

Low burden sensors for in-field detection of water contaminants based on synthetic biology and cell-free expression systems

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Abstract

The ability to detect harmful contaminants in water is critical for health and safety in both field-forward and resource-poor environments. Microorganisms have naturally devised mechanisms for sensing and responding to a variety of analytes in the environment, and this molecular machinery can be exploited and further engineered via synthetic biology to generate sensors against a multitude of threats. Despite the potential, deployment of these sensors remains a challenge. Sensors based on cell-free expression (CFE) systems, however, overcome many of the limitations of whole-cell sensors, enable detection of contaminants toxic to living systems, and hold great promise for providing rapid, in-field detection capabilities. CFE systems can be freeze-dried onto paper substrates, and following rehydration at the point of need, a measurable signal is produced in response to the target analyte. These paper-based sensors are low size, weight, power, and cost (SWaP-C), disposable, simple to use, and multiplexable.

Here, we present a tri-service effort to develop CFE sensors to detect the toxic water contaminants arsenic, cadmium, and mercury. Under laboratory conditions, all three sensors can detect heavy metals at or below Military Exposure Guideline (MEG) levels, and the arsenic and mercury sensors achieve detection limits below the World Health Organization (WHO) recommended levels within 30 minutes. The sensors were subsequently tested and optimized for field-relevant conditions enabling detection at target concentrations in spiked environmental water. Current efforts aim to further develop and manufacture these sensors and continue to push the technology into the field. A key feature of the underlying biotechnology presented here is its adaptability to other sensor and detection modalities enabling sensing of other chemical and biological threats, including pathogens, proteins, small molecules, and toxins. In the future, this approach could be expanded to detect a wider range of contaminants and allow for simultaneous in-field detection of multiple water hazards in a low SWaP-C package.

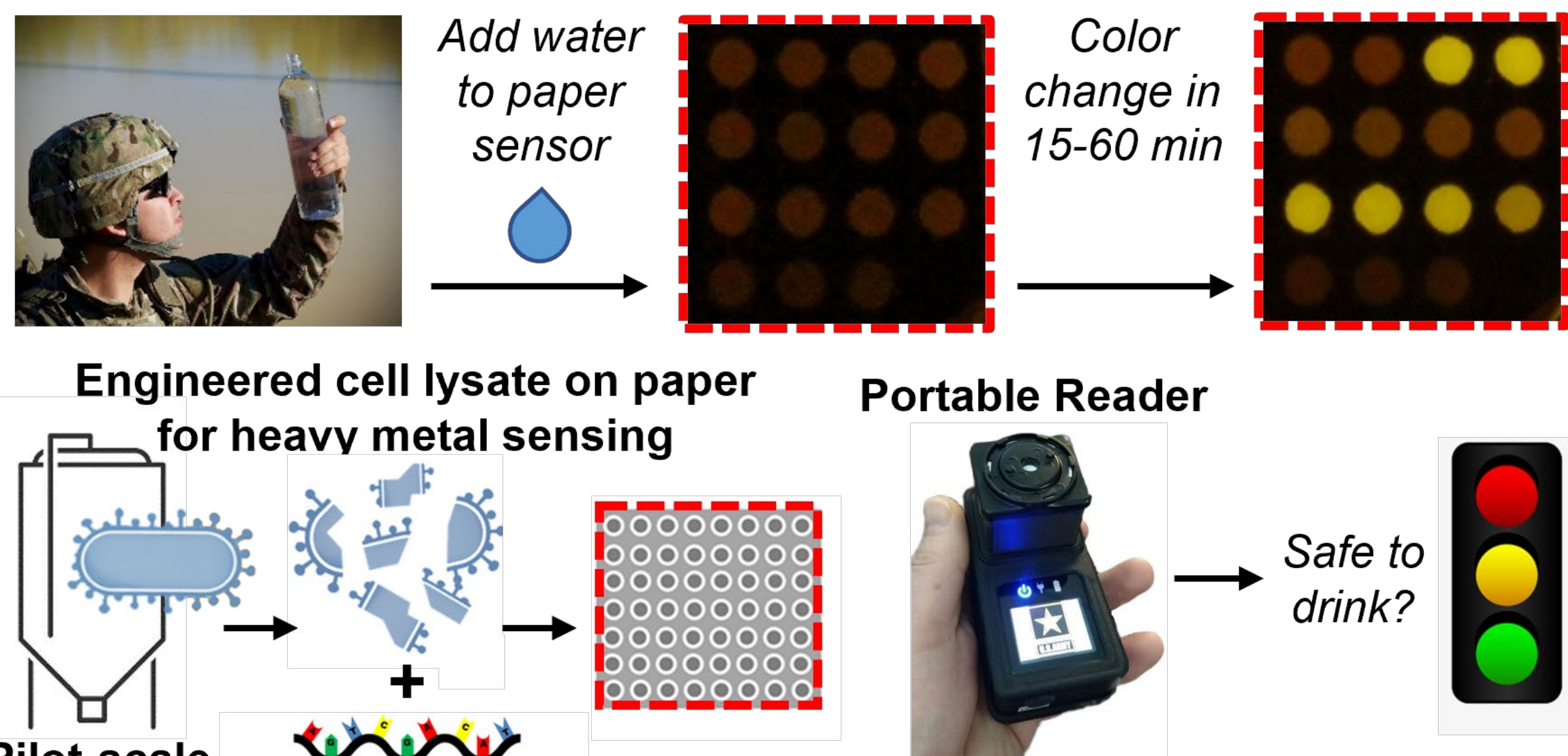


Figure 1. Overview of heavy metal detection using paper-based, cell-free sensors.



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Cell-Free Heavy Metal Sensors

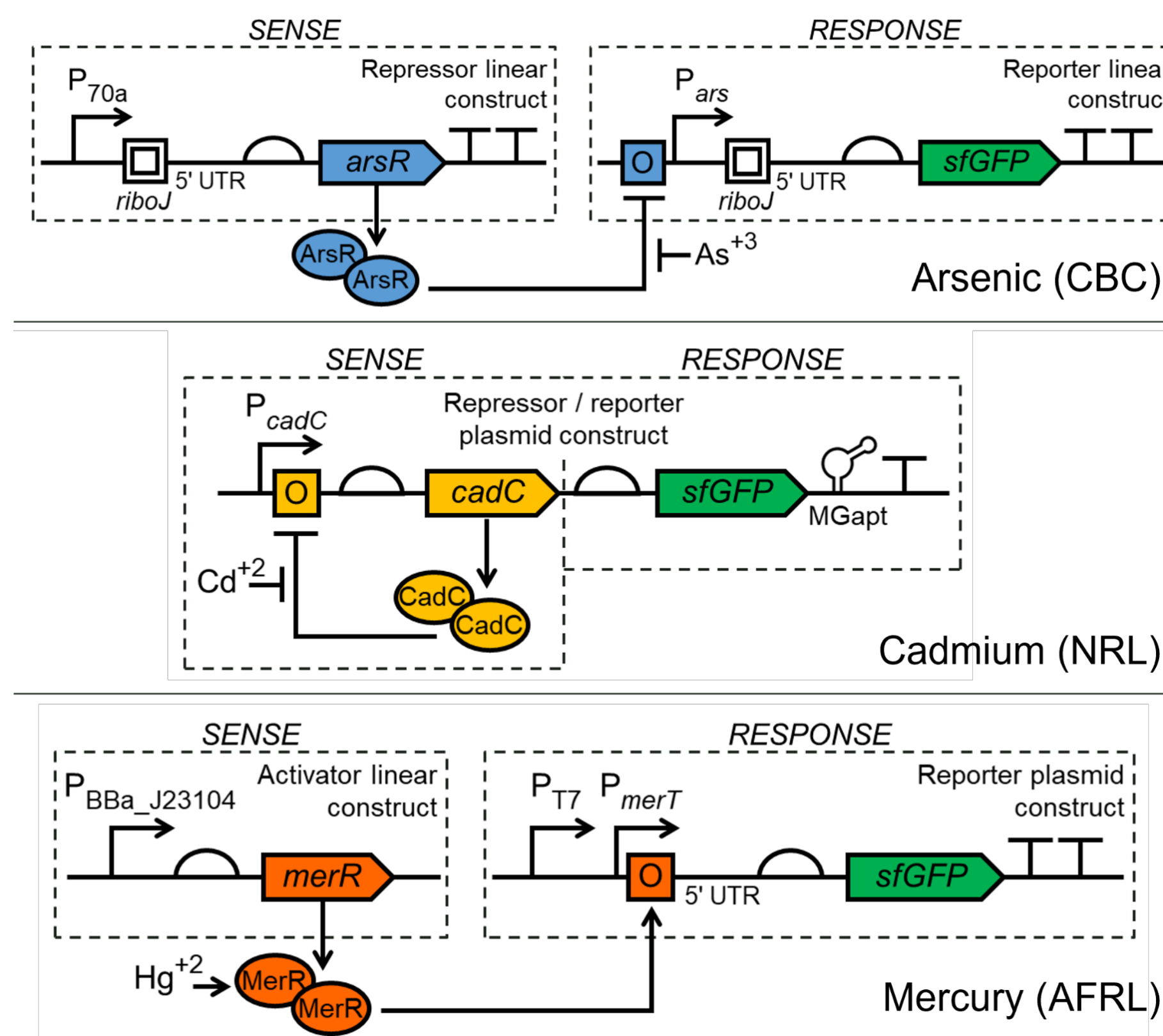


Figure 2. Heavy metal-responsive circuits for cell-free expression. We designed biosensors to detect the toxic heavy metal contaminants arsenic, cadmium, and mercury. The developed sensors utilize engineered genetic circuits consisting of bacterial transcription factors and regulatory elements that are naturally responsive to heavy metals, paired with reporter proteins that produce a fluorescence signal in response to the target analyte.

Table 1. Cell-free sensors detect heavy metals at relevant levels. Military Exposure Guidelines (MEG) and World Health Organization (WHO) guidelines for hazardous levels of heavy metal contamination in drinking water are shown along with the limit of detection (LOD) for each cell-free heavy metal sensor. Highlighted values are the target MEG concentrations for detection under this effort. Under laboratory conditions, all three sensors can detect heavy metals at or below MEG target levels within 30 min. Additionally, the arsenic and mercury sensors achieve detection limits below the WHO recommended levels.

Water Quality Standard	Elemental Arsenic		Elemental Cadmium		Mercury Chloride	
	µg/L	µM	µg/L	µM	µg/L	µM
MEG (7 Days, 5 L/Day)	300	4	56	0.5	—	—
MEG (1 Year, 5 L/Day)	60	0.8	7	0.062	42	0.155
WHO Guideline	10	0.133	3	0.027	6	0.022
Cell-Free Sensor LOD	9.4	0.125	56	0.5	3	0.011

Detection of Arsenic in Environmental Water

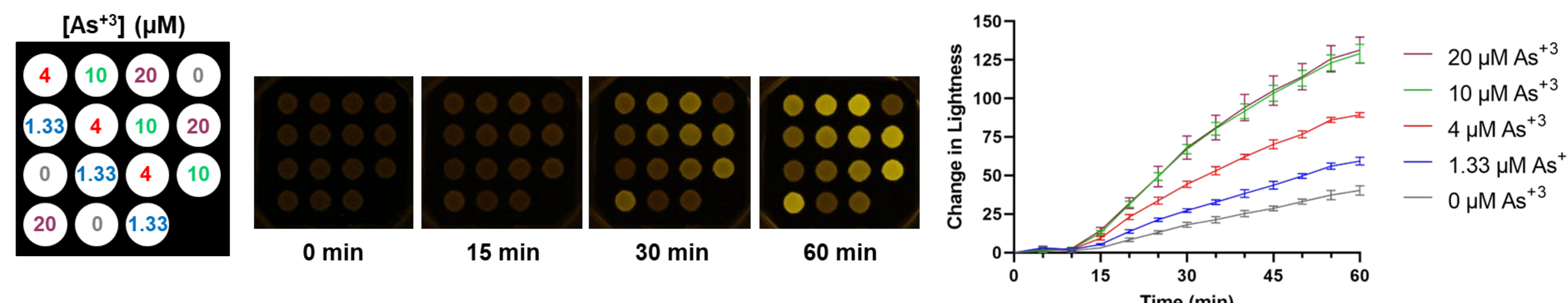


Figure 3. Arsenic is detected below target level in environmental water. Lyophilized reactions on paper were rehydrated with environmental water spiked with arsenic. Imaging was performed with a fluorescent camera rig and quantified with in-house software. 1.33 µM (100 µg/L) is detected in 15 min. Limit of detection is 0.8 µM (60 µg/L) in 35 min (data not shown). Target MEG = 4 µM (300 µg/L).

Arsenic Sensor Device and Optical Reader

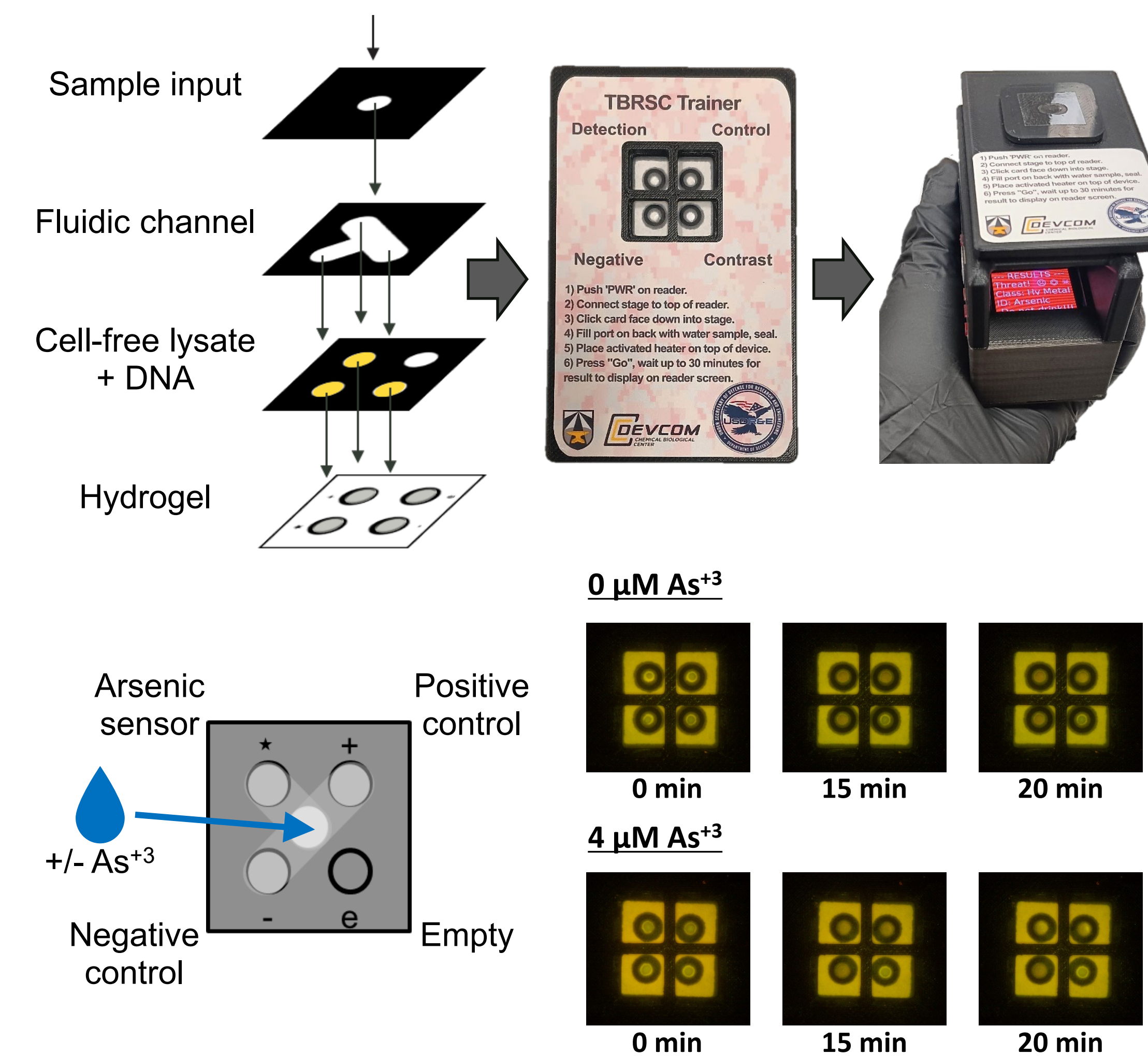


Figure 4. Paperfluidic device and integration with optical reader. To improve fieldability and ease of use, cell-free sensors were moved to a paperfluidic device where a water sample can be added to a single port and flow through paper layers to rehydrate lyophilized reaction spots (Left). The 3D-printed device that houses the paper layers (Middle) connects to the MACAW optical reader for image analysis and decision making (Right).

Figure 5. Arsenic is detected at target level with paperfluidic device and optical reader. Water containing 0 µM (Top) or 4 µM (MEG; Bottom) arsenic was added to arsenic sensor devices. Images were captured with the MACAW reader. Arsenic is detected at the target MEG level in 15-30 min.

Future Work

Under the current Tri-Service Biotechnology for a Resilient Supply Chain (T-BRSC) program, this project aims to develop:

- 1) A drop-in replacement for an existing COTS arsenic sensor fielded by the DoD
- 2) A wholly novel version that will detect mercury and cadmium in addition to arsenic on the same device

Future plans of this effort include:

- Optimization for shelf-stability and manufacture of sensors by industry
- Demonstrations in simulated operational environments
- Integrated heating
- Semi-quantitative sensing
- Expanding cell-free sensing platform to detect more water contaminants of concern (e.g., TIC/TIMs, toxins)

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