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Reflection Imaging X-Ray Laser Microscope (RIXRALM) and its Biological Applications

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I. Summary

The concept of the Reflection Imaging X-Ray Laser Microscope (RIXRALM), for which we received a US patent # 5,177,774 (in 1993, see Appendix A), is based on the observation a significant reflection of the Soft X-ray Laser (SXL) beam from surfaces of materials. This was confirmed by calculation of the reflection coefficient and contrast for the 10 - 20 nm wavelength range for different materials and different angles of incidence. This wavelength range, which perfectly matches the operating wavelengths of our soft X-ray laser (SXL), is the most suitable for a reflection soft X-ray (SX) microscope: at shorter wavelengths, the reflectivity of materials rapidly decreases (as well as contrast), whereas at longer wavelengths, the resolution of the microscope would decrease.

The main stimulus for the development of the proposed microscope (RIXRALM) is the possibility to view the surface and near surface structure of biological materials, such as cell membranes at much higher resolution than an optical (confocal) microscope. Although the predicted resolution of RIXRALM was lower than a Scanning Electron Microscope (SEM), the possibility to obtain images of cells (membranes) in a more natural, hydrated state and, in many cases, without staining, made the idea of a reflection X-ray microscope very attractive. The specimen can be in an H₂O saturated He atmosphere at atmospheric pressure. As the image can be obtained quickly (nsec exposure, occurring within seconds of insertion into such an environment), the cell surface can be seen in a state which is very close to its natural condition. Besides, the short exposure time eliminates the effect of motional blurring on the images. Our X-ray reflection microscope fit well in the very large gap in the size of biological objects studied in light microscopy (sub-micron size) and electron microscope (down to a few nanometers size).

With the support from the Office of Health and Environmental Research (OHER) of DOE, RIXRALM was transformed from a concept into a real device. Although RIXRALM needs further development in order to become a useful and practical x-ray microscope, we have demonstrated its feasibility and obtained preliminary images.*

We have proceeded with the development of RIXRALM along two paths using (a) a microzone plate, and (b) a multilayer-coated Schwarzschild objective as image magnifying elements. After experiencing a number of difficulties with the development of ellipsoidal zone plates and with geometrical problems in adapting a circular zone plate to the reflection microscope, we concentrated our effort on the second path. The first prototype X-ray reflection microscope with our original (relatively large) SXL as a source of X-ray radiation has provided proof-of-principle of this concept (publication in Optics Letters; in Appendix A). With the slightly better quality Schwarzschild objective we were able to obtain the first images of biological cells (resolution of the images are still quite low due to the quality of the Schwarzschild objective - a high quality objective costs ten times more than the one we are using).

*It should be noted here that due to OHER's budget constraints we were granted only 60% of requested budget, which was not sufficient to purchase a high quality Schwarzschild objective and develop a high resolution microscope.

Principle of the Reflection SXL Microscope.

not to scale

Fig. 1.

Principle of Reflection SXL Microscope

Fig. 2

In order to proceed with the development of a very compact RIXRALM as well as to explore the possibilities of its improvement while conducting biological experiments, we have also constructed a second prototype of the reflection microscope and installed it on a new, "table top" SXL (which is under development). In the future (depending only on the possibility to obtain appropriate financial support) we plan to have the whole system, including SXL and its pumping laser, on one 4' x 10' optical table.

In the process of developing the RIXRALM, we came up with a new idea of using this microscope for mapping of chemical elements in cells. Our initial theoretical analysis indicated the strong differences between elements in the dependence of the reflectivity on (a) angle of incidence and (b) illumination wavelength (the wavelength of a soft X-ray laser can be changed by changing the target material). For example, at the wavelength 18.2 nm calcium has almost 2x higher reflectivity than protein at 10° angle of incidence, whereas at 13 nm calcium has about 3x lower reflectivity than protein. It will be possible to exploit these differences to map a particular element in the cell membrane. We should note here that our SXL operating at several wavelengths in region of 13 - 18 nm is most suitable for RIXRALM because of the ideal match of the high reflectivity multilayer coating of the Schwarzschild objective and the significant reflectivity of biological materials at these wavelengths. Due to the short SXL pulses, RIXRALM is also an instrument with a very short exposure time ($\sim 10^{-9}$ - 10^{-8} sec).

The RIXRALM project is part of an ongoing program at Princeton University on the development of the soft X-ray lasers and their applications to SX microscopy. We have successfully used the SXL for contact microscopy and more recently for SX transmission microscopy. This new type of soft X-ray microscope - the reflection imaging microscope - is expected to be a very important tool for biologists and biochemists for cell studies, especially when the whole instrument will be a very compact device.

In close collaboration with biologists at Rutgers University, University of Miami and Princeton University, we are going to use RIXRALM to study the proteins in cell surface membranes. These surface proteins are of great interest to biologists in relation to cancer and immunology studies, which are connected to our earlier collaborative works with biologists using the contact X-ray laser microscope (COXRALM), which presently is replaced by the RIXRALM.

II. Principle of Reflection SXL Microscope

Microscope with zone plates objective and Schwarzschild condenser. Initially we designed the SX reflection microscope based on a micro zone plate. We had chosen zone plates at that time, as a proven method of 50 nm resolution imaging at 18.2 nm wavelength. (A soft X-ray laser, SXL, operating at a single wavelength is well suited for zone plate operation). The zone plates have been used very successfully as X-ray focusing elements in soft X-ray transmission scanning microscopes with synchrotron radiation¹⁻³ as well as for a transmission imaging microscope with a plasma source⁴. They are particularly well suited for transmission microscopy in the "water window", where multilayer mirrors do not have yet sufficiently high reflectivity (still much less than the theoretically predicted 20-30%)^{5,6}. We planned to use an ellipsoidal

zone plate, which would be specially developed for our project by a group at King's College (London). Such a zone plate could be placed parallel to an object and the reflected SX beam would be focused on a film or CCD detector as shown in Fig. 1. Also shown in this figure is a multilayer coated reversed Schwarzschild objective used as a condenser.

Unfortunately, the group at King's College discovered that the development of an ellipsoidal microzone plate was much more time consuming than initially estimated and therefore too expensive for us, taking into account budget constraints. Instead, however we obtained from King's College 3 circular zone plates for 18.2 nm wavelength in order to pursue the scheme presented in Fig. 2. These are fabricated by carbon contamination writing using a modified Scanning Electron Microscope (SEM), and operate at 18.2 nm without a gold coating. We have conducted very intensive work with these circular zone plates, initially in a transmission configuration in order to compare with some earlier results and to have a reference point when using them in the reflection mode. The reflection mode, however, is much more difficult technically due to very close distance of zone plate to object (focal length $\sim 100\ \mu\text{m}$), the small depth of focus ($\sim 0.1\ \mu\text{m}$) and complicated alignment. In fact, the alignment of the zone plate for the reflection mode using single pulse SXL (i.e. very low repetition rate: 1 pulse in 3 min.) was very difficult and not reliable.

In parallel, we constructed a condenser based on a multilayer coated Schwarzschild system. As it is well known, in order to maximize the efficiency of the optical system, it is necessary to have the numerical aperture (NA) of the condensing system match that of the imaging system. This is even more important when the imaging optic has a large central obstruction, as is the case for both zone plates (where it is necessary to eliminate "zero order" or undiffracted radiation) and Schwarzschild objectives. Although a multilayer, off-axis conic would be the minimum surface solution, such optics are extremely expensive to polish to the very high surface finish (less than 0.5 nm rms surface roughness) required for near normal incidence soft x-ray optics. We therefore settled on a Schwarzschild mirror condenser, with both of the mirrors being spherical. [More recently we developed the concept of a Soft X-ray Diffraction Plate, SX DP (patent pending), which combined with a grazing incidence toroidal mirror may serve as a quite efficient condenser and may simplify the construction of RIXRALM while adding spectral flexibility].

It is worth noting here the tremendous progress in the development of multilayer coatings for normal incidence (large angle of incidence) X-ray optics, and the potential for applications⁶. In particular, it makes possible for the construction of a reflection SX microscope, that would have been impossible a decade ago.

The Schwarzschild optics used in our experiments were manufactured by T.R. Optics, Ltd., in England⁷. The optical surfaces were polished to a high surface finish ($\sim 0.5\ \text{nm rms}$ surface roughness). The Schwarzschild mirror system comprised of a concave and convex mirror. The diameter of the large concave mirror was 66.3 mm and its radius of curvature was 68.5 mm. The small convex mirror was 14.5 mm in diameter with a 23 mm radius of curvature. The Schwarzschild mirror system had a numerical aperture $\text{NA} \approx 0.4$ and a focal length $f = 14\ \text{mm}$. The multilayer coating and reflectivity measurements were done by the Advance Optics Group of the

Lawrence Livermore National Laboratory under the direction of Dr. N. Ceglio. The multilayer coating consists of 15 layer-pairs of molybdenum and silicon of thickness ~ 3 nm and ~ 9 nm respectively. Fig. 3 shows the measured and calculated reflectivity of the multilayer-coated mirror in the vicinity of 18.2 nm corresponding to our SXL wavelength. The calculation of the reflectivity is done using a code developed by Oxford Research Group⁸. This calculation assumes the abrupt transition and perfect smoothness between Mo and Si layers. Although this code is capable of modeling interdiffusion between layers, we choose to ignore this option because of the lack of experimental data. The important conclusions are the matching of the shape of the curves: especially, the location of the peak B, and the side lobes, A and C, and other structures which can be seen in Fig. 3. The discrepancy of the factor of 2 in magnitude between the measured and calculated reflectivity is assumed to be due to scattering due to surface roughness of the optics and to interlayer diffusion in the multilayer coating.

Fig. 4 shows schematically the set up for Schwarzschild condenser testing. For simplicity this was done in transmission mode (the results of such a test can easily be related to the reflection mode as well). The goal of these tests was to determine how well the Schwarzschild condenser could be adapted to the reflection soft X-ray microscope. A filter of 80 nm Al covered with 10 nm C was used to block visible UV and VUV radiation. Our "old" (developed in 1984-85) soft X-ray laser⁹ (pumped by CO₂ laser in strong magnetic field) operating at 18.2 nm was used as a source. On Fig. 5a and b are shown images obtained with the Schwarzschild condenser (a) without the Al filter at 10 mm from focal plane and (b) with the Al filter at distance significantly closer to the focus (hence the smaller central ring).

We were quite satisfied with the performance of the Schwarzschild condenser, which well concentrated the X-rays. Aberrations in the condenser system are of secondary importance as they do not affect the final resolution of the image and are only a problem if they are severe enough to cause non-uniform illumination of the specimen. Therefore it was quite easy to work with such a condenser, which did not require very precise alignment of the mirrors.

Schwarzschild objective. Major difficulties with using zone plates as mentioned earlier, and very encouraging progress in the fabrication of high precision objectives together with rapid progress in the development of multilayer coatings reflective in the soft X-ray region has made Schwarzschild objective an attractive alternative to zone plates for X-ray imaging, especially for 18.2 nm wavelength (e.g. AT&T research group has achieved 0.05 μ m resolution using Schwarzschild objective for X-ray projection lithography program¹⁰). Initially we did not have the precision adjustment of the mirrors required for diffraction limited imaging, since our Schwarzschild had been designed for the less demanding task of a condenser (where aberrations are not important). Nevertheless we decided to try out our Schwarzschild mirrors as an alternative (second) path to obtain magnification and test the concept of the reflection SX microscope. Because we had no matching Schwarzschild optics to serve as a condenser, we had to use the optics off-axis to avoid the central obstruction.

Fig. 3 (a) The measured reflectivity of the Mo/Si multilayer-coated mirror in the vicinity of 18.2 nm. The reflectivity of the mirror is about 20% at 18.2 nm. (b) The calculated reflectivity of the mirror. The abrupt transition and perfect smoothness between Mo and Si layers are assumed in this calculation. The discrepancy between the measured and calculated reflectivity may be attributed to the interdiffusion of one layer into another and the possible surface roughness in the interface.

Fig. 4 A schematic diagram showing the use of the Schwarzschild mirror set as a condenser, collecting light onto a transmission specimen as the first stage of an imaging X-ray microscope. The 80 nm Al, 10 nm C filter is used to eliminate visible and ultra violet light. The source is the Princeton University 18.2 nm laser, relayed via a 1:1 imaging gold coated, grazing incidence ellipsoid.

Fig. 5 The Schwarzschild as a condenser. On the left is the image 10 mm from the focal plane taken without the Al filter, and on the right is the image with the filter in place. The film is Kodak 101-07 X-ray film. The four bright spots on the left hand side are visible light coming through tears in the Al filter from four locating screws. The image on the right is closer to the focus, hence the smaller central ring.

The Schwarzschild mirror system was used in two sets of experiments. The purpose of the first set of experiments was to test the Schwarzschild optics in the transmission imaging configuration in order to have a reference point in comparing to existing results; on the other hand, the goal of the second set of experiments (which was closely related to the goal of the whole project) was to demonstrate the principle of a soft X-ray reflection microscope. Fig. 6 shows the experimental set-up in which transmission imaging experiments were performed. The 18.2 nm soft X-ray laser has a 5-10 mrad divergence¹¹ due to the single-pass amplification (it does not have cavity mirrors around the gain medium). An ellipsoidal grazing incidence mirror was used to collect and image the 18.2 nm radiation onto a TEM (Transmission Electron Microscope) #440 grid. The transfer mirror was made from an Al substrate, figured to a ellipsoidal shape with diamond tool, plated with nickel and coated with rhodium. The mirror assembly had four micrometers for the precise control of the mirror position. The numerical aperture of this ellipsoidal mirror was 0.1. Since the numerical aperture of a central of the Schwarzschild mirror system was larger (0.17) than that of the ellipsoidal mirror, the Schwarzschild mirror system was tilted at 15° of axis. An 80 nm thick Al filter, with a coating of 10 nm of C was placed before the Cu TEM #440 grid (period is 63 μm) to block out UV, VUV and visible light. The transmission of the filter is about 56% at 18.2 nm. The TEM grid was illuminated by light passing through the filter and imaged by the Mo/Si multilayer-coated Schwarzschild mirror system with a magnification of 22 onto Kodak 101-07 X-ray film.

Figure 7 shows the recorded picture. After we obtained the image as shown in Fig. 7, we intentionally reversed an X-ray film to double check that the exposure was due to X-rays. Any longer wavelength light present (especially, visible light) would have exposed the reversed X-ray film (the film is thick so that all X-rays would be absorbed in the backside of the film and not reach the emulsion surface). We did not observe any exposure with the film reversed. The specimen field of view was 1.3 mm by 0.7 mm.

III. Proof of principle and modeling for improvement of RIXRALM resolution.

Proof of principle. Once the Schwarzschild optics was verified to function properly, the optics were rearranged in the reflection imaging configuration as shown in Fig. 8. The 18.2 nm beam was aligned to a reflection object which was constructed by evaporating gold onto polished glass surface through a TEM #200 grid. The angle of incidence of the 18.2 nm laser beam to the reflection object was limited by our vacuum chamber adjustment to 70° (or the angle of 20° between the reflection surface and the incident beam). The difference in the reflectivity at this angle between gold and glass is large enough to produce a good contrast image. The image of the reflection object recorded on Kodak 101-07 X-ray film is shown in Fig. 9. The dark lines represent the glass part of the reflection object, while the white areas represent the gold part¹².

The importance of these results was that they show the feasibility of X20 reflection microscopy using the existing soft X-ray laser at Princeton University and indicated the possibility of increasing the magnification up to X(50 - 100) by improvements in multilayer mirrors reflectivity and the precision of the Schwarzschild objective.

Fig. 6 The arrangement used in the X20 magnification 18.2 nm imaging experiment.

Fig. 7 The result of the X20 imaging experiment. The object is a 63 μm period Cu electron microscope grid. This is imaged onto Kodak 101-07 X-ray film at an X-ray magnification of X20 using an off axis, multilayer coated Schwarzschild reflection system. The source of light is a single 20 nsec long pulse from the Princeton University 18.2 nm soft X-ray laser.

Fig. 8 The arrangement used in the reflection imaging experiments.

Fig. 9. The result of the X20 reflection imaging experiment. The object was a gold on glass grid with $63\ \mu\text{m}$ period. The angle of incidence was 20 degrees.

Modeling. In order to design a high resolution Schwarzschild objective for RIXRALM, a ray tracing computer code (developed in Lebedev Institute in Moscow) was used. This work was done in collaboration with Prof. a. Vinogradov and Dr. N. Zorev from Lebedev Institute. The code simulated the reflective imaging of X-ray optical systems including the effect of surface roughness. The calculation of resolution as a function of distance between the large mirror (convex mirror) and small mirror (concave mirror) indicated a very high sensitivity of the resolution on precision of relative location of these two mirrors. From Fig. 10, which presents calculations for one example of a set of two mirrors, one may see rapid degradation of resolution when the mirror separation deviates more than 10 μm from the optimum distance. The calculations also gave us a limit on the surface roughness and the tolerance (very small) in the deviation of mirror curvature from an ideal (spherical) one in order to obtain better the $\sim 0.05 \mu\text{m}$ and magnification X100. (In the future resolution of the order of $0.025 \mu\text{m}$ may become possible for a SXL operating at wavelength 13.5 nm). We also obtained the tolerance on off-axis misalignment of the mirrors, which should not exceed 3 μm in order to maintain desired resolution and magnification for a field of view of $100 \mu\text{m} \times 100 \mu\text{m}$. However this field of view is further limited and depends on the reflectivity of multilayer coating, the flux of the soft X-ray laser beam, the reflectivity and contrast of the object, transmissivity of Al- and C-filters, the sensitivity of the soft X-ray film and size of the grains (relatively good sensitivity SX films with grain size $\approx 0.2\text{-}0.3 \mu\text{m}$ are now available) or the sensitivity and pixel size of the Charge Coupled Device (CCD) which will replace film. Taking into account all of these parameters, the field of view will be limited to $\approx 30 \mu\text{m} \times 30 \mu\text{m}$ in a single pulse SXL.

CCD detector. Thinned back illuminated CCDs have recently emerged as the detector of choice for the soft X-ray wavelength range¹³. Their detection efficiency is two orders of magnitude higher than film or phosphor coated front illuminated CCDs and is very close to quantum limited (up to 80% of incident soft X-ray photons being detected). Work on these was initiated in our laboratory initially in collaboration with Princeton Instrument Inc. (PI Inc.) and further developed in collaboration with Princeton Scientific Instrument, PSI. These systems are presently available commercially from these two companies.

Although our current CCD has pixel size $\sim 25 \mu\text{m}$, however, there are already available CCD chips of pixel size 9 μm (from KODAK). Due to a very rapid progress with decreasing size of the pixels (e.g. SRI Sarnoff Center of Princeton decreased size of the pixels down to 3-5 μm in experimental devices) such cooled SX CCD will be an excellent detector for real time image capture and direct storage into computer readable memory. Due to much higher sensitivity of CCD than SX film it will be possible to obtain images of area $50 \mu\text{m} \times 50 \mu\text{m}$ in single shot with resolution better than $0.05 \mu\text{m}$ using CCD with pixel size smaller than $5 \mu\text{m}$ and 100X magnification of Schwarzschild objective.

Testing systems. We should mention here that testing of the SX CCD was conducted on the 4'x 10' optical table in our table-top soft X-ray laser experimental set up, which is shown on Fig. 11. The soft X-ray radiation is directed to the CCD by inserting a flat multilayer mirror at 45° in the beam path. The mirror was coated for peak reflectivity at 18.2 nm at 45° by the group of Prof. C. Falco's (Univ. of Arizona). On Fig. 11 is also shown the electronic system

Fig. 10. Resolution of the Schwarzschild microscope as a function of distance between the objective mirrors.

("electronic box") for interconnection between CCD and computer. On the back of the first table is located reflection SX microscope, which is connected to the vacuum pipe. This way by turning our flat multilayer mirror by 90° , the soft X-ray beam could be directed to the reflection SX microscope. The soft X-ray radiation in the vicinity of the 18.2 nm lasing line is measured by the SX grazing incidence monochromator with multichannel detector (located on the right hand side of the picture, above the high vacuum pumping system) connected to the data acquisition system (electronics & computer). Behind the microscope, on the second 4'x 10' table, are located major parts of 10 J, 1.5 ns Nd/Glass laser, which is pumping laser for SXL. This laser will be replaced by one approximately half the physical size but the same energy and pulse length.

A better view of the reflection SX microscope can be obtained from Fig. 12 with the microscope high vacuum pumping system, which is attached to a condenser's vacuum chamber. As a condenser we were using the grazing incidence toroidal mirror for illumination of Schwarzschild objective through Diffraction Plate. The focal length of the toroidal mirror is adjustable with 3 vacuum fee-through micrometers. The microscope by itself is presented on the next picture (Fig. 13). On this picture is presented a slightly different reflection SX microscope, which is installed on our original ("old"), much larger (and much more powerful) SXL. While both microscopes are very similar their roles were very different. The microscope on Fig. 13 was prepared mainly for experiments with biological cells such as testing different modification (improvements) of the environmental chamber ("ant farm"), the observation of the effect of soft X-ray radiation on biological cells, monitoring reflectivity of dry and wet cells, effect of multi-pulse exposure on cell degradation. The role of the second reflection microscope (Fig. 12) was as a test-stand for: Schwarzschild objective and condenser, Diffraction Plate as a possible replacement of the Schwarzschild condenser, CCD array, comparison of film and CCD for S/N ratio, etc.

The use of two systems for the development of RIXRALM for biological research was very productive. The first system was used to study aspects related to imaging of cells with minimum changes in the microscope set-up. The role of the second system was to broadly explore the quality and performance of optical components of the microscope, improvement of its performance and explore new designs and ideas.

Diffraction Plate. When we started the RIXRALM project we considered several types of condensers, beside the multilayer coated reversed Schwarzschild objective already discussed, such as zone plates, spherical mirrors, off-axis spherical mirrors, paraboloidal mirrors. After theoretical analysis and a number of experimental tests we chose the reversed Schwarzschild objective, taking into account its perfect match to the numerical aperture (NA) of the magnifying Schwarzschild objective, the very good reflectivity multilayer coating available for RIXRALM's wavelength region 10-20 nm and the less stringent requirements on the precision of Schwarzschild mirrors (hence, moderate cost). However, since that time, we came also to a new idea of a SX condenser based on a Diffraction Plate, DP, which may in the future, simplify the construction of the reflection SX microscope, and adds spectral flexibility. (The DP operation is much less sensitive to wavelength than multilayer mirror). Preliminary tests of DP, developed in our laboratory, provided very encouraging results¹⁴ (please see Appendix A): its efficiency was

Fig. 11

Reflection Microscope Experimental setup

- 1) CCD
- 2) CCD "electronic box"
- 3) Soft X-ray Spectrometer
- 4) 45° Multilayer mirror controller
- 5) Reflection Soft X-ray Microscope
- 6) Nd/Glass pumping laser

Fig. 12

Reflection Microscope Experimental setup

- 1) Reflection Soft X-ray Microscope
- 2) Grazing incidence toroidal mirror
- 3) Microscope Vacuum pumping system
- 4) 45° multilayer mirror positioner
- 5) Soft X-ray Spectrometer
- 6) CCD

Fig. 13 Side view of Reflection Microscope

1) Schwarzschild objective holder	4) Microfiber/microplate system
2) Objective position control	5) Vacuum pumping system
3) Fiber bundle and detector holder	

approximately 30% and in agreement with theoretical calculations conducted in collaboration with Dr. N. Zorev for Lebedev Institute (Moscow). The DP is based on the idea that a film that has irregular sub-micron scale features, placed in close proximity to the specimen, will diffract the soft X-rays into the required angle.

In Fig. 14 is shown the diffraction plate (DP) as a condenser for Schwarzschild objective for the reflection SX microscope. The soft X-rays, after passing through an Al-filter, are diffracted by the DP and reflected by the surface and near surface layers of material, so as to fill the aperture of the microscope objective, which provides images on the detector. Initial tests used a collimated beam of visible light. After transmission through the specimen this beam was blocked by the central optic in a Schwarzschild objective and there was no image. However, on the insertion of the diffraction plate, a light was diffracted to a large solid angle and a good image was formed. In the second step the SX beam from the 18,2 nm laser was used. A grazing incidence ellipsoidal mirror was used to focus the X-rays onto the diffraction plate. The DP (as shown in Fig. 16a) diffracted the light into a solid angle of 0.34 radian. (Fig. 15a, shows SX spot without DP and Fig. 15b with DP). We plan to continue work on DO and incorporate it in to experiments on the second RIXRALM set up at the "table-top" SXL.

Pulse duration effect. One of the major problems with the application of X-ray microscopes for biological use is the effect of radiation damage to the cells. This is a general problem, which is faced by any of the X-ray microscopes both with continuum type of radiation (synchrotron) or pulse radiation (SXL, laser generated plasma). In the case of pulse radiation we have to address the problem of blurring due to hydrodynamic motion as a result of the relatively large dose of radiation required for good images (good S/N ratio). Therefore the question which we have to consider: is the speed of imaging fast enough to "outrun" radiation damage? This question was addressed by R. London et al.¹⁵ for holographic SX imaging, but so far little experimental work has been done. London et al. derived a maximum exposure time ~ 50 psec for a 15 nm protein sphere. Here there is a strong scaling of dose, D , on the required resolution, R ($D \sim R^{-4}$) and the maximum pulse length scales as R^3 . By scaling the numbers to 50 nm resolution the maximum exposure time is ~ 2 nsec. Taking into account the heat conductivity of an environmental medium (e.g. water) we can assume $t \approx 10$ nsec. During such time, a model 15 nm diameter protein can be displaced due to kinetic energy acquired by the cell components. Assuming linear movement (for simplicity), from the kinetic equation:

$$1/3 kT = 1/2 mV^2$$

we obtain $V \approx 1$ m/sec and corresponding displacement $d \approx 0.01 \mu\text{m}$ during 10 nsec for temperature $T \approx 300^\circ \text{K}$, which should have a very small effect on the quality of image. However in order to develop a better understanding of SX radiation damage on biological cells, and particularly SX pulse shortening as possible method to decrease such a damage, we conducted a series of cells imaging experiments using ultra-short SX pulses.

As a source of ultra-short SX pulse we used a plasma produced by our powerful subpicosecond (300 fsec) KrF laser (PSPL). The plasma was produced in target chamber, which was specially

Fig. 14 Diffraction plate as a condenser for reflection SX microscope.

Fig. 15 Diffraction result with x-ray illumination. Left-beam without diffraction plate. Right-beam with tilted diffraction plate.

built for this experiment. In order to obtain information about the effect of pulse duration on cell imaging we decided to use contact microscopy, which would allow us to compare cell images using ultra-short SX pulses with our earlier cell images obtained with contact microscope COXRALM¹⁶ using a 20 nsec SXL pulse. In both series of experiments images of fixed and dried whole HeLa cancer cells were recorded on photoresist. In both cases resolution was better than 50 nm. We did not see any significant difference in resolution or in contrast between images obtained with 20 nsec and ~ 1 -10 psec exposures.

However this result cannot be applied directly to the reflection SX microscope. Since the reflectivity of the cell is lower than its transmissivity, the dose of photons required for reflection SX microscope is expected to be significantly higher than for transmission SX microscope. This difference in photon dose requirement may be significantly compensated by the longer operating wavelength of the reflection microscope (hence lower deposited energy per photon). Therefore similar measurements using different pulse durations will have to be conducted for reflection images, including "wet" cells. It is worth noting here that it is much easier to work with the soft X-ray laser as a source of radiation since it can be located far away from the microscope and provides a more "clean" beam than the SX from the laser generated plasma (LGP). Of course, for imaging microscopy, a tremendous advantage of the SXL in comparison to LGP is its much higher brightness and single line radiation.

Compact SXL. In our effort to develop a very compact ("table top") SXL we have made significant progress during the reported period due to the implementation of a new target configuration and more efficient plasma cooling during fast recombination.¹⁷ (Appendix B). This compact SXL is based on the interaction of a line-focused Nd/Glass laser beam with a carbon target. The plasma column created along the target consists of highly ionized carbon ions, which rapidly recombines and provides a lasing medium with lasing transitions at 18.2 nm or 13.5 nm in CVI. Using Al or Si targets, the lasing action can be generated in AlXI at 15.4 nm and in SiXIII at 13.0 nm. The pumping laser beam is relatively low energy (4-5 J in 1.5-2 nsec), which can lead to a very compact system. A new target has very fine grooves on the surface, which allows for additional (limited) plasma expansion in axial direction in the process of creation of the plasma column. With this new target and improved design of iron cooling blades we have already demonstrated a gain-length product $GL \approx 4.2$. Very recently a very exciting avenue for a very compact soft x-ray laser was opened by the demonstration of lasing action at 13.5 nm in transition to ground state of Li III ions¹⁸ in transition to ground state of Li III ions (see Appendix B). This laser is operating at 2 Hz repetition rate and with improvement of the beam output energy to 10 μ J/pulse it will suit well as a source of radiation for RIXRALM.

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