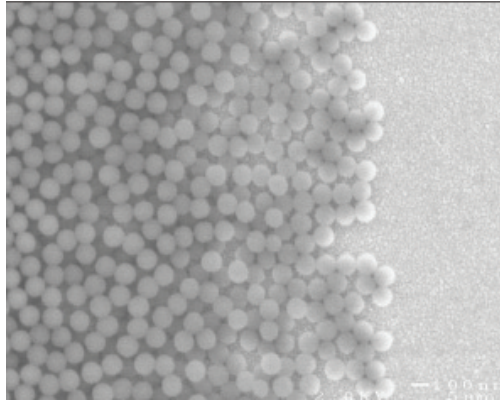# Microsphere Characteristics for Rapid Test Development

Presented by Mary Meza
at Diagnostic Support 2001

Chicago, IL
April 24-25, 2001

Polymeric Microspheres

- Established technology

- Large scale of manufacture

- Broad range of possibilities

2

Choosing Microspheres

- Color
    - Red, dark blue, and black most common
    - Can combine colors

- Size
    - Good flow rates with diameters $\leq$ 1/10 the membrane pore size
    - Capture bead $\geq$ 1/3 the pore size

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Choosing Microspheres

- Surface properties
    - Polystyrene for adsorption
    - Surface functional groups for covalent attachment
    - Binding proteins

- Special properties
    - Absorbance of specific wavelength
    - Fluorescence
    - Magnetic
    - Density, refractive index, etc.

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Coupling-Chemical Groups on Ligands

- Carboxyl from C-terminal amino acids, aspartic & glutamic acids

- Amino from N-terminal amino acids, lysine, and amine-terminated oligos

- Sulfhydryl from cysteine

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Coupling-Chemical Groups on Particles

- Carboxylate
- Amine
- Amide
- Hydrazide
- Hydroxyl

- Chloromethyl
- Aldehyde
- Epoxy
- Others

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Gold Colloid vs. Polymer Beads

- Colloidal Gold
  - Intense red color
  - 4-50nm
  - Adsorption or disulfide coupling
  - 19 g/cm^3
  - Color change / fading

- Microspheres
  - Any color / shade / fluorescent / magnetic / etc.
  - 20nm - 200µm
  - Adsorption / covalent binding
  - ~1.05 g/cm^3
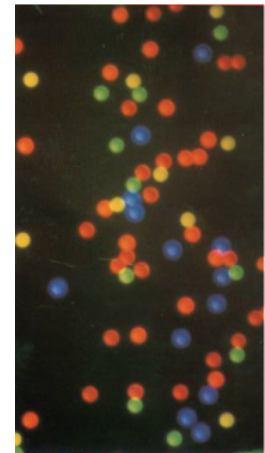 - Stable color

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Quantitative Technologies

- Reflectance / transmission
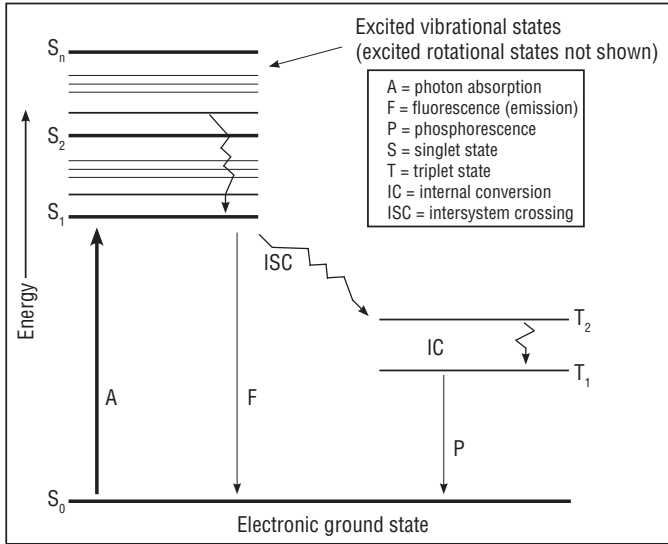- Fluorescence
- Proximity
- Magnetic properties

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Quantitative Technologies - Reflectance / Transmittance

- Reflectance
  - 10µm read depth

- Transmittance
  - read through membrane
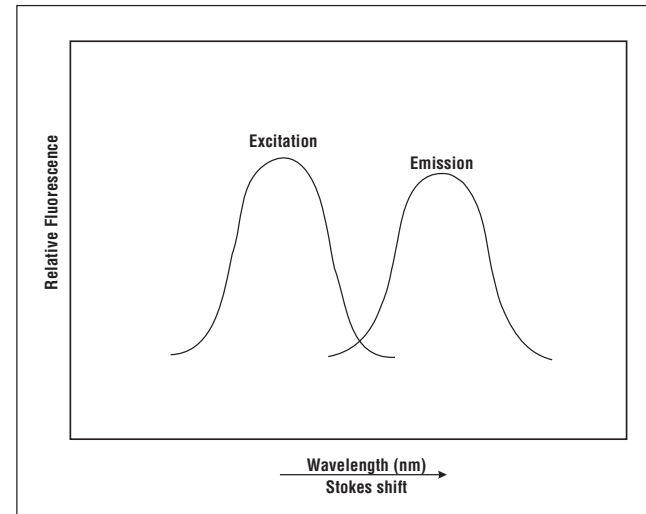
- Currently most common type
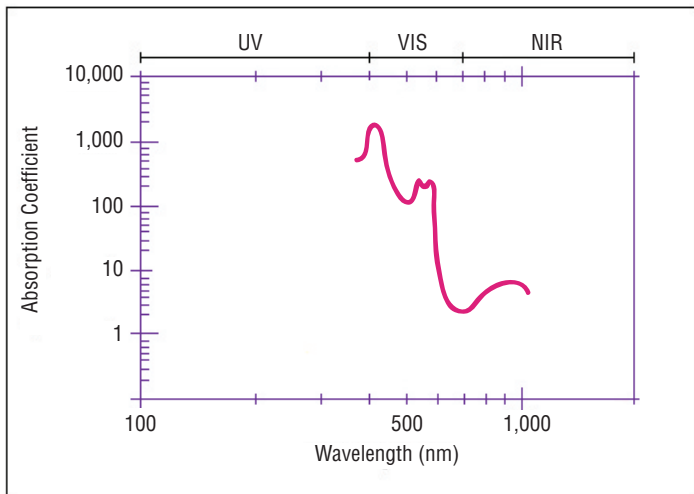

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Quantitative Technologies - Fluorescence

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Quantitative Technologies - Fluorescence

- λ max em - λ max ex = Stokes shift
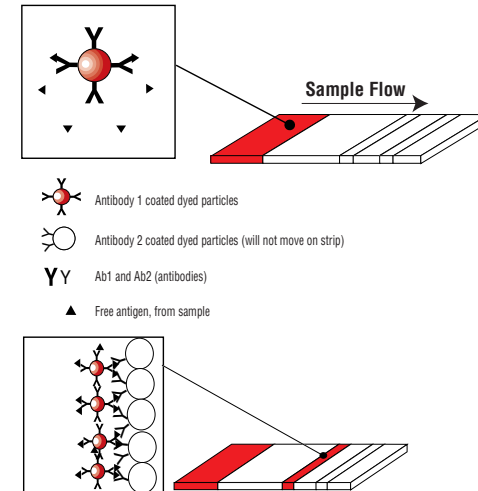
- Larger shifts are more desirable

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Quantitative Technologies - Fluorescence

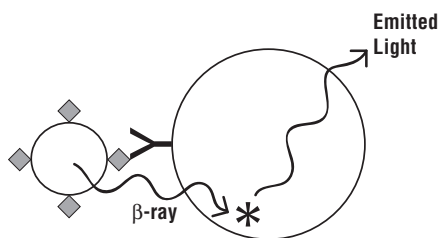

Absorbance of Whole Blood

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Quantitative Technologies - Proximity Assays

"Boulders-in-a-Stream" Configuration

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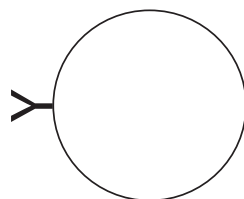# Quantitative Technologies - Proximity Assays

Assays

Scintillation Proximity Assays (SPA): If Ag/Ab reaction binds particles together, light will be given off when β-rays emitted from Ag-coated microspheres enter Ab-coated, scintillator-dyed microspheres. Free Ag in sample interferes with the two microspheres coming together and decreases light output. (Amersham)

Ag-Coated, Radiolabeled Microspheres

Ab-Coated, Scintillator-Dyed Microspheres

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# Quantitative Technologies - Proximity Assays

Technology	Particle 1	Particle 2	Signal
Scintillation Proximity Assay (SPA)	β emitter "dyed" bead	Scintillator dyed beads	Light
Luminescent Oxygen Channeling Immunoassay (LOCI)	Singlet oxygen donor	Singlet oxygen acceptor	Light
Fluorescence Resonance Energy Transfer (FRET)	Fluorophore donor	Fluorophore acceptor	Fluorescence at λ emission of acceptor
Fluorescence Inhibition	Fluorophore	Quencher	Reduced fluorescence
Up-converting Phosphors	Yb^{3+} absorber	ER^{3+} emitter	Fluorescence at increased λ

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Quantitative Technologies - Magnetic

- Quantum design
  - Measures local magnetic field expressed by the total mass of iron

- Ericomp
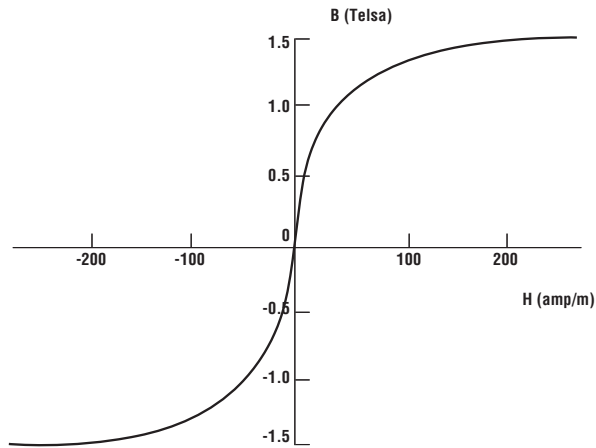 - Measures residual magnetism of magnetic beads

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Quantitative Technologies - Magnetic Hysteresis

- Ferromagnetic hysteresis typical of iron oxides

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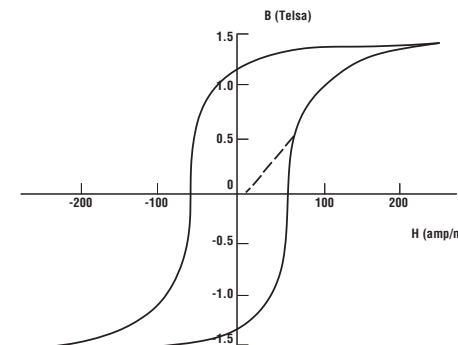
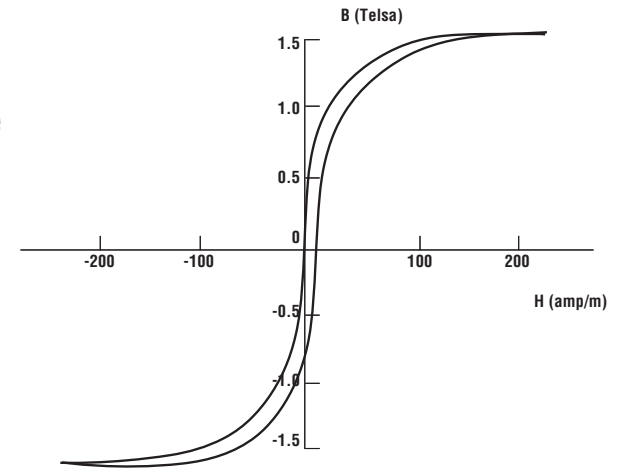## Quantitative Technologies - Magnetic Hysteresis

- Anhysteresis - no residual magnetism

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Quantitative Technologies - Magnetic Hysteresis

- Hysteresis curve typical of commercial paramagnetic beads

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Quantitative Technologies - Magnetic Particle Types

Type	Matrix Material	Functional Groups	Diameter
Synthetic Polymer (encapsulated or not)	Polystyrene, divinylbenzene, polyvinyltoluene, polyester, polyurethane	None, SO_3, COOH, NH_2, Epoxy, OH, CH_2Cl, Toysl, CHO, SH	>0.3-5µm Usually spherical
Silane coated iron oxide	Silica	SiO_2 and derivatives (OH, NH_2, COOH...)	0.5-3µm Spherical and non-spherical
Polysaccharide particles	Dextran	OH and activated derivatives	20-50nm Usually spherical
Polysaccharide / Silane	Crosslinked dextran - silica fortified	OH and activated derivatives	250nm Non-spherical
Polysaccharide particles	Agarose, cellulose	OH and activated derivatives	1-10µm Usually spherical
Protein particles	Gelatin, albumin	NH_2, COOH, SH, and OH	>10µm Usually spherical
Liposome	Phospholipids	None	>10µm Spherical
Chemically activated iron oxide	None	Dimercaptosuccinic acid	5-300nm Non-spherical?

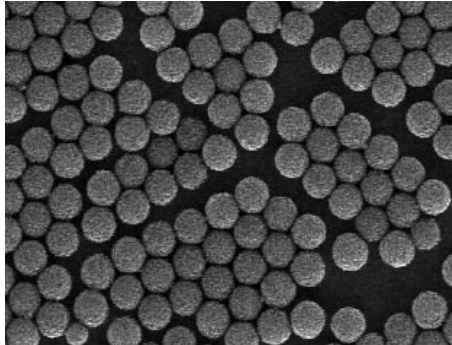## Quantitative Technologies - Magnetic - Estapor® Classical Beads

- Homogeneous Fe_3O_4 distribution

- Large magnetite content

- Broad size distribution
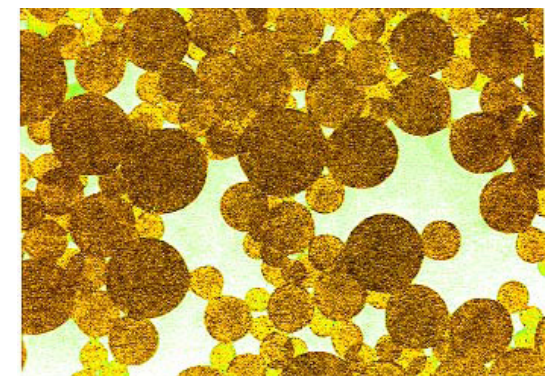
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Quantitative Technologies - Magnetic - Estapor® Classical Beads

- Uniform size distribution

- Predictable surface area and separation

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Summary

- Polymeric microsphere technology is mature and economical.

- Quantitative assays increase technical demands on the label.

- Many labeling options exist within the range of microspheres

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