

MINIATURIZED DEVICES FOR BIO/CHEMICAL SAMPLE PREPARATION

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ABSTRACT

Micro scale biochemical sample preparation technologies are relevant to many application areas ranging from environmental monitoring systems to miniaturized biomedical analysis systems. In this paper, selected micro fluidic systems are discussed for use as approaches to micro scale sample preparation for biomedical applications. The three systems include a) micro electrical field flow fractionation; b) micro needle technology, and c) micromachined pipette technology. Micro electrical field flow fractionation provides a tool for separation (and fractionation) of particles based on size and zeta potential for particles in a size range of approximately 1 nanometer to 1 micrometer. The micro needle technology is an enabling technology base for the development of needles with micro scale cross sectional dimensions and added functionality. The micro needles are used for conventional drug delivery and sample extraction applications as well as for integration with other miniaturized biochemical analysis systems. The micromachined pipettes are enabling for the manipulation of pL– μ L volumes of samples as well as for parallel distribution of samples or reagents on a close (<500 μ m) center-to-center spacing.

INTRODUCTION

The miniaturization of biochemical analysis systems has been a topic of growing interest over the past decade. During this time, a large majority of the technical effort has been invested in the development of the primary separation (or amplification) component of the various analysis systems such as the micro columns used in the miniaturized chromatographic systems (e.g. electrophoresis, gas chromatography, liquid chromatography) or the chambers used for miniaturized polymerase chain reaction (PCR) systems. Less efforts have been directed toward the development of technologies for micro scale sample preparation (with the exception of PCR). Sample preparation technologies include methods for purifying, manipulating, interfacing, amplifying, and chemically modifying sub-micro liter volumes of samples for analysis in a miniaturized format. While each of these technologies is available in a macro scale format, most have not been available on the micro scale until recently. During the past two to three years, there has been a significant increase in

the worldwide efforts to improve the technology base for integrated miniaturized sample preparation. These efforts have resulted in integrated systems for purifying and sorting samples, manipulating samples, interfacing samples and modular analysis system components, and sample amplification [1].

In this paper, we will focus on the use of micro systems fabrication technologies for the development of three different micro systems for sample preparation. The three systems include: a) micro electrical field flow fractionation; b) micro needle technology, and c) micromachined pipette technology

MICROMACHINED NEEDLE ARRAYS

Micro instrumentation is a rapidly growing area of interest for a broad spectrum of engineering applications. One application of interest to the biomedical industry is the development of microneedles. Some of the smallest hollow needles that are available today have inner diameters of over 200 μ m. A demand exists for a hollow needle device that can withstand typical handling and subcutaneously deliver medication without the usual discomfort associated with current needles. A schematic representation of such a device, a fluid coupled hollow metallic micromachined needle array, is presented in Figure 1.

Each array is composed of hollow metallic microneedles fabricated on top of a silicon substrate using surface micromachining fabrication techniques [2]. Every microneedle of the array consists of input shafts and cantilevered output shafts. A novel cross-flow design (needle coupling channels) is incorporated to equalize pressure

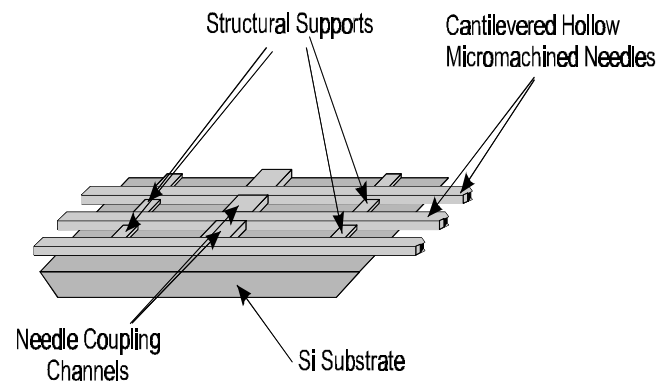


Figure 1. Schematic representation of the fluid coupled micromachined needle array.

distribution and minimize the effects of clogged passages within the needles. The optimum design for the needle coupling channels has been investigated using an ANSYS finite element numerical model [1]. The process used to fabricate the fluid coupled micromachined needle array includes p^+ etch-stop membrane technology, anisotropic etching of silicon in potassium hydroxide, sacrificial thick photoresist micromolding technology, and micro-electrodeposition technology. The fabrication process is low temperature and is compatible with integrated circuit (IC) technology as a post process. The microneedles are fabricated on top of 3-inch silicon wafers using microelectroformed palladium and are subsequently released from the substrate before packaging.

A scanning electron micrograph of a fabricated micromachined needle array released from silicon substrate is shown in Figure 2. The needle coupling channels are positioned at the center of each needle and are 100 μm wide. Two sets of $60 \times 100 \mu\text{m}^2$ structural supports are 250 μm from

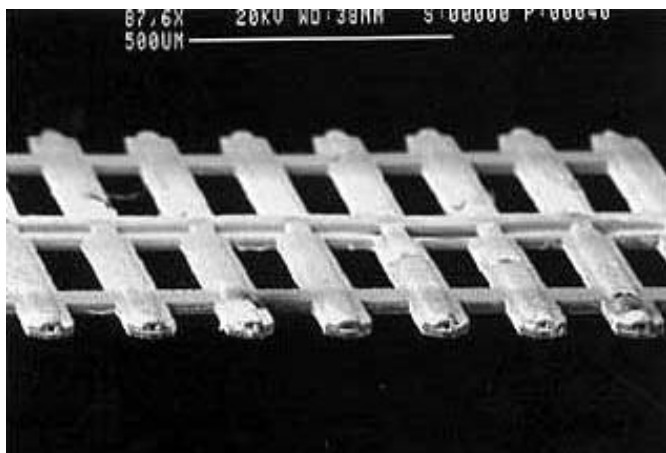


Figure 2. SEM of micromachined needle array. Individual needle channels are 2 mm long and have center-to-center spacing of 200 μm . The inner dimensions are approximately $20 \times 40 \mu\text{m}^2$. The total needle array width is 5.2 mm. Needle coupling channels are centered along the length of each needle and are 100 μm wide.

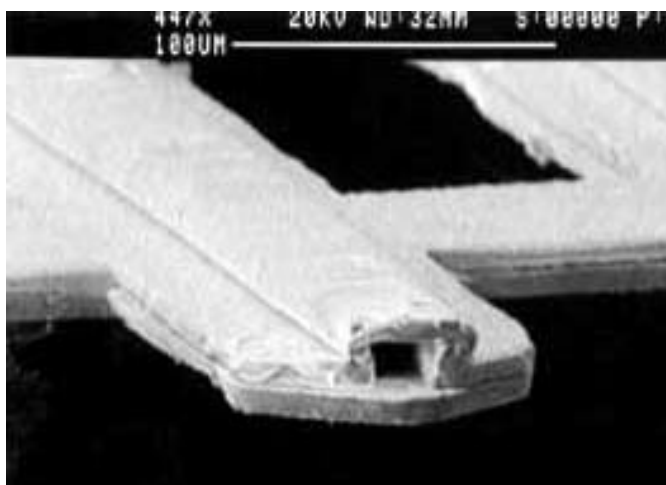


Figure 3. SEM of a micromachined microneedle output tip. The inner dimensions are approximately $30 \times 20 \mu\text{m}^2$, while the outer dimensions are approximately $80 \times 60 \mu\text{m}^2$.

each needle end. The needle wall thickness is approximately 20 μm of electroformed palladium. Each needle channel is 2 mm long, while the total width of the 25-needle array is 5.2 mm. The center-to-center spacing of individual needles is 200 μm .

It is especially important to note the quality of the fabrication of the tip of each needle, since this is the part that will be inserted through skin for drug delivery. Figure 3 is a SEM showing a close-up of one of the needle tips. The inner dimensions are approximately $30 \times 20 \mu\text{m}^2$, while the outer dimensions are approximately $80 \times 60 \mu\text{m}^2$. The distance from the needle tip to the structural supports is 250 μm . The needle tip is formed by a 45° angle for ease of penetration.

Packaging of the micromachined needle arrays is accomplished using machined acrylic that serves as an interface between a standard syringe and the array. The input end of the interface has a tapered luer fitting that allows connection to a standard syringe. The midsection of the interface contains a region that serves as a fluid reservoir that allows development of flow into individual microneedles and ensures an equal pressure distribution between microneedle inputs. The output end of the interface contains a machined slot in which the micromachined needle array is inserted and permanently mounted using a biocompatible polymeric adhesive.

MICROMACHINED PIPETTE ARRAYS

One of the challenges of future miniaturized biological/chemical analysis laboratories is to manipulate small (sub- μL) samples on a macro-scale in a parallel fashion. Today's commercially available sample handling systems for biochemical analysis are limited to wide center-to-center spacing (3.0 mm) and cannot handle sample volumes less than approximately 0.5 μL . In addition, these systems are able to dispense in the range of 6 to 12 pipettes at one time. With the current trends of miniaturizing biochemical analysis techniques (e.g., electrophoresis, chromatography, PCR) and the drive toward the development of a $\mu\text{-TAS}$, techniques must be developed to allow precise macro-scale manipulation of pL to nL range sample volumes in a highly parallel manner.

Therefore, it is important to develop a method for highly parallel macroscale sample loading of pL to μL volumes that would allow precise handling of samples in the pL to μL range and still be compatible with the size dimensions (center-to-center spacing) of the micromachined biochemical analysis systems. One technique that addresses these problems uses micromachined pipette arrays for sample loading of miniaturized biochemical analysis systems.

The micromachined pipette arrays are fabricated using extensions of previously reported surface micromachining fabrication technologies in a manner similar to the process described for the microneedles [3,4].

Each array typically consists of 5 or 7 pipettes. While each pipette had an individual input port, the acrylic interface provides a single pressure source through a manifold. The volume of the manifold is many times greater than the volume of each pipette, allowing a uniform pressure

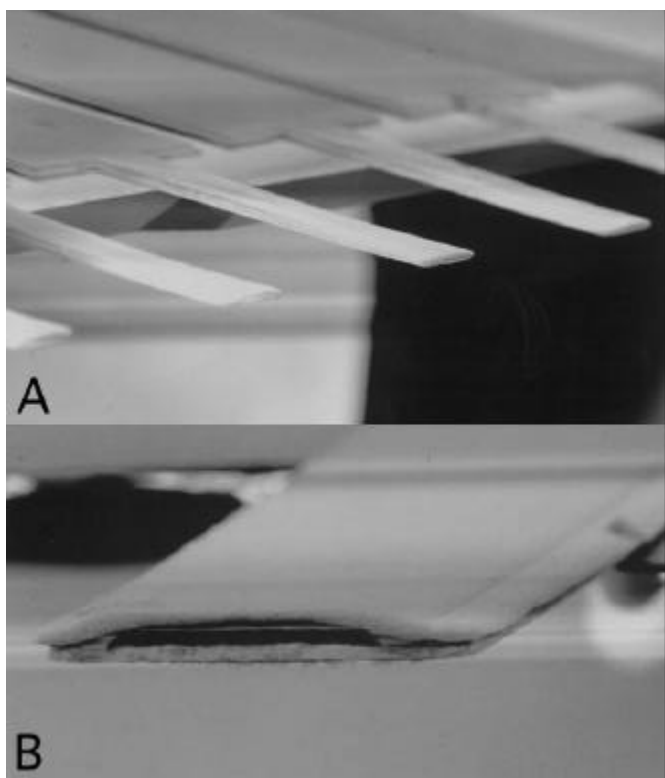


Figure 4. A. SEM micrograph of an array of pipettes extending from the silicon substrate. The wide sections are $12750 \times 1500 \times 30 \mu\text{m}^3$ (L \times W \times H). Pipettes extend 1.5 mm from the substrate and are $500 \mu\text{m}$ wide. The structural material is electroformed nickel with wall thickness of $15 \mu\text{m}$. B. Close-up of the end of a pipette. The inner cross-sectional area is $500 \times 30 \mu\text{m}^2$.

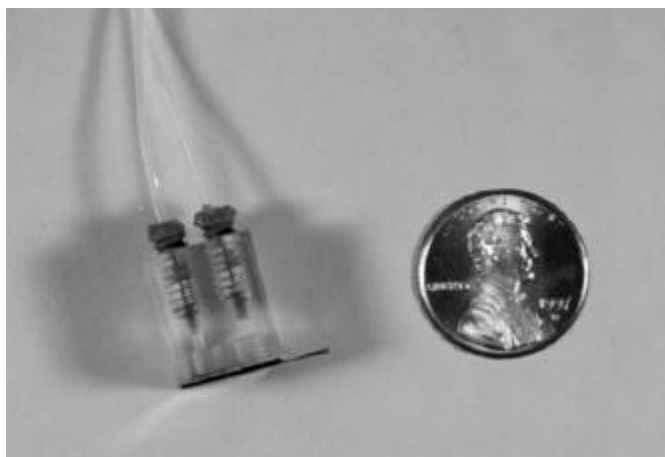


Figure 5. Photograph of a double-input micromachined pipette array interfaced from the backside. Teflon tubing with a $397 \mu\text{m}$ inner diameter connects the acrylic interface to an external pressure source. A penny is provided for size comparison.

distribution across input ports of the entire array. Thus, the flow through each pipette is assumed to be equal.

Individual pipettes are accessed through ports that are fabricated by etching through the substrate for backside access. The use of bottom input ports allowed the macroscale interface between microchannels and macro-tubing to be moved from the front of the device to the back, resulting in a more robust interconnect. The interface can also be modified to provide downstream ports for static pressure measurement in addition to the inputs for fluid flow.

An example of a micromachined pipette array fabricated on top of a silicon substrate is shown in 4. These pipettes are 7 mm in length with 2 mm extending from the substrate, while 3 mm separates the pressure ports from the pipette ends. The inner width of individual pipettes is $600 \mu\text{m}$ while the inner height (the thick photoresist thickness) is $30 \mu\text{m}$. The electroplated nickel walls are $20 \mu\text{m}$ thick.

Interfaces machined from acrylic are used to connect the fabricated micromachined pipette arrays with $397 \mu\text{m}$ inner diameter Teflon tubing. The acrylic interfaces are attached to the micromachined pipette arrays using an ultraviolet (UV) curable adhesive. Teflon tubing connects the acrylic interfaces to either a pressure source or a pressure transducer. Photographs of interfaced single-input and double-input micromachined pipette arrays are shown in Figure 5.

Micromachined Electrical Field- Flow Fractionation System

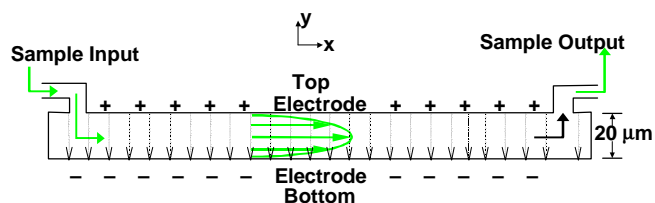


Figure 6. Schematic diagram of the operation of an EFFF system.

Another system that has some unique packaging requirements with which we have been working is the micromachined electrical field- flow fractionation system (μ -EFFF). Electrical Field-Flow Fractionation is a particle separation technique that relies on an electric field perpendicular to the direction of flow and separation as shown in Figure 6. The separations are performed in a low-viscosity liquid (typically an aqueous buffer solution) which is pumped through the separation channel. EFFF controls the relative velocity of particles by forcing particles towards the wall of the channel. Particles with high charge density pack closer to the wall and move more slowly compared to particles of lower charge density that form a more diffuse cloud and move more quickly through the channel. The channels for the miniaturized EFFF system are fabricated by bonding a silicon substrate and a glass substrate together around a photolithographically defined polyimide

spacer. Both substrates have metal thin films on their surface, which are patterned to define the electrodes for the channel and an electrical impedance detector. The input and output ports are fabricated in the silicon substrate using KOH etching and are 200 μm square on the interior of the channel and about 1 mm square on the external face of the silicon substrate. The channel dimensions are typically about 6 cm long, 1-6 mm wide, and 10-50 μm in height. Fabrication and other information about the system has been reported previously [5]. The system also has an integrated electrical impedance detector that has been incorporated at the exit end of the channel. The $\mu\text{-EFFF}$ system, therefore, requires both electrical and liquid connections. The electrical connections are rather straightforward with the only complication being that connections must be made to two parallel substrates separated by a very small distance and covered by polyimide. The fluid flow connections are much more demanding and include a need for sample injection into the system. The electrical and fluid connections will be covered separately. The electrical connections for the prototype $\mu\text{-EFFF}$ system were made by bonding small diameter wire to the metal bond pads using an electrically conducting adhesive. The resistance of the connection was typically 2-3 Ω with very good strength and flexibility. The requirements for the fluid flow connections in the $\mu\text{-EFFF}$ were much more demanding and included the following, a) ability to withstand up to 20 psi (140 kPa), b) flow area match with channel, c) accurate positioning of connections, c) flexible connections to allow component changes, and d) biocompatibility and no leaching components. In addition to these requirements, the two ends of the channel do not have identical requirements. At the entrance of the channel there is a requirement for sample injection while the exit of the channel connects only to a drain or fraction collector. The ideal though, we be to have the same basic configuration that can be modified for either purpose. The connection is made by bonding a polypropylene ferrule for 1/16" tubing from Upchurch Scientific over the port using a biocompatible UV cured adhesive (Loctite 3301). Just a small amount of adhesive can be used to position the ferrule since further bonding will be performed later. This step is critical in that the position of the ferrule determines the position of the tubing later on. To facilitate accurate placement of the ferrule (which can usually be done visually), some large bore tubing can be placed in the ferrule with either some wire or a needle through the center of the tubing. The wire or needle can then be placed into the port to insure that the tubing is centered over the port. Once the ferrule has been adequately positioned, tubing can be placed into the ferrule and seated against the silicon substrate. At low pressures the friction fit is sufficient to prevent leakage, but for most applications additional adhesive can be applied to prevent leakage even at high pressures. The tubing of choice for this application is Tefzel tubing from Upchurch Scientific. Tefzel can be securely bonded using most adhesives while Teflon tubing is nearly impossible to bond

with any strength. Steel and titanium tubing have also been used in this application, but they tend to transmit torque and bending moments very well which can cause loss of adhesion or outright fracture of the adhesive or the silicon (due to the small diameter and long moment arms, the torques can be very high even at low applied forces). This setup meets all of the requirements specified earlier and can be assembled in about five minutes due to the fast curing of the UV-cured adhesive. An external sample injection system is used since the flow into which the injection is made is pressurized and the addition of valves would greatly increase the complexity, cost, and losses in the system. Additionally, the external system eliminates losses in analyte and still functions adequately, though an on-chip injection system would likely improve the resolution of the separation system. A schematic of the injection system is shown in Figure 7. This configuration has several advantages including ease of setup and reconfiguration for other attachments, sample injection in or very near the entrance of the channel (which improves resolution in the system and eliminates the time required for sample transport to the channel), there is no flow blockage during injection which eliminates pressure buildup, and the connections are very robust with high resistance to applied forces.

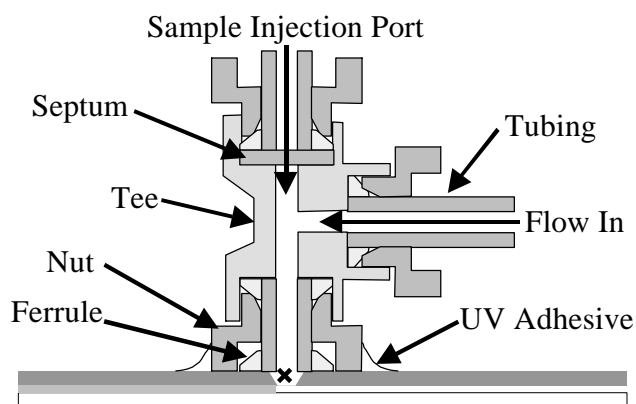


Figure 7. Cross section of the sample injection system showing the inverted nut bonded to the silicon substrate, the injection tee, and the configuration of the attached components. The X marks the point of sample injection.

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