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Concepts for Micro analysis in Diagnostics. The Influence of Detrimental Volumes on Bio-Analytics and Technological Solutions to Minimize Volume Effects

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The Influence of Detrimental Volumes
on Bio-Analytics and Technological Solutions
to Minimize Volume Effects



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Introduction

This work is about a modular microsystem for use in analytical applications. The modular construction kit as proposed in [MENZ95] is a basic approach for modularization of microsystems and its transferability to fluidic analysis systems will be evaluated regarding efficiency and reproducibility. This should result in a user-friendly concept for daily use.

Due to the practical orientation of this work, not only preciseness but also reliability, efficiency and practicality are aimed at. Keeping the strict requirements of high-standard analysis in mind, the focus of the work is on the function of the analysis rather than on the system itself.

Several micro analysis systems have been presented previously. Furthermore, the first micro analysis system for medical use is already commercially available, the I-Stat by Agilent. It can measure 7 blood values automatically and must be considered a real micro-system. For several reasons, the moment has come for microsystem-technology in health care:

- ✗ public health is becoming too expensive. To save costs, the number of patient stays in hospital must be reduced, home stays with private care will further be propagated. A mobile health watch system can provide the safety required to permit the patient to stay at home. The most important patient data will be surveyed automatically, informing the physician in charge while the patient can stay in his familiar surroundings;
- ✗ many homogeneous antibody assays (HAA) for diagnosis are available; those HAA are suitable for microsystems, since no washing step is necessary [LOT98], making the procedure easy;
- ✗ biochips and genechips have started to be offered commercially [KLAP01] and some of them can be combined with a fully integrated micro liquid measuring system.

The problem that all micro total analysis systems share to date is the diversity of tasks to be fulfilled: the fluid must be aspirated, spread, mixed, applied to the sensing facility, analyzed and discarded. This is called the **fluid delivery**.

The amount of components needed to fulfill those tasks is considerable, and each component can fail its service. In addition to bad reliability, the overall system volume and dead volumes of complex systems are large and hence the danger of cross-contamination is high. Moreover, interfaces are difficult to achieve and create additional dead volume. It will be shown in this work that for analysis reasons, **dead volume** and **overall system volume** are crucial points in analysis, as they can cause cross-contamination.

A very important issue in re-usable systems is the **prevention of cross-contamination**. Therefore, thorough rinsing must be guaranteed. As long as the concentration of a functional group after rinsing is only some orders order of magnitude smaller than its natural concentration, a false diagnosis is

probable. This is called the dynamic range of the detection limit. For tumor markers, the reduction must be $R=10^{-7}$, other common markers even require a factor of $R=10^{-9}$ [GREN02].

To be commercially successful, a point-of-care system must be precise and reliable as mentioned before, but also has to be highly rugged and applicable for everyday use. Redundancy will increase reliability, while taking its toll on system costs. However, only a low system price will finally lead to commercial success.

The problems encountered by recent approaches can be attributed to the adoption of macroscopic concepts to the micro-world by miniaturization. In existing micro analysis systems, complex constructions were built up to fulfill the very simple task of doing an analysis inside an analysis chamber. In this work, the logic behind such constructions is reconsidered in order to avoid the need for constructing complex systems. Hence, the point is to remove the cause but not the symptoms [FURT75]. Not the change of interfaces or components is sought but a solution, which is convincing due to its simplicity. A visionary concept was looked for, providing minimized complexity but full functionality. As the right question is often the most important step to the answer, the question was quickly put into words:

If only the analysis chamber is needed for the analysis itself, how can such an analysis chamber be designed to perform the sub-task of fluid delivery by itself?

To show the importance of a new concept, first the amount of dead volumes and their influence on analysis in standard fluidic microsystems are shown using simulations with exemplary devices. It is shown in this work, that for bio-analytical analysis, the disadvantages of complex systems threaten their application in this field. While complexity may not lead to a successful system, a system with maximum simplicity is developed in this work and called the Capillary Force Filled Mixer. This solution entails replacement of the whole system by a single component, which can be produced in plastics and is very cheap. The new device is introduced, simulated and tested.

In this investigation, the word 'micro' is used with great care. In most publications, fluid components are called 'micro', when micro-fabrication technologies are used for their fabrication. Their dimensions, however, are absolutely 'macro' and therefore they can not really be called micro-systems. Those components can be defined as tools for micro-fluidics and therefore make way for the understanding of the latter. A 'micro-pump' measuring more than 10 mm x 10 mm x 1 mm is much smaller than conventional pumps with the same pump rate and maximum pressure. Using the term "micro components" thus seems misleading. The author prefers the definition in which a technology receives the attribute 'micro', when at least two dimensions are in the micrometer-range. In fact, it should rather be three dimensions than two. This work about micro-systems will avoid the word 'micro' unless it is necessary for the understanding.

1 A Microsystem for Standard Analytical Applications

In this study, a microsystem for mobile or laboratory analysis using homogeneous assays for spectroscopic detection is developed. The detection facility is separated from the analysis component, which is designed regarding maximum simplicity and best usability. Both the fields of application as well as the type of analysis are perfectly suitable to a microsystem, and are explained using the following focuses:

- ✗ analysis: point of care testing and laboratory testing using homogeneous assays
- ✗ modularization and system complexity
- ✗ detection limits due to miniaturization
- ✗ miniaturized fluid volumes and reagent costs
- ✗ separated analysis and detection units

1.1 Point of Care Testing and Laboratory Testing

Miniaturization of all components may not be the appropriate way for the development of microsystems analysis, if maximum sensitivity or mobility is required. Two main areas of application have to be distinguished: mobile (point of care testing, POCT) or laboratory use. The main characteristics, which those systems have to provide, can be seen in table 1.1. Both share the need for reliability but show very different demands with regard to required features.

Throughput is only necessary for laboratory use, where there is no need for miniaturization of size and weight. Volumes should be kept small for both fields of applications, but for different reasons: whereas in POCT only the fast reaction times are of interest, expensive reagents are of major concern in laboratories. Reducing performance against tough handling is a means to reduce running costs of laboratory facilities. In fact, ruggedness against failure rather than against mistreatment is asked for. Investment in analysis apparatus can be a lot higher than in mobile use because of its frequent and permanent use. Laboratory personnel are professionals, they are used to sophisticated devices (nonetheless, fail-safe systems are welcome). Precision is an important feature.

POCT is very often used for vital tests or for summary checks, thus lower precision may suffice. In contrast to laboratory personnel, those using mobile analysis are in general not professionals and use it as a tool without background knowledge. All systems are supposed to be small and light. POCT systems perform only a few tests per day, the consumer application requires low system costs.

For hospital use, POCT systems will play an important role in the future. In this area, the characteristic use is both, mobile as well as laboratory. Although the personnel has received expert training, they are still not analysis professionals. The systems should be small because of the lack of space, but not to the detriment of performance.

	Point of Care Systems		Laboratory Systems	
size and weight	***	mobile use		
fast results (minutes range)	***	decision base in emergency situations	**	low personnel costs
ruggedness	***	no pre-analysis and mobile use	*	professional pre-analysis
systems costs	**	a few tests per day / lifetime	*	a lot of tests per day / life cycle
small volumes	***	small reaction times	***	economic use of reagents (costs)
prevention of maloperation	***	non-professional mobile use	*	professional use
prevention of malfunction	***	protection against false medication	***	costs of down-time
comfort	**	non-professional mobile use	*	professional use
precision	*	for emergency situations	***	precise values for diagnosis
throughput			***	time is money

table 1.1: Features required by POC and laboratory systems. Rating: from *** (indispensable) to no star (unnecessary).

New investigations show a side effect of inexpensive POCT: physicians tend to perform more tests than necessary, requiring even cheaper tests.

1.1.1 Clinical Diagnostics by Biochemical Analysis

The general use of analytic testing in diagnostics is to find ingredients, which are distributed statistically in a sample and to determine their concentration. To fulfill this task, the ingredient can be detected directly (NMR, IR-spectroscopy, chromatography, direct sensors, etc.) or indirectly by using an additional reagent, which reacts with the ingredient and can be detected spectroscopically or using chromatography etc. (e.g. genetics, antibody testing and assays). Depending on the method, even single molecules can be found. The large number of substances to be analyzed has stimulated various detection methods optimized for their application. Molecular, antibody and genetic analysis in clinical diagnostics, pharmaceutical research, pollution control, industrial monitoring and process control are just some of the most important and quickly growing economic fields.

Homogeneous assays are standard for most antibody assays or enzyme microsystem assays. The expensive washing step which is needed for inhomogeneous assays is not applied [LOTT98] [ZECK01]. Instead, the markers which are detected by the analyzer become active only after reaction. The detected signal is a direct gauge of the concentration of the substance to be analyzed. In inhomogeneous assays, on the other hand, markers are detectable, both before as well as after binding.