# X-ray micobeam facility for single cells irradiations

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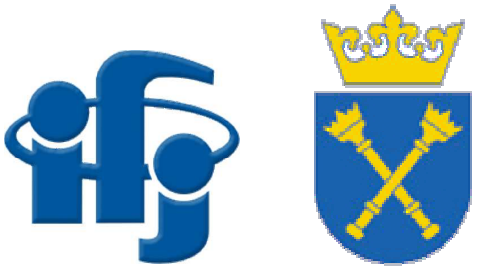

Krakow, Poland

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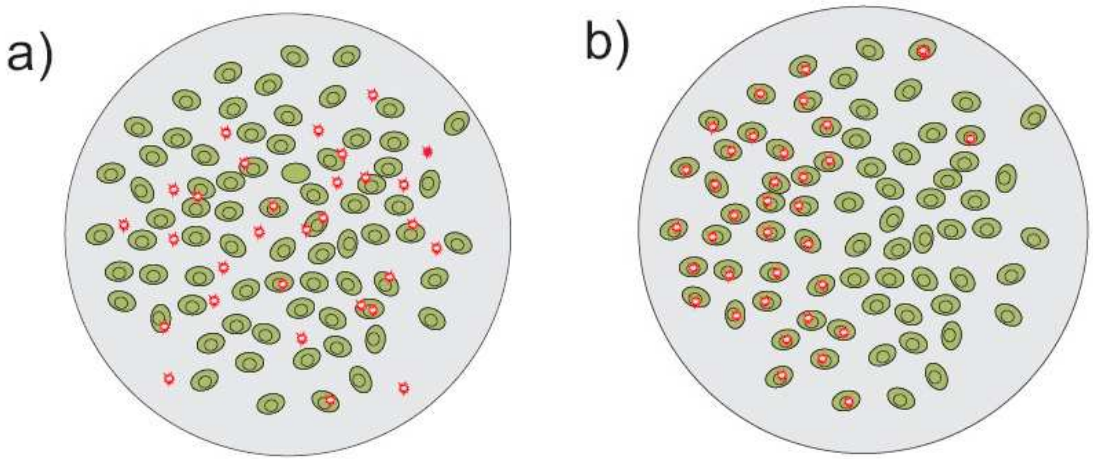
# Outline

1. Motivation
2. Facility overview
3. X-ray focusing
4. System aligment
5. Irradiation procedure movies

# Motivation

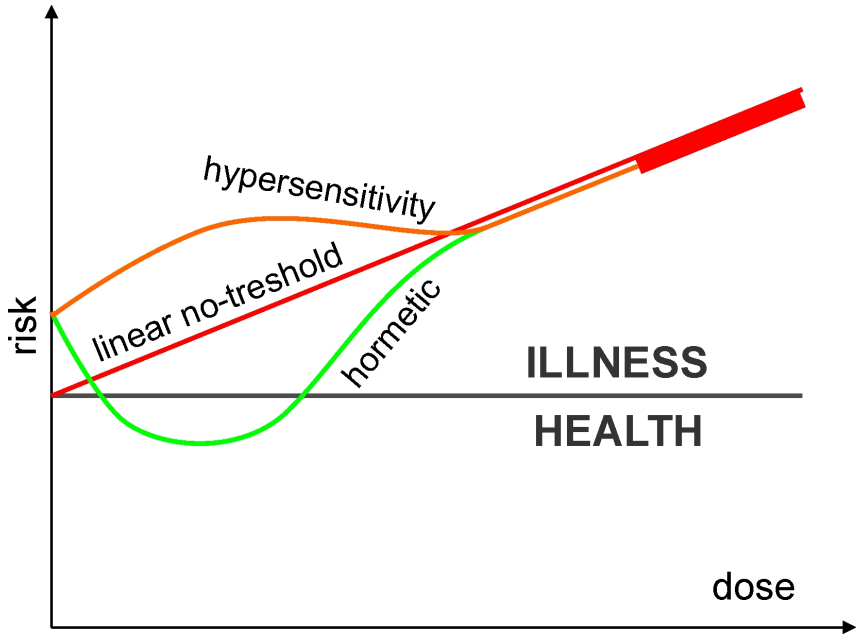
Quantitative analysis of the response of living organisms to the **radiation** at the **cellular level** is facilitated with **microbeams**.




Cells irradiated with

a) classic source

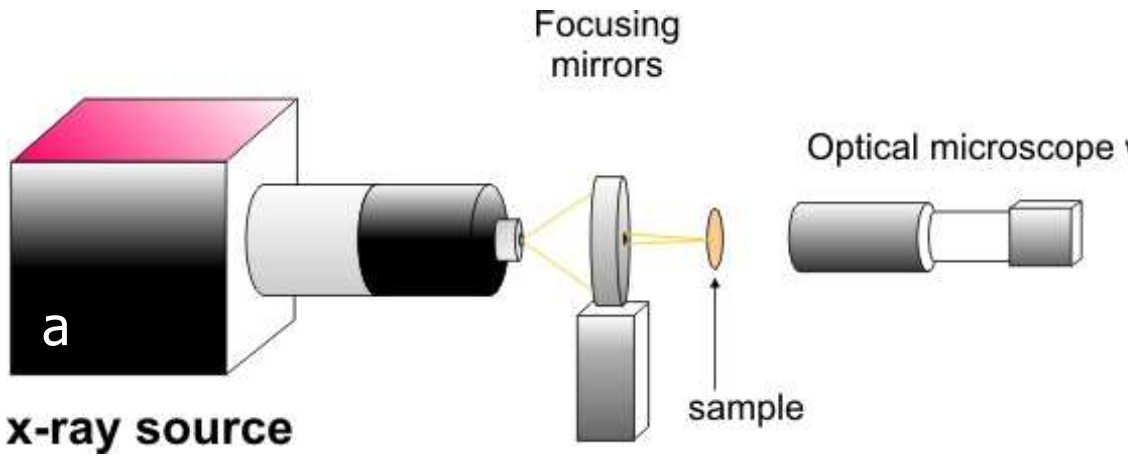
b) microbeam




A microbeam enable to study:

- ❑ adaptive response
- ❑ bystander effect
- ❑ kinetics of DNA damage repair

# Facility overview




**Open-type x-ray source**

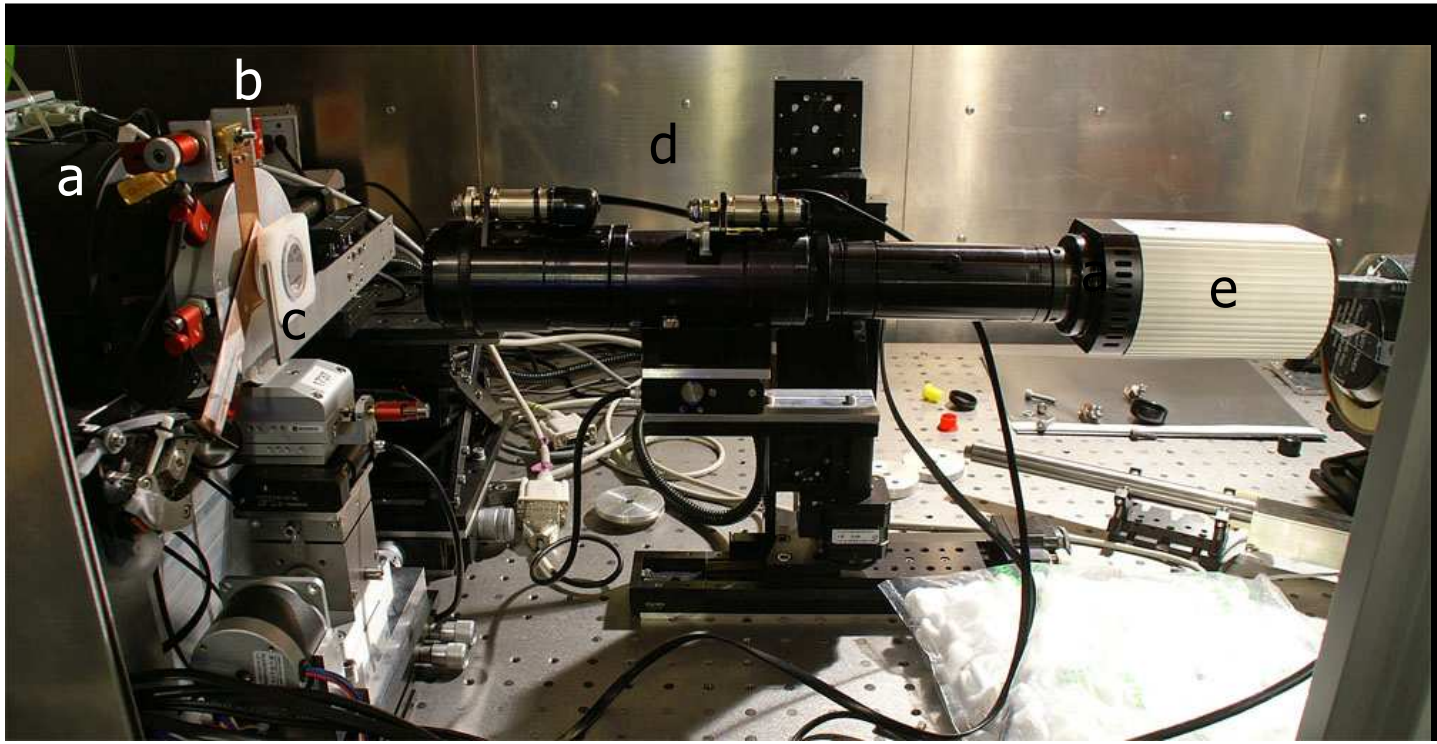
Titanium anode $K_\alpha$ of 4.5 keV

Accelerating voltage 20-160 kV

Target current 0.1 - ~24 uA

120° cone beam

X-ray spot emission ~ 3 μm



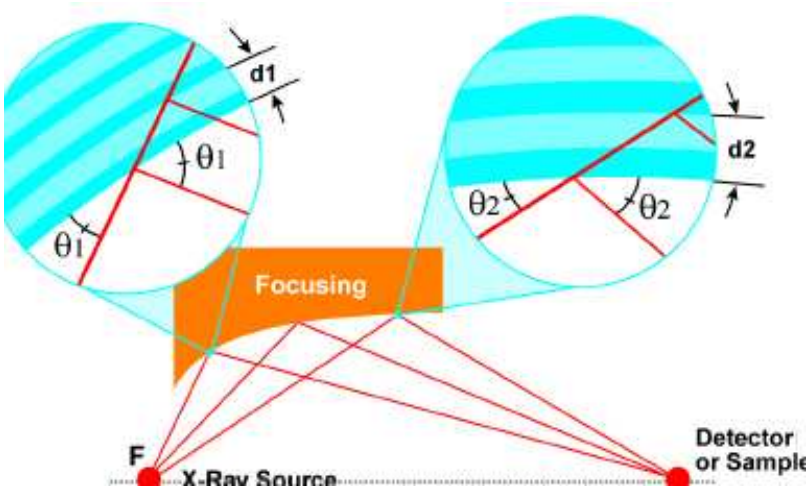

e – digital camera

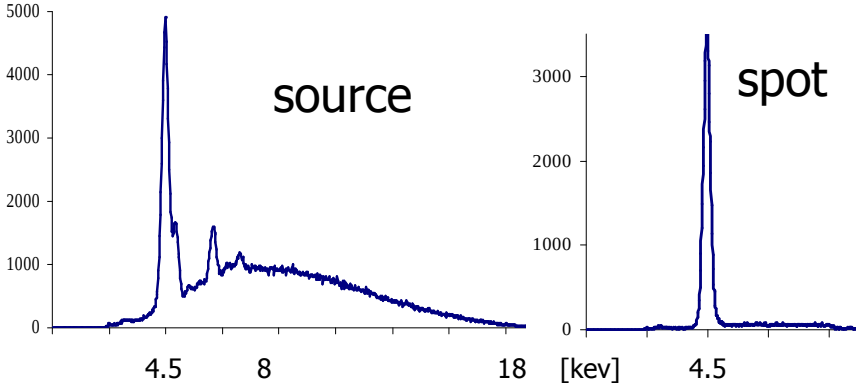
Source is fixed

The mirrors, sample, and microscope have 3D precise movement ability

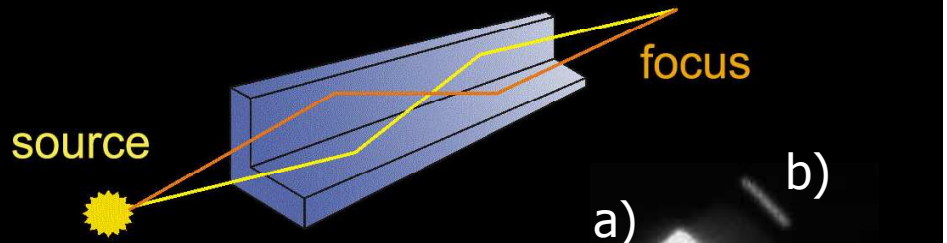
# X-ray focusing

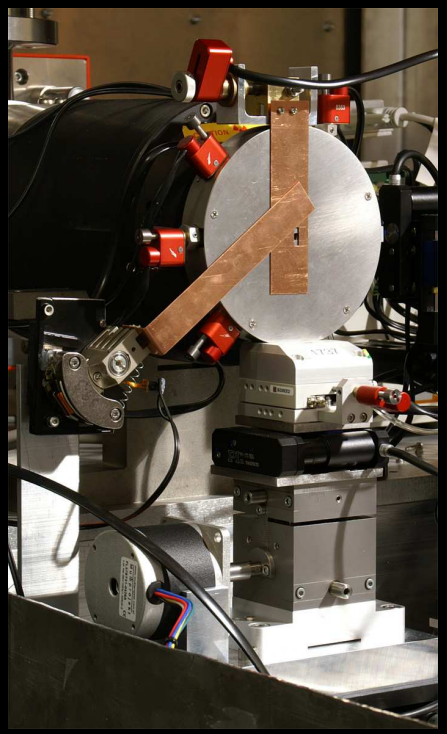
The beam is focused with a multilayer optics.




Two multilayer elements in perpendicular arrangement. The beam image shows:
a) direct beam
b) beam reflected from single mirror
c) beam reflected from both mirrors (the spot)

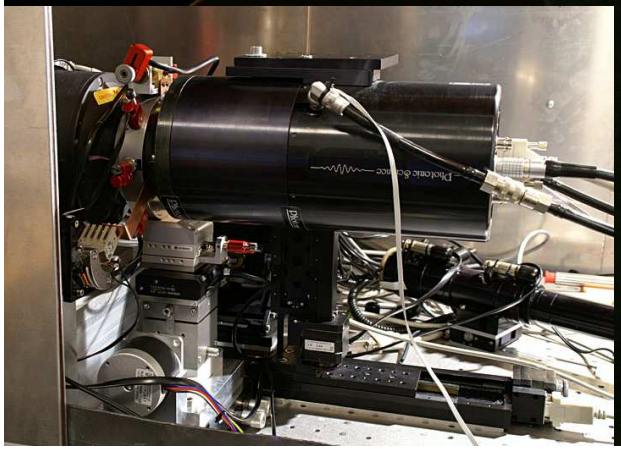


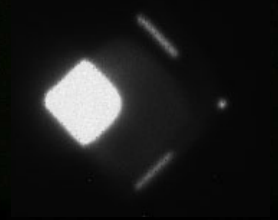



# Beam preview

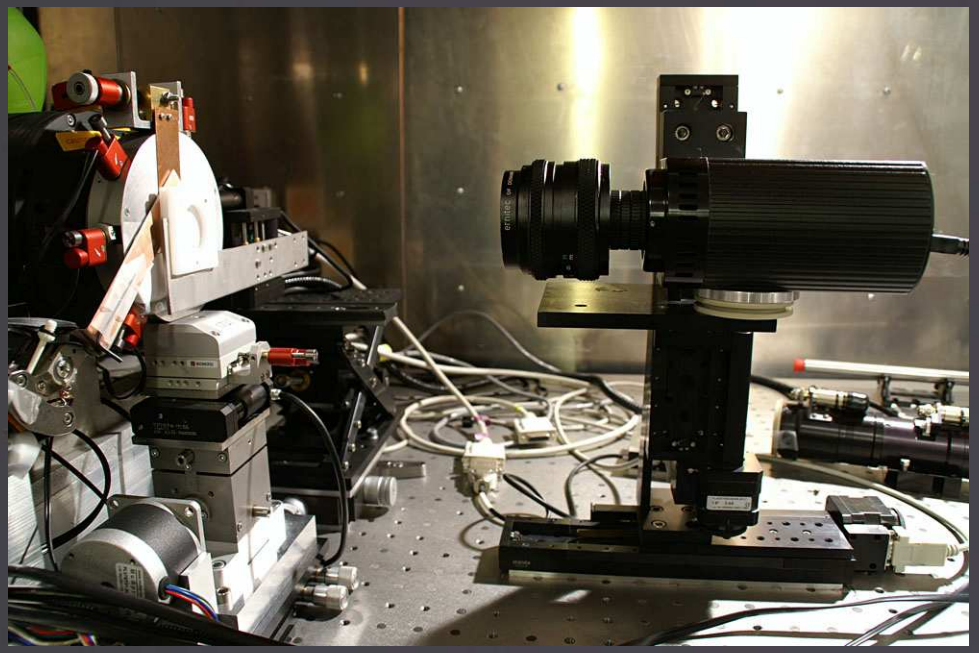
Scintillator material
- 4 um layer of P43 phosphor
- 2 um aluminum coating

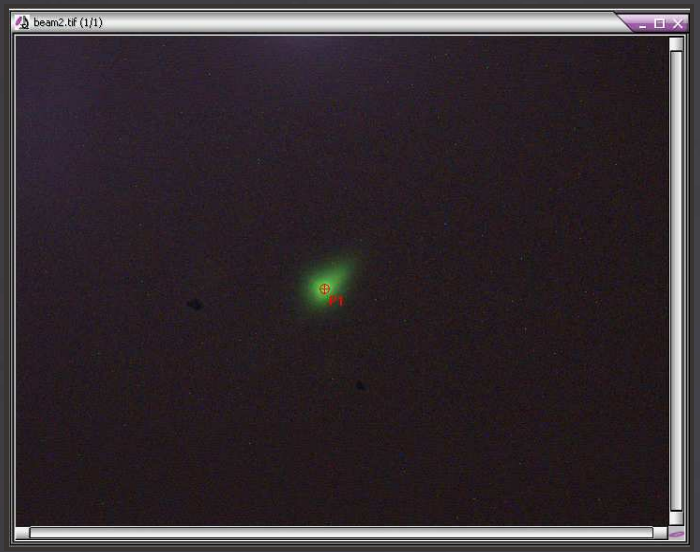
Focusing process is realised with
x-ray sensitive CCD camera

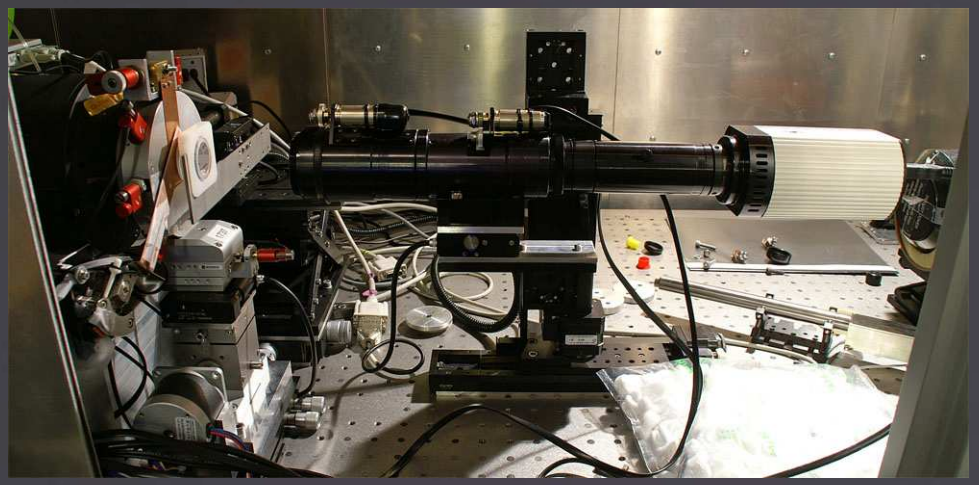




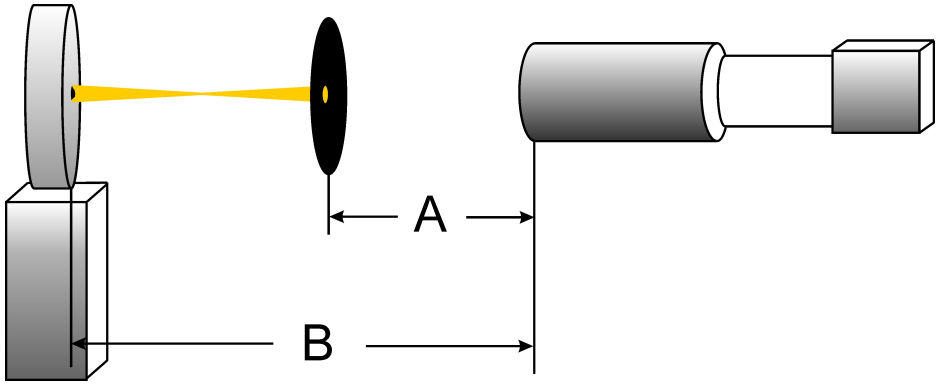
# Microscope alignment in the plane perpendicular to the beam



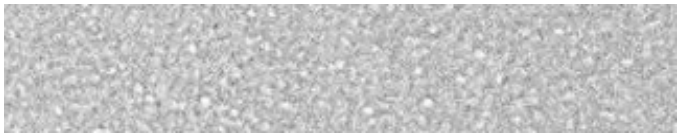


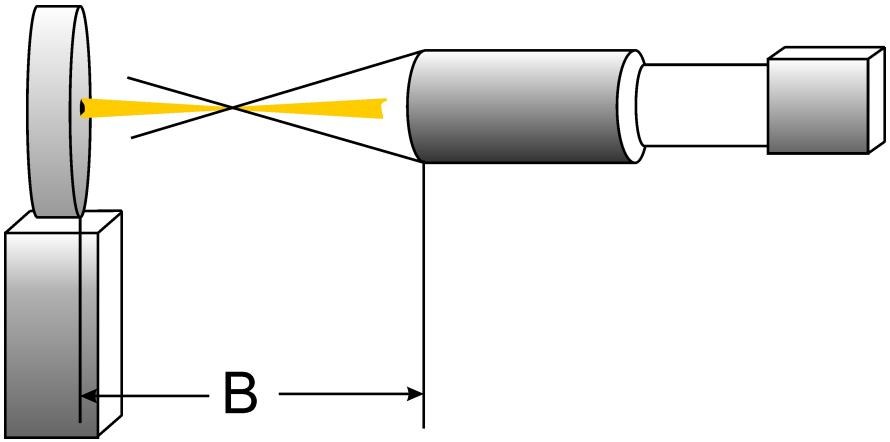
# Microscope alignment in the beam longitudinal direction

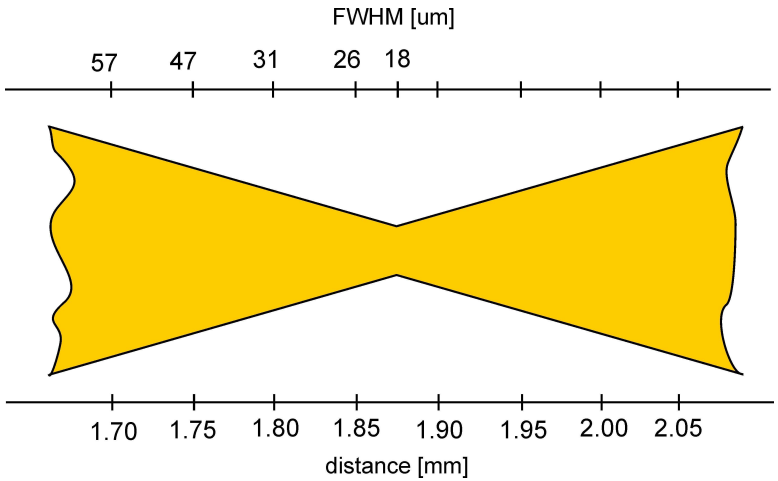



1. The distance **A** is optimized for the highest magnification of the microscope (scintillator crystal grains are sharply visible).



2. The scintillator and microscope move along the beam direction with a constant distance **A** until the smallest spot in the screen is obtained. Then the focal plane of the microscope is the focal plane of the beam. Focal plane of the beam is found by observing the beam profile.



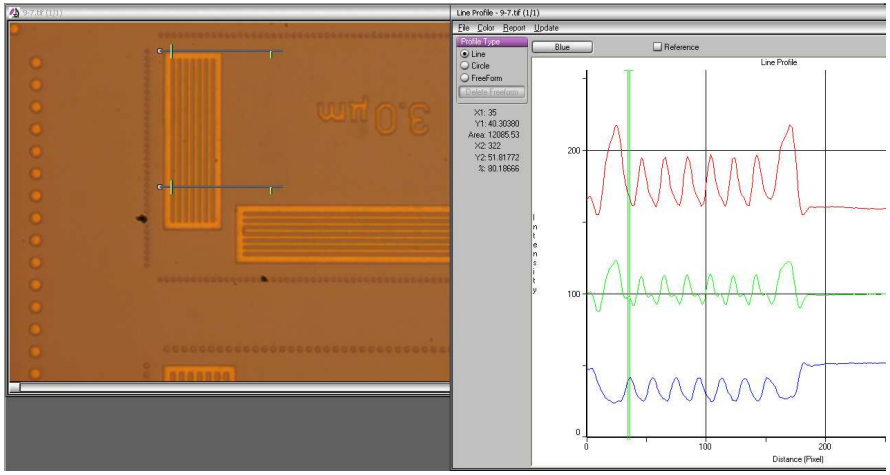
# CELLS

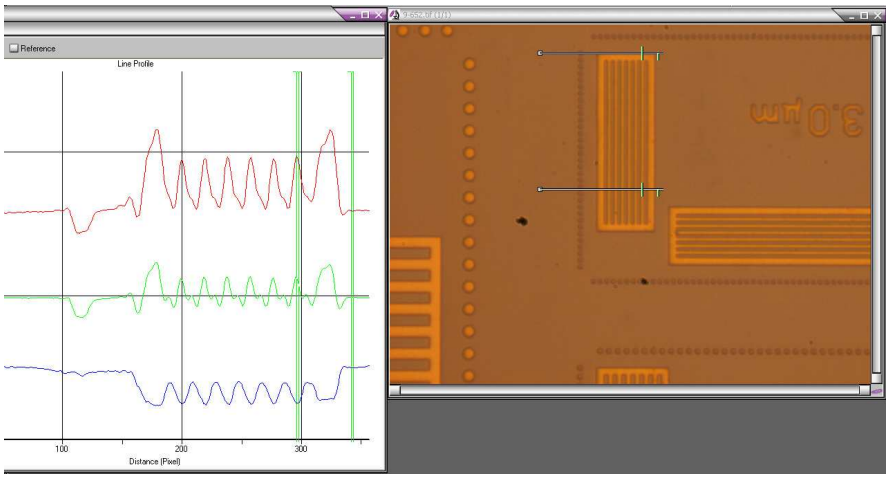
Cells are seeded and irradiated on **35 mm** diameter **Petri dishes** with 10 mm round holes in the central part of the bottom.

The bottom is covered with the **1.5 µm thick Mylar foil**.

A population of about **$10^5$ cells** in **4 µl medium** is seeded on the central part of the Mylar foil **16–18 hours before** the experiments.

# Positioning calibration




A **resolution pattern** enables precise determination of the **micrometer per pixel calibration ratio**, as well as the resolution of the sample positioning system.

In the figure the average distance between maxima is **19,3 ± 0.2 px**. The distance between centers of sticks is **6 um**.

⇒ **0.311 um/px** calibration ratio

⇒ **60 nm** positioning resolution.

| Positioner coordinates [mm] | | | | | | | |
|---|---|---|---|---|---|---|---|
| **9,7** | **9,694** | **9,688** | **9,682** | **9,676** | **9,67** | **9,664** | **9,658** |
| Position of maxima in the pixels readout | | | | | | | |
| 46 | 63 | 83 | 103 | 121 | **141** | **162** | 181 |
| 66 | 83 | 103 | 122 | **141** | **160** | 181 | 199 |
| 84 | 102 | 121 | **142** | **160** | 179 | 200 | 219 |
| 104 | 121 | **141** | **161** | 179 | 199 | 220 | 238 |
| 123 | **141** | **161** | 180 | 198 | 218 | 238 | 257 |
| **142** | **160** | 180 | 199 | 218 | 237 | 258 | 277 |

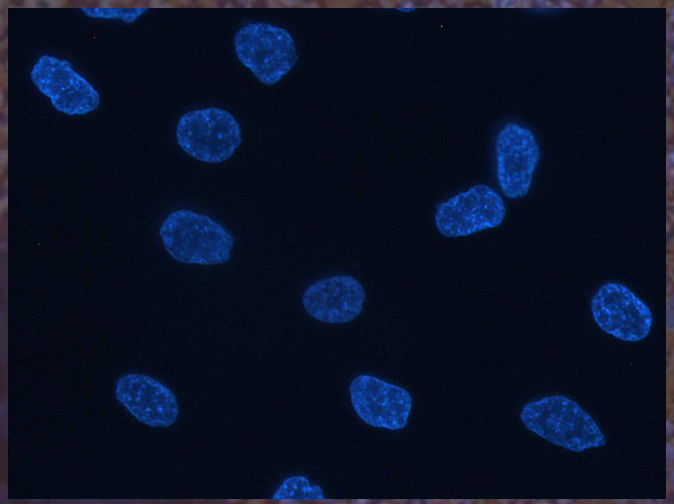
Irradiation procedure movie

# Biological analysis

After irradiation, cells are being visualized under a fluorescent microscope.

Blue marker – living cells

Red marker – dead cells



PC3 cancer cells irradiated with the proton microbeam

# Acknowledgements

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- Foundation for Polish Science and Technology
- European Cooperation in Science and Technology
- Polish Ministry of Science and Higher Education
- National Science Centre


THANK YOU FOR YOUR ATTENTION
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# Dose calculation - first approach with the NIST XCOM database

For **4.5 keV** mass attenuation coefficient for water is **58.34 $cm^2/g$**

Linear attenuation coefficient is 58.34 $cm^2/g \cdot 1g/cm^3$ = 58.34 / cm

**$\mu = 58.34 \cdot 10^{-4}$ / um**

Cell thickness **x = 10 um**

For parallel monochromatic beam **$I = I_0 \cdot e^{-\mu \cdot x} = 0.94\ I_0$**

The spot intensity is **5000 counts / sec**

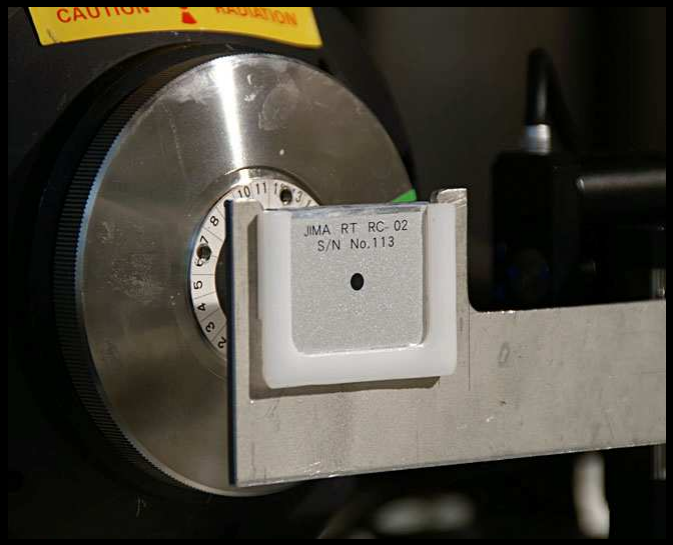
5000 · 6% = **300 photons/sec** deposited in cell.

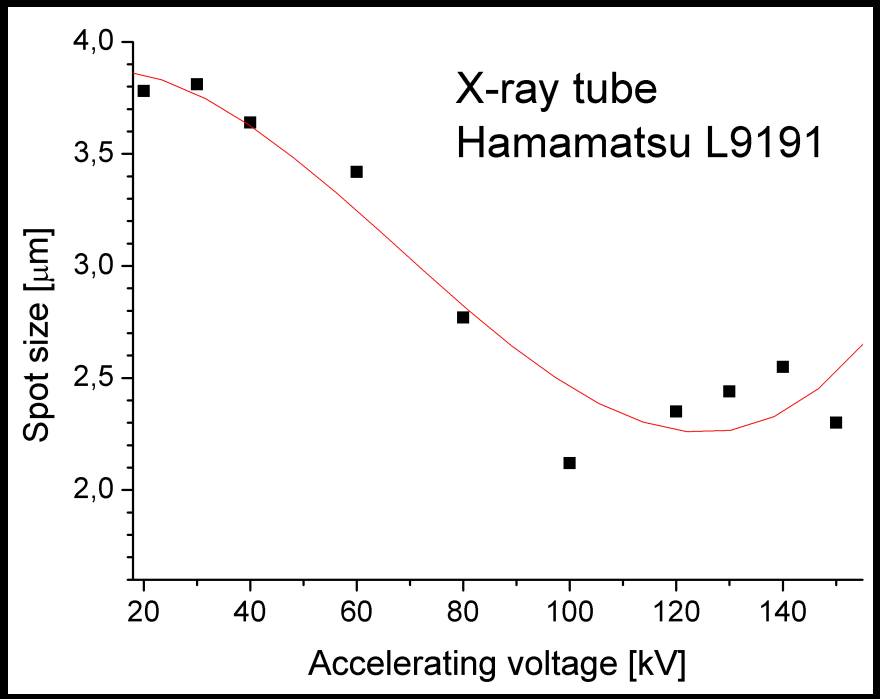
300 ·4.5 keV = 1350 keV/s = **$2{,}16 \cdot 10^{-12}$ J/s**

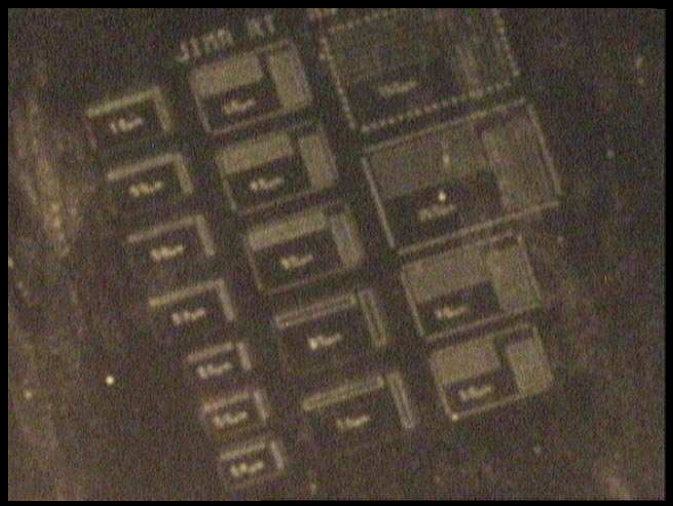
Mass of 10 um in diameter water ball is **$4{,}19 \cdot 10^{-12}$ kg**

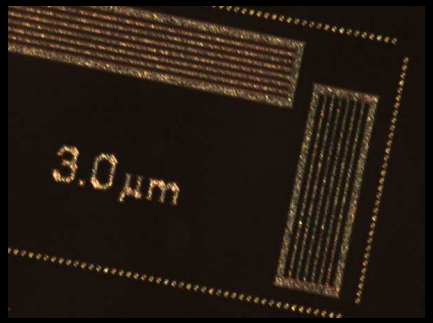
The dose is about **500 mGy/s**

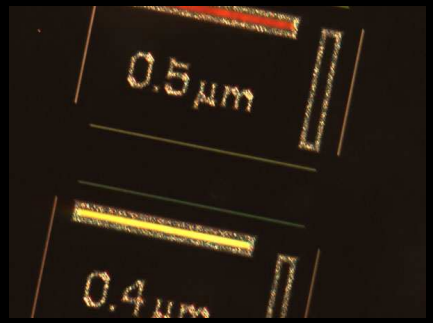
# Source resolution measurements



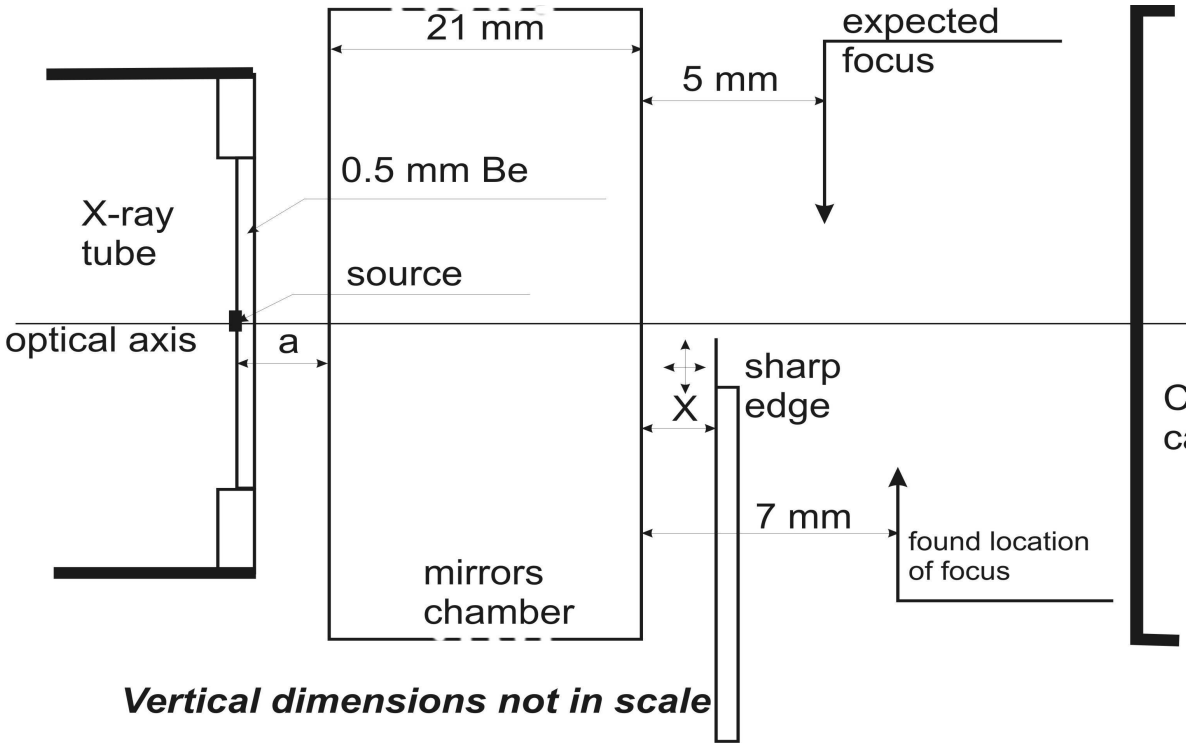





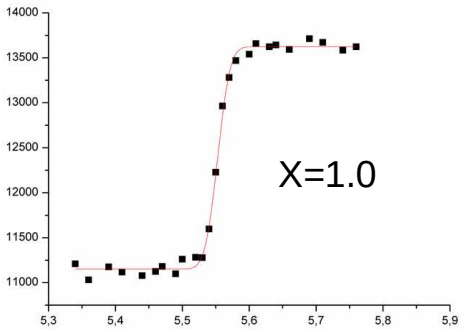



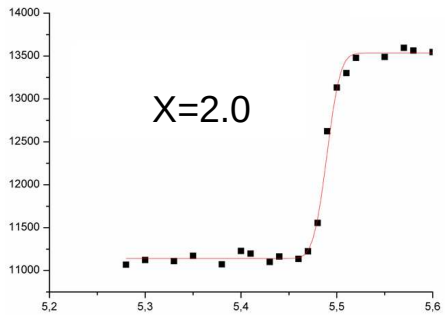
# Focal spot size measurements

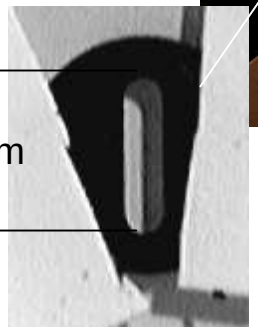


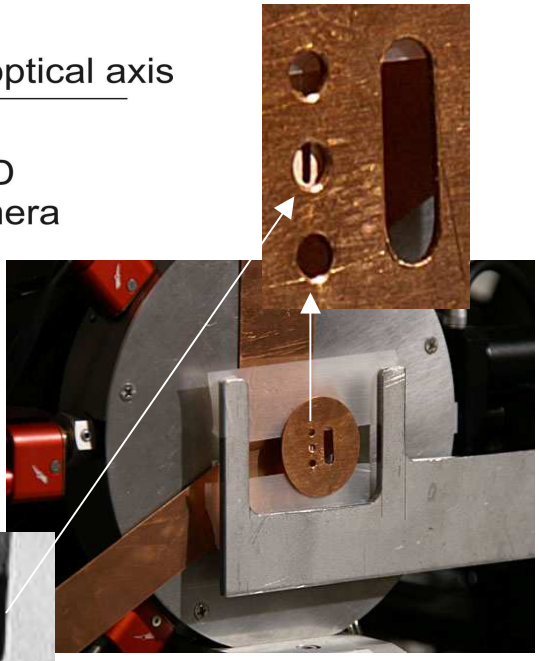

Knife edge method

***Vertical dimensions not in scale***



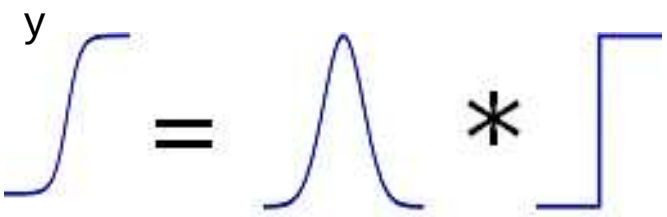



Precise thin copper foil as the edge

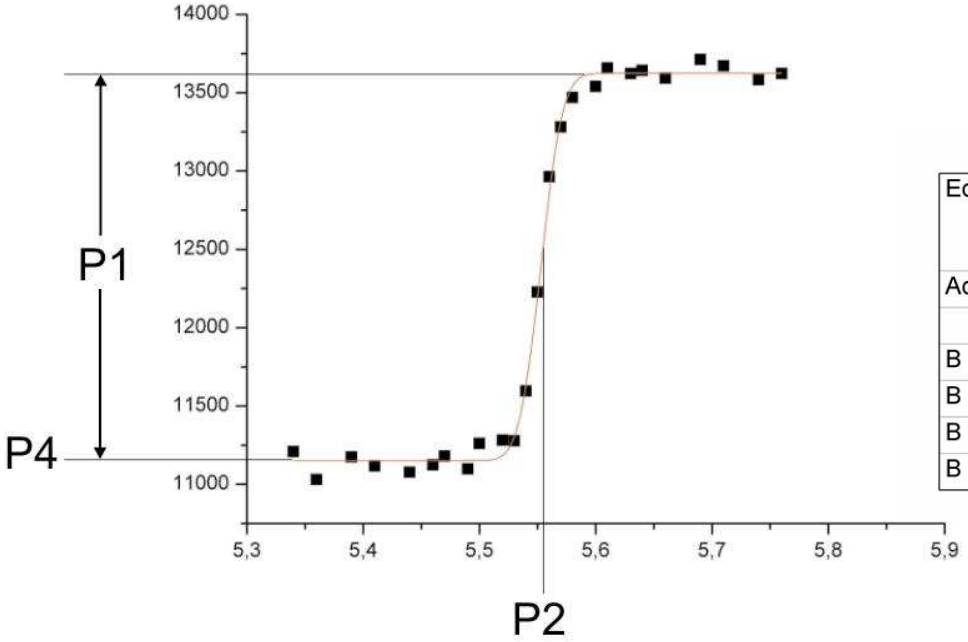
# Calculation of the spot size

Obtained curve **y** is the result of convolution of Gaussian distribution and step function.



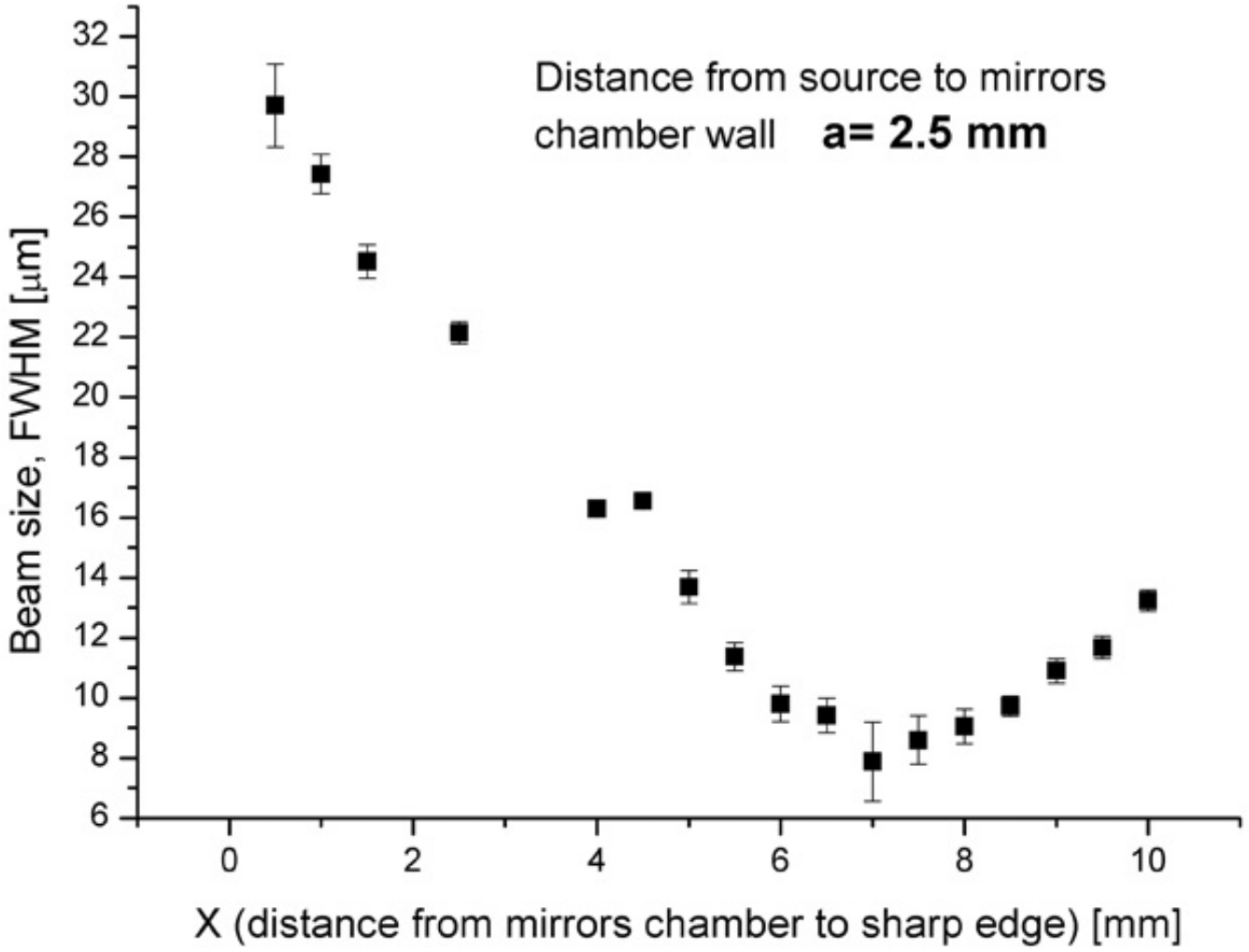

$$y = \frac{P1}{2}\left(1 + erf\left(\frac{x - P2}{P3}\right)\right) + P4$$

P1 – beam intensity
P2 – coordinates of the peak maximum
P3 – width of the peak
P4 – bias




| Equation | y =(P1/2)*(1+erf((x - P2)/P3))+P4 | | |
|---|---|---|---|
| Adj. R-Square | 0,99677 | | |
| | | Value | Standard Error |
| B | P1 | 2472,35801 | 30,92598 |
| B | P2 | 5,55279 | 8,98788E-4 |
| B | P3 | 0,02081 | 0,00153 |
| B | P4 | 11151,82192 | 21,49682 |

# Spot size


Distance from source to mirrors
chamber wall a= 2.5 mm
Beam size, FWHM [μm]
32
30
28
26
24
22
20
18
16
14
12
10
8
6
0
2
4
6
8
10
X (distance from mirrors chamber to sharp edge) [mm]