



AnALyT

**Drinking water -
Analysis of Bacterial Contamination**



Drinking Water Analysis - State-of-the-Art

Standard evaluation methods - minimum 18 hours depending on bacterial species

- low bacterial concentrations
- high input volumes
- human factor



Contents

Project aim

Actual state

Filtration

Bacterial lysis

Luminescence biosensor

Outlook



Aim of the Project

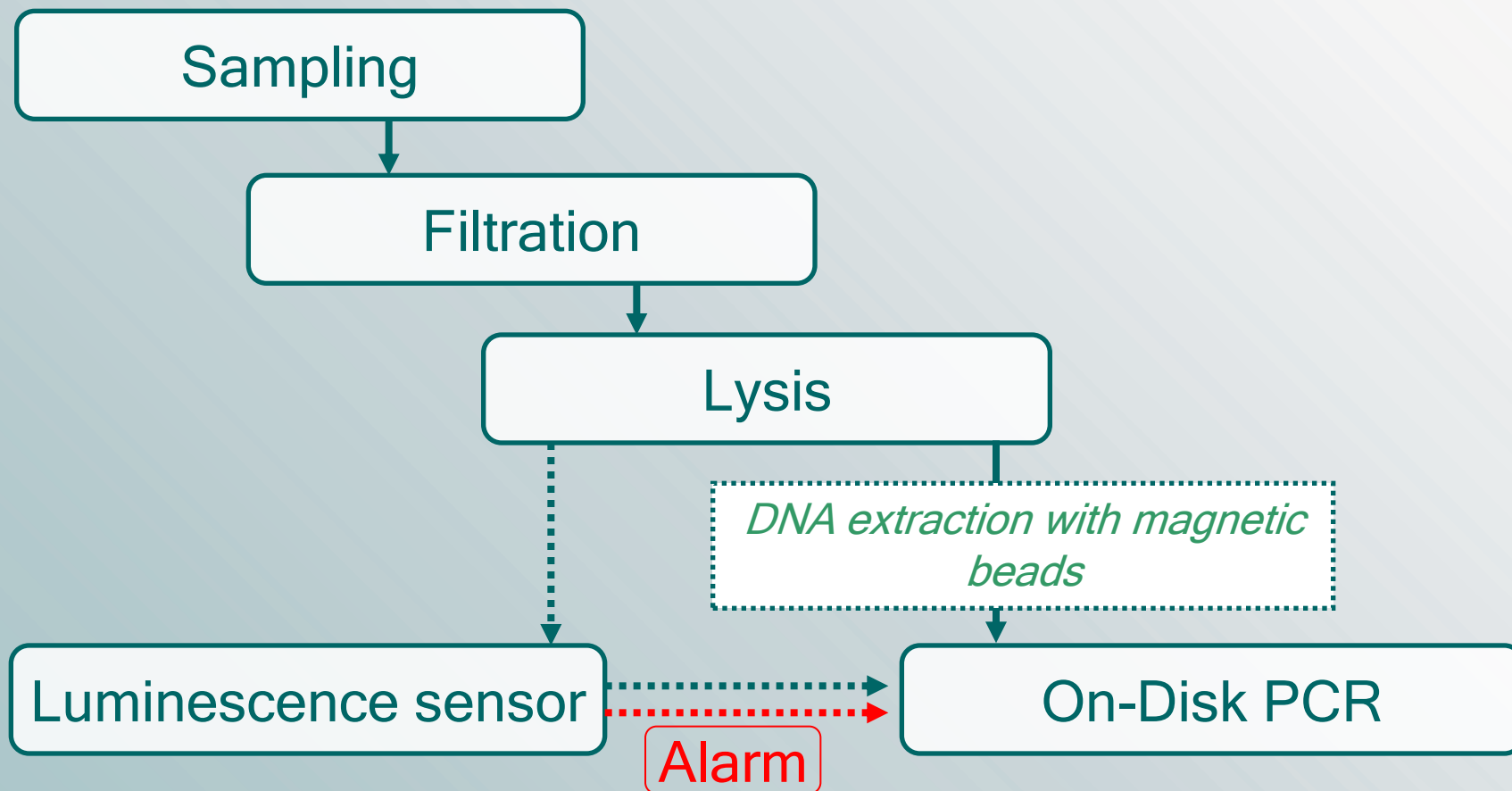
SOLUTION - online system

ENSURING fast filtration of larger water volumes in order to increase the concentration of bacteria

DETECTING bacterial contamination via a luciferase-based alarm system and subsequent discrimination of 4 different bacterial species (PCR)



Water Analytical Process





FILTRATION - Concentrating Bacteria

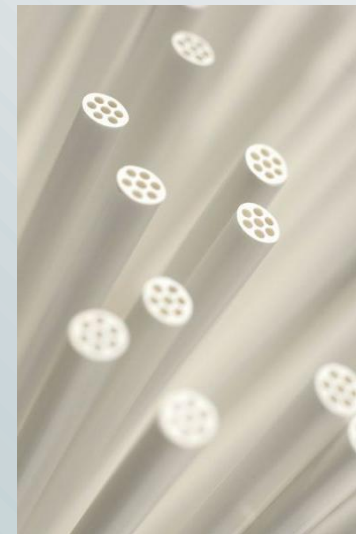
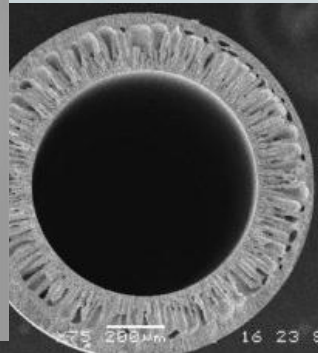


Filtration Modules

Different filter elements



Hollow fibre



PESM membrane



ultra- and microfiltration membrane
($0.01\mu\text{m}$ or $0.2\mu\text{m}$ pore size)



How does the Filtration Work?

Two filtration steps

- water sample (manually adjustable between 1 - 100L) is forwarded to the 1st filter by a peristaltic pump
- semi dead-end filtration
- the concentrate is back-flushed by an air-water mixture
- 2nd filtration step is triggered automatically

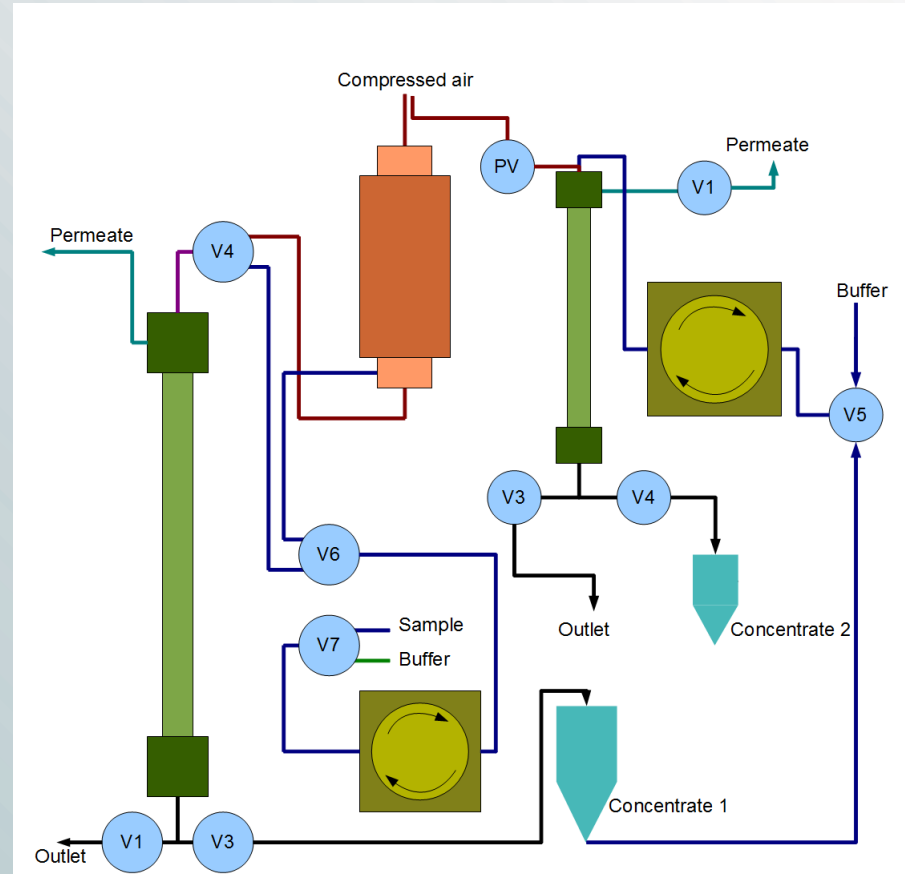
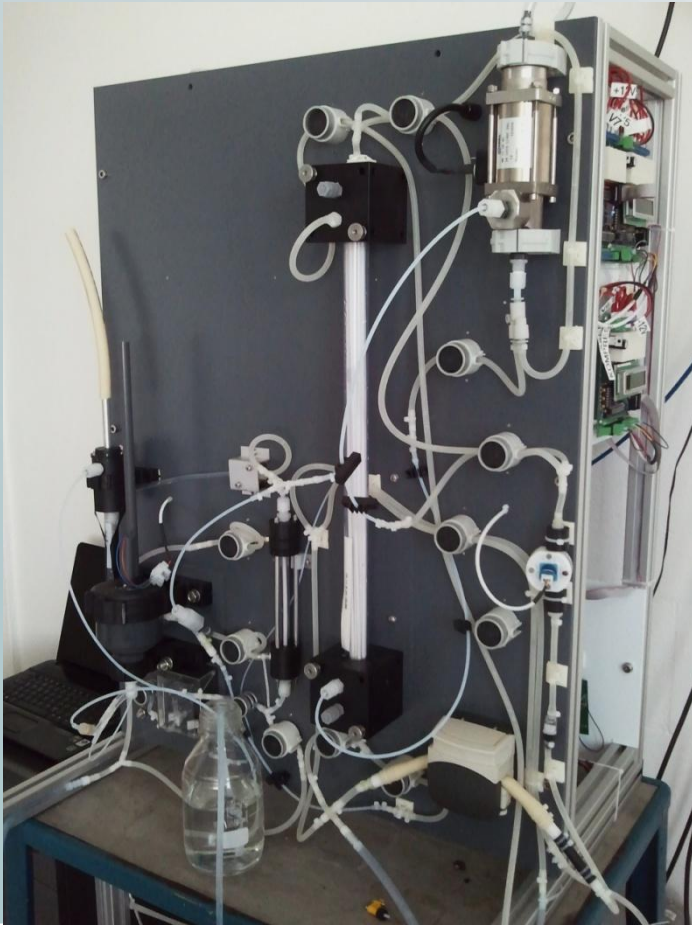


Components

- use of peristaltic pump
- fast coaxial valve switching between compressed air and water for back-flushing
- use of inner-tube valves - keeping the dead volume small
- collecting tray for both filtration steps to solve hydraulic/pneumatic problems



Filtration Module (1st and 2nd Filtration Unit)





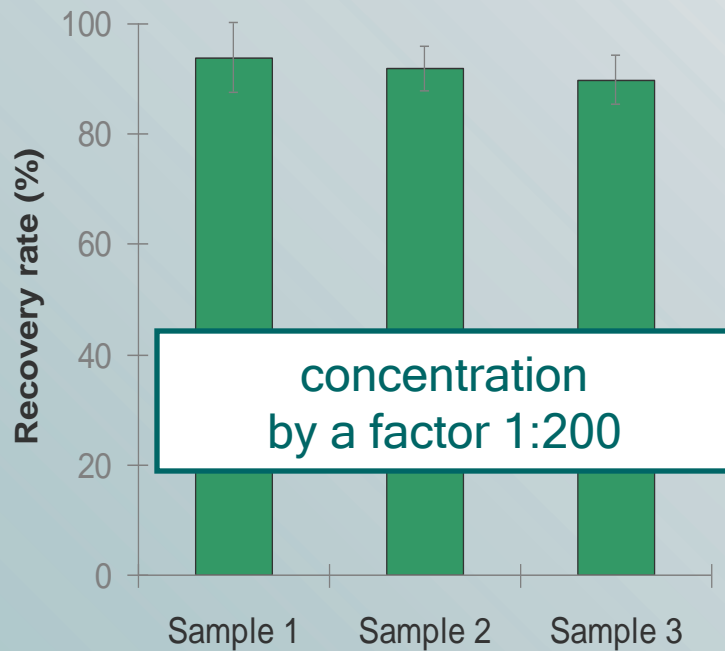
Tests of Recovery Rate

Tests with *Escherichia coli*

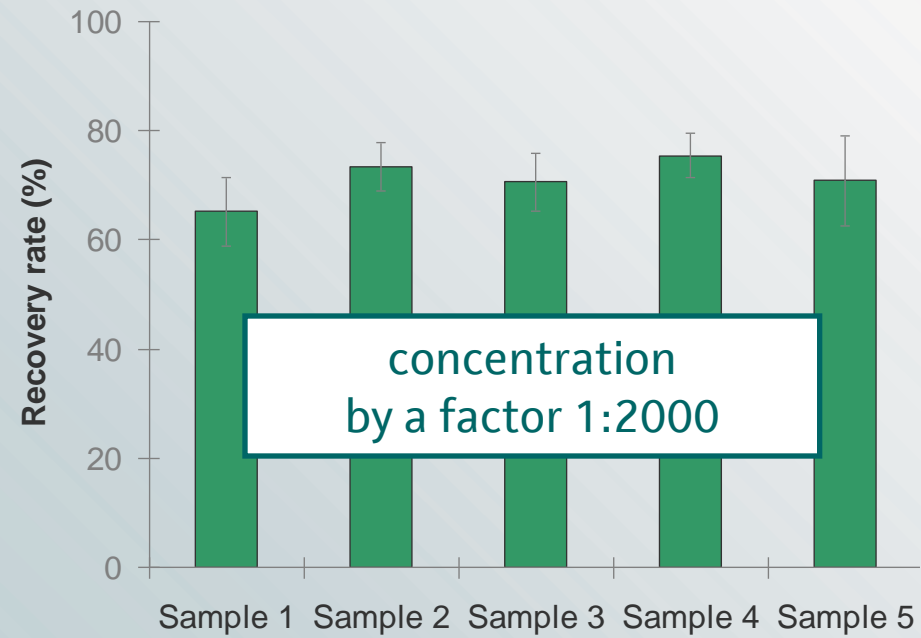
- bacteria grown on minimal medium
- preset bacterial concentration approx. 10000 cfu/10 liter
- Tests of recovery rate by following methods:
 - β -Galactosidase_MUG_assay
 - Luciferase_ATP_assay
 - Colony counting on lactose_TTC_agar



Tests of Recovery Rate



November 2011



May 2012



Current Situation

Minimal input volume - 10L

- concentrate 10mL
- concentration factor 1:2000 (10000 cfu/10ml)
- time approx. 40 min
- recovery rate with *E.coli* ca. 65%

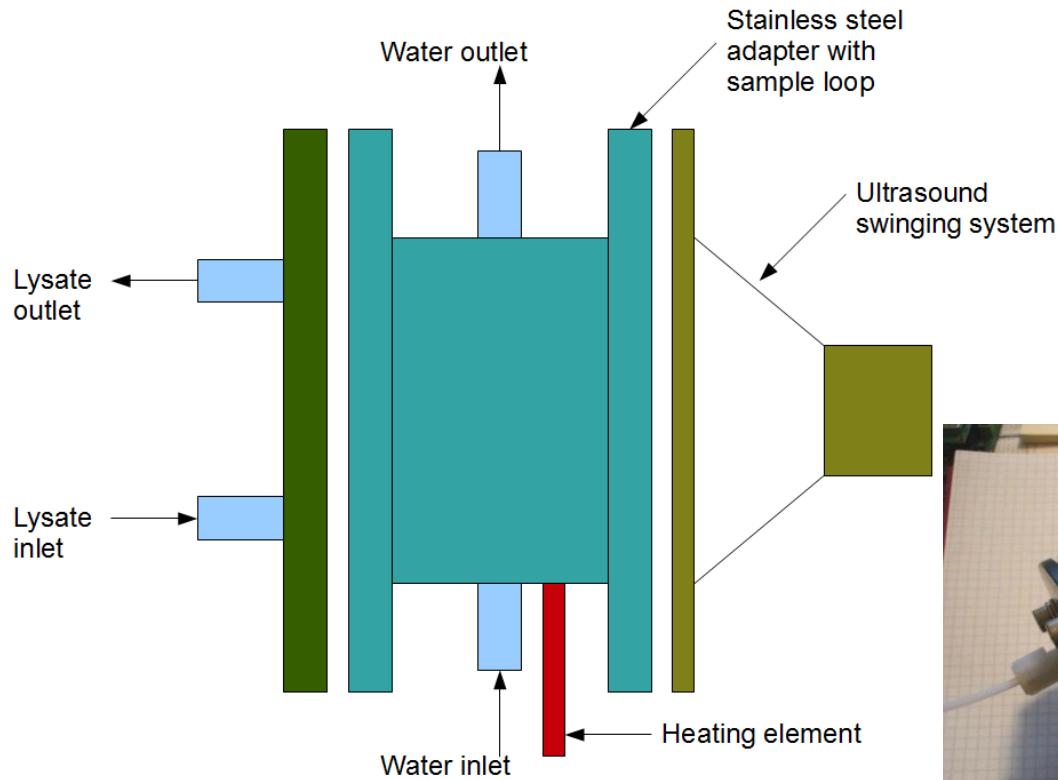


BACTERIAL LYSIS

Recovery of ATP and DNA

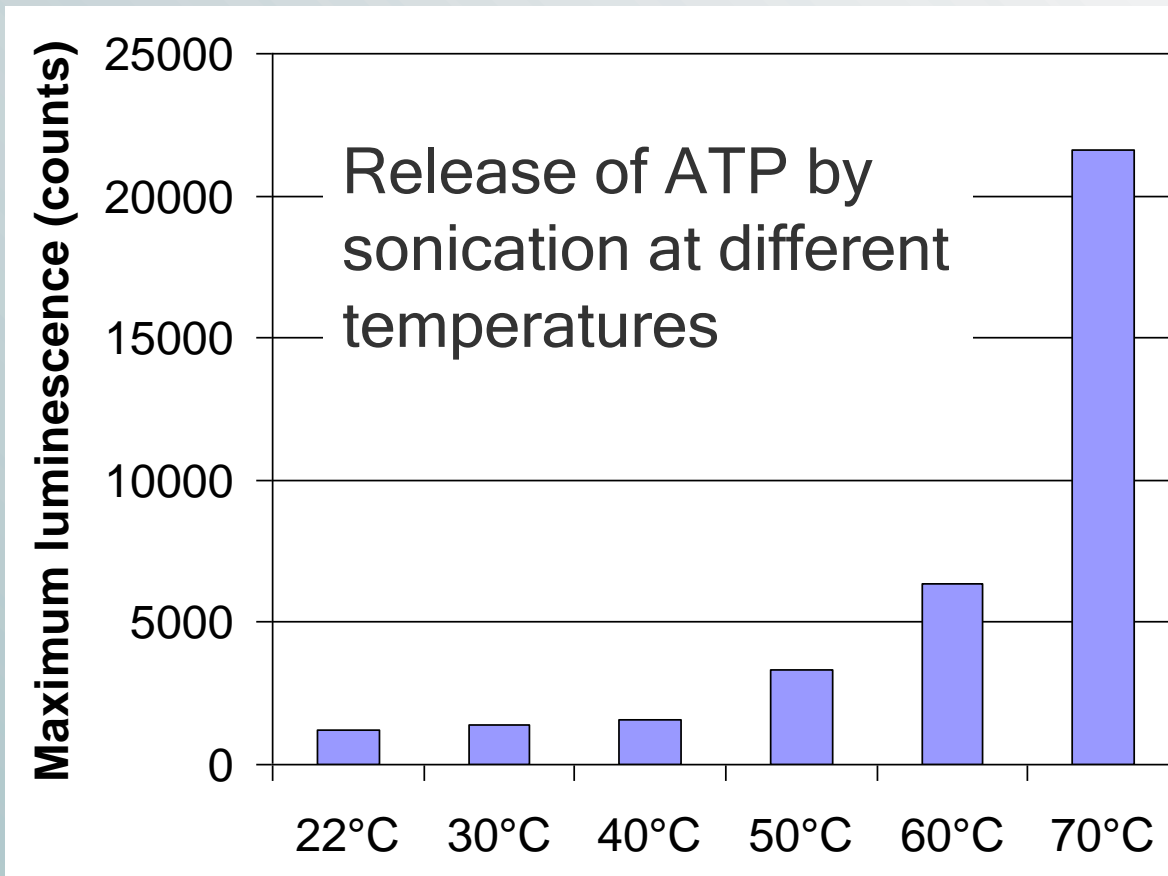


Sonication Device





Lysis of Bacteria





BIOSENSOR - Measuring Bacterial ATP by Luminescence



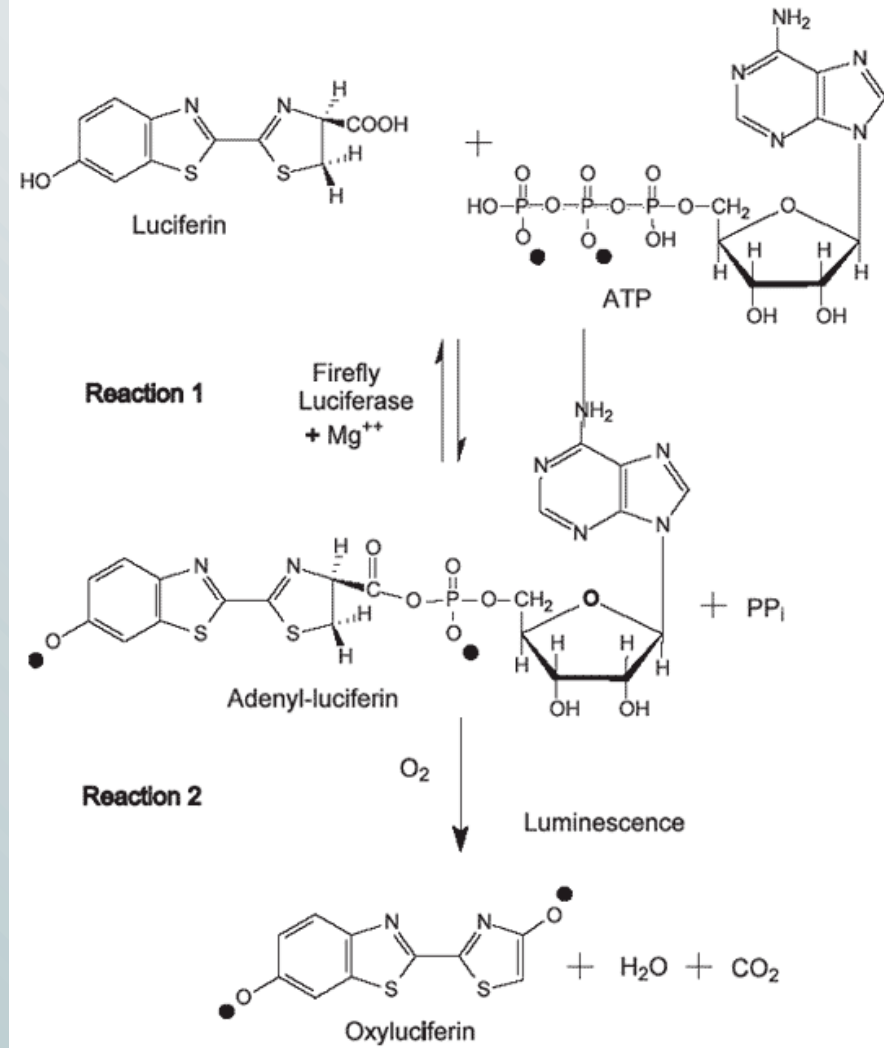
Luciferase

- Firefly (*Photinus pyralis*)
- luciferin-4-monooxygenase
- bifunctional enzyme
- 61kDa protein
- localised in peroxisomes



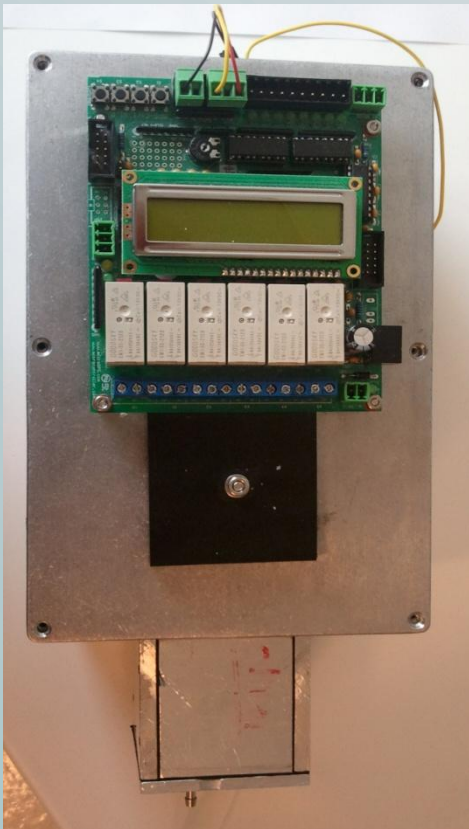


Reaction Mechanism





Luminescence Biosensor



Flow-through chamber

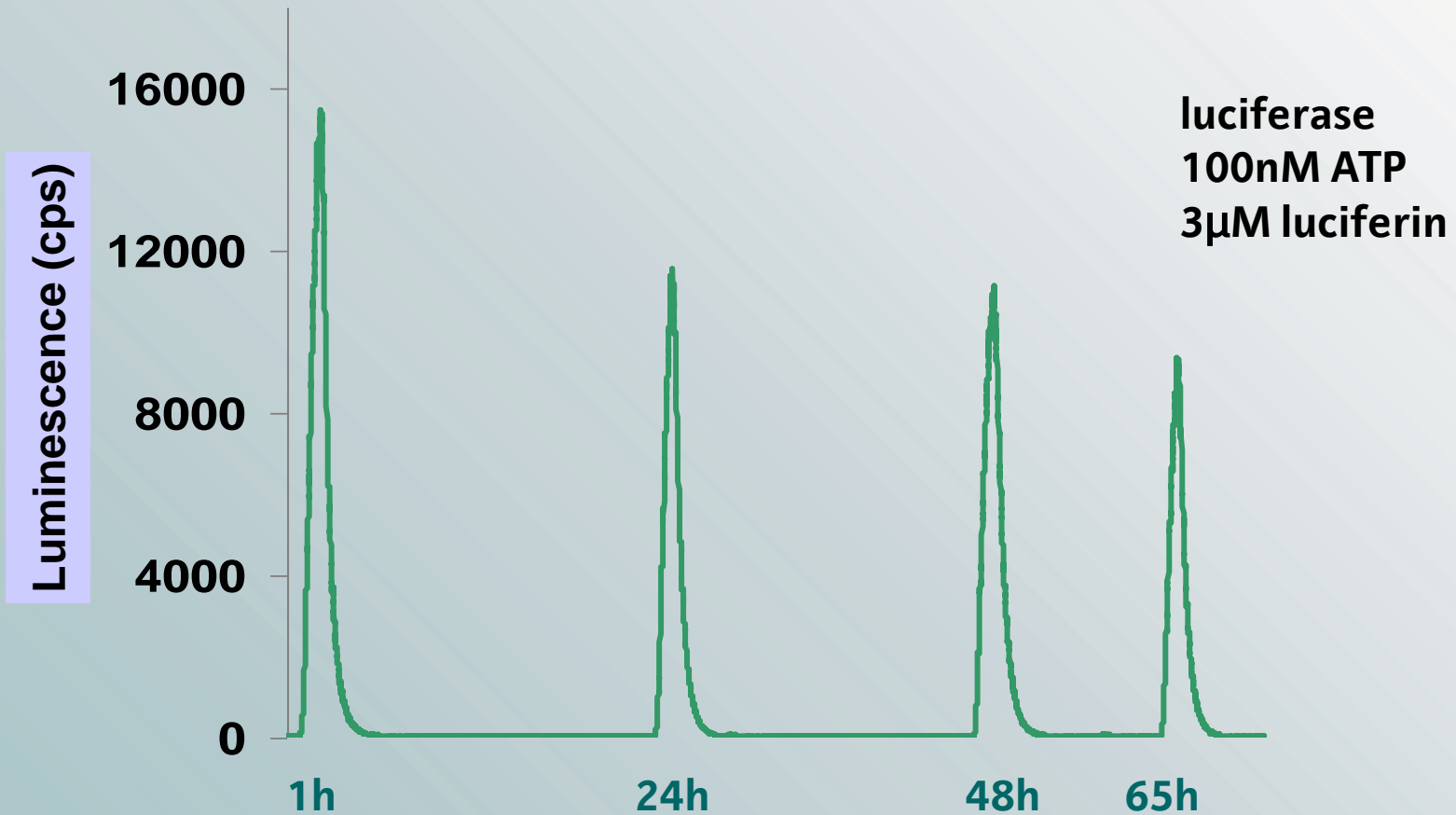
- luciferase coupled on beads
- application of bacterial ATP and luciferin

luminescence detector - photomultiplier





Long-Term Measurements





Integration of All Components

Robotic device with linear actuator



PCR device

Foil disk





Outlook

- increase the stability of luciferase
- test other bacterial species
- long-term measurements in whole-device configuration
- integration of sample preparation with PCR



Project Partners

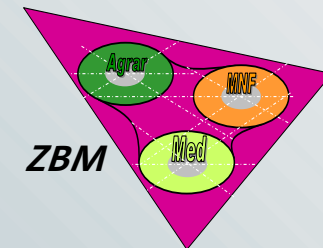
ZBM



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Thank you for your attention!!!

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