Journal of Instrumentation

SISSA

OPEN ACCESS

X-ray imaging of bio/medical samples using laser-plasma-based X-ray sources and LiF detector

To cite this article: F. Flora *et al* 2019 *JINST* **14** C10006

Published by IOP Publishing for Sissa Medialab

Received: *June 21, 2019*
Accepted: *August 8, 2019*
Published: *October 3, 2019*

3rd European Conference on Plasma Diagnostics (ECPD2019)
6–10 May 2019
Lisbon, Portugal

X-ray imaging of bio/medical samples using laser-plasma-based X-ray sources and LiF detector

F. Flora,[a,1] S. Almaviva,[a] G. Baldacchini,[a] D. Batani,[f] S. Bollanti,[a] F. Bonfigli,[a] P. Di Lazzaro,[a] M. Francucci,[a] P. Gaudio,[e] A. Lai,[a] T. Letardi,[a] L. Mezi,[a] R.M. Montereali,[a] D. Murra,[a] E. Nichelatti,[a,h] A. Poma,[d] A. Scafati,[d] L. Palladino,[d] T. Pikuz,[b,i] A. Reale,[d] L. Reale,[c] M. Richetta,[e] A. Ritucci,[d] A. Rydzy,[a] M.A. Vincenti[a] and P. Zuppella[g]

[a] *ENEA, Frascati Research Center, Via E. Fermi 45, 00044 Frascati, Italy*
[b] *Osaka University, 2–6 Yamadaoka, Suita, Osaka, 565-0871, Japan*
[c] *Campus Bio-Medico University, Via Álvaro del Portillo 200, 00128 Roma, Italy*
[d] *University of L'Aquila, Coppito, 67010 L'Aquila, Italy*
[e] *Department of Industrial Engineering, University of Rome Tor Vergata, Via del Politecnico 1, 00133 Rome, Italy*
[f] *Université Bordeaux, CNRS, CEA, CELIA, UMR 5107, F-33405 Talence, France*
[g] *National Research Council — Insitute for Photonics and Nanotechnology, Via Trasea 7, 35128 Padova, Italy*
[h] *ENEA, Casaccia Research Center, Via Anguillarese 301, 00123 S. Maria di Galeria, Rome, Italy*
[i] *JIHT RAS, 13-2 Izhorskaya st., Moscow, 125412, Russia*

E-mail: francesco.flora@enea.it

[1] Corresponding author.

https://doi.org/10.1088/1748-0221/14/10/C10006

Abstract: This contribution to ECPD2019 is dedicated to the memory of Anatoly Faenov. During a period of approximately thirteen years 1994–2006, Anatoly and his wife Tatiana Pikuz (simply "Tania" for friends), accepting the frequent invitations of the National Institute for Nuclear Physics (INFN) and of the Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), cooperated with many Italian research laboratories dedicated to EUV and soft X-ray generation, spread in different towns (L'Aquila, Frascati, Milano, Padova, Pisa, Roma, etc.). In spite of the fact that they could stay in Italy only about one or two months per year, their activity was so intense that more than 50 peer- reviewed publications were generated from their experimental and theoretical work (just considering only the results obtained at L'Aquila and Tor Vergata — Rome Universities and at the ENEA Research Center of Frascati), without mentioning the cultural atmosphere that they stimulated in the field of Science and Humanity.

The numerous experimental spectra obtained at ENEA by means of their spherically bent mica spectrometers, together with the corresponding theoretical simulations performed in Moscow, allowed to study the changing role of different excitations mechanisms for various plasma conditions, and to characterize at best the ENEA laser-plasma source for different applications: polychromatic and monochromatic micro-radiography of dried biological samples at 1 keV, soft X-ray contact microscopy (SXCM) of living cells in the water-window spectral region, spectroscopy of hollow atoms, etc.

In this memorial paper, the main results of biological samples imaging on lithium fluoride (LiF) detectors, obtained with the ENEA and Tor Vergata University laser-plasma sources, are presented. In particular, the improvement of the micro-radiography and of the SXCM techniques obtained after moving from photoresist detectors and photographic films to lithium fluoride (LiF) detectors are discussed, for both dried and wet biological samples.

Keywords: X-ray detectors; Plasma diagnostics - interferometry, spectroscopy and imaging; Plasma generation (laser-produced, RF, x ray-produced)

2019 JINST 14 C10006

Contents

2019 JINST 14 C10006

1 The ENEA source and its spectra

Most of the results which will be presented, were obtained on a laser-plasma source pumped by a XeCl excimer laser called "Hercules" (λ = 308 nm), designed and realized at ENEA. With its pulse energy of ~ 5 J and pulse duration of 120 ns, this UV laser can warm up solid tape targets reaching a laser peak intensity of 5×10^{12} W/cm^2 at best focusing.

The source was developed in the frame of a cooperation between ENEA and L'Aquila University. As shown in figure 1, most of the laser energy is converted in the Extreme Ultraviolet (EUV) spectral region reaching an EUV pulse energy emission of 0.5 J (or up to 1 J by defocusing the laser down to 5*10^{10} W/cm^2), while the emissions in the water-window (W.W.) and in the 1–1.5 keV region have a pulse energy of 6 mJ and 1 mJ, respectively [1].

Just three months after the first laser-plasma shot (February 1994), Anatoly and Tania came to ENEA for the first time, invited by Prof. Armando Reale of L'Aquila University.

In that occasion, they could stay just two weeks in ENEA; but, in spite of the short time, using their spectrometers based on spherically bent mica crystals and comparing the copper spectrum with the simulations performed at the VNIIFTRI institute of Moscow, it was possible to determine the plasma density and temperature [2]: 10^{22} cm^{-3} and 120 eV, respectively.

After the first measurements in 1994, the cooperation with Anatoly and Tania never finished. From 1994 till 2006 (when they were invited to join the Japan Atomic Energy Agency) they came to ENEA for 1 month/year. Different funding sources were exploited for that purpose: ENEA internal funds for cooperation, L'Aquila University funds, funds from the Italian-Russian cooperation agreement.

The efficient combination between the experimental results obtained by Anatoly and Tania at ENEA on X-ray spectroscopy and the spectra simulations performed by their colleagues of the VNIIFTRI institute, Lawrence Livermore National Laboratory, Los Alamos National Laboratory, and Darmstadt Technical University, allowed:

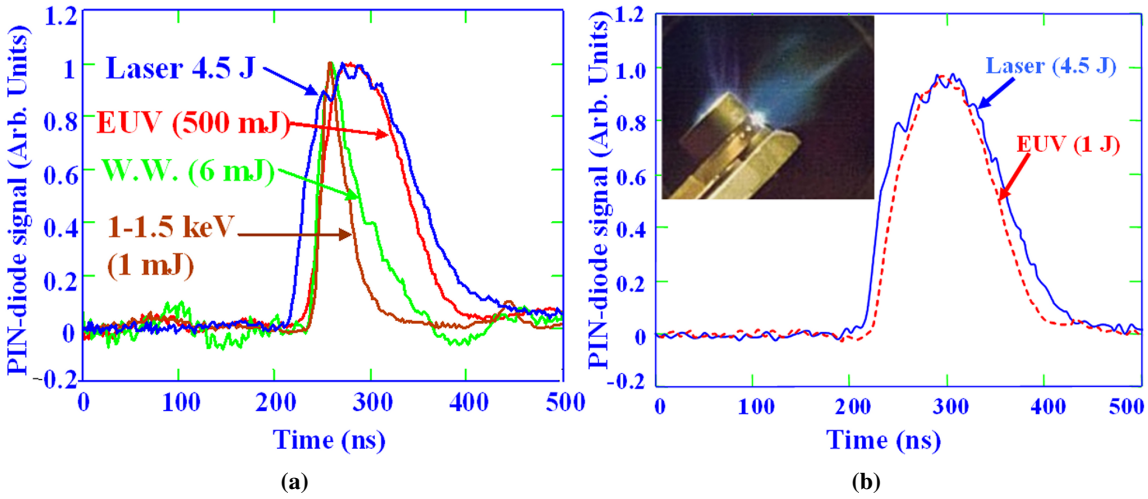

Figure 1. Temporal evolution of the Hercules laser pulse and of the plasma emission in different spectral regions when a solid copper target (100 μm thick) is placed at best focusing (a) or 2 mm behind the focal plane (b), reaching a laser peak intensity of 5×10^{12} W/cm^2 and 5×10^{10} W/cm^2, respectively [1]. No signal is observed in the W.W. and 1 keV ranges when the target is 2 mm out of focus. A picture of the plasma source (time integrated) is inserted.

- to study the changing role of different excitations mechanisms for various plasma conditions
- to characterize at best the ENEA laser-plasma source for different applications:
  - polychromatic and monochromatic micro-radiography of biological samples at 1 keV,
  - Soft X-ray Contact Microscopy (SXCM) of living cells in the water-window region,
  - studies of DNA exposure to soft X-rays,
  - spectroscopy of multiply charged hollow ions, inner-shell satellites transitions, etc.

As an example of the numerous spectra obtained in that period, the magnesium one is shown in figure 2. In this case the spherically bent crystal was aligned in such a way to produce a 1D

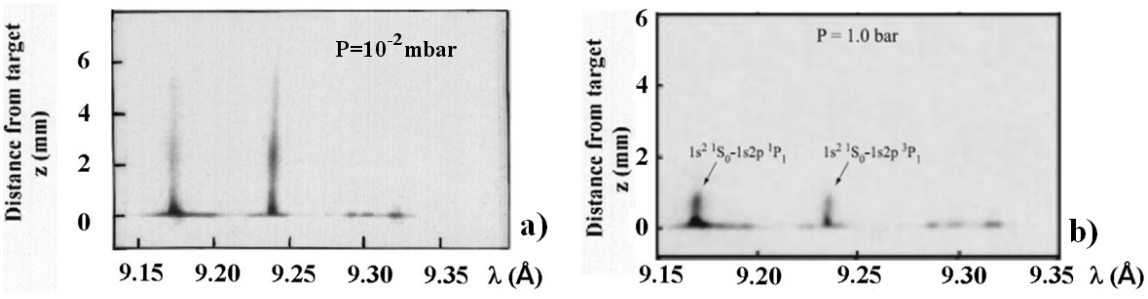

Figure 2. Spectra of a 175 μm thick Mg tape target in the 1 keV region [1, 3], obtained with the Hercules laser-plasma source for vacuum conditions (a) and for atmospheric pressure helium environment (b). In the last case the emission length is shorter and somewhere stronger (see the $1s^2{}^1S_0$-$1s2p^1P_1$ line darkness at 0.8 mm), with kind permission of Società Italiana di Fisica.

2019 JINST 14 C10006

imaging of the source in the direction perpendicular to the Rowland circle (i.e. to the spectrum direction) [1, 3]. It is interesting to observe that the emission column of the transitions $1s^2\,^1S_0$-$1s2p\,^1P_1$ and $1s^2\,^1S_0$-$1s2p\,^3P_1$, is very long (up to 6 mm from the target) for the vacuum case while they becomes shorter and, in some cases, more intense for the He environment case (see, in particular, figure 12b in ref. [1]).

2 Micro-radiography on photographic films

The following micro-radiographies of insects were performed in the 1–1.5 keV spectral region using commercial photographic films as imaging detector.

2.1 Polychromatic micro-radiography

Our first experiment of polychromatic microradiography was done in 1998 using a copper tape as target material [1]. The insect was in air environment at atmospheric pressure (so that it could be alive), separated from atmospheric pressure helium by two thin mylar foils, as shown in figure 3a.

The spectral region below 1 keV was cut by the helium gas, by the two mylar foils and by a 1.5-μm thick aluminum filter (used to protect the DEF photographic film from visible light).

To enhance (by one order of magnitude) the source emission in the 1–1.5 keV region, a 10-ns Lambda-Physik laser pulse was injected and amplified in the Hercules laser. A single laser shot was sufficient for a proper exposure of the DEF film, so that even alive samples could be imaged without motion blur issues.

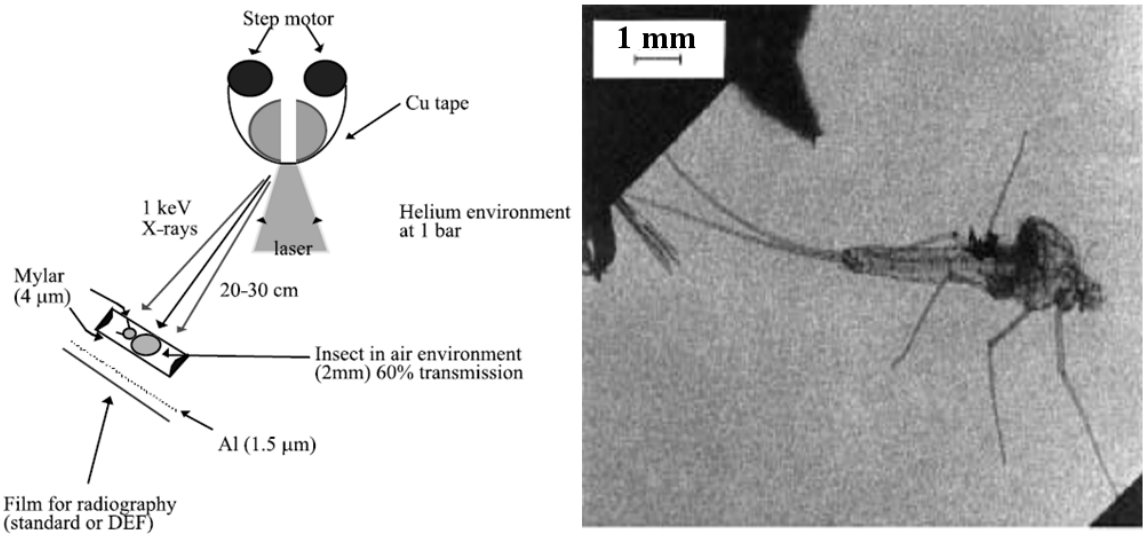

Figure 3. Polychromatic microradiography of a libelluloidea in the 1–1.5 keV spectral region: experimental set-up (on the left) and imaging results (on the right) [1], with kind permission of Società Italiana di Fisica.

2.2 Monochromatic micro-radiography

Two years later, in 2000, following a proposal of Anatoly Faenov and Tania Pikuz, we obtained our first monochromatic microradiography at ENEA. For this experiment, the spherical mica crystal

2019 JINST 14 C10006

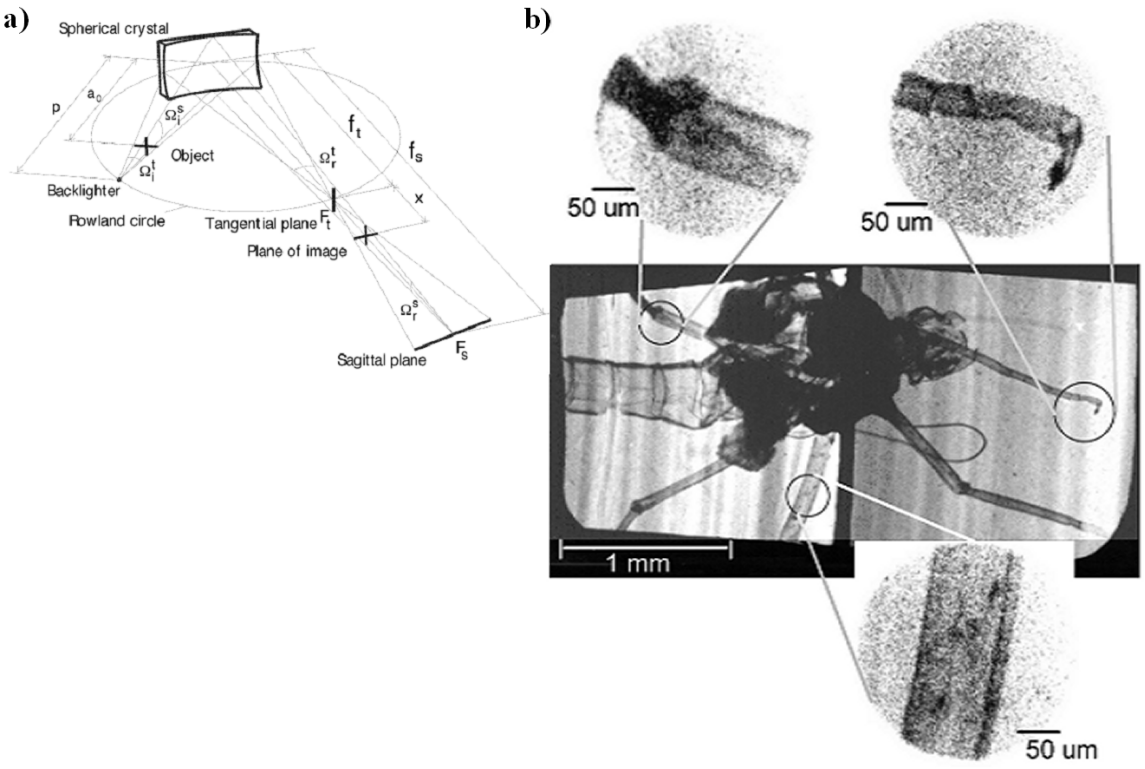

Figure 4. Monochromatic micro-radiography of a libelluloidea in the 1 keV spectral region obtained by shadow monochromatic backlighting: experimental set-up (a) and imaging results (b) [5]. Reprinted with permission. Authors acknowledge *Laser Part. Beams* journal.

was used for a double purpose: to monochromatize the spectrum of the plasma radiation in the 1 keV region and to project a 2D image of a biological sample placed between the Rowland circle and the crystal, as shown in figure 4.

The alignment geometry is called "shadow monochromatic backlighting scheme" [4, 5] — a modification of the traditional monochromatic X-ray backlighting approach — which gives important advantages: (a) the scheme works down to very low Bragg angles (35° or even less), thus allowing to choose wavelengths values far away from the crystal 2D spacing structure, and (b) the depth of focus is much larger.

In the shadow monochromatic backlighting scheme, the plasma source is placed very close to the Rowland circle in order to get almost monochromatic radiation. In our case [5] we selected hν ~0.9 keV, where the Ne-like ions of the nickel target have many dense emission lines (which can be recognized as background behind the insect in figure 4).

Many tiny details can be recognized in the libelluloidea radiography; the monochromaticity of the radiation enhances the contrast because of the phase-contrast effect.

3 Contact X-ray microscopy in the water-window

As it is well known, Soft X-Ray Contact Microscopy (SXCM), if compared with projection microscopy based on Fresnel zone-plate lenses, allows for a modest resolution, around 50–100 nm

2019 JINST 14 C10006

(limited by many effects such as penumbra blurring, X-ray diffraction, etc. [6]), but a much wider field of view (mm^2) can be reached and the polychromatic X-ray dose can be released in a single shot by table-top laser-plasma sources, so that the biological samples don't need any treatment at all.

3.1 SXCM on photoresists

Our preliminary SXCM experiments at ENEA were done in 1994 using small Si wafers ($5 \times 5\,mm^2$) coated by PMMA photoresist as imaging detectors. Our first imaged biological samples were green algae, like chlamydomonas [6, 7] and leptolyngbya [7].

Photoresists, being developed for microelectronic purposes, have a very good spatial resolution ($< 10\,nm$), but they present a rather poor dynamic range (low number of grey levels), so that the image easily saturates and the imaging result strongly depends on the resist developing conditions. For example, in the leptolyngbya case, sometime the images show only the external shape of the cells, as shown in figure 5a, and the envelope containing the cells (see figure 6a of ref. [7]), while sometime it is possible to see the internal structure, as shown in figure 5b. We realized that a new type of high resolution detector was needed for SXCM.

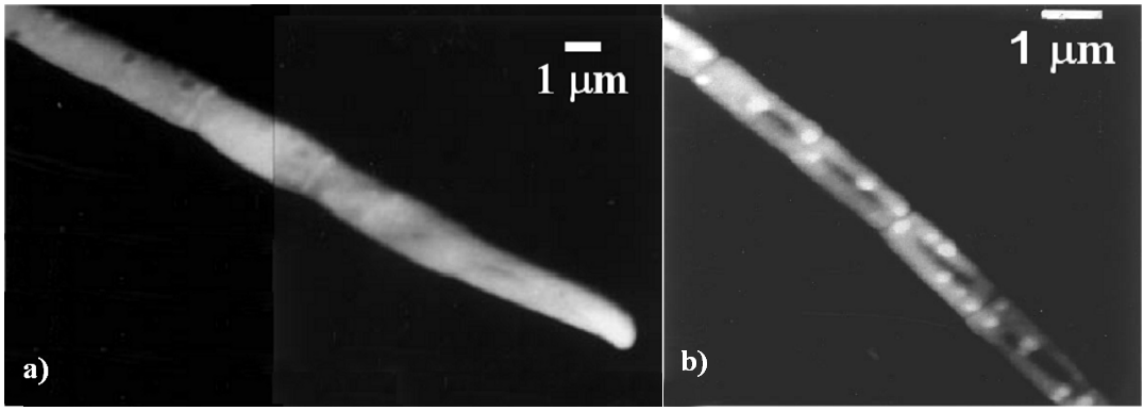

Figure 5. Contact X-ray microscopy of leptolyngbya green algae on PMMA photoresist after a short development (a) and after an additional longer development (b): only in the second case the cells internal structure appears [7]. Authors acknowledge *J. of Microscopy* journal.

3.2 LiF as new high resolution imaging detector

One year later, in 2001, the excimer laser group and the nanophotonics group of ENEA combined their expertise, on soft X-ray generation and on LiF as photonic material, respectively, in order to try, together with Anatoly and Tania, the generation of luminescent patterns on a LiF crystal by irradiation with 1 keV photons [8]. Ionizing radiation can indeed generate point defects in the LiF crystal lattice, called "color centers", which change the optical properties of the crystal. In particular, the F_2 and F_3^+ color centers (a group of two and three, respectively, anion vacancies filled by two electrons) can absorb blue light at 450 nm and emit red (F_2) and green (F_3^+) light by fluorescence, as shown in [9]. Hence, assuming a proportionality between the concentration of color centers and the dose of X-rays that generated them, the observation of the fluorescence map provided by the crystal after being exposed to soft X-rays gives an indication of X-ray dose

2019 JINST 14 C10006

spatial distribution. The response of LiF covers orders of magnitude of X-ray dose, so that the corresponding dynamic range is as wide as that of photographic films or even wider [9].

As a first experiment of generation of color centers in a LiF crystal by 1 keV radiation, we exposed the crystal to the radiation generated by the Hercules laser-plasma, equipped with a Cu tape target, at 10 cm from the source [8], as shown in figure 6a. A copper mesh was placed in contact with the crystal in order to get a pattern.

After the exposure, the crystal was observed at an optical microscope in fluorescence mode, exciting the F_2 and F_3^+ color centers with blue light and observing the fluorescent image through a yellow filter to cut the reflections/diffusions of the pumping blue light and transmit the green and red fluorescence of the F_3^+ and F_2 centers.

As it can be seen in figure 6, the LiF crystal presented a strong yellow (red+green) luminescence in the whole exposed region, the shadow of the copper mesh was clearly visible and the spatial resolution (see zooming in figure 6c) of the generated patterns was below 400 nm (the estimation of the patterns resolution was limited by that of the optical microscope used for the LiF reading, which was 400 nm). This demonstration of the high resolution imaging capability of LiF using soft X-ray, done with Anatoly Faenov, has been the first ever.

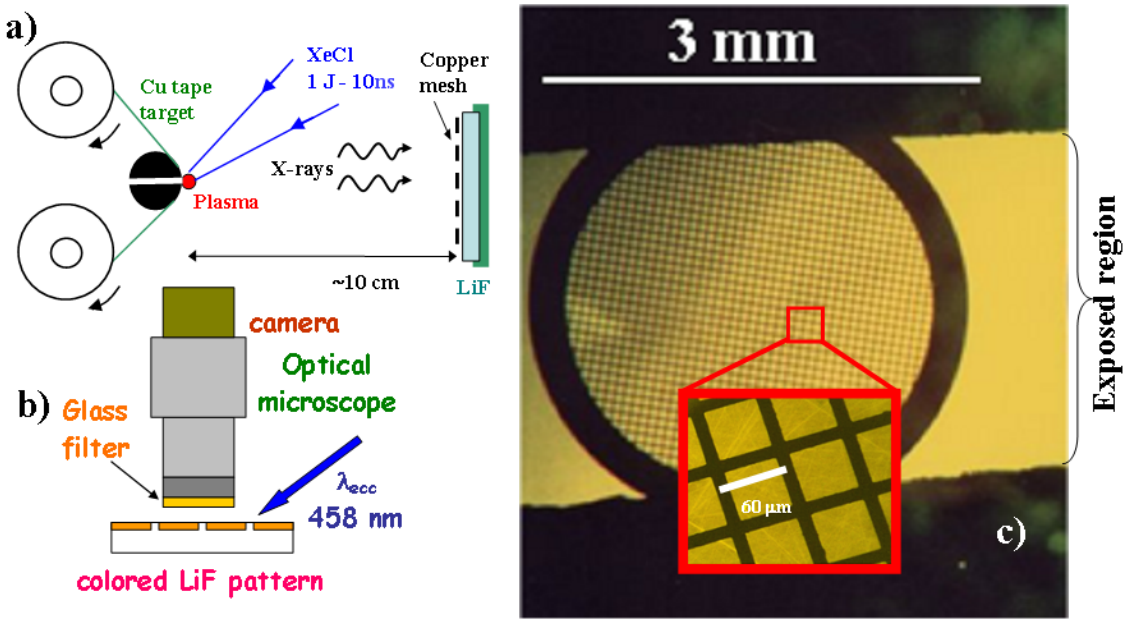

Figure 6. First experiment of luminescent patterns generation on a LiF crystal by 1–1.5 keV X-rays: experimental setup for X-ray exposure (a), reading procedure in fluorescence mode after the exposure (b), and result of the crystal reading (c). A 60 µm bar is inserted for scale reference. Reproduced from ref. [8], with the permission of AIP Publishing.

Right after the good results obtained for mesh replication in a LiF crystal, we repeated the experiment replacing the copper mesh with dry biological samples, such as the wing of a dragonfly or of a mosquito. The wide dynamic range of LiF and the sub-micron resolution of these first micro-radiographies were definitively confirmed [9, 10], as shown in figure 7.

We also successfully proved (see figure 8) the use of LiF thin films, deposited by thermal evaporation on a glass substrate, as high resolution detectors for X-ray laser beams characterization [9].

2019 JINST 14 C10006

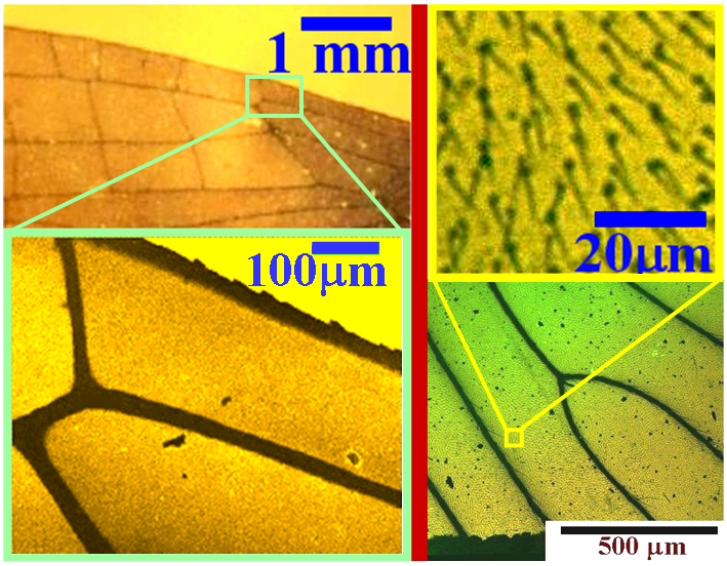

Figure 7. First ever microradiography on LiF thin film of a dragonfly (left) and of a mosquito (right) wings observed with a conventional and with a confocal microscope, respectively. [10].

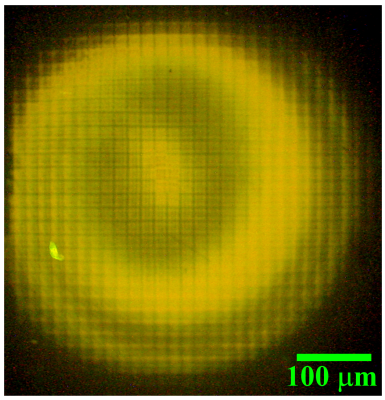

Figure 8. Fluorescent spot on a LiF thin film, of thickness 200 nm, obtained by irradiation with a capillary discharge X-ray laser based on Ne-like Ar ions (λ = 46.9 nm). The beam is partly focused by a multilayer concave mirror. A 360-µm period nickel mesh, placed just before the mirror, generates interference patterns on the LiF detector [9].

3.3 SXCM on LiF

Few years later, in 2006, we were ready for the SXCM experiments using LiF crystals as high spatial resolution imaging detector. In order to protect the crystals from erosion due to the water solution containing the biological specimens, we coated them with a 50 nm thick Si_3N_4 layer. The exposure experiments were performed at the Engineering Dept. of Tor Vergata University of Rome, where a laser-plasma source based on a 10 J – 10 ns Nd-YAG laser provided a larger X-ray fluence in the water-window with respect to the ENEA one.

High resolution SXCM images of alive green algae (Chlamydomonas, Leptolyngbya and Chlorella) by using LiF crystals as imaging detectors were obtained for a single shot of the plasma source [11, 12] as shown in figures 9 and 10.

2019 JINST 14 C10006

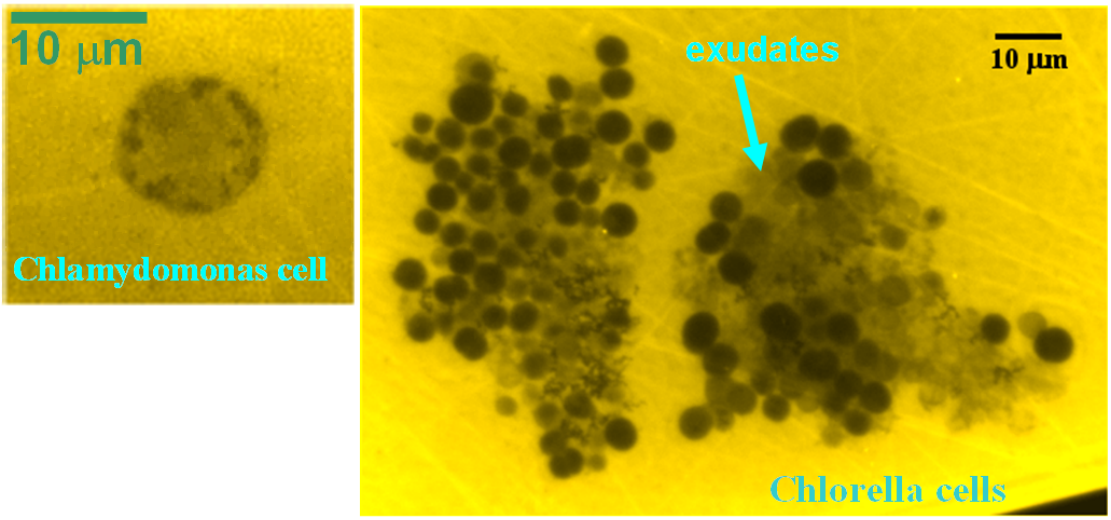

Figure 9. First ever SXCM images on LiF of Chlamydomonas (left) and Chlorella cells (right) observed with a conventional and with a confocal microscope, respectively. Beside the shadow of the chlorella cells (dark bowls) the exudates are well visible [11].

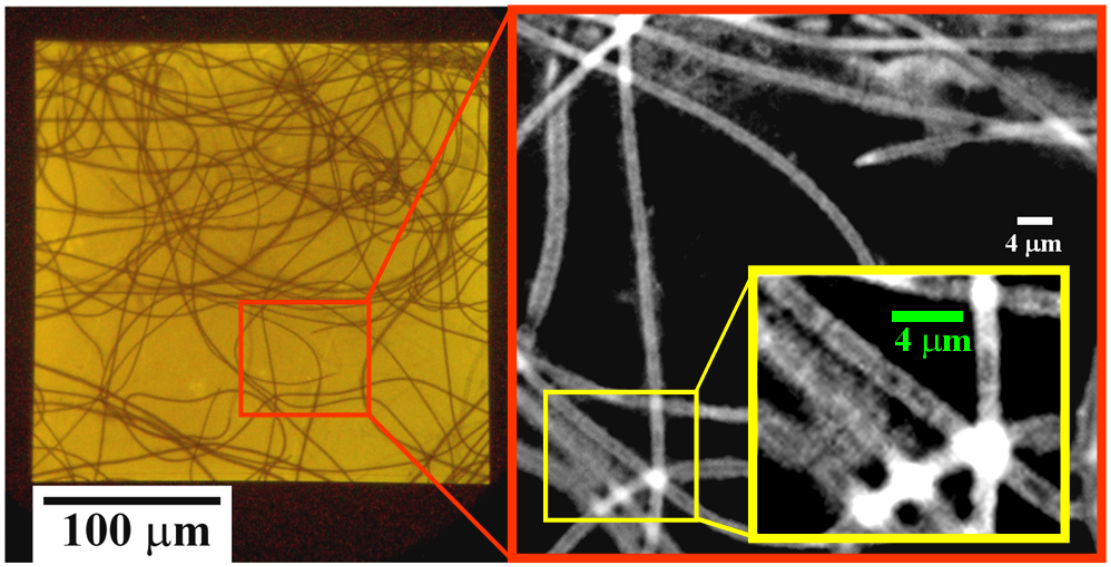

Figure 10. First ever SXCM images on LiF of Leptolyngbya filaments: the fluorescence image corresponding to the full size ($250 \times 250\,\mu m^2$) of the Si_3N_4 window (left) and a zooming of a detail in the negative image (right): the cells of the Leptolyngbya chain can easily be recognized [12]. Authors acknowledge *J. of Microscopy* journal.

In particular, for the Chlorella cells case, the cells exudates, extremely difficult to be seen in the visible range because of the poor absorption contrast, could be observed very clearly. The resolution of these images, as for the mesh case, is approximately 400 nm, mainly limited by the resolution of the optical microscope used for the LiF reading process (see figure 6b).

The present limit in the LiF imaging spatial resolution, due to the optical microscope used for the fluorescence reading and to the light wavelength diffraction limit, might be overcome by a non

2019 JINST 14 C10006

optical reading. Accidentally, in fact, while sealing a colored LiF films (the one shown in figure 7a) inside a glass box with Attack glue, we noticed that a layer of glue vapors got deposited on the LiF film in a non uniform way, following the shape of the dragonfly wing image. . . Work is still in progress.

4 Conclusions

In spite of the fact that Anatoly (see figure 11) and Tania could stay in Italy only for one month per year, during the 25 years of cooperation with them an amazing amount of scientific results was obtained at the ENEA, L'Aquila and Tor Vergata Universities, published in more than 100 publications (50% of them in peer reviewed journals).

Their high intellectual value has been always accompanied by a big pleasant friendship and enrichment for all who have been so lucky to work with them. The ENEA, L'Aquila University and Tor Vergata University teams will never forget Anatoly, his smart brain and his rich soul.

Figure 11. Anatoly Faenov.

Acknowledgments

Authors emphasize the fundamental scientific and economic contribution of INFN for the cooperation with Anatoly and Tania. Authors thank Prof. Giuseppe Tomassetti (†) for his fundamental contribution to the experiments of X-ray laser spot imaging on LiF and Dr. Alberto Renieri (†) for his important support while he was the Director of the New Technologies Dept. of ENEA-Frascati Research Center.

Authors thank Dr. Moreno Vaselli, the former scientific attaché of the Italian Embassy in Moscow, for his support to the scientific cooperation between ENEA and the VNIIFTRI Institute of Moscow in the frame of the Italian-Russian Cooperation Agreement.

2019 JINST 14 C10006

References

[1] S. Bollanti, P. Albertano, M. Belli, P. Di Lazzaro, A.Ya. Faenov et al., *Soft X-ray plasma source for atmospheric pressure microscopy, radiobiology and other applications*, *Nuovo Cim.* **D 20** (1998) 1685.

[2] S. Bollanti, P. D. Lazzaro, F. Flora, T. Letardi, L. Palladino, A. Reale et al., *Na-like autoionization states of copper ions in plasma, heated by excimer laser*, *Phys. Scripta* **51** (1995) 326.

[3] A. Stepanov, A. Starostin, V. Roerich, V. Makhrov, A. Faenov, A. Magunov et al., *Modeling of the He-like magnesium spectral lines radiation from the plasma created by XeCl and Nd-glass lasers*, *J. Quant. Spectr. Radiat. Transfer* **58** (1997) 937.

[4] M. Sanchez del Rio, M. Fraenkel, A. Zigler, A.Ya. Faenov and T.A. Pikuz, *Collimation of plasma-produced X-rays by spherical crystals: Ray-tracing simulations and experimental results*, *Rev. Sci. Instrum.* **70** (1999) 1614.

[5] T. Pikuz, A.Y. Faenov, M. Fraenkel, A. Zigler, F. Flora, S. Bollanti et al., *Shadow monochromatic backlighting: Large-field high resolution X-ray shadowgraphy with improved spectral tunability*, *Laser Part. Beams* **19** (2001) 285.

[6] S. Bollanti et al., *Long-duration soft X-ray pulses by XeCl laser driven plasmas and applications*, *J. X-Ray Sci. Technol.* **5** (1995) 261.

[7] P. Albertano, L. Reale, L. Palladino, A. Reale, R. Cotton, S. Bollanti et al., *X-ray contact microscopy using an excimer laser plasma source with different target materials and laser pulse durations*, *J. Microsc.* **187** (1997) 96.

[8] G. Baldacchini, F. Bonfigli, F. Flora, R. M. Montereali, D. Murra, E. Nichelatti et al., *High-contrast photoluminescent patterns in lithium fluoride crystals produced by soft X-rays from a laser-plasma source*, *Appl. Phys. Lett.* **80** (2002) 4810.

[9] G. Baldacchini, S. Bollanti, F. Bonfigli, P. Di Lazzaro, A. Faenov, F. Flora et al., *Point defects in lithium fluoride by EUV and soft X-rays exposure for X-ray microscopy and optical applications*, *IEEE J. Select. Topics Quantum Electron.* **10** (2004) 1435.

[10] G. Baldacchini, F. Bonfigli, A. Faenov, F. Flora, R.M. Montereali, A. Pace et al., *Lithium fluoride as a novel X-ray image detector for biological* μ*-world capture*, *J. Nanosci. Nanotechnol.* **3** (2003) 483.

[11] F. Bonfigli, A. Faenov, F. Flora, M. Francucci, P. Gaudio, A. Lai et al., *High-resolution water window X-ray imaging of in vivo cells and their products using LiF crystal detectors*, *Microsc. Res. Techn.* **71** (2007) 35.

[12] L. Reale, F. Bonfigli, A. Lai, F. Flora, P. Albertano, M.L. Di Giorgio et al., *Contact X-ray microscopy of living cells by using LiF crystal as imaging detector*, *J. Microsc.* **258** (2015) 127.

2019 JINST 14 C10006