminated volume. This makes high intensity even more important as many virus data collection experiments must be "flash bulb": data are collected from a given volume of a crystal by taking a few oscillation photographs with a total exposure time of 10-15 seconds. Fortunately virus crystals are often of sizes in excess of 0.5 mm so it is possible to translate the crystals and obtain multiple exposures from each crystal. In this regard, the ability to more finely focus the beam without loss of intensity will be an enormous advantage of SPEAR3.

Ultra-High Resolution Data

More intense sources allow feasible exposure times for experiments designed to measure very weak, ultra-high resolution data for detailed studies of protein structure and function. Measurement of data to resolutions past about 1.2 Å Bragg spacing allows study of proton positions (of interest, for example, in enzyme mechanisms) and permits individual anisotropic temperature factor refinement to examine anisotropic motions. The high quality and reliability of these structures will impact related disciplines such as structure-based drug design and computational chemistry.

There has been an ongoing effort at SSRL to obtain high quality, very high resolution data. In recent months Peter Kuhn of the SSRL staff has measured data from a number of different protein crystals to resolutions significantly higher than previously possible, using the new, high flux beam line 9-1. Even with well-diffracting crystals, these experiments require long exposure times and multi-pass data collection in which one or more lower-dose data sets are required to stay within the dynamic range of the detector for the lower-angle data. A standard exposure time at the SSRL beam line 7-1 is around 10 to 20 seconds and considerably less on beam line 9-1. In contrast, average exposure times of 3 to 5 minutes were needed to collect very high resolution

data on a variety of systems, including human topoisomerase (1.93 Å), phosphoglycerate kinase (1.56 Å), cholera toxin B5 pentamer (1.25 Å), photoactive yellow protein (0.85 Å), and subtilisin (0.81 Å). In each case the resolution limit was extended by more than 20% relative to previous synchrotron data sets. Reduction of exposure times will make these experiments practically feasible given limited beam time and improve data quality for the reasons outlined above. With SPEAR3 such experiments will become more routine, and provide more accurate, higher-resolution structures.

An exciting new area opened up by the ability to collect ultra-high resolution data is *de novo* macromolecular structure determination by direct methods for determination of protein structures solely from atomic-resolution diffraction data. Direct methods have progressed to the point where they have been successfully used to phase macromolecular structures, provided ~1 Å resolution data can be collected[11]. Future development of these methods and application to large systems will require brighter synchrotron sources such as SPEAR3.

MAD Phasing

The enhanced brightness of SPEAR3 will be essential for future applications and development of MAD phasing. The MAD method exploits the variation of anomalous scattering factors with energy to obtain phase information from a single crystalline species, and does not depend on isomorphism between crystals. Given the presence of enough anomalous scattering centers (commonly Se, which can be incorporated biosynthetically into proteins as selenomethionine, or traditional heavy atom derivatives such as Pt and Hg), MAD provides experimental electron density maps without the need for time-consuming screens for heavy atom derivative crystals isomorphous with native crystals, which is often the rate-limiting step in structure solution. Importantly, the method is theoretically applicable to problems of any size. Because MAD phases extend to the limit of accurately measured data, rather than where isomorphism breaks down, phases extend to resolutions well past those typically provided by multiple isomorphous replacement, which greatly facilitates accurate chain tracing and assignment of the sequence to the electron density. These factors should make MAD the first choice method for many problems. Indeed, a recent survey by W.A. Hendrickson (Figure 3, personal communication) shows the steep rise in the number of new structures determined by MAD phasing.

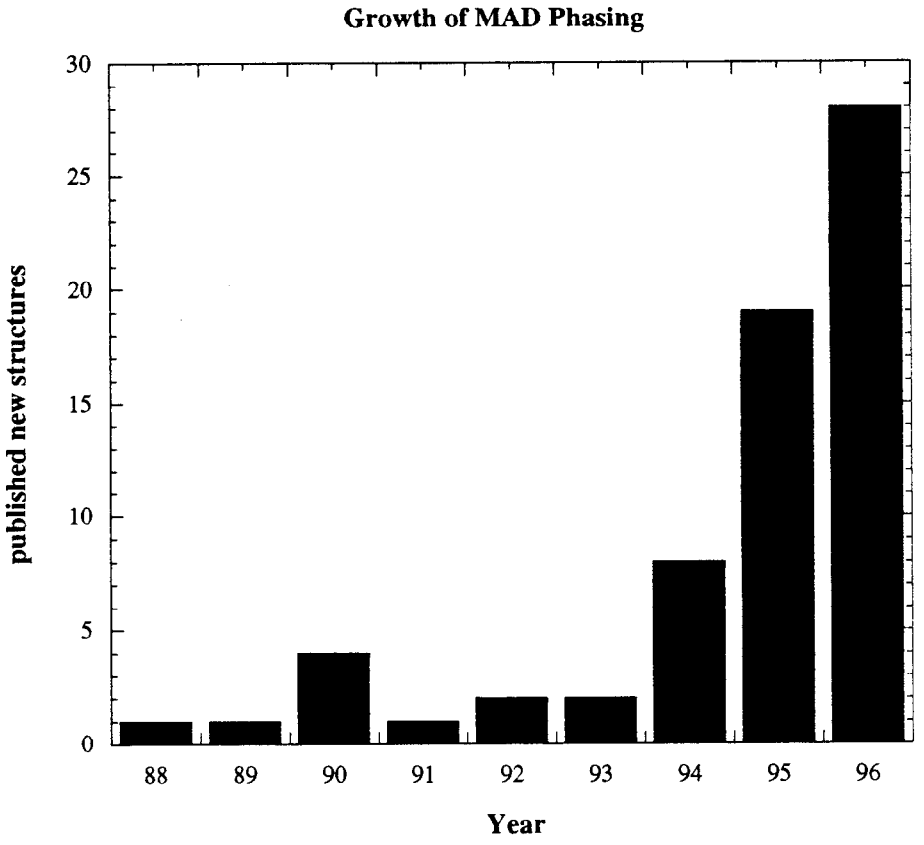

Figure 3. Number of new structures determined by MAD phasing

Long lifetime and beam stability, which will be maintained with the SPEAR3 upgrade, are important advantages of MAD phasing experiments at SSRL. Highly reproducible energy selection is critical to successful MAD phasing, as drifts of even 1 or 2 eV can dramatically alter the anomalous scattering factors and cause a loss of the anomalous scattering signals; monochromator instability or improper recalibration after beam fills are significant causes of failure in MAD experiments. The extremely stable beam at SSRL allows the user to change wavelengths often during data collection without recalibrating the monochromator. Another important and not-often-appreciated advantage of the SSRL MAD lines is the use of double flat monochromators. At many facilities, use of sagittal focus geometries or placement of a channel cut monochromator after a bent crystal monochromator restricts how far from the edge data can

be measured without refocusing the beam. However, optimization of dispersive differences depends on obtaining the largest difference in the real part of the anomalous scattering factors $\Delta f'$, which is achieved by measuring data at the absorption edge (minimum of $\Delta f'$) and one or more wavelengths remote from the edge (near a maximum of $\Delta f'$). Because $\Delta f'$ increases asymptotically away from the edge, significant enhancement of the dispersive signal can be achieved by measuring data far from the absorption edge. For example, data a full keV away from the absorption edge of Se provides a dispersive signal 30% higher than data measured only 200 eV off-edge.

Despite the many advantages of the MAD method and its implementation at SSRL, a limitation has been that intensity is sacrificed to achieve high energy resolution: the phasing signals are directly proportional to the anomalous scattering factors, and to achieve extrema of $\Delta f'$ and $\Delta f''$ the energy bandpass should be less than the width of the absorption edge (ideally 1-2 eV, typically 3-6 eV in practice). This has made MAD collection from large unit cell and/or small samples difficult. *Bright, tunable beam lines with high energy resolution will permit routine MAD phasing experiments on small samples or those with large unit cells.* Moreover, MAD experiments are extremely time-consuming, as they require measurement of complete diffraction data sets at each of three or four different energies. The enhanced flux and change in critical energy of SPEAR3 will provide much faster throughput critical for keeping up with the growth of this method. For example, beam line 1-5 will see improvements of about 80 - 150 x in flux density at energies commonly used for MAD experiments. In particular, experiments presently difficult, such as the K edge of Br (which is easily incorporated into nucleic acids as brominated uridine), or not feasible, such as the L_{III} edge of uranium (which is extremely well-suited for MAD[12]), will be well-suited for this beam line.

Enhanced flux at lower energies will allow new developments in MAD phasing. The development of techniques for using high-pressure Xe gas as a derivative by Michael Soltis of the SSRL staff in collaboration with the Rees group at CalTech[13] provides the opportunity to do MAD phasing at the Xe L_{III} edge, which is at about 4.8 keV. With the use of small crystals to minimize absorption and helium beam paths to reduce background scatter at these long wavelengths, a new class of MAD experiments should be possible. This will be especially important for cases where traditional heavy atom derivatives have failed, and in cases where

incorporation of Se is not possible (*i.e.*, when the protein is made as a recombinant molecule in certain cell culture systems, or there are not a sufficient number of methionine residues). Iodinated derivatives of nucleic acids or proteins will similarly benefit from such a beam line (I L_{III} edge at 4.6 keV). The advantage of such experiments is that the anomalous scattering factors are much larger at the L_{III} edge than the K edge, leading to enhanced signals for large problems.

Time-Resolved Studies

Intense, wide spectrum x-ray sources have many advantages for time-resolved studies with macromolecular crystals through the use of Laue and rapid data collection methods. Applications include studying structural changes that occur during enzyme-substrate reactions, photo-induced reactions or during phase transitions at atomic resolution. In these studies, data sets are collected at defined time intervals after initiation of a reaction, to monitor the progression of structural changes. Under SPEAR, beam line 9-2 will have a broad-band Laue capability. For a decently diffracting crystal, the exposure time for a diffraction image will be on the order of 1 msec. The time resolution for the same crystal would be on the order of 100 μsec in the SPEAR3 configuration.

The bright SPEAR3 source will also make feasible time-resolved monochromatic data collection experiments in which an entire data set can be measured in seconds or minutes. With the anticipated advancements in solid state x-ray detectors such as pixel detectors, which can be electronically gated on and off and have a very fast readout time, monochromatic time resolved experiments will be possible. As in the Laue case, time resolution is proportional to the flux density, so an increase in flux density would result in an increase in time resolution and expand the time range of experiments that could be performed. An especially exciting area made possible by SPEAR3 is the possibility of time-resolved single crystal studies on viruses. Virus particles are dynamic assemblies that usually form in a non-infectious configuration, which subsequently matures to an infectious form. The purpose of the provirion to virion transition is to prevent the virus particle from infecting the cell of its origin. Dr. Jack Johnson's laboratory has studied two families of RNA insect viruses called Nodaviruses[14] and Tetraviruses[15], and a double-stranded DNA phage called HK97, which are representative of more complex viruses. In these systems, extracellular factors such as divalent metal ions or intercellular proteases trigger

virus maturation. The time regimes of these maturation events are relatively slow, with a half-life on the order of an hour. Understanding the structural basis of these events will require time-resolved monochromatic data. In particular, caged metal ions or protons can be incorporated in the crystals or, given the slow kinetics, crystals can be permeated with the ligands that change the virus state and the transition followed by crystallography. Although some of these transitions are large enough to disrupt the crystal lattice, time-resolved studies may provide structural information for the initial events in virus maturation. These methods should also be applicable to the study of virus entry into cells, which involve structural transitions activated by receptor binding or by the low pH of the endosome.

Dedicated Undulator Beam Line for Protein Crystallography

The 4 m long straight sections created by the proposed SPEAR3 upgrade, combined with the large reduction in SPEAR emittance, provides the possibility of using a 4 m long undulator on SPEAR to generate a very bright beam at x-ray energies useful to protein crystallography. From the undulator curves shown in the technical section, the brightness appears to be comparable to the new wiggler beam line 9-2 at 10 keV, and as much as an order of magnitude higher brightness below 2 keV. Since an undulator beam line provides an x-ray beam that is quasi-parallel in both the vertical and horizontal planes (~10 microradians), it would be ideal for recording data from crystals with very large unit cells: it would be straightforward to produce a good quality focus over an extended range of sample-to-detector distances, with very little convergence in the beam. As discussed above, the brightness of the line would also be ideal for the collection of very high resolution data. A very bright undulator beam will also be of great importance for data collection from microcrystals.

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Biological Small-Angle X-ray Scattering and Diffraction

Introduction

Studies of structure-function relationships have become a central part of modern biology. There are a number of important biological molecular assemblies whose characteristic lengths are in the range of nanometer to micrometer, such as virus particles and muscle fibers. Many of those complex molecular assemblies are in a partially ordered or randomly oriented state that is not suitable for high resolution crystallographic analysis. Studies of dynamical structural changes of such large molecular assemblies that are linked to certain biological functions are required to expand our knowledge of atomic resolution protein structures to more complex biological systems which have remarkably diverse and important roles in enabling life processes. Small angle x-ray scattering/diffraction (SAXS/D) is a technique suitable for studying non-crystalline biological structures, and the use of a synchrotron radiation source has made it possible to conduct time-resolved SAXS/D studies of non-crystalline biological systems. The source characteristics of the proposed SPEAR3 would play a major role in making SAXS/D experiments even more effective in structural biology through:

- improved accessibility to shorter time scales by a factor of at least 10, reaching to the submillisecond regime, in time-resolved experiments because of higher flux delivered to the sample
- enhanced ability to examine smaller and hard-to-obtain biological samples due to the smaller beam size
- improved accessibility to smaller angles by a factor of 5 in the horizontal diffraction plane and 2 in the vertical plane, covering larger structural periodicities because the direct beam can be separated more easily from scattering at very small angles
- increased flux by a factor of approximately 5 for anomalous scattering experiments with biological specimens

In the sections that follow the positive impact of the proposed SPEAR3 upgrade is given in the context of specific experimental problems now being examined at SSRL along with new scientific opportunities presented by the new technology. Some of technological considerations are also given.

Small Angle Scattering from Biomolecules in Solution

Small angle scattering from a protein randomly oriented in solution can still give useful structural information at a low resolution, for instance radius of gyration, molecular weight, largest dimension within a molecule, volume and folding state. There are a number of important biological problems that can be addressed in terms of such quantities. Solution scattering can be calculated from a set of atomic coordinates when a crystal structure is known, and comparison of the crystal structure with the solution scattering curves that are obtained in different solution conditions can provide important insights of a molecular species that is not amenable to crystallization[1].

a) Protein folding

One of the major challenges in the structural molecular biology field is understanding how proteins fold from a linear chain of amino acids into compact three-dimensional structures which are the engines for many life processes. Protein folding constitutes a class of problems in which small angle solution scattering is beginning to play a unique and major role. This is largely due to the fact that solution scattering is very sensitive to changes in average electron density of a protein which undergoes unfolding or folding of its three-dimensional structure. Solution scattering also provides a quantitative way of distinguishing folded state with complete tertiary structures from unfolded state with random coil, and detects partially folded "molten globule" states[2].

The increase in flux by a factor of at least 10 would improve data quality in particular at higher angles that are sensitive to tertiary structure change. Solution samples in this class of experiments usually contain high concentrations of denaturant such as urea, thus excess electron density contrast of protein over solvent is rather low, resulting in a weak scattering signal. The increased flux would be very helpful in improving overall data statistics.

Aggregation of small proteins during the folding process is often considered to be a nuisance. However, it is also a problem of considerable biomedical interest due to pathologies involving protein misfolding and aggregation such as prion diseases, Alzheimer's disease, and other related phenomena. The ability to gain structural information on chemical equilibrium between monomers and dimers, and also on kinetics of conformational changes induced by protein-

protein association, will be of considerable interest in the coming years. Although measuring solution scattering from small proteins or peptides is always a challenge due to weak signal, future improvements in reducing background such as the use of an evacuated sample cell[3] and high-flux beam provided by SPEAR3 are expected to have a very positive impact on this area of research.

Stopped-flow time-resolved measurements: Currently at SSRL a stopped-flow system is being used to resolve time-dependent changes in biomolecular structure as a result of changing chemical conditions through rapid mixing. This system could definitely make use of increased flux since several hundred mixing events have to be averaged in order to get even reasonable signal-to-noise for radius of gyration measurements due to the weak signal in this class of studies. Radiation damage can become a problem on a time scale of minutes, although stopped-flow experiments on the scale of tens of seconds do not show signs of radiation damage. An increase in flux of an order of magnitude would make these experiments much more practical since by using a smaller number of mixing events, a smaller amount of protein could be used. The main limitation on the current experiments is quantity of protein needed, which runs into gram quantities. By reducing quantities needed into the tens of milligrams from gram or sub-gram quantities, this experiment would become practical for a much wider range of protein samples such as those produced by recombinant techniques. This is where the biochemistry is going in this field since the way protein folding depends on the primary sequence can be tested by mutations of a given protein. Improving the technique so that measurements can be made using much smaller quantities of protein would open it up to studies on a much wider range of different proteins.

Continuous flow mixing: Very recently, in collaboration with J. Hofrichter and W. Eaton of the NIH, SSRL has been able to demonstrate that a continuous flow mixer delivering solution into a 300 micron inner diameter capillary can lead to time-resolved measurements extending down from the current limitations of the stopped-flow mixing machine (around 100 ms with small proteins) to tens of ms. The quality of the scattering data is comparable to that from static samples since, by positioning the mixer head at different distances from the x-ray beam, one can measure scattering from protein samples at a range of time intervals following the initial mixing. Through the velocity of the jet emerging from the mixing head, this experiment thus converts the

time since mixing into the position from the head. Hence scattering data giving structural information on the state of a protein a few tens of milliseconds after mixing can be acquired over a period of many minutes by appropriate positioning of the mixing head relative to the x-ray beam. The problem with this experiment is that it consumes large quantities of protein because the capillary used for the SAXS/D measurements is about 300 microns as opposed to 50-100 microns used for similar measurements with laser induced fluorescence[4]. In order both to use less protein and to work with a faster jet and, hence achieve shorter times since mixing, one needs to narrow down the jet diameter by a factor of 5 to 10 and hence the consumed volume by a factor of 25 to 100. This experiment will thus benefit enormously from a higher brightness source allowing the x-rays to be focused down to 50-100 microns, assuming that appropriate optical elements can be put into the beam line. By pushing the time scale of observation back towards the 50 microsecond dead time limit caused by the mixing time in the micron-sized mixing chamber, SAXS/D can be used as a structural probe of the kinetics of folding of small proteins under pH and denaturant conditions where they approach the ultimate protein folding speed which is estimated to be on the scale of tens of microseconds. Protein folding is a fundamental problem which is of interest for a very large range of different proteins, and the need for increasingly sophisticated measurements of protein folding will become increasingly important in the next five to ten years as one of the key experimental measurements providing metrical details important to understanding the nature of intermediates in folding pathways.

b) Solution scattering studies of protein assemblies and subunit interactions

Changes in quaternary structures of oligomeric proteins are effectively studied with solution scattering. For instance the quaternary structure change associated with the allosteric transition of *E. coli* transcarbamoylase has been studied with static and time-resolved solution scattering[5]. Time-resolved solution scattering from a mutant version of *E. coli* aspartate transcarbamoylase was recently studied at SSRL. Since the mutant enzyme is much harder to obtain than the wild type enzyme, only a few solution conditions have been so far tested. The smaller and brighter beam from SPEAR3 would allow one to use a smaller sample volume while achieving improved statistics for the scattering data. The improvement in time-resolution would then be about 10 times on the order of 10 ms, approaching to the mixing dead time of about 5 ms.

The Ca^{2+}-promoted hexamer formation of annexin XII, a key protein involved in Ca^{2+}-mediated membrane fusion and voltage-gated Ca^{2+} channels, has recently been confirmed by solution scattering studies at SSRL. This was first proposed by a crystallographic study[6]. By screening varieties of solution conditions (different pH and ionic strength), the conditions where the hexamerization is optimized were found. Several different mutant versions of annexin XII were studied by solution scattering, aimed at identifying key amino acid residues for hexamerization. Stopped-flow time-resolved studies were also done, though the hexamerization takes place in the time range faster than one second. With the improved beam characteristics SPEAR3 would bring, one would be able to study the hexamerization process in a shorter time scale and would be able to study even more precious mutant versions.

Other larger proteins have also been studied at the BL 4-2 SAXS/D station: Trewhella *et al.* studied cGMP-dependent protein kinase (R_g 45-60 Å) and Thiyagarajan *et al.* GroEL (R_g 63-66 Å, Mw ~803 kDa). They are studying large quaternary structure changes when such proteins bind a substrate or substrate analogs. In order to measure radii of gyration of these large oligomeric proteins one would need to have a good access to small angles beyond 1000 Å. This number corresponds to the smallest angle one can reach with the current source in vertical direction. Relatively large source size in the horizontal direction currently prevents one from reaching very small angles in that direction. This also deteriorates data quality to some extent when a two-dimensional solution scattering pattern is circularly averaged. SPEAR3 would provide a smaller beam size in particular in the horizontal direction, making the existing instrument more suitable for the use of a 2D detector for solution scattering. The small angle resolution of the instrument would be improved by at least a factor of two in the vertical plane and several in the horizontal plane.

c) Virus solution scattering

Several virus and phage systems have been studied on BL 4-2 during the past few years. Many of the studies were aimed at complementing other structural techniques. For instance, the application of the spherical harmonics algorithm in analysis of solution scattering yields the determination of the molecular envelope of icosahedral virus particles[7]. We have collaborated with electron microscopists to obtain much more accurate scattering amplitudes of a virus particle in the smallest angular range to 5-10 Å. The determination of the contrast transfer

function in electron microscopy (EM) is often inaccurate at the high angle range and we intend to improve EM structures by combining with solution scattering amplitudes. Another important category in virus solution scattering is time-resolved studies of phage/virus maturation. Most viruses and phages undergo morphological changes as they assemble themselves into matured infectious viruses or phages from protein capsids. We have studied a maturation phase of the DNA phage HK97 head particle with the stopped-flow time-resolved technique.

Virus/phage particles are very large compared to most proteins: at least a few hundreds of Ångströms in diameter, thus covering very small angles is essential for accurate shape determination. Viral samples are usually very hard to obtain in larger quantities compared to many protein samples, and the ability to obtain high-quality data in a shorter time with a small amount of sample is very crucial. The smaller beam size afforded by the SPEAR3 upgrade would allow easier access to much smaller scattering angles than now obtained, and would allow one to measure even more hard-to-obtain viral samples. Higher flux delivered to the samples will enable time-resolved studies of virus/phage maturation in a much shorter time scale, possibly sub-millisecond.

Low Angle Single Crystal Diffraction Studies

The small angle scattering instrument on SSRL BL 4-2 has been modified to enable it to also record low angle single crystal diffraction data. Low resolution data sets thus far recorded have been proven to be very useful in crystallographic analysis of large unit cell protein crystals, in particular virus crystallography. In high resolution structure analysis of virus crystals, initial phases are often obtained from low resolution structure models typically deduced from cryoelectronmicrographs. Successful phase extension to higher resolution depends on the amount of low resolution diffraction data accurately recorded, accuracy of the contrast transfer function determination in electronmicroscopy, and shape of the virus particle of interest, among other quantities. The low angle diffraction data recorded at BL 4-2 effectively fills in the "resolution gap" between high-resolution data and low-resolution structure models. A DNA phage HK97 structure has just been solved using the low angle diffraction data in combination with high-resolution data that had been recorded separately (Wikoff *et al.* 1997 in preparation). The other advantage of studying low resolution diffraction spots is to visualize disordered structures which are not contained in high resolution data. For instance, we have visualized

nucleic acid structural organization inside a flock horse virus-like particle which was not seen with the high-resolution structure (Tsuruta *et al.* 1997 in preparation). This is based on an approach similar to that previously used to visualize internal structure of polyoma virus[8]. However, the use of the advanced synchrotron small angle scattering instrument played a major role in the improvements to data quality. We expect to have more research projects of this type to be performed on BL 4-2 in coming years.

The beam size at the BL 4-2 is currently limited primarily by two factors: a relatively large source size projected by the 1:1 demagnifying toroidal mirror, and the non-optimal performance of the focusing mirror. The current beam size is often larger than many virus crystals. By combining the increased brightness and an optimized focusing mirror, the SPEAR3 upgrade would provide a much smaller beam size with higher intensity, thus allowing one to measure much smaller crystals. Hence the low angle single crystal diffraction technique could be applied to a greater range of biological and medical problems.

Muscle Fiber Diffraction

The modern concept of the mechanism of muscle contraction is based on structural, mechanical and biochemical studies, all indicating that relative sliding between the two filament types in the sarcomere depends on cyclic ATPase-coupled crossbridge interactions between myosin heads on the thick filaments and actin in the thin filaments. The exact nature of the force-generating myosin-actin interaction, the ATP-driven power stroke, remains unclear. Fiber diffraction of muscle has a key role in approaching this problem because of its ability to study muscle fibers under hydrated, physiological conditions, in fact even in the living state[9]. Furthermore, it has the ability to detect global changes in sarcomere structure at the physiologically relevant millisecond and sub-millisecond time scale.

Small angle diffraction of muscle is most effectively used in combination with other structural techniques. Correlation of low-angle x-ray diffraction with EM images and image transforms has been a key approach in past work. X-ray diffraction plays a critical role in establishing the fidelity of the EM preparations to the true physiological state. Since the sampling and averaging are different in the two techniques, the information is of complementary nature and of immense value in constructing structural models. The other most powerful use of x-ray diffraction is in

combination with mechanical measurements on muscle fibers. One use is to look for intensity changes in specific x-ray reflections during rapid tension transients that can be correlated with specific structural models.

Need for higher brightness sources for time-resolved fiber diffraction: Small angle x-ray diffraction studies of muscle make unusually high demands on x-ray sources and optics. Muscles diffract relatively weakly, have many components with similar long spacings and the changes of physiological state in muscle and the accompanying structural changes occur in a time scale of milliseconds or a few tens of milliseconds. It has been over 20 years since the ~100-fold intensity advantage of synchrotron sources for x-ray diffraction first excited K. Holmes and G. Rosenbaum, motivating them to begin development of the first synchrotron biological x-ray diffraction facilities at Hamburg. It took until the late 1970's for the first truly useful results from experiments using synchrotron radiation to be obtained. Since then, synchrotron radiation studies of muscle have told us much of what we know about the chronology of structural changes accompanying force development.

The future promise of time resolved x-ray diffraction studies of muscle can be realized in studies that produce full two-dimensional diffraction data at high time resolution during rapid mechanical transients. The major impediment to reaching this goal has been lack of flux. Lack of flux has meant that most of the work so far has been done from large leg muscle of the frog. It is not possible to do adequate (by today's standards) mechanical experiments on whole muscle and large fiber bundles. State-of-the-art work is done with single muscle fibers and even myofibrils. Some of the reasons for excluding larger preparations are fiber to fiber variation, difficulty of synchronizing and ensuring uniformity of contraction, limitations imposed by diffusion rates of substrates and products, difficulty of imposing servo-control of sarcomere length and force which (in most cases) are necessary to have interpretable results. Muscle fibers are typically a few mm long by 50-200 μm wide. Somehow as much flux as possible has to be delivered to the sample while maintaining acceptable beam divergence. Since in small angle diffraction one is trying to resolve closely resolved spots, it is necessary to have low beam divergence, the lower the better but less than 0.1 mrad in both directions highly desirable.

Prospect for the SPEAR3 upgrade in muscle fiber diffraction: Current level of flux obtained at the BL 4-2 SAXS/D facility with the use of multilayer monochromator is comparable to that

obtained at the CHESS wiggler station A-1. The proposed SPEAR3 upgrade with the upgraded wiggler will make the SSRL SAXS/D facility competitive with other 3rd generation facilities such as the APS or ESRF in terms of flux density that can be delivered to the sample. This will allow most of the experiments possible at those existing 3rd generation facilities to be done at SSRL, greatly enhancing access to beam for muscle researchers. That would also give opportunities to study wider varieties of muscle fibers and tissues.

Anomalous Scattering

A preliminary anomalous scattering experiment has just been conducted, aimed at studying conformation of cytochrome *c* in different oxidation states. Anomalous scattering at the Fe K absorption edge (7112 eV) requires rather narrow energy bandpass (~2 eV) which is currently achieved by using a narrow pre-mirror aperture. This is to minimize optical aberration of the toroidal focusing mirror. This, however, reduces the beam flux incident on the sample significantly. The small source size and small beam divergence achieved by the SPEAR3 upgrade would provide higher flux to the experiment. Separation of vertical and horizontal focusing would further improve energy resolution without reducing flux, though a large change in beam line optics configuration is not part of the SPEAR3 upgrade plan. However, the use of the proposed undulator would produce a smaller divergence beam which is even more suitable for anomalous small angle scattering in particular in the energy range of 1-4 keV which covers K absorption edges of biologically important elements S, P and Cl. The potential use of the undulator is discussed below.

New Non-Crystalline Biological Systems

Amyloid fibers, which are thought to be the major cause of the Alzheimer's disease, are usually oriented inside a small quartz capillary as a thin disk. The disk can be as thin as about 100 μm, requiring a very small beam size to reduce background as well as small beam divergence in order to study long range interaction and structural organization of the fibers, which are not well understood to date. The small beam size at specimen provided by SPEAR3 would facilitate studies of this clinically very important system.

Many viral systems and some of the most important components of molecular machinery in cellular motility such as flagellin form fibers. Fibers are usually much less ordered than protein

crystals, thus giving significantly strong diffraction spots only at relatively small angles. These fibers can also be quite small. The small beam size from SPEAR3 would thus enable the application of fiber diffraction techniques to many fiber systems that are difficult to study with a larger beam size.

Among relatively recent time-resolved studies, studies of lipid-based model membrane systems using the laser or microwave temperature-jump technique, and studies of proteins under high pressure or using pressure-jump perturbation would also benefit significantly from the SPEAR3 upgrade. These systems could be studied with a shorter time-resolution due to the increased flux. The smaller beam size would allow one to use smaller sample area to irradiate with a laser or microwave beam, allowing a temperature-jump with a larger magnitude. A smaller high-pressure window area could be used with the smaller beam size, thus expanding the range of pressures to be used.

Required Optics Improvements

To take full advantage of the upgraded SPEAR3 source, it will be necessary to upgrade the beam line optics for improved stability under the expected high heat load and to optimize photon beam characteristics for small angle scattering experiments. Current SAXS/D measurements on 4-2 are very sensitive to temperature changes in the mirror as the incident flux varies, in particular immediately after filling the storage ring. Improvements in the mirror system to deal with increased flux will be critical in order to take advantage of the improved SPEAR3 characteristics. The current monochromator cooling scheme has to be upgraded as one sometimes sees very small thermal effects. At minimum, a cooled mirror and an improved mirror bender system should be implemented in order to preserve the brilliance of the source. We intend to upgrade BL 4-2 optics components to meet the SPEAR3 requirements as soon as sufficient funds become available.

Recording scattering at very small angles requires minimum angular divergence and small spot sizes in at least one direction, so a high degree of demagnification cannot be tolerated in those cases. For highly anisotropic systems such as oriented fibers and capillaries in flow systems, the criteria may differ in vertical and horizontal directions. The existing 1:1 focusing system using a toroidal mirror is, in principle, appropriate to preserve intrinsically small beam divergence of synchrotron beams. It, however, has the disadvantage that the horizontal focal point can not be

adjusted. It would be ideal to separate horizontal and vertical focusing elements. That would provide independent control to permit optimal matching of beam dimensions to the sample and the detector. For instance the combination of a 1:1 focusing cylindrical mirror for vertical focusing and a sagittal focusing monochromator for horizontal focusing may realize this situation. Optics schemes such as this should be considered when the optics upgrade actually takes place.

New Wiggler on BL 4-2 and Potential Use of An Undulator

It is proposed that the existing electromagnet wiggler be replaced with a new wiggler based on the latest permanent magnet technologies. This has important consequences as the large horizontal electron beam excursion in the existing wiggler limits the source size reduction that would be otherwise achieved with the low emittance of the SPEAR3 upgrade plan. Smaller beam size and smaller beam divergence would have major impact in the way SAXS/D experiments are conducted at SSRL. For experiments using photon energies less than ~10 keV, an undulator would be a brighter source on SPEAR3 than the new wiggler, and would provide a source which is easier to focus than the wiggler. Most of the new SAXS/D stations at third generation synchrotron sources use undulator sources. A new undulator beam line would offer the greatest flexibility in independent horizontal and vertical focusing and would allow scattering down to smaller momentum transfers than would be possible with a wiggler.

An undulator source would be especially exciting for anomalous SAXS at elemental edges in the 1-4 keV range, where a SPEAR3 undulator could be expected to have superior brightness. Anomalous small angle scattering from phosphorus, a major component of nucleic acid, can be much more effectively studied due to the increase in flux density by a few orders of magnitudes compared to that obtained at the HASYLAB A-1 bending magnet station, which has been the sole instrument for this type of research[10]. At low beam energies, most photons are absorbed by window materials and the sample, and only few photons reach a detector. The dramatic increase in flux density in this energy range with the use of the proposed undulator would facilitate studies of protein-nucleic acid complexes. The iodine L_{III} edge may be used for the same purpose when a part of protein-DNA complex can be iodinated.

It is outside of the scope of the SPEAR3 upgrade to replace the BL 4-2 wiggler with an undulator source. However, the potential use of a new undulator beam line for small angle scattering on part time basis should be seriously considered, given the potential benefits described above.

Need for New Detector Technologies

New detector technologies have to be introduced to overcome the high detector count rates arising from the increased beam flux. Even now it is sometimes necessary to attenuate the beam in order to keep detector count rates below 100,000 counts per second, beyond which our linear gas chamber detector system has significantly lower counting efficiency. This situation was brought on primarily by the introduction of multilayer monochromator crystals a few years ago. An image plate detector has recently been characterized for low-angle diffraction and encouraging results have been obtained. SSRL has also been involved in characterizing several different types of advanced CCD x-ray detectors in last few years. These detectors function better at high count rates, but some of their detector characteristics such as dark current, readout noise and quantum efficiency have to be examined and evaluated critically before their routine use for small angle scattering experiments. In the last few months a cooled CCD x-ray detector on loan from the Photon Factory, National Laboratory for High Energy Physics, Japan has been in use on BL 4-2[11]. This detector has been tested in time-resolved and static solution scattering studies of proteins as well as time-resolved muscle fiber diffraction experiments. It has been proven to have high sensitivity with very low noise, and has a relatively fast read out time of 50 ms with 2x2 binning for time-resolved data acquisition. Such a 2D detector system for high count rate SAXS/D applications would enable a large number of new experiments and implementation of such a system should be considered on BL 4-2. Other detector technologies may be developed in the future for very fast time-resolved applications.

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Biological X-ray Absorption Spectroscopy

Introduction

Metal ions are essential constituents of many biological assemblies, with roles that range from a simple architectural element in creating a particular shape (*e.g.*, Zn in the finger proteins that control gene-switching) to being the essential catalytic reaction site (*e.g.*, Fe in the cytochrome P-450s that oxidize drugs and other xenobiotics). Understanding how function is determined by a given metal ion is closely coupled to understanding its electronic and metrical structure in the resting and catalytic (where applicable) states. Biological X-ray absorption spectroscopy (XAS) is a synchrotron based technique that enables element-specific, direct investigation of the electronic configuration and chemical coordination environment of such metals (distances to, numbers of, and types of atoms surrounding the metal)[1]. Unlike crystallography, the technique can be applied to solutions and the results directly provide information such as oxidation state and accurate metal-ligand bond distances. Hence XAS has proven to be an extremely valuable tool in understanding structure and function in metalloproteins and other systems involving metals in biology. There have been numerous applications to the first and second row transition metals as well as to some of the alkali and alkaline earth metals. XAS has grown to be an essential tool and in the following sections the role of increased flux and flux density that would derive from a SPEAR upgrade are considered in light of different classes of experiments that are, or could be, done.

Conventional Samples

XAS accounts for a significant amount of the beam time demand at SSRL (and nationwide). Although some of these experiments, such as transmission XAS, are not flux limited, most are. Such experiments dominate both in terms of numbers of experiments, and to an even greater extent, the amount of beam time used. Flux limited experiments will benefit by a factor of 2-6 in various ways under SPEAR3, as discussed below. Although this is a relatively modest improvement, it will have a significant impact on the science that is done using "conventional" XAS, affecting concentration, sample size, data quality, and synchrotron access.

- *Lower concentrations.* The practical lower limit for solution XAS at SSRL is *ca.* 0.5 mM in absorbing atom concentration, with some variation depending on the element of interest. The SPEAR3 increase in flux, when coupled with anticipated improvements in detectors, will make measurements of ~0.1 mM absorber concentrations in solution feasible, and allow studies of metalloproteins that are presently too insoluble to be studied in solution (for example, many DNA binding proteins) or that cannot easily be concentrated (for example, membrane-proteins). Even when proteins are soluble to give 1 mM absorber concentration, it is sometimes necessary to work with diluted samples, for example in freeze-quench studies, where solution viscosity mandates the use of dilute samples. This is a current limitation in studies of kinetic intermediates in enzymes such as methane monooxygenase and ribonucleotide reductase, where an understanding of the catalytic mechanism can be expected to have important implications for improving gas-conversion technologies, and in understanding certain pathologies.

- *Sample size.* In some cases only limited amounts of biological samples are available. This has diminished as an issue with the development of molecular biological methods for protein over-expression, but is still an issue in some cases where viable expression systems have not been found. The higher brightness of SPEAR3 will be especially important for such samples, as it will allow studies of smaller volumes.

- *Data quality.* Even today, a great deal of published biological EXAFS data suffer from serious noise problems and/or severely limited k range. The higher flux of SPEAR3 will enable noticeable improvements in the quality of data that can be acquired, and extension to higher k, in a given period of time. The resolution of the EXAFS is directly determined by the k-range, and often the questions that can be answered by XAS depend directly on the resolution. One example is the discrimination of sulfur ligands located *cis* or *trans* to an oxo in molybdenum enzymes such as arsenite oxidase. This enzyme has important environmental implications, converting the highly toxic As(III) to the much less toxic As(V).

- *Increased availability of biological spectroscopy.* The SSRL wiggler beam lines remain oversubscribed by a factor of ~2. An increase of even a factor of 2 in productivity (*i.e.*, time

required to measure the present samples with data of the present quality) would have an enormous impact (equivalent, effectively, to constructing several more beam lines). In reality, this factor of 2 increase in throughput is unlikely to be realized, because users will probably collect higher quality data when given the opportunity. There will be a second increase in throughput, however, because the SPEAR bending magnet beam lines will become a valuable resource. While with SPEAR they do not have sufficient flux for most protein EXAFS experiments, the *ca.* 6-fold increase under SPEAR3 will make the bending magnet beam lines useful for many experiments that currently can only be done on wiggler beam lines.

Single Crystal X-ray Absorption Spectroscopy

The application of XAS to single crystals will take advantage of the polarization properties of synchrotron radiation, and yield bond-specific metrical information and the means to determine electronic structure, including properties such as oxidation state, spin state, and covalency[2]. While protein crystallography provides three-dimensional structural information, including the location and coordination of metal active sites, the accuracy of the metrical information is often limited by the resolution of the data and the size of the structure. Distances determined by protein crystallography are thus typically determined to an accuracy of only a few tenths of an Ångström, which from a chemical viewpoint does not provide enough detail for understanding changes that occur in, for example, a redox-based electron transfer process. XAS focuses on short-range order and determines local structure to an order of magnitude higher accuracy, typical first-shell distance determination to within an accuracy of 0.01-0.02 Å. Longer range order can also be studied, out to distances of about 5 Å from the absorber. Together with the information from the XAS edge, one has a comprehensive probe of metal site structure and the ability to determine function through the preparation and investigation of intermediates, inhibitor complexes, or other perturbations at the active site metal center.

Different aspects of this approach can be illustrated with specific scientific applications:

- In copper-containing electron transfer proteins, for example, the ability to determine ligation of and distances to Cu to an accuracy of *ca.* 0.01 Å is essential since the changes in Cu-L

distances between the Cu(I) and Cu(II) states will be in the range of 0.1 Å. Further, through crystal orientation it is possible to look selectively at different Cu-L bonds and study their relative contribution to the determination of the electronic structure, and hence electron transfer ability, of the Cu site[3].

- Single crystal XAS also establishes directly in the crystal the electronic structure of the metals in the active site, something only implied by crystallography studies. For example, in Cu oxidases (such as galactose oxidase) it would be possible to directly probe the possible role of the unusual oxidation state of Cu(III) in the catalytic mechanism, and to distinguish between Cu(II) plus a ligand radical *vs.* Cu(III).

- The question of whether there is a difference between an active site in a protein solution and in the crystalline state can be addressed by performing comparative measurements. For example, the enzyme methane monooxygenase can have different bridging ligands at the binuclear Fe site depending upon how it was crystallized, and single crystal XAS compared with solution XAS can directly probe this question and provide insight into the actual catalytic form of the binuclear center. Another example where a combined approach would be useful is dimethylsulfoxide reductase. The molybdenum site of this enzyme has robust solution properties, as indicated by XAS. Four different crystal structures for this enzyme are now known. While these have nearly identical protein folds they have radically different structures at the molybdenum active site. Combined XAS and protein crystallography would resolve questions about the relationship of the crystallographic structures to solution structures.

- XAS can also be used with single crystals to directly investigate changes in electronic and metrical structure when substrates and/or inhibitors are bound. This can be achieved through the use of flow cells, freeze-trapping or by utilizing the high pressure gas/rapid freeze technique developed for crystallography. For example, the MoFe protein of nitrogenase contains the active center where N_2 is bound and reduced. N_2 and acetylene are substrates whereas CO is an inhibitor, and gas pressurization studies could probe the molecular

mechanism of the interaction of these small molecules with the catalytic center, hence leading to a chemical understanding of this important process.

Single crystal XAS experiments would benefit dramatically from the proposed gain in flux density through the SPEAR3 upgrade because of the small volume of the samples compared to normal solution XAS. It is estimated that high quality XAS data could be collected on crystals of ~ 200 microns, and single crystal diffraction data could be measured on the same samples. The experimental requirements would be a beam line combining characteristics of a protein crystallographic beam line (well collimated, small spot size, high flux density beam) with that of an x-ray absorption spectroscopy beam line (tunable monochromator, high energy resolution, high mechanical and beam stability). The experimental setup would include the capability to do crystallography (goniometer, collimator, crystal cooling system, imaging plates) to record enough diffraction data to ensure knowledge of crystal orientation, proper crystal form, and check of structure *vs.* known structure, and ion chambers and fluorescence detectors for x-ray absorption experiments. Development of such a beam line would make SSRL a unique resource.

Other Oriented Samples

While crystallographic orientation is preferred where possible, there are a variety of other ways in which proteins can be oriented when crystals are unavailable. Some examples include DNA regulatory proteins, that can be bound to DNA fibers, and membrane proteins, that can be oriented as monolayers and studied by grazing incidence XAS and x-ray standing wave methods[4]. EXAFS measurements on these samples would provide novel insights into molecular orientations of metal cofactors while x-ray absorption edge structure would provide insight into the electronic structure of the metal ion that is not available from isotropic XAS.

Intact, Living Systems

The availability of a higher flux density will allow exciting new opportunities for studying intact cells and tissues[5,6]. There are several reasons for doing this:

- A large number of enzymes have been studied as isolated, purified proteins. In favorable cases it should be possible to examine these enzymes *in vivo*, or at least in unfractionated

organelles. For example, probably most of the manganese in chloroplasts is associated with the water-splitting enzyme, and it is important to verify that our understanding of the system, gained principally from isolated preparations, is indeed correct.

- The possibility of determining both the spatial location, and the chemical nature, of specific elements in biological systems will be an exciting advance. Important examples include understanding spatial localization and chemical nature of elements involved in pathologies such as Alzheimer's disease, and determining the spatial location and chemical nature of elements moving in plants during phytoremediation of contaminated soils. Both location and speciation can be done at present, but with lower spatial resolution than desirable. Combining the techniques will open new avenues for research and the high flux density of SPEAR3 will provide for spatial resolution in the 10 μm range.

- Some metal ions, such as calcium and zinc, are important messengers and modulators in cell biology. The ability to follow the changes of metal coordination during major metabolic events, such as egg fertilization, will open new windows on how such processes proceed.

Kinetics

As discussed above, the increased flux of SPEAR3 will allow experiments with more dilute samples than has previously been possible. These include kinetic studies involving rapid mixing and freezing[7], where the viscosity of concentrated protein solutions is limiting. Another class of kinetic experiments will be made possible by the high-flux densities of SPEAR3. Continuous-flow techniques can provide information on intermediates of catalytic turnover, with potential time resolution significantly faster than rapid freezing (*e.g.* <1 msec., compared to >5 msec.). Unfortunately, this technique has been little used in XAS because of the substantial sample sizes presently required. The high flux densities from SPEAR3 will make these experiments feasible on much smaller samples, and we envision early applications to the study of catalytic intermediates in enzymes such as sulfite oxidase, a key enzyme in mammalian sulfur metabolism, and nitrate reductase, a key enzyme in the global nitrogen cycle.

Diffraction Anomalous Fine Structure

The Diffraction Anomalous Fine Structure experiment effectively provides XAS-like information by monitoring the energy-dependent intensity changes in diffraction peaks, and can provide site-selective information in crystalline materials[8]. This experiment is severely flux-density limited and the SPEAR3 upgrade would make SSRL an excellent facility for DAFS experiments. For example, site-selective studies of superconducting ceramics should provide important insights to materials. A variety of simple biological crystalline proteins might also be studied, such as site-selective studies of the two different metal atoms in Fe_2S_2 ferredoxins, important cofactors in photosynthesis, and mixed valence binuclear manganese clusters in catalyses, enzymes that detoxify peroxides.

High Resolution X-ray Fluorescence

The K-fluorescence lines (specifically K_β and $K_{\beta'}$) of the transition metals exhibit chemical shifts due to oxidation and spin state. In addition, K_β emission arises predominantly from a spin down 3p hole while $K_{\beta'}$ arises from a spin up hole. These fluorescence lines can be measured at high resolution (<1 eV) using a spectrometer employing spherically bent crystal optics placed on a Rowland circle with the sample and detector[9]. The resolution of the spectrometer allows selective observation of fluorescence arising primarily from one oxidation/spin state in the presence of others. This site selectivity based on oxidation or spin state has direct application to many metalloproteins containing clusters of two or more metal ions which vary in oxidation/spin state depending upon their position in the protein or stage of the catalytic cycle. The manganese cluster of photosystem II, for example, consists of a mixed valence cluster of four manganese ions which cycles though a series of oxidation states during the conversion of water to dioxygen. A detailed understanding of the environment around metal ions of different oxidation state (as opposed to conventional EXAFS which gives the average of all four ions) would be very useful in understanding the structural intermediates and reaction mechanism in photosynthetic water oxidation.

The spin up/spin down nature of the K_β lines also allows measurement of spin polarized EXAFS (SPEXAFS) without the use of circularly polarized radiation. One possible application of this

would be to allow one to distinguish diamagnetic from paramagnetic scatterers, possibly simplifying the often-complicated XAS spectra of metal clusters in proteins, and increasing our understanding of their electronic structure.

The inherently small acceptance of the spectrometer and a requirement for a small vertical beamsize (<0.5 mm) have resulted in extremely low counting rates and have made work on metalloproteins difficult at current synchrotron sources (NSLS and SSRL). The reduced beamsize and higher flux density resulting from the SPEAR3 upgrade will make these experiments possible on a wide array of biological and materials-science samples.

Low Energy Spectroscopy

While the SPEAR3 upgrade will impact a broad range of experiments, it is in the 1-4 keV energy range that the greatest benefits of brightness from an undulator source can be realized. This energy range includes the K-edges of Na through Ca, the L-edges of Cu through Cd, and the M-edges of I through Pu. Despite the wealth of information available from this energy range, experimental access has to date been very limited. The SPEAR3 upgrade with existing beam lines provides access in the 2-4 keV region (BL 6-2) and the 1-2 keV region (BL 3-3 with YB_{66} or InSb monochromator crystals). To date, the greatest potential has been realized at the sulfur K-edge, with important progress in understanding the electronic structure, and thus metal-ligand covalency, of sulfur ligands in metalloproteins[10], and the speciation of sulfur in complex samples such as microbial cells[5], oils, coals[11], soils, and sediments. Similar experiments with phosphorus, aluminum, *etc.* are much more difficult, but the SPEAR3 upgrade will facilitate them, and in the long run provide the basis for an improved insertion device that will make this region of spectroscopy readily accessible. Such an upgrade, especially if coupled with a new undulator beam line, would make SSRL the premier facility for spectroscopy in this energy range.

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Molecular Environmental Science

Introduction

A large and growing molecular environmental sciences (MES) user community has evolved at SSRL over the past seven years in response to the need for quantitative molecular-scale information on the chemical speciation of metal-ion contaminants in dilute natural samples and on the fundamental chemical and biological processes that control their transformations, mobility, toxicity, and bioavailability. X-ray absorption spectroscopy, in bulk and high-spatial resolution modes, can provide much of this information on complex, heterogeneous samples, including those containing solid-liquid-gas interfaces, under natural conditions (*i.e.*, with contaminants at low concentrations, bulk water present, and at ambient temperature and pressure). The SPEAR3 upgrade will substantially improve these capabilities relative to current wiggler beam lines at SSRL commonly used for MES research (BL 4-1, 4-2, 4-3) and will provide new opportunities for MES research at SSRL. For example, the Molecular Environmental Sciences Beam Line Facility, BL 11, which will be completed in early 1999, when coupled with the SPEAR 3 upgrade will provide an order of magnitude greater flux than these beam lines and will permit focusing of the incident beam, using Kirkpatrick-Baez mirrors, to a spot size of 7 x 10 μm with a flux of 10^{10} photons s^{-1}. These improvements will permit micro-XAS studies of dilute contaminant speciation in spatially and chemically heterogeneous environmental samples.

The SPEAR3 upgrade will also improve beam stability, facilitating the analysis of smaller, thus less hazardous samples containing radionuclides or carcinogens than is currently possible, and will substantially improve the ability to characterize environmentally important chemical reactions and transformations at the surfaces of natural materials. In particular, the higher flux will permit *in-situ* grazing-incidence and conventional XAS studies of defect-level coverages of adsorbates at environmentally relevant metal oxide- and metal sulfide-aqueous solution interfaces. Surface defects, which are often at concentrations of less than 5% of the total surface sites, control surface reactivity in these systems, and existing fluxes on MES wiggler beam lines at SSRL are not sufficient to probe this low coverage level. These and other SPEAR3-generated enhancements in MES capability were identified by a subcommittee of scientists performing research in the field of MES at a number of academic institutions and national laboratories,

including other DOE synchrotron light sources (ALS, APS). The conclusions of the subcommittee are discussed below.

XAS Studies of Natural Samples and Model Systems

The key use of XAS in MES research is characterizaton of the chemical speciation of contaminants in natural samples and the study of adsorbate-surface interactions in simplified model systems where variables can be independently studied and processes understood at a fundamental level. XAS is unique among other spectroscopic methods in providing this information for most elements of the periodic table under environmentally relevant conditions (*i.e.*, dilute element concentrations, the presence of bulk water, ambient P-T conditions). The primary limitation of XAS studies on available wiggler beam lines at SSRL and NSLS is insufficient flux to probe metal-ion species at natural concentration levels, which may be a few parts per million or less. Such studies are most often flux limited rather than brightness limited because the sample volume is typically large and the entire sample can be illuminated by the x-ray beam. Depending upon the element being studied, the type of sample matrix, the type of x-ray detector used, and beam conditions, we are currently limited to about 10-20 ppm of an element in the optimum case for x-ray absorption near edge structure (XANES) studies. Considering (1) thc SPEAR3 upgrade, which will increase total flux by a factor of two, (2) the availability of MES BL 11, which will increase flux by a factor of 5-10 relative to other MES wiggler beam lines at SSRL, and (3) a new solid-state array detector for BL 11, which will increase sensitivity by a factor of about five relative to currently available array detectors, the enhancement in sensitivity to dilute concentrations of metal ions in natural environmental samples should be a factor of 50-100. This enhancement will also permit studies of metal-ion adsorbates in synthetic model systems at total metal-ion concentrations and surface coverages low enough to mimic solute concentrations and surface coverages of contaminants in many natural aquatic systems.

This increase in flux has important implications for MES studies, particularly the lower metal-ion concentration thresholds that would be accessible using XAS. Many studies of metal ions in aqueous systems are sensitive to the *absolute* concentrations of the metal ions. For example, groundwater-born An(V,VI) (An = U, Np, Pu) solutes occur predominantly as mononuclear AnO_2^{n+} (n = valence - 4) species at typical contaminant concentration levels. If the aqueous

concentrations of these metal ions are increased by factors of two or three, however, they can hydrolyze and oligomerize into large multimeric species, which react differently with soils and may have different transport properties than mononuclear species. Currently, it is often necessary to use concentrated model systems samples to collect usable EXAFS spectra, which may alter the speciation of the contaminant ion relative to real systems. Thus, an increase in sensitivity of 50-100 will substantially enhance XAS investigations under environmentally relevant conditions.

As mentioned earlier, the enhancement in total flux provided by the SPEAR3 upgrade will also make it possible to carry out XAS studies of chemical interactions at metal oxide- and metal sulfide-aqueous solution interfaces at defect-level surface concentrations of reaction products. It is often necessary to conduct polarized grazing-incidence (GI) XAS studies of aqueous metal-ion reactions on oriented single crystals, since many important aspects of interfacial chemistry can not be resolved by studying such reactions on high-surface-area powders. Single-crystal GIXAS measurements result in at least an order of magnitude lower background noise relative to XAS measurements on high-surface-area powders, and they also provide information on the orientation of molecules on surfaces.

Grazing-incidence XAS experiments on environmentally relevant solid-solution interfaces are flux- *and* flux density-limited. Currently, crystals larger than approximately 1 cm diameter and having $\geq$ 0.1 monolayer sorption density are required to collect useable XAS data on wiggler beam lines at SSRL. However, the vast majority of natural single-crystal samples are smaller than 1 cm, and it is often difficult to obtain high quality synthetic oriented single crystals of many environmentally relevant materials much larger than this diameter. The anticipated order-of-magnitude increase in flux density due to the SPEAR3 upgrade will make it possible to perform experiments on millimeter-diameter synthetic samples and will allow polarization-dependent studies of adsorbates on natural samples. Furthermore, it will be possible to collect EXAFS spectra from metal ions adsorbed on single crystals at sorption densities low enough to probe their interaction with defect sites.

The increases in flux provided by SPEAR3 will substantially reduce the time required to collect usable XAS from samples currently measured at SSRL. These reductions in time will facilitate the routine measurement of samples from contaminated field sites for science-based risk

assessment and environmental monitoring purposes. Time-resolved XAS measurements on dilute samples undergoing transformations are currently flux limited, and the minimum time interval that can be sampled is of the order of a few minutes using conventional fluorescence-yield XAS methods. Thus, the increases in flux provided by SPEAR3, BL 11, and new detectors will significantly improve the time-resolution that can be obtained for XAS studies of chemical reactions in natural samples.

Micro-XAS Investigations of Natural Samples

Metal-ion contaminants in soils, microbes, plants, and anthropogenic materials often have heterogeneous spatial distributions in grain coatings, crack fillings, colloids, precipitates, and organelles with length scales from 500 μm to less than 10 Å. In addition, a given contaminant may be present as more than one chemical species. Thus micro-XAS measurements are essential for characterizing the spatial and chemical species heterogeneity of such samples. The proposed reduction in emittance and increase in current of SPEAR, combined with micro-focusing optics planned for BL 11, will permit micro-EXAFS measurements to be performed on these samples to spot sizes of ~7 x 10 μm at a flux of ~10^{10} photons/sec, which is comparable to the total flux currently available on unfocused SSRL XAS beam lines, and micro-XANES measurements to spot sizes of ~2 x 2 μm with ~10^{8} photons/sec. These capabilities will be surpassed by only a few beam lines at the APS and will exceed the capabilities of bending magnet BL X-26A at NSLS, which is used primarily for micro-XANES studies of environmental samples due to flux limitations.

Because of the recognition that high spatial resolution XAS measurements of metal-ion contaminants in heterogeneous environmental samples provide critical information needed for design of remediation methods, the demand for such facilities has grown significantly over the past few years and will continue to grow as new MES users seek access to synchrotron light sources. The need for additional hard x-ray beam lines with high spatial resolution XAS capabilities is discussed in the 1995 DOE-Airlie and 1997 DOE-SSRL workshop reports on synchrotron radiation applications to MES.

Radioactive and Hazardous Samples

The increased capability to focus x-rays reduces the need for large samples (currently, the typical MES sample volume at SSRL is approximately 0.1 mL, or about 0.5 g), which is of practical significance to the preparation of samples containing radionuclides and other hazardous elements at SSRL. Smaller samples will be safer to prepare, pose less risk, and result in smaller volumes of hazardous waste than conventional samples.

Beam Stability

Noise created by positional instabilities of electron and x-ray beams can be a limiting factor for some MES experiments and will assume greater importance as the capability to illuminate small volumes of structurally heterogeneous natural samples is realized. The SPEAR3 upgrade plan includes many aspects which specifically address beam stability. In parallel with the upgrade of storage ring components, many of the x-ray beam line optical elements for the older beam lines, which currently suffer from thermal affects, will be replaced with actively cooled components optimized to remain stable under high x-ray power densities. These upgrades will improve the stability of x-ray beams at SSRL, thus enhancing the experimental capabilities of SPEAR for MES research.

Oxidation State-Selective Fluorescence-Yield XAS

The energy positions of K_β emission lines for different oxidation states of given element can vary by several eV. These oxidation state-dependent K_β lines can be isolated from the total fluorescence yield of an element during XAS measurements by use of appropriate monochromator crystals, placed between the sample and the detector. By monitoring K_β fluorescence over a specific energy range characteristic of a given oxidation state of an element during XAS scans, it is possible to obtain XAS spectra that are particularly sensitive to a given oxidation state of an element, even when more than one oxidation state of that element is present (Bergmann *et al.*, 1996, unpublished results). This technique is particularly useful for transition elements (*e.g.*, Mn(II,III,IV), Fe(II,III,IV), Ni(II,III)) in mixed-valence and redox-active samples such as plant matter, soils, proteins, and microbially-precipitated microcrystalline oxides. For example, the mechanisms by which plant pathogens (*e.g.*, rice blast disease, wheat take-all disease) attack and necrotize tissue in important crops are related to the oxidation of

Mn(II) in plant tissue to Mn(IV), which interferes with the plant's metabolic pathways. Oxidation state-selective XAS could be used to study *in-situ* the progress of the pathogen-driven transformation of Mn in living plant tissue. The ability to make such measurements and the resulting knowledge would be useful to the design of pathogen-resistant crops.

The x-ray emission spectrometers used to resolve and isolate individual K_β lines in oxidation state-selective XAS measurements currently collect very small solid angles (10^{-1} to 10^{-2} sr) of the total sample fluorescence and require a small vertical beam dimension (about 400 μm). Given this small solid angle of collection and the weakness of K_β lines, high x-ray fluxes are necessary for oxidation state-selective XAS measurements. In addition, since focused beams are currently about 1 mm in height on high-flux wiggler end stations at SSRL, it is necessary to define the vertical beam size at 400 μm using tantalum apertures, which results in significant losses of incident photons. The loss of flux is more severe for experiments using high-resolution monochromator crystals, *e.g.*, Si (400), which have small vertical acceptances. Currently, it is possible to measure oxidation state-selective XAS from metals in concentrated samples (*i.e.*, approximately ≥ 500 ppm) having moderately absorbing matrices (*e.g.*, water, wet Al- and Si-oxide samples). The SPEAR3-generated improvements in flux, focusing capability, beam line optics and their thermal stability should provide about an order-of-magnitude improvement in photon flux on samples, which will significantly enhance the capability to perform oxidation state-selective XAS measurements on environmental samples.

Specific Examples of the Impacts of SPEAR3 on Environmental Studies at SSRL

Speciation of plutonium in soils

The DOE is currently conducting cleanup activities at its nuclear weapons development sites, many of which have accumulated plutonium (Pu) in soils for 50 years. There is scientific uncertainty about the levels of risk to human health posed by this accumulation and about the possible migration of Pu from Federal reserves onto public lands. The Rocky Flats Environmental Technology Site (RFETS) is considered to be one of the most significant threats to public safety among DOE sites[1]. Two decades of routine monitoring have shown that the environment around RFETS is contaminated to the degree of placing RFETS on the Superfund National Priorities List (NPL)[2]. Plutonium activities in surface soils range from 1,450 to 0.05

pCi/g,[3] and there is new evidence that Pu in RFETS surface and subsurface soils may not be as geochemically immobile as previously believed[2]. There is great public concern regarding the potential movement of Pu in soils at RFETS, where more than 1.2 million people live within 20 miles of the plant[4].

Recent data indicate that redox changes in settling ponds used to accumulate contaminated waters at nuclear materials production sites are closely associated with whether the Pu is mobilized or immobilized[2], which underscores the fundamental importance of chemical speciation in controlling the fate and transport of Pu in soils. Using XANES spectroscopy, the widely held belief that Pu in RFETS soils exists as PuO_2 or as colloidal Pu(IV) can be tested. Recent studies of Pu speciation at the Nevada Nuclear Test Site suggest that transport of Pu hydroxide colloids by surface waters is responsible for Pu movement up to several km[5]. However, the distribution of Pu contamination in RFETS soils is inhomogeneous, believed to be associated with minor fractions of the soil matrix, such as small particles, grain coatings and crack fillings, which are difficult to separate and concentrate. Furthermore, the average concentrations of Pu in most samples are too low to collect useable XANES spectra using current facilities. The SPEAR3 upgrade will make a significant contribution to the study of such samples. Microfocusing optics on BL 11 at SSRL will make it possible to measure XANES and possibly EXAFS spectra on the Pu-bearing "hot particles" dispersed in the soils, allowing determination of plutonium speciation in soils at RFETS.

Radionuclide transport at Yucca Mountain, NV

The vast majority of transuranic elements are produced in commercial nuclear reactors utilizing uranium-based fuels, and the spent fuel and decay products are expected to be stored in deep geologic repositories[6]. Neptunium is the most problematic actinide element with respect to environmental migration[6,7] because its solubility under typical groundwater conditions is expected to be high enough to cause radiological concern[8], and its sorption on common minerals is expected to be relatively low[9]. Under most environmental conditions, Np will be present as the pentavalent trans dioxo (*i.e.*, neptunyl) cation, NpO_2^+. Carbonate is an important natural ligand because of its relatively high concentration and strong actinide complexing ability[7,10]. Therefore, carbonato complexes of neptunium(V) are expected to play an important role in the fate and transport of Np in natural aquatic environments. In the vicinity of Yucca Mountain, NV, the US

candidate site for long-term storage of high-level radioactive wastes, Np shows very little tendency to sorb to the natural Tuff, except along fractures that are rich in carbonate-containing minerals such as calcite. It is unknown whether Np is complexed with carbonate ligands on the surface of the calcite, similar to the aqueous Np(V) species previously identified using EXAFS and NIR spectroscopy[11], or whether other surface processes are responsible for Np sequestration. A microfocusing capability provided by a SPEAR3 upgrade would allow for micro-XANES and micro-EXAFS experiments to probe the nature of the Np complex along the fracture surfaces. Such information would be invaluable in understanding fundamental mechanisms controlling the migration of Np in Yucca Mountain environs.

Speciation of lead and arsenic in mine wastes and sediments: implications for risk assessment and remediation strategies

Mining operations often result in the contamination of soils, surface waters, and aquifers with heavy metals, which are concentrated in mine drainage effluent and/or are leached out of mine tailings by rainwater. Due to the toxicity and large quantities of heavy metal contamination in such locations, many mining areas have been designated as US Superfund sites, *e.g.*, the Leadville, CO mining district. In such areas it is necessary to assess the risk posed to potable water due to migration of heavy metal contaminants in groundwater and remediate the affected soils and groundwaters. Knowledge of the molecular speciation of metal ions (*i.e.*, their oxidation states; distribution among dissolved, adsorbed, and solid phases; and the presence and effects of complexing ligands) in these environments is necessary to describe the mobility and bioavailability of heavy metals in groundwater.

Recently, XAS has been used at SSRL to characterize the speciation of arsenic and lead in contaminated sediments from mine wastes at Trona, Jackson, and Marysville, CA (arsenic) and Leadville, CO (lead). XAS is well suited to this problem, since it can provide information on the oxidation state and local coordination environments of arsenic in dilute natural samples. In the case of arsenic-contaminated mine samples, edge and EXAFS analyses showed that the dominant forms of arsenic are As(V) and As(0)[12]. The more toxic and mobile As(III) species was not detected in this work. Moreover, As(V) was found in both crystalline arsenate phases and as chemisorbed species on mineral surfaces. Similarly, it was found that mine tailings from Leadville, CO, contained lead as both adsorbed species and in crystalline solids[13]. In each case it is difficult to define conclusively the identities of the arsenic- and lead-bearing solid species

because a variety of phases occur that typically have grain sizes less than 500 μm diameter. It is currently not possible to collect XAS data from individual grains using current SSRL wiggler beam lines. Adsorbed lead and arsenic species are often associated with Fe- and Mn-oxide grain coatings and crack fillings, which also have spatial dimensions smaller than 500 μm. Thus, the SPEAR3-generated improvements in focusing capability and flux density will substantially improve the ability to determine the speciation of heavy metals in these complex natural samples.

Np sequestration in bone

An illuminating example of the importance of the SPEAR3 upgrade is the ongoing study of Np incorporation in mammals designed to understand the mechanisms of Np uptake, metabolism, and deposition. The speciation and spatial distribution of Np deposited in bones is completely unknown. Knowledge of these mechanisms is necessary to understanding the long-term health effects of actinide poisoning. XANES results from the initial studies of Np in mouse femurs are shown below[14]. Long data acquisition times (approximately 2.5 h./sample) were necessary in

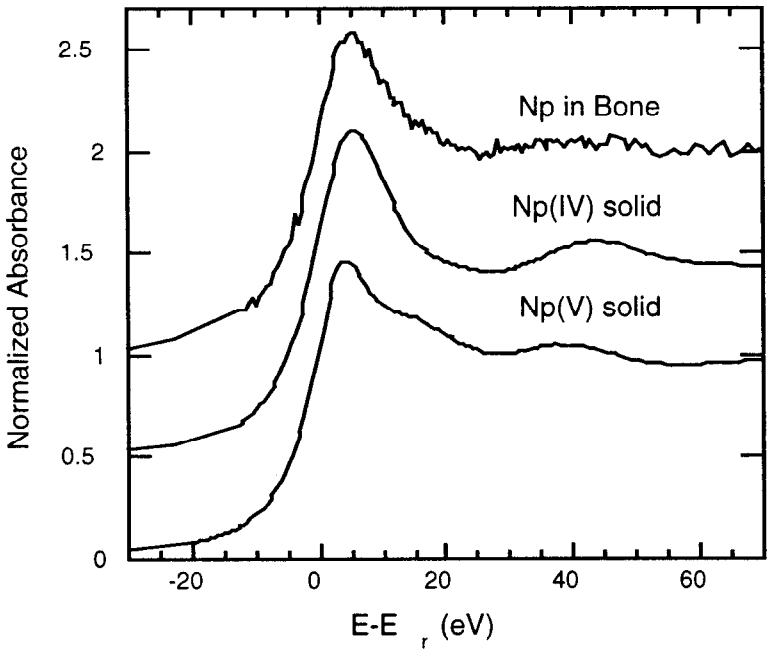

Figure 4. Np L_{III} edge spectra collected in fluorescence mode from mouse femurs compared to well-characterized Np oxidation state references. Np concentration in the bone sample was approximately 1 ppm.

order to collect these spectra, due to the low concentrations of Np in the samples (approximately 1 ppm), which is too low to permit analysis by EXAFS spectroscopy using existing facilities. Improvements in flux density and spatial resolution would permit investigation of Np oxidation state, speciation, and distribution in thin layers of bone, where it is believed to accumulate in proximity to blood supply.

Tc speciation in cementitious waste forms

The study of proposed nuclear waste form materials (glass, ceramic, and cementitious) using XAS is another area that would benefit from the SPEAR3 upgrade and the resulting improvement in microfocusing capability. For example, XAS studies have documented that Tc reduction occurs in cementitious waste forms, but it is spatially inhomogeneous[15]. Complete and long-term reduction of Tc is essential for the implementation of cement waste forms that can be used for the safe long-term internment of high-level radioactive waste. Better spatial resolution is required to understand the local chemical reasons for incomplete reduction. The SPEAR3 upgrade will significantly improve the ability to characterize actinide speciation in fractures and in phase-separated regions in the waste forms, which affect waste form durability.

New opportunities for XAS studies in the 1-4 keV region

The SPEAR3 lattice will permit the development of a new high brightness undulator beam line in the 1-4 keV energy range that would be brighter than any existing or planned beam line covering this energy range. This spectral region contains a number of low-Z elements (Na, Mg, Al, Si, P, S, Cl and K) that are major constituents in a wide range of geo-(clays), environmental (soils), biological, and technological (zeolites, metal silicides) materials. The ability to perform Al- and P- K-edge XAS on natural samples would have a major impact on environmental sciences. For example, Al^{3+} is a ubiquitous component of soils and groundwaters, where it occurs as solute species, Al-oxide and hydroxide colloids and grain coatings, and as aluminosilicate minerals. These phases are highly reactive and affect the pHs of soils, the bioavailability of nutrients, and the solubilities and transport of contaminant metal ions in soils and aquifers. XAS studies of the speciation of Al^{3+} and Al^{3+}-bearing phases would provide information necessary to develop accurate geochemical models of Al cycling in the environment and contaminant remediation technologies. In spite of the importance of these elements, their K-edges largely have been unexplored until recently[16,17], due to a lack of soft x-ray monochromator materials that are stable under high vacuum and high power-load conditions. Thus, the soft x-ray region has been a "no-

man's land" for XAS studies. With the successful development of a new YB_{66} double-crystal monochromator on the JUMBO beam line (BL 3-3), however, SSRL now has the unique capability of probing the 1-2 keV region[16,17].

The MES working group strongly recommend that a new soft x-ray undulator beam line be developed to take advantage of the scientific opportunities in the 1-4 keV region. The low emittance of SPEAR3 would dramatically improve the ability to generate high flux from undulator beam lines. Thus, using the YB_{66} monochromator, a host of new soft x-ray experiments could be performed, including:

a. XAS spectroscopic studies of materials containing Na, Mg through K
b. *In-situ* studies of catalytic processes containing zeolites
c. Chemical dynamics and kinetic studies of dehydration process in clays, dealumination in zeolites, synthetic paths of meso-structured materials.
d. Soft x-ray SAXS measurements
e. Soft x-ray XAS measurements on biological materials
f. Chemical microscopic/tomographic imaging of Si devices.

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Materials X-ray Absorption Spectroscopy

Introduction

Materials Science is an enormously broad field which has greatly benefited from existing synchrotron research capabilities and will continue to do so with future improvements including those envisioned with the SPEAR3 upgrade. The increase in focused flux density that would be available with SPEAR3 would enhance the opportunities for a number of XAS experiments on materials problems that use small beams, either for spatial resolution or for very small samples such as those studied with diamond anvil cells or on the surfaces or interfaces of solids and liquids.

Fusion Welding

One of the experiments currently being performed on beam line 10-2 is the phase mapping and measurement of phase transformations in the heat-affected zone of the fusion welding processes[1,2]. With current capabilities, a sample area of ~200 μm can be measured using an integration time of ~10 seconds. The smaller source size of SPEAR3 will enable a reduction of the sample size by approximately an order of magnitude with no loss of flux, which should enable us to look at ~60 μm spots with the same flux density. This will greatly improve our ability to resolve transition regions between zones in the welding process.

High Pressure XAS

Current diamond anvil cell (DAC) experiments typically utilize an ~100 μm beam which is comparable to the diameter of the diamond anvil faces. Because of the quasi-hydrostatic mode of the DAC, large pressure gradients (~50% at Mbar pressures) exist which affect the accuracy of the P-V-T data and induce large errors in the *equation of state* so derived. The increased flux density of SPEAR3 will enable ~30 μm beams into the DAC with the same flux density. This minimizes the uncertainty of the pressure gradient in the sampled region and provides much needed basic data for more realistic simulation studies in the science-based stockpile stewardship (SBSS).

Atomic structure at the interfaces of solids with other solids, liquids and gases is currently of great interest in many aspects of materials science. It is at the interface that many important phenomena occur including those that cause interactions of the material with real environments or with industrially important reactions. Some examples include:

Corrosion Interfaces

Both studies of the formation of corrosion layers on important materials and the adhesion of coatings to protect these materials are areas of study by synchrotron techniques and these techniques will be greatly enhanced by the SPEAR3 upgrade. The increased flux density will result in being able to study more realistic concentrations of corrosives on surfaces as well as looking at the spatial variation of the corrosion process.

Catalytic Surfaces and Interfaces

Catalytic surfaces have become accessible to synchrotron techniques, particularly in real environments (operating gas streams, high temperature and pressure *etc.*)[3]. High vacuum techniques generally study surfaces that are not stable in real catalytic environments. For example, through XANES studies of transition metal sulfide catalysts (important catalysts for removing sulfur from petroleum hydrocarbons) such as ruthenium disulfide, it is now known that the active stable catalytic surfaces are carbides. This could not be determined from high vacuum studies since the surfaces are not stable in high vacuum and revert to metal surfaces. This new knowledge is leading to a new generation of environmentally important hydrotreating catalysts. The increased focused flux density will enable lower concentrations of dispersed catalysts on surfaces to be studied.

Catalytic interfaces are also very important. Again the transition metal sulfide desulfurization catalysts are an important example. Molybdenum disulfide and cobalt sulfide are active hydrodesulfurization catalysts. However, when combined as they are industrially, they have an order of magnitude higher activity, which has puzzled researchers for over 50 years. By combining HRTEM (High Resolution Transmission Electron Microscopy) with synchrotron techniques such as EXAFS and XANES it has been determined that this effect occurs at the interface between the two phases. This finding at the nanoscale level is an important

breakthrough that will again assist in producing a new generation of hydrodesulfurization catalysts demanded by future environmental demands of hydrocarbon fuels.

Atmospheric Nanoparticulates[4]

Following the above catalytic examples, it is now recognized that airborne particulates in the less than 2.5 μm range are significant health hazards leading to recently proposed EPA regulations. In addition, particulates in this size range are potentially photocatalytic. Materials such as iron oxides, iron sulfides and manganese oxides have band-gaps which are in the right range to adsorb sunlight. Their role in atmospheric chemistry is currently unknown. Synchrotron techniques such as XAS applied to these particulates may become practical with the SPEAR3 upgrade and thus could have significant impact on the understanding of the structure and role of these particles. For example, it would be interesting to study the property of these solids *in-situ* because when suspended they have liquid covered surfaces. SPEAR3 would make more feasible performance of such an *in-situ* experiment in which the atmospheric nanoparticles are suspended in air under conditions which approximate environmental conditions. Such experiments would require long x-ray path lengths to create appreciable absorption or scattering at real particulate concentrations. Also, in this category are many smoke and soot studies which are currently not understood. For example, it is known that ferrocene effectively suppresses smoke in hydrocarbon fires. The mechanism for this is unknown and could be addressed by researchers using the enhanced capabilities of SPEAR3.

Nanomaterials

Nanomaterials are currently of extreme interest throughout the materials science world[5]. Again the field is extremely broad and only a few examples are given below.

In-situ synthesis of nanomaterials: Nanoparticles are now being regularly produced industrially and the industry is rapidly growing. Yet the production processes are poorly understood and empirically derived. *In-situ* synchrotron studies of these processes can significantly enhance our understanding of how to control and optimize these processes. For example, Co metal is mixed with tungsten carbide to form hard drill bits used in many industries such as the oil drilling industry. Forming such drill bits requires carefully formed two phase nano-particles for superior

properties. These particles are produced by gas phase reactions which could be followed by *in-situ* XAS and scattering processes.

Nanomachines: The production of nanomachines such as nanobatteries, nanomotors and nanosprings is now believed to be feasible and provides examples of a developing industry based on detailed knowledge of atomic and longer range structure. For example, metals which are intercalating in layered materials such as titanium disulfide compounds dynamically "bend the layers" during intercalation. This bending relaxes in real time upon completion of the intercalation process, thus providing a "nanospring" model. Fundamental understanding of the elastic constants of the material can be derived as well as direct correlation of these properties to atomic process such as compressing and stretching of the bonds of the layered materials. These properties and effects can be studied *in-situ* efficiently using the enhanced capabilities which will become possible with the SPEAR3 upgrade.

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Imaging/Tomography/Topography

X-ray Microbeam Diffraction

High resolution synchrotron radiation micro-beam diffraction was developed for mapping of three-dimensionally varying microtexture in polycrystalline materials to understand the evolution of strains in the vicinity of strain concentrators (cracks) using imaging plates as a detector[1]. The last ten years of experience on SPEAR beam line 2-2 has shown that intensities in the polychromatic beam drop below what is practical to use at about 0.5 Å, *i.e.*, about 25 keV. This empirical limit applies to x-ray diffraction topography of highly perfect as well as heavily deformed crystals as well as for microbeam diffraction from polycrystalline samples. There is approximately 1 x 10^{11} photons/sec/ring current/mrad/0.1%BW at 25 keV in SPEAR bending magnet radiation. For SPEAR3 this intensity is calculated to extend to 45-50 keV for bending magnet radiation. This roughly doubled maximum usable energy makes diffraction studies possible for interior volumes of bulk samples of important engineering materials, including Ti, Fe, Ni, Cu and Pb.

Table 2 lists linear attenuation coefficients μ and thickness t of materials for which μt = 1, 2, 3 or 4 (corresponding to 37, 13, 5 and 2% transmissivities, respectively). Diffraction from depths of 1 mm or deeper beneath the sample surface should be obtainable from samples of Ti-6Al-4V; in steels this depth should certainly be 0.4 mm.

Table 1. Linear Attenuation Coefficients, Material Thicknesses and Material Transmissivities

		t (mm) for μt=			
Material	μ (cm^{-1})	1	2	3	4
Ti	9.5	1.1	2.1	3.2	
Fe	26.5	0.38	0.75	1.1	
Ni	36.5	0.27	0.55	0.82	
Cu	40	0.25	0.5	0.75	1.0
Pb	145	0.07	0.14	0.21	0.28

Important reliability issues remain with new generations of turbine blades. Single-crystal blades are in service commercially. Damage accumulation mechanisms and rates within these blades crystals are largely uncertain. Transmission polychromatic topography, for example, would reveal all of the accumulated damage within a blade; nondestructive interrogation of it would allow the same blade to be examined periodically throughout its life. Damage accumulation models could be validated, for example, by quantifying plastic deformation in material adjacent to the various stress concentrators in a given type of blade. In such an ambitious program, many measurements on different blades would be required over a period of time. It is doubtful whether the APS beam lines, even bending magnet lines, would be able to provide the necessary access. Further, ultra-small source size and emittance of APS are not needed for these experiments and may actually be a disadvantage.

Current models of texture development in response to different loading histories agree qualitatively with experimentally observed textures but over-predict the amount and rate of sharpening of the texture[2]. In many fcc metals, for example, sharp microtexture *within* each grain develops at large strains[3]; relatively large rotations develop between domains separated by persistent slip bands. Texture evolution in highly absorbing samples such as Cu, Ni or Ti currently can be studied only by destructive sample preparation. A hollow, tube-like sample geometry is required for obtaining constant deformation across the sample cross-section, and the wall thicknesses are typically 1 mm or larger. The increased flux at high energy (45 keV) makes it possible to nondestructively quantify texture within the sample[4]. Thus, the same portion of a sample could be observed multiple times, providing for the first time data to which any texture evolution model would have to conform. While such data could, in principle, be obtained by testing multiple samples and destructively preparing many thin sections for x-ray or TEM microtexture quantification, large sample-to-sample variability is the rule in mechanical properties research. This frequently obscures trends in the data which might be observed if a given sample could be studied throughout the process in question. Determining the correct parametric forms to represent this effect in models of texture evolution is a crucial step in improving modeling of metallurgical forming operations.

Solder joints consist of a mixture of lead and tin, and improved understanding of damage accumulation in such joints would lead to improved joint design and better reliability of the material. Microbeam diffraction is an ideal method for following damage accumulation in solder

joints, and SPEAR3 would allow samples with bulk dimensions to be examined through their life cycle (nondestructively).

Tomography

X-ray Tomography Microscopy (XTM) uses a 2D, thermoelectrically–cooled charge coupled device (CCD) array detector to take x–ray radiographs of a specimen as it is rotated in an x–ray beam. These data are then reconstructed to form a three-dimensional image of the specimen. Synchrotron radiation offers tremendous advantages over conventional radiation sources for microtomography of biological tissues. The intensity of a synchrotron beam is several orders of magnitude greater than conventional x–ray generators can provide; this makes it practical to select optimal x-ray energies for imaging. Not only does energy selection greatly improve the signal-to-noise ratio and decrease the radiation dose, but it makes it possible to convert the reconstructed image data into absolute values of the local tissue composition without any corrections for polychromatic beam hardening effects. Thus, the three-dimensional images not only show tissue structure, but also provide a quantitative map of the tissue composition and density with a spatial resolution of a few cubic micrometers.

The XTM was developed in the mid 1980s, and used the most effective technology then available to acquire high–resolution x–ray images. The heart of this system is a two–dimensional CCD (charge coupled device) detector. This detector is thermoelectrically-cooled to reduce dark current, and is read out more slowly than video rates in order to reduce read-out noise. Spatial resolution is greatly limited by the synchrotron source size. The proposed SPEAR3 upgrades will increase spatial resolution by two- to three-fold, enabling new types of biological imaging applications that are impractical with the present source. In addition, *in-vivo* biomedical applications are limited by the x–ray optics (the lack of good cooling on the crystal monochromators). The improvements to the beam line infrastructure will, therefore, be a great enhancement to SSRL capabilities.

One of the most significant research efforts using XTM is a study of osteoporosis. It is known that estrogen deficiency results in accelerated bone remodeling with loss of bone and increased bone fragility. It is directly implied that preservation of bone mass preserves trabecular microstructure, but maintenance of bone mass does not always lead to good bone quality[5]. For

example, treatment with fluoride increases bone mass, but can result in a mineralization defect that increases bone fragility. Therefore, a better morphologic understanding of how bone preserving agents work, and when they need to be administered to prevent bone fragility, is required. The proposed SPEAR3 upgrade will enhance the ability of XTM to image trabecular bone loss at multiple time points after estrogen depletion in the rat model of osteoporosis. 3D XTM images will confirm or reject our hypothesis that the first change of consequence in trabecular bone after estrogen depletion is loss of connectivity, and that this loss in connectivity is irreversible. Also, we hypothesize that if estrogen is replaced before significant changes in trabecular structure occur, then trabecular structure and strength should be preserved. The SPEAR3 enhancements will greatly increase the amount of bone tissue that can be imaged in a given time frame, making it possible to image the entire tibial metaphysis of the anesthetized rat. The increase in data acquisition speed will also greatly reduce the length of the anesthesia now required for *in-vivo* imaging, and increase the number of animals that can be imaged in a day.

X-ray Diffraction Topography

X-ray topography allows imaging of the defects and long-range strains present in semi perfect bulk crystals and thin films. This is a very useful, non-destructive technique that requires large beam size and high geometrical resolution. There are no dedicated x-ray imaging lines at APS; while microbeam diffraction is an important component of several CAT's programs, wide field-of-view imaging has been neglected. It is an extremely valuable capability, especially for sources having usable x-ray intensities above 25 keV. With SPEAR3 one will be able to image with energies up to 60 keV (bending magnet) or 100 keV (wiggler lines) and vertical field of view of 3-4 mm; this combination will be unique and will be extremely valuable for crystal characterization of highly absorbing materials, particularly in the transmission setting. Another advantage of the harder radiation is that it will make *in situ* studies of growth, environmental degradation, *etc.* much more practical. Unless APS builds an imaging line 500-1000 m long, there will be no other US source with this capability. Thus we envision a unique resource for crystal characterization.

The much smaller source size will improve geometrical resolution significantly. This would allow larger sample-to-detector distance without degrading the resolution, important for any type of experiment using an environmental chamber. With increased flux, quasi-plane wave

topography would be possible at SPEAR3, allowing also "weak beam" imaging (*i.e.*, imaging using the tails of the rocking curve, where the diffracted intensity is very low) to obtain higher spatial resolution and strain sensitivity. This would be important for misfit dislocation studies

Flux on the bending magnet topography line, which does not utilize focussing geometry, will increase by only about a factor of 3-5 for the energy range up to 10 keV, but by a factor of 10 to 20 for energies between 40-60 keV, which is not adequate for most dynamic experiments but will be very helpful with some slow dynamic experiments that have been already tried.

The overall improvements of SPEAR will allow the study of a wider range of problems in opto-electronic materials such as threading and misfit dislocations in thin films and microdefects in bulk materials, domains in magnetic materials and highly absorbing crystal, where use of the higher energies will allow one to obtain $\mu t<1$ for kinematic imaging.

Soft X-ray Imaging

SPEAR3 offers a state-of-the-art capability to operate soft x-ray undulators and thus to implement the brightness oriented techniques of zone-plate microscopy[6], holography[7] and microanalysis[8]. At 3 GeV one should be able to get from the water window to about 4-5 keV using the first and third harmonic which would include the K edges of many of the light elements, including several which are of high interest for life science research and are hard to reach efficiently now. The list is C, N, O, F, Na, Mg, Al, Si, P, S, Cl, K, and Ca. In the water window region SPEAR3 would be at least as bright a source as the X1A beam line at NSLS where the principal US soft x-ray imaging program is currently operating[9]. Examples of programs there are biological zone plate scanning microscopy with microXANES, dark-field, visible fluorescence, cryoprotection of samples, polarization contrast, *etc.* Another important direction is material science which is pursued in part using the biological microscope for polymer research via μXANES[10] at the carbon and other edges but there is also a UHV compatible zone-plate scanning microscope system for a range of spectromicroscopies including XANES, photoemission and μXPS (small-spot ESCA). Such experiments have now become a major growth industry at soft x-ray facilities such as ALS and ELETTRA as well. The particular contribution of SPEAR3 would be in opening up the 1.5-4 keV range for these types of experiments because it would be in that range that 3 GeV would be optimum. A particularly

interesting area is to perform microscopy in a resolution regime at or below the line width of microcircuits and investigate elemental and chemical state distributions of the above elements in device manufacturing situations. One reason why research in the energy range 1.5-4 keV is scarce is monochromator problems. However, SSRL is especially well positioned to move into the range based on its development of the YB_{66} system. An alternative scheme for covering the entire range would be a multilayer-coated grating (R&D needed).

Apart from operating the above types of experiments at higher energies, one type of beam line that would be quite unique is proposed. This is a scanning microprobe with both a zone plate (ZP) and an elliptical Kirkpatrick-Baez (K-B) mirror pair: K-B for best light gathering, ZP for best resolution. The main detection mode would be x-ray fluorescence (for which detector R&D to get element recognition over large solid angles would be needed) and one should be able to push toward parts per billion sensitivity at minimum possible dose to the sample. A further way to trade flux for resolution would be to make a real image of the source at adjustable-size "pinhole" at, say, the hutch entrance and then demagnify this with the above optics. This facility would open up significant new parameter space in the atomic-number/sensitivity/resolution space and would be likely to appeal to a wide range of scientific communities. Moreover, the adjustable focus in the hutch would provide valuable flexibility in designing new set-ups.

ALS experience is that interesting versions of these experiments using both ZP's and both hard and soft x-ray specular K-B's can also be done on bending magnets. This is important strategically because such sources are inexpensive and plentiful.

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Condensed Matter, Materials Science and Technology

The upgrade to SPEAR3 will have a dramatic impact on the fields of condensed matter, materials science and technology. Because the field is so diverse, individual examples have been chosen to illustrate how the reduced source size and increased flux will impact some aspect of this field.

Roughness of the Silicon-SiO_2 Interface

Roughness of the silicon-SiO_2 interface is of technological importance due to its impact on device characteristics. It has been shown that increased roughness causes both a lower charge-to-breakdown voltage and lower time-dependent dielectric breakdown as well as a decrease in the electron channel mobility. As integrated circuits shrink in size, controlling the roughness of the Si- SiO_2 interface is crucial for obtaining faster performance and device reliability. Crystal Truncation Rod (CTR) Scattering has been shown[1-3] to be very effective in non-destructively measuring the roughness of this interface. A less-well understood aspect of the Si-SiO_2 interface is whether there is relaxation (expansion or contraction) of the layer(s) of silicon closest to the interface. This information can be obtained by measuring the CTR further away from the Bragg peaks. Relaxation can be observed as an asymmetry in the CTR between the (2,0,2) and (2,0,6) reflections. The problem with attempting such a measurement is that the CTR scattering is very weak, as much as 10 decades less intense than the Bragg peak itself. The higher flux density that is possible with SPEAR3 will directly improve our ability to map out the intensity of the CTR between the Bragg peaks. Note that the CTR is not a sharp feature in reciprocal space, but is extended. Thus the ~1 mrad divergence that a wiggler beam line produces is not a drawback to doing these measurements.

Thin Films in the Computer Industries

The SPEAR3 upgrade would make bending magnet beam lines essentially equivalent to present SPEAR wiggler beam lines. These bending magnet beam lines will be extraordinarily useful for x-ray diffraction and reflectivity measurements of the thin films that are used in the semiconductor, display and magnetic storage industries. Such measurements would greatly benefit from high-intensity synchrotron radiation for several reasons: First, these films are typically polycrystalline and only a few nanometers to a few tens nanometers thick, and thus,

they diffract only weakly. The higher intensity available with synchrotron radiation provides significantly better data in shorter times compared with lab-based sources. Second, for many materials (*e.g.*, magnetic media), it is important to accurately characterize the grain size, which requires measurements of multiple orders of diffraction peaks. Synchrotron radiation allows one to obtain data to high enough Q that these multiple orders can be measured. Such high Q values are typically larger than can be obtained at most lab sources. Third, more samples can be measured in a finite time. This is very important for materials development where many combinations of processing parameters must be varied to obtain optimum structures. Fourth, read heads in magnetic storage devices are made up of multilayers of alloys of third row transition metals, and the functioning of these heads depends critically on the structure of the interfaces in these multilayers. However, in most cases, this structure is unknown. The use of anomalous specular and diffuse reflectivity will be valuable for structure-property studies of such materials.

For these reflectivity and scattering beam lines to be useful to Silicon Valley industries, it is important that the users are able to get fast (*ca.* a few days) access to the beam lines without going through the (usually long) proposal process.

Recent experiments at NSLS have been done to study the formation of silicides (*e.g.*, $TiSi_2$, $CoSi_2$) on patterned Si wafers[4]. These silicides are the local interconnections in VLSI circuits, and it is important that low resistance phases are formed. The time-resolved measurements at the NSLS were performed as the wafer is quickly ramped up in temperature, as is done in semiconductor processing. These measurements have provided valuable insight into the formation of various silicide phases as a function, for example, of interconnect linewidth. However, due to intensity limitations, the temperature ramp rates in the NSLS experiments are about five times slower than those used in present chip processing. Since the silicide formation process is affected by the ramp rate, it is important to do these experiments at realistic ramp rates. The SPEAR3 wiggler beam lines (*e.g.*, 7-2 and 10-2), if used with multilayer monochromators, will have sufficient flux so that experiments at these ramp rate can be conducted.

The structural evolution of films during growth is becoming increasingly important as more and

more technology is based on films with thickness in the atomic scale. Measuring the structure of these materials presents a challenge to researchers working to correlate structure and properties. A higher brightness source will make possible new types of measurements to study these films *in-situ*. Current research at SSRL is handicapped by the long count times required for *in-situ* GIXS scans of sub nm thickness films. This long count time allows for possible contamination and relaxation, making the measurements of less value for comparison with non-interrupted film growth. Furthermore the long count times puts practical limits on the range of *k*-space which can be investigated and necessitates the use of epitaxial films to enhance diffracted intensity.

X-ray Scattering from 2D Structures

During the last fifteen years, x-ray scattering has proven to be a powerful tool in understanding the structure and evolution of two-dimensional structures on surfaces. Such structures include the very thin oxide layers essential to the operation of modern integrated circuits, thin liquid layers adsorbed on surfaces, surface roughness, and islands on surfaces. There have been numerous review articles written on this subject[5,6] in recent years and their contents will not be repeated here.

However, we will discuss two general classes of problems whose study would be greatly enhanced by the SPEAR3 upgrade and, in particular, the development of a vertically polarized, high brightness beam line. The two classes of problems are thin amorphous (or highly disordered) layers on surfaces and diffuse scattering studies of surfaces during processing.

Thin Amorphous Layers

There are a large number of very thin amorphous layers (<100 Å thick) for which a detailed understanding of their structural properties and the relationship of those properties to the underlying substrate would be very useful. For example, the Si-SiO_2 interface is very important in the production of semiconductor devices. Early experiments indicated that this interface was atomically sharp and had very few dangling bonds indicating a high degree of order at the interface. However, x-ray studies over the last 10 years have shown that the structure and the decay of order away from the interface is much more complicated that originally thought. These studies have been limited by the low signal rates available with the current x-ray sources and

researchers have not been able to systematically study large regions of reciprocal space. The development of a vertically polarized undulator or low-divergence wiggler coupled with the SPEAR3 upgrade would result in an increase in signal rate of roughly 240. This dramatic increase in intensity would allow for much more systematic study of these important amorphous thin films.

Another classic problem on which little progress has been made despite several attempts is understanding the premelting of surfaces thought to occur near (but below) the bulk melting point. There have been several studies of the premelting of Pb(110) surfaces which have disagreed about the thickness dependence of the liquid layer as a function of temperature and about the nature of this layer. These experiments are challenging because of the difficulty of measuring the extremely weak signal (a monolayer of liquid Pb on a 2nd generation source gives signal rates of roughly 1 photon/second) from the bulk diffuse background. The brighter source proposed for SPEAR3 would increase this signal rate by two orders of magnitude. Experience furthermore suggests that the much higher brightness of the SPEAR3 beam lines would lead to much better control of diffuse scattering from the substrate. The increased signal and decreased background using SPEAR3 would enable accurate studies of the nature of surfaces close to the melting temperature. However, these experiments would probably not be unambiguous unless large blocks of beam time were made available to carefully characterize the diffuse scattering and to make systematic measurements.

Diffuse X-ray Scattering from 2D Structures on Surfaces

An example of this class of problems is layer-by-layer epitaxial growth of semiconductor thin films. During layer-by-layer growth of GaAs, for example, small, monolayer high islands are nucleated on the surface of the crystal. These islands then grow and coalesce into a complete layer and the process repeats to build up macroscopic crystals. X-ray scattering studies of growth can yield valuable information about the diffusion and incorporation processes which control the island sizes and the quality of the resulting crystal. Measurement of this diffuse scattering is currently difficult because of the low signal rates and the necessity to make real-time measurements (the growth of each monolayer typically takes about 1 second).

This class of experiments suffers from three main problems at the moment. First, the experiments are required to be run with the substrate horizontal (because of convection in the gas flow). The high horizontal divergence of BL 6-2 means that long length scales cannot be resolved. Thus, the lineshape before growth starts is determined by the beam line's resolution function and is not a measure of the correlations on the surface.

Second, the initial, smallest islands cannot be detected because the diffuse scattering is spread over too much of reciprocal space. The more intense beams developed for SPEAR3 would allow the diffuse scattering to be detected from smaller islands and a much more detailed picture of these processes could be developed. The higher momentum range would also allow fitting of more complicated and complete models to the diffuse scattering.

Finally, these experiments have been greatly hampered by lack of reliable access to synchrotron radiation. The necessary infrastructure to deal both with the natural complexity of the experiment and to deal with the toxic and pyrophoric nature of the highly reactive organometallic molecules that are used in these growth processes necessarily implies a long and laborious setup. This long setup has meant that the experiments could only be run once a year (at most). This infrequent running has made it difficult to follow up on exciting possibilities which come both internally from the experimental results and externally from the rapidly evolving crystal growth technology.

X-ray Diffraction Studies of Transition Metal Oxides in High Magnetic Fields[7]

Transition metal oxides (TMO) provide a myriad of possibilities for studies of complex quantum many-body phenomena. In particular, the strong electron-electron correlations in charge-carrier-doped antiferromagnetic insulators with perovskite-like structures lead to properties such as metal-insulator transitions, high-temperature superconductivity, colossal magnetoresistance, spin-ordered phases, and charge-coupled phases. High-resolution x-ray diffraction using synchrotron radiation is one powerful tool for studying these systems. Structural, magnetic, and electronic properties are often intimately coupled in TMO. The presence of a magnetic field often leads to structural phase transitions in these materials. Therefore, the ability to carry out x-ray diffraction measurements in fairly high magnetic fields (>10 T) is desirable.

Superconducting magnets producing high fields always have a vertical field orientation, and access for x-ray beams lies in the horizontal plane. The optimum x-ray source for such experiments would have a sub-mm horizontal size, sub-mrad horizontal divergence, and vertical or circular polarization.

One class of materials to be studied are the doped rare-earth manganese perovskites $RE^{3+}_{1-x}X^{2+}_{x}(Mn^{3+}_{1-x}Mn^{4+}_{x})O^{2-}_{3}$ (where RE = rare earth, 0.15<x<0.5, and X = Ca, Sr, Ba, or Pb). The discovery of colossal magnetoresistance in these materials has attracted interest partly due to potential applications in sensors, and also due to fundamental interest in the intriguing interplay among magnetic, lattice, and charge degrees of freedom. Upon lowering the temperature or applying a magnetic field, the Mn^{3+} spins order ferromagnetically at the Curie temperature $T_C(x)$, the lattice contracts, and the system undergoes an almost simultaneous insulator-metal transition. Traditionally, the electronic properties of these systems have been understood to arise from the double-exchange effect: the hopping of the doped charge carriers (which are e_g holes) between two Mn sites depends on the relative alignment of the localized t_{2g} core spins, being maximal (minimal) when they are parallel (antiparallel). This interaction favors ferromagnetic order. However, it has been argued recently that double-exchange alone can neither explain the large resistivity in the paramagnetic phase nor its dramatic drop just below $T_C(x)$. It has been suggested that the additional mechanism is a strong electron-phonon coupling which localizes the conduction band electrons as polarons above $T_C(x)$.

A recent neutron-scattering study of the ferromagnetic metal $La_{1-x}Pb_xMnO_3$ (x=0.3) found that the low-temperature behavior is simply explained by a cubic nearest-neighbor Heisenberg Hamiltonian. Given the strong double-exchange interactions and the close proximity of the ferromagnetic metal phase to the antiferromagnetic insulator phase, the simplicity of this effective low-temperature spin Hamiltonian is remarkable. In order to arrive at a satisfactory understanding of the rich phase diagram of this material, it will be important to carry out systematic structural and magnetic scattering studies, both in zero and non-zero magnetic fields, covering the entire doping range.

The 3d TMO are narrow-band systems with strong electron-electron correlations. When the

long-range Coulomb interactions between the charge carriers overcome the kinetic energy, real-space charge ordering of the carriers may occur. In the manganese perovskites, charge ordering is accompanied by structural changes, and is one of many instabilities that compete with the double-exchange. Charge ordering is favored when the one-electron bandwidth is sufficiently small and the ratio Mn^{3+}:Mn^{4+} is 1:1 (*i.e.*, x=0.5). It is possible to control the transfer interaction of the e_g holes by varying the ionic radius of the perovskite *A* site (the site of the rare earth and X ions), which results in changes of the Mn-O-Mn bond angle and consequently in the one-electron bandwidth.

For example, $La_{1-x}Sr_xMnO_3$ has no charge-ordering instability and is a ferromagnetic metal for $0.175<x<0.5$. On the other hand, $Pr_{1-x}Ca_xMnO_3$ exhibits a charge-ordered insulating phase for $0.3<x<0.5$, but no metallic behavior. Depending on the exact Ca concentration and the temperature, the magnetism in the charge-ordered insulating state is either paramagnetic, antiferromagnetic, or canted antiferromagnetic. It was recently discovered that both the application of a magnetic field and illumination by x-rays induces a first-order insulator-metal transition in this material. This transition is accompanied by a melting of the charge-ordered state and by dramatic changes in magnetic properties.

Progress in this area would be greatly assisted by the ability to carry out systematic x-ray diffraction studies of sets of these materials at low temperatures and in magnetic fields. Currently, there are very few synchrotron-based facilities for this type of measurement. A source with good stability, high flux, low emittance, and small horizontal source size, such as the proposed SPEAR3, would be ideal. If the source could be vertically polarized, it would be perfect.

Research using High Pressure[8]

Since the reduction of the emittance of SPEAR in 1991, SSRL has become a center for research on structure of inorganic materials at high pressure. The West Coast has an active community of scientists engaged in high pressure research, with the focus ranging from fundamental solid state chemistry to physics and geology. This research is conducted by a number of academic research groups, and also by a number of scientists from the Lawrence Livermore National Laboratory,

which has a long tradition of high pressure research.

This high pressure research effort will greatly benefit from the upgrade to SPEAR3. High pressure diffraction experiments generally are conducted in diamond anvil cells, and this imposes some restrictions:

The sample is typically in a volume of $(100\ \mu m)^3$, with a cross section of about $100\mu m^2$. Photons with energy above 10 kV are required for transmission through the diamonds. The vast majority of high pressure structural work at SSRL is currently conducted on beam line10-2, because a high flux is required through the small aperture of the diamond cell. The research groups doing high pressure work are coordinating their efforts: they currently request consecutive beam time, and they share some important pieces of equipment (for example, the Alivisatos group brings a Ruby fluorimeter, a microscope, drill press, *etc.*, for loading diamond cells and measuring pressure, and they typically leave it at SSRL until all the high pressure users are done). It is anticipated that the upgrade to SPEAR3 will result in at least an order of magnitude improvement in signal-to-noise for the current experiments.

An example of a specific research effort is the investigation of the influence of particle size on the phase diagrams of inorganic solids[9,10]. These studies take advantage of the tremendous developments in the synthesis of large amounts of high quality, monodisperse, nanometer size crystals of many inorganic solids. These enable investigation of changes in both the thermodynamics and the kinetics of solid-solid phase transitions which occur when a crystal is very small. The increased flux density of SPEAR3 will result in a dramatic improvement in the diffracted beam intensity of these experiments, so that smaller samples (and thus samples with more uniform pressure), can be measured.

Powder Diffraction

For the focused bending magnet beam line 2-1, the proposed upgrade of SPEAR would have a dramatic impact on the throughput and data quality of the samples run. The increase in focused flux density would directly impact the signal rate of experiments. Signal rates with the present system are limited by a mirror that needs to be replaced, so it is difficult to compare the current

signal rate to that with the proposed upgrade, but it is likely that a factor of 50 increase in count rate would occur. This would enable researchers to use the highest angular-resolution optics (a diffracted beam monochromator) while collecting good signal-to-noise ratio data in a reasonable length of time. The increased beam stability of SPEAR3 would also impact the data quality, which is extremely sensitive to small changes in electron source position.

Reduction in the horizontal spot size would also have a positive impact on the quality of the structures determined from powder diffraction data. Powder diffraction peaks on BL 2-1 high resolution diffractometer are highly asymmetric and broaden the effective peak by a factor of 2. It is likely that this asymmetry is predominantly due to axial divergence from the width of the beam on the sample. Besides degrading the resolution, asymmetry also makes the task of profile-fitting, essential for most structural analysis, more difficult. At present we reduce the peak asymmetry by either reducing horizontal width of the beam on the sample or by limiting the portion of the diffracted beam that enters the detector. Either remedy reduces the effective number of photons utilized in an experiment. On the other hand, small beam footprint would require particular care that the samples are finely ground and sufficient attention is paid to rocking and rotating the sample that even at the highest sample inclination sufficient number of grains are sampled by the beam.

The increase in the source critical energy of the SPEAR3 bending magnets (with corresponding changes in the beam line optics) would make many new powder diffraction experiments possible on the BL 2-1 (or any bending magnet based) diffractometer. For a routine powder diffraction experiments it is unlikely that the diffractometer would be operated at photon energies much higher than 10 keV (1.2 Å). However, there are two types of measurements that would become possible with the higher energy photons that would be available on a SPEAR3 bending magnet beam line. By performing scattering experiments in the vicinity of an absorption edge (resonance scattering), one can not only obtain element specific structural information, but also probe different electronic states of an element. With the present energy window on BL 2-1, one can access only (the K edges of) the first row transition metals and (the L edges of) the lanthanides. However, with an increase in the critical energy to 7 keV (and in either unfocused operation or with a mirror with a higher cut-off energy) one could easily access photon energies

up to 25 and perhaps even 35 keV. An accessible energy range of 5 to 30 keV would provide easy access to the absorption edges of most elements. (Note: compression of the reciprocal space into a smaller 2θ space with increasing energy enables one to compensate for some of the high energy reduction in flux by counting longer at individual data points but keep the overall scan time reasonably low.)

The second advantage of scattering at high energies is accessibility to a much larger reciprocal (Q) space. Structural analysis from high quality, very-high-Q diffraction data would make many novel crystal chemical investigations feasible. With very high Q diffraction data one can begin to "see" structural details at sub-Ångström resolution. With such spatial resolution one could decipher the arrangement of outer shell and bonding electrons around atoms. Fox example, an ability to observe the actual "aspherical" arrangement of electrons around atoms would provide an invaluable tool in understanding the chemical and electromagnetic properties of many of the transition and rare earth metal compounds. The least reliably determined parameters in structure determination are the thermal displacement factors. Thermal displacement factors contain a wealth of information on short range order and dynamics of atoms and molecules. High resolution high Q diffraction data contain many more independent diffraction peaks. Structural solutions from such data would yield very accurate and robust thermal displacement parameters, and knowledge of lattice dynamics.

High Intensity Micro-Beams for Microelectronics

Feature sizes in microelectronics circuits are currently in the sub-micron regime and the need for diagnostic probes that match these features becomes compelling. Charged particle probes in the sub-micron range are available, however in many cases their use is inappropriate or inapplicable. The development of high intensity neutral beam probes, such as x-rays, becomes a matter of some urgency. Their primary use is expected to be element-specific analysis using fluorescence (preferably in the scanning mode), diffraction or structural analysis on the micron scale or less and grazing incidence defect characterization in thin films or diffused surface layers. The methods proposed for producing micron-sized beams are (a) zone plates, (b) Bragg-Fresnel lenses, (c) capillary optics, (d) Kirkpatrick-Baez (K-B) reflection optics. Current efforts are directed towards developing the last two options, primarily because advanced expertise is

available both in producing glass capillaries and in K-B development at the ALS. Preliminary experience with these systems both at SSRL and ALS show that either of these techniques is capable of materials fluorescence analysis or diffraction studies on a micron scale. In particular beam lines at both SSRL and ALS have been used to characterize these optics and also to perform preliminary diffraction experiments. A factor of ten increase in source brightness would have a dramatic impact on the utility of these optics, especially for focal spots approaching 0.1 μm.

Capillary Optics

Using linearly tapered capillaries from various sources we find that beam lines 4-2 and 6-2 at SSRL using multilayer monochromators yield 10^8 photons/μm^2 for a 3 μm capillary and somewhat less for a 1 μm capillary at 8.9 keV. The average gain achieved with these capillaries is 100. Using these beams we have measured diffraction patterns on image plates from micron-size tungsten lines on a silicon substrate and also from blanket W films. The lattice parameters determined in these tests are consistent with results using a diffractometer which averages over many lines. The preliminary work is promising enough to support the development of a capillary pulling facility at ARACOR (INTEL initiative supplemented by SBIR and NIH grants). Also under development is a simple back-reflection diffractometer accurate enough to measure lattice parameters with changes in the 10^{-4} to 10^{-5} range. Current film exposures are on the order of hours; with a 10-fold increase in flux density at the above beam lines expected with the SPEAR3 upgrade and the use of a K-B mirror coupled with improved capillary optics should result in a flux density of nearly 10^{10} photons/μm^2, which compares favorably with results from other 3rd generation sources.

Kirkpatrick - Baez Optics

At the ALS a diffractometer for microbeam diffraction experiments based on Kirkpatrick-Baez optics is currently under development. As matters stand at the moment the mirror pair configuration is a more reliable and reproducible system than the current state-of-the-art in capillaries. Preliminary tests show that beam sizes in the 2 μm range can be produced (the goal for the project being 0.5 μm diameter beams). Using unfocused white beam from a bending

magnet line at ALS (10.3.2) diffraction patterns from 1 μm size Cu lines were collected in about 1 hour. With the proposed new lattice for SPEAR direct experiments with K-B mirror pairs become attractive. It is likely that as development proceeds a hybrid of K-B mirrors and capillary optics will be needed to achieve usable beams of 0.1 μm. While such beam sizes will be required in the next generation of microelectronics development, we are already at a stage where 0.3 μm beams would be an appropriate match for design features in existing semiconductor circuits.

X-ray Diffuse Scattering

Grazing incidence X-Ray Diffuse Scattering is a powerful tool for studying point defects and defect clusters in implanted silicon. Current studies of defect-mediated Transient Enhanced Diffusion (TED) of implanted species during thermal treatment have been performed on beam lines 7-2 and 10-2 to study the scattering in the vicinity of Bragg reflections. In the current configuration poor counting statistics far out in reciprocal space limit analysis of the data. For these regions in reciprocal space higher brilliance and a factor of 10 in flux density would greatly enhance the counting statistics in the tails of the Bragg peak as well as speed the data accumulation. It will also allow the examination of implants at lower doses.

Another technique which could be used is the x-ray standing wave (XSW) technique, which can be used to locate atoms relative to a diffracting matrix very accurately as well as determine the fraction of atoms on a given site. Normally one does not think of standing waves as a high brilliance experiment and this is true for UHV surface studies on thick samples. However, implanted wafers are always curved which broadens the Darwin reflectivity and reduces resolution by factors of two or three. This can be overcome by irradiating a small region of the wafer so that the effect of curvature is minimal. Normally this would result in a reduced fluorescence signal which is usually in the counts/sec range. With a factor of 10 increase in focused flux density such experiments could be undertaken on the focused wiggler beam lines of SPEAR3.

Impact of SPEAR3 on Microcontamination Measurements

The area of trace element analysis on wafer surfaces ("microcontamination") using total

reflection x-ray fluorescence (TXRF) would benefit from the SPEAR3 upgrade by taking advantage of both the reduced source size as well as the increase in ring current. A quantification of this improvement is the minimum detectability of trace metals. Table 1 shows the improved sensitivity which would be obtained in several configurations. The minimum detection limit (MDL) of $3x10^8$ atoms/cm^2 can be compared to a monolayer of Si at about $1x10^{15}$ Si atoms/cm^2. The MDL is proportional to Signal/$\sqrt{}$(Background) and is calculated from the estimated flux and amount of beam subtended by the sample compared to the present configuration on BL 6-2. For the case of an undulator insertion device instead of a wiggler, there is an additional MDL improvement from background reduction due to a smaller beam divergence. As can seen from the table, there is about a factor of 2 improvement in the MDL by going from SPEAR to SPEAR3 and about an order of magnitude by the additional use of a vertically polarizing undulator. The big improvement associated with a vertically polarizing insertion device is due to a more optimum sample orientation with respect to the beam shape and divergence.

Table 2. Estimated MDL for Ni in various configurations

	Relative Signal	$\sqrt{}$Relative Signal	MDL decrease due to smaller beam divergence	MDL (atoms/cm^2)
SPEAR BL 6-2	1	1		$3x10^8$
SPEAR3 BL 6-2	5.4	2.3		$1.3x10^8$
SPEAR3 Undulator	2.3	1.5	2	$1x10^8$
SPEAR3 Vertically Polarizing Undulator	36	6	2	$2.5x10^7$
SPEAR3 Vertically Polarizing Wiggler	64	8		$4x10^7$

The current detection limit using synchrotron radiation ($3x10^8$/cm^2) is in a very useful range for semiconductor process evaluation and is a capability that has been difficult to achieve with other

methods. Presently, the best alternative method, which can have comparable sensitivity, requires chemically removing the impurities from the surface using ultra-pure chemicals and subsequent analysis. The advantage of synchrotron radiation is that it allows for mapping the location of the impurities on the wafer, measurement repeatability and (in principle) no spurious sources of impurities. An improved detection limit, even by a factor of two, is a capability which cannot be achieved by any other method.

Another significant benefit from SPEAR3 would be measurement throughput (number of measurements/ unit time). If cleaning chemistry is to be evaluated with the goal of modifying a manufacturing process, it is important to get statistically significant results through multiple measurements. Benchmark MDL's are based on a 1000 sec. (detector live-time) measurement. For fully automated in-house x-ray equipment, this time period limits the maximum number of measurements to about a 25 wafer cassette (3 points/ wafer) in 24 hours. If the present detection limit were maintained, the measurement time could be decreased by a factor of 5 (assuming an appropriately fast detector). With associated automation, this improvement would be very considerable.

While the current detection limit using synchrotron radiation is quite useful, there are several driving forces for a lower one. The foremost is that in state-of-the-art cleaning processes, the ability to clean the wafer still exceeds our ability to measure it. In addition, we still have a poor understanding of exactly which aspects of the cleaning process are most technologically important. If the MDL using synchrotron radiation can be brought down another order of magnitude to the low 10^7/atoms/cm^2 range without any additional wafer processing, this capability would aid the understanding semiconductor process development for a considerable time to come.

Finally, microcontamination efforts are being developed at the 3rd generation synchrotron radiation sources in Europe and in Japan. The continued improvement of this expertise and capability in the US, with the momentum presently being generated by the collaboration between SSRL and the Sematech member companies should be a tremendous advantage for the semiconductor industry.

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VUV and Soft X-ray Science

Overview

The VUV working group unanimously and wholeheartedly supports the SPEAR3 upgrade. The group felt that the upgrade would significantly impact both the science and the technology that would be performed at SSRL during the foreseeable future. Also, the group wished to stress that the VUV community feels a strong need for efforts at *both* SSRL and at the ALS - while certain experiments (mostly micro-spectroscopy types) will always be better performed at the ALS, SSRL with the SPEAR3 upgrade will be strongly competitive for the majority of experiments and preferable to the ALS for some (particularly those that need exceptional stability, such as magnetic circular dichroism experiments). Finally, the time-consuming nature of the experiments and great demand for high performance VUV beamtime speaks for strong support of the VUV facilities at both the ALS and at SSRL.

Background

SSRL has had a very strong history of VUV/soft x-ray science. The strengths that were already developed there will be further strengthened by the SPEAR3 upgrade. These strengths are due to strong local groups at universities or industries, and the coupling of these local groups to the national and international communities. The contributions have been in many scientific areas:

Condensed matter

The high resolution angle-resolved photoemission studies on high T_c superconductors which gave the first evidence for the anisotropy of the superconducting energy gap[1] and the first observation of the pseudogap in the normal state[2]. Both of these have lead to a new paradigm in our way of thinking about these materials. One of the papers received a scientific impact ranking (based upon citation frequency) as high as #2 in all of physics[3], and news articles highlighting the various advances have been published in the "Search and Discovery" sections of Physics Today[4,5].

Surface and interface science

Valence band and core level photoemission studies of semiconductor surfaces and interfaces. Development of soft x-ray core level spectroscopy[6,7], including scanned energy photoelectron

diffraction[8], for the understanding of interfacial chemistry and atomic structures was pioneered at SSRL. This was tied into electronic structure studies which lead to fundamental understanding of semiconductor surface passivation and Schottky barriers[9].

Materials science, especially magnetic thin films and interfaces

The use of circularly polarized x-rays enabled for the first time the quantitative determination of element specific moments in thin metal films and metallic multilayers[10]. These studies have proven the existence of magnetic moments in "non-magnetic" sandwich layers like Cu and the fact that thin layers of magnetic materials like Fe may become magnetically dead. These findings have important implications for optimizing the performance of spin-valve magnetic sensors used in the magnetic recording industry. The work has also led to a clear understanding of the microscopic origin of magnetic anisotropy, one of the fundamental problems in magnetism.

Chemical sciences

VUV and x-ray photoemission and x-ray absorption studies of the geometric and electronic structure of surface complexes and their contributions to physical properties and reactivity. This work has led to a systematic understanding of molecular bonding geometries on metal surfaces[11]. An example is the definition of the geometric and electronic structure of the surface intermediates in the reaction mechanism of CO hydrogenation by the CO/ZnO methanol synthesis catalyst[12]. Much important work in catalysis and other fields[13] has been carried out.

Biological sciences

Variable-energy photoemission, ligand K- and metal L-edge absorption and x-ray MCD definition of metal sites in biology, both intrinsic active sites and metallo-drugs and diagnostics. An example is the definition of the unique electronic structure of copper sites in proteins and the role of the protein in controlling active site structure which regulates reactivity[14].

The work at SSRL has also had broad technological impacts:

- The work on semiconductor surfaces has led to an understanding of surface reactions and atomic structures which are important to semiconductor processing[15,16].
- Work on thin magnetic films has helped to direct the development of improved spin-valve magnetic recording heads[17].

• Work on rubbed polymer surfaces has revealed the origin of liquid crystal alignment on such surfaces, leading to new materials and manufacturing methods for liquid crystal displays[18].

In addition, over the years the local VUV community has trained on the order of 100 graduate students many of whom now hold prominent positions in both academia and industry.

Pioneering work at SSRL in the soft x-ray region, especially core level photoemission and x-ray absorption studies in the 100-1000 eV range containing most of the sharp atomic core levels such as the important K edges of C, N and O laid the scientific foundation for the construction of many third generation soft x-ray light sources around the world, notably the ALS[19].

The Case for SPEAR3

SPEAR3 will allow a competitive VUV program to continue at SSRL, and will help alleviate the pressing demands for high performance VUV beamtime, as well as allowing many new types of experiments in the VUV range.

Recent advancements in the sophistication of the VUV experiments have dramatically altered the types of experiments that can be performed, and these experiments touch at the very heart of some of the most basic problems in science and technology in these fields. The flipside to the sophistication is in many cases increased complexity and lengthiness, exacerbating the need for increased beamtime.

It is noted that the flavor of the experiments in the low energy regime are different from those in the higher energy regimes. One of the main effects of these differences is in the amount of time required to successfully carry out an experiment. This is due to the difficulty of working in the UHV environment, the necessity for *in-situ* sample preparation, and the necessity to repeat the experiments many times due to surface degradation. The net result is that it often takes months rather than days to complete a careful experiment, and that the VUV community is facing a clear deficiency in high performance (high resolution/high flux) beam time.

In the VUV/soft x-ray regime SPEAR3 will give:

- Increased photon fluxes on the sample. This will have many effects on the experiments that can be performed. It will allow for improved spectral resolution giving much greater information content to all experiments, allow the study of smaller or short-lived samples, and allow for new types of experiments, *e.g.* spin-resolved photoemission or x-ray absorption on very dilute samples.

- Long lifetimes and state-of-the-art beam stability for stable uninterrupted experiments. This is especially important for long scan experiments, experiments that need a very large number of spectra from the same sample surface, and experiments looking for very small effects, such as the dichroism experiments.

These improvements are critically important both for science and for technology. Scientific efforts continue strongly in the 5 areas mentioned above (condensed matter, surface and interface science, materials science, chemical science, and biological science), and a sixth area (environmental science) is opening up as an increasingly important field. There are at least 15 strong groups with important VUV programs at SSRL.

Finally, the application of synchrotron radiation to industrial problems is envisioned to become increasingly important. SSRL is centrally located in Silicon Valley and its facilities continue to be used by companies like IBM. Here the emphasis is on state-of-the-art materials characterization; x-ray absorption with variable polarization and core level photoemission techniques have proven to be important methods for obtaining definitive and unique answers to complex materials science problems.

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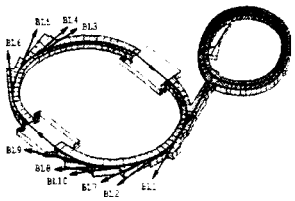

Appendix II

"SPEAR III - A Brighter Source at SSRL"

by

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SPEAR III – A BRIGHTER SOURCE AT SSRL*

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Abstract

By replacing the magnets and vacuum chamber for the 3 GeV SPEAR II storage ring, the natural emittance of the machine can be reduced from 130 to 18 nm-rad and the stored current can be raised from 100 to 200 mA with a 50 h lifetime. This configuration increases focused photon flux for insertion device beamlines by an order of magnitude and the photon brightness for future undulators would exceed 10^{18} at 5 keV. Due to a higher critical energy, the photon flux in the 20 keV range for bending magnet beamlines increases by more than two orders of magnitude. We present preliminary SPEAR III design study results and plans to implement the facility upgrade with minimal downtime for SSRL users.

1 INTRODUCTION

For the last 25 years the SPEAR storage ring has served both the high energy physics (HEP) and synchrotron radiation (SR) scientific communities, first as SPEAR I operating at a maximum energy of 2.4 GeV, and then as SPEAR II (1974), operating at up to 3.5 GeV. In 1990 SPEAR II became a dedicated 3 GeV, 100 mA light source with beam injected from a newly commissioned booster synchrotron. To this day, the SPEAR septum magnet limits the injection energy to 2.37 GeV and energy ramping to the 3 GeV user configuration is required. While other studies were made to reduce SPEAR emittance in the 1970s and 80s [1,2], the addition of a third injection kicker enabled a practical alteration the FODO lattice magnet settings in 1991 that reduced the emittance from ~500 nm-rad used for HEP to 130 nm-rad [3]. Alternative lower emittance lattices, which require new magnets and vacuum chamber, have since been considered and proposed [3,4]. These studies are now being extended for the 3 GeV, 200 mA low emittance SPEAR III proposal.

SPEAR II has eighteen magnet girders and eighteen straight sections. Seven straight sections are presently used for ~2 m insertion devices (IDs) and four more are available for future IDs, including one of the two long interaction region (IR) straight sections that could accommodate up to 17 m of ID. Four beamlines have bending magnet sources.

SPEAR III upgrade goals include:

- Reduce natural emittance to 18 nm-rad
- Increase stored beam current to 200 mA
- Achieve high lifetime at 200 mA (~50 h)
- Achieve high beam stability
- Maintain existing beamline alignment
- Create more long straight sections (~4 m)
- Inject at 3 GeV
- Maintain high operational reliability
- Reduce operating power costs
- Limit conversion downtime to <6 months
- Permit future upgrade possibilities

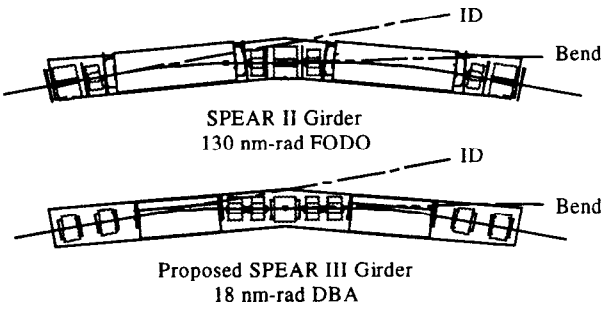

Figure 1: Magnet girders for SPEAR II and III

The basic upgrade plan is to replace the SPEAR II magnets with new magnets on the existing girders in a double bend achromat (DBA) configuration, leaving SR source points and beamline alignment virtually unchanged (Fig. 1). The four magnet girders flanking the two long IRs will be moved closer to the interaction points to increase the lengths of four straight sections from 2.7 m to 4.2 m while reducing the IR straight length to 12 m. The two existing RF cavities can be moved to the West IR (or an adjacent straight section) to create two new ID sites. The vacuum chamber will be replaced with a smaller aperture chamber rated for 500 mA to permit future higher current operation. A key aspect of the upgrade strategy is to limit the conversion period to one long downtime of six months (or less) together with normal two month shutdowns in order to minimize the impact on user programs. In the following sections we discuss photon and electron beam properties for SPEAR III and present preliminary design plans for beamline and accelerator systems.

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2 SPEAR III PHOTON BEAMS

2.1 Photon Beam Properties

For a broad range of experiments, focused flux density (photons/sec/mm^2/0.1%BW), or the number of photons one can fit through a small aperture at the sample, is a more important beam parameter than brightness (photons/sec/mm^2/mrad^2/0.1%BW). The reduced beam size and increased current and critical energy of SPEAR III (Table 1) result in an order-of-magnitude increase in focused flux density for ID beamlines, and a two order-of-magnitude increase for bend magnet lines, making them comparable to SPEAR II ID lines (Fig.2). A 4 m undulator in SPEAR III could produce a brightness of $>10^{18}$ in the 5 keV range, and $>10^{17}$ in the 10 keV range.

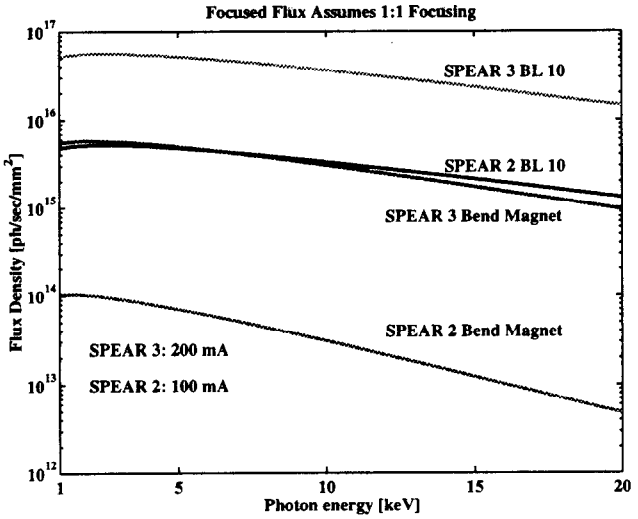

Figure 2: Flux densities for bend and wiggler (BL10) lines for SPEAR II and III.

2.2 Beamline Upgrades

SPEAR III provides two challenges for photon beamlines: (1) increased power loading on masks, slits and windows, and (2) the need for enhanced beamline optical performance in order to fully exploit beam source improvements. All beamline masks and windows will be upgraded for 200 mA operation; few changes are anticipated for ID beamlines built in recent years. Replacement components will utilize concepts developed for SSRL's newest and most powerful beamline 11 which develops a 4.5 kW/mrad^2 peak power density. Monochromators and mirrors on most ID lines will be replaced with versions using cooling technologies developed for third generation SR sources, which include pinpost and LN-cooled Si monochromator crystals, and internally and side clamp-cooled Si mirrors. Where possible, optics upgrades will extend beamline capabilities, for example, by changing mirror cutoff energies or optics acceptances.

	SPEAR II	SPEAR III
Current	100 mA	200 mA
Natural emittance	130 nm-rad	18 nm-rad
H-V coupling	1%	1%
Energy spread	.00074	.00087
Momentum compact.	.015	.0012
Nat. chromaticity (x,y)	-12, -14	-20, -20
Betatron tunes (x, y)	7.18, 5.28	14.75, 5.85
Critical energy	4.8 keV	7.1 keV
Lifetime at max. curr.	~30 h	~50 h
Average ring pressure	1 nTorr	1 nTorr
Beam sigma (x,y) – ID	1.85,.05 mm	.51,.04 mm
Beam sigma (x,y)-bend	.72,.18 mm	.16,.04 mm

Table 1: Machine parameters for 3 GeV SPEAR II and SPEAR III (approximate).

3 LATTICE

The 12.8 m bending radius SPEAR II FODO lattice will be replaced with an 8.38 m bending radius DBA lattice in SPEAR III. Since the new lattice is constrained by girder, ID and beamline locations, as well as by the RF-determined path length of 234.12 m, options for the numbers and placement of magnets are limited. The most conservative option uses separated function magnets with doublet quadrupoles and four sextupoles per cell (Figs. 1,3). Additional quadrupoles are placed in the long IRs for tune and optics control. Detailed study and optimization of this lattice is in progress. A combined function lattice that would increase arc straight section lengths by up to 0.5 m and reduce the horizontal beta functions in them by a factor of two is also being considered. A reduced horizontal beta is desired by users because of the smaller focused beam size, but it makes injection more difficult and may reduce the Touschek lifetime.

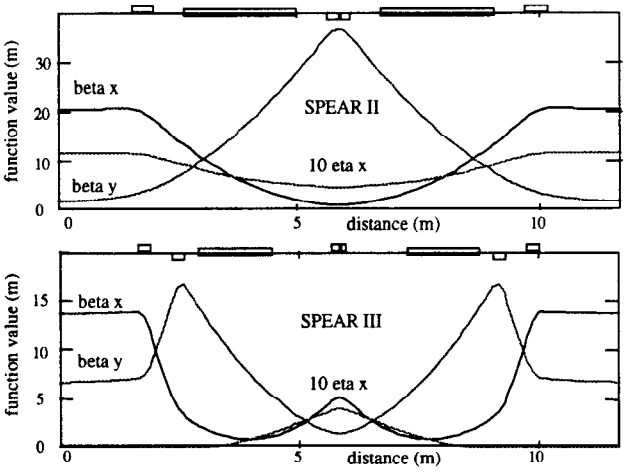

Figure 3: Lattice functions for SPEAR II and III cells.

4 COLLECTIVE EFFECTS AND LIFETIME [5]

The two SPEAR II 5-cell RF cavities will be used initially for SPEAR III. These cavities have numerous high order mode resonances, necessitating longitudinal and transverse feedback systems to damp multibunch beam instabilities. Computed instability thresholds are below 10 mA, assuming zero chromaticity and the overlapping of synchrotron sidebands with HOM resonances. A future upgrade possibility, especially if the stored current is increased beyond 200 mA, is to install single-cell, mode-damped cavities to reduce or eliminate the need for feedback.

The broadband impedance of the ESRF was scaled to the SPEAR circumference to yield |Z/n| = ~2.5 ohms and a Q=1 resonance centered at 30 GHz. This impedance will initiate a turbulent regime at 0.6 mA. Bunch lengthening and widening coefficients will be 1.9 and 1.5 respectively for a 2.8 mA single bunch current (200 mA in 70 out of 280 RF buckets).

A 70 h Touschek lifetime has been computed assuming a 3% momentum acceptance, 2.7 MV RF voltage, and 200 mA in 70 bunches. This value can be increased by filling the same current in more buckets (i.e. a factor of 4 increase for the maximum 280 bunches) or by using a bunch lengthening cavity.

A 100 h Coulomb scattering lifetime has been calculated for an N_2-equivalent pressure of 0.25 nTorr and a minimum vertical full aperture of 12 mm in one ID chamber. The bremsstrahlung lifetime is 300 h assuming a 3% momentum acceptance.

The total 200 mA lifetime is 36 h for 70 bunches and 60 h for 280 bunches.

5 ACCELERATOR COMPONENTS

5.1 Vacuum System

The girder vacuum chambers will be designed to accommodate smaller magnet gaps and higher SR power loads. The chamber cross section has ~36x90 mm inner dimensions. Many of the existing straight section chambers will be kept, including those for the IDs, kicker magnets, and some diagnostic components. Tapered transitions from new to old chambers will be required in some cases to reduce impedance. RF-shielded bellows elements will be designed to minimize parasitic mode losses. Beamline front end masks and absorbers will also be upgraded for higher SR power.

To maximize beam lifetime, we seek an average ring pressure of order 1 nTorr. An antechamber design with discrete photon absorbers would achieve this goal and maximize chamber stability under varying SR power load. Since an antechamber design may be more costly and may necessitate more expensive C-core or Collins-type magnets, we are also considering a narrow chamber design. Since absorbed SR power may cause this chamber to move, beam position monitor modules would need decoupling bellows and stable supports to reduce transverse motion to the 10 μm level as requred by the orbit stabilizing system.

5.2 Magnets and Supports

The preliminary separated function SPEAR III lattice requires 36 dipoles (50 mm gap), 94 quadrupoles (70 mm bore diameter), and 72 sextupoles (80 mm bore diameter), and 54 pairs of horizontal and vertical correctors. The operating field for the 1.5 m, 10.6° dipoles will be 1.19 T at 3 GeV. Quadrupole gradients are ~20 T/m at 3 GeV. Sextupole strengths are on the order of 300 T/m^2. These field designs will permit 3.5 GeV operation with acceptable core saturation. A C-core dipole accommodates the SR beamline exit chamber. It has not been determined if open-core quadrupoles and sextupoles will be needed.

The new magnets will be mounted on existing 10 m concrete girders, each of which is now supported by three piers sunk 1.5 m into the ground. These girders have a rotational oscillation mode at ~5 Hz about the long axis that could be stabilized with a fourth support. The magnets will be mounted on the girders using kinematic struts. New girders will be installed for the four repositioned magnet cells flanking the IRs.

5.3 Injection

The booster synchrotron and booster-to-SPEAR transport lines will be upgraded for 3 GeV injection. A new septum magnet will be needed for the higher injection energy and the reduced displacement between incoming and stored beams (~15 mm). The three existing vacuum-core kicker magnets will be reused.

A possible future upgrade is to move the injection point to a long straight section unsuitable for beamline use and to install shorter ferrite-core kickers. This would liberate two arc straight sections and the second 12 m IR straight section for IDs.

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