

On-Chip Aptamer-Based Sandwich Assay For Thrombin Detection Employing Magnetic Beads And Quantum Dots

Dr. Hutanu Daniela

Diaspora în cercetarea științifică și învățământul superior din România 2010

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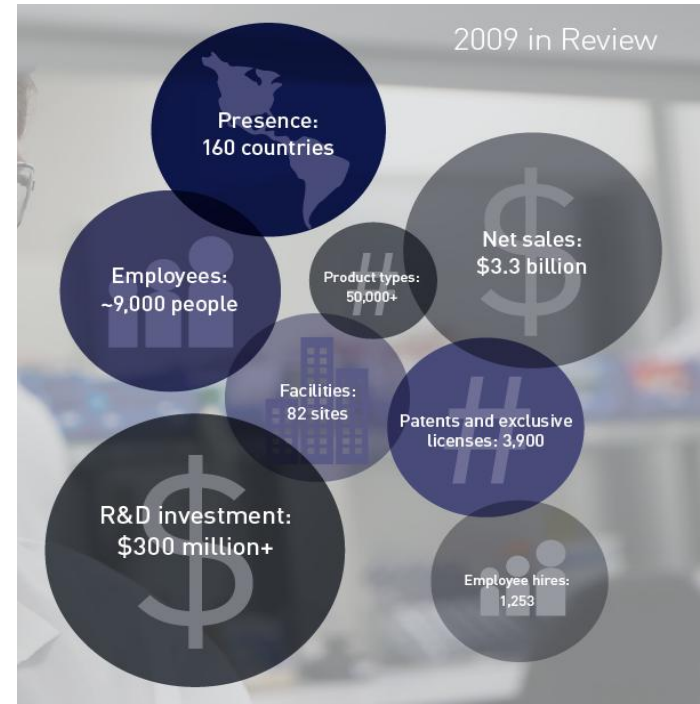
Presentation Overview

- About Life Technologies, and Oregon State University collaborator
- Introduction to the collaborative project
- Properties of reagents and devices
- Assay workflow and results
- Conclusions
- Acknowledgements

About Life Technologies

Life Technologies is a global biotechnology tools company dedicated to improving the human condition. Life Technologies customers do their work across the biological spectrum, working to advance personalized medicine, regenerative science, molecular diagnostics, agricultural and environmental research, and 21st century forensics.

Each year, the company sponsors 20–25 collaboration projects that allow Life Technologies researchers to connect with external thought leaders and forge relationships that last long after the six-month compact ends.



About Academic Collaborator from Oregon State University (OSU)



Vincent T. Remcho

Email: Vincent.Remcho@oregonstate.edu

Oregon State University, Corvallis, OR

Professor of Chemistry and of Materials Science

Adjunct Professor of Biochemistry & Biophysics

Founding member of the Oregon Nanoscience and Microtechnologies Institute (ONAMI)

OSU Oregon State University



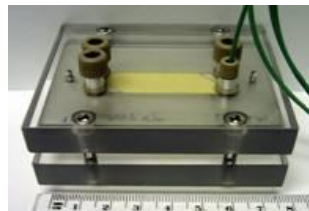
Lab-on-a-chip technology - fabrication and implementation of separations systems in microchip format

Molecular recognition technologies - high selectivity sorbents for separations

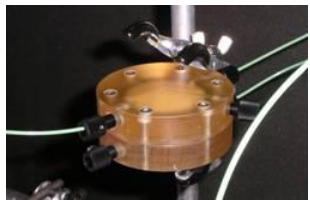
Proteomics - separation and measurement of proteins from complex mixtures

Biothreat analysis

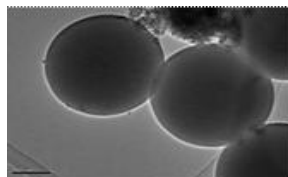
Environmental monitoring



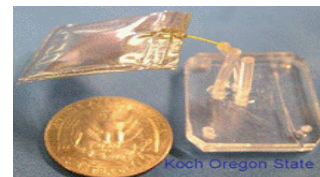
Microfluidic Nanofiltration Device



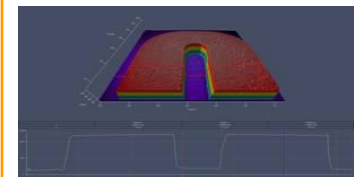
Macroscale Fixture



Functionalized Magnetic Nanoparticles



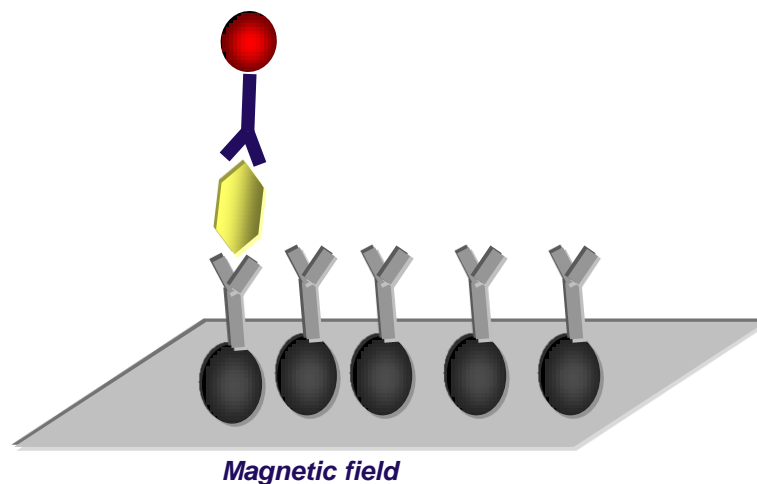
Microfluidics for In-situ Water Quality Monitoring



3-D Image of a Microfluidic Channel

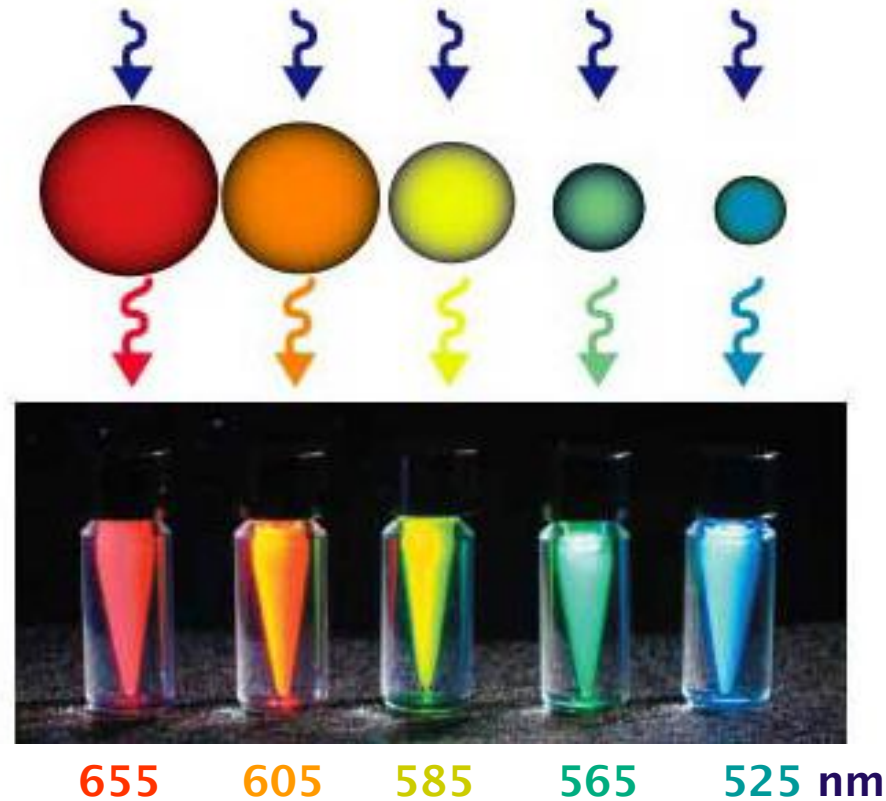
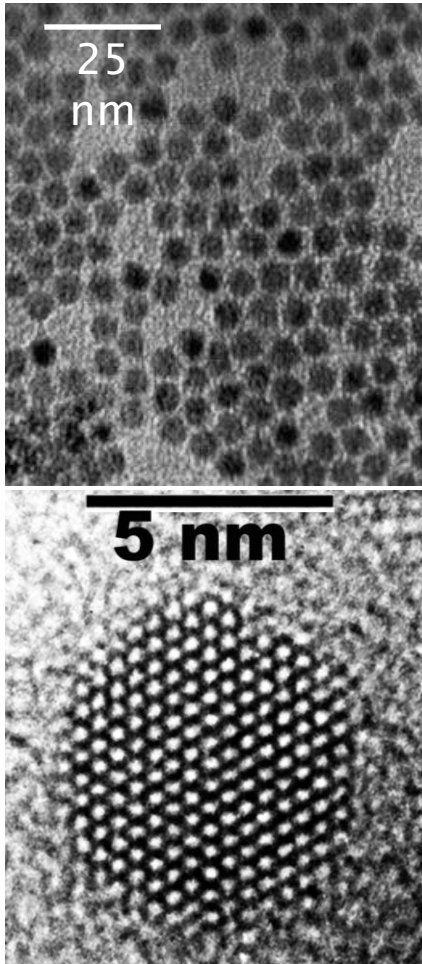
Introduction to the Collaborative Research Compacts (CRC) Project with OSU

- We report the development of an on-chip aptamer-based fluorescence bio-sensor assay for protein detection and quantification based on sandwich ELISA principles.
- **Aptamer-functionalized magnetic beads** were utilized to capture the target analyte (alpha-thrombin), while a second aptamer, functionalized with **quantum dots**, was employed for detection by fluorescence microscopy in **microchip** format.



Why Quantum Dots?

Highly fluorescent, nanometer-sized, single crystals of semiconductor materials.

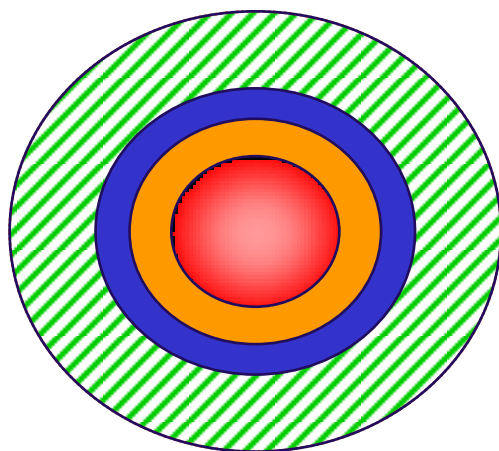


Size of the nanocrystal determines the color.

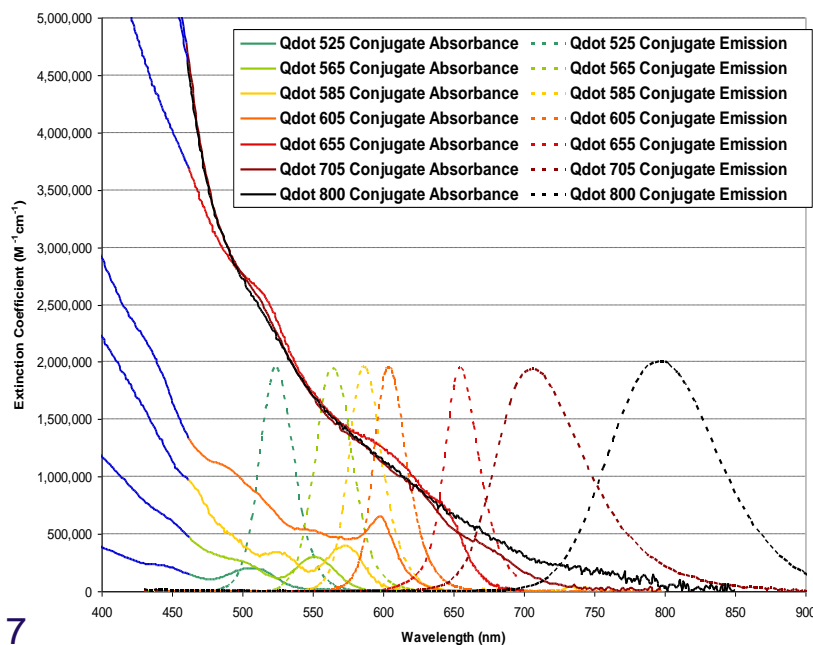
Size is tunable from ~2-15 nm ($\pm 3\%$).

Size distribution determines the spectral width.

Qdot® Nanocrystal Structure and Properties



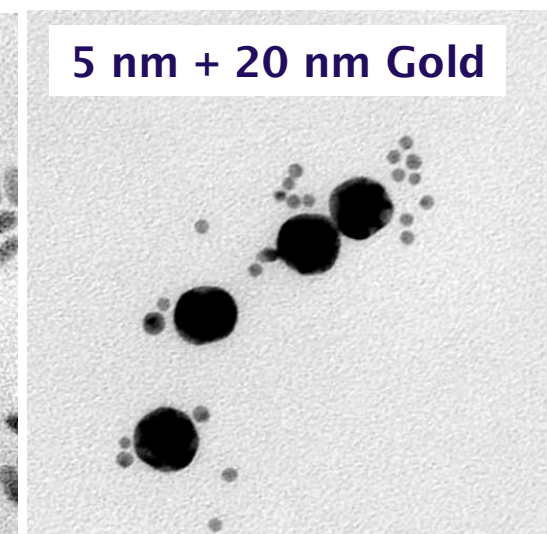
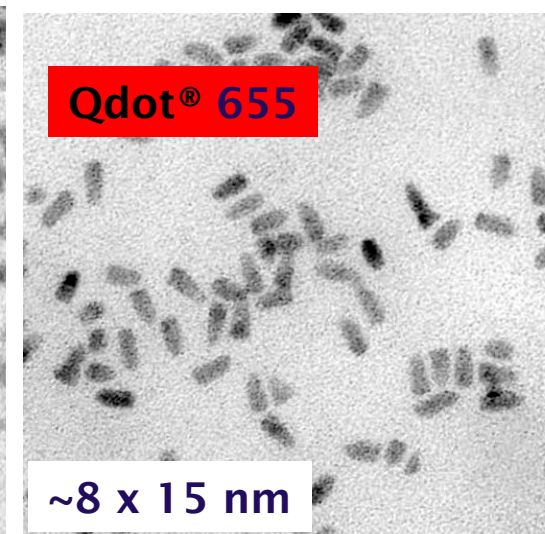
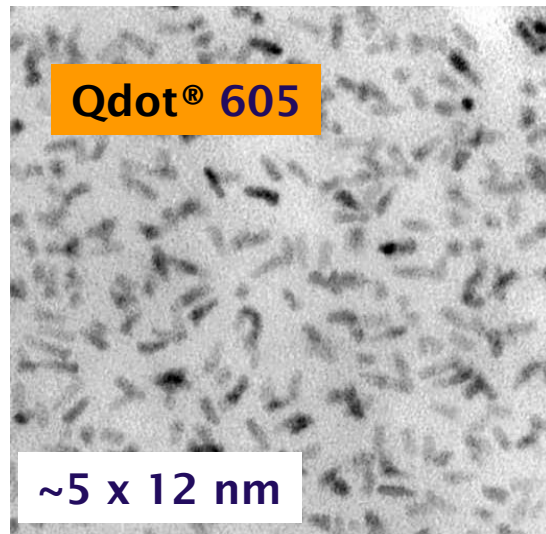
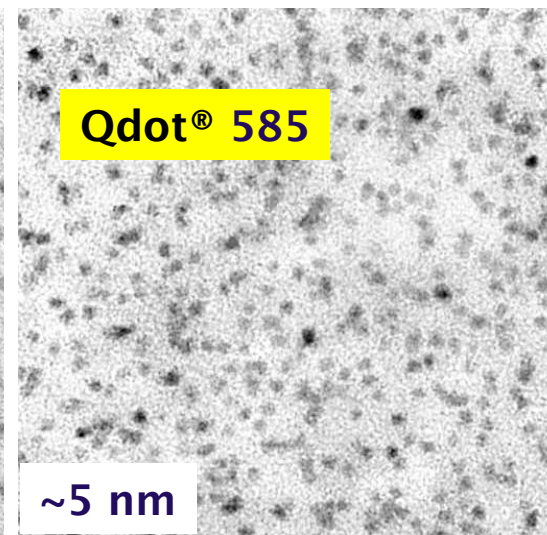
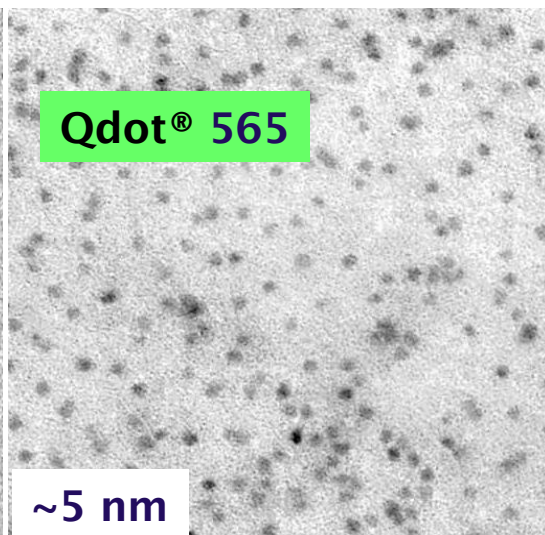
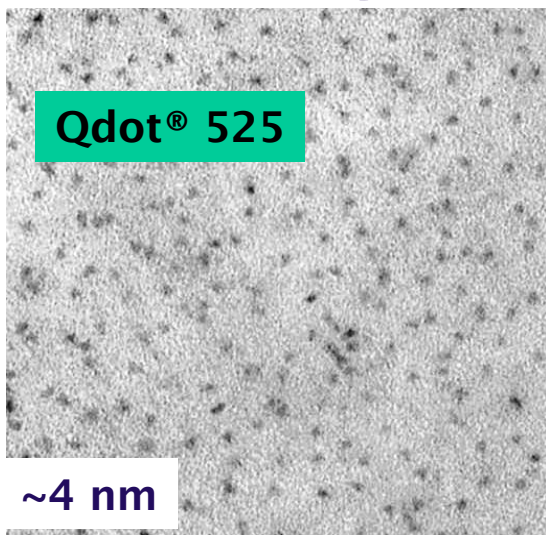
- Core nanocrystal (CdSe)**
 - Determines color
- Inorganic shell (ZnS)**
 - Improves brightness and stability
- Organic coating**
 - Provides water solubility and functional groups for conjugation
- Biomolecule**
 - Covalently attached to polymer shell
 - Immunoglobulins
 - Streptavidin, Protein A
 - Receptor ligands
 - Oligonucleotides



Advantages over fluorescent dyes

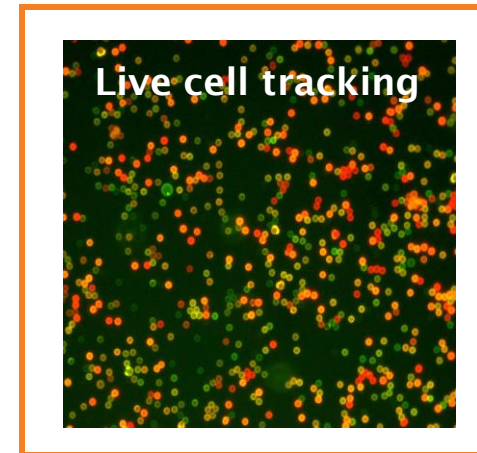
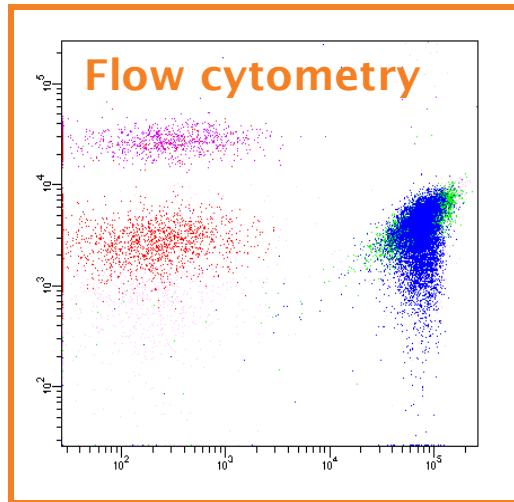
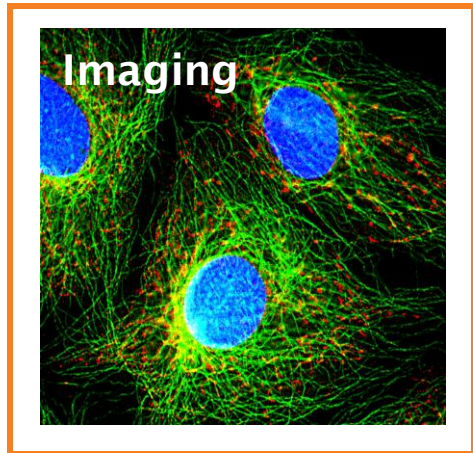
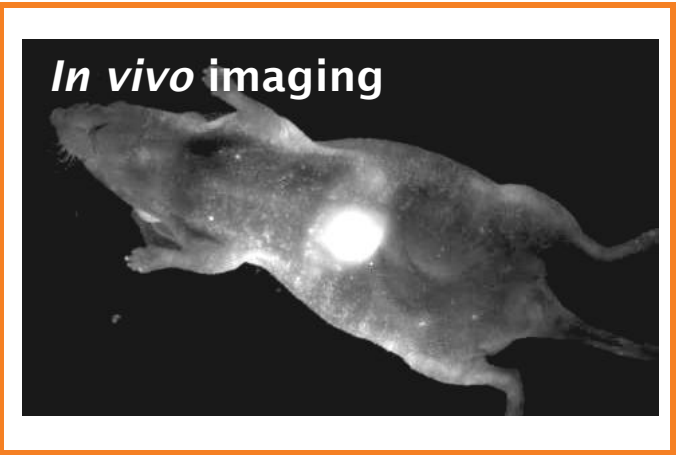
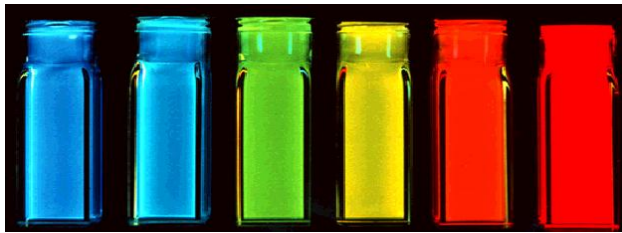
- Single source excitation
- Narrow emission (multiplexing)
- Excellent photostability

TEM Images of Qdot® Nanocrystals

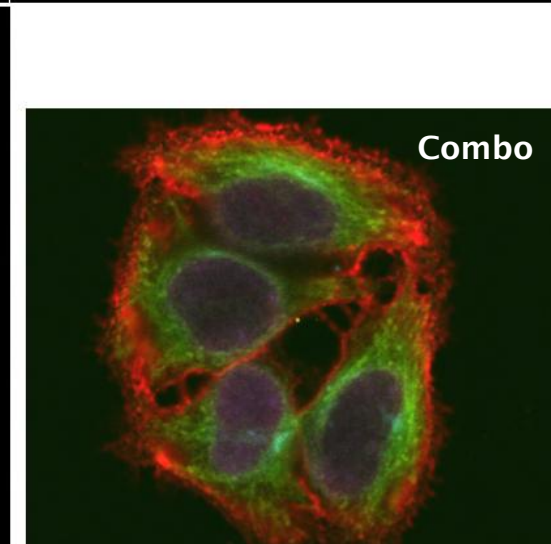
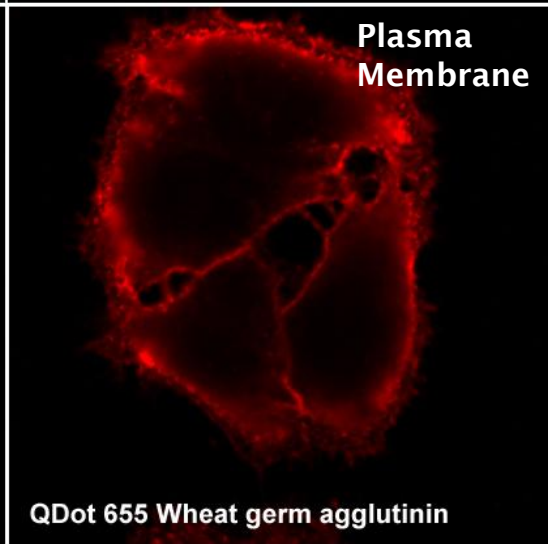
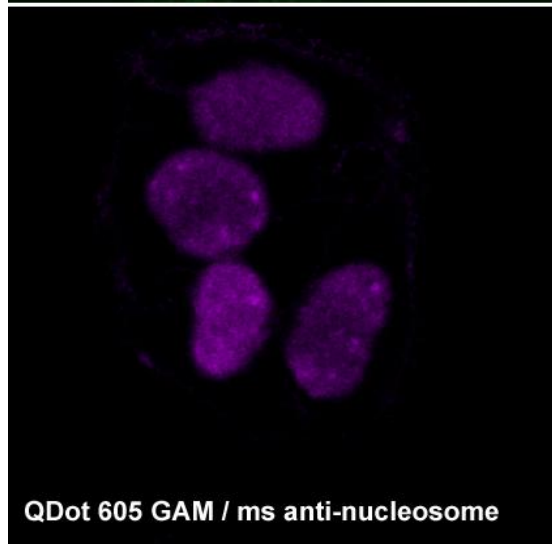
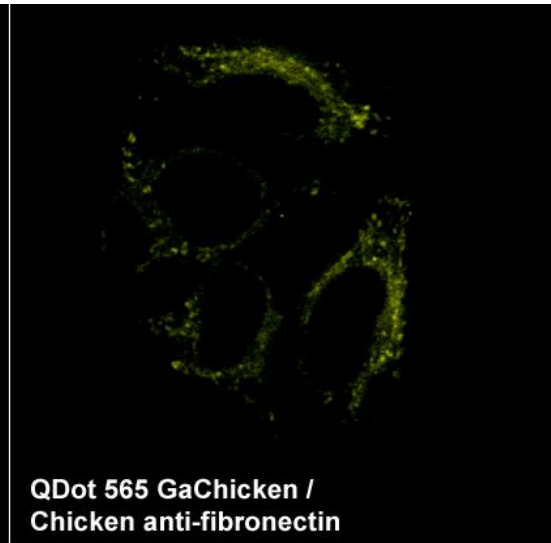
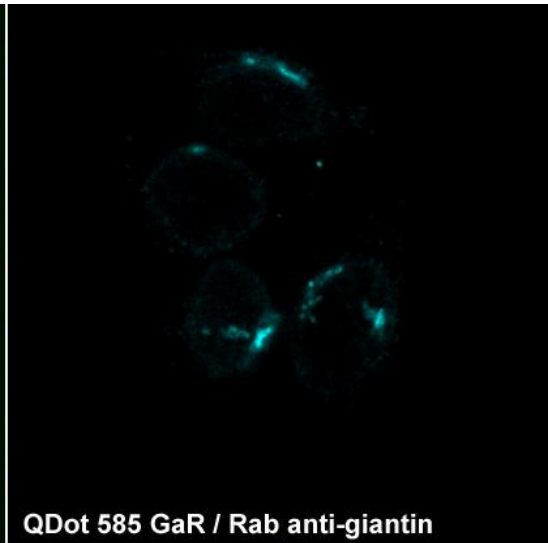
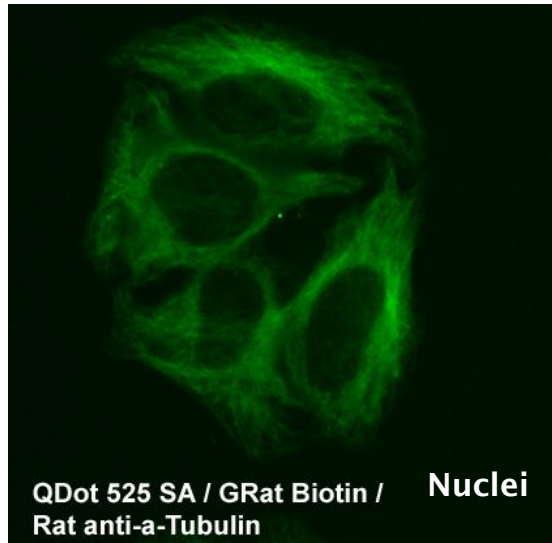


Images provided by Mark Ellisman, National Center for Microscopy and Imaging Research, UCSD, San Diego, CA

Some Applications of Qdot® Nanocrystals



Example of Multiplexed Experiments with Qdot® Nanocrystals



Images provided by Jason Kilgore, Life Technologies.

Why Dynabeads® Magnetic Particles

Dynabeads® magnetic particles are superparamagnetic particles; they exhibit magnetic properties when placed in a magnetic field, with no residual magnetism once removed from the magnetic field.

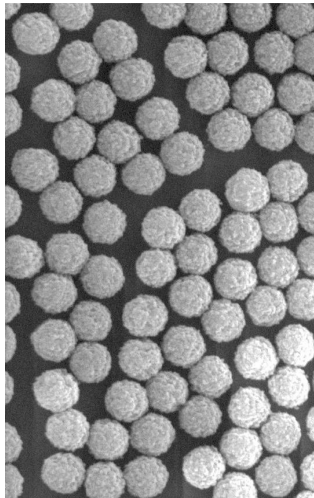
Size: 1 μm , 2.7 μm , 4.5 μm

Magnetic parameters

Binding Capacity

Signal/Noise

Readout Compatibility



Hydrophilicity / Hydrophobicity

Charge: -ve, neutral, +ve

Polarity: δ^- , neutral, δ^+

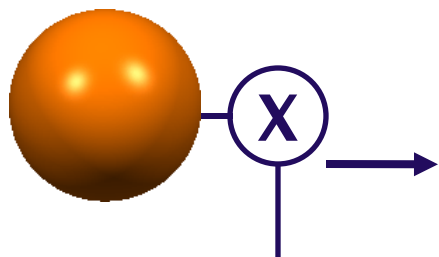
Attachment chemistry

Pre-coupled

e.g; Streptavidin, Antibody, Protein A/G

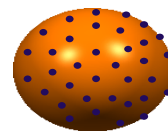


Dynabeads® Magnetic Particles Employed for Isolation

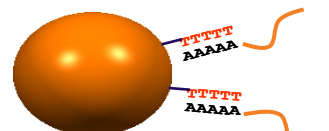


- Surface activated
- Primary Antibody (Ab)
- Secondary Ab
- Protein A/G
- Talon (His-tag)
- **Streptavidin**
- Oligo dT (deoxy-thymine nucleotides)

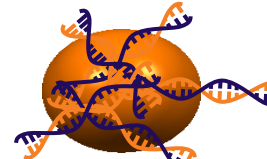
- Small molecules
- Specific nucleic acids
- Total nucleic acids
- Peptides/proteins
- Immunoassay
- Organelles
- Cells



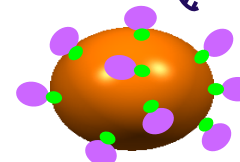
Surface Activated



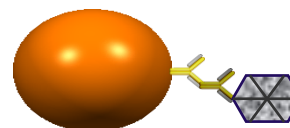
Oligo(dT)



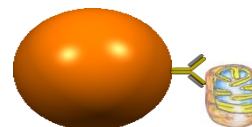
Silane



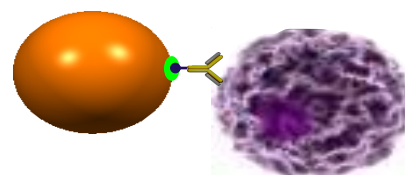
Talon His-tag



Sheep-anti-rabbit



Protein A/G



FlowComp™ (StA)



Aptamers

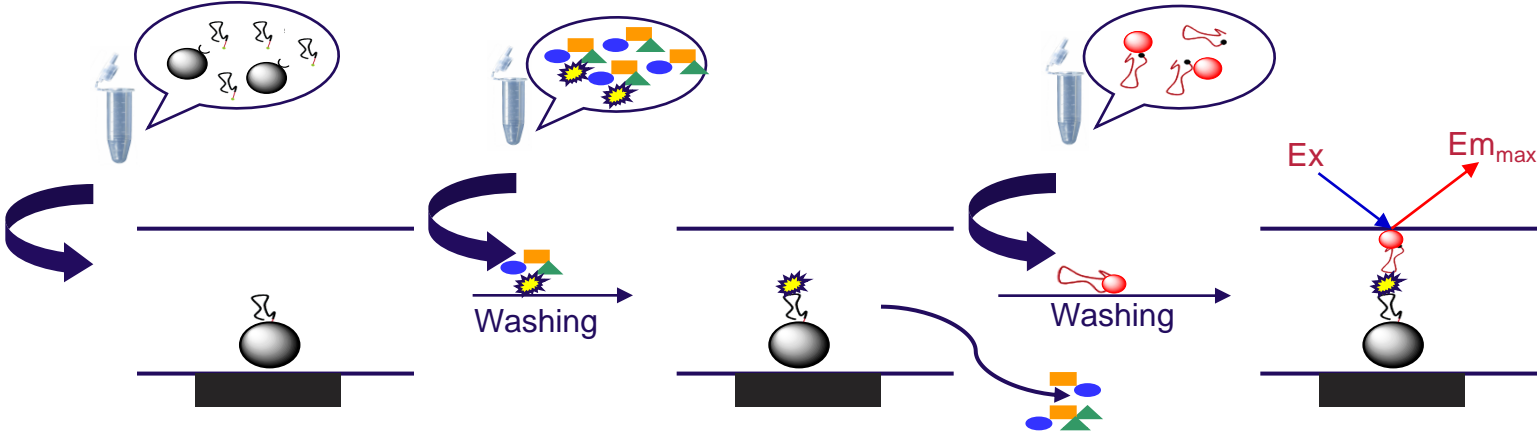
- Single stranded DNA, or RNA molecules
- High specificity, comparable to antibodies
- Relative ease of synthesis & chemical modification
- Tailored binding affinity
- Resistance against denaturation

Microfluidics

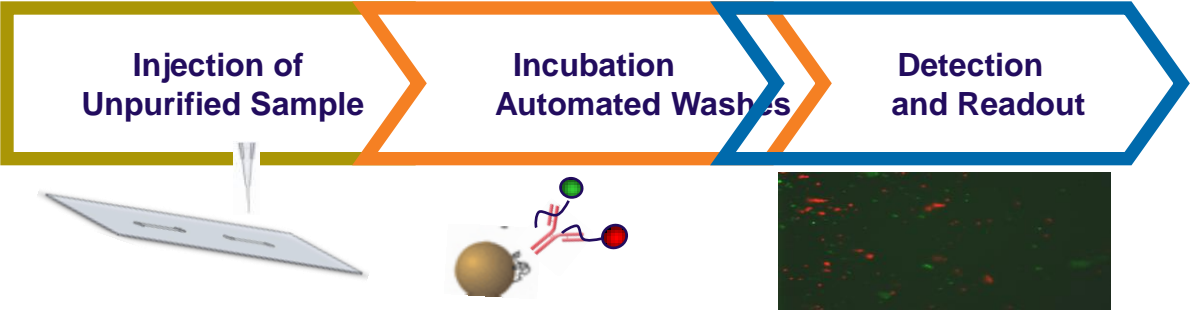
- Small sample and reagent volume (μL)
- Efficient washing in automated continuous flow
- Large surface area-to-volume ratio
- Decreased total analysis time (minutes)
- Inexpensive fabrication of disposable microchips

Hutanu, D., and Remcho, V.T., *Advances in Chromatography*, 2007, 45, pp 173-196.

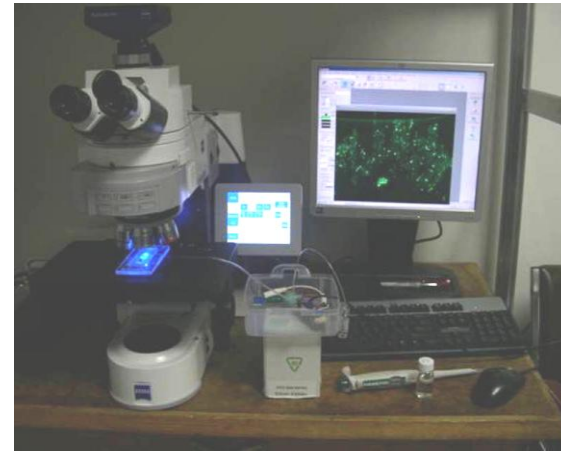
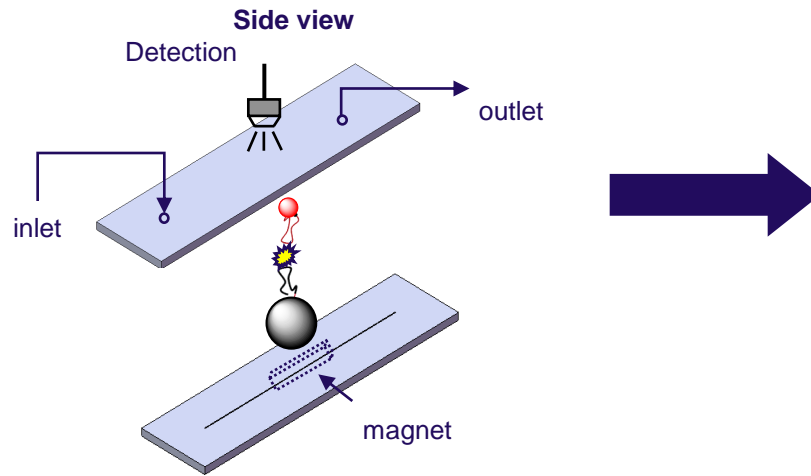
Developed Assay Workflow



	= Streptavidin-coated magnetic beads		= Thrombin
	= Biotin-Aptamer A		= Streptavidin-coated quantum dots
	= Magnet		= Biotin-Aptamer B

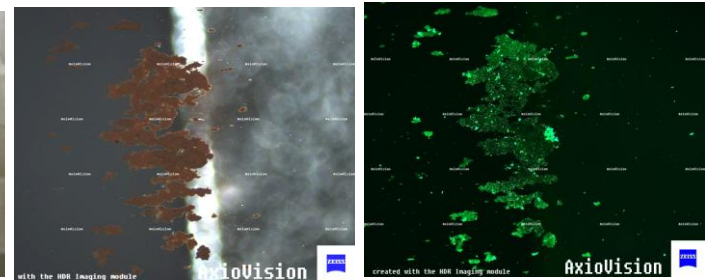
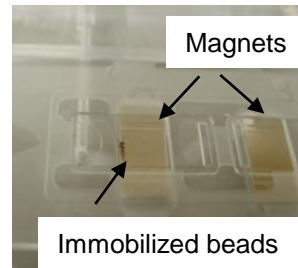


Fluorescence Detection of Thrombin with Developed Assay



Zeiss Axiomager m1M fluorescence microscope

The magnetic beads were trapped by magnets underneath the channel. Thrombin detection was performed on a fluorescence microscope to capture fluorescence images and intensity measurements.

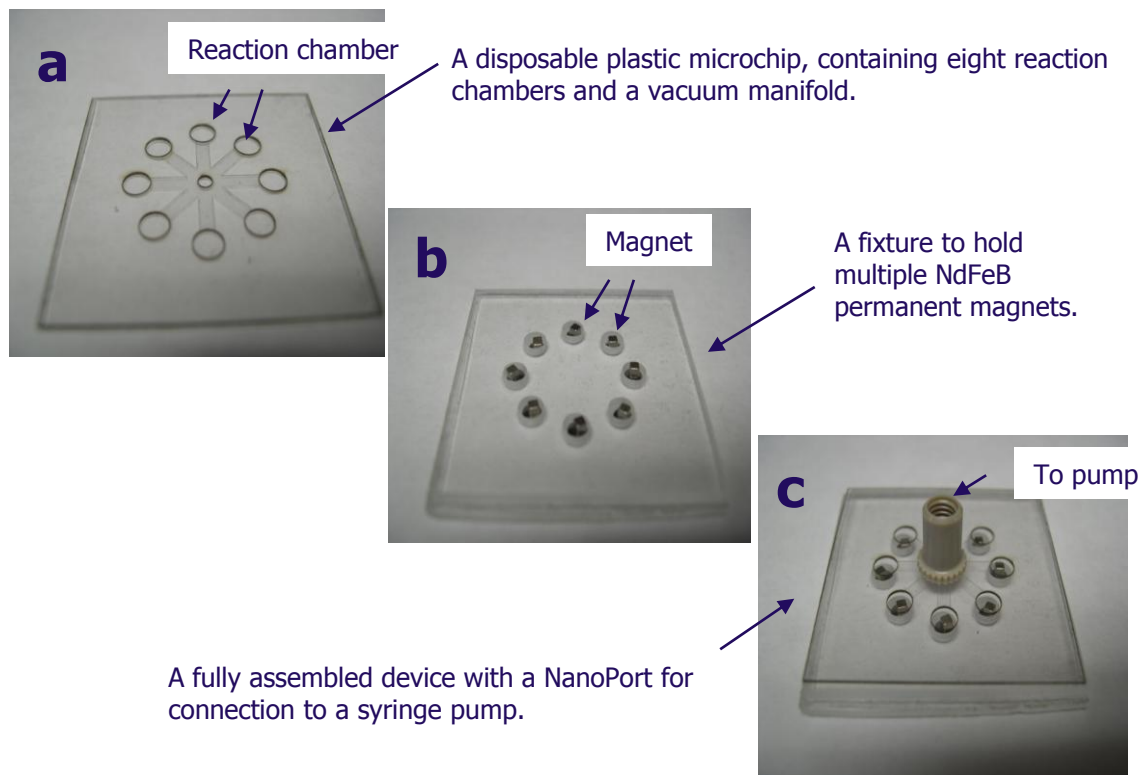


Dark field

Fluorescence

Qdot® 525 immobilized on 1 µm Dynabeads® MyOne™ Streptavidin C1 via aptamer-based sandwich assay

Microchip Design for High-Throughput Thrombin Detection and Quantification



Microchip components:
Top layer, made of polycarbonate (PC) or polymethylmethacrylate (PMMA).

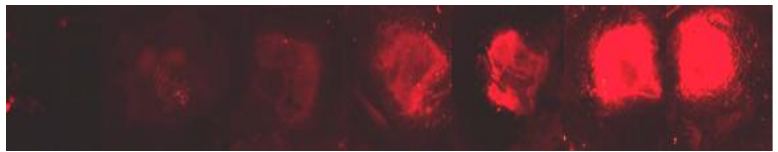
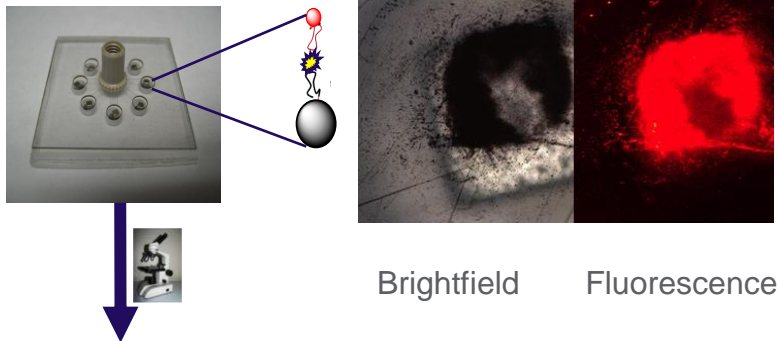
Middle layer, double-sided adhesives, for channel fabrication.

Bottom layer, made of PC or PMMA, as the enclosure.

Channels were cut with laser on 3M Optically Clear Adhesive 8272 double-sided adhesives, and then sealed with plastic polymers on both sides.

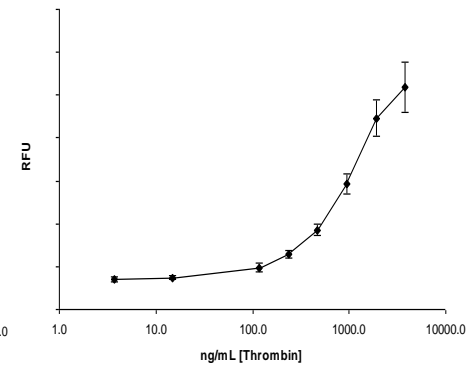
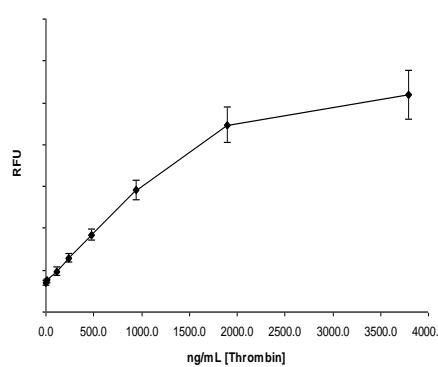
Patterning was done on a 5 watt 355nm ESI UV laser tool designed for micro-machining.

On-Chip Thrombin Detection Results with Developed Assay



Fluorescence images of aptamer-coated beads incubated with increasing concentrations of thrombin (from left to right).

	On-Chip	96 Well-Plate
Total assay time	minutes	hours
Sample volume	μL	mL
Reagent consumption	low	medium
Linear range	100 – 1000 ng/mL	100 – 950 ng/mL
Limit of detection	10 ng/mL	18 ng/mL
Mean standard deviation	8%	14%



On-chip dose-response curve for thrombin.


Conclusions

- Successful application of on-chip aptamer-based sandwich assays, with Qdot® nanocrystals and Dynabeads®, for detection of target proteins of biomedical importance.
- Experimental conditions, such as reagent consumption and incubation time, were optimized in the microchip platform for the lowest limit of detection, highest specificity and shortest assay time.
- The microfluidic chip proved to be a rapid and efficient system for aptamer-based thrombin assays, requiring only minimal (microliter) reagent use.

For Research Use Only. Not intended for any animal or human therapeutic or diagnostic use.

Acknowledgements

- *Funding:* Life Technologies, 2009 Collaborative Research Compacts.
- *CRC Collaborators:* Yolanda Tennico and Cheryl Moody Bartel (Life Technologies, Eugene, OR, USA); Myra Koesdjojo and Vincent Remcho (Oregon State University, Corvallis, OR, USA).
- *Reagents, instrumentation and assay development:* Schuyler Corry, Jason Dallwig, Jim Hirsch, David Wright, Kari Haley, Joe Bartel, Birte Aggeler, Shawn Starkenburg, Dean Tsou, Vanessa Adams, Matt Beaudet, Shula Jaron, Laurel Stone, Ameet Juriani (Life Technologies, USA).



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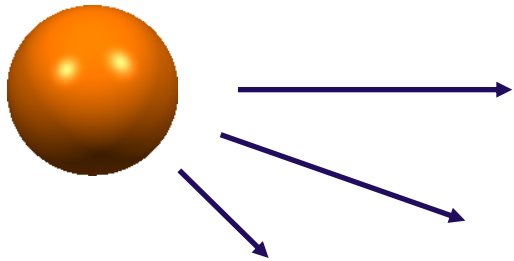
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Extra Slides

Dynabeads® Magnetic Particles Surfaces



Nucleic acids

Oligo dT / dX
(Specific Capture)

SILANE
(Total Capture)

Surface Activated

Tosyl

Epoxy

COOH

Amine

Coated beads

Protein A

Protein G

Sheep α -mouse

Streptavidin
(neutral)

Streptavidin
(neg. charge)

FlowComp Flexi
("detachable biotin-StA")

Invitrogen™ Magnets

DynaMag™-50
(12302D)



DynaMag™-15
(12301D)



DynaMag™-2
(123-21D)

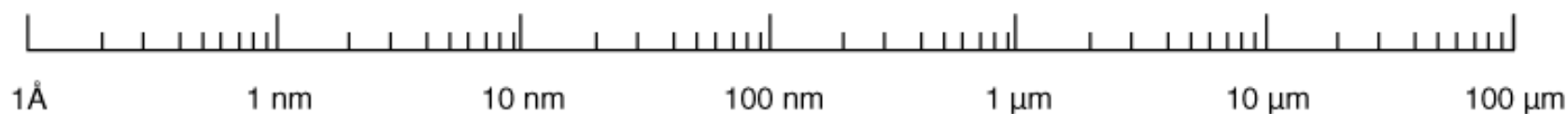


DynaMag™-Spin
(123-20D)

Relative Size of Qdot® Nanocrystals



Qdot® nanocrystal
10 nm–20 nm



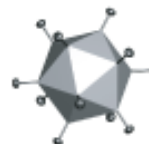
atom
0.05 nm–0.5 nm



small dye
molecule
0.5 nm–10 nm



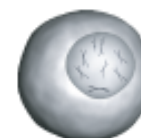
fluorescent protein
10 nm–20 nm




virus
20 nm–400 nm



bacterium
500 nm–10 μm



animal cell
10 μm–100 μm



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Why Dynabeads® Magnetic Particles?

Dynabeads® magnetic particles are superparamagnetic particles; they exhibit magnetic properties when placed in a magnetic field, with no residual magnetism once removed from the magnetic field.



Dynabeads (MyOne)®

