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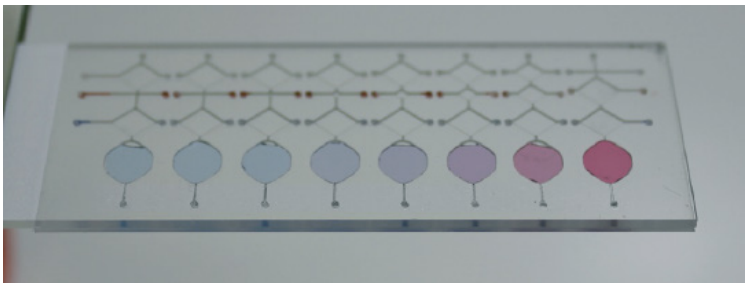
A New Dilution Tool to Facilitate High-Throughput Assay Techniques

A new tool provides a quick and easy way to dilute samples for biochemical and biological analyses. The microfluidic dilution generator, developed by researchers led by Tingrui Pan, Ph.D., at the University of California, Davis Superfund Research Program Center, can serve as a simple dilution device in research laboratories, point-of-care clinical settings, and low-resource environments.

Generating a series of dilutions is a routine practice in labs and a gold standard to obtain quantitative readings for a variety of biological and biochemical assays. For example, serial dilutions are used to dilute blood serum to determine the exact amount of proteins and antibodies, develop standard curves to detect and quantify anything from environmental chemicals to DNA, and create dose-response curves based on enzyme activity.

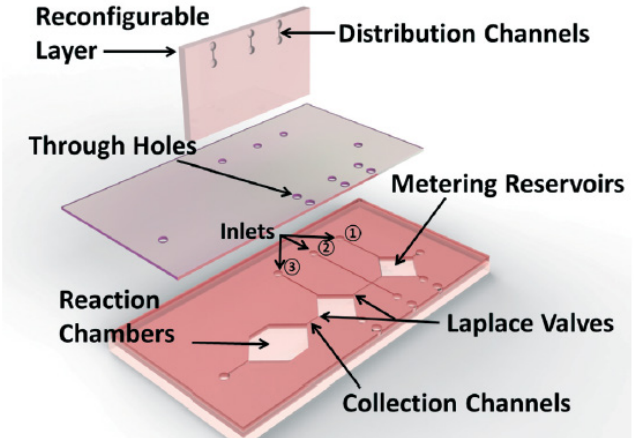


A serial dilution characterized using red dye as the sample, deionized water as the diluent, and blue dye as the detection reagent for a visual demonstration of the device. (Image reproduced from Fan et. al. 2015 with permission of The Royal Society of Chemistry)

To date, standard dilutions rely heavily on manual dilution or expensive robotic automation. The new tool developed by Pan and his team provides a simple way to produce serial dilutions without the need for expensive tools or highly trained expertise.

Device Design

The microfluidic dilution device consists of three layers (as shown in the figure). The bottom layer contains metering reservoirs for the sample, liquid to dilute the sample, also called the diluent, and any reagents that need to be added in equal amounts to the serial dilution. The middle layer contains a through-hole membrane with horizontal channels that connect the reservoirs in the bottom layer. The top layer is a reconfigurable layer for valve switching. The device's flexible design allows the user to create linear, logarithmic, or arbitrary dilution profiles by adjusting the dimensions of the reservoirs.

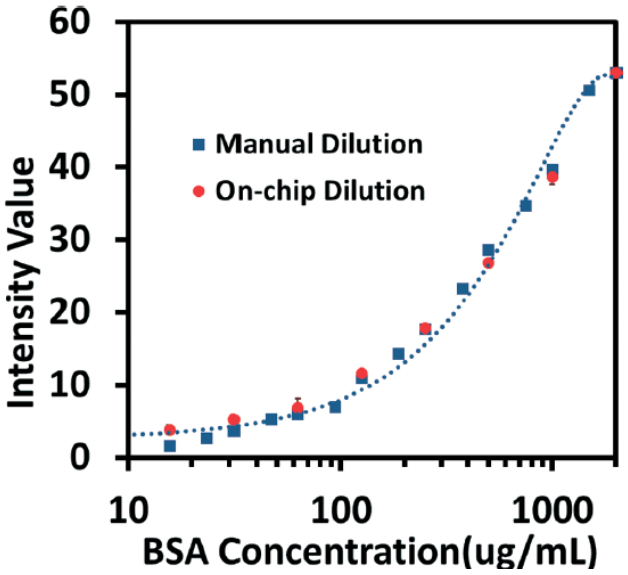


The figure above shows the structural assembly of one dilution unit in the device. The numbered inlets indicate the entry point for the diluent (1), sample (2), and reagent (3). (Image reproduced from Fan et. al. 2015 with permission of The Royal Society of Chemistry)

Only three steps are needed to create dilutions, which can be applied in a wide variety of biological and biochemical assays for high-throughput and quantitative outcomes. In the first step, a pipette is used to load the sample, diluent, and reagent into the inlets, from where they move horizontally through the distribution channels and fill all reservoirs. The device is then reconfigured by manually removing the top reconfigurable layer, separating each horizontal distribution channel and leaving only the vertical passages connecting the sample, diluent, and reagent. Finally, a pipette is used to vertically pull the sample, diluent, and reagent into the reaction chambers, mixing them together. For a demonstration of the dilution device, see the supplementary video provided by the authors.

A Simple Alternative

The device can easily produce discrete concentration profiles ranging from 1 to 100-fold dilution from a fixed small sample volume of 10 microliters. Although several approaches that dilute samples using a diffusive method are emerging, all of them require a pump or vacuum, which is often unavailable in clinics or point-of-care settings and may require higher volumes of samples. This device reduces the time required and is completely compatible with standard biological protocols for a wide variety of quantitative assays in laboratories, clinical settings, and in-field testing. Requiring limited steps, this device also makes it easy to use for personnel without extensive training and complicated equipment.



Researchers tested the device with the commonly used quantitative bicinchoninic acid assay, which quantifies protein concentrations in human tissue extracts. They generated a standard curve for bovine serum albumin using this chip and verified the concentrations of each dilution by measuring color intensity. They found that the on-chip dilution, using their new device, produced a very similar serial logarithmic dilution compared to the manual dilution method. (Image reproduced from Fan et. al. 2015 with permission of The Royal Society of Chemistry)

For more information, contact:

Tingrui Pan, Ph.D.
University of California, Davis
Department of Biomedical Engineering
Davis, CA 95616
Tel: 530-754-9508
Email: tingrui@ucdavis.edu

To learn more about this research, please refer to the following source:

Fan J, Li B, Xing S, Pan T. 2015. Reconfigurable microfluidic dilution for high-throughput quantitative assays. Lab Chip 15(12):2670-2679. doi: 10.1039/C5LC00432B.