# Immunological and cellular reactions influenced by microgravity and space irradiation – development of a biosensor with phagocytes (TRIPLE-LUX B)


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**www.tu-berlin.de/~oekotox**

**BMFT/DLR – esa/ESTEC:**
**TRIPLE-LUX-B project**

# Immunological and cellular reactions influenced by microgravity and space irradiation – development of a biosensor with phagocytosis active cells (phagocytes)

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# Overview

- **Objectives**
- **Immunological Endpoint: Phagocytosis**
- **Cryoconservation of the Immuncells**
- **Experimental frame criteria and the measurement concept**
- **Results**


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# The TRIPLE-LUX-B Project contributes to risk assessment concerning immunotoxicity under space flight conditions

## The assay system of the TRIPLELUX-B Experiment will be performed with a well-defined quantification and evaluation of the immune function phagocytosis

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**The results expected will allow us to conclude whether the observed responses are caused by microgravity and/or radiation**


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**The definition of the immune response by mussel hemocytes is the selective reaction to particles which are indentified as foreign by its immune system shown by phagocytosis**

**Adhesion**
**Ingestion**
**Phagosome formation**


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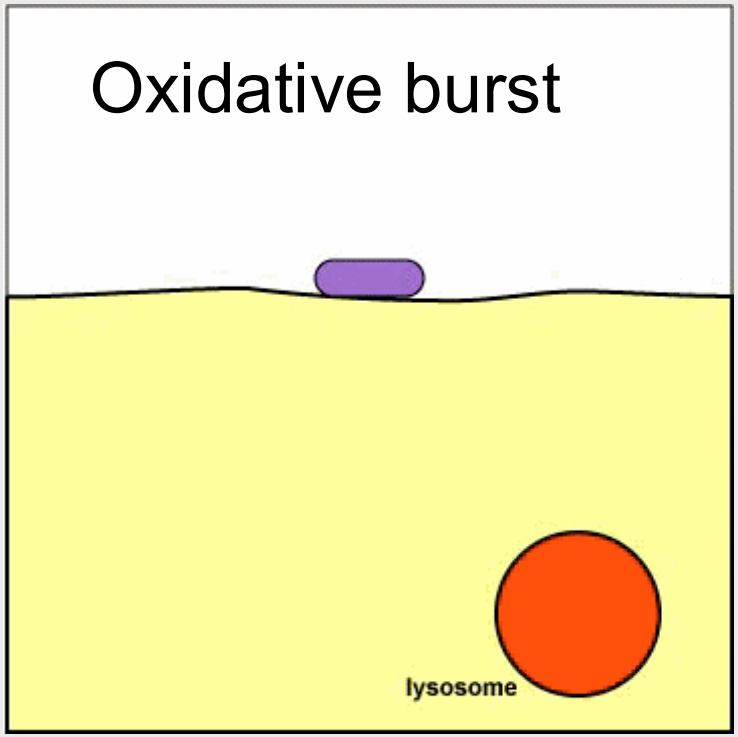
# Assays for phagocytotic activities are based on

- Chemotaxis → Migration assays
- Uptake of labelled particles → Fluorescent assays
- Oxidative burst → Chemiluminescent assays





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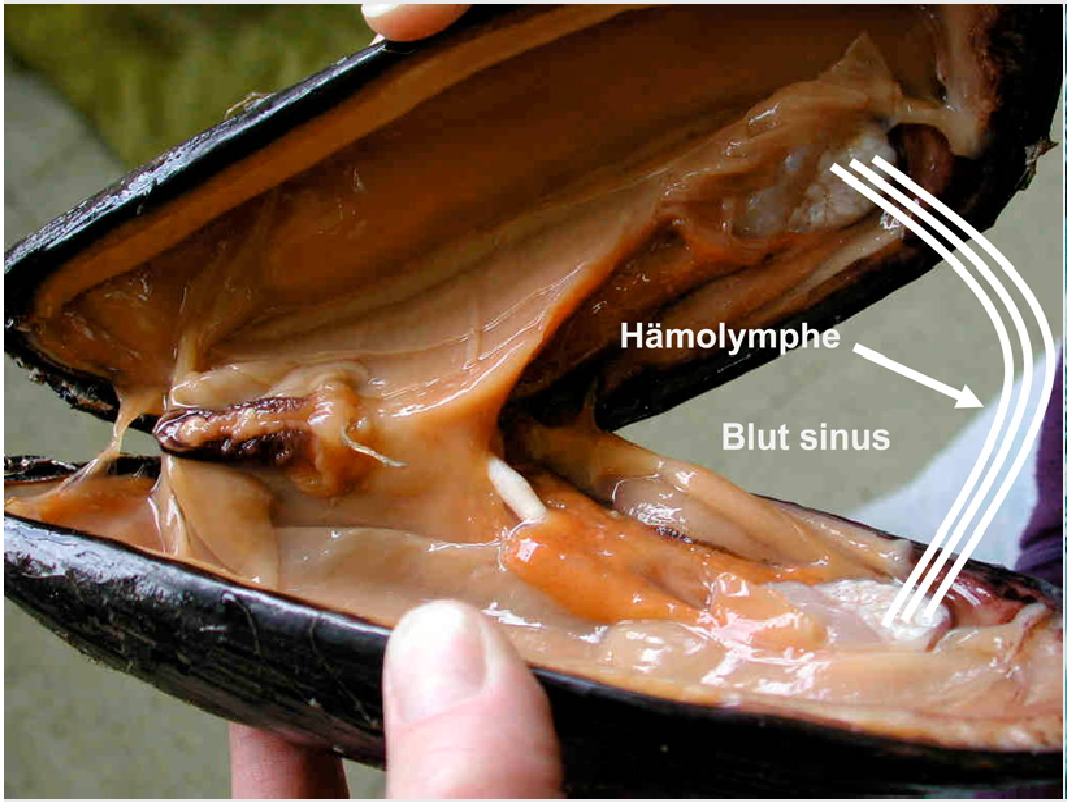
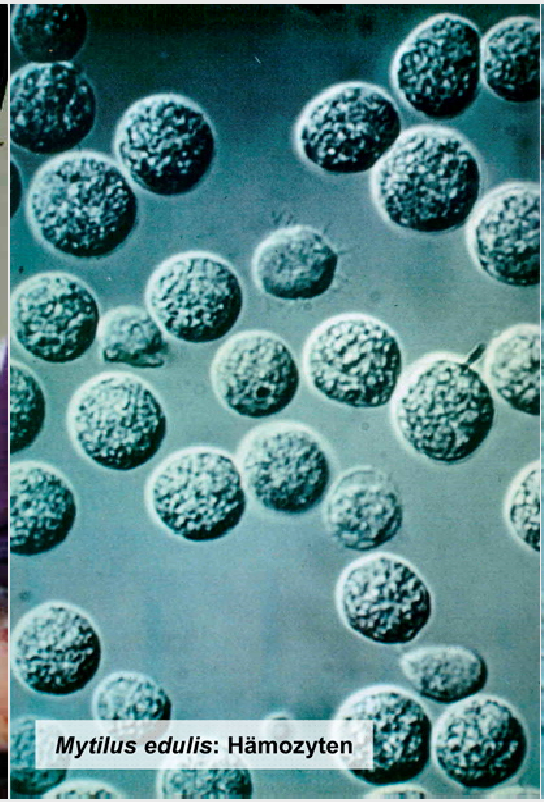
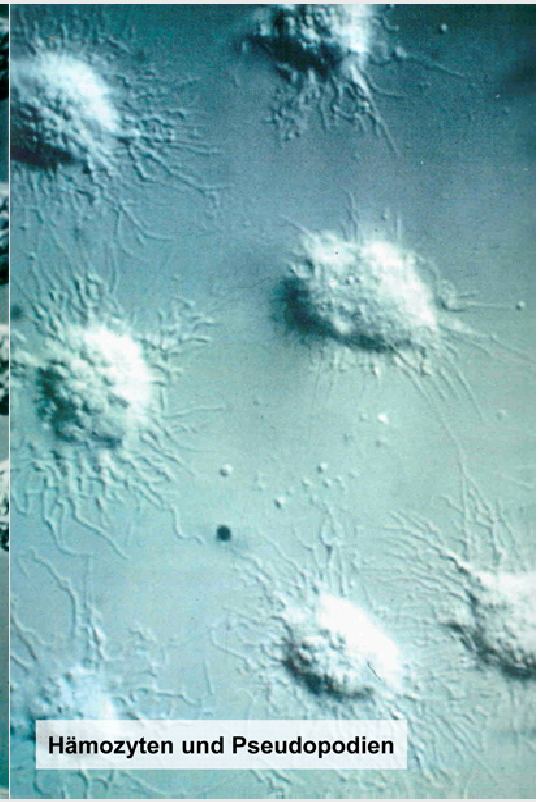
# Immuncells = Hemocytes





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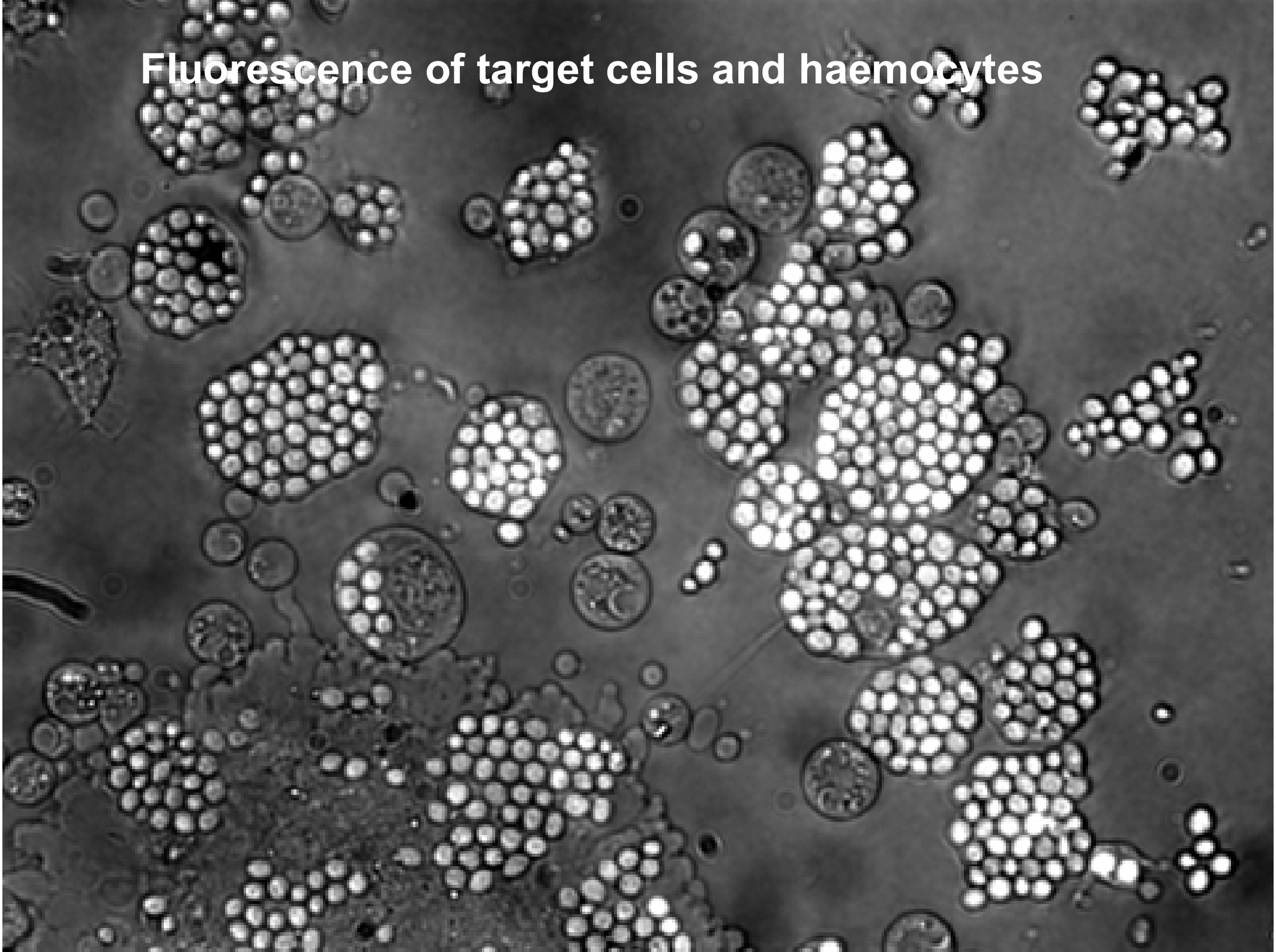
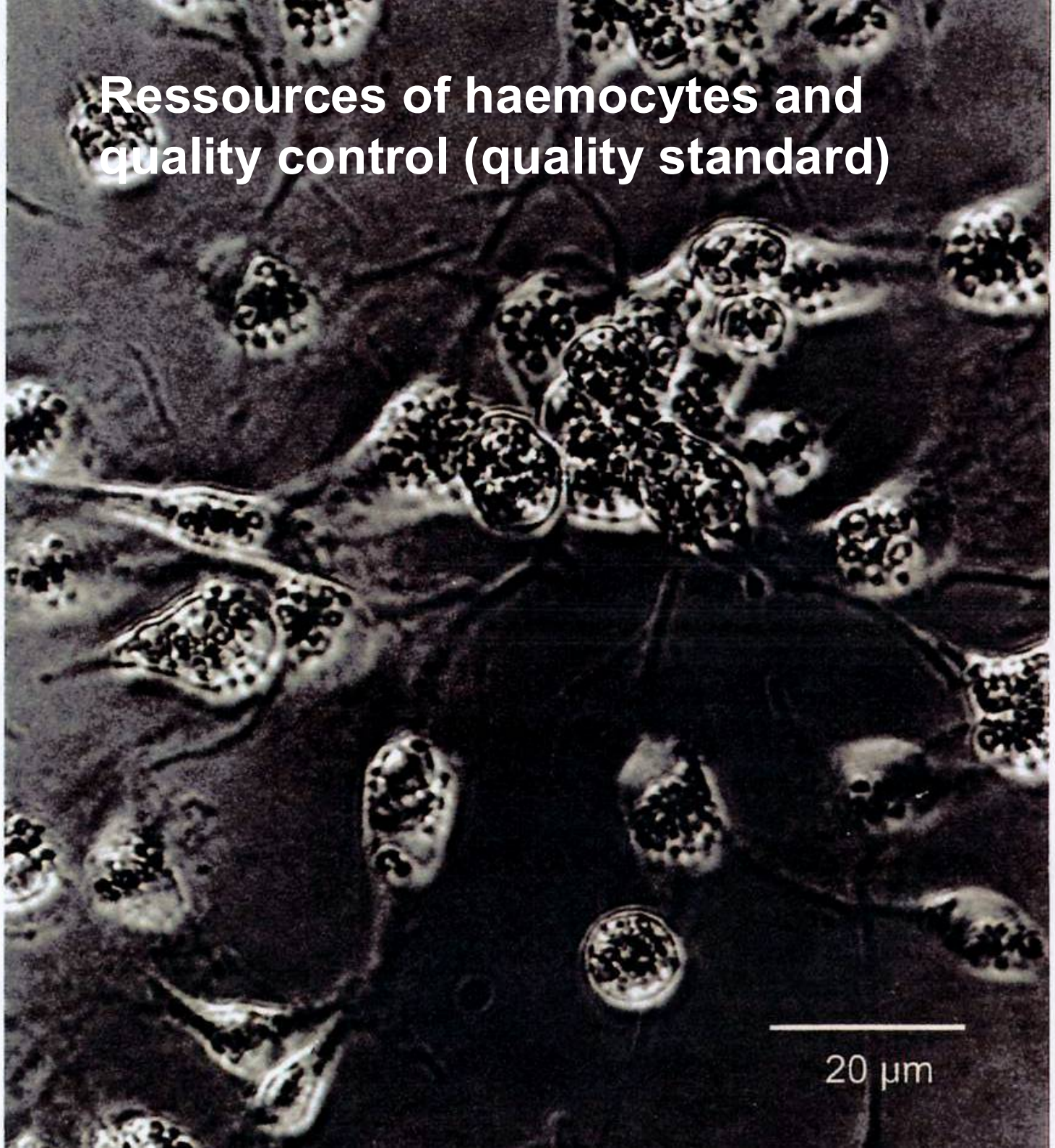
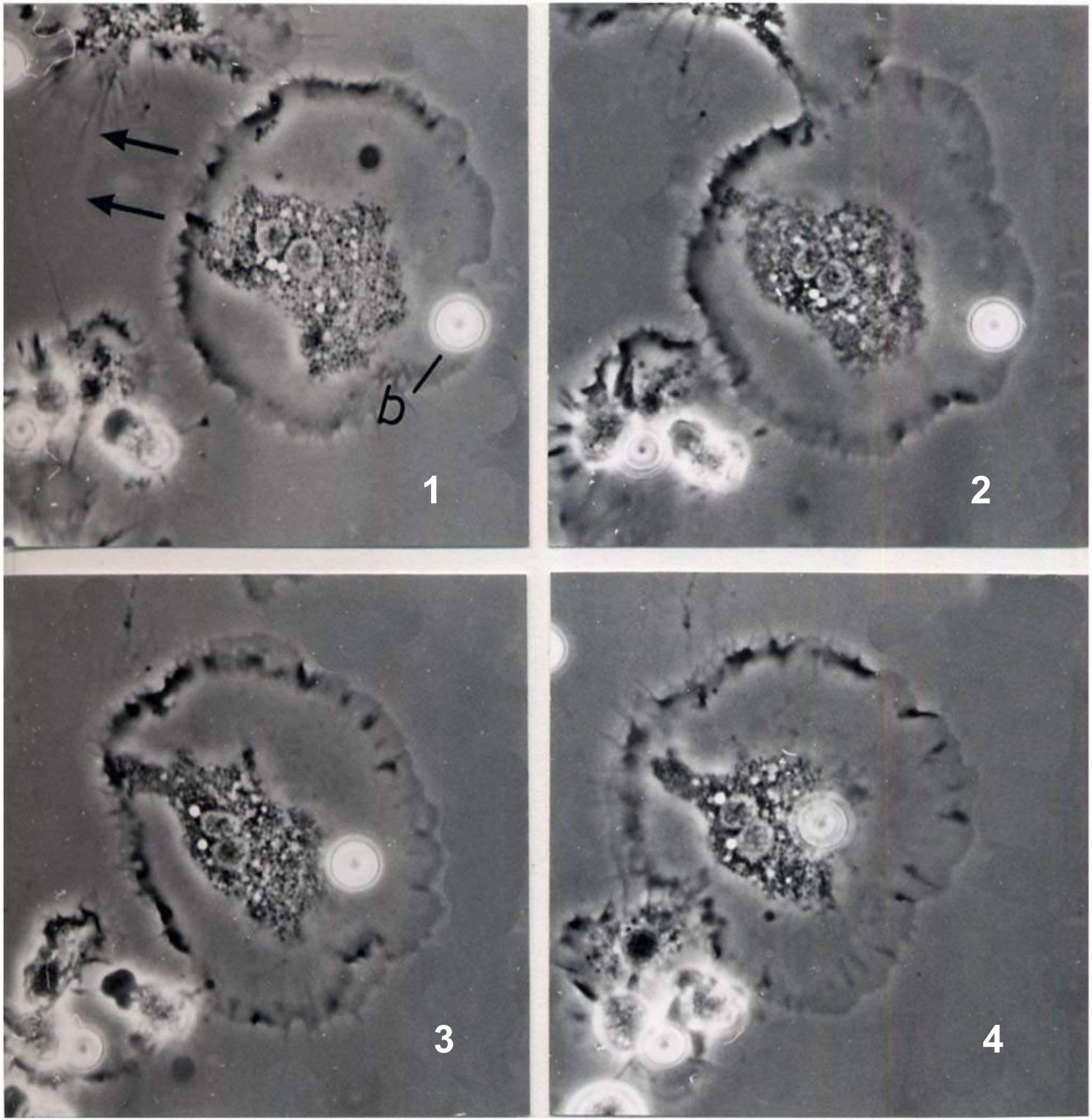

Fluorescence of target cells and haemocytes

# Ressources of haemocytes and quality control (quality standard)




**Haemocytes after 11 months in primary culture**

**Siegmund 2002**




A latex bead (b) attaches to the ectoplasma of a spread haemocyte of *Mytilus edulis* and is transported on the surface of the cell to its endoplasmatic area.

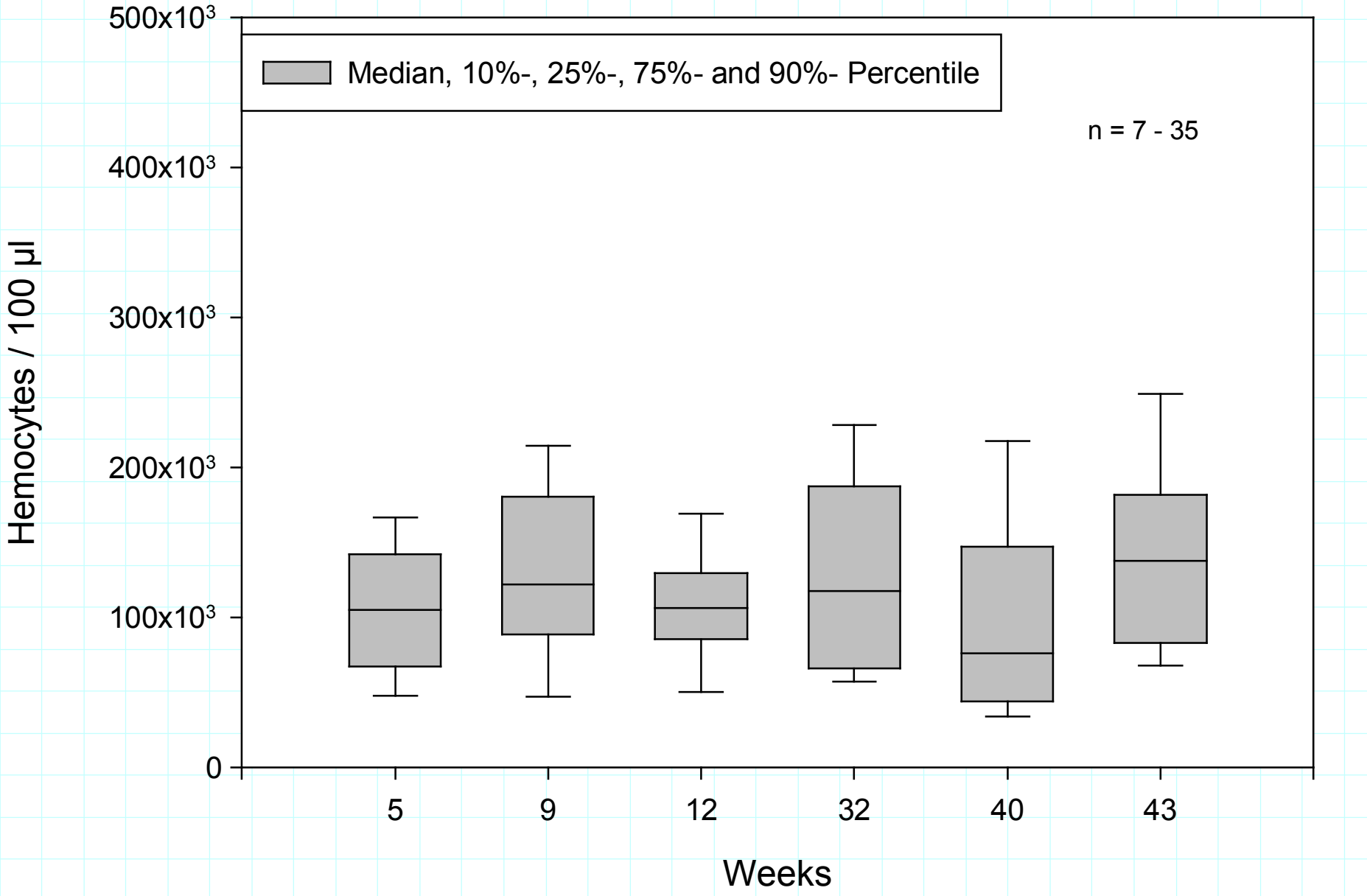
Zeiss PhaCo, 530x – Latexbeads: 7.6 µm

Time range **1-2-3-4 = 3-6-3 min.**

Renwrantz, L. 1990. Internal defence system of *Mytilus edulis* In: Neurobiology of *Mytilus edulis*, Ed. G.B. Stefano, Manchester University Press, 269

erlin

# Haemocytes in mussels kept under standardised laboratory conditions



# Phagocytosis

- Adhesion at the surface of the hemocytes (carbon-specific surface receptor)

- incorporation and formation of the phagosome
- melting of phagosome and lysosome
- oxidative destruction – “Oxidative burst“ (ROS)
- enzymatic degradation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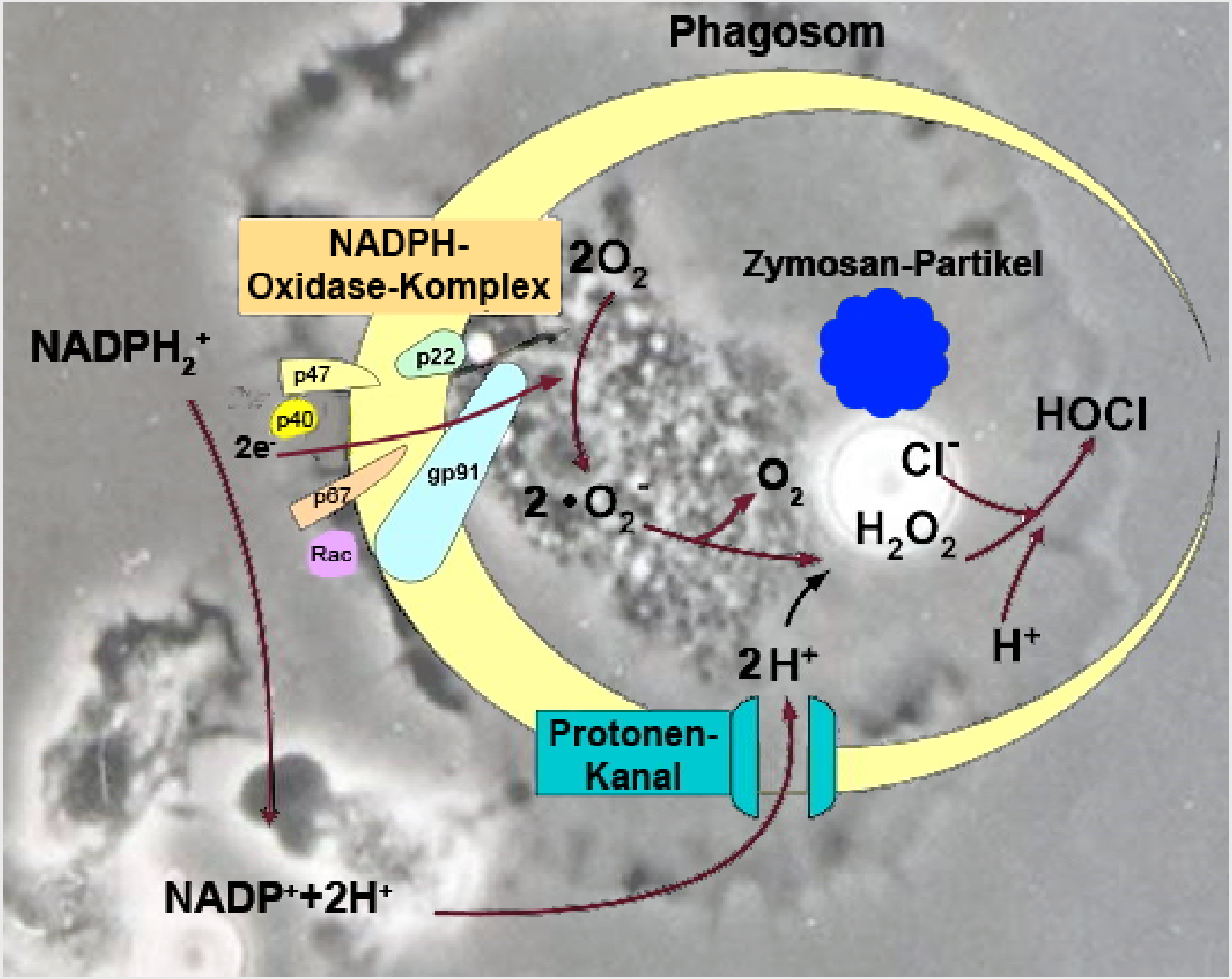


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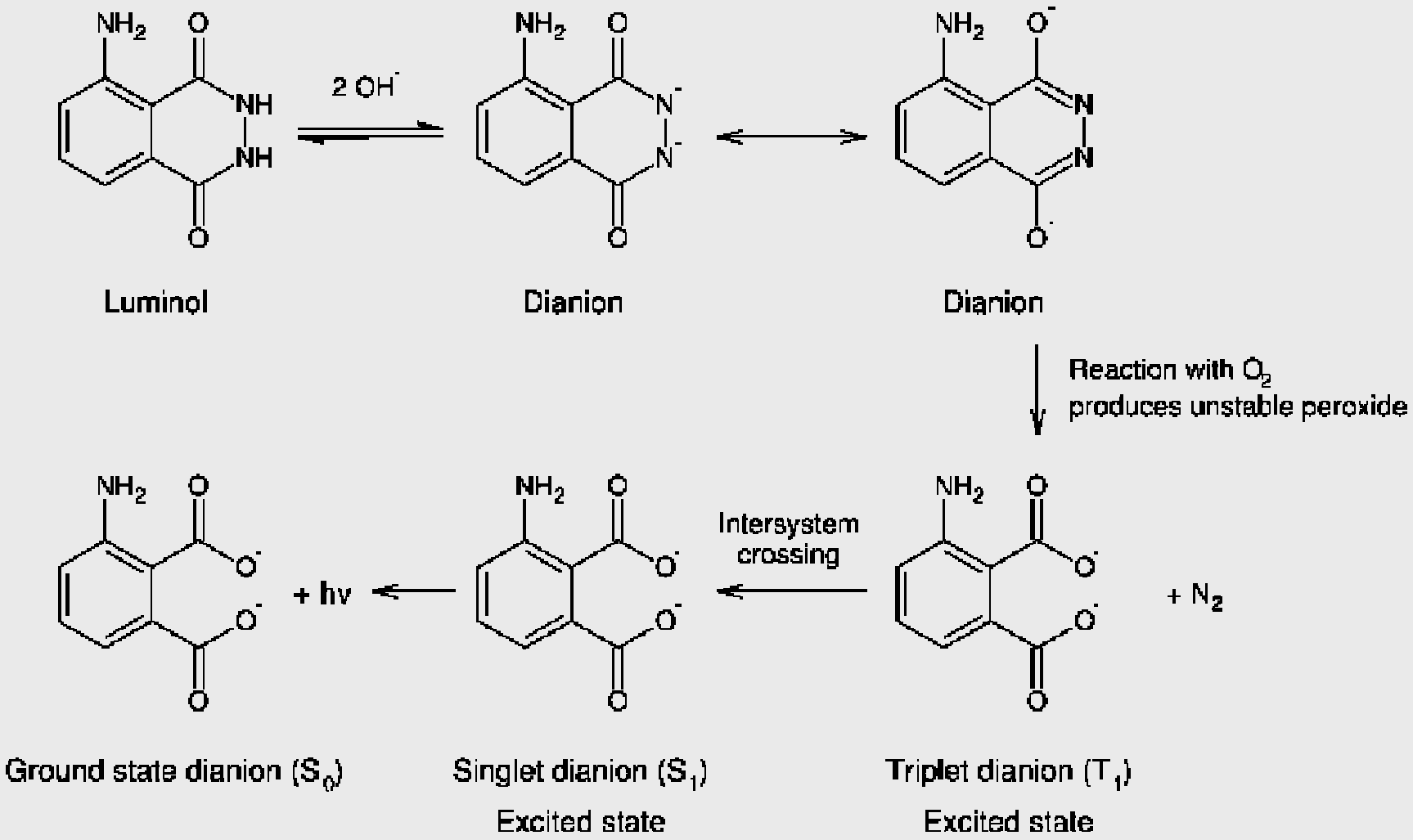
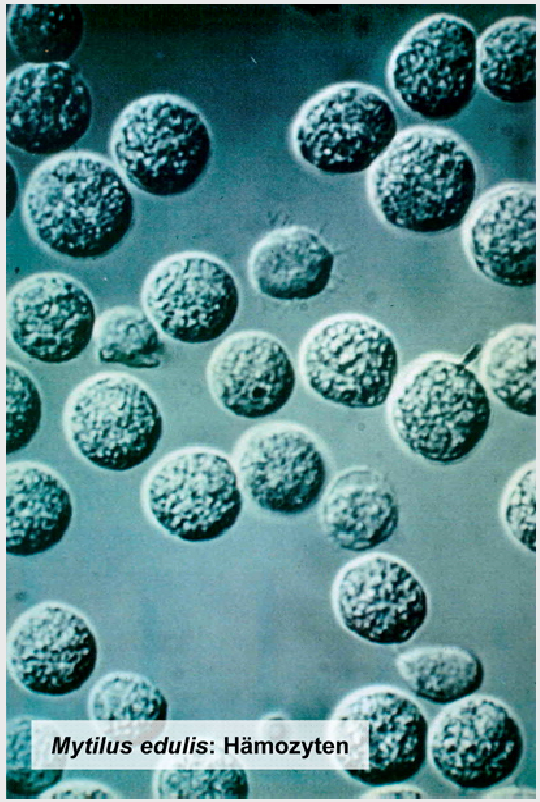
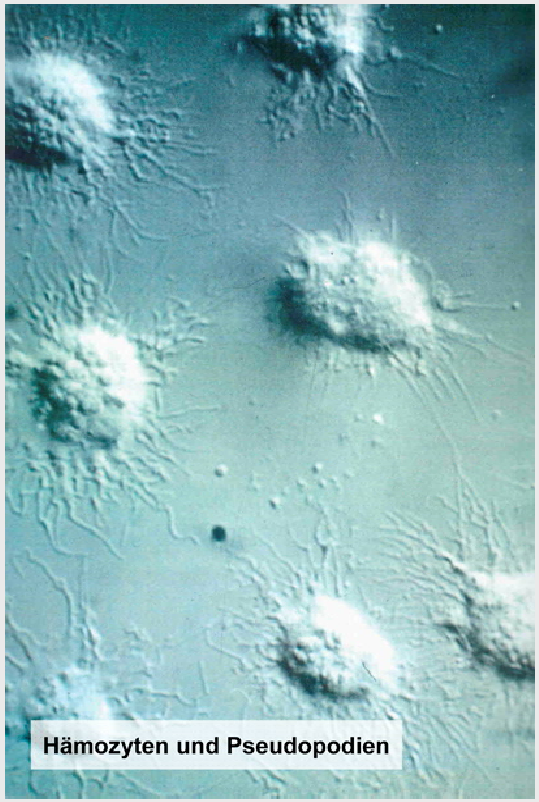

NH2
O
NH
NH
O
2 OH-
NH2
O
N-
N-
O
NH2
O-
N
N
O-
Luminol
Dianion
Dianion
Reaction with O2
produces unstable peroxide
NH2
O
O-
O-
O
+ hv
NH2
O
O-
O-
O
Intersystem
crossing
NH2
O
O-
O-
O
+ N2
Ground state dianion (S0)
Singlet dianion (S1)
Excited state
Triplet dianion (T1)
Excited state

# Luminescence Measurements with native, fresh cells and cryopreserved / reconstituted cells

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## Phagocytosis a key-function in immunology

**Adjustment and conditioning of the cells (Hemocytes) to given experimental conditions in the AEC (advanced experimental containment):**

- **Hemocytes in suspension**
- **Cryokonservation of the Hemocytes and Phagocytosis Activity**



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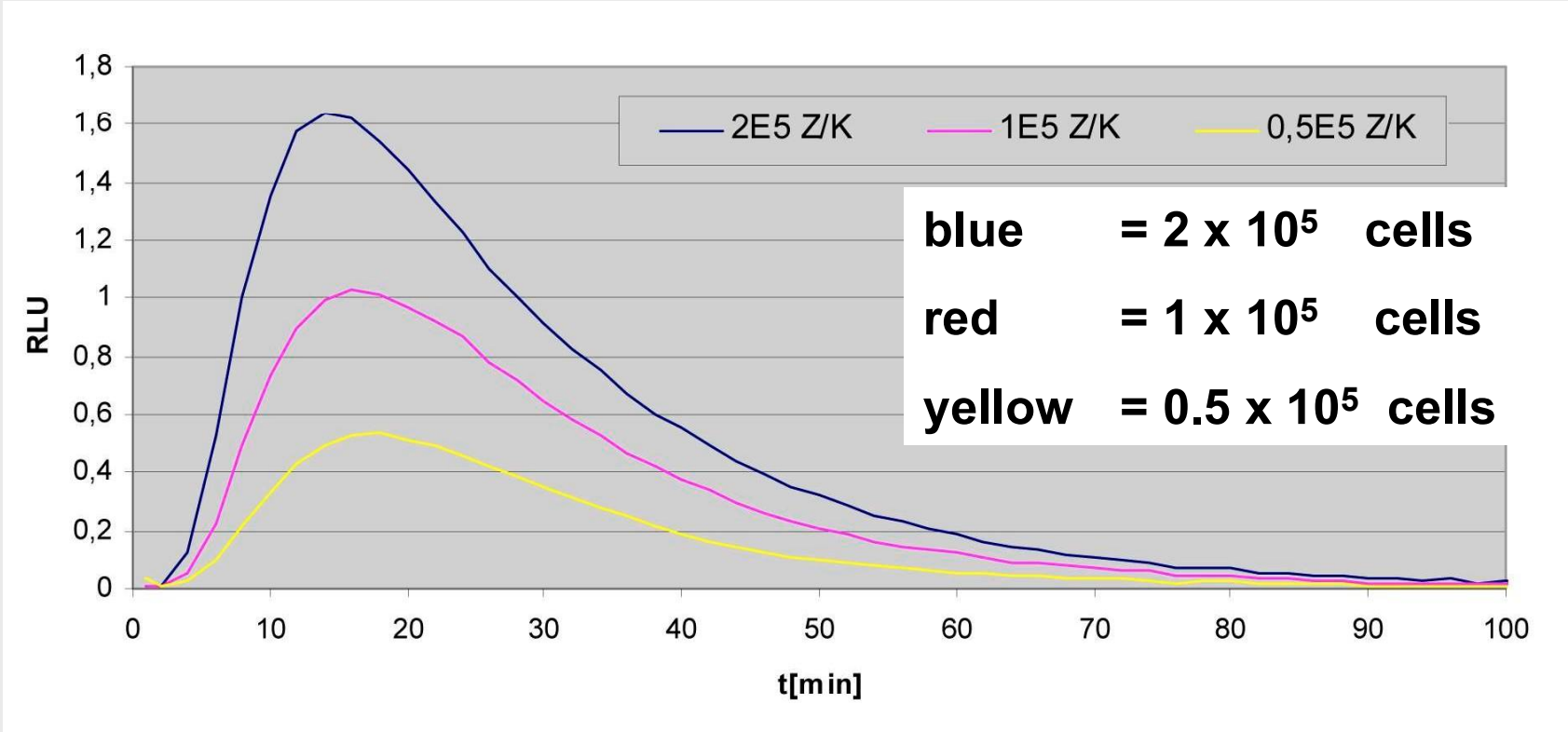
# Luminol luminescence in pools of freshly collected haemocytes after addition of peroxydase and zymosan fresh cells:
## Cell number and resulting bioluminescence signal

micro testplate (white); different cell concentrations per well; Dynatech ML3000

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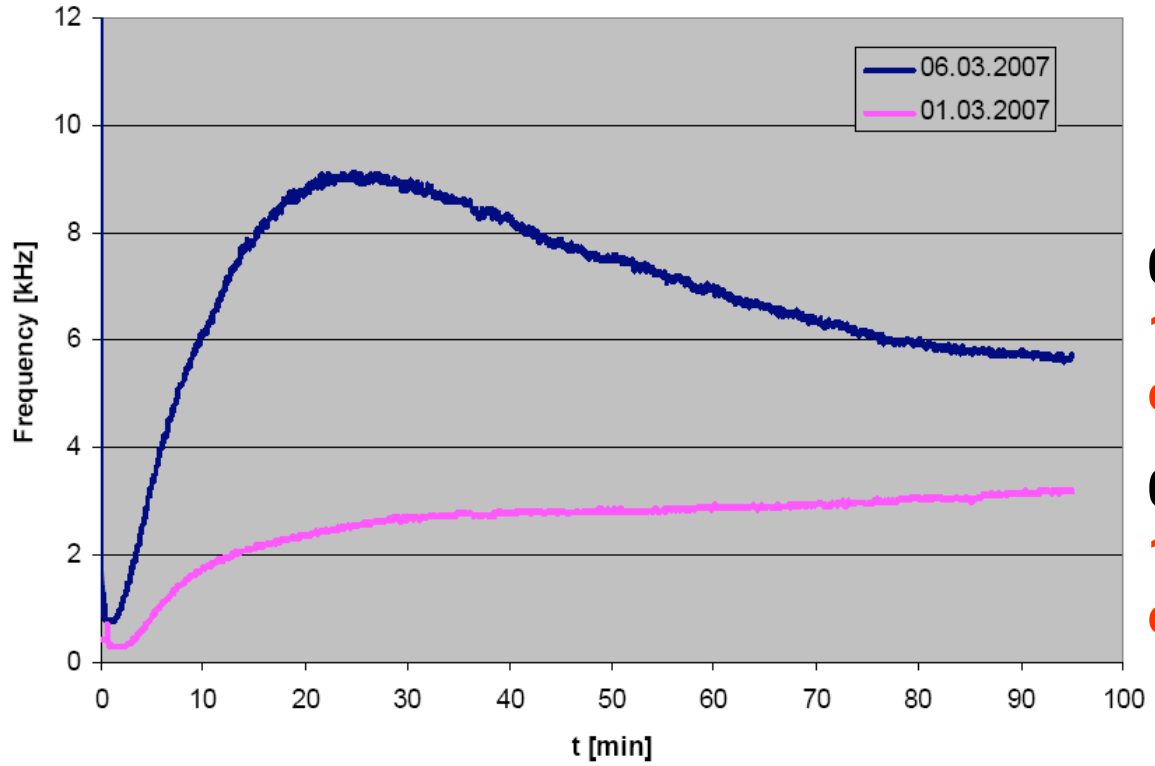
# Luminol luminescence in pools of freshly collected haemocytes after addition of peroxydase and zymosan (AEC/breadboard measurements for in-flight simulation)

## Cuvette with Stirrer, Photon counter




**06.03.07**
**1,670000 cells/ml**

**01.03.07**
**1,250000 cells/ml**

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# Cryokonservation of the Immune Cells

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# Media for Cryoconservation our first trial: classical way

- In Serum with 10 % DMSO, 2% Glycerol, 1% ß-Mercaptoetanol
- In Serum with 10 % DMSO, 2% Glycerol,
- In Serum with 10 % DMSO, 1% ß-Mercaptoethanol

# Media (new) for Cryoconservation

- Serum + Polyvinylpyrrholidone (PVP) +
- Serum + PVP und DMSO
- Culturemedium + PVP + Serum (cellfree Hämolymph)
- Culturemedium + PVP + Serum + DMSO


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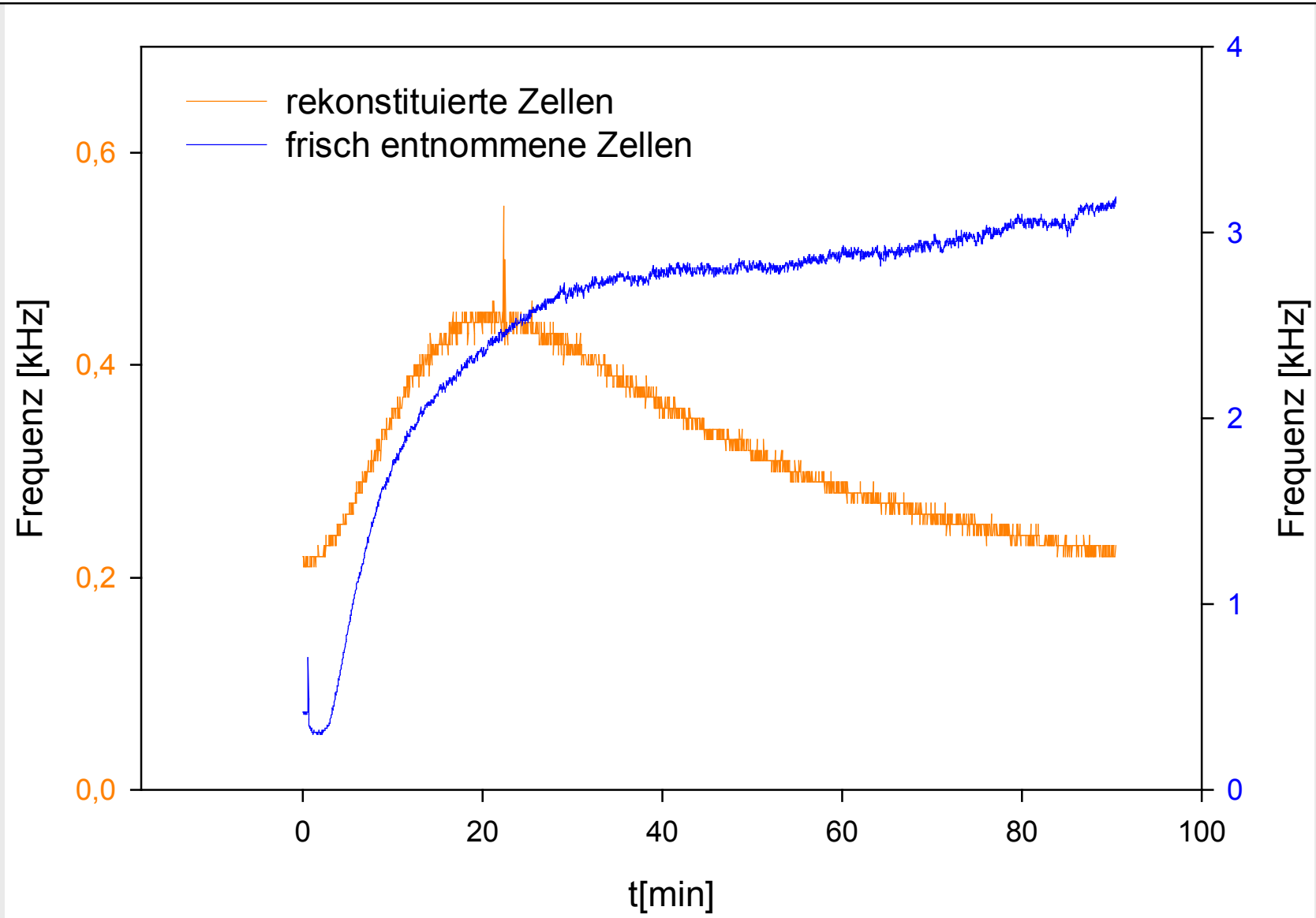
# Native and reconstituted Hemocytes





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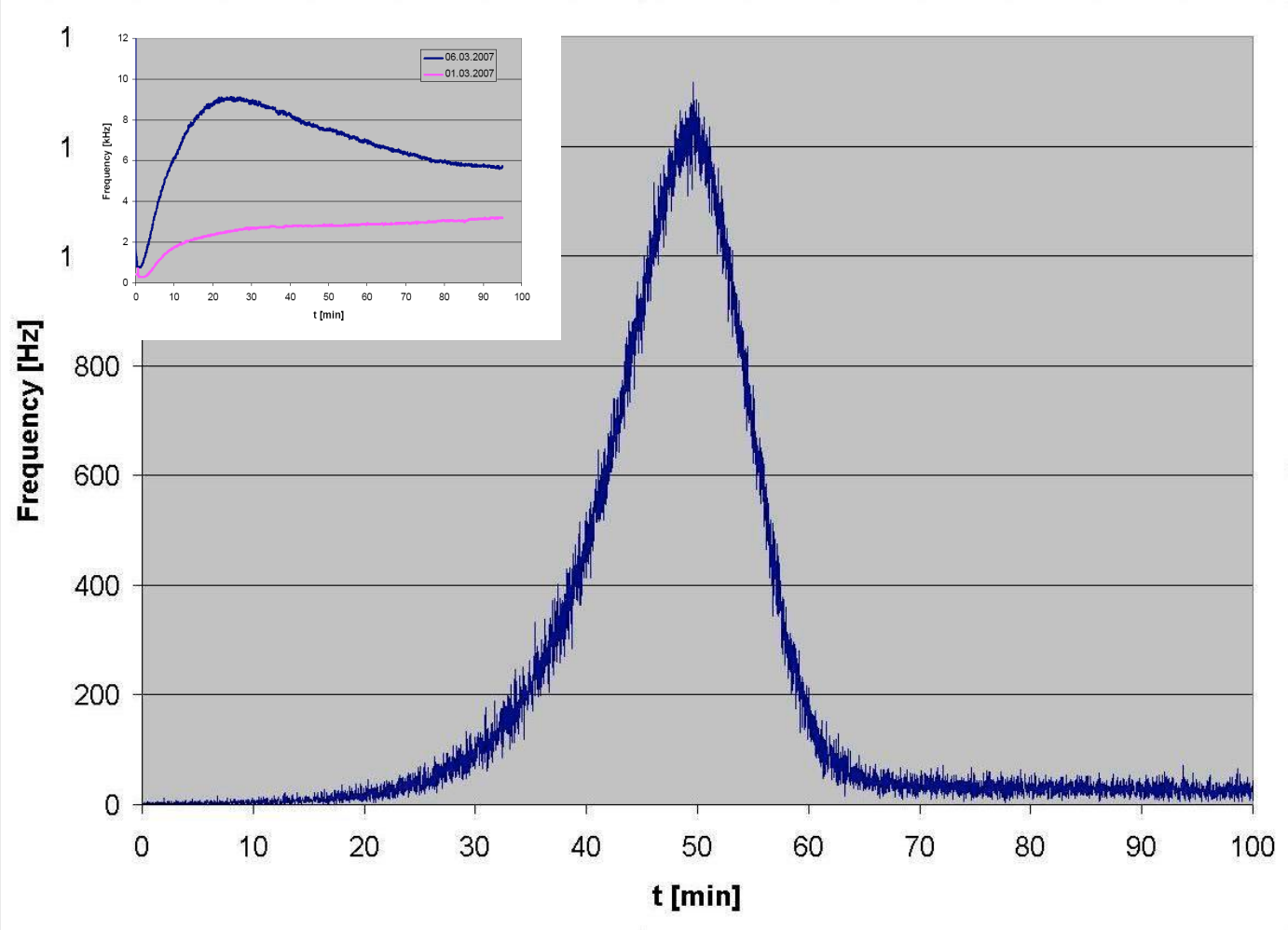
# Luminol luminescence in reconstituted hemocytes after addition of Peroxydase and Zymosan with cryoconservated cells and delayed signal




Ca. 1 Mio Zellen/ml, Küvette mit Rührer, Photoncounter



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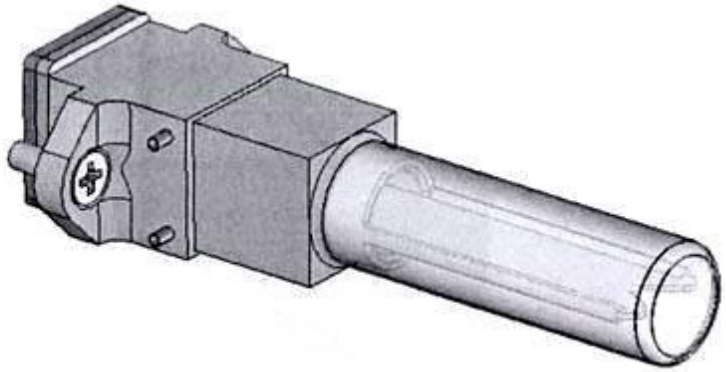
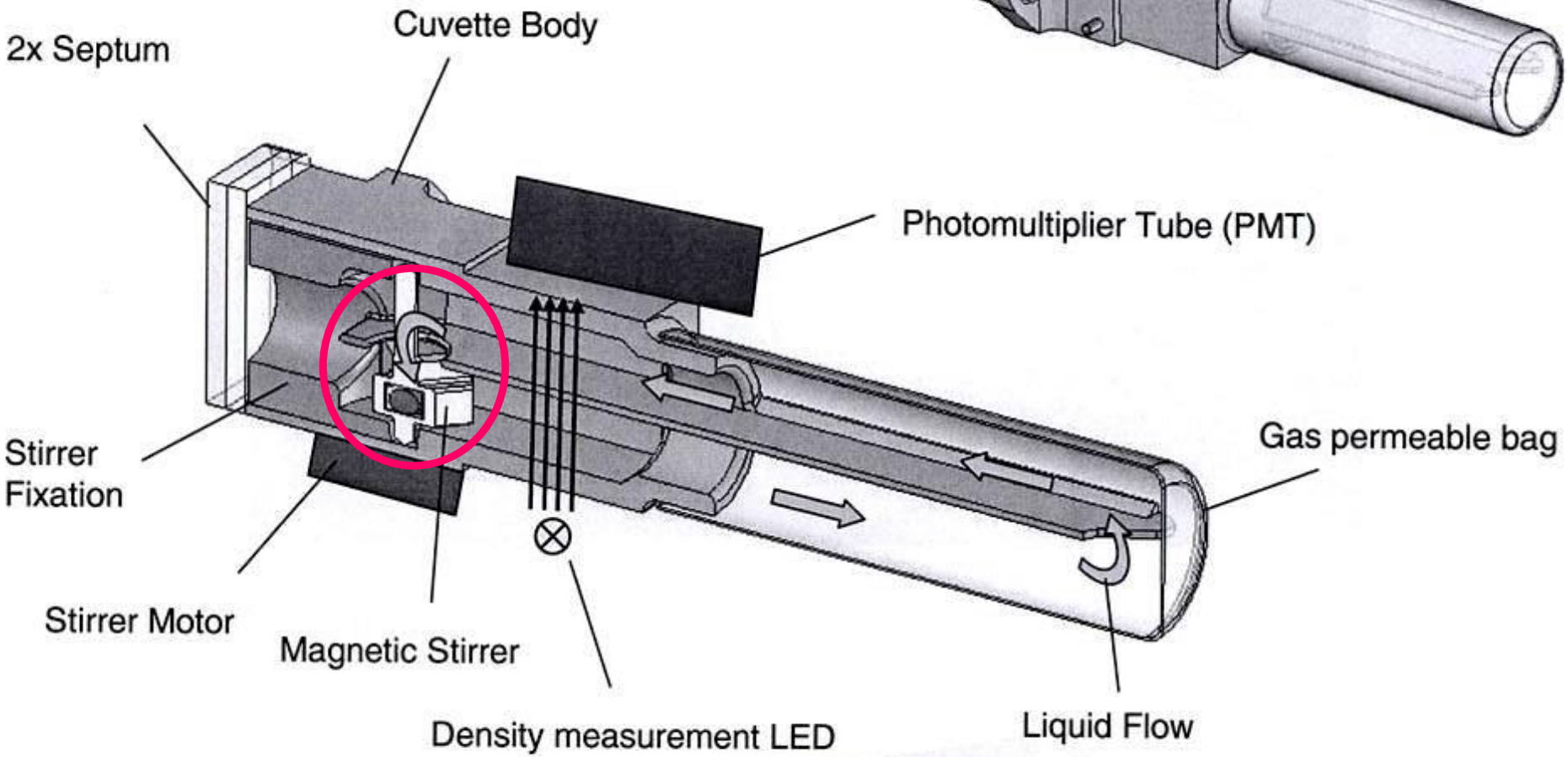
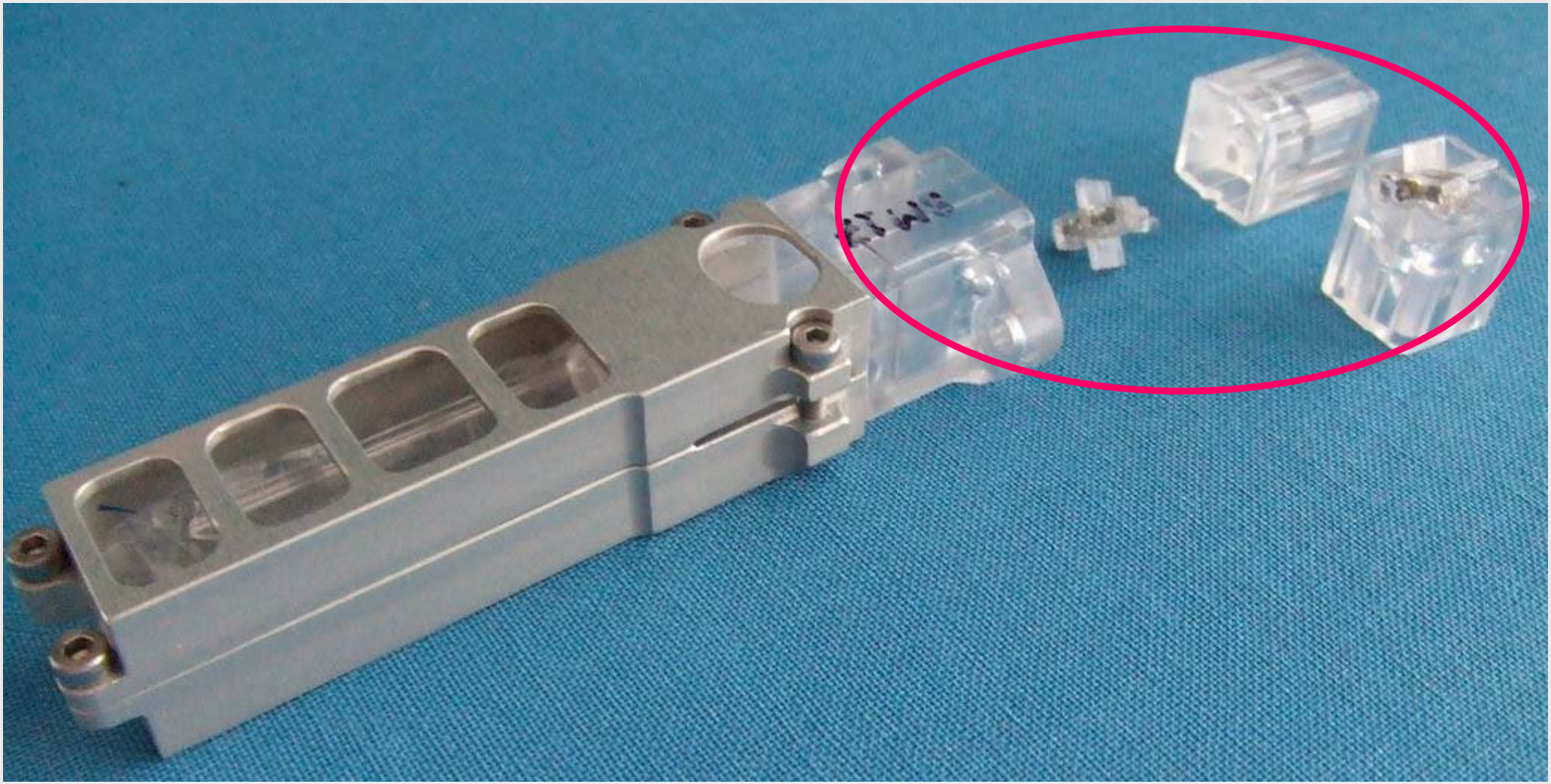
## Measurement Bag (MB)

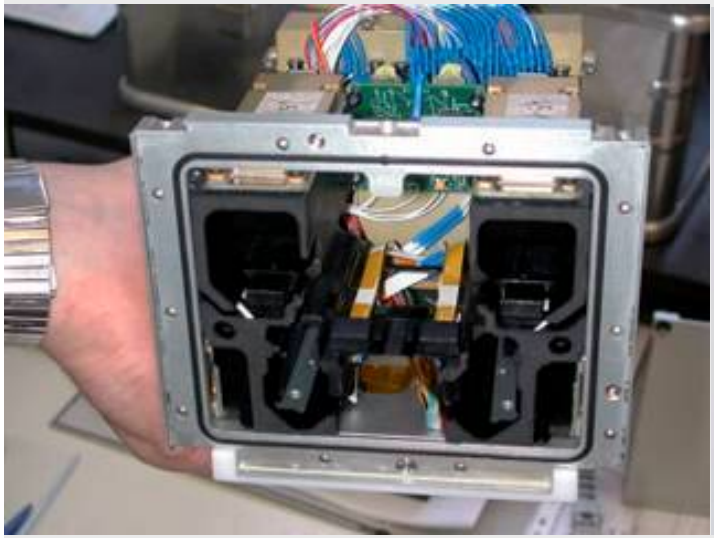


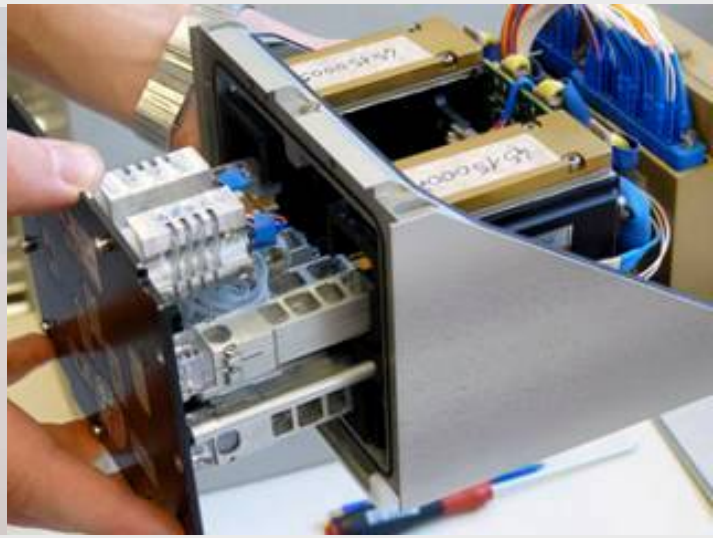


# Stirrer



# Phagozytosis Monitor with cryoconservated Mussel-Hemocytes





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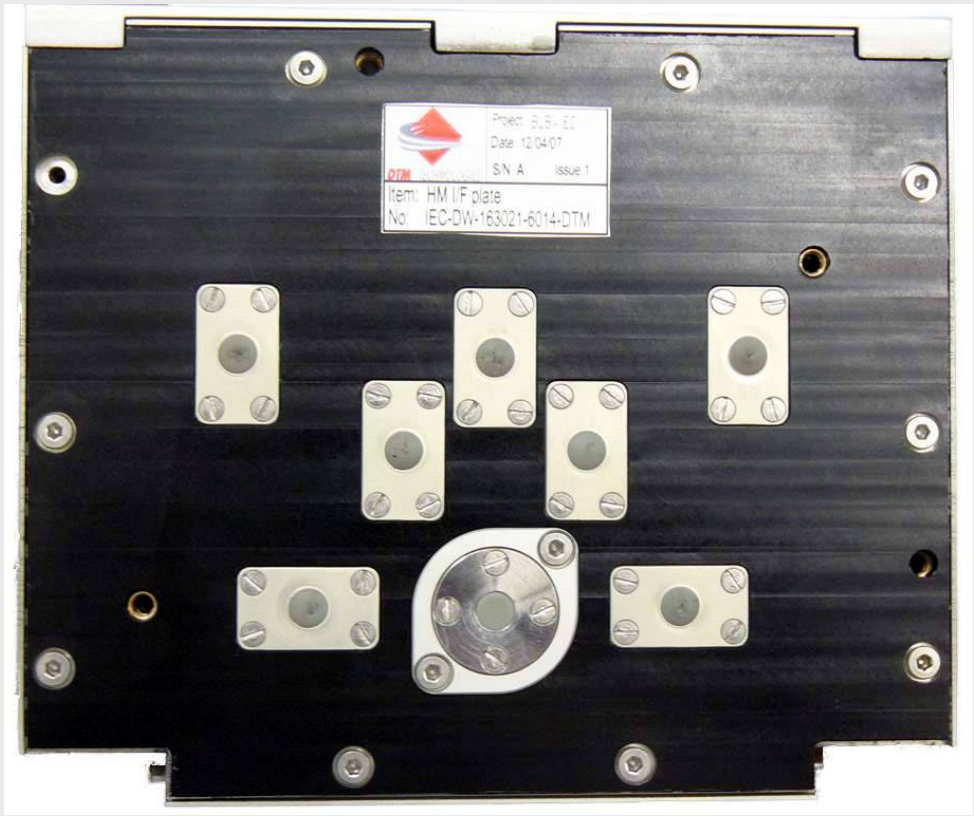
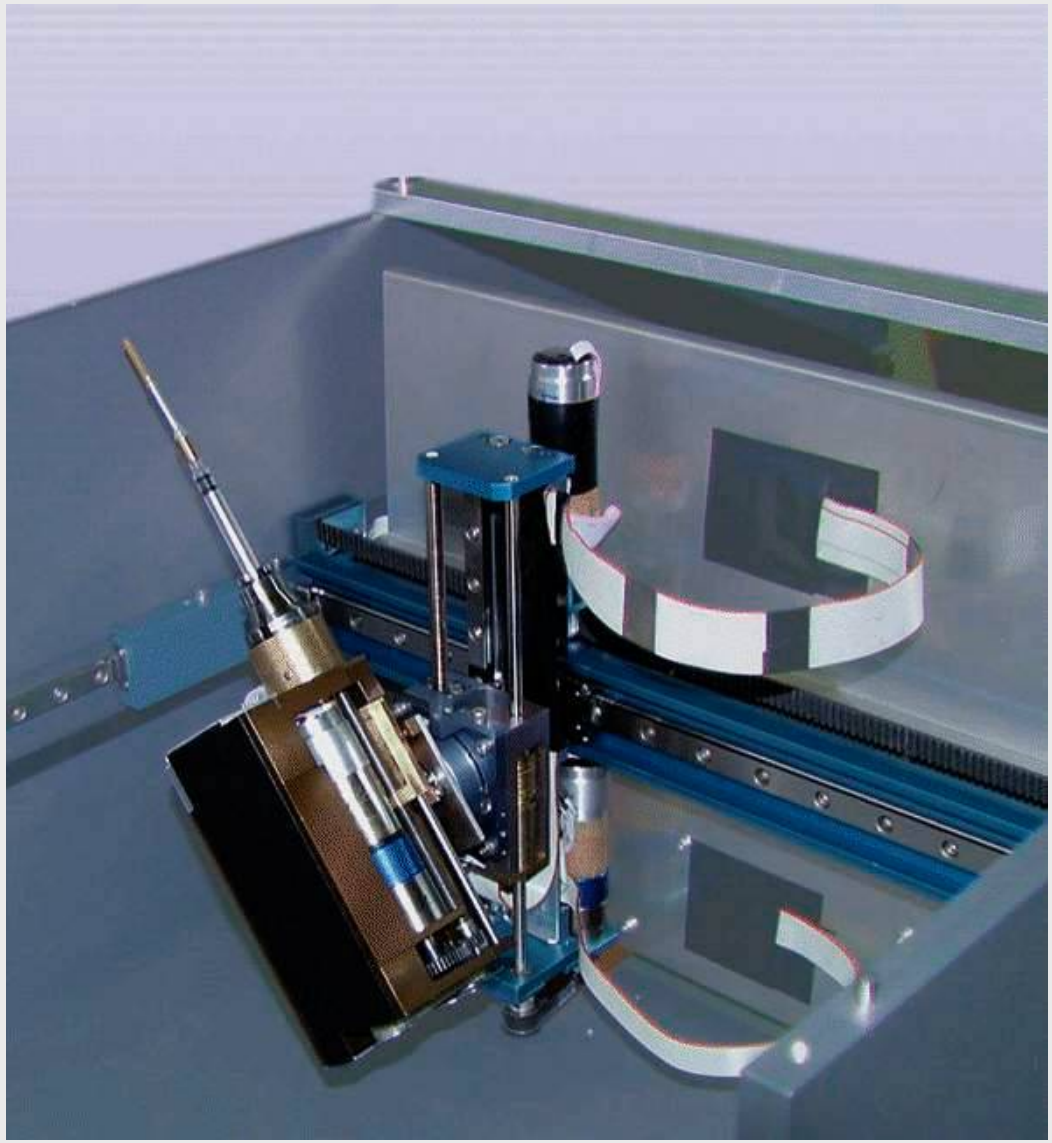



**Frontplate and “ports of the AEC**



**Handling mechanism (robot system)**

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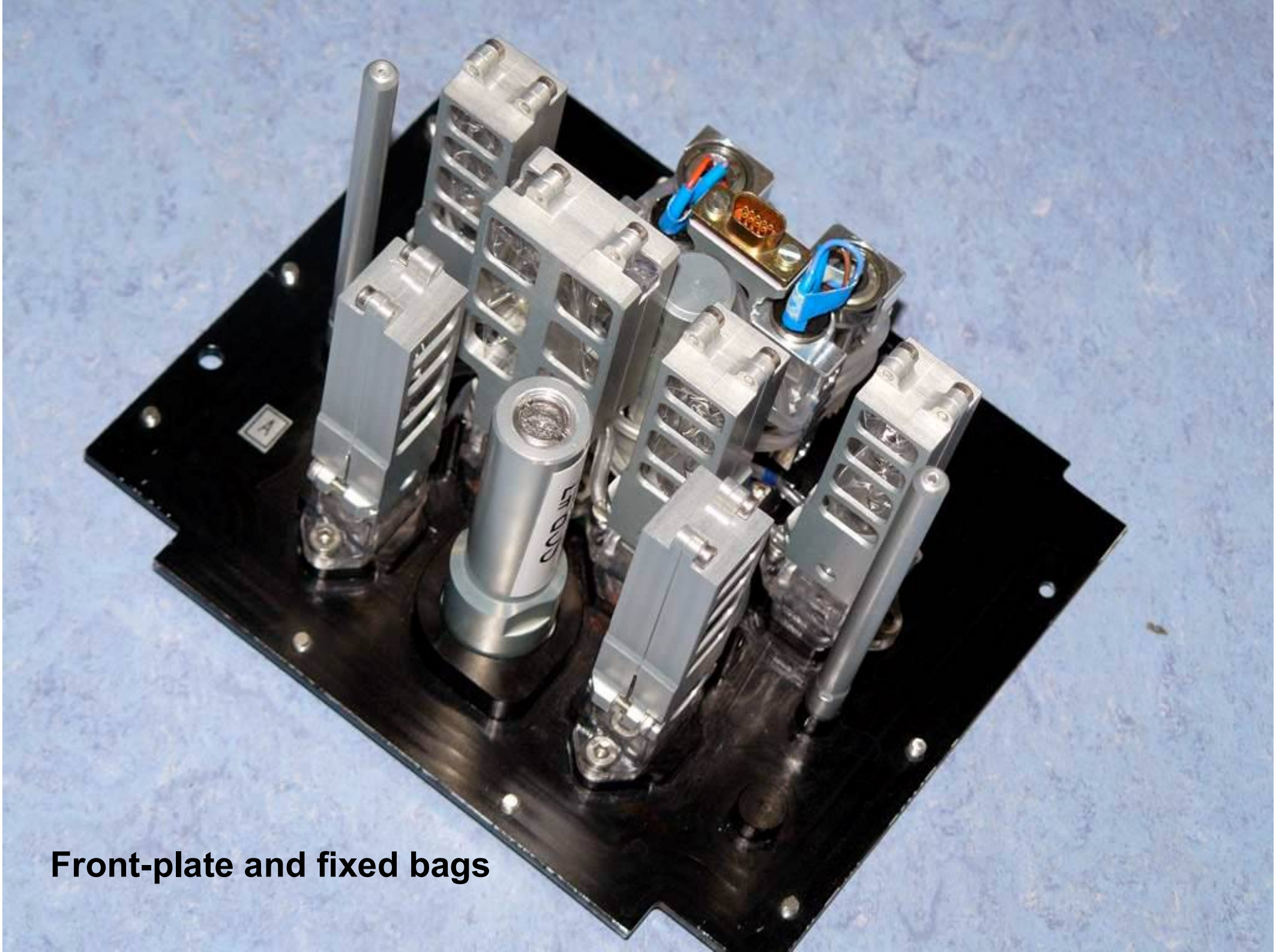
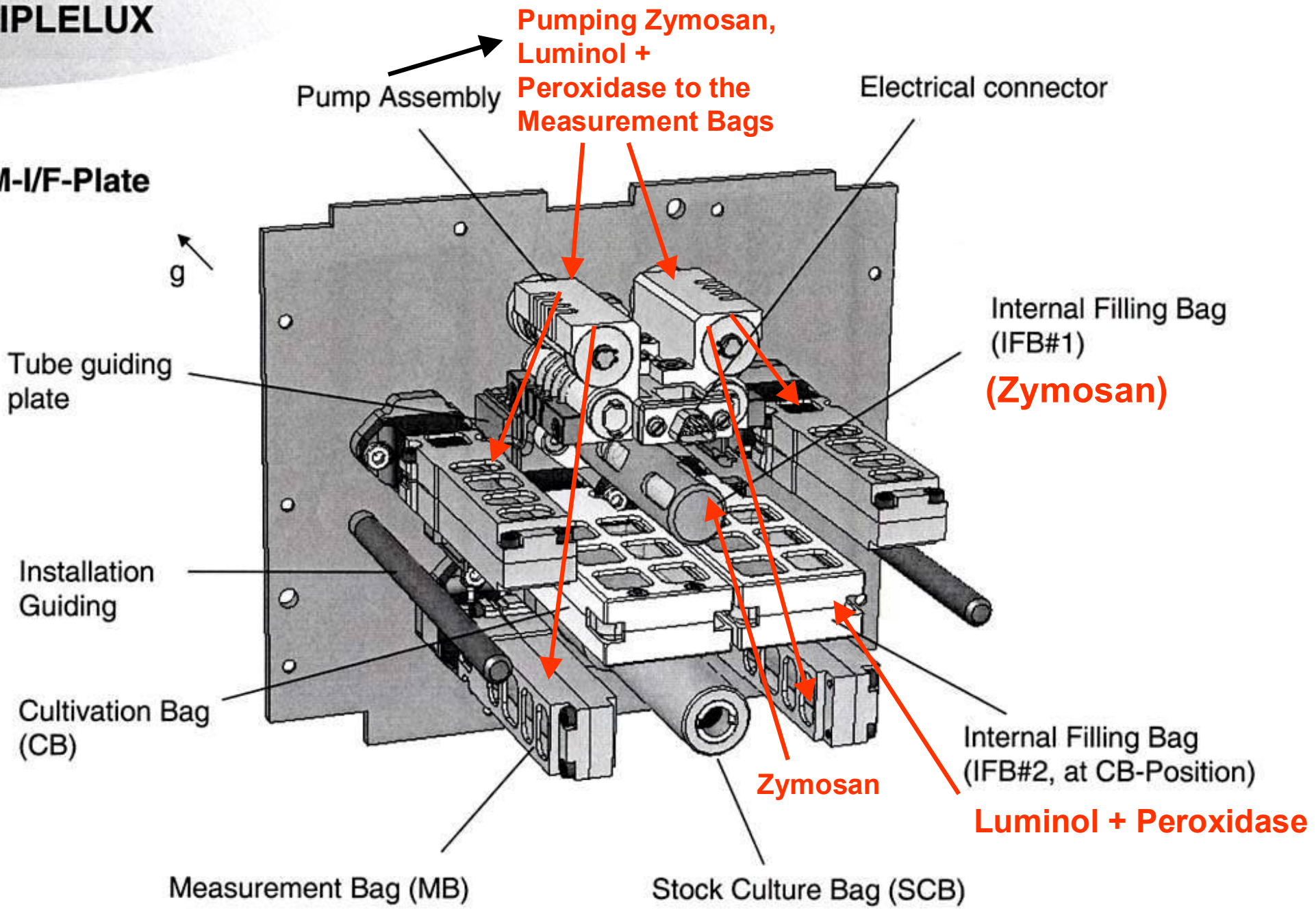


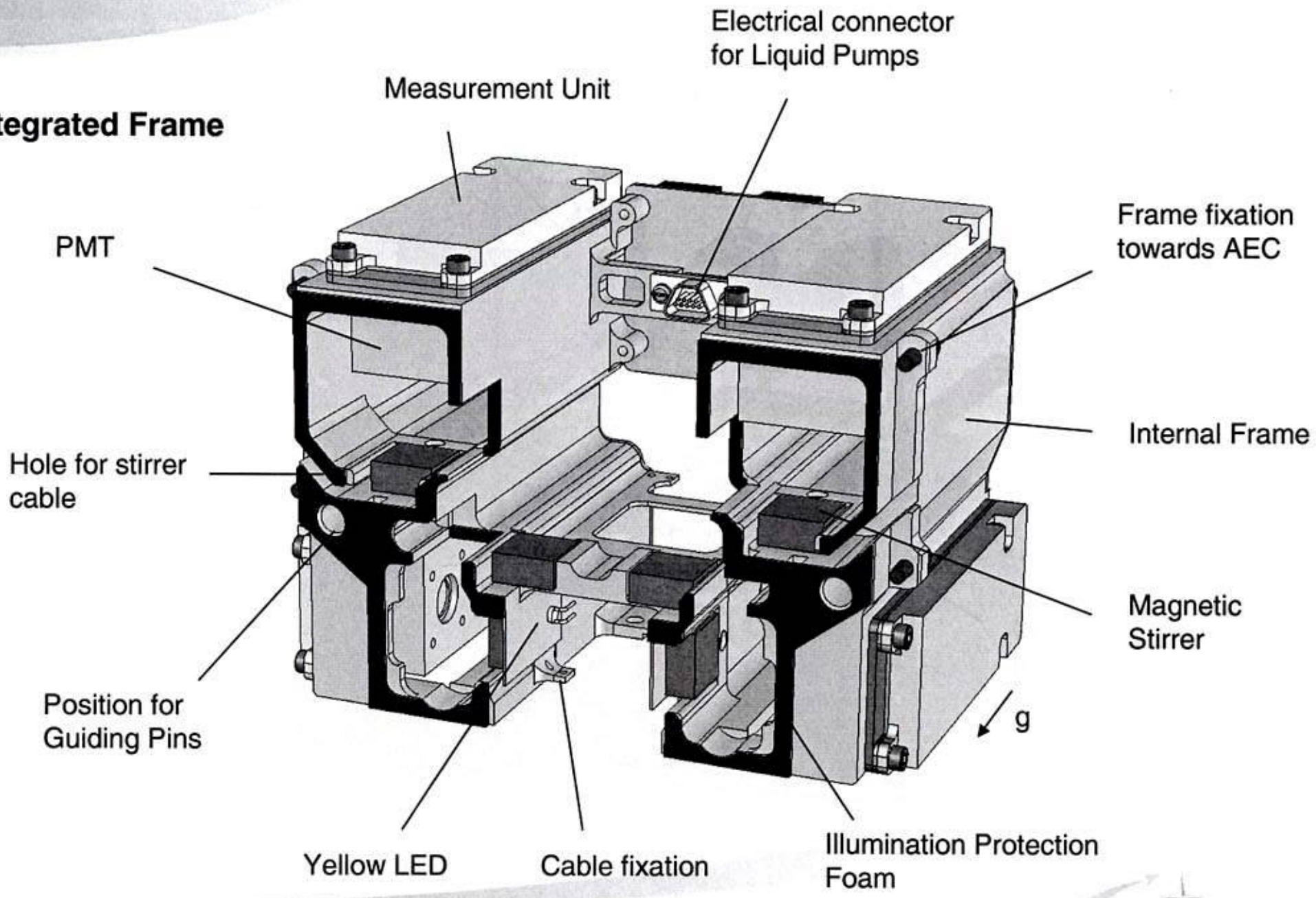
Front-plate and fixed bags

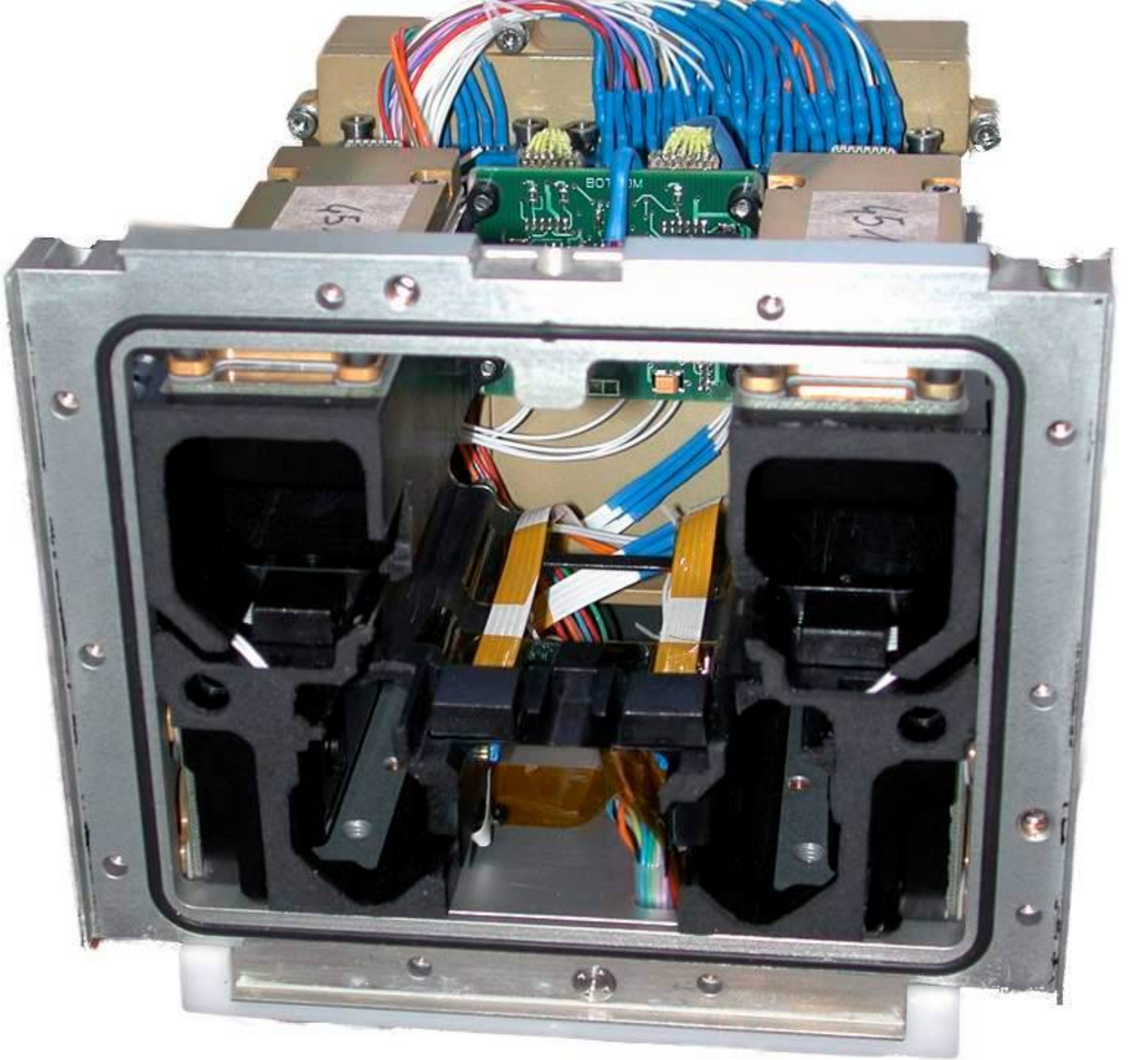
# TRIPLELUX

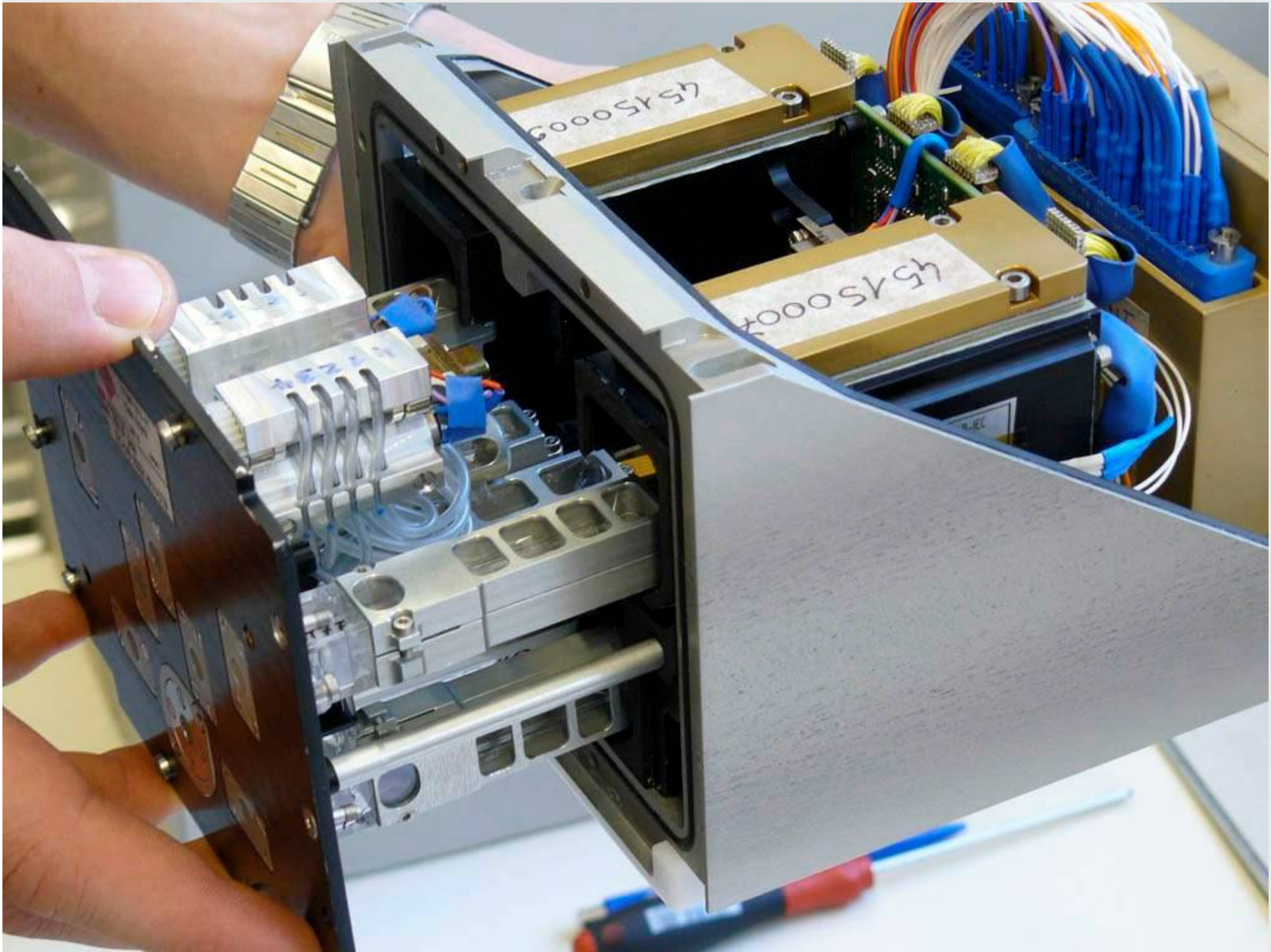

Pumping Zymosan, Luminol + Peroxidase to the Measurement Bags
Pump Assembly
Electrical connector
HM-I/F-Plate
g
Internal Filling Bag (IFB#1)
(Zymosan)
Tube guiding plate
Installation Guiding
Cultivation Bag (CB)
Zymosan
Internal Filling Bag (IFB#2, at CB-Position)
Luminol + Peroxidase
Measurement Bag (MB)
Stock Culture Bag (SCB)

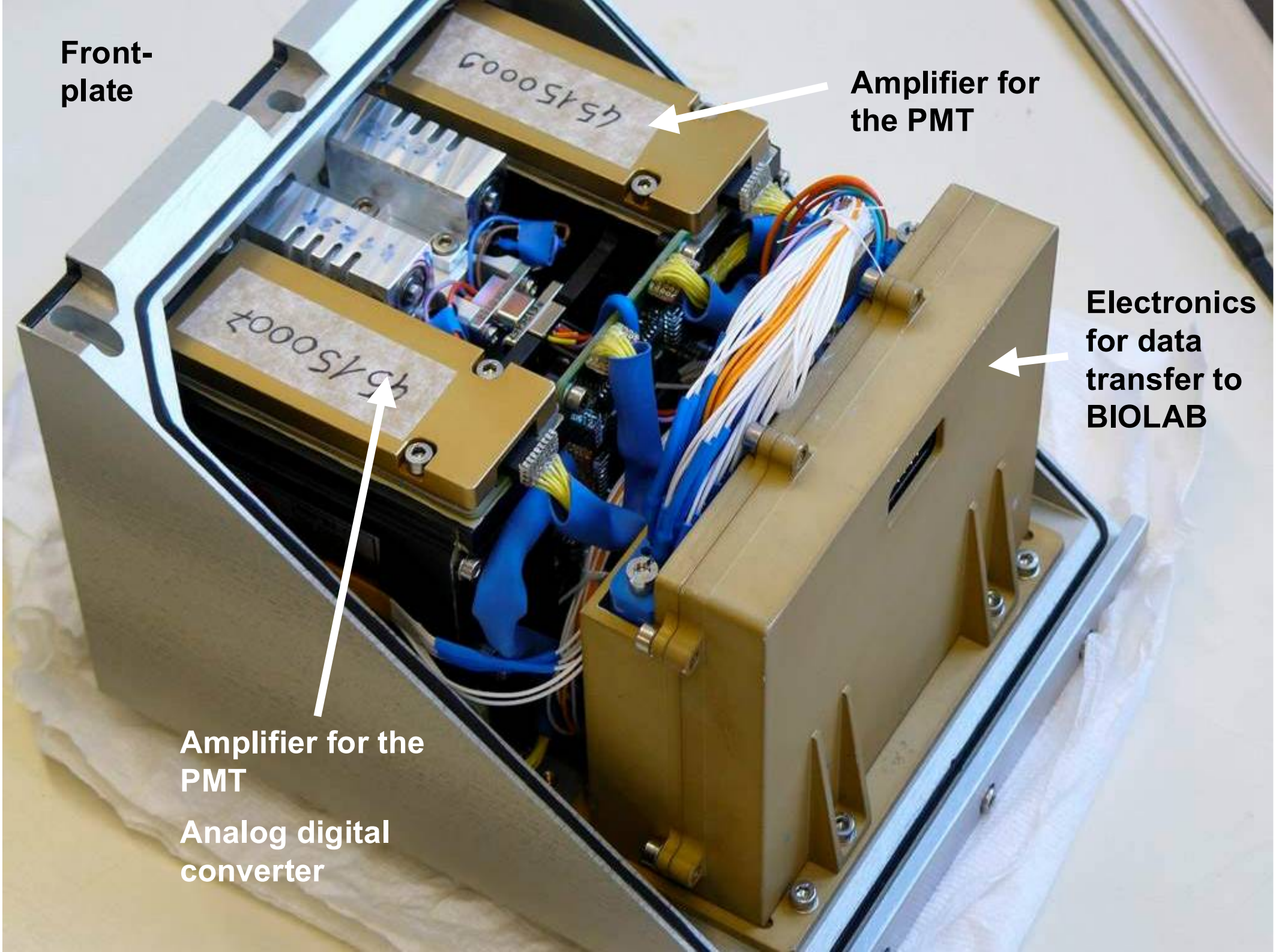
# TRIPLELUX

## Integrated Frame





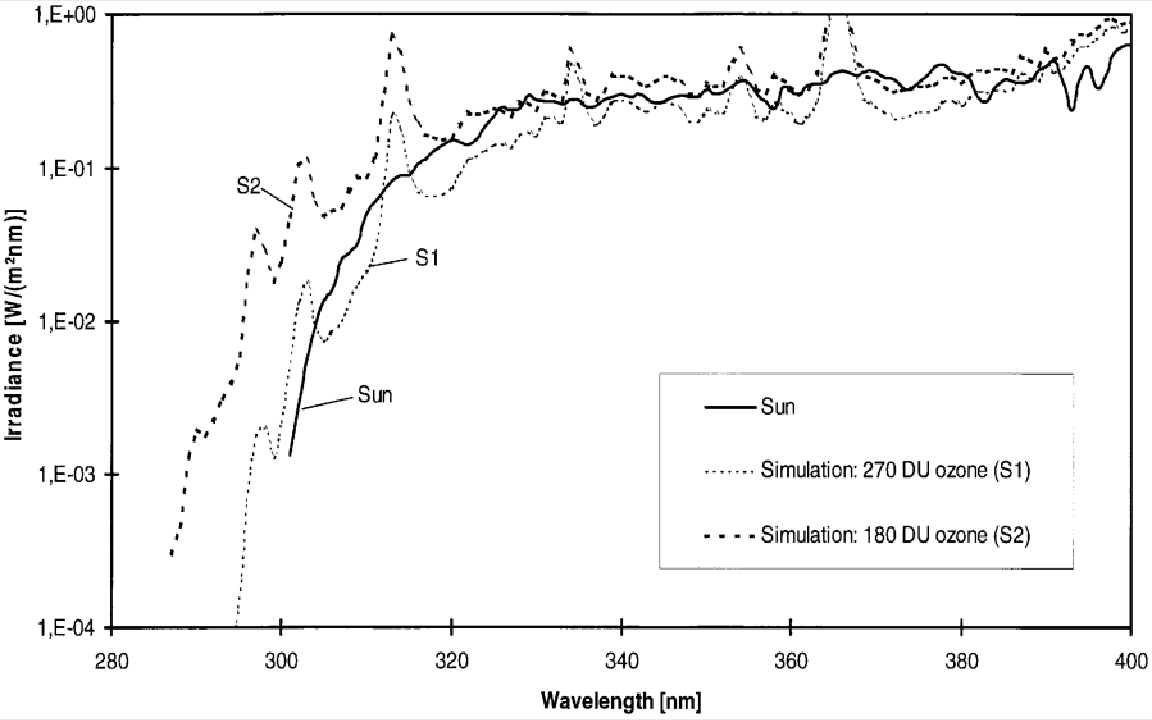


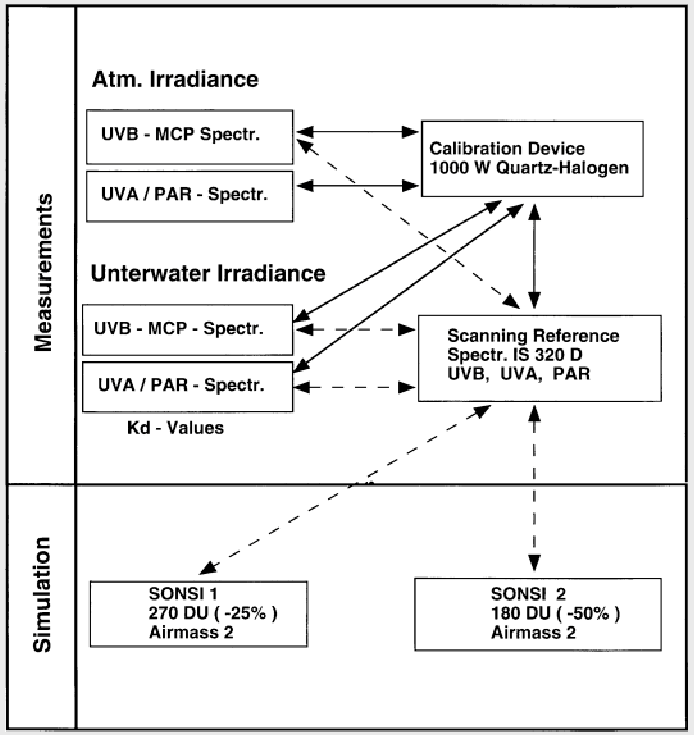

Front-plate
Amplifier for the PMT
Electronics for data transfer to BIOLAB
Amplifier for the PMT
Analog digital converter


**Measurement bags and hardcover for protection of the teflon bags**

**Testbed on ground for the in-flight hardware and final set up for in-flight position at the centrifuge surface**

# Influence of space irradiation
## dosimeter and download

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## Why “Download“ of the exposed Hemocytes?

- **Accumulation of DNA-damages**

  **Detection**

  **DNA-unwinding and Apoptossis-Marker**

2. **Phagozytosis-events without ROS- Induction in case of a weak Luminescence-Signal**

   **Glutaraldehyde preserved material to measure Phagocytosis events on ground**


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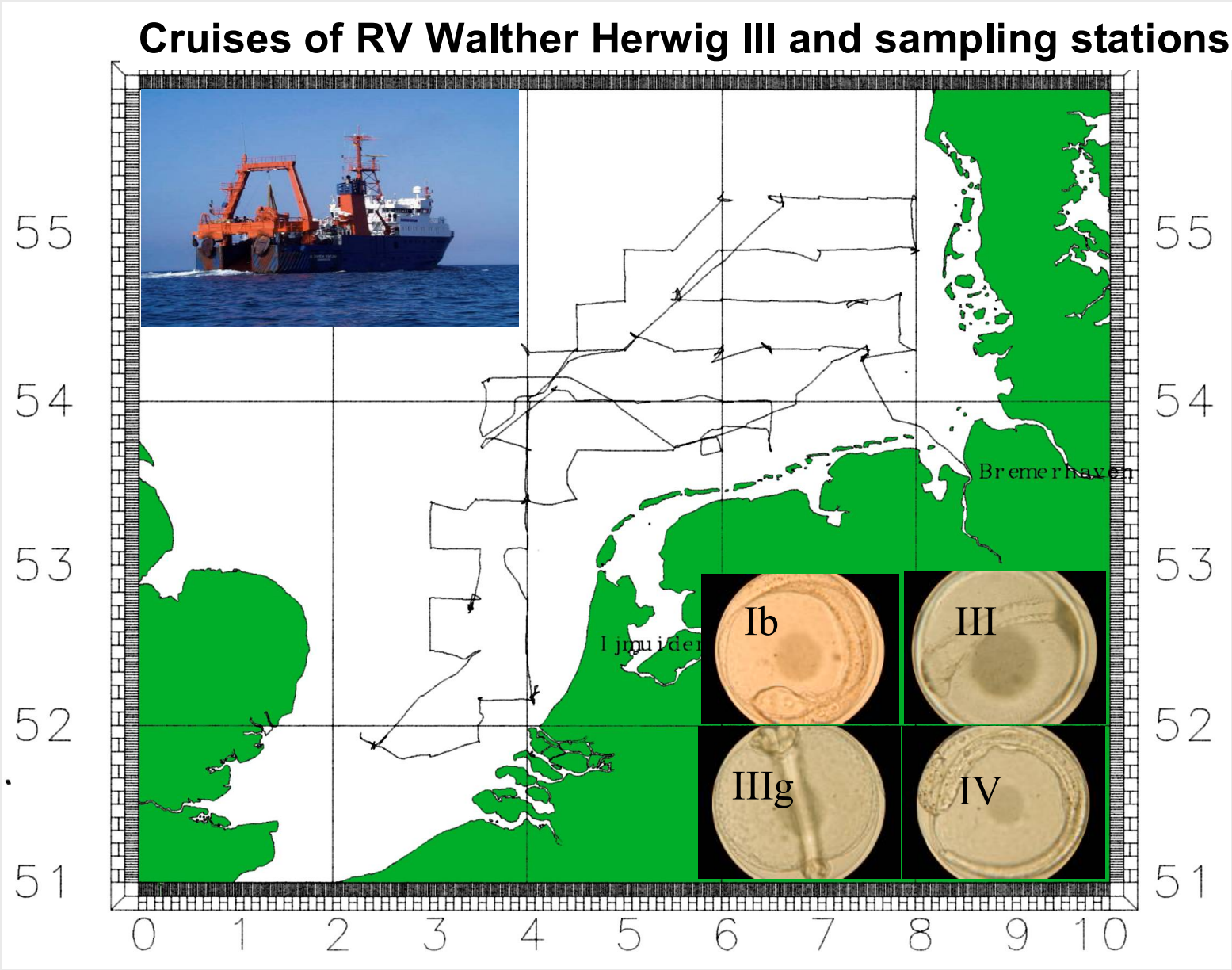
# Cruises of RV Walther Herwig III and sampling stations




***Limanda limanda:*** **ontogenetic stages Ib-IV; in total 9 day of incubation**

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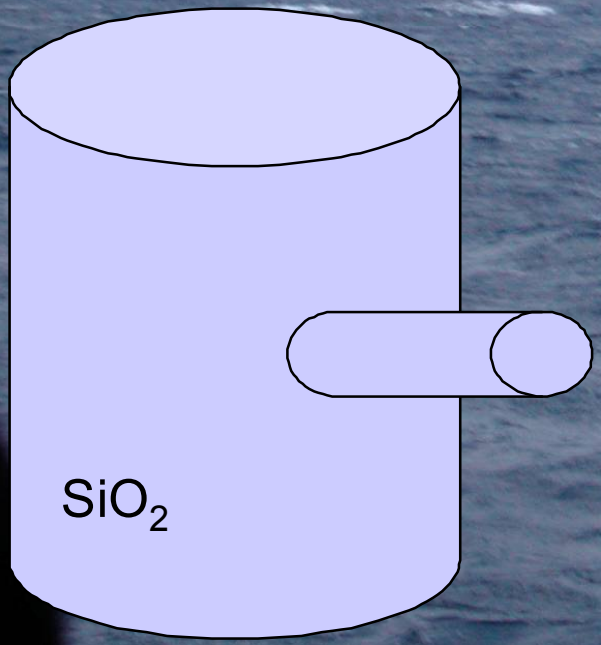
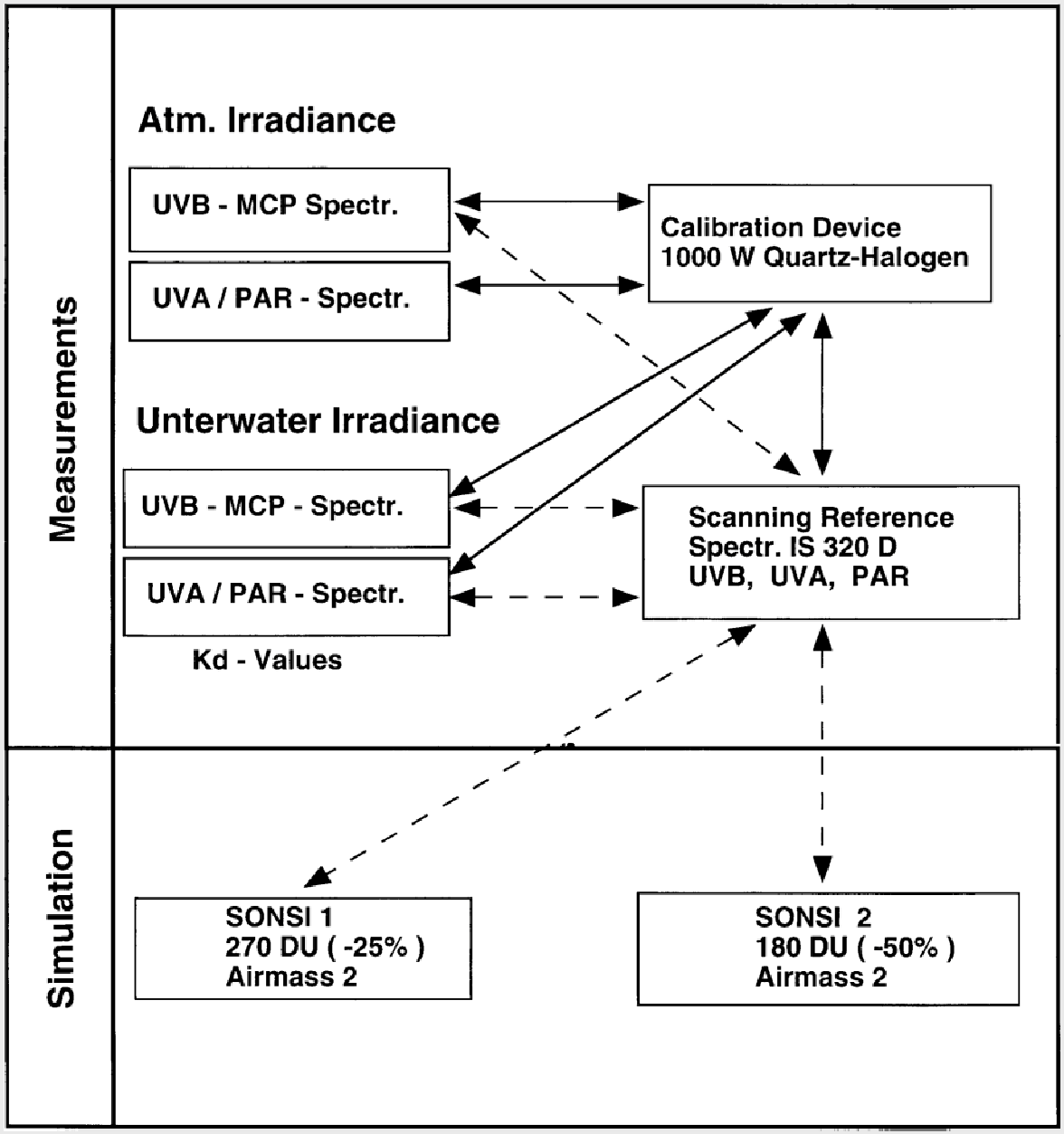
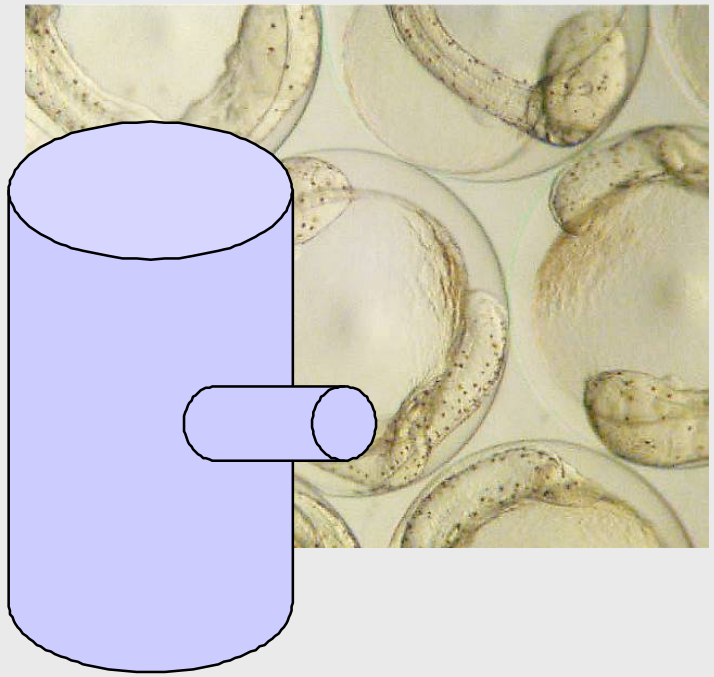



**250 eggs,**
**UV-B, 290 nm**
**4645-6568 Ws/m²**



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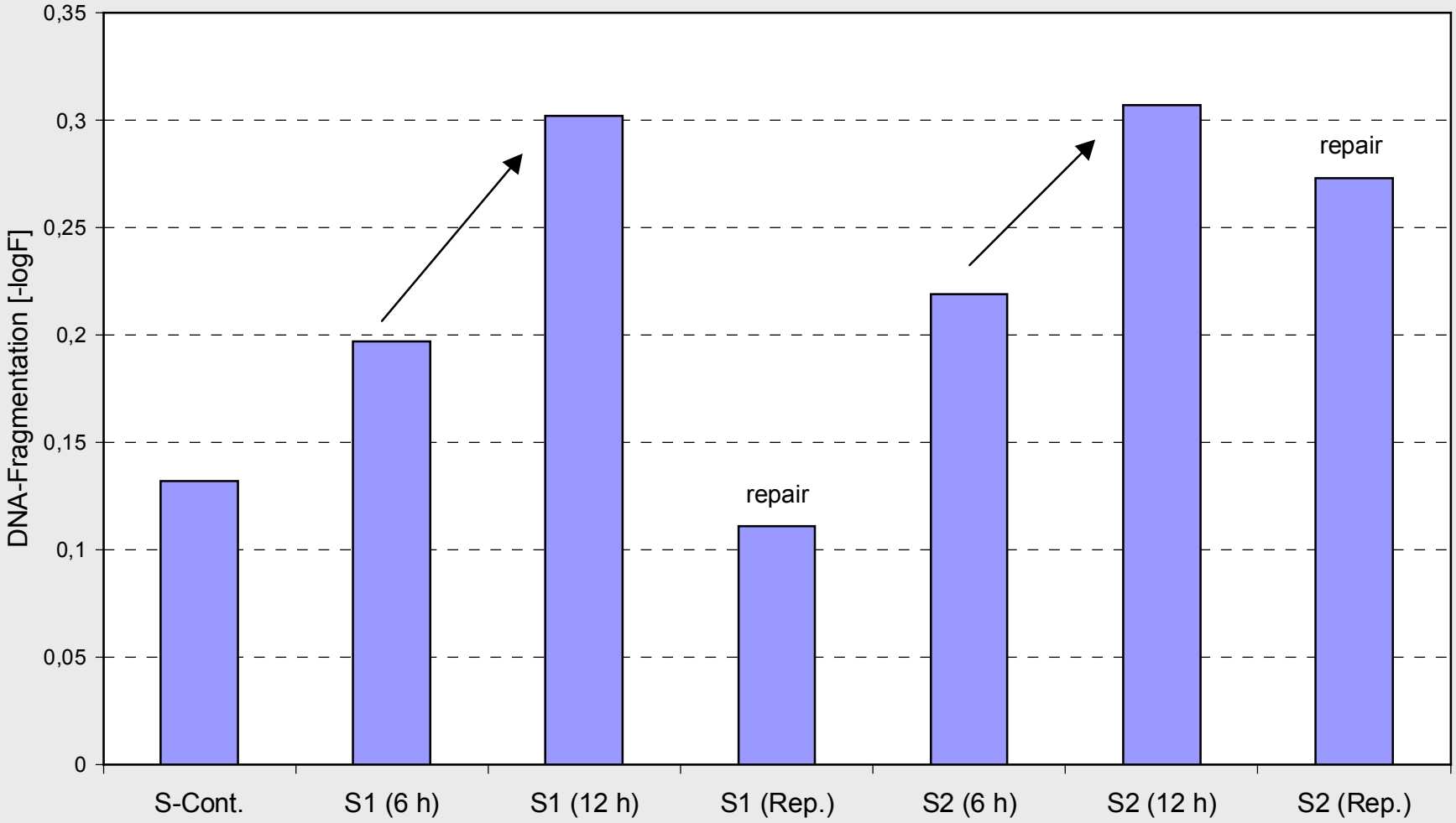
# SONSI 1 and SONSI 2 exposure: DNA Fragmentation of exposed embryos (*L. limanda*) and repairment

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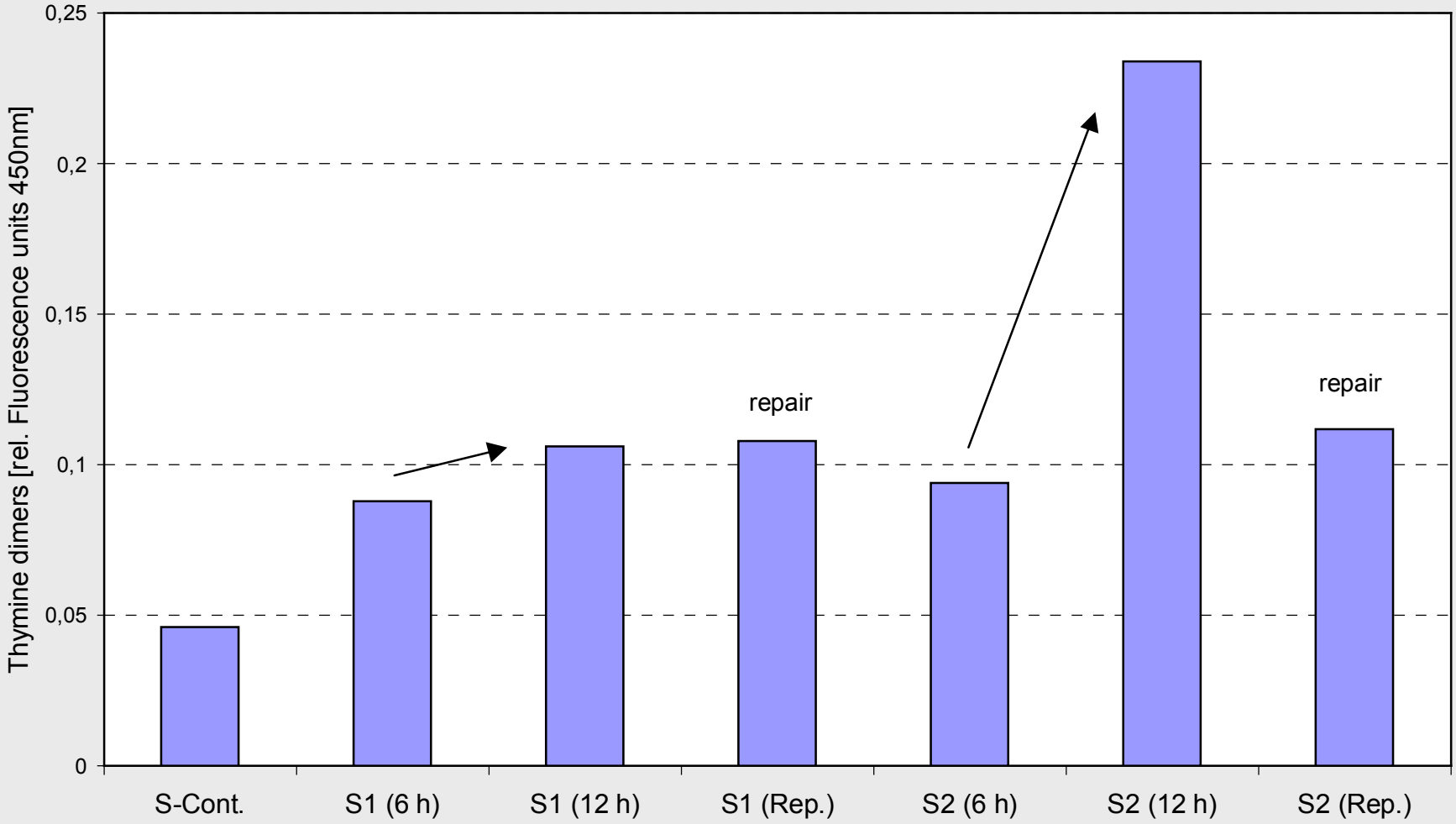
# SONSI 1 and SONSI 2 exposure: Thymine dimeres of exposed embryos (*L. limanda*), early ontogenetic stages Ib-II, 2 times 6h in total 12h exposure and 18h repairment

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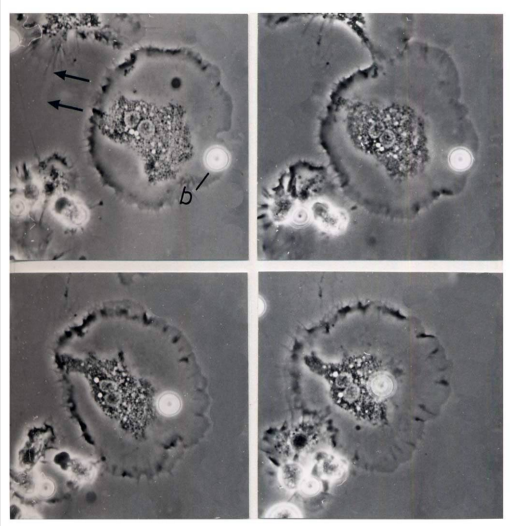
# Development of the biosensor / bioanalytical system with phagocytosis active cells for - real time Monitoring

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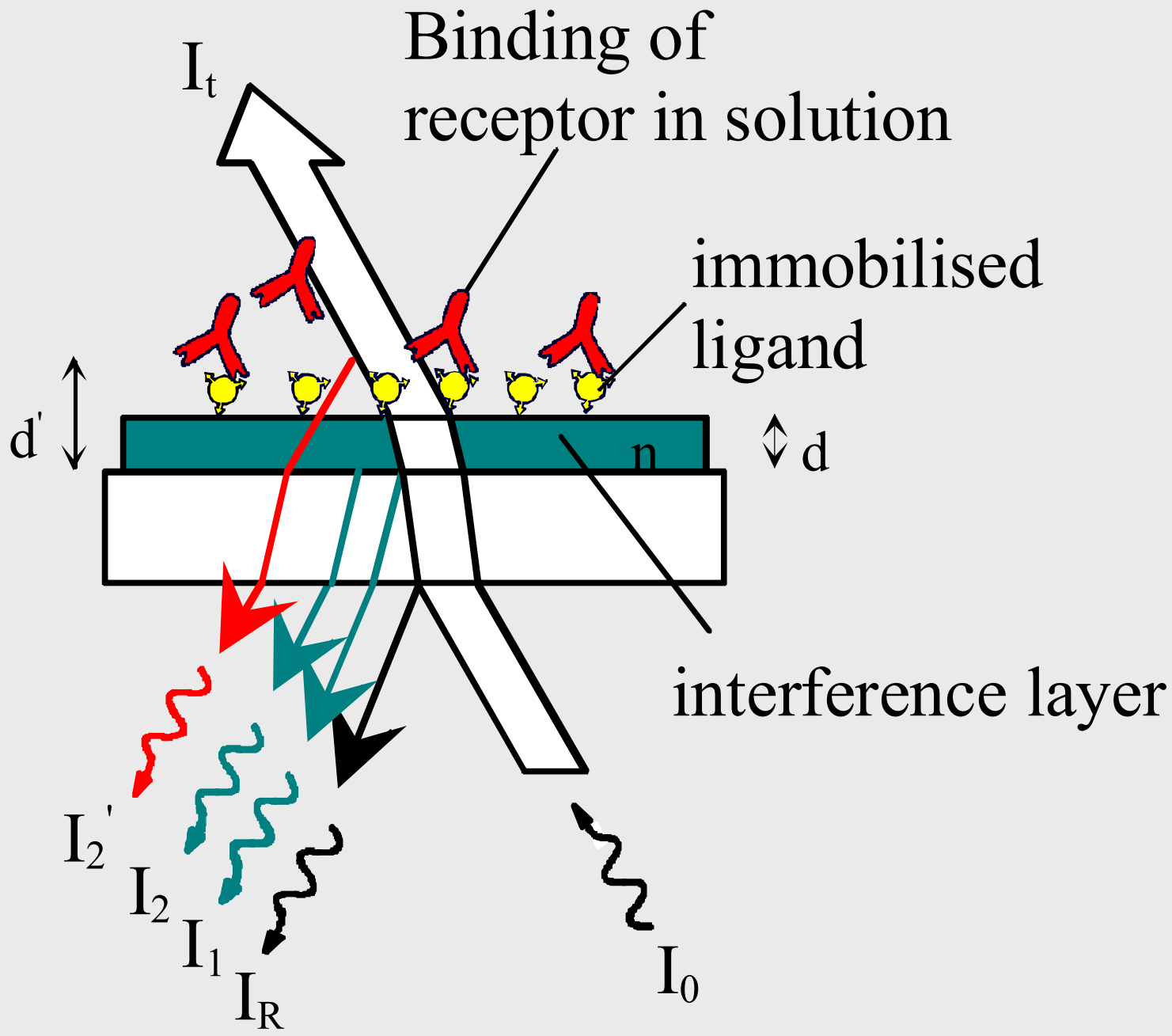


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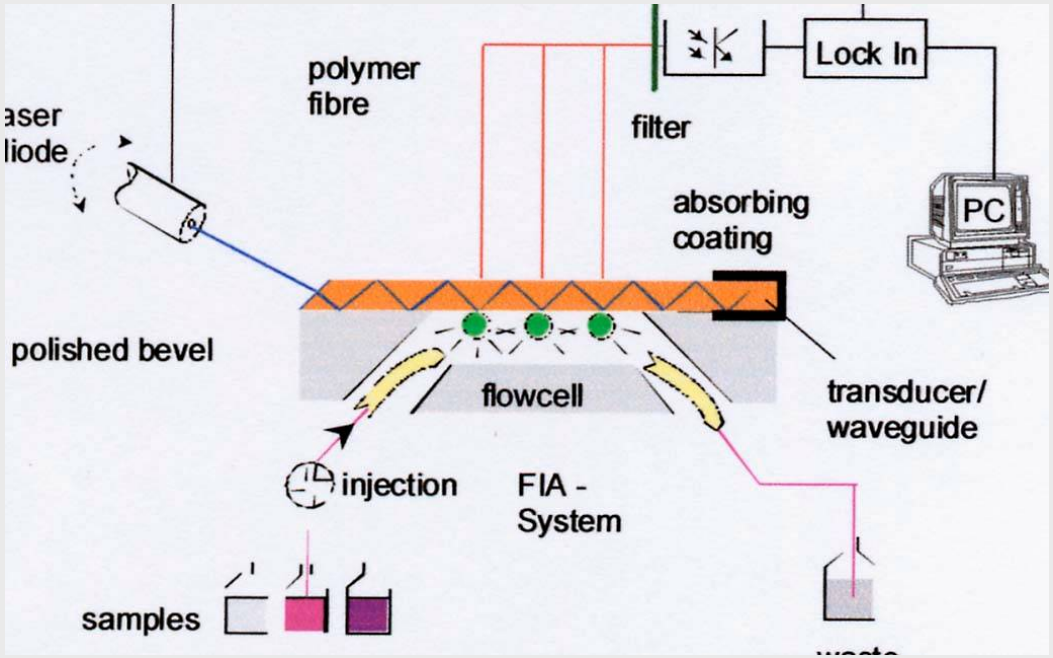


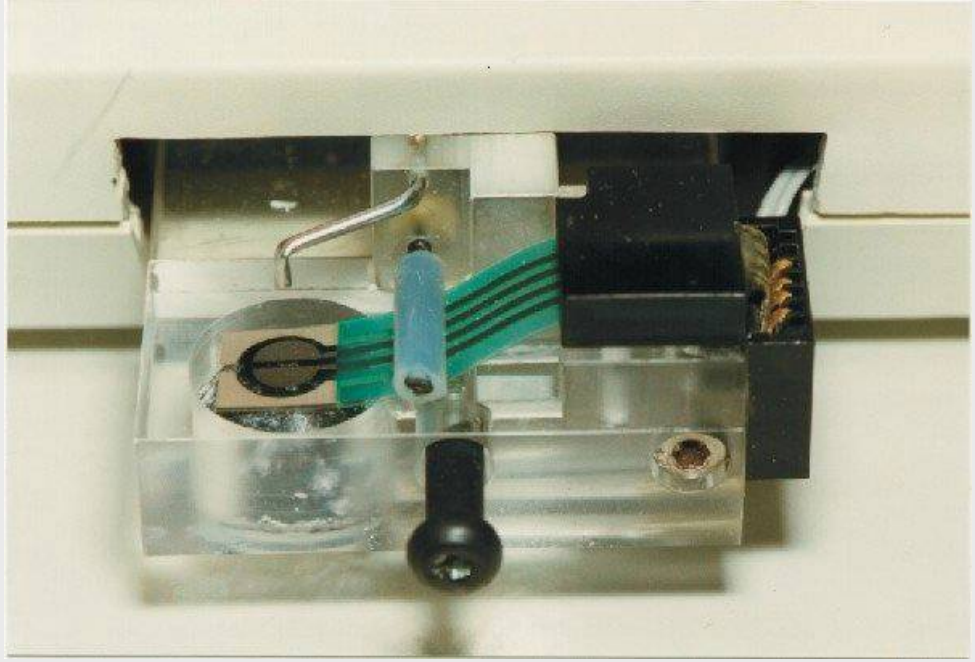

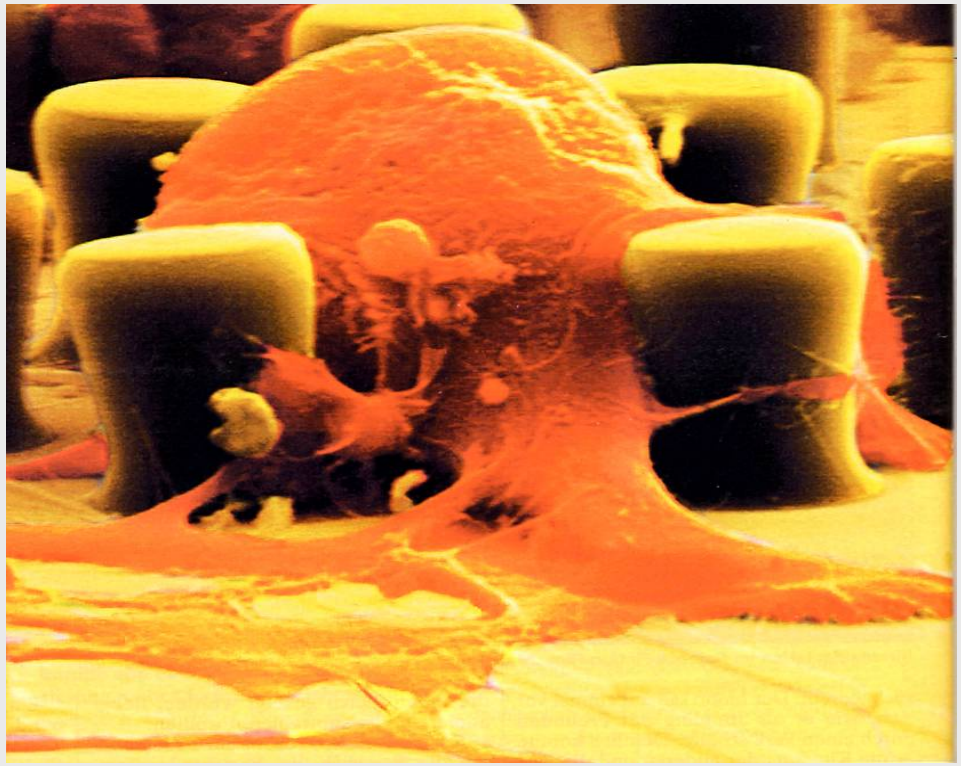

**Photon counting**


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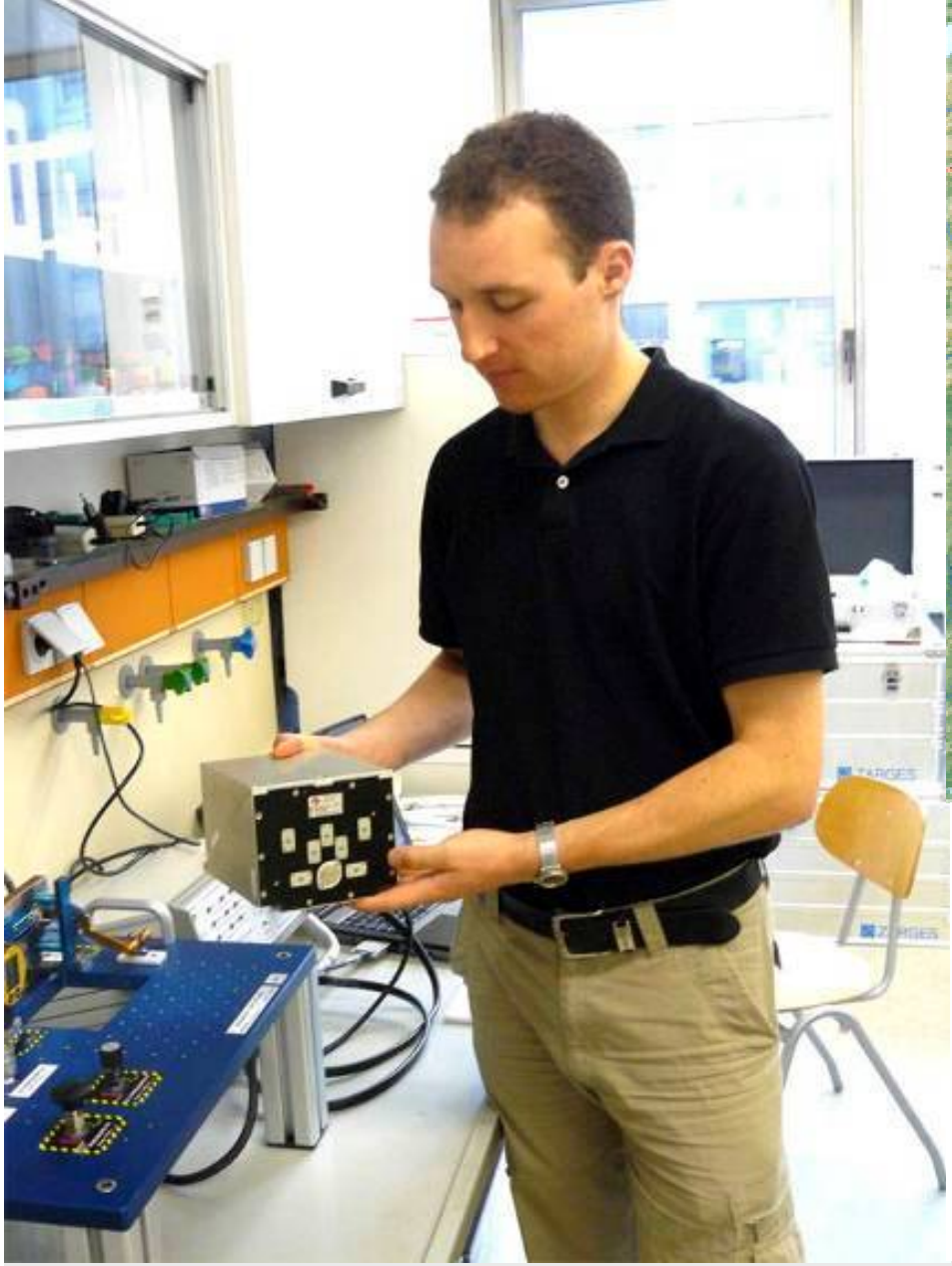


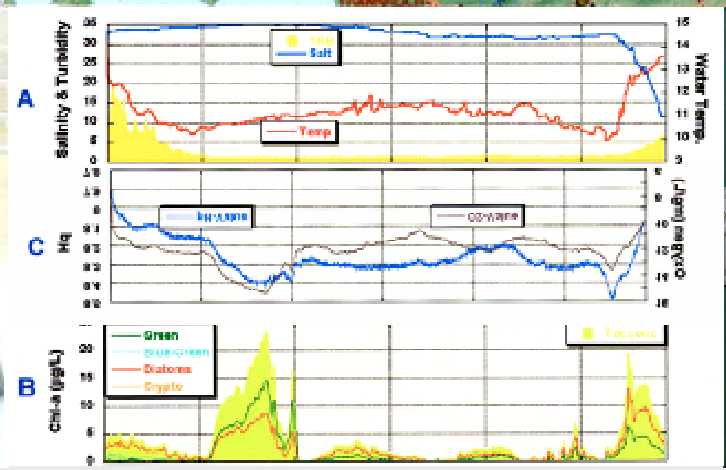



## Operational Monitoring -WFD

## Real time bioassays, FerryBox 4E Jena - bbe

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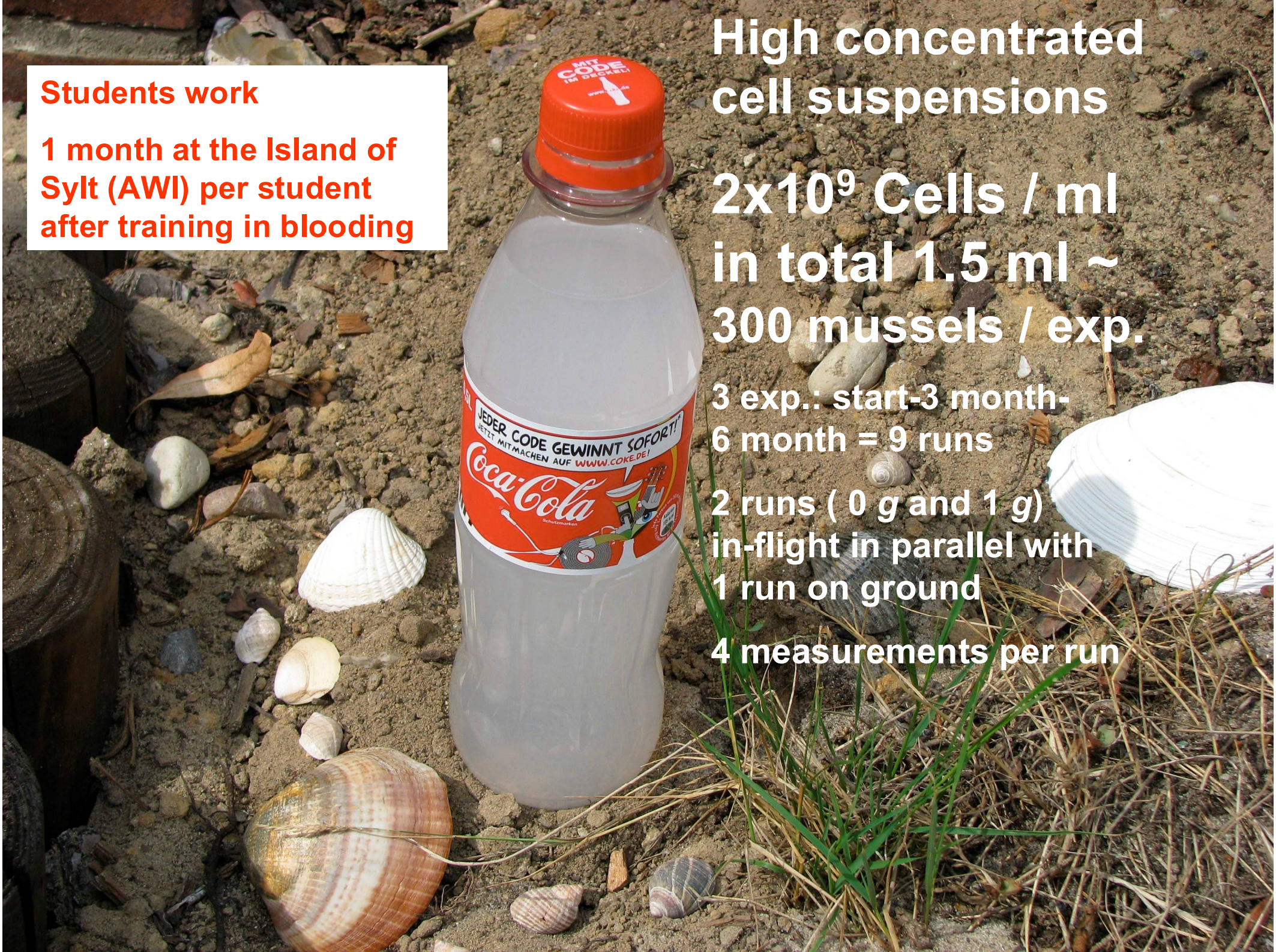



**Students work**

**1 month at the Island of Sylt (AWI) per student after training in blooding**

# High concentrated cell suspensions

## $2x10^9$ Cells / ml in total 1.5 ml ~ 300 mussels / exp.

3 exp.: start-3 month-6 month = 9 runs

2 runs ( 0 *g* and 1 *g*) in-flight in parallel with 1 run on ground

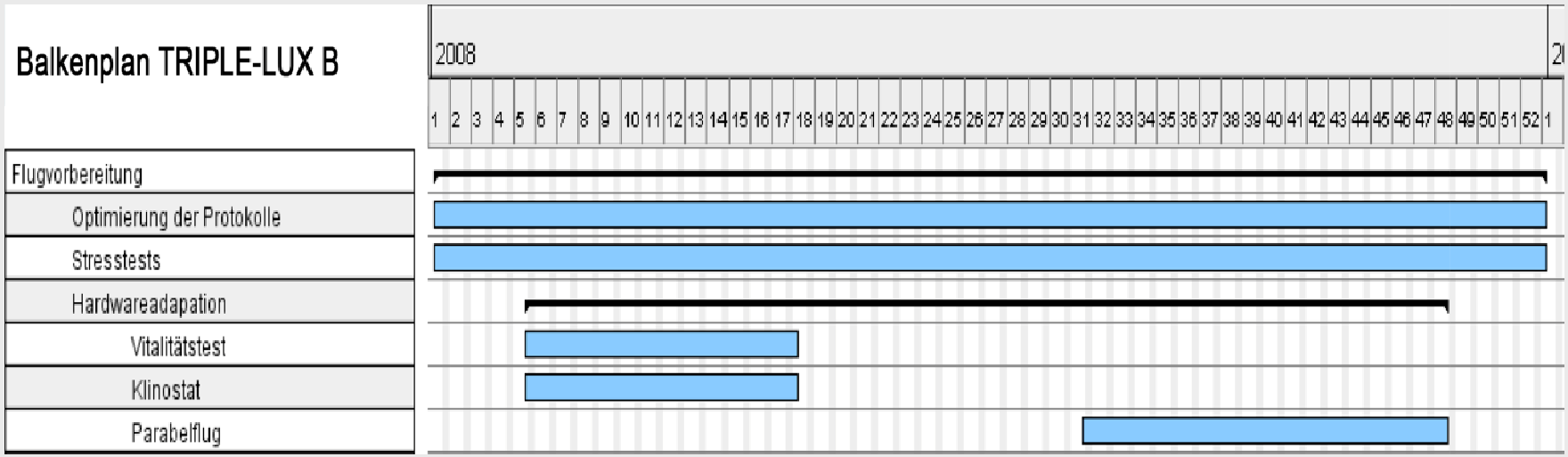
4 measurements per run

# Meilensteinplan für das Jahr 2008

## Flugvorbereitung

**Optimierung der Protokolle:** Optimierung der Protokolle zu Kryokonservierung und Rekonstitution der Zellen
**Stresstests:** Stresstests mit rekonstituierten Zellen und mit Zellen in Primärkultur (0g pyper-g und Bestrahlung)
**Hardwareadapation**: Hardwareadapation und Testung in Zusammenarbeit mit DLR, ESA und Astrium
**Vitalitätstest:** Praktische Erprobung des Vitalitätstest mit dem Mikroskop mit Durchflußkammer der DLR

**Klinostat (2009):** µG Simulation auf dem Klinostaten (BSSC), diese Arbeiten werden separat bei der ESA beantragt.
**Parabelflug (Okt./Nov. 2008):** µG Simulation im Parabelflug (BSSC)





P.-D. Hansen u. E. Unruh, TU Berlin, Faculty VI, Dept. of Ecotoxicology, 10587 Berlin

**Literature:**

Hansen, P.D. and E. Unruh. 2005. TRIPLE LUX-B: Phagocytosis in Mussel Hemocytes, Proceedings of the 9th European Symposium on Life Sciences Research in Space /26th Annual International Gravitational Physiology Meeting, Cologne Germany, 26 June-1 July 2005 (ESA SP-585, August 2005)

Hansen, P-.D. 2008. Biosensors and Ecotoxicology, Eng. Life Sci., 8, 1, 1-7

**Phagocytosis and Hemocytes:**

Renwrantz, L., Daniel, I. and Hansen, P.-D. (1985).
Lectin - Binding to Hemocytes of *Mytilus edulis*. Developmental and Comparative Immunology (DCI), 9, 203-210

Hansen, P.-D., Bock, R. and Brauer, F. (1991).
Investigations of phagocytosis concerning the immunological defence mechanism of *Mytilus edulis* using a sublethal luminescent bacterial assay (Photobacterium phosphoreum). Comp. Biochem. Physiol. Vol. 100C, No 1/2, 129-132


P.-D. Hansen u. E. Unruh, TU Berlin, Faculty VI, Dept. of Ecotoxicology, 10587 Berlin

Hansen, P.-D. (1993). Schadstoffwirkungen auf das Immunsystem. In: Biochemische Methoden zur Schadstoff-erfassung im Wasser - Möglichkeiten und Grenzen. Hrsg. Fachgruppe Wasserchemie in der GDCh, VCH Verlagsgesellschaft, Weinheim (1993), 107-121

Dizer, H., B. Fischer, A.S.A. Harabawy, M.-C. Hennion, P.-D. Hansen 2001. Toxicity of domoic acid in the marine mussel *Mytilus edulis*, Aquatic Toxicology, 55, 3-4, 149-156

Blaise, C., Gagné, F., Pellerin J., Hansen, P-D., Trottier, S. 2002. Molluscan shellfish biomarker study of the Saguenay Fjord (Quebec, Canada) with the soft-shell clam, Mya arenaria. Environ. Toxicol. 17,3, 170-186. Blaise, C.,

Trottier, S., Gagné, F., Lallement, C., Hansen, P.-D. 2002. Immunocompetence of bivalve hemocytes by a miniaturized phagocytosis assay. Environ. Toxicol. 17,3, 160-169.

Gagné, F., C. Blaise, M. Fournier, P.D. Hansen. 2006. Effects of selected pharmaceutical products on phagocytic activity in *Elliptio complanata* mussels. Comp. Biochem. Physiol. C Toxicol Pharmacol, 143 (2), 179-186

## Summary

- **Blue mussels kept in artificial seawater are keeping their haemocyte quality and concentrations fairly stabil and are used as the source for the cryoconservation experiments. A SOP for the quality control of the standardisation of cryoconservated haemocytes is in progress.**
- **Haemocyte-pools can be stored frozen in either -80°C or $LN_2$ for at least half a year. Best results so far can be achieved when the enriched cell-suspension are frozen with PVP in their own plasma.**
- **After reconstitution the cells can be stimulated to perform phagocytosis and produce ROS.**
- **The components of the phagocytosis test system for the BIOLAB are now established under ground conditions. The adaptation of the biological component (phagocytosis) to the technical set up for the micro gravity experiments in space is in progress.**
- **The results expected will contribute to risk assessment and management of immunotoxic effects under space flight conditions.**
- **The AEC = Advanced Experimental Containment will be applied for in-flight and on ground exposure- and effects- monitoring.**
- **The AEC will be complementary to advanced real time bioassys for *in situ* effects monitoring.**

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# Acknowledgements


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**Project 96-HEDS-04/05-101**

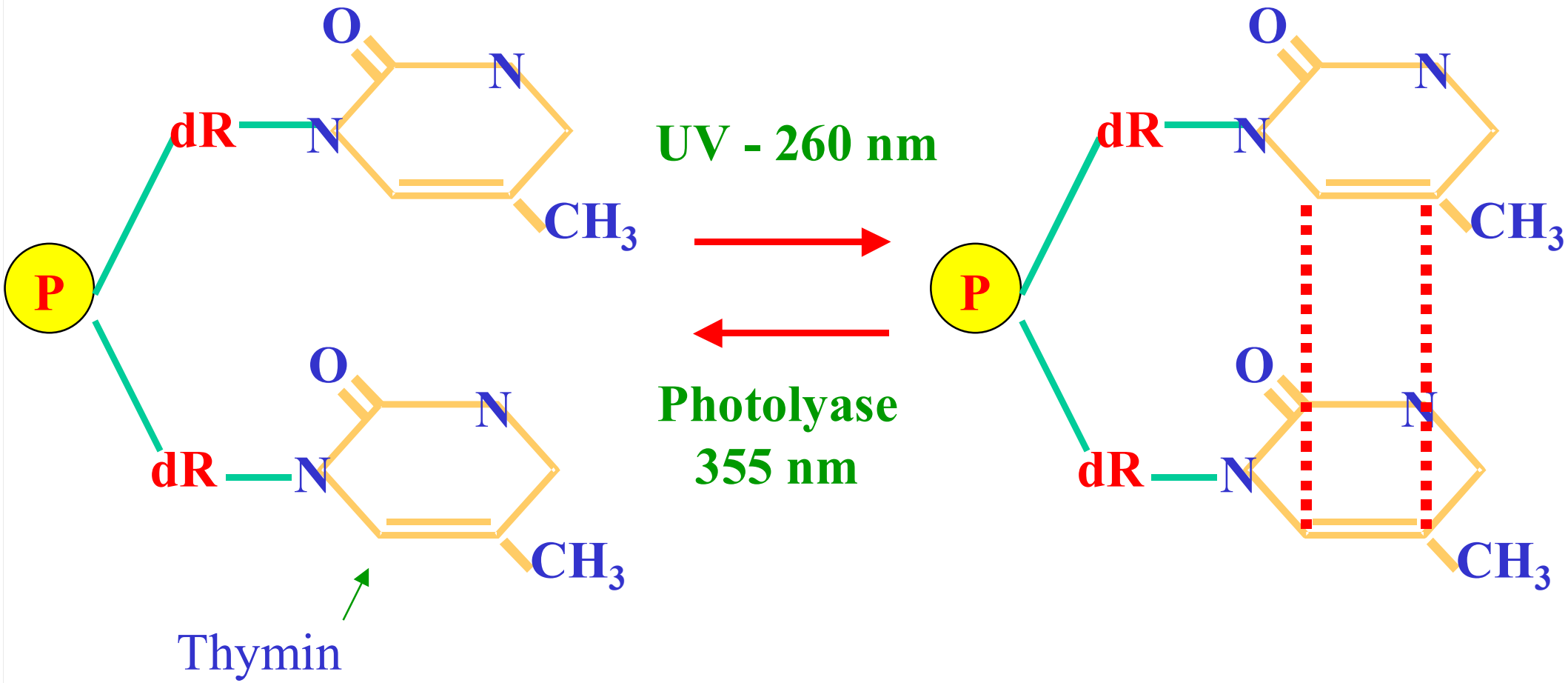
**BMBF Federal Minister for Education, Research and Technology**

**European Commision: BEEP**
**EVK3-CT-2000-00025.33**

# Thank you very much for your attention!

Dr. E. Unruh & Prof. Dr. Peter Hansen

# Thymin-Dimerisierung durch UV führt zu Verzerrung der DNA-Doppelhelix und Störung der Basenpaare. DNA-Polymerasen bleiben an Thymindimeren hängen.




P = Phosphat dR = Desoxyribose