

REPORT DOCUMENTATION PAGE			Form Approved OMB NO. 0704-0188		
<p>The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA, 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</p>					
1. REPORT DATE (DD-MM-YYYY) 23-10-2014		2. REPORT TYPE Final Report		3. DATES COVERED (From - To) 1-Aug-2010 - 31-Jul-2014	
4. TITLE AND SUBTITLE Final Report: Micro/nano-particles and cells: Manipulation, transport, and self-assembly			5a. CONTRACT NUMBER W911NF-10-1-0353		
			5b. GRANT NUMBER		
			5c. PROGRAM ELEMENT NUMBER 611102		
6. AUTHORS R. Sooryakumar			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAMES AND ADDRESSES Ohio State University 1960 Kenny Road Columbus, OH 43210 -1016			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES) U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211			10. SPONSOR/MONITOR'S ACRONYM(S) ARO		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S) 56755-MS.20		
12. DISTRIBUTION AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.					
14. ABSTRACT Technologies that control nano- and micron-sized inert as well as biological materials are crucial to realizing engineered systems that can assemble, transport, and manipulate objects at these length scales. Two principles, (a) the domain wall structure of patterned magnetic structures and (b) the superparamagnetic properties of nanoparticles, were used to apply directed forces that maneuver, transport, sort and configure these tiny entities on a platform. Convenient, remotely activated, changes to the local energy landscape of the platform that were developed underlie the ability to: a) generate high (10,000 T/m) local field gradients, b) selectively apply forces to					
15. SUBJECT TERMS magnetic dipoles, domain walls, remote manipulation, microfluidics, superparamagnetism					
16. SECURITY CLASSIFICATION OF:		17. LIMITATION OF ABSTRACT		15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT UU	b. ABSTRACT UU	c. THIS PAGE UU	UU		R. Sooryakumar
				19b. TELEPHONE NUMBER 614-292-3130	

Report Title

Final Report: Micro/nano-particles and cells: Manipulation, transport, and self-assembly

ABSTRACT

Technologies that control nano- and micron-sized inert as well as biological materials are crucial to realizing engineered systems that can assemble, transport, and manipulate objects at these length scales. Two principles, (a) the domain wall structure of patterned magnetic structures and (b) the superparamagnetic properties of nanoparticles, were used to apply directed forces that maneuver, transport, sort and configure these tiny entities on a platform. Convenient, remotely activated, changes to the local energy landscape of the platform that were developed underlie the ability to, a) generate high (10,000 T/m) local field gradients, b) selectively apply femto- to pico-Newton scale forces to objects with designed magnetic signatures and c) direct these forces along desired pathways. A broad range of problems, both fundamental and applied, also benefited from control over the stochastic (Brownian) trajectories of trap-confined micro- and nano-particles. The projects ranged from activation of soft confinement barriers through creation of novel time-orbiting magnetic potentials that provide a new framework to stabilize intricate and design-specific behavior of interacting magnetic dipoles, formation of magnetic colloidal rotor pumps actuated within microfluidic channels, and the high-throughput transfection of genes into living cells for screening cellular heterogeneity.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

<u>Received</u>	<u>Paper</u>
08/21/2012	4.00 G. Vieira, A. Chen, T. Henighan, J. Lucy, F. Yang, R. Sooryakumar. Transport of magnetic microparticles via tunable stationary magnetic traps in patterned wires, Physical Review B, (05 2012): 174440. doi: 10.1103/PhysRevB.85.174440
08/24/2011	2.00 T. Henighan, D. Giglio, A. Chen, G. Vieira, R. Sooryakumar. Patterned magnetic traps for magnetophoretic assembly and actuation of microrotor pumps, Applied Physics Letters, (03 2011): 103505. doi: 10.1063/1.3562037
08/24/2011	3.00 Fengyuan_Yang, Michael_Poirier, Ciriám_Jayaprakash, Ratnasingham_Sooryakumar, Aron_Chen, Greg_Vieira, Thomas_Henighan, Marci_Howdyshell, Justin_North, Adam_Hauser. Regulating Brownian Fluctuations with Tunable Microscopic Magnetic Traps, Physical Review Letters, (08 2011): 87206. doi: 10.1103/PhysRevLett.107.087206
10/03/2013	7.00 Aaron Chen, Tom Byvank, Woo-Jin Chang, Atul Bharde, Greg Vieira, Brandon L. Miller, Jeffrey J. Chalmers, Rashid Bashir, Ratnasingham Sooryakumar. On-chip magnetic separation and encapsulation of cells in droplets, Lab on a Chip, (02 2013): 1172. doi: 10.1039/c2lc41201b
10/03/2013	8.00 Aaron Chen, Tom Byvank, Gregory B. Vieira, R. Sooryakumar. Magnetic Microstructures for Control of Brownian Motion and Microparticle Transport, IEEE Transactions on Magnetics, (01 2013): 300. doi: 10.1109/TMAG.2012.2224850
10/07/2013	13.00 Nima Jokilaakso, Eric Salm, Aaron Chen, Larry Millet, Carlos Duarte Guevara, Brian Dorvel, Bobby Reddy, Amelie Eriksson Karlstrom, Yu Chen, Hongmiao Ji, Yu Chen, Ratnasingham Sooryakumar, Rashid Bashir. Ultra-localized single cell electroporation using silicon nanowires, Lab on a Chip, (02 2013): 0. doi: 10.1039/c2lc40837f
10/08/2014	14.00 Michael_Prikockis, Aaron_Chen, Tom_Byvank, Gregory_Vieira, Brian_Peters, Fengyuan_Yang, Ratnasingham_Sooryakumar. Programmable Self-Assembly, Disassembly, Transport, and Reconstruction of Ordered Planar Magnetic Micro-Constructs, IEEE Transactions on Magnetics, (05 2014): 1. doi: 10.1109/TMAG.2013.2292601
10/08/2014	16.00 R. Sooryakumar, A. Chen. Patterned time-orbiting potentials for the confinement and assembly of magnetic dipoles, Scientific Reports, (11 2013): 0. doi: 10.1038/srep03124
TOTAL:	8

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received

Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

1. A new dimension in magnetic tweezers: Research tool to biomedical applications, R. Sooryakumar, IEEE International Magnetism Conference, Dresden, Germany May 2014. (Invited Speaker)
2. The role of hydrodynamic forces in the confinement and assembly of magnetic dipoles, M. Prikockis, A. Chen, R. Sooryakumar, Meeting of the American Physical Society, Denver, Colorado, March 2014.
3. High throughput transfection of cells: nano-electroporation and mobile magnetic traps, M. Howdyshell, D. Gallego-Perez, G. Vieira, V. Malkoc, L.J. Lee, R. Sooryakumar, Meeting of the American Physical Society, Denver, Colorado, March 2014.
4. Magnetic micro- and nano-transporters: Applications in Bioengineering, R. Sooryakumar, NT4D conference, Tucson, Arizona, November 2013. (Invited Speaker)
5. On-chip Cell Separation and Encapsulation: Mobile magnetic traps and droplet microfluidics, R. Sooryakumar, International Conference on Materials for Advanced Technologies 2013 (ICMAT 2013), Singapore, July 2013. (Invited Speaker)
6. Magnetically actuated micro-robots: Self-assembly, disassembly, transport and reassembly of planar constructs, R. Sooryakumar, Materials Week, The Ohio State University, May 2013. (Invited Speaker)
7. Magnetic microstructures for regulating Brownian motion, R. Sooryakumar, Meeting of the American Physical Society, Baltimore, Maryland, March 2013. (Invited Speaker)
8. Multiplexing nano-electroporation for simultaneous transfection of multiple cells M. Howdyshell, G. Vieira, D. Gallego-Perez, X. Zhao, L. J. Lee, R. Sooryakumar Meeting of the American Physical Society, Baltimore, Maryland, March 2013.
9. Assembly and manipulation of planar ordered magnetic micro-bead clusters, M. Prikockis, A. Chen T. Byvank G. Vieira R. Sooryakumar, Meeting of the American Physical Society, Baltimore, Maryland, March 2013.
10. Nanoengineered Platform for Molecular Capture, Detection, and Manipulation, K.D. Mahajan, G. Vieira, G. Ruan, N. Bouxsein, M. Lustberg, G. Bachand, J.J. Chalmers, R. Sooryakumar and J.O. Winter, 4th International Conference on Bioengineering, Fort Lauderdale, FL, January 13-16, 2013.
11. Magnetic Micro-transporters: Applications in Bioengineering, R. Sooryakumar, Korean Magnetic Society, International Symposium on Magnetism and Magnetic Materials 2012, Pyeongchang, Korea, December 2012. (Invited Speaker)
12. Magnetic Micro-transporters: Applications in Bioengineering, R. Sooryakumar, Center for NanoBioEngineering and Spintronics, Chungnam National University, Daejeon, Korea, November 2012. (Invited Speaker)
13. Mobile Magnetic Tweezers: From Research Tool to Engineering Applications and Biomedical Diagnostics R. Sooryakumar, Ninth International Conference on the Scientific and Clinical Applications of Magnetic Carriers, Minneapolis, Minnesota, May 2012. (Invited Speaker)
14. Magnetic wire trap arrays for biomarker-based molecular detection Gregory Vieira, Kalpesh Mahajan, Gang Ruan, Jessica Winter, Meeting of the American Physical Society, Boston, Massachusetts, March 2012.
15. On-chip Magnetic Separation and Cell Encapsulation in Droplets A. Chen, T. Byvank, A. Bharde, B.L. Miller, J.J. Chalmers, R. Sooryakumar, W.-J. Chang, R. Bashir, Meeting of the American Physical Society, Boston, Massachusetts, March 2012.
16. Dual microbead-labeled DNA manipulation with magnetic traps in a microfluidic device M. Howdyshell, M. Simon, M. Poirier R. Sooryakumar, Meeting of the American Physical Society, Boston, Massachusetts, March 2012.
17. Magnetic Micro- and Nano-transporters: Applications in science, engineering and medicine, R. Sooryakumar, International Conference on VLSI, MEMS and NEMS (VMN-2012), Noida, India, January 2012. (Invited Speaker)
18. Harnessing nano-magnetism for engineering and medicine, R. Sooryakumar International Conference on Nanoscience and Technology (ICONSAT-2012), Hyderabad, India, January 2012. (Invited Speaker)
19. Magnetic micropatterns for biological cell manipulation and transport, R. Sooryakumar, 13th International Conference on Electromagnetics in Advanced Applications (ICEAA 2011), Turin, Italy (declined) September 2011. (Invited Speaker)
20. Magnets within a Tunable Micro-bowl: Self-assembly and structure, A. Chen, R. Sooryakumar, MRS Workshop on Directed Self-assembly of Materials, Nashville, Tennessee, September 2011.
21. Magnetic Micro-transporters: New Tools for Engineering and Medicine R. Sooryakumar, National Technical University of Singapore,

August 2011. (Invited Speaker)

22. MAGNETIC MICRO-TRANSPORTERS: New Tools for Engineering and Medicine, R. Sooryakumar, National University of Singapore, August 2011. (Invited Speaker)

23. Nano-magnetism and micro-transporters: New tools for engineering and medicine, International Conference on Low Dimensional Structures and Devices (LDS2011), Telchec, Mexico, May 26, 2011. (Invited Speaker)

24. Forces due to Patterned Magnetic Traps within Microfluidic Channels, M. Howdyshell, G. Vieira, A. Chen, M. Simon, M. Poirier, R. Sooryakumar, Meeting of the American Physical Society, Dallas, Texas, March 2011.

25. Interacting Superparamagnetic Brownian Particles in an Array of 2D Asymmetric Magnetic Traps, G. Vieira, A. Chen, R. Sooryakumar, Meeting of the American Physical Society, Dallas, Texas, March 2011.

26. Realization of a Bowl-like Potential and Its Confinement of Magnetic Microspheres, A. Chen, T. Henighan, G. Vieira, R. Sooryakumar, Meeting of the American Physical Society, Dallas, Texas, March 2011.

27. Separating Magnetically Labeled and Unlabeled Biological Cells within Microfluidic Channels T Byvank, Greg Vieira, B. Miller, B. Yu, J. Chalmers, L. J Lee, R. Sooryakumar, Meeting of the American Physical Society, Dallas, Texas, March 2011.

Number of Presentations: 27.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

<u>Received</u>	<u>Paper</u>
08/21/2012	5.00 Nima Jokilaakso, Eric Salm, Aaron Chen, Larry Millet, Carlos Duarte Guevara, Brian Dorvel, Bobby Reddy, Amelie Eriksson Karlstrom, Yu Chen, Hongmiao Ji, Yu Chen, Ratnasingham Sooryakumar, and Rashid Bashir. Ultra-Localized Single Cell Lysis Using Silicon Nanowires, Lab on a Chip (08 2012)
08/21/2012	6.00 Aaron Chen, Tom Byvank, Gregory B. Vieira, and R. Sooryakumar. Magnetic Microstructures for Control of Brownian Motion and Microparticle Transport, IEEE Trans Mag (07 2012)
10/04/2013	10.00 M. Prikockis, A. Chen, T. Byvank, G. Vieira, B. Peters, F.Y. Yang, R. Sooryakumar. Programmable Self-assembly, Disassembly, Transport and Reconstruction of Ordered Planar Magnetic Micro-constructs, IEEE Transactions on Magnetism (09 2013)
10/04/2013	9.00 Aaron Chen, R. Sooryakumar. Patterned Time-orbiting potentials for the confinement and assembly of magnetic dipoles, Scientific reports (09 2013)
10/17/2014	17.00 Lingqian Chang, Marci Howdyshell, Wei-Ching Liao, Chi-Ling Chiang, Daniel Gallego-Perez, Zhaogang Yang, Wu Lu, John C. Byrd, Natarajan Muthusamy, L. James. Lee, Ratnasingham Sooryakumar. Magnetic Tweezers-based 3D Microchannel Electroporation for High-Throughput Gene Transfection in Living Cells, Small (08 2014)
10/22/2014	18.00 Marci L. Howdyshell, Michael Prikockis, Stephanie Lauback, Gregory B. Vieira, Kalpesh Mahajan, Jessica Winter, R. Sooryakumar. Deterministic and stochastic trajectories of magnetic particles: Mapping energy landscapes for technology and biology, IEEE Transactions on Magnetism (03 2014)
TOTAL:	6

Number of Manuscripts:

Books

Received Book

TOTAL:

Received

Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Aaron Chen (graduate student), awarded the 2012 PhD Dissertation Research Award presented by the American Physical Society Tropical Group on Magnetism and its Applications. This award recognizes outstanding dissertation research in Magnetism.

Aaron Chen (graduate student), awarded 2012 Ohio State University Presidential Fellowship that embodies the highest scholarship in the university graduate programs.

Greg Vieira (graduate student), awarded 2011 Ohio State University Presidential Fellowship that embodies the highest scholarship in the university graduate programs.

Marci Howdysshell (graduate student), recipient of Ray Travel Award (2011), (2012).

Sam Stuard (undergraduate), recipient of DAAD RISE scholarship for Summer research in Germany (2011).

Sam Stuard (undergraduate), Fulbright scholarship in Physics for research in Brazil (2013).

Graduate Students

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	<u>Discipline</u>
Aaron Chen	0.40	
Greg Vieira	0.40	
Marci Howdysshell	0.25	
Mike Prikockis	0.10	
Jonathan Zizka	0.15	
FTE Equivalent:	1.30	
Total Number:	5	

Names of Post Doctorates

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
Atul Bhadre	0.20
FTE Equivalent:	0.20
Total Number:	1

Names of Faculty Supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	National Academy Member
R. Sooryakumar	0.22	
FTE Equivalent:	0.22	
Total Number:	1	

Names of Under Graduate students supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	Discipline
Paul Zivik	0.20	
Grant Savage	0.10	
Tom Byvank	0.30	
Sam Stuard	0.00	
George Vogt	0.20	
FTE Equivalent:	0.80	
Total Number:	5	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 3.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 3.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 3.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 3.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: 3.00

Names of Personnel receiving masters degrees

<u>NAME</u>	
Mike Prikockis	
Total Number:	1

Names of personnel receiving PHDs

<u>NAME</u>	
Greg Vieira	
Aaron Chen	
Marci Howdysshell	
Total Number:	3

Names of other research staff

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See Attachment

Technology Transfer

During this grant period, diverse projects based on patterned microscopic magnetic surface topographies were advanced. Some of the highlights include:

- (1) Integrated (for the first time) the mobile magnetic trap array to compartmentalize individual cells in droplets with reagents at well-defined pick-up and drop-off locations;
- (2) Created a nano-enabled technology to perform parallel detection and separation of multiple targets (e.g., cells, molecules);
- (3) Regulated the spatial extent of Brownian trajectories of microscopic magnetic particles solely by weak fields;
- (4) Stabilized *high-symmetry* ordered structures of fluid-borne superparamagnetic particles that are subsequently disassembled, transported and reconstructed back into the ordered phase;
- (5) Designed and constructed time orbiting magnetic potentials to confine and assemble magnetic dipoles;
- (6) Integrated Si nanowires with the magnetic tweezers to lyse individual cells;
- (7) Stabilized stationary domain walls and tunable energy landscapes for micro-/ nano-transport;
- (8) Developed a novel high-throughput magnetic tweezers based chip for gene delivery and screening cellular heterogeneity in living cells.

These advances are described briefly below. A complete list of papers published based on research findings of the grant is also provided at the end.

1. **On-chip Magnetic Separation and Encapsulation of Cells in Droplets**, A. Chen, et.al., Lab on a Chip 13, 1172 (2013).

While many cell and molecular biology techniques present diagnostics and therapeutic systems, most of these methods rely on ensemble measurements obtained from heterogeneous cell populations. However, it has been demonstrated that even within an isogenic cell population, stochastic gene expressions exist among cells.¹ Analyzing an ensemble of cells at an individual level with high spatiotemporal resolutions can thus lead to a better understanding of such cell-to-cell variations. Two key processes required prior to performing single-cell analyses are (i) the sorting of cells into subpopulations and (ii) the compartmentalization of these cells of interest with dedicated reagents into individually isolated environments.

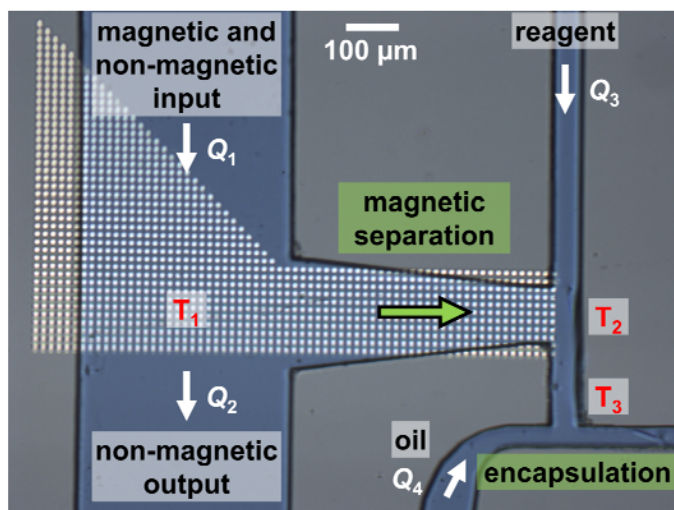


Figure 1: Integration of magnetic tweezers with droplet microfluidics.

While various schemes have been utilized to contain single cells when delivering reagents through pumps and valves, the nature of these rigid confining structures limits the ability to

multiplex. In contrast, in microfluidic droplet based devices, the containers (droplets) are created anew during the encapsulation of single cells yielding a practically unlimited number of droplets that allow easy multiplexing. In order to compartmentalize the cell while it still maintains the property as derived from a native heterogeneous environment, it is advantageous to perform the compartmentalization immediately following sorting in the same setup. However, existing single-cell analysis generally require transfer between instruments from one step to another or purification of the samples elsewhere.

In this study we integrated, for the first time, the magnetic sorting capability of our mobile magnetic trap array^{2,3} immediately before the compartmentalization of cells provided by droplet microfluidics on the same device. The well-defined pick-up and drop-off locations of the mobile trap array and its ability to actively manipulate cells against the flow are unique features enabling the separation of magnetically labelled cells and their encapsulation into droplets with the reagents. Preliminary assay on the viability of encapsulated cells through fluorescence detection demonstrates the potential to further integrate the device with downstream on-chip analysis schemes. With the combined advantages of low cost, ability to multiplex and the biocompatible nature of magnetic forces, this separation-encapsulation device could become a key component in future single-cell analysis platforms.

2. **MagDot-Nanoconveyor Assay for Detection and Isolation of Molecular Biomarkers (Invited)** K.D. Mahajan, et.al., Chemical Engineering Progress, December 41- 51 (2012).

“Biomarkers” are small molecules (e.g. proteins, deoxyribonucleic acids (DNAs), or various ribonucleic acids (RNAs), such as messenger RNAs (mRNAs) micro RNAs (miRs)) that can be objectively measured as an indicator of normal biological processes, disease conditions, or response to drug treatment. A widely-used simple biomarker is the concentration of small molecule glucose, which is used to monitor diabetes.

Accurate detection of biomarkers is valuable in medical diagnostics. These analytes are often present at very low concentration (e.g., as few as 1 copy per cell). Ideally, biomarker assays would identify analytes at levels approaching that of a single molecule. Unfortunately, most bulk measurement techniques (e.g., absorbance) cannot meet this requirement. Moreover, in isolating extremely rare cells, such as circulating tumor cells (CTCs), it is difficult to employ techniques such as flow cytometry to isolate the small number of cells that may be present in a highly heterogeneous population of cells.

One possible solution is the use of nanotechnology-based molecular detection schemes because of the size similarity between nanomaterials and the analytes they are designed to detect. Also, nanomaterials have many unique properties, such as the potential for superparamagnetic properties, fluorescence, and surface plasmon resonance that can be directly converted into a detectable signal. The ability to manipulate molecules in particular is an important component of the nanoengineering of molecular structures and for small scale synthesis (e.g., supramolecular chemistry). A single, one-pot assay for detection, characterization, quantification, and separation of molecular and cellular biomarkers is therefore highly desired.

In this effort we investigated a possible nano-enabled technology that can perform parallel detection and separation of multiple targets (e.g., cells, molecules). This assay is based on two platform technologies: patterned magnetic nanowires, i.e., magnetic conveyers³ and fluorescent-

magnetic nanoparticles, i.e., MagDots⁴. The former demonstrated the first simultaneous detection and manipulation of sub-100 nm nanoparticles⁴. The detection scheme for bound molecules uses a fluorescent-magnetic composite nanoparticle targeted to the biomarker of interest, whereas free molecule detection is based on biomarker induced aggregation of individual fluorescent and magnetic nanoparticles. Molecules and cells are then recovered and transported on magnetic nanowires controlled by weak external magnetic fields.

3. **Magnetic microstructures for control of Brownian motion and microparticle transport**, A. Chen, T. Byvank, G. Vieira & R. Sooryakumar, IEEE Transactions in Magnetism 49, 300 (2013).

In advancing techniques that provide pico- to femto-Newton scale forces at the micro- and nano-scale several challenges must often be addressed. These challenges arise from: (a) the need for non-contact manipulation, (b) the necessity, especially with diminishing object size, for large localized optical fields, or electrical/magnetic fields with high gradients that do not adversely affect the targeted object, (c) stochastic forces such as those arising from Brownian fluctuations that hinder directed movement, (d) parallel manipulation for improved throughput and (e) selectiveness to maneuver objects with desired characteristics across different surfaces.

In this study we apply forces on fluid borne magnetic micro-particles or labeled biological entities⁵⁻⁸ by combining a remotely activated weak external field ($H_{\text{ext}} \sim 100$ Oe) with fields generated from domain walls (H_{DW}) residing in ferromagnetic wires or magnetic disks (H_{Disk}) patterned on the surface. The changes in the energy landscape induced by H_{ext} enable manipulation of the objects with gentle forces that do not cause damage to typical soft materials and biological cells. A key enabling feature of the magnetic arrays is the very large, tunable, magnetic field gradients ($> 10^4$ T/m) that can be designed within a planar geometry on various opaque and transparent substrates and flexible membranes or films.

The platform also offers the attractive possibility of regulating Brownian fluctuations – the “white noise” of random collisions from molecular movements whose collective influence on a much larger suspended object leads to its inevitable chaotic motion. We illustrate that not only are the random Brownian fluctuations of microscopic magnetic objects sensitive to details of the magnetization profile of the designed patterns, but the spatial extent of their trajectories can also be controlled to a high degree solely by fields less than 100 Oe.

4. **Programmable Self-assembly, Disassembly, Transport and Reconstruction of Ordered Planar Magnetic Micro-constructs**, M. Prikockis, et.al., IEEE Transactions on Magnetism 50(5), 1 – 6 (2014).

Technologies to sequentially assemble, configure, disassemble, transport, and reconstruct multiple nano- and micro-scale structures in parallel remains a daunting challenge. Unlike the case of objects at the macroscopic scale or the natural workings of biological constructs such as the molecular assembly and disassembly of virus capsids⁹, the engineering control needed to accomplish all of these functionalities at the mesoscopic length scale (100 nm to 10 μm) requires a suite of precisely integrated tasks. Although individual strategies (e.g., nanomaterial assembly

via molecular interactions,¹⁰⁻¹³ transport through atomic force microscopy^{14,15} or optical-tweezers^{16,17}) have been developed to separately address one or more of these distinct tasks, techniques that permit an encompassing approach spanning from assembly to reconstruction have yet to be achieved.

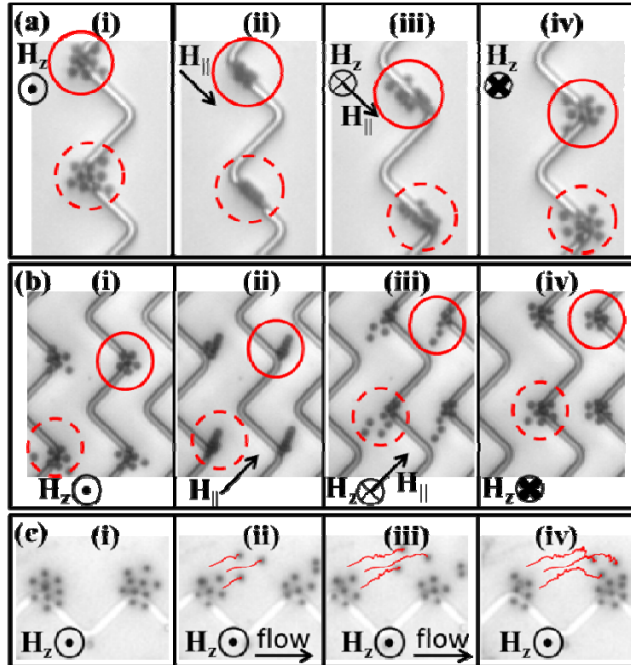


Figure 2: Transport of clusters. Sequential applications of external fields transport entire clusters (indicated in red solid and dashed circles) (a) from vertex to vertex on the same wire, and (b) from wire to adjacent wire. \mathbf{H}_{\parallel} and \mathbf{H}_z denote the in-plane and out-of-plane fields respectively with the thickness of the into-the-page crosses indicating the relative magnitude of \mathbf{H}_z . (c) Sequential photographs taken from a different experiment with similar parameters showing the transport of outer-ring particles to adjacent cluster with a pulse of fluid flow. Trajectories of three transferred particles are shown in red.

Micro- and nano-scale particles offer several advantages as building blocks of artificial materials. The relative ease of modifying their charge states, tunability of cluster sizes with approaches such as evaporation of colloidal suspensions, as well as promoting dipolar interactions through external fields are some of their benefits. Patterned templates further permit “colloidal epitaxy” to create nearly perfect crystals of tailored lattice structure, orientation and size. Superparamagnetic (SPM) particles offer added benefits: (1) rapid response to weak magnetic fields which enables particles to act as miniature force transmitting handles, (2) zero remanence which discourages undesirable aggregation in the absence of a field, and (3) the insensitivity of magnetic fields to ionic content in bio-compatible environments.

In this study the self-assembly of SPM microspheres is used to create arrays of geometrically ordered planar clusters in solution. The present study discusses the stabilization of *entire high-symmetry structures* of ordered, interacting, fluid-borne SPM particles that are subsequently seamlessly disassembled, transported and reconstructed back into the ordered phase by an externally applied weak magnetic field H_{ext} . This approach thus offers an excellent avenue to study competing deterministic and stochastic forces that control interactions amongst a collection of particles. The contending forces stabilize ordered mesoscale structures of tunable sizes and symmetries, with the added advantage of allowing the assembled particles to be transported along specific conveyor pathways to targeted destinations through remotely activated protocols. By integrating this platform with microfluidic technology, the removal and replacement of individual components within the cluster is also demonstrated.

5. Patterned Time-orbiting Potentials for the Confinement and Assembly of Magnetic Dipoles, A. Chen and R. Sooryakumar, Scientific Reports 3, 3124 (2013).

In contrast to their atomic and molecular counterparts, the diverse interactions among macroscopic particles have led to new equilibrium phases, novel phenomena and opportune applications.¹⁸⁻²⁰ Magnetic interactions are particularly advantageous in this regard because they are (i) unimpeded within most environments, (ii) non-contact, (iii) easily controlled through external means and (iv) can yield distinct stable structures with small energy differences. However, despite parallel advances in semiconductor devices where interactions between electrons and the confining crystal are electrically manipulated to provide astounding functionalities, a purely magnetic analogy for concomitant control on the interplaying forces between magnetic dipoles and their confinement has remained elusive.

We have developed a straightforward approach to achieve such control by taking advantage of micro-patterned thin-film materials in conjunction with a time-orbiting magnetic field. Highlights of the scheme are demonstrated through self-arrangements of fluid-borne microspheres tunable over a wide range within pattern-coded confinements, providing access to fundamental phenomena such as first order transitions that are associated with nucleation,²¹ the glass transition due to jamming,^{22,23} and frustrated constructs arising from competing interactions.²⁴ We also envision broad applications of this all-magnetic format in biomedical devices,²⁵ assembling functional materials such as field-tunable photonic crystals,²⁶ and, when appropriately scaled, trapping of cold quantum gases.^{27,28}

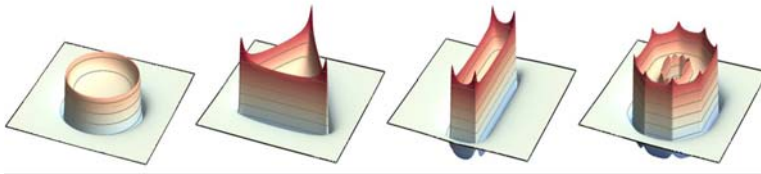


Figure 3: Time-orbiting potentials of various shapes. Time-averaged energy landscapes generated from various Permalloy patterns are calculated for (from left to right) Circle, triangle, long rectangle, and octagonal ring.

The time-orbiting potential can be readily adapted to various shaped confining potentials with surrounding barriers and trenches defined by Permalloy patterns (e.g. circle, triangle, long rectangle, and more complex octagonal ring; Figure 3) while maintaining independent control on the confining and dipolar forces. The advantages of an all-magnetic approach include easy, non-contact manipulation of the dipoles by weak external magnetic fields, and absence of intricate wiring patterns and complex logic for addressing specific electrodes as well as screening effects that are generally present in charge-based approaches. The clearly defined confinement and dipolar interaction that are readily tunable by a single precessing external field render the platform as a perfect experimental realization for various theoretical studies on the structure and dynamics of a cluster of confined repelling objects as well as nucleation, jamming, and frustration effects. In addition to fundamental studies, such a platform can be exploited for numerous applications as well as the basis for strategies to build new functional materials.

6. **Ultra-Localized Cell Lysis Using Silicon Nanowires**, N. Jokilaakso, A. Chen et. al. Lab-on-chip 13(3), 313 (2013).

Analysis of cell-to-cell variation can further the understanding of intracellular processes and the role of individual cell function within a larger cell population. The ability to precisely lyse single cells can be used to release cellular components to resolve cellular heterogeneity that might be obscured when whole populations are examined. We developed a method to position and lyse individual cells on silicon nanowire and nanoribbon biological field effect transistors.

In order to target and electroporate single cells using the transistors, the cells must be in immediate proximity of the device surface. A variety of methods have been used to controllably position cells including patterning,²⁹ fluidic traps and wells,³⁰ optical traps,³¹ dielectrophoresis,³² and others. In this study, label-free and trap-less technique is used to manipulate magnetic beads to push and pull untethered or non-specifically-bound cells into desired positions. In particular HT-29 cancer cells were positioned on top of transistors by manipulating magnetic beads using weak external magnetic fields. Ultra-rapid cell lysis was subsequently performed by applying 600-1200 mV_{pp} at 10 MHz for 2 ms across the transistor channel and the bulk substrate. We show that the fringing electric field at the device surface disrupts the cell membrane, leading to lysis from irreversible electroporation.

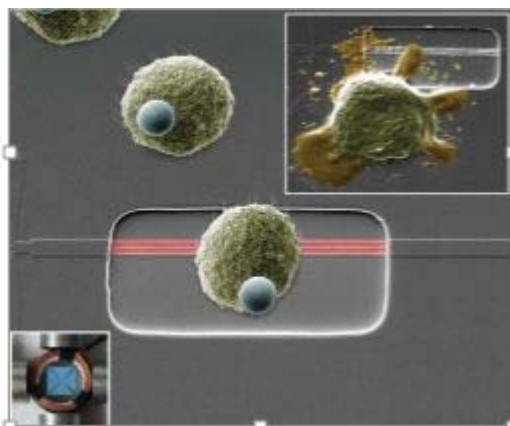


Figure 4: Cover page Lab-on-chip: Illustration of cell lysis using magnetic tweezers and Si nanowires.

We demonstrate integration of the magnetic manipulation techniques with a robust cell lysis by using field effect transistors with potential for field effect sensing of released cellular components. This would enhance the usability and portability of lab-on-a-chip devices by minimizing loss of biological molecules. We envision this system being used in single cell analysis studies that focus on cell-to-cell variation within a population. The methodology allows rapid and specific single cell lysis and analysis with potential applications in medical diagnostics, proteome analysis and developmental biology studies.

7. **Transport of magnetic microparticles via tunable stationary magnetic traps in patterned wires**, Vieira et.al Phys. Rev. B 85, 174440 (2012).

Magnetism-based manipulation, separation, and detection methods for engineering and biological applications have seen rapid growth in their use. Among these methods, techniques that utilize superparamagnetic particles as the force-transmitting handle have been particularly promising. The non-hysteric magnetization loops and absence of remanence or coercivity at room temperature when the particle size lies below the single domain limit (~ 20 nm for Fe₃O₄) are attractive features that render predictable forces and do not promote particle clustering in the

absence of an external field. On the other hand, the corresponding magnetic tractive force for such a tiny particle in a field gradient, is greatly diminished, requiring very large applied fields and/or high gradients to maneuver them. Although the use of macroscopic magnets in such situations is possible, they offer limited accuracy and are unpractical in many settings for manipulation of micron- and smaller-scale entities.

In contrast to transporting particles by moving the DWs via application of external magnetic fields, static domain walls offer a viable means to maneuver particles and labeled cells across a surface.⁷ The benefits of static domain walls include the ability to (a) maneuver and transport particles away from the ferromagnetic wire conduits (in addition to transporting particles along the wire), (b) generate the required directed forces for transport along specific surface trajectories through weak external fields (<100 Oe) produced by inexpensive miniature electromagnets, (c) weaken or strengthen the trapping potential to control Brownian fluctuations⁶ that become more pronounced with diminishing particle size, and (d) multiplex trapping and transport of particle ensembles across a surface, thereby enabling efficient outcomes related to transfer and conveyor applications. In addition, since in this approach the DWs are stationary, they are not susceptible to pinning at topographic imperfections or defects that could hinder particle transport.

Two types of engineered surfaces were investigated for the purpose of creating traps: (a) stationary domain walls in patterned zigzag wires with regular turns (vertices) for trapping particles⁷ and (b) a new particle trapping technique, based on straight wires with periodic indentations (notches). The models developed in this study provide qualitative explanations of some of the more subtle characteristics, such as the ability to (i) steer particles with weak external fields (<100 Oe) away from the wires or along them in predetermined directions, (ii) localize the fluid-borne particles within a trap for extended time periods (tens of minutes), and (iii) provide rapid particle transit times that are limited only by protocols that modulate the external fields and the fluid environment. Furthermore, since the weak external magnetic fields necessary for manipulation generally do not interfere with chemical or biological interactions, this approach lends itself to be integrated into microfluidic devices for biological cell and microparticle sorters, as well as next-generation biomedical devices. We envision scale up for transporting $\sim 10^6$ magnetic particles on a single centimeter-sized platform.

8. Magnetic tweezers based high-throughput gene delivery in living cells, Chang, Howdyshell et.al, Accepted for publication, Small (2014).

Current methods for gene delivery are limited in either efficiency or throughput, and are thereby not applicable for clinical studies. We present a novel platform that combines two technologies: micro-channel based 3D electroporation (3D MEP) and remotely-controlled micromagnetic tweezers (MT) for high-throughput, safe, and rapid gene delivery into living cells *in vitro*. We demonstrate precise alignment of individual cells into large ordered planar arrays using only weak magnetic fields (< 100 G) and accomplish gene delivery with low voltages (< 10 V), resulting in $\sim 90\%$ cell viability. Genetic-coded molecular beacons are introduced into living cells to detect the presence of specific populations of messenger RNA in each cell, thus providing details about cellular heterogeneities that cannot be acquired by surface markers.

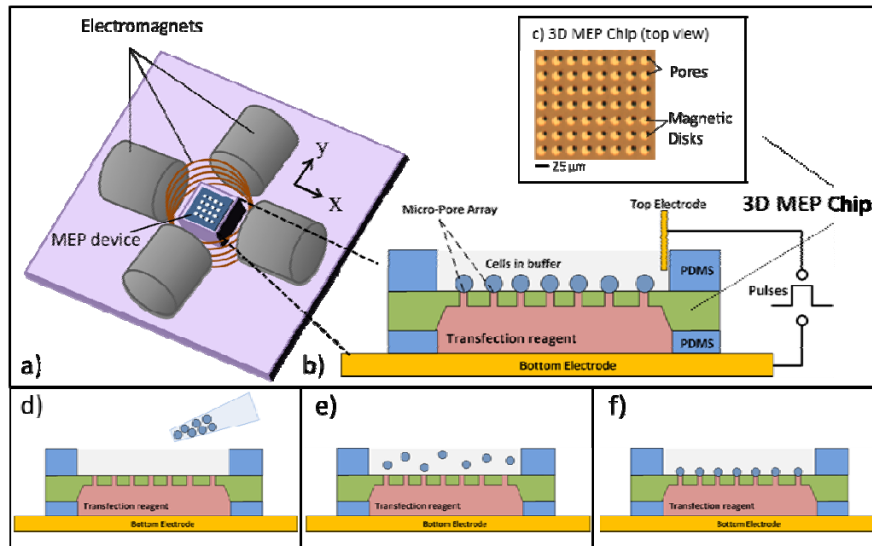


Figure 5: The 3D MEP-MT system setup. (a) Electromagnet setup consists of 5 orthogonal electromagnets which are used to create the x-, y-, and z-components of the external magnetic field. (b) The 3D MEP device sits at the center of the magnets. A gold substrate serves as the bottom electrode. A PDMS spacer holds the transfection reagents in solution. The 3D MEP wafer sits above the spacer with etched pores and magnetic disks. Another PDMS spacer sits on top to hold cells in solution, and a platinum electrode is located above. (c). Micrograph of a 3D MEP wafer, showing Permalloy disks aligned with 5 micron diameter pores. Cell seeding is performed by simply pipetting (d) cells in PBS buffer solution into upper chamber and allowing them to settle (e) with magnetic fields turned on so that they fall into an aligned pattern with the pores (f).

The 3D MEP-MT system is capable of rapid, effective cell alignment resulting in high-throughput electroporation. The main chip (Figure 5) that is central to the platform consists of an ordered array of micropores (pore diameter: $\sim 5 \mu\text{m}$) with each pore aligned closely to an individual magnetic disk. The chip has two major functions: (1) Provide a high throughput platform that seats a large number of cells ($10^4 \sim 10^6$ cells, depending on the geometry and density of micropores); (2) Focuses the electrical field through the micropores so that a low voltage is sufficient for membrane poration. The transfection achieved through low voltages ($< 10 \text{ V}$), as well as the weak magnetic fields used to remotely control cell alignment and movement do not harm the cells. This platform provides a promising high-throughput gene transfection device with the advantages of high cell viability and uniform transfection at the single-cell level.

The present MT-based approach illustrates parallel manipulation, localization, transfection, and subsequent transport of the transfected cells. The versatility of the approach is shown with several distinct cell types and transfection reagents, including delivery of the intracellular probe, GATA2 molecular beacon (MB) for detection of GATA2 mRNA expression. GATA2 family of transcription factors play important roles in the proliferation and differentiation of pluripotent hematopoietic stem cells (HSCs).³³ Among them GATA2 is highly expressed in HSCs and progenitors regulating hematopoietic development and its disorder has been implicated in the onset of leukemia.³⁴ Detection of GATA2 is thus of great significance for the study of heterogeneities of HSCs. As a transcription factor, however, few accessible technologies to date can achieve intracellular detection for GATA2 within living cells. Future studies will include delivery of therapeutic genetic materials, as well as intracellular probes for a variety of purposes that range from gene therapy to intracellular marker detection.

References

- 1 P. Dalerba, T. Kalisky, D. Sahoo, P. S. Rajendran, M. E. Rothenberg, A. A. Leyrat, S. Sim, J. Okamoto, D. M. Johnston, D. Qian, M. Zabala, J. Bueno, N. F. Neff, J. Wang, A. A. Shelton, B. Visser, S. Hisamori, Y. Shimono, M. van de Wetering, H. Clevers, M. F. Clarke and S. R. Quake, *Nat. Biotechnol.*, **29**, 1120. 2011.
- 2 T. Henighan, A. Chen, G. Vieira, A. J. Hauser, F. Y. Yang, J. J. Chalmers and R. Sooryakumar, *Biophys. J.*, **98**, 412, 2010.
- 3 Vieira, G., T. Henighan, A. Chen, A.J. Hauser, F.Y. Yang, J.J. Chalmers, and R. Sooryakumar, *Magnetic Wire Traps and Programmable Manipulation of Biological Cells*. Physical Review Letters, **103**(12): p. 128101, 2009.
- 4 Ruan, G., G. Vieira, T. Henighan, A. Chen, D. Thakur, R. Sooryakumar, and J.O. Winter, *Simultaneous Magnetic Manipulation and Fluorescent Tracking of Multiple Individual Hybrid Nanostructures*. Nano Letters, **10**(6): 2220–2224, 2010.
- 5 M. Howdyshell, M. Priokkis, S. Laubak, G. Vieira, K. Mahajan, J. Winter and R. Sooryakumar, *Deterministic and stochastic trajectories of magnetic particles: Mapping energy landscapes for technology and biology*. to appear Transactions on Magnetics, (2014).
- 6 A. Chen, G. Vieira, T. Henighan, M. Howdyshell, J. A. North, A. J. Hauser, F. Y. Yang, M. G. Poirier, C. Jayaprakash, and R. Sooryakumar, “*Regulating Brownian Fluctuations with Tunable Microscopic Magnetic Traps*,” *Phys. Rev. Lett.* Vol. 107, 087206, 2011.
- 7 G. Vieira, T. Henighan, A. Chen, A.J. Hauser, F.Y. Yang, J.J. Chalmers and R. Sooryakumar, *Magnetic Wire Traps and Programmable Manipulation of Biological Cells*, *Phys. Rev. Lett.* 103, 128101, 2009.
- 8 G. Vieira, A. Chen, T. Henighan, J. Lucy, F. Y. Yang, and R. Sooryakumar, *Transport of magnetic microparticles via tunable stationary magnetic traps in patterned wires*, *Phys. Rev. B*, 85, 174440, 2012.
- 9 B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, *Molecular Biology of the Cell*, Garland Science, New York, 2002.
- 10 M. Shimomura, T. Sawadaishi, *Curr. Opin. Colloid Interface Sci.* 6, 11–16, 2001.
- 11 J. S. Mohammed, W. L. Murphy, *Adv. Mater. (Weinheim, Ger.)* 21, 2361–2374, 2009.
- 12 S. Zhang, *Nat. Biotechnol.* 21, 1171–8, 2003.
- 13 Y. Lin, C. Mao, *Front. Mater. Sci.* 5, 247–265, 2011.
- 14 T. Junno, K. Deppert, L. Montelius, L. Samuelson, *Appl. Phys. Lett.* 66, 3627, 1995.

- 15 F. A. Carvalho, N. C. Santos, *IUBMB Life* **64**, 465–72, 2012.
- 16 D. G. Grier, *Nature* **424**, 810–6, 2003.
- 17 G.M Whitesides, & M. Boncheva, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 4769 (2002).
- 18 D.Issadore, et. al., *Lab Chip*. **11**, 147-41, 2011.
- 19 S.C. Glotzer, *Science* **306**, 419 (2004).
- 20 F. Li, D. P. Josephson, & A. Stein, *Angew. Chem. Int. Ed.* **50**, 360–388 (2011).
- 21 U. Gasser, E.R. Weeks, A. Schofield, P.N. Pusey, & D. A. Weitz, *Science* **292**, 258–262 (2001).
- 22 A.J. Liu, & S. R. Nagel, *Nature* **396**, 21-22 (1998).
- 23 P.N. Segrè, V. Prasad, A.B. Schofield & D. A. Weitz, *Phys. Rev. Lett.* **86**, 6042–6045 (2001).
- 24 V. Lubchenko, *Proc. Natl. Acad. Sci.* **105**, 10635-10636 (2008).
- 25 N. Pamme, *Lab Chip* **6**, 24-38 (2006).
- 26 J. Ge & Y. Yin, *Angew. Chem. Int. Ed.* **50**, 1492–1522 (2011).
- 27 T. Lahaye, et al. *Nature (London)* **448**, 672 (2007).
- 28 M. Lu, S.H. Youn, S. H. & B.L. Lev, *Phys. Rev. Lett.* **104**, 063001 (2010).
- 29 S. Bhattacharya, S. Salamat, D. Morisette, P. Banada, D. Akin, Y.-S. Liu, A. K. Bhunia, M. Ladisch and R. Bashir, *Lab on a Chip*, **8**, 1130 (2008).
- 30 S. Lindström, K. Mori, T. Ohashi and H. Andersson-Svahn, *Electrophoresis*, **30**, 4166 (2009).
- 31 K. Ramser and D. Hanstorp, *Journal of Biophotonics*, **3**, 187 (2010).
- 32 P. Bajaj, B. Reddy, L. Millet, C. Wei, P. Zorlutuna, and R. Bashir, *Integrative Biology*, **3**, 897 (2011).

Papers Published During Grant:

- 1 **Magnetic Tweezers-based 3D Microchannel Electroporation for High-Throughput Gene Transfection in Living Cells**, L. Chang, M. Howdyshell, Wei-Ching Liao, C-L Chiang, D. Gallego-Perez, Z. Yang, W. Lu, J. C. Byrd, N. Muthusamy, L. J. Lee, R. Sooryakumar, Accepted to appear, Small (2014)
- 2 **Deterministic and stochastic trajectories of magnetic particles: Mapping energy landscapes for technology and biology**, M. Howdyshell, M Prikockis, S. Laubak, G. Vieira, K. Mahajan, J. Winter and R. Sooryakumar, Accepted to appear, Transactions on Magnetism, (2014).
- 3 **Programmable Self-assembly, Disassembly, Transport and Reconstruction of Ordered Planar Magnetic Micro-constructs**, M. Prikockis, A. Chen, T. Byvank, G. Vieira, B. Peters, F. Y. Yang, and R. Sooryakumar, Transactions on Magnetism, 50(5), 1 - 6 (2014).
- 4 **Patterned Time-orbiting potentials for the confinement and assembly of magnetic dipoles**, A. Chen and R. Sooryakumar, Scientific Reports 3, 3124 (2013).
- 5 **Magnetic microstructures for control of Brownian motion and microparticle transport**, A. Chen, T. Byvank, G. Vieira and R. Sooryakumar, IEEE Transactions in Magnetism 49(1), 300 (2013).
- 6 **On-chip Magnetic Separation and Encapsulation of Cells in Droplets**, A. Chen, T. Byvank, W-J Chang, A. Bharde, G. Vieira, B. Miller, J. J. Chalmers, R. Bashir and R. Sooryakumar, Lab on a Chip 13 (6), 1172 (2013).
- 7 **Ultra-Localized Cell Lysis Using Silicon Nanowires**, N. Jokilaakso, E. Salm, A. Chen, L. Millet, C. D Guevara, B. Dorvel, B. Reddy, Jr., A. E. Karlstrom, Y. Chen, H. Ji, C. Yu, R. Sooryakumar, and R. Bashir, Lab on a Chip 13(3), 336 (2013).
- 8 **MagDot-Nanoconveyer Assay for Detection and Isolation of Molecular Biomarkers (Invited)** K.D. Mahajan, G. Vieira, G. Ruan, B.L. Miller, M. Lustberg, J.J. Chalmers, R. Sooryakumar, J.O. Winter, Chemical Engineering Progress December (2012)
- 9 **Transport of magnetic micro-particles via tunable stationary magnetic traps in patterned wires**, G. Vieira, A. Chen, T. Henighan, J. Lucy, F.Y. Yang, R. Sooryakumar, Physical Review B 85, 174440 (2012).
- 10 **Patterned magnetic traps for magnetophoretic assembly and actuation of micro-rotor pumps**, T. Henighan, D. Giglio, A. Chen, G. Vieira and R. Sooryakumar, Applied Physics Letters 98, 103505 (2011).
- 11 **Regulating Brownian Fluctuations with Tunable Microscopic Magnetic Traps**, A. Chen, G. Vieira, T. Henighan, M. Howdyshell, J. North, A. Hauser, F. Y. Yang, M. Poirier, C. Jayaprakash, and R. Sooryakumar, Physical Review Letters 107, 087206 (2011).