

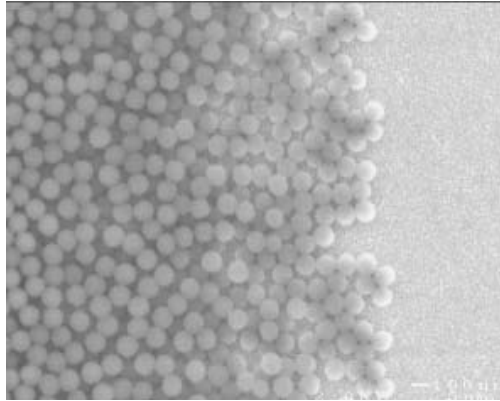
Microsphere Characteristics for Rapid Test Development

**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



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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/cm³
 - Color change / fading
- Microspheres
 - Any color / shade / fluorescent / magnetic / etc.
 - 20nm - 200µm
 - Adsorption / covalent binding
 - ~1.05 g/cm³
 - Stable color

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Quantitative Technologies

- Reflectance / transmission
- Fluorescence
- Proximity
- Magnetic properties

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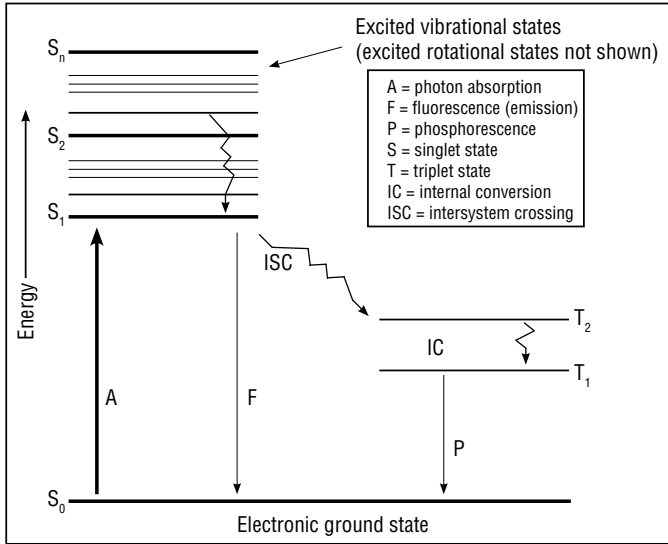
Quantitative Technologies - Reflectance / Transmittance

- Reflectance
 - 10µm read depth
- Transmittance
 - read through membrane
- Currently most common type

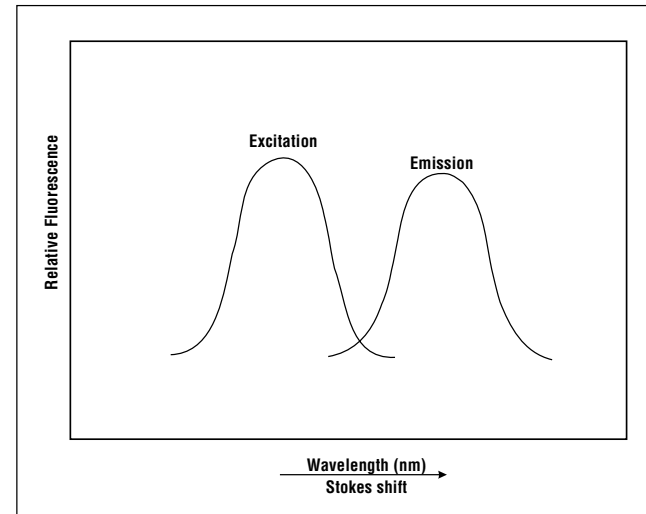


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Quantitative Technologies - Fluorescence

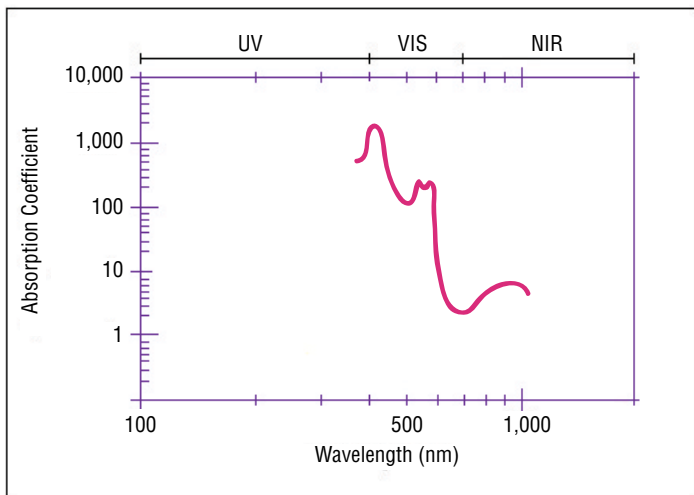


Quantitative Technologies - Fluorescence



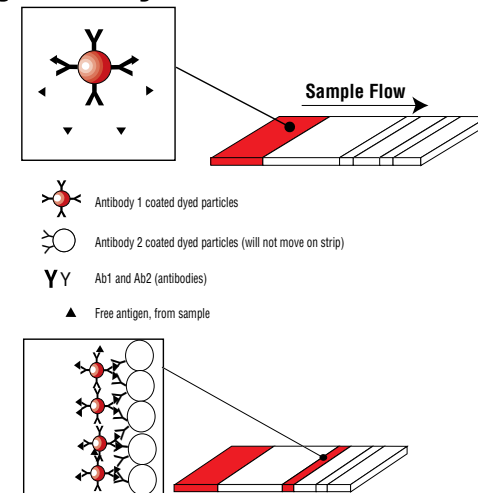
- λ max em - λ max ex = Stokes shift
- Larger shifts are more desirable

Quantitative Technologies - Fluorescence



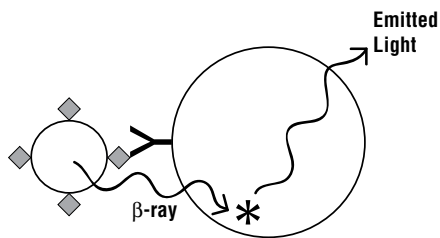
Absorbance of Whole Blood

Quantitative Technologies - Proximity Assays



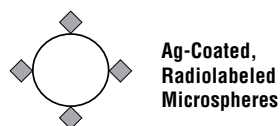
"Boulders-in-a-Stream" Configuration

Quantitative Technologies - Proximity Assays

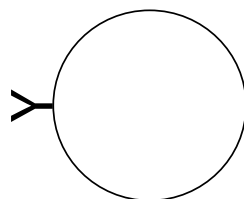


Assays

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

Quantitative Technologies - Proximity Assays

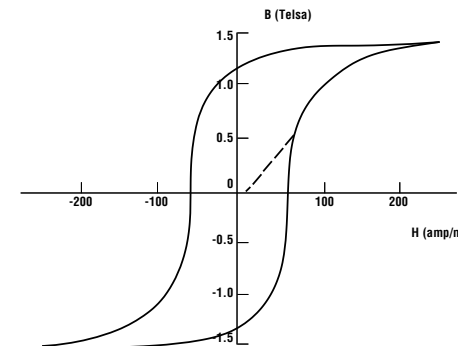
Technology	Particle 1	Particle 2	Signal
Scintillation Proximity Assay (SPA)	β emitter "dyed" bead	Scintillator dyed beads	Light
Luminescent Oxygen Channeling Immunoassay (LOCI)	Singlet oxygen donor	Singlet oxygen acceptor	Light
Fluorescence Resonance Energy Transfer (FRET)	Fluorophore donor	Fluorophore 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

Quantitative Technologies - Magnetic

- Quantum design
 - Measures local magnetic field expressed by the total mass of iron
- Ericomp
 - Measures residual magnetism of magnetic beads

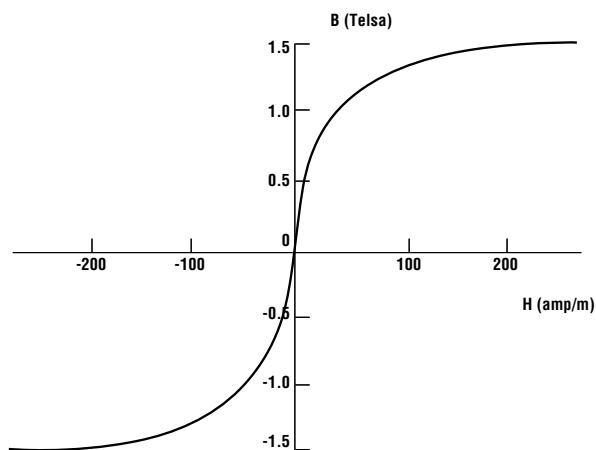
Quantitative Technologies - Magnetic Hysteresis

- Ferromagnetic hysteresis typical of iron oxides



Quantitative Technologies - Magnetic Hysteresis

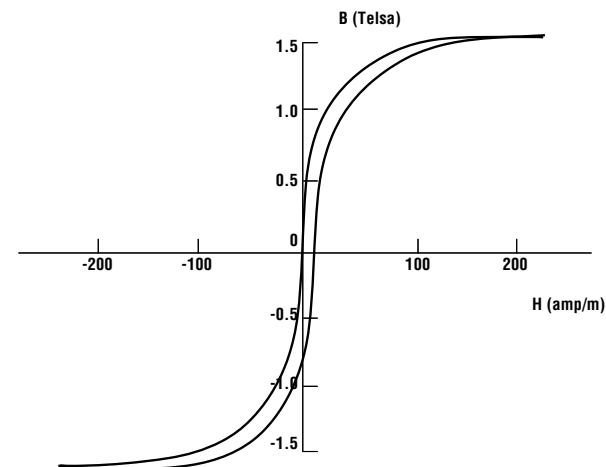
- Anhysteresis - no residual magnetism



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Quantitative Technologies - Magnetic Hysteresis

- Hysteresis curve typical of commercial paramagnetic beads



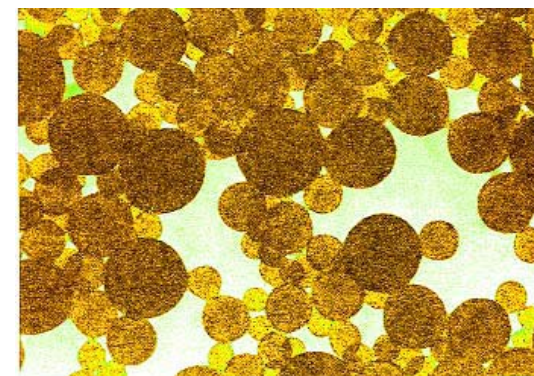
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Quantitative Technologies - Magnetic Particle Types

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 ₂ Cl, 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

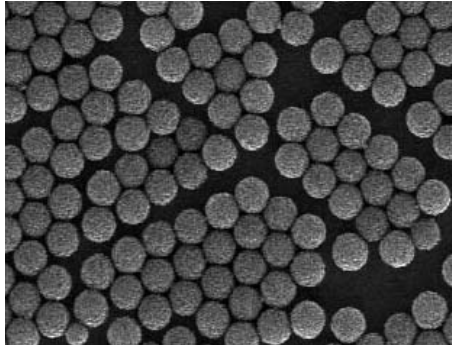
- Homogeneous Fe₃O₄ distribution
- Large magnetite content
- Broad size distribution



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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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