Nanoscale Electrostatic Trap for Charged Particles

(OTT ID 1162)

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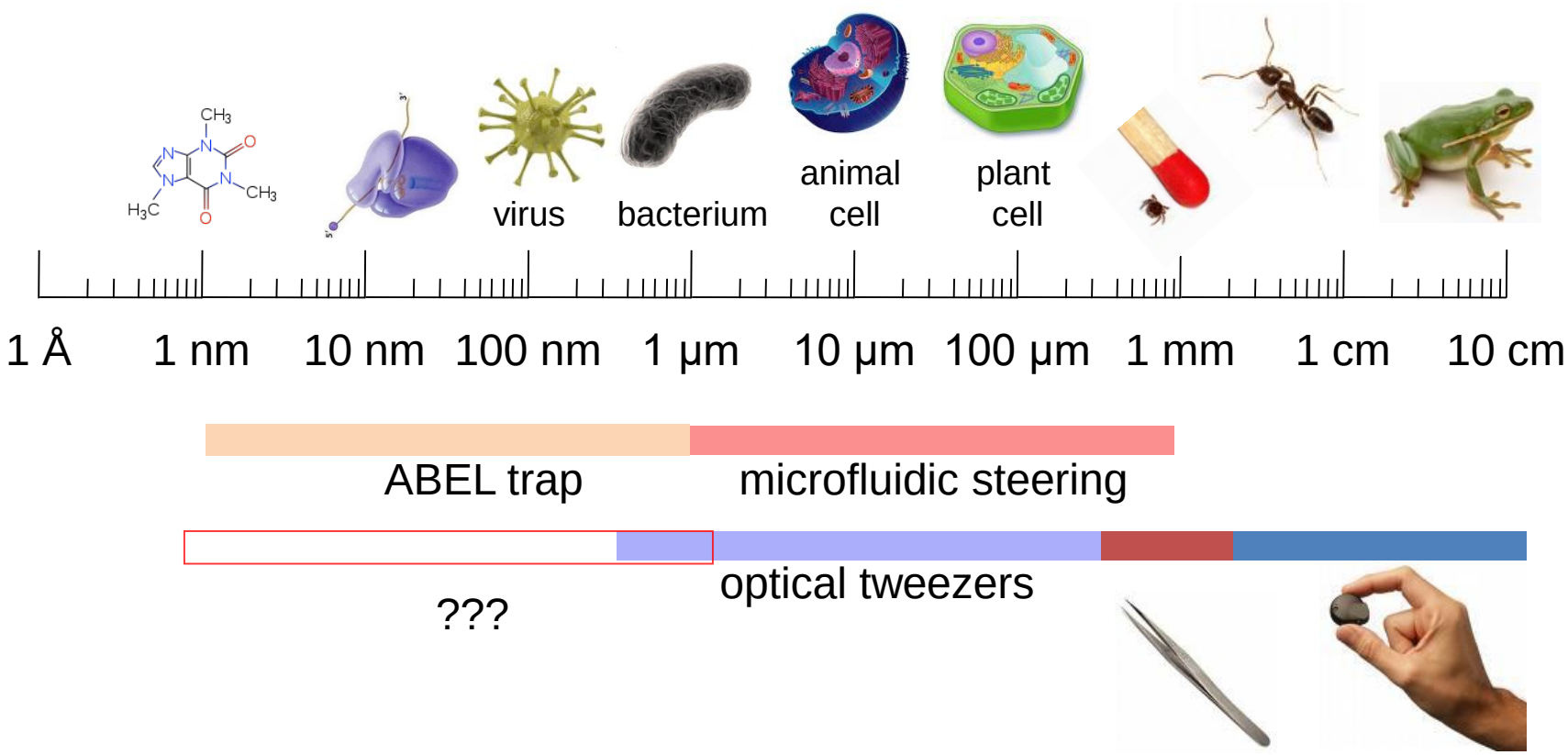
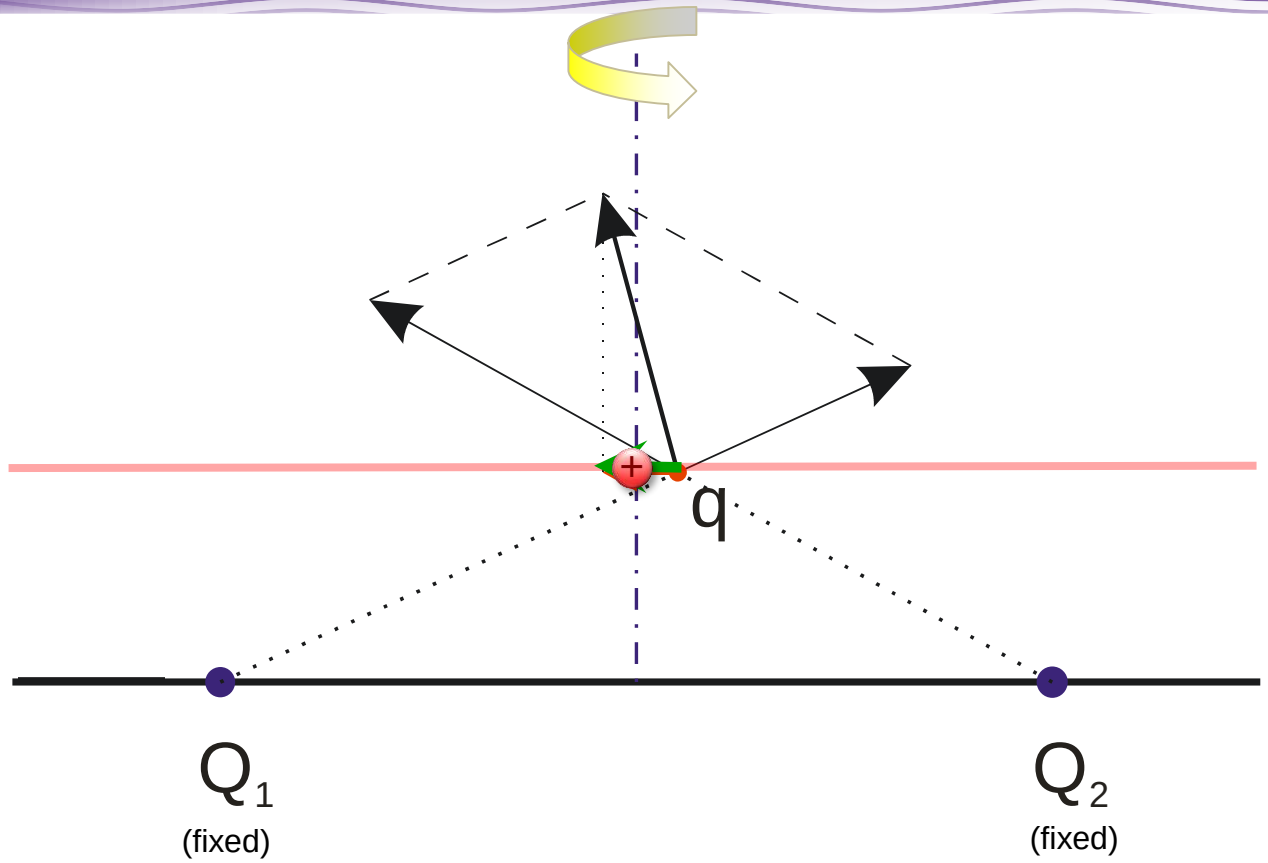
Shortfalls of Current Trapping Tools



Problems:

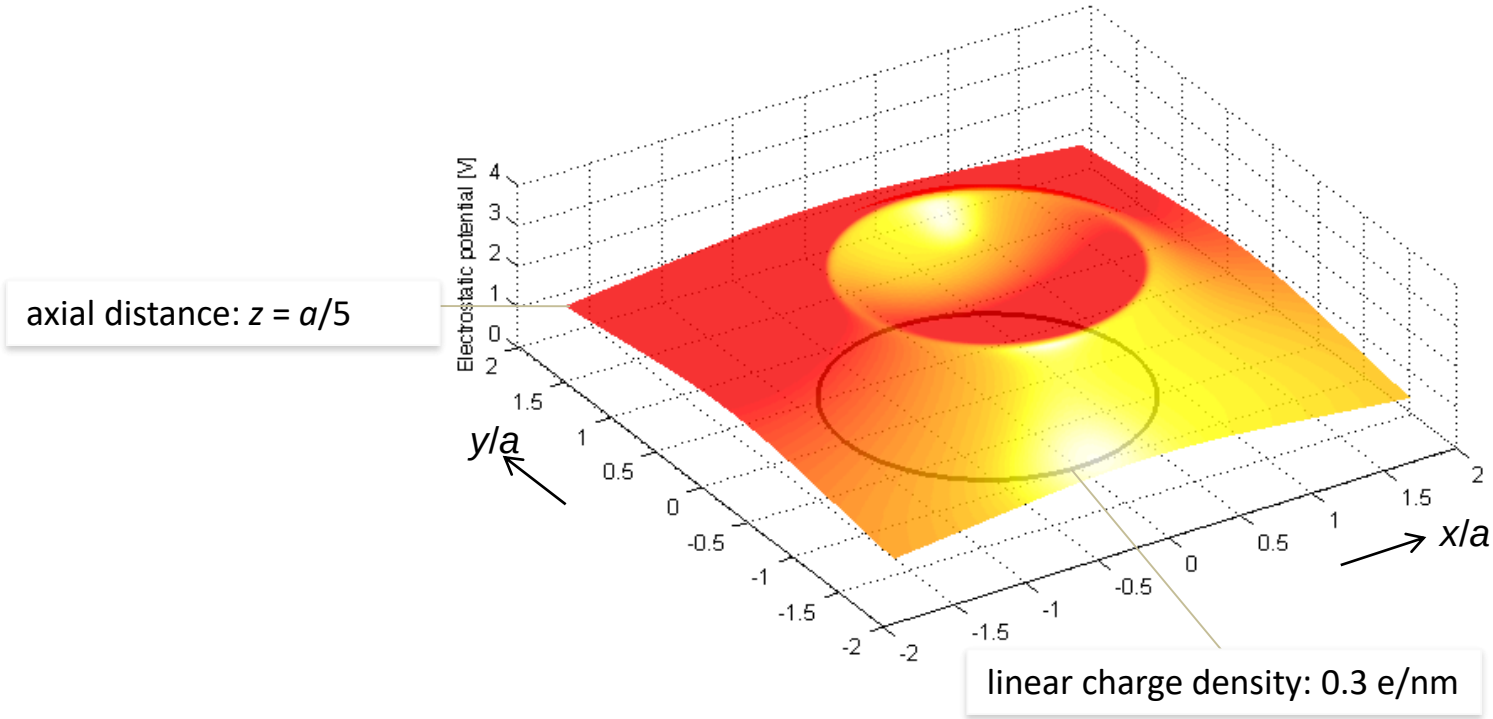
- Popular methods like optical tweezers are not adequate for nanoscale particles and molecules
- ABEL trap requires complex hard- and software, continuous particle monitoring, and only acts on one particle or molecule at a time

Technological Solution: The Conception



- Imagine that you have two fixed positive charges on a substrate, and another positive charge that is free to move along the red line
- If the charge is located on the axis of symmetry, the Coulomb forces acting on it will lead to a net force in an axial direction
- If the particle moves away from this position, however, the symmetry is broken and as a result, the net force now has a lateral component that pulls the particle back to the original position

Technological Solution: The Electrostatic Corral



- This one-dimensional trap (previous slide) can be made into its two-dimensional analog by rotation about the axis of symmetry.
- Instead of two fixed charges, we now have a ring or corral of charges on the substrate, but the essential forces remain the same.

Technological Solution: The Electrostatic Corral

Advantages:

- Electrostatic trapping scales favorably with particle size
- No active feedback system required (stable trap)
- Illuminate only when needed
- A particle will remain trapped for as long as the ring of charges is present
- Multi-particle trapping capability

Limitations:

- No axial confinement (yet)
- But this is not really an issue since the axial motion of the sample is restricted through physical boundaries
- No positioning capability (yet)
- Currently working on an implementation of a positionable corral trap based on optical tips

Drawbacks of the ABEL Trap versus the Corral Trap

Continuous particle trapping

A particle can no longer be held in the trap when it "disappears from view", for example due to photobleaching, blinking, or transition into a "dark state" in the case of single molecules. The corral trap, in contrast, does not rely on particle monitoring for its operation (only on the application of a trapping potential), which means that single molecules, for example, can be held in the corral trap without monitoring its position.
continuous particle monitoring usually implies continuous illumination by a laser source, which can limit the observation time due to photobleaching in the case of single molecules. Again, the corral trap does not need continuous illumination to keep the molecule trapped.

Complex Hardware/Software

Complex hardware and software setup is necessary for the ABEL trap, consisting of automatic image capture and analysis (for software-based position readout) or a rotating laser beam setup with lock-in detection (for hardware-based position readout), PID feedback system, and a trapping chamber with two integrated and individually addressable electrode pairs

Destruction of Sample

The ABEL trap operates by applying a voltage to electrode pairs that are exposed to electrolytes, and electrolysis of water is therefore a concern; the ABEL trap chamber therefore includes secondary chambers from which electrolytically formed gas bubbles can escape (in other words, the sample is to some extent destroyed). The corral trap only operates with a single electrode, and bubble formation has never been observed.

Trapping of only one molecule

The ABEL trap can only operate on one particle at a time (by counteracting its random Brownian diffusion), but cannot trap two or more particles in the same volume. The corral trap is based on a stable potential well and can trap multiple particles simultaneously, which extends the range of applications (e.g. to study chemical reactions or recognition events between biomolecules)

Road to Commercialization: Market, Intellectual Property, and Partnering

Market

This trapping system in a flow cell design could be utilized in diagnostic and biomedical applications such as DNA genotyping, DNA sequencing, forensic DNA analysis, and other DNA hybridization applications.

Trapping of charged biological samples including: viruses, single cells, proteins, and DNA.

BCC Research also reports that worldwide market for DNA sequencing products will grow to more than $3.3 billion by 2015. The largest markets for sequencing are life science research and drug discovery and development.

New emerging applications include personal genomics and clinical diagnostics which are forecasted to reach $541 million by 2015 as compared to $15.5 million in 2010.

Research use and microscopy:

The field of microscopy continues to evolve rapidly, as new requirements and imaging technologies are developed. Microscopy sales are predicted to reach $3.1 billion by 2014 with microscope accessories and supplies predicted to reach $513 million.

Intellectual Property

U.S. Utility Patent Application in prosecution; US 2011-0180701 A1

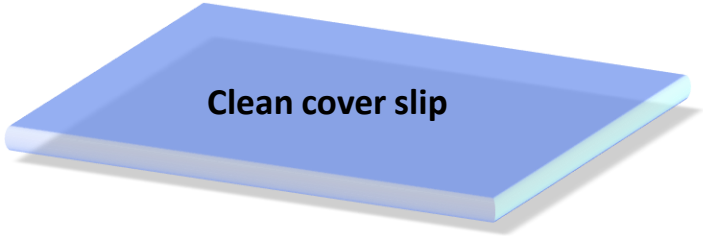
A prototype flow cell device was recently completed and a corral trap will be integrated into the unit as well as an array of multiple corral traps for potential applications in the biomedical sector

Partnering

Looking for a development partner to aid in the development of a final product

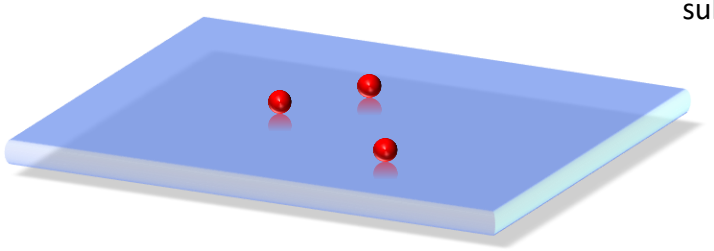
This technology is available for licensing under exclusive or non-exclusive terms

Corral Trap Fabrication Using Shadow Evaporation

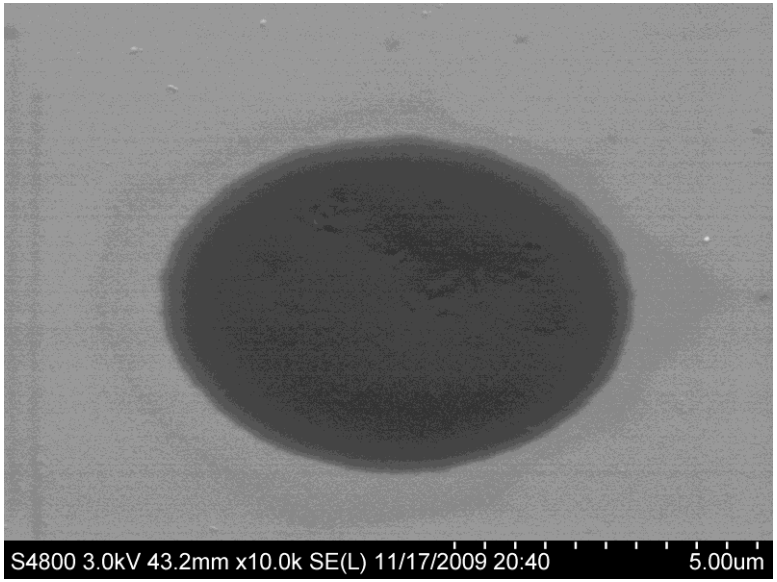


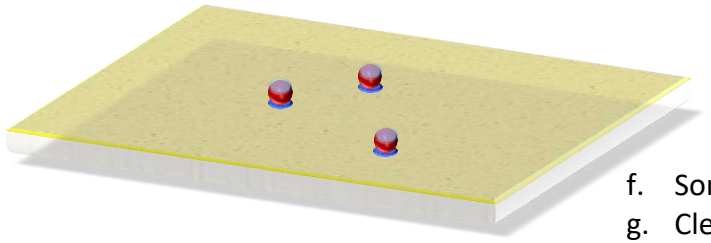
a. Cleaning with polar organic solvents by sonication
b. Drying under stream of N_2

c. Deposition of 1 µL drops of dilute, aqueous solution of unlabeled polystyrene microspheres (Ø 10.0 µm)
d. Drying in clean environment; beads are the masking substrate

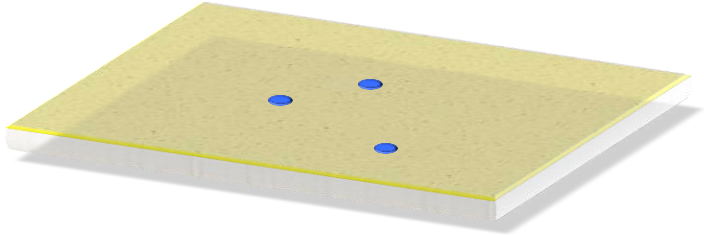


e. Metallization through thermal evaporation (5 nm of 60-40 Au-Pd)





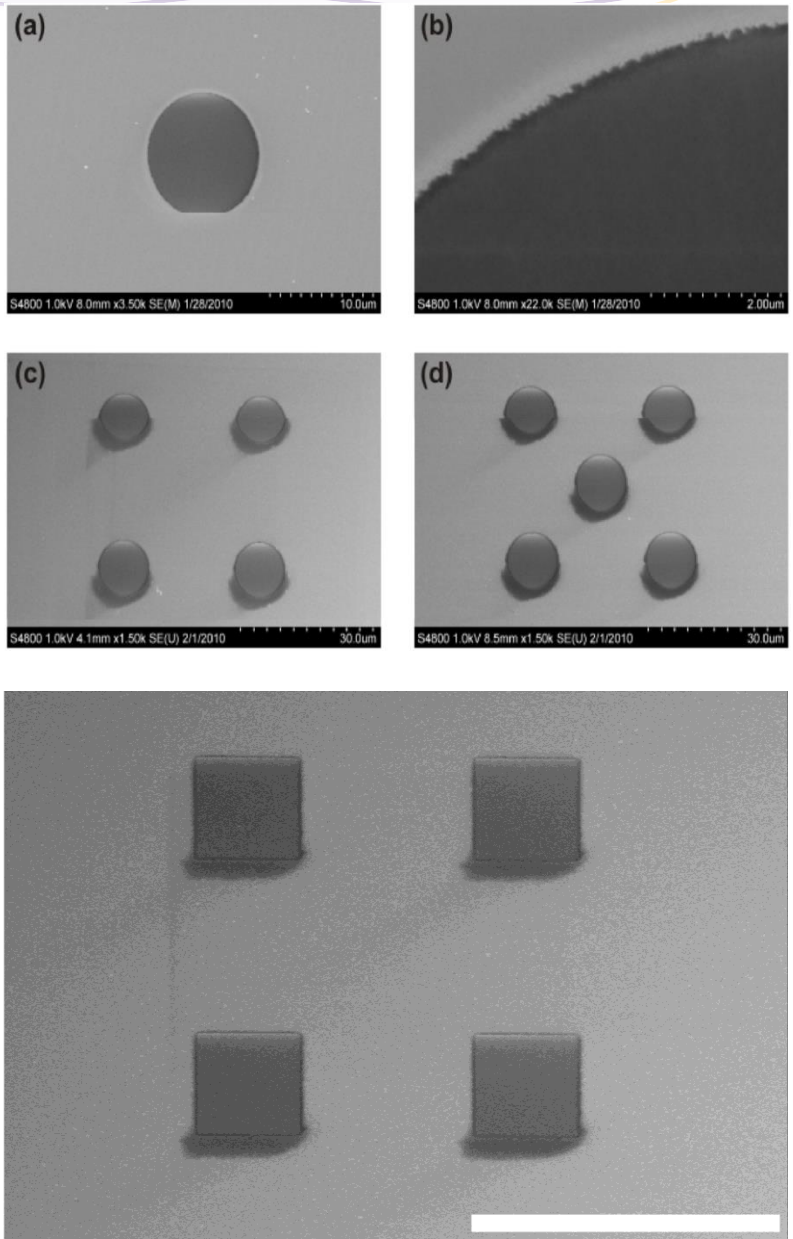
f. Sonication in toluene
g. Cleaning with *iso*-propanol
h. Drying under clean N_2



Left with an uncoated circular area in the metal film

Corral Trap Fabrication by Focused Ion Beam Milling

- The placement of the beads cannot be controlled
- Current focus is on ion beam milling and photolithography in order to produce well-defined patterns of corral traps and explore different shapes.
- All experiments in the following slides were obtained using the simple shadow evaporation technique.

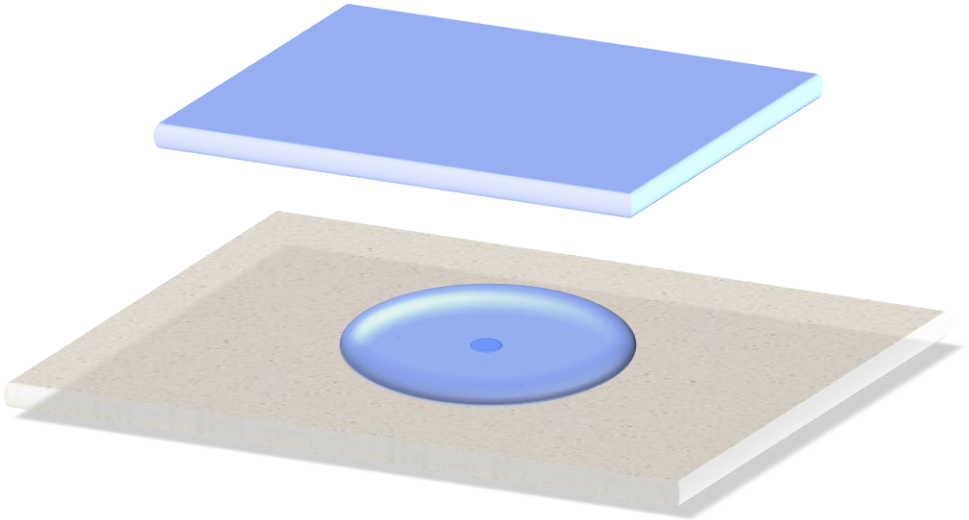


Proof of Principle Experiments

- **Particle**: 2.0±0.13 µm carboxylate-modified polystyrene beads (~10^8 charges)
- **Solution:** aqueous glycerol (1:1 v/v), pH 10 (NaOH); increased pH deprotonates the carboxylic acids on the beads to leave them negatively charged
- **Axial confinement** (~2-3 µm) with "grounded" top coverslip
- **Induced flow:** pressure was exerted on one side of the assembly
- **Image Capture:** brightfield images: 1 fps @ 100 ms/frame
- Movie shows the first successful trapping of the 2 micron bead in a 10 micron corral trap by applying a voltage of -10V when bead moves into the trap

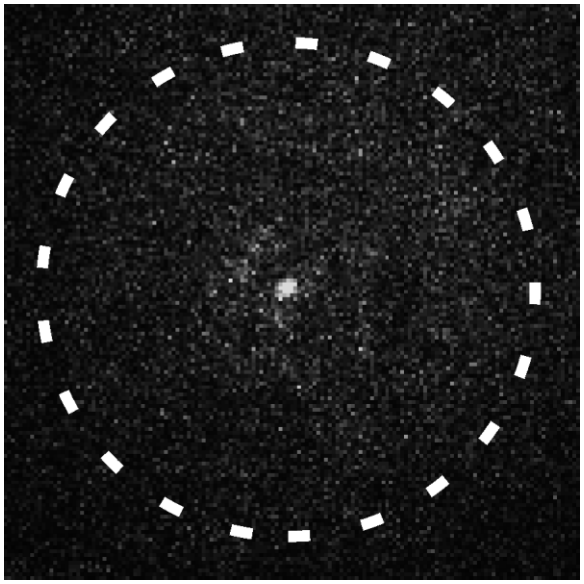
- See the movie for the trap at (apple quick time is needed for viewing): https://pantherfile.uwm.edu/woehl/public/Movie1.mov

*The strong correlation between applied potential and particle motion shows that adsorption of the particle on the glass substrate is not responsible for trapping.



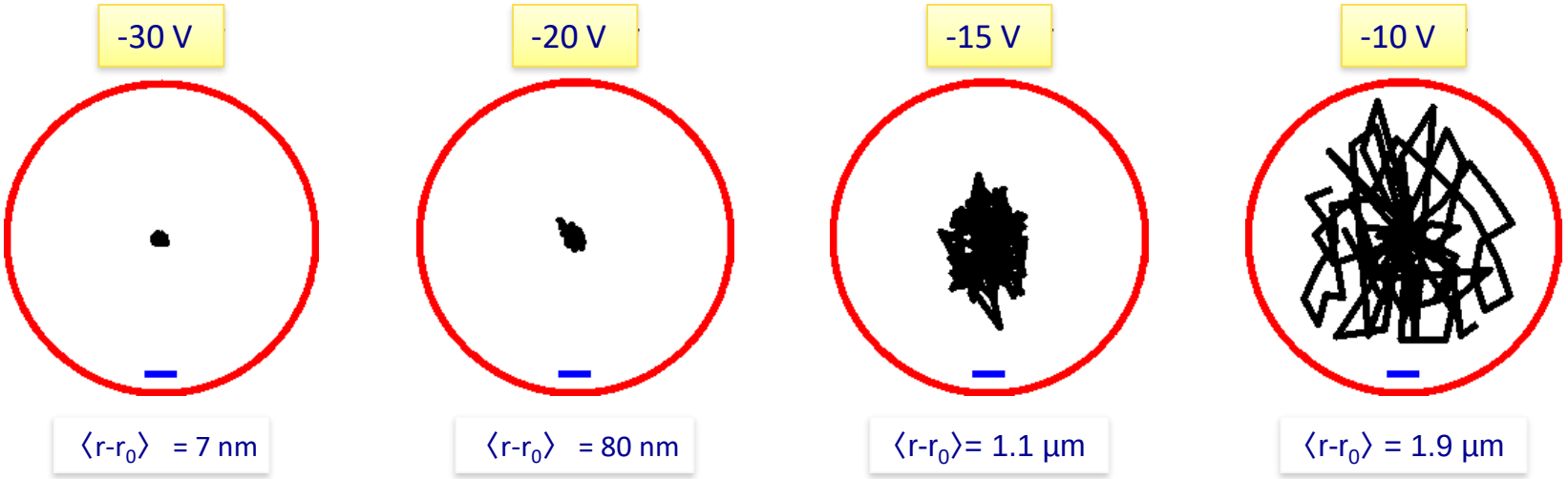
Trapping of Virus-size Particles

- Fluorescent 21±3 nm CML (carboxylate modified) nanobeads
- 5% v/v aqueous glycerol, pH 10 (NaOH)
- ~950 charges/particle
- Axial confinement by spin-coating 1 µL for 30 s at 8000 rpm (<1 µm)
- Induced flow
- Trapping voltage varied; 100 fluorescence images/voltage (3.3 fps; 100 ms/frame)
- **A single bead is successfully trapped in the corral trap below**



20nm Beads with Variable Trapping Voltage

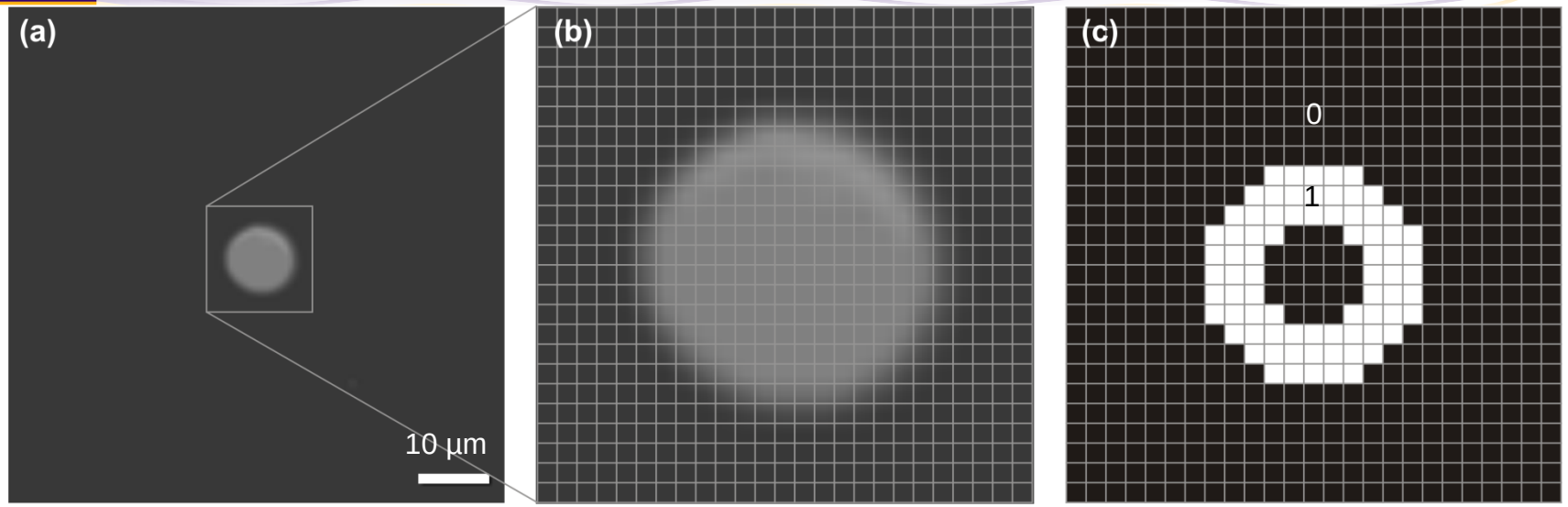
- The trapping voltage below is varied from -30 V (the limit of the dc power supply) to -10 V
- The decrease in trapping voltage leads to a more pronounced motion of the particle within the trap, as expected from the simple electrostatic model

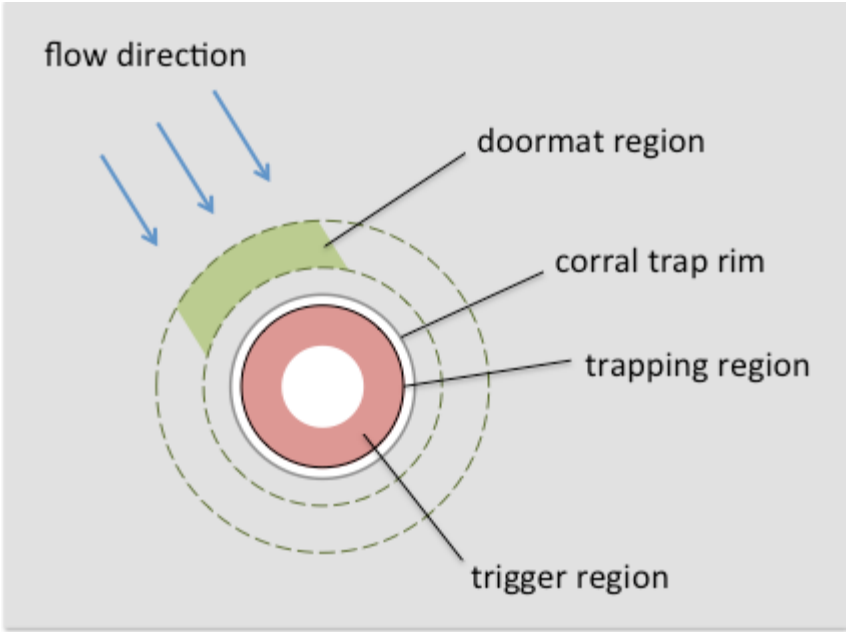


Automated Particle Trapping

- 800-nt ssDNA labeled with a single Cy3 fluorophore
- TBE buffer: 44 mM TRIS, 44 mM boric acid, 1 mM EDTA
- pH found to be 8-9 → ~800 charges/molecule
- Axial confinement < 2 µm
- Induced flow
- Imaging conditions: 7.7 fps; 100 ms/frame; on-chip gain ~1000
- DNA nucleotides carry a negative charge, thus the length of the DNA molecule dictates its charge and behavior in the trap
- **Observe the movie at** (apple quick time is needed for viewing)**:** **https://pantherfile.uwm.edu/woehl/public/ssDNAMovie/Resources/ssDNAMovie.mov**
- The dashed red circle in the movie corresponds to the outline of the corral trap, which was obtained from a brightfield image of the same region
- The white spot corresponds to a single ssDNA molecule labeled with a single Cy3 molecule

Automated Particle Trapping

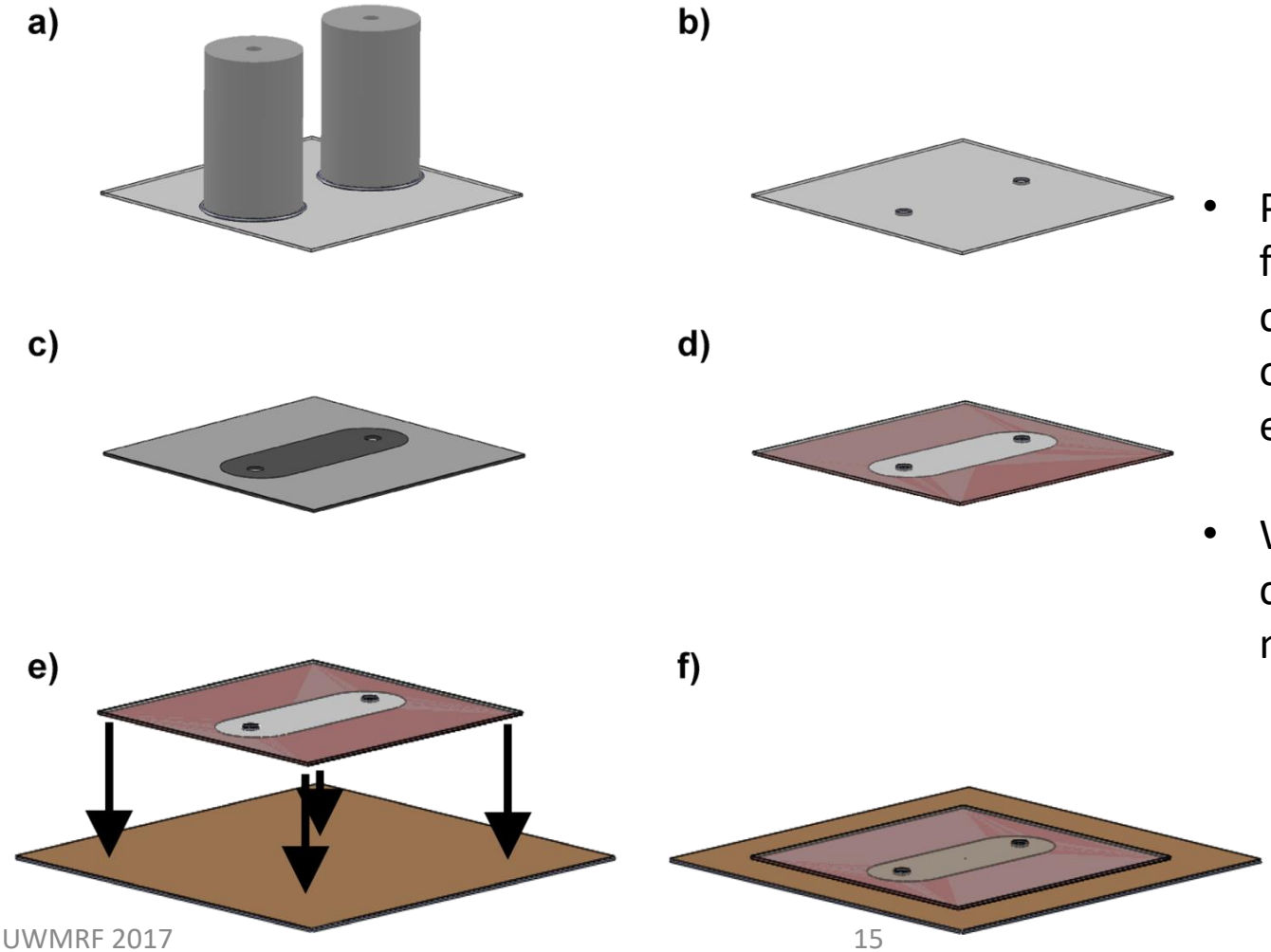




- The inventors have developed a fully automated system based on a real-time, rapid image analysis algorithm
- They can trap one, two, or any number of particles successively by automatically turning the voltage on or off at the right time, all of this without letting particles that are already trapped escape from the trap.

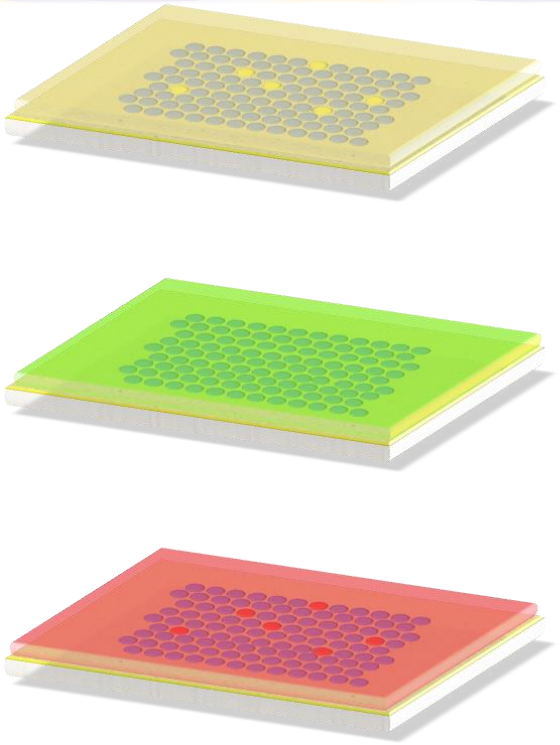
New Corral Trap Design: Microfluidic Chamber

- Improve flow dynamics
- Provide axial confinement
- Rapid sample exchange
- Reusability



- Photolithographically fabricated 800nm deep channels with inlet and outlet for solution exchange
- Working now to bond channels onto the metalized trap coverslips

Ultrasensitive Biomedical Analysis Application



1. Target DNA “pull-down”

2. Fluorescent probe A

3. Fluorescent probe B etc.

- One promising application involving confinement of single molecules in solution is DNA hybridization without PCR

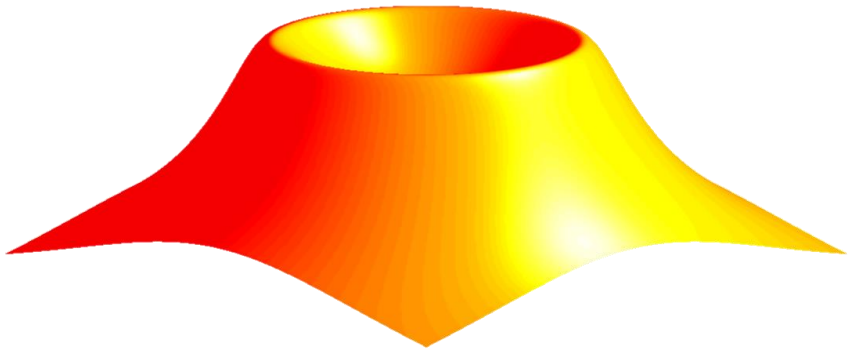
- A simple microfluidic device for this purpose could consist of a large array of corral traps, which are used to “pull down” or confine target ssDNA molecules in their native solution state. The traps containing DNA can be identified by labeling with a fluorescent marker.

Ultrasensitive Biomedical Analysis Application

- Once the ssDNA molecules are spatially confined, they can then be successively exposed to different fluorescent oligonucleotide probes.
- The much shorter probes carry only few charges and will therefore not be retained by the corral traps, so that after washing only probes that have hybridized with target DNA would give rise to a fluorescence signal.
- Any hybridized probes are then be thermally dissociated, washed away, and the trapped target DNA could is exposed to another sequence probe
- These cycles could be repeated over and over again to test for the presence of different sequences on a single DNA molecule.

Summary

- A new nanoparticle trap, the **corral trap**, has been conceived and fabricated
- Simple experimental setup; trap precisely controlled by voltage
- Trapping scales favorably with particle size, only depends on charge
- No particle monitoring or active feedback system necessary during trap operation – illuminate only when needed (stable trap)
- **Single micro- and nanoparticles** carrying multiple charges have been successfully trapped
- **Single DNA molecules** have been successfully trapped under normal buffer conditions
- **Multiple-particle trapping** has been demonstrated
- Parallelization possible for device fabrication



Next Steps

Further investigations

- Integration of a corral trap into the microfluidic device and testing for nanoparticle trapping
- Fabrication of an corral trap array for integration into the microfluidic device
- Development of software for automated image analysis of particles trapped in corral trap arrays for forensic analysis
- Development of a genetic profiling assay of a single, trapped DNA target for the presence of multiple genetic markers (SNPs)

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