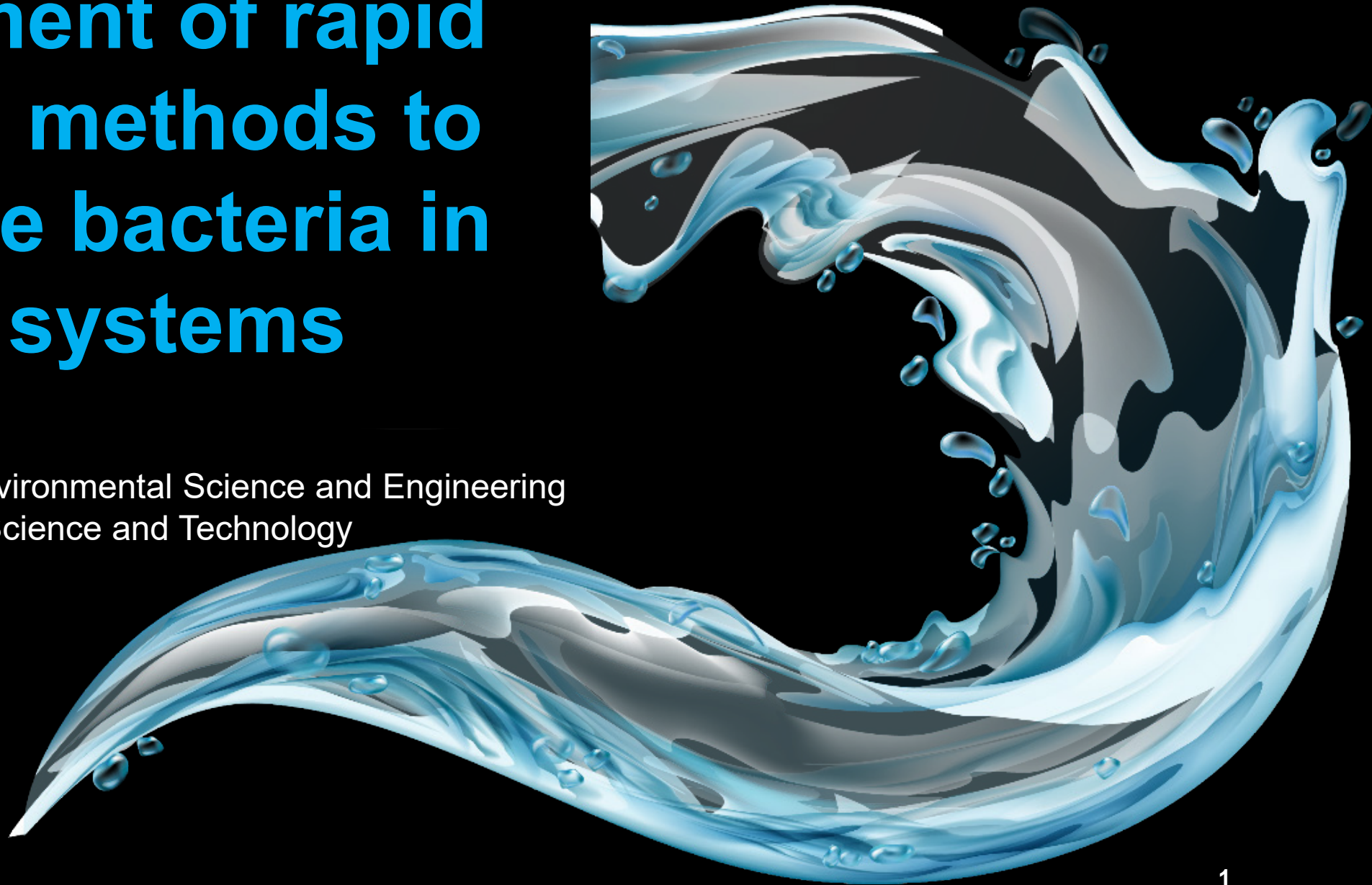


Development of rapid detection methods to enumerate bacteria in water systems

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King Abdullah University of Science and Technology







SUSTAINABLE DEVELOPMENT GOALS



Specific targets within SDG6:

6.1. Safe and affordable drinking water

6.2. End open defecation and provide access to sanitation and hygiene

6.3. Improve water quality, wastewater treatment and **safe** reuse

6.4. Increase water-use efficiency and ensure freshwater supplies

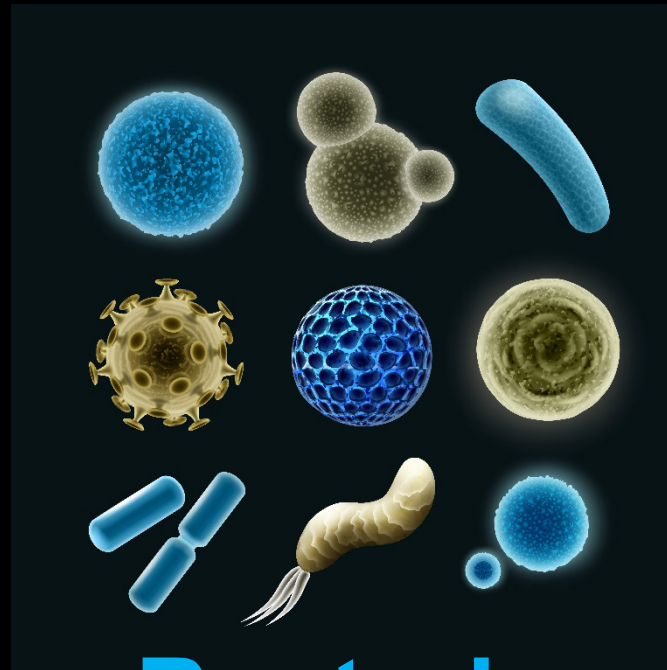
6.5. Implement integrated water resources management

6.6. Protect and restore water-related ecosystems

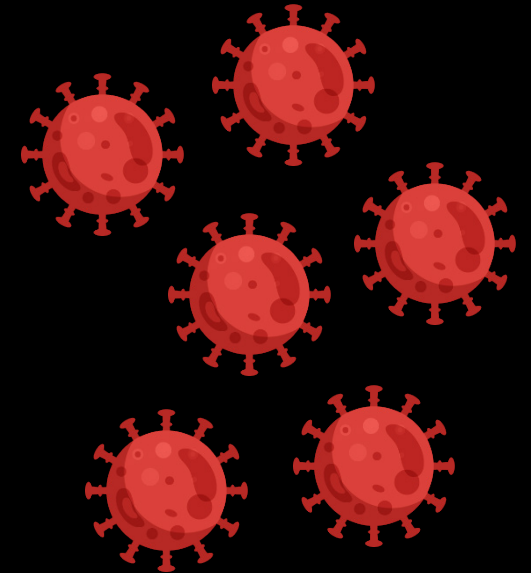
Myriad of biological contaminants



Protozoa



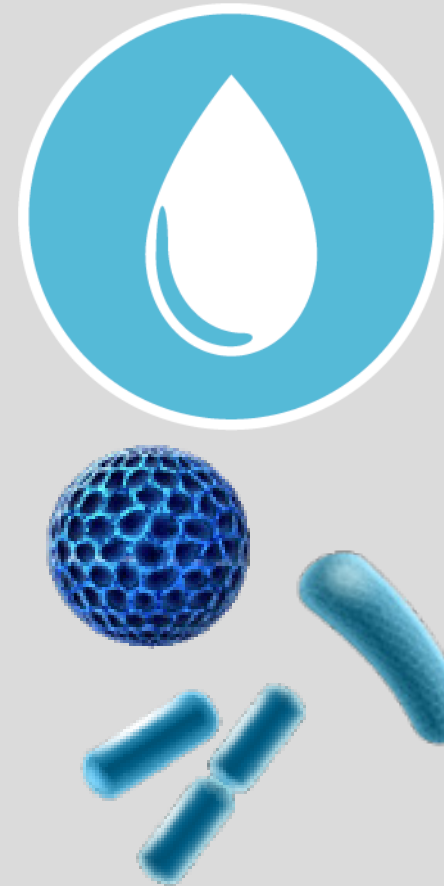
Bacteria



Virus

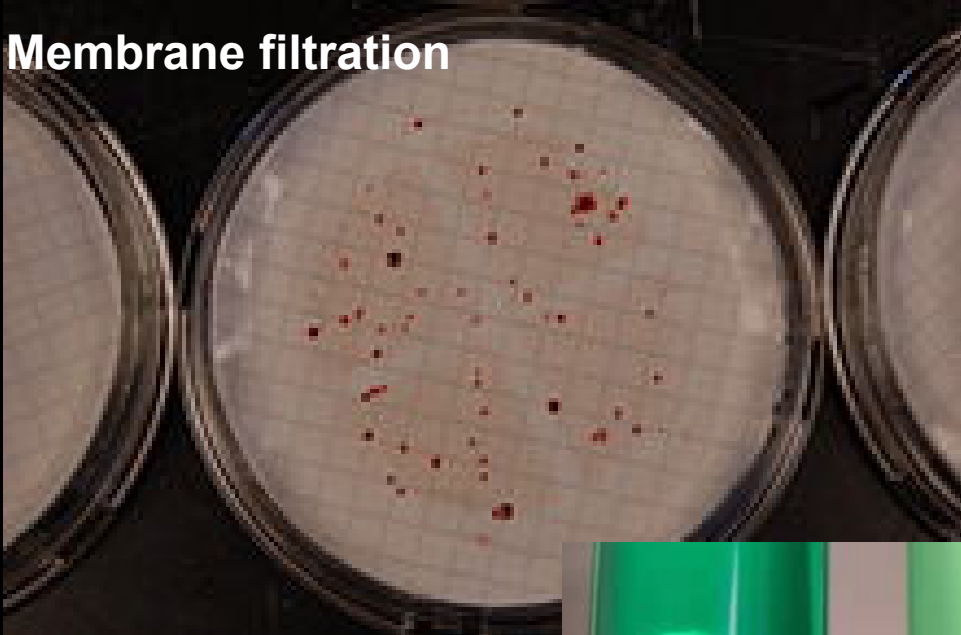
Smart water systems

- Current smart network mainly detects
 - Water leakage
 - Water usage
 - Chemical or physical water quality (feasible for parameters for which sensors are already available)
- Sensors to detect microbial contaminants not available

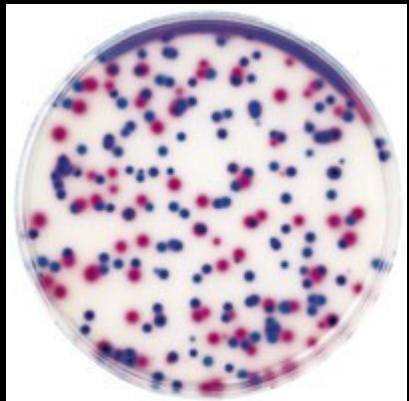


Conventional monitoring methods

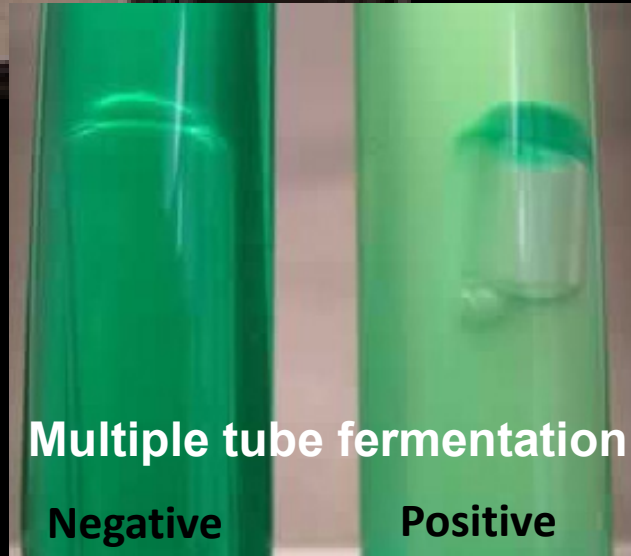
Membrane filtration



Simultaneous enzyme



Multiple tube fermentation



- **Cultivation of bacteria**

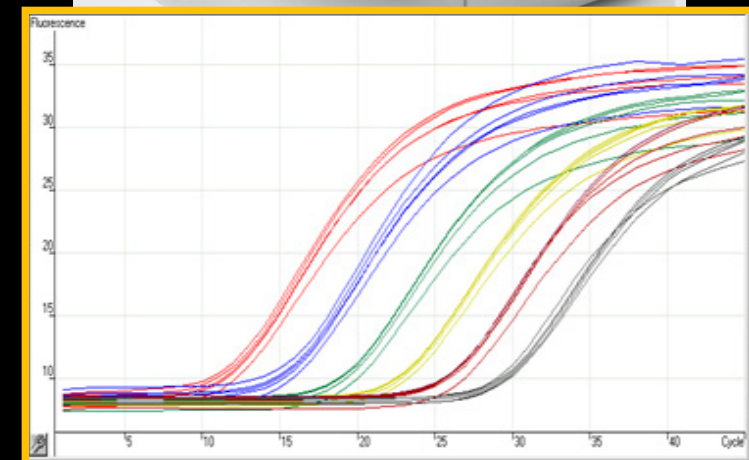
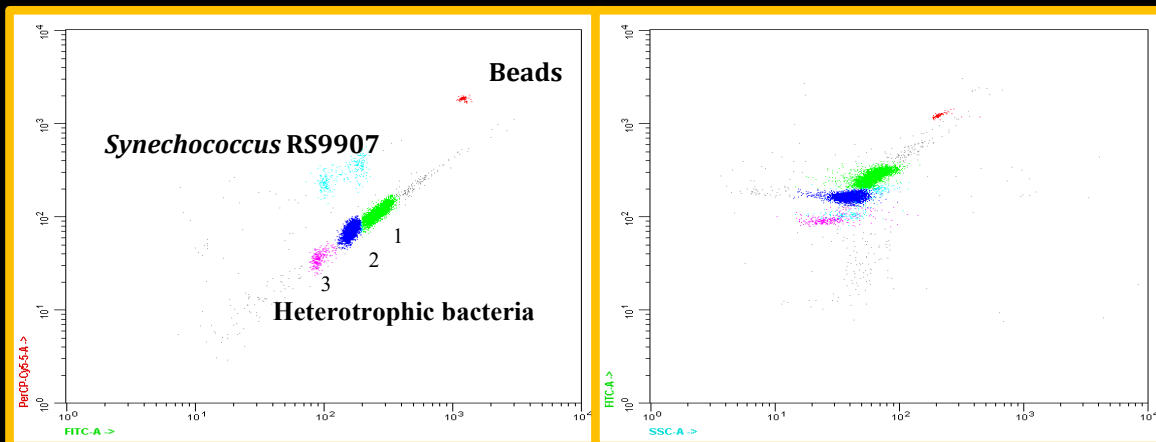
- Usually heterotrophic bacteria, coliforms, enterococci etc in accordance to standard approved methods
- Viable but non-culturable bacteria can bias results
- Time-consuming and does not permit real-time continuous monitoring

Advanced monitoring methods

Flow cytometry



Quantitative PCR



Goal and study objectives

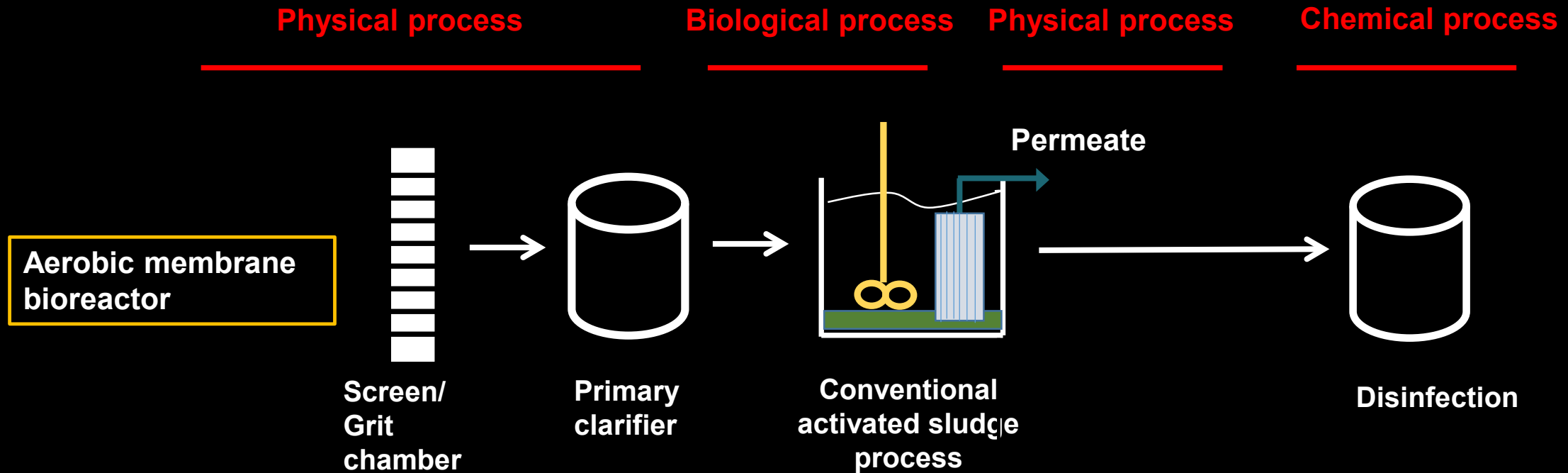
- **Aim:**

To establish continuous/semi-continuous biosensors so as to work towards a smart water system

- **Objectives:**

- A neural-network based approach to estimate cell density based on easily measurable parameters
- A DNAzyme-based microfluidics device to determine presence of *E. coli* in water matrices

Approach 1: Neural network-based sensing approach for wastewater treatment plants



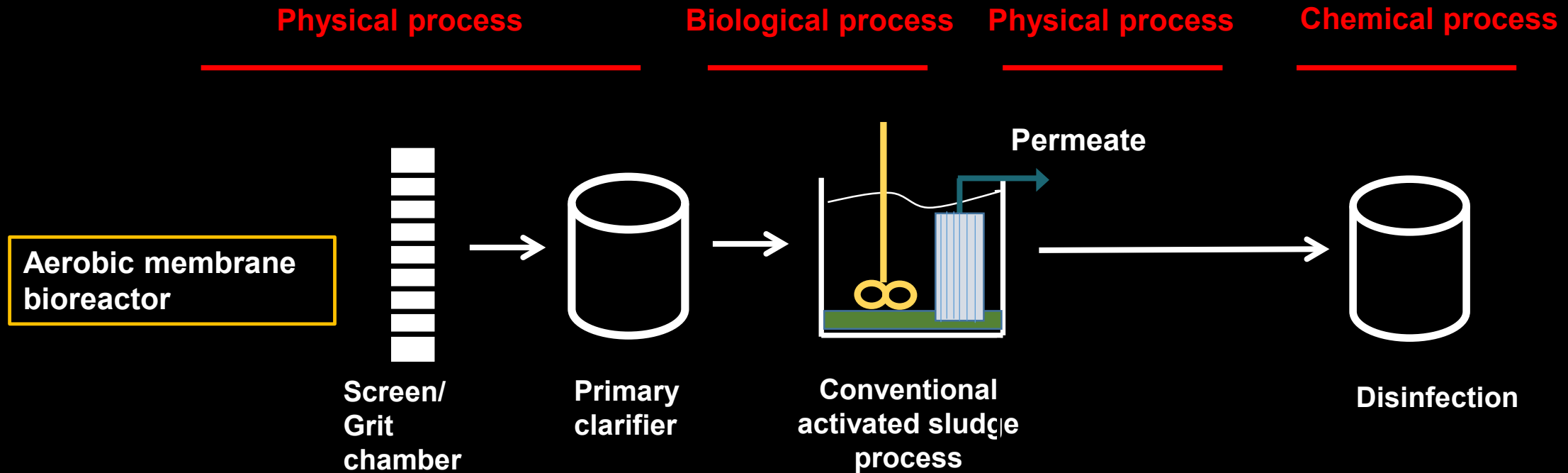
Monitoring log reduction value (LRV) is particularly important, for example, CAL EPA imposes a 12/10/10 rule for treatment to facilitate indirect potable reuse
Specifically 12-log removal for enteric virus; 10-log removal for *Cryptosporidium* and *Giardia*

Current approach

Table 3.3 Potential Microbiological Surrogates for *Cryptosporidium*

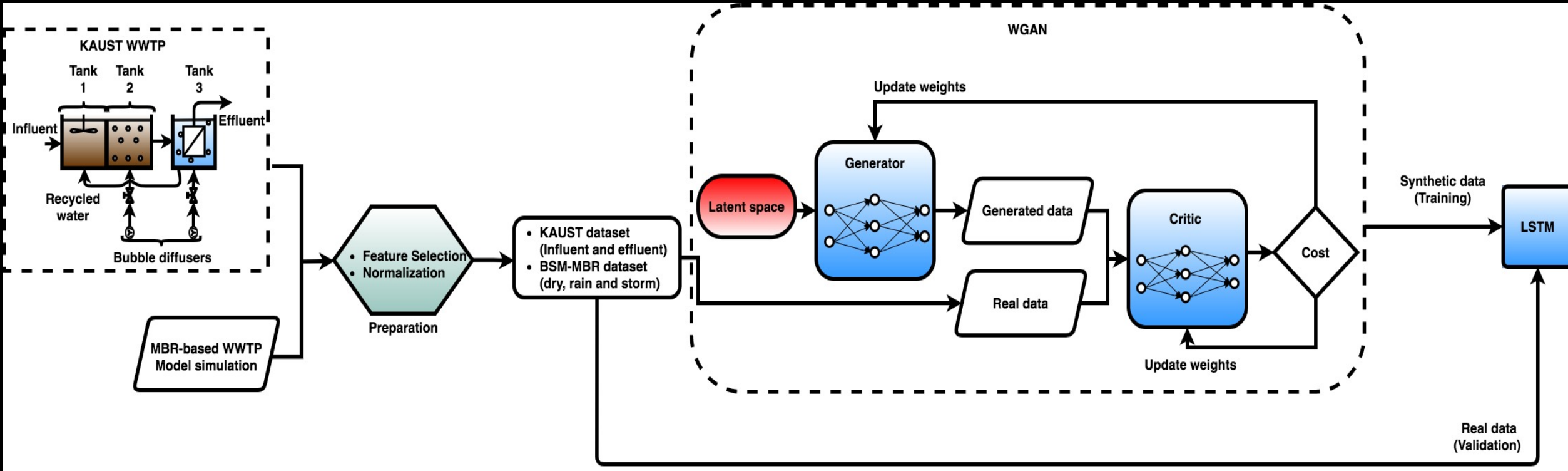
Microorganism	Size Range (μm)	Target Organism	Enumeration Method
<i>Micrococcus l.</i>	7 - 12	<i>Giardia</i>	Standard Methods 9222
<i>Bacillus subtilis</i>	~ 1	<i>Cryptosporidium</i>	Barbeau et al. (1997)
<i>E. coli</i>	1 - 4	<i>Cryptosporidium</i>	Standard Methods 9222
<i>P. dimunita</i>	0.3	<i>Cryptosporidium</i>	Standard Methods 9222
<i>S. marcessans</i>	0.5	<i>Cryptosporidium</i>	Standard Methods 9222
MS2 bacteriophage	0.01	Enteric virus	Adams (1959)

Approach 1: Neural network-based sensing approach for wastewater treatment plants



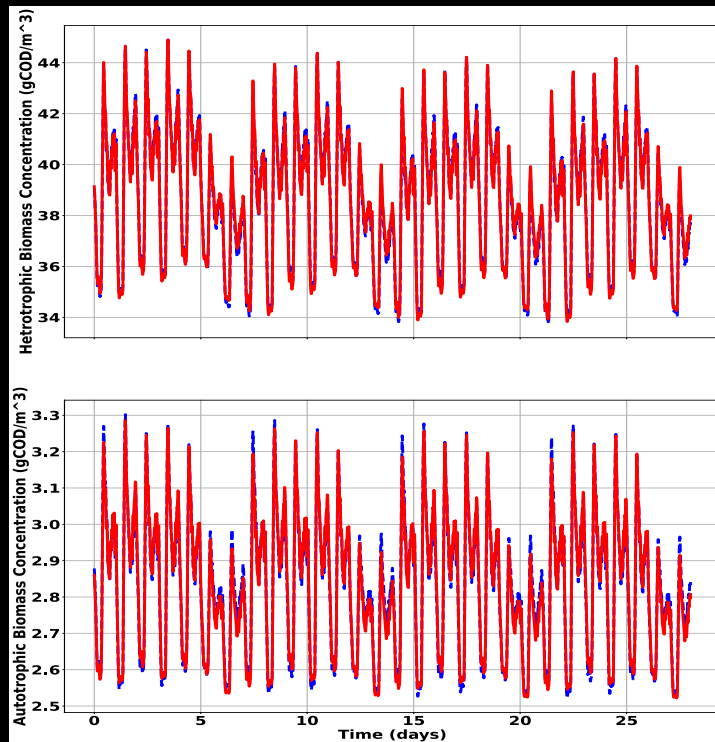
Parameters easily measured because of availability of online sensors:
turbidity, conductivity, total dissolved solids, total suspended solids, chemical oxygen demand

Developing the neural network based sensing approach

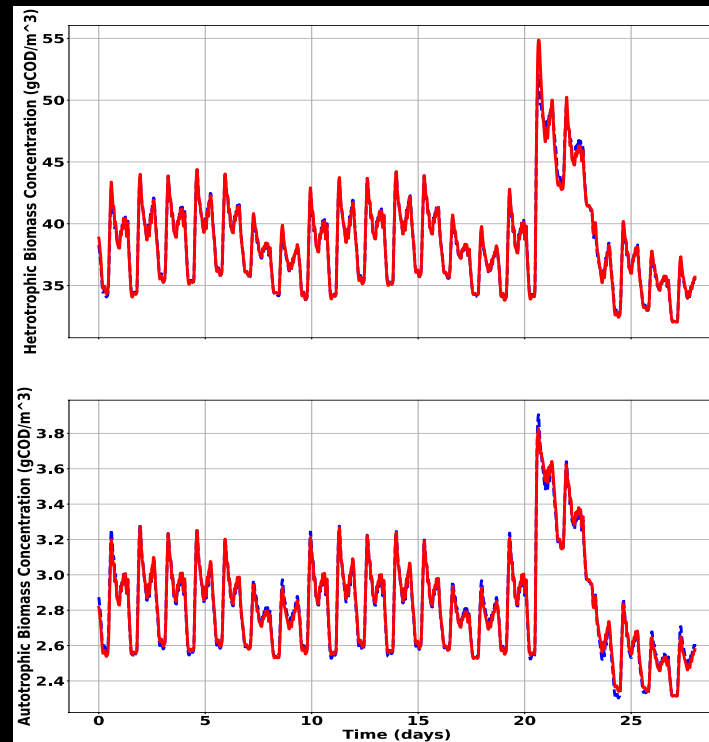


Prediction results for bacterial cell concentrations in effluent of BSM-MBR

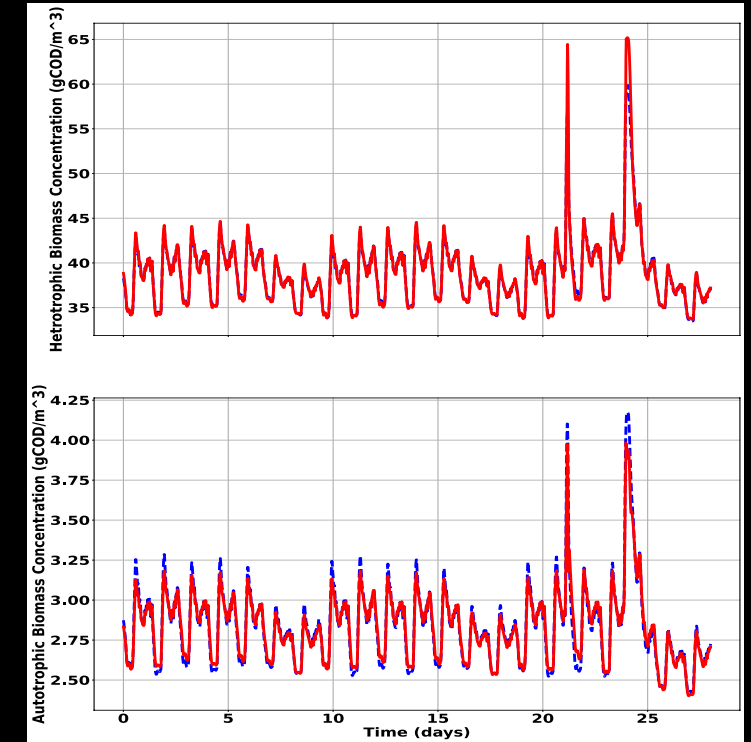
Dry conditions



Rain conditions

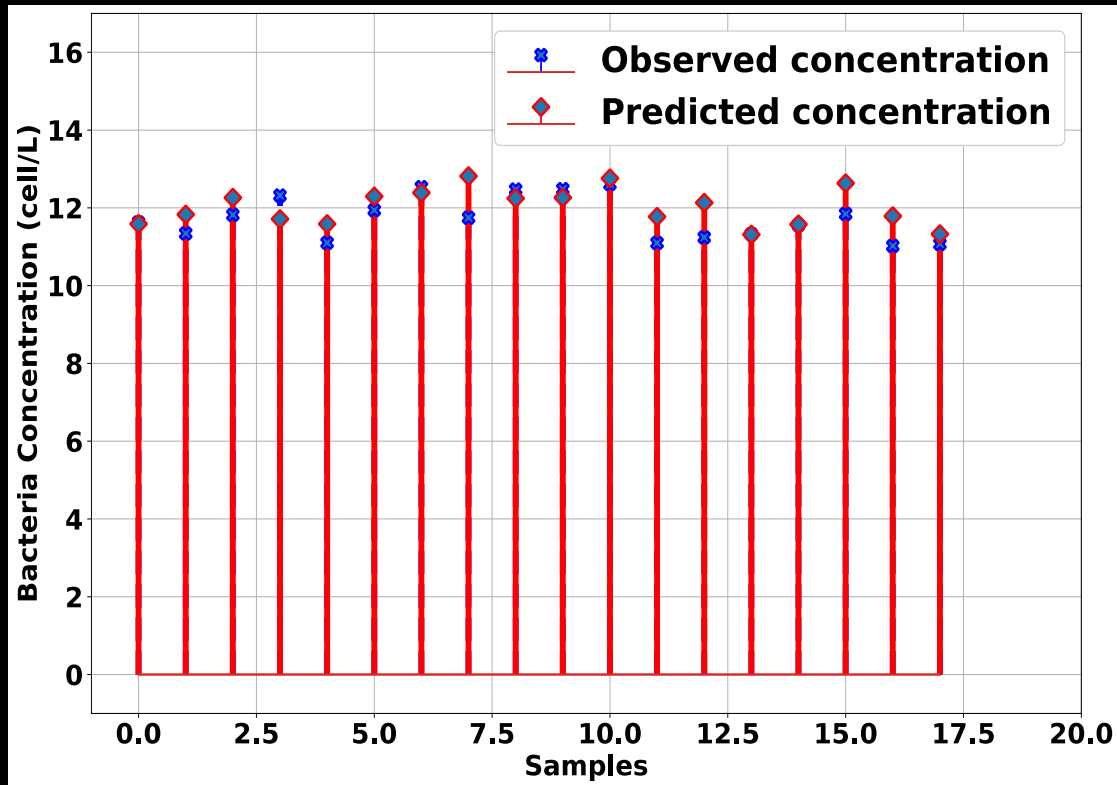


Storm conditions

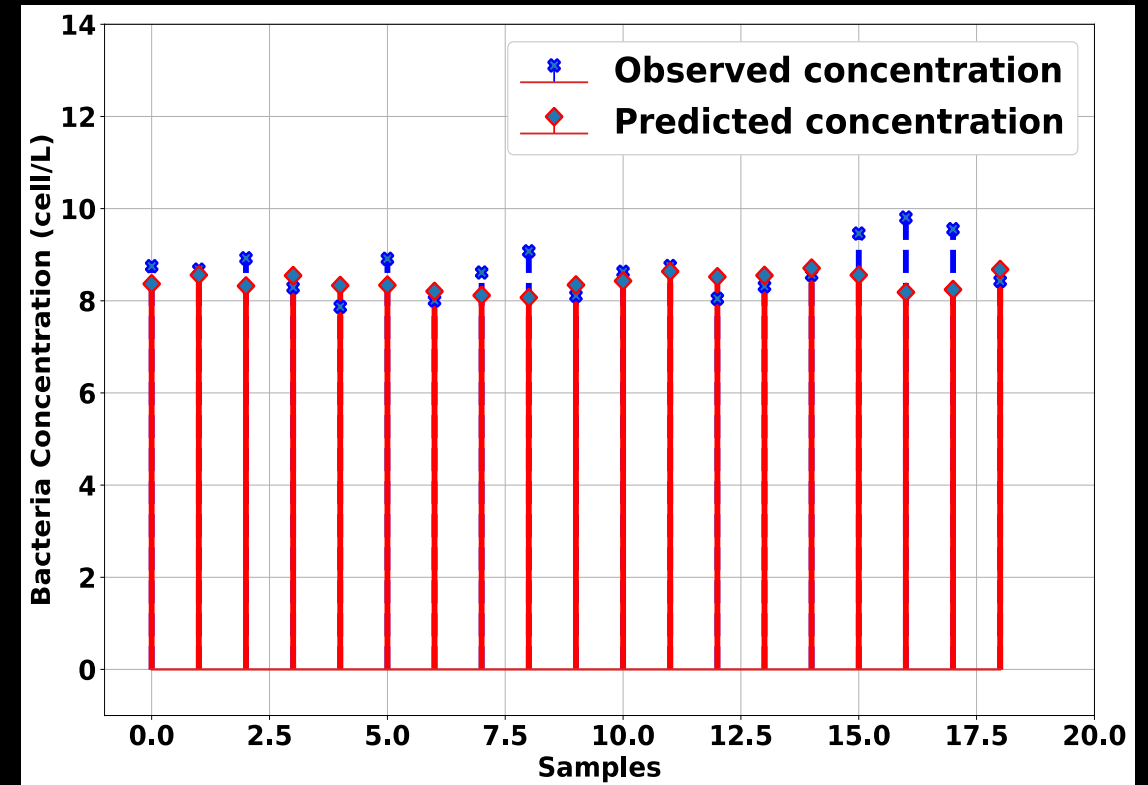


Prediction results for bacterial cell concentrations in KAUST WWTP

Influent



Effluent



Implications (Part 1)

- Utilize parameters already available from online sensors present in wastewater treatment plant, input them into model
- And use it to predict cell concentrations at different stages of the wastewater treatment plant
- This allows plant operators to rapidly determine log removal values of their treatment units, thus assessing the treatment efficiency

Work in progress

- The current neural network-based sensing approach will need to be calibrated for different wastewater treatment plants and different water types (e.g. drinking water)
- A neural network-based sensing approach will need to be developed for viral cell density enumeration
- Not able to denote presence of pathogens or fecal contamination events

Goal and study objectives

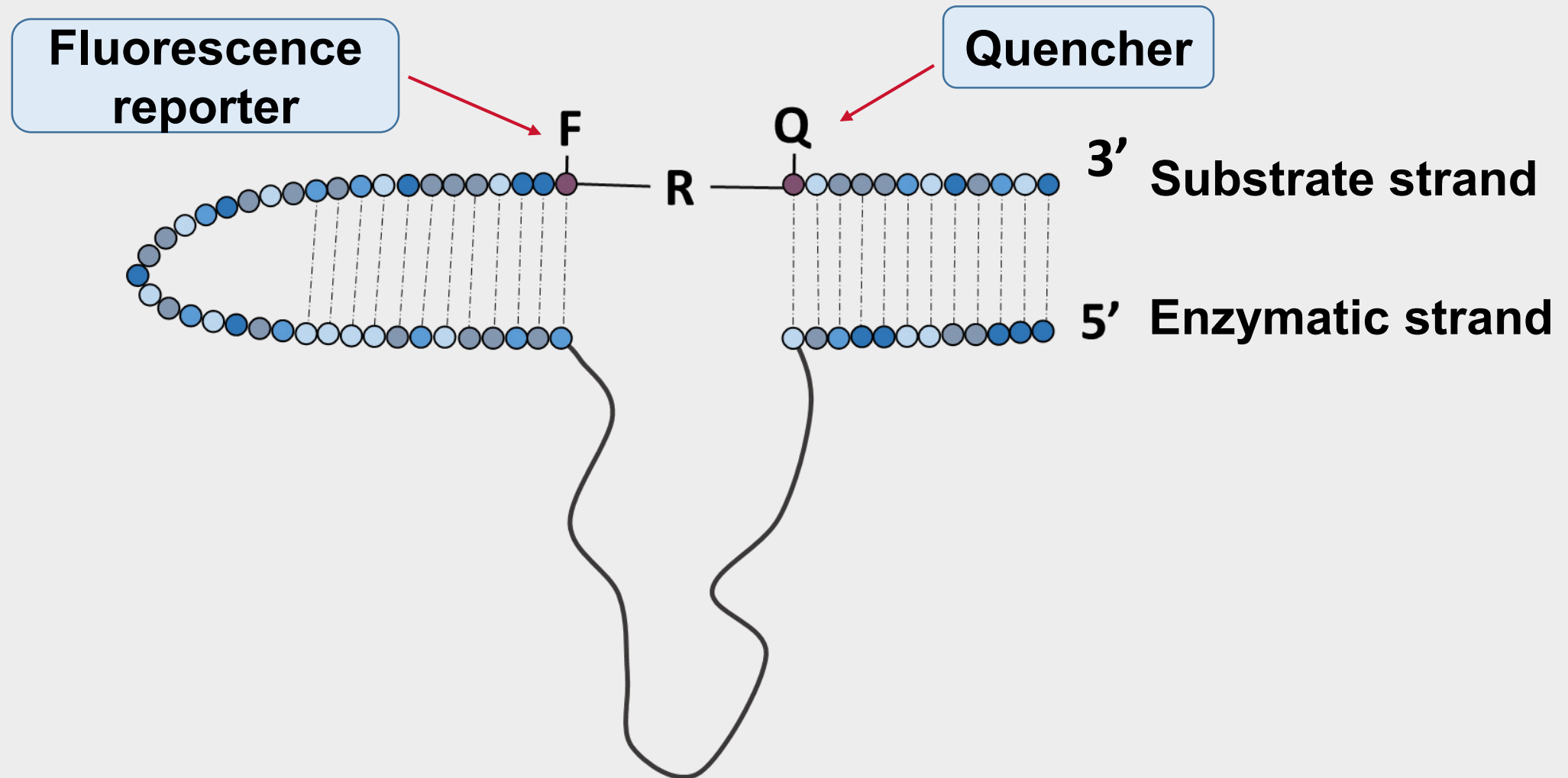
- **Aim:**

To establish continuous/semi-continuous biosensors so as to work towards a smart water system

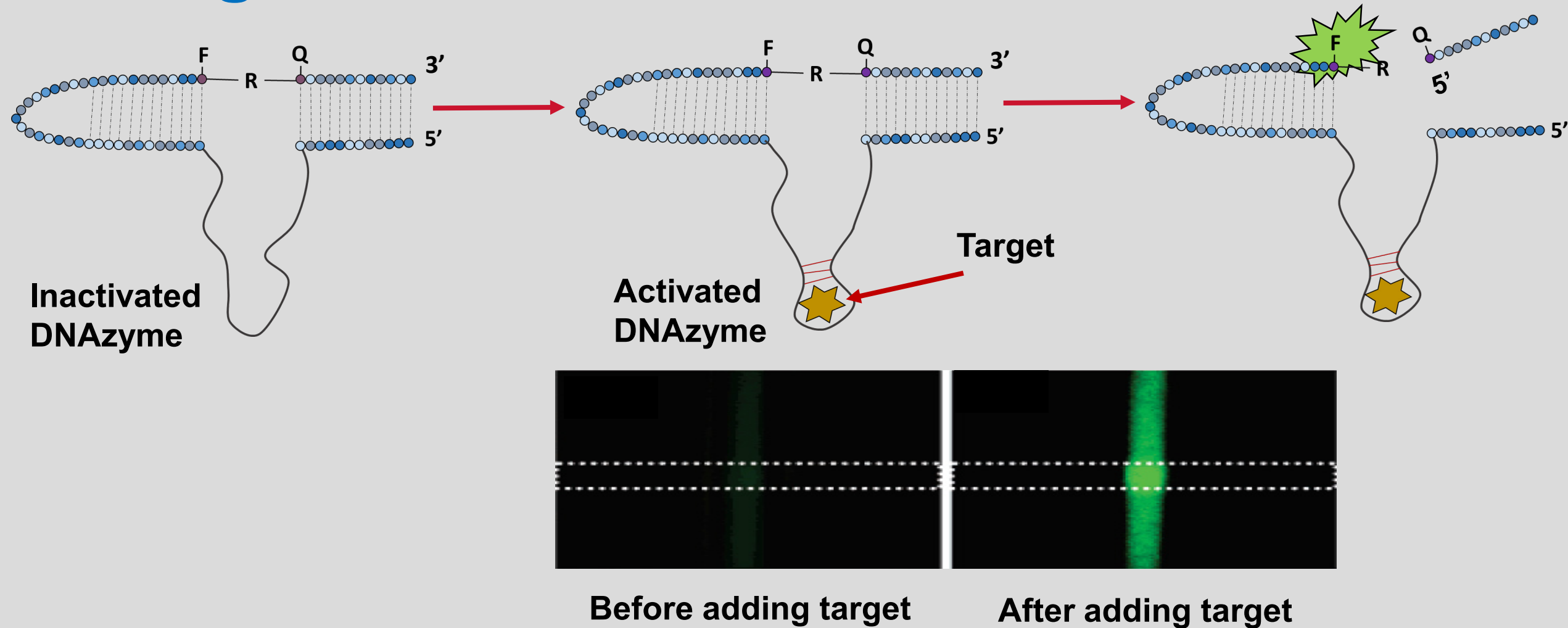
- **Objectives:**

- A neural-network based approach to estimate cell density based on easily measurable parameters
- A DNAzyme-based microfluidics device to determine presence of *E. coli* in water matrices

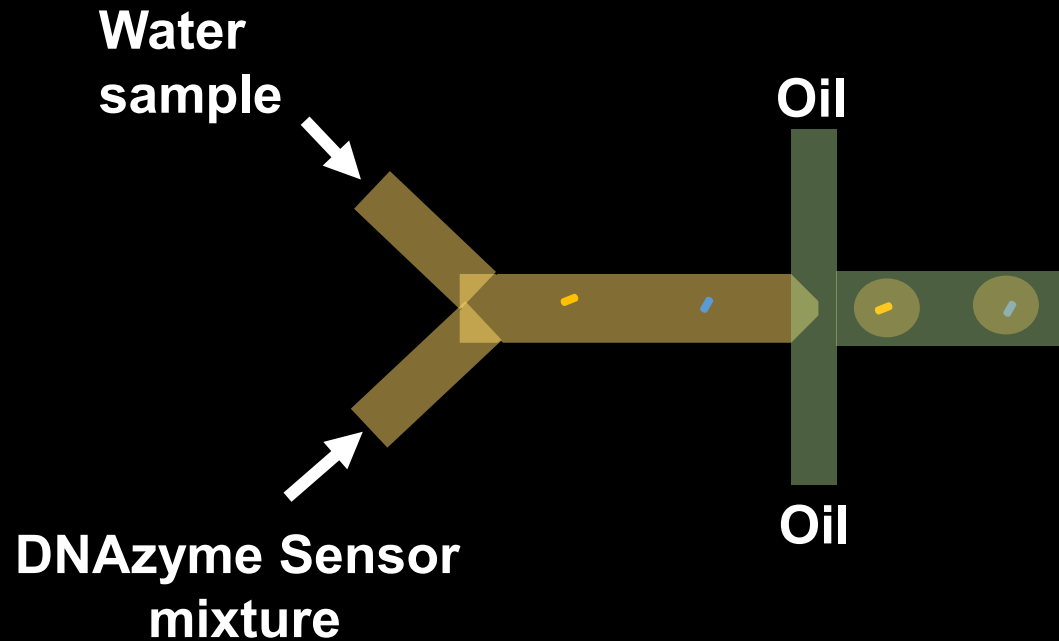
Approach 2: DNzyme-based microfluidic device



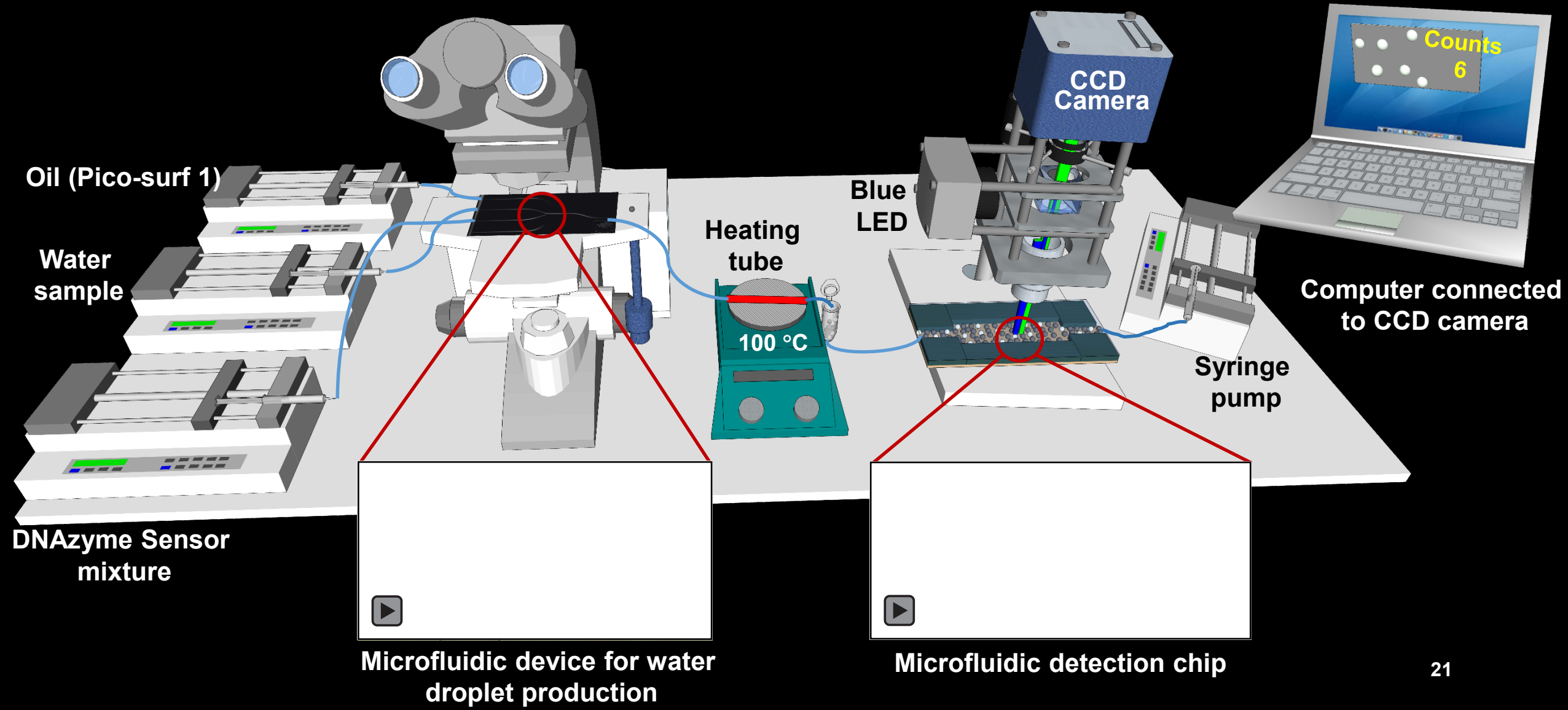
How does DNzyme detect bacterial target?



Coupling it to a microfluidics platform

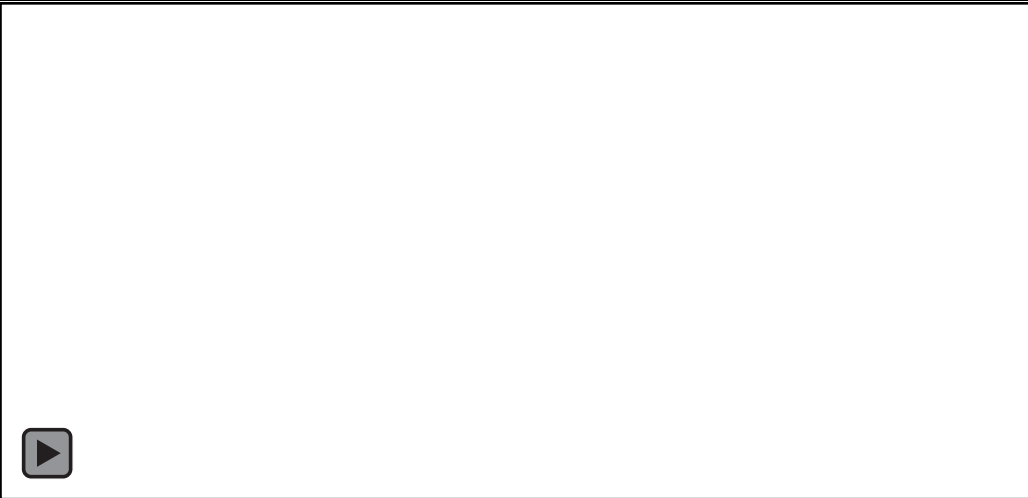


Proof-of-concept for capture system

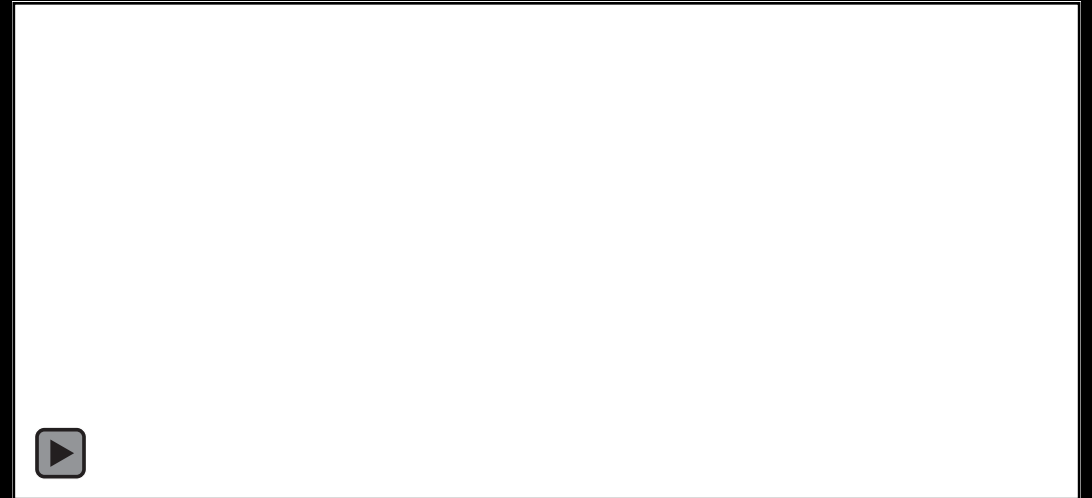


Example of real-time capture results

Without *E. coli*



With *E. coli*



A combination of *E. coli* and non-*E. coli* coliforms, namely *Klebsiella* (EPA 193), *Morganella* (MA-35-Ready-2), and *Serratia* (EPA 74) was used to test the specificity. Each bacteria was grown under the same conditions.

Fluorescence droplet counting using image processing and deep learning

https://youtu.be/PbLhTI_dW7s

Implications (Part 2)

- DNAzyme-based sensor is able to identify *E. coli* (the golden standard for fecal contamination) in a specific manner
- By further coupling with microfluidics-based online system and image recognition software, we demonstrated proof-of-concept to monitor for *E. coli* in a continuous manner

Work in progress

- Tentative limit of detection (LOD) of this entire system is estimated at 100 bead particles per 50 μL of volume (the volume sorted through our system).
- Further improvements to the detection sensitivity needed:
 - Improving the image recognition software and sorting rates,
 - Developing sampling processing step that involves the concentration of large water volumes to the 50 μL needed to sort through the system
- Expand it to facilitate direct detection of other types of pathogens

Take-home messages

- A neural-network based approach to estimate cell density in wastewater matrices based on easily measurable parameters (e.g. turbidity, conductivity, chemical oxygen demand)
- A DNAzyme-based microfluidics device to determine presence of *E. coli* in water matrices, thus providing an immediate indication on water quality
- Both facilitate attempts to work towards a smart water system

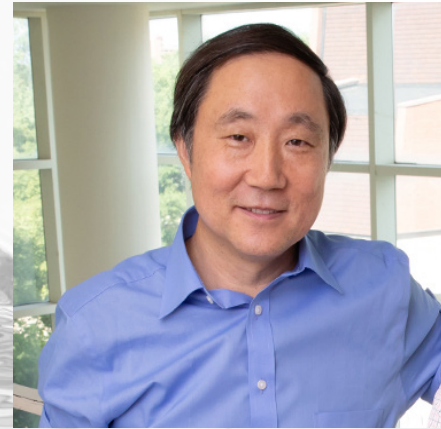
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Chemistry, U Texas at Austin



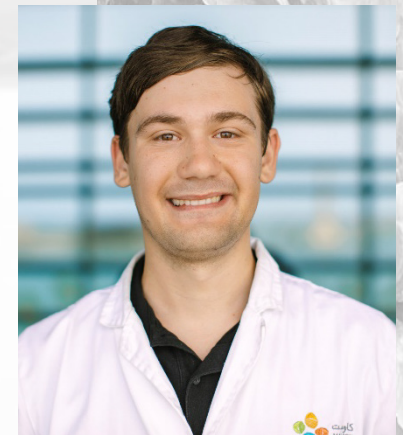
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