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# Integrated Cell-Based Sensors and "Cell Clinics" Utilizing Conjugated Polymer Actuators

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## ABSTRACT

Cell-based sensors are being developed to harness the specificity and sensitivity of biological systems for sensing applications, from odor detection to pathogen classification. These integrated systems consist of CMOS chips containing sensors and circuitry onto which microstructures have been fabricated to transport, contain, and nurture the cells. The structures for confining the cells are micro-vials that can be opened and closed using polypyrrole bilayer actuators. The system integration issues and advances involved in the fabrication and operation of the actuators are described.

**Keywords:** conjugated polymers, polypyrrole, system integration, microsystem, sensing, CMOS

## 1. INTRODUCTION

We are developing cell-based sensors, which utilize living cells as sensing elements, to exploit the unparalleled specificity and selectivity of biological systems. Some cells are specialized for such sensing tasks. Olfactory neurons, for example, transduce chemical stimuli into electrical outputs, and a cell-based nose-on-a-chip can be envisioned on which different cells responding to various odorants are cultured and monitored. Electronic noses have a wide range of potential applications: monitoring food freshness, sniffing the bouquet of wine, detecting counterfeit perfume, determining food origin, drug and explosives detection, disease diagnosis, search and rescue, process control, and chemical ID. The interest in cell-based sensors goes beyond that, however, to monitoring cells for their responses to drugs (for new drug development and personalized medicine) or pathogens (for detection and classification of toxins and diseases).

The same technology is also being developed to realize "cell clinics" that can continuously monitor small groups of cells or even single cells. Such lab-on-a-chip devices are of interest for numerous basic biological studies, as well as for rapid screening, for example of nanoparticle toxicity. Such sensors would allow continuous data collection for weeks at a time, and each chip surface could be covered with many sensors. Not only would this allow multiplexed monitoring of cell response to a large number of agents, but it would also allow monitoring different types of cells as well as monitoring with a spectrum of different types of sensors. In addition to allowing massively parallel studies, miniaturization also brings the potential for portability and low cost.

The cell-based sensing technology is based on the combination of complementary metal oxide semiconductor (CMOS) integrated circuitry with micro-electro-mechanical systems (MEMS) [1-6]. Optical, electrical, electrochemical, chemical, and other sensors can be realized in CMOS, together with the appropriate circuitry for switching, signal conditioning, communication, and control. On top of the CMOS chip, the MEMS devices will be used to steer cells to the correct locations on chip and then hold them over the sensors, introduce stimulants, and regulate the cell medium. The microfluidic system includes lidded vials to separate the cells cultured on the surface into different populations and to control the timing, dose, and type of exposure to various agents (Figure 1). In principle, a unique experiment could be done in each vial.

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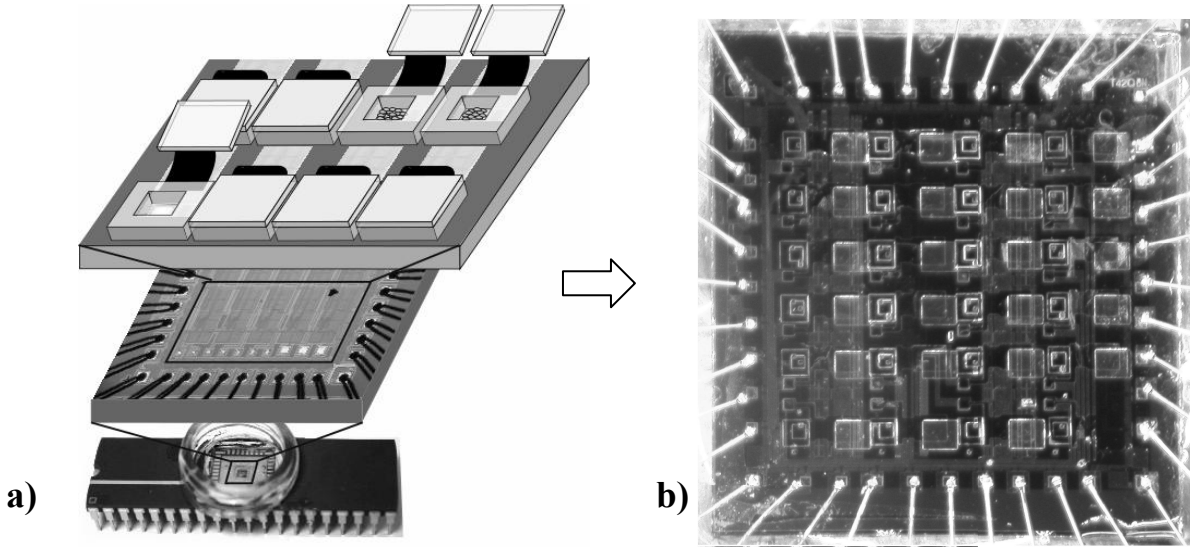

**Figure 1.** a) Schematic illustration of the cell-based sensor concept. Vials with individually addressable lids are fabricated over the sensors (top). Different cells can be loaded into each vial, and the populations in each vial monitored continuously and independently. Eight vials are shown for illustration, but the number of vials scales up with the size of the chip. A close-up of the chip surface with bond wires and circuitry (actual photo, center). The sensing chip is bonded to a DIP package surmounted by a well containing cell medium (bottom). b) Close-up photograph of an actual chip (right) 3*3 $mm^2$, showing the bond wires on the outer edge (packaged inside a transparent, photopatternable polymer) and the lidded vials in the center of the chip.

This paper focuses on the system integration issues needed to achieve these devices from the actuator perspective. In particular, it discusses actuator fabrication and packaging, control of actuator position, and operation in cell medium.

## 2. METHODOLOGY

The various components of the cell-based sensors not only need to function individually, but also together (Figure 2a). The MEMS structures must function when fabricated on top of the chip, without the fabrication process disturbing the CMOS sensors and circuitry. The circuitry needs to control the position of the actuators, and the MEMS structures need to control the position of the cells over the sensors. The packaging must protect the circuitry but allow the sensors access to the fluid environment, and the packaging process must not damage either the MEMS or the CMOS structures. The cells need to make appropriate contact with the sensors, and all of the components must be biocompatible.

As described previously [7], the CMOS sensors and circuitry are custom designed, and the chips are fabricated by a commercial foundry through the MOSIS service. Any exposed Al is subsequently plated with Au, and then microstructures are fabricated on top of the chip. This is followed by wire bonding. The wire bonds are next encapsulated in a polymer, the chip is mounted to a DIP, and the chip is surmounted with a well for containing cell medium (Figure 1a). The system is then ready for plating with cells and testing of the sensors and actuators.

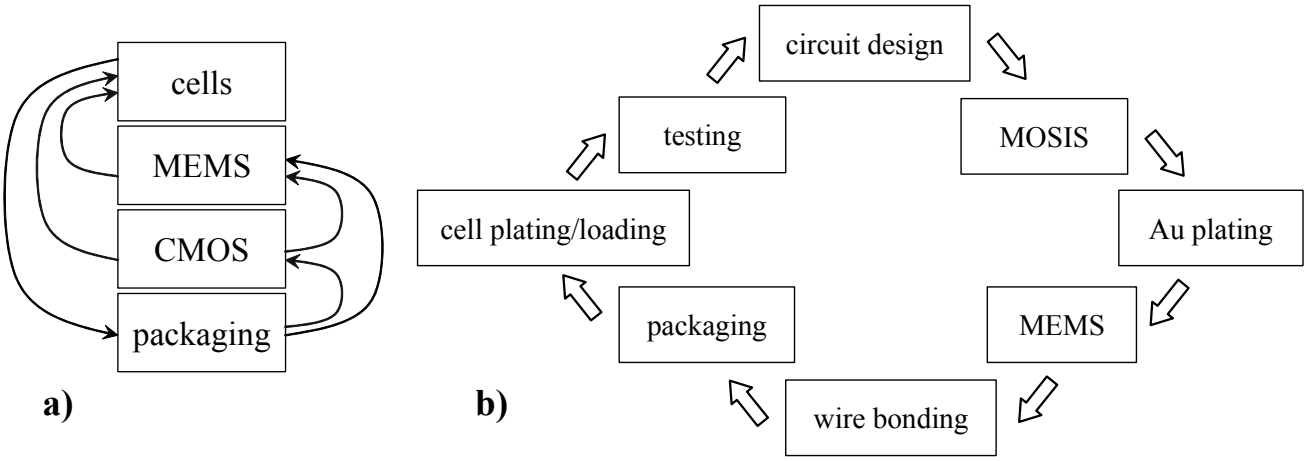


**Figure 2.** a) Not only must each component of the system function individually, but they must work together. b) The device realization process consists of design, fabrication, and testing, leading to modifications and subsequent rounds.

### 2.1 Fabrication of the actuators on the CMOS chip

Microfabrication of the PPy actuators on the CMOS surface entails a number of challenges, including the small size of the chips (we have been working with two chip sizes, 1.5*1.5 $mm^2$ "tiny chips" for the contact imager, capacitance sensor , fluorescence sensor, and potentiostat, and 3*3 $mm^2$ bioamplifier chips), variations in chip size from batch to batch, surface topography and roughness, and Al electrodes. A procedure for handling the chips has been developed that involves placing them into an exactly-sized well in a 4" diameter Si handle wafer during fabrication. The oxynitride passivation layer on the chip surface is covered with $SiO_2$ to allow the differential adhesion method to be used [8]. The Al electrodes are coated with gold using either electroless plating or by metal film deposition (e.g. evaporation or sputtering) and patterning. The lids and the vials are made of SU8, the former 5 μm in height and the latter 50 μm. The microactuators to rotate the lids are PPy(DBS)/gold bilayers [9]. A close-up of a chip surface is shown in Figure 3a.

The uppermost metal layer of the CMOS process is avoided in the sensor and circuitry designs. The chips are fabricated in a 2-poly, 3-metal process, in which metal 3 is the uppermost metal layer and is therefore not planarized (only the conformal passivation layer goes over this). The ~1 μm height of metal 3 (Figure 3b) is significant compared with the actuator thicknesses (0.5 – 1 μm). If lids are fabricated over such features, they cannot be released. Thus, no metal 3 is used underneath the actuators or lids, which constrains the chip design. The first step of the process for electrolessly Au plating the exposed Al removes some of the Al, and if only a single metal is used, there is a risk that all the Al will be lost. Thus, on areas which are plated, it is preferable to use both metal 1 and metal 2 in combination (one on top of the other), connected by small vias, to allow sufficient thickness of the Al for the plating process.

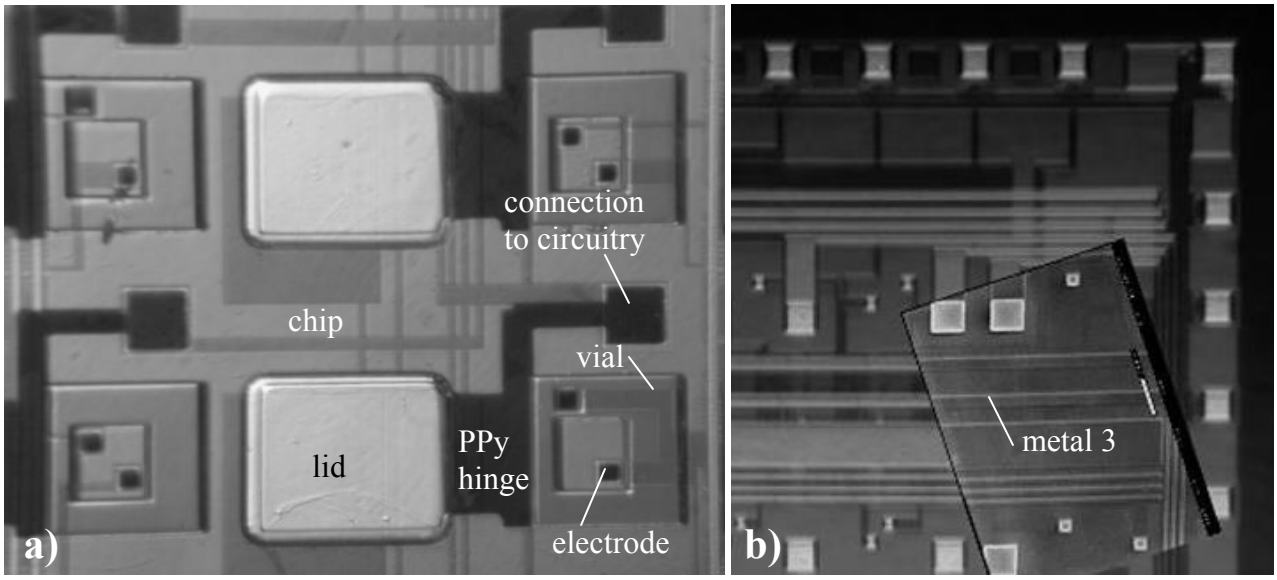


**Figure 3.** a) Optical micrograph of two lidded vials on the surface of the bio-amplifier chip, which has electrodes inside the vials for making recordings of electrical activity. The lids are fully open, and not yet actuated, in this image. The PPy actuators are electrically connected to the chip circuitry which allows for individual addressing and control. b) Optical micrograph and overlapping SEM micrograph (tilted rectangle) of a previous-generation bio-amplifier chip, without any MEMS structures on it. The metal 3 layer is 1 μm thick, and the passivation layer that overlies it is conformal, so the surface has a 1 μm bump over metal 3 features.

### 2.2 Packaging

The microsystem is packaged to function in an incubator. The packaging is challenging because the bond wires, which must be kept completely dry, are ~1 mm high and only ~25 μm away from features that need to be exposed to cell medium. Thus, a high-aspect ratio, microfabricatable material is needed whose processing is compatible with both the MEMS and the CMOS. Since this step is done after MEMS fabrication, temperatures must be kept below ~100 °C in order not to damage the PPy. Mechanical contact with the microstructures needs to be avoided, or the MEMS structures could be sheared off. Likewise, acids, bases, and solvents could cause damage to the Al or the SU8.

Currently, the chips are packaged using Loctite 3340, a UV-curable, and thus photopatternable, adhesive (Figure 4a) [10]. This material is compatible with the cell cultures we are using and can withstand up to a week in the incubator before the package fails. (Package failure occurs due to swelling by water, which can electrically short the wires as well as introduce mechanical stress that pulls the wires off the surface). While this has allowed us to collect data continuously for reasonable periods of time, we are pursuing a longer-term solution that eliminates the bond wires from the face of the chip. The goal is to contact the circuitry from the bottom of the chip by etching vias to metal 1, passivating the Si sidewalls with an insulating layer, and then metalizing the vias by plating. The chip would be mounted to a fan-out substrate for electrical contacts, and these contacts encapsulated in a more water-tight, not-necessarily-biocompatible potting compound. This would also free up the front for access to microfluidic ports.

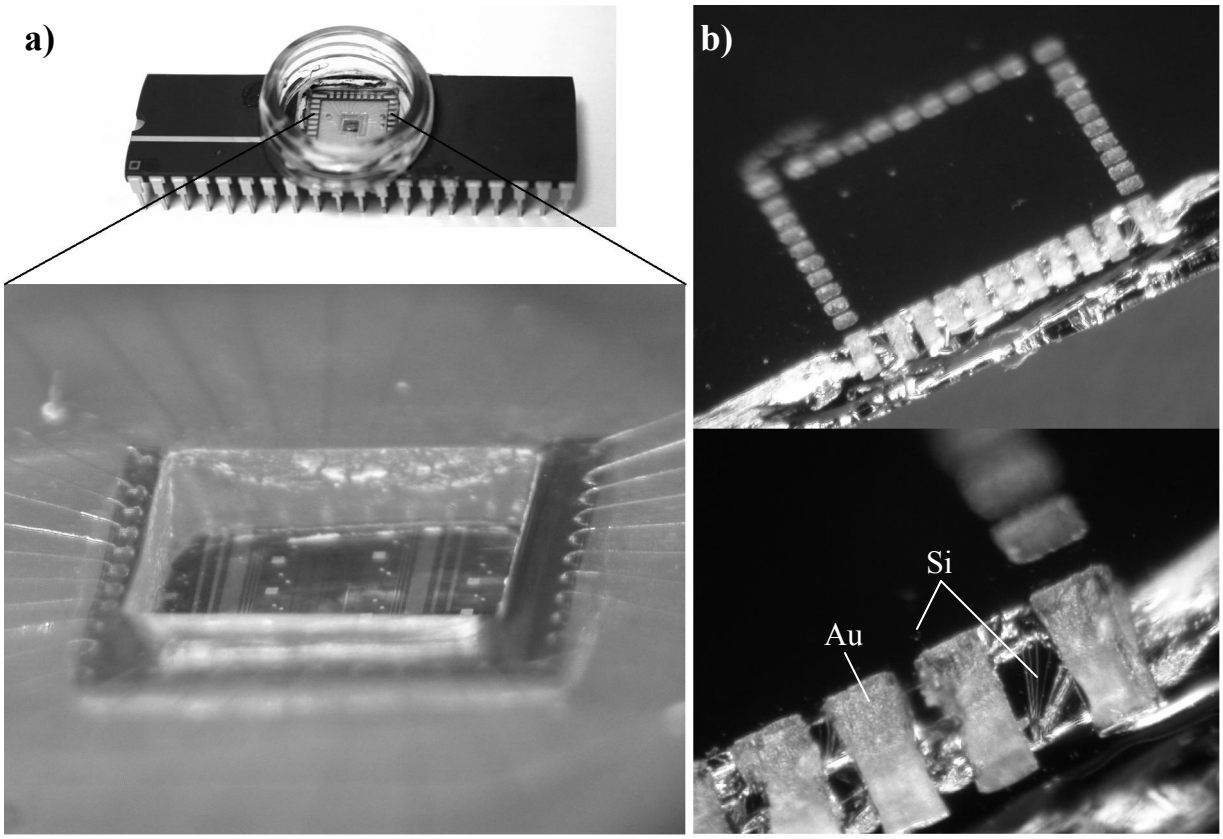


**Figure 4.** a) (Top) Chip mounted and bonded to a DIP 40 package, then surmounted by a well for cell medium. (Bottom) Close-up of the chip with bond wires encapsulated in Loctite 3340. b) Vias etched into the back of a Si wafer and then electrolessly plated with Au to the surface.

## 3. RESULTS

### 3.1 Driving the actuators with an on-chip potentiostat

A CMOS chip comprising a potentiostat circuit and on-chip working, reference, and counter electrodes has been developed for controlling the PPy/Au hinge actuators in future generations of the cell-based sensors [11,12]. While on-chip potentiostats have previously been reported, those devices were current detectors designed for high-sensitivity measurement of biochemical analytes (see for example [13,14]). In contrast, this actuation application requires the potentiostat to serve as a robust current driver (μA to mA) and potential controller.

Operation of the potentiostat chip has been demonstrated both by depositing PPy onto the working electrode and by cycling the PPy (Figure 5). Cyclic voltammograms typical of PPy were recorded, and in addition, during electrochemical cycling electrochromic changes were observed in the films, confirming that the PPy was switched between fully oxidized and reduced states. This was the first demonstration of such an integrated system for electrochemical actuator applications.

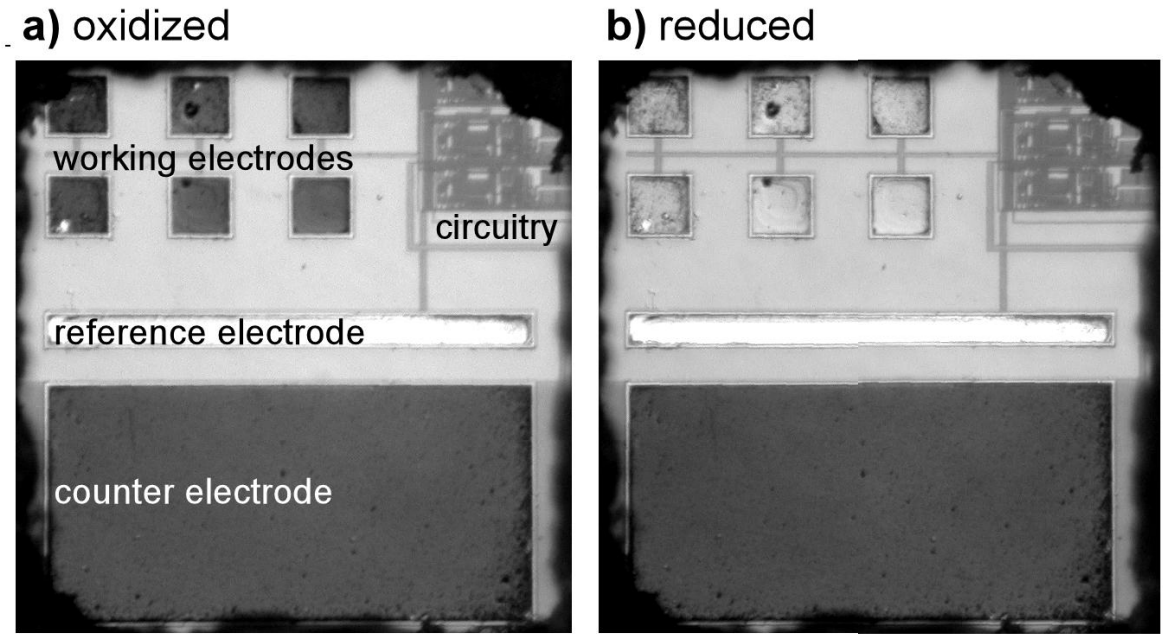


**Figure 5.** Photographs of the packaged potentiostat chip; the Loctite 3340 polymer used for packaging can be seen at the edges. The PPy that is being switched is on the 6 working electrodes at the upper left. The counter electrode, here also coated with PPy to increase its surface area, is on the bottom. The reference electrode is in the center, and is an Au pseudo-reference. The circuitry can be seen in the upper right corner. The PPy on the working electrodes is a) oxidized and b) reduced. The potential on the counter electrode is not significantly altered, as indicated by the relatively unchanged color of that PPy film.

### 3.2 Lidded vials

In order to achieve lids that close the vial by rotating 180° in cell medium at room temperature, several key studies have been completed over the last several years, including research on how to prevent delamination of the PPy from the Au [15], measurement of bending angle as a function of PPy and Au thickness [16,17], bending angle as a function of temperature [18], and actuation in ion mixtures [19]. This has allowed us to design [20] the actuators so that the lids function as intended (Figure 6a,b). (If incorrectly designed, one obtains results such as in (Figure 6c.) Studies are currently underway on actuator force and speed. In addition, basic studies are being done to develop the constitutive equations describing the volume change in PPy(DBS) so that the actuators can be controlled [21,22].

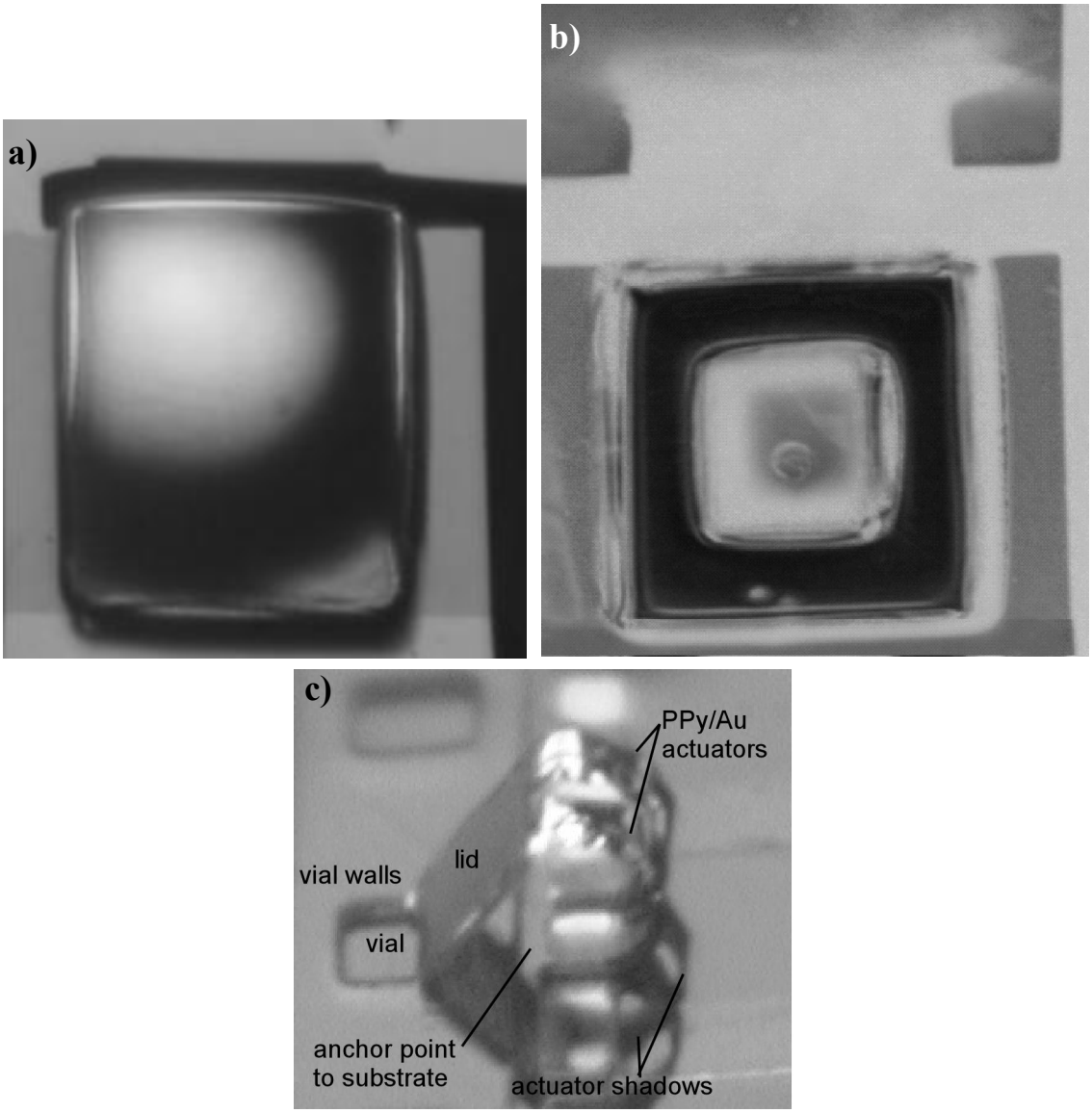


**Figure 6.** Properly designed and functioning lidded vials on a Si wafer in the a) closed position rotated 180°, and b) open to ~90° in Hanks buffered saline, showing a cell in the vial (the vertically-standing lid is out of focus at the top). c) Vials with actuators that are too long. In this device, the actuator design consisted of two parallel bilayers.

### 3.3 Actuation in electrolytes with various cations

Cell medium contains a number of different ions necessary for cell viability, so the operation of the actuators in the presence of these ions must be understood. We have previously presented data showing that for the series of alkali cations, Li produced the largest volume change and moved the most quickly, following the order Li > Na > K > Rb > Cs, so that Cs produced the smallest strain and moved the most slowly [19]. Those data were for out-of-plane volume change and inplane ion velocity.

Measurements of out-of-plane ion velocity and of actuator movement show that the story is more complicated. Not only are in-plane and out-of-plane velocities different for a given ion, but also the *relative order* of the speed for the alkali cations is different out-of-plane than in-plane. This would suggest that the speed of a micro-actuator is thus partially determined by its width, since that determines the relative contribution of in-plane to out-of-plane ion transport. Narrow actuators designed for in-plane ion transport were therefore fabricated and tested. Preliminary results show that, unlike in stationary strips, the speed in solutions with different cations follows the out-of-plane transport order. The fact that the movement of the bilayer affects its ion transport properties shows the strong connection to the chain movements, as Otero et al. have demonstrated with their conformational relaxation model. These effects will need to be taken into account in a complete model of actuator behavior in order to be able to implement control algorithms for these devices.

## 4. SUMMARY

The development of a complex system that integrates electroactive polymers as one component imposes a number of constraints: the use of the EAP constrains the system, and the system places requirements on the actuators. In order to meet these challenges, the actuation must be thoroughly understood (i.e., we must have predictive mathematical models of the behavior) so that the actuators can be properly designed and controlled. In addition, the actuator metrics must be known under the required operating conditions (film thickness, temperature, electrolyte contents, etc.). This requires extensive basic characterization studies.

This work is ongoing, and has progressed rapidly over the last several years. Many of the individual components have been fabricated and tested, and the next step is to integrate these components together into a working system.

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