A High-Sensitivity Micromachined Biosensor

DAVID R. BASELT, GIL U LEE, KAROLYN M. HANSEN, LINDA A. CHRISEY, AND RICHARD J. COLTON

Invited Paper

The Force Amplified Biological Sensor (FABS) is a desktop or portable instrument currently under development at the Naval Research Laboratory. FABS will use a rapid, automated immunoassay to detect analytes such as proteins, viruses, and bacteria. The assay uses forces produced by micron-sized magnetic particles to pull on antibody–antigen bonds. Microfabricated piezoresistive cantilevers measure the resulting piconewton-level forces with sufficient sensitivity to detect single antibody–antigen bonds. These forces also serve to characterize the bonds, allowing FABS to distinguish specific antibody–antigen bonds from nonspecific interactions.

I. Introduction

A. Immunoassays

Immunoassays are a group of methods that use antibodies to measure the concentration of an analyte. In the classic radioimmunoassay, antibodies are attached to a solid surface. A sample solution is flowed over this surface, and the antibodies "capture" analyte from the solution by binding to a specific location on the molecules. Radiolabeled antibodies in solution then bind to a second location on the analyte, becoming part of antibody-analyte-antibody "sandwiches" that are attached to the solid surface. After washing off excess radiolabeled antibodies, the amount of radioactivity remaining on the surface is proportional to the analyte concentration. The unique ability of antibodies to bind only one specific species of molecule within a sample containing millions of other species has made the immunoassay an invaluable tool for medicine, environmental testing, and biological research.

Mainly through the use of novel labeling techniques, certain experimental immunoassays have achieved zeptomole sensitivity, i.e. they can detect ~1000 molecules in a ~10 microliter sample [1]. Substantial effort has also been devoted to producing "point-of-care" immunoassay-based devices that are simple to operate and rugged enough to be used, not only by laboratories, but also by nurses, field personnel, and patients. Such devices generally use an electronic transducer to directly or indirectly detect the bound antibodies, in which case they are referred to as "immunosensors" [2]. The sensitivity of immunosensors is generally limited either by the transducer sensitivity or by nonspecific binding of molecules to the sensor.

Unfortunately, the cost, size, and mechanical complexity of immunosensors often precludes their use in settings other than clinical laboratories, where they provide only minimal advantages over existing techniques [3].

B. Atomic force microscopy

First described in 1986 [4], the atomic force microscope (AFM; also known as the scanning force microscope or SFM) can image surfaces both in air and under liquids at nanometer resolutions. In its repulsive or contact mode, the AFM lightly touches a tip at the end of a 50–300 μm long leaf spring (the "cantilever") to the sample. As a raster scan drags the tip over the sample, a detector measures the vertical deflection of the cantilever, which indicates the local sample height. The detector typically consists of a laser reflected off the cantilever and into a position-sensitive detector ("optical lever," Fig. 1) [5].

AFM can obtain atomic-resolution images on certain samples [6]. It also has the ability to measure how hard or sticky a sample is by pushing the tip down into the sample or pulling the tip up off the sample. Most significantly for the present work, AFM can measure the adhesion between the tip and sample with enough sensitivity to resolve single 10 pN hydrogen bonds [7], the weakest type of chemical bond.

C. AFM studies of interactions between biomolecules

If the tip and sample are coated with two types of molecules, an AFM can measure the force of attraction or repulsion between them, potentially at the level of single pairs of molecules. This type of measurement is of particular interest when applied to biomolecules (such as DNA or antibody/antigen

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D.R. Baselt, G.U Lee, and R.J. Colton are with the Naval Research Laboratory, code 6177, Washington, DC 20375 USA (e-mail: dbaselt@stm2.nrl.navy.mil; glee@stm2.nrl.navy.mil; rcolton@stm2.nrl.navy.mil).

K.M. Hansen is with Geo-Centers, Inc., Ft. Washington, MD 20375 (e-mail: khansen@stm2.nrl.navy.mil).

L.A. Chrisey is with the Naval Research Laboratory, code 6950, Washington, DC 20375 (e-mail: lchrisey@cbmse.nrl.navy.mil).

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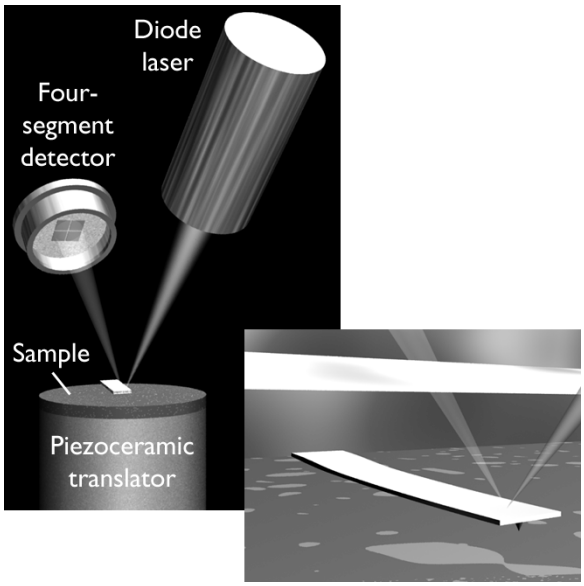

Fig. 1. Concept of AFM and the optical lever: (left) the optical lever and (right) close-up of the cantilever touching the sample. The sample, attached to the piezoceramic translator, moves underneath the cantilever. Scale drawing; the piezoceramic measures 24 mm in diameter, while the cantilever is 100 μm long.

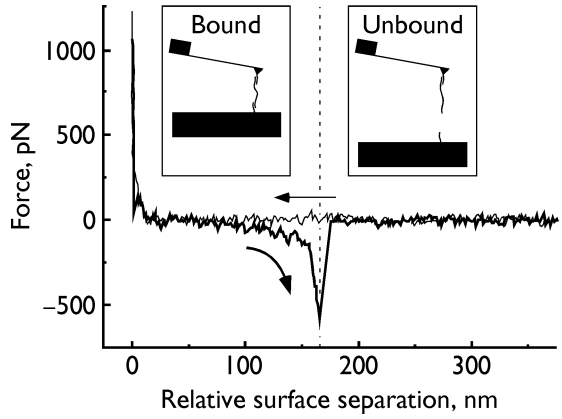

Fig. 2. Interaction force between two complementary strands of DNA, measured by AFM. "Relative surface displacement" is the distance between the tip and sample relative to the position at which 1000 pN of force is reached. Measurements are recorded both as the tip and sample are brought together (thin trace) and as they are separated (thick trace).

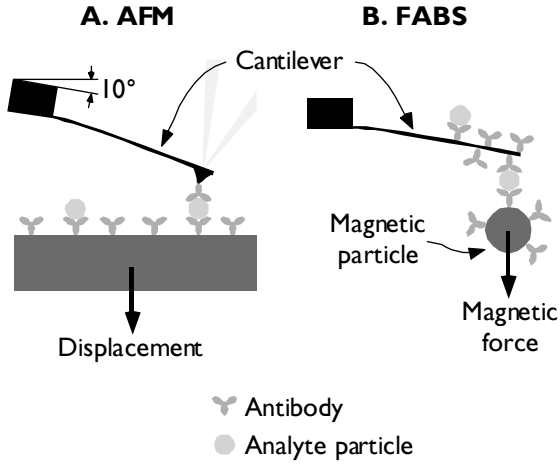

Fig. 3. Detection of antibody–antigen interaction forces with AFM and with FABS. A. In AFM, a piezoceramic translator moves an antibody-derivitized surface away from a cantilever until a single antibody–antigen bond breaks. B. In FABS, a magnetic field pulls on antibody–derivitized magnetic particles. The cantilever can bear thousands of particles, although the figure only shows one.

pairs) that specifically bind to or "recognize" each other. The technique is still experimental, but the ability to measure specific binding forces could offer unique insights into the structure and function of these highly-important molecules, and furthermore make it possible to detect or "map" specific molecules on the surfaces of living cells with unprecedented sensitivity and spatial resolution. To date, authors at the Naval Research Laboratory and elsewhere have used AFM to measure interaction forces between single pairs of DNA nucleotides [8], complementary DNA strands [9, 10], streptavidin and biotin [11–15], adhesion proteoglycans [16], and antibodies and their antigens [17, 18].

For example, Lee *et al.* have measured the force required to tear two complementary strands of DNA apart. In one such experiment (Fig. 2) [9], 20-base-pair long strands of polycytosine (i.e., single-stranded DNA) were covalently attached to the tip and sample. Free strands of polyinosine averaging 160 base pairs long were introduced. When the tip and sample were brought together, these strands would sometimes bind to both the polycytosine on the tip and that on the sample, bridging the tip and sample. The tip and sample were then pulled apart. The cantilever does not sense any force until the slack in the DNA is taken up, at which point tension on the DNA begins to pull the cantilever down. This pull is registered as negative force. When the force is large enough (–600 pN in Fig. 2), the DNA–DNA bonds at either the tip or sample break, and the force on the cantilever returns to zero. The fact that only one negative peak or break point is seen in Fig. 2 indicates that only a single polyinosine strand bridged the tip and sample. No adhesion force is observed if polyinosine is not present, so although this experiment was actually carried out to characterize DNA–DNA binding, it could also be said that it has detected the presence of polyinosine.

The nonideal geometry of most AFMs can produce artifacts [19]; for example, the ~10° tilt of the cantilever relative to the piezoceramic (Fig. 3A) produces lateral motion of tip versus sample during a measurement. Nonetheless, as expected from theory, intermolecular forces measured by AFM have a linear relationship to the enthalpy of bond formation (Table 1), and also depend on the speed of bond rupture [11].

D. Cantilever-based chemical sensors

Microfabricated cantilever beams for AFM were first constructed by Albrecht et al. in 1989 [20] and have helped make the AFM a successful laboratory instrument. The piconewton

Interaction	Force pN	ΔG kcal/mole	ΔH	Ref.
Avidin–desthiobiotin	90 ± 10	16.5	13.5	13
Avidin–biotin	160 ± 20	14.3	21.5	13
Antibiotin–biotin	240 ± 20	varies	varies	17
Streptavidin–biotin	260 ± 20	18.3	32.0	11
$(ACTG)_5$–$(CAGT)_5$	1500 ± 200	28	102.7	9

Table 1. Intermolecular adhesion forces measured by AFM compared with free energies (DG) and enthalpies (DH) of bond formation. Antibiotin is a polyclonal antibody against biotin; $(ACTG)_5$–$(CAGT)_5$ is an interaction between two complementary DNA strands.

force sensitivity, commercial availability, and reasonable cost of micromachined cantilevers contribute significantly to the ease of use and nanometer-scale resolution of AFMs.

These attributes have also inspired various researchers to use AFM cantilevers in novel, non-AFM chemical sensing applications. Gimzewski *et al.*[21] have used a bimetallic cantilever to measure, with a sensitivity of 1 pJ, the heat of reactions occurring on the cantilever. Thundat *et al.* [22] have coated cantilevers with various "sensitizing layers," the elastic modulus of which changes upon exposure to a gaseous sample (as a simple example, water vapor will soften a sensitizing layer of gelatin). By measuring the resulting resonant frequency shift of the cantilever Thundat *et al.* have detected humidity, mercury vapor, natural gas, and mercaptans with picogram resolution. O'Shea *et al.* were the first to use AFM-cantilever-based sensors in liquid, detecting surface stresses created by electrochemical processes [23].

II. FABS CONCEPT

FABS [24] is a cantilever-based immunosensor. It works by measuring intermolecular interactions in much the same way as an AFM, but it has a greatly simplified configuration. Rather than using a piezoceramic translator to pull on intermolecular bonds, FABS uses magnetic particles (Fig. 3), which eliminates the need to manually position a tip and sample next to each other with picometer precision and stability. The cantilever-beam force transducer is the only element of AFM that FABS retains, but (as described below) to further simplify the instrument we have replaced the optical lever with piezoresistive detection.

The prototype FABS will measure antibody–antigen interactions. One or more cantilevers with attached antibodies will capture antigen (i.e., the analyte) from a sample solution. 2 μm particles that also have attached antibodies will then bind to the captured antigen. The particles are made of a magnetic material, but to avoid aggregation they must not be magnetized at this stage. After they have bound to the cantilever, a large magnetic field will magnetize the particles while a modulated field gradient exerts force on them. This force will cause particles bound via antibody-antigen bonds to pull on and bend the cantilever, while dislodging nonspecifically-bound particles ("force discrimination"). The amount that the cantilever bends

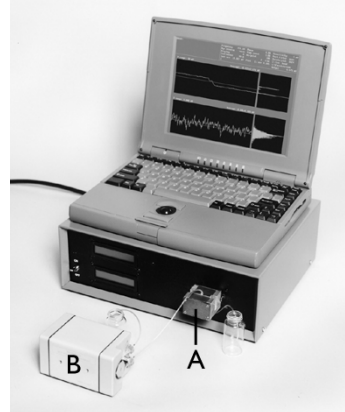

Fig. 4. Prototype FABS instrumentation. The FABS flow cell containing the cantilevers is mounted on the front (A). The electronics box contains a preamplifier, a lock-in amplifier, and a current source. The instrument also includes a small pump (B) and a laptop computer.

will indicate the number of particles bound to the cantilever and, therefore, the concentration of analyte in the sample. We have designed FABS to be capable of detecting a single bound particle, potentially corresponding to a single antigen molecule.

A second detection mode might involve gradually increasing the force on the magnetic particles and determining the number of particles that detach at the expected antibody–antigen interaction force. This method would provide more stringent force discrimination against nonspecifically-bound magnetic particles, but would also require magnetic fields 3–10 times larger than the FABS device currently generates.

A. Benefits of FABS

With its ability to detect a single bound molecule and distinguish specific from nonspecific interactions, FABS could potentially have 6–8 orders of magnitude more sensitivity than commonly-used immunoassays [24]. This ability would be of value for environmental monitoring. Testing for airborne bacteria or viruses, for example, can presently require several days of air collection to accumulate detectable amounts of analyte. The high sensitivity of FABS could dramatically reduce sampling times and speed the detection of dangerous microorganisms or chemicals.

Furthermore, FABS devices will be small, simple, and rugged, and therefore potentially suitable for point-of-care applications. The highly-miniaturized nature of FABS should also permit the development of multiple-analyte sensors, as will be described below.

The prototype FABS device is a small desktop unit (Fig. 4). The following sections discuss its critical components.

III. DETECTION HARDWARE

A. Helmholtz coils

The FABS sensor is essentially a miniaturized vibrating reed magnetometer [25] and, like this instrument, uses a two-component magnet assembly. A "C" shaped permanent magnet

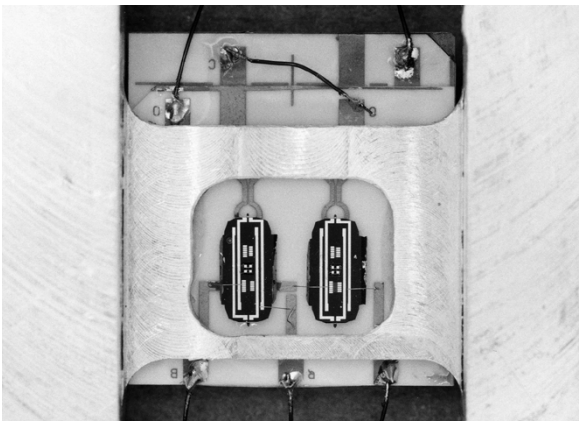

Fig. 5. FABS cell containing signal and reference Piezolevers™ mounted to a Helmholtz coil stack, which in turn is attached to an aluminum spacer. The cantilevers are glued onto the top coil substrate, which measures 12.5 x 12.5 mm. The bottom coil substrate, which measures 12.5 x 13.5 mm, is partially visible at the top of the photo. Normally, the Helmholtz coils would be hidden by a ground plane layer, but this particular FABS cell is an older design that lacks the ground plane. A second coil stack (not shown) mounts above the cantilevers and seals off the 37 µl liquid cell.

produces a 7300 G field that magnetizes the particles, while an antiparallel Helmholtz coil pair with radius r=0.5 mm produces a 200 Hz, 531 G/cm RMS oscillating field gradient that exerts force on the magnetized particles. The gradient produced at the cantilever, midway between the two coils, depends on coil current I, the number of turns in the coil N, and coil radius r as follows (SI units):

$$\frac{dB}{dZ} = 10^{-6} CNIr^{-2}$$

This equation assumes that the coil wire has no width. C is a constant that depends on coil separation s: C=1.079 if s=r; C=0.664 if s=2r as in our sensor. Smaller coils therefore provide greater field gradients and, because they have less resistance, require less power.

We make the coils by photolithography of 8 µm thick gold films deposited on both the front and back of 125 µm thick alumina substrates. Lithography is carried out on both the front and back of the substrates, and we glue two substrates together to form a four-layer stack. The layer closest to the cantilever is a ground plane that shields the cantilever from electric interference, while the other three layers are coils; i.e., N=3. The coil traces are 200 µm wide. Two coil stacks, separated by 1 mm, form a Helmholtz pair; the cantilever is midway between the two coil stacks. Each FABS cell currently contains two side-by-side Helmholtz pairs, one for a signal cantilever and one for a reference (Fig. 5). The total resistance of the coil assembly is 2.0 Ω; the 0.5 A RMS coil current dissipates 0.5 W of power. Without heat-sinking the coil assembly heats up to 60° C, so each coil is glued to a 25 × 25 × 3 mm aluminum backing that is in contact with the permanent magnet assembly. This arrangement keeps the temperature to 30° C, suitable for the antibody-antigen chemistry.

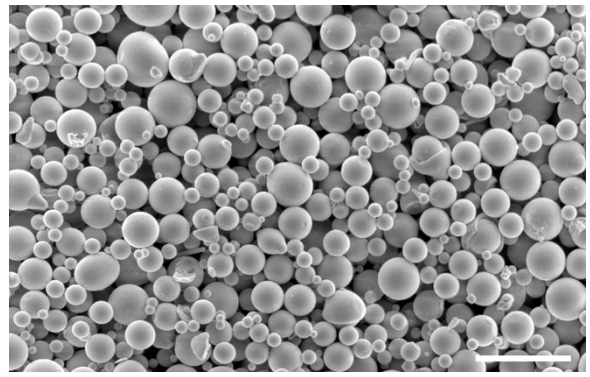

Fig. 6. Electron micrograph of gas-atomized NdFeBLa particles. Bar is 10 µm long.

Finite-element calculations indicate that, because the FABS coils have a finite width (200 µm), the field that they produce is 87% of the value calculated from the above equation.

The electric current requirements of the coils and the size of the permanent magnets will ultimately be the main factors that determine to what extent FABS can be miniaturized. Improvements in other FABS components — the magnetic particles and the cantilevers — will be needed to reduce these requirements.

B. Magnetic particles

The force generated by each magnetic particle is:

$$F = 0.524 Md^3 \frac{dB}{dZ}$$

where M is the volume magnetization of the magnetic material, d is the particle diameter, and dB/dZ is the field gradient generated by the Helmholtz coils.

Our intention is to use Dynabeads (Dynal, Inc., Lake Success, NY), commercially-available superparamagnetic particles consisting of 12–20% maghemite crystallites dispersed in 2.8 or 4.5 µm diameter polystyrene spheres. Dynabeads are

Type	*d* µm	density g/cm³	*M* cgs	*F* pN RMS
NdFeBLa	2.0 ± 1.1	8.24	731.3	11.1
Dynabeads M–280	2.8 ± 0.2	1.34	14.8	0.59
BioMag 8-4100B	nonspherical	2.5	273.2	?
Estapor 66% 1 µm	0.35 ± 0.15	2.24	116.2	0.0091

Table 2. Properties of several types of magnetic particles considered for FABS. Units for volume magnetization M are emu/cm³. The ideal particle would have small values of numerical average diameter d (to reduce drag) and density (to slow settling from solution), but high magnetization M and force F (to produce high signal levels). Values of M and F are for the 7300 G, 347 G/cm field in the FABS cell. BioMag particles are needle-shaped and the force that they exert will vary greatly depending on their orientation.

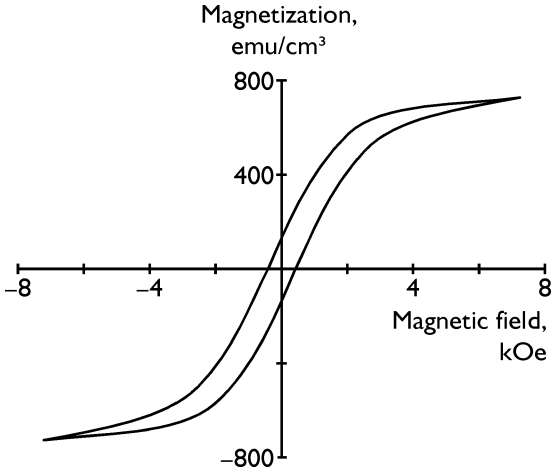

Fig. 7. Hysteresis loop showing magnetization of NdFeBLa particles as a function of magnetic field. Obtained by vibrating sample magnetometry.

remarkably uniform in diameter and can be obtained with covalently-attached streptavidin, so biotin-conjugated antibodies are easily attached (see "Antibody attachment chemistry", below). However, Dynabeads and other commercial particles such as Estapor® beads (Bangs Laboratories, Carmel, IN) and BioMag® particles (PerSeptive Biosystems, Framingham, MA) are only weakly magnetic compared to most ferromagnetic materials (table 2).

We have tested a number of ferromagnetic particles, the most magnetic of which is a gas-atomized $Nd_{1.3}Fe_{13}BLa_{0.002}$ powder (Fig. 6) [26] custom-made by UltraFine Powder Technology (Woonsocket, RI). The powder has been aerodynamically size-classified to a measured numerically-weighted mean diameter of 2.0±1.1 μm (specified mass-weighted mean diameter, 6.35 μm). It is 50 times more magnetic than 2.8 μm Dynabeads (Table 2 and Fig. 7). Although NdFeB-type materials corrode easily, tests showed no detectable change in magnetization after 12 hours in solution.

Developing these custom particles is a major research challenge. The initially-unmagnetized particles tend to magnetize each other during stirring or sonicating operations, resulting in excessive aggregation. Also, the particles quickly settle out of solution because of their high density. Unlike Dynabeads, gas-atomized particles do not have a uniform diameter, so they will have to be further size-classified by sedimentation. Finally, as discussed below, any custom particles will require chemical modifications to attach antibodies.

C. Cantilevers

FABS uses micromachined piezoresistive cantilevers [27], which are single-crystal silicon structures with a boron-doped surface layer. This layer is conductive, and its resistance changes by a few percent per micron of cantilever deflection. Piezoresistive cantilevers thus have the considerable advantage that the deflection-sensing element is integral to the cantilever, so unlike the optical lever, they require no external lasers and detectors that the user must manually adjust.

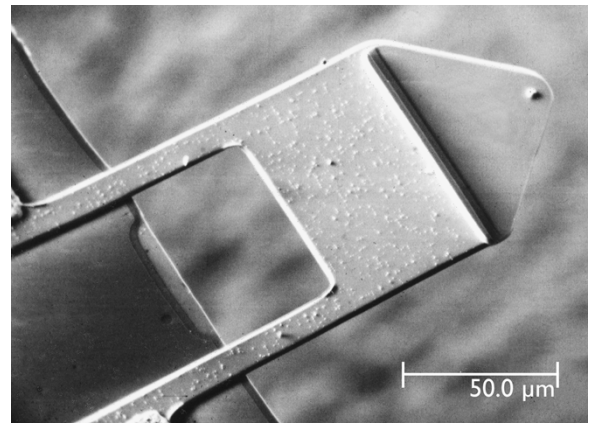

Fig. 8. Electron micrograph of a Park Scientific Instruments Piezolever™. The cantilever measures 90 × 150 μm.

In the FABS device, the resistance of the piezoresistive cantilever is measured with a Wheatstone bridge. This arrangement produces an output voltage given by:

$$V = \frac{FV_b}{4k}\frac{\Delta R}{R}$$

where F is the force exerted on the cantilever, V_b is the Wheatstone bridge bias voltage, k is the cantilever spring constant, and $\Delta R/R$ is the resistance change of the cantilever per unit deflection (ΔR) divided by the resistance of the cantilever (R).

The bias voltage V_b is set to 5 V. When the metal leads attached to a piezoresistive cantilever are immersed in physiological salt solution (containing 150–300 mM NaCl), V_b drives both corrosion of the leads and electrolysis of the solution. Aluminum leads in particular cannot be protected by a reasonable thickness (<1 μm) of coatings such as silicon nitride [28]. Gold leads are preferable; tests with electrochemical cells have demonstrated that neither gold nor the cantilever material corrode when V_b is less than or equal to 5 V. The only effective method we have found to protect aluminum leads involves gluing a small rectangle of 175 μm thick glass over them.

The FABS device presently uses piezoresistive cantilevers from Park Scientific Instruments (PSI; Sunnyvale, CA). Several types of Piezolevers™ are available, but the ones we have obtained (Fig. 8) are 150 μm long and 2 μm thick, with k=2.5 N/m and $\Delta R/R$=2.5×10^{-6} per nm. These cantilevers are intended for AFM and are therefore optimized for deflection sensing: their noise level in terms of deflection is 5.0 pm; in terms of force, 12.5 pN.

By making the cantilevers longer and thinner, we expect to obtain significantly force higher sensitivity. Deflection sensitivity is correspondingly lowered, but this is not important for FABS. Piezoresistive cantilevers have been fabricated that are 300 μm long and 1 μm thick, with k=0.0073 N/m and $\Delta R/R$=0.21×10^{-6} per nm; their noise level is 84 pm or 0.616 pN [29].

To cancel out noise from external vibrations, FABS uses a reference cantilever that is identical to the signal cantilever

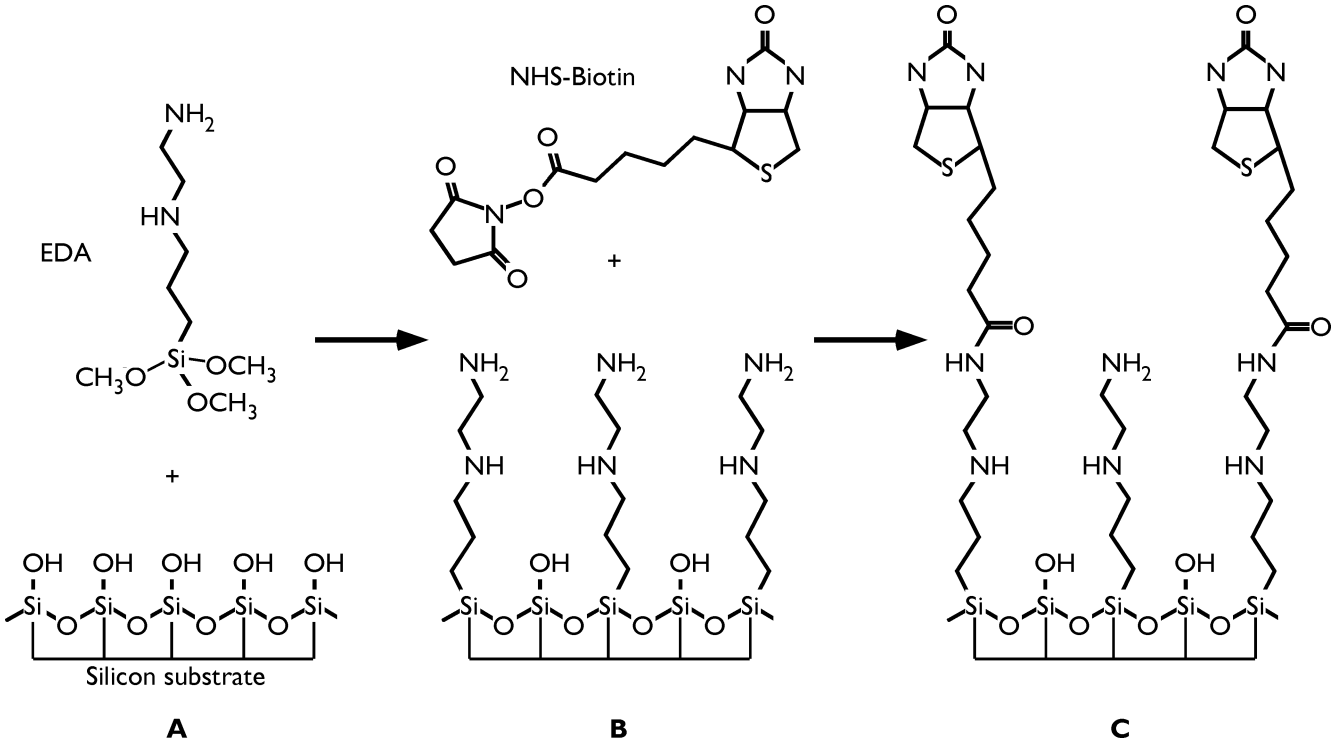

Fig. 9. Strategy for biotinylating the silica surface of a cantilever. A. N-2-aminoethyl-3-aminopropyltrimethoxysilane (EDA; Huls of America, Piscataway, NJ) films are formed on the silica surface by immersion in an acidic solution of 1% silane for 30 min at room temperature. The surfaces are subsequently rinsed and heated to 120° for 3 min to drive covalent condensation of the silanes. B. The silane films are immersed in 1 nM biotin-N-hydroxysuccinimide ester (NHS-biotin) in dry DMSO. NHS-biotin reacts with the primary amine of EDA. C. Streptavidin-conjugated antibodies will bind tightly to the resulting biotinylated surface.

except that it does not have an antibody coating. Although each cantilever presently has its own Helmholtz coil, finite-element magnetic field calculations indicate that four 100 μm wide cantilevers could share a coil and still experience a field gradient homogenous to within ±4%.

IV. Antibody attachment chemistry

The development of a method to immobilize antibodies on cantilevers and magnetic particles is a critical component of the FABS program. Many immunoassays use antibodies that have simply been absorbed onto a solid substrate, but the resulting bond is not strong enough to withstand the magnetic forces used for FABS detection and for the force discrimination method described above. For FABS the antibodies must be strongly attached, leaving the antibody–antigen bond as the weakest in the immunoassay sandwich.

Furthermore, attachment of antibodies to substrates frequently reduces their ability to bind antigen. By binding the antibodies in a controlled orientation and/or allowing them mobility on the end of a "tether" molecule [17, 30], a well-designed covalent attachment strategy can enhance the fraction of antibodies that remain fully functional, potentially improving the sensitivity of the immunoassay.

Although the cantilever and NdFeBLa magnetic particle surfaces have different compositions (SiO_2 and iron/neodymium oxide, respectively), they can be chemically modified using similar protocols. Our approach is based on organosilane films, which self-assemble onto hydroxylated surfaces and can present a wide variety of functional groups at their surface. Certain silanes are photolabile and can thus be photo-patterned by ablating with ultraviolet radiation [31]. Since ablated regions can then be resilanized, the procedure can be repeated to pattern a number of different functional groups or physical properties [32] onto a single surface. Several laboratories have used organosilane chemistry to create patterned surfaces of nucleic acids [33, 34] and antibodies [35–37].

Surface modification of FABS cantilevers begins by generating an aminosilane film. A heterobifunctional crosslinker is then attached to the aminosilane; the crosslinker can in turn react with thiols or amines on the antibody or with other biomolecules. Alternatively, the aminosilane film may be functionalized with a biotin-N-hydroxysuccinimide ester (Fig. 9). The resulting biotinylated surface binds the protein streptavidin with high specificity and bond strength. Many antibodies are commercially available as streptavidin (or biotin) conjugates; streptavidin-biotin attachment is widely used in immunoassays.

Photopatterning of the aminosilane films will allow us to confine antibody attachment to a "active region" of about 100 × 100 μm at the end of the signal cantilever, while covering reference cantilevers and other regions with a second silane such as polyethylene glycol (PEG) silane that reduces nonspecific adsorption of analyte molecules. Photopatterning will also be essential to the fabrication of multianalyte arrays of cantilevers.

VIII. Sensitivity and dynamic range

Our initial demonstrations of the FABS equipment have used NdFeBLa particles, since at present the equipment is not capable of detecting Dynabeads with the desired single-bead sensitivity. As calculated from the above equations, and accounting for a differential amplifier with 10^4 gain, the prototype FABS instrument should yield 0.15 mV of signal per 2 μm NdFeBLa particle. The measured noise level, when detecting with a lock-in amplifier at 200 Hz, is 0.044 mV/√Hz; signal averaging for 102.4 seconds produces a 99% confidence interval of ±0.021 mV. Most of this noise is due to Johnson noise in the piezoresistive cantilever [27].

Although we have not yet integrated chemistry and hardware to perform a complete FABS assay, we have glued a NdFeBLa particle onto a FABS cantilever and measured the resulting signal. In air the measured signal is almost exactly the calculated value; with the cantilever immersed in water, the signal drops to about half the calculated value, perhaps because of damping.

A single bound particle, though detectable, has little statistical significance for a quantitative assay. On the other hand, only about 5000 2 μm particles will fit on each cantilever. Each cantilever will therefore have a dynamic range on the order of 10^2 (~10–1000 particles). This limited range will require combining, for example, three cantilevers with different antibody densities to obtain a more reasonable dynamic range of 10^6. Thus a 1 × 1 cm array of nine Helmholtz coils, each containing one reference and three signal cantilevers, would be capable of detecting nine analytes.

The sensitivity of FABS is also limited by the number of analyte molecules that come into contact with the active area during the assay. In the limit in which few analyte molecules have bound to the surface, adsorption studies indicate that antibody-antigen bonding is irreversible — i.e. the bonds do not spontaneously dissociate [38] — and mass transport considerations limit the rate of antibody–antigen bond formation. Assuming the cantilever does not affect the flow of fluid at its surface, the number of molecules N that come into contact with the active region is:

$$N = kAt\left(C - C_0\right)$$

where k is the mass transport coefficient (~0.001 m/sec [39]), A is the active area, t is the amount of time, C is the concentration (in molecules/m^3) of the analyte in solution, and C_0 is the concentration at the cantilever surface (initially 0). If we assume that N=1000 contacts produces 10 detected antibody–antigen bonds, and given the 2×10^{-8} m^2 active area of a cantilever and t=600 s, the minimum detectable concentration of analyte is 1×10^{-16} M. This value could be improved by using a larger cantilever, or several cantilevers in parallel, to increase the active area.

V. Conclusion

Although development of FABS is still in its early stages, the sensor promises a unique combination of chemical sensitivity and mechanical simplicity. We have developed hardware capable of detecting a single 2.0 μm NdFeBLa magnetic particle and are developing covalent attachment chemistry. Our present goals include demonstrating detection of streptavidin–biotin binding in a FABS cell, followed by a complete assay using antibody–antigen interactions.

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References

[1] L.J. Kricka, "Selected strategies for improving sensitivity and reliability of immunoassays," *Clin. Chem.* vol. 40, pp. 347–357, 1994.

[2] C.L. Morgan, D.J. Newman, and C.P. Price, "Immunosensors: technology and opportunities in laboratory medicine," *Clin. Chem.* vol. 42, pp. 193–209, 1996.

[3] B. Manning and T. Maley, "Immunosensors in medical diagnostics — major hurdles to commercial success," *Biosensors and Bioelectronics* vol. 7, pp. 391–395, 1996.

[4] G. Binnig, C.F. Quate, and Ch. Gerber, "Atomic force microscope," *Phys. Rev. Lett.* vol. 56, pp. 930–933, 1986.

[5] G. Meyer and N.M. Amer, "Novel optical approach to atomic force microscopy," *Appl. Phys. Lett.* vol. 53, pp. 1045–1047, 1988.

[6] Y. Sugawara, M. Ohta, H. Ueyama, S. Morita, F. Osaka, S. Ohkouchi, M. Suzuki, and S. Mishima, "Atomic resolution imaging of InP(110) surface observed with ultrahigh vacuum atomic force microscope in noncontact mode," *J. Vac. Sci. Technol. B* vol. 14, pp. 953–956, 1996.

[7] J.H. Hoh, J.P. Cleveland, C.B. Prater, J.-P. Revel, and P.K. Hansma, "Quantized adhesion detected with the atomic force microscope," *J. Am. Chem. Soc.* vol. 114, pp. 4917–4918, 1992.

[8] T. Boland and B.D. Ratner, "Direct measurement of hydrogen bonding in the DNA nucleotide bases by atomic force microscopy," *Proc. Natl. Acad. Sci. USA* vol. 92, pp. 5297–5301, 1995.

[9] G.U Lee, L.A. Chrisey, and R.J. Colton, "Direct measurement of the forces between complementary strands of DNA," *Science* vol. 266, pp. 771–773, 1994.

[10] E.-L. Florin, M. Rief, H. Lehmann, M. Ludwig, C. Dornmair, V.T. Moy, and H.E. Gaub, "Sensing specific molecular interactions with the atomic force microscope," *Biosens. Bioelectron.* vol. 10, pp. 895–901, 1995.

[11] G.U Lee, D.A. Kidwell, and R.J. Colton, "Sensing discrete streptavidin–biotin interactions with atomic force microscopy," *Langmuir* vol. 10, pp. 354–357, 1994

[12] E.-L. Florin, V.T. Moy, and H.E. Gaub, "Adhesion forces between individual ligand-receptor pairs," *Science* vol. 264, pp. 415–417, 1994.

[13] V.T. Moy, E.-L. Florin, and H.E. Gaub, "Intermolecular forces and energies between ligands and receptors", *Science* vol. 266, pp. 257–259, 1994

[14] U. Dammer, D. Anselmetti, M. Dreier, M. Hegner, W. Huber, J. Hurst, G. Misevic, and H.-J. Güntherodt, "Measuring molecular adhesion with force microscopy," in *Forces in Scanning Probe Methods*, H.-J. Güntherodt, D. Anselmetti, and E. Meyer, Ed.: Kluwer Academic Publishers, 1995, pp. 625–631.

[15] A. Chilkoti, T. Boland, B.D. Ratner, and P.S. Stayton, "The relationship between ligand-binding thermodynamics and protein-ligand interaction forces measured by atomic force microscopy," *Biophys. J.* vol. 69, pp. 2125–2130, 1995.

[16] U. Dammer, O. Popescu, P. Wagner, D. Anselmetti, H.-J. Güntherodt, and G.N. Misevic, "Binding strength between cell adhesion proteoglycans measured by atomic force microscopy," *Science* vol. 267, pp. 1173–1175.

[17] P. Hinterdorfer, W. Baumgartner, H.J. Gruber, K. Schilcher, and H. Schindler, "Detection and localization of individual antibody-antigen recognition events by atomic force microscopy," *Proc. Natl. Acad. Sci. USA* vol. 93, pp. 3477–3481, 1996.

[18] U. Dammer, M. Hegner, D. Anselmetti, P. Wagner, M. Dreier, W. Huber, and H.-J. Güntherodt, "Specific antigen/antibody interactions measured by force microscopy," *Biophys. J.* vol. 70, pp. 2437–2411, 1996.

[19] J.K. Stuart and V. Hlady, "Effects of discrete protein–surface interactions in scanning force microscopy adhesion force measurements," *Langmuir* vol. 11, pp. 1368–1374, 1995.

[20] T.R. Albrecht, S. Akamine, T.E. Carver, and C.F. Quate, "Microfabrication of cantilever styli for the atomic force microscope," *J. Vac. Sci. Technol. A* vol. 8, pp. 3386–3396, 1990.

[21] J.K. Gimzewski, Ch. Gerber, E. Meyer, and R.R. Schlitter, "Observation of a chemical reaction using a micromechanical sensor," *Chem. Phys. Lett.* vol. 217, pp. 589–594,1994.

[22] T. Thundat, G.Y. Chen, R.J. Warmack, D.P. Allison, and E.A. Wachter, "Vapor detection using resonating microcantilevers," *Anal. Chem.* vol. 67, pp. 519–521, 1995.

[23] S.J. O'Shea, M.E. Welland, T.A. Brunt, A.R. Ramadan, and T. Rayment, "Atomic force microscopy stress sensors for studies in liquids," *J. Vac. Sci. Technol. B* vol. 14, pp. 1383–1385, 1996

[24] Previously described in D.R. Baselt, G.U Lee, and R.J. Colton, "Biosensor based on force microscope technology," *J. Vac. Sci. Technol. B*, vol. 14, pp. 789–793.

[25] See for example H.J. Richter, K.A. Hempel, and J. Pfeiffer, "Improvement of sensitivity of the vibrating reed magnetometer," *Rev. Sci. Instrum.* vol. 59, pp. 1388–1393, 1988.

[26] See for example Y. Sakaguchi, T. Harada, and T. Kuji, "Microstructural studies of Nd–Fe–B powders produced by gas atomization," *Mat. Sci. Eng.* vol. A181/A182, pp. 1232–1236, 1994.

[27] M. Tortonese, R.C. Barrett, and C.F. Quate, "Atomic resolution with an atomic force microscope using piezoresistive detection," *Appl. Phys. Lett.* vol. 62, pp. 834–836, 1993.

[28] P.M. Natishan, E. McCafferty, E.P. Donovan, D.W. Brown, and G.K. Hubler, "The pitting behavior of silicon nitride ion beam assisted deposited coatings on aluminum," *Surf. Coatings Tech.* vol. 51, pp. 30–34, 1992.

[29] J. Harley and T. Kenny, personal communication. The process by which these cantilevers are made is described in B.W. Chui, T.D. Stowe, T.W. Kenny, H.J. Mamin, B.D.Terris, and D.Rugar, "Low-stiffness silicon cantilevers for thermal writing and piezoresistive readback with the atomic force microscope," *Appl. Phys. Lett.* vol. 69, pp. 2767–2769, 1996

[30] G.U Lee, L.A. Chrisey, C.E. O'Ferrall, D.E. Pilloff, N.H. Turner, and R.J. Colton, "Chemically specific probes for the atomic force microscope," *Israeli J. Chem.* (in press).

[31] J.M. Calvert, "Lithographically patterned self-assembled films," in *Thin Films, vol. 20: Organic Thin Films and Surfaces*, A. Ulman, Ed. Boston: Academic Press, 1995, pp. 109–141.

[32] C.S. Dulcey, J.H. Georger, V. Krauthamer, T.L. Fare, D.A. Stenger, and J.M. Calvert, "Deep UV photochemistry of chemisorbed monolayers: patterned coplanar molecular assemblies," *Science* vol. 252, pp. 551–554.

[33] L.A. Chrisey, P.M. Roberts, V.I. Benezra, W.J. Dressick, C.S. Dulcey, and J.M. Calvert, "Selective attachment of synthetic DNA to self-assembled monolayer functionalized surfaces," in *Mat. Res. Soc. Symp. Proc.: Biomolecular Materials by Design* vol. 300, pp. 179–184, 1994.

[34] L.A. Chrisey, C.E. O'Ferrall, B.J. Spargo, C.S. Dulcey, and J.M. Calvert, "Fabrication of patterned DNA surfaces," *Nucleic Acids Research*, in press.

[35] S.K. Bhatia, L.C. Shriver-Lake, K.J. Prior, J.H. Georger, J.M. Calvert, R. Bredehorst, and F.S. Ligler, "Use of thiol-terminal silanes and heterobifunctional crosslinkers for immobilization of antibodies on silica surfaces," *Anal. Biochem.* vol. 178, pp. 408–413, 1992.

[36] S.K. Bhatia, J.J. Hickman, and F.S. Ligler, "New approach to producing patterned biomolecular assemblies," J. Am. Chem. Soc. vol. 114, pp. 4432–4433, 1992.

[37] S.K. Bhatia, J.L. Teixera, M. Anderson, L.C. Shriver-Lake, J.M. Calvert, J.H. Georger, J.J. Hickman, C.S. Dulcey, P.E. Schoen, and F.S. Ligler, "Fabrication of surfaces resistant to protein adsorption and application to two dimensional protein patterning," *Anal. Biochem.* vol. 208, pp. 197–205, 1993.

[38] M. Stenberg and H. Nygren, "Kinetics of antigen–antibody reactions at solid–liquid interfaces", *J. Immun. Meth.* vol. 113, pp. 5–5, 1988.

[39] The mass transport coefficient is for a molecule with a diffusion coefficient of 10^{-7} cm²/s flowing through a cylindrical tube of diameter 0.2 mm at a velocity of 1 cm/s. E.L. Cussler, *Mass Transfer in Fluid Systems*, New York: Cambridge University Press, 1994, p. 230.

David R. Baselt leads the detection hardware aspect of the FABS project. He received a B.S. in chemistry from the University of California at Berkeley in 1988 and a Ph.D. in physical chemistry from the California Institute of Technology (1993), where after constructing an early atomic force microscope, he used novel imaging modes to study tip–sample interactions in atomic force microscopy and their effects on biological samples. He spent a year as a postdoctoral associate in cell biology at Yale University and then joined the Naval Research Laboratory as a postdoctoral fellow in 1994. His research is focused on the development of novel sensors.

Gil U Lee leads the immunoassay aspect of the FABS project and is a co-inventor of the FABS concept. He received a B.S. degree in chemical engineering from Purdue University (1987) and a Ph.D. degree in chemical engineering from the University of Minnesota (1992), where he studied adsorption of proteins at interfaces. He joined the Naval Research Laboratory as a postdoctoral fellow in 1992 and became a staff member in 1995. His research is focused on the study of surface, inter- and intramolecular forces in complex systems and their use in novel sensor and materials applications.

Karolyn M. Hansen is developing surface modification and immunoassay protocols for FABS. She received a B.S. degree in biology from The Pennsylvania State University (1980), an M.S. in environmental science and toxicology from Drexel University (1984), and a Ph.D. in marine biology and biochemistry from the University of Delaware (1990). During this time she studied adhesion proteins and biomineralization in molluscs. She joined Geo-Centers, Inc. in 1996.

Linda A. Chrisey is developing surface modification and patterning methods for FABS. She received her B.S. in biochemistry from the State University of New York at Binghamton (1983), followed by a Ph. D. in bioorganic chemistry from the University of Virginia (1988), where she isolated and characterized natural products that mediate DNA cleavage. Dr. Chrisey completed a postdoctoral appointment at the National Institutes of Health, synthesizing pharmacologically relevant anti-inflammatory and psychoactive alkaloids. She then joined Synthecell Corp. where as Senior Scientist she supervised production of chemically modified nucleic acids and helped develop the company's research and development efforts in antisense therapeutics. Dr. Chrisey joined the Naval Research Laboratory's Center for BioMolecular Science and Engineering in 1991, and became a full-time staff member in 1993. Her research program focuses on the use of microlithographic techniques to create surfaces patterned with biomolecules such as DNA and proteins, and on development of novel biomaterials.

Richard J. Colton is a supervisory research chemist and Head of the Advanced Surface Spectroscopy section at the Naval Research Laboratory (NRL), Washington, DC. He earned his B.S. and Ph.D. degrees from the University of Pittsburgh in 1972 and 1976, respectively. He performed his graduate work in the areas of ultraviolet and X-ray photoelectron spectroscopy. In 1976, he was a National Research Council Resident Research Associate at NRL working on secondary ion mass spectrometry (SIMS). Dr. Colton joined the NRL staff in 1977 and conducts basic and applied research in surface chemistry. His current research interests include the measurement of the nanomechanical properties of materials using atomic force microscopy and the development of new sensors using electron tunneling and force transducers. He has published over 130 technical papers included three book chapters and five patents. Dr. Colton is a member of the American Chemical Society (ACS), Sigma Xi, American Vacuum Society (AVS), American Physical Society (APS), and Materials Research Society (MRS). He is the recipient of the 1992 Hillebrand Prize awarded by the Chemical Society of Washington and was elected AVS Fellow in 1995.