DENSE PLASMA FOCUS USE FOR X-RAY PULSED BACKLIGHTING AT MICROSCOPIC RADIOGRAPHY OF BIO-OBJECTS IN A COURSE OF THEIR VITAL ACTIVITY

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Introduction

Spatial resolution of various methods of visualization of microscopic objects is restricted by the wavelength of the backlighting radiation

Because of this it is impossible to see with optical microscope any object having size notably less than 1 micrometer

Moreover visible light is absorbed within many of these micro-objects in a very high degree

Modern microscopy methods – electron microscopy and scanning probe microscopy – achieved during the last 10-20 years impressive results in visualization and investigation of a *surface* of various miniature objects

Spatial resolution of these methods is on a ***sub-nanometer level***; moreover, scanning probe microscopy, used in two modifications – atomic-force and tunnel microscopy, – allows measuring not only the surface profile, but also local friction forces, adhesion degree, elastic and viscous properties of the surface, and chemical element contents of objects under investigation as well

Side by side with advantages, which are specific for each of the above-mentioned methods, these contemporary means of micro-object investigations have also their intrinsic drawbacks

E.g. the electron microscopy method doesn’t give high precision in measuring of the relief tallness

Electron and tunnel microscopy demand using of electro-conductive surfaces or their preliminary metallization

Atomic-force microscopy can lead to production of contact deformations on some not too rigid objects and as a consequence to various artifacts appearance

At the same time the ***general features*** of all above-mentioned methods are:

- Gaining of information related only to the properties of a ***surface*** of an object under investigation
- Using a ***substrate*** (e.g. an atomically smooth surface of a chip of mica)
- Applying of adequate methods of ***immobilization*** of structures under investigation upon the substrates

These preparation processes of a bio-object to its scanning ***do not allow counting the objects live***

All these features lead to a search of new more informative methods of investigation of microscopic objects and in particular of living matter

In principle, it is clear that using of ***soft X-Rays*** may deliver various data on ***internal structure*** of tiny little bio-objects

It could be done in a manner as it is realized by the help of hard X-Ray radiography operating with ***macroscopic*** objects having ***strongly discriminative sub-structures*** (e.g. bones and soft tissues)

The problem is how to make this technique applicable to a ***very small*** bio-objects (preferably of micrometer's and nanometers' scale) having almost ***homogeneous*** internal composition

Visualization and contrasting in X-Ray micro-radiology

Generally speaking electromagnetic waves (including soft X-Rays – SXR) passing through matter may be changed in their ***frequency, amplitude, phase, polarization***, and ***direction*** of its propagation

Frequency change is a non-linear effect not observing at present level of radiation power in X-Ray range; as for changes of other e/m wave characteristics, ruled by ***permittivity*** of materials, the corresponding effects are: ***absorption, refraction*** (+birefringence), ***diffraction*** (+interference), and ***polarization change***

Unfortunately, real part of the refraction index in the X-Ray range for the majority of elements is close to unity and imaginary part of it – to zero; it means that propagation velocity of SXR inside objects is close to the speed of light in vacuum whereas absorption is negligible

It results in an ***infinitesimal angle*** of ***refraction*** and in a ***very low contrast*** of ***shadow*** pictures of images correspondingly

In particular, the situation becomes even more complicated in relation to ***microscopic biological*** objects, which consist of elements with low atomic number, saturated by water with high concentration

And what's more – the difference between various macro-molecules and bio-structures one from another and from water by the effective charge (that determines absorption) is very small

All of this makes utterly difficult visualization of an object in X-Rays and leads to a ***very high necessary dosage***, usually resulted in a ***partial evaporation*** of the object ***during*** the radiology process

To increase contrast of its details and decrease radiation load onto the object we have to resort to various tricks

The most important between them are as follows

- ***phase contrasting*** of an image using ***refraction with interference*** of refracted photons with the straight beam; it is achievable with the beam of photons from a quasi-monochromatic source within the region of its spatial coherence according to the theorem of van Cittert-Zernike:

$$dh/a < \lambda/2;\ d < \beta a/2b$$

where d – size of the source, h – size of the object under visualization, a and b – distances from the bio-object to the source and to the X-Ray film correspondingly, λ – wavelength of the radiation used for X-Ray examination, β – shift of the refracted beam at the image plane (fringe-shift)

- the same due to *Fresnel **zone** and **phase** plates*
- the same due to ***diffraction*** on micro-structures:

$$\beta \sim b\lambda/h$$

One can add to the above-mentioned methods the following means:

- the same as above, but because of SXR *grazing incidence **reflection*** from the object surface at the border of two media (e.g. water and cells)
- use of a ***high contrast γ*** – i.e. a sharp slope of the characteristic curve of the film, when small change in the radiation exposure ***H*** results in the drastic change of the film's internal transmission density ***D***:

$$D = \gamma \cdot lgH$$

- use of a strong dependence of the ***Bragg's reflection coefficient*** on the SXR angle with the crystal (operation at the slope of the beam swinging curve of a crystal) at the low-angle refraction of the beam inside the object under investigation, when the crystal is positioned after an object
- the same due to ***polarization change*** measurements of the polarized SXR (previously reflected from the first crystal) accomplished with the help of the second crystal
- use of ***characteristic frequencies and absorption bands*** (e.g. *K*-, *L*-… shells or edges) as well as "transparency windows" of materials preferably at the slopes of their wavelength dependencies

Nanosecond X-ray micro-radiography and surviving of bio-objects

Side by side with the above-mentioned methods that decrease demands to an intensity of a source and preserve an object from high radiation doses, there is another approach to the problem; it lies in use of ***ultra-short X-Ray pulses*** for micro-radiography

Very short pulses give an opportunity to irradiate any photomaterial “simultaneously” by all photons of the X-Ray flash – in the sense that the photon action interval is comparable with the process of formation of centers of a hidden image

At this "***collective***" action of photons because of various *synergetic* effects the irradiation duration, which is necessary for the exposure of a photomaterial, is decreased by several times thus automatically decreasing the necessary dose

New generation of modern radiation devices can produce pulses in the femto-…nano-second time range, thus the well-known term "flash radiation chemistry" could be applicable now in ***its perfect sense*** to the interaction of radiation beams with objects, provided that the time of this interaction is *short compared with the duration of corresponding physical processes or chemical reactions*

We shall operate now with beam ***energy*** and ***power flux density*** of the irradiating beam at the target, which are the **'physical'** parameters of an irradiation process

Taking into consideration ***mean free path*** and ***pulse duration*** of the X-ray beam photons within a biological tissue, we shall eventually discuss the problem in terms of ***concentration of fast particles within the biological object during the irradiation process,*** which are the **'chemical'** characteristics of the process

The most important point is that during irradiation, a concentration of ***"effective interaction micro-volumes"*** (spurs and blobs) should be sufficiently dense to ***allow micro-volumes to overlap*** each other

And as it was mentioned above, this condition should be realized ***during a time interval*** (radiation pulse duration), *which is* ***less compared with the corresponding biochemical process***

Besides the process of visualization as a whole takes a time interval short compared with the period of ***evaporation*** and ***spread*** of the irradiated object (i.e. during the ***inertial confinement time*** of matter)

So in this case our bio-object hasn't be moved or changed during the exposure period

However some new problems of gas-dynamical description of the process of a ***collective volumetric action*** of SXR photons upon matter and the conception of a bio-object's ***"death"*** at its pulsed irradiation are arisen

It appears that the ***survival threshold*** depends not only on ***a dose*** of X-Ray radiation, received by a bio-object, but also on the ***dose power***, i.e. on the time of the dose embedding into the irradiated sample

The micro-radiological photographing has to be implemented with **high temporal** ***(pico- and nanosecond)*** and **spatial** ***(micrometer and nanometer)*** **resolutions** and with a **high contrast**

Problems arisen here are as follows

- selection the SXR ***wavelength range*** giving the best contrast and resolution at the visualization procedure
- determining the ***minimal number*** of SXR photons necessary for a picture (with noises consideration)
- selection of the particular ***micro-radiological scheme*** with phase contrasting of an image
- determining the ***dose*** *and* ***dose power thresholds*** in relation to the bio-object's ***survival***
- clarifying the ***conception of "survival"*** for a different level of bio-objects organization
- elaboration a SXR ***source*** and a ***registration (visualization) system*** satisfying the above-mentioned demands

Mean charge of elements in the contents of bio-objects is not very high; so the range of SXR photon energy for objects with ***characteristic size less than 1000*** nanometer (DNA, viruses, intra-cell structures, etc.) is ***below 1 keV (the wavelength range 1-100 nm)***

The necessity to ***distinguish these objects with water*** results in use of the SXR photons within the range ***2.3-4.4 nm (water transparency window)***

For structures with a size of ***units*** and ***tens of μm*** (e.g. cells, the smallest organs of insects, etc.) the optimum energy of X-Ray photons lies ***near 1 keV***

For greater objects (***up to 1 mm***) it is necessary to use SXR in the range from ***1 to 10 keV***

- Best ***X-Ray photo-materials*** have ***spatial resolution*** on the level of ***1 μm***; usual figure for their ***sensitivity*** is circa ***10^10 photons/sq.cm*** (about ***1-10 μJ/sq.cm***)

- ***Below the resolution of 1 μm*** one has to use ***photoresists;*** these materials provide higher spatial resolution (restricted by size of monomers, which they are consisted off – circa ***a few nm***); yet they have much lower sensitivity (from ***1 J/sq.cm*** for PMA till ***10 mJ/sq.cm*** for the resist with chemical amplification, e.g. SU-8); it means that in this case demands to the SXR source power increase by a very high degree

Sources able to generate such SXR photon beams may be based on ***X-Ray tubes***, ***classical accelerators***, including synchrotrons, ***isotopes***, ***X-Ray lasers***, and ***plasma sources***, in particular, ***Dense Plasma Focus*** (DPF) and ***laser produced plasma*** (LPP)

However X-Ray tubes, X-Ray lasers and synchrotrons have ***low power*** and cannot produce SXR pulses of nanosecond time duration, which would be ***intensive enough to take a "single-shot" X-Ray image***

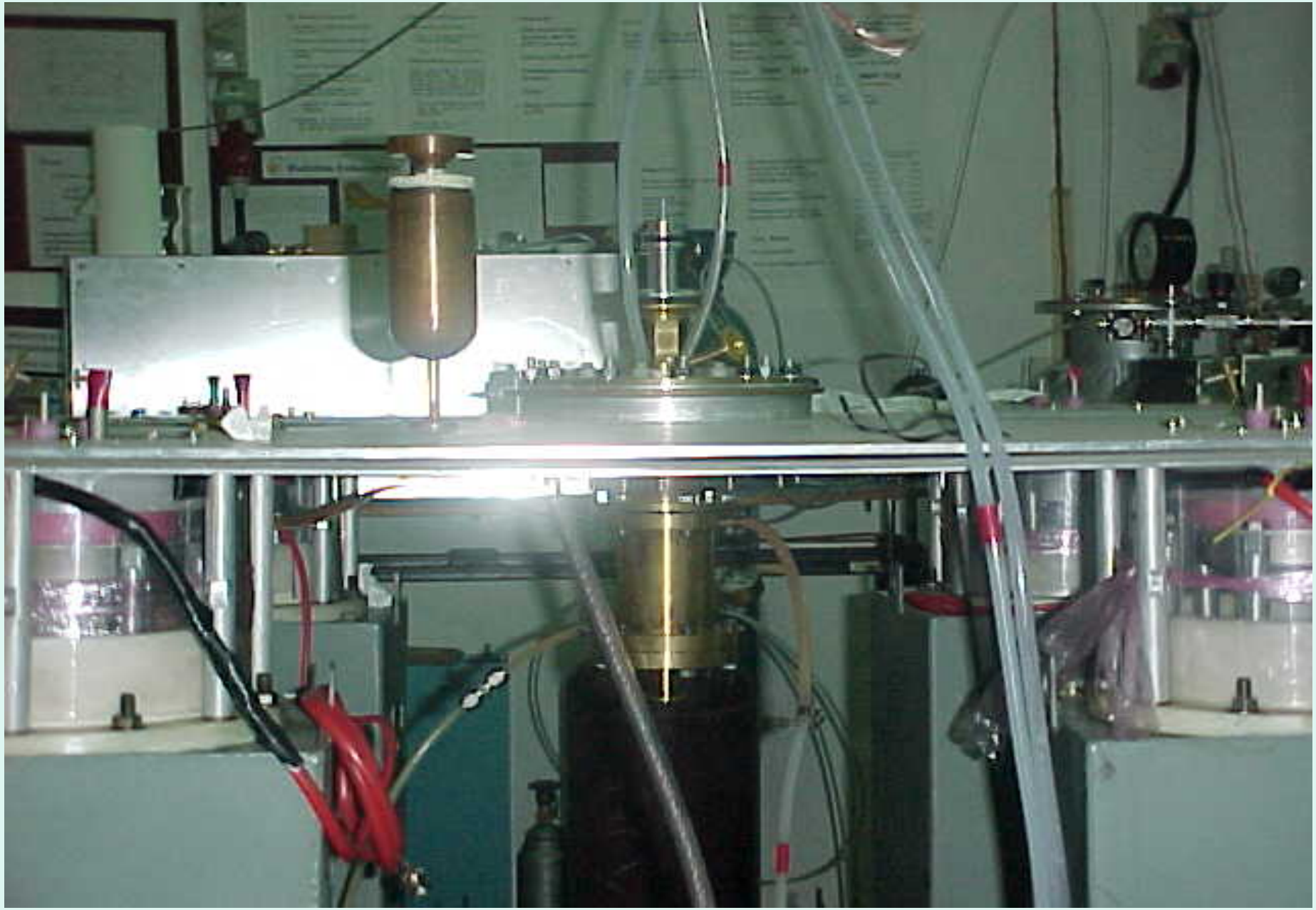
High-current accelerators are ***cumbersome, expensive***, and they cannot operate in a ***repetition rate mode***

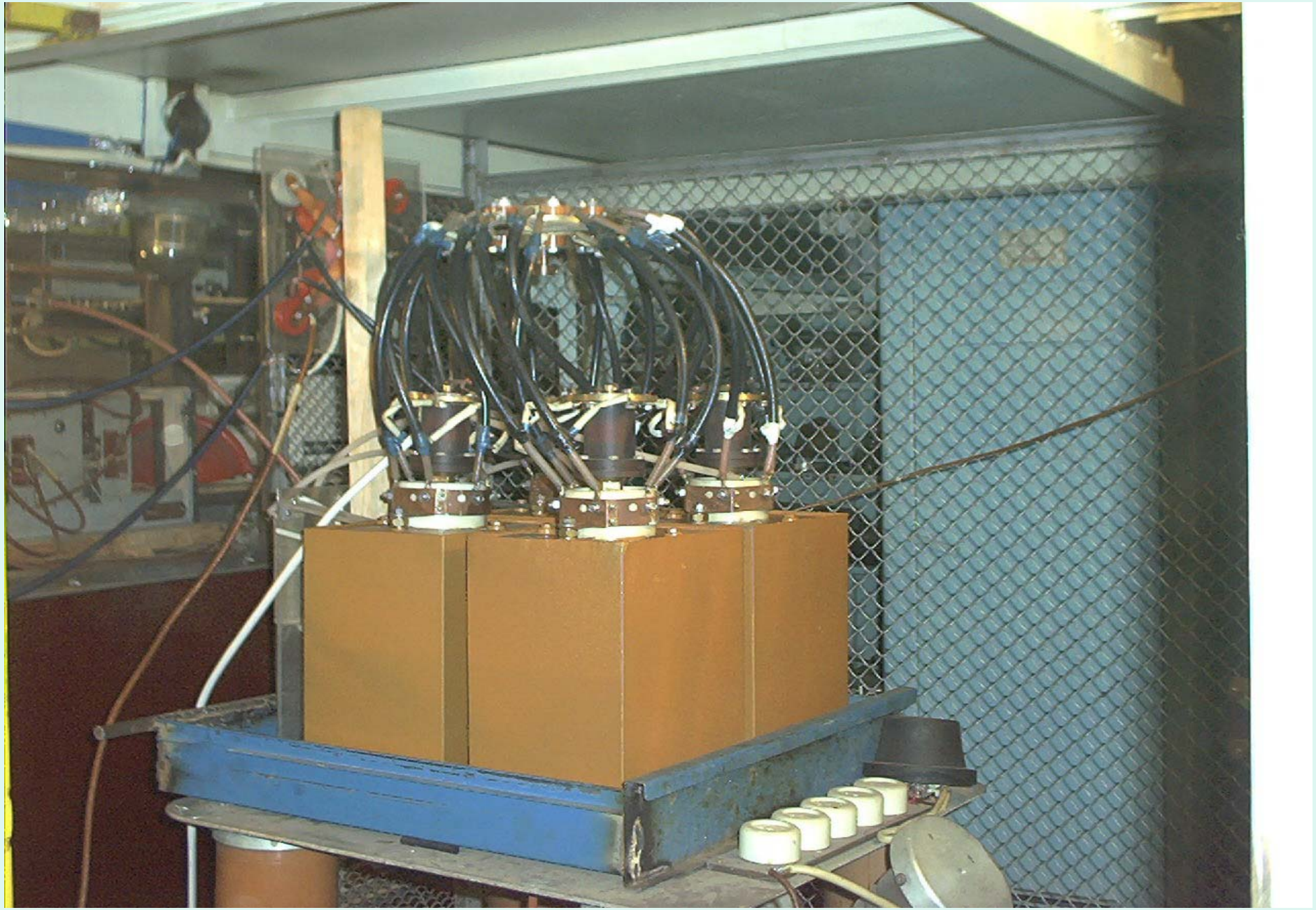
We shall concentrate here our attention namely on the DPF device, which possess necessary features

Apparatus

We have used for ***X-ray backlighting*** of different microscopic objects three DPF:

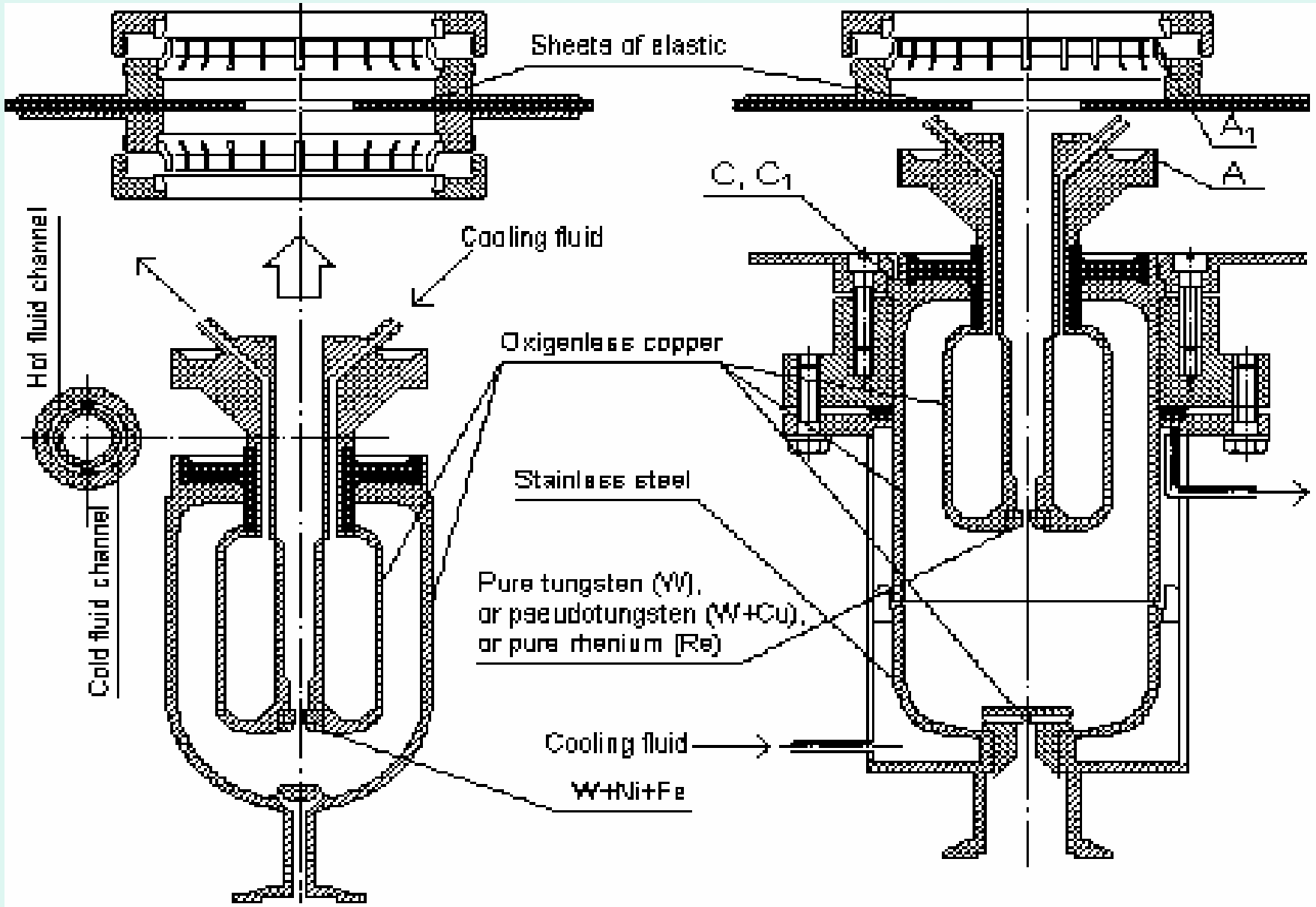
- **NX1**, belonging to the ***Institute of Education, Nanyang Technological University, Singapore***, (2.5 kJ, 250 kA), has *vertical DPF chamber*
- **PF-6**, designed at the ***Institute of Plasma Physics and Laser Microfusion, Poland*** (7 kJ, 750 kA), has a horizontal direction of its chamber
- **PF-5** ***(Institute of Theoretical and Experimental Physics)*** is a device (5.7 kJ, 450 kA), which has much lower weight (150 kg) than the above two





All of these devices have special chambers cooled by water and giving a possibility to irradiate SXR photons through a hole made in the centre of its anode in the ***upward direction*** (except PF-6); it gives the following advantages:

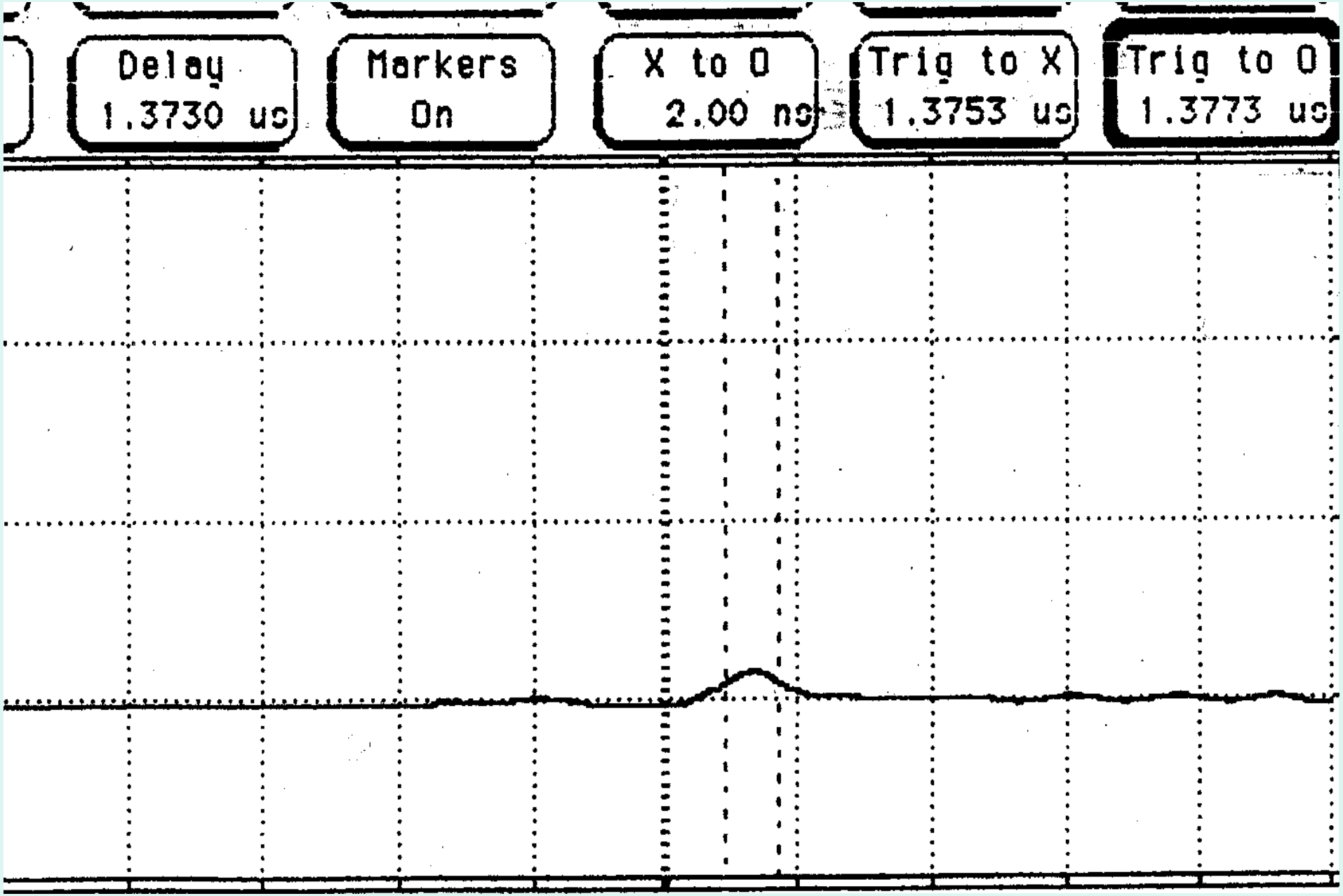
- ***electron beam*** (same propagation direction) can be deflected by a magnet much easier than the ion beam propagated in the opposite direction
- ***metal debris*** produced in each DPF shot may not fall down to the outlet window covered by berillium or mylar foil; it is so because the gravity force is directed downward whereas our outlet window is positioned in the upward direction

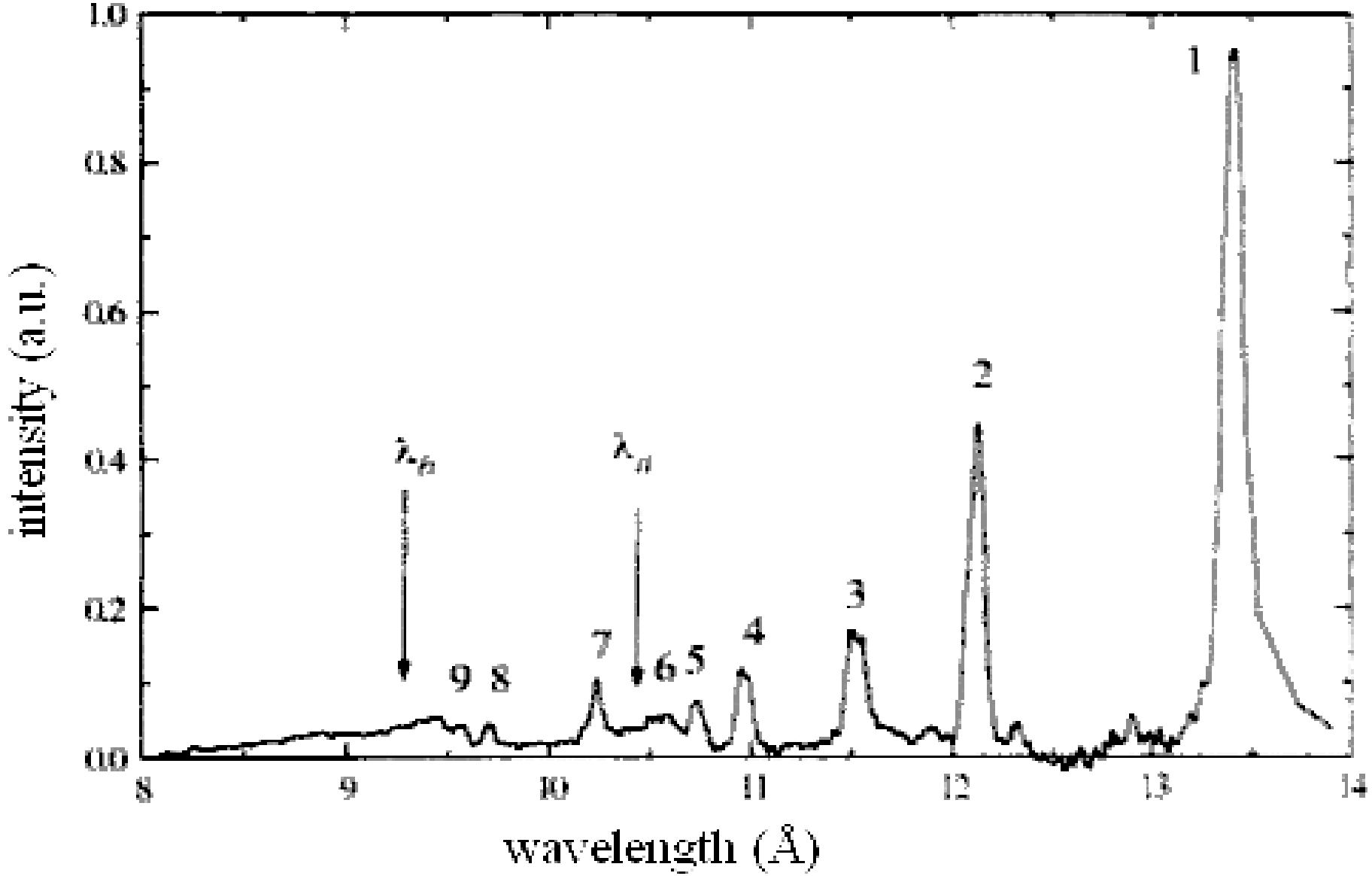

Sheets of elastic
A1
A
C, C1
Cooling fluid
Hot fluid channel
Oxigenless copper
Stainless steel
Cold fluid channel
Pure tungsten (W),
or pseudotungsten (W+Cu),
or pure rhenium (Re)
Cooling fluid
W+Ni+Fe

These devices can generate SXR pulses of ***nanosecond*** time duration with narrow bandwidth

E.g. the ratio of the FWHM of the main line to its wavelength is $\Delta\lambda/\lambda \cong$ **0.015** near $\lambda \cong 13.5$ Å for the **NX1** device operating with neon as a working gas

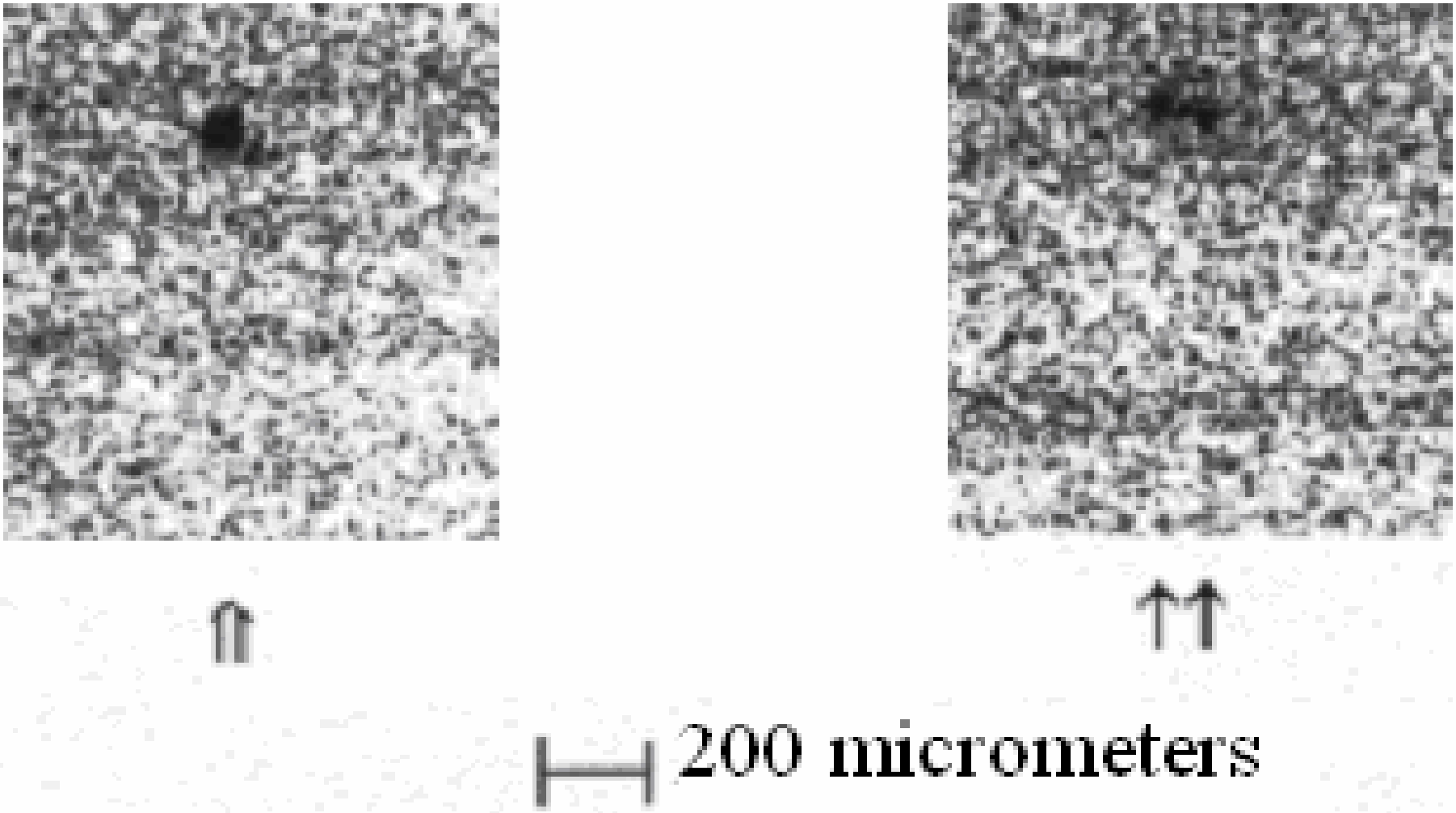
It gives the ***coherence length circa 0.1 μm*** for the main (brightest) spectral line – the resonance line of the H-like neon ion


Delay
1.3730 us
Markers
On
X to O
2.00 ns
Trig to X
1.3753 us
Trig to O
1.3773 us


1.0
0.8
0.6
0.4
0.2
0.0
intensity (a.u.)
8
9
10
11
12
13
14
wavelength (Å)
λ_6
λ_n
1
2
3
4
5
6
7
8
9

Depending on the working gas pressure and some other parameters DPF can operate either in ***pinch regime*** or in so-called ***"hot-spot" mode***

In the first case (with neon as a working gas) the ***spatial size*** of the SXR source has a diameter about **100 μm** (with **100 J** irradiated into a solid angle) whereas in the second mode it decreases down to a **few μm** (with a **few J** in a 4π-angle)


200 micrometers

When the ***higher energy of the SXR photons*** is needed for a radiographic experiment it is possible to use two possibilities

First is the use of ***higher atomic number gases or their mixtures*** as a working gas: argon, krypton, etc.

Second opportunity is given by the fact that the very powerful ***electron beam*** generated in a DPF is self-focused on the anode surface with a focal spot of a few hundred μm thus producing a ***point-like source*** of strong radiation of characteristic lines of the anode material (*K*-, *L*-…lines)

E.g. *Cu Kα* line having $\hbar\omega \cong 9\ keV$ may be irradiated with a very high efficiency (higher than 1%)

Just opposite to this case - when the ***lower energy of X-Ray photons*** is demanded - surprisingly also the ***same*** methods may be used

But in this case - gases with ***much higher*** atomic numbers are used to excite ***lower degrees of ionization*** of the gases (e.g. Xe for production radiation with the wavelength 11.3 nm), or ***lower atomic number elements*** on the anode are utilized (e.g. carbon to produce radiation in the "water window" bandwidth)

In current experiments to test this method we use **neon as a working gas** for DP foci working in the hot-spot mode of operation to minimize the source size for better spatial coherence

Experimental scheme

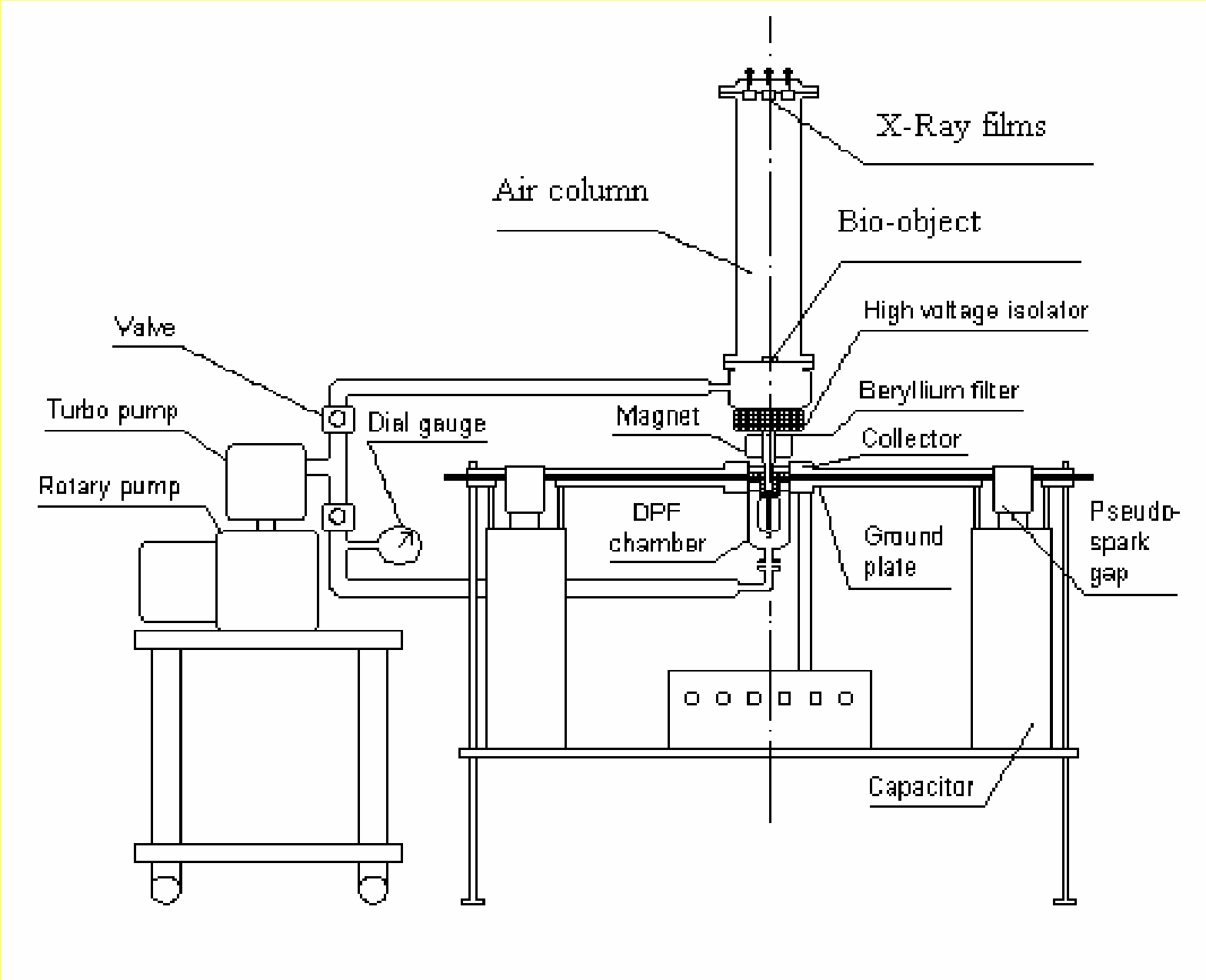
SXR radiation was extracted from the anode pipe covered by a vacuum-tight ***beryllium foil of 10-μm thickness***

We use ***aluminum foil*** to wrap up a small piece of a usual ***X-Ray film*** to preserve it from the visible light; thickness of the foil was ***7.5 micrometers***

Distance from the source till the object was about 20 cm

Objects for radiography were positioned at ***various distances*** from the outlet window depending on the structure size intended to be visualized with accordance to the ***above formulas***

Sensitivity of our X-Ray film was circa 10^9-10^10 photons/sq.cm; it is more than enough to visualize our object in spite of high absorption coefficient for 1-keV photons in both foils (Be and Al), in neon gas filled the space between the SXR source and the outlet window and in the air column separated an object and the film


X-Ray films
Air column
Bio-object
High voltage isolator
Valve
Beryllium filter
Turbo pump
Dial gauge
Magnet
Collector
Rotary pump
DPF
chamber
Ground
plate
Pseudo-
spark
gap
Capacitor

RESULTS

We have tested this method in different situations:

- visualizing object sizes from tens of ***micrometers*** down to ***50 nanometers***;
- static and moving (live) objects;
- biological and inanimate (inorganic) items

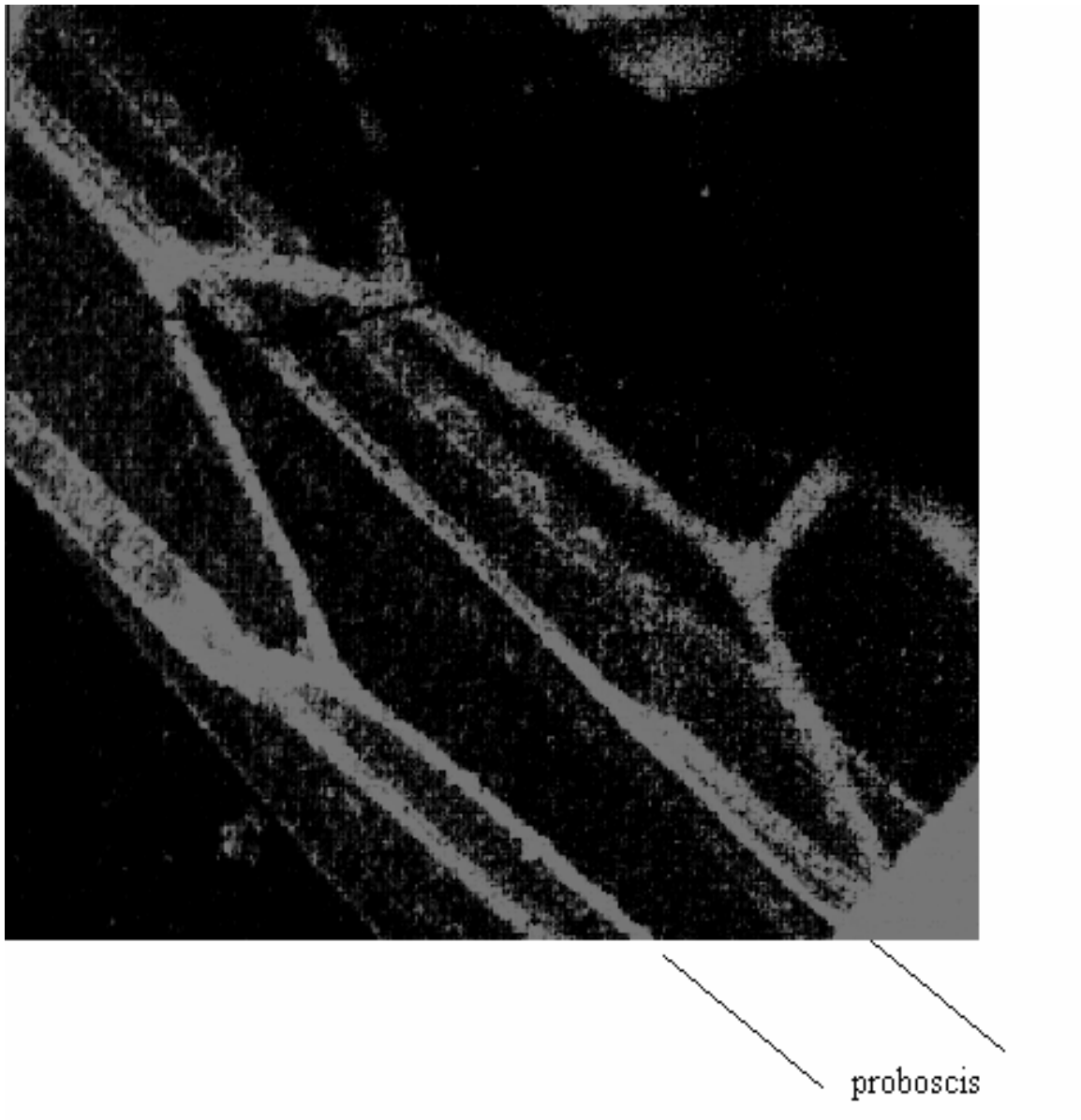
A few examples of the tests are shown in the pictures where X-Ray films are presented with their visualization by an optical microscope *Swift Instruments International S.A.* with lenses 10/0.25, 25/0.4 or 40/0.65 or by scanning electron microscope

Sometimes side by side with these X-Ray films ***real objects*** are presented as they are seen in the same microscope

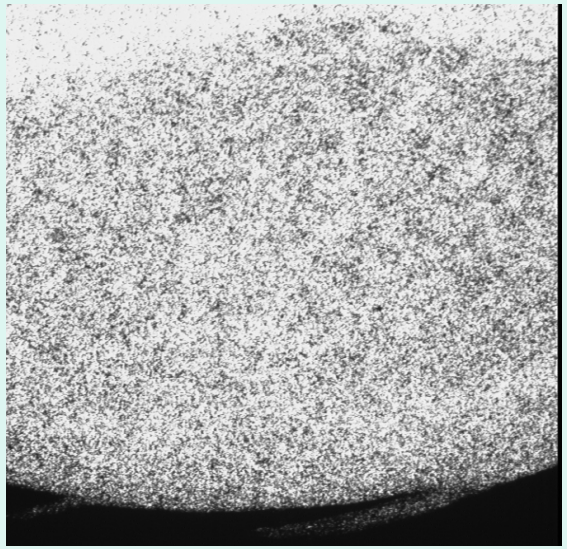
One has to take into account that ***in visible light*** these ***objects themselves*** are seen ***directly*** whereas ***SXR films*** presents ***negative image***

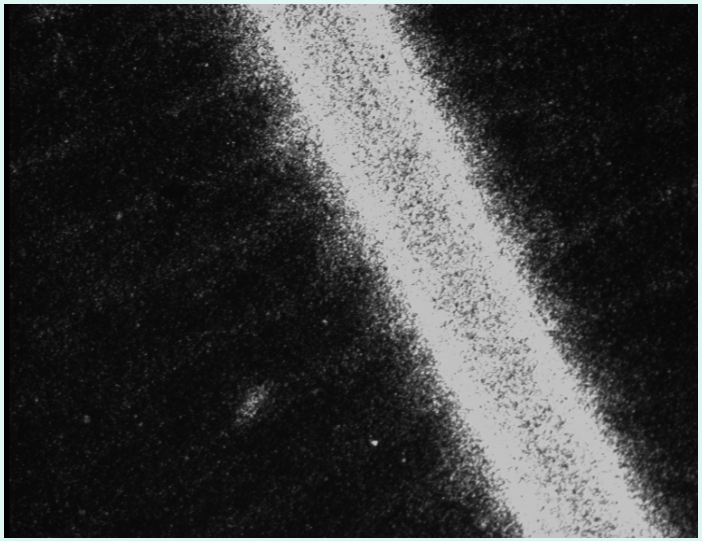
So the black color and the white one on these pictures are ***opposite (additive)*** to each other

A mosquito proboscis in its “open” position

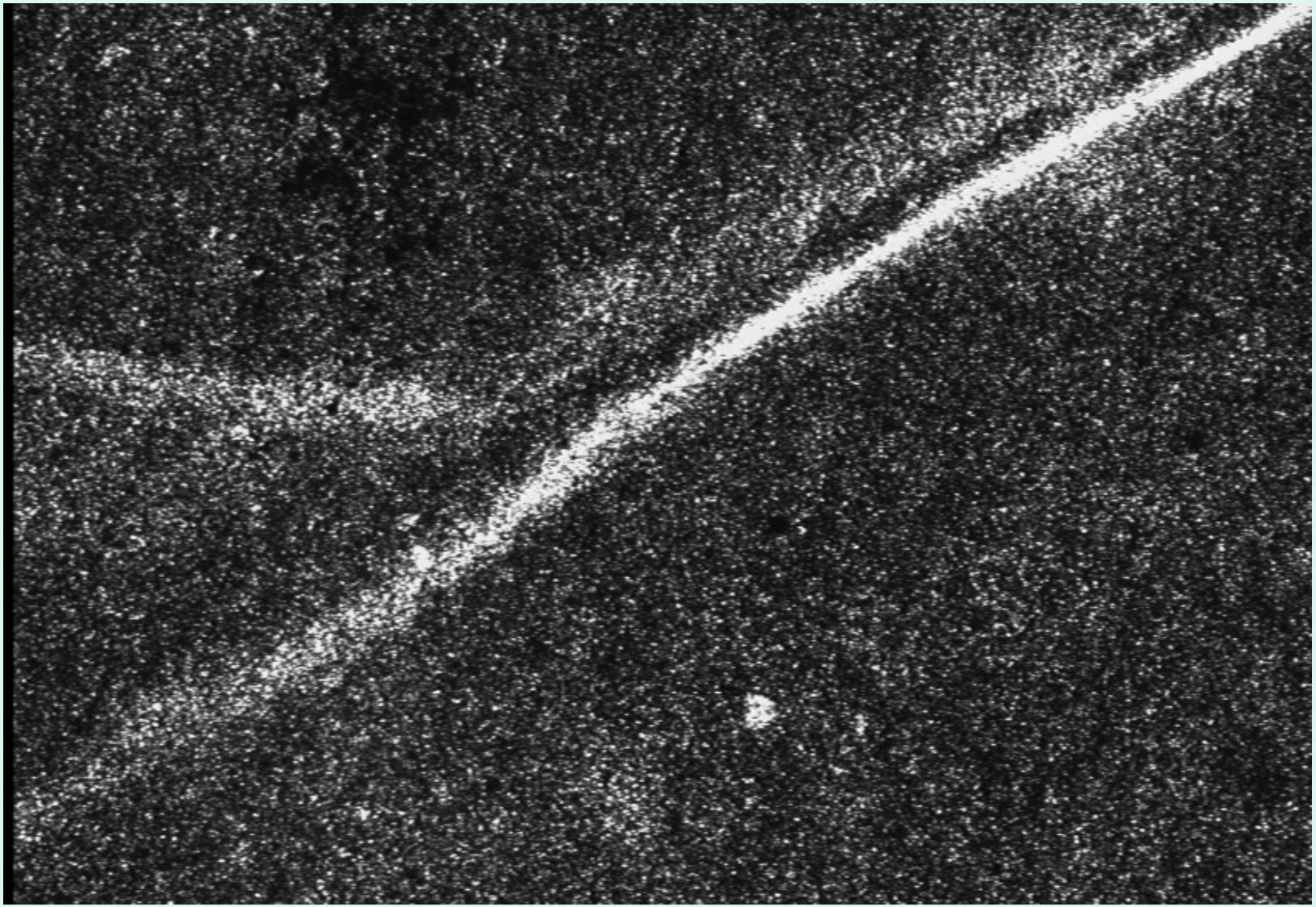


Three pictures of a bird's feather – direct optical image and SXR visualizations of it





An intermediate phase of the closing process of a mimosa leaf

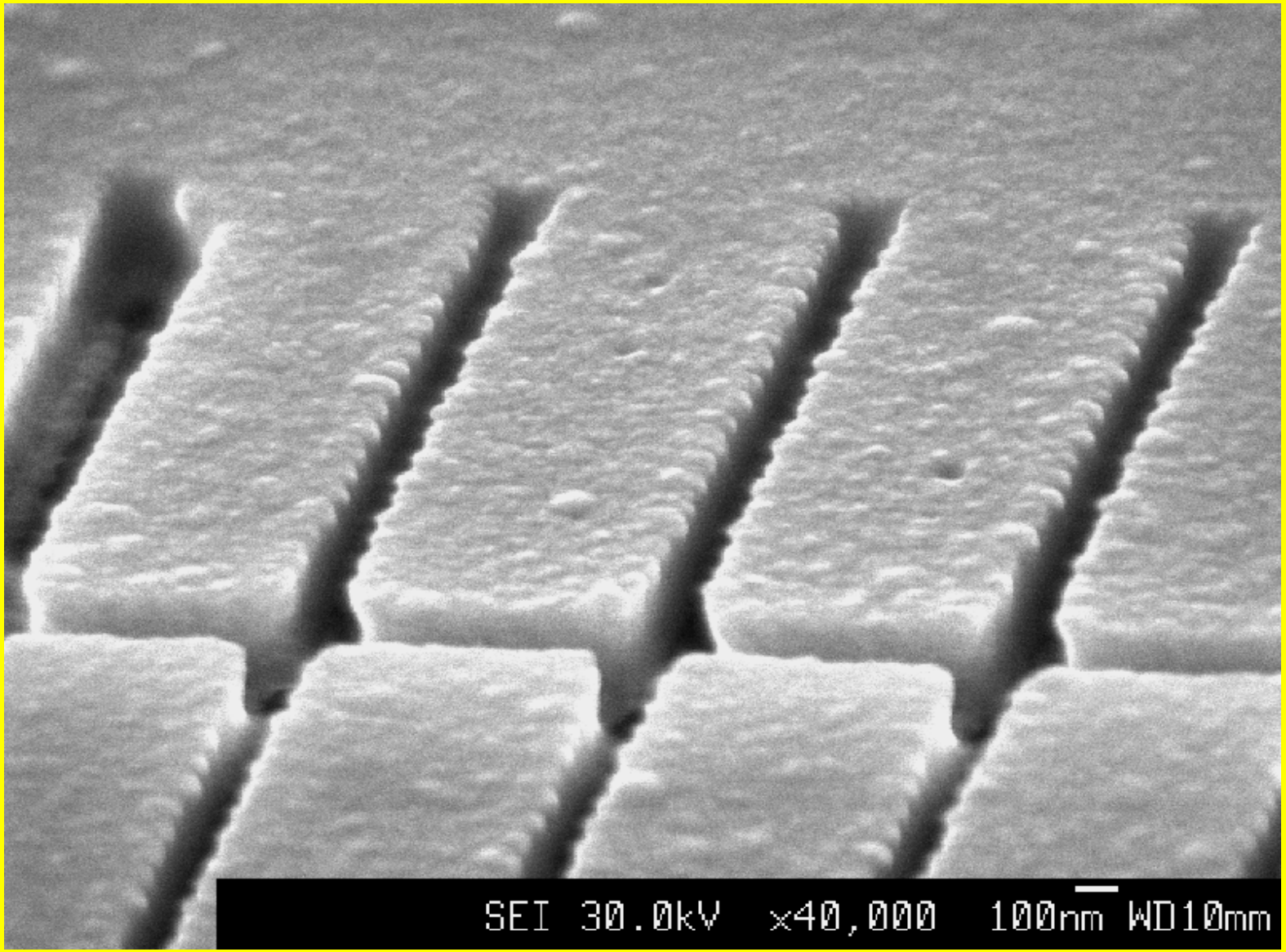


In all these pictures ***spatial resolution*** of images was circa **1 μm**, which has been specially tested by photographing of a golden wire of 15 μm thickness (with and without phase contrast)

When one needs a spatial resolution ***better than this*** various ***photoresists*** should be used instead of X-Ray films; we have tested ***SU-8 photoresist*** with chemical amplification to visualize a special mask made by gold

We've done it in the so-called "***proximity X-Ray lithography***" scheme

Mask on SU-8 photoresist



A dose necessary to produce the image on this resist ***appeared to be several times (almost 10 times) less*** than it was estimated for and checked by classical X-Ray tube; yet we had to produce in our case *about 500 shots* of DPF; **resolution in this case was 50 nm**

We also investigated by these technique ***diamond-like films***, ***embryo of reptiles*** as well as their ***organs***, various ***insects***, a ***butterfly,*** etc.

We intend to present results on these experiments in details elsewhere

DISCUSSION AND CONCLUSION

From the above-mentioned one may see that this method is reliable for the goals of ***micro-radiography of live bio-objects in a course of their vital functioning***

Backlighting of the objects by nanosecond flashes of SXR photons may give information on their activity with a spatial precision up to tens nanometers

We have received this accuracy in present experiments by means of ***500 shots*** (appr. 2 min) with the visualization of a micro-object on a photoresist

But simple estimations have shown that ***if the distance*** from the source till the mask (and resist) would be ***decreased by two times*** and if ***the space*** between the anode outlet hole and the Be foil will be ***differentially pumped*** thus excluding neon gas in this zone it would be possible to decrease the number of shots down to ***a single one*** for the best SXR yield of the DPF of the type **NX1** or **PF-6**

It means that it will be possible to use this method in nanometers range for ***dynamical investigation of bio-objects***

Some other interesting results received here are:

- certain structures, which are ***transparent*** *in* *visible light*, may be partly ***invisible*** *in* *SXR range* thus giving additional information on the imaginary part of the permittivity
- movement of mimosa leaves is ruled by ***water pumping/injection*** within the leaves' tissue;
- diamond-like films grow by disks having ***thickness of the same order*** of dimension as the film itself but their ***diameter is more than ten times larger***, etc.

It is clear that to develop this method of ***pulsed radiography of bio-objects in the sub-micrometer and nanometer ranges in the regime of its inertial confinement*** one must ***improve DPF devices*** and to apply the above-mentioned technique for parallel ***testing of radiation survival of lower level of organization (miniature) bio-objects*** during these types of experiments

ACKNOWLEDGEMENTS

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Thank you!