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# Fully automated and long term stable biological Toxicity Sensor

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## MOTIVATION AND AIMS

§ **Aim: Enhancement of the biosensor prototype *AquaBioTox* for broadband detection of chemical contaminants**

à from a laboratory device with high maintenance effort to a fully automated prototype (targeted maintenance effort ~ 4 weeks)

§ **Fraunhofer IGB:**  
biological part

**Fraunhofer IOSB:**  
low cost fluorescence sensor;  
automation and integration



AquaBioTox prototype (Fraunhofer IGB + IOSB, 2010)

# MOTIVATION AND AIMS

## Motivation

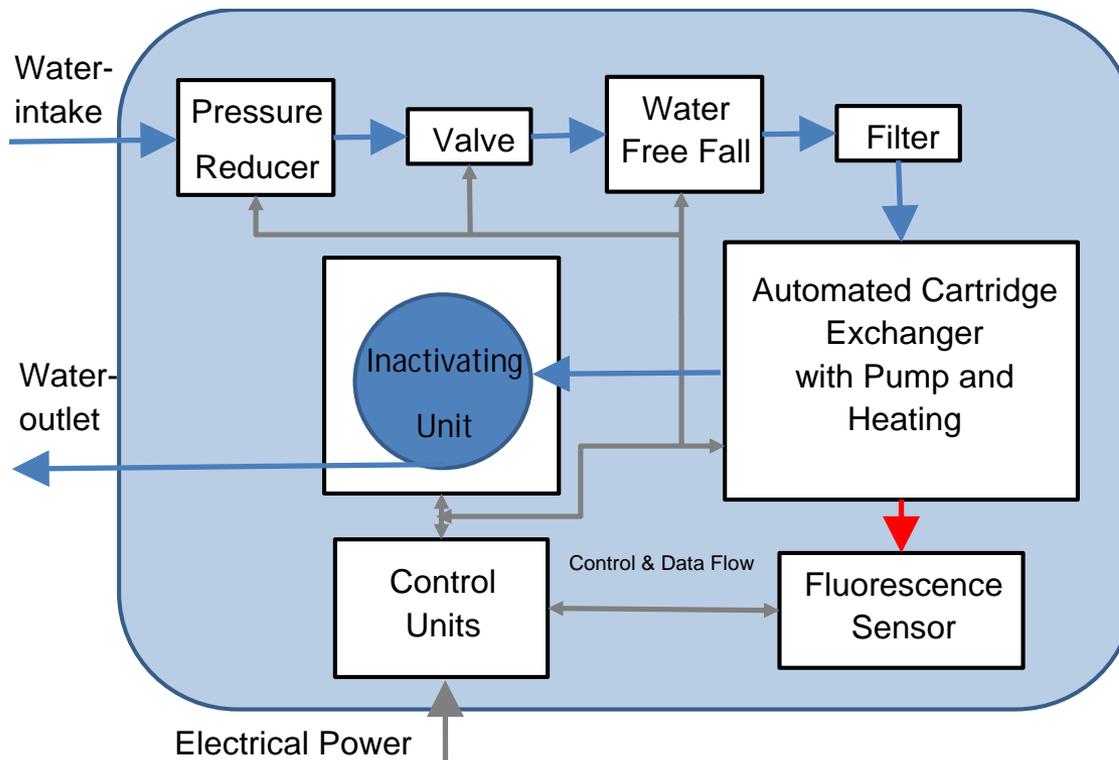
- § Online monitoring of water quality by measuring fluorescence from genetic engineered bacteria (e.g. *Ecoli* and *Caulo bacter*)
- § Industrial Fluorescence measurement systems are very costly (8 – 14 k€, e.g. Algaetorch, bbe Moldenke or FP 360, Hach Lange)
- § Targeted costs for online water toxin meters are < 7.000€ for the complete system

## Principle of AquaBioTox biosensor:

- § Genetically constructed strains of Escherichia coli and Caulobacter vibrioides
- § Non pathogenic bacteria (risk group 1)
- § Adapted to the normal conditions in water

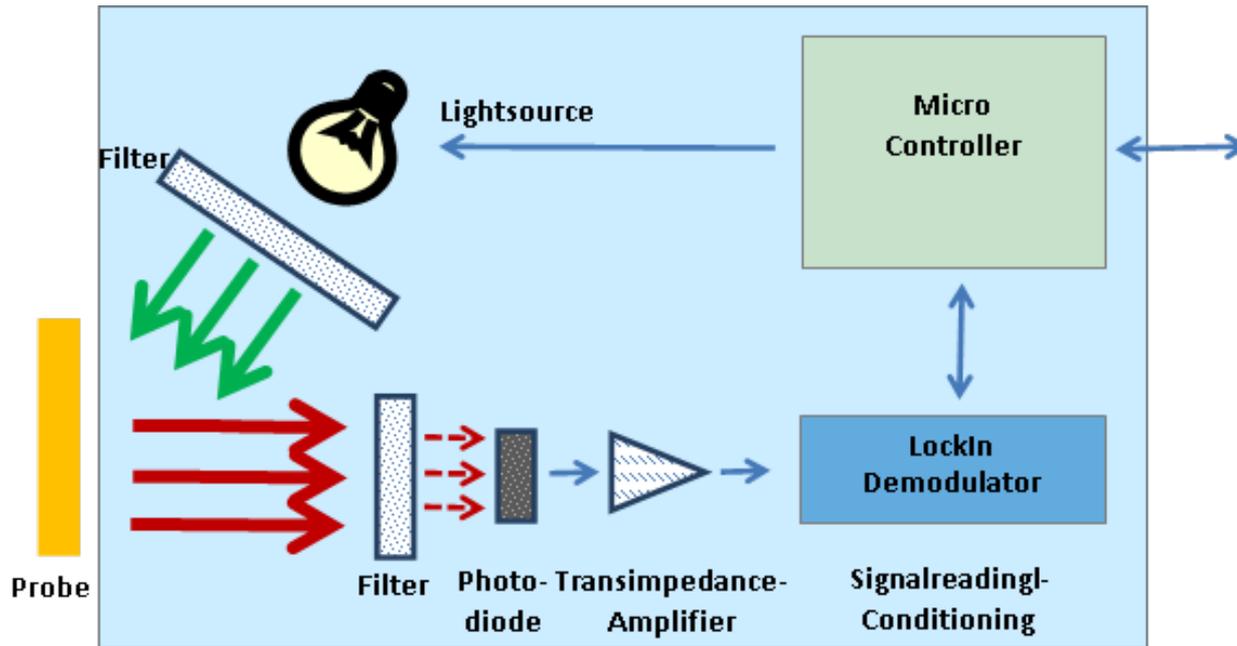
# SENSOR SYSTEM - OVERVIEW

## Main components of the automated sensor system:



# LOW-COST FLUORESCENCE SENSOR

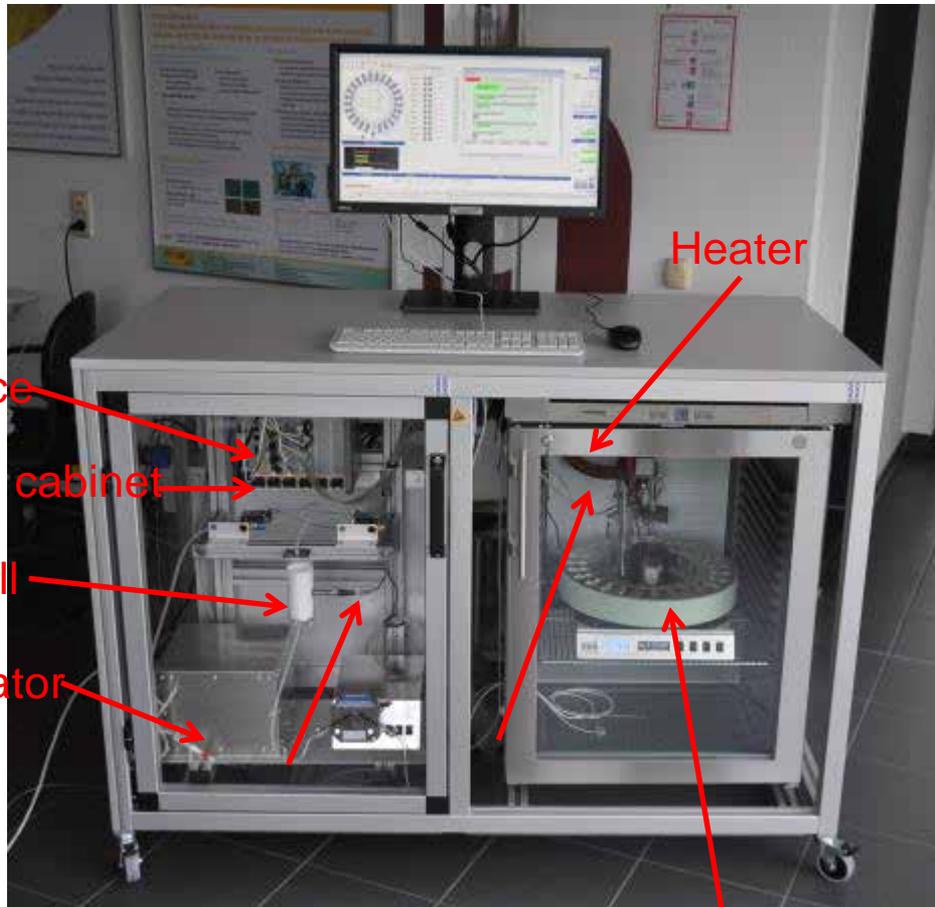
## Principle of the low-cost Fluorescence Sensor



- Robust rugged case IP65
- 2 versions of the optical unit
  - Coaxial setup with dichroitic filter
  - 20 ° Angle setup
- stable long term behavior



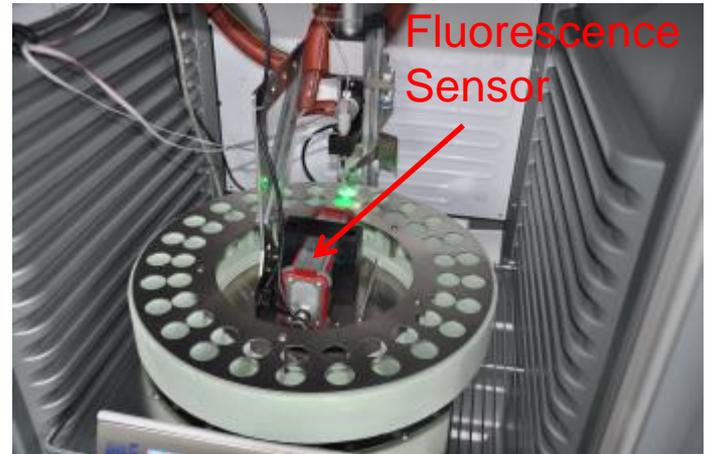
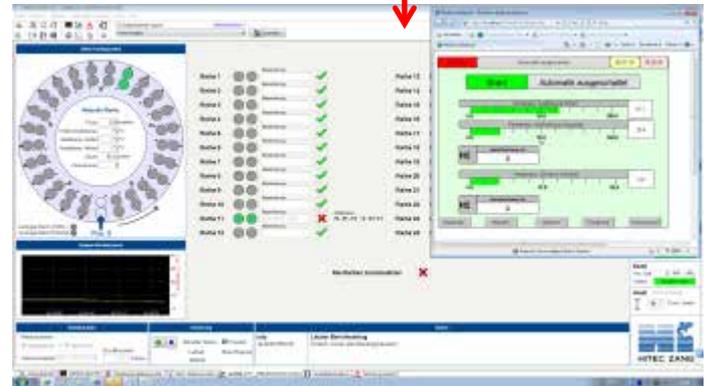
# FINAL DEMONSTRATOR



Interface  
Control cabinet  
Free fall  
Inactivator

Pump  
Autosampler  
Refrigerator

HMI

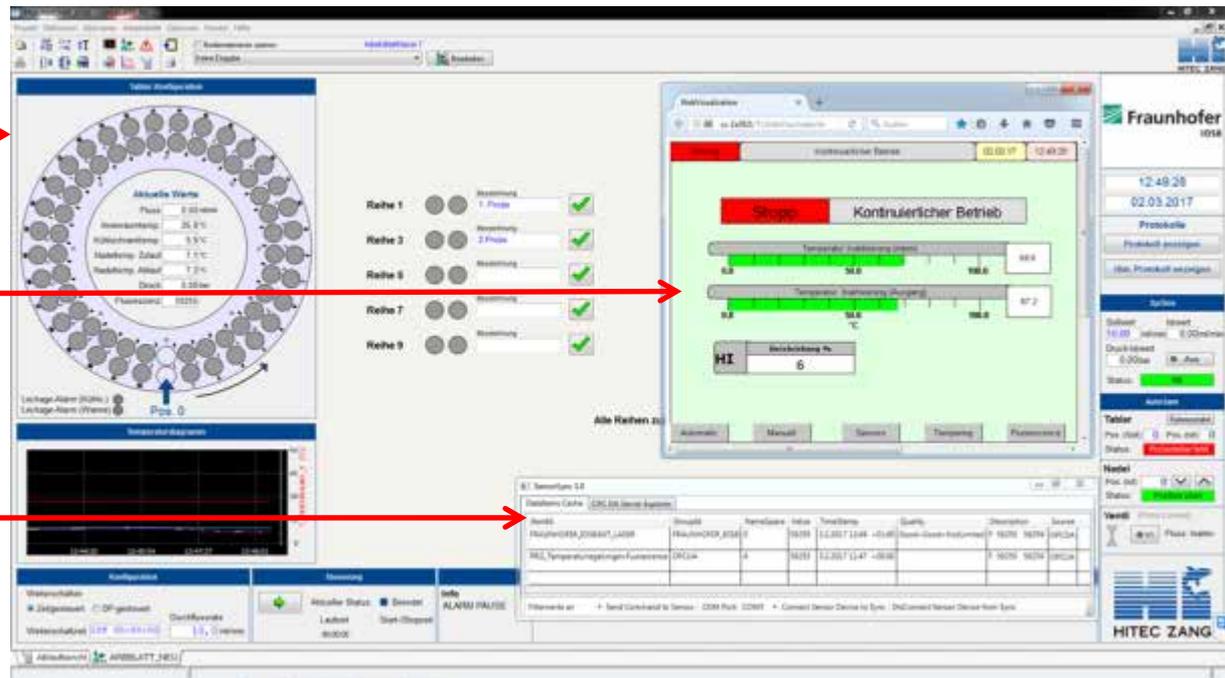


Fluorescence Sensor

# FINAL DEMONSTRATOR

## Software modules:

- § Lab-Vision with ResiWater project
- § PLC program for free fall and inactivator
- § Module for fluorescence sensor and data exchange



## SAFETY ASPECTS

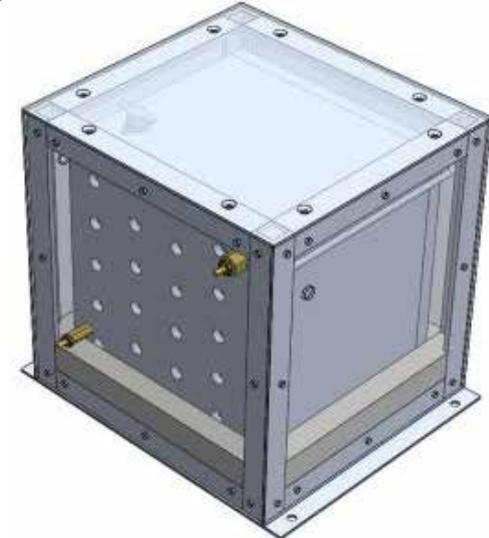
### § Prevention of reflow of contaminations into the water distribution system:

- ∅ No direct connection to the water pipe
- ∅ Water free fall with sensor based level control integrated in the water intake system of the demonstrator



### § Prevention of contamination of the environment with micro-organisms by an inactivation unit:

- ∅ Inactivation temperature: 90°C, transient time: 100min
- ∅ Experimental results of microbiological testing: no augmentable bacteria leave the system



# BIOSENSOR SYSTEM

## § Biosensors:

### Bacterial strains:

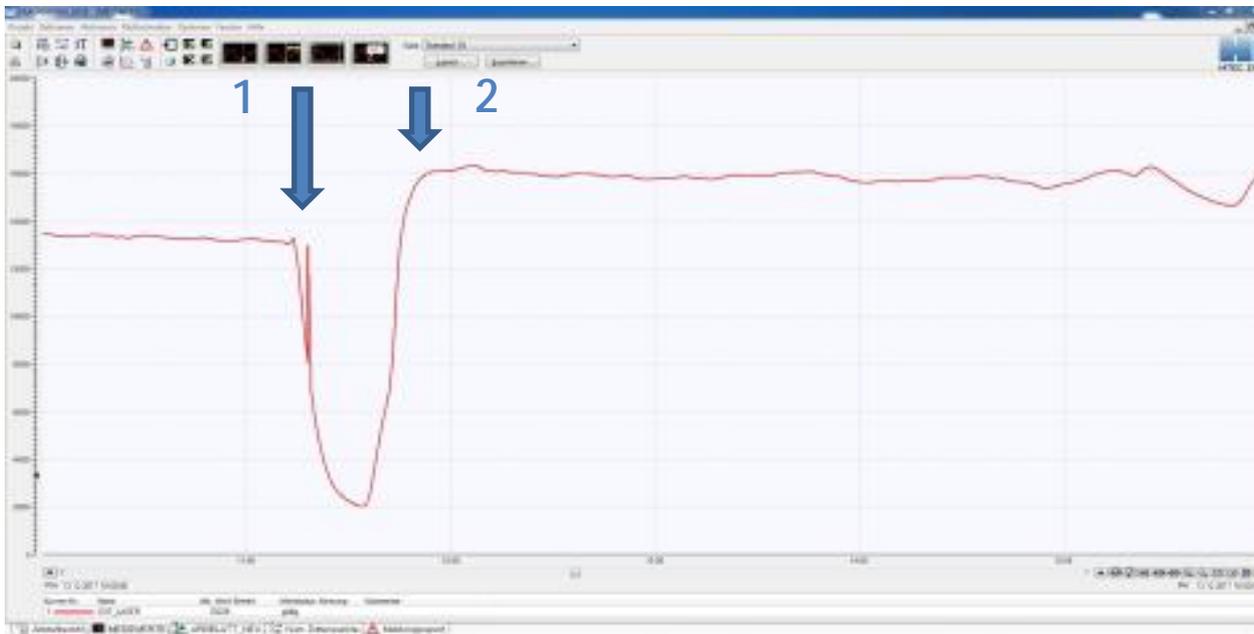
- ∅ strains react on different substances: extended application
- ∅ easily handling
- ∅ red fluorescence by genetically engineering
- ∅ Real time measurement by newly developed fluorescence sensor



## EXPERIMENTAL RESULTS

### § Experimental testing of influence of toxic substances on biosensors in the automated system:

Example: Influence of pH effect on biosensor (*E. coli* RFP)



Addition of acetic acid  
pH 4 (1)  
Regeneration of  
fluorescence by  
addition of water  
(pH 7) (2)

➔ Reaction time:  
< 1 min

# EXPERIMENTAL RESULTS

## Results with selected substances and toxins:

tested by Fraunhofer IGB and TZW

Agens		Biosensor	
		<i>E. coli</i> RFP	<i>Caulobacter vibrioides</i> RFP
Buffered solution	pH 4 pH 5 - 7	84 % < 3 - 8 %	80 %
Ethanol	30 % 50 %	4,5 % 40 %	Not tested
Substance #1*	42 mg/L	< 2 %	40 %
Substance #2*	430 mg/L	54 %	< 2 %
Substance #3*	1 g/L	26% <small>↑</small>	< 2 % <small>↑</small>

\*) Name hidden (confidential)

decrease of fluorescence in percentage (%)

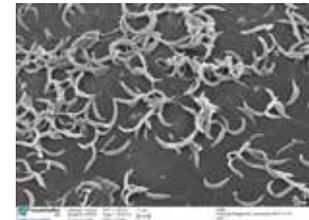
# EXPERIMENTAL RESULTS

## Characterization of the biosensors:

- ü Biosensor types: 2  
*Escherichia coli RFP*  
*Caulobacter vibrioides RFP*



- ü Method of application: immobilisation on carrier material



- ü Time for response: < 1 min → real time measurement
- ü Long term stability tested: 3 weeks
- ü Storage of biosensors under cooling conditions (8°C): > 6 months
- ü Availability in the demonstrator:  
about 6 measurement cells (more are possible) → operation of system  
theoretically possible for about 6 months
- ü Before leaving the demonstrator: cells are effectively inactivated by heating (90 °C)

# CONCLUSIONS

- § The biosensor prototype *AquaBioTox-II* for broadband detection of chemical contaminants has been developed to a fully automated and long term stable system
- § A stable operation for more than 30 days was achieved
- § The targeted maintenance effort of 4 weeks was fulfilled

## Recommendations for future work:

- § For robust continuous operation the hydraulic part of the sensor system has to be optimised (pipes, pumps)
- § The size of the sensor system could be minimised