The LabCD™: A Centrifuge-Based Microfluidic Platform for Diagnostics

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ABSTRACT

Diagnostics for point-of-care (POC) and field use requires the integration of fluid processes with means of detection in a user-friendly, portable package. A drawback to the use of many current analyzers for POC and field applications is their reliance on expensive and fragile robotic technology for automation, lack of portability, and incomplete integration of sample processing into the device. As a result, a number of microfluidic technologies are being developed for diagnostics applications outside of central laboratories. We compare several of these technologies with our own preferred centrifugal flow system, the LabCD™, with an emphasis on fluid propulsion. LabCD™ has been developed to perform a variety of fluidic processes necessary in diagnostics while dispensing with traditional pumps and valves. The use of the CD-ROM model provides a natural division of the system into an instrument and a disposable component, each with well-defined functions. The CD format also allows for the use of encoded information to integrate process control, data acquisition, and analysis. Finally, the "solid state" nature of the microfluidics and use of standard manufacturing techniques should yield a low-cost platform.

Keywords: microfabrication, centrifuge, point of care, microfluidics, diagnostics, drug discovery.

1. Introduction

Current trends in diagnostics and drug discovery suggest an explosive growth in the need for chemical detection and compound synthesis and screening. As our understanding of disease pathology continues to increase, the number of species which must be monitored continues to expand. Concurrently, the search for new therapeutics continues to accelerate, currently requiring the synthesis and characterization of hundreds of thousands of candidate compounds in a slow hit-or-miss process to find one with the right ability to bind to a particular biological target.

At the same time that our knowledge of diagnostics and treatment is growing there is continuing pressure to contain health care costs. These two trends are driving diagnostic and drug discovery technologies toward increased automation, integration and miniaturization.

Automation has long been a part of diagnostics. High-throughput clinical analyzers can, for example, measure 300 samples/hour for basic blood chemistry. These large, standalone systems do not usually fully automate or integrate analysis: trained technicians and several steps on different machines are involved. The lack of full automation and integration is not a real disadvantage in these cases: the tremendously high throughput ensures that total costs remain relatively low. There is a growing realization though of the value of decentralized diagnostics. In this scenario, analysis of clinical samples is carried out at or near the point-of-care, for example, in the doctor's office, in the operating suite, or in the emergency room. Current high-throughput systems are inappropriate for the obvious reasons: They require significant sample preparation, skilled users to monitor their function, and are usually large and expensive. It is in this application area we will explore the usage of our miniaturized analytical chemistry platform, the LabCD™.

Automation in drug discovery and synthesis has been significant, but identification, screening and synthesis of potentially useful pharmaceutical compounds remains a daunting, unpredictable and time consuming challenge. Companies like Affymax use miniaturization and parallel processing techniques to produce and screen very large numbers of novel compounds [1].

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LabCD™ can also be used for drug discovery applications, but we have chosen to concentrate on diagnostic applications in the current paper.

For both diagnostics and drug discovery, miniaturization of large parallel arrays of sensors and fluidic systems promise to provide the solution for further progress. Microinstrumentation based on integrating miniaturized fluid handling together with the required sensors has indeed emerged as a better approach to solve chemistry and biology problems. There are several reasons why such an integrated instrument might be preferable to disposable chemical sensors alone. These include overcoming shortcomings of chemical sensors by enabling calibration, separating bound and unbound species, cleaning the detectors, filtering out unwanted compounds and obviating the need for highly selective chemical sensors. In addition to reduction in reagent volumes, basic theory of hydrodynamics and diffusion predicts faster and more efficient chromatographic and electrophoretic separations in miniaturized biomedical and analytical equipment [2]. Performance is expected to increase with miniaturization because of the favorable scaling properties of some important instrument processes (for example, heating and cooling are faster while the effect of diffusion is reduced). Micromachining might also allow co-fabrication of many interconnected functional instrument blocks. Tasks that are now performed in a series of conventional benchtop instruments could then be combined into one unit, reducing labor and risk of sample contamination. And, because microinstruments could potentially be batch fabricated at low cost, they might be used only once and then thrown away to prevent sample contamination.

While micromachined microfluidics has made significant advances, they still suffer from four drawbacks: Unresolved valving and connector problems, high manufacturing costs, and a lack of overall integration which results in the need for significant "up-front" sample processing in most systems. In this paper we will review the centrifuge-based LabCD™ instrument as a microfluidic diagnostic platform as an attractive option. This micro-centrifuge approach will be contrasted with microfluidics based on acoustic, pressure, electro-osmotic, electrowetting and electrohydrodynamic pumping.

2. Generic Challenges for Miniaturized, Integrated Diagnostic Platforms

The generic technical challenges in making a miniature biomedical instrument include: sample introduction (and in some cases, sample preparation); propulsion of fluids, including sample to be analyzed, reagents and wash and calibration fluids, through tiny conduits; valving; mixing fluids when desired and isolating them when they are to be kept separate; temperature control of the fluids; and signal generation and detection. A further challenge is the integration of these functions in as complete a manner as possible, with the ultimate goal of complete automation from the taking of the raw sample through the display and storage of results. Overriding these concerns is the need for cost-effective manufacture.

Beyond answering the technical questions of how to miniaturize an instrument with all these functions, one may ask how to partition all of these functions into disposables and a fixed instrument. At one extreme is the totally integrated option in which there are no boundaries between the disposable and the reader instrument, such as proposed in the micro total analysis system (μ-TAS) approach, promoted mainly in Europe [3]. More realistically, one might look for a division of functions as sketched in Figure 1 [2]. For most micro-instruments, a disposable "cassette" incorporating the specific reagents needed for a set of tests and a separate permanent reader instrument may be a better solution. An important design step is then to determine which functions should be contained within the disposable cassette and which to incorporate in the reader, *i.e.* how to partition the electrical and mechanical functions of instrument and disposable. In the following, we will discuss the technical approaches of the LabCD™ to meeting these challenges.

3. LabCD™ Platform

Introduction

The LabCD™ platform consists of a disposable plastic cartridge (disc) and an associated instrument (reader) (see Figure 2). The primary fluidics technology is propulsion through the application of centrifugal force. Rather than interfacing a chip with an external pump, using integral micromachined pumps, or using electric forces for pumping, the rotation of the disc and the inertia of the fluid contained in it provide the pumping force. Such an approach is inherently "low tech" in its demands on both the manufacture of the fluid structures, which can be molded in conventional fashion from plastic, and

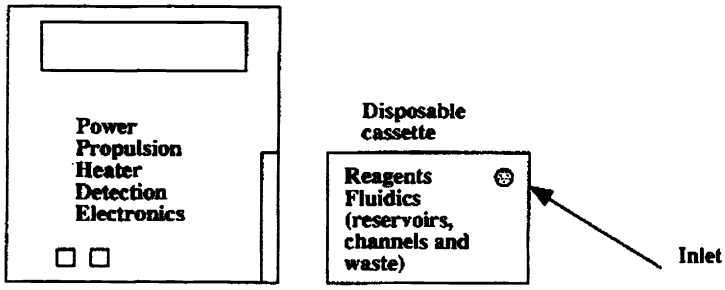


Figure 1. Partitioning of functions between instrument and disposable. Generic microinstrument, with inlet, valves, fluid propulsion, detection, waste, and thermal control. Various options exist for partitioning between cassette and reader [3].

on the reader, which simply needs a power supply, motor, and CPU to control processes. This type of centrifugal pumping was in a larger format implemented in Abaxis's Piccolo™ blood analyzer [4] and in a variety of large-scale clinical analyzers stretching back to the 1970s.

The LabCD™ takes this idea further by using the precise control of flow in small channels to provide a large set of fluidic processes which can be used to automate assays. The fluidics, while the focus of this paper, are only one component of the full system. By also storing CD-ROM information on the disc, a smart disposable is created which incorporates test-specific data, process control parameters and software, and databases of related information. In the future, through writeable CD technology, the system will be able to archive data and enhance information management. Ideally, the CD laser is used both to read this information and to obtain analytical results (*e.g.* through absorption spectropohotometry, light scattering, fluorescence,...). In other applications, independent optical systems will be used for data acquisition.

The partitioning of the system between instrument and disposable is indicated in Table 1 and Figure 2. The disposable consists of the fluid-handling manifold, other special fluidic structures, information and little else. Importantly, the chemistries--reagents necessary for clinical chemistries, antibodies necessary for ELISAs, probes necessary for DNA analysis, etc.--are also contained on the disposable in either liquid or dried form. All "control" systems--power, the spindle motor, heaters, actuators for any valves, and the detection system--are contained in the instrument.

Some exceptions to the above partitioning scheme are envisioned. As illustrated below, heating elements may be made part of the disposable, to allow for localized heating; similarly, in the case of electrochemical sensors, signals may be fed from the (rotating) sensors to the stationary instrument.

Table 1. LabCD™ System: Disposable and Instrument

Disc	Reader
Fluidics manifolds and structures (channels, reservoirs, "capillary" valves, sacrificial valves, on-disc electronics)	Rotary motor to pump fluids and overcome capillary valves; electrical contacts (for sacrificial valves, heating...)
Chemistries	Detection and analysis of chemistry results
CD-ROM format information	Process control using disc-specific information

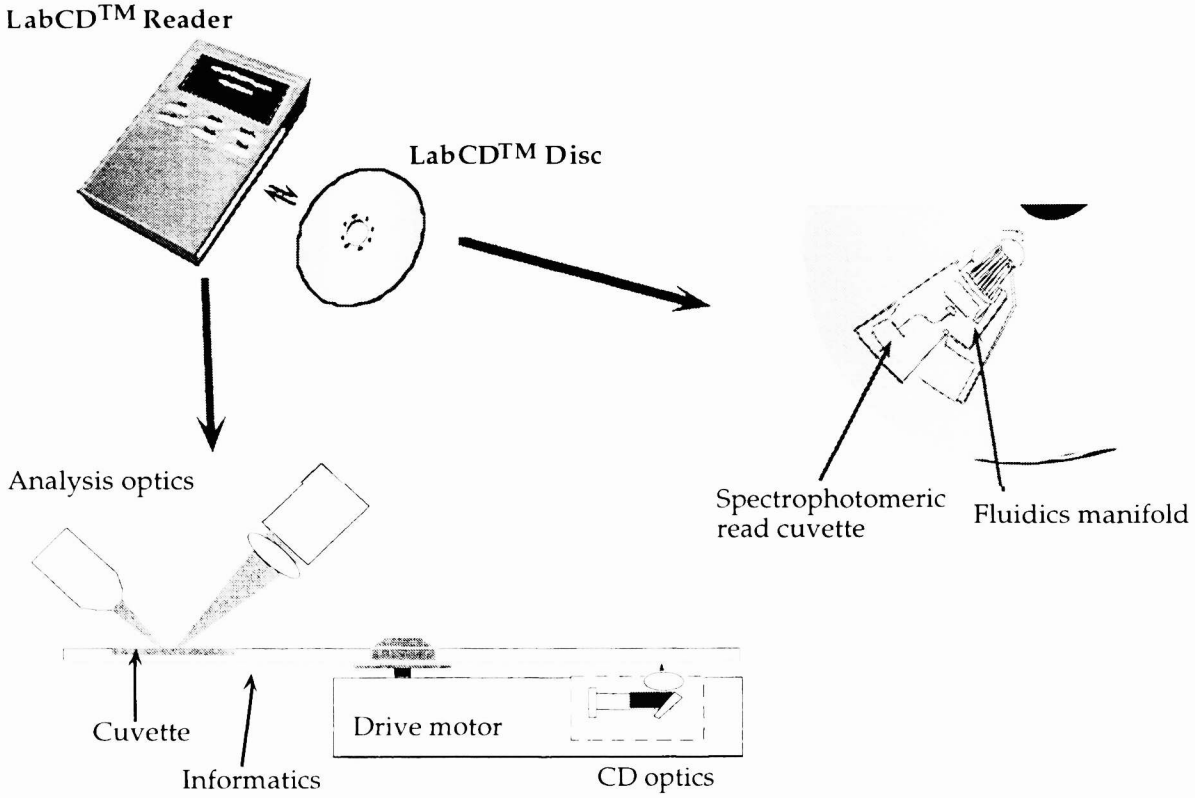


FIGURE 2. LabCD™ Instrument and Disposable. Here, the analytic result is obtained through reflection spectrophotometry at a reference wavelength.

LabCD™ Fluidic Components

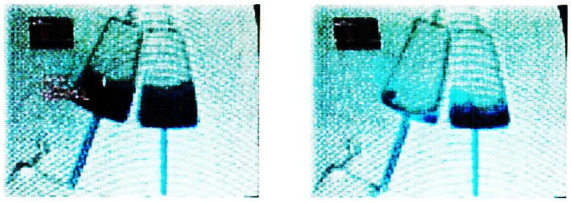
As an overview of LabCD™ capabilities, we present a brief summary of some of the processes necessary for diagnostics automation which have been implemented in the fluidic CD system already . In all cases, the prototype discs were prepared by machining fluid handling elements (chambers, channels, etc.) into acrylic using conventional CNC milling, then covering the structures with adhesive-backed tape

1. Propulsion (Figure 3A). Fluid propulsion is achieved through rotationally-induced hydrostatic pressure and depends on rotation rate, geometry and location of channels, and fluid properties. The figure illustrates chambers under centrifugation dispensing fluid simultaneously through two channels, that on the left having a hydrodynamic radius of ~1.17 times that on the right. As a result, the left-hand structure drains approximately twice as quickly as that on the right.

2. Valving (Figure 3 B). Passive and actively-controlled sacrificial valves have been use to regulate flow of fluids under rotationally-induced hydrostatic pressure. The figure shows a time sequence of capillaries being used as "valves" with a monotonic increase in RPM required to overcome capillary forces. Passive valves are discussed more fully below.

3. Mixing (Figure 3C). Mixing in micro-scale devices is often inhibited due to the laminar nature of the flow. The figure illustrates a mixing process which takes advantage of the "violent" (turbulent) flow which can be attained in a system which allows bubbles and air pockets; here, the two fluids are mixed by passing through a small chamber on the way to the final reservoir.

3A: Pumping

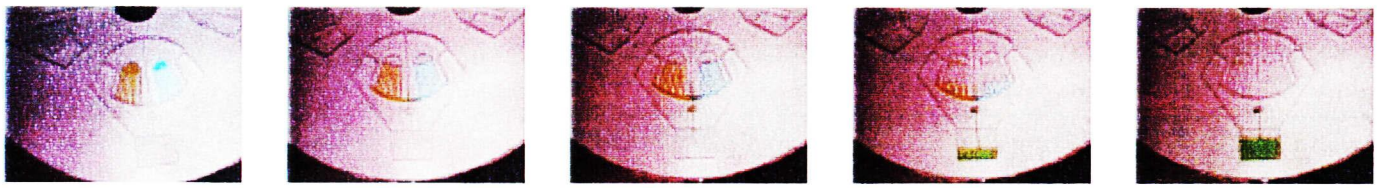


Initially: Volumes equal Upon spin: larger channel dispenses more rapidly

3B: Valving

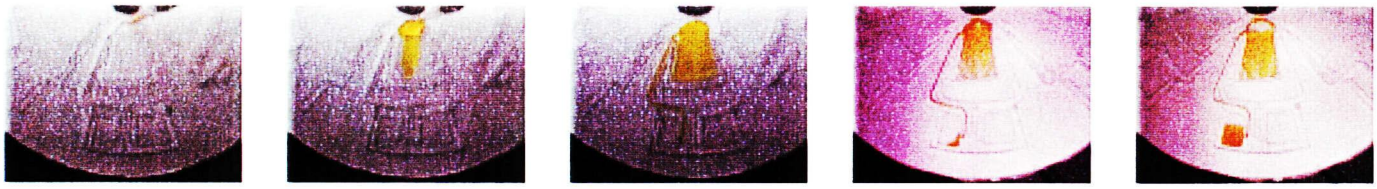
Initially: 4 filled volumes | Release of valve 1 at RPM=F1 | Release of valve 2 at F2>F1 | Release of valve 3 at F3>F2 | Release of valve 4 at F4>F3

3C: Mixing



Initially: unmixed dyes | Dyes flow into small capillary | Dyes enter small mixing chamber | Mixed dye released into reservoir

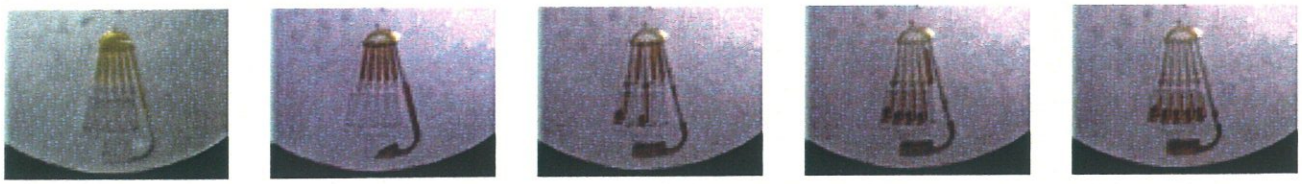
3D: Sample entry/metering



Sample placed in entry port | Capillary action draws fluid into metering capillaries | Spin: excess enters overflow chamber | Capillaries contain metered volume

FIGURE 3(PART 1)

3E: Sample splitting



Fluid drawn into capillaries

Spin: 10μl aliquots delivered to 5 cuvettes; excess fluid to overflow chamber

3F: Washing

Concentrated dye flanked by dilutions of 0.1% and 0.01%

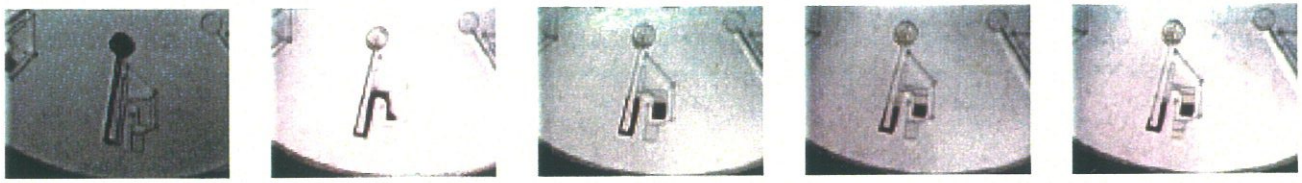
3X wash upon spinning

Wash better than 0.1%

3G: Heating

Power supplied to thin-film resistive heater through armature at hub. Increase in temperature visualized with liquid crystal patch.

3H: Centrifugation



Blood enters capillary

Spin: pre-determined volume left in column

Centfiguation at high RPM

Serum delivered at third RPM

FIGURE 3 (PART 2)

4. Sample metering (Figure 3D). Sample metering may be necessary for two reasons: The delivery of precise volumes to assays which require them from samples of imprecise, excess volume; and delivery of accurately-determined sub-volumes (see no. 5). The figure illustrates the use of an overflow reservoir which removes excess sample before further processing.

5. Sample splitting (Figure 3E). A given sample may need to be divided for different assays. The figure illustrates a simple splitting structure which uses an overflow chamber and small capillaries to deliver identical 10μl aliquots.

6. Washing (Figure 3F). Many solid-phase processes (*e.g.,* immunoassays, combinatorial chemistry, etc.) involve binding of an analyte to a support and subsequent flushing of unbound analyte from the reaction chamber. Depending on kinetics of binding processes, transport of analyte to the binding surface, and surface area/volume ratio of binding surface to chamber volume, washing must be able to remove contents of the chamber to the level of 99-99.9% or better. Using dye as an indicator, it can be seen that a single wash of 3X the chamber volume removes greater than 99.9% of the dye.

7. Heating (Figure 3G). Elevated temperatures are required for numerous assays, usually at 37°C (body temperature) or near 100°C (denaturing of nucleic acids). The figure shows localized heating through a resistive heater using power supplied through the hub of the rotor.

8. Centrifugation (Figure 3H). LabCD™ is at its heart a centrifuge. The figure illustrates the centrifugation of whole blood into serum and cellular components, followed by dispensing of serum into another reservoir for further processing.

9. Particle detection (not shown). By making the fluidic channel of the size of particles of interest (say a biological cell at 2-15 μm), particle sorting and counting is possible [5]. A diode laser is capable of counting and identifying cells as they pass through the light beam path [6]; extension of this to a CD laser should be possible.

10. Detection (not shown). The results of diagnostic assays must be read by the instrument with the precision necessary for clinical relevance. A variety of detection methods can be envisioned. Figure 2 shows a simple spectrophotometric system which uses a reflection from the rear side of the cell, an approach which is adequate for many clinical assays. Modifications which have been explored include multiple reflection within the cuvette, which has been shown to increase sensitivity by over 30-fold.

4. Fluid Propulsion

General Considerations

Before discussing the LabCD™ in relation to other fluid propulsion methods, a few general equations in fluidics must be introduced. The two most important parameters characterizing a fluid are density and viscosity. The density, ρ, of a fluid is the mass per unit volume, and the viscosity, η, is the fluid property that causes the shear stresses when the fluid is moving; without viscosity in a fluid there would be no fluid resistance.

There are two general categories of flow in viscous fluids: *laminar flow,* in which fluid moves smoothly, and *turbulent flow,* in which the fluid does not move along predictable streamlines, but forms complex, non-deterministic patterns. By adding to the density and viscosity two further parameters, the fluid velocity U and a characteristic dimension of the flow system L, one forms the dimensionless Reynolds number:

$$R_e = \frac{\rho U L}{\eta} \tag{1}$$

The characteristic length, as the term implies, is the length which best represents the body under consideration. In a capillary, for example, the diameter d is far more important in determining the nature of the flow than the length l, and we identify $L=d/2$ (the channel radius). The Reynolds number may be viewed as the ratio of shear stress due to turbulence (i.e. inertial forces, or the forces set up by acceleration or deceleration of the fluid) to shear stress due to viscosity. Under ordinary conditions, laminar flow exists in a regime with Reynolds numbers $R_e \leq 2300$.

A simple calculation illustrates what is expected for flow in microfluidic channels. Applying Equation 1 to calculate the Reynolds number for flow of water ($\eta=1.5\times10^{-2}$ cm^2/s) in a 50 μm diameter capillary, assuming U= 0.05cm/s (ten times the diameter of the pipe per sec) we obtain 0. 025, considerably less than 2300. This indicates that fluids in a microsystem will operate in the viscosity-dominated, so-called Stokes regime.

For laminar flow, the pressure gradient in a circular capillary of diameter d is given as:

$$\frac{\Delta p}{\Delta x} = -\frac{32\eta U}{d^2} \tag{2}$$

Since flow occurs from a higher pressure to a lower pressure, the pressure change over l is negative in sign. U is directly proportional to the pressure gradient. The pressure change over a length l of the pipe, based on Equation 2, is :

$$\Delta p = -\frac{32\eta U l}{d^2} \tag{3}$$

so that the pressure scales as the ld^{-2}; for laminar flow in a channel of length l much larger than the characteristic dimension L ($=d$), this pressure scaling is lL^{-2}. Because of this scaling, the pressure drop over narrow capillaries is expected to be very high. Consequently, the use of many pumps, with each pump responsible for only a fraction of the total load, is expected to be important in pressure driven microsystems--effectively reducing the length l into a series of much shorter segments. The required high pressures are a good reason to look at alternative propulsion techniques which scale differently. These include for example surface force scaling, such as piezeoelectric, osmotic, electrowetting, and electrohydrodynamic pumping. Alternatively, centrifugal force removes the scaling in l for appropriate choices of geometry (see below).

Centrifuge Theory

The parameters affecting fluid pumping in the LabCD™ are illustrated in Figure 4: a circular channel of diameter d and length l extends from the distal end of a reservoir at radius r_0 to an outer radius r_1 at the proximal end of a receiving reservoir. The channel need not be arranged along the radius of the disc, but may be inclined or take a variety of shapes. The inner reservoir may have a volume of fluid in it such that the "head" of liquid over the channel is H. While the following is developed for a circular channel, the scaling with these parameters (with the hydrodynamic diameter replacing the circular diameter) should be independent of the cross-sectional geometry of the channel.

Flow through the channel will not be steady-state in this situation, beginning with a transient and decreasing as the head H is decreased due to draining of fluid from the inner reservoir. If the channel is narrow enough that flow is established without substantial depletion of H, laminar flow is expected. The mean velocity can be shown to be given by

$$U = \frac{d^2}{32\eta}\frac{\rho\omega^2\bar{r}\Delta r}{l} \tag{4}$$

where $\bar{r} = (r_0 - H + r_1)/2$ and $\Delta r = (r_1 - r_0 - H)$. The flow rate is then

$$Q = \frac{\pi d^4}{128\eta}\frac{\rho\omega^2\bar{r}\Delta r}{l} \tag{5}$$

An important observation is that, for radially arrayed channels, the fluid velocity and flow rate are independent of channel length, since $\Delta r = l$:

$$U \sim \frac{\rho}{\eta} d^2 \omega^2 \bar{r}$$
$$Q \sim \frac{\rho}{\eta} d^4 \omega^2 \bar{r} \tag{6}$$

From Equations 4 and 5, it can be seen that the velocity and flow rate are expressible in terms of the geometric variables $d^2\bar{r}\Delta r/l$ and $d^4\bar{r}\Delta r/l$, respectively. Figure 5 (left) shows velocities calculated for water for choices of $d^2\bar{r}\Delta r/l = 7.5\times10^{-5}\text{cm}^3$, $3\times10^{-4}\text{ cm}^3$, and $1.2\times10^{-3}\text{ cm}^3$, corresponding to 50μm, 100μm, and 200μm channels along

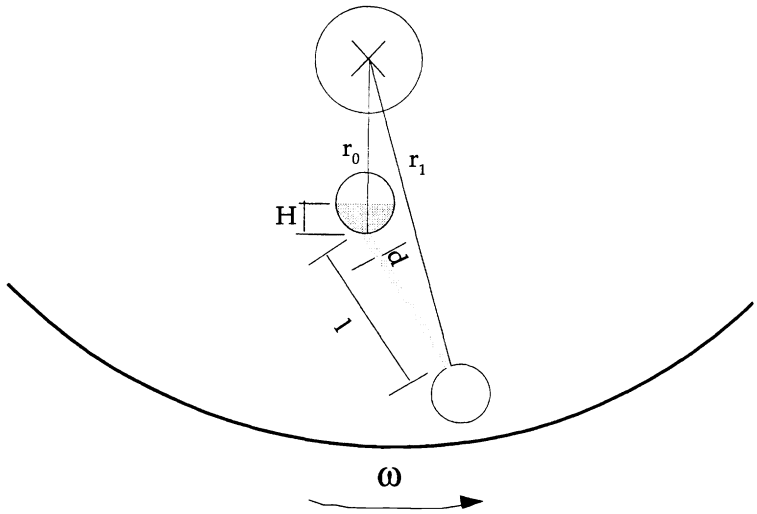


FIGURE 4. Simple LabCD™ microstructure with a channel connecting two reservoirs. The figure shows the parameters which are used to develop theory on fluid flow and "capillary valving": r_0, the inner radius of fluid undergoing flow; r_1, the outer radius of fluid undergoing flow; d, the diameter of the flow channel; l, the length of the flow channel. The structure spins at angular frequency ω.

the radius at an average position of $\bar{r}$=3cm. Figure 5 (right) illustrates flow rates for the same choices of channel dimension and radial position.

To check the consistency of the assumption of laminar flow which gives Equation 4, the Reynolds number can be calculated:

$$R_e = \frac{\rho^2 \omega^2 d^3 \bar{r} \Delta r}{64 \eta^2 l} \tag{7}$$

For water in a 100μm channel at an average radius of $\bar{r}$=3cm, Δr=l, spinning at 3000 RPM produces a R_e=46. Clearly, steady-state flow in these structures will be predominantly laminar.

To automate a diagnostic assay, one must be able to valve fluids as well as pump them, so that sample movement, wash fluid movement, and reagent movement are all controlled. Valving of fluids in a centrifugal platform can be accomplished in a number of ways, including the use of heat-activated sacrificial valves. Here we focus on a single simple, passive system which uses capillary forces in conjunction with geometry and rotational rate to form RPM-dependent "valves".

Capillary forces act to inhibit flow where narrow capillaries open into larger diameter sections (in the case of hydrophilic forces) or constrict into narrower passageways (for hydrophobic surface forces). In order to overcome capillary force and induce flow, an external hydrostatic pressure must be applied. There has been use of capillary forces in fluidic devices, both for capillary stop-junctions and capillary flow devices [7]. The ability of capillary forces to provide a means for multi-step fluid processing in centrifugal devices has until now been relatively unexploited.

Referring to figure 4, if the contact angle of the fluid on the material of the disk is less than 90°, liquid will wick into the channel by capillary action but be retained at the exit of the capillary into the outer reservoir. It can be shown that fluid flow will be initiated into the outer reservoir only when the pressure due to rotation overcomes that due to capillary forces and that

this pressure is a function of the geometry and rotational rate of the disk. Since the easiest way to sequentially valve fluids is through a monotonic increase of rotational rate, we are interested designing structures with progressively higher "burst frequencies" at which fluid flows. We can express this frequency as

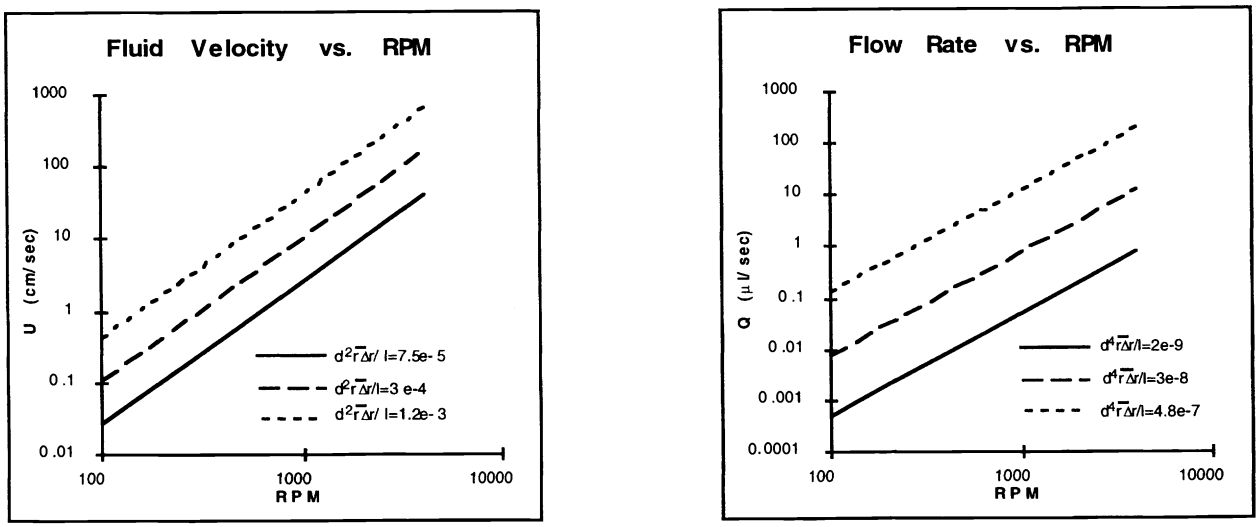


FIGURE 5. The left figure shows mean fluid velocity for laminar flow in LabCD™ for various choices of $\overline{d^2 r}\Delta r/$ (cm³) and $\overline{d^4 r}\Delta r/l$ (cm⁵).

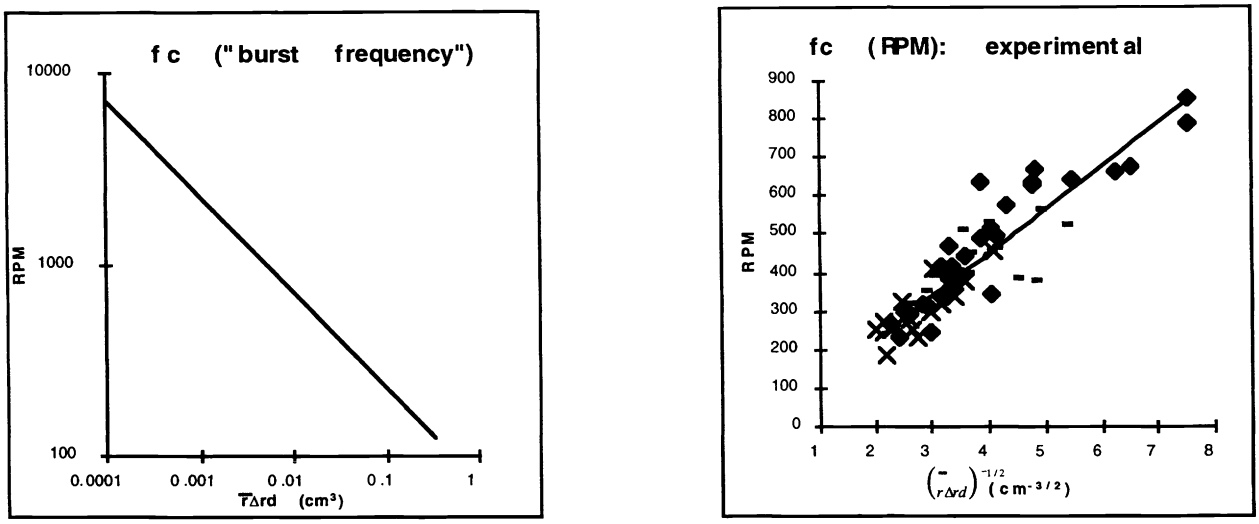


FIGURE 6. Capillary valving in the LabCD™. The left-hand figure shows a theoretical plot of capillary release RPM as a function $\bar{r}\Delta rd$ (d=hydrodynamic radius) for a choice of $\gamma\cos\theta$=28dyne/cm² which might be typical of biological fluids on plastic. The origin of the x-axis corresponds roughly to a small reservoir emptying through a 100μm channel near the center of a compact-disc sized platform. In the right-hand figure we plot experimental release RPM vs. $\left(\bar{r}\Delta rd_H\right)^{-1/2}$ for machined acrylic devices; the best-line fit is for $\gamma\cos\theta$=34.8dyne/cm², consistent with water and a contact angle of θ~61.5⁰. The various symbols correspond to different choices of channel size.

$$f_c = \left[\frac{\gamma \cos\theta}{\pi^2 \rho \bar{r} \Delta r d_H} \right]^{1/2} \tag{8}$$

Here, f_c=frequency in revolutions/sec at which fluid flows, γ=surface tension in dyne/cm^2, θ=contact angle of the fluid with the material of the disc, d_H is the hydrodynamic diameter of the channel, and the other parameters are as above. Figure 6 (left) illustrates f_c as a function of the geometric variable $\bar{r}\Delta r d$. Figure 6 (right) shows experimental results of this burst RPM for machined discs. By an appropriate choice of channel sizes and reservoir dimensions and locations, we have been able to show feasibility of assays with up to 5 sequential fluid movement steps.

5. Comparisons of Microfluidic Pumping Technologies

In Table 2 we compare various fluidic platforms. The most important parameters for *practical* comparisons are probably whether the technology is generic with regard to the kinds of fluids which may be employed, the variety of materials and manufacturing methods which can be used, and the current level of maturity of the technology (i.e., how many unsolved problems are in the way to useful devices). Another important consideration, related to how the systems scale with miniaturization, is the achievable volumetric flow-rates. As an example, manipulation of 10µl liquid volumes with flows of 0.01µl/sec would make little sense in most cases.

One way in which pressure-based propulsion in microfluidics can be realized is by coupling external, macroscopic pumps to the miniaturized fluid manifold. Due to its simplicity and the ready availability of the technology this is a serious contender for diagnostics using relatively few steps and simple processes. It is also the only method commercialized so far. The Johnson & Johnson blister pouch for HIV testing incorporates a plastic bag featuring a number of filled and empty blisters and connecting channels. A mechanical roller pushes fluids through the channels and ruptures small plastic nips (passive, sacrificial valves) in the channels to release reagents. The system is simple, capable of pumping a wide range of biological fluids, is cheaply made in plastic, and is practical today. The blister pouch design, with only fluidic channels and reservoirs and reagents in the disposable and all the other components contained in the reader instrument, is a good example of clever partitioning; more integrated approaches will have a tough time competing on cost with this design [8]. One major disadvantage of the blister approach, however, is that there remains little opportunity for further miniaturization or for multiple tests. This is largely due to the fact that the driving force for the blister system, the roller, is in itself macroscopic, making it difficult to imagine how one would create complex manifolds capable of many processes.

The other general pressure-based approach, that of miniaturizing mechanical pumps, has proven difficult to realize. This is to a large degree because of the incompatible scaling of pressure and fluid velocity relative to the material properties of the pump. It is easy to show that miniaturization which maintains the same process rates leaves the pumping pressure required in Equation (3) unchanged, while at the same time the mechanical strength of the pump, and hence its maximum pumping pressure, scales as l^3. As a result, micro-pumps cannot achieve the same performance as their macroscopic counterparts.

There is another mechanical pumping method, however. This is acoustic pumping. In a vibrating tube, an axially-directed acoustic-streaming force may be generated along the inner surface [9]. This driving force can be generated along the entire length of a narrow tube through the use of piezoelectric actuators to create a distributed pump that moves fluids without an externally applied pressure. The flexural plate wave is an embodiment of an acoustic pump and this elegant solution scales very well into the microdomain, leading to larger pumping speeds for smaller fluidic channels [10]. A separate valving solution must be sought in the case of acoustic pumping, but it does have the attractive feature of being quite generic with regard to the kinds of fluids which can be pumped. The choice of materials is severely limited to piezoelectrics and cost of devices is likely to be high, at least initially. In terms of maturity this is a rather novel technique mostly in the research stage.

Electro-osmosis scales favorably into the microdomain. Electro-osmosis as a technique has, to a large degree, solved the problem of propulsion in a manner which scales favorably for miniaturization. Electro-osmotic flow results from the motion of charges in a fluid in response to an applied electric field. For large field strengths, the ions move without appreciable movement of surrounding fluid, resulting in electrophoresis. For smaller fields, however, collisions between the charges and

Table 2. Microfluidics Platforms Compared

	Mechanisms					
Properties	Centrifuge	Pressure: blister pouch	Acoustic	Electro-osmosis	Electrohydro-dynamic	Electrowetting
Valving solved?	Yes	Blister pouches	No	Yes	Yes	No
Maturity	R&D	Mature	R	R&D	R	R
Materials	e.g. plastic	e.g. Plastic	e.g. Si/ZnO (Piezoelectric needed)	e.g. Glass		
Generic propulsion?	Yes	Yes	Yes	No (depends on pH and ions)	No	No
Power source	Rotary motor	Mechanical roller	5 to 40 V (peak to peak)	10 kV	10kV	1-10s V
Flow rate	1-100μl /sec		e.g. 20μl/sec	0.001-1μl/sec		e.g.0.3μl/sec

carrier fluid impart electro-osmotic flow to the fluid itself. The technology for electro-osmosis is simple, does not involve moving parts, and is easily implemented in micromachined channels. One needs only an electrode in a reservoir at each end of the flow channel. Harrison et al. [11] have achieved electro-osmotic pumping with fluid velocities of up to 1 cm/sec in 20 μm capillaries micromachined in glass. Since the manifolds are "open", without physical valves, the possibility of uncontrolled mixing at intersecting capillaries through convective and diffusion effects might arise. It has been demonstrated that these effects can be controlled by the appropriate application of voltages to the intersecting channels simultaneously, allowing injection, mixing, and reaction of fluids without physical valves[11]. Unfortunately, electro-osmosis does suffer from a strong dependence on the properties of the fluid, making it somewhat less generic than pressure as a propulsion method. The method depends on the pH and existence of ions or dipoles in the fluid of sufficient concentration to form a Helmholtz layer (diffuse double layer) along the flow path but not so concentrated as to lead to conduction. Organic compounds and solvents may not be able to meet the charge and pH requirements [12]. Surface impurities are also expected to affect the formation of the Helmholtz layer . Finally, the choice of materials for devices is limited to those which are capable of presenting negatively charged groups at the surface in contact with the fluid. From a practical point of view the connection between the outside world and the capillaries still needs to be addressed: Gluing glass reservoirs for holding the platinum bias electrodes to the glass plate substrate with RTV silicone is not an attractive option [13].

A similar technique is electrohydrodynamic (EHD) pumping, in which fluid forces are generated by the interaction of electric fields with *induced* electrical charges in the fluid (as opposed to ionic species as in electro-osmosis) [13,15]. In order to induce free charge in the pumping fluid, large electric fields must be generated, something which is practical at acceptable electrode voltages only within microstructures. Therefore this principle becomes more and more effective with decreasing size. A requirement for the continued existence of free charge is the presence of a spatial gradient in the conductivity or permittivity. Free charge generated in a region without a gradient will relax in a characteristic relaxation time. One way to develop a conductivity gradient in the bulk of a slightly conductive fluid is by imposing a temperature gradient. A limitation of EHD is its reduced effectiveness with conductive fluids such as biological environments. EHD work has also been confined thus far to glass. This technology is still in the early research phase.

Electrical control of interfacial tension between a liquid and a solid in electrowetting provides yet another means of direct fluid pumping with no moving mechanical parts[16]. To increase the surface area of any liquid requires effort, and this work per unit surface area is equal to the surface tension of the liquid multiplied by the cosine of the contact angle ($\gamma\cos\theta$). By modifying the contact angle through application of a potential, capillary forces may be manipulated to provide a driving pressure. The pressure produced in a capillary by electrowetting, calculated from typical values of gas-liquid surface tension and a channel of radius of 10 μm, is in the range of 0.01 Mpa, roughly the same as achieved with piezoelectric pumps which are much larger. As in the case of electro-osmotic pumping, subtle, uncontrollable changes at the solid/fluid interface are likely to make this actuation principle difficult to control and electrolyte specific. We further note that this approach seems sound in theory but has not been carried out in the laboratory.

Finally, in the case of the phase-change pump, periodic phase changes of a fluid are used for pumping. The pressure here arises from the volume change which accompanies the phase change from liquid to gas as the liquid is heated. Considering the speed of heat exchange and the value of the Reynolds number in narrow capillaries, this mechanism scales well into the microdomain [17]. This approach is only in the very early research stage and was not listed in Table 2.

6. Conclusions

Acoustic, electrohydrodynamic, electrowetting, and phase-change propulsion methods appear to be too difficult or in too early a stage of development to implement at this time in a generic fluidic platform. Pressure-based solutions are mature in the sense that simple devices like blister pouches have already been commercialized but, due to their poor scaling at microscopic dimensions, this route is unattractive for further miniaturization.

This leaves electro-osmosis and centrifuge-based microfluidics as the most mature contenders for wide acceptance. The attractiveness of electro-osmosis is attested to by the ongoing efforts of several companies to commercialize the technology and the government's high level of funding through DARPA. There are still problems with electro-osmosis, however, revolving around its susceptibility to fluid properties and the level of sophistication required in manufacture of the disposable (manufacture in glass, with electrodes, etc.). Devices using this method will certainly be developed and sold, though it is unclear how universal or affordable they will be.

The LabCD™ has been shown to successfully fulfill some of the basic needs for a microfluidics platform. Its pumping system is simple, using a single low-cost motor, but is capable of fine flow control through appropriate choices of channel and reservoir dimensions and geometries. The system has also addressed the issue of valving using passive (capillary) forces and the same motor used in pumping. Fluid properties--namely, viscosity, surface tension, and contact angle--should not affect the system's behavior in ways that cannot be predicted. Finally, a wide range of diagnostic assays can be done using discs produced through mature manufacturing techniques, such as injection molding, and using expensive materials. But beyond its fluid-handling capabilities, the LabCD™ has already demonstrated many of the functions necessary to field a complete assay, such as sample preparation, mixing, and washing. The integration of these processes with the information-carrying capacity of the CD yield a truly flexible and affordable diagnostic system.

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