

Biosensor based on force microscope technology

David R. Baselt, Gil U Lee, and Richard J. Colton

Naval Research Laboratory, Code 6177, Washington, DC 20375-5342

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We are developing a sensor capable of detecting biological species such as cells, proteins, toxins, and DNA at concentrations as low as 10^{-18} M. The force amplified biological sensor will take advantage of the high sensitivity of force microscope cantilevers to detect the presence of as little as one superparamagnetic particle bound to a cantilever by a sandwich immunoassay technique. The device, which will ultimately be small enough for hand-held use, will perform an assay in about 10 min. Lock-in detection and use of a reference cantilever will provide a high degree of vibration immunity. An array of ten or more cantilevers will provide greater sensitivity and the capability to detect multiple species simultaneously. The force amplified biological sensor also offers the potential of distinguishing and studying chemical species via its ability to measure binding forces.

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I. INTRODUCTION

The force amplified biological sensor (FABS) is primarily motivated by the need for a highly-sensitive assay capable of monitoring the concentration of various biological species in the environment. Ideally, such an assay would operate remotely, and would therefore be fully-automated, compact, and rugged. Since the assay may warn of a potential threat to human health, it should also be rapid.

These requirements — sensitivity, speed, automatic operation, and ruggedness — are also of value in more common clinical applications. We therefore believe that FABS will prove useful in a variety of settings.

II. FABS CHEMISTRY

FABS could potentially detect a wide variety of biologically-active materials, including toxins, proteins, viruses, and bacteria, in concentrations approaching a single particle per 10–100 μ l sample volume. To accomplish this it uses a sandwich assay, a proven technique found in various commercially-available assays such as the Enzyme-Linked Immunosorbent Assay (ELISA).¹

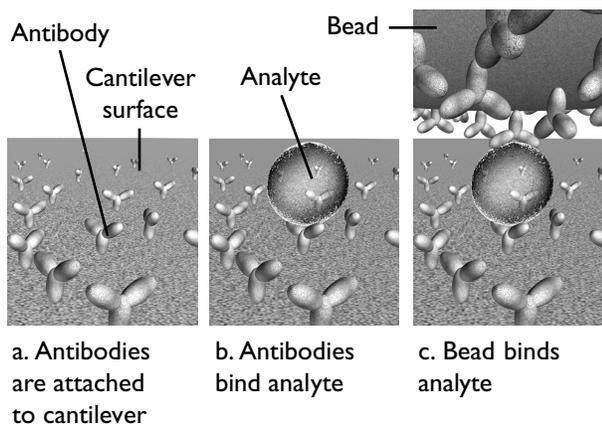


Figure 1. Immunobead sandwich assay.

In the implementation of the sandwich assay that we use, antibodies against a particular protein, virus, or bacterium are covalently bound to a solid surface [Fig. 1(a)]. For this example, assume we are trying to detect a virus. The sample solution flows over the surface, and the antibodies capture any of the virus present [Fig. 1(b)]. Next, superparamagnetic beads, also coated with an antibody against the virus, flow through the liquid cell and bind to the analyte [Fig. 1(c)]. After washing away excess beads, a number of beads remain bound to the surface through the virus. By determining this number of beads, we can calculate the concentration of virus in the original sample.

III. FABS HARDWARE

A. Force transducer

The beads are large enough to count with an optical microscope, but such a detection method is neither auto-

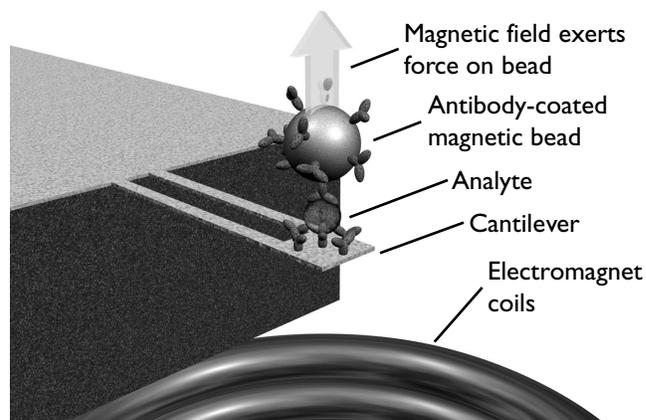


Figure 2. FABS concept. A cantilever-beam force transducer senses the presence of magnetic beads, the number of which is proportional to the concentration of analyte in the sample. Not to scale; in general, not one but many beads will attach to the cantilever.

matic, compact, nor rugged. Therefore, we have decided to use a cantilever-beam force transducer (Fig. 2) developed for atomic force microscopy² (AFM). The surface of the cantilever is coated with antibodies in the first step of the sandwich assay. The beads (Dynal, Inc., Lake Success, NY) are superparamagnetic — 2.8 or 4.5 μm diam polystyrene spheres impregnated with 60% magnetite (Fe_3O_4). Ferromagnetic beads would provide more signal, but they could also aggregate. After the beads become bound to the cantilever, we turn an electromagnet on. The magnetic field pulls on the beads, which pull on the cantilever and make it bend.

AFM technology provides a number of ways to measure the bending of the cantilever. Currently, we are using piezoresistive cantilevers³ (PiezoleversTM) from Park Scientific Instruments (Sunnyvale, CA). These measure 150 μm long, 90 μm wide, and 2 μm thick. They have a nominal spring constant of 2.5 N/m, resonant frequency of 60 kHz, and a resistance of about 2 k Ω that changes by about 0.5 m Ω for each Ångström of deflection.⁴ Unlike the optical detection methods commonly used in AFM, piezoresistive cantilevers do not require external sensing hardware. This is an important advantage for FABS, since such hardware usually requires manual alignment to the cantilever and tends to be large and easily damaged.

Unlike AFM, FABS does not have a scanning element, feedback, or tip-sample approach. The only element that FABS has in common with AFM is the cantilever.

Using a cantilever, and specifically a piezoresistive cantilever, provides several benefits. First, since the cantilever is micromachined, it satisfies our requirement for a miniature sensor. Second, we can easily make an array of, for example, ten cantilevers. The array could sense either ten different species, or a single species with ten times the sensitivity. Third, since the cantilever is a force transducer, we could increase the magnetic field until the antibody-antigen bond breaks and measure the antibody binding forces. The same principle could be used to study other types of intermolecular interaction.⁵ Such experiments would require about twice the magnetic force that we currently use.

We have previously demonstrated the viability of cantilever-based FABS detection by attaching 2.8 μm diam streptavidin-coated beads to a biotin-coated cantilever. Waving a small magnet over the cantilever produced a measurable deflection, as detected with an optical lever. Counting the number (270) of beads with a microscope and dividing the observed signal by this number demonstrated that sufficient signal-to-noise existed to measure the presence of a single bead.

B. Instrumental noise

PiezoleversTM are about an order of magnitude less sensitive than optical lever detection. More significantly, in a “real-world” setting, vibrations will often interfere with deflection measurements. For these reasons, we have to use AC detection (Fig. 3) to obtain reasonable FABS noise levels. A waveform generator oscillates the electromagnet current at about 100–200 Hz, and a lock-in amplifier measures the root-

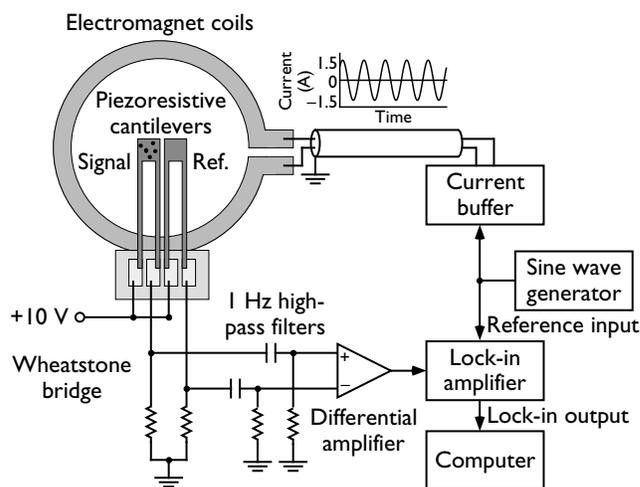


Figure 3. FABS electronic schematic showing reference cantilever and lock-in detection. Differential amplifier gain is currently 10⁴.

mean-square (rms) cantilever deflection. For single-bead detection, a measurement bandwidth of 1 Hz should give a signal-to-noise ratio of about one even without vibration isolation. Since the cantilever is in a solution, it seems unlikely that detecting at cantilever resonance (60 kHz) would significantly improve the signal.

A reference cantilever (Fig. 3), identical to the signal cantilever but without the covalently-bound antibodies, further improves performance. External vibrations cause both the signal and the reference cantilever to vibrate. Without the reference cantilever, such vibrations might, in severe conditions, overwhelm the minute forces exerted by the beads. Subtracting the reference from the signal helps reduce such interference. The reference also cancels out some electromagnetic interference (discussed below).

Assuming detection at 100–200 Hz with a 1 Hz bandwidth, the expected electronic noise of a PiezoleverTM is 5 μm .⁴ We operate the instrument on a tabletop without vibration isola-

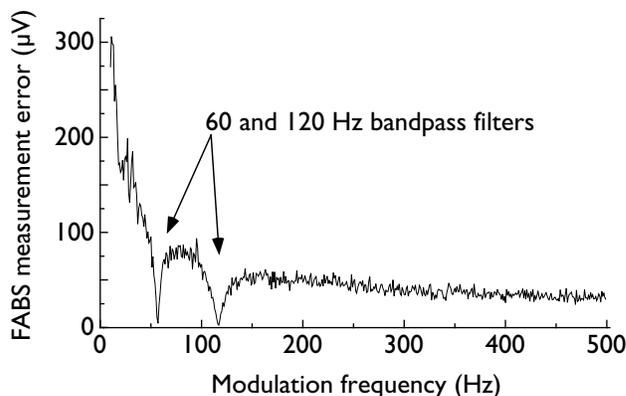


Figure 4. Measured noise of the prototype FABS as a function of frequency. Each point in the graph is the 95% confidence interval for a 102.4-second average of lock-in amplifier output. Bandpass filters on the lock-in amplifiers create the two notches. Nominal calibration: 20 $\mu\text{V}/\text{pN}$.

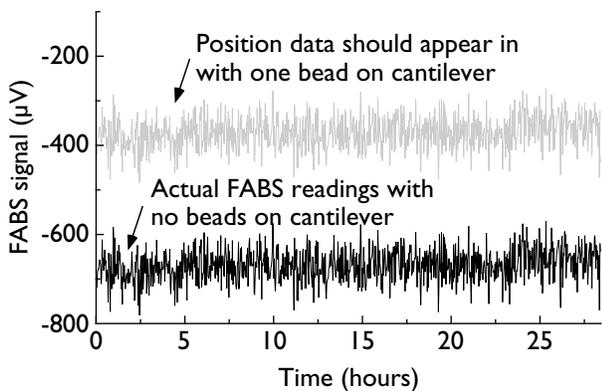


Figure 5. Graph of 1,000 consecutive FABS readings, showing sufficient signal-to-noise and long-term stability to detect a single bead. Nominal calibration: 20 µV/pN.

tion; given these conditions and the vibrational properties of Piezolevers™, we expect 0.55 pm of vibrational noise. Combining the two sources of noise and multiplying by the nominal 2.5 N/m spring constant gives a theoretical 12.5 pN rms noise. The measured noise of the prototype is also 12.5 pN rms.

Since the antibody-antigen binding chemistry will take about ten min, it is not unreasonable to improve signal-to-noise by using a further 1 or 2 min for signal averaging. At present we signal average for 102.4 s to obtain each FABS reading. The resulting measurement has a 99% confidence

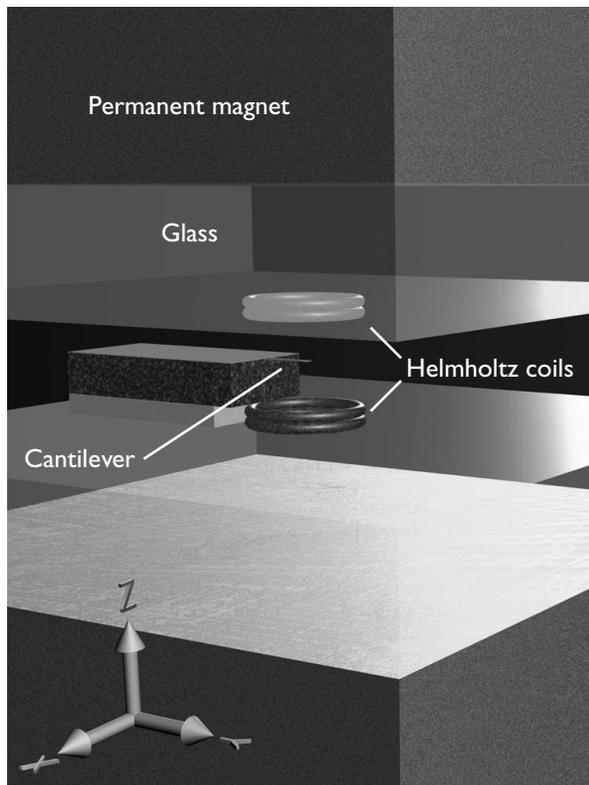


Figure 6. The FABS magnetic assembly consists of two rare-earth magnets and a miniature antiparallel Helmholtz pair. Note the definition of the X, Y, and Z axes.

interval of ±5 pN (105 µV), about one-third the signal that a single 4.5-µm diam bead should produce (Figs. 4 and 5).

In the completed sensor, a software package will control data acquisition and will signal average until the confidence interval (95%, 99%, 99.9%, etc., as determined by the user) falls below the single-bead level.

C. Magnet assembly

Superparamagnetic particles only have a dipole moment when they are exposed to a magnetic field. Once the dipole moment appears, a field gradient can exert a force on the bead. To exert a force on a bead, we therefore need both a field and a field gradient; i.e., $F = \chi \cdot B \cdot dB/dZ$, where F is the force, χ is the magnetic susceptibility, B is the magnetic field, and dB/dZ is the field gradient.

The FABS prototype uses a two-component electromagnet similar to that used in a number of previous instruments.⁶ The first component consists of two 25 x 25 x 13 mm Neodymium Iron Boron magnets (Magnet Sales & Mfg. Co., Culver City, CA), separated by 6.4 mm and backed with steel, that

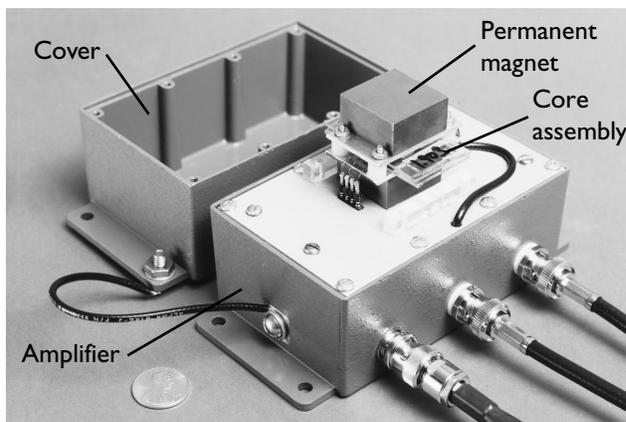


Figure 7. Overall view of the prototype FABS device showing the magnetic assembly (magnets not steel-backed) and connections to power, coil current, and lock-in amplifier.

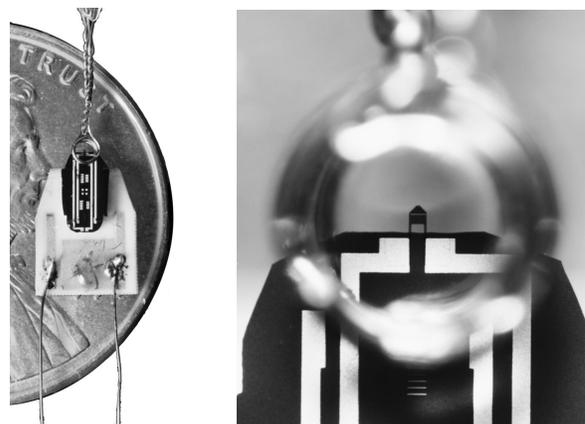


Figure 8. Two views of the core assembly, consisting of the piezoresistive cantilever and the 1 mm diameter Helmholtz coils.

Dia.	Separation	Turns	A p-p	Power
12 mm	12 mm	250	1	100 W
6 mm	3 mm	25	5	17 W
*1 mm	1 mm	2	3	0.08 W

*Prototype specifications

Table 1. RMS power required to generate a field gradient of 160 G/mm peak-to-peak with three Helmholtz pair designs. All use 125 μm diameter OFHC copper wire.

produce a field of 7,200 G (Figs. 6 and 7). As a function of Z (as defined in Fig. 6), the field is uniform to within 110 G. The second component is an antiparallel Helmholtz pair (Figs. 6 and 8). Two 1 mm diam coils separated by 1 mm, each containing two turns of 125 μm diam OFHC copper wire, generate an oscillating field gradient of 160 G/mm peak-to-peak when provided a current of 3 A peak-to-peak. Together, the field and the gradient should produce a force of 45 pN peak-to-peak or 15 pN RMS on one 4.5- μm diam bead.

Small Helmholtz coils complement our goal of producing a miniaturized sensor and could be micromachined for greater consistency. Small coils not only occupy less space; they also require less power, and therefore a smaller power supply, to generate the required 160 G/mm peak-to-peak field gradient. Table 1 shows three coil specifications considered for the FABS device. Although we could make even smaller coils, they would not produce a sufficiently uniform field over the active area ($\sim 90 \times 90 \mu\text{m}$) of the cantilever.

D. Electromagnetic coupling

Inductive and magnetic force coupling were a concern during early development of the FABS prototype. The piezoresistive cantilever is actually a small current loop. Set next to the two larger Helmholtz coils, the device becomes a transformer: induction creates an oscillating current in the cantilever that could overwhelm the piezoresistive signal. Furthermore, the cantilever carries a small piezoresistive detection current that generates a magnetic field. This field will interact with the oscillating magnetic field generated by the Helmholtz coils and exert an oscillating force on the cantilever.

Fortunately, inductive and magnetic force coupling can be reduced to manageable levels. First, although the gradient oscillates, the field is ideally constant at the cantilever (Fig. 9). At a given instant, the field may be positive above the cantilever and negative below, and later, the polarity will switch; but the field is always zero at the cantilever. Second, inductive coupling is 90° out of phase with the signal. Third, the reference cantilever cancels out any coupling.

The first prototype shows about 1000 μV RMS of electromagnetic coupling without and 300 μV of electromagnetic coupling with the reference cantilever (Fig. 10). Since the response is nominally 20 $\mu\text{V}/\text{pN}$, the signal from one bead should measure about 300 μV rms. However, the coils are hand wound and the device hand-assembled; the microma-

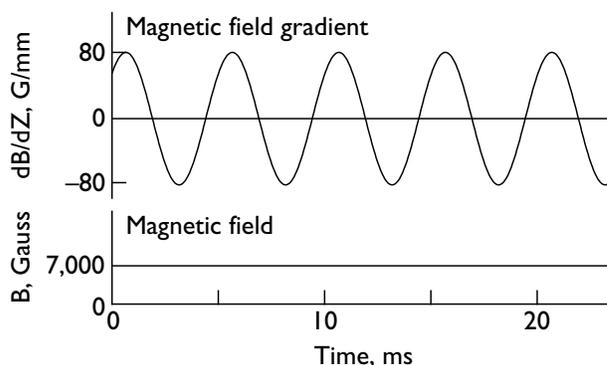


Figure 9. Theoretical magnetic field and gradient produced at the cantilever by the prototype electromagnet.

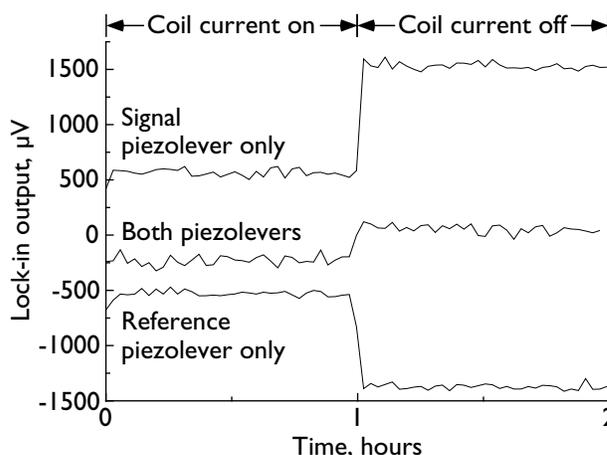


Figure 10. Measured electromagnetic pickup with and without reference cantilever (no beads present).

chined coils we plan to use in the future should yield better consistency and alignment and, therefore, better canceling of electromagnetic coupling from the reference cantilever.

Accurately counting the beads on the cantilever requires knowing the cantilever spring constant. This could, at least in principle, be measured by taking advantage of magnetic force coupling. Passing a known current through the cantilever while exposing it to a known magnetic field will produce a known force. The field can be generated with two wires, one passing in the X direction (as defined in Fig. 6) above the cantilever, the other below.

E. Chemical sensitivity

Figure 11 compares the predicted sensitivities of FABS and two commonly-used sandwich assays, illustrating the dependence on antibody binding affinity.⁷ Recently, the evanescent-wave fiber optic biosensor⁸ has demonstrated a hundred times more sensitivity than the methods shown in Fig. 11, but FABS still promises yet a million times more sensitivity. This sensitivity arises from the ability to sense a single bound analyte particle.

We can illustrate the usefulness of such sensitivity by considering the application of FABS to environmental moni-

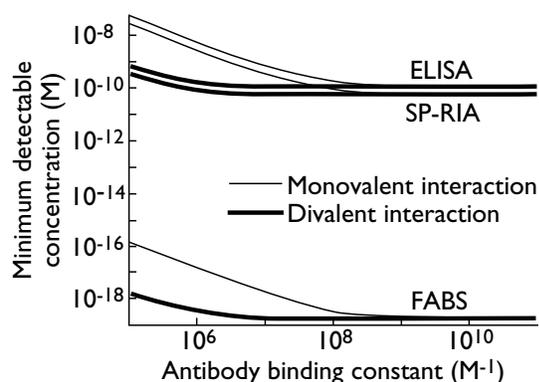


Figure 11. Sensitivity (expressed as minimum detectable concentration) of ELISA, solid-phase radioimmunoassay (SP-RIA), and FABS.

toring. Typically, for this type of application, a cyclone or other aerosol collector the size of a small refrigerator collects the sample, filtering $0.1 \text{ m}^3/\text{minute}$ with the aid of a large fan. Even using such a large filtering capacity combined with novel high-sensitivity assays, the device must operate for several days before it collects enough sample for a barely-incapacitating concentration of virus to be detected. In the case of a potentially lethal virus, such a delay would be unacceptable. However, the same collector would only have to operate for a fraction of a second to collect adequate sample for FABS. In fact, a much smaller, portable collector filtering perhaps $0.001 \text{ m}^3/\text{minute}$ would still collect enough sample if allowed to operate for about a minute.

IV. CURRENT AND FUTURE EFFORTS

Our immediate goal is to record a reproducible change in signal upon binding a single bead to the cantilever. Although the FABS signal exhibits long-term stability (Fig. 5), it can change dramatically upon disturbing the device, probably due to inadequate shielding of the sensor and electronics from the coil current. We are also micromachining multi-layer Helmholtz coils to improve reproducibility, durability, and ease of construction.

Chemistry is an important and challenging aspect of the FABS sensor that this paper has not addressed; the cantilever must efficiently bind the analyte without interference from nonspecific binding. Research at NRL and elsewhere is addressing this issue.

Efforts in the more distant future may involve developing new forms of FABS. FABS was originally conceived as a general-purpose biosensor that obtains high chemical sensitivity by taking advantage of the high force sensitivity of AFM cantilevers. Because they are familiar to us and commercially

available, we have chosen to implement the assay using magnets, paramagnetic immunobeads, and piezoresistive cantilevers, but the FABS concept is not limited to this particular form. We hope to eventually extend the usefulness of FABS by developing assays that use elements such as DNA-DNA interactions, electrostatic forces, or ferromagnetic powders.

ACKNOWLEDGEMENTS

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Note added in proof: Superconducting Quantum Interference Device (SQUID) magnetometry has demonstrated that, due to magnetic saturation, the Dynabeads generate only one-tenth the estimated force under the conditions in the FABS cell. We have replaced the Dynabeads with an initially-demagnetized ferromagnetic powder, and subsequently demonstrated single-particle detection at signal-to-noise levels similar to those originally anticipated for Dynabeads.

We would like to acknowledge Norm Koon and Valerie Browning for obtaining the SQUID measurements.