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ANALYSIS ON DEMONTRATION OF MICROMETER-SCALE BIOCHEMICAL INDUCTION OF BACTERIAL CELLS VIA TRANSPORT OF SIGNALING MOLECULES

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ABSTRACT

Levitation has evolved into a top choice for a variety of tasks due to its contact-free nature and reduced danger of contamination. Beyond Earnshaw's theorem, magnetic levitation utilizing diamagnetism is a sort of energy-free passive stable levitation that is possible even at room temperature. Intriguingly, even materials that don't appear to be magnetic can be lifted in a magnetic field and used to stabilise the free levitate of magnetic materials. A simple approach of controlling complex magnetic fields using thermo-magnetic and optical material features is introduced. In a closed loop control system, the user can specify the positions of milli-robots that are automatically detected and actuated to the appropriate positions and work to maintain them there. In this paper we have used several diamagnetically levitated robots, which can show how to distribute dissolved bio-chemicals in liquid while still being able to view them under a light microscope. Fluorescent molecules are loaded into hydrogels with microscale precision and released in a diffusive manner, and synthetically created bacterial cells are activated with signalling molecules. This platform can be used in a wide range of fields, including synthetic biology, developmental biology, and tissue engineering.

1. INTRODUCTION

Mobile small-scale systems are plagued by difficulties like friction, stickiness, and wear, all of which decrease performance and complicate control. Diamagnetic levitation with wireless actuation allows for frictionless transit, which is useful for integrating assembly and manipulation tasks. There is no magnetic hysteresis, and there is no need for an intermediary medium to connect its moving components to its fixed ones, hence it necessitates simple control techniques. The use of acoustic waves1 and optical tweezers in assembly and manipulation are two examples of common wireless or contactless actuation techniques. A diamagnetic layer can also be used to generate controllable electromagnetic fields for a levitated magnet array.

In order to transfer dissolved molecules necessary for growth, development, and communication, MULTICELLULAR biological systems have devised a variety of transport methods. It's a never-ending problem to design fluidic delivery systems that work well with cells and tissues. For research and diagnostics, existing automated systems for fluid delivery include automated pipetting robots for loading and dispensing at the microliter scale, as well as microfluidic designs for delivering volumes as small as sub-microliters.

For microscopy and control at the microscale, bulky commercially available pipetting robots are preferable because of their flexible programming, high throughput, and quick operation. Each well in the storage plate contains a separate experiment, and the systems used to handle them are typically 96-well storage plates. For example, each well can contain a few microliters of biological cues (such hormones or nutrients) or signalling molecules (like morphogens).

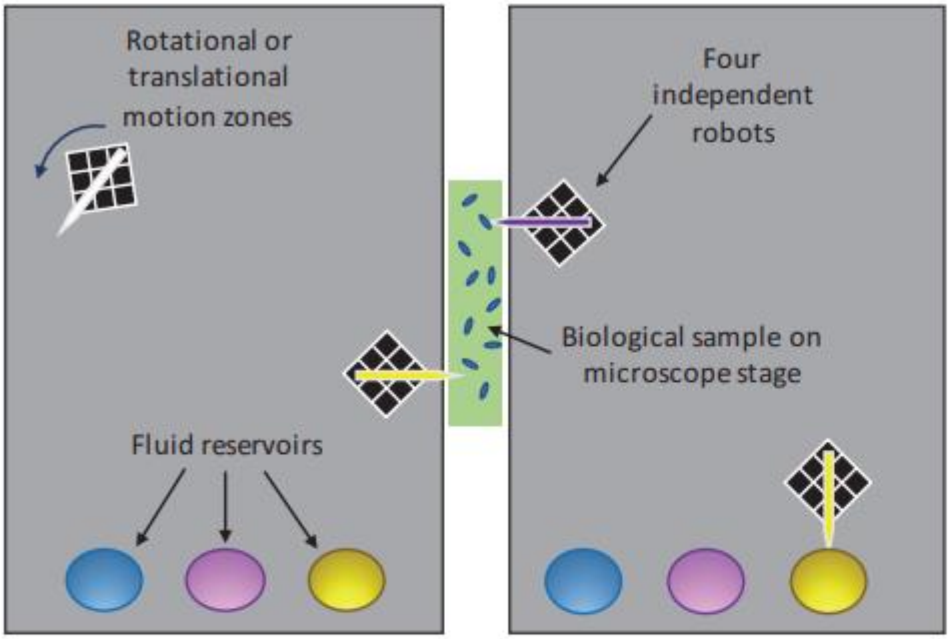


Fig. 1. System for microbiological experiments using microrobots. It is possible to load, transport, and release microscale biochemical payloads using four fully programmable robots.

Diamagnetically levitated robots driven by magnetic forces generated by current traces on printed circuit boards under the MicroFactory system were previously developed (PCBs). Although it can carry loads of a few milligrammes, there are design constraints, such as weight and size limits, that limit its usefulness. These considerations make it difficult to design systems that are actively pumped and equipped with electronic components. The levitating robot's micropipette was therefore chosen to load and deliver fluids and water-soluble molecules into the device (Fig. 1). Microfluidic devices have successfully demonstrated passive loading.

This method takes use of surface tension's supremacy as a source of forces at tiny scales. This means that only Laplace pressure can be used to load fluids into capillary tubes and micropipettes. Another passive method is used to transport molecules. The payload is delivered and released using diffusion after the pipette tip is guided to the target. It is possible to replicate intercellular delivery systems that rely on cell-to-cell contact and diffusion using this microbiology technique for chemotaxis tests. The transfer of molecular species can be controlled by altering the pipette aperture, molecular concentration, or dwell duration.

2. LITERATURE REVIEW

Gerber, 2017, Biotechnology and the life sciences benefit greatly from liquid-handling robots, and their impact on daily life is growing. While educational robotics such as Lego Mindstorms play a significant role in mechatronics and programming initiatives, no comparable links exist in the life sciences. There are a variety of experiments that these robots can help with, from education to research. With the help of common, low-cost household items, pipetting routines programmed into robot designs, and modifications to robot designs, we created a dynamic work environment.

Whitehead, 2018, The use of robotic automation in synthetic biology is especially important for experiments involving large amounts of liquid. Research tasks that aren't perfectly standardised, on the other hand, aren't automated very often. Molecular biological protocols require significant investments to translate into robot programmes, and the resulting programmes are frequently too specific to be easily reused and shared are two major reasons for this. Recently, some aspects of protocol portability and user friendliness for liquid handling have been addressed. They are, however, either too simple or too complicated for the average user to use, and thus require a great deal of time and effort to set up and use.

Enten, 2016, Cross-linking ligands to microbead surfaces is critical in the biomedical industries of diagnostics, drug delivery, and other applications. Multi-step liquid exchange, incubation, and mixing are required for microbead functionalization, all of which are time consuming and laborious processes. Automation in biomedical laboratories is limited by the high cost and complexity of automated systems. We created a benchtop robotic system for the functionalization of microparticles and sample preparation. Microcontroller functions can be programmed into the robot's microcontroller to perform incubation and mixing. This can be avoided by using filters with pore diameters smaller than the smallest diameter of the beads. A batch of 10(7) microbeads can be processed by the robot using three liquid reagents.

Marzo A, 2015, Aerodynamic levitation utilises a jet nozzle to create a gas stream that lifts a sample off the ground. The divergence of the jet can provide vertical stability, while the increased Bernoulli force at faster flow rates can ensure stability in the transverse direction by providing a centering force. However, in a vacuum, this method will not be able to lift a sample. This technique suspends an object in mid-air by utilising the force generated by acoustic radiation.

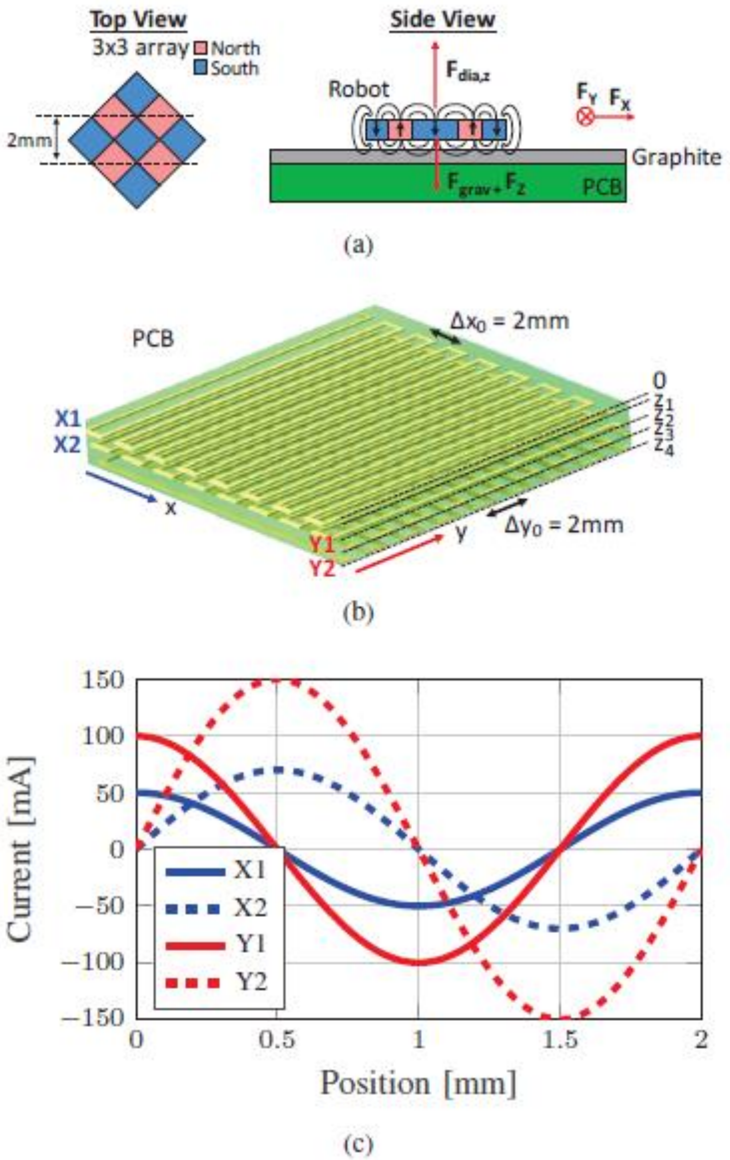
3. SYSTEM DESIGN

3.1 Diamagnetic Levitation and Robot Theory

NdFeB magnets (N52M, 1.4 mm) are used in the robots described in this paper, which are arranged in a north-south checkerboard pattern (3 x 3 arrays of NdFeB magnets) (a). The diamagnetic force generated by the magnet's magnetic field interaction with the underlying graphite provides passive levitation of these magnetic arrays.

$$F_{dia} = \frac{\chi V}{2\mu_o} \nabla\left(\vec{B}_{magnet} \cdot \vec{B}_{magnet}\right)$$

F_{dia} is the upward force on the robot, χ is the magnetic susceptibility of the graphite, B is the magnetic field generated by the permanent magnet, V is the volume, and µ0 is the vacuum permeability. An electrical trace pattern (Fig. 2b) is used to drive these arrays around in the X, Y, and Z directions through the PCB's 24 mm x 24 mm square pattern. The x and y directions each have two sets of serpentine traces.



(a)

(b)

(c)

Fig. 2. MicroFactory diamagnetically levitated robot system diagram and schematic.

Robots are made up of magnetic squares arranged in a checkerboard pattern. (a) By emitting magnetic fields, the array produces a diamagnetic force on the magnet. (b) PCB schematic showing pitch-matched magnetic traces with serpentine patterns. (c) Drive currents should be used for each serpentine layer or trace. The pitch of the magnetic traces determines the frequency of the movement. By driving two traces in quadrature, analogue motion can be achieved.

3.2 Diamagnetic materials

The orbital motion of electrons around their nucleus and their intrinsic spin, which determines magnetism, are both influenced by the atom's structure and temperature. Because they are the most common type, ferroelectric materials are referred to as both paramagnets and diamagnets. Magnetic domains in ferromagnetic materials are aligned by an external magnetic field, which results in their vector sum being manifested as magnetization. Ferroelectric materials exhibit nonlinear magnetization behaviour that is influenced by applied magnetic fields and is characterised by B-H curves because of the domain sizes and structures as well as their interactions with each other The induction Br remains even after the external magnetic field is removed. The magnetization of weak magnetic materials, in contrast to that of ferromagnetic materials, increases linearly in the presence of an external magnetic field and returns to zero when the magnetic field is removed.

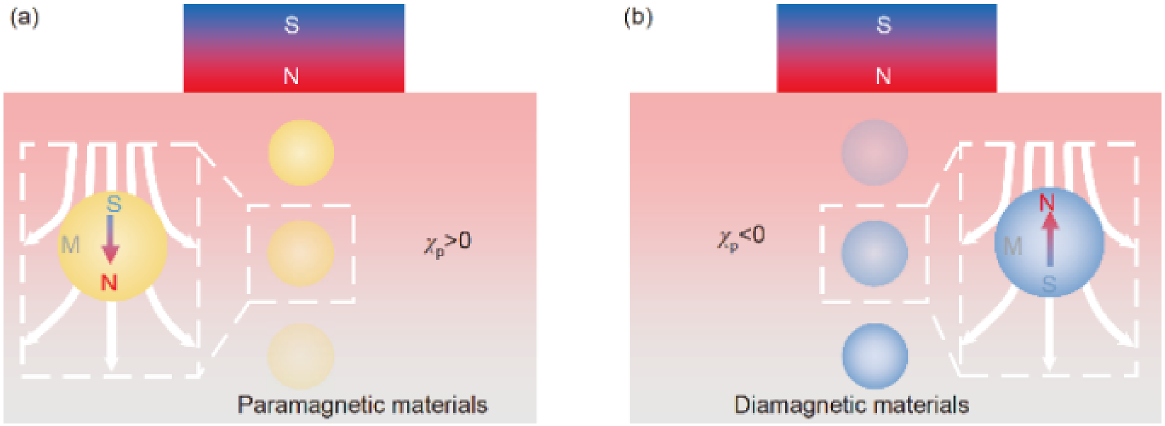


Figure 3: Under the influence of an external magnetic field, magnetic effects can be observed. (a) An attractive force is exerted on paramagnetic materials whose magnetization direction resembles that of the external magnetic field (b) In materials whose magnetization is antiparallel to the direction of the external magnetic field, a repelling force is experienced by diamagnetic materials.

Paramagnetic materials align themselves along the external magnetic field and are drawn to the strongest magnetic field (Figure 3(a)) when their susceptibilities are positive. However, diamagnetic materials have a negative susceptibility, which can be described as an external magnetic field that induces an induced magnetic moment in opposition to the external field. It is because of this that they are compelled to move toward the lowest possible magnetic field (Figure 3(b)).

4. METHODS AND APPLICATIONS

Due to the absence of mechanical friction, wear, and thermal dilatations, diamagnetic levitation has proven its worth in recent years. Passive levitation, on the other hand, does not require any active control or power supply, resulting in a longer service life and improved reliability.

4.1 Levitation mechanism and configurations

In order to levitate diamagnetic materials, the structure must be able to support the object against gravity but also maintain levitation stability in the face of external disturbances. Each dipole of the diamagnetic material must be taken into account when calculating the total diamagnetic force acting on the levitated materials. For diamagnetic force, the entire volume can be expressed in terms of

$$F_d = \frac{\chi_p}{2\mu_o} \iiint \nabla B^2 dv$$

For stable levitation, the distribution of the magnetic field is critical. With the use of Bitter magnets, superconductors or hybrid magnets one can achieve an extremely high and varying magnetic field. Electromagnets, magnetic configurations, or a combination of both can produce a weaker magnetic field. With permanent magnets, diamagnetic levitation is achieved by creating a local magnetic field minimum with a high gradient.

4.2 Actuating and micromanipulation

Scale-reduction effects from magnetic interactions improve integrated MEMS systems' performance. It's possible to design diamagnetic bearings in a variety of ways. Diamagnetic motors and a magnetic array stator are the most fundamental parts. Research on diamagnetic bearing dynamics and rotational properties was conducted by Chen et al. Vertical, lateral, inclination, inclination damping, and rotational losses of the eddy current effect were studied using the thin-sheet model and the image method.

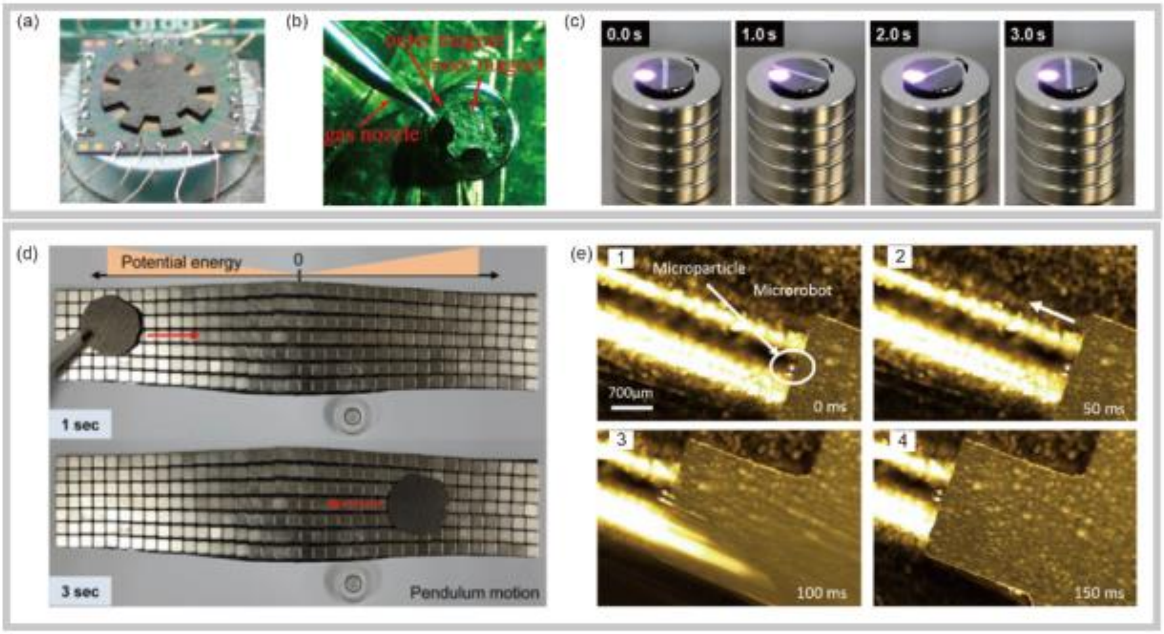


Figure 4 Representations of actuators using diamagnetic levitation are shown (Color online). (a) A image of an electrostatically driven pyrolytic graphite rotor. (b) with permission, a micromachined diamagnetic rotator driven by a gas flow,(c) levitated pyrolytic graphite rotatory motion, reprinted with permission from the American Chemical Society copyright 2012; (d) Controlling the trajectory of a levitated diamagnetic graphite by manipulating the magnetic field distribution; (e) For this experiment, the microrobot was used to carry the microparticle over 1000 nm.

Openloop and closed-loop control were used to design an electrostatic micromotor based on the axial variable-capacitance motor principle (Figure 4(a)). Rotor speed was 70.0 RPM when levitated above the magnets, with the gear-shape graphite rotor.

4.3 Method

4.3.1 MicroFactory System

Design changes from previous work allow the MicroFactory to be easily integrated into an optical microscope. Analog outputs (VIN) from NI-DAQ 6343 data acquisition boards are synchronised with custom-built analogue power amplifier based on Python code. When using dual-channel APEX PA74 power amplifiers, voltage is converted from voltage supply rails (VDD and VSS) or 12 V. One zone can be controlled by two of these operational amplifiers.

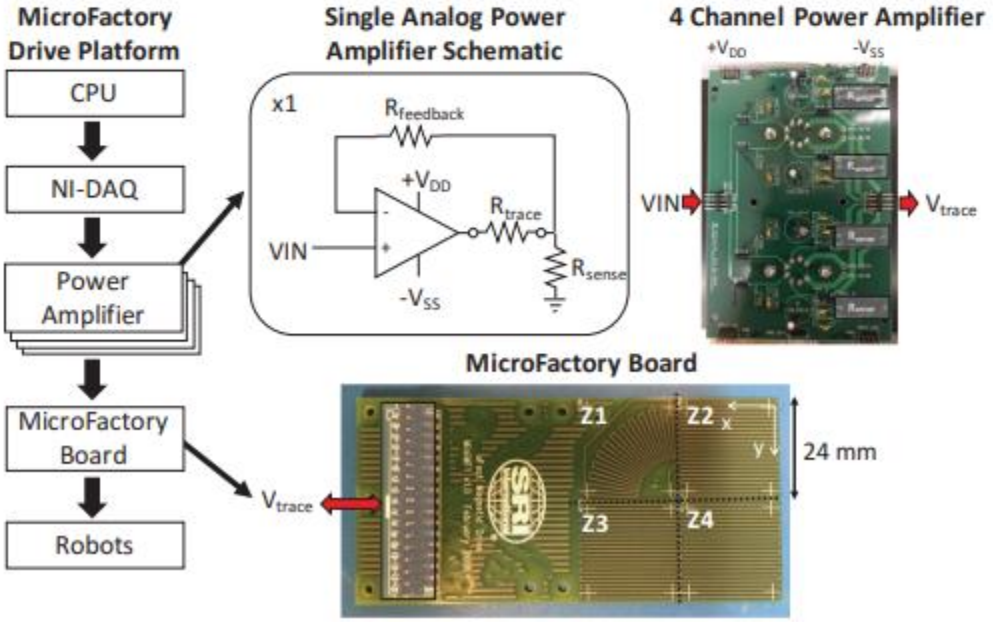


Fig. 5. MicroFactory control system diagram for a dimagnetically levitated robot. The voltage signals from the data acquisition boards are transformed into large currents by current amplifiers (0.2-0.7 A). Each of the four channels on the 4 channel power amplifier board has its own independent current control. Rewiring a 2x2 array into 4 independent zones or two pairs of clustered independent PCBs is possible (Z1-Z3, Z2-Z4). The robot's orientation can be changed only in one place on the board (Z1).

The PCB connects the Vtrace output of the power amplifiers to the board's current traces. The MicroFactory allows for simultaneous control of multiple robots. A PCB board without pyrolytic graphite plate is shown in Figure 5 bottom, which helps to better illustrate the control areas (Z1, Z2, Z3, Z4). Figure 2(b) depicts a Vtrace connector connecting four separate traces tiled within the plane. Multiple independent control zones can be created by tiling these sets of serpentine traces in 2D space using NI-DAQ and amplifiers.

CONCLUSION

Diamagnetic levitation, a rising and powerful tool, has inspired a number of research efforts. Pyrolytic graphite milli-robots were used to demonstrate an automated position control system for optically activated, passively levitating samples. Multiple milli-robot array configurations, including sequential control of multiple milli-robots, were successfully tested and demonstrated to work Using the milli-robot to magnet array grid spacing ratio, motion control was found to be directly related to milli-robot size Our research also demonstrates that the induction of signalling molecules within a cell can be tailored to fit its specific needs. It is possible to carry out these tasks simultaneously using four autonomous robots, each with a different cargo Cargo positioning, loading, and unloading were demonstrated even though this open loop system was used. There are plans to incorporate cell-state data into this platform, which would allow for microscale automation in nanomedicine and developmental biology, as well as real-time monitoring of cell activity.

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