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An integrated microfluidic sensor for real-time detection of RNA in seawater using preserved reagents

M.-N. Tsaloglou (1), C.M. Loukas (1), J.M. Ruano-López (2), H. Morgan (3), and M.C. Mowlem (1)

(1) Sensors Development, Ocean Technology and Engineering Group, Natural Environment Research Council, National Oceanography Centre, Southampton, SO14 3ZH, UK., (2) IKERLAN J. M. Arizmendiarrieta, 2, 20500 Arrasate-Mondragón, Guipuzcoa, Spain., (3) School of Electronics and Computer Science, University of Southampton, SO17 1BJ, UK.

Quantitation of RNA sequences coding either for key metabolic proteins or highly conserved ribosomal subunits can provide insight on cell abundance, speciation and viability. Nucleic sequence-based amplification (NASBA) is an isothermal alternative to traditional nucleic acid amplification methods, such as quantitative PCR. We present here an integrated microfluidic sensor for cell concentration and lysis, RNA extraction/purification and quantitative RNA detection for environmental applications.

The portable system uses pre-loaded reagents, stored as a gel on a disposable microfluidic cartridge, which is manufactured using low-cost injection moulding. The NASBA reaction is monitored real-time using a bespoke control unit which includes: an external fluorescence detector, three peristaltic micro-pumps, two heaters and temperature sensors, a battery, seven pin actuated micro-motors (or valve actuators), and an automatic cartridge insertion mechanism. The system has USB connectivity and none of the expensive components require replacing between reactions.

Long-term storage of reagents is critically important for any diagnostic tool that will be used in the field, whether for medical or environmental analysis and has not been previously demonstrated for NASBA reagents on-chip. We have shown effective amplification, for as little as 500 cells of the toxic microalga Karenia brevis using reagents which had been preserved as a gel for 45 days. This is the first reported real-time isothermal RNA amplification using with on-chip preservation. Annealing of primers, amplification at 41 °C and real-time fluorescence detection using, also for the first time, an internal control and sequence-specific molecular beacons was all performed on our microfluidic sensor. Our results show excellent promise as a future quantitative tool of in situ phytoplankton analysis and other environmental applications, where long-term reagent storage and low power consumption is essential.