Soft X-ray microscopy on a Lithium Fluoride-based novel imaging detector

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A proposal is presented for exploiting the peculiarities of soft X-ray radiation produced by SPARX in the field of biological investigation by using single-shot contact microscopy and holography [1, 2] on an innovative imaging detector.

Extreme ultraviolet (EUV) radiation and soft X-rays (XUV) are currently used by physicists and biologists to obtain images of living biological samples at a spatial resolution lower than 100 nm, which is an intermediate resolution between optical microscope and electron transmission microscopes (TEM). As compared with these traditional types of microscopes, the X-ray microscopy has the potential to allow a better resolution (due to the smaller wavelength) while still keeping the sample alive. This is not possible for TEM, since the samples require to be stained, dehydrated and sectioned into thin (100 nm) slabs. Another important difference between optical and X-ray μ-radiography (or X-ray microscopy) is obviously given by the different way in which the observed matter and the probing radiation interact with each other. In the visible spectral range, photons are absorbed from the external electrons of atoms, so that chemical bonds mainly influence the absorption coefficient; on the contrary, X-rays are absorbed from the core electrons, so that the absorption coefficient is independent of the chemical bonds. The (2.2-4.4) nm wavelength interval (called water-window) is generally used [3,4], because of the strong absorption of carbon (as compared with that of water) which allows to obtain a natural contrast in cells imaging. The carbon window region (4.4-5 nm) is another attractive spectral range for X-ray images[5]. This spectral interval is characterized by a largest radiation-penetration depth into carbon-containing compounds and the difference in the absorption coefficients of cellular structures and substances in the carbon window is 5-6 times larger than in the water window. This means that it is possible to study much thicker sections of fixed and dried biological object placed also in paraffin. The large difference in the absorption coefficients of various element produces a high differential contrast of different compounds.

Actually, intense soft X-ray sources for microscopy are synchrotrons, laser plasma sources and micropinch discharge sources. X-rays from laser plasma sources are not monochromatic and are not coherent so they can be used only for contact microscopy. The sample is located in tight contact with a sensitive material and it is illuminated by the X-ray beam. Single-shot experiments are possible in this experimental configuration, but the spatial resolution of the images obtained by contact microscopy is limited by diffraction effects, penumbra blurring effects and resolution of the detector. The penumbra blurring, δ_s, is related to the diameter Φ of the X-rays source, to the gap G between mask and sample and to the distance d between sample and

X-rays source by the formula: $\delta_s = \frac{\Phi \cdot G}{d}$. On the other hand, the diffraction blurring, δ_r, due to the wave nature of the incident radiation, of wavelength λ, approximately equals $\sqrt{\lambda \cdot G}$. In contact microscopy the spatial resolution of the recorded images is also limited by the detector. Due to their high spatial resolution, polymeric photoresists (PMMA and similar materials) are the most commonly used detectors in contact microscopy. The image is formed through photoabsorption process: the recorded pattern is the relief map on the photoresist of the variation in the X-ray photoabsorption coefficient of the biological sample. After a chemical development, the PMMA is thinner in the more transparent regions. The recorded pattern can then be read by an atomic force microscope.

With the advent of synchrotron sources, more sophisticated microscopy configurations based on projection methods (Full Field and Scanning Microscopy) have been exploited. The projection microscopy configurations need optics (Fresnel Zone Plates FZP) to focus radiation on the sample and to project a magnified image of the object onto detectors which can possess low spatial resolution, like CCD camera or photographic plates. The use of FZP optics requires monochromatic radiation, with a consequent reduction of the photon flux on the samples. This means long exposure times and need of cooling the biological specimens at cryogenic temperature, making impossible dynamic studies and in-vivo observations. At synchrotron sources, X-ray holography experiments to obtain 3D-informations of the observed objects have been also performed. Holographic techniques need a spatial filtering of the synchrotron radiation to select the coherent part of the beam with a consequent loss of intensity and long exposure times.

Coherence, monochromaticity and high brillance of a X-ray free electron laser (FEL) as SPARX will overcome the limitations of the actual soft X-rays sources and will allow to obtain images of biological samples in single-shot experiments both in contact and in holographic configuration with very high spatial resolution. Due to the short duration (~ 100 fs) of the X-FEL pulse, it could be possible to study living biological specimens by recording images in a very short exposure time, before radiation damage occurs.

The spatial resolution and the dynamic range of the selected detector plays a fundamental role in all x-ray microscopy configurations. For the contact X-ray microscopy two types of detectors have mainly been used so far: photographic plates and polymeric photoresists. Photographic films have a large dynamic range (8-10 bits), but with a resolution limited to a few microns by the grain sizes of the emulsion itself. Photoresists allow a very good spatial resolution (the polymer molecule size is just 10 nm), but the dynamic range is very poor (5-6 bits), because of a limited linearity of the photoresist response versus X-ray dose. Both this detectors require a developing procedure. The other class of electronic solid state detectors, consisting of CCDs and their more recent developments, has a very large dynamic range (10-16 bits) but a very poor spatial resolution (around 10 μm), so that μ-radiographs require projection mode operation.

We propose to use for such applications at SPARX a novel X-ray imaging detector based on optically stimulated luminescence (OSL) of color centers in lithium fluoride (LiF) [6] that possesses very peculiar performances without requiring a development process. The simplicity of the OSL reading technique allows for an extremely large field of view without limiting the final detectable resolution. For these peculiarities LiF-based sensors are promising candidates for suitable application in biology. This material is optically transparent from the near ultraviolet to the near infrared spectral

interval, practically not hygroscopic, and can be grown through versatile and well-assessed deposition techniques as a thin film on different substrates, such as glass, silicon and plastic [7]. It is a radiation-sensitive salt, which has been utilized and is still being used in radiation dosimetry [8], optoelectronics [9], integrated optics[10]. It has also been deeply studied as far as basic optical properties of color centers (CCs) are concerned [11]. It is well known that different ionizing radiation, such as charged particles (ions and electrons) and energetic photons (X-rays and γ-rays), can efficiently generate optically active CCs that are stable at room temperature in LiF. Among them we focus our attention on F_2 and F_3^+ defects, which consist of two electrons bound to two and three close anion vacancies, respectively. These centers have almost overlapping absorption bands (M band) centered at around 450 nm [12] and, therefore, can be simultaneously excited with a single pump wavelength. On the other hand, they exhibit two different broad emission bands in the green (F_3^+) and red (F_2) spectral ranges. After X-ray exposure of biological samples, placed in contact with the LiF surface, the image is stored in the radiation-sensitive material and can be read (after removing the sample) just by illuminating the detector with a blue light and observing its visible photoluminescence with an optical microscope [13]. By using advanced fluorescence optical microscopes as readout systems, like Confocal Light Scanning Microscope (CLSM) and Scanning Near field Optical Microscope (SNOM), images with sub-micrometric and nanometre spatial resolution can be respectively detected [14]. A LiF-based soft X-ray imaging plate could overcome the limitations (i.e. resolution, dynamic range, reading times,…) of other detector types and fully exploit the potentialities offered by SPARX's peculiar characteristics.

Fig. 1 shows the micro-radiography of a dragonfly (Pyrrhesoma nymphula) wing obtained by placing the sample in contact with a 2 μm thick LiF film grown by thermal evaporation on a glass substrate at the Solid State Laboratory (C.R. ENEA Frascati) [7] and by exposing it to soft X-rays from a laser plasma source developed at the Excimer Laser Laboratory (C.R. ENEA Frascati) [15]. The X-ray emission covers the spectral interval from 0.8 to 60 nm (hν = 20 eV - 1.5 keV), which corresponds to the full EUV region and part of the soft X-rays region. The X-ray dose delivered to the sample is about 0.2 J/cm^2. Images of cells in their living state have been also obtained by a single shot experiment (20 mJ/cm^2).

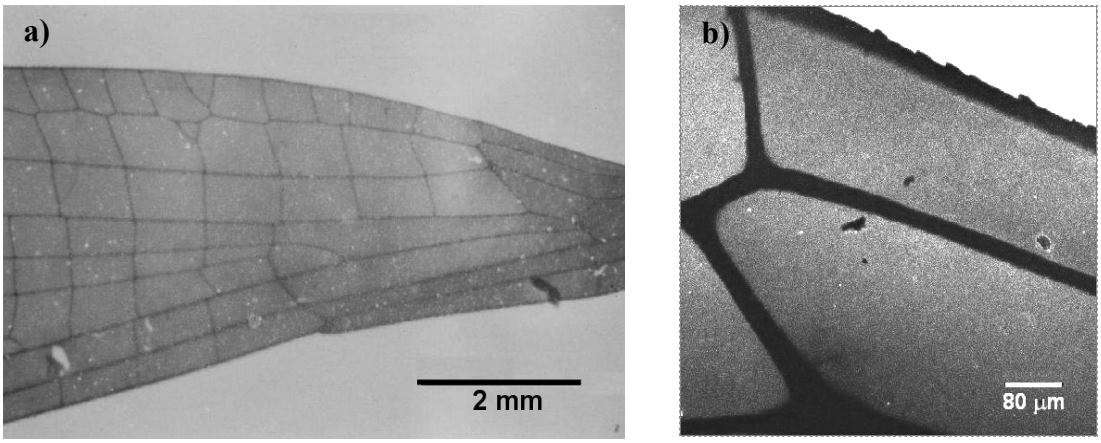

Fig. 1 a) Dragonfly (*Pyrrhesoma nymphula*) wing radiograph stored on a LiF film observed with an optical fluorescence microscope illuminating with blue ligth. b) Detail of Fig. 3a) observed under a LEICA fluorescence confocal optical microscope.

The spatial resolution of LiF imaging plate, in principle limited only by the CC dimension, that is at atomic scale (~ 1nm), is comparable with that of photoresists (< 10 nm), but the dynamic range of LiF is orders of magnitude wider than that of photoresists, as shown in Fig. 2.

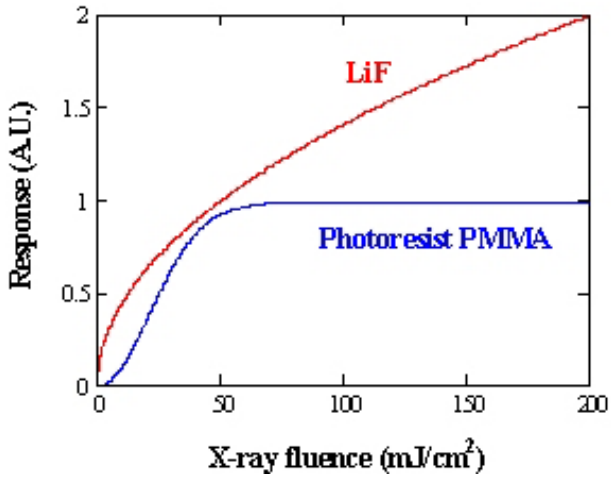

Fig. 2: Response of LiF and PMMA detectors versus X-ray fluence

High irradiation doses are usually required in X-ray microscopy, in order to obtain high-resolution images. The graph of Fig. 3 shows the relation between spatial resolution and the source fluence based on a Poisson statistics. In principle the high fluence of X FEL (~ 20J/cm^2) allows to reach spatial resolution forbidden with actual sources, especially if the attenuation induced by the monochromatization of a synchrotron radiation source in order to select the coherent part of the beam is taken into account.

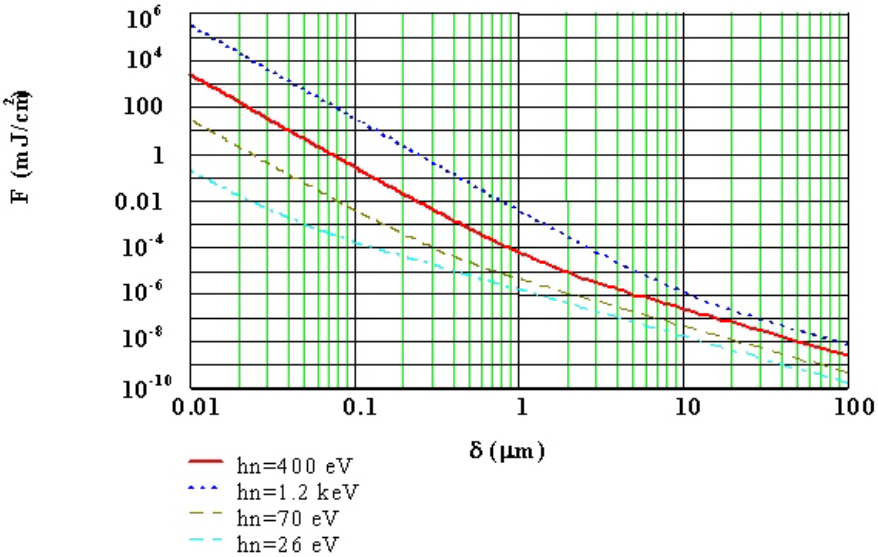

Fig. 6: Spatial resolution versus source fluence for different energies.

We propose to exploit X FEL radiation, at 3-5 nm emission wavelengths (falling in the water-carbon window), to perform X-ray contact microscopy of biological samples in single-shot configuration by using an innovative imaging detector based on LiF material.

Due to the short duration (~ 100 fs) of the X-FEL pulse, it could be possible to study living biological specimens by recording images in a very short exposure time, before radiation damage occurs. The high and unique brightness (3-8x10^{30} Phot/s/0.1%bw/(mm-mrad)^2) will ensure a nominal spatial resolution unreachable with current sources. Moreover, according to the point-like nature of X FEL source, the penumbra blurring can be completely neglected. The high resolution and the large dynamic range of the LiF detector could fully exploit the potentialities offered by the SPARX peculiarities.
Due the coherence of the X-FEL beam (30-40 μm with a seeding configuration) and the high brightness, biological investigation can be performed by single shot holography experiments as a method for a high resolution 3D imaging. If these peculiar characteristics of X FEL beam are combined with the high spatial resolution of the LiF detector, a very simple in-line Gabor configuration can be proposed (Fig. 7a)). A plane wave hits directly the object and the interference between reference and object waves on the plane detector form a hologram consisting in a system of circular fringes. The resolution of the reconstructed images depends on the information recorded in the hologram which is limited by the size of the detector and by its resolution. A LiF recording material can be used to record fine fringes that can allow a high spatial resolution for the reconstructed 3D images of the object.
Due the coherence of X FEL beam also complex holographic circuits can be proposed. With an off-line holography set up (shown in Fig. 7b) it is possible to control parameters, such as the relative intensity of reference and object wave or the angular separation of the beams, in order to increase the resolution of the reconstructed image of the object.

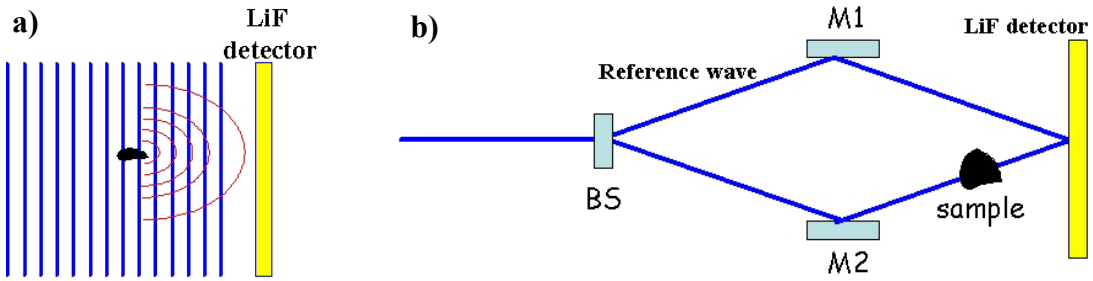

Fig 7: a) In-line Gabor holography ; b) Off-line holography: BS (beam splitter), M_1,M_2 (mirrors)

We have presented a proposal for exploiting the soft X-ray radiation produced by SPARX in the field of biological investigation by using single-shot contact microscopy and holography on an innovative imaging detector based on OSL of CCs in LiF.
The high brightness and short duration of SPARX source could allow to obtain imaging of biological specimens in their living state with very high spatial and time resolution.
Due the coherence of X FEL beam, biological investigation can be performed by single shot holography experiments as a method for a high resolution 3D imaging, also with complex holographic circuits.
A LiF-based soft X-ray imaging sensor could overcome the limitations of the standard detectors and fully exploit the potentialities offered by SPARX peculiar characteristics.

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