

# Painless Particles®

Quarterly Global Newsletter  
Volume 15, #1, March 2002



A DIVISION OF POLYSCIENCES, INC.

**B E A D S ● A B O V E T H E R E S T™**

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## The Latex Course™ 2002

### "Designing Microsphere-Based Tests and Assays"

**June 10-12, Indianapolis:** We have all the details now and the brochure is being printed. If you can't wait for your printed copy, you can find it at our website: [www.bangslabs.com/2002LatexCourse](http://www.bangslabs.com/2002LatexCourse). And if you *really* can't wait, then here is some essential information.

**When:** Mon.-Wed., June 10-12 (actually starts with a wine/cheese reception Sunday evening)

**Where:** Indianapolis Marriott Hotel (\$129/night through May 10) Tel: 877-640-7666

**Who:** Eleven experts in various aspects of microsphere or latex particle manufacture, reagent preparation, and application. Introducing three new speakers this year. (See bios on website.)

**Why:** Get up-to-speed or get caught up on the latest uses and handling techniques for beads.

**How Much:** \$1375 (\$1275 if paid before 4/27/02) and worth every penny! Includes reception, course dinner at Indy's Eiteljorg Museum, all breakfasts, lunches, and refreshments at breaks.

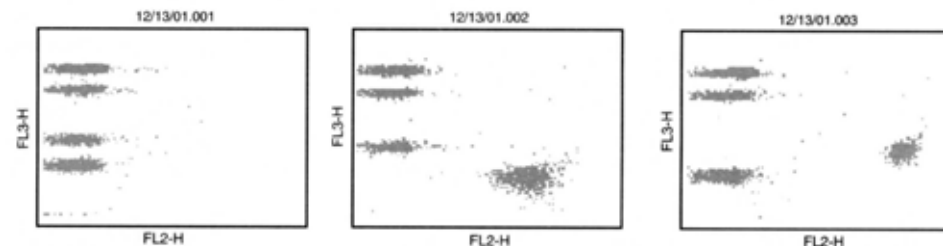
**What:** Twelve lectures plus the >500 page course book (It's heavy!). (See website for lecture titles.) (San Diego, May 2001)

## ISO-9002 Certification



BLI has been certified by TRA Certification as having a Registered Quality System—ISO 9002.

## QuantumPlex™ for Cytokine Analysis



The dot plots above were provided by an investigator using our QuantumPlex beads in a cytokine assay. Samples containing the cytokine of interest are identified by their increased fluorescence response. For more information on QuantumPlex, check out p. Flow-1 of our enclosed Product Selection Guide.

## Looking for Blue Light Specials? (We call them Bangs Bargain Beads!)

See our online list for regular BLI beads (at [www.bangslabs.com/products/bangs/guide.php](http://www.bangslabs.com/products/bangs/guide.php)) for special prices on small quantities of end-of-run, "close-outs", or left-over lots of our microspheres – all sizes, colors, and "flavors" – flow beads, too!

## QC3™ Calibration: 4-Color Standards

This month marks the release of our new four-color QC3 calibration product. The four-color kit consists of two bead populations, each surface-labeled with two fluorochromes: the first labeled with FITC and PE, the second with Cy5 and APC. Surface-labeling allows the beads to exhibit the same excitation and emission properties as stained cell samples, and alerts you to adverse conditions in the system, such as contaminants or changes in pH. The four-color standards are also available with a Certified Blank® population in the popular QC Windows® format.

## "On the Road Again!"

Below is the BLI meeting/show schedule for the first part of 2002. If you will be attending any of these shows, please stop by or booth and meet our fabulous folks, a.k.a., *The Bangs Gang!* (There are more meetings to come.)

- ❖ American Association for Cancer Research 93rd Annual Meeting. April 7-10, San Francisco, CA. Booth 317. <http://www.aacr.org>
- ❖ Experimental Biology 2002. April 20-24, New Orleans, LA. Booth 1442. <http://www.faseb.org/meetings>
- ❖ ISAC XXI International Congress. May 4-9, San Diego, CA. Booth 501. <http://www.isac-net.org>
- ❖ American Society for Microbiology. May 19-23, Salt Lake City, UT. Booth 1710. <http://www.asmsa.org>
- ❖ AACCClinical Lab Expo. July 30-August 1, Orlando, FL. Booth not yet assigned. <http://www.aacc.org/2002am/>
- ❖ Drug Discovery Technology 2002. August 4-9, Boston, MA. Booth 3003. <http://www.drugdisc.com/us/>



"WELL, I WON'T BELIEVE IN AN ANTI-PARTICLE UNTIL I SEE ONE."

## P(articles)<sub>2</sub> = Particle Articles

- ❖ "Silanized Nucleic Acids: a General Platform for DNA Immobilization," Kumar A, Larsson O, Parodi D, Liang Z, *Nucleic Acids Res.*, **28**(14), i-vi (2000). Binding silyl moieties onto oligos or DNA creates silanized nucleic acids which can be instantly bound to glass surfaces – should be useful for binding DNA to silica beads.
- ❖ "Sensitive Detection of *Escherichia coli* O157:H7 in Food and Water by Immunomagnetic Separation and Solid-Phase Laser Cytometry," Pyle BH, Broadway SC, McFeters GA, *Appl. and Environmental Microbiol.*, **65**(5), 1966-1972 (1999). Mag beads coated with Rabbit Ab were used to recover *E. coli* O157:H7 from meat. After staining, the captured bacterial cells were quantified by laser cytometry or fluorescence microscopy. Sensitivity was found to be better than plating. Cool SEM pics of mag beads bound to bugs.
- ❖ "A Latex Bead-Based Flow Cytometric Immunoassay Capable of Simultaneous Typing of Multiple Pneumococcal Serotypes (Multibead Assay)," Park MK, Briles DE, Nahm MN, *Clin. and Diag. Lab. Immunology*, **7**(3), 486-489 (2000). Fifteen latex beads of different sizes and different levels of red fluorescent dyes were coated with different serotypes of pneumococcal capsular polysaccharide to build a flow cytometric assay. The method distinguished cross-reactive serotypes and correctly identified 100% of 86 pneumococcal isolates tested.
- ❖ "Silver-enhanced colloidal gold electrochemical stripping detection of DNA hybridization," Wang J, Polsky R, Xu D, *Langmuir*, **17**(19), 5739-41 (2001). A biotinylated probe was bound to streptavidin (SA)-coated beads from BLI; hybridization occurs between the bead-bound probe and a biotinylated target; SA-coated colloidal gold particle is then bound to biotinylated target; silver is deposited on gold particle and subsequently dissolved and detected for determination of hybridization event.

## Strangely Believe It!

### ❖ Actual Technical Service Telephone Call to BLI on 2/13/02

Caller: "Do you guys have Rhodamine beads?"

Nathan Foushee: "Yes, we have beads dyed with Rhodamine B, Rhodamine 123, tetramethyl-Rhodamine, and Rhodamine WT in several different diameters. What do you need?"

Caller: "Holy s---!" (And then he hung up.)

← **We at Bangs Labs are definitely Pro-Particle and hope you are too! Please ask us to help to supply your "particular" needs – for service or BEADS • ABOVE THE REST™** (Cartoon reprinted with special permission from Sidney Harris <SHarris777@aol.com> and [www.sciencecartoonsplus.com](http://www.sciencecartoonsplus.com).)

## Ask “The Particle Doctor<sup>®</sup>”

### Quenching vs. Photobleaching

**Q** : What is quenching? Is it the same as photobleaching?

**A** : Quenching and photobleaching are two different phenomena. Quenching is the loss of fluorescence intensity due to interference between fluorochromes. It usually occurs when fluorochromes are closer together than 50 angstroms – too many dye molecules per bead. Quenching is a reversible proximity issue. Photobleaching, on the other hand, is an actual alteration or destruction of the fluorochrome by the excitation energy or ambient light – like your favorite shirt that just fades in sunlight. That said, if you can find a way of quenching photobleaching, please let us know!



our website, [www.bangslabs.com](http://www.bangslabs.com).

### Meaning of MESF

**Q** : What is the value in knowing the brightness of a sample in **Molecules of Equivalent Soluble Fluorochrome (MESF)**?

**A** : Equal numbers of fluorochrome molecules do not necessarily have the same brightness. Brightness needs to be corrected for changes in extinction coefficient, quenching and small spectral shifts. MESF units account for most of these environmental corrections. For example, a cell with a very high expression of a given marker may be labeled with 2,000,000 FITC molecules, but due to quenching, may exhibit the fluorescence intensity of only 1,500,000 FITC molecules in solution.



### Streptavidin / Biotin Complex Stability vs. pH

**Q** : What do you know about the stability of the streptavidin/biotin complex vs. pH? After binding of SA/B, I want to do a reaction at acidic pH ~3.5.

**A** : You should be OK. The book **Avidin-Biotin Chemistry: A Handbook** notes: “This complex is not significantly affected by pH values between 2 and 13 nor by concentrations of guanidine HCl up to 8M at neutral pH’s.” This information was originally reported in “Avidin”, Green, N.M., 1975. *Advances in Protein Chemistry*, 29: 85-133. 1975. New York: Academic Press (Eds. C.B. Anfinsen, J. T. Edsall, F.M. Richards).

### Building a Latex Agglutination Test (LAT)

**Q** : How do I make a latex agglutination test for an internal QC check?

**A** : Here is some general information regarding latex agglutination tests: 1) LATs make use of microspheres in the range of 0.2-1.0µm. 2) You can calculate material needs (latex, antibody and antigen) for each test from the following data: a) it takes ~100 latex clumps to judge agglutination, b) each clump must be ~50µm in size to be seen by the eye, c) ~10 bonds are required per microsphere to agglutinate them, and d) sample size can be as small as 10µL. See TechNote 301, *Immunological Applications*, for more details.

When you want to bind IgG antibody to microspheres, there are a variety of options. Included are: direct adsorption or covalent coupling of the antibody to the microsphere. Or, you might want to use beads that are precoated with a generic binding protein - perhaps streptavidin plus a biotinylated IgG of your choosing. A discussion of binding strategies is provided in our TechNote 201, *Working with Microspheres*. TechNotes 101, *ProActive® Microspheres*, 204, *Adsorption to Microspheres*, and 205, *Covalent Coupling*, may also help as you consider the development of a coating protocol. Each contains sample protocols. A complete list of TechNotes can be found and TechNotes can be downloaded from

## Mail Bonding (Subscribers "do the 'write' thing"!)

❖ *"We do indeed like the beads and have already run them through several of our assays. I can't tell you enough about how helpful Nathan Foushee was throughout the whole process. We were in the process of selecting a vendor for our bead solution. Nathan was the only rep who didn't quote the company line and try to make a sale outright. He had the knowledge and patience to learn our application and make an excellent recommendation based on the products you had available. Where we didn't have enough information, he identified the issue and sent a sample for us to try. With that new data, we were able to get exactly the beads we needed. Between that kind of customer service and the protocols available on the website, it shows you, as a company, care more about the researcher and cultivating a long term relationship than making a few dollars on protocols or samples.*

*We are currently in the development phase of a major project. While the beads are an intrinsic part of this project, there are many other 'challenges' that need to be resolved. Although I can't say when we will need to purchase more beads, I do know it will be in the relatively near future. When that time comes, I plan on making only one phone call. As long as I have a say, our days of shopping bead vendors are over now that I have found Bangs." (SC, Frederick, MD) Many thanks from Nathan (and from all of us at BLI) for your kind words.*

❖ *"We are very interested in your latex products, I learn a lot from the technical notes in your website. Can you add me to your newsletter mailing list? I want to know more." (XL, Xiangzhou, China) We are happy to send you our newsletter. You might also like to attend our short course, "The Latex Course - 2002" (Details are on p.1).*

❖ *"As always, your company is phenomenally helpful and friendly!" (KBM, Bozeman) Thanks, Mom. We'll keep trying.*

"We become what we repeatedly do. Excellence then, is not a single act, but a habit." – Aristotle

Technical References – See our website ([www.bangslabs.com](http://www.bangslabs.com)) for "downloadable" TechNotes and Product Data Sheets or ask for copies by mail or fax. We continually update and add new TechNotes and Product Data Sheets to our website.

### Product-Specific TechNotes:

101. **ProActive® Microspheres** – Handling tips plus protocols for streptavidin, Protein A, and goat anti-Mouse coated microspheres.
102. **Magnetic Microparticles** – Data plus handling tips for >11 varieties of superparamagnetic particles; COOH- and NH<sub>2</sub>-modified; classical, encapsulated (low surface iron content), etc.
103. **Fluorescent/Dyed Microspheres** – Applications, fluorescence spectra, and product descriptions. Includes confocal microscopy standards.
104. **Silica Microspheres** – For immunoassays, nucleic acid capture, velocimetry (LDV, PIV), flat panel display spacers, and others.
105. **Microsphere Size Standards** – Data for 9 sizes (0.2-20µm), available singly or in kits, with certificates of analysis.
106. **Confocal Standards** – Using our three, bright, single-label 60nm fluorescent beads in confocal microscopy.

### Handling-Specific TechNotes:

201. **Working with Microspheres** – Choosing, cleaning, characterizing, coating beads, etc.
202. **Microsphere Aggregation** – Preventing, detecting, and reversing aggregation. Chemicals and equipment sources.
203. **Washing Microspheres** – Variety of methods for cleaning microspheres; advantages/disadvantages of methods; suppliers of equipment.
204. **Adsorption to Microspheres** – Adsorbing protein onto particles; use of "surface diluents" (blockers); recipes and references.
205. **Covalent Coupling** – Chemical attachment of proteins, nucleic acids, etc. to various types of surface-functionalized microspheres; recipes for buffers, blockers; miscellaneous coupling ideas, vendor information, and references.
206. **Equations** – For calculating particles/mL, area/g, "parking area", settling velocity @ 1G and in centrifuge, etc.
208. **Microsphere Sizing** – Various manual and automated methods are described and discussed, with references and supplier list.

**Flow Cytometry Standards?** See the "flow" portion of our website for lots of technical information about flow cytometry standardization in general and our expanding line of flow cytometry standards products in particular.

### Application-Specific TechNotes:

301. **Immunological Applications** – Review of commercial applications of microspheres.
302. **Molecular Biology** – Overview of purification and solid phase separation methods.
303. **Lateral Flow Tests** – Putting dyed particles on membranes so they will move properly.
304. **Light-Scattering Assays** – Turbidimetric and nephelometric applications of microspheres.

### Reprints:

402. **Microspheres, part 1: Selection, cleaning, and characterization, and part 2: Ligand attachment and test formulation** – LB Bangs & Mary Meza, *IVD Technology (in Medical Device & Diagnostic Industry)*, **17**, #3, 18-26, March, and #4, 20-26, April, 1995. (Note that you can download these papers at the IVDT website: [www.devicelink.com/ivdt/archive/95/03/009.html](http://www.devicelink.com/ivdt/archive/95/03/009.html) and [.../95/04/006.html](http://www.devicelink.com/ivdt/archive/95/04/006.html)).
403. **New Developments in Particle-Based Immunoassays** – Leigh B. Bangs, *Pure & Appl. Chem.*, **68**, #10, 1873-1879 (1996). Review of 40 years of diagnostic uses of microspheres – from LATs to biosensors.
405. **Applications of Magnetic Particles in Immunoassays** – Mary Meza, Ch. 22 (pp. 303-309) in *Scientific and Clinical Applications of Magnetic Carriers*, U. Häfeli, *et al*, Eds., Plenum Press, New York, 1997.
406. **Measuring Microsphere Binding Capacity** – JM Duffy, JV Wall, MB Meza, LJ Jenki, *IVD Technology*, **4**, #7, 28-34 (1988). (No reprints are available; you can download from our website.)
407. **Bead-based HTS Applications in Drug Discovery** – MB Meza, *Drug Discovery Today: HTS Supplement*, **1**, #1, 38-41 (2000).

**BLI Presentations and References** See our website for copies of the latest public presentations by the technical people at BLI and for publications that cite use of Bangs Beads or were authored by BLI personnel.

**Ask us for help to find...** cleaning equipment, big beads, slides, membranes for strip tests, etc. (Or see the "Hot Links" page at our website.)

**Free Literature for you!** What information do *you* need? We freely share our library: >1000 papers about microspheres, cross referenced, so we can search for types of particles, coupling methods, uses, author, etc. New papers are added as we get them.  
**Help from you?** Please tell *us* about good papers which we should have as you find them. And please send us any good bead art that you find – photos, drawings, etc. showing microspheres or their applications.