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RAL-TR-98-007

Laser Plasma X-Ray Contact Microscopy of Living Specimens Using a Chemically Amplified Epoxy Resist

TMR Large-Scale Facilities Access Programme

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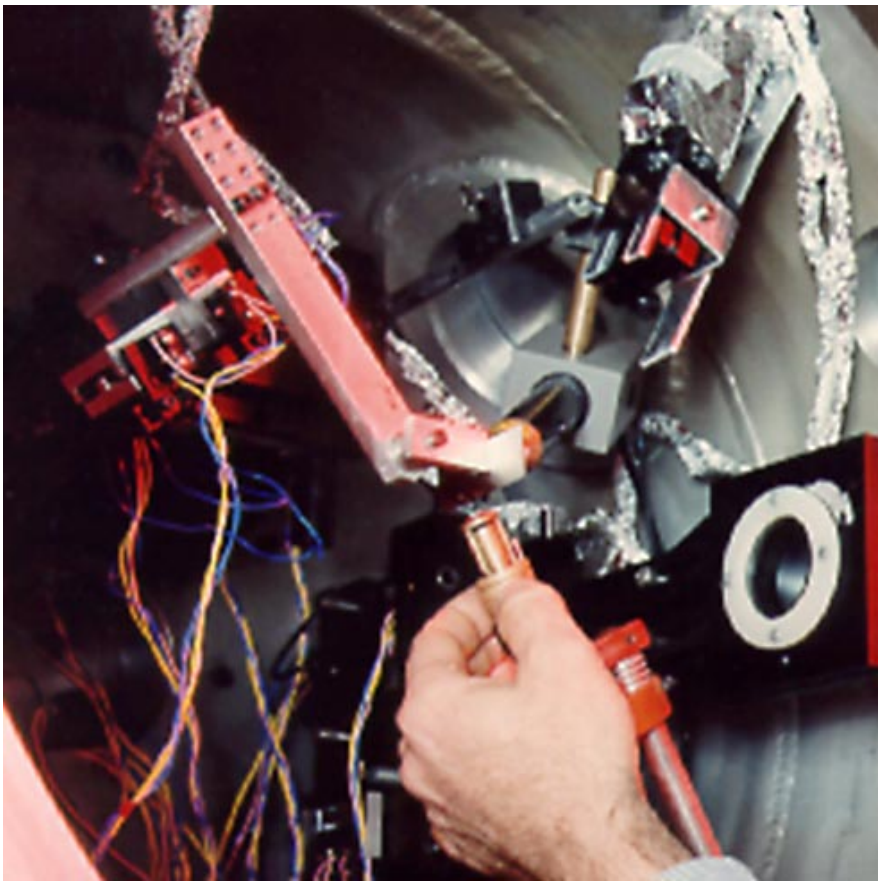

Laser Plasma X-Ray Contact Microscopy of Living Specimens Using a Chemically Amplified Epoxy Resist

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Access to Lasers at the Central Laser Facility
Rutherford Appleton Laboratory
Contract No. ERBFMGECT950053

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SUMMARY

This report describes the experiment entitled ‘Laser Plasma X-Ray Contact Microscopy of Living Specimens Using a Chemically Amplified Epoxy Resist’; carried out at the Central Laser Facility (CLF) from the 3rd to the 9th February 1997. The experiment, funded by the Framework IV Large-Scale Facilities Access Scheme, was proposed by Dr AC Cefalas, Theoretical and Physical Chemistry Institute, National Hellenic Research Foundation, Athens, Greece, and carried out by visiting researchers from the Institute. They were supported by researchers from the UK from Royal Holloway College, University of London and the Central Laser Facility, Rutherford Appleton Laboratory.

Experimental Results

- Investigated the use of an epoxy novolac chemically amplified photoresist to produce X-ray images of living biological specimens in the water window using laser plasma generated soft X-rays (2.4 - 4.4 nm).
- The photoresist response was at least one order of magnitude “faster” than the standard PMMA (polymethyl methacrylate) previously used in soft X-ray contact microscopy (SXCM).
- Obtained images of biological specimens clearly showed the flagella of the motile green alga, *Chlamydomonas*, suggesting a lateral resolution better than 300 nm, whilst the AFM was capable of discriminating height features of 20 nm in depth profiles.
- Perhaps, more significantly however, the experiment has demonstrated that such photoresists could be used with less intense X-ray sources and could therefore be the basis of development of a small scale soft X-ray microscope using a small commercial laser.

The CLF makes beam time at its facilities available to European Researchers with funding from DG-XII, CEC under the Large-Scale Facilities Access Scheme. For further information contact Dr. Chris Edwards at the CLF. Tel: (0)1235 445582, e-mail: c.b.edwards@rl.ac.uk

Dr Costas Cefalas loads up a sample prior to a laser shot.

The EU researchers with collaborators and RAL support staff.

From left to right
Top row: C Beckwith, D Neely, C Cefalas, P Argitis
Bottom row: J Knott, A Stead

Arising Publications

“Laser plasma X-ray contact microscopy of living specimens using a chemically amplified epoxy resist.”
P Argitis, AC Cefalas, Z Kollia, E Sarantopoulou, TW Ford, AD Stead, A Maranka, CN Danson, J Knott, D Neely.
Submited to Appl.Phys.Letters.

“Single pulse, high resolution X - ray contact microscopy with an advanced epoxy novolac resist.”
A Argitis, AC Cefalas, Z Kollia, E Sarantopoulou, TW Ford and AD Stead.
SPIE proceedings of the 2nd Gr - I international conference on new laser technologies and applications, Ancient Olympia, Greece, 1-4 June 1997.

"Fast, high resolution X - ray contact microscopy with an advanced epoxy novolac resist."
AC Cefalas, P Argitis, Z Kollia, E Sarantopoulou, TW Ford, AD Stead, A Marranca CN Danson, J Knott and D Neely.
Central Laser Facility Annual Report, RAL-TR-97-045.

“Laser plasma X-ray contact microscopy of living specimens using a chemically amplified epoxy resist.”
AC Cefalas, P Argitis, Z Kollia, E Sarantopoulou, TW Ford, AD Stead, A Maranka, CN Danson, J Knott, D Neely.
Rutherford Appleton Laboratory Technical Report, RAL-TR-98-007

"Fast, high resolution X - ray contact microscopy with an advanced epoxy novolac resist."
AC Cefalas, P Argitis, Z Kollia, E Sarantopoulou, TW Ford, AD Stead, A Marranca CN Danson, J Knott and D Neely.
Submitted to Euro-CLEO, Glasgow, Scotland, UK, 13-18 Sept 1998.

Laser Plasma X-Ray Contact Microscopy of Living Specimens Using a Chemically Amplified Epoxy Resist

ABSTRACT

We report on the use of an epoxy novolac chemically amplified photoresist to produce X-ray images of living biological specimens in the water window using laser plasma generated soft X-rays (2.4 - 4.4 nm). The photoresist response was at least one order of magnitude "faster" than the standard PMMA (polymethyl methacrylate) previously used in soft X-ray contact microscopy (SXCM). After chemical development of the exposed resists, atomic force microscopy (AFM) of the relief images obtained of biological specimens clearly showed the flagella of the motile green alga, *Chlamydomonas*, suggesting a lateral resolution better than 300 nm, whilst the AFM was capable of discriminating height features of 20 nm in depth profiles.

INTRODUCTION

Soft X-ray contact microscopy (SXCM) is an interdisciplinary technique that has many applications in both life and material sciences. For life sciences SXCM enables the ultrastructure of living hydrated specimens to be studied without the need of dehydration or other chemical pretreatment of the living specimen by using suitable pulsed X-ray sources such as laser plasmas[1,2]. The interest in using soft X-rays, in the so called "water window" (2.3 - 4.4 nm or 280 - 530 eV), is based on the low attenuation at these wavelengths caused by water as compared to the attenuation caused by organic matter. Indeed, just below the oxygen K edge (2.4 nm), 1 m water has only 20% absorption while the carbon containing proteins have distinctively higher absorbance[3]. Therefore, images with good contrast can be produced by SXCM, provided that a high contrast photoresist is available. Furthermore, the shorter wavelength of the X-ray light, relative to visible light, allows for better resolution than with light microscopy whilst the path length of the 3 - 4.4 nm X-ray photons is 20 times higher than that of the 10 KeV electrons. Thus, unlike electron microscopy, images of specimens up to 10 μm thick can be obtained if their carbon content is low.

To be successful the technique requires the development of sensitive photoresist materials for image recording; these should have optimised contrast, high resolution and an extended greyscale. Up to now the only known photoresist used successfully in SXCM has been polymethyl methacrylate (PMMA). This is a high resolution photoresist with good contrast but it is a relatively slow photoresist and therefore requires a very large fluence of X-rays for imaging. This has limited the range of X-ray sources that can be used, mainly to those of large national facilities.

Laser plasma sources are devices that can efficiently generate X-rays. The intensity and the spectral distribution of the X-ray emission from the plasma depends on many parameters, such as the energy of the laser pulse, its wavelength, its pulse duration, the focusing of the beam on the target and the atomic number of the target. In this experiment we used the Nd:glass laser facility at Rutherford Appleton Laboratory at 1.06 µm to generate soft X-rays in the water window. Using the same geometry and development procedure for all of our photoresist samples, we have compared the performance of PMMA (a positive resist) to two forms of a novel negative, epoxy novolac photoresist, which we call EPR (Epoxy resist)[4].

MATERIAL AND METHODS

The experimental apparatus for producing soft X-ray contact images consisted of the laser source and the vacuum chamber (Target Area 4), where the holder containing the biological samples and the photoresist was placed[5], shown in figure 1. The laser source was the Nd:glass laser of the Rutherford Appleton Laboratory that can deliver 16 J at 1.06 µm. The vacuum chamber was evacuated at a pressure of 10^{-5} mbar and the laser beam was focused with a lens of 40 cm focal length on a planar yttrium foil target (Goodfellow, Cambridge, UK). The X-rays were monitored with a pin photodiode and an X-ray diode array[6]. After initial tests to compare the sensitivity of PMMA and EPR in the absence of either water or a biological specimen cells of the motile green alga, *Chlamydomonas*, were placed in a droplet of medium on the silicon nitride window (120 nm thick; FaSTec, Silverstone, UK). The photoresist was placed onto this and the holder assembled and tightened to ensure that the specimens were in close contact with the photoresists[1].

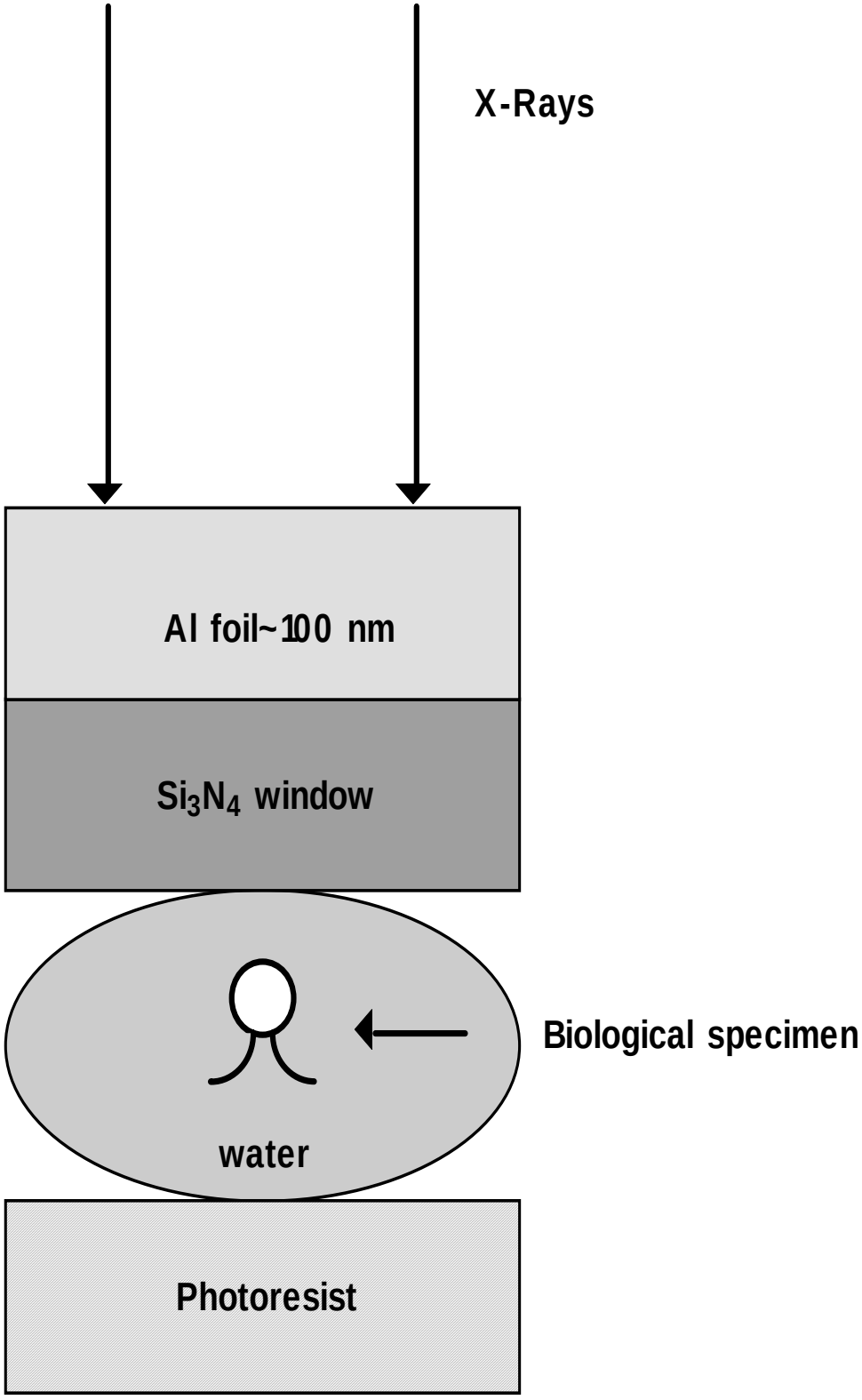

Fig1. A schematic of the experimental apparatus for producing soft X-ray contact images.

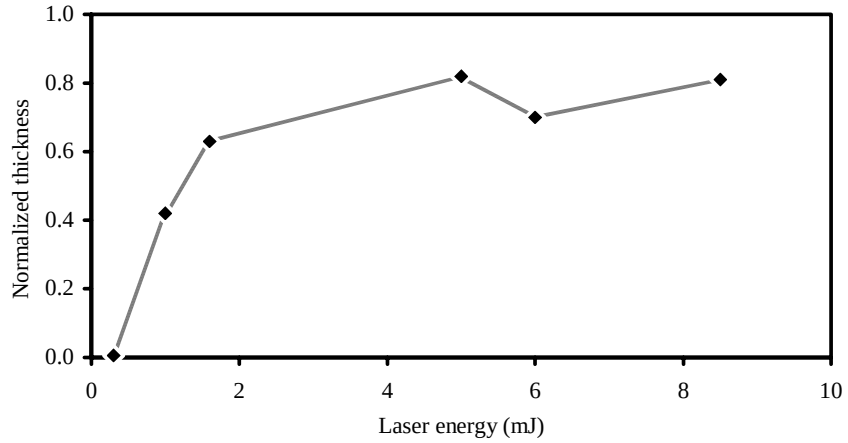

Fig.2 Normalised thickness as a function of the input laser energy for the EPR resist.

The thickness of the water was monitored by light microscopy prior to placing the specimen holder into the vacuum chamber. To filter out the 1 KeV photons a thin (0.1 µm) aluminium film was placed in front of the silicon nitride window. Throughout the experiments the distance between the target and the front surface (i.e. silicon nitride window) was maintained at 15 mm.

A Du Pont Elvacite 2041 (MW 443,000) polymer was used for PMMA resist formulation. 500 nm films were spun from a propylene glycol methyl ether acetate solution and prebaked at 160^{o} C for 60 mins. Methyl isobutyl ketone/ Isopropanol 1:1 was used as developer at developing times from 45 secs to 105 secs. The EPR resist formulation described elsewhere[4)] was used as the standard EPR. The modified epoxy novolac resist version was formulated with a higher MW polymer (MW 3300 vs MW 2250). For both the EPR and modified EPR resists, a 4 min post exposure bake at 90^{o} C was used, and methyl isobutyl ketone was used as developer (1 min) followed by a rinse with isopropanol (45 secs). The developed photoresists were examined with a Burleigh SPM atomic force microscope with a 75 µm scan head.

RESULTS

Initial images taken either without a silicon nitride window or, when a window was present, without a biological specimen, revealed that the negative, epoxy novolac photoresist, which

we call EPR[4)], is at least one order of magnitude more sensitive in getting useful images than PMMA. A fluence of 50 $mJcm^{-2}$ in the water window, was found to be the threshold for a blank image to appear on the PMMA, although such images were very shallow, with just 20 nm maximum depth difference; this agrees with previously published figures[7,8)]. In comparison the threshold for EPR in a similar experiment was only 3 - 5 $mJcm^{-2}$ and fluences such as this were obtained with a laser energy of just 300 mJ (Fig. 2). This value was even lower for a slightly modified epoxy novolac based resist (0.5 $mJcm^{-2}$) which was produced using a related material formulated with a higher MW polymer.

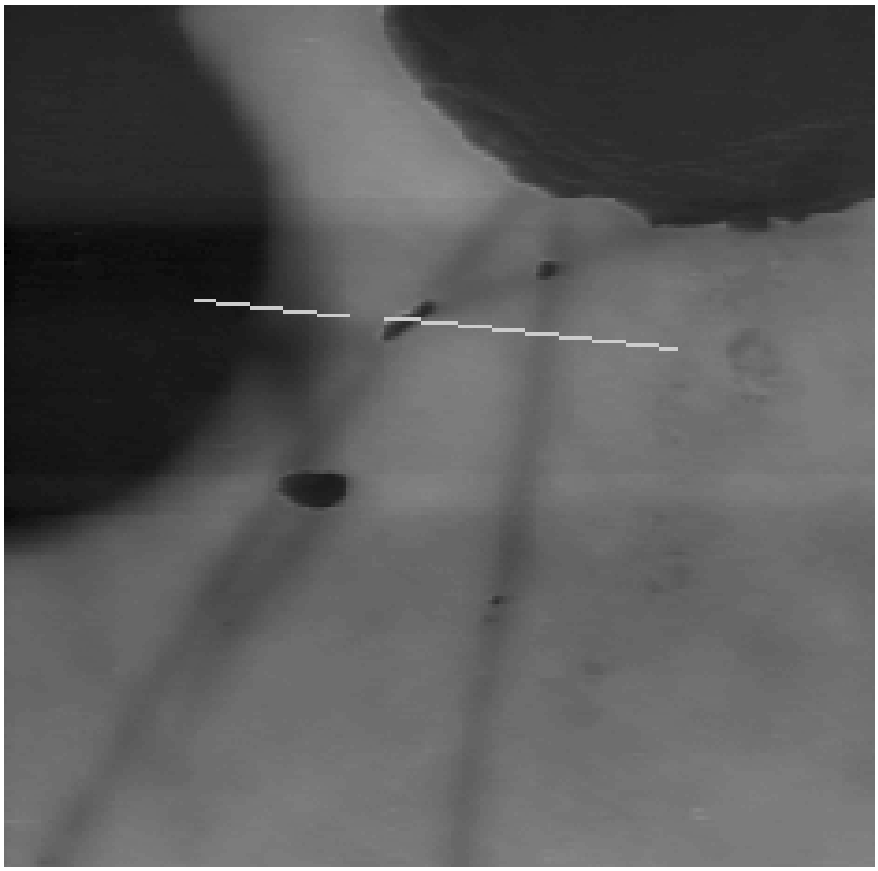

Fig. 3 Image of *Chlamydomonas* using the EPR resist. The laser energy was 10.6 J and the development time was 1 min. The cell body and the flagella are distinct but no structural details are visible within the cell body.

Images of *Chlamydomonas* cells were successfully obtained using the negative epoxy novolac photoresist (Fig. 3), these images clearly show the cell body and the flagella. Using PMMA in conjunction with a similar source to specimen distance, biological imaging was not possible when the specimen was behind an 0.1 µm aluminium filter even at the maximum energy pulse obtained from the laser. This suggests that in previous imaging experiments[9,10)] a

significant contribution to image formation may have been from higher energy photons or from UV which is also filtered out by an aluminium coating[11].

The biological images (Figs. 3,4) recorded with EPR suggest a lateral resolution considerably better than 300 nm as the diameter of flagella are approximately 600 nm but these are clearly distinguishable. For successful biological imaging it is also necessary to be able to distinguish adjacent areas of the specimen which may differ only marginally in their ability to absorb soft X-rays, this is in effect the contrast characteristics of the photoresist. The present images, unlike previous studies using PMMA[12,13], show no ability to discriminate structures within the cell, that is to say the absorption by the cell body was sufficient to result in the complete dissolution of the photoresist when it was developed. However, the response of the photoresist was able to differentiate between a single flagellum and areas where two flagella overlapped (Fig. 5). In this case the photoresist area corresponding to the overlapping regions was c.80 nm deep, whereas images of single flagella were only c.20 nm. Since the depth of the images of the cell body were c.100 nm deep it is concluded that two flagella also absorbed sufficient X-rays to produce a maximum response.

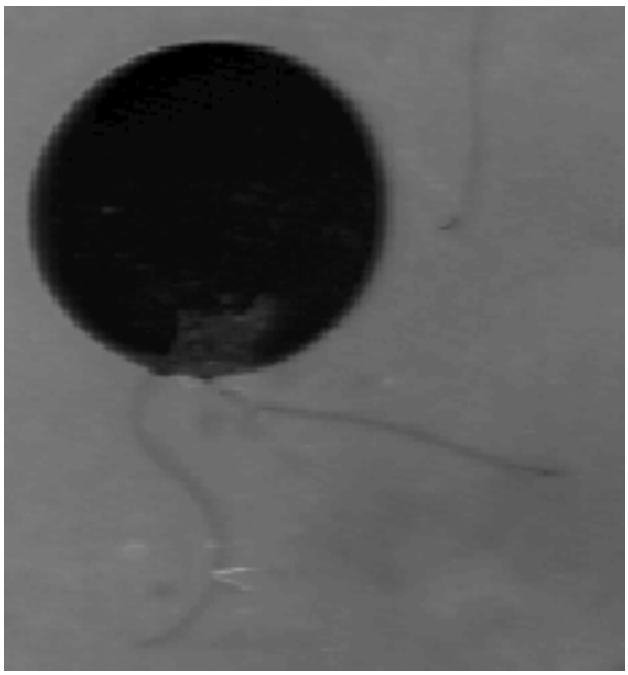

Fig. 4 Image of *Chlamydomonas* using the EPR resist. The laser energy was 10.6 J and the development time was 1 min.

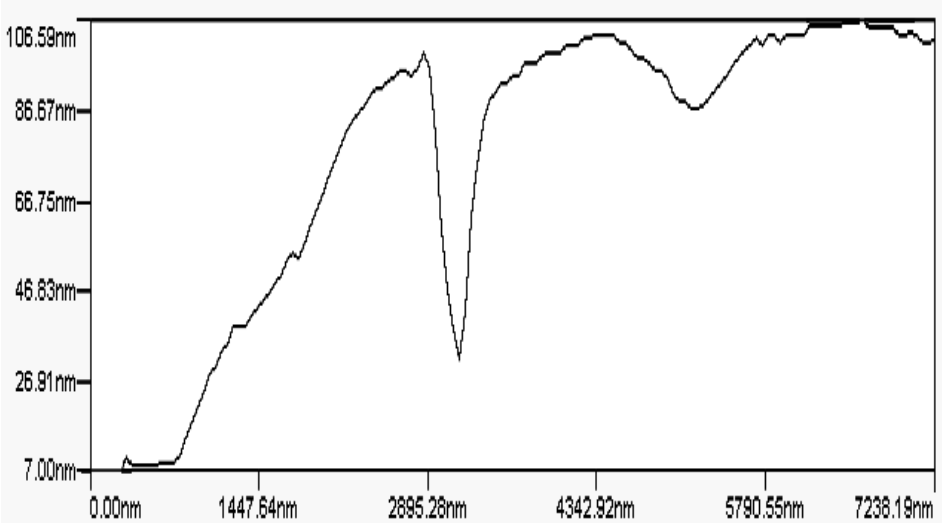

Fig. 5 The depth of the area corresponding to single flagella is approximately 20 nm deep (open arrow) whereas those of overlapping flagella are 80 nm.

In conclusion we have therefore demonstrated that the novel photoresist used in this study are significantly more sensitive than the traditionally used PMMA and, whilst their range of sensitivity may not be as great as PMMA, such materials can be used in imaging experiments. Perhaps, more significantly however, is that such photoresists could be used with less intense X-ray sources and could therefore be the basis of development of a small scale soft X-ray microscope using a small commercial laser.

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