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CONTACT MICROSCOPY WITH A SOFT X-RAY LASER

By

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Contact Microscopy with a Soft X-Ray Laser

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ABSTRACT

A soft X-ray laser of output energy 1-3 mJ at 18.2 nm has been used to record high resolution images of biological specimens. The contact images were recorded on photoresist which was later viewed in a scanning electron microscope. We also present a Composite Optical X-Ray Laser Microscope "COXRALM" of novel design.

EP

I. Introduction

The emerging technology of X-ray lasers [1,2] will have increasing impact in a number of fields. Microscopy using soft X-ray lasers offers the exciting prospect of imaging live cells at high resolution, thereby bridging the gap between electron microscope images of non-live cells that have undergone extensive specimen preparation, and low resolution but high fidelity images of live cells recorded with light microscopes [3,4]. In this paper we present high resolution images of several different biological specimens obtained with a soft X-ray laser. The soft X-ray laser at Princeton Plasma Physics Laboratory (PPPL) is well suited to contact microscopy as the pulse length (10-30 nsec) and pulse energy (1-3 mJ) are sufficient to record a flash image of a biological specimen on photoresist in a single shot. At the laser wavelength of 18.2 nm the absorption cross section of oxygen is three times that of carbon, providing good contrast between carbon in the cell proteins and oxygen in the surrounding water.

To be of maximum utility to biologists a soft X-ray laser contact microscope should be suitable for everyday use on fragile, living biological specimens. We are developing a novel microscope design which will enable the operator to view and manipulate the specimen just prior to exposure.

II. X-Ray Laser Development at PPPL

The soft X-ray laser at PPPL is based on a rapidly recombining plasma confined in a magnetic field. A commercial CO₂ laser with maximum energy of 1 kJ and duration 70 nsec is focussed onto a carbon disc located in a strong (up to 90 kG) magnetic field, creating a plasma of sufficient temperature so that a substantial fraction of the ions are stripped

of electrons. Radiation cooling after the laser pulse produces rapid three-body recombination into highly excited states in hydrogen-like carbon. The $m = 2$ level is rapidly depopulated by the strong $2 \rightarrow 1$ radiative transition and in this way a population inversion is built up between levels 3 and 2, leading to amplified spontaneous emission and lasing at 18.2 nm (Fig. 1). The strong confining magnetic field provides several advantages. It enables the electron density to be controlled to the optimum value and forms the plasma into a long thin geometry suitable for a laser. The geometry is also suited to fast radiation cooling, and the radiation cooling can be enhanced by the addition of high Z materials.

The CO_2 laser is focussed onto a target chamber inside a solenoidal magnet that is surrounded by diagnostic spectrometers (see Fig. 2). The spectrometers are calibrated absolutely and measure the soft X-ray gain by measuring the directionality of the emitted radiation. In a long thin plasma most of the stimulated emission is along the plasma axis and the gain is measured by comparing the axial to transverse intensity.

The target is a carbon disc with one to four blades attached perpendicular to the target surface. The blades help to generate an elongated plasma. They also help to cool the plasma by thermal conduction and additional radiation losses. An important feature is the radial profile of the plasma. The plasma pressure is balanced by the magnetic field pressure so that on axis there is a high temperature due to heating by the laser and corresponding low density. In the outer cooler regions the density is higher and it is in these outer regions that the conditions are most favorable for fast recombination. The gain is generated in an annular region around the center of the cylindrical plasma. An

off-axis slot in the target transmits the stimulated emission to the axial spectrometer. The system has a very accessible output beam, can be fired every three minutes, and operated by only two people.

A gain-length of $GL \approx 8$ on the 3-2 transition at 18.2 nm has been achieved with an output energy of 1-3 mJ in a low divergence (5 mrad) beam (see Table 1). This system has been described previously in a number of publications, see e.g. [5] - [7]. More recently, efforts to obtain gain at wavelengths shorter than 18.2 nm have resulted in measurements of gain-length $GL \approx 3-4$ at 15.4 nm in Al^{IV} and $GL \approx 1-2$ in Si^{XII} [8]. Current research is focussed on increasing the output energy of the 18.2 nm laser by additional amplifiers [9] and exploring a new approach to developing X-ray lasers in the wavelength region from 5 nm down to 1 nm [10].

III. Contact Microscopy Using a Soft X-Ray Laser

Much progress has been made in Biology and Medicine due to the high resolution images obtained from electron microscopes. However, in order to be viewed by an electron microscope the specimen must be dried, stained, and sectioned, and it is clear that some information about the living cell is lost in the process. One can view a live cell with a light microscope with high fidelity but the resolution is, of course, limited. Soft X-ray contact microscopy offers a new method to obtain high resolution images of live cells. The specimen is contained in an environmental cell isolated from the X-ray laser vacuum system by a 120 nm thick silicon nitride window. The image is recorded on photoresist which is later viewed by an electron microscope [11]. This approach has been used recently with plasma light sources and synchrotrons [3,12]. The highly collimated output beam of the soft X-ray laser

compared to a conventional plasma light source has the advantage of less penumbral blurring of the image and more flexible microscope design. The soft X-ray laser also has the advantage of a 10-30 nsec exposure time enabling flash images of live cells to be recorded, unlike the several minutes needed for synchrotron sources which effectively prevents the imaging of live cells with synchrotrons.

The soft X-ray laser output beam originates from an annular gain region 50-100 μm wide in the plasma. The beam is emitted along the magnetic field axis and is normally detected with a grazing-incidence spectrograph equipped with a multichannel detector. For microscopy experiments, an ellipsoidal mirror is used to deflect the soft X-ray beam to the photoresist in a contact microscope. The mirror is a diamond-turned, post-polished, rhodium-coated ellipsoid which provides an image of the soft X-ray gain region in the plasma on the photoresist.

In the first experiments a contact image of # 100 mesh was made on both Kodak 101 film and copolymer photoresist P(MMA co MAA). While the film was much more sensitive, the inevitable grain structure degraded the resolution to a few microns. Much higher resolution was obtained on the photoresist. Noteworthy here is that although the photoresist is much less sensitive than film, needing of the order of 100 mJ cm^{-2} for exposure, the available energy in the soft X-ray laser beam was sufficient to expose the resist in one shot.

In contact microscopy the shadow of the specimen is recorded in the resist in the following way. Exposure to X-rays produces chain scission in the long polymer molecules in the photoresist and hence the exposed areas dissolve faster in a suitable solvent. After development, a relief map of the X-ray absorption by the specimen is formed on the surface of the resist. The resist is then coated with

a thin layer of Au/Pd and viewed at high resolution in a scanning electron microscope.

A suspension of diatoms was placed on photoresist (silicified skeletons of planktonic algae) which was left to dry. After exposure by the soft X-ray laser the copolymer photoresist was developed for one minute in a mix of equal parts methyl isobutyl ketone and isopropyl alcohol and viewed in a scanning electron microscope (SEM). The SEM image is shown in Fig. 3 and a resolution of 0.1 μm is apparent. Since the diatoms are high contrast objects, this technique can also be described as microlithography and illustrates the potential application of X-ray lasers to microelectronics.

It was found that the photoresist PBS (polybutene-1-sulfone) was a factor of three more sensitive than the copolymer resist and was, therefore, used for subsequent exposures of biological specimens. The PBS resist was developed in methyl ethyl ketone (2 parts) and isopropanol (1 part) for 15 seconds. In initial experiments with biological specimens placed in contact with the resist, it proved difficult to remove the specimens from the resist after the exposure. This opened the possibility of confusion of remnants left on the resist with the relief image formed in the resist [13]. In order to avoid this problem the biological specimens were mounted on a thin (5 nm) carbon film supported on a standard TEM grid (Fig. 4). This was placed in contact with the resist for the exposure and could be easily removed and if necessary reused in later exposures.

One topic of considerable current interest in biology is immunology. Since this involves the dynamic response of immune cells to foreign matter, it is a natural subject for investigation by soft X-ray laser microscopy. One of the most primitive, and hence simplest, immune systems

is found in the horseshoe crab (*Limulus Polyphemus*) where there is only one type of immune cell to fulfill several functions. A sample of blood from the horseshoe crab was dehydrated in an ethanol series, critical point dried using the standard procedure, but not fixed or stained and an exposure taken with the soft X-ray laser contact microscope. The resulting images viewed in an JEOL 840 scanning electron microscope are shown in Fig. 5. The depressions apparent in the granulocyte are known to exhibit immune functions from work in conventional electron microscopy. The smaller white features covering the cell are new and were not apparent on images obtained in conventional electron microscopy. Their identity and function is presently unknown.

One of the difficulties of reading a relief pattern in a photoresist with an SEM is that the electron beam in the SEM is capable of writing a raster pattern in the resist and destroying the image. In the above images the accelerating voltage was reduced to 5 kV to avoid this effect, but the resulting readout process is lengthy. We have developed a technique which enables the resist image to be quickly acquired and read out at a much lower beam current (2×10^{-12} A compared to the 2×10^{-11} A used previously). In this technique the resist has a 1000 Å thick gold underlay which increases the flux of backscattered electrons. The SEM image is then predominantly formed by backscattered electrons rather than secondary electrons from the surface. There is a minor decrease in the ultimate achievable resolution of the SEM with this technique, but this does not appear to be a significant factor at present.

This technique was used in the image of Hela cells shown in Fig. 6. This is an embedment-free section of a monolayer of extracted Hela cells on a carbon/formvar base. Hela cells are cultured human cervical cancer cells and the clumping feature apparent in the irregularly shaped nuclei are the result of invasion by a virus.

IV. Microscope Development

Perhaps the greatest potential for soft X-ray laser microscopy is in the study of dynamic processes in live cells. To maintain and manipulate cells in a living state is a considerable experimental challenge. For novel microscopic techniques to have a significant impact in biology, they must be practical and convenient to use. To this end we have developed a Composite Optical Soft X-ray Laser Microscope, "COXRALM" (see Figs. 7 and 8). This is a new type of microscope combining an inverted phase contrast optical microscope with a soft X-ray laser contact microscope. The inverted optical microscope has already been used [14] as a microspectrofluorometer for the study of transient coenzyme fluorescence changes in living cells as a result of the intracellular microinjection of metabolites. This research is aimed at understanding the pathology and physiology of cancer cells. However, the inevitable limitations in resolution of the optical microscope meant that many features of the metabolic processes inside the cells remained obscure.

COXRALM offers the possibility of making an X-ray contact image of these cells while preserving the ability to select and observe through the optical microscope and perform microinjection of metabolites and/or inhibitors and xenobiotics just prior to X-ray exposure.

The cells will be mounted on resist on a transparent substrate. When a contact X-ray image is desired, the X-ray laser beam tube will be lowered until the silicon nitride vacuum window at the tip is in contact with the cell and then the X-ray laser is triggered. The system has been installed on the X-ray laser and was used in initial experiments without the optical microscope to generate the contact images shown in Figs. 5 and 6.

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Table 1Soft X-Ray Laser Output Parameters

Gain - Length	8
Wavelength	18.2 nm
Energy	1-3 mJ
Power	100 kW
Duration	10-30 nsec
Divergence	5 mrad
Efficiency	10^{-5}
Shortest Wavelength	12.9 nm
Repetition Time	3 min.

Figure Captions

- Fig. 1: Principle of generation of population inversion and lasing action in hydrogen-like ions in a rapidly recombining plasma.
- Fig. 2: Contact microscope installed on the soft X-ray laser.
- Fig. 3: SEM image of replica of diatom fragment (silicified skeleton of planktonic algae).
- Fig. 4: Contact microscopy of biological specimens.
- Fig. 5: SEM image of replica of Granulocytes from *Limulus Polyphemus* (Horseshoe Crab).
- Fig. 6: SEM image of replica of hela cells (Helen Lane cervical cancer cell).
- Fig. 7: Schematic of Composite X-Ray Laser Microscope.
- Fig. 8: Composite of X-Ray Laser Microscope.

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Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

87X0675

COMPOSITE X-RAY LASER MICROSCOPE (COXRALM)

Fig. 7

Fig. 8

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