COST 726 Training School October 6 – 10, 2008

UV-Effects on Microorganisms

Alexander Cabaj

Veterinärmedizinische Universität
Abteilung für Medizinische Physik und Biostatistik

Overview

Technical UV-sources

Fluence and fluence rate

Inactivation of microorganisms, survival functions, different survival functions, EHEC

Spectral response

Photoreactivation

Microorganisms as biodosimeter

Disinfection of drinking water

Nea Michaniona, EU-project

Technical UV-sources

Mercury low pressure lamps

Quasi-monochromatic radiation at 253,7 nm.

Hg-gas pressure about 0,8 Pa (in operation)

Electric power about 60 W - 200 W.

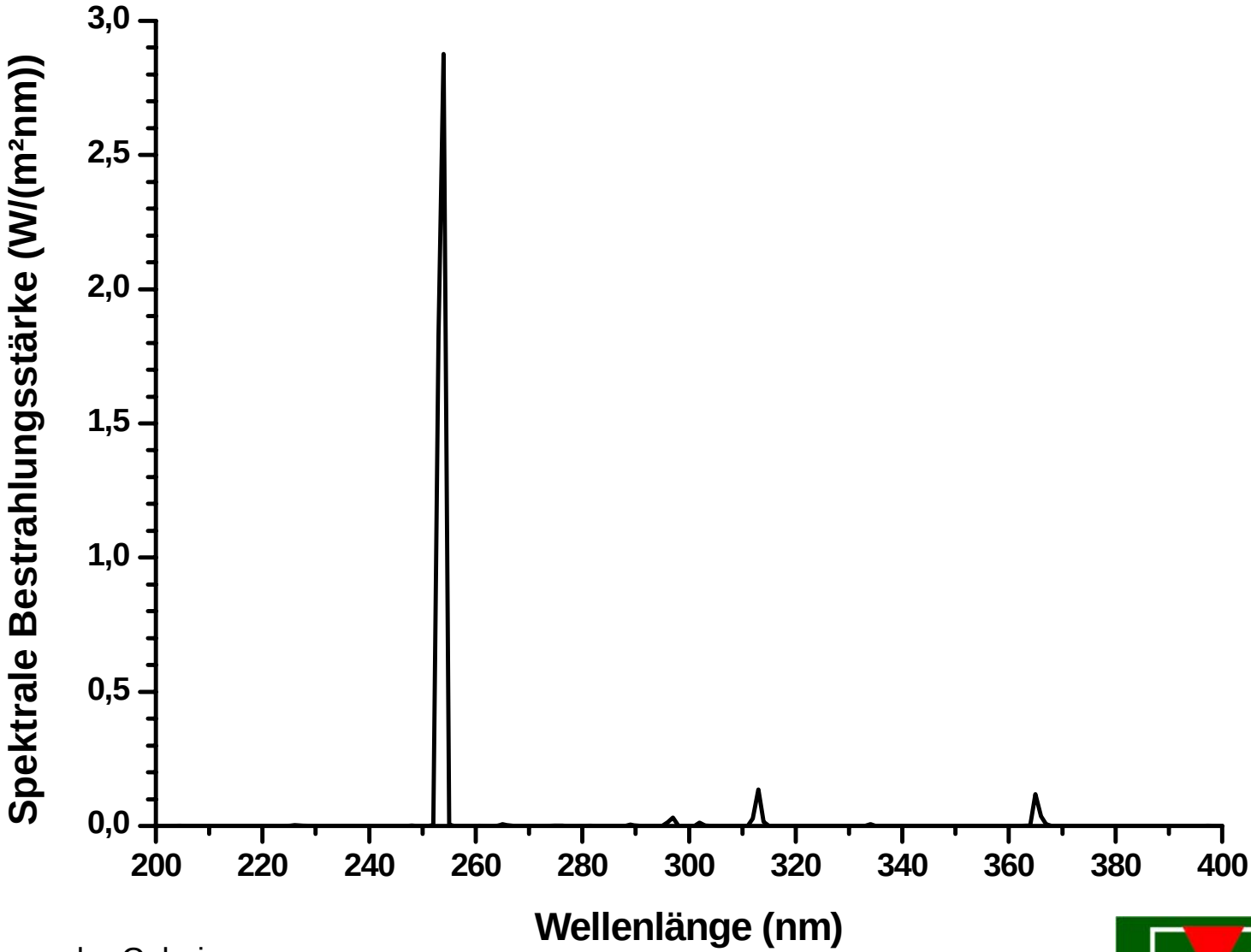
Amalgam lamps

Hg-low pressure lamp with higher UV-power

Spectrum has higher content of longwave UV-radiation.

Electric power up to 600 W

Spectrum of Hg-low pressure lamp



E:/cabrett/Publikation/Kurse/OEMORM-Seminar2004/Hg-NiederdrucklampeUV-TechnikO3erzeugend.opj graph2

Technical UV-sources

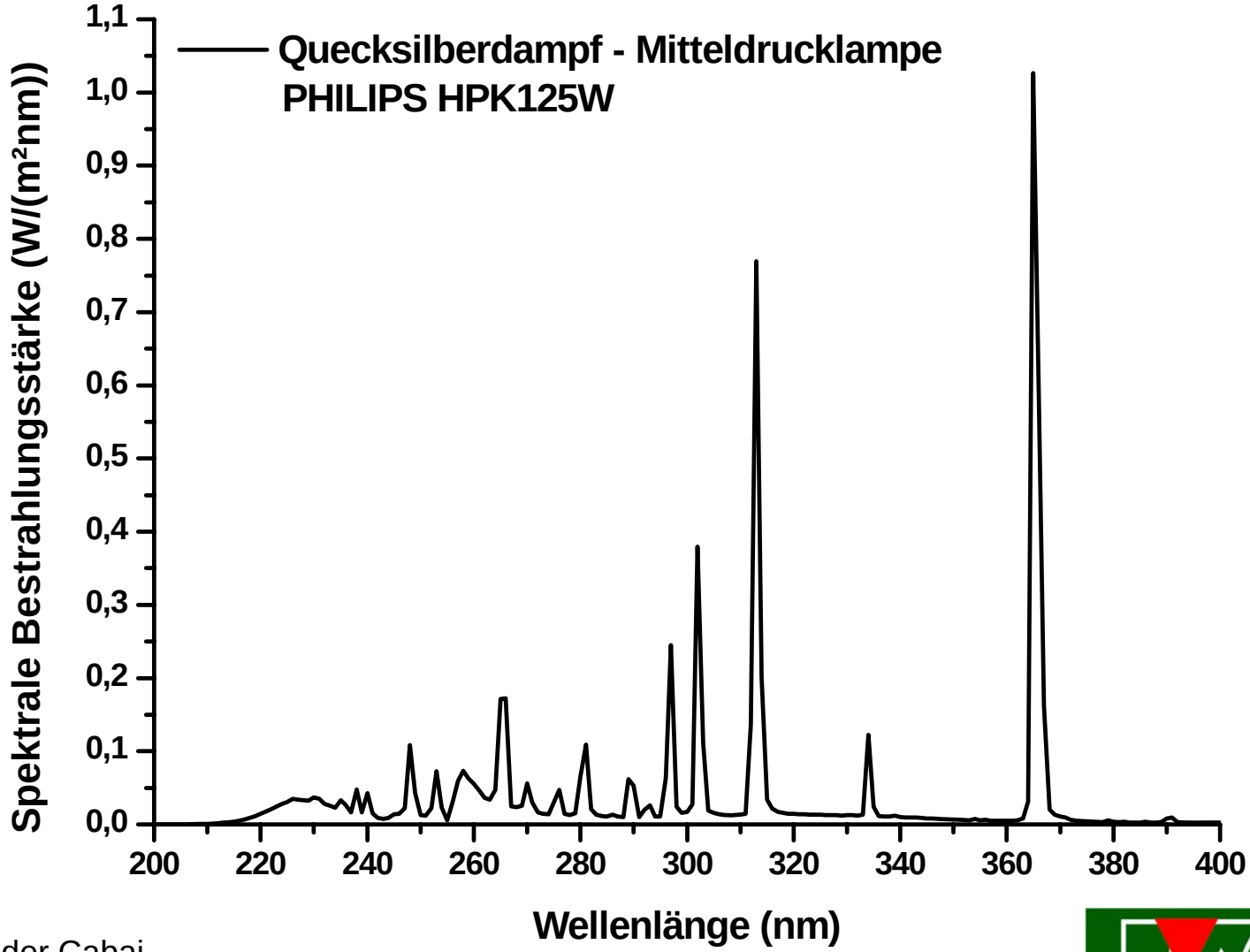
Mercury medium pressure lamps

Polychromatic UV-radiation with wavelengths from below 200 nm to 400 nm (plus visible light).

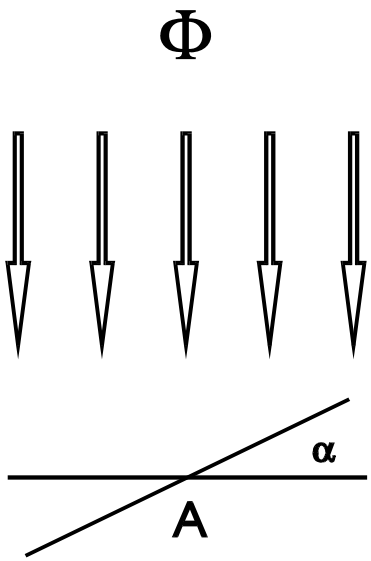
Hg-gas pressure 0,5 to 10 bar.

Electric power up to some tenthousand Watt.

Spectrum of Hg-medium pressure lamp



What does a sensor with flat entrance optic measure ?
Irradiance E (with cosine response)



$$E = \frac{\Phi \cdot \cos\alpha}{A}$$

E: Irradiance (W/m²)

Φ: Radiant power (W)

A: Area (m²)

Integration of E over time gives the radiant exposure H in J/m²

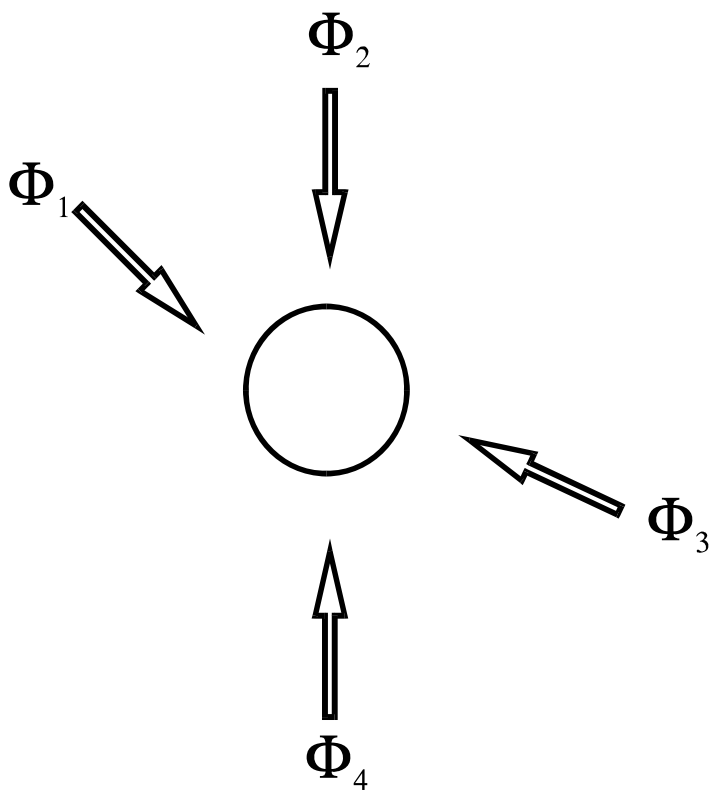
$$H = \int E(t) \cdot dt$$

If E is constant:

$$H = E \cdot t$$

Fluence rate E_o

$$E_0 = \frac{\Phi_1 + \Phi_2 + \Phi_3 + \Phi_4}{r^2 \pi}$$



E_o Fluence rate in W/m²

Φ_i Radiant power in W

r Radius of sphere in m (very small)

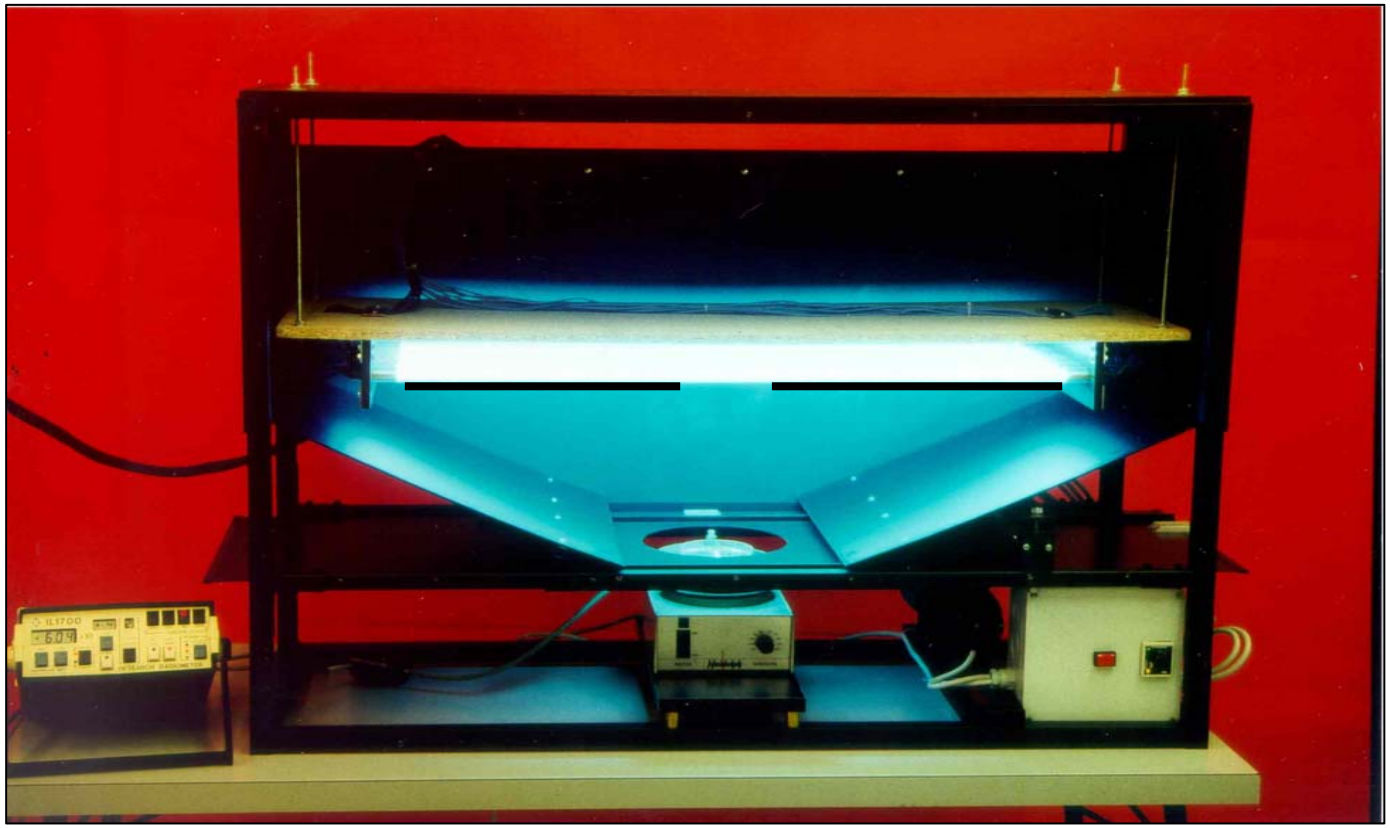
Integration of E_0 over time gives the fluence H_o in J/m²:

$$H_o = \int E_o(t) \cdot dt$$

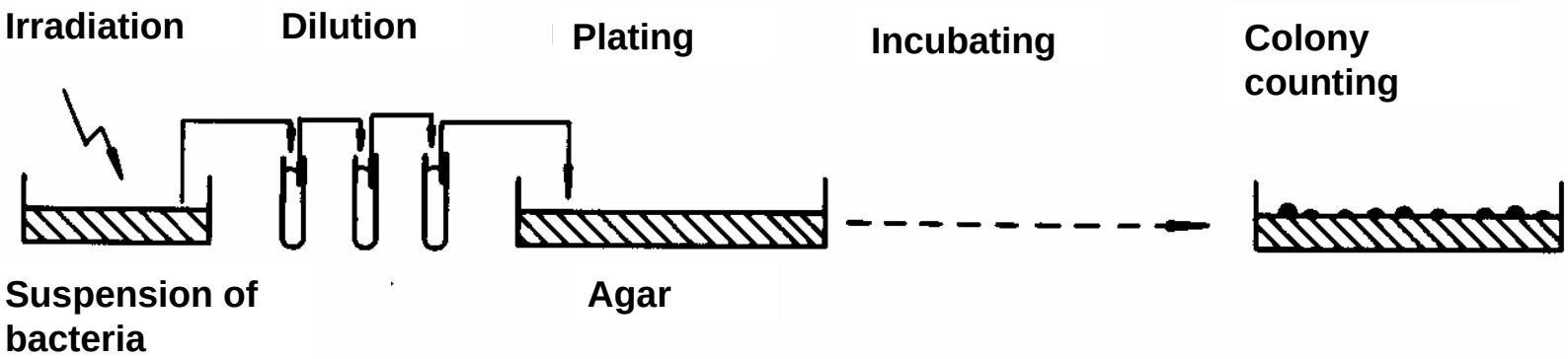
If E_0 is constant: $H_0 = E_0 \cdot t$

Laboratory irradiation apparatus with low pressure lamps

Measurement of survival curves at wavelength 253,7 nm

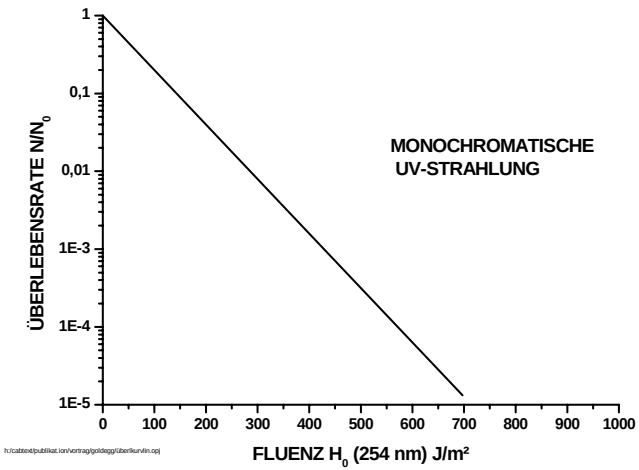


Measurement of survival curves



- **Stirring of bacterial suspension**
- **Measurement of UV-irradiance before and after irradiation, quasi-parallel beam**
- **Calculation of mean UV-irradiance (equal to mean fluence) over the irradiated volume**

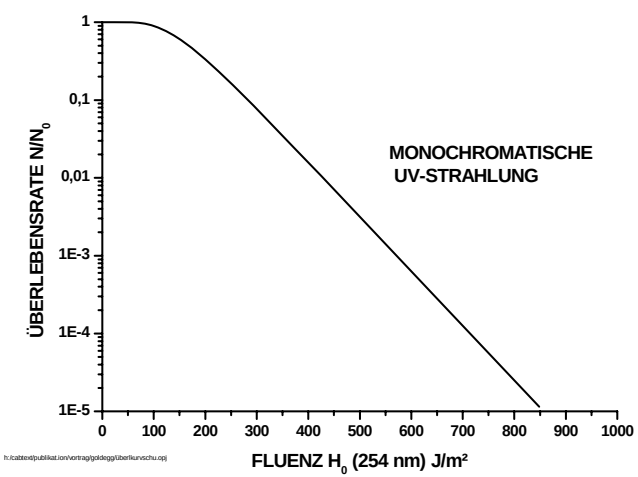
Survival functions of microorganisms



$$\frac{N}{N_0} = 10^{-k \cdot H_0}$$

Unshouldered survival curves

Virus



$$\frac{N}{N_0} = 1 - (1 - 10^{-k \cdot H_0})^{10^d}$$

Shouldered survival curves

Bacteria, spores

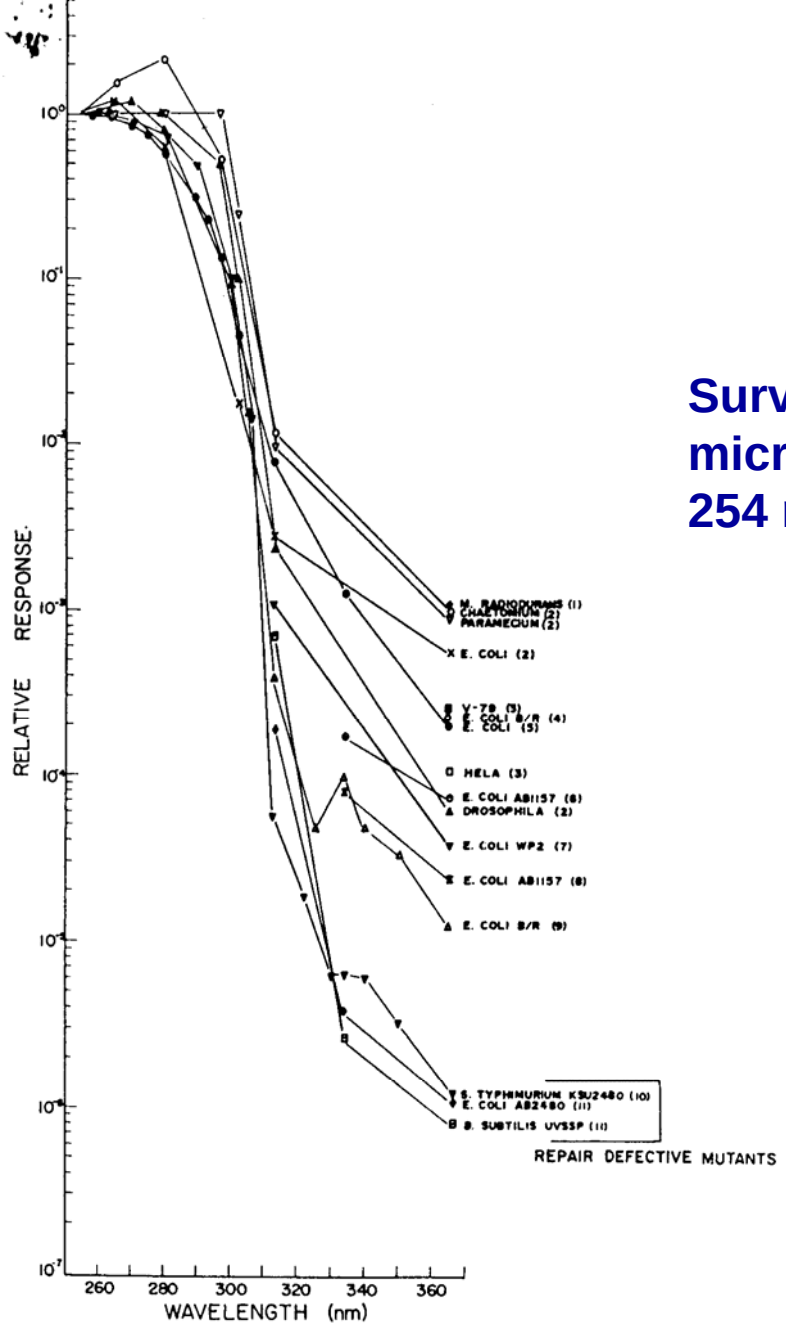


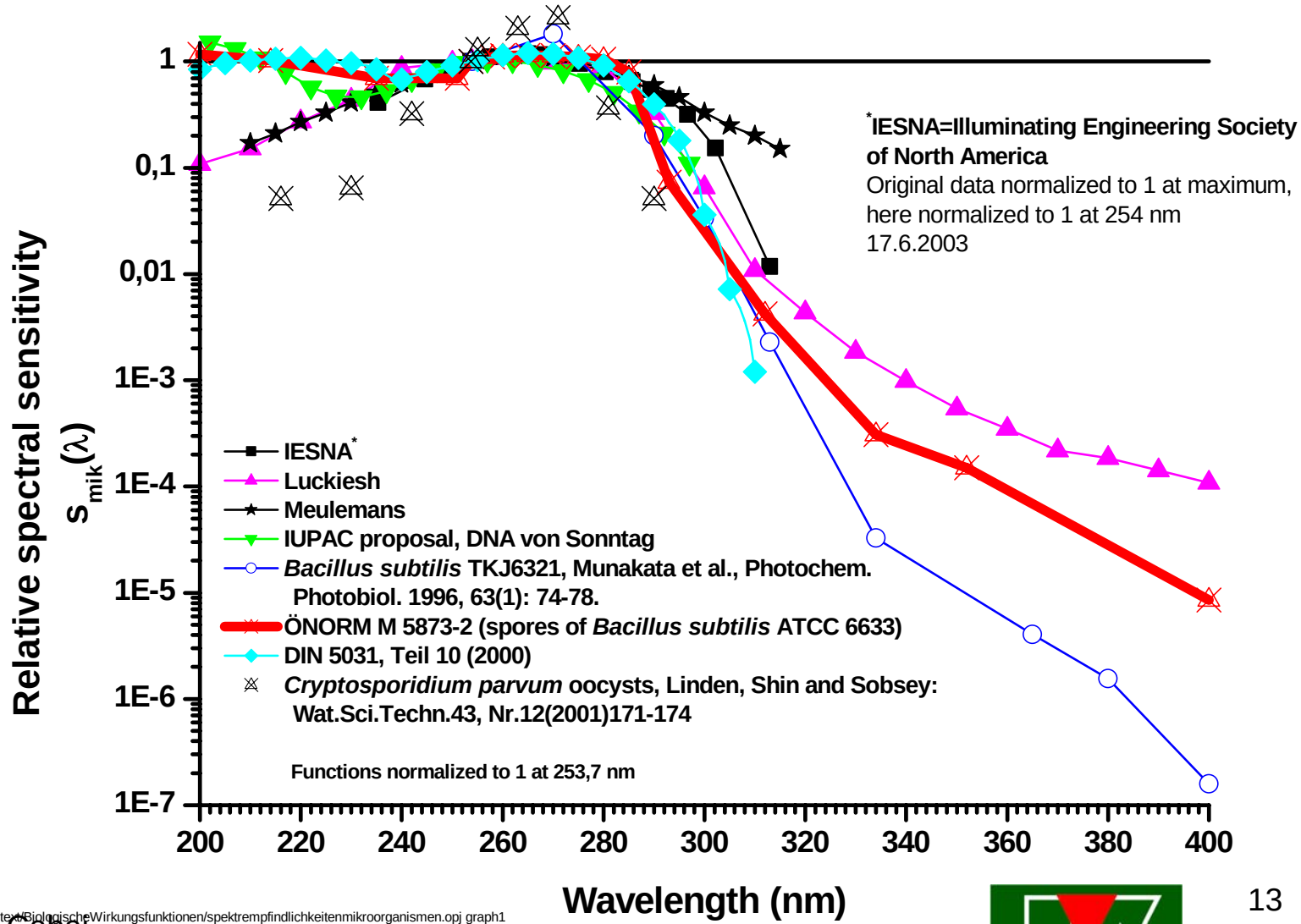
Figure 1. A compendium of action spectra for UV-induced lethality assembled from various published action spectra of diverse microorganisms. All spectra include observations of response to the two mercury lines 254 nm (far UV) and 365 nm (near UV). Results have been normalized to the response at 254 nm; where survival curves were available the 90%-lethal effects were utilized as the criterion of

Survival curves of different microorganisms normalised to 1 at 254 nm.

J. Calkins and J. Barcelot: Some Further Considerations on the Use of Repair-Defective Organisms as Biological Dosimeters for Broad-Band Ultraviolet Radiation Sources.

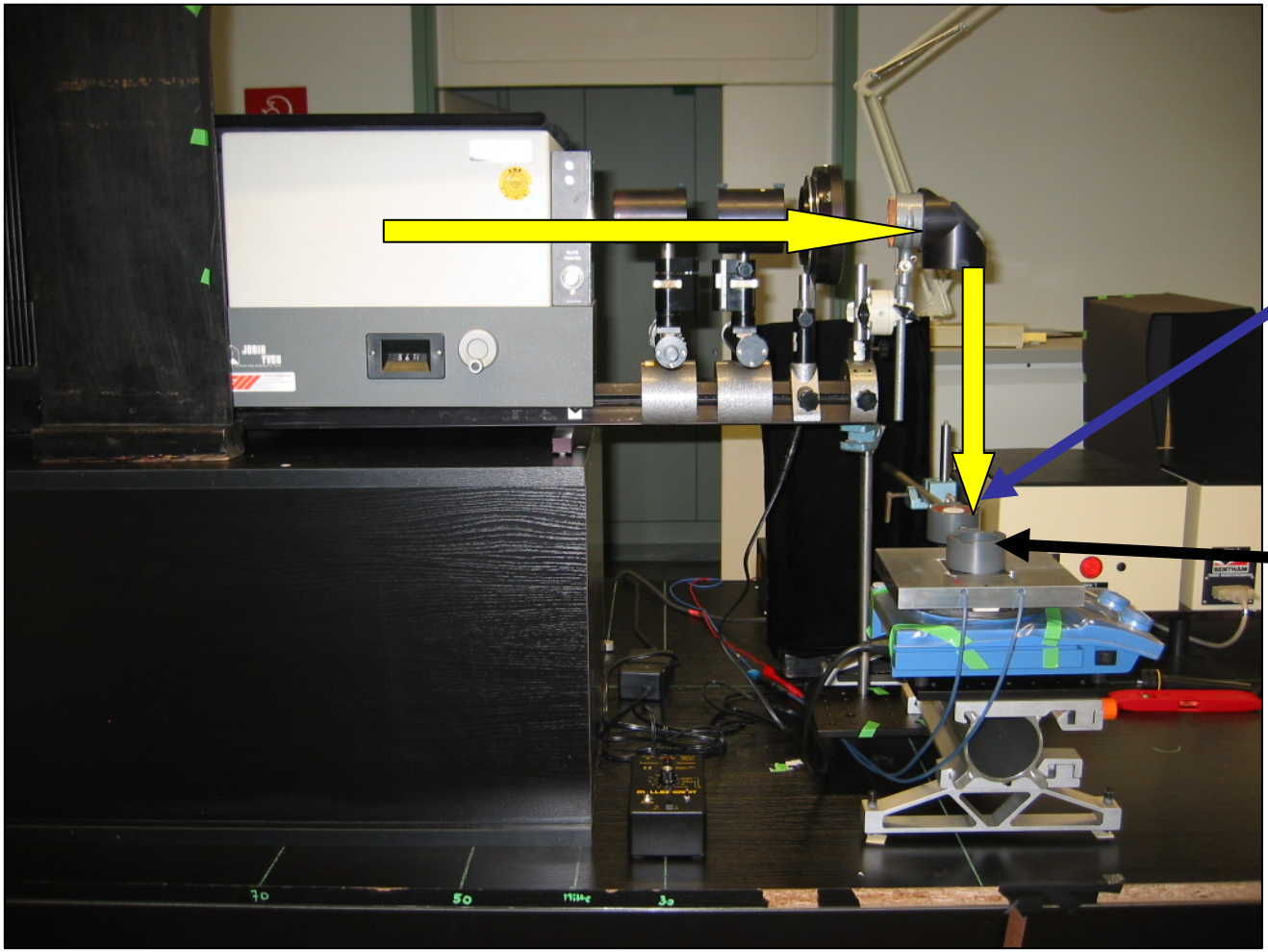
Photochemistry and Photobiology 1979, 30, 733-737

Collection of action spectra for microorganisms



h:/cabtex/BiologischeWirkungsfunktionen/spektrempfindlichkeitenmikroorganismen.opj graph1

Investigation of the spectral Sensitivity of Microorganisms

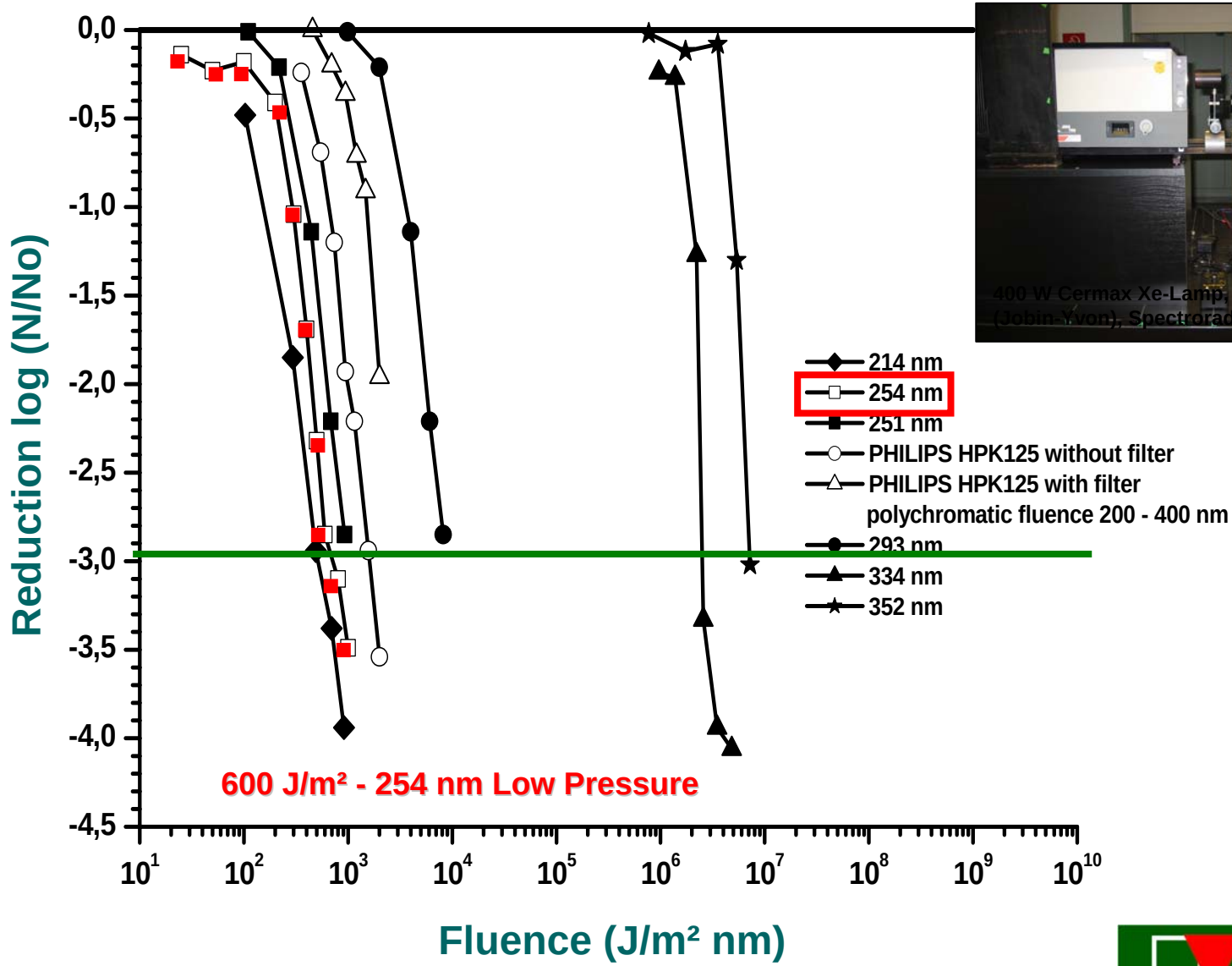


Sensor of the spectroradiometer

Irradiation vessel (stirrer) with the suspension of MO

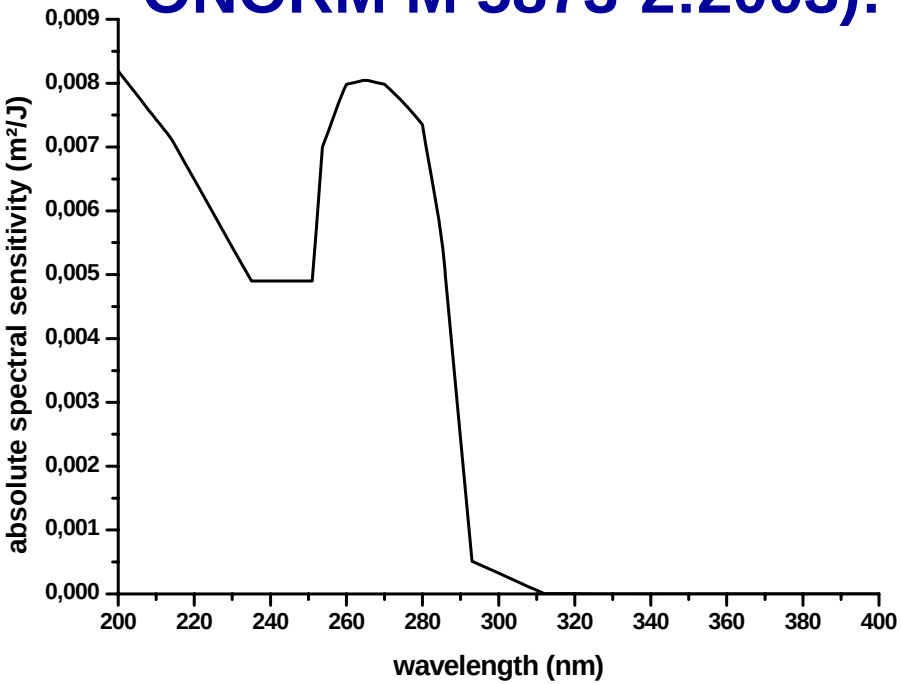
400 W Cermax Xe-Lamp, Monochromator (Jobin-Yvon), Spectroradiometer (Bentham)

UV Inactivation of *B. subtilis* spores (ATCC 6633) at different wavelengths

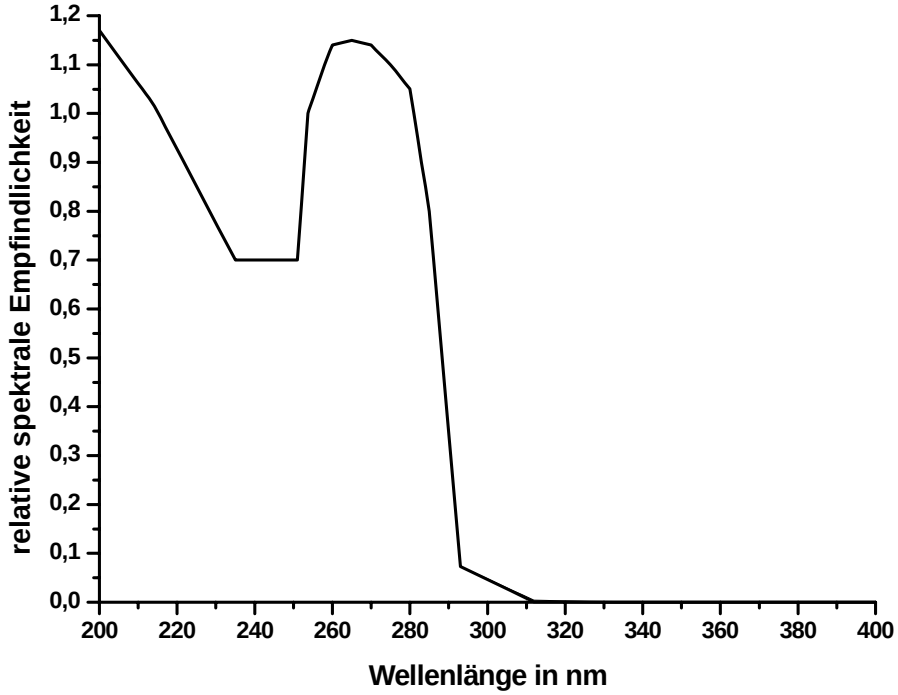


Cabaj, Sommer, Pribil, Haider (Wat. Science Techn., 2002)

Absolute and relative spectral sensitivity of spores of *Bacillus subtilis* ATCC 6633 (standardised in ÖNORM M 5873-2:2003):



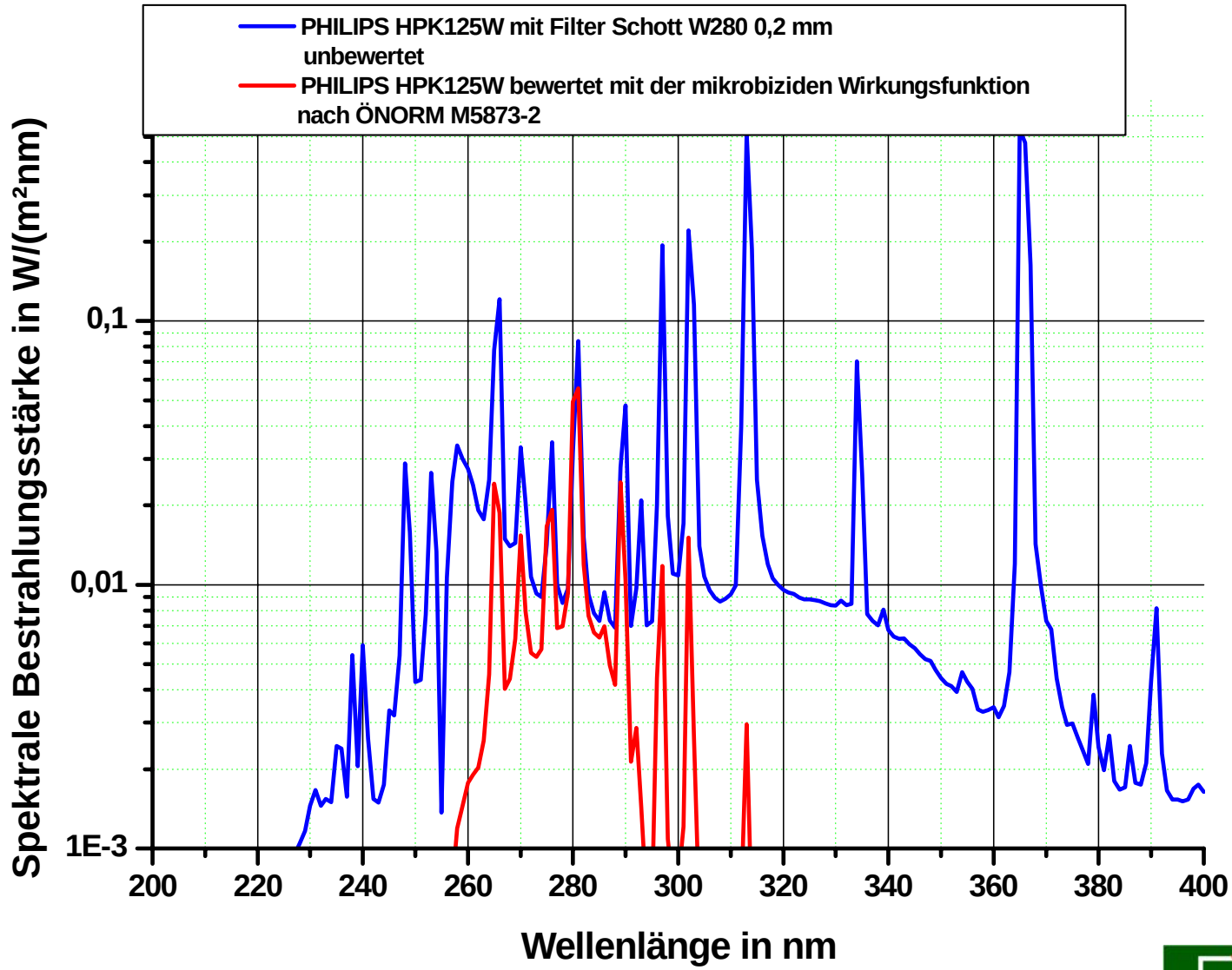
H:/cabtext/OENORM/Teil2/Bild1spektrale mikrobizide Empfindlichkeitneu.opj graph4



Absolute sensitivity:
0,007 m²/J at 253,7 nm

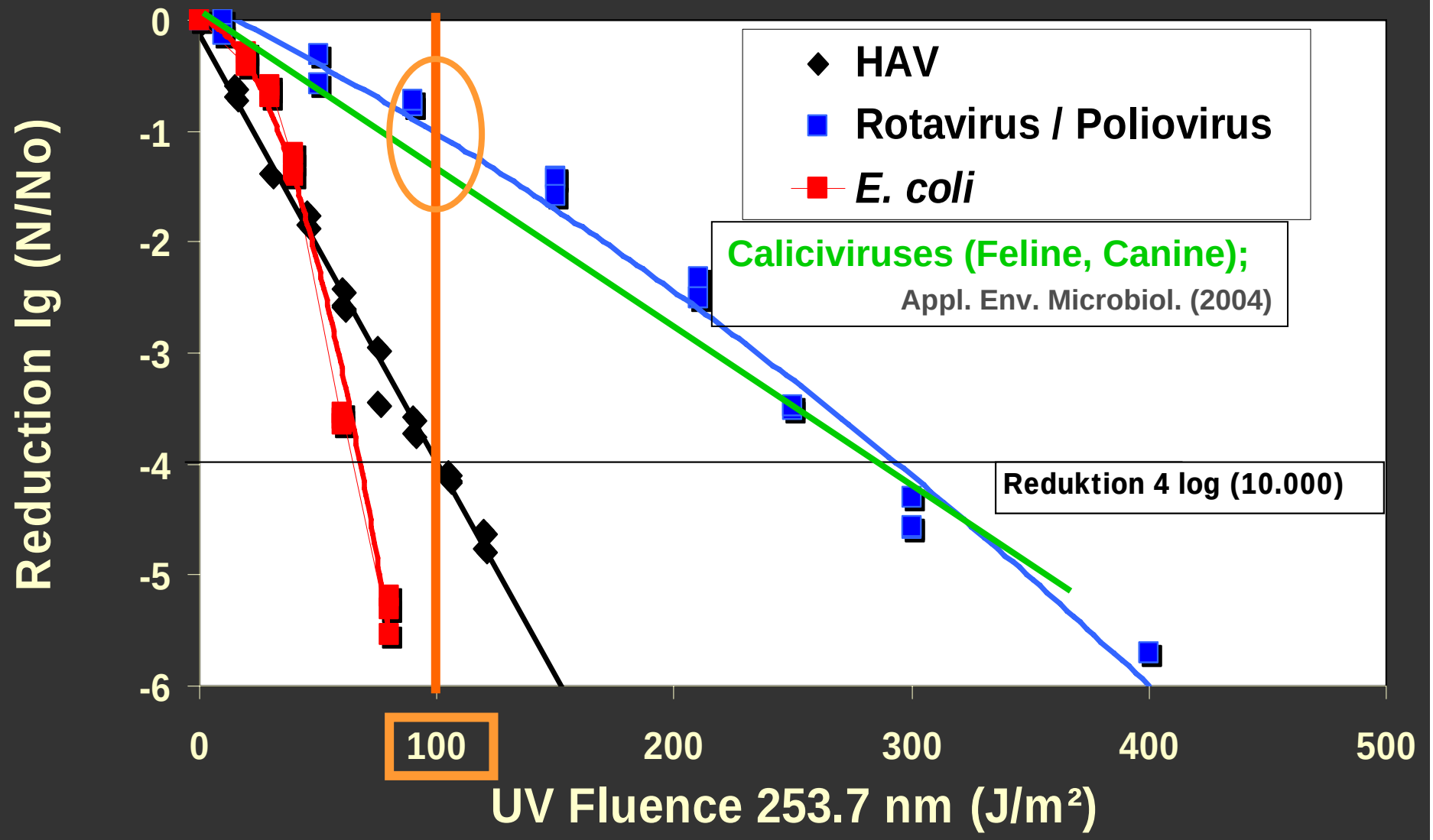
Relative sensitivity:
1 bei 253,7 nm

Spectrum of medium pressure lamp weighted with microbicidal weighting function according to ÖNORM M 5873-2:2003



H:/cadek/publikation/kurse/KursOEV2WT/haur12.und13.6.2008/HPK125mF.opj

Low pressure UV-253,7 nm inactivation of important, water related viruses
E. coli is given as comparison



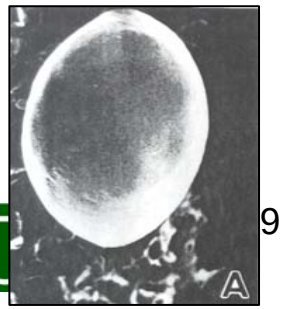
Cryptosporidiosis Epidemic 1993

- **Milwaukee (1 600 000 inhabitants)**
- **403.000 ill, heavy diarrhea (4.000 in hospital, 100 deaths, cost 131 Mill USD)**
- **public drinking water**

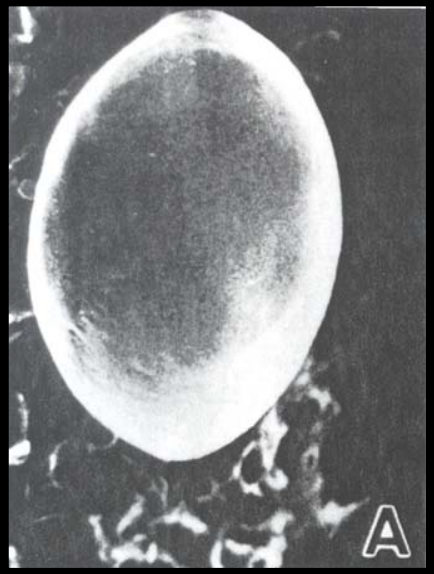
  Lake Michigan, flocculation, filtration chlorination

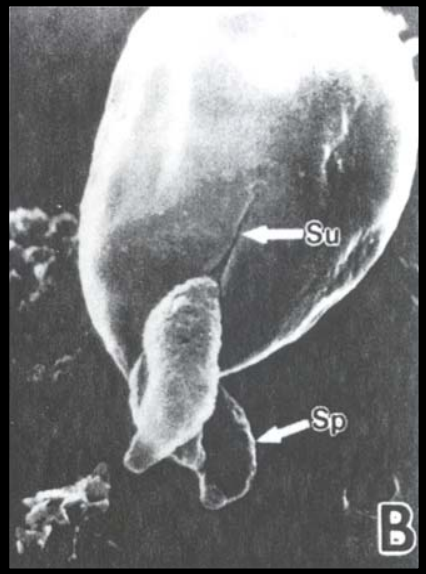
- **Heavy rain, increased fecal contamination, (waste water influent), concentration of chlorine increased**

Bacteriologic results (*E. coli*) negative !



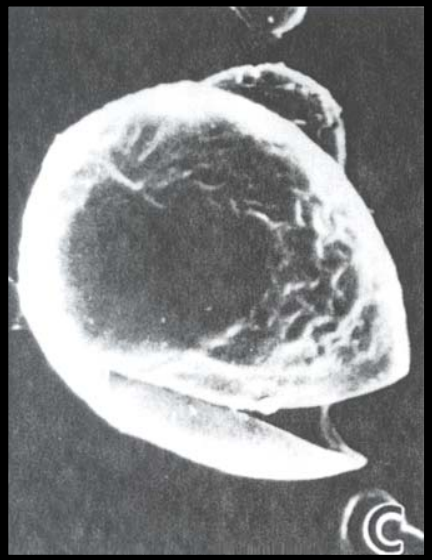
Cryptosporidium parvum (protozoan parasite)



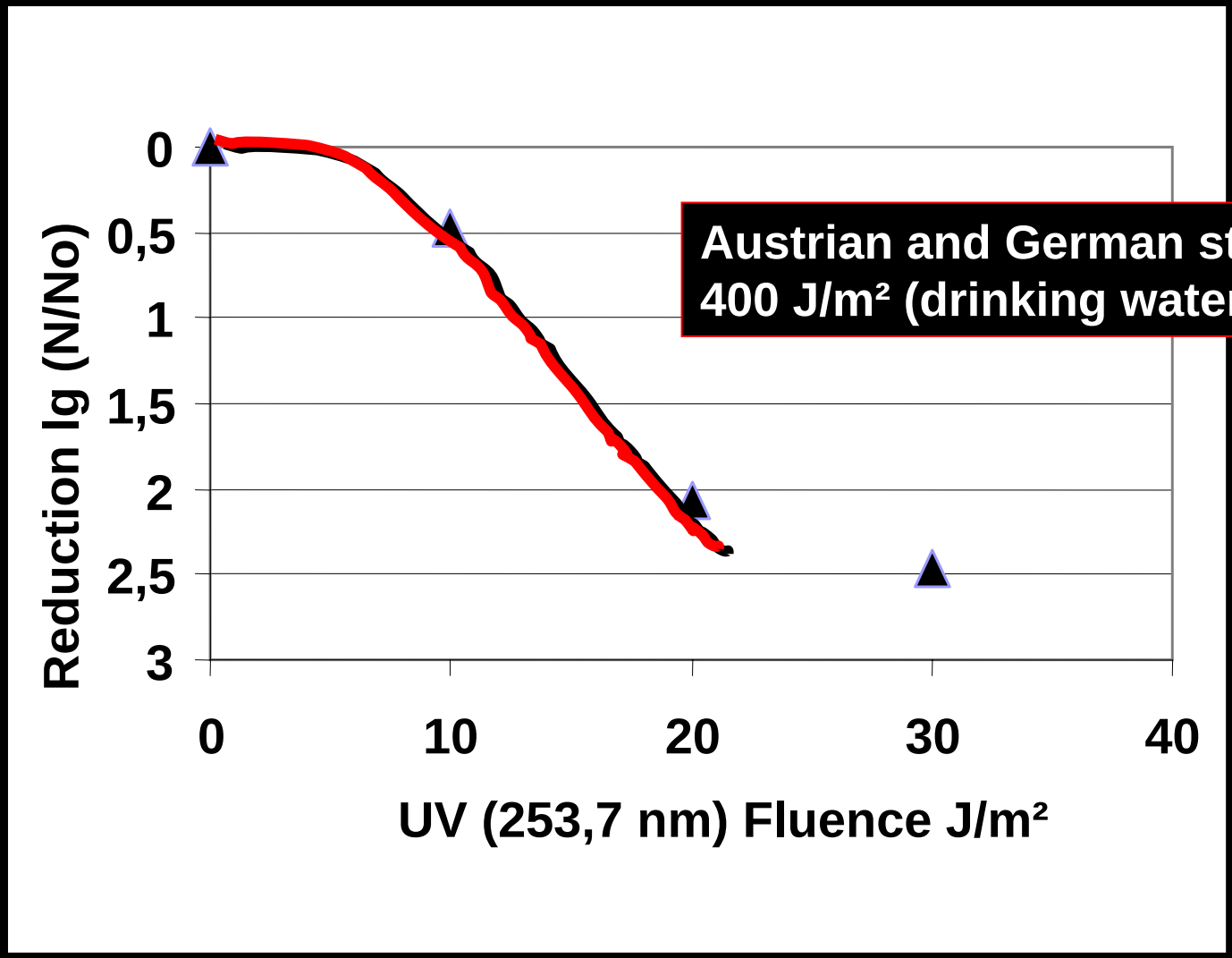


Oocyst

Trophozoit



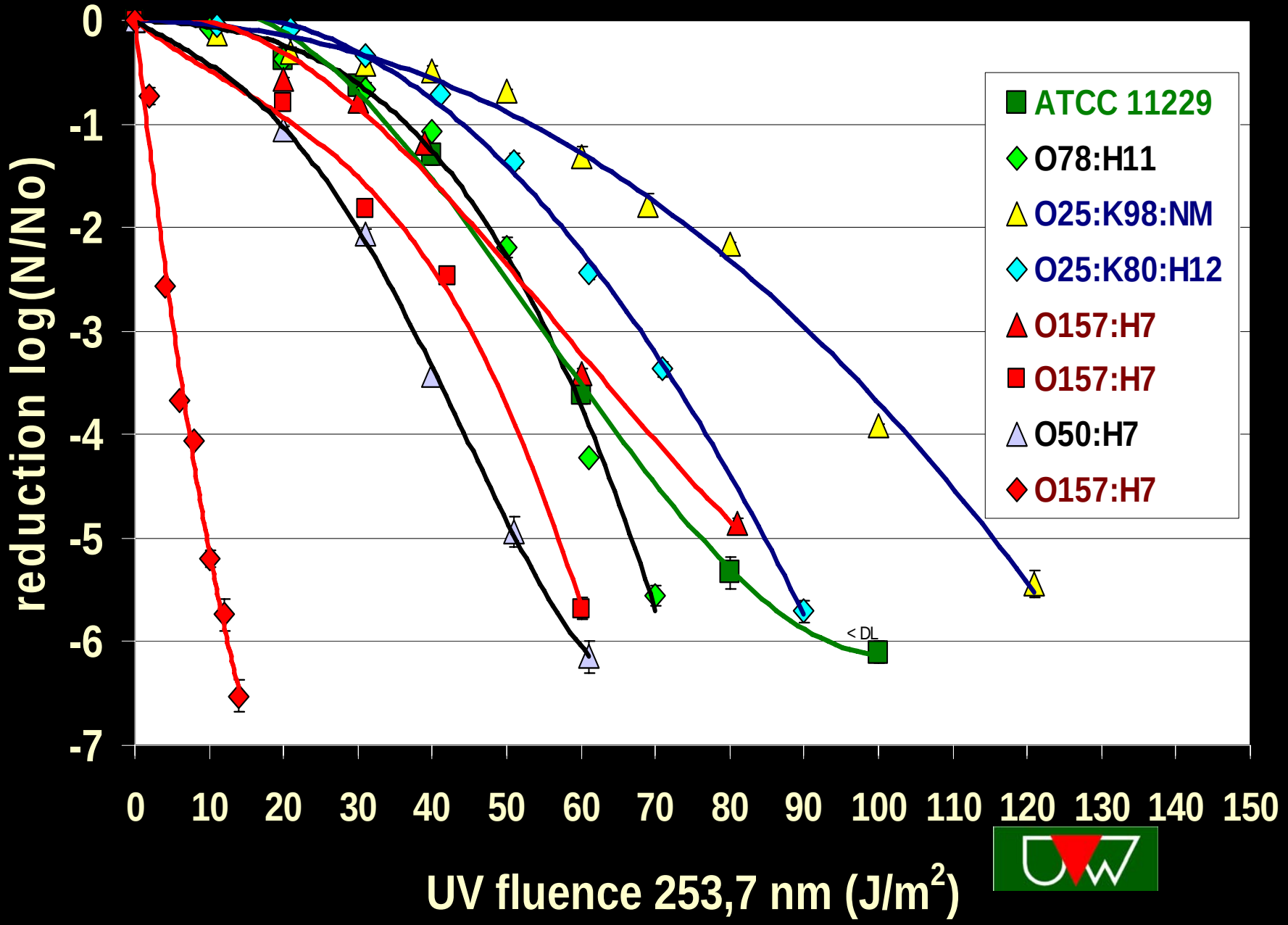
Inactivation of oocysts of *Cryptosporidium parvum* by UV (253.7 nm) radiation



Austrian and German standard:
400 J/m² (drinking water disinfection)

Appelt, Slifko, Cabaj and Sommer (2000)

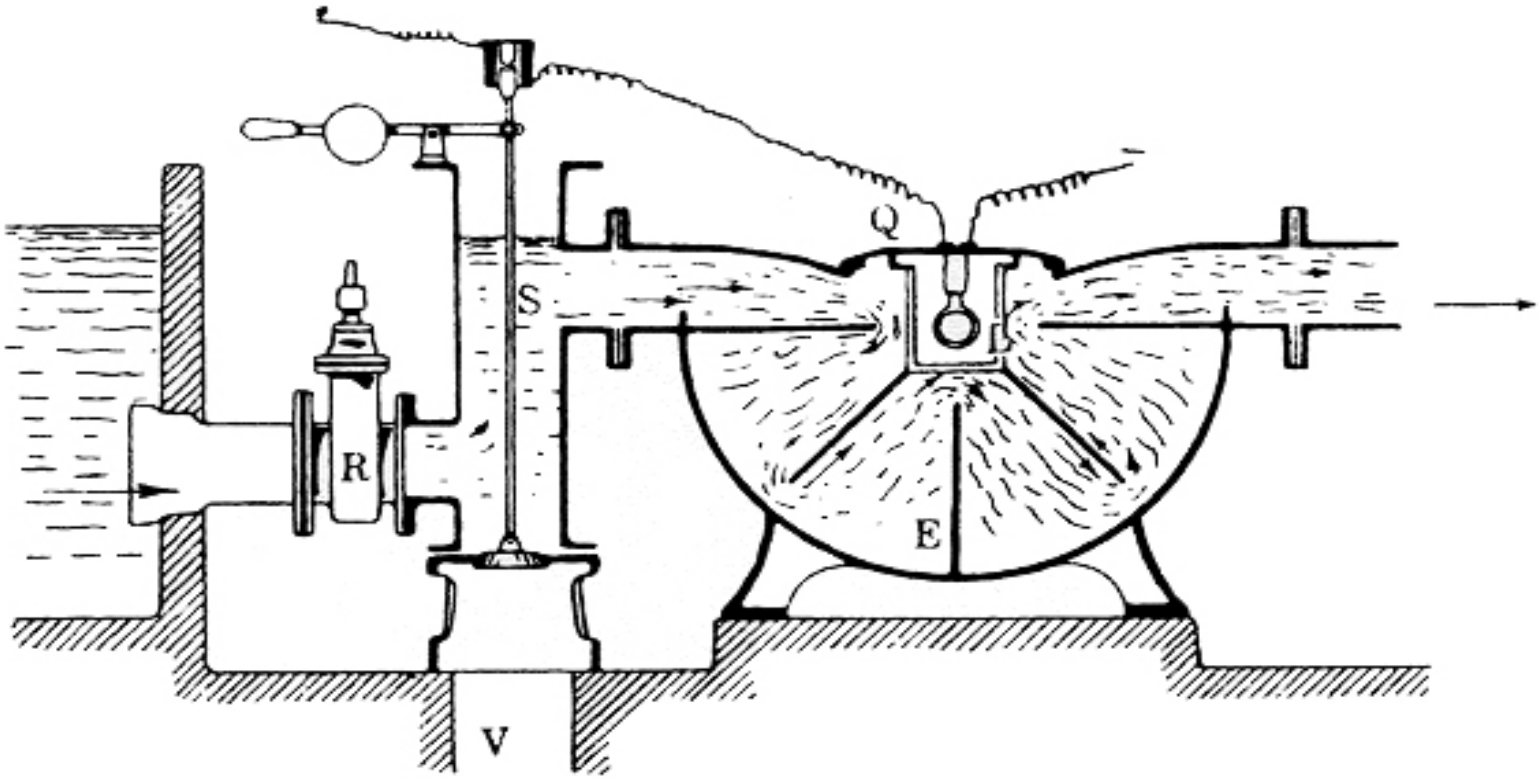
Inactivation of pathogenic strains of *Escherichia coli* in water

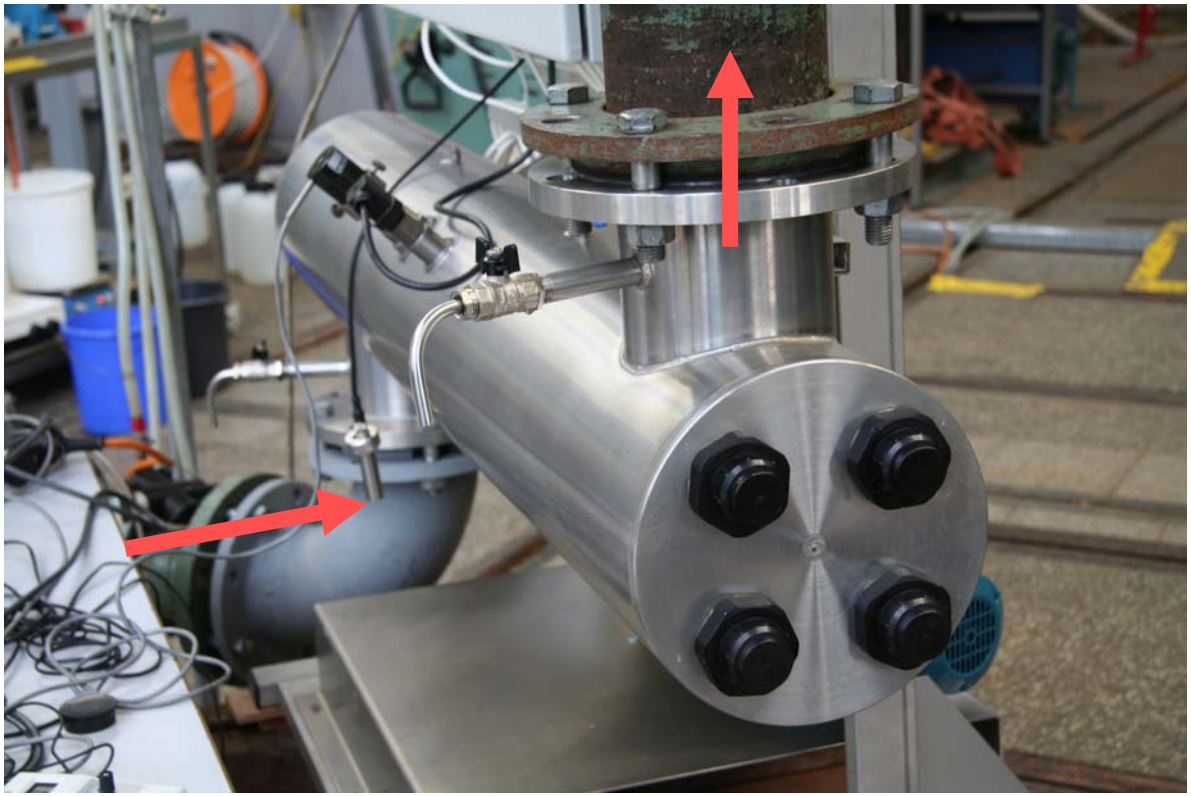


1910: first UV-plant in Marseille, France

(Henri *et al*., Compt. Rend. Acad. Sci. 151, 677-680)

Many technical problems, unreliable UV-lamps, instability, short life time of lamp, REF unknown, …





UV-disinfection plant for drinking water

4 low pressure lamps

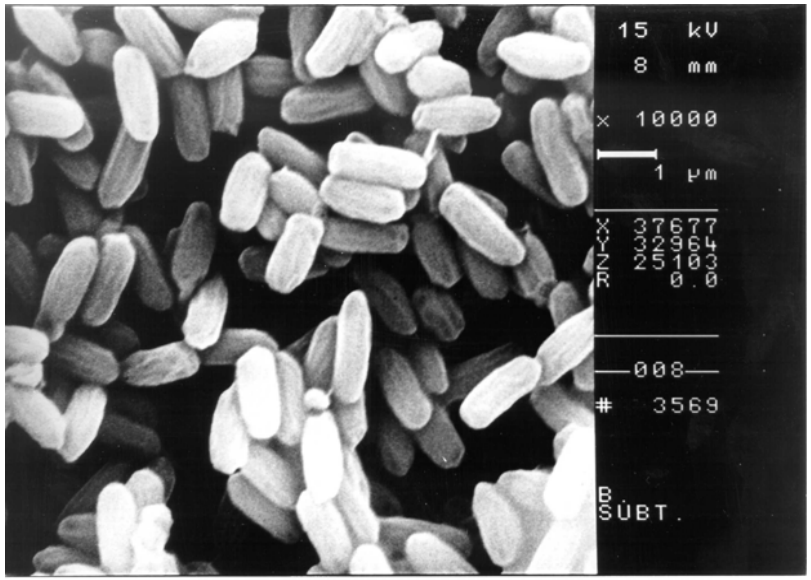
Water flow parallel to lamps

1 sensor (radiometer)

Biodosimetry

Use of microorganisms (calibrated for UV-sensitivity) to measure the “dose” which is applied to the water in a UV-disinfection plant.

The result of this measurement is the Reduction Equivalent Fluence (REF). Originally called Reduction Equivalent Dose (RED). Unit: J/m²



A. CABAJ, R. SOMMER and D. SCHOENEN:
Biodosimetry: Model Calculations for u.v. water disinfection devices with regard to dose distributions
Water Research 30 (1996) 1003-1009.

Spores of *Bacillus subtilis* (Biodosimeter)

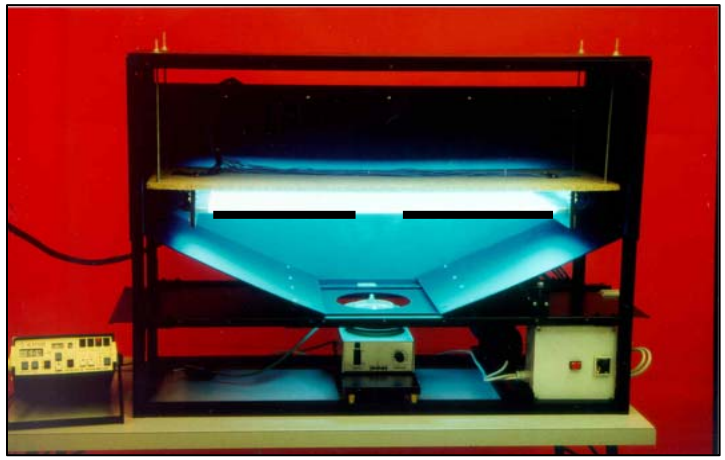
Alexander Cabaj

Practical measurement of REF

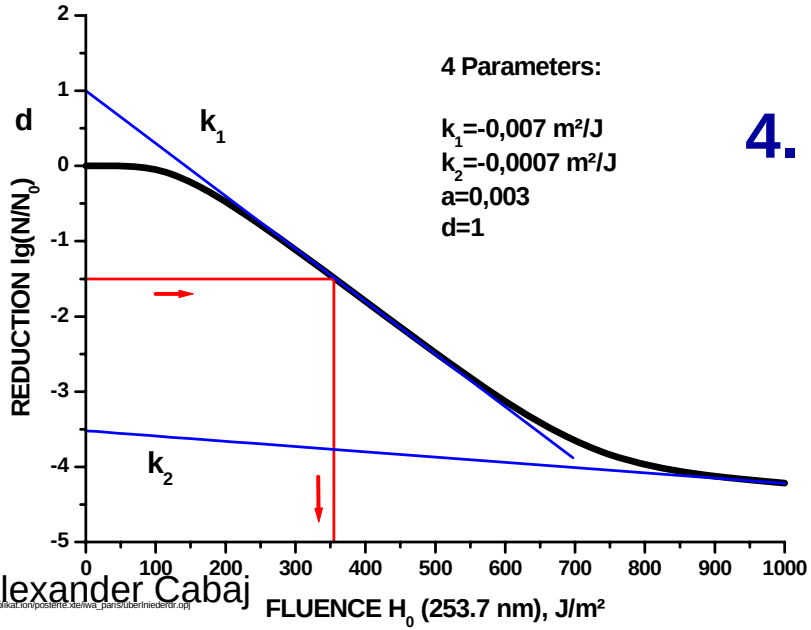
1. Calibration of biodosimeter

2. Measure reduction log(N/No) caused by UV-plant

3. Use calibration curve to determine REF



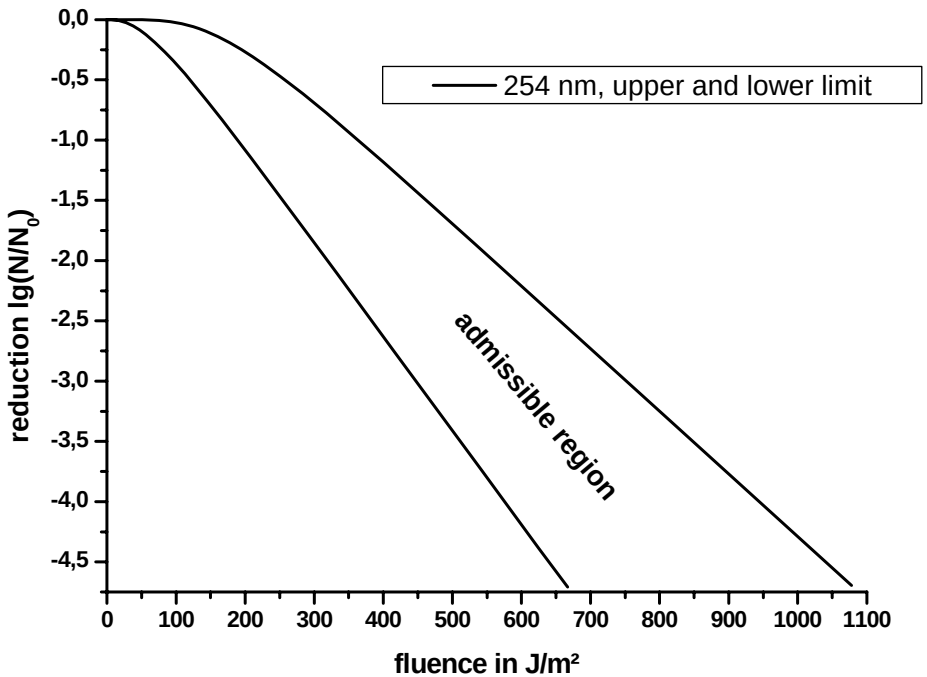
Laboratory irradiation device



4. Or: calculation of REF

$$REF = -\frac{1}{k_{253.7}} \cdot \lg\left[1-\left(1-10^{\lg\frac{N}{N_0}}\right)^{10^{-d_{253,7}}}\right]$$

The UV-sensitivity of the biodosimeter must be defined



ÖNORM M 5873-1:2001

ÖNORM M 5873-2:2003

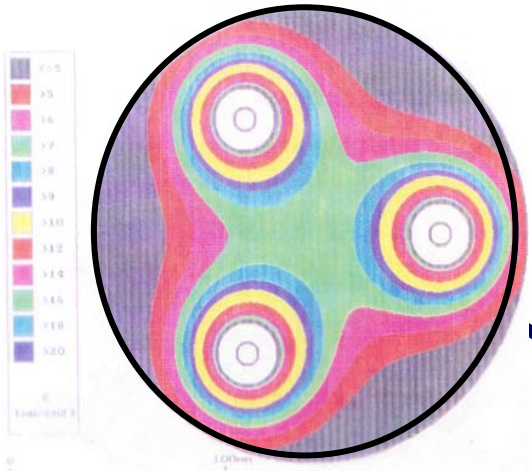
The constants k and d shall be in the following range:

$k = 0.0072 \pm 20\ \%\ m^2/J$

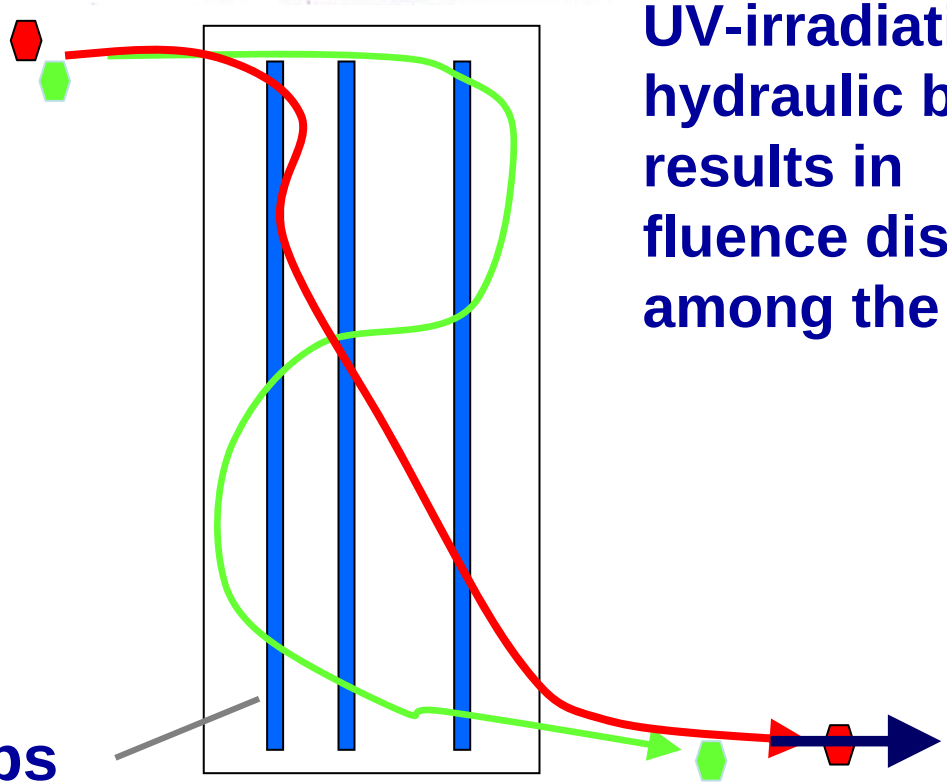
This means $0.0058\ m^2/J \leq k \leq 0.0087\ m^2/J$

$d = 0.87 \pm 30\ \%$

This means $0.61 \leq d \leq 1.13$)



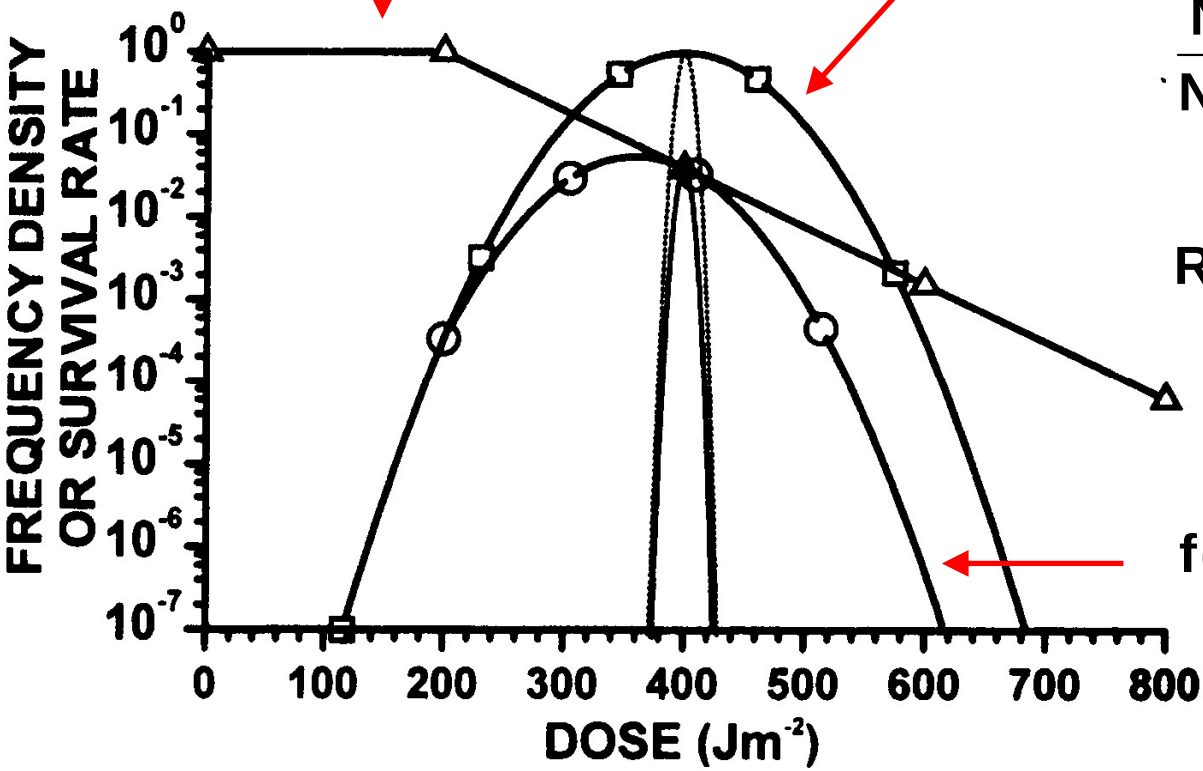
UV-irradiation field + hydraulic behaviour results in fluence distribution among the microorganisms



Definition of REF

Density function of the fluence distribution $f(H_o)$

Survival curve: $g(H_0) = \frac{N(H_0)}{N_0}$

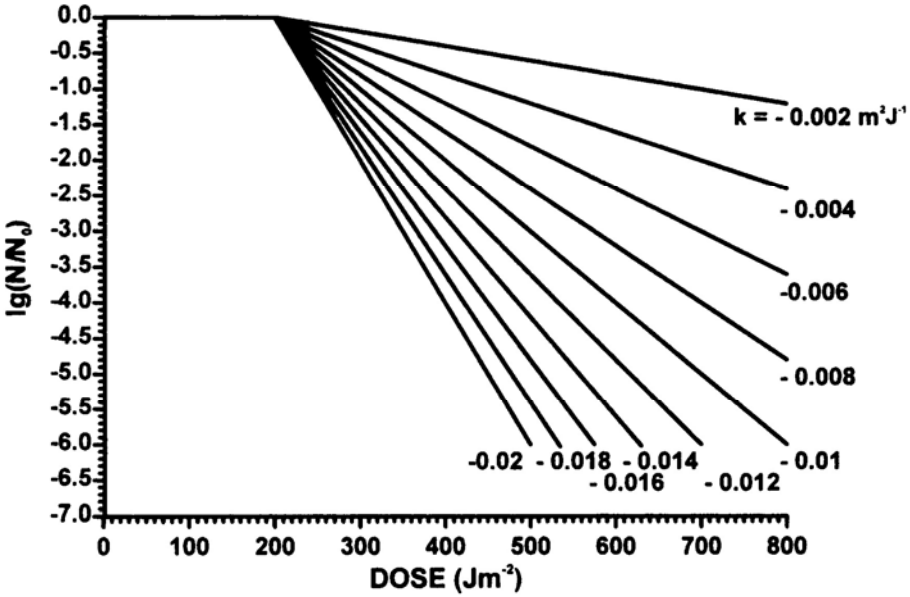


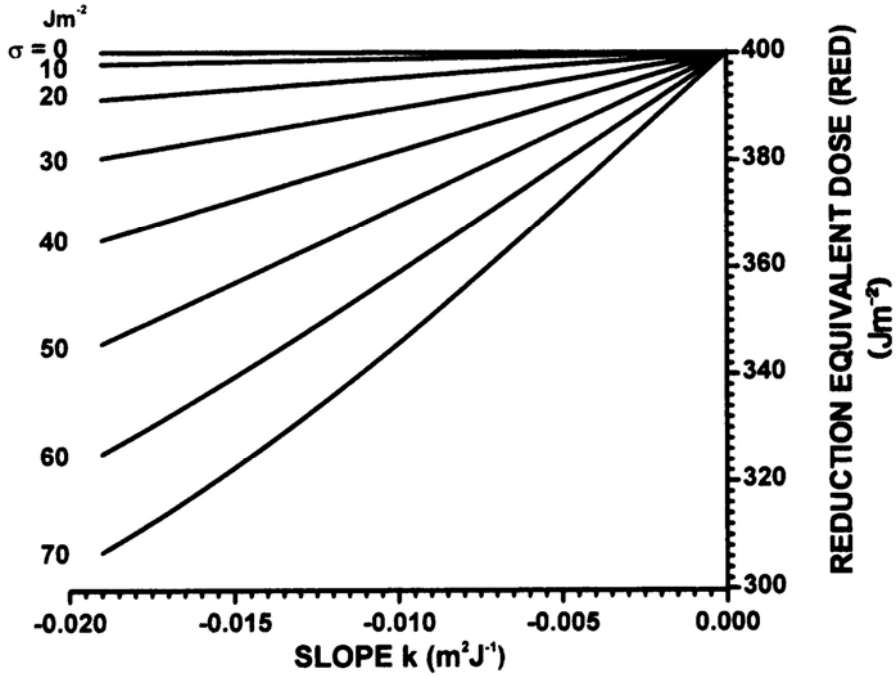
$$\frac{N}{N_0} = \int_0^\infty f(H_0) \cdot g(H_0) \cdot dH_0$$

$$REF = g^{-1}\left[\frac{N}{N_0}\right] \qquad \frac{J}{m^2}$$

$f(H_o) \cdot g(H_o)$

Definition of REF (continued)





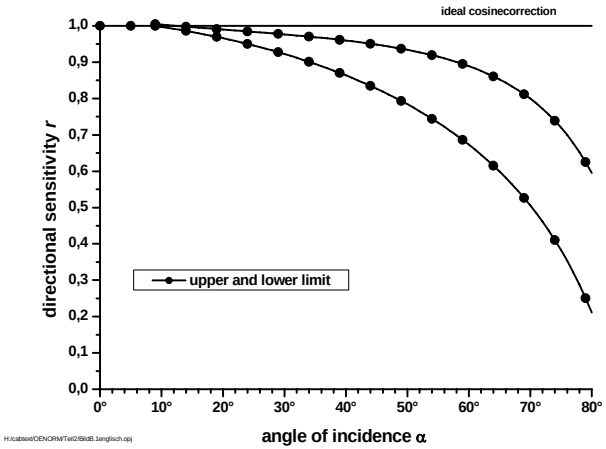
Survival curves with different slopes (UV-sensitivities) k (m²/J)

The REF measured with biodosimeters with different UV-sensitivities k is decreasing with increasing k.

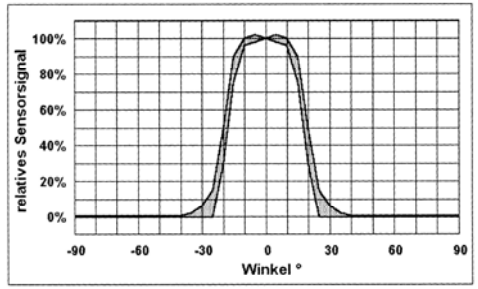
Broader distributions give lower REFs

Angular response of some sensor types for the measurement of „irradiance“

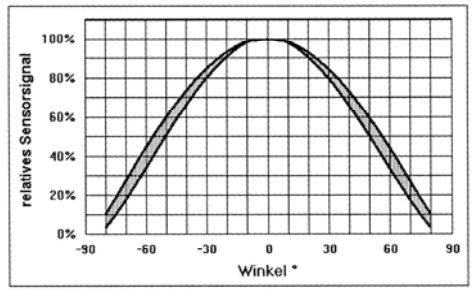
ÖNORM M 5873-1 und -2



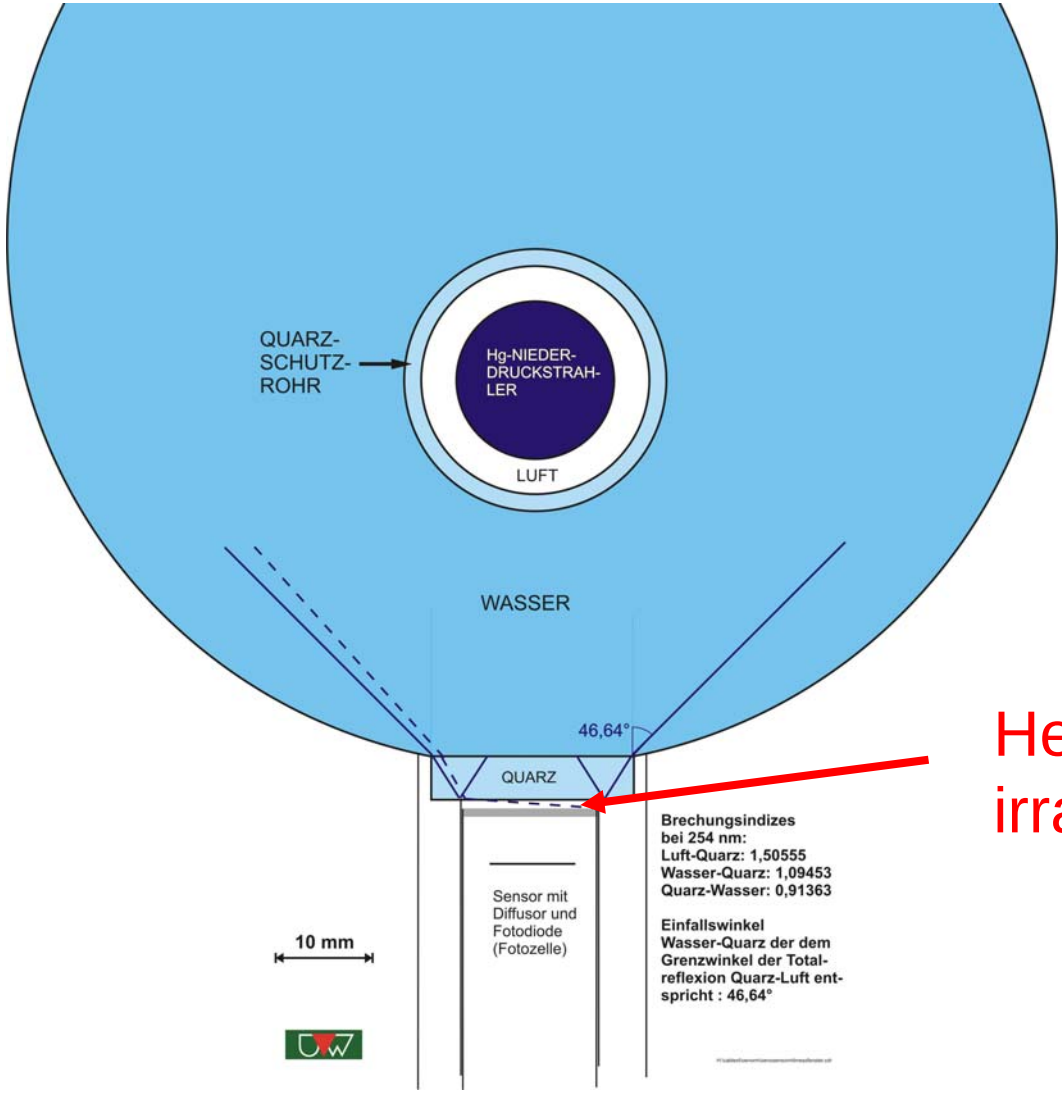
DVGW W 294-3



40° Sensor: Winkelabhängigkeit des Sensorsignals mit Toleranzbereich. (Daten erfüllen auch W 294 10-97)



160° Sensor: Winkelabhängigkeit des Sensorsignals mit Toleranzbereich (Daten erfüllen auch M5873 2001-03)



Here: reference irradiance

UV-plants from the following companies were type-tested according to ÖNORM M5873-1 and -2:

BWT, Austria

ITT-Wedeco, Germany

Hanovia, Great Britain

Davey Water Products, New Zealand

SITA, Italy

LIT, Russia

BIO-UV, France

Aquatech Solution, Switzerland

Visa, Austria

Umex Kirchheim, Germany

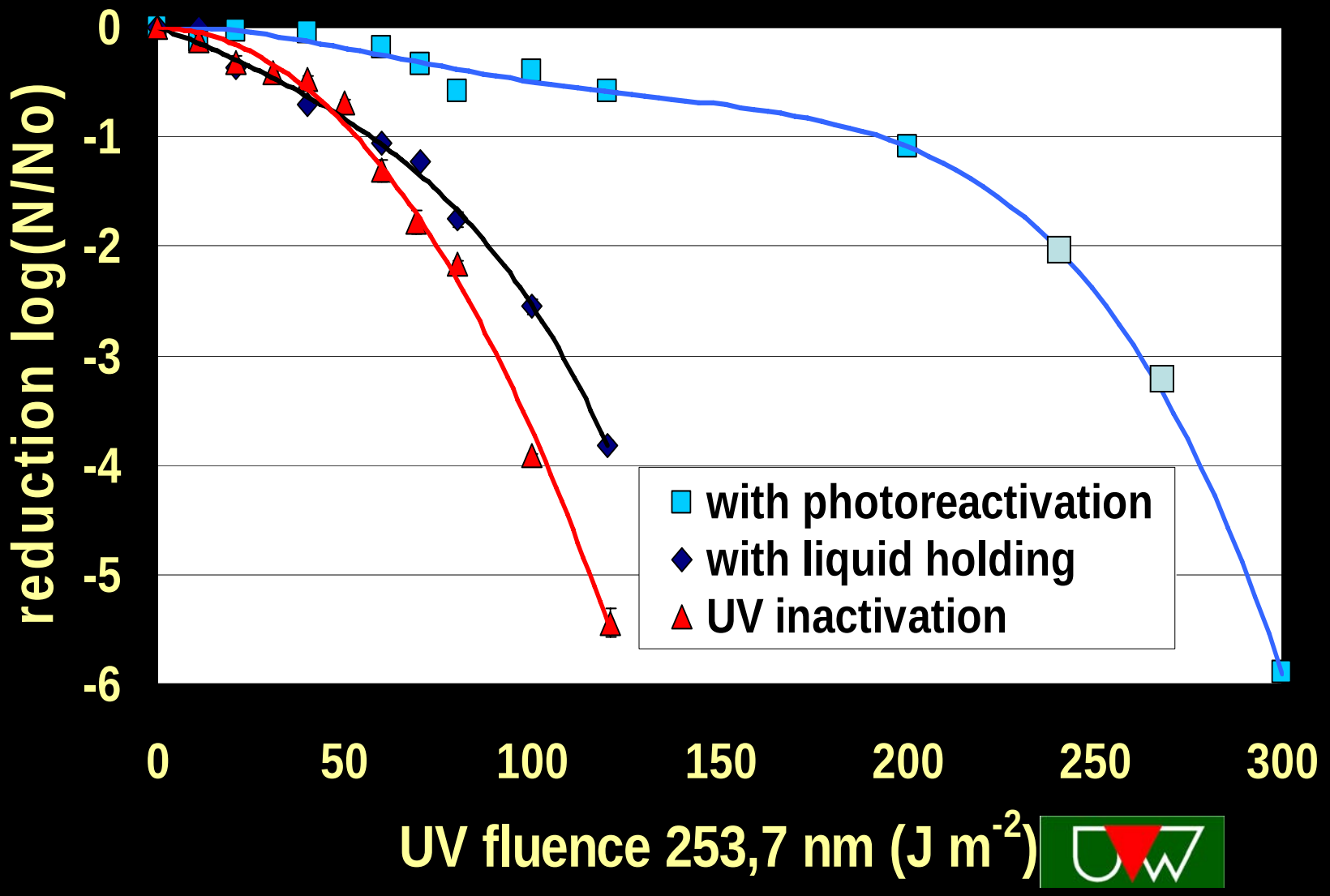
Umex Dresden, Germany

Berson, Netherlands

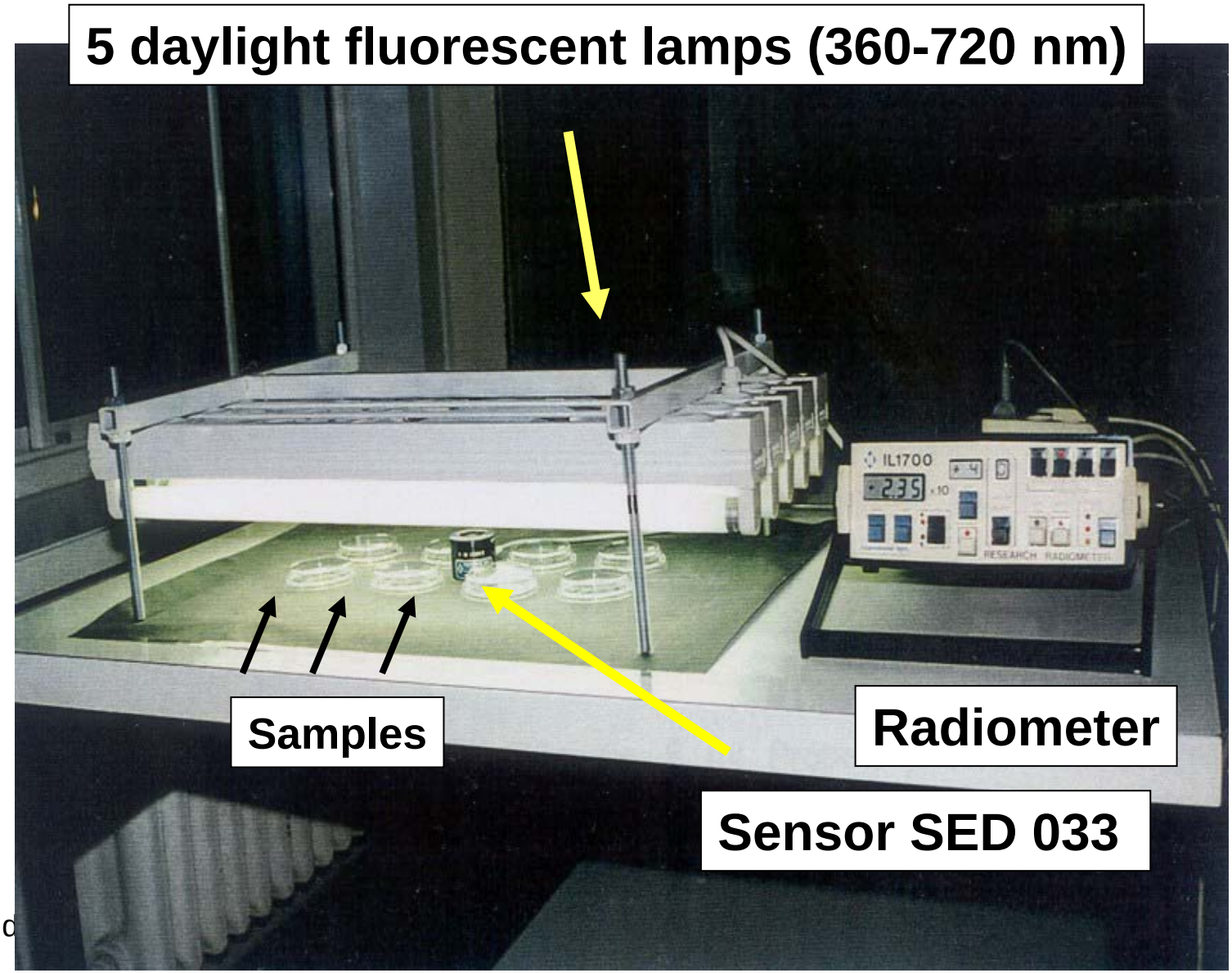
and others

Photoreactivation and dark repair

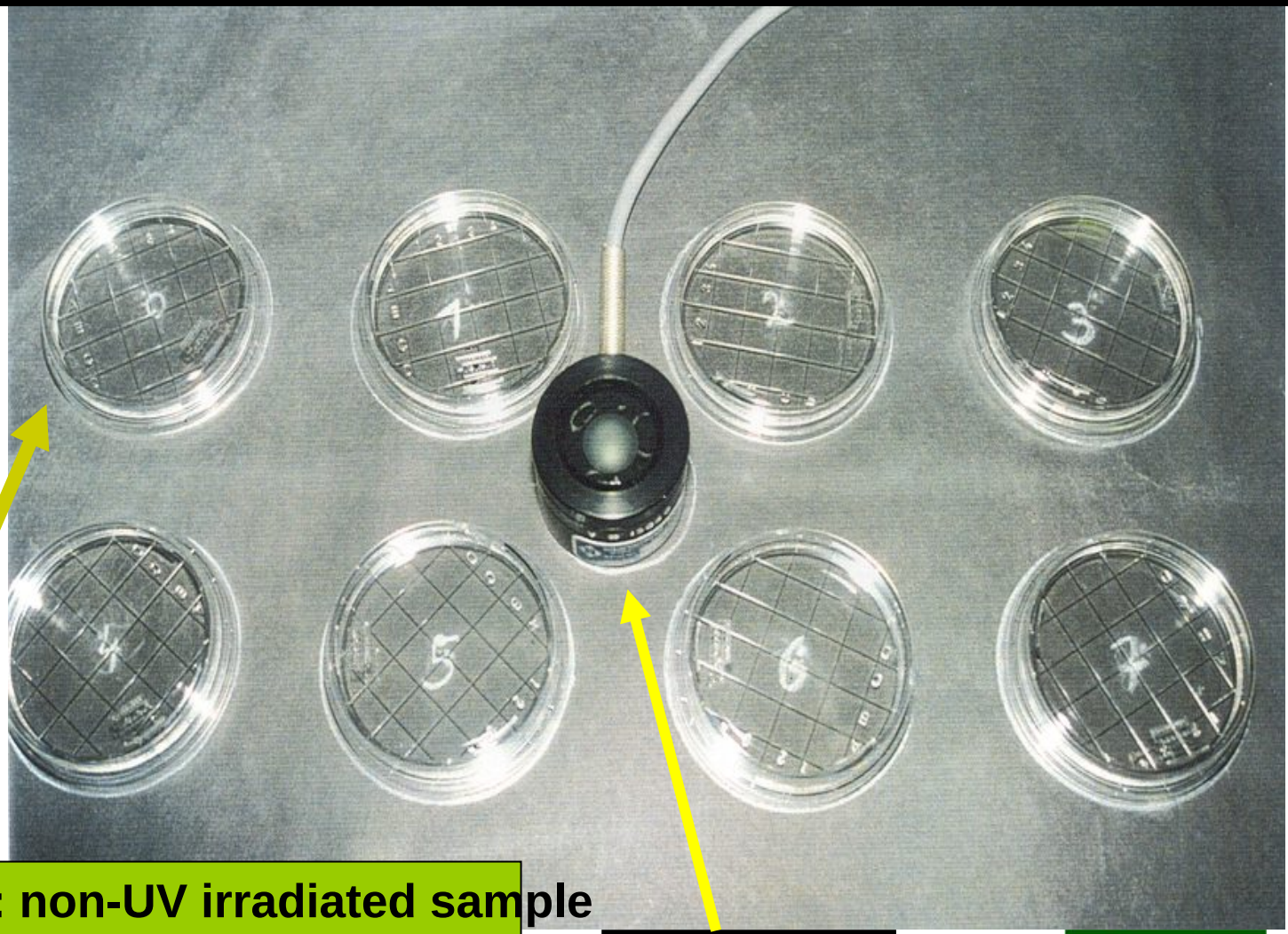
Pathogenic strain of *E. coli* (EHEC)



Illumination apparatus



Illumination of samples (23.500 Lux; overcast summer day in Middle Europe, 2 h)



Control: non-UV irradiated sample

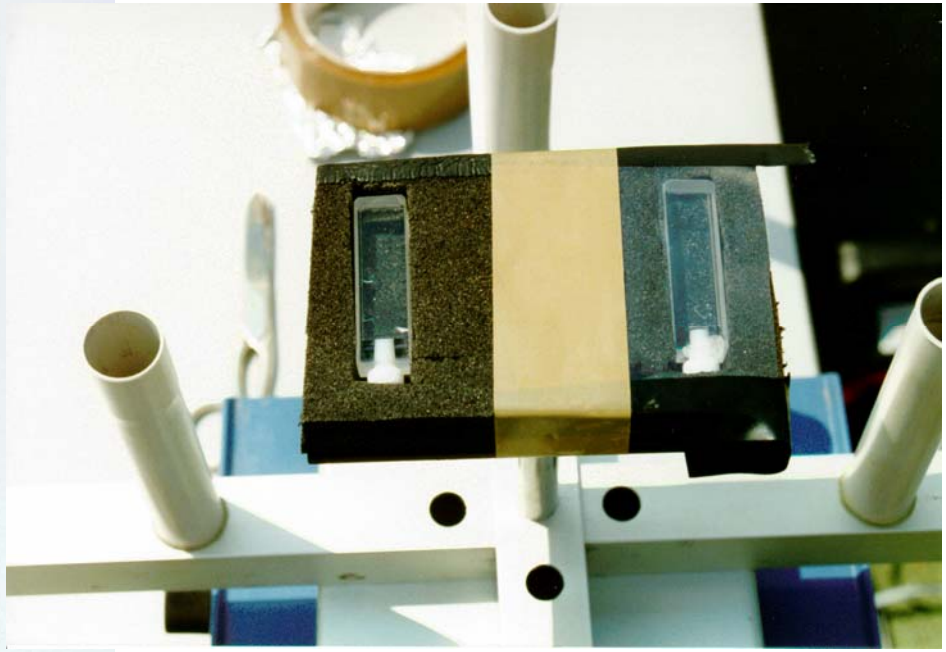
Sensor

Nea Michaniona, EU-project

The following two pictures visualise the inhomogeneity of the radiation field in the volume of the irradiation vessel if irradiated by a collimated beam:

Fig. 3.1/2: Spherical Suprasil-vessel filled with aqueous solution of fluorescein irradiated horizontally with a collimated beam of 254 nm radiation from left. Because of refraction the beam is concentrated on the right side in the solution. Up and down darker regions can be seen. Due to schlieren in the material of the vessel rays of radiation are formed.

Fig. 3.1/3: Same arrangement as above but for semi-spherical vessel. In this case radiation comes under an angle of about 45° up from left



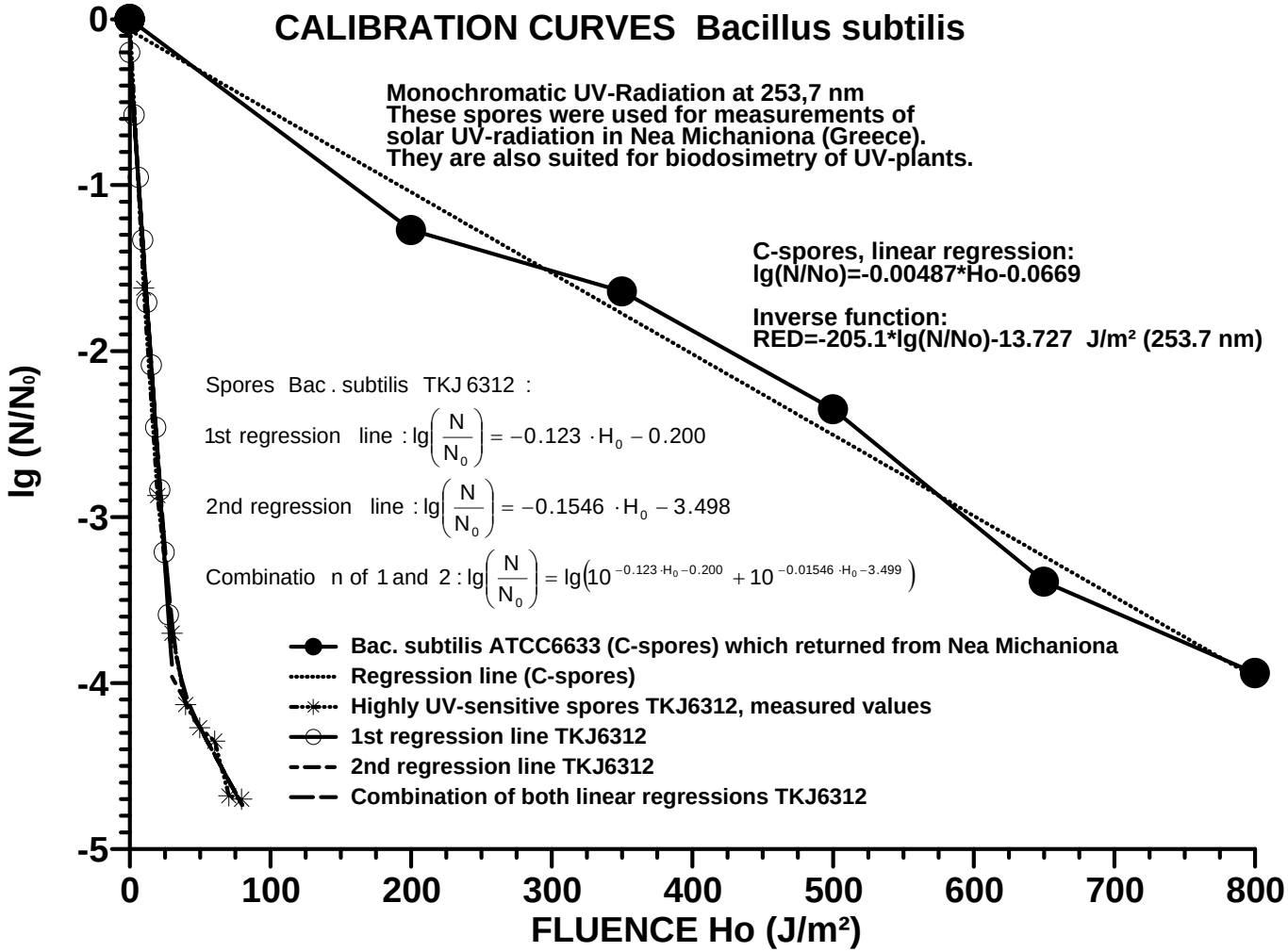
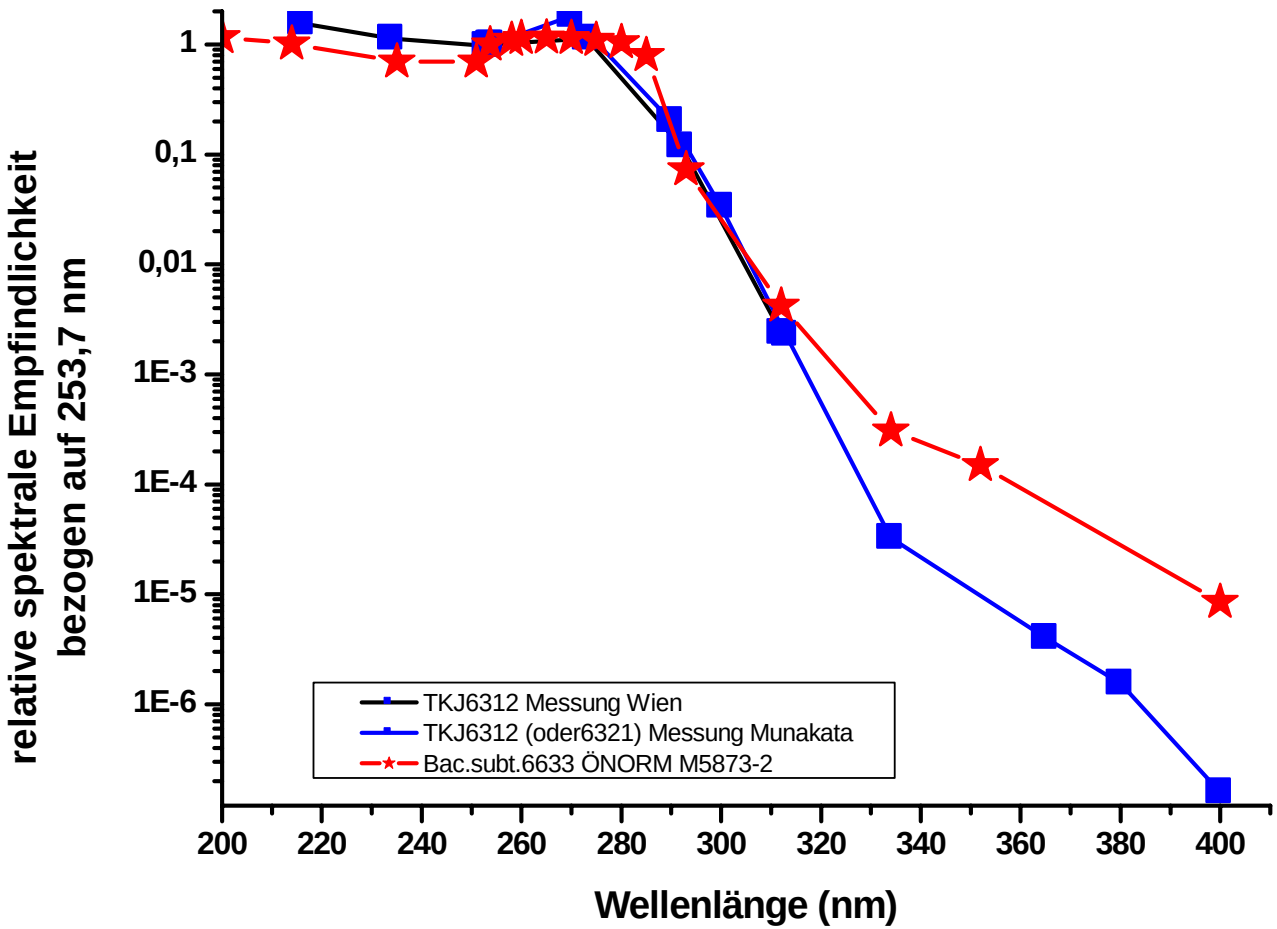


Fig. 4.2.3/17: Calibration curves for measurements in Nea Michaniona

10/01/98 15:27:18 C:\CABTEXT\EUPROJEK.T\BERICHTE\ENDBER~1\CSPORK.SPF

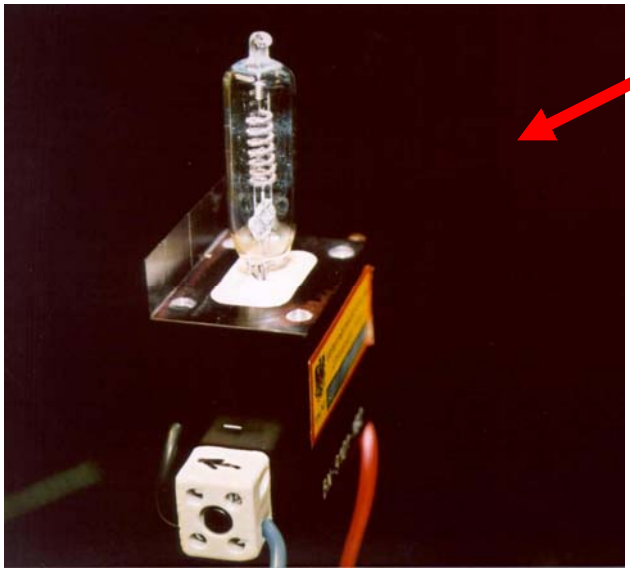
Solar UV-radiation	Irradiance 250 - 400 nm	Fluence or REF within 3 h 250 - 400 nm
	W/m²	J/m²
Measurement with spectro-radiometer	60,9	658 000
$REF_{253,7}$ measured with spores of *Bacillus subtilis ATCC 6633*	0,04523	489
$REF_{253,7}$ measured with spores of *Bacillus subtilis TKJ 6312*	0,0268	290

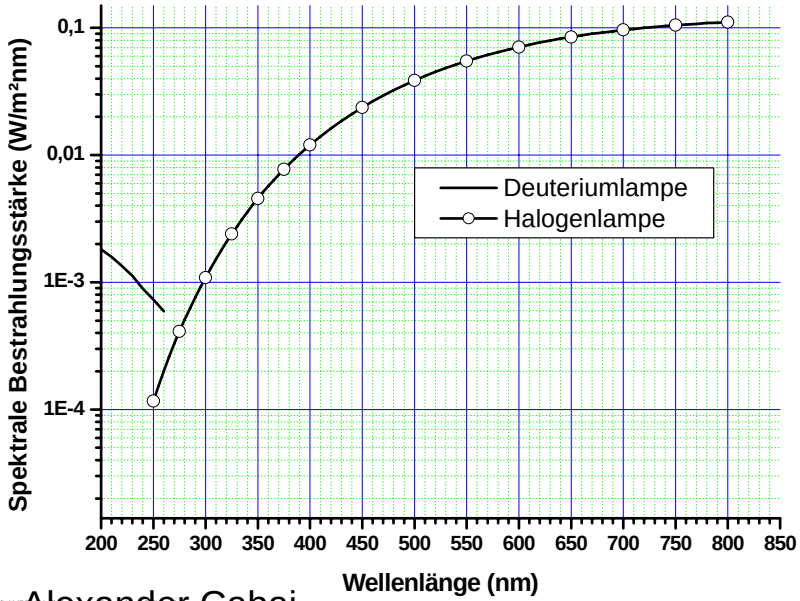


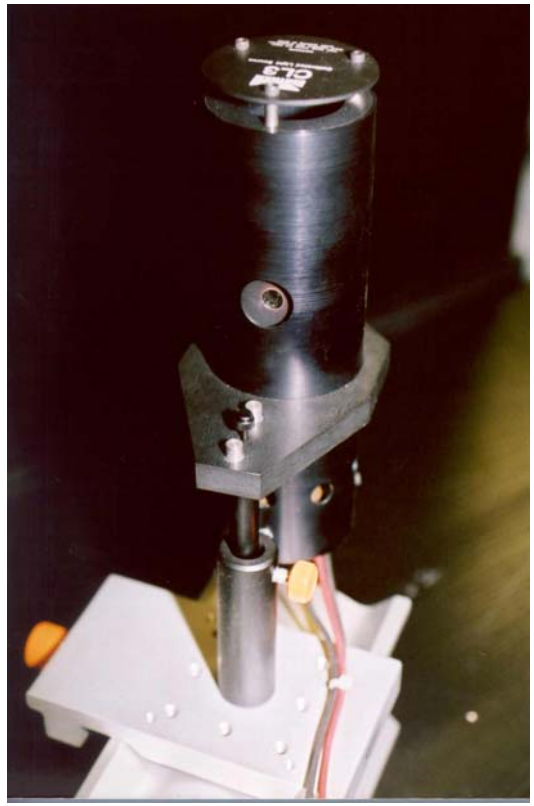
H:/cabtext/BiologischeWirkungsfunktionen/spektrempfindlichkeitenmikroorganismen.opj graph 4

Calibration lamps

FEL-lamp: 110 V; 8,0000 A
250 nm – 800 nm







Deuterium lamp: 200 nm – 400 nm