

# Use of a Piezoelectric Actuator to Study the Mechanical Oscillatory Behavior of Living Cells

By

Jack Yu

Submitted to the Department of  
Mechanical Engineering in  
Partial Fulfillment of the Requirements  
For the Degree of

Bachelors of Science

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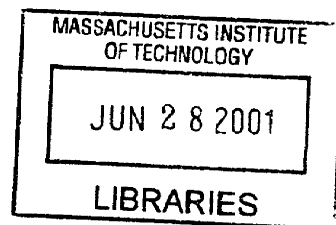
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## Abstract

Mechanical forces are known to directly influence the structure and function of living cells in a frequency-specific manner, however, the mechanism behind this frequency sensitivity remains unknown. The effects of high frequency mechanical perturbations were investigated in cultured capillary endothelial cells using a piezoelectric actuator. The actuator was used to displace fibronectin-coated coverslip on which the cells were cultured the associated displacement of sub-cellular regions was examined using surface-bound microbeads in conjunction with real-time light microscopy and image analysis. The role of different cytoskeletal microtubules and microfilaments in the cellular response to stress was examined using specific chemical disruptors (nocodazole and cytochalasin D, respectively); the level of prestress (preexisting tension) in the cell was altered by addition of the constrictor agent, thrombin. The results showed that the experimental method is effective for determining the effects of chemical disruptors on the mechanical oscillatory behavior of cells. Specifically, in the presence of nocodazole, absolute displacement of beads on cells peaked at 1100 Hz whereas it peaked at approximately 200 Hz in the presence of cytochalasin-D. Finally, error inherent in the original system was reduced by refocusing the cells and beads on the microscope and optimizing image exposure time, which both yielded more definitive results. This optimized technique may be useful for future studies analyzing changes in cell structure and cellular biochemistry in response to different frequencies of mechanical stimulation.

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## **1 Introduction**

### **1.1 Cytoskeleton**

The cytoskeleton (CSK), which occupies the cytoplasmic space of eukaryotic cells, plays an important role in cellular mechanics, contributing to cell function, motility, adhesion to the extracellular matrix (ECM), and mechanotransduction - the response of a cell to mechanical stimuli. The CSK consists of three main filament systems: microfilaments, microtubules, and intermediate filaments (Fig. 1A). Each type of filament is formed from a different protein subunit:  $\gamma$ -actin for actin filaments,  $\alpha$ - and  $\beta$ -tubulin for microtubules, and a family of fibrous proteins, such as vimentin or desmin, for intermediate filaments. The filament systems have certain functions: actin microfilaments, especially those close to the surface of the cell, are responsible for cell motility, microtubules organize the CSK, and intermediate filaments provide cells with mechanical strength (1, 13).

### **1.2 Transmembrane Integrin Receptors**

The plasma membrane attaches to the ECM at focal adhesion contacts. Focal adhesion contacts contain clusters of transmembrane integrin receptors, whose external portion binds to the ECM and cytoplasmic portion links to the actin filaments. The linkage is indirect and mediated by a number of attachment proteins (Fig. 1B). The cytoplasmic portion of the integrin binds to the protein talin, which binds to vinculin. Vinculin attaches to  $\alpha$ -actinin and thus links to an actin filament. This entire system is referred to as the focal adhesion complex (FAC) (14, 17).

Integrins also function as signal transducers, activating various intracellular signaling pathways when specifically binding to the ECM. Although the molecular mechanisms are not known, it is widely believed that clustering integrins generate intracellular signals by initiating the assembly of a signaling complex on the cytoskeletal backbone of the focal adhesion complex on the cytoplasmic face of the plasma membrane. Signaling by both integrins and growth factor receptors seems to be required for an optimal response of ultimate cell fate including cell growth and apoptosis. Further, many cells only proliferate in response to growth factors if cells are attached to ECM via integrin receptors (14, 17).

### **1.3 Mechanotransduction**

Recent studies have shown that transmembrane integrin receptors can transmit changes in the balance of forces, allowing a cell to sense mechanical stresses. By attaching surface-bound, ligand-coated, magnetic microbeads to specific cell surface receptors, a magnetic twisting device and magnetic tweezers have been used to apply controlled mechanical stresses. Employment of these methods revealed that cells respond differently to stresses applied to integrins or other adhesion receptors that mechanically couple to the CSK than to those applied to other types of transmembrane receptors (e.g., metabolic receptors, HLA antigen). For instance, twisting magnetic microbeads bound to integrins results in activation of signal transduction mechanisms (e.g. cAMP pathway) leading to gene transcription, whereas the same stresses applied to other surface receptors produce little effect. Furthermore, integrins must be in an "activated" state that promotes

formation of intact focal adhesion complexes linked to the CSK in order for the mechanical signal to be effectively transduced into a chemical response (10, 18).

#### **1.4 Tensegrity**

The mechanism by which these mechanical signals are transduced and converted into a biochemical response could be based, in part, on the finding that the CSK is organized and stabilized with tensegrity architecture. According to tensegrity, actin filaments extend throughout the cell, exerting tension pulling the membrane and the internal constituents of the cell toward its nucleus, while microtubules and large bundles of cross-linked actin filaments (along with the ECM) act as compressive elements that oppose this inward pull. Certain cellular responses to stress differ depending upon the level of pre-stress (pre-existing tension) in the CSK and the mechanical integrity of the different CSK filament systems. Cytoskeletal responses to stress involve all three CSK filament systems as well as nuclear scaffolds. Recent studies confirm that alterations in the cellular force balance can influence intracellular biochemistry within focal adhesion complexes that form at the site of integrin binding and gene expression in the nucleus as well as switch cells between different phenotypes (e.g., growth and apoptosis). These results suggest that mechanosensation may not result from direct activation of any single "mechanoreceptor" or transduction molecule. Instead, mechanical forces may be experienced by individual cells in the living organism as a result of stress-dependent changes in cell, tissue, or organ structure that alter ECM mechanics, cell shape, CSK organization, or internal pre-stress in the cell-tissue matrix (11, 12).

## **1.5 Objective of Study**

The purpose of this study is to investigate the effects of high frequency mechanical perturbations on cells. Our working hypothesis is that the CSK may exhibit a sensitivity to a particular frequency of mechanical stress application in terms of producing changes in cytoskeletal organization and cell shape which, in turn, may influence cellular biochemistry. This frequency sensitivity would be expected to change if cell structure were altered by modulating cytoskeletal integrity or prestress. Nocodazole and cytochalasin-D are chemicals which depolymerize microtubules and disrupt actin filaments, respectively, while thrombin increases cytoskeletal tension (prestress).

## **2 Background**

### **2.1 Techniques Applying Mechanical Forces on Cells**

A number of techniques have been used to study cell behavior in response to mechanical forces, including optical tweezers, magnetic tweezers, magnetic twisting cytometry (MTC), atomic force microscopy (AFM), and micropipette aspiration. None of these techniques, however, mechanically vibrated the entire cell through basal FACs at high frequency.

#### **2.1.1 Optical tweezers**

Optical tweezers, also known as the single-beam gradient trap, consists of a focused laser beam, which can manipulate microscopic particles in the size range 100 nm - 100  $\mu\text{m}$  by drawing transparent dielectric particles towards its focal region. The laser beam is introduced into a conventional optical microscope such that the same objective is used to view and trap particles immersed in a liquid. In biological applications, optical tweezers can successfully trap, move and position a wide variety of single cells and sub-cellular particles. Optical tweezers can also oscillate ligand-coated beads attached to specific cell surface receptors in a linear motion and provides a means of measuring the resulting displacement at force ranges up to 10 piconewtons (3, 9).

#### **2.1.2 Magnetic tweezers**

Magnetic tweezers consists of three sets of coiled copper wire, each connected to a power source. The coils are positioned to produce electromagnetic fields in three dimensions: backward and forward, side to side, and up and down. By shifting the

amount of electricity flowing in each coil, a magnetic object may be moved in any direction. Similar to optical tweezers, magnetic tweezers can oscillate ligand-coated magnetic beads attached to specific cell surface receptors in a linear motion and provides control of the force applied by the bead or multiple beads attached to the apical side of cells (2, 13).

### **2.1.3 Magnetic Twisting Cytometry (MTC)**

MTC involves using a strong magnetic field in a specific orientation to magnetize ligand-coated microbeads attached to specific cell surface receptors. A second weaker magnetic field, perpendicular to the first, is activated to apply a twisting torque (shear stress) to the beads and cause them to rotate. Rotation of the beads causes deformation of both the cell membrane receptors and attached cytoskeletal filaments. Through sophisticated imaging techniques, the motion of the bead can be inferred (4, 15).

### **2.1.4 Atomic Force Microscopy (AFM)**

AFM is a Scanning Probe Microscopy (SPM) technique in which a fine tip is brought into atomically close contact with a sample surface. The repulsive force between the tip and the atoms of the sample surface (about 1 nanonewton) causes a deflection of the cantilever where the tip is mounted. When there is a change in the surface topography, a change in the cantilever deflection is detected by spectroscopic deflection of the laser beam. A scan of the sample surface measures the topography with accuracy on the order of 0.1 nm along the vertical scale. Using beam bending calculations the force and displacement be quantified on the apical surface of a cell (6, 8).

### **2.1.5 Micropipette Aspiration**

Micropipette aspiration involves using a pressure differential to draw a cell into a small-bore pipette. The pipettes are typically on the order of a few microns in diameter. Thus, non-specific cell membrane deflection can be probed. Under the assumptions that the cell membrane contributes little to the stiffness of the cell and that the cell is homogeneous and linearly elastic with Young's modulus, this test can determine the localized elasticity of the cytoskeletal elements (7, 16).

### **2.2 Oscillatory Effects on Cells**

The oscillatory effects on cells have been investigated to a certain extent by employing the quartz crystal microbalance (QCM). The QCM contains two gold-coated quartz crystals (a sample and a reference). When driven by an electrical current, each crystal resonates at a frequency of about 15 MHz. As material is deposited on the sample crystal, the resonant frequency changes. The Sauerbrey equation dictates that this change in frequency is directly proportional to the change in mass. The study utilizing this technique showed that different cell species generate an individual response on the QCM at high frequencies. Moreover, the QCM technique is only sensitive to those parts of the cell that are involved in cell substrate adhesion, i.e. close to the resonator surface. That study was not, however, able to identify the underlying mechanisms that determined the QCM signal for a particular cell type or extend the study outside a narrow range of oscillations (19).

### **3 Materials and Methods**

#### **3.1 Experimental Procedure**

The displacement of localized regions of a cell was determined at different frequencies of stress application since its amplitude of displacement would help reveal the structural response to mechanical forces. The first step in finding the displacement was attaching cells to a cover slip by using fibronectin and then attaching fluorescent beads to both the cells and the cover slip (Fig. 3A). The next step was placing the cell and bead-coated cover slip in the piezoelectric actuator (Fig. 3B), which was positioned under a microscope equipped with a digital camera. The actuator vibrated the cover slip at frequencies of 10 and 100-1500 Hz for increments of 100 Hz. A function generator and amplifier provided the signal and voltage needed to drive the actuator (Fig. 3C).

Using Image Processing Laboratory (IPLab) software, an image of the total displacement traversed by a bead at each frequency (Fig. 3D thru 3F) was acquired. The displacement appeared as a blurring of fluorescent light emitted by the bead thus capturing the total displacement of the bead at a specified time period. IPLab was also used to measure the displacement. Drawing a segment through the blur and performing a SUM COLUMN command from the MATH menu resulted in a histogram of light intensity versus position (in pixels) (Fig. 3G). The maximum achievable light intensity had a value of 4095. Displacement was defined as the difference between the two opposite positions where light intensity was half the maximum minus the minimum value. Scripts for IPLab and MS Excel allowed us to determine displacement as a function of frequency (Appendix 1). Absolute displacement was determined by subtracting the initial bead diameter at 0 Hz from the measured displacement, and then



plotting the values obtained as a function of frequency. The data were also presented normalized with respect to the absolute displacement of the control bead. Normalization was done to negate the mechanical oscillation characteristics inherent in the system, independently of the cell. Defined mathematically,

$$n = \frac{|d_{bead}|}{|d_{control}|} \quad (1)$$

where  $n$  is the normalized value,  $|d_{bead}|$  is the absolute displacement of the bead on the cell, and  $|d_{control}|$  is the displacement of the control bead.

## **3.2 Cell Preparation**

### **3.2.1 Cell Culture**

Prior to experiments, bovine capillary endothelial cells (BCE) were cultured in standard growth media under 10% CO<sub>2</sub> on gelatin-coated plastic in Dulbecco's Modified Eagle's medium (DMEM) containing 10% calf serum, 2mM glutamine, 100 µg/mL streptomycin, 100 µg/mL penicillin, and 1 ng/mL basic fibro blast growth factor (bFGF). When placed in the piezoelectric actuator, BCE cells were studied in CO<sub>2</sub> independent medium, containing DMEM supplemented with basic fibroblast growth factor (5 ng/mL), human high-density lipoprotein (10 µg/mL), and transferrin (10 µg/mL) (5).

### **3.2.2 ECM Coating**

To attach cells to coverslips, coverslips were coated with fibronectin (FN). 2 µl of FN (5mg/mL) was added to 2 mL of sterile phosphate-buffered saline to give a ratio of 1:1000. 250 µL drops of this solution were placed in a sterile petri dish, and a sterilized

square cover slip (25 mm<sup>2</sup>) was placed on each of these drops. After allowing 1 h for the FN to coat the cover slips, each cover slip was transferred to a culture dish well filled with 1.5 mL of CO<sub>2</sub> independent media. The cover slips were placed upright (with the FN coated side facing up) to allow for cell attachment. Cells were trypsinized, pelleted, aspirated and resuspended in fresh medium; cells were added at 15,000 cells/coverslip.

### **3.2.3 Bead Attachment**

Beads (Polysciences) coated overnight with 50 µg/mL of FN in 0.1 M carbonate buffer, pH = 9.4, were incubated with the cells at a 10:1 ratio (10<sup>7</sup> beads/mL) for 1 h to allow beads to attach to the cells (5).

### **3.2.4 Chemical Disruptors**

After images of cell vibration under normal conditions were acquired, a chemical disruptor was added by using a micropipette. 2 µl of either nocodazole (100 ng/ul; 100 µg/ml final concentration when diluted) or cytochalasin D (1 µg/ml) was injected into the medium and allowed to incubate for 20 minutes before cells were again perturbed through the established frequency range.

## **3.3 Oscillatory Device**

### **3.3.1 Function Generator**

A function generator provided a sine waveform signal at 10 and 100-1500 Hz for increments of 100 Hz (Fig. 3H).

### **3.3.2 Amplifier**

A low frequency amplifier Model 7500 (Krohn-Hite) amplified the signal provided by the function generator to yield up to 100 V (Fig. 3I).

### **3.3.3 Piezoelectric Actuator**

The piezoelectric actuator (Physike Instrumente) developed by Vassilios Bezzerides and Phil LeDuc in the Ingber labororaty consisted of a 50 layer piezo stack that measured 6 mm X 6 mm X 18 mm and had a resonant frequency of 28 kHz. The maximum axial displacement was 15  $\mu\text{m}$  and maximum force was 1000 N for an input of 100 V. This stack was housed in an aluminum shell for protection and reduction of tensile forces on the piezo element. The housing also isolated the cells from the system with respect to the liquid medium. A specifically designed clamp with two screws was attached to the actuator in order to lock down the cover slip with adherent cells (Fig. 3J and 3K).

## **4 Results**

### **4.1 Frequency Sweep**

To determine a frequency range at which differences in sub-cellular structure were noticeable, data for absolute displacement as a function of frequency were collected and plotted for beads attached to cells and compared with control beads attached directly to the coverslip (Fig. 4A). The absolute displacement of the control bead hovered around 7 to 8 pixels (1 pixel = 1  $\mu\text{m}$ ) before peaking at 100 Hz and then decreasing in a linear fashion. The absolute displacement of the bead attached to the cell was around 8.25 pixels for lower frequencies and then decreased for frequencies greater than 100 Hz. The decreasing section of this data curve had a break at around 1000 Hz. In general, the absolute displacement of the bead attached to the cell appeared to have a greater absolute displacement than the control bead for all data points except at 100, 1000, and 50000 Hz. Its absolute displacement was greater by approximately 1 pixel.

### **4.2 Nocodazole**

Data was collected for two beads attached to the same cell and a control bead attached directly to the coverslip (Fig. 4B). According to the data, the first bead had a greater absolute displacement than the control at nearly all frequencies except 10, 400, 900, and 1300 Hz. The absolute displacement of the bead varied by as much as approximately 1.7 pixels for consecutive data points (500 and 600 Hz) and fluctuated by more than 1 pixel from each data point between 400 to 800 Hz. To a lesser magnitude, this fluctuation also occurred for the control. For frequencies below 300 Hz, the absolute displacement of the bead was about 2.5 pixels but then settled around 1.6 pixels from 800

to 1500 Hz. The second bead adherent to the cell did not show a consistent trend with that on the first, for its absolute displacement was lower than or equal to that of the control bead beyond 200 Hz with the exception at 1100 Hz. The absolute displacement of the bead dropped sharply from 200 to 300 Hz and fluctuated by up to 1 pixel for each data point collected between 400 to 800 Hz. From 800 to 1500 Hz, the pixel absolute displacement hovered around 0.8 pixels.

After nocodazole was added to the system to depolymerize microtubules, the data collected showed that the absolute displacement of the first bead was greater for nearly all frequencies when the cell was in the presence of nocodazole with exceptions at 1, 300 and 700 Hz (Fig. 4C). The absolute displacement of the bead was greater by as much as approximately 3 pixels and fluctuated by as much as approximately 2.5 pixels for consecutive data points spanning the entire frequency range.

These data may also be presented normalized with respect to the absolute displacement of the control bead (Fig. 4D). This plot reveals that the bead displacement increased in the presence of nocodazole and clarifies that the larger displacement at lower frequencies and smaller displacement at higher frequencies are characteristic to the system. More importantly, this plot shows a jump in displacement at 1100 Hz.

According to the graph shown in Figure F, the data collected showed somewhat different results for the second bead. Specifically, the absolute displacement did not vary by more than 1 pixel for most of the frequency range above 200 Hz except at 700 Hz. Normalized with respect to the absolute displacement of the control bead, however, the absolute displacement of the bead was shown to increase in the presence of nocodazole and to peak at 1100 Hz - similar results obtained for the first bead (Figure 4E).

To ensure that adding nocodazole did not affect the attachment of the beads, data was also collected for the control in the presence of nocodazole (Fig. 4G). The absolute displacement did not vary by more than 1 pixel for the entire frequency range except at 100 and 700 Hz.

### **4.3 Cytochalasin-D**

Data were again collected for two beads attached to the same cell and a control bead attached directly to the coverslip (Fig. 4H). According to the data, the absolute displacement of the first bead was lower than that of the control for most of the frequency range by up to about as much as 3 pixels. The absolute displacement was greatest at 100 Hz but dropped sharply from 200 to 300 Hz and settled down to around 1 pixel from 800 to 1500 Hz. In the 800 to 1500 Hz range, the absolute displacement fluctuated by as much as 1.5 pixel for consecutive points. The absolute displacement of the second bead was also consistently lower than that of the control but only by about 1.5 pixels at most. The absolute displacement was greatest at 10 Hz and then decreased to hover around 1.75 pixels from 500 to 1400 Hz. There was an unexpected jump at 1500 Hz. The fluctuation in the 500 to 1400 Hz range was less than 1 pixel for consecutive points.

After the cytochalasin-D was added to the system, the absolute displacement of the first bead was lower for frequencies below 500 Hz (with the an exception at 300 Hz) by as much as about 2.75 pixels and as small as about 0.35 pixels. The absolute displacement was greater for frequencies above 600 Hz (with exceptions at 900 and 1100 Hz) by as much as about 2 pixels and as small as about 0.25 pixels (Fig. 4I).

Normalized with respect to the absolute displacement of the control bead, the absolute displacement of the bead was shown to increase in the presence of cytochalasin-D for much of the frequency range and peaked twice, once at 200 Hz and to a lesser degree at 1200 Hz (Fig. 4J).

In the presence of cytochalasin-D, the absolute displacement of the second bead was not consistently below or above its displacement compared to when cytochalasin-D was not present (Fig. 4K).

Normalized with respect to the absolute displacement of the control bead the displacement of the second bead in the presence of cytochalasin-D was shown to peak at 200 Hz (Fig. 4L) as in the case of the first bead (Fig. 4J).

To ensure that adding cytochalasin-D did not affect the attachment of the beads, data was also collected for the control in the presence of cytochalasin-D (Fig. 4M). The variation of the absolute displacement was less than 1 pixel for most of the frequency range.

#### **4.4 Source of Error**

To determine possible discrepancy in the results obtained in sections 4.2 and 4.3, a number of tests were conducted attempting to identify sources of error. These tests included 1) comparing the displacement of different control beads, 2) increasing the mass of the piezoelectric actuator, 3) using IPLab to sample the displacement multiple times, 4) recapturing and then reprocessing an image of a control bead after power to the function generator is turned off and then on again, and 5) capturing images of beads at different light exposures.

#### **4.4.1 Displacement of Different Control Beads**

To determine the variation of displacement for different control beads, data were collected for displacement as a function of frequency for two control beads (Fig. 4N). Other than at 200 Hz, the displacement of the beads did not vary by more than 1 pixel.

#### **4.4.2 Adding Mass to the Piezoelectric Actuator**

Mass of the piezoelectric actuator was increased to reduce possible vibration of the entire system. Displacement as a function of frequency was determined for four control beads attached directly to the cover slip without additional mass on the system and another bead attached directly to the cover slip with additional mass (Fig. 4O). The bead with additional mass had a consistently larger displacement than three of the four control beads (ctrl1, ctrl2, and ctrl3) between 300 and 1000 Hz, but displacements for all beads hovered around 3 pixels from 700 to 1000 Hz. The bead with additional mass did not have a consistently larger or smaller displacement than the fourth control bead (ctrl4), which had dramatic increase in displacement from 900 to 1000 Hz.

#### **4.4.3 Reprocessing Captured Image of Bead**

To determine the error associated with using IPLab to measure the displacement, the displacement was measured twice for two beads attached to the cover slip. A plot of displacement versus frequency shows that re-measured displacements did not differ from original measurements by more than 0.5 pixels for the first bead (image a) except at 200 Hz or for the second bead (image b) (Fig. 4P).



#### **4.4.4 Recapturing Images of Bead at Different Exposures**

Images of beads were recaptured after shutting the function generator off and then on again to investigate discrepancy due to suddenly stopping and starting vibration. Capturing and recapturing images of the beads was conducted at three different light exposures: 500, 750, and 2000 as quantified by IPLab, to determine displacement as a function of frequency (Fig. 4Q). At an exposure of 500, the peak displacement never reached above 3.5 pixels with larger displacement for frequencies below 500 Hz and a smaller displacement of about 2 pixels for frequencies above 500 Hz. The discrepancy between the displacements of captured and recaptured images was as much as 1 pixel for lower frequencies, i.e. below 400 Hz, but less than 0.5 pixels for higher frequencies, i.e. above 400 Hz. At exposure of 750, the peak displacement never reached above 4 pixels with larger displacement for frequencies below 500 Hz and a smaller displacement of about 2 pixels for frequencies above 500 Hz. The discrepancy between the displacements of captured and recaptured images was as much as 1 pixel for lower frequencies, i.e. below 400 Hz, but less than 0.5 pixels for higher frequencies, i.e. above 400 Hz. At exposure of 2000, the peak displacement was greater than 6 pixels with larger displacement for frequencies below 200 Hz and a smaller displacement of about 3.5 to 4 pixels for frequencies above 200 Hz. The discrepancy between the displacements of captured and recaptured images was less than 0.5 pixels for the entire frequency range.

#### **4.5 Cytochalasin-D (after error analysis)**

After conducting tests investigating possible sources of discrepancy in the results obtained in sections 4.2 and 4.3, testing the effect of cytochalasin-D was repeated. Data for absolute displacement as a function of frequency was collected for a bead attached to the cell and a control bead attached directly to the cover slip (Fig. 4R). The error bars represent displacement values from captured and recaptured images of each bead as described in section 4.4.4. The average of those values are the plotted data points. The plot shows that all three curves exhibit decreasing absolute displacement for increasing frequency. The absolute displacement of the bead was larger for most of the frequency range when the cell was treated with cytochalasin-D than when the cell was not. Note that the absolute displacement of the control bead was rarely larger than that of the bead on the cell treated or untreated with the chemical disruptor.

Normalized with respect to the absolute displacement of the control bead (Fig. 4S), the absolute displacement of the bead on the cell was shown to be consistently larger in the presence of cytochalasin-D. It was also shown to peak at 400 Hz and slightly less at 1100 Hz.

#### **4.6 Inverting Cover Slip**

To ensure that the chemical disruptors were able to diffuse into the gap spaced region between the cover slip in the piezoelectric actuator and the aluminum shell, the cover slip was placed inverted in the piezoelectric device, such that the cells were now facing up allowing for more immediate exposure to dose of cytochalasin-D. Data for absolute displacement as a function of frequency was collected for a bead attached to the cell and a control bead attached directly to the cover slip (Fig. 4T). The error bars

represent displacement values from captured and recaptured images of each bead as described in section 4.4.4. The average of those values are the plotted data points. The plot differed significantly in shape from previous plots. Specifically, all three data curves hovered around 2.75 to 3 pixels below 600 Hz, peaked at 3.5 to 4.5 pixels at 800 Hz, dropped to about 2 pixels at 1100 Hz, and then steadied for the remainder of the frequency range at about 3 to 3.5 pixels. The plot also indicates that the absolute displacement of the bead on the cell when treated with cytochalasin-D was not consistently larger or smaller than when the cell was untreated.

Normalized with respect to the absolute displacement of the control bead, the absolute displacement of the bead on the cell was shown to have about the same value as that of the control (Fig. 4U).

#### **4.7 Thrombin**

Data was collected for a bead attached to a cell and a control bead attached directly to the cover slip (Fig. 4V). Two sets of data were obtained for each bead: one for the cell and system untreated and the other for the cell and system treated with Thrombin. The error bars represent displacement values from captured and recaptured images of each bead as described in section 4.4.4. The average of those values provide the plotted data points. For all curves, displacement tended to be greatest for lower frequency values and decreased as frequency increased. From 1300 Hz and greater, the absolute displacement of all beads under all conditions was around 2.5 pixels. The absolute displacement of the bead on the cell when treated with Thrombin was not consistently larger or smaller than when it was untreated.

Normalized with respect to the absolute displacement of the control bead, the absolute displacement of the bead on the cell was shown to have about the same value as that of the control (Fig. 4W).

## 5 Discussion

Determining the localized differences in sub-cellular structure for the frequency range proved to be extremely difficult since initial results revealed that the beads did not exhibit consistent displacement behavior (Fig. 4B and 4H). For instance, in the testing described in section 4.2 the first bead attached to the cell had an absolute displacement that was larger than that of the control bead for most of the frequency range whereas the second bead had one that was smaller (Fig. 4B). Furthermore, the absolute displacement of the bead on the cell was expected to be greater than that off the cell because the cell was expected to deform due to the forces induced by the motion of the piezoelectric actuator. According to the data from testing described in sections 4.2 and 4.3 (Fig. 4B and 4H), this was not the case.

Beads also showed inconsistent displacement behavior when cells were treated with the chemical disruptors nocodazole and cytochalasin-D. In the presence of nocodazole, the first bead on the cell had greater absolute displacement than when not in the presence of nocodazole, whereas the second bead had about the same absolute displacement (Fig. 4C). Similarly, in the presence of cytochalasin-D, the first bead on the cell had greater absolute displacement for at least part of the frequency range than when not in the presence of cytochalasin-D, whereas the second bead did not show a consistently larger or smaller absolute displacement (Fig. 4I). Since nocodazole and cytochalasin-D depolymerize microtubules and actin filaments respectively and thus affect the structural stiffness of the cell, the absolute displacement of the beads on the cells was expected to increase or decrease in the presence of chemical disruptors. The

results from sections 4.2 and 4.3 (Fig. 4C and 4I) did not conclusively show this to be true.

Normalizing data for these initial tests, however, showed some consistent trends in terms of displacement behavior due to chemical disruptors. Specifically, in the presence of nocodazole, the absolute displacement of the beads located on the cell increased and peaked at the same frequency - 1100 Hz (Fig. 4D and 4F). In the presence of cytochalasin-D, the absolute displacement of the beads located on the cell also peaked at the same frequency of 1100Hz, but this time also at 200 Hz (Fig. 4J and 4L). Since chemical disruptors affect the frequency at which the cell displacement peaks, the concept of the CSK as a structure with a behavior-altering frequency could have some important consequences. Moreover, since the chemical disruptors affect different filament systems of the CSK, the CSK seems to be highly dependent on the interaction of those systems.

The inconsistency and unexpected displacement behavior of the beads suggested to a certain degree that the location of sub-cellular microdomain where the bead attaches to the cell would affect displacement. Also the beads may not have attached properly to either the cell or cover slip, which may have been another source of error inherent in the experimental method. Out of the numerous experiments investigating possible sources of experimental error, recapturing and then reprocessing an image of a control bead (after power to the function generator is turned off and then on again) proved to reveal the greatest amount of variance in bead displacement (Fig. 4Q). To reduce this error, continued testing involved taking two measurements for bead displacement at each frequency; averaging those values improves the accuracy of this method.

Using this additional step, the effects of cytochalasin-D were tested again. Results seemed to meet expectations, i.e. in the presence of the chemical disruptor the absolute displacement for the bead was consistently larger (Fig. 4R and 4S). Normalizing this data showed that absolute displacement was greatest at 400 Hz, indicating cell resonance at this frequency (Fig. 4S). Though this value differs from the 200 Hz peak frequency determined from the testing described in section 4.3, the two frequency values are close enough to suggest that a possible resonant frequency of a cell with depolymerized actin filaments is in that approximate frequency range. Possible explanations for discrepancy include experimental error (discussed previously) and difference of structure among cell populations.

In another test in which the cover slip in the piezoelectric actuator was inverted to ensure enhanced diffusion of the chemical disruptor, the absolute displacement for the bead was not consistently larger or smaller due to the addition of cytochalasin-D and no peak frequency occurred when data was normalized (Fig. 4U). Significant shear forces may not have been generated since the cell was not confined to the gap space. Alternatively, some other unknown microenvironmental factor may contribute to these different responses.

Results for testing the effects of Thrombin showed that it did not affect absolute bead displacement since displacement values did not vary significantly (Fig. 4V and 4W), though it was expected that the displacement would decrease since Thrombin stiffens the CSK. The CSK and its filament systems may not actually deform much during mechanical oscillation, thus stiffening microfilaments which already do not undergo much change would not yield different results. More likely, the concentration of the

Thrombin dosage may not have been high enough to affect the actin filaments or, again, the position of the cells on the apparatus may not have been optimal.

In summary, the results, while preliminary, seem to show that the experimental method is adequate for determining the effects of chemical disruptors on the mechanical oscillatory behavior of living cells. If the difference in response we measured between beads attached to the same cell are correct, then this method also may be able to detect localized differences in sub-cellular structure. Alternatively, this different measurements may be due to poor control over the degree of bead adhesion or even to problems inherent in the piezoelectric actuator system. A number of observations made during testing also suggest the possibility of significant improvements in the future. First and foremost, the oscillation of beads at higher frequencies, i.e. beyond 400 Hz, displaced for larger distances initially but then displaced for smaller distances as time continued. This observation seems to indicate internal impedance within the function generator; thus, a new function generator should be used. Also, the image of the vibrating bead often moves out of focus. Since the sensitivity of the displacement depends significantly on the image captured by the microscope camera such that a bead slightly out of resolution might be processed as having a skewed displacement, the image of the bead should be refocused during testing to ensure that the clearest image is captured. Further testing will be conducted using this exciting novel system to probe cell and sub-cellular mechanics at high oscillatory frequencies.



## 6 References

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# 7 Figures

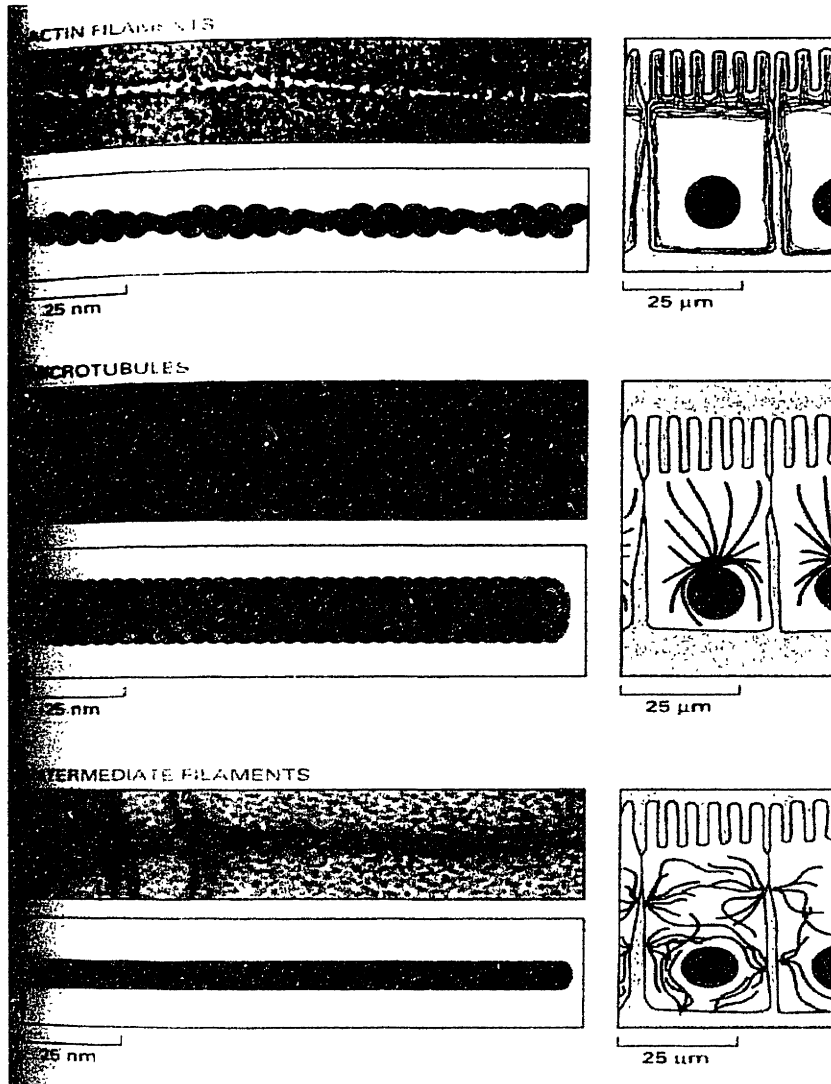


Figure 1A: Actin filaments, microtubules, and intermediate filaments

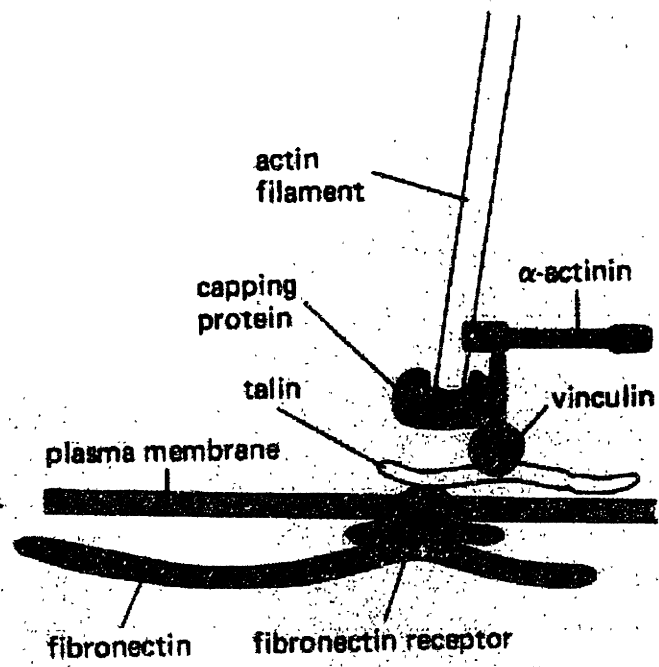
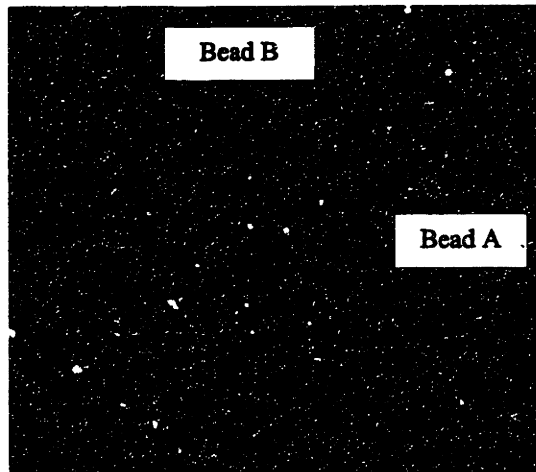


Figure 1B: Model of focal adhesion complex



**Figure 3A: Fluorescent beads attached to cell and cover slip  
(Bead A is on cover slip, and Bead B is on cell)**

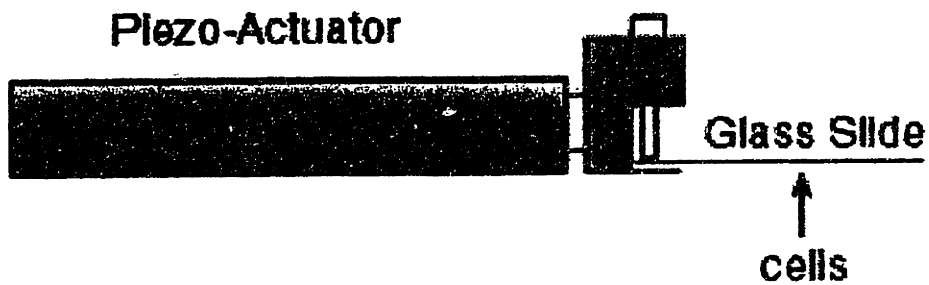


Figure 3B: Schematic of cell and bead coated cover slip in piezoelectric actuator

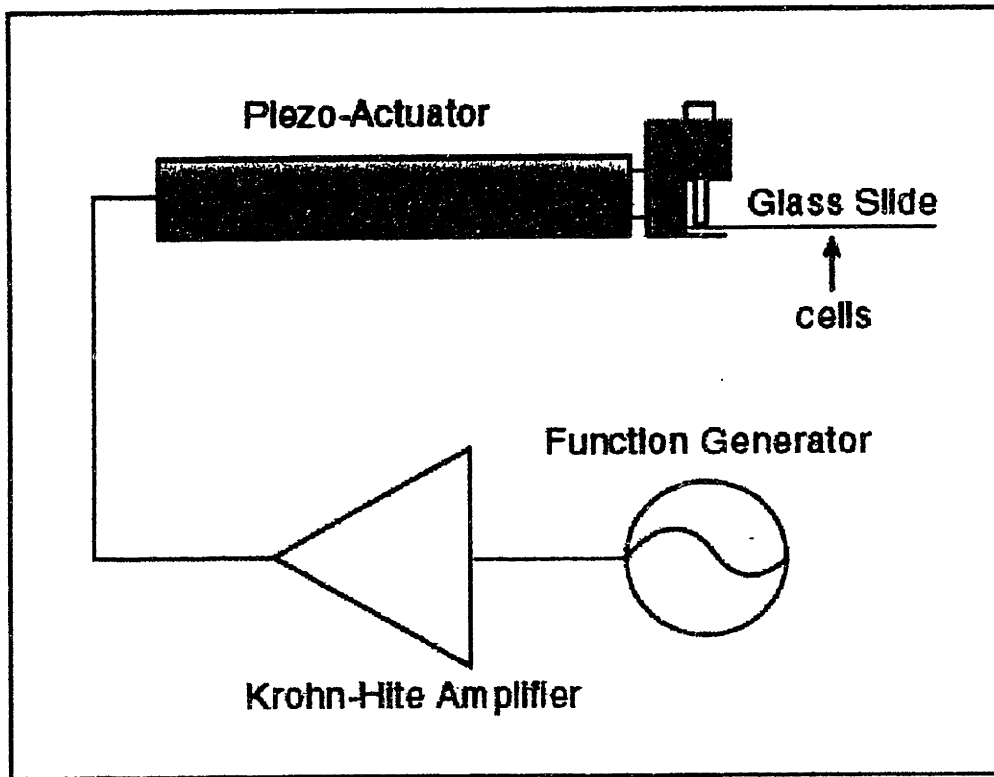


Figure 3C: Schematic of piezoelectric actuator, function generator, and amplifier setup

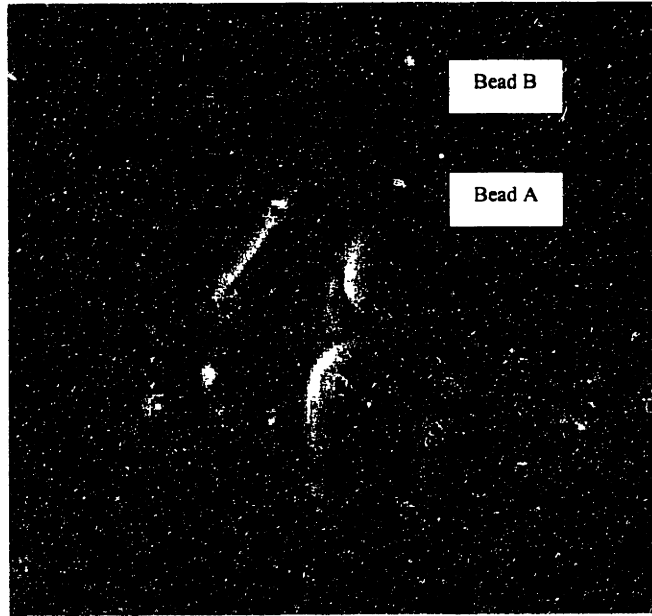


Figure 3D: Fluorescent beads attached to cells and cover slip  
(Bead A is on cover slip, and Bead B is on cell)



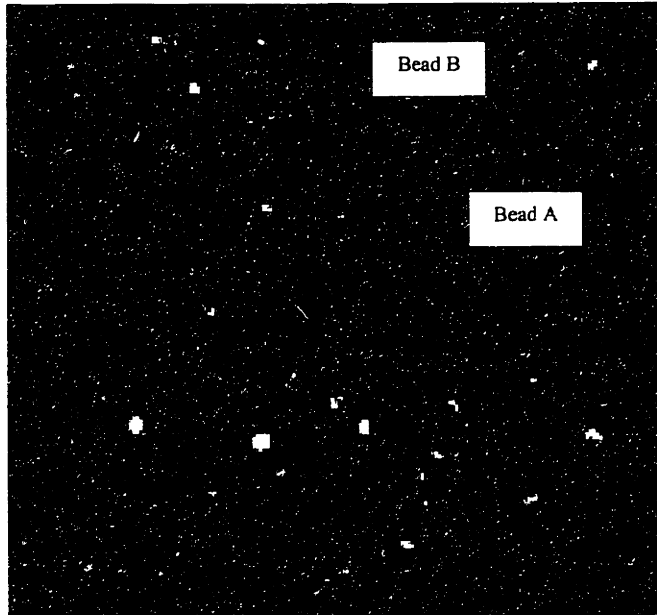


Figure 3E: Image of fluorescent beads at 0 Hz  
(Bead A is on cover slip, and Bead B is on cell)

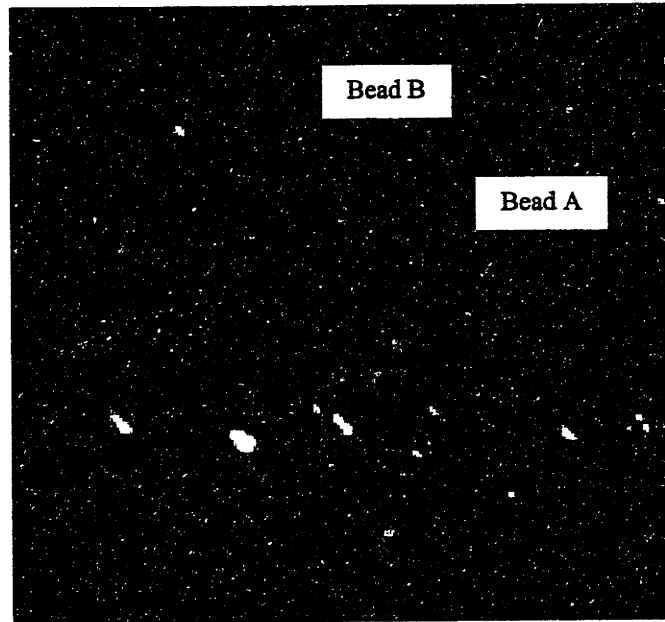
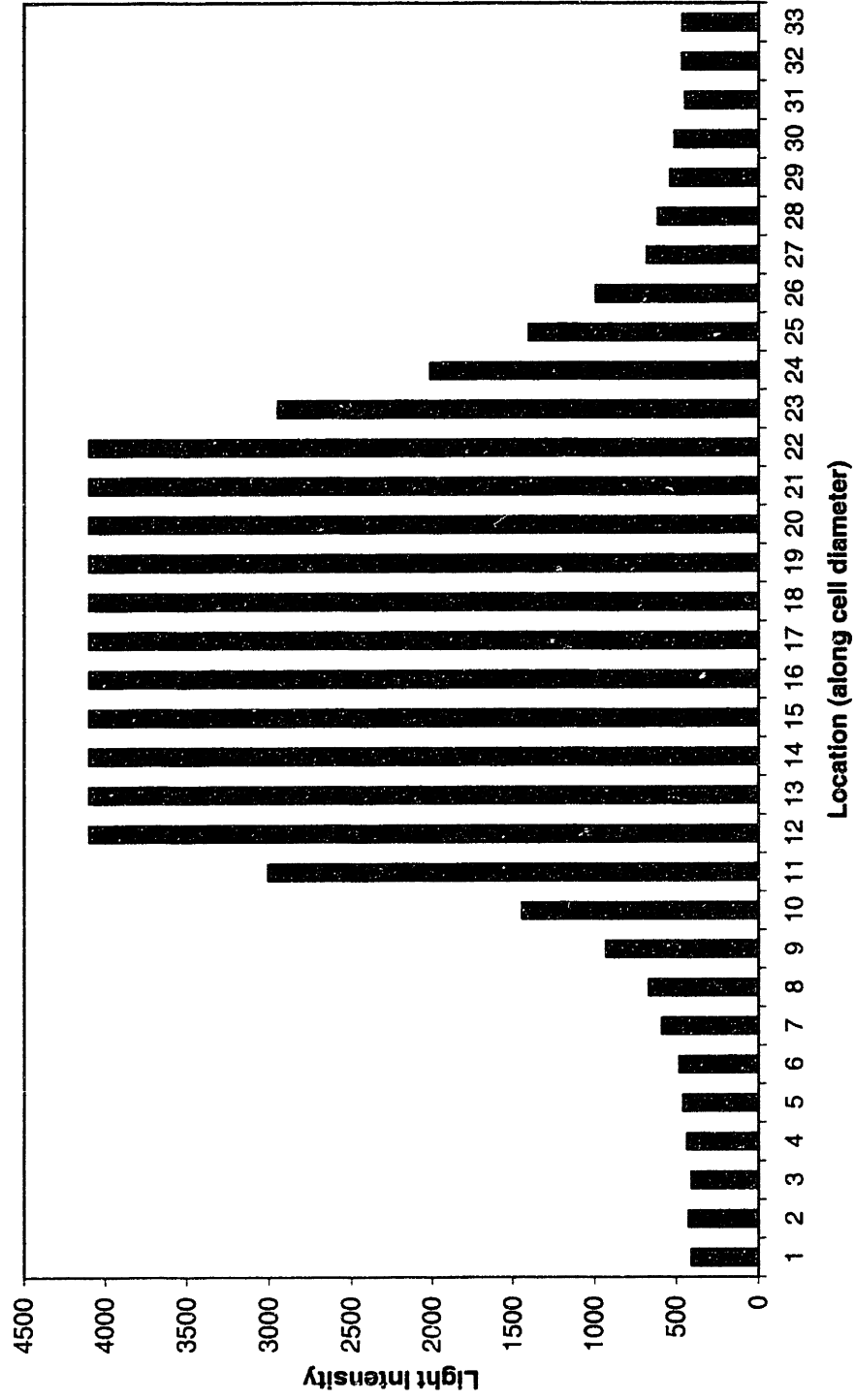


Figure 3F: Image of fluorescent beads at 10 Hz  
(Bead A is on cover slip, and Bead B is on cell)

Figure 3G: Light intensity versus position (in pixels)

### Light Intensity vs Location (1 Hz Off Cell)



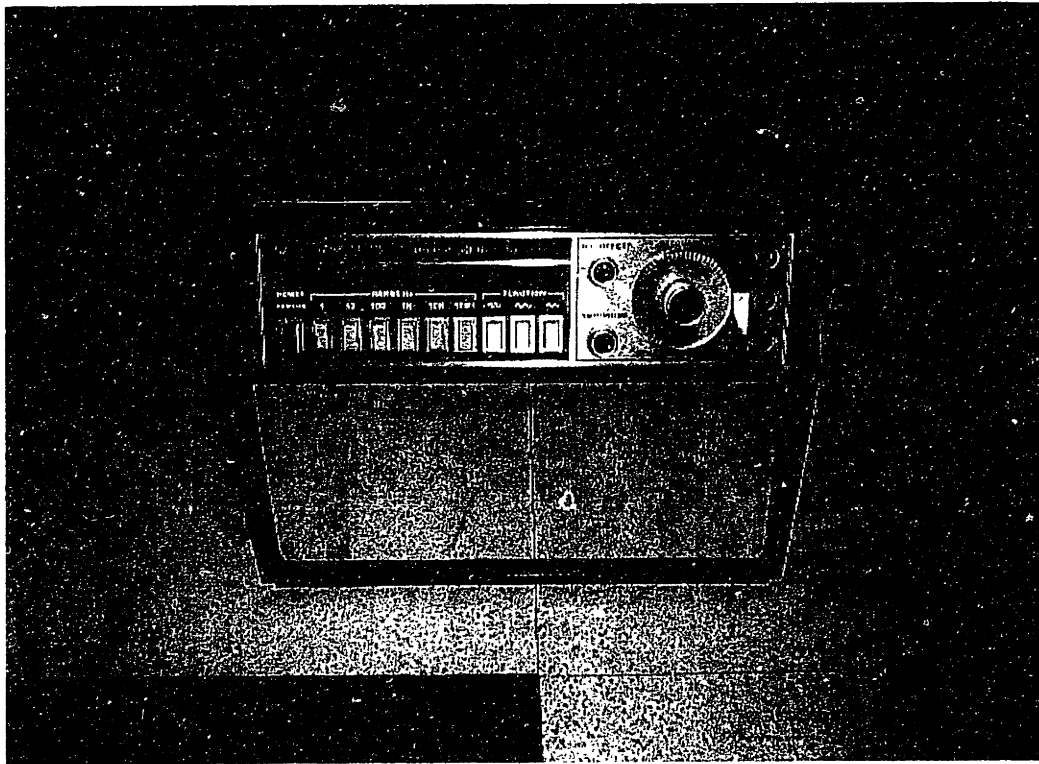


Figure 3H: Picture of function generator

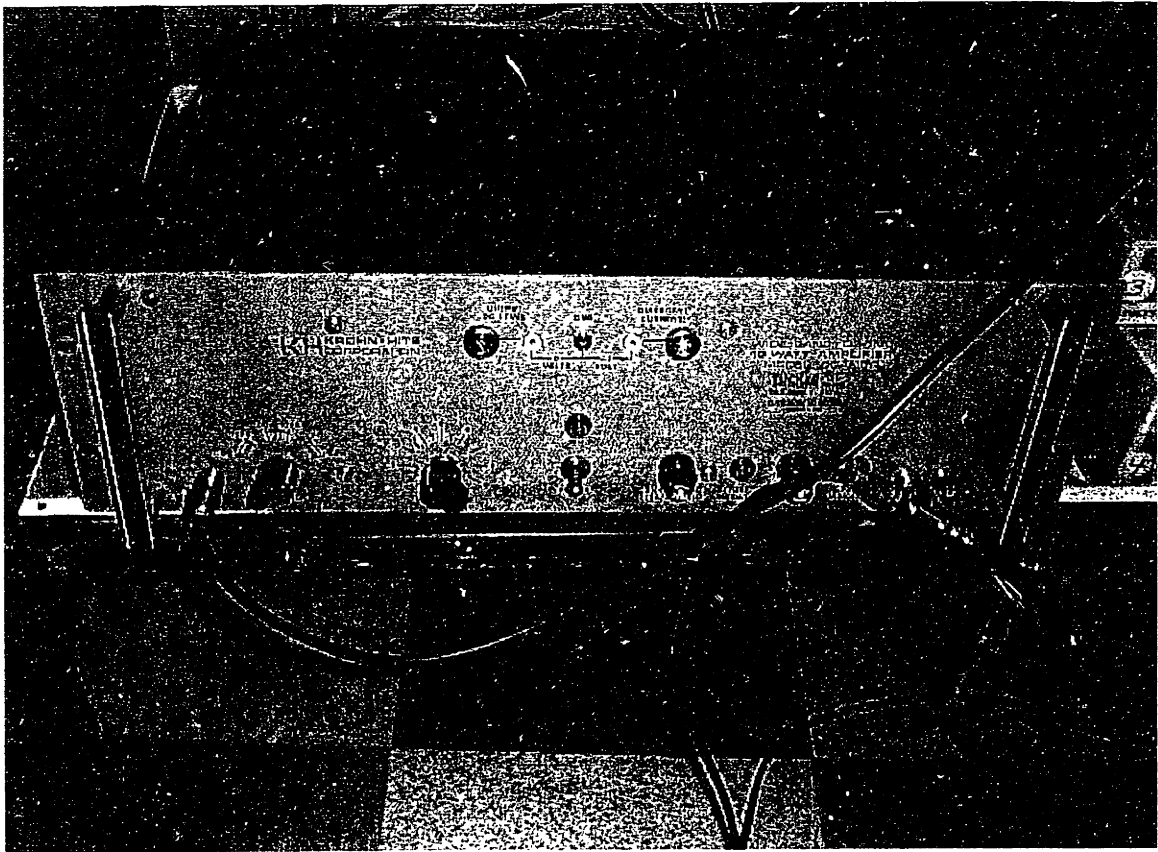


Figure 31: Picture of amplifier

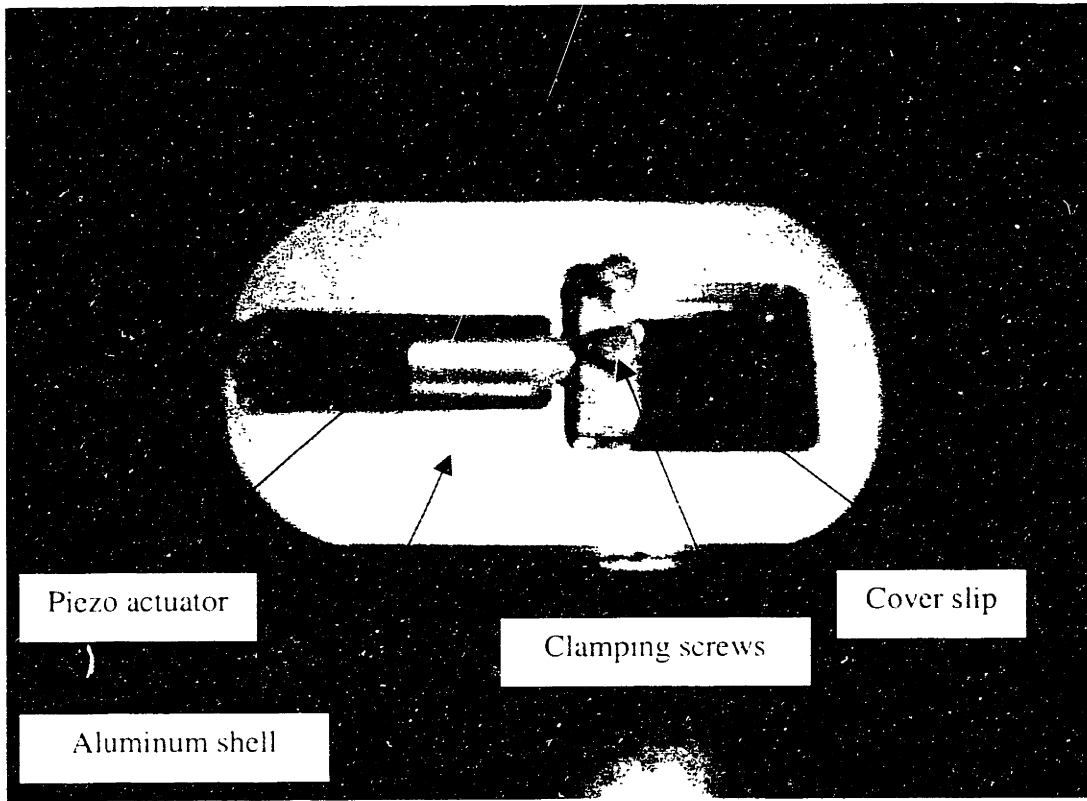


Figure 3J: Picture of piezoelectric actuator

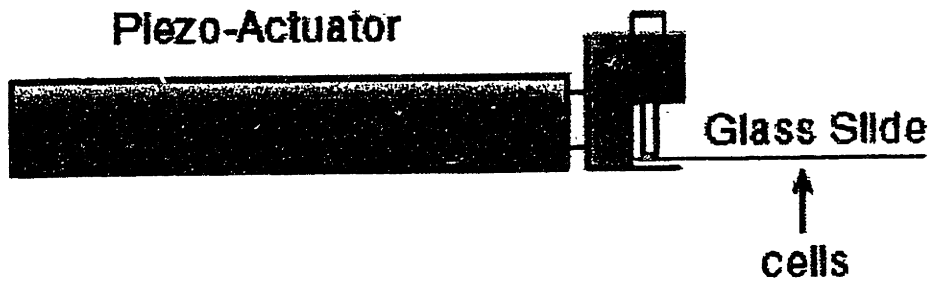


Figure 3K: Schematic of piezoelectric actuator

Figure 4A: Frequency versus absolute displacement (frequency sweep)

Frequency vs Absolute Displacement

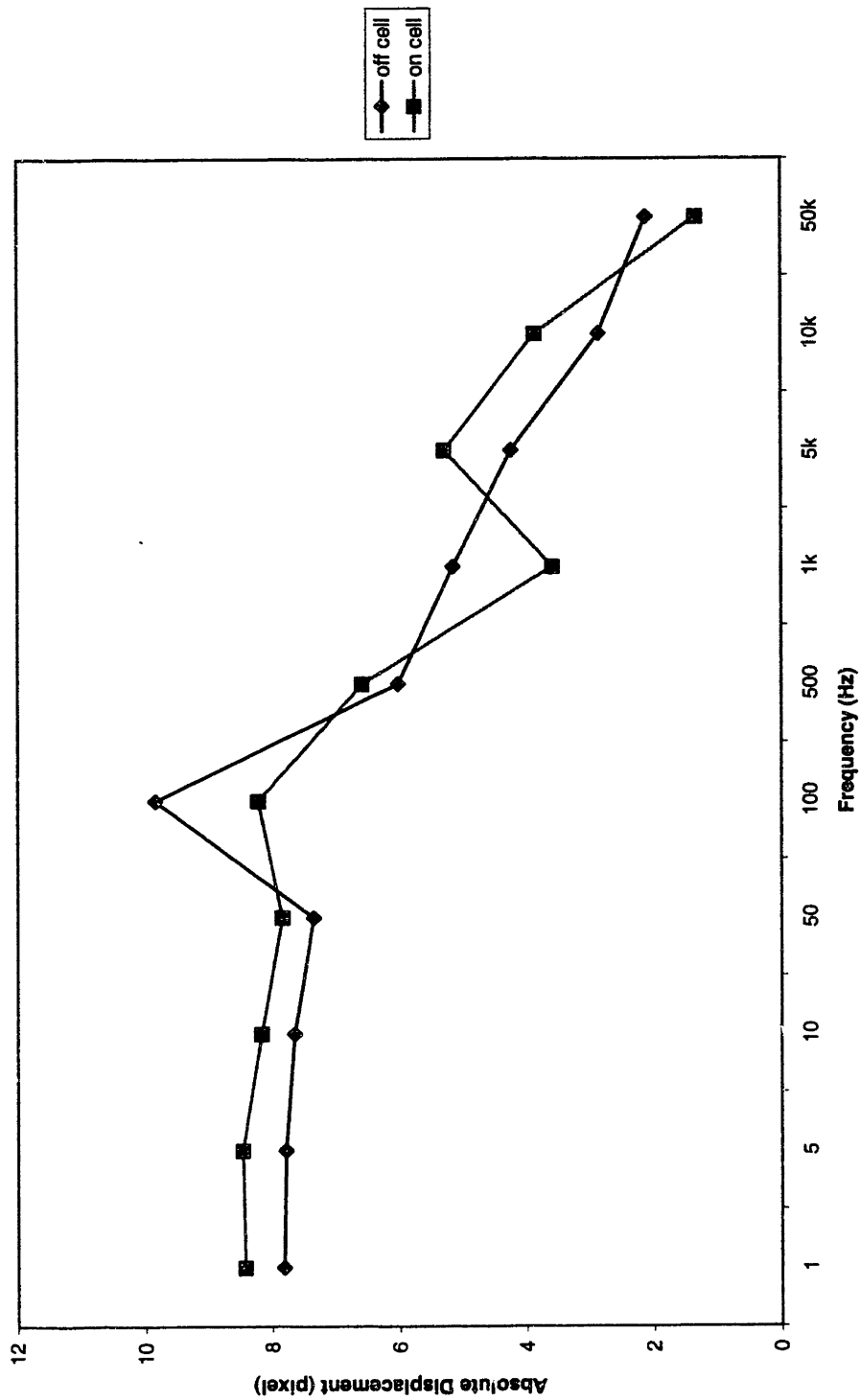




Figure 4B: Frequency versus absolute displacement

Frequency vs Absolute Displacement  
Cell w/o Noc

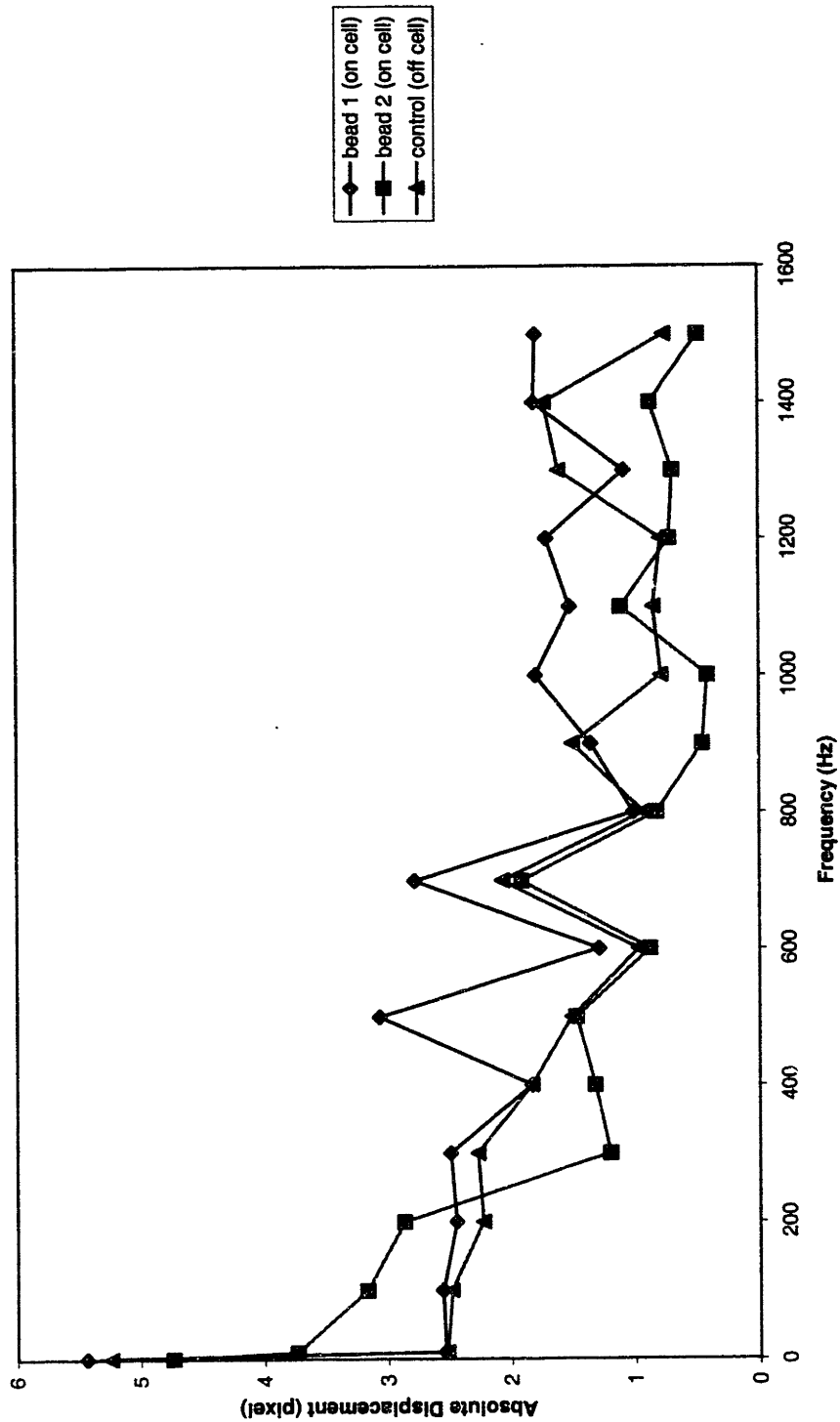


Figure 4C: Frequency versus absolute displacement for w/ nocodazole versus w/o nocodazole (bead 1)

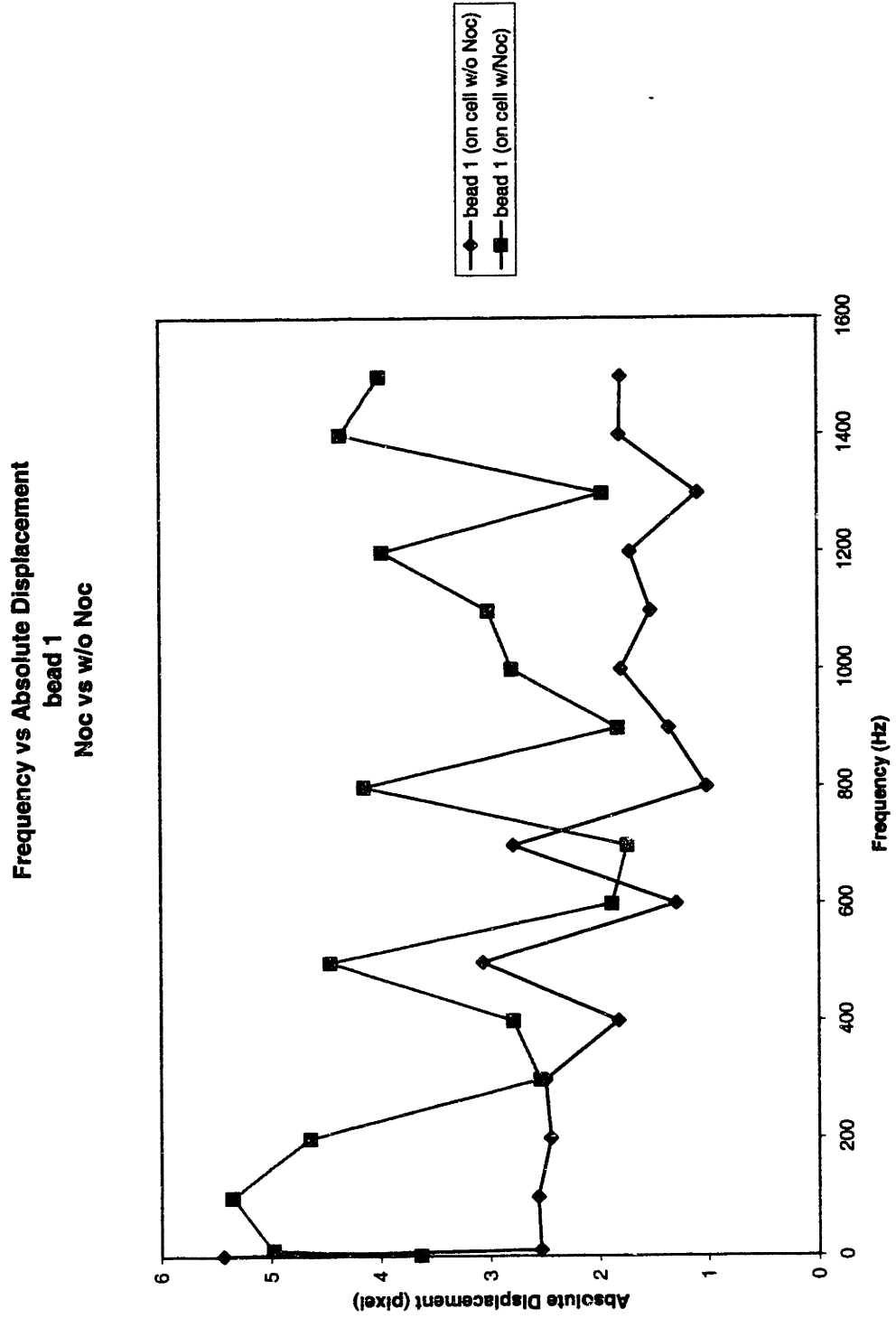


Figure 4D: Normalized displacement versus frequency for w/ nocodazole versus w/o nocodazole (bead 1)

Frequency vs Normalized Displacement  
bead 1  
Noc vs w/o Noc

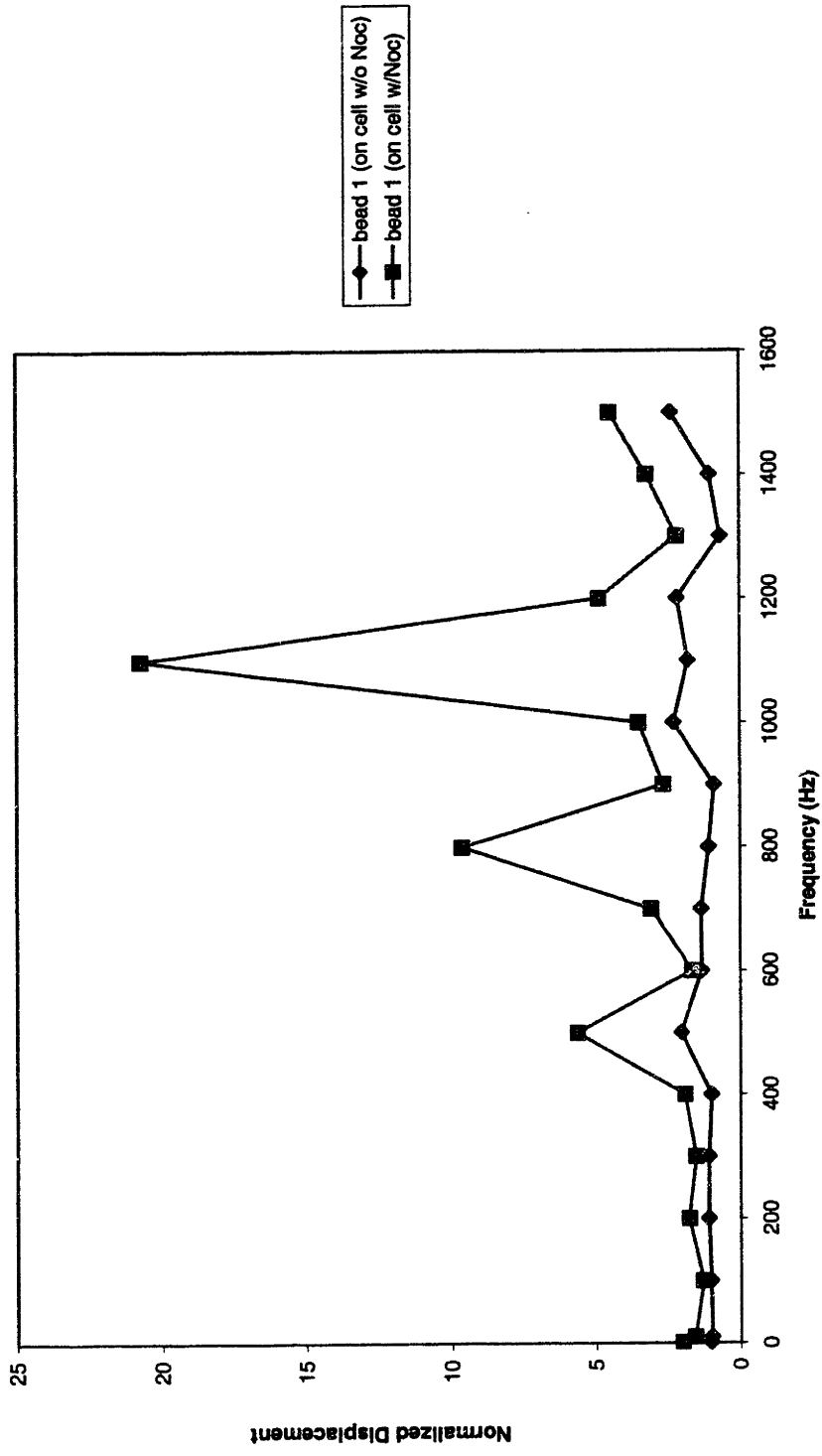


Figure 4E: Frequency versus absolute displacement for w/ nocodazole versus w/o nocodazole (bead 2)

Frequency vs Absolute Displacement  
bead 2  
Noc vs w/o Noc

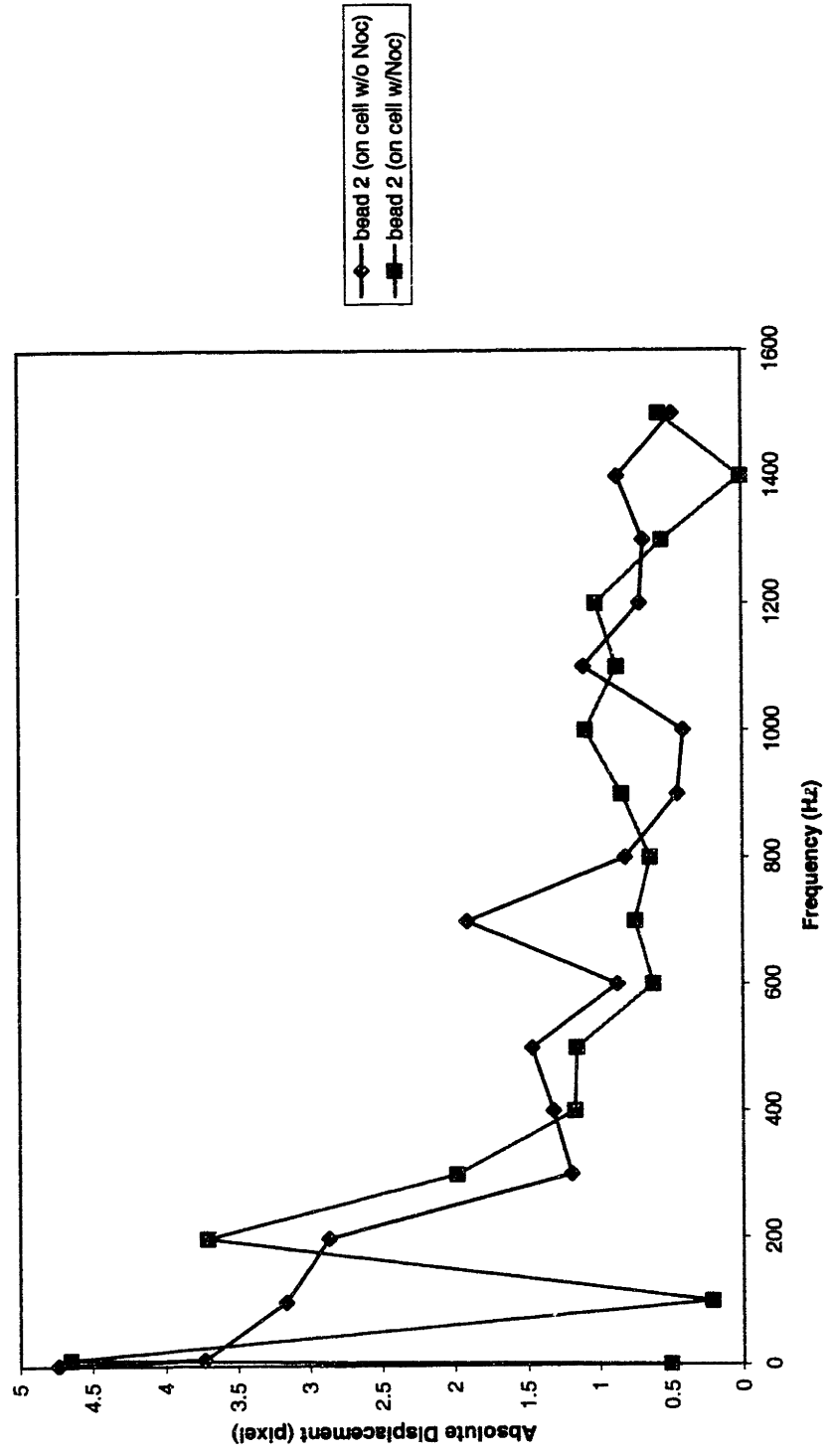


Figure 4F: Normalized displacement versus frequency for w/ nocodazole versus w/o nocodazole (bead 2)

Frequency vs Normalized Displacement  
bead 2  
Noc vs w/o Noc

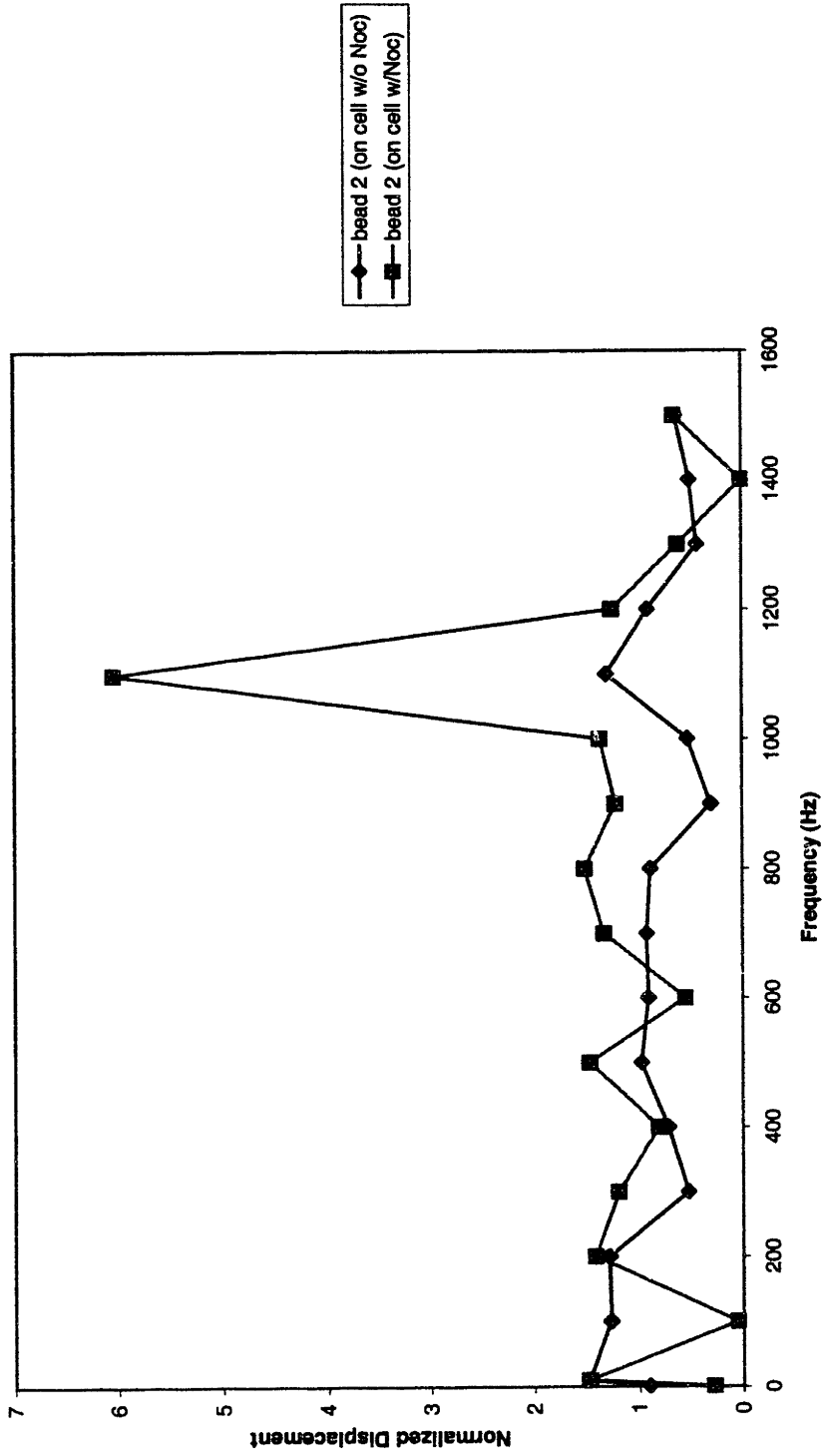


Figure 4G: Frequency versus absolute displacement for w/ nocodazole versus w/o nocodazole (control)

Frequency vs Absolute Displacement  
control  
Noc vs w/o Noc

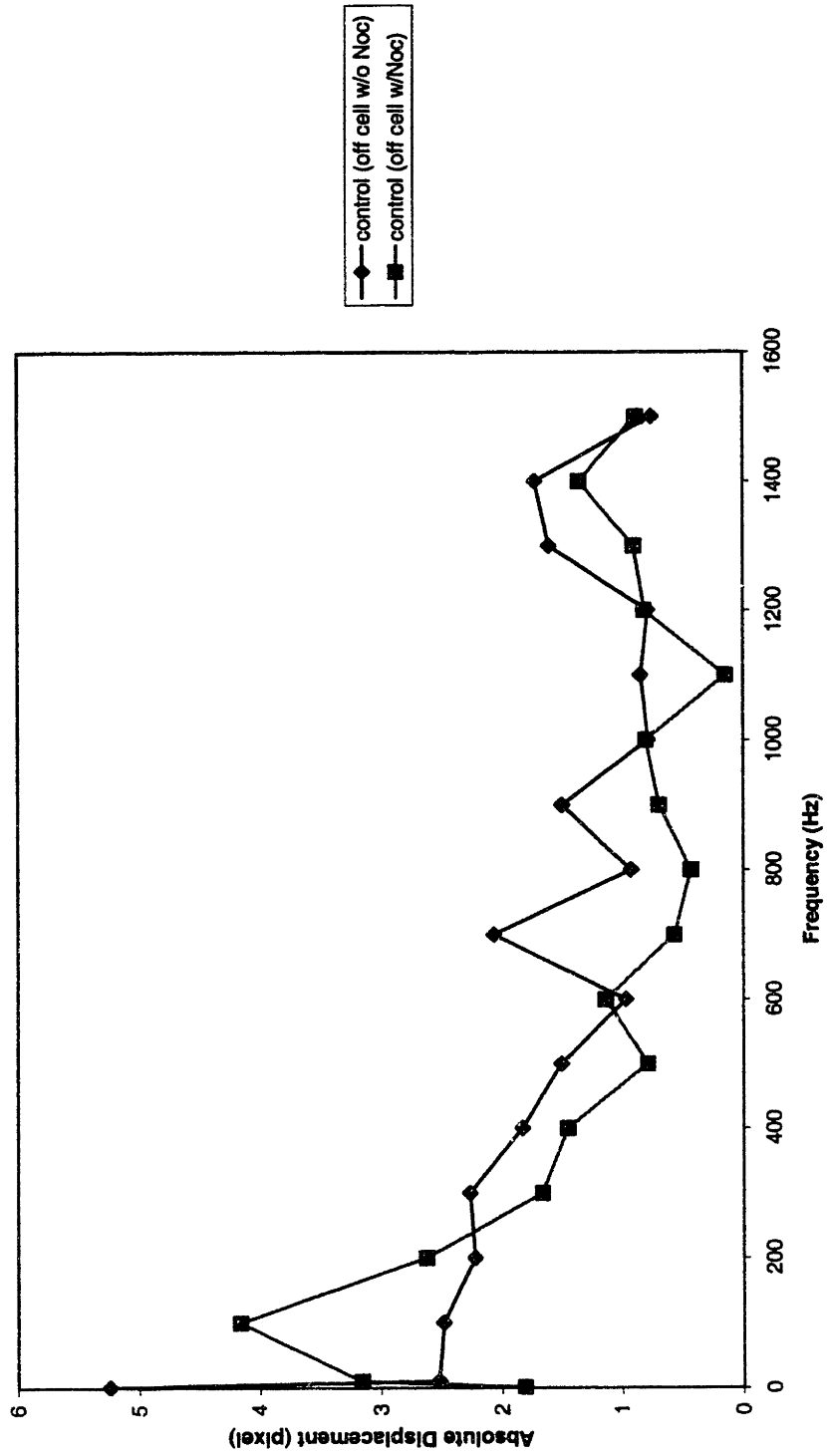


Figure 4H: Frequency versus absolute displacement

Frequency vs Absolute Displacement  
Cell w/o Cyto

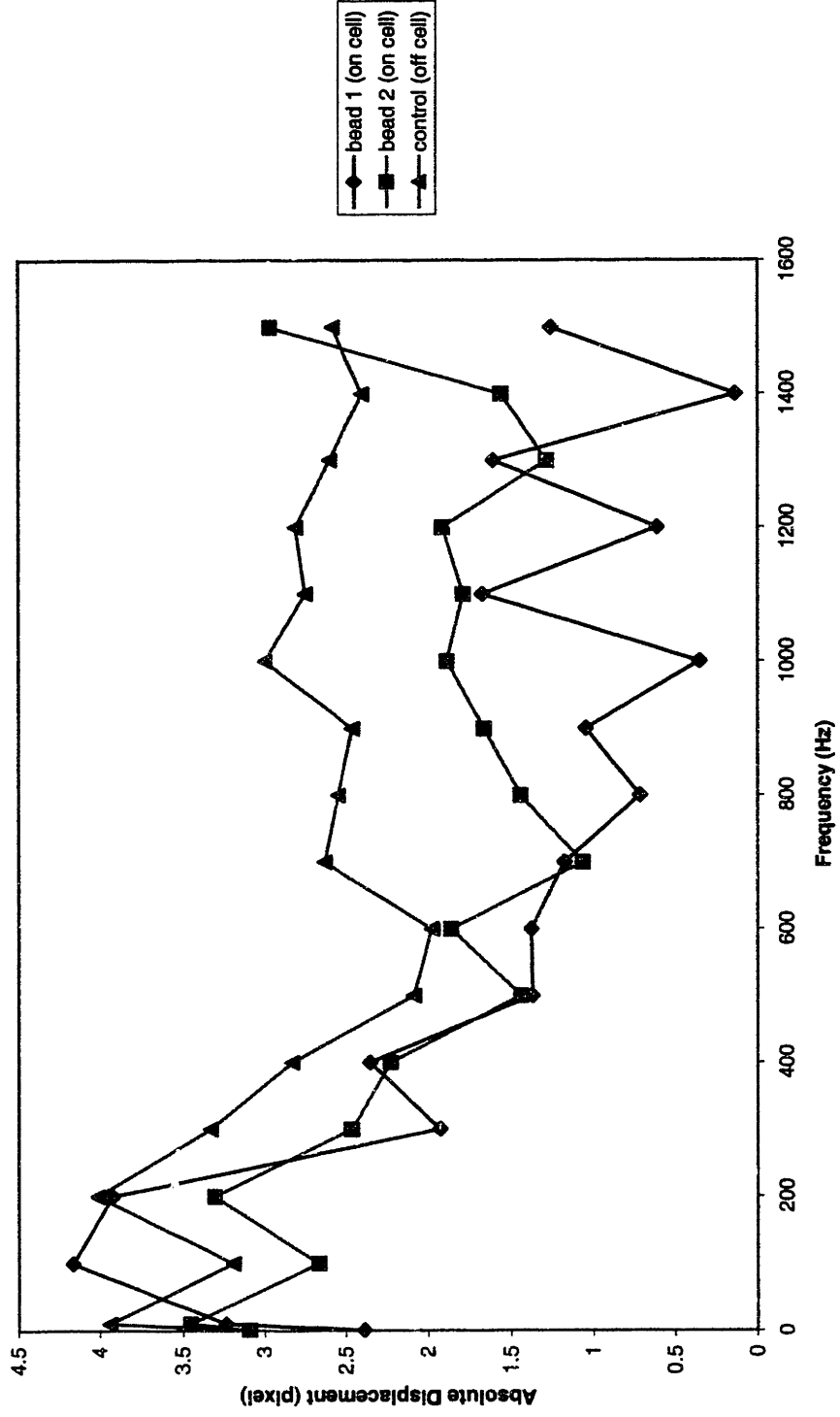


Figure 41: Frequency versus absolute displacement for w/ cytochalasin-D versus w/o cytochalasin-D (bead 1)

Frequency vs Absolute Displacement  
bead 1  
Cyto vs w/o Cyto

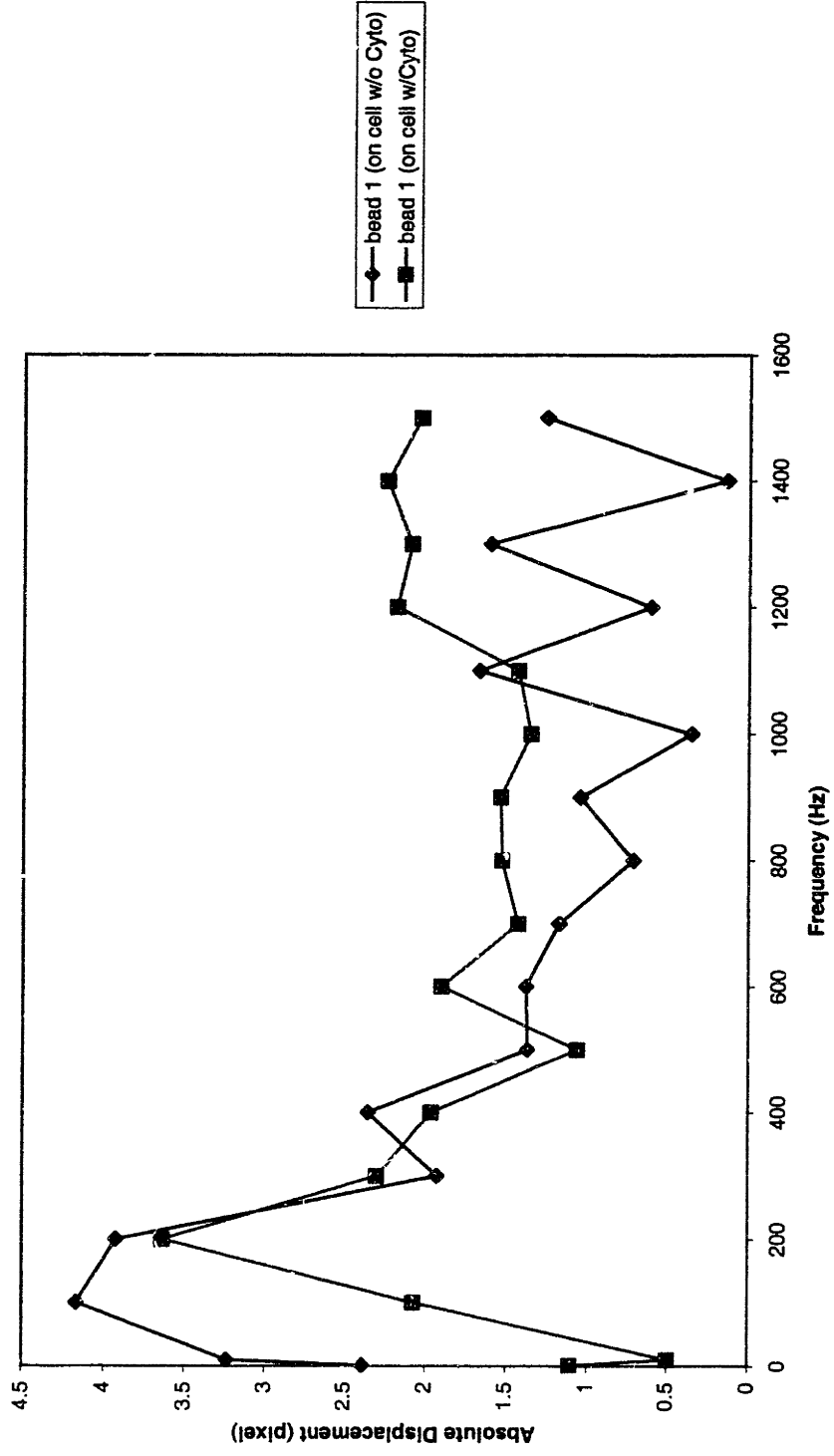




Figure 4J: Normalized displacement versus frequency for w/ cytochalasin-D versus w/o cytochalasin-D (bead 1)

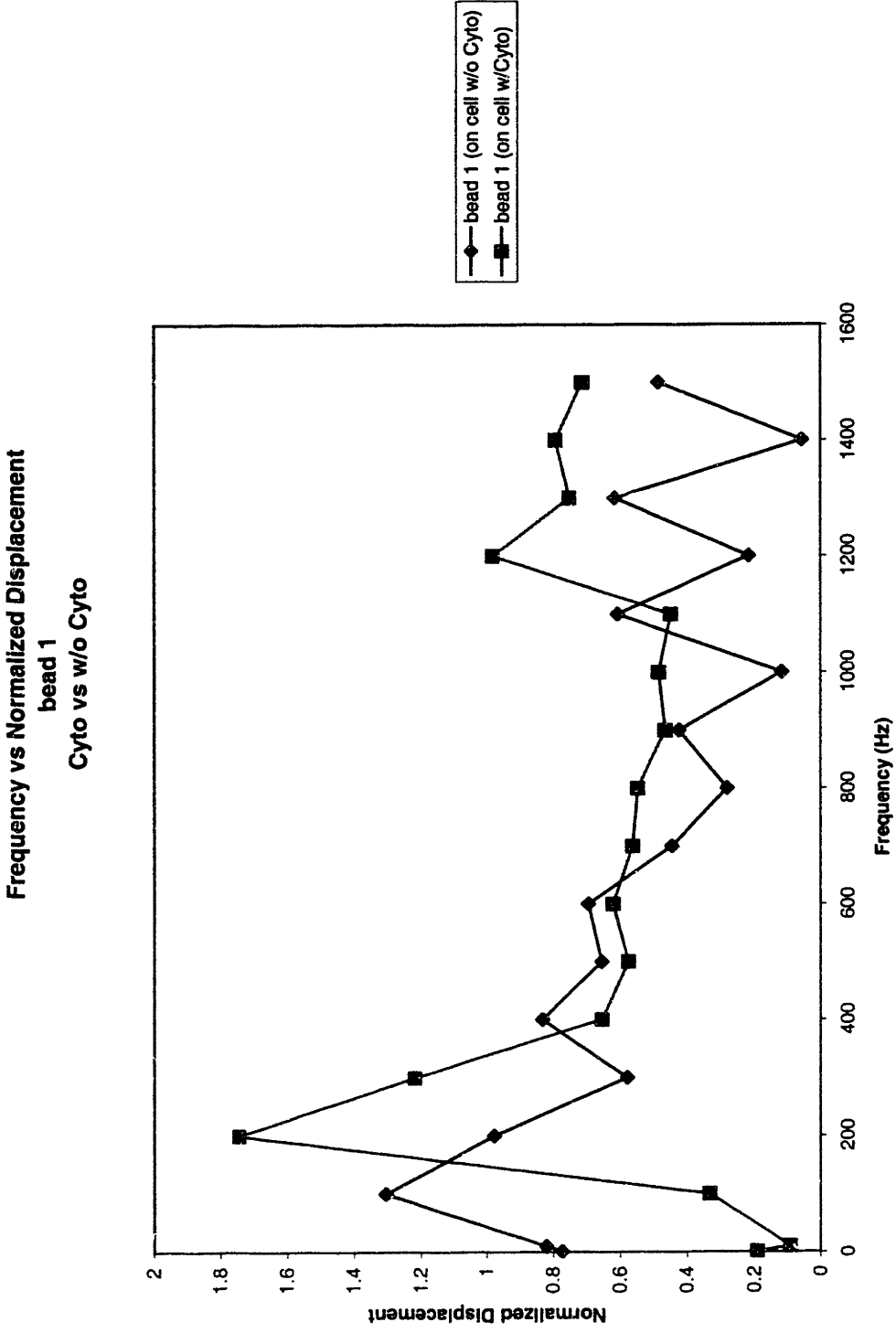


Figure 4K: Frequency versus absolute displacement for w/ cytochalasin-D versus w/o cytochalasin-D (bead 2)

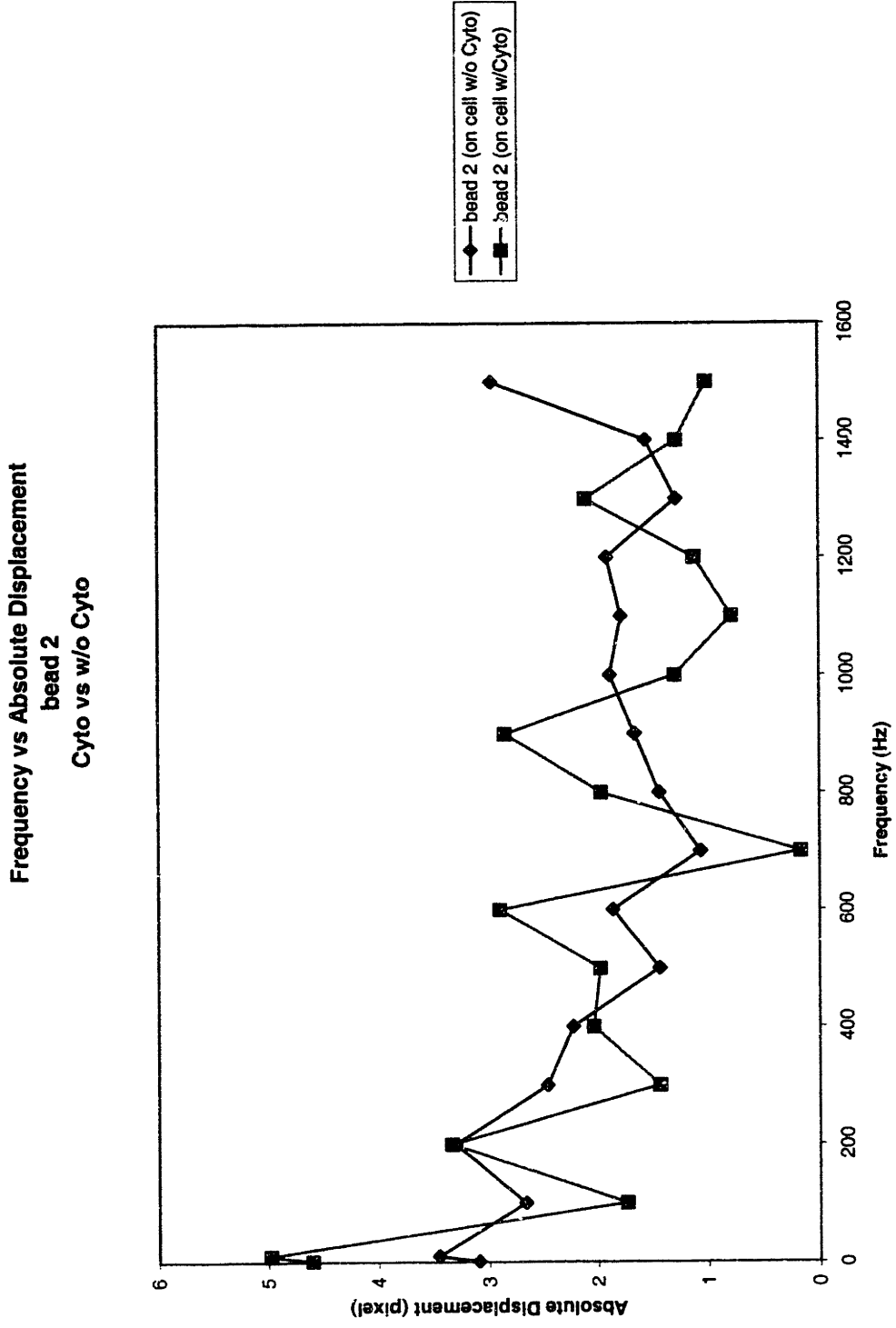


Figure 4L: Normalized displacement versus frequency for w/ cytochalasin-D versus w/o cytochalasin-D (bead 2)

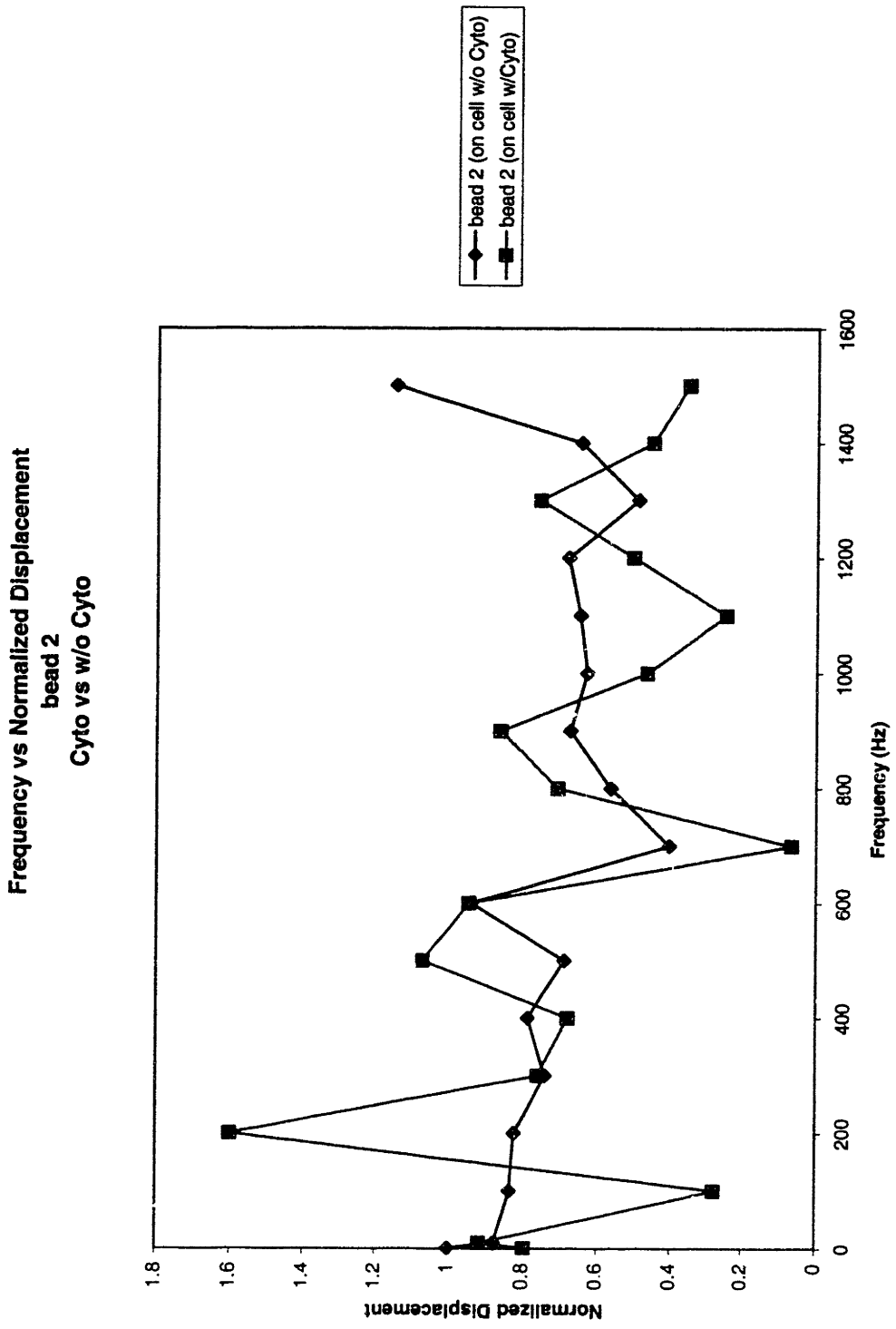


Figure 4M: Frequency versus absolute displacement for w/ cytochalasin-D versus w/o cytochalasin-D (control)

Frequency vs Absolute Displacement  
control  
Cyto vs w/o Cyto

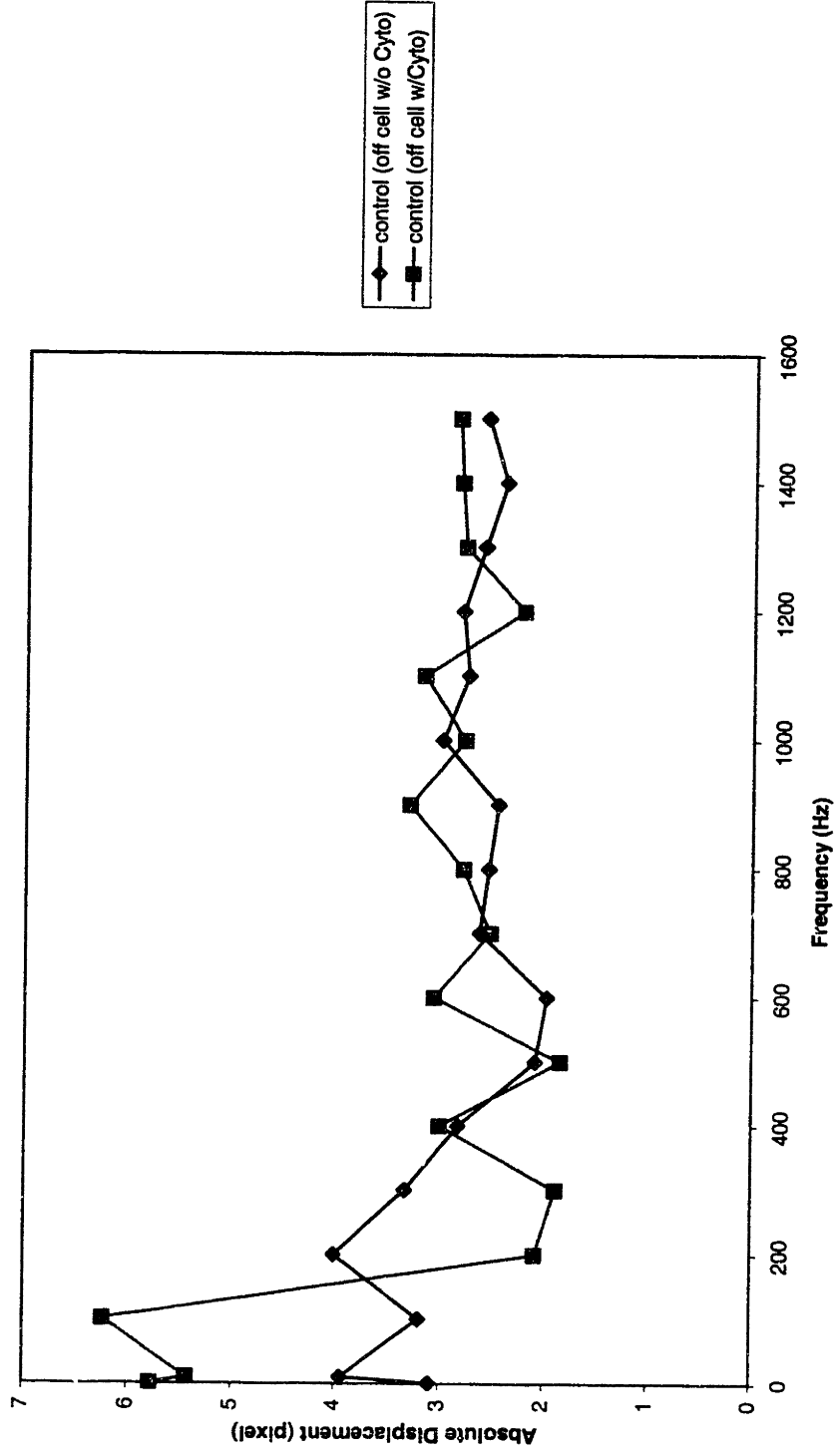


Figure 4N: Displacement versus frequency for different control beads

### Frequency vs Displacement

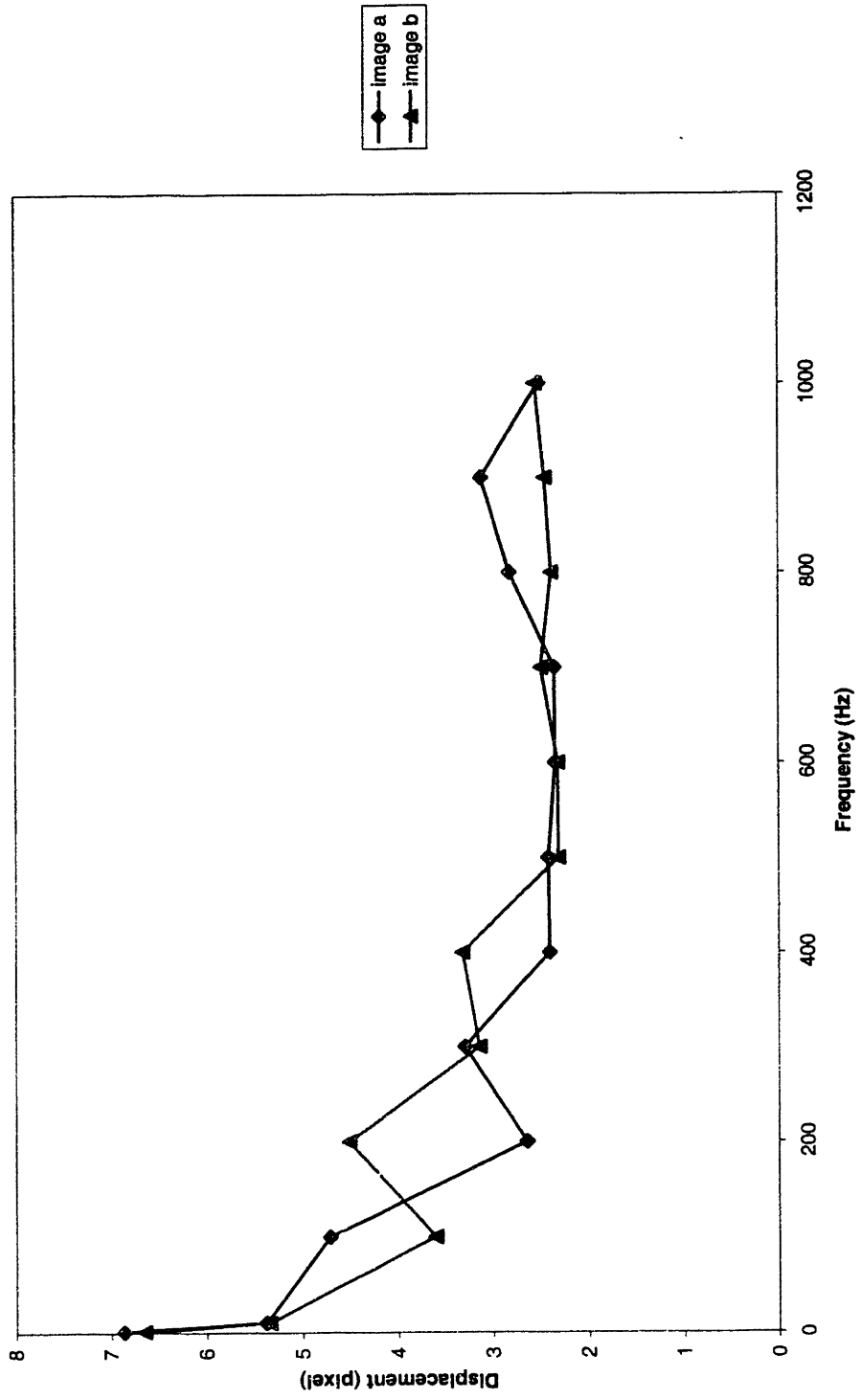


Figure 4O: Displacement versus frequency w/ mass added to piezoelectric actuator

### Frequency vs Displacement

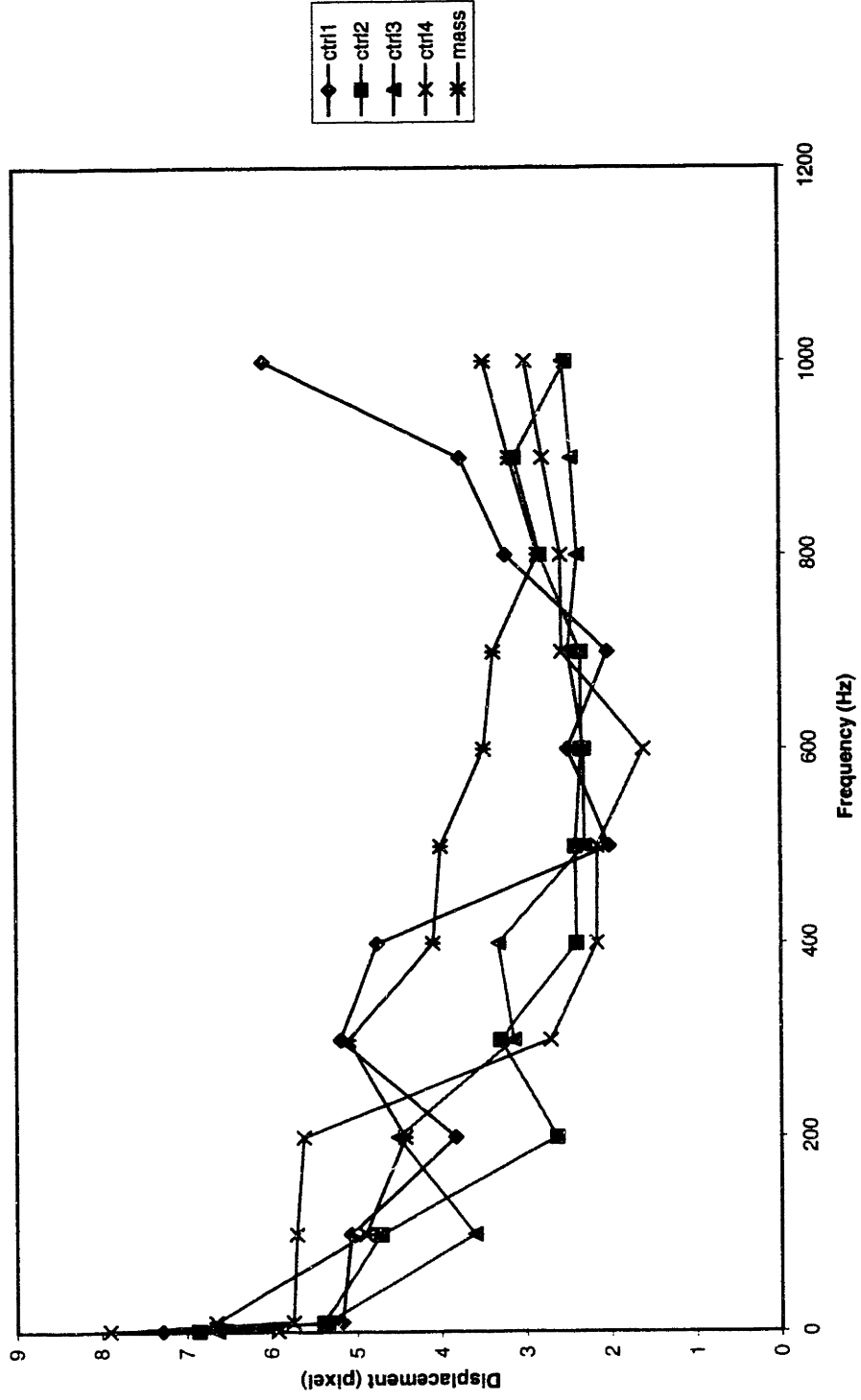


Figure 4P: Displacement versus frequency for reprocessed image of bead

### Frequency vs Displacement

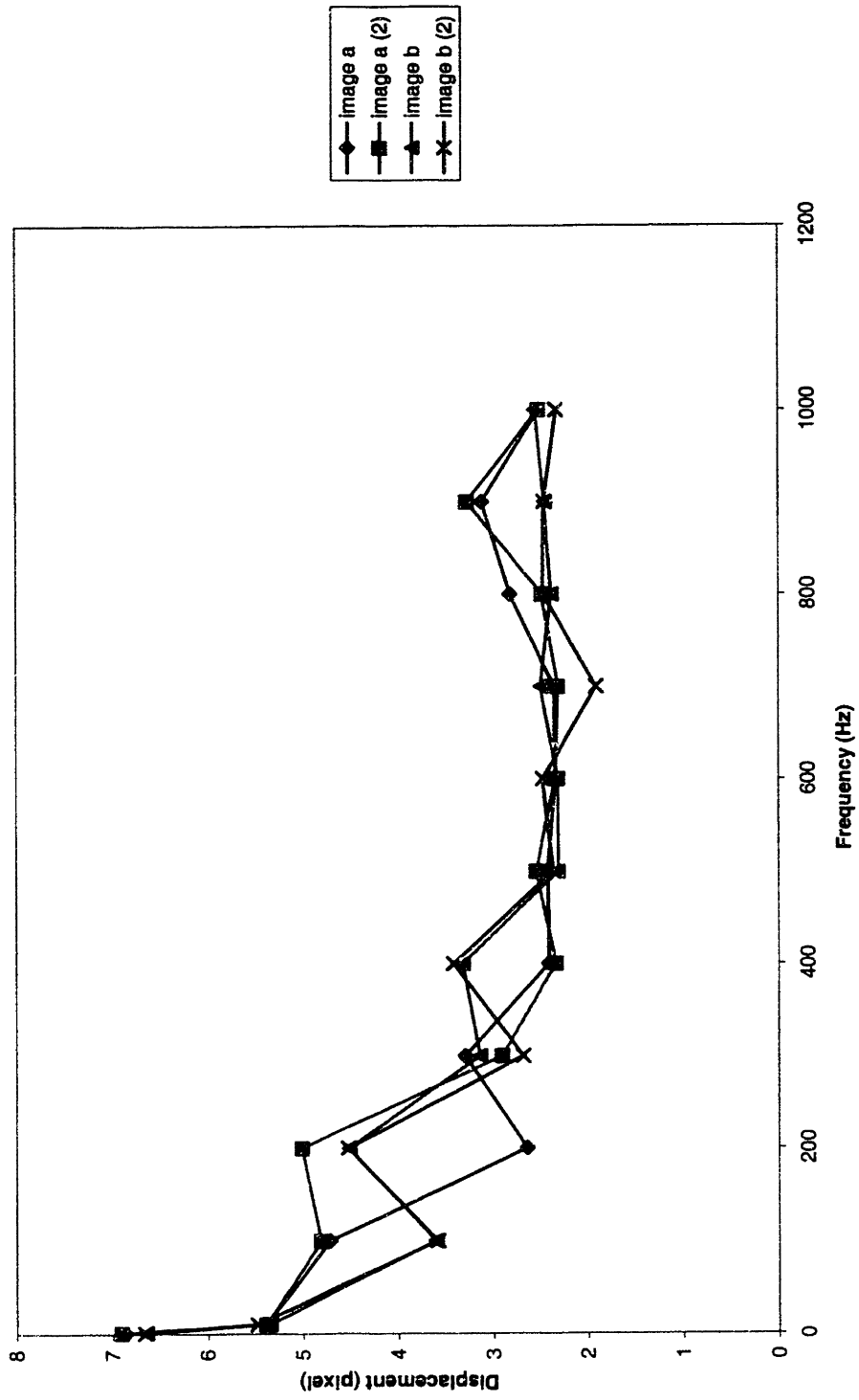


Figure 4Q: Displacement versus frequency for recaptured image of bead at different exposures (500, 750, and 2000)

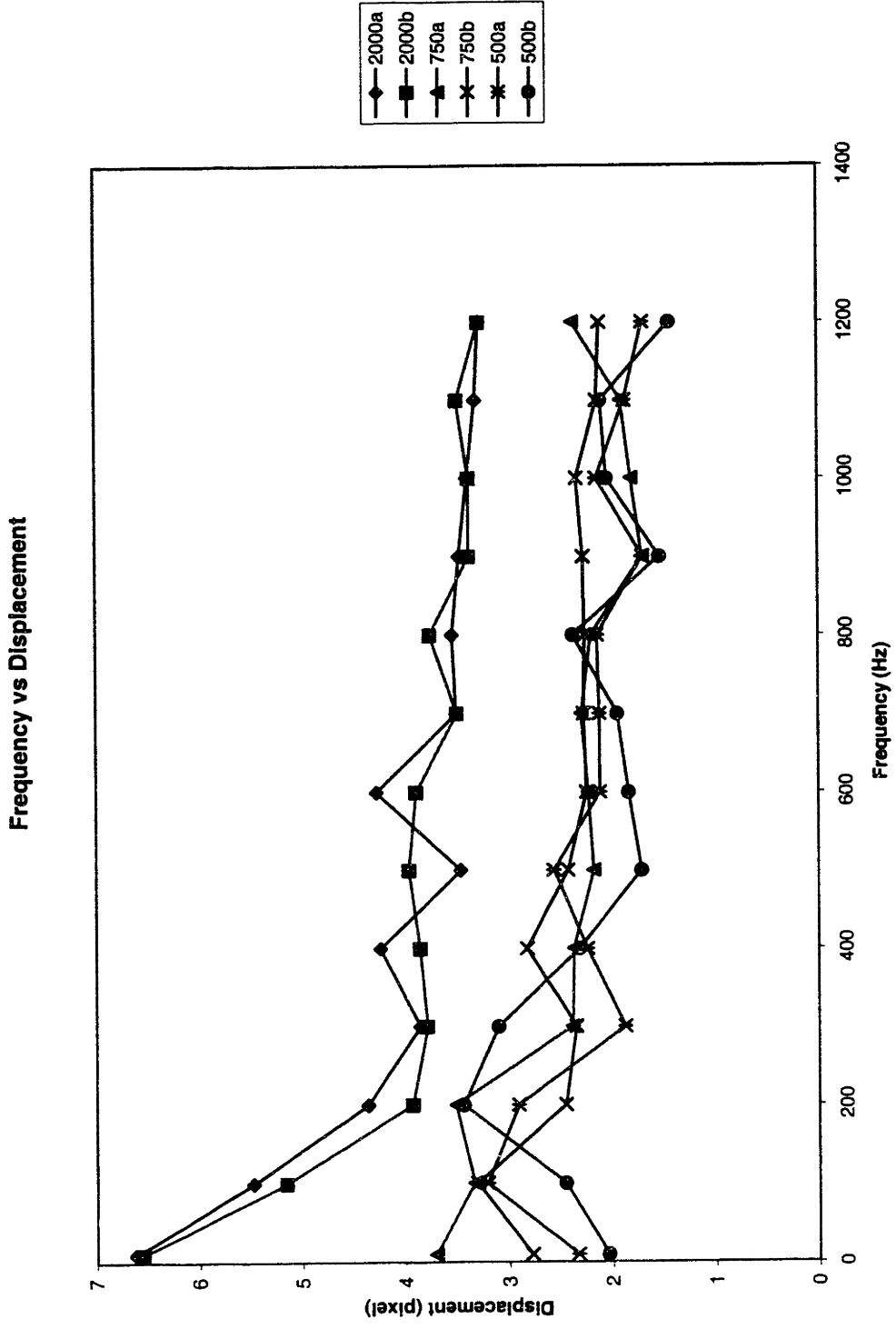




Figure 4R: Frequency versus absolute displacement

### Frequency vs Absolute Displacement

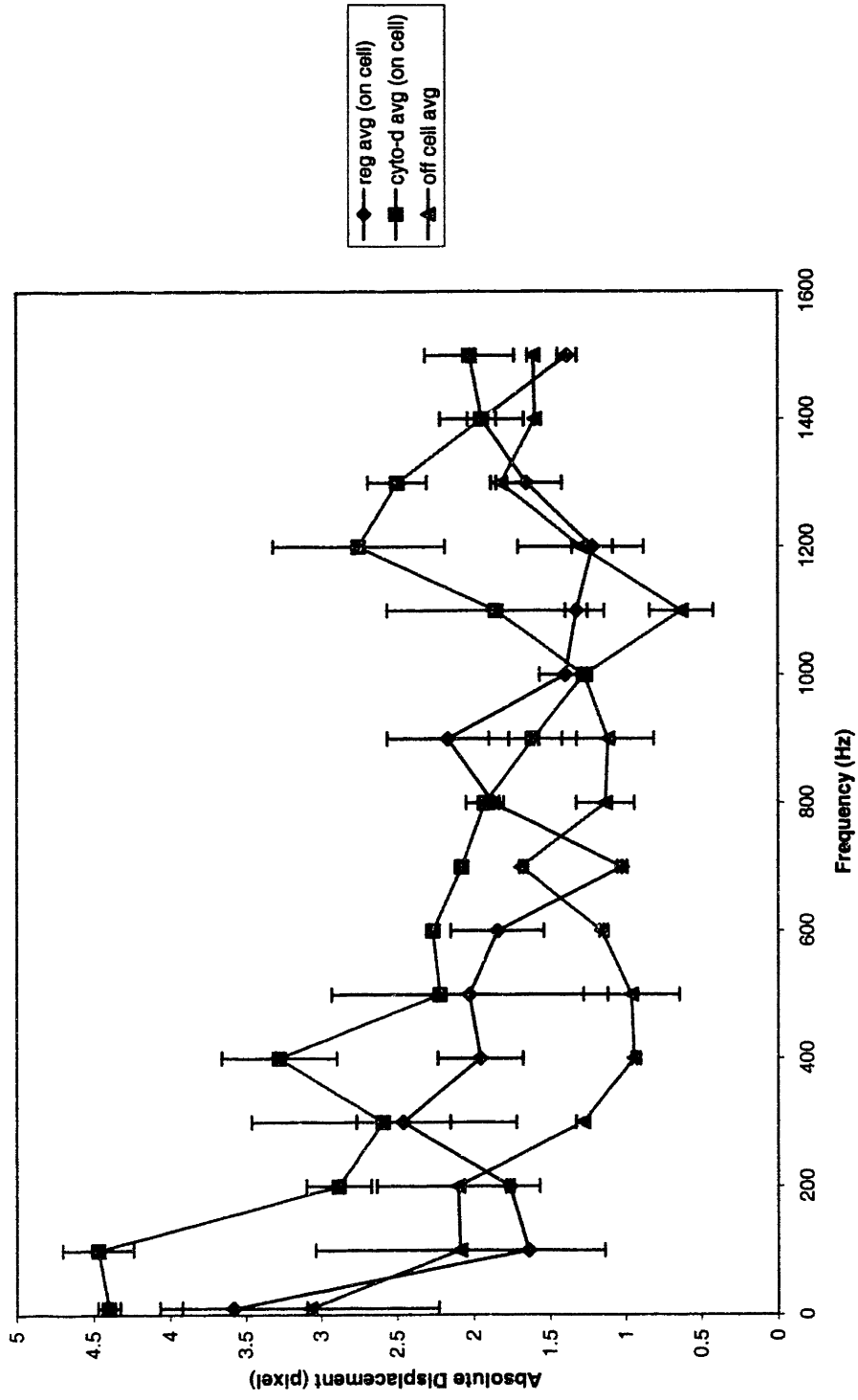


Figure 4S: Normalized displacement versus frequency for w/ cytochalasin-D versus w/o cytochalasin-D

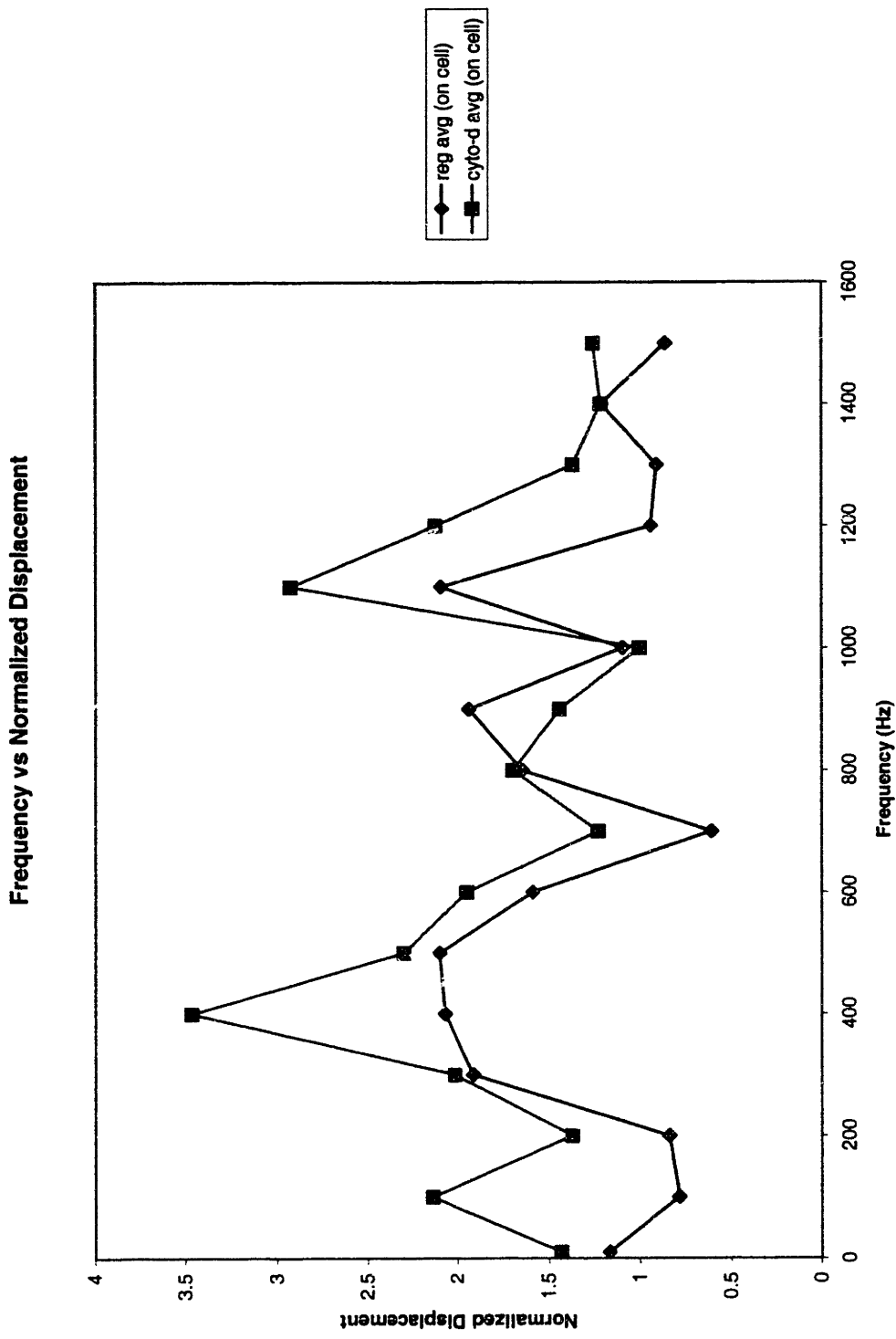


Figure 4T: Frequency versus absolute displacement for inverted cover slip

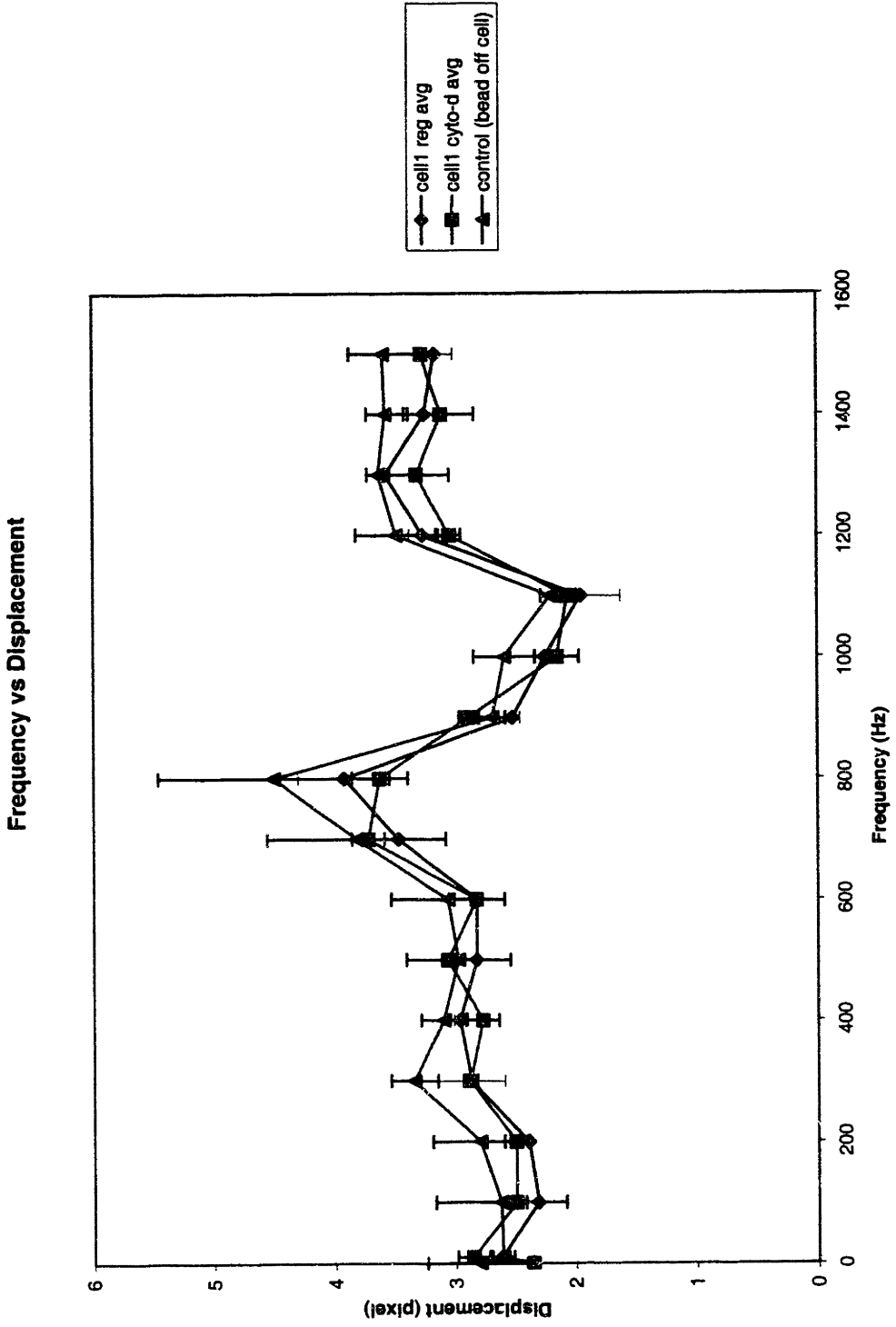


Figure 4U: Normalized displacement versus frequency for w/ cytochalasin-D versus w/o cytochalasin-D for inverted cover slip

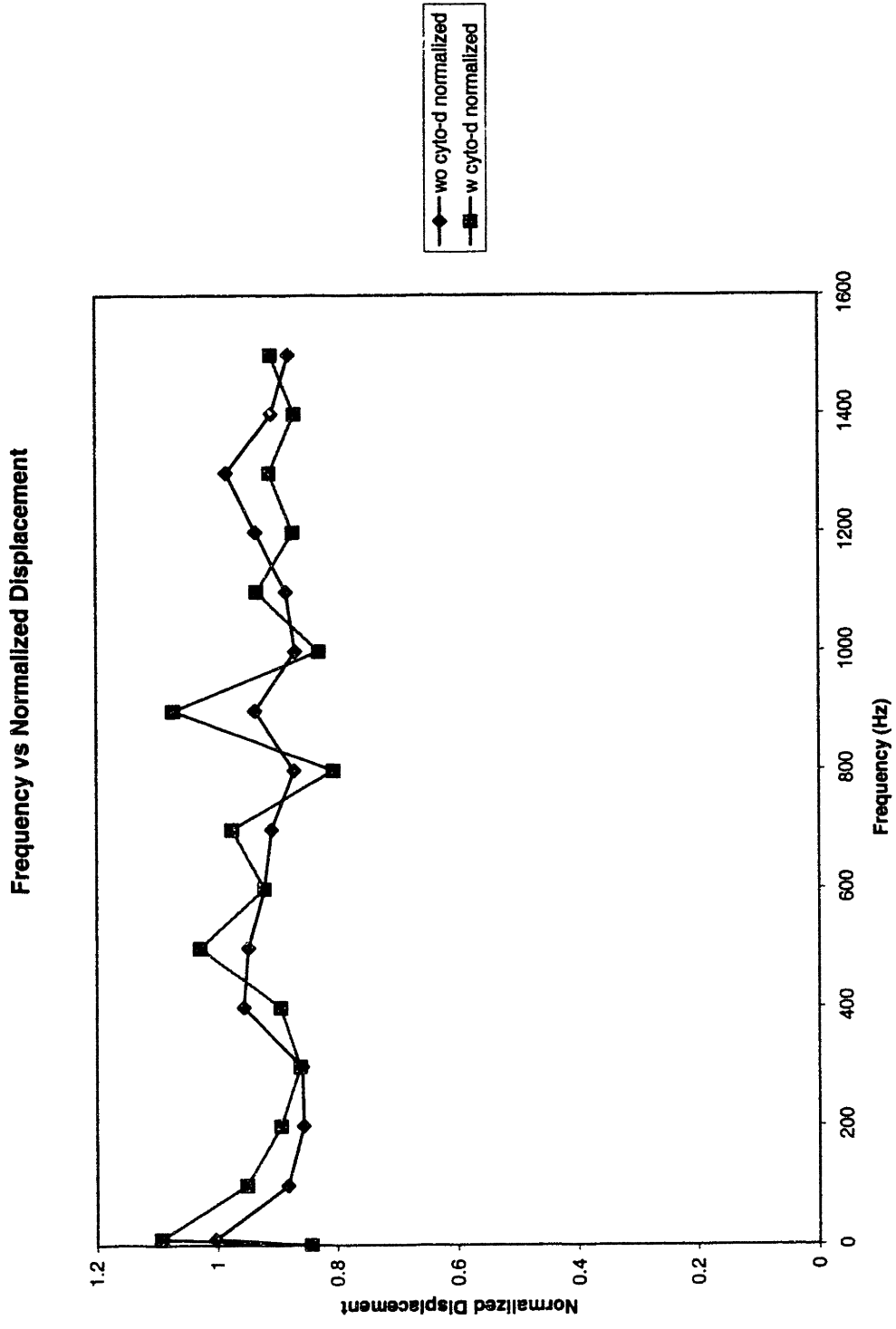


Figure 4V: Frequency versus absolute displacement

Frequency vs Displacement

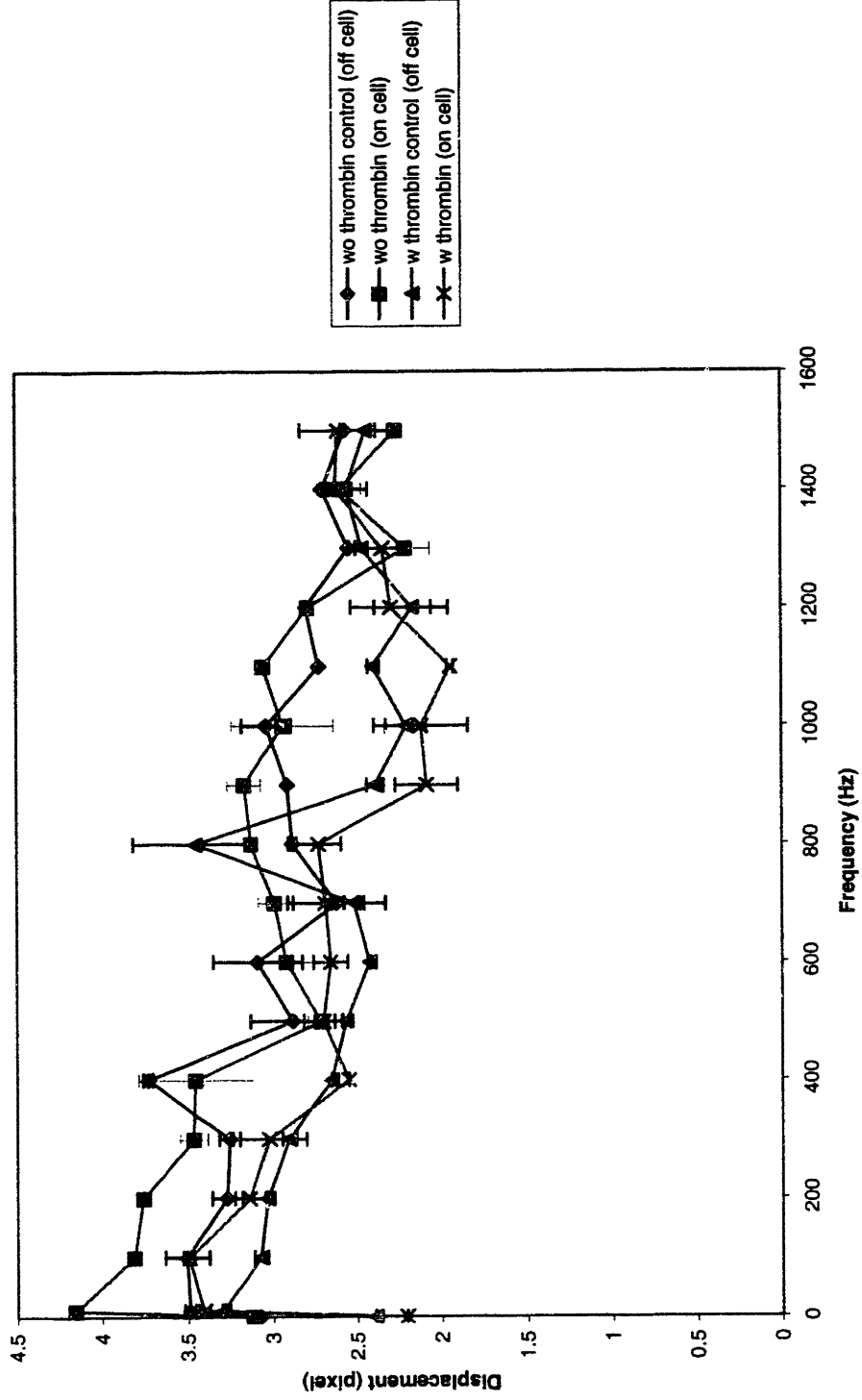
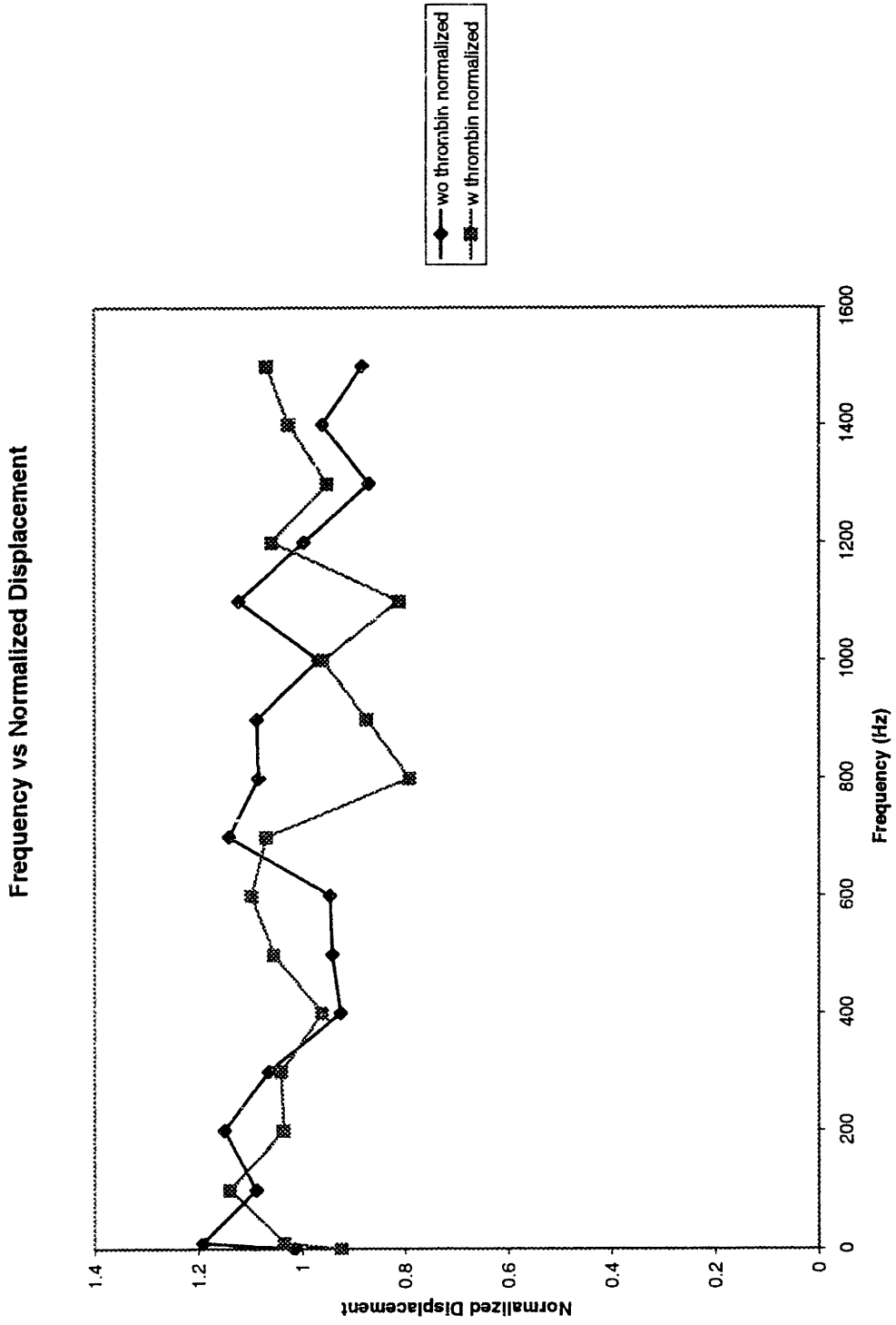


Figure 4W: Normalized displacement versus frequency for w/ thrombin versus w/o thrombin



## Appendix

EXCEL Script 'Above'

Sub Above()

' THIS IS GOOD FOR AVERAGE REPLACEMENT 1/18/01

' Macro2 Macro

' MACRO FOR CHECKING LARGE NUMBERS (4096 and above)

'

low = 100000

high = 0

numlow = 0

numhigh = 0

num = 0

tempcount = 0

middle1 = 0

middle2 = 0

'     OUTERLOOP.....

Do

'LOOP1.....

Do

temp1 = ActiveCell.Value

ActiveCell.Offset(0, 1).Activate

tempafter = ActiveCell.Value

ActiveCell.Offset(0, -1).Activate

If temp1 > 4096 Then ActiveCell.Value = temp1 / 2

'ActiveCell.Offset(1, 0).Activate

'ActiveCell.Value = temp1

'ActiveCell.Offset(1, 0).Activate

'ActiveCell.Value = high

'ActiveCell.Offset(1, 0).Activate

'ActiveCell.Value = low

'ActiveCell.Offset(-3, 1).Activate

ActiveCell.Offset(0, 1).Activate

num = num + 1

Loop While tempafter <> ""



```
ActiveCell.Offset(1, -(num)).Activate
temp1 = ActiveCell.Value
ActiveCell.Offset(0, 1).Activate
tempafter = ActiveCell.Value
ActiveCell.Offset(0, -1).Activate
```

```
num = 0
```

```
Loop While tempafter <> ""
```

```
End Sub
```

## EXCEL Script 'Oscillatory Deformation'

Sub OscillatoryDeformation\_2()

' THIS IS THE RIGHT ONE...PROCESSED 12/14/00

,

' Macro2 Macro

' Macro recorded 11/07/2000 by Li Yan

,

' MACRO FOR OSCILLATORY DEFORMATION (11/12/00)

' MAKE SURE THAT THE END OF THE DATA IS "CLEAR" OTHERWISE THERE  
WILL BE AN ERROR

,

low = 100000

high = 0

numlow = 0

numhigh = 0

num = 0

tempcount = 0

middle1 = 0

middle2 = 0

' OUTERLOOP.....

Do

' LOOP1.....

Do

temp1 = ActiveCell.Value

ActiveCell.Offset(0, 1).Activate

tempafter = ActiveCell.Value

ActiveCell.Offset(0, -1).Activate

If temp1 > high Then high = temp1

If temp1 < low Then low = temp1

'ActiveCell.Offset(1, 0).Activate

'ActiveCell.Value = temp1

'ActiveCell.Offset(1, 0).Activate

'ActiveCell.Value = high

'ActiveCell.Offset(1, 0).Activate

'ActiveCell.Value = low

'ActiveCell.Offset(-3, 1).Activate

ActiveCell.Offset(0, 1).Activate

num = num + 1

middle = (high + low) / 2

Loop While tempafter <> ""

ActiveCell.Offset(0, -(num - 1)).Activate

'LOOP2.....

Do

tempcount = tempcount + 1

tempbefore = ActiveCell.Value

ActiveCell.Offset(0, 1).Activate

temp1 = ActiveCell.Value

ActiveCell.Offset(0, -1).Activate

If (temp1 > middle) And (tempbefore < middle) Then middle1 = 1 + tempcount +  
((middle - tempbefore) / (temp1 - tempbefore))

If (temp1 < middle) And (tempbefore > middle) Then middle2 = 1 + tempcount +  
((middle - tempbefore) / (temp1 - tempbefore))

'ActiveCell.Offset(4, 0).Activate

'ActiveCell.Value = middle

'ActiveCell.Offset(1, 0).Activate

'ActiveCell.Value = temp1

'ActiveCell.Offset(1, 0).Activate

'ActiveCell.Value = tempbefore

'ActiveCell.Offset(1, 0).Activate

'ActiveCell.Value = middle1

'ActiveCell.Offset(1, 0).Activate

'ActiveCell.Value = middle2

'ActiveCell.Offset(-8, 0).Activate

ActiveCell.Offset(0, 1).Activate

Loop While temp1 < middle

ActiveCell.Offset(0, (-tempcount - 2)).Activate

ActiveCell.Offset(0, num).Activate

tempcount = 0

'LOOP FROM RIGHT SIDE OF GRAPH

Do

```
tempcount = tempcount + 1
temp1 = ActiveCell.Value
ActiveCell.Offset(0, -1).Activate
tempbefore = ActiveCell.Value
ActiveCell.Offset(0, 1).Activate
```

```
'If (temp1 > middle) And (tempbefore < middle) Then middle1 = 1 + tempcount +
((middle - tempbefore) / (temp1 - tempbefore))
If (temp1 < middle) And (tempbefore > middle) Then middle2 = (num - tempcount) +
((middle - tempbefore) / (temp1 - tempbefore))
```

```
'ActiveCell.Offset(2, 0).Activate
'ActiveCell.Value = middle1
'ActiveCell.Offset(1, 0).Activate
'ActiveCell.Value = middle2
'ActiveCell.Offset(1, 0).Activate
'ActiveCell.Value = temp1
'ActiveCell.Offset(1, 0).Activate
'ActiveCell.Value = middle1
'ActiveCell.Offset(1, 0).Activate
'ActiveCell.Value = middle2
'ActiveCell.Offset(-4, 0).Activate
```

```
ActiveCell.Offset(0, -1).Activate
```

Loop While temp1 < middle

```
ActiveCell.Offset(0, (tempcount + 2)).Activate
```

```
ActiveCell.Offset(1, -num).Activate
temp1 = ActiveCell.Value
```

```
ActiveCell.Offset(-1, 100).Activate
ActiveCell.Value = middle
ActiveCell.Offset(0, 1).Activate
displacement = middle2 - middle1
ActiveCell.Value = displacement
```

```
ActiveCell.Offset(1, -102).Activate
```

```
low = 100000
high = 0
numlow = 0
numhigh = 0
```

```
num = 0  
tempcount = 0  
middle1 = 0  
middle2 = 0
```

```
Loop While temp1 < ""
```

```
End Sub
```

IPLab script 'process line displacement'

Row/Col Plot

View as text

Select all

Copy

End

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