5th Korea - US Nano Forum

April 17, 2008

Electric Field Devices for Manipulation, Directed Assembly, Isolation and Detection of BioDerivatized Nanoparticles

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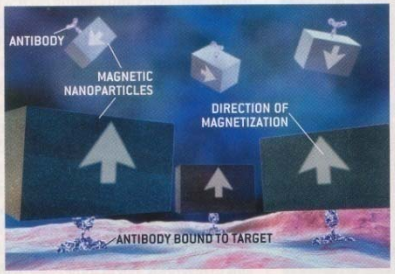
NCI Alliance for Nanotechnology in Cancer

Rebecca and John Moores UCSD Cancer Center
An NCI-Designated Comprehensive Cancer Center

Nanotechnology for Next Generation BioSensors , In-Vivo Drug Delivery Motherships and Other Applications

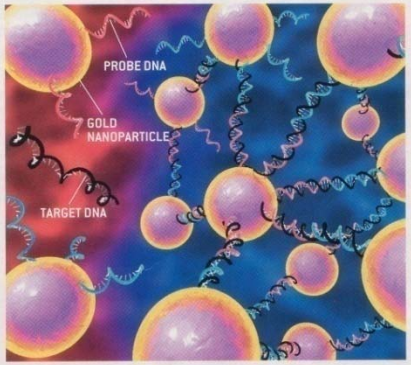
BIO-NANOTECH IN ACTION

The items here could one day enhance the speed and power of biomedical tests, such as those used to screen small samples of material for the presence of particular genetic sequences. For clarity, the images have not been drawn to scale.



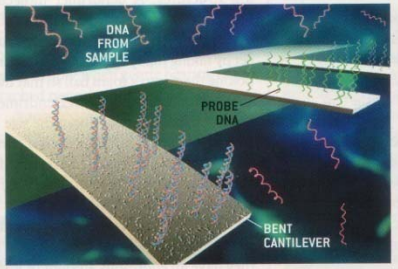
MAGNETIC TAGS

Many tests reveal the presence of a molecule or disease-causing organism by detecting the binding of an antibody to that target. When antibodies labeled with magnetic nanoparticles bind to their target on a surface (*foreground*), brief exposure to a magnetic field causes these probes collectively to give off a strong magnetic signal. Meanwhile unbound antibodies tumble about in all directions, producing no net signal. This last property makes it possible to read the results without first washing away any probes that fail to find their target.



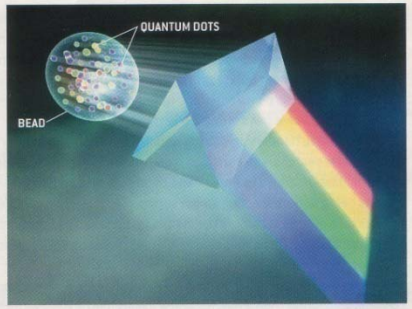
GOLD PARTICLES

Gold nanoparticles studded with short segments of DNA could form the basis of an easy-to-read test for the presence of a genetic sequence (*black*) in a sample under study. DNA complementary to half of such a sequence (*red*) is attached to one set of particles in solution, and DNA complementary to the other half (*blue*) is attached to a second set of particles. If the sequence of interest is present in the sample, it will bind to the DNA tentacles on both sets of spheres, trapping the balls in a dense web. This agglomeration will cause the solution to change color (*from red to blue*).



CLEVER CANTILEVERS

Biological samples can be screened for the presence of particular genetic sequences using small beams (cantilevers) of the type employed in atomic force microscopes. The surface of each cantilever is coated with DNA able to bind to one particular target sequence. A sample is then applied to the beams. Binding induces a surface stress, which bends the affected beams by nanometers—not much, but enough to reveal that the bent beams found their specific targets in a sample.

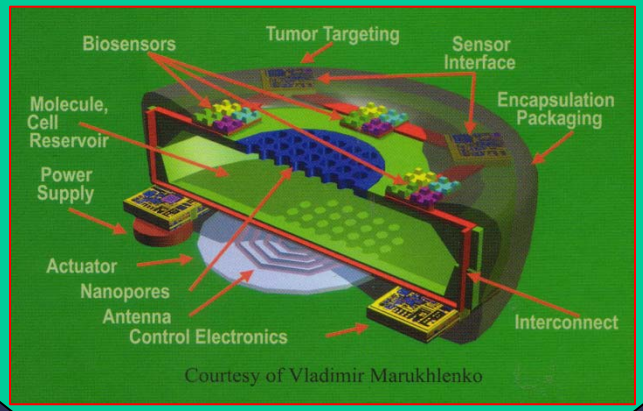


NANO BAR CODES

Latex beads filled with several colors of nanoscale semiconductors known as quantum dots can potentially serve as unique labels for any number of different probes. In response to light, the beads would identify themselves (and, thus, their linked probes) by emitting light that separates into a distinctive spectrum of colors and intensities—a kind of spectral bar code.

MINIATURIZED PEOPLE in the 1966 movie *Fantastic Voyage* swim like nanobot surgeons in a patient's bloodstream.

Ultimate Goal

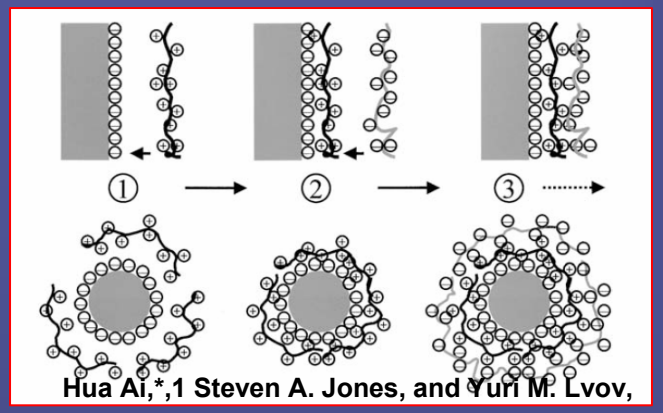


Courtesy of Vladimir Marukhlenko

Directed Self-Assembly Nanofabrication

Potential Applications

- ***Bio/Chem Sensors***
- ***Drug Delivery NanoVesicles***
- Photovoltaics
- Fuel Cells
- Batteries
- Optical films
- Nanophotonic films/devices
- Ceramic materials
- Morphing nanocomposites



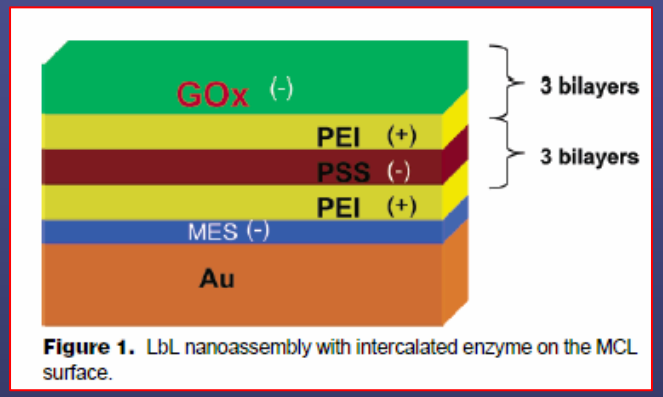
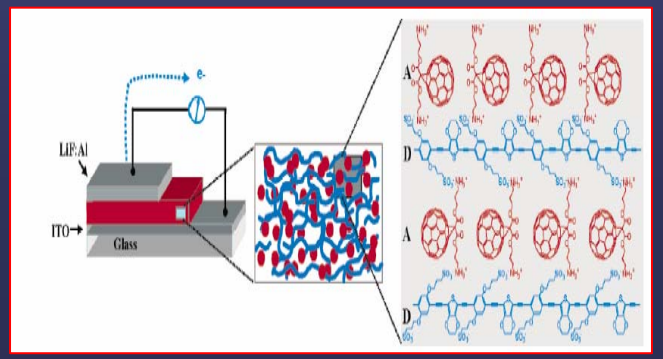


Figure 1. LbL nanoassembly with intercalated enzyme on the MCL surface.

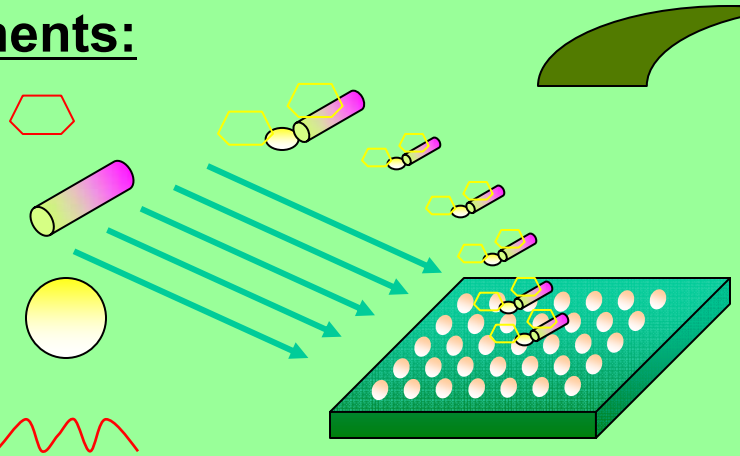


Electric Field Directed Self-Assembly and Heterogeneous Integration for 3D Hierarchical Nanomanufacturing

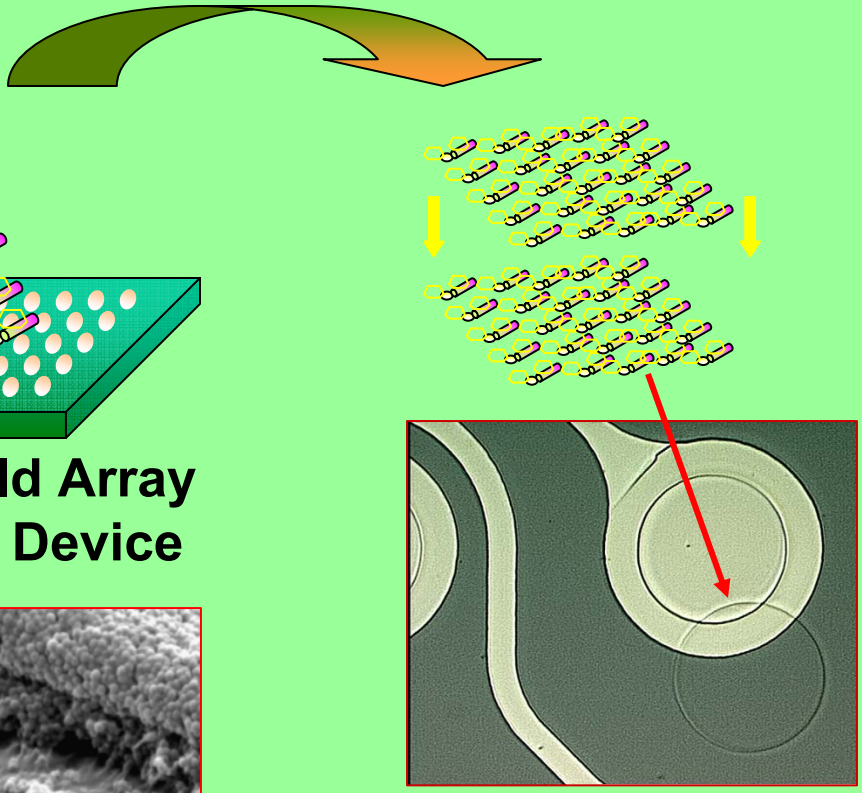
"Synergy of Top-Down and Bottom-Up Processes"

Molecular Lego Nanocomponents:

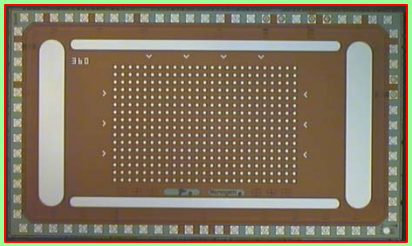
- Biomolecules
- Nanotubes
- Nanofilaments
- Nanoparticles
- Quantum-Dots
- Polymers
- Fullerenes
- Dendrimers
- Cells
- CMOS Lift-Off Devices



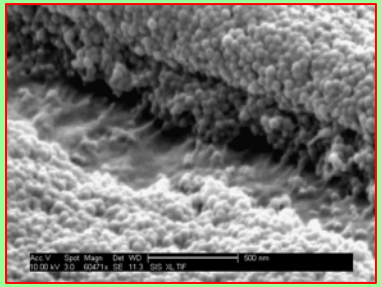
Electric Field Array Assembler Device



Component Release and Further Assembly

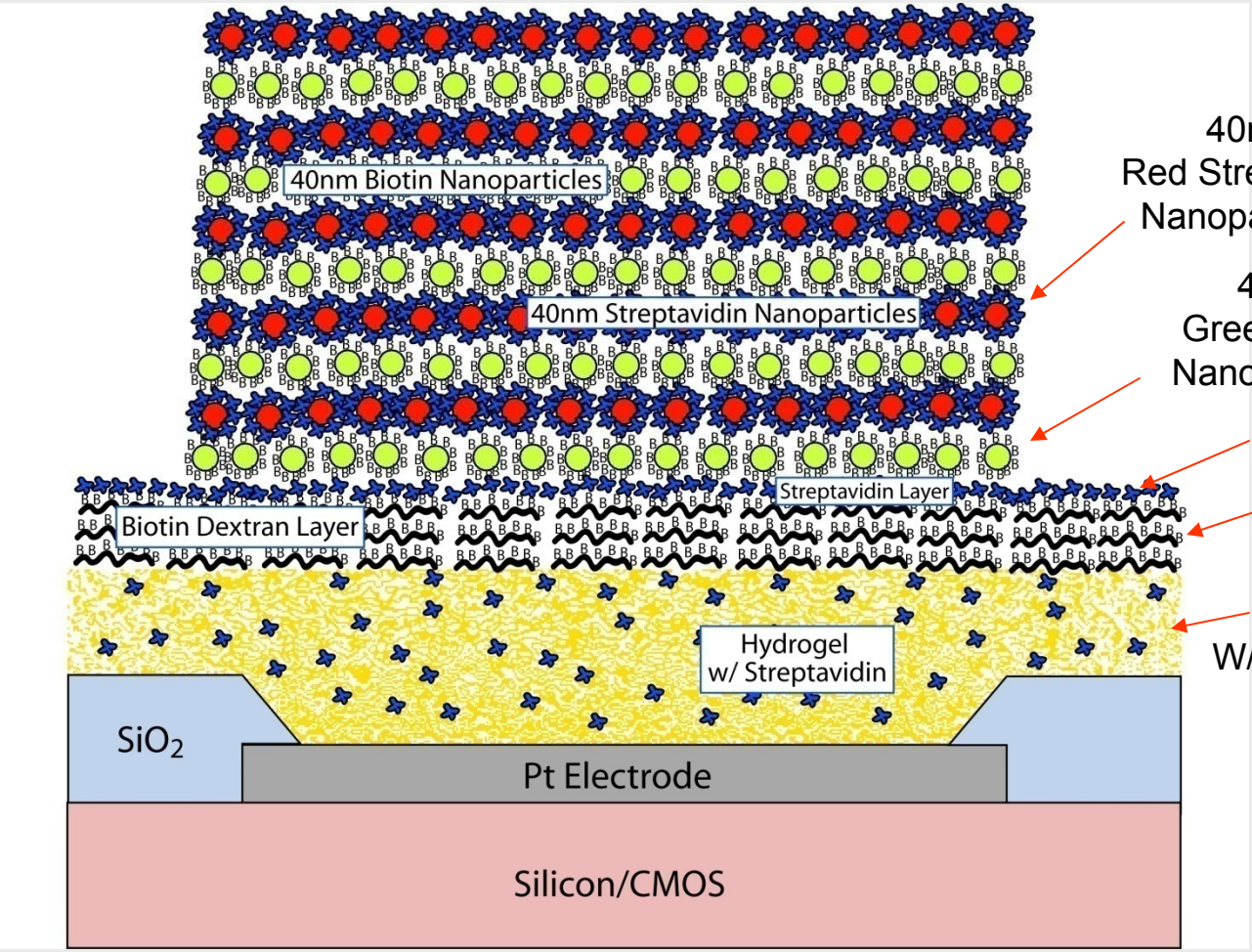


Electric Field Array Device



Integrated 3D NanoStructures

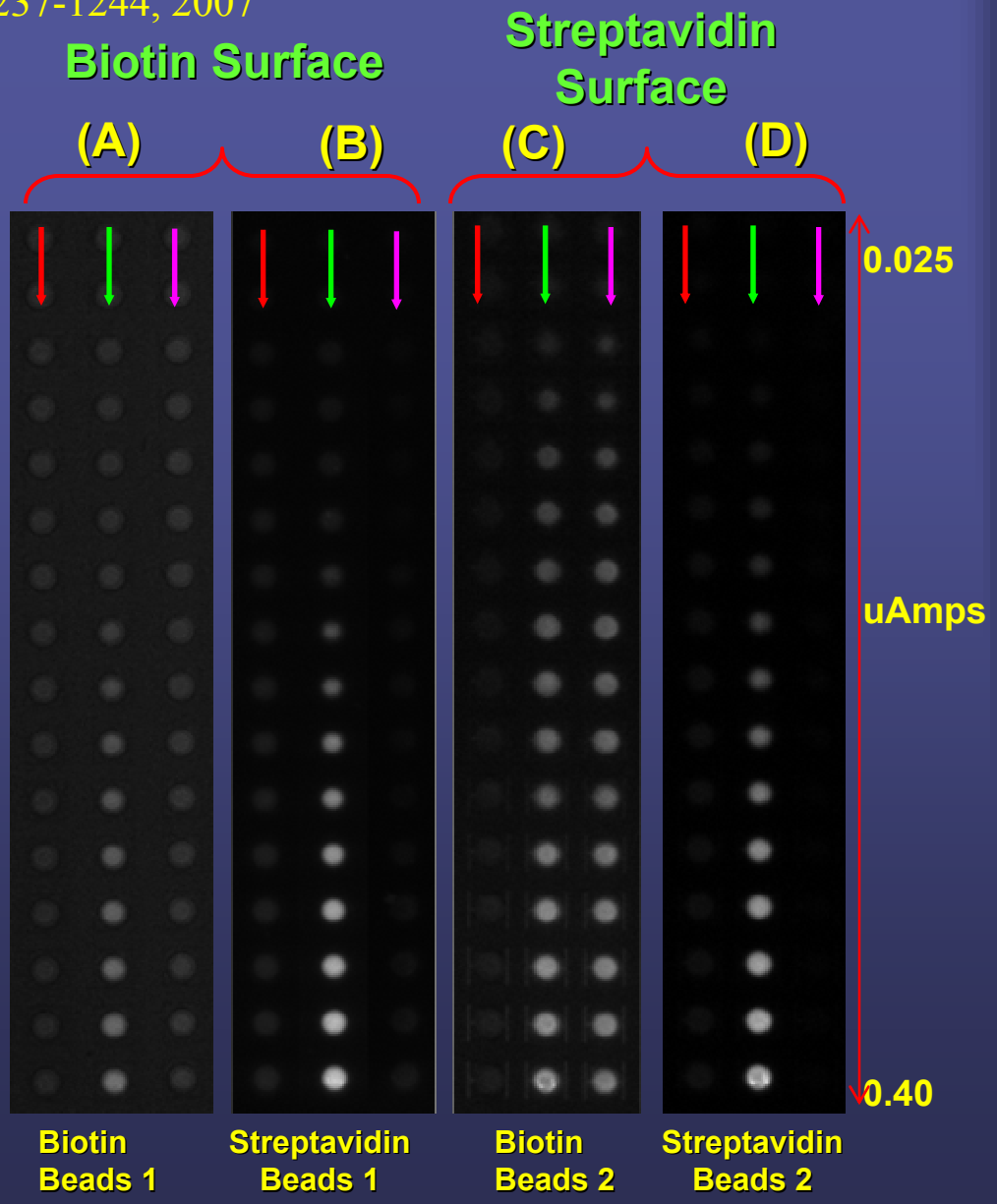
Electric Field Directed Self-Assembly Nanofabrication



Electric Field Directed Nanoparticle Assembly

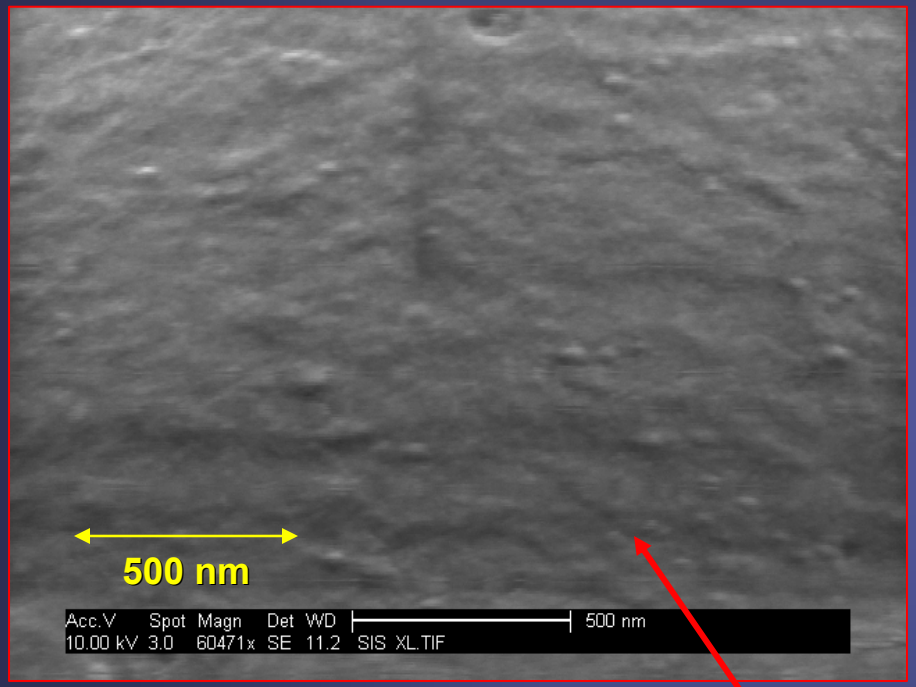
Dehlinger DA, et al., J. Assoc. Lab Automation, October 2007; Dehlinger DA, et al., SMALL, V3, #7, pp. 1237-1244, 2007

- A and B show the results of a biotin starting surface, C and D the results of a streptavidin starting surface. Both experiments were run for 20 layers.
- Chip activated in sets of three columns
  - Streptavidin nanoparticles only (left)
  - Biotin nanoparticles only (right)
  - Both Biotin and Streptavidin nanoparticles (center)
- A and B are the same electrodes under different filter sets With a biotin surface. C and D are a different set of electrodes with a streptavidin surface. A and C show biotin beads, B and D show streptavidin
- Streptavidin beads show good stringency, Biotin beads show moderate stringency, possibly the result of the bead spectra

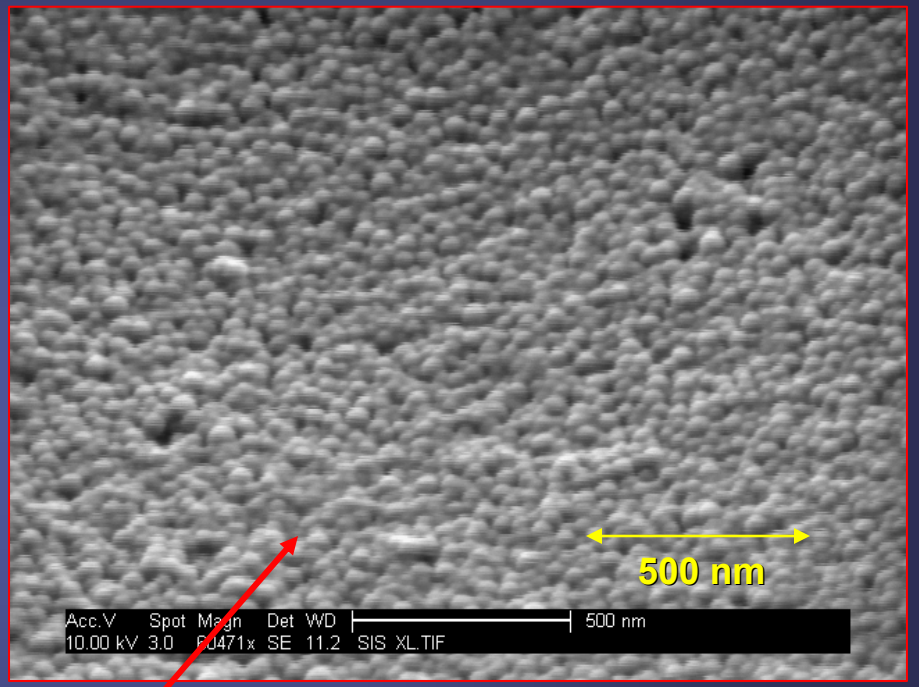


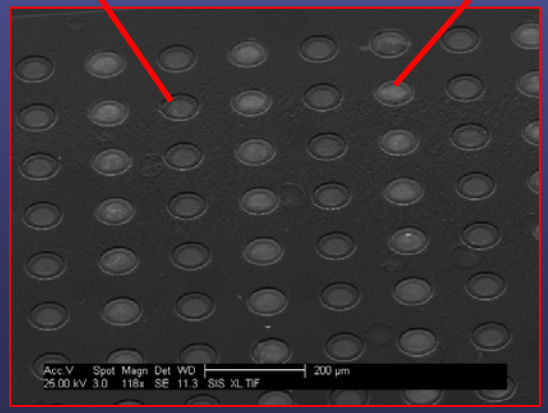
3D Nanoparticle Structures With Up To 100 Alternating Layers of 40 nm Biotin and Streptavidin Nanoparticles

SEM top view surface w/o nanoparticles



SEM top view layered structure

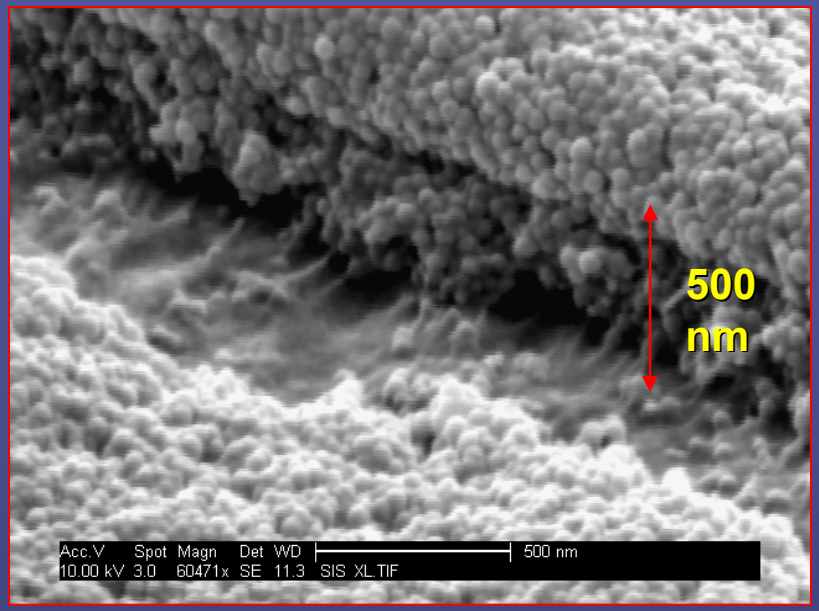


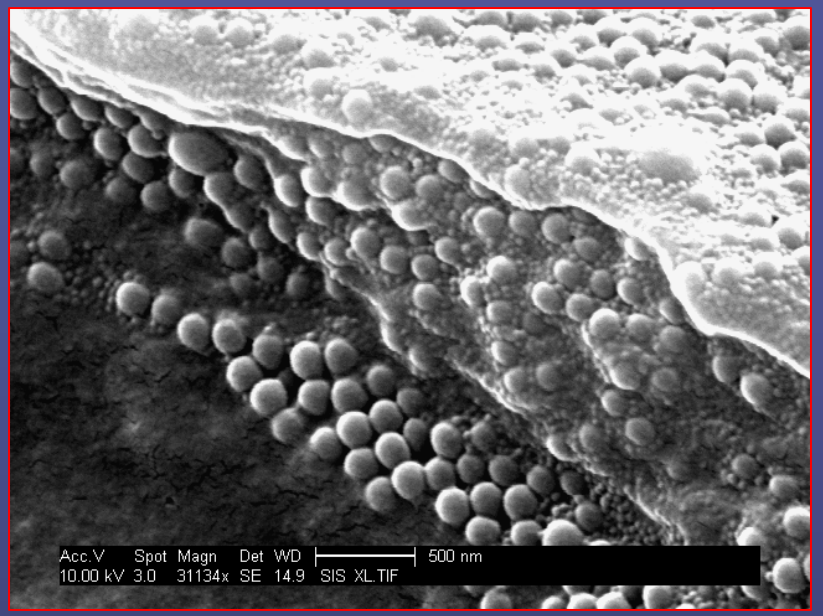


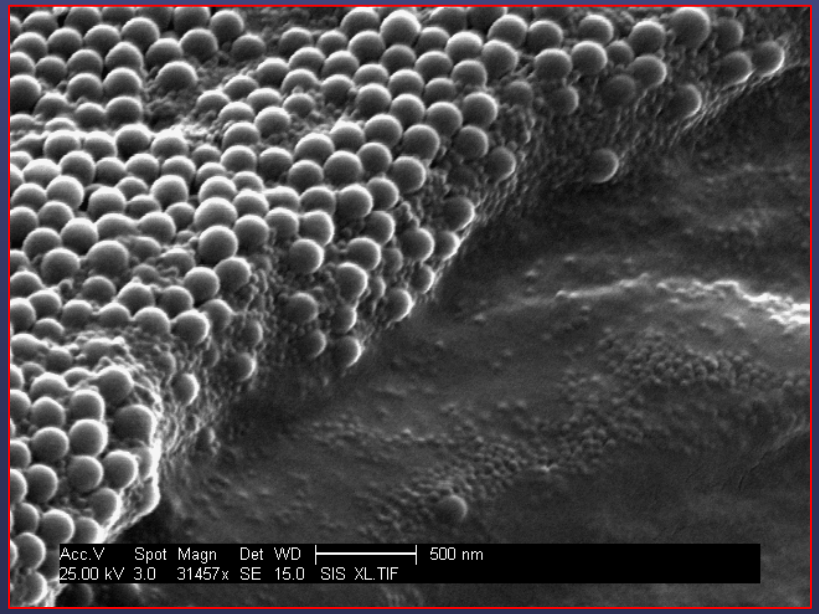
Very little non-specific binding even after 50 exposures to biotin nanoparticles

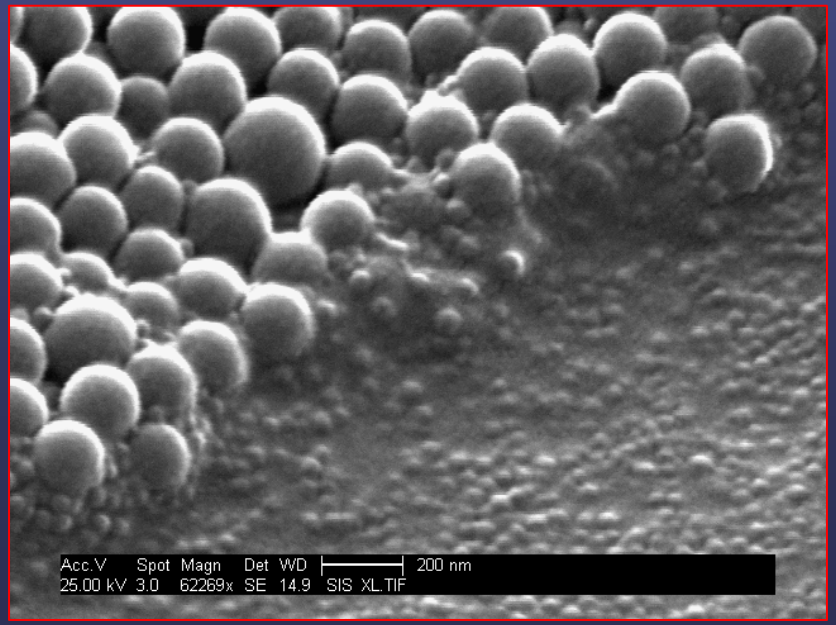
Relatively smooth surface after 100 nanoparticle addressings

Layers with 40 nm and 40nm/200 nm Biotin-Streptavidin Nanoparticles





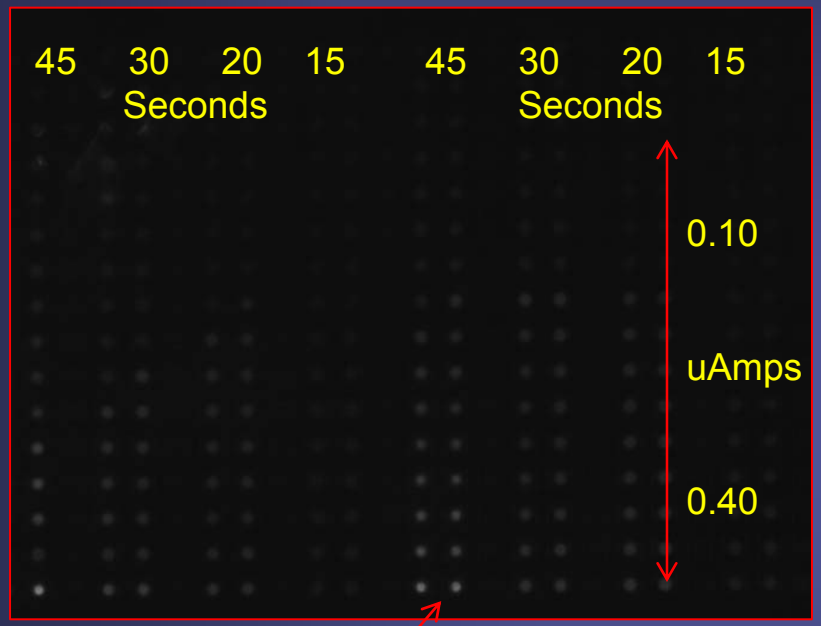




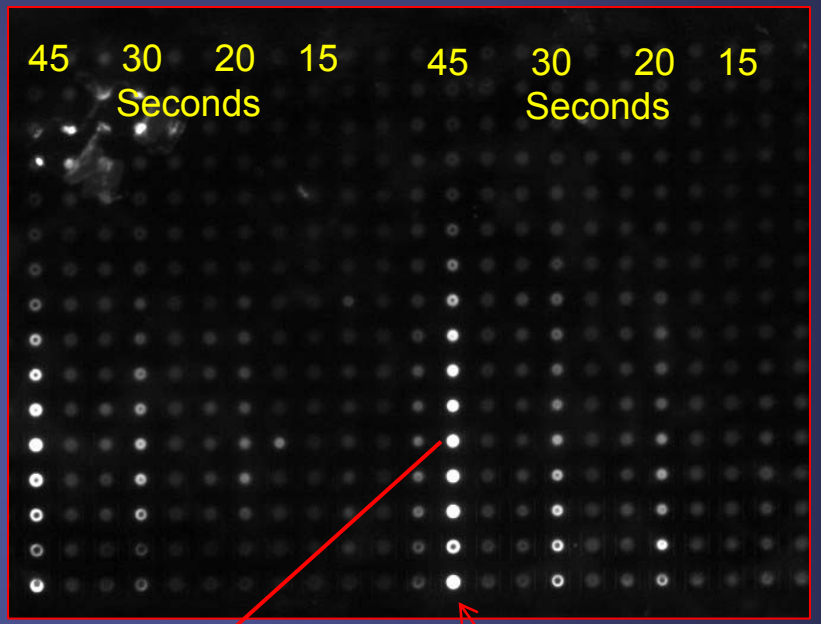
20 Layer DNA Derivatized Nanoparticles Structures

Using 51mer DNA Template and Complement on 40 nm Red Fluorescent Nanoparticles

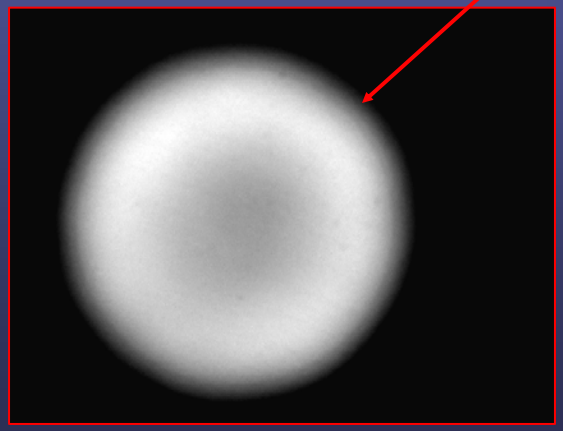
Initial



Final



B-DNA Template Complementary DNA and Template DNA Nanoparticles



B-DNA Template Complementary DNA and Template DNA Nanoparticles

Heterogeneous Nanoconstruction Materials

Streptavidin
Polyacrylamide Streptavidin
Dextran-Biotin Polymer (10,000 MW)
Silica Particles (~10nm-20nm)

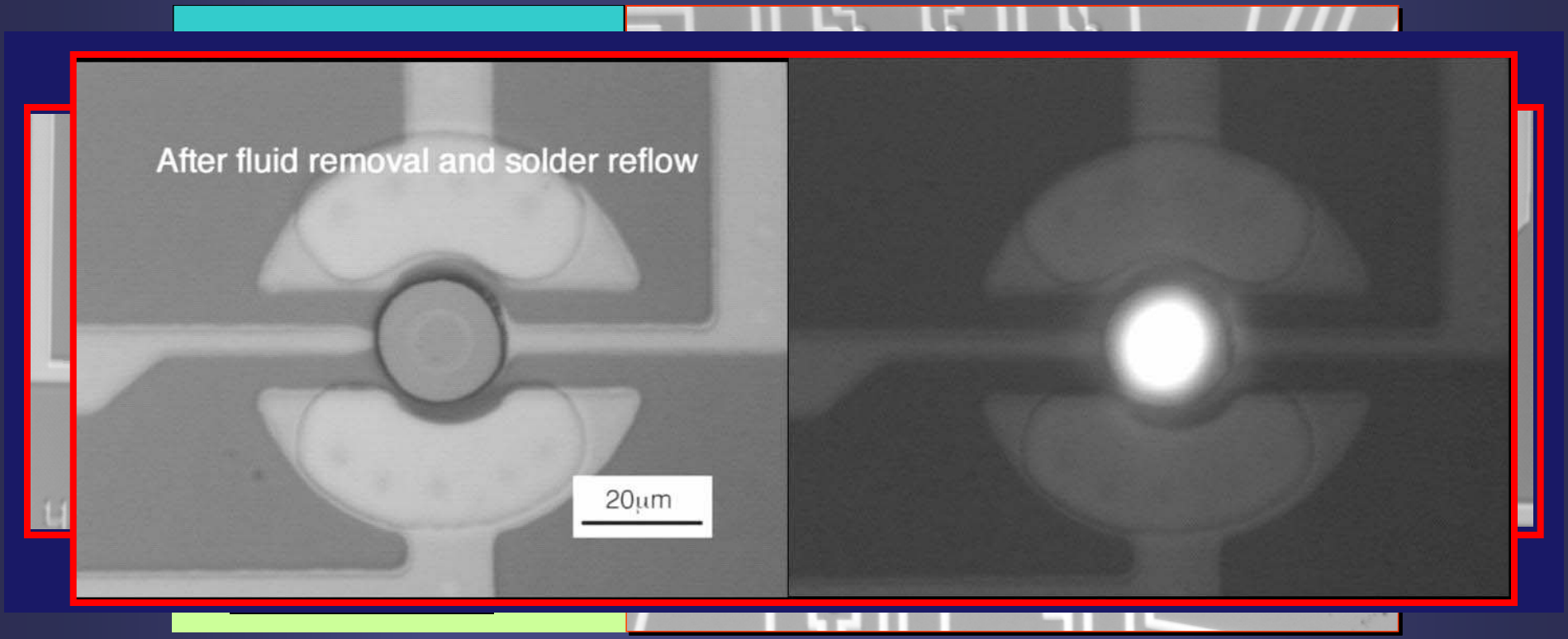
40 nm Nanoparticles (Biotin, Green Fluorescence)
40 nm Nanoparticles (Streptavidin, Red Fluorescence)
200 nm Nanoparticles (Streptavidin, Red Fluorescence)

15 nm Quantum Dots (Streptavidin, Red Emission 610nm)
12 nm Quantum Dots (Biotin, Green Emission 506nm)
50 nm Gold Nanoparticles (Streptavidin)

5'-Biotin-GAA-CAG-CTT-TGA-GGT-GCG-TG-3' (Initial Template)
40nm Streptavidin Nanoparticle-5'-Biotin-GAA-CAG-CTT-TGA-GGT-GCG-TG-3'
40nm Streptavidin Nanoparticle-5'-Biotin-CAC-GCA-CCT-CAA-AGC-TGT-TC-3"

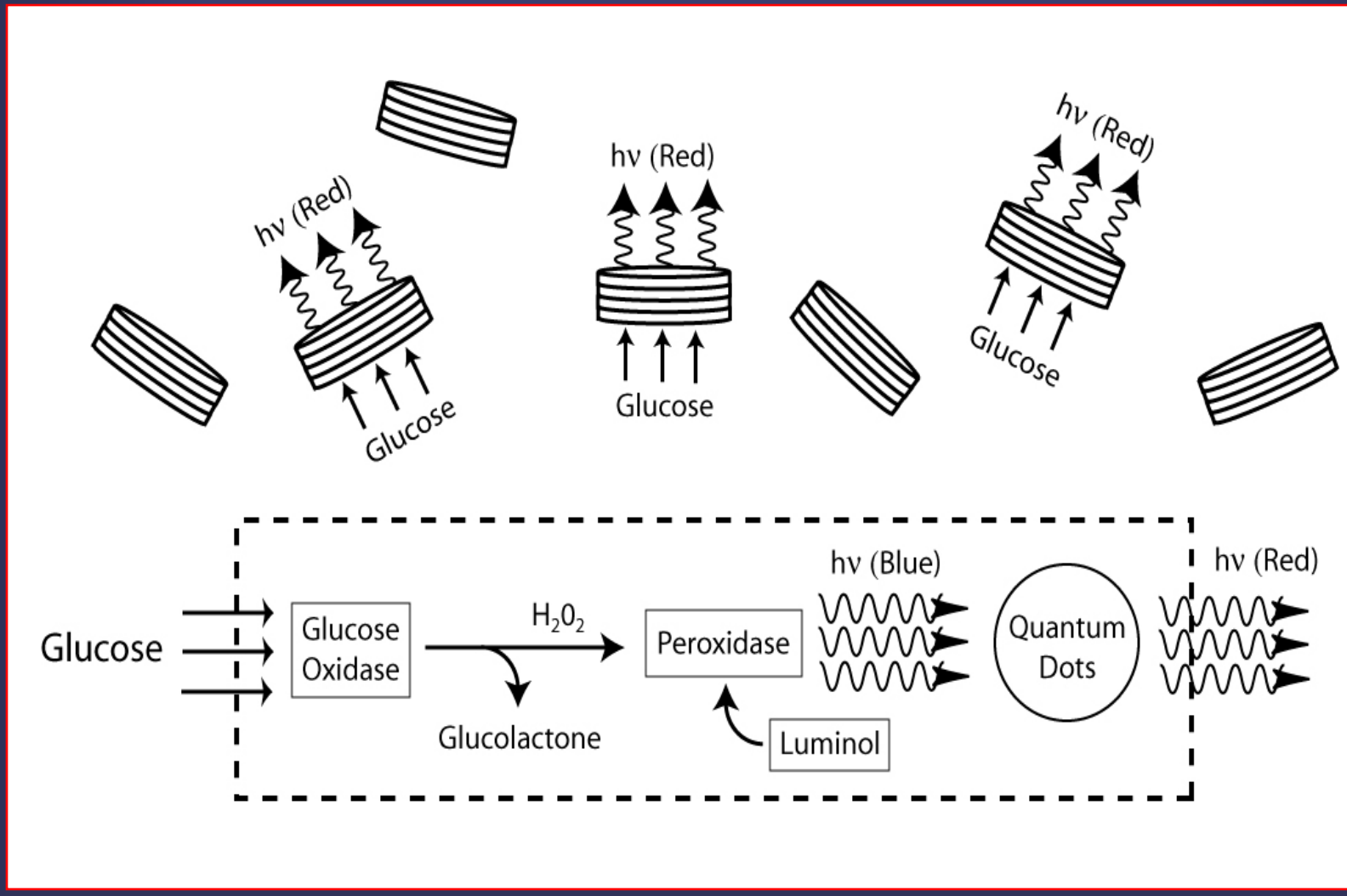
5'-Biotin-GAA-CAG-CTT-TGA-GGT-GCG-TGT-TTG-TGC-CTG-TCC-TGG-GAG-AGA-CCG-GCG-CAC-3' (Initial Template)
40nm Streptavidin Nanoparticle-5'-Biotin-GAA-CAG-CTT-TGA-GGT-GCG-TGT-TTG-TGC-CTG-TCC-TGG-GAG-AGA-CCG-GCG-CAC-3'
40nm Streptavidin Nanoparticle-5'-Biotin-GTG-CGC-CGG-TCT-CTC-CCA-GGA-CAG-GCA-CAA-ACA-CGC-ACC-TCA-AAG-CTG-TTC-3"

Electric Field Integration of Lift-Off CMOS Devices and Directed Nanoparticle Assembly to Create Novel BioSensores and in-Vivo Drug Delivery Devices



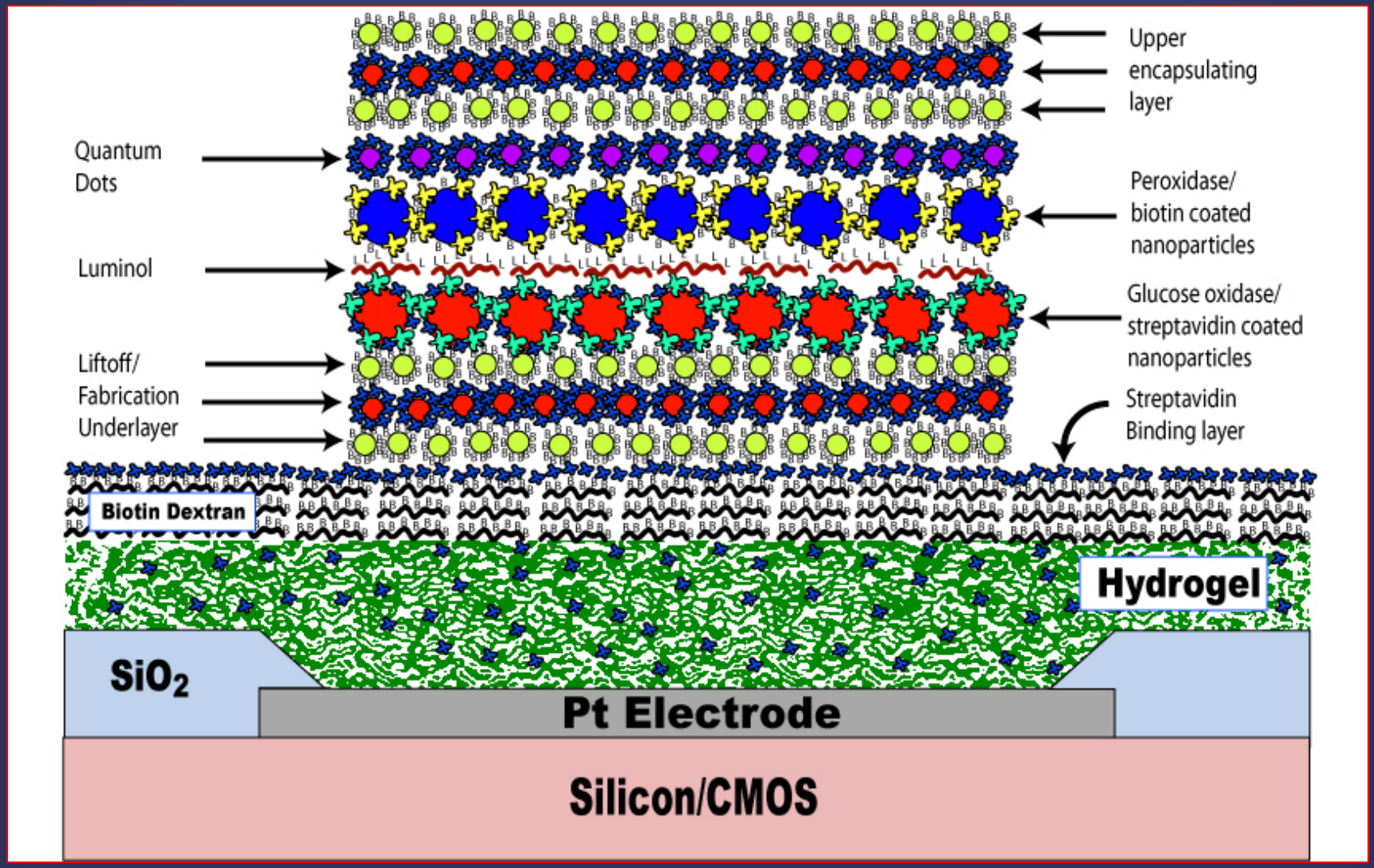
Shows electric field transport, positioning and activation of an LED Lift-Off device on an electronic array platform. (Edman CF, Gurtner, C, Formosa RE, Coleman JJ, Heller MJ. 2000. Electric-Field-Directed Pick-and-Place Assembly. HDI. (3)10: 30-35; and Edman CF, Swint RB, Gurthner C, Formosa RE, Roh SD, Lee KE, Swanson PD, Ackley DE, Colman JJ. Heller MJ, 2000. Electric Field Directed Assembly of an InGaAs LED onto Silicon Circuitry. IEEE Photonics Tech. Letters, 12(9):1198-1200).

Micronsize Bio/Chem Sensors and Lab-on-Chip Devices by Nanoparticle Assembly



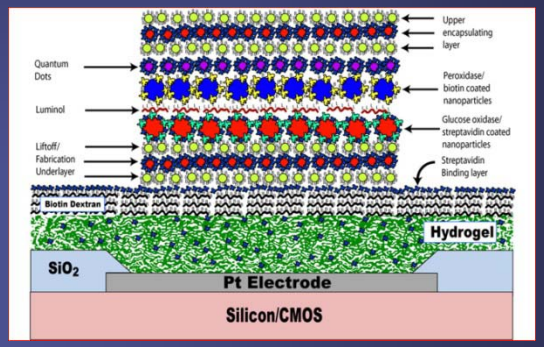
Active Glucose Sensor (Micronsize/Dispersable)

(HRP and GO Nanoparticle Activity Retained)

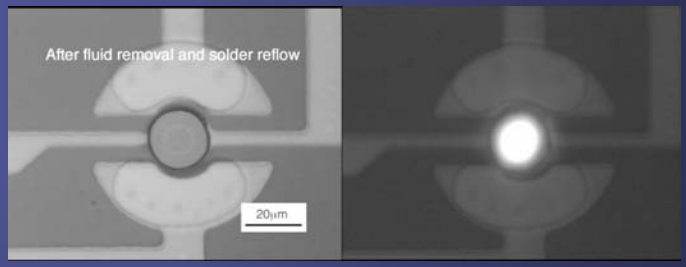


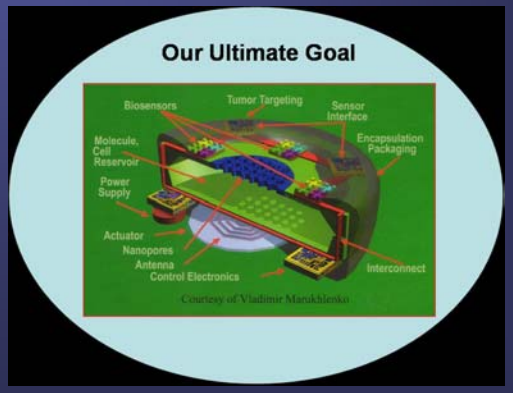
Electric Field Integration of Lift-Off CMOS Devices with Nanoparticle Assembly to Create Micron Scale In-Vivo Drug Delivery/Biosensors Devices

Creating viable In-Vivo Drug Delivery/Biosensor Devices "Fantastic Voyage Motherships" by present fabrication technologies is an integration nightmare and not cost effective!

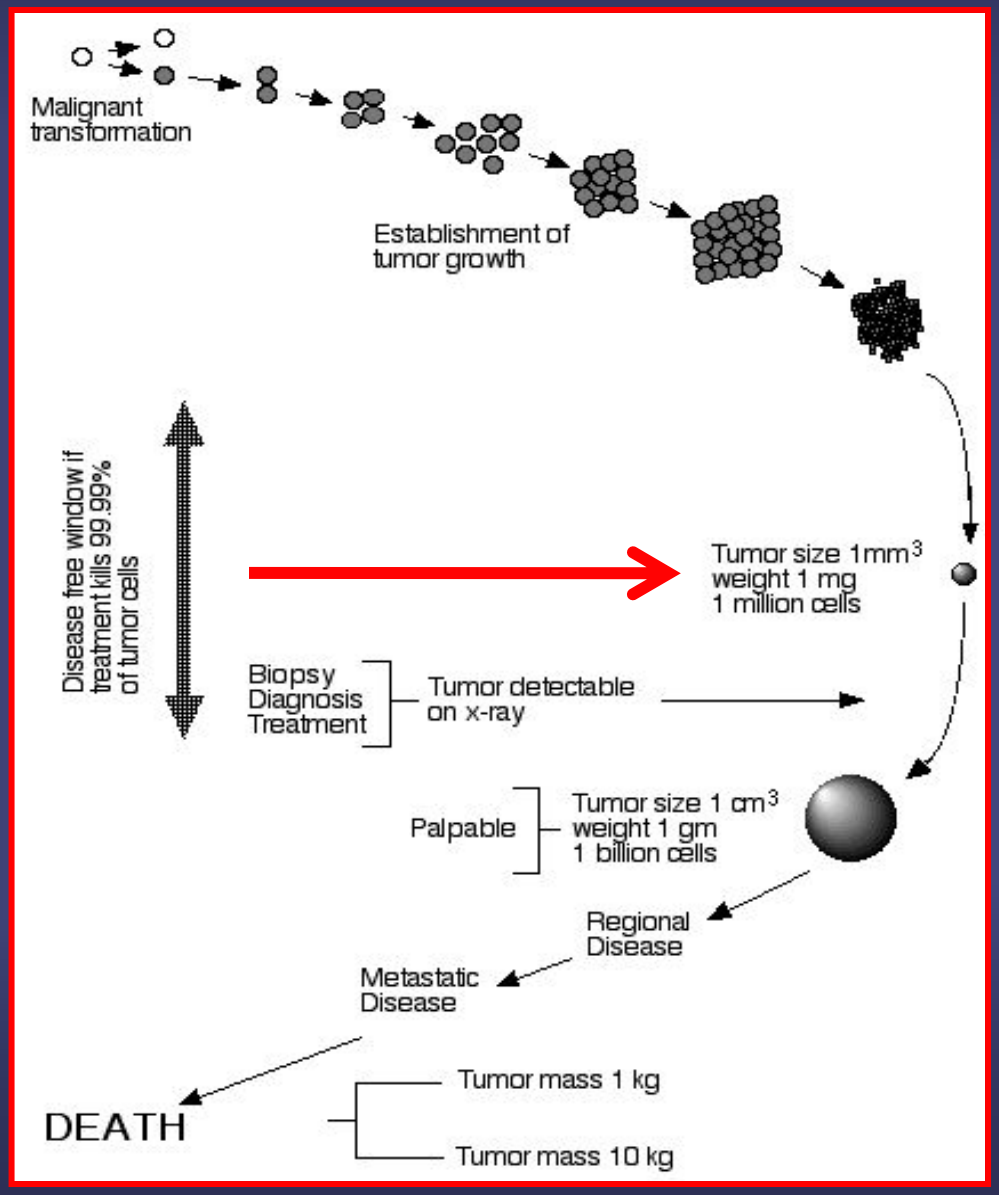


- *Controlled Propulsion/Motion*
- *Sensing/Diagnostics*
- *Drug delivery mechanism*
- *Logic/Communication*
- *Power supply*
- *Soft flexible encapsulation*
- *Biocompatible outer coating*
- *Not larger than 10 microns*





Cancer: Evolution of a Cell that Grows Uncontrollably
(Dr. Dennis Carson, UCSD)



NCI Alliance for Nanotechnology in Cancer

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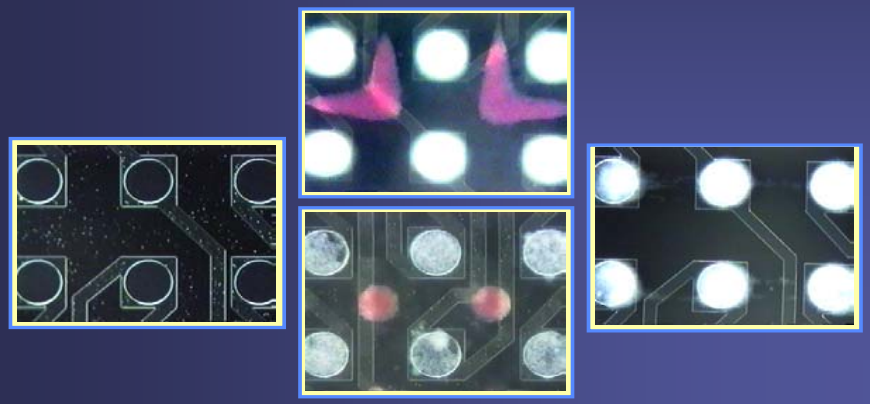
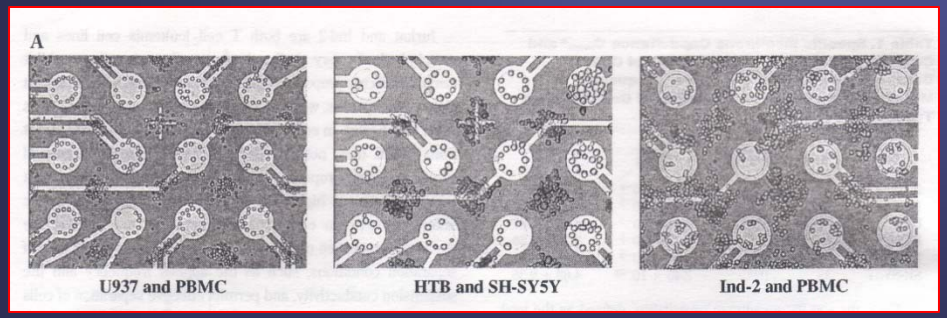
An NCI-Designated Comprehensive Cancer Center

Earlier DEP Work Demonstrating Separation of Bacteria from Blood and Cancer Cell Separation



DEP separation of E. coli from human blood
(Nature Biotechnology Vol. 16, 541-546, 1998)



DEP separation of closely related cell types /(monocytes U937, human T lymphoma cells (Jurkat), HTLV-1 tax-transformed human T cells (Ind-2), peripheral blood mononuclear cells (PBMC), glioma cells (HTB), and neuroblastoma cells SH-SY5Y Anal. Chem. 74, 3362-3371, 2002

NanoTumor Center Ex-Vivo DEP System Goals

1. Separation and identification of cancer cells, hmw-DNA nanoparticulates and therapeutic/drug delivery nanoparticles in blood/plasma (advanced disease and chemotherapy monitoring ~100ng hmw-DNA /ml blood or plasma)

2. Early detection and residual disease monitoring of hmw-DNA nanoparticulates and cancer cells from blood/plasma (<1ng hmw-DNA/ml blood or plasma)

3. Ex-Vivo Diagnostic/Chemotherapy Monitoring Systems (blood tested rapidly, directly, with no dilution and returned to patient)

Overcome the basic limitation of DEP "requiring all separations of cells, nanoparticles, DNA and proteins to carried out at low ionic strength /conductances <1-10 mS/M"

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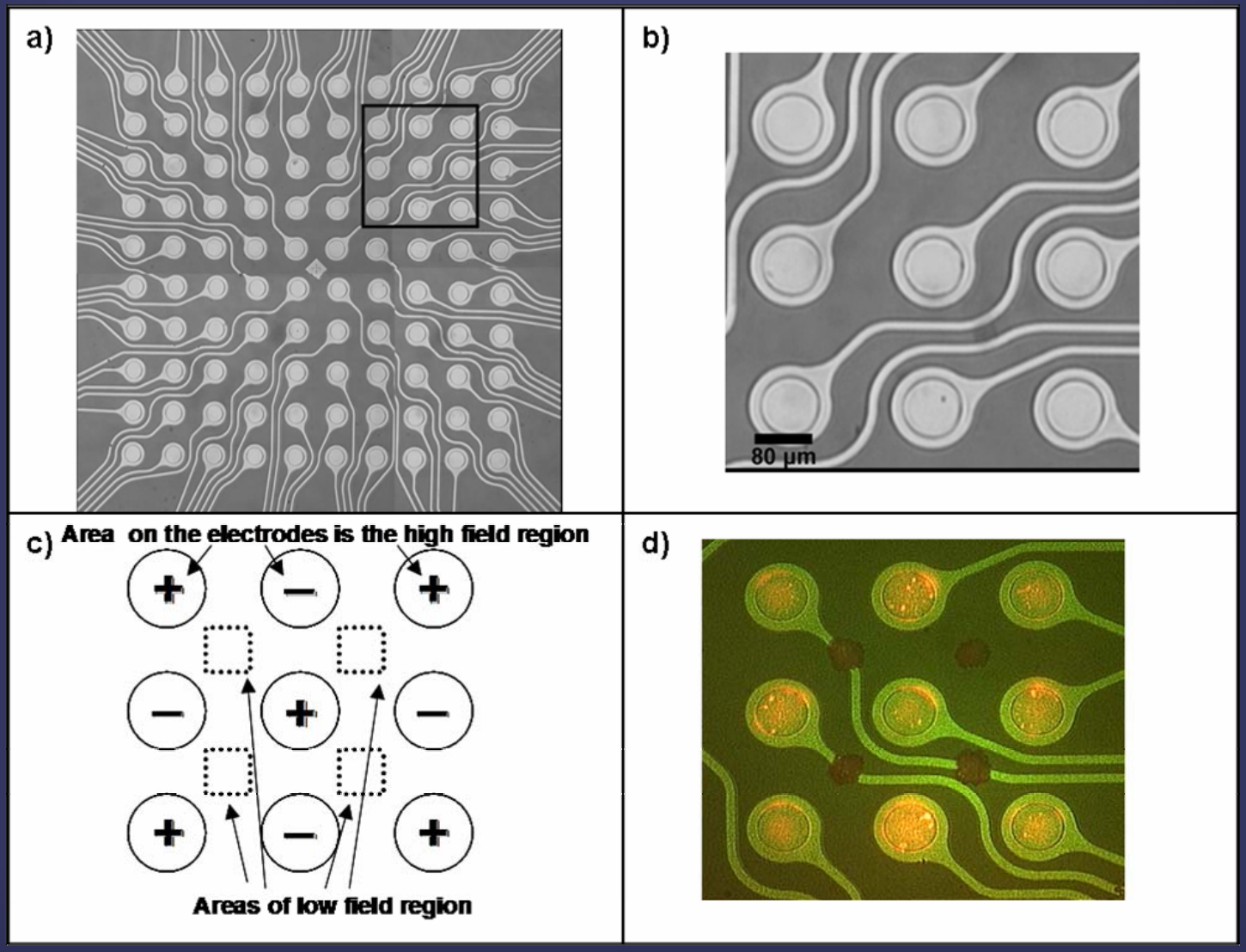
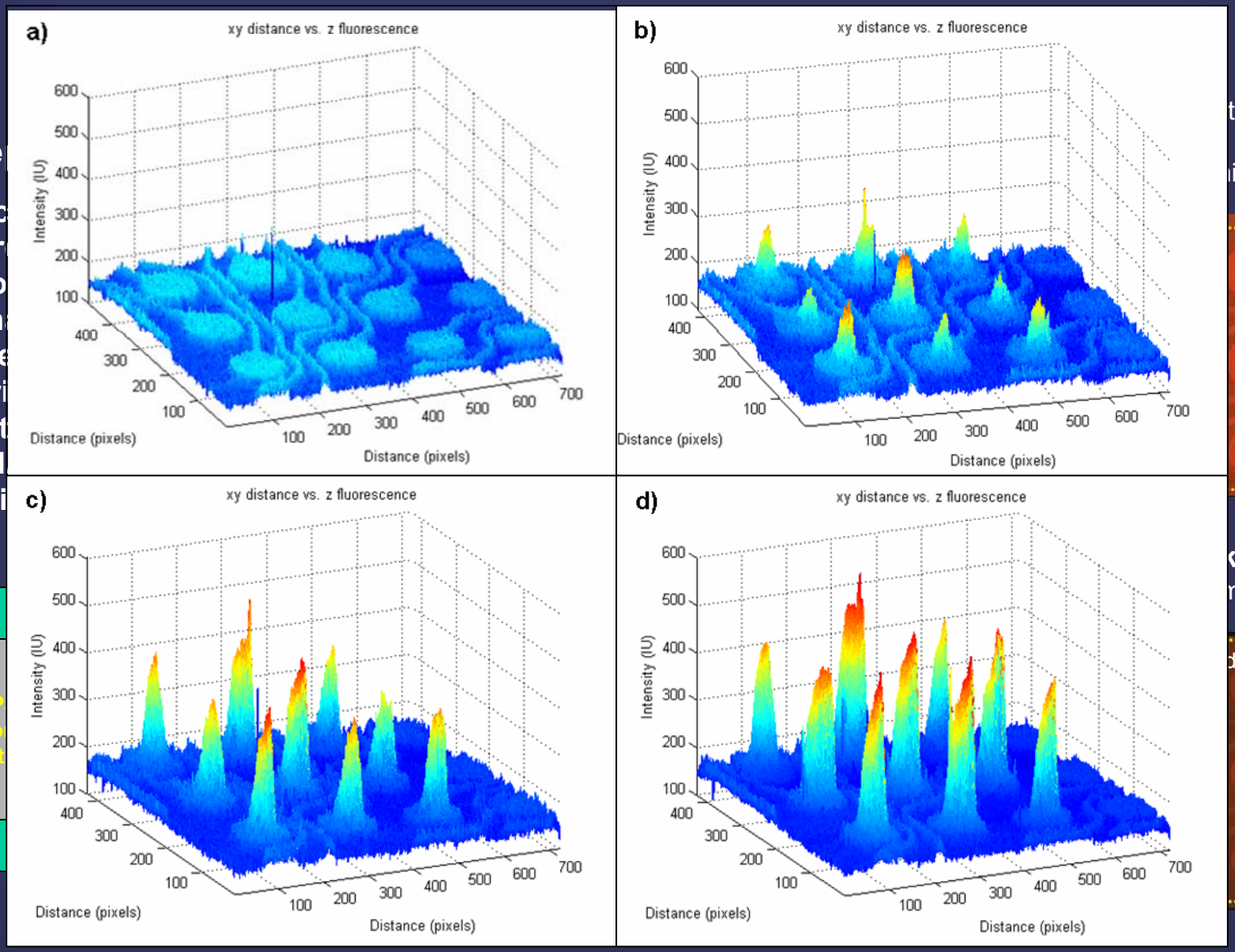
DEP Microarray System Used for Cancer Cell, DNA BioMarkers and Nanoparticle Experiments



Separation of 60nm DNA Nanoparticles and 10 Micron Particles



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nanopart
hMW-DN
drug deli

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ission)

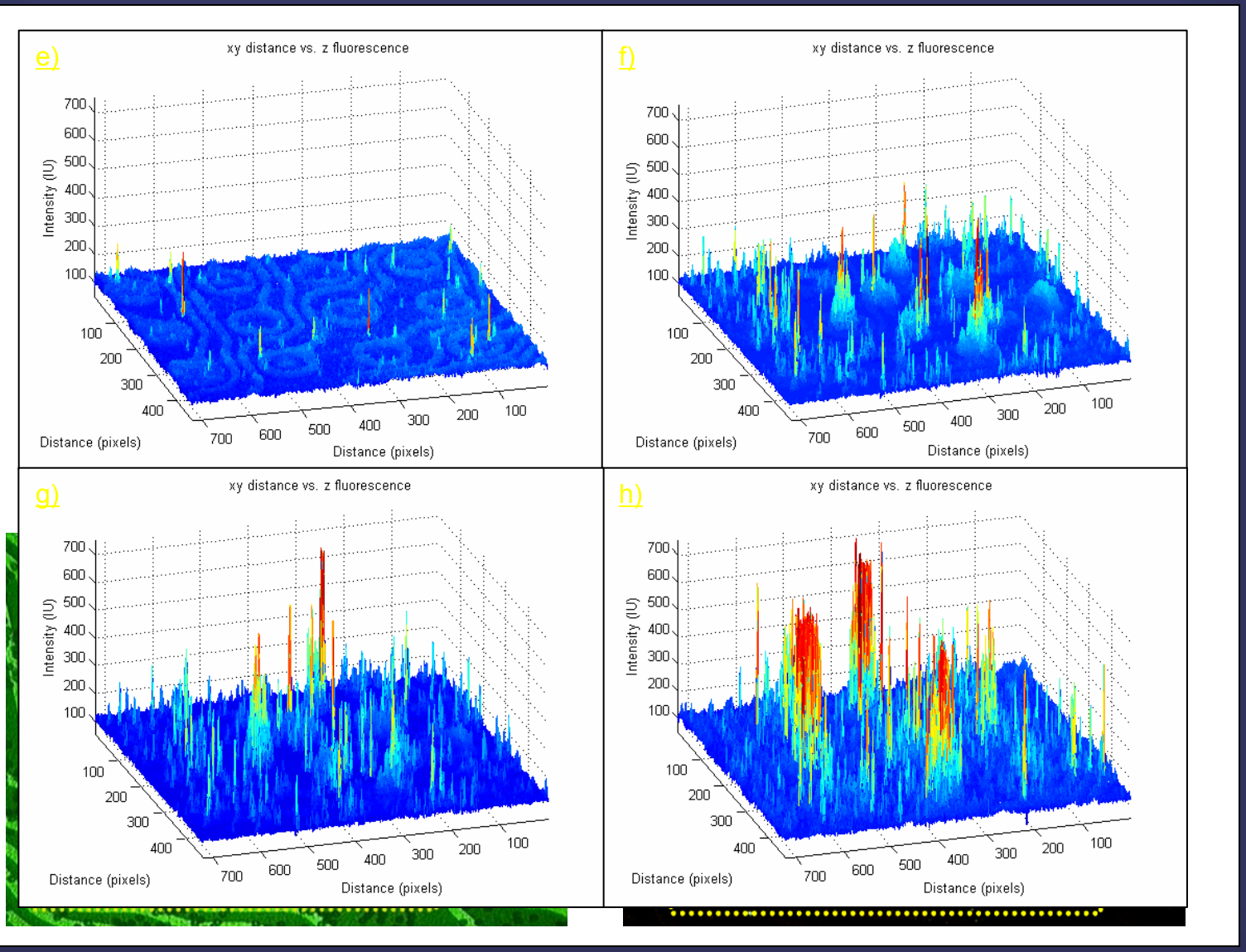
kHz/10 V
nission)

d fluorescent
nanoparticles

10 µm P
o
Jurkat

19

Separation of 200 nm Nanoparticles and 10 Micron Particles in High Conductance Solutions (1XPBS)



Separation of 60nm/200 nm Nanoparticles and 10 Micron Particles in High Conductance Solutions

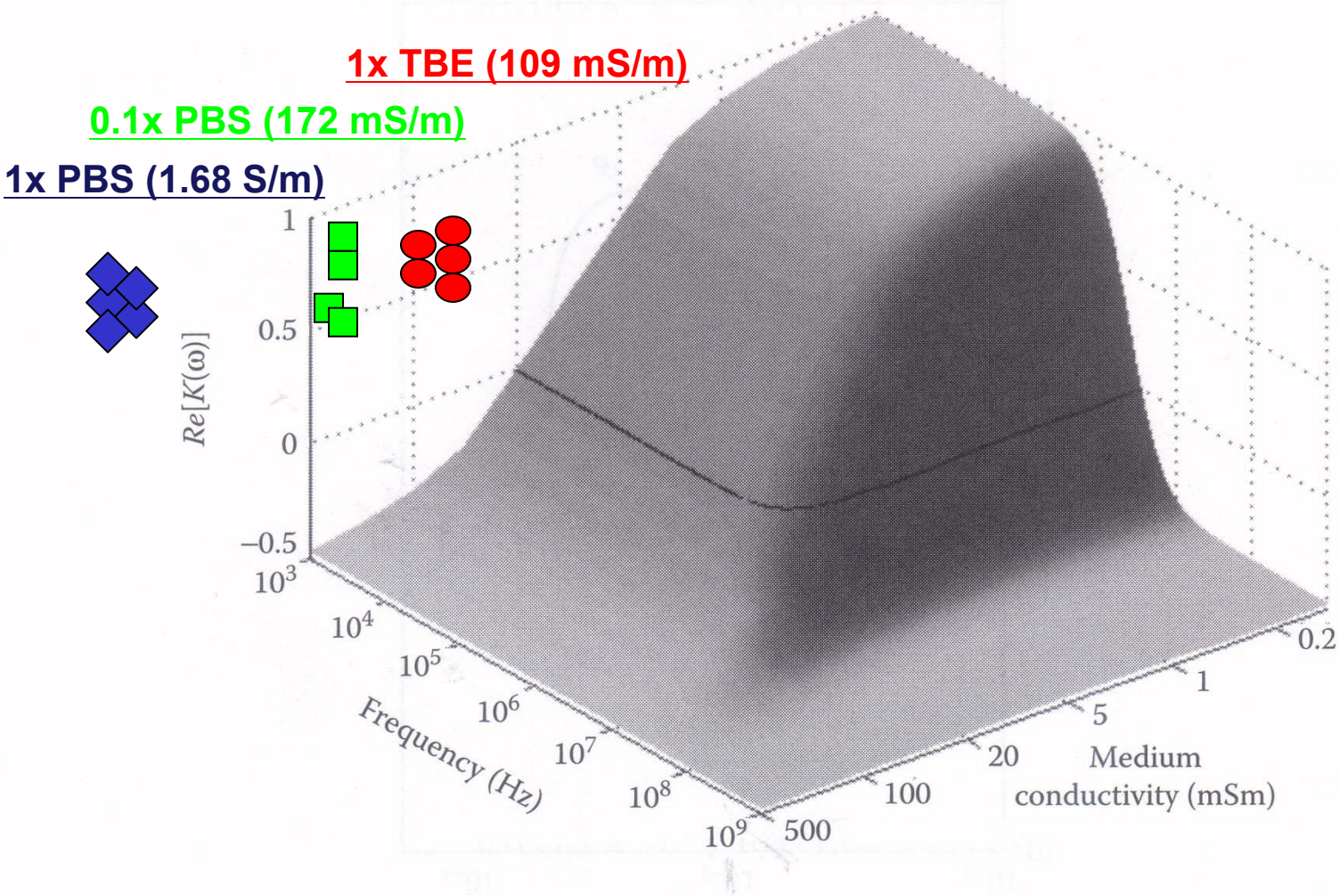


FIGURE 16.6 The data presented in Figure 16.3 plotted with conductivity on a third axis. The combinations of frequency and conductivity where $\mathrm{Re}[K(\omega)] = 0$ form a distinct shape, indicated by the black line.

Electrophoresis 2008, *29*, 0000–0000

Fast Track

Rajaram Krishnan
Benjamin D. Sullivan
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Alternating current electrokinetic separation and detection of DNA nanoparticles in high-conductance solutions

In biomedical research and diagnostics, it is a significant challenge to directly isolate and identify rare cells and potential biomarkers in blood, plasma and other clinical samples. Additionally, the advent of bionanotechnology is leading to numerous drug delivery approaches that involve encapsulation of drugs and imaging agents within nanoparticles, which now will also have to be identified and separated from blood and plasma. Alternating current (AC) electrokinetic techniques such as dielectrophoresis (DEP) offer a particularly attractive mechanism for the separation of cells and nanoparticles. Unfortunately, present DEP techniques require the dilution of blood/plasma, thus making the technology less suitable for clinical sample preparation. Using array devices with microelectrodes overcoated with porous hydrogel layers, AC electric field conditions have been found which allow the separation of DNA nanoparticles to be achieved under high-conductance (ionic strength) conditions. At AC frequencies in the 3000 Hz to 10 000 Hz range and 10 volts peak-to-peak, the separation of 10-μm polystyrene particles into low field regions, and 60-nm DNA-derivatized nanoparticles and 200-nm nanoparticles into high-field regions was carried out in 149 mM 1× PBS buffer (1.68 S/m). These results may allow AC electrokinetic systems to be developed that can be used with clinically relevant samples under physiological conditions.

Keywords:
Alternating current electrokinetics / Dielectrophoresis / DNA / High-conductance solution / Nanoparticles
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1 Introduction

In clinical diagnostics and many areas of biomedical research it is both important and frequently a challenge to separate and identify rare cells (cancer), low numbers of bacteria and virus, low concentrations of DNA biomarkers, antibodies and other entities in complex samples like blood [1], plasma [2], serum [3], saliva [4] and urine [5]. Additionally, the advent of bionanotechnology is leading to numerous drug delivery approaches that involve encapsulation of drugs and imaging agents within nanovesicles and nanoparticles [6]. Thus, it will now also be important to identify and monitor residual nanovesicles and nanoparticles that remain in the blood.

A variety of physical, electronic and biological methods and techniques can be used for the isolation of cells, biomarkers and nanoparticles from complex samples like blood. These include centrifugation, gel filtration, affinity binding, magnetic beads, electrophoresis, flow cytometry and various combinations thereof incorporated into lab-on-a-chip, microfluidic devices and sample to answer systems [7, 8]. Nevertheless, many of these techniques remain relatively time-consuming processes that are not without problems and limitations. Alternating current electrokinetic techniques which involve the use of alternating current (AC) fields to manipulate particles offer a particularly attractive mechanism for rapid separation and analysis of cells [9–11], biomarkers such as cell-free circulating high-molecular weight DNA [12–15] and proteins [16], and ultimately drug

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Abbreviations: AC, alternating current; DEP, dielectrophoresis; pk-pk, peak to peak

Summary

New DEP Theory/Concepts

New DEP Parameters

New DEP Devices

New Loss-Less & Label-Less Applications

- Cancer & Stem Cells
- Drug Delivery Nanoparticles
- hMW DNA and RNA
- Cellular Nanoparticulates
- Bacteria & Virus
- Nanoparticle Assembly

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