The Silicon that Moves and Feels Small Living Things

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Silicon microelectronic chips that make today's computers possible are emerging as powerful new tools for rapid and sensitive analysis of small biological objects, including cells, proteins, DNA, and viruses. The interface between electronic systems & biological systems is aimed at revolutionary advances in the life sciences and human healthcare, e.g., early cancer detection.

The present article is an attempt to share some exciting developments in this burgeoning area with this newsletter's readership. A large amount of high quality research is being done in the field. Therefore, the selected topics and references are far from being exhaustive, but we think they are examples which comprise an effective exposure to the field.

How does one go about interfacing solid-state circuits with squishy biological objects? The theoretical underpinning is that biological entities possess inherent electric properties (DNA carries intrinsic charges; cells are dielectric; nerve cells fire electrical pulses; etc.) and also that biological species can be attached to certain molecules or artificial particles of pronounced electric or magnetic characters. It is these electric or magnetic properties with which silicon circuitries can be structurally and functionally configured to interact. To gain one concrete picture for this broad and very widely applicable statement, let us begin our brief journey into this field with one of the most fascinating silicon-biology interfaces, *electronic DNA microarrays*.

Electronic DNA microarrays

DNA contains and issues the language of life. It gives cells instructions for living, and tells living organisms about their hereditary traits. This language is coded into the DNA's famous double helix structure: Fig 1(a). Each helical strand exhibits a sequence of four chemical bases, adenine (A), guanine (G), cytosine (C), & thymine (T), e.g., CAAGTG. The two twisted strands are bound together by pairing base A always with base T, and G always with C. Due to this pairing rule, within any section of DNA, once the sequence of one strand is identified, that of the other strand is easily inferred: e.g., the complementary sequence of CAAGTG is GTTCAC. DNA sequences are the language of life. Reading them, therefore, is of prime importance.

Electronic DNA microarrays are CMOS integrated circuits (ICs) that can rapidly decipher unknown DNA sequences [1-6]. A double-stranded DNA molecule can unzip into two complementary strands. A single-stranded DNA molecule thus obtained can bind back to its complementary sequence (either the old mate or a new one), forming again a double-stranded DNA molecule. This binding of two complementary strands, or *hybridization*, underlies the genetic sequencing operation of the electronic DNA chip.

The electronic DNA microarray is constructed by immobilizing single-stranded DNA molecules of different identified sequences onto different grid points on a CMOS IC [Fig. 1(b)]. The grid points are often defined by post-fabricated gold electrodes that are electrically connected to the underlying CMOS IC. Different grid points represent distinct DNA sequences. These single-stranded DNA molecules of known sequences making up the array are called *DNA probes*. Now consider single-stranded DNA molecules of an unknown sequence, or *DNA targets*. When a solution of DNA targets is introduced onto the DNA array, the target strands wander around to eventually hybridize to their complementary probe strands at a specific grid point [Fig. 1(b)]. Locating the hybridization position reveals the target sequence, for we already know the probe sequence at that position, which must be complementary to the target sequence. The CMOS IC underneath is used for electronic detection of the hybridization point.

One well-established electronic detection technique is to label DNA targets with reporter molecules of a distinctive electronic signature and to search for them. Redox enzymes are an example of such electronic labels. If a voltage is suddenly applied between an electrode where hybridization occurred and the electrolyte (DNA target solution), redox labels attached to target molecules will give up electrons to the electrode, thereby increasing the current through the electrode. The same voltage step in an electrode with no hybridization would cause no such current increase as redox labels are absent at that electrode. By applying the voltage step and monitoring current change fast across the whole array using the underlying IC, hybridization positions are rapidly detected, leading to target sequence identification. Redox-label-based CMOS DNA chip examples are found in [1-3].

While the label-based detection

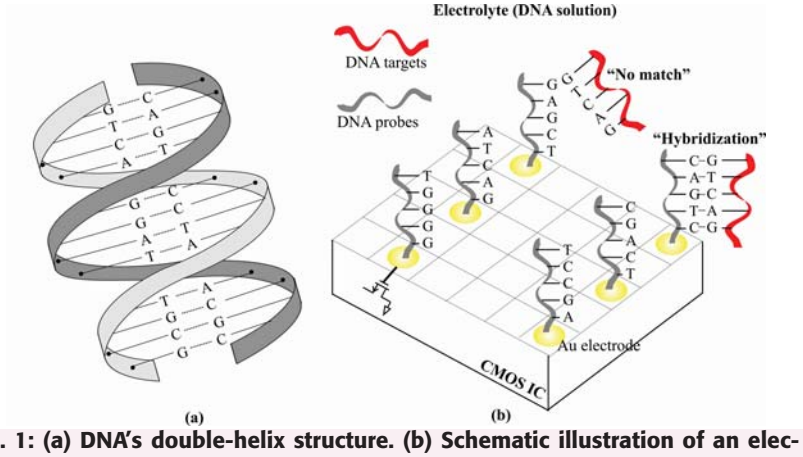

Fig. 1: (a) DNA's double-helix structure. (b) Schematic illustration of an electronic DNA microarray.

offers excellent sensitivity, significant efforts are being placed to develop label-free electronic DNA microarrays [4,5] because elimination of labeling steps would simplify the sample preparation. In [4], for example, the capacitance of an electrode immersed in the electrolyte is monitored to sense hybridization. No labeling is needed, as target strands added to an electrode during hybridization naturally lead to a dielectric constant change, or, capacitance change.

Field effect transistors (FETs), the basic commodity of CMOS ICs, can be also used *directly* for label-free electronic detection of DNA hybridization [7-9]. Underlying this sensing modality is the exploitation of the impact of DNA's intrinsic negative charges upon the FET behavior. Imagine that underneath the DNA array there is a corresponding FET array integrated in the CMOS IC. The gate dielectric of each FET is linked to DNA probe strands of the same sequence in a corresponding site in the DNA array. When a target strand hybridizes to its complementary probe strand anchored to a specific FET gate, the target's intrinsic charge alters the channel conductance and capacitance of the FET. Therefore, by monitoring the channel of each FET in the array, one can attain label-free electronic readout of target sequences. While field effect sensors per se are widely used [9], there is a lot of room for development in their use in DNA microarrays. Post-processing to expose gate dielectrics to electrolyte may pose a challenge. The smallest possible FET width must be used to maximize the impact of the DNA's charge on channel properties.

Hybridization is at the heart of many other DNA sequencing techniques. What makes electronic DNA microarrays unique is their massively parallel operation. Distinct probe sequences numbering as many as hundreds of thousands can be simultaneously available across an array. The CMOS IC monitors each site of the array fast across at a gigahertz speed, and hence, its operation may be regarded as parallel to human eyes. This parallelism allows for rapid collection of vast amounts of genetic information (far faster than non-microarray techniques), accelerating the speed at which we probe the secrets of living organisms. The parallelism is a direct outcome of using CMOS microfabrication techniques to build large microarrays, and is enhanced by the use of integrated electronics.

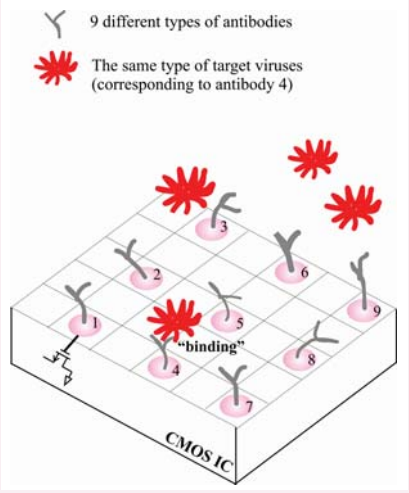

Fig. 2: Schematic illustration of a CMOS biosensor microarray to detect viruses.

In the original invention, which is still the commercially dominant form of the DNA microarray, hybridization is sensed by optical means [10]: fluorescent dyes labeling target strands light up upon illumination, reporting hybridization points. This optical machine boasts sensitivity superior to, and parallelism similar to, its much smaller electronic cousin. Although considerable work is needed to develop a high-performance electronic DNA chip as an alternative to the optical type, decisive advantages of using ICs (small size, low cost, programmability, real-time, label-free options) are the cogent reason for the ever-growing efforts in the development of electronic DNA chips.

Other electronic biosensor microarrays

Generalizing the concepts of the electronic DNA chip, one can readily consider CMOS biosensors that can detect other biological objects such as viruses and disease marker proteins [11]. Just like a DNA strand sticks specifically to its complementary strand, a virus or a protein binds specifically to its unique biochemical mate, an antibody. This highly specific binding is analogous to the way different keys fit into different locks. Fig. 2 illustrates a scenario where nine different types of viruses can be detected by using an array of nine specifically corresponding types of antibodies. When the array is exposed to a target solution containing type-4 viruses, for instance, the viruses will specifically bind to antibodies in array point 4. This binding can be detected using the CMOS IC below in much the same ways DNA hybridization is detected. By reading the location of the binding, the presence and type of viruses are determined. The multiplexed array platform can be especially useful for the diagnostics of complex diseases like cancer, where samples should be screened for multiple disease markers.

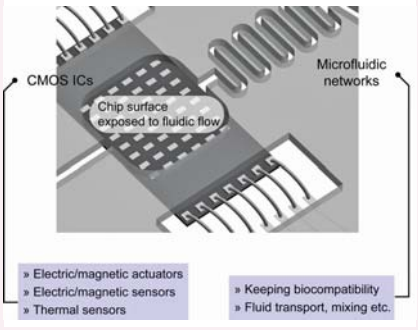

Fig. 3: Conceptual illustration of a CMOS/microfluidic hybrid system.

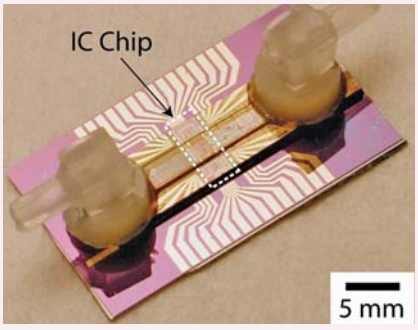

Fig. 4: Our first IC/microfluidic hybrid prototype.

One relevant design goal of electronic microarrays to sense viruses and disease marker proteins should be to maximize the ultimate sensitivity, because such capabilities would enable early disease detection when the pathogens are still sparse, which is of central importance in medical diagnostics. As transistors continue to become smaller with the scaling of CMOS technology, such ultra sensitive biosensors will become very feasible especially in the form of field effect sensors.

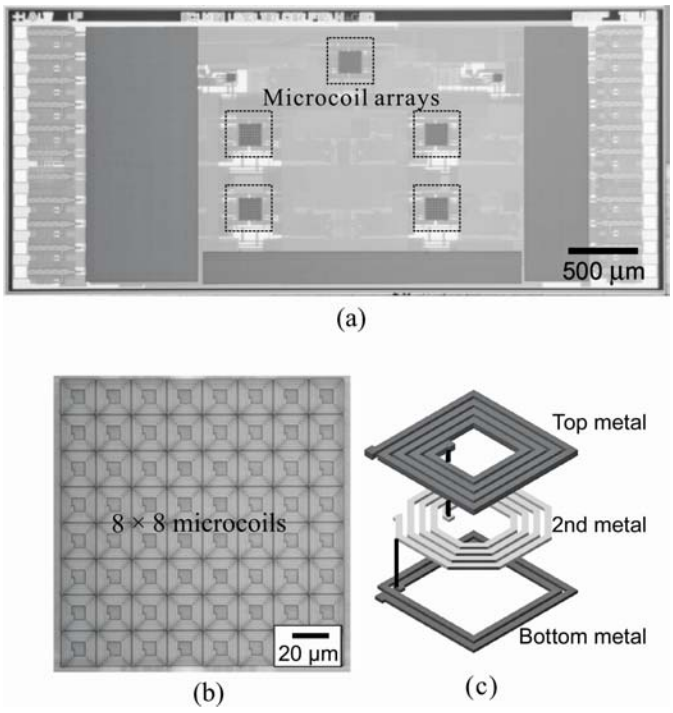

Fig. 5: (a) CMOS microcoil array IC. (b) Microcoil array close-up. (c) Schematic depiction of a single microcoil.

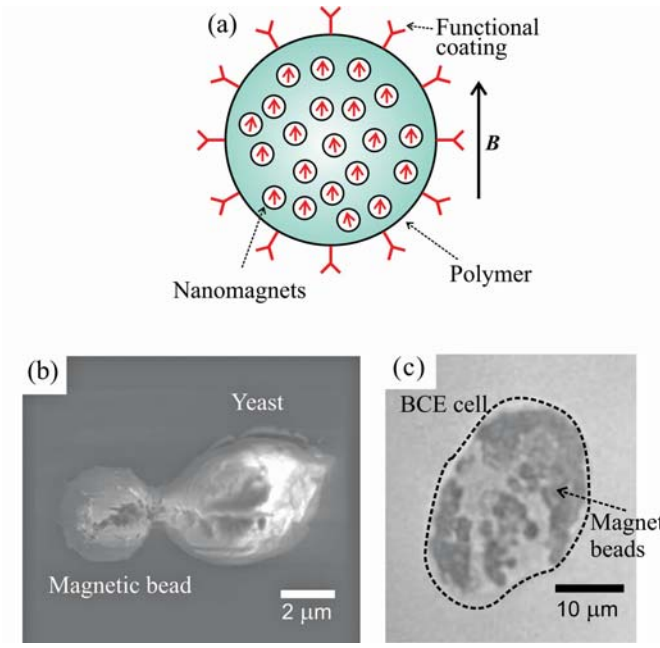

Fig. 6: (a) Illustration of a magnetic bead. (b) Yeast cell attached to a magnetic bead. (c) BCE cell that has engulfed multiple magnetic beads.

CMOS/microfluidic hybrid system

We have seen how solid-state circuits can be electrically interfaced with biological systems for their analysis. To make all this possible, however, biological systems ought to be introduced and maintained on top of a CMOS IC in a wet, biocompatible environment. Preserving a sample drop on the chip using a glass cover is one way, and it works out well for a variety of experiments as seen in many papers. Placing a microfluidic system on top of the CMOS IC, however, represents a superb approach. It allows valve-controlled precision for the introduction & removal of samples, consistent sustenance of biocompatibility, sophisticated flow control for sample mixing, reaction, & separation, and robust system packaging.

Together our two groups systematically developed such a hybrid system combining a microfluidic system on top of a CMOS IC [12-15]. Fig. 3 is a conceptual illustration of the hybrid system. Fig. 4 shows our first hybrid prototype used for magnetic actuation of cells [12] as discussed shortly. We refer interested readers to [16] for our recipes in fabricating microfluidic systems on CMOS ICs.

So far we have focused on using CMOS ICs for electronically "feeling" bio objects. We will now venture into new territory, exploring how to "move" biological cells, in the course of which we will introduce magnetic labels to attach to bio objects. We will later return to the business of sensing, but this time using the magnetic labels in conjunction with magnetic resonance.

Magnetic manipulation of biological cells

The ability to simultaneously control the motions of multiple individual cells along different paths with tight spatial control is desired for complex cell sorting operations. Such ability may also enable new types of investigations in systems biology, e.g., one can assemble an artificial microscale tissue by bringing together cells one by one into a desired geometry.

Various means are used to actuate cells, but simultaneous & independent addressing of individual cells with tight position control is not easy. For example, optical tweezers boast high-precision, 3D control of single cells, but are not suitable for handling multiple cells simultaneously. For another example, one can pull many cells attached to magnetic beads along the same direction using a magnetic tweezer (elongated electromagnet with a sharp tip), but control resolution is low and parallel manipulation of individual cells along different paths is difficult.

To attain the capability to simultaneously move multiple individual cells along different paths with tight position control, together our groups developed integrated *microcoil array* circuits within the CMOS/microfluidic hybrid structure

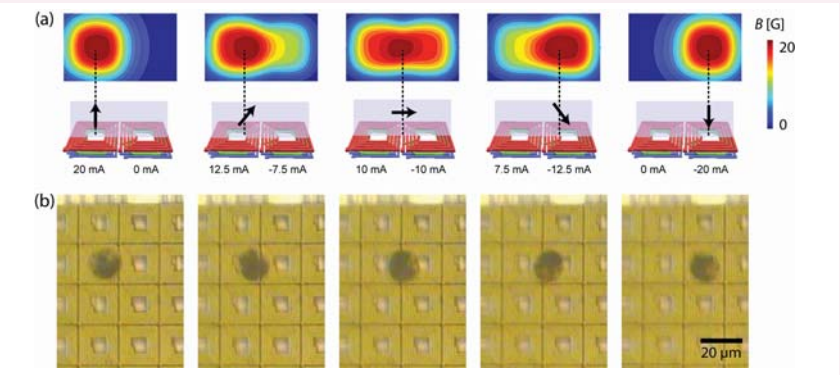

Fig. 7: 2-coil manipulation example: (a) Calculated field patterns for different current distributions. (b) Corresponding experiment using a single BCE cell. Reprinted from [15] with permission of the Royal Society of Chemistry.

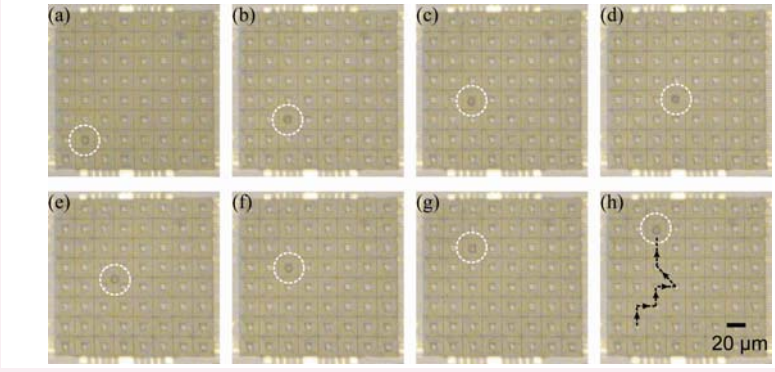

Fig. 8: Manipulation of a single magnetic bead. The alphabetical subfigure indices (a) through (h) represent images at different times, in chronological order.

[12-15]. The CMOS IC consists of a microcoil array and its control electronics: Fig. 5 shows an example IC we built. When the current distribution in the microcoil array is given, the array produces a certain spatial pattern of microscopic magnetic fields. In a given field pattern, magnetic dipoles (e.g., magnetic beads we will discuss shortly) move towards local field magnitude peak positions and get trapped there. Now by changing the current magnitude and direction in each microcoil independently, the field pattern is reconfigured and magnitude peak positions are moved independently. Magnetic-bead-bound cells suspended inside the microfluidic system on top of the IC then can be transported along different paths simultaneously. The spatial manipulation resolution is set by the dimension of the microcoil, which can be made comparable to or smaller than most cells. The parallel operation of multiple microcoils is what enables independent addressing of individual cells. The programmability of the CMOS IC makes the manipulation versatile and efficient. The detailed design of microcoil array ICs can be found in [14,16].

A brief discussion of magnetic beads would be useful. A magnetic bead is a polymer microsphere containing nanomagnets [Fig. 6(a)]. When subject to a magnetic field, the nanomagnets line up and the bead develops a net magnetic moment. It is this magnetic moment that interacts with magnetic fields in our manipulation. The bead surface can be modified with antibodies for specific bindings to target objects, e.g., yeast [Fig. 6(b)]. Fig. 6(c) shows a bovine capillary endothelial (BCE) cell that has engulfed multiple beads (~ 250 nm).

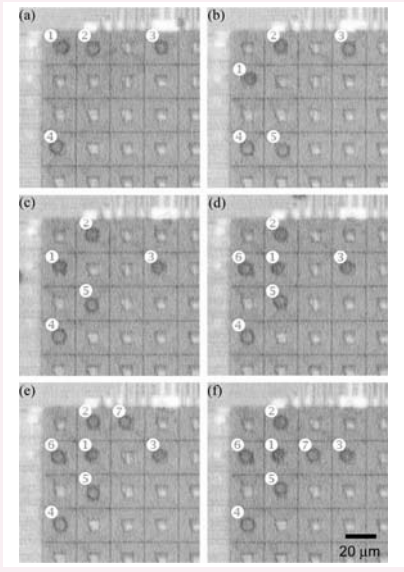

Fig. 9: Manipulation of multiple magnetic beads.

Figs. 7 ~ 10 show our magnetic manipulation experiments using the microcoil array IC [14, 15]. Fig. 7 elucidates the principle of magnetic manipulation with two microcoils. As current distribution changes, the calculated field peak moves from one coil to the other [Fig. 7(a)]. Fig. 7(b) is a matching experiment using a BCE cell. The cell rolls during the transport as the field direction also changes [Fig. 7(a)].

Fig. 8 shows the manipulation of a single magnetic bead (8.5 μm). By moving a field magnitude peak along a prescribed path, the bead was transported with an average speed of 11 μm/s subject to an average force of 40 pN.

Fig. 9 shows simultaneous independent manipulation of multiple beads to arrange them in a cross shape. One current source is shared sequentially in time among all coils to minimize power consumption [14]. This is possible

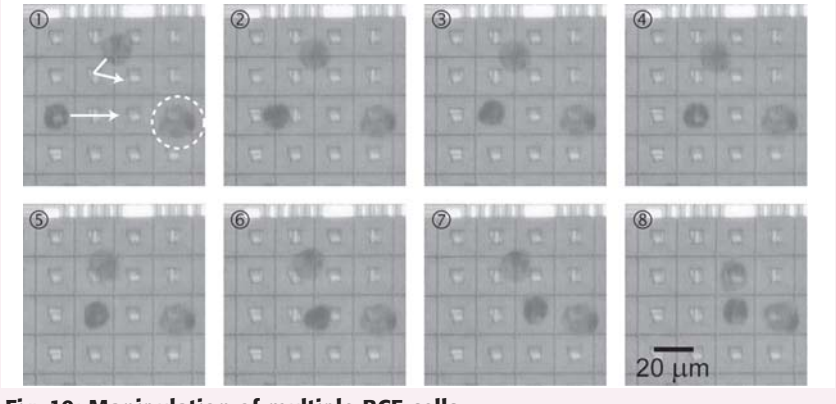

Fig. 10: Manipulation of multiple BCE cells.

because electronics are much faster than the motion of objects in fluid.

Fig. 10 shows manipulation of three BCE cells. The cell in the circle is held still; the rest cells are independently moved.

Electric manipulation of biological cells

In the same way that the spatially patterned magnetic fields move magnetic dipoles, spatially non-uniform electric fields produced by a microelectrode array can move dielectric objects (e.g., cells). Roberto Guerrieri's group at U. Bologna first implemented this *dielectrophoresis* in CMOS ICs [17]. We (the Westervelt group) also developed CMOS IC arrays in conjunction with a robust microfluidic system on top [18]. Depending on specific needs, a proper choice can be made between the electric and magnetic method. Each approach has advantages and disadvantages: while the magnetic method is more biocompatible as magnetic fields are transparent to cells, it requires more sample preparation steps (bead attachment).

Magnetic resonance based biosensors

Nanoscale magnetic beads (~ 30 nm) can be utilized for biosensing in a very fascinating way. Consider putting magnetic nanoparticles whose surfaces are modified with specific DNA strands into a bio-sample. If complementary strands exist in the sample, hybridizations occur and the magnetic nanoparticles self-assemble into clusters [Fig. 11(a)], as found by Ralph Weissleder's group at the Massachusetts General Hospital [19]. Similarly, magnetic nanoparticles coated with antibodies can self-assemble into clusters in the presence of specific target proteins [Fig. 11(b)]. This self-assembly can be detected using nuclear magnetic resonance (NMR) [19].

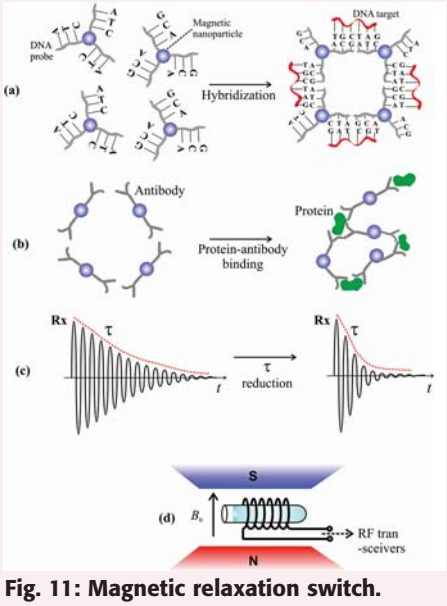

Fig. 11: Magnetic relaxation switch.

In a standard NMR experiment [Fig. 11(d)], nuclei spins within a sample (e.g., proton spins of hydrogen atoms within water) excited by a pulsed RF signal sent through the coil will initially precess about a common axis and at a common frequency, both of which are defined by the static magnetic field B_0. Spin-spin interactions, however, will interfere with this uniform precession, causing variations in precession frequencies among different spins. On a macroscopic average, the resultant loss of phase coherence manifests as an exponential relaxation (damping) in the precession of the net magnetic moment. During this precession and relaxation, the coil picks up a damped sinusoidal signal. The relaxation's characteristic time called T_2 is a measure of how fast coherence is lost.

The clusters (bigger magnets) that are formed from magnetic nanoparticles in the presence of target objects produce pronounced local magnetic fields that introduce spatial and temporal modulations on top of the static field B_0. This modulation introduces more precession frequency variations on top of those caused by the basic spin-spin interactions, accelerating the rate at which the system loses phase coherence. Therefore, the resultant reduction in the relaxation time T_2 [Fig. 11(c)] indicates the presence of target objects. This technique by Weissleder, which he aptly calls *magnetic relaxation switch*, is a new electronic biosensing modality [19,20].

Currently we (the Ham group with the Weissleder group) are miniaturizing the magnetic resonance biosensor [21,22]. Full NMR RF transceivers incorporating pulse-sequence techniques are integrated on CMOS ICs, along with an array of NMR microcoils. Again we encounter a microarray, whose effective operation is enabled by CMOS ICs. We believe that the sensitivity of our parallel NMR measurements of small divided samples on the microcoil array will dwarf that of the standard NMR measurement of one larger sample in statistically a very fascinating manner. Such high sensitivity would facilitate early disease detection.

CMOS biotechnology

With several implemented examples and feasible implementation ideas, we have illustrated how a CMOS IC can be utilized to electronically actuate and analyze micro and nanoscale biological objects in a sample placed on top. Here CMOS ICs play active roles in front-end sensing and actuation in direct contact with the biological world.

One powerful feature of the CMOS-bio interface uniquely derived from the use of CMOS technology is parallelism seen through various microarrays, combined with programmability. This enables rapid, sensitive, and selective detection and versatile actuation. Possibilities for label-free detection are an additional merit. As transistors become smaller with technology scaling, they will become more suitable for highly sensitive direct detection as field effect sensors. Sensitive front-end analog ICs will be an integral part of the CMOS-bio interface.

This field, which we call CMOS biotechnology, brings together various disciplines of engineering and science. There are many exciting developments, and we looked at only a small fraction of them, omitting fascinating subjects like neuron-CMOS interfaces [23-26] to study brain dynamics or to aid vision processes. Though limited, we hope this review has conveyed a meaningful perspective of this new fertile ground of research.

Contributors: Yong Liu and Hakho Lee enabled the magnetic manipulator work. Liu and Nan Sun in our group work with Lee, now at MGH with Weissleder, on the CMOS MR biosensor.

Acknowledgements: We thank William Andress of Harvard, Larry DeVito of ADI, NSF, and NIH for suggestions and supports.

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Robert M. Westervelt received the Ph.D. degree in physics from UC Berkeley in 1977. Following a postdoctoral appointment at Berkeley, he moved to Harvard University, where he is currently the Mallinckrodt Professor of Applied Physics and Physics.

His group investigates the quantum behavior of electrons inside nanoscale semiconductor structures and develops tools for the manipulation of biological systems. Current research topics include: imaging electron flow through nanoscale devices at low temperatures using scanning probe microscopy, making artificial molecules composed of few-electron quantum dots for quantum information processing, and the development of hybrid integrated circuits/microfluidic chips to trap, move, assemble, and sort biological cells and small particles in fluids. He is Director of the NSF-funded Nanoscale Science and Engineering Center at Harvard University, which includes participants from the Massachusetts Institute of Technology, UC Santa Barbara, and the Museum of Science in Boston. Previously, Prof. Westervelt was Director of the Materials Research Science and Engineering Center, and Co-Director of the Joint Services Electronics Program at Harvard.