A VERSATILE MACRO-TO-MICRO DISPENSING SYSTEM
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Abstract
Presentation of a system for rapid transfer of different liquids in the µL- and nL-range from a microplate to a micro-device (in this case a Gyrolab Bioaffy™ CD) using a flow-through dispenser.

Keywords: Dispenser, CD, interfacing, macro-to-micro

1. Introduction
Rapid transfer of one liquid to multiple positions on a chip have been demonstrated [1] in many systems, but few allow rapid dispensing of different liquids into different microstructures as required when performing multiple assays. A CD microlaboratory format (Gyros AB) has been developed that permits parallel processing of up to 384 different samples [2]. Such a high degree of parallelism is possible since there is no need for connectors to the CD. However, this generates the need for new tools that can rapidly transfer multiple samples from the macroworld into microstructures within the CD. Here we present a system that allows rapid, contact-free dispensing of a wide range of sample and reagent volumes into a microfluidic CD.

2. Experimental
The basic system configuration is depicted in Figure 1 and 2. This dispensing system contains one flow-through dispenser. The dispenser is directed upwards and is static while the CD and the MTP are mobile. A capillary, 2 cm long with 250 µm inner diameter, is connected to the inlet of the dispenser. A syringe-pump and a vacuum system connected to the outlet are used to prime the dispenser and aspirate the samples and washing solutions. Approximately 500 nL volumes of a liquid were dispensed for carry-over and minimal filling experiments. In these experiments the aspiration time was identical for the samples, blank and washing solution. A fluorescently-labeled antibody (in 15 mM PBS, 0.01% Tween) was dispensed into one CD microstructure followed by filling with a blank solution. When the microstructures were filled a spin program was executed and the liquid was volume defined to 200 nL and spun through the reverse phase, nanoliter scale column in the structure. A laser-induced fluorescence detector (in a Gyrolab™ Workstation, Gyros AB) was used for detection. Washing solution (15 mM PBS, 0.05% Tween) was aspirated through the dispenser after every application. A myoglobin sandwich-based immunoassay was performed in a Gyrolab Bioaffy™ CD using the dispenser to apply samples and reagents. This assay concept has been described previously [3].
3. Results and discussion
A single flow-through dispenser was used to load 48 structures with 500 nL of different samples within 5 minutes with a carry-over of less than 1/10000 (Figure 3). No contamination was detected when the dispenser system was used to perform a myoglobin sandwich-based immunoassay (Figure 4). Dispensing of solutions containing 0.01% Tween and 1% bovine serum albumin (BSA) confirmed compatibility with different additives. In this system approximately 7-8 μL of washing solution, at 2.5 seconds aspiration intervals, are necessary to ensure minimal carry over.

Other system configurations can be designed in order to reduce the total volume required for sample application. Microfluidic simulation, using multiphysics finite element analysis software, FEMLAB®, and practical experimentation where samples were pumped by dispensing confirmed this possibility. Using this pumping by dispensing required 3 μL total liquid in order to dispense 500 nL (Figure 5). Reduction of the dispenser’s internal volume will further reduce the volume needed [4].

4. Conclusion
A rapid, versatile macro-to-micro dispensing system using a flow-through dispenser has been demonstrated. Several dispensers used in parallel would provide a macro-to-micro interface that can load 384 microstructures in a CD with different samples within one minute and with a cross contamination lower than 1 per 10000.

References
Figure 1. Schematic of the dispensing system.

Figure 2. Photograph of the system.
Figure 3 on the left. Carry-over during dispensing of labeled antibody and blank solutions versus liquid aspiration time (vacuum -15 mbar). Black bars represent blank controls and gray bars represent labeled antibody solutions (4, 21 and 85 nM).

Figure 4 on the right. Calibration curve for myoglobin sandwich assay performed on a Gyrolab Bioaffy CD (blank controls (n=3) are at 1 on the x-axis).

Figure 5. Minimum volume needed for a sample to fill the dispenser using the dispensing principle. Note that the total filling volume including the dispenser and its inlet capillary is approx. 1.5 µL.