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

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Giornate di Studio SPARX e Applicazioni

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Outline

• Soft X-ray microscopy:
✓ Configurations
✓ Water and carbon window
✓ Standard imaging detectors

• Proposal:
➢Single-shot Contact Soft X-ray microscopy on a Lithium Fluoride-based novel imaging detector
➢Single Shot X-ray holography with coherent X-FEL radiation on LiF detector

• Biological images on LiF detector

• Conclusions

Soft X-ray microscopy

Traditional technique for biological samples imaging

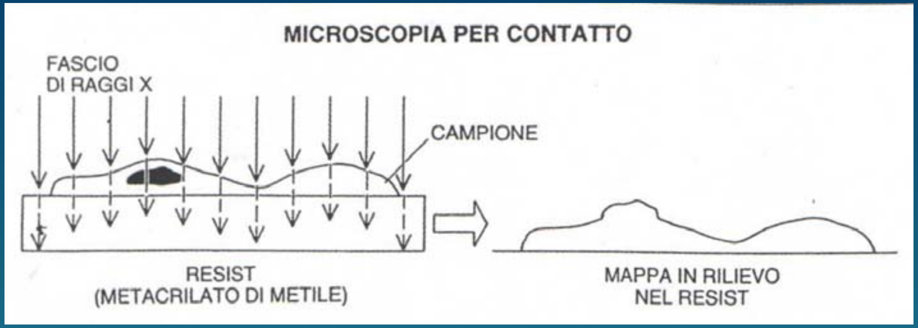
Light Microscopy (LM)
Limitation:
resolution limit due to the wavelength of visible light

Electron Microscopy (SEM or TEM)
Limitation:
preliminary treatments of sample (dehydration, fixing and dying with electron dense substances)

Soft X-Ray Microscopy
($\lambda > 1$ nm , $E < 1$ keV)
were introduced for imaging of internal structure of biological samples in their normal living state, at a high spatial resolution and avoiding all specimen preparation

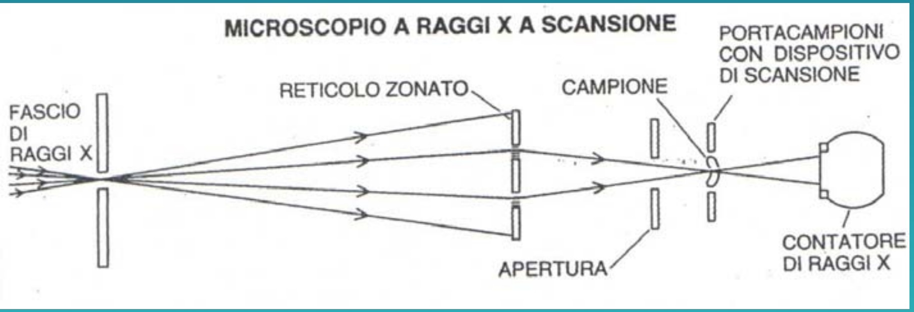
X-ray sources for Soft X-ray microscopy:
Synchrotrons
Laser Plasma Sources
Micropinch discharges

X-ray microscopy configurations

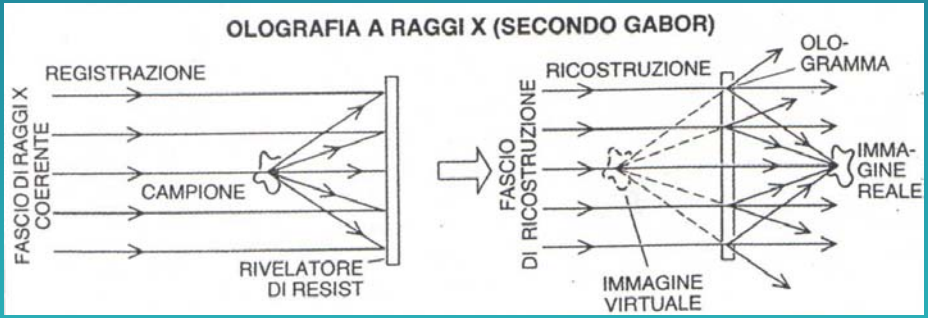


Contact X-ray microscopy:
- No need of optical elements
- No coherent radiation
- Imaging of biological samples in their living state in single shot exposure
- Spatial resolution limited by: diffraction effect, penumbra blurring, detector.

Focusing X-ray microscopy:
- Need of optical elements
- Need of monochromatic radiation
- Imaging of biological samples in their living state in single shot exposure only with high intensity sources.



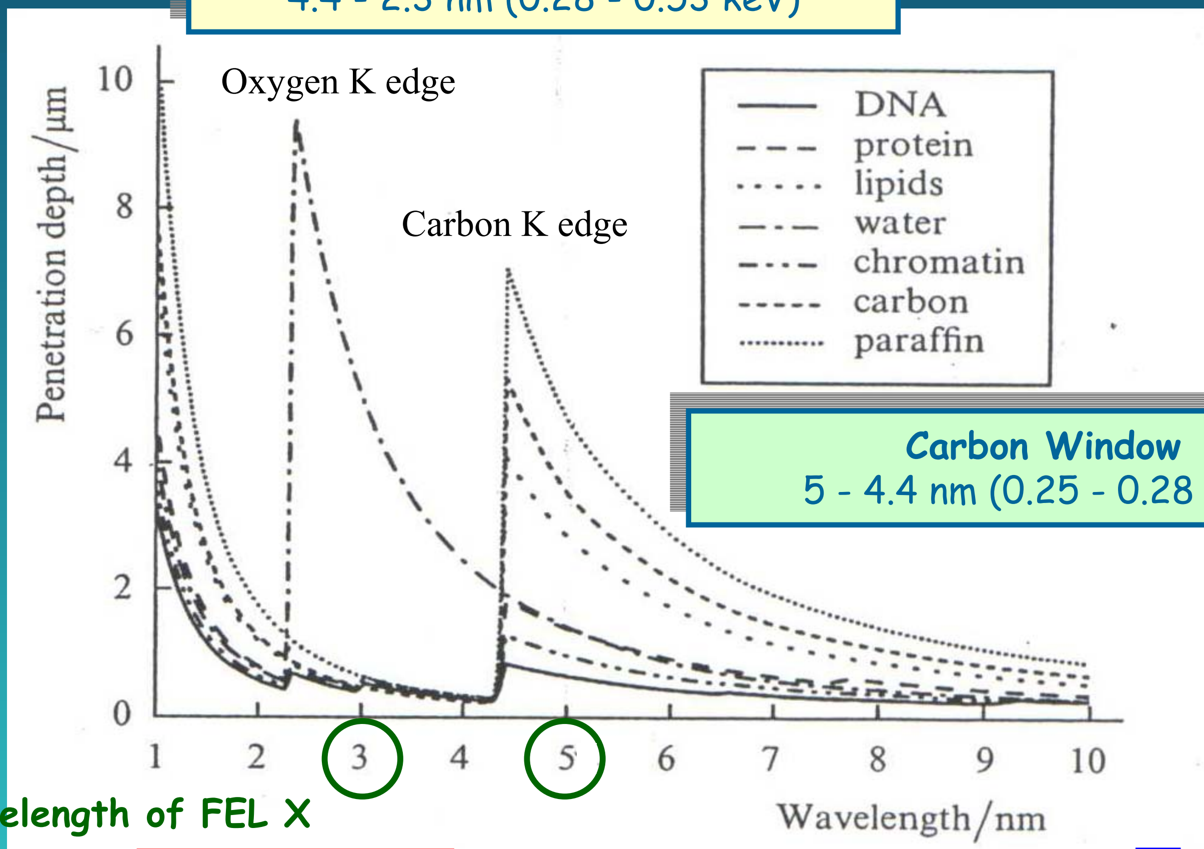
Scanning X-ray microscopy:
- Need of optical elements
- Need of monochromatic radiation
- Need of long exposure time



Holography:
- Need of coherent radiation
- Imaging of biological samples in their living state in single shot exposure only with high intensity coherent sources.

Penetration depth in water and in biological material

Water Window
4.4 - 2.3 nm (0.28 - 0.53 keV)



Carbon Window
5 - 4.4 nm (0.25 - 0.28 keV)

Emission wavelength of FEL X

Soft X-ray
1-4 nm (1000 – 300 eV)

EUV
4 – 60 nm (300 – 20 eV)

Imaging detectors for Soft X-ray Microscopy

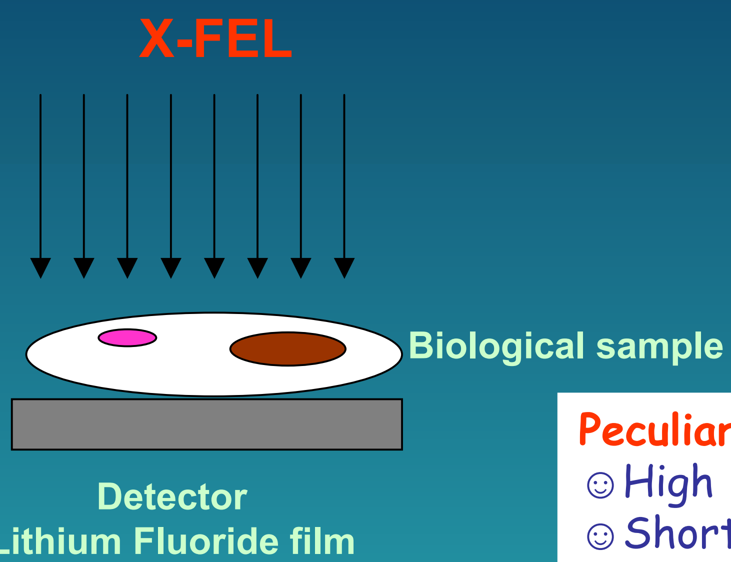
Photographic film
Pixel size ~ 5 μm
Fluence dynamic range: 1 nJ/cm^2 - 1 μJ/cm^2
Readout system: optical microscope

CCD (Charge Coupled Devices)
Pixel size ~ 2 μm
Fluence dynamic range: 0.1 nJ/cm^2 - 10 μJ/cm^2
Readout system: Analogical/Digital Conversion

Photoresist PMMA
Pixel size ~ 0.01 μm
Fluence dynamic range: 1 mJ/cm^2 – 50 mJ/cm^2
Readout system: Atomic Force microscope

Innovative imaging detector based on Optically Stimulated Luminescence (OSL) of color centers in Lithium Fluoride (LiF): high spatial resolution, high dynamic range, efficient detection and redout process.

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



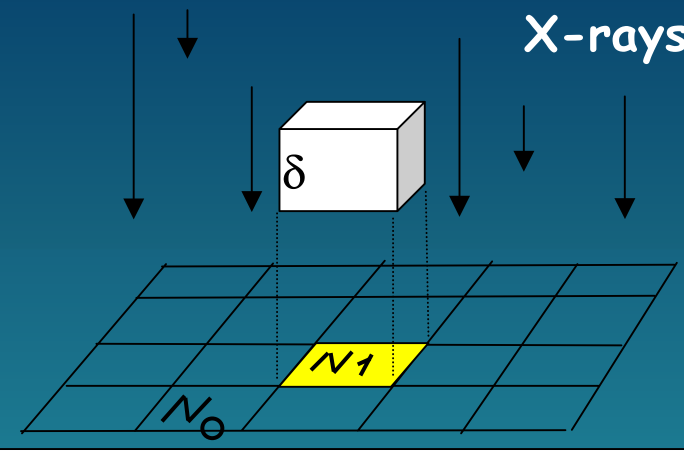
Contact X-ray microscopy:
- No need of optical elements
- No coherent radiation
- Imaging of biological samples in their living state in single shot exposure
- Spatial resolution limited by: diffraction effect ($(\lambda \times d)^{1/2}$), penumbra blurring ($P = S \times d/D$), detector.

Peculiarity of SPARX source
☺ High Brightness: 3-8x10^{30} Phot/s/0.1%bw/(mm-mrad)^2
☺ Short duration: ~ 100 fs
☺ λ = 3-5 nm (water and carbon window)

The peculiarity of SPARX source allows to obtain images of biological samples with very high spatial and time resolution.
The **high brightness and the short duration** allows to obtain images:
- in a single shot of living biological samples. The short duration allows to study living biological specimens in a very short exposure time, before radiation damage occurs. The fs-FEL pulse allows a high time resolution.
- with very high spatial resolution

LiF-based soft X-ray detector could fully exploit the potentiality offered by SPARX peculiar characterisitcs.

X-ray fluence vs spatial resolution



F= source fluence
μ = absorption coefficient

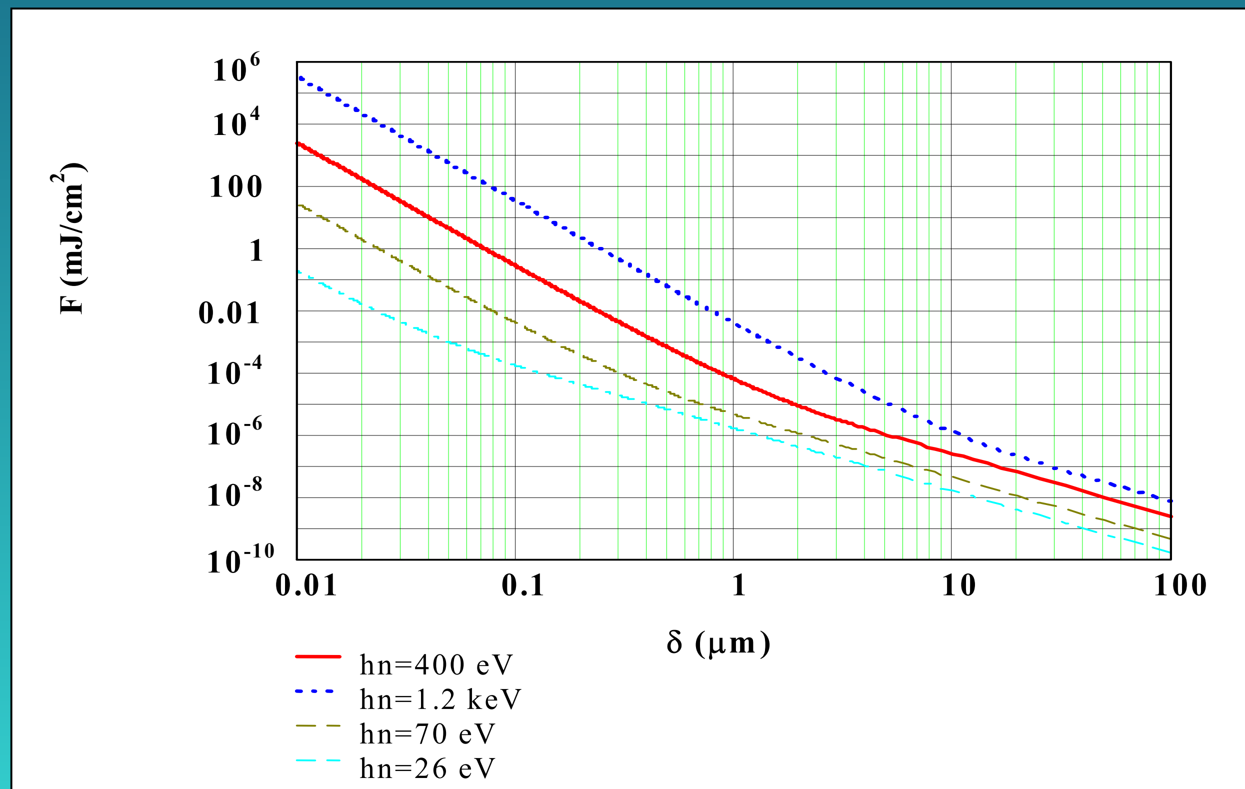
$$N_0=\delta^2 \cdot F/h\nu$$
$$N_1= \delta^2 \cdot F \cdot e^{-\mu\delta} /h\nu$$

Condition to see the shadow:

$$N_1 - N_0 \geq 2\sqrt{N_1} + 2\sqrt{N_0}$$

from which:

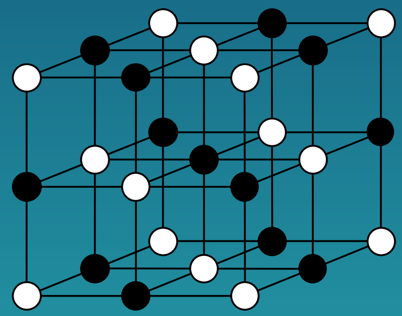
$$F = h\nu \cdot \frac{4}{\delta^2} \cdot \left(\frac{1+\sqrt{e^{-\mu\delta}}}{1-e^{-\mu\delta}} \right)^2$$



A novel imaging detector
for Soft X-ray Microscopy based on LiF

We propose to use a novel X-ray imaging detector based on optically stimulated luminescence (OSL) of color centers in Lithium Fluoride (LiF)

Alkali halides: ionic crystals with face centered cubic structure, optically transparent from near UV to IR



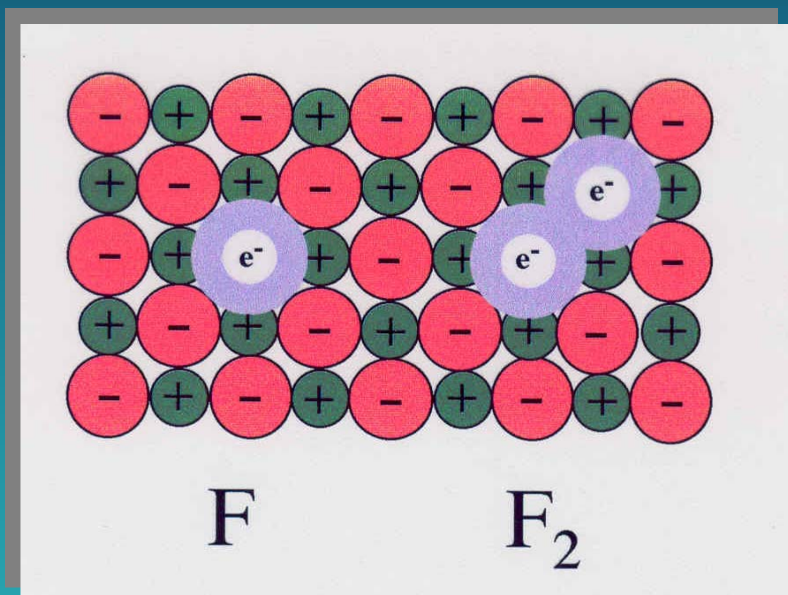
LiF is of particular interest because :
- it is almost ***non-hygroscopic***
- it can host point defects ***stable at room temperature***
- it can host ***laser active color centers*** tunable in a broad wavelength range in the ***VIS*** and ***NIR***.

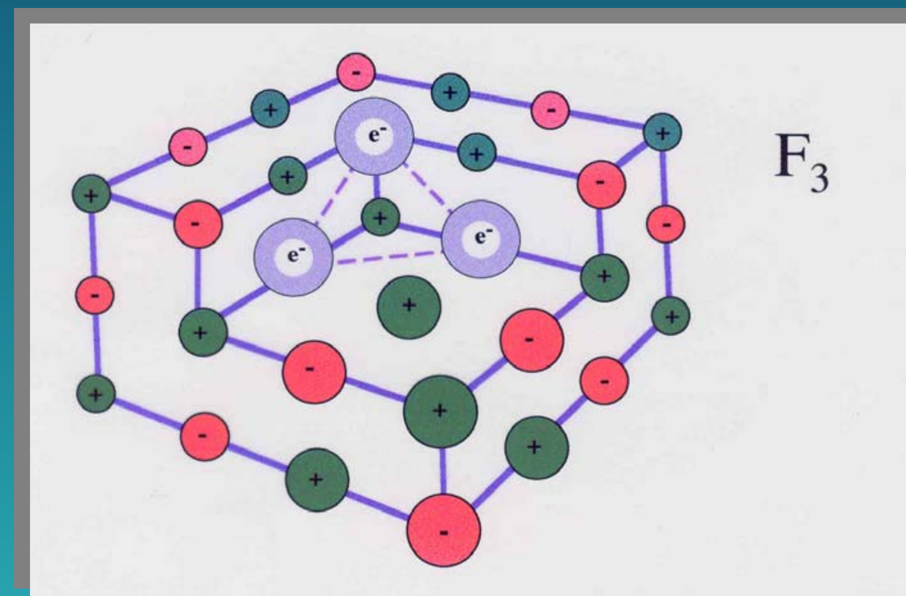
Polycrystalline LiF films can be grown by thermal evaporation on different substrates

Nearest neighbour distance (Å)	2.013
Melting point (°C)	848.2
Density (g/cm^3)	2.640
Molecular weight	25.939
Refractive index @ 640 nm, RT	1.3912
Solubility (g/100g H_2O @ 25°C)	0.134
Transmission range (μm)	0.12 - 7

Color centers in LiF

Optically active defects in LiF can be generated by ionizing radiation like charged particles (electrons and ions), as well as gamma and X-rays.

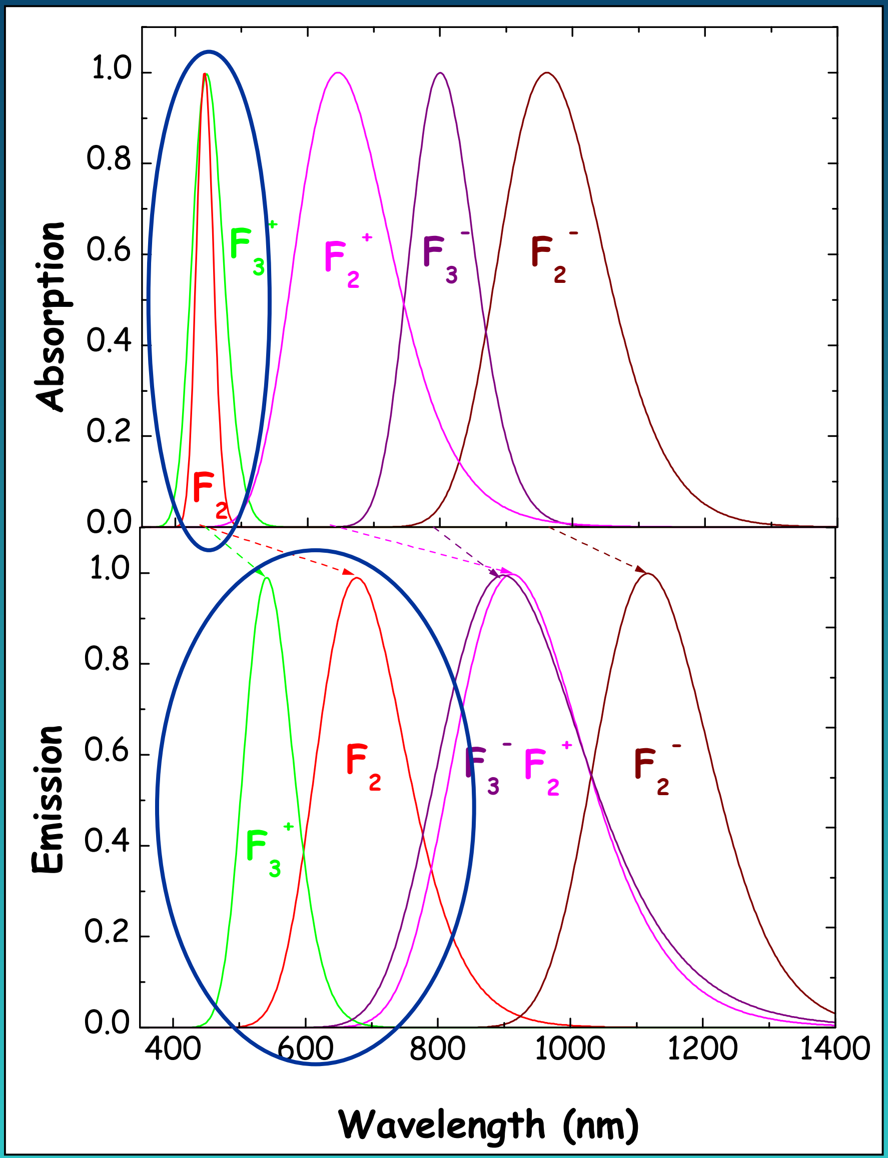




F center is an anion vacancy occupied by an electron; they are not optically active centers

F_2 and **F_3^+ centers** are optically active F-aggregates consisting in two electrons bound to two and three close anion vacancies, respectively

Laser active in LiF at RT



Center	Ea, eV,nm	Ee, eV,nm	HWa, eV	HWe, eV
F	5.00, 248	-	0.76	
F_2	2.78, 445	1.85, 670	0.186	0.279
F_3^+	2.70, 458	2.30, 539	0.347	0.347
F_2^+	1.97, 630	1.36, 910	0.434	0.29
F_2^-	1.29, 960	1.1, 1120	0.21	0.17
$F_3(R_1)$	3.92, 316			
$F_3(R_2)$	3.31, 374			
$F_4(N_1)$	2.40, 517			
$F_4(N_2)$	2.26, 547			
Li colloids	2.75, 450			

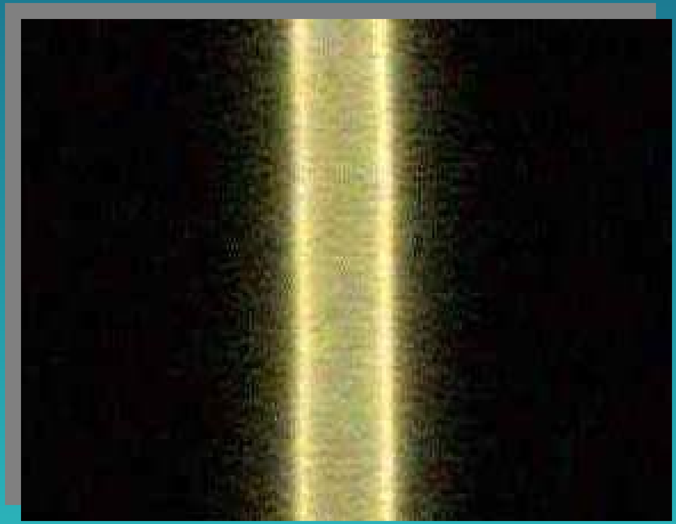
Miniaturized photonic devices based on CCs in LiF by using Electron Beam Litography

The use of versatile, well-assessed and low-cost fabrication techinques consisting of physical vapor deposition of LiF films combined with direct writing lithographic processes based on electron-beam allows the realization of miniaturized optical structures, like:

Vertical microcavities

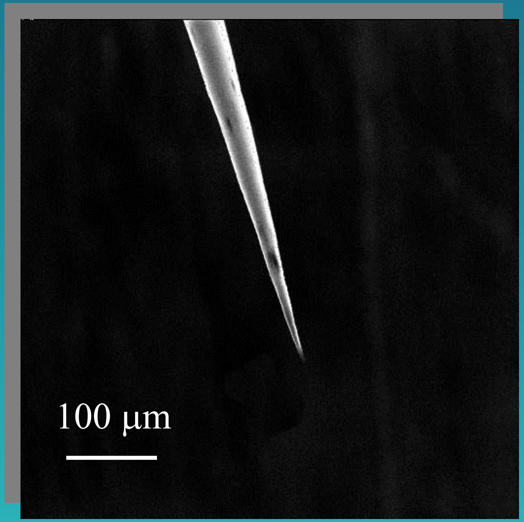
F. Bonfigli, B. Jacquier, F. Menchini, R.M. Montereali, P. Moretti, E. Nichelatti, M. Piccinini, H. Rigneault, F. Somma Radiation Effects & Defects in Solids, Vol. 158, 185-190 (2003).

Channel waveguides



R.M. Montereali, A. Mancini, G.C. Righini, S. Pelli, Opt. Commun. ***153****, 223 (1998).*

Point light sources



F. Bonfigli, S. Loreti, T. Marolo, R.M. Montereali, A. Pace, A. Santoni, M. Piccinini, S. Sekatskii, Abstracts Nanocose 2, Frascati (Roma), 13-15 Ottobre 2003, p.25.

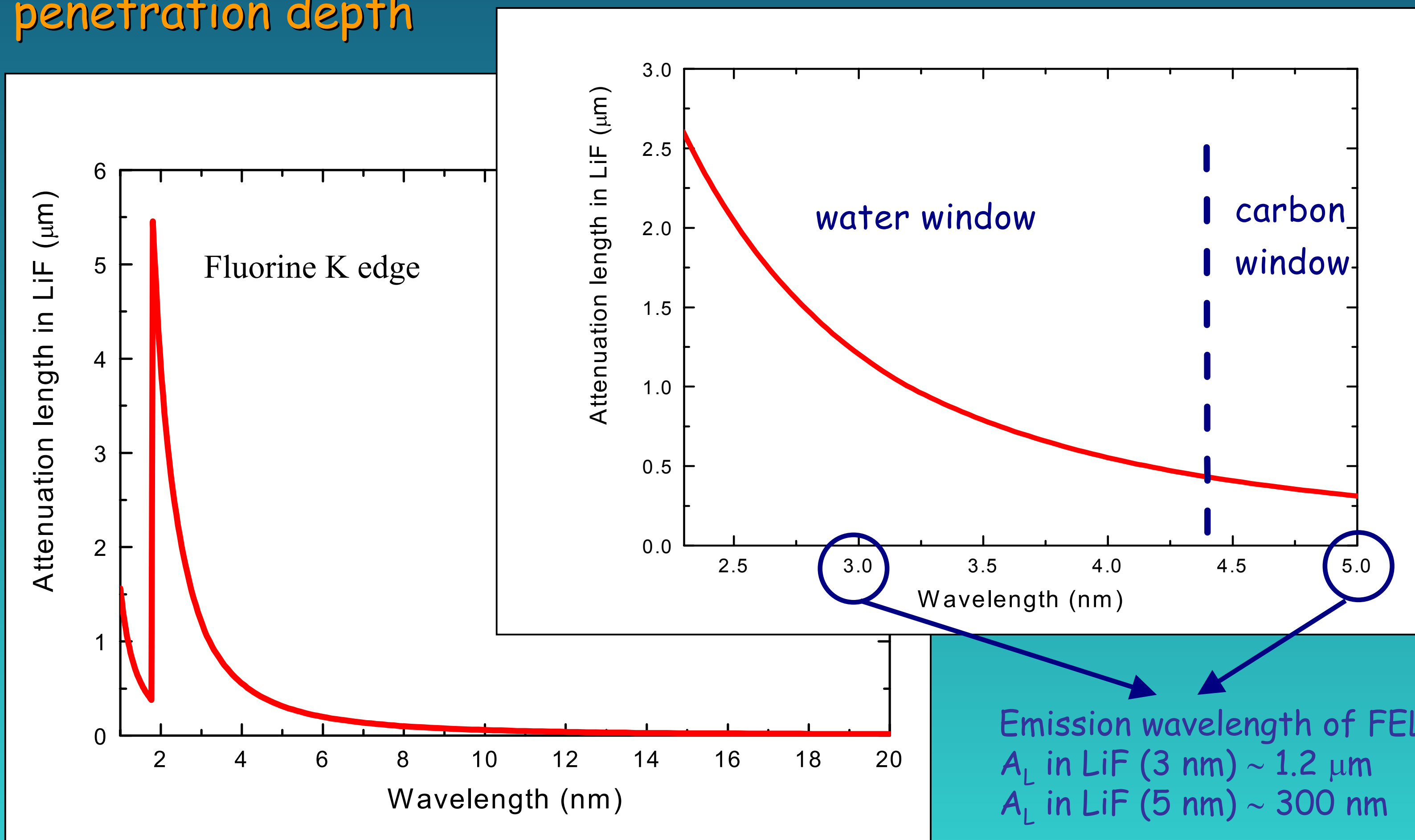
Peculiarity of soft X-rays in LiF coloration

Short wavelength

Neutrality

Low penetration depth

formation of active color centers with high spatial resolution

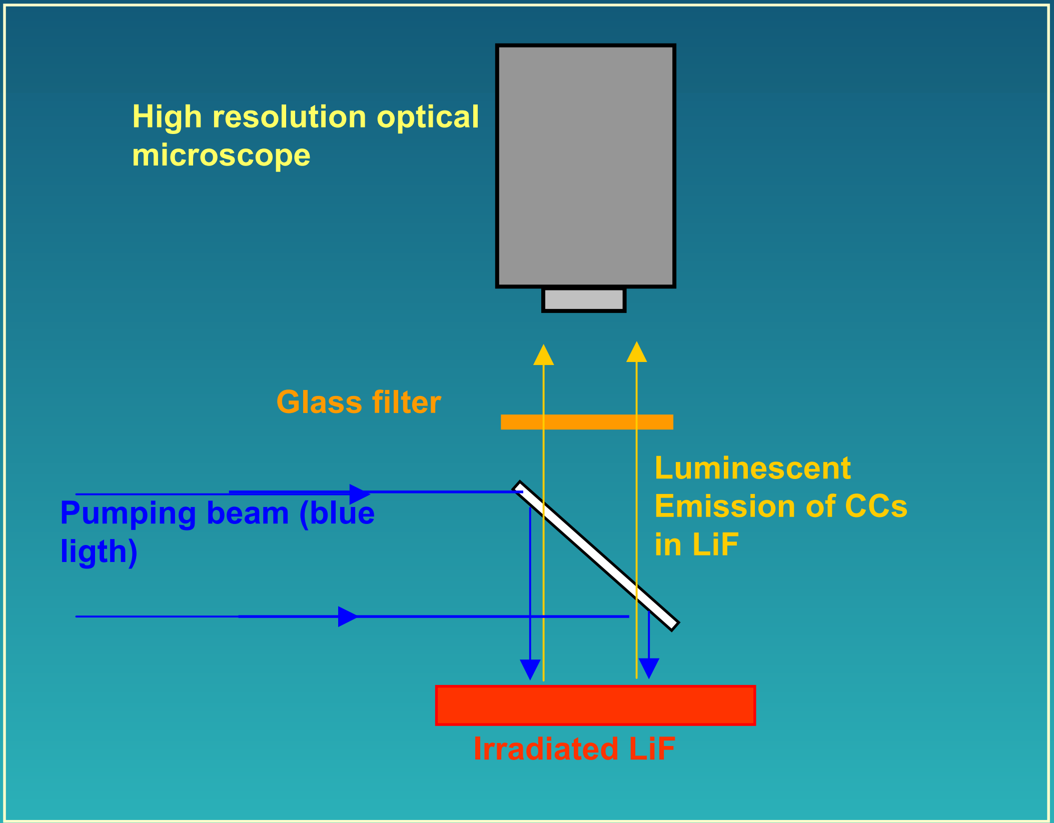


Emission wavelength of FEL X

A_L in LiF (3 nm) ~ 1.2 µm

A_L in LiF (5 nm) ~ 300 nm

Readout process of Optically Stimulated Luminescence (OSL) from Lithium Fluoride detector



Images stored in the irradiated LiF samples are observed by using optical microscopes in fluorescent mode. Irradiation with blue light excites the visible photoluminescence of the F_2 and F_3^+ defects locally created in the areas previously exposed to the X-ray beam.
With appropriate laser excitation sources and time-resolved detection, the luminescence sensitivity maybe virtually unlimited: under suitable conditions even single luminescence center present in the sample can be detected.

Lithium Fluoride-based novel imaging detector

LiF detector
Pixel size ~ 0.001 μm
Fluence dynamic range: 0.1 mJ/cm² – 1 J/cm²
Readout system: advanced optical fluorescence microscope

CCD (Charge Coupled Devices)
Pixel size ~ 2 μm
Fluence dynamic range: 0.1 nJ/cm² - 10 μJ/cm²
Readout system: Analogical/Digital Conversion

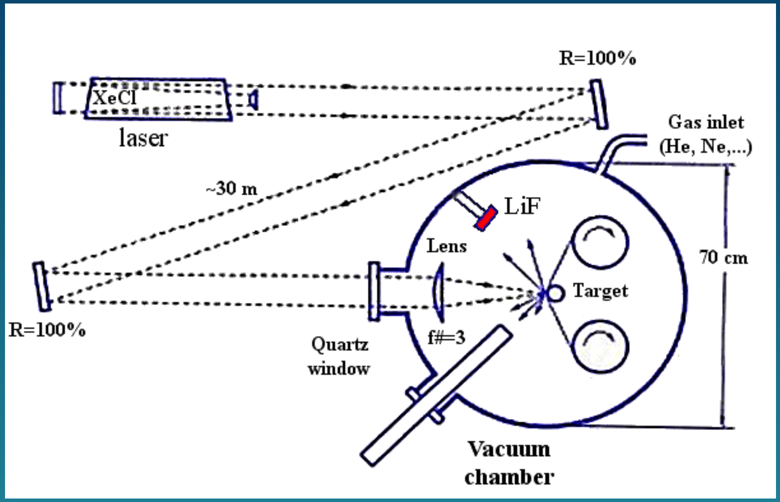
Photographic film
Pixel size ~ 5 μm
Fluence dynamic range: 1 nJ/cm² - 1 μJ/cm²
Readout system: optical microscope

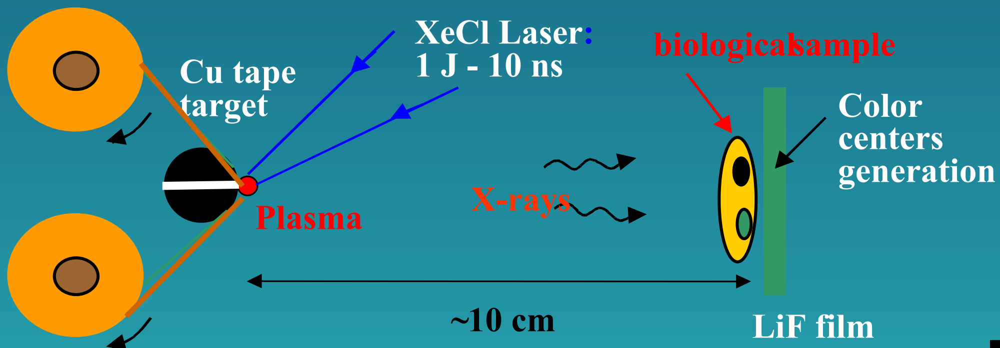
Photoresist PMMA
Pixel size ~ 0.01 μm
Fluence dynamic range: 1 mJ/cm² – 50 mJ/cm²
Readout system: Atomic Force microscope

Micro-radiography of a dragonfly (*Pyrrhesoma nymphula*) wing on LiF

Laser plasma source of Excimer Laser Laboratory (FIS-ACC, C.R. ENEA Frascati)

Excimer laser	EUV and soft X-ray source
$\lambda = 308$ nm	λ = 0.8 - 60 nm
Δt = 10 ns	$h\nu$ = 1.5 keV - 20 eV
E_L = 2 J	E_{EUV} = 130 mJ/shot
	E_{ww} = 20 mJ/shot
	E_{1keV} = 2 mJ/shot

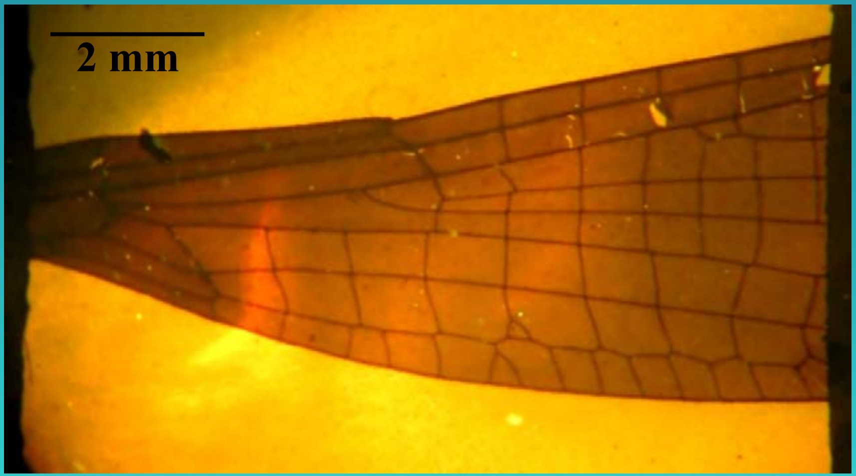




Biological sample: dragonfly (*Pyrrhesoma nymphula*) wing

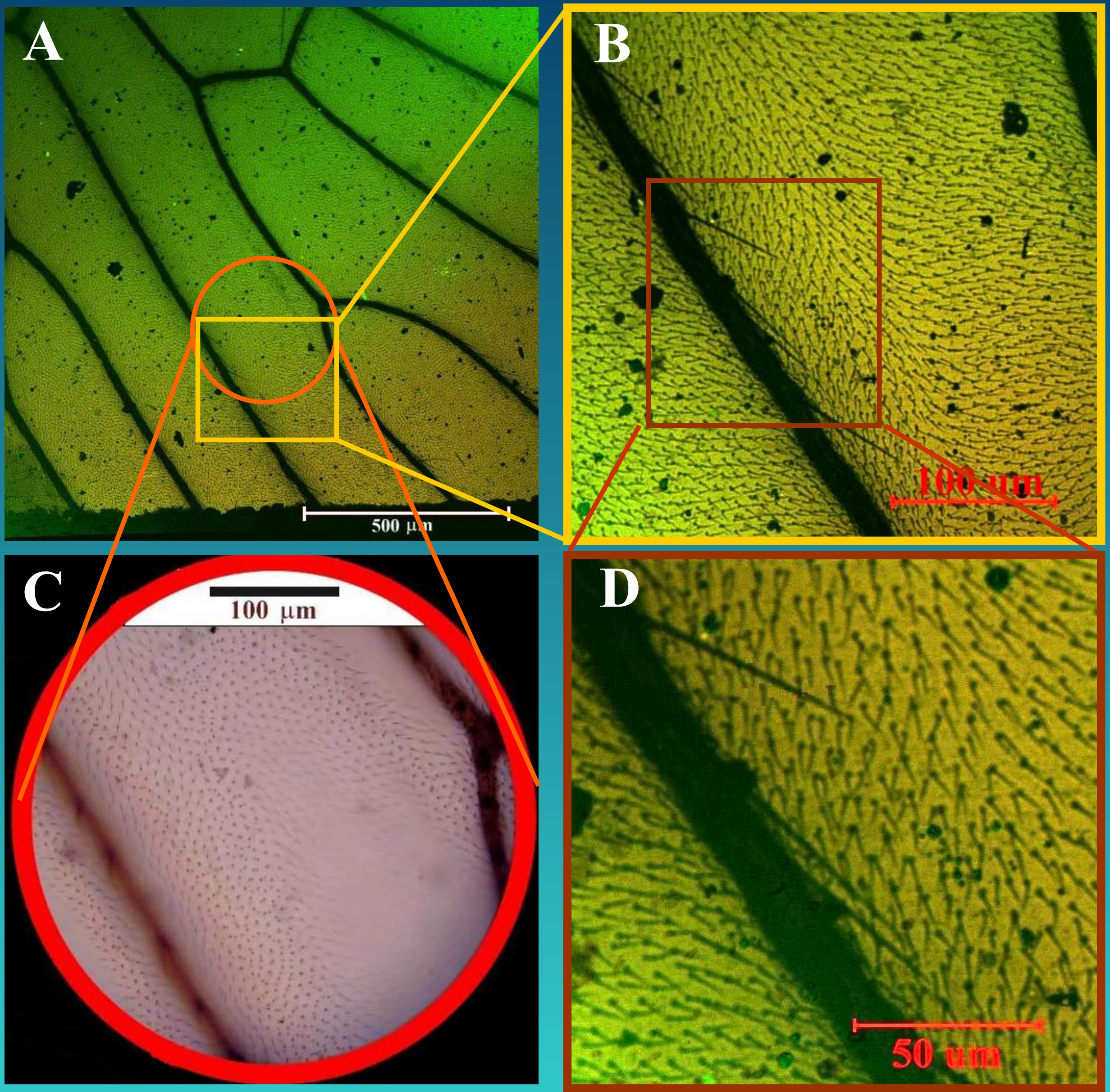
LiF sample: 2 μm film, thermally evaporated on glass

Wing micro-radiography on 2 μm thick LiF film at optical microscope obtained by observing the Optically Stimulated Luminescence (OSL). Pumping wavelength: 458 nm



ENEA patent n° TO2002A000575

Micro-radiography of a mosquito (*Diptera*) wing on LiF



A, B, D: Wing radiograph stored on a LiF film (120 nm) and observed under a ZEISS LSM 510 fluorescence confocal optical microscope at different magnification values

C: Image of the same wing, directly observed under a conventional optical microscope in transmission mode

Micro-radiography of a Lylium pollen on LiF

Laser plasma source of University Tor Vergata

Nd:YAG laser

$\lambda = 1064$ nm

Δt = 15 ns

E_L = 8 J

EUV and soft X-ray source

l = 0.8 - 60 nm

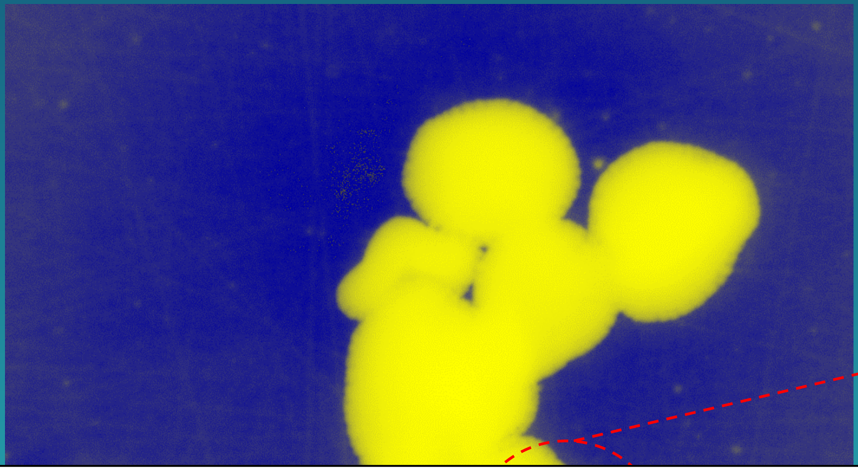
$h\nu$ = 1.5 keV - 20 eV

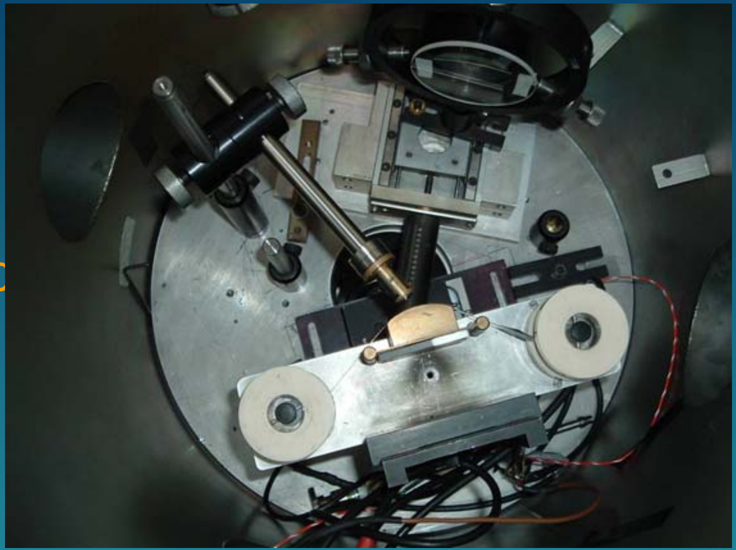
E_{ww} = 200 mJ/shot

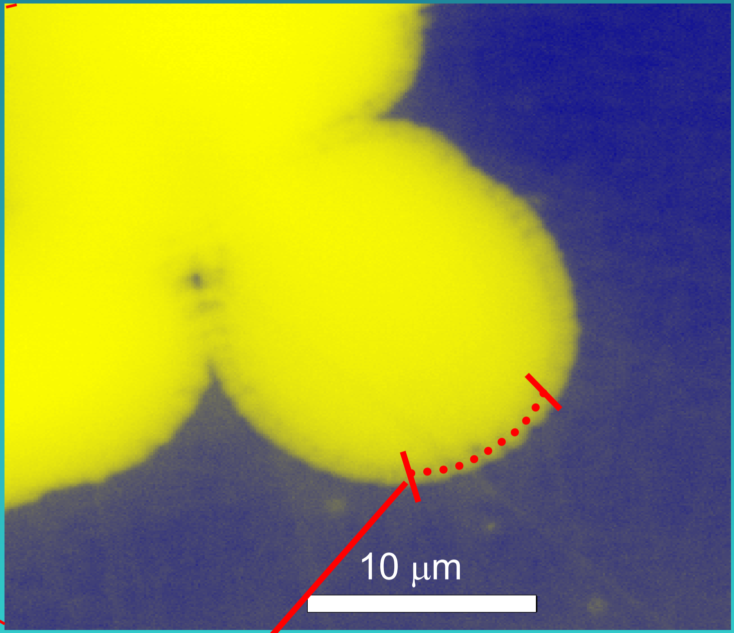
E_{1keV} = 100 mJ/shot

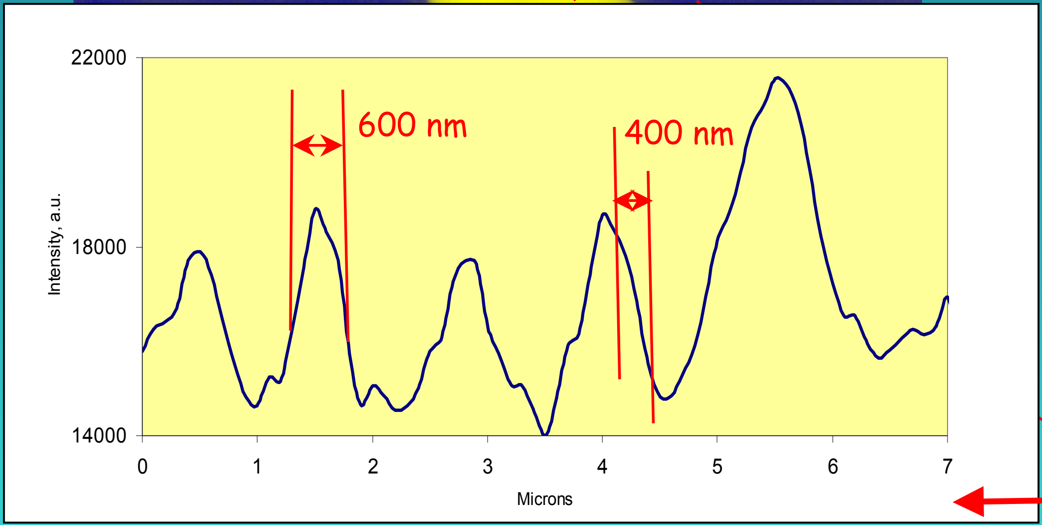
N shot = 10

Target: Cu

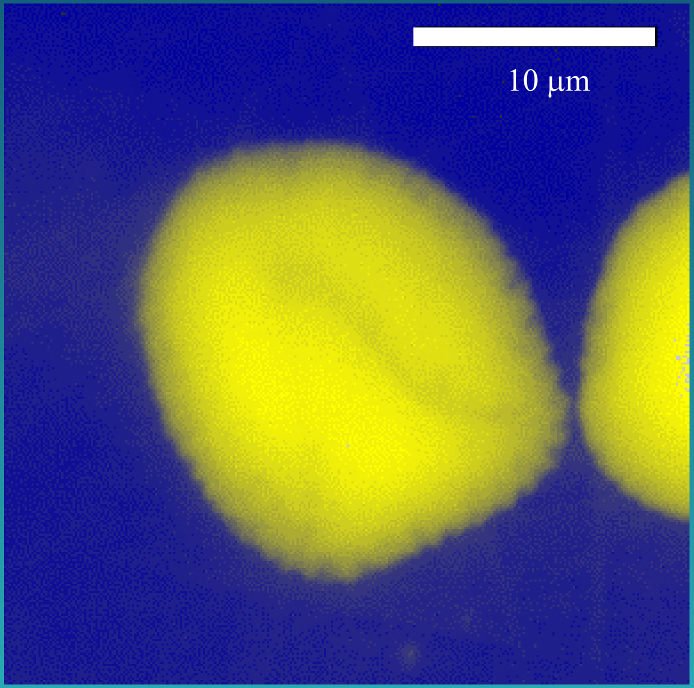


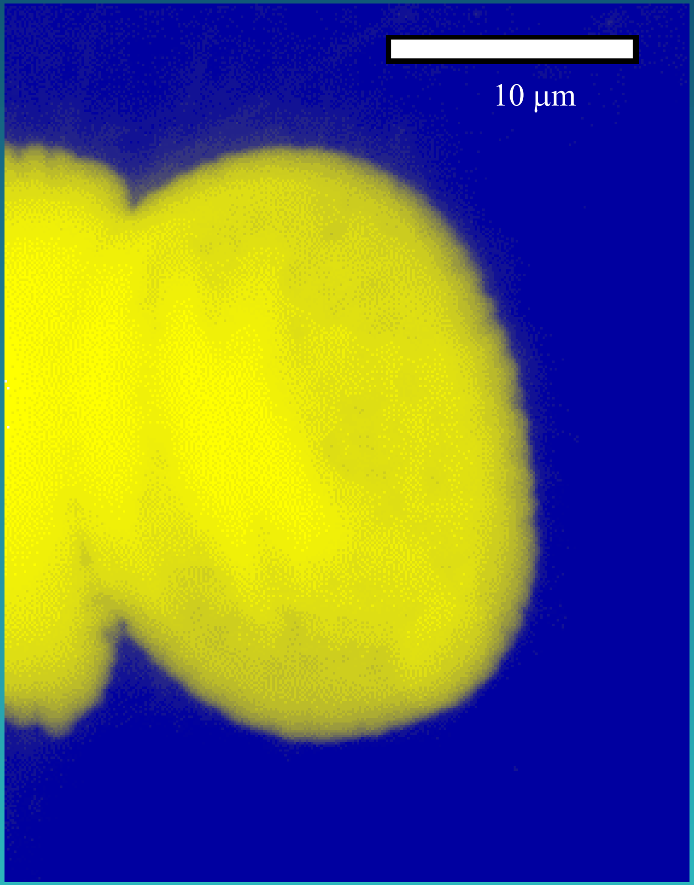






Micro-radiography of a Lylium pollen on LiF





Microscopy of chlorella cells

Laser plasma source of University Tor Vergata

Nd:YAG laser

$\lambda = 1064$ nm

Δt = 15 ns

E_L = 8 J

EUV and soft X-ray source

l = 0.8 - 60 nm

$h\nu$ = 1.5 keV - 20 eV

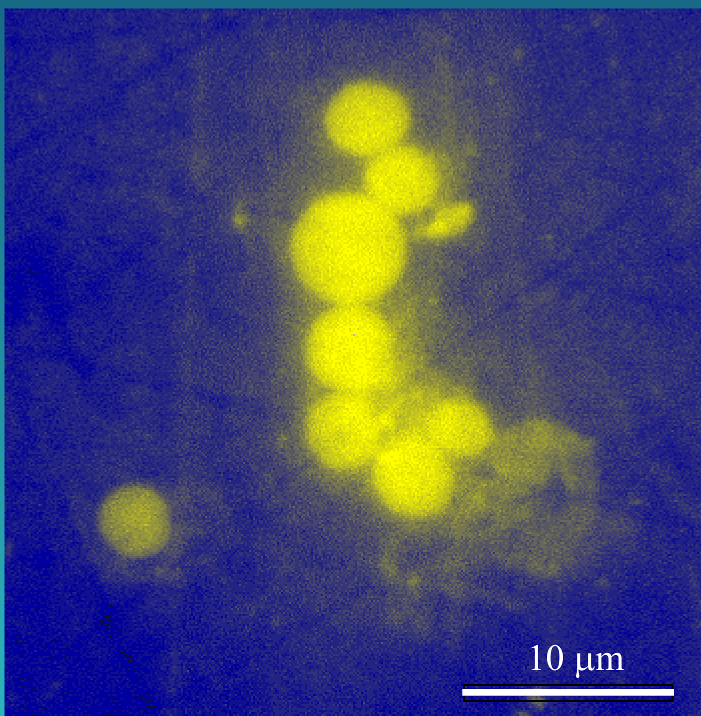
E_{ww} = 200 mJ/shot

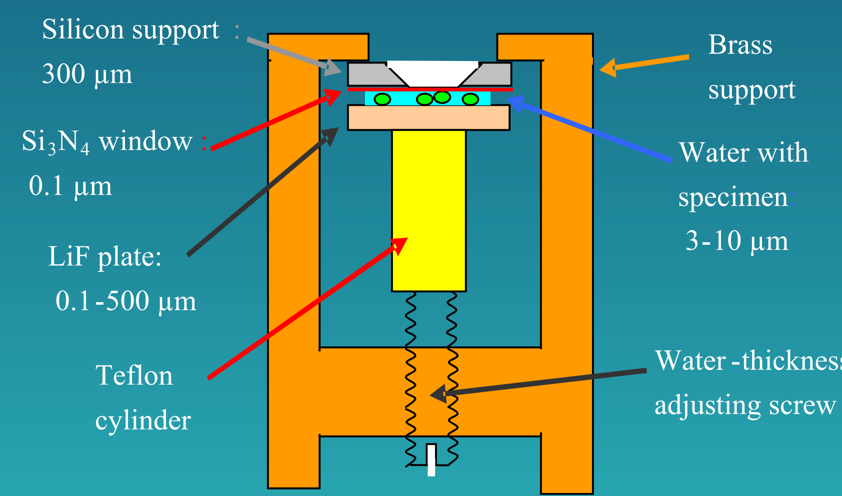
E_{1keV} = 100 mJ/shot

N shot = 1

Target = Y

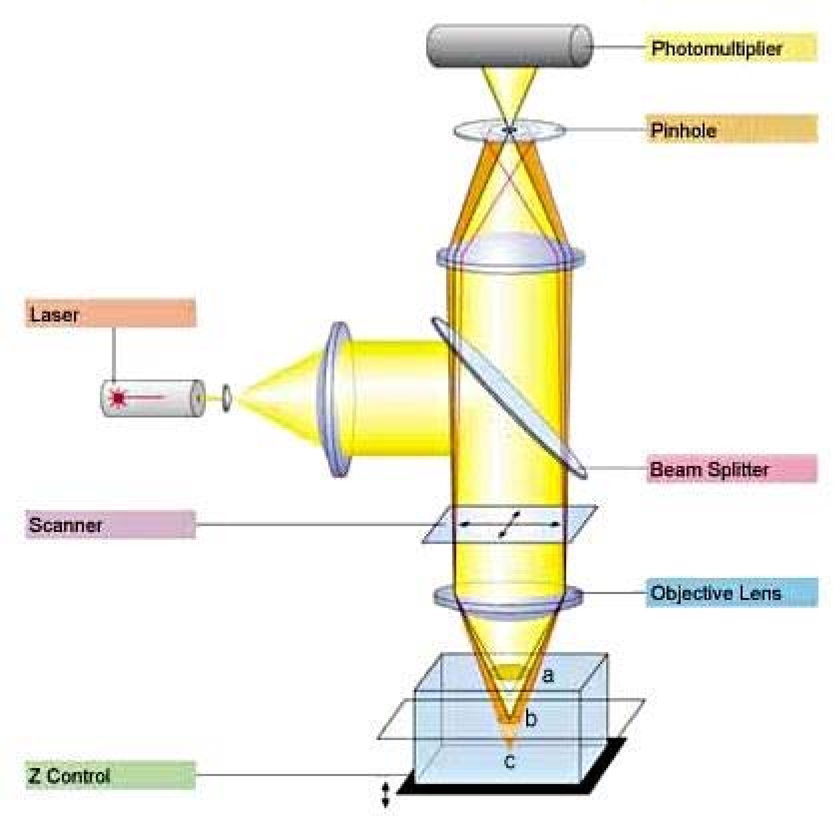
LiF Protection layer - Benzene



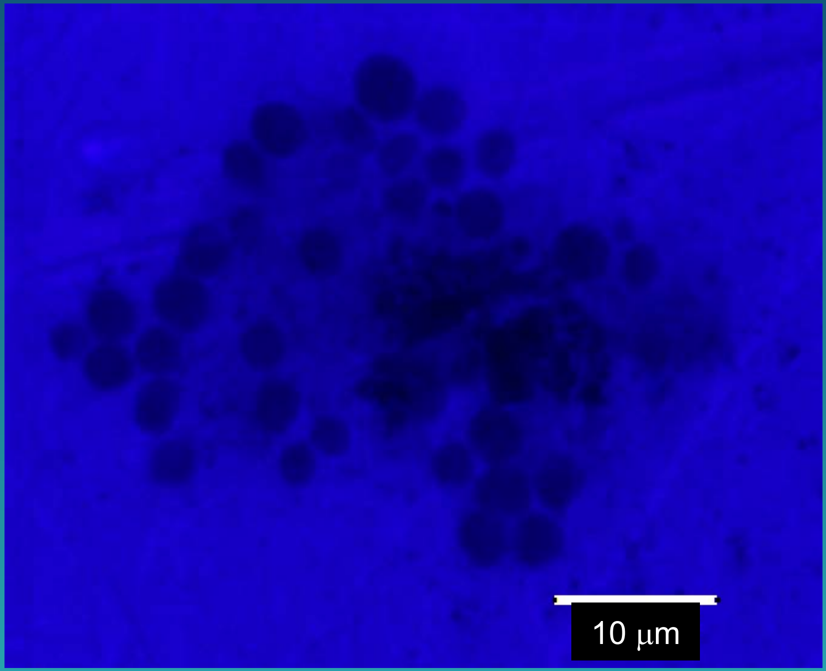


Chlorella cells image on a LiF crystal at optical microscope obtained by observing the Optically Stimulated Luminescence (OSL).

Confocal Laser Scanning Microscope (CLSM)



Only in-focus light (b) arrives at the detector; all out-of-focus light (a,c) is eliminated.



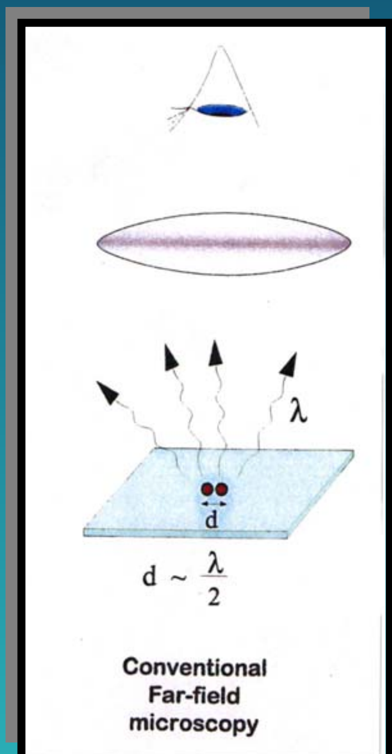
Chlorella cells image on a LiF crystal at CLSM obtained by observing the Optically Stimulated Luminescence (OSL). Pumping wavelength: 458 nm

Advanced Optical Microscope

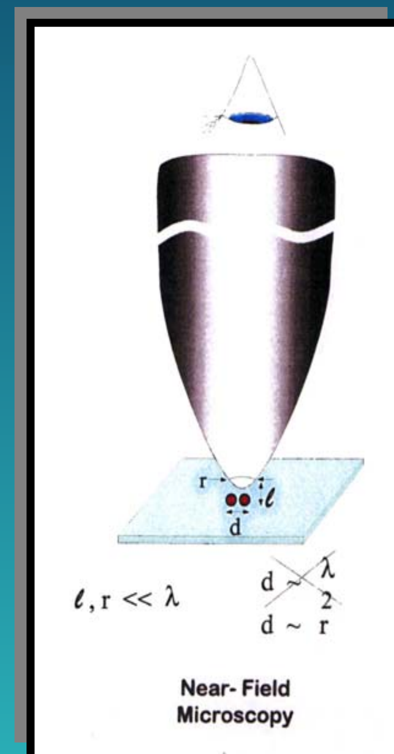
Conventional Far-field microscopy

Near-field microscopy

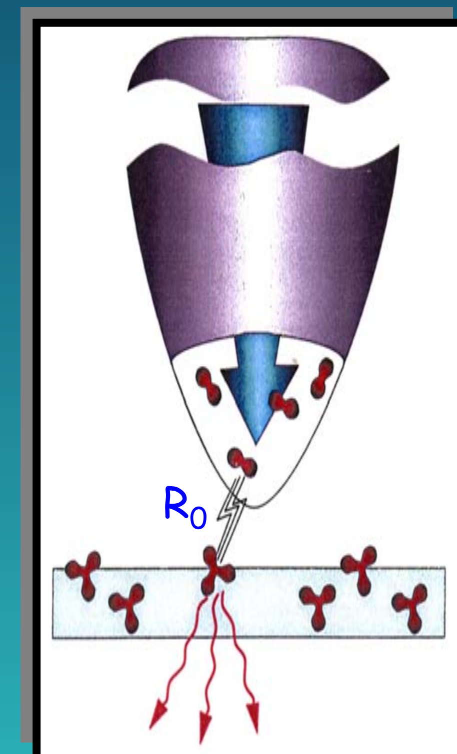
FRET SNOM



Spatial resolution:
~ 250-300 nm



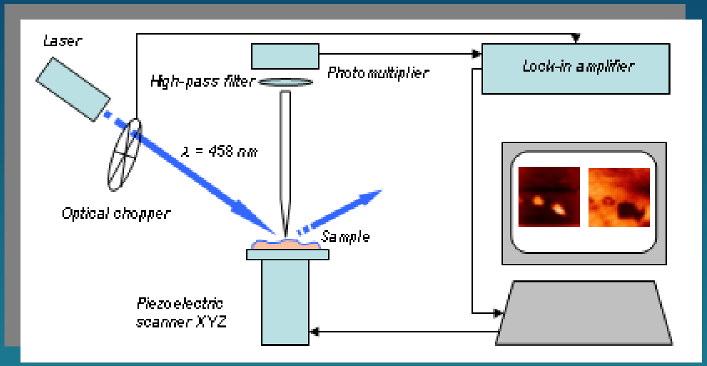
Spatial resolution:
~ 20-50 nm



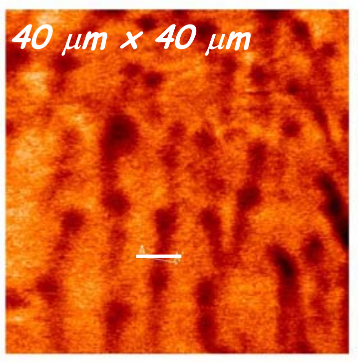
R_0 ~ 1-5 nm

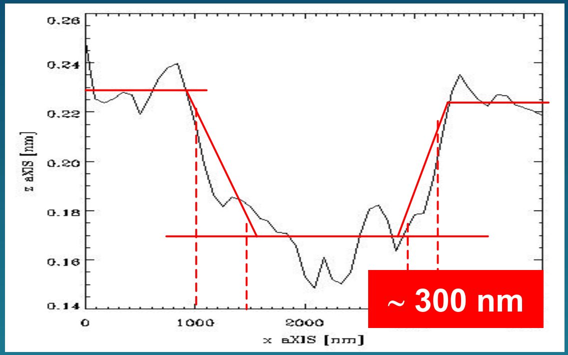
S.K. Sekatskii, V.S. Letokhov, *JEPT Lett.* 63, 319 (1996)

Scanning Near Field Microscope (SNOM) observation

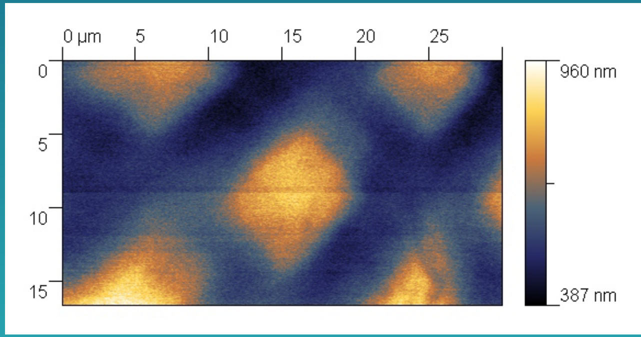


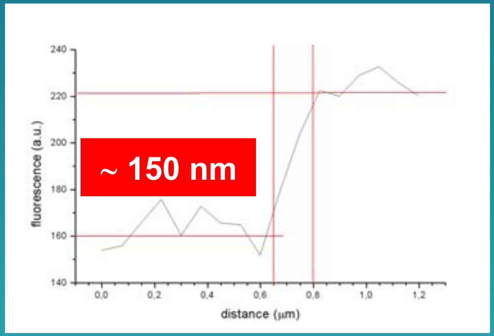
Wing micro-radiography on LiF

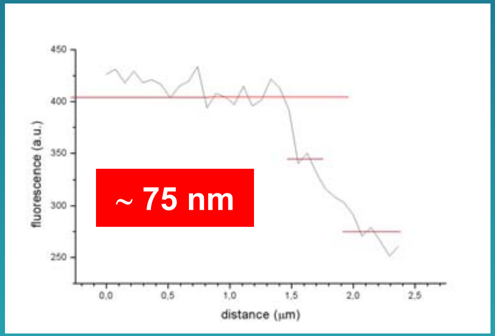




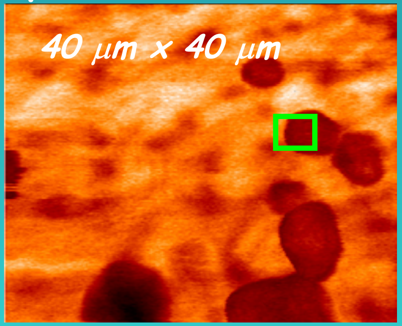
Regular luminescent pattern on LiF

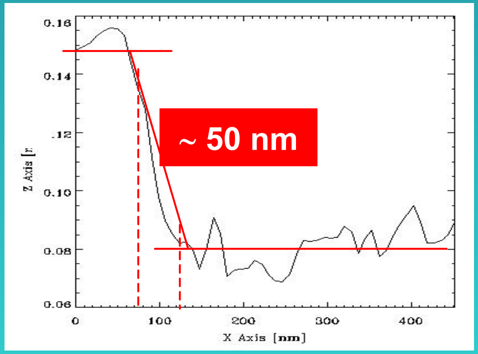






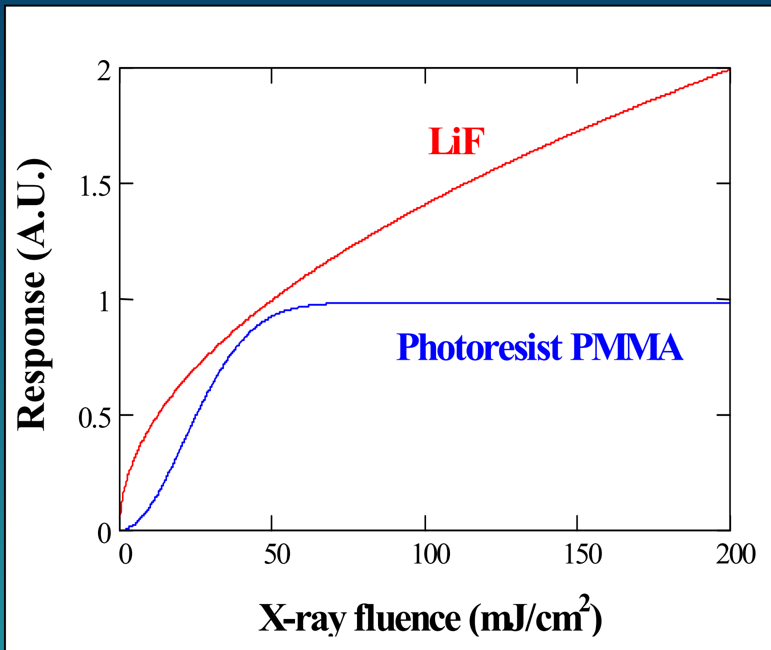
Laser plasma source debris on LiF





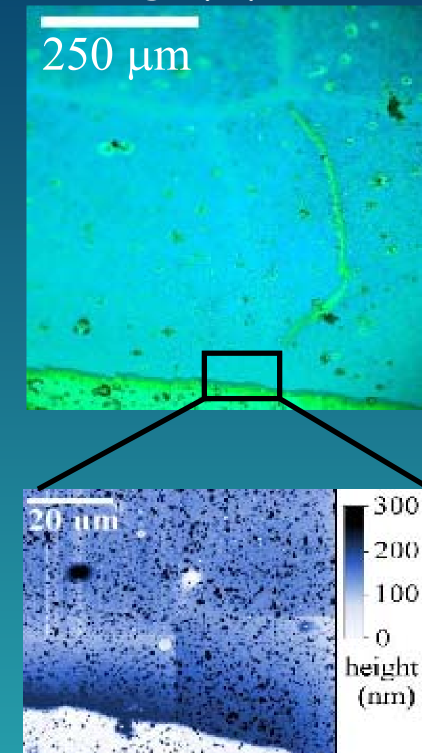
Courtesy of Dr. A. Cricenti , A. Ustione
CNR-ISM Tor Vergata

Dynamic range of LiF detector and of Photoresist



Wing micro-radiography on LiF

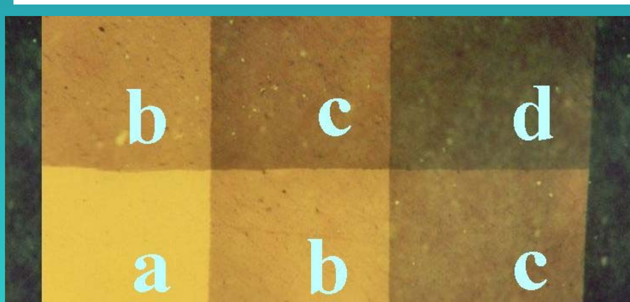
Wing micro-radiography on PMMA



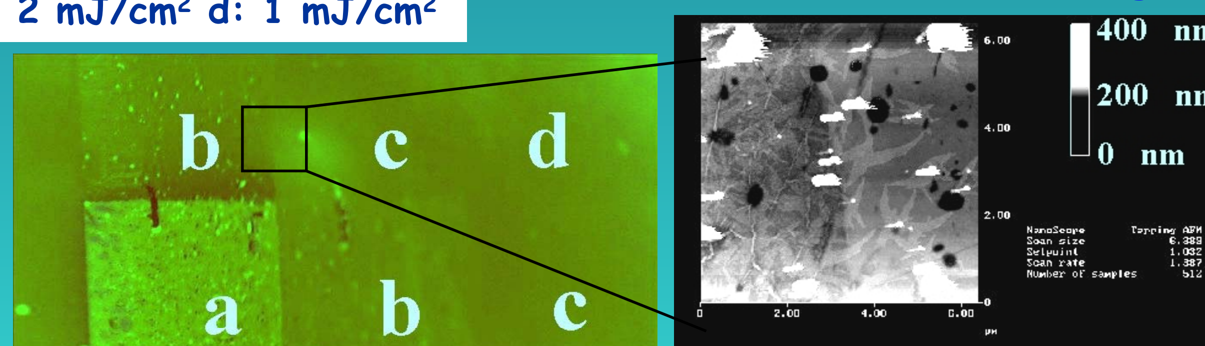
PMMA AFM image

Polypropylene phantom radiography

a: 600 mJ/cm² b: 4 mJ/cm² c: 2 mJ/cm² d: 1 mJ/cm²



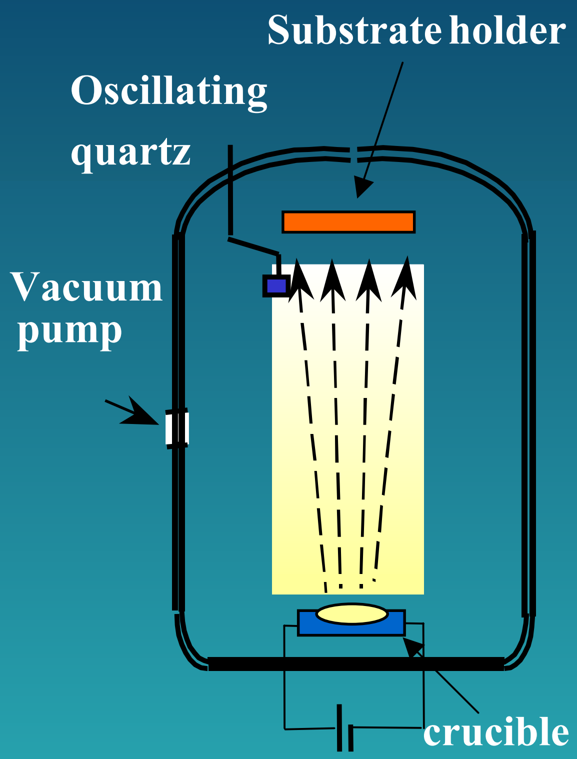
LiF optical image

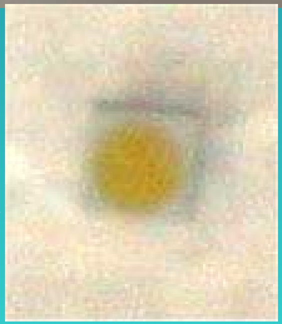


PMMA optical image

Detail of PMMA at AFM

LiF film deposition by physical vapour deposition



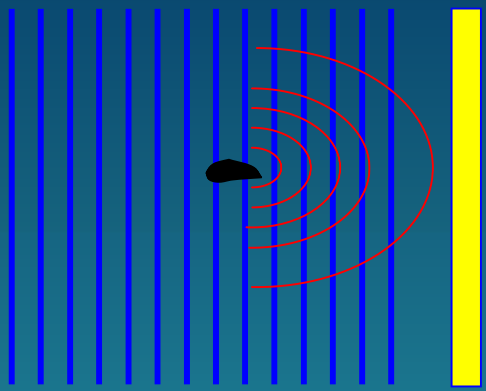


Deposition parameters

Pressure P < 10-6 mbar
Evaporation rate R = 0.5-2 nm/s
Total film thickness t = 5 nm - 5 μm
Substrate temperature Ts = 250 °C

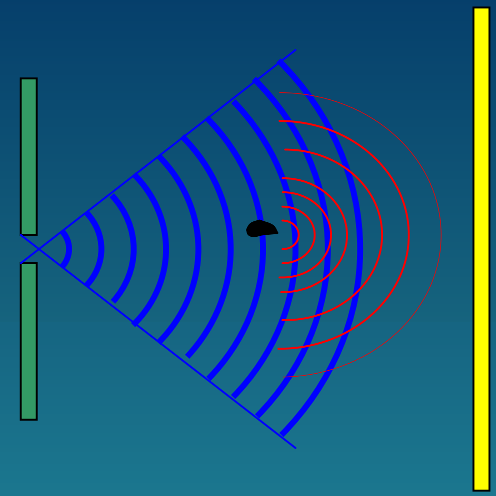
X-ray holography with coherent radiation

Gabor in-line holography



detector

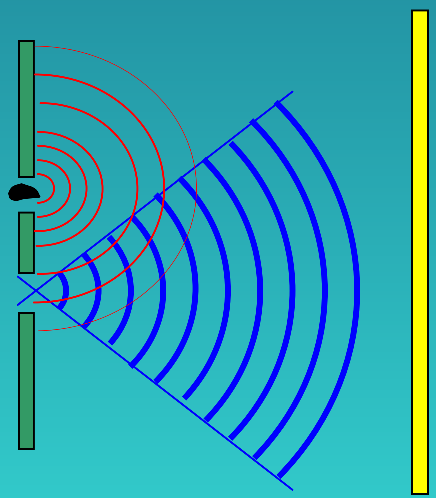
- The reference beam is a plane wave.
- No need of optical elements
- The detector must have a good spatial resolution.



detector

- The reference beam is a spherical wave
- Need of optical elements
- The detector’s spatial resolution is not crucial.

Fourier Transform holography



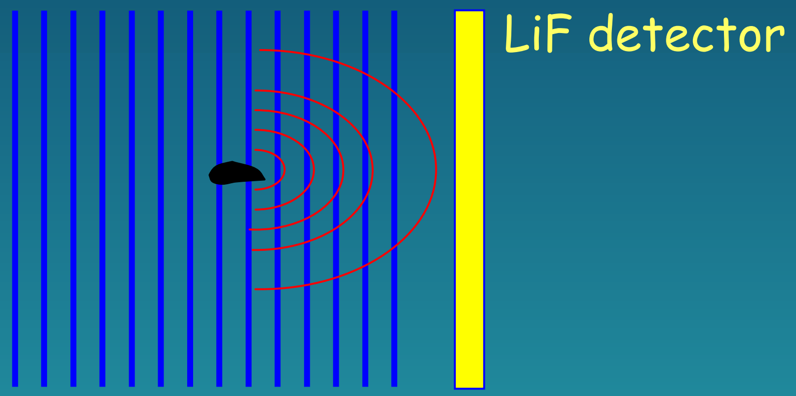
detector

- The reference beam (spherical wave) is placed in the same plane as the object
- Need of optical elements

The resolution of the reconstructured images depends on the information recorded in the hologram which is limited by thesize of the detector and its resolution

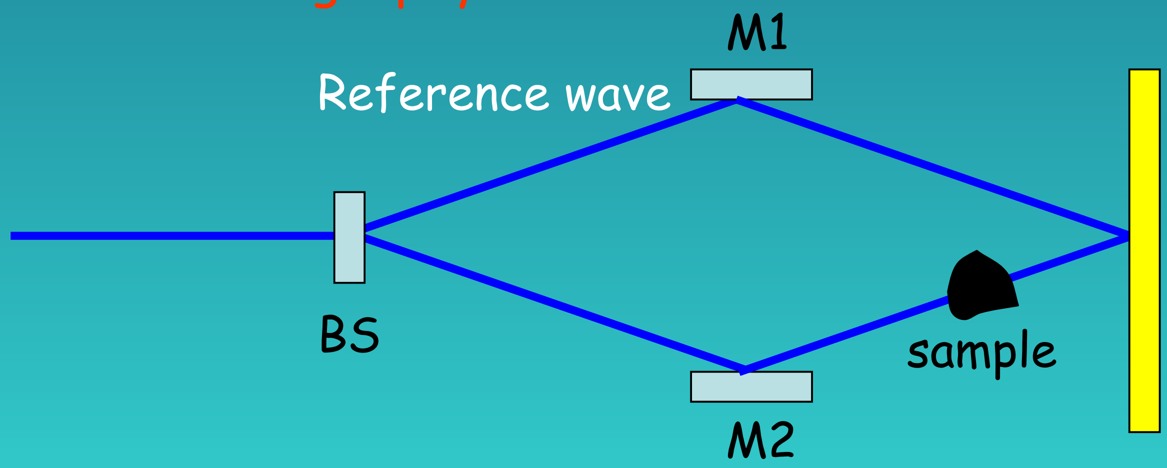
Single Shot X-ray holography with coherent X-FEL radiation on LiF detector

Gabor in-line holography



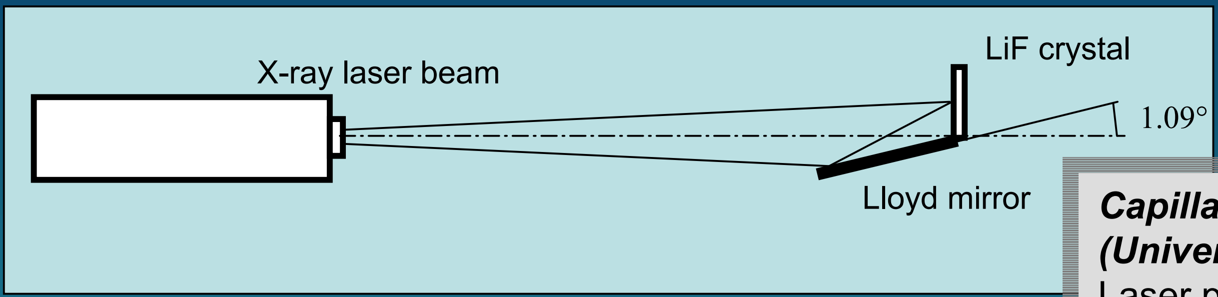
Combining the coherence (30-45 μm) of the X FEL beam and the peculiarity of the LiF detector (spatial resolution and dynamic range) fine fringes can be stored in the hologram plane and a high spatially reconstructed images can be obtained in a single shot experiment

Off-line holography



Due to the coherence of the beam, more complex holographic circuit can be proposed.

Interferometric pattern on LiF by capillary discharge pumped soft X-ray laser



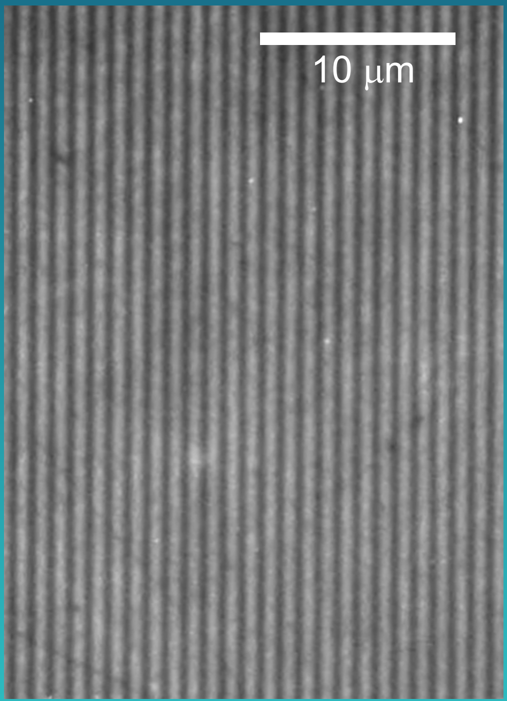
Capillary discharge pumped Soft X-ray laser (University of Aquila)

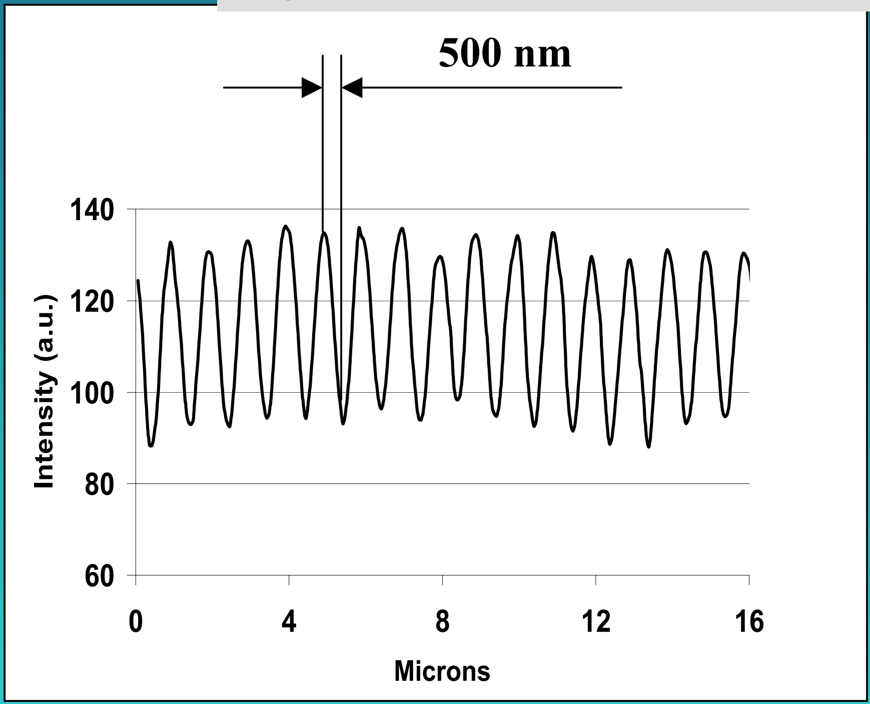
Laser pulse: 0.1-0.2 mJ/pulse

Δt: 2.3 ns

λ: 46.9 nm

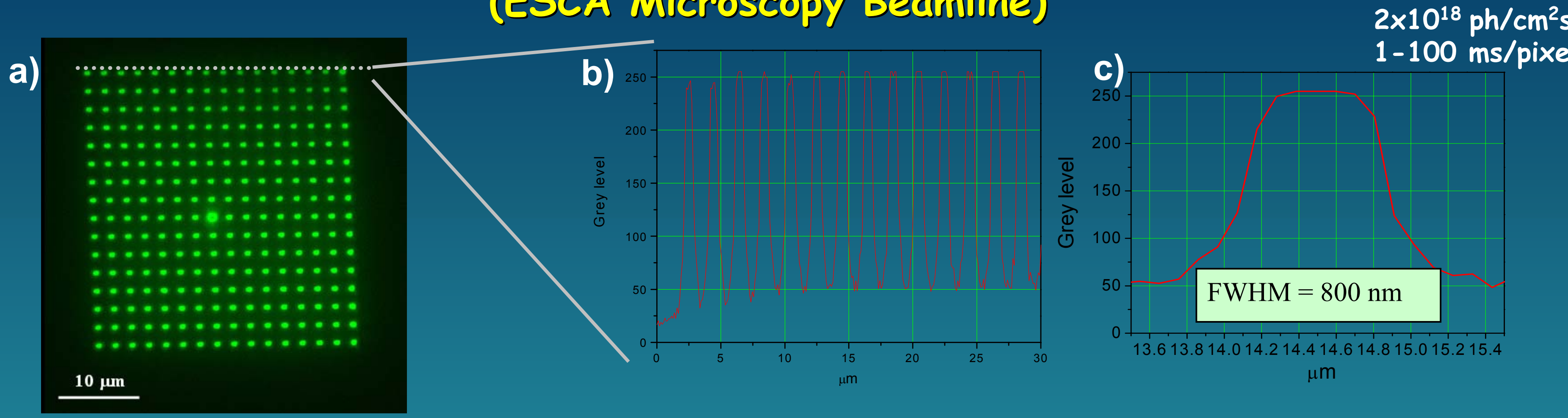
Repetion rate: 0.1 Hz



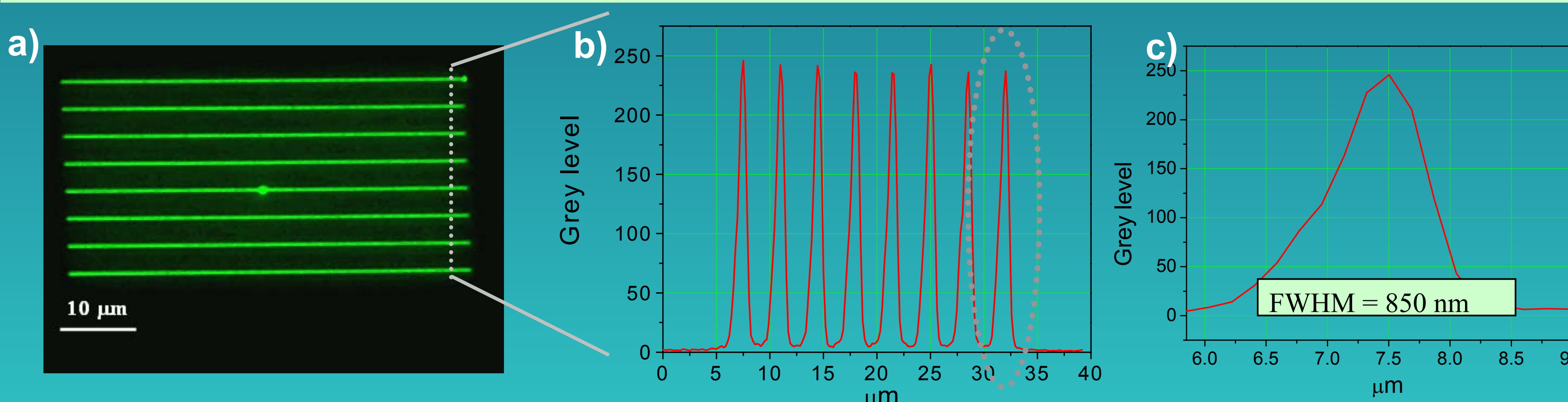


Courtesy of University of Aquila: Prof. A. Reale, Prof. G. Tomassetti, Dr. A. Ritucci

Periodic luminescent patterns on LiF produced by X-ray microprobe irradiation at the *ELETTRA* Synchrotron (ESCA Microscopy Beamline)



A typical fluorescent grating based on CCs directly written on a 324 nm thick LiF film on Si, E=450eV. CLSM (Nikon) image a), its intensity profile, b) and detail of a single line, c).



Fluorescence microscope (Leica) images of a regular array (32x32) μm^2 of 16x16 dots 2.0 μm spaced, realized on 324 nm thick LiF film on Si, a), its intensity profile, b) and detail of a single line, c).

CONCLUSIONS

We 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 charactestics.

LiF detector peculiarities

- High dynamic range (10-12 bits)
- High resolution
- Efficient detection and readout process (OSL)
- Large field of view
- No development needs
- Compatible with permanent protective layers