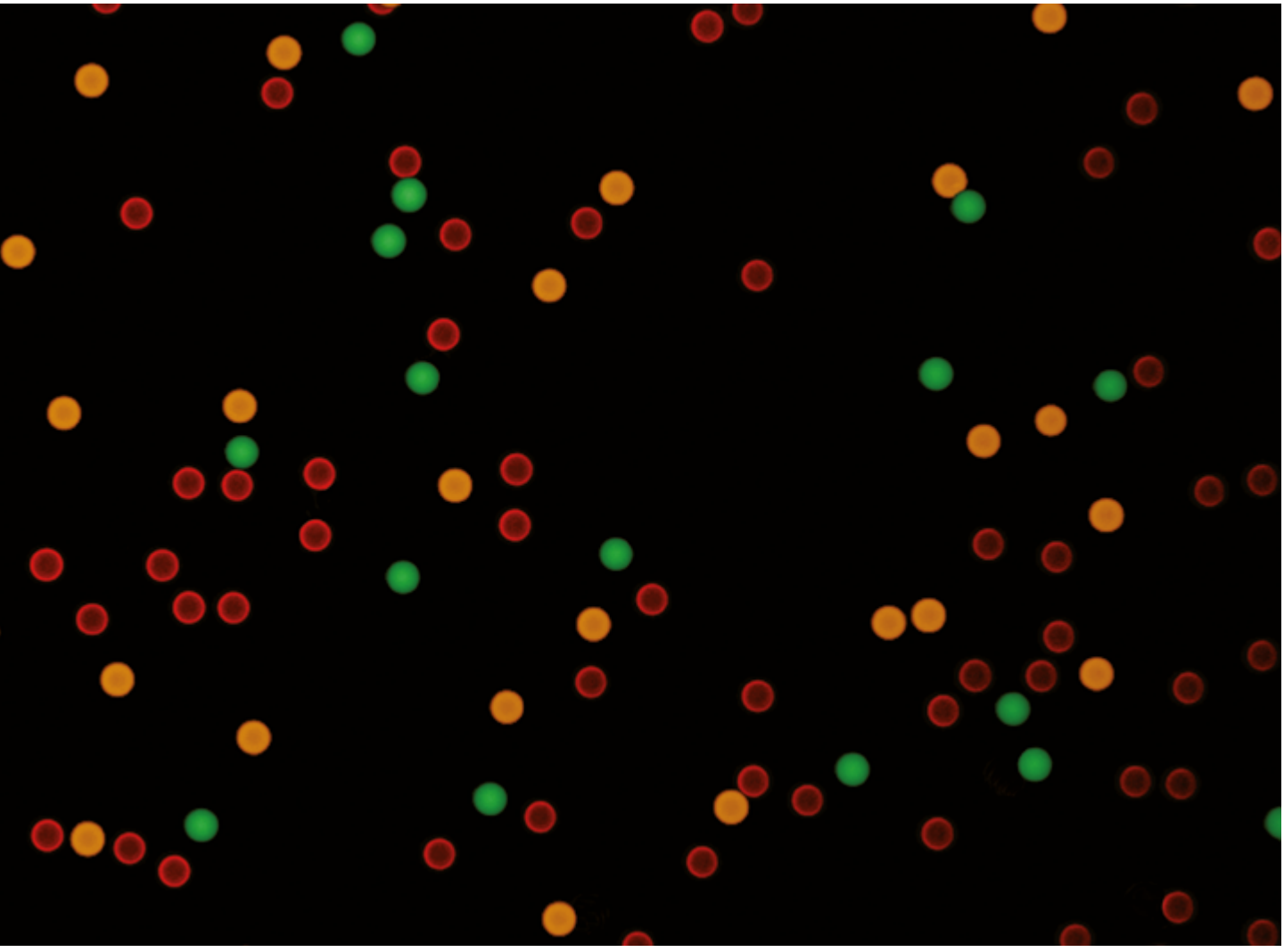
Polymer Microparticles &
Submicron Particles

PolyAn

molecular
surface
engineering

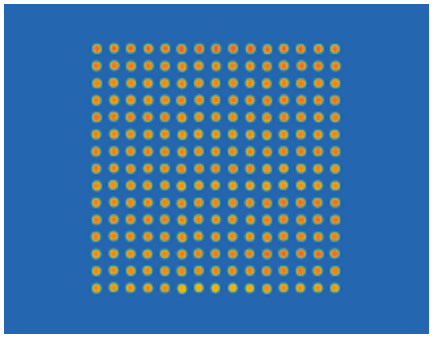


www.poly-an.de

About PolyAn

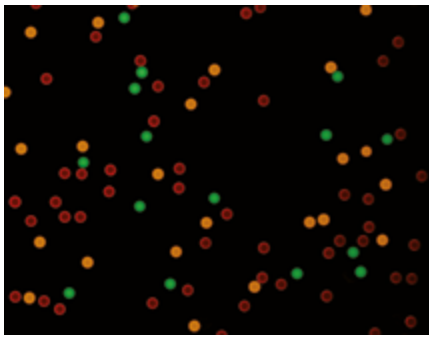
Surface functionalized consumables for LifeScience applications

PolyAn is a nanotechnology company specialized in the modification of surfaces using Molecular Surface Engineering (MSE). Since 1996 PolyAn develops and manufactures high-performance consumables for multiplex diagnostics and LifeScience research.



Functionalized Surfaces for Microarrays

PolyAn is one of the leading producers of functionalized surfaces for microarrays. Our wide range of surfaces, substrates and handling tools for microarrays enables our customers to easily select the most suitable material for their specific application.



Fluorescent Micro- and Nanoparticles

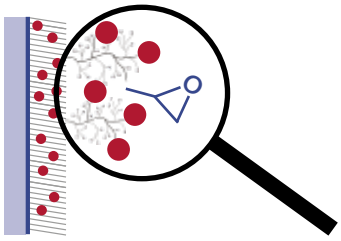
PolyAn is offering a portfolio of monodisperse PMMA micro- and nanoparticles for (bio)applications such as multiplexed bead assays, and for calibration of flow cytometers and fluorescence imaging systems. PolyAn's beads can be color encoded with a wide range of fluorescent dyes and functionalized with PolyAn's reactive 3D-matrices.

Functionalized Microplates

PolyAn's microplates are used for the covalent binding of biomolecules that cannot be immobilized efficiently by passive adsorption. PolyAn offers Amine-binding plates, Click chemistry plates, and Streptavidin-coated plates for demanding assay applications.

Molecular Surface Engineering Services

PolyAn is able to equip almost any substrate with our reactive matrices for selective immobilization and with antifouling surfaces for the reduction of cell adhesion and unspecific binding, respectively. As part of our Molecular Surface Engineering services, we offer functionalized consumables for OEM applications, which are tailored to specified customer requirements.



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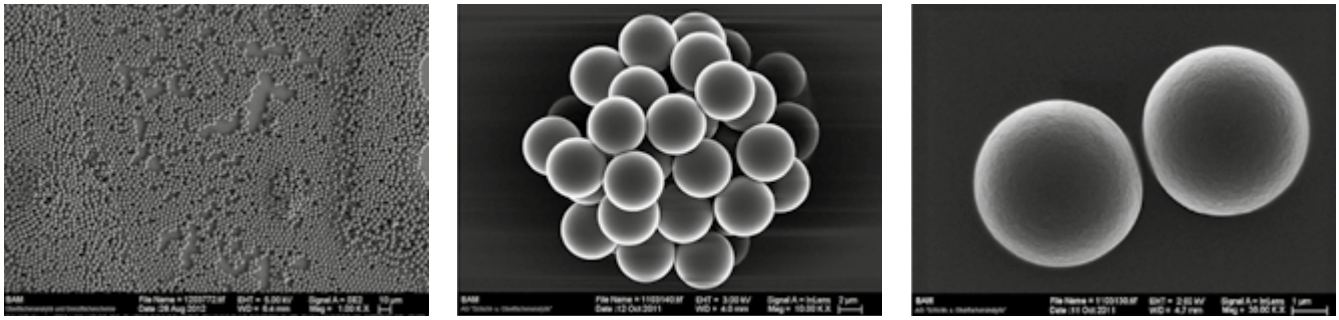
1. Functionalized Micro- and Nanoparticles

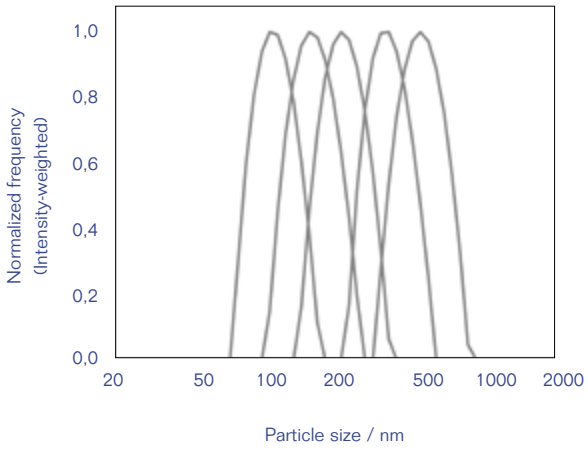
1.1 PMMA Particles

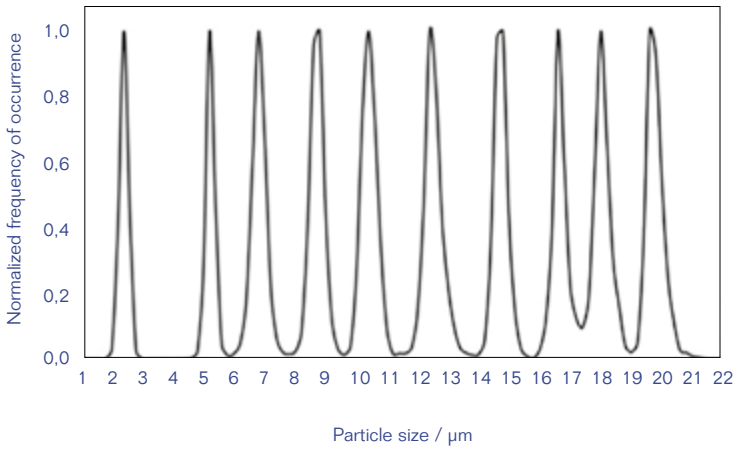
PolyAn's microparticles (microbeads) and submicron particles (nanobeads) are comprised of PMMA (poly methyl methacrylate). Using PMMA ensures an excellent optical brilliance and low autofluorescence compared to other particle materials.The refractive index of PMMA is 1.48, which is close to the refractive index of biomaterials such as cells (ca. 1.38). Our PMMA beads have a density of 1.18 g/cm³, a glass transition temperature (Tg) of about 110°C, and are of biocompatible grade.

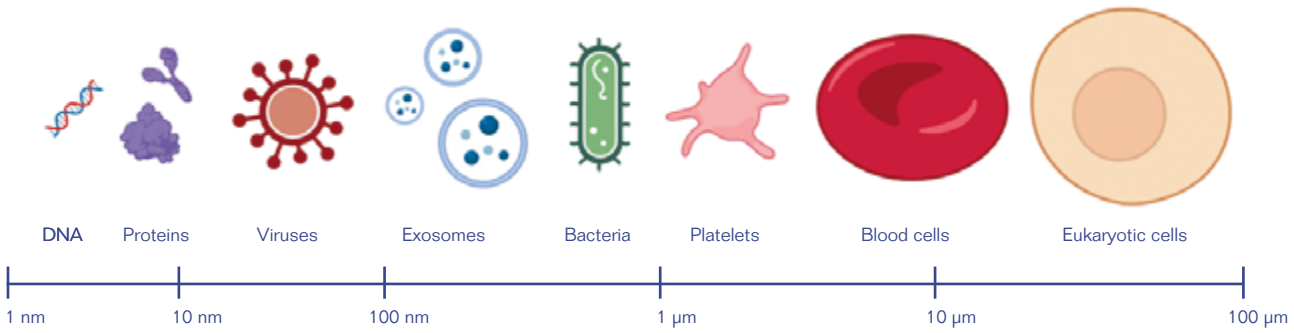


SEM images of PolyAn PMMA beads at different magnifications

PolyAn's nanobeads and microbeads cover the size range of many biological particles, ranging from extracellular vesicles (exosomes) and bacteria to human blood and cancer cells. Thus, PolyAn beads can be used to mimic biological particles in test measurements, to set up and monitor corresponding detection methods, and/or to analyze these entities in various kinds of in bioassays.

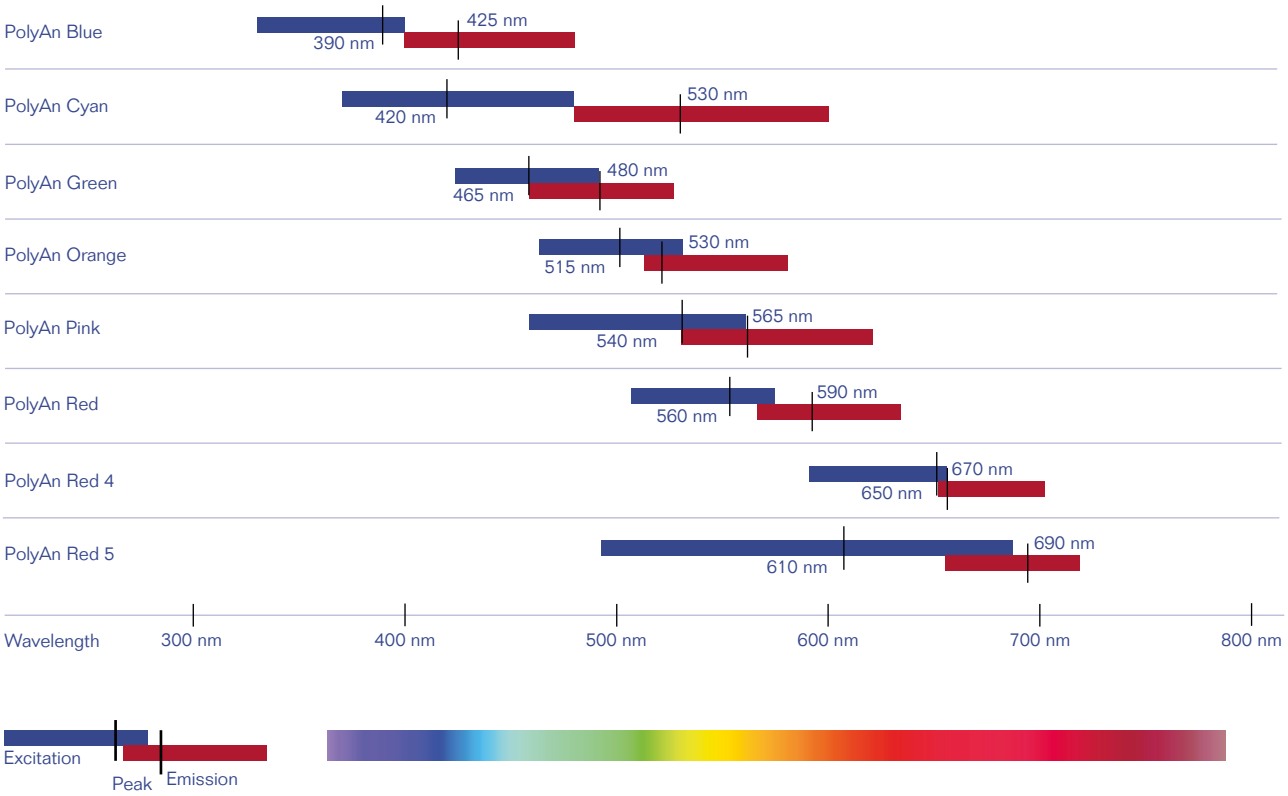






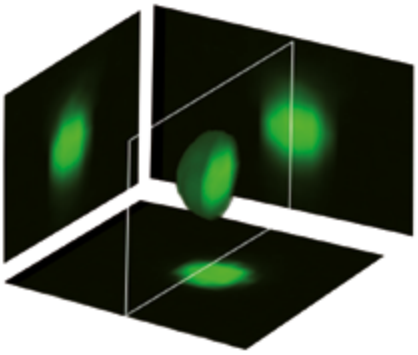
1.2 Fluorescent PMMA Particles

PolyAn's fluorescent micro- and nanobeads are encoded with either one fluorophore (single-color) or multiple fluorophores (multi-color) at excitation/emission wavelengths and emission intensities of your choice (see chart below). PolyAn PMMA beads can be color-encoded with up to six fluorescent dyes. Multiplex beads with specific intensity ratios or fluorescence lifetimes are also available.



The fluorescent dyes PolyAn Plex C (excitation 420–475 nm, emission 480–560 nm) and PolyAn Plex R (excitation 460–545 nm, emission 540–600 nm) are also available for our microbeads.

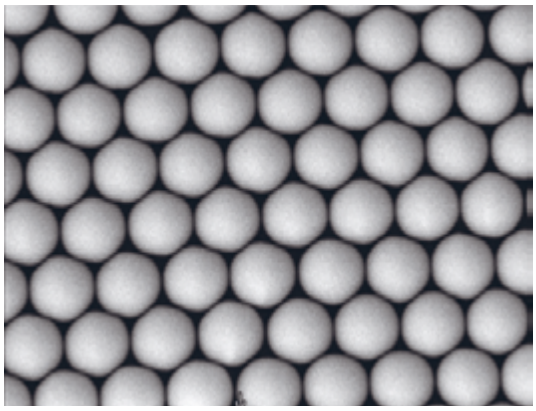
Homogeneous Distribution of Fluorophores



Confocal Laser Scanning Microscope (CLSM) image, 3D-Z-stack of PolyAn Orange bead*

With PolyAn's production process the fluorophores are incorporated directly into the core of the beads during particle formation. This ensures a more homogeneous distribution of the dye within the beads when compared to conventional diffusion controlled dyeing processes. Additionally, the fluorophores are caged within the PMMA matrix, and thus, are less likely to leak-out.

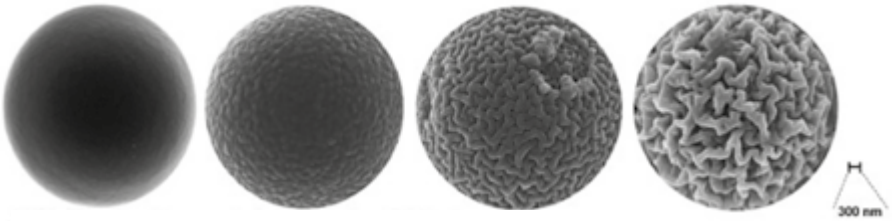
Transparent Beads and Dye-colored Beads



PolyAn also offers transparent as well as dye-colored nano- and microparticles. These non-fluorescent beads can be used for colorimetric assays, as calibration tools for particle analysis, and for various other applications.

* Image courtesy of Bundesanstalt für Materialforschung und -prüfung (BAM)

1.3 Functionalized PMMA Particles

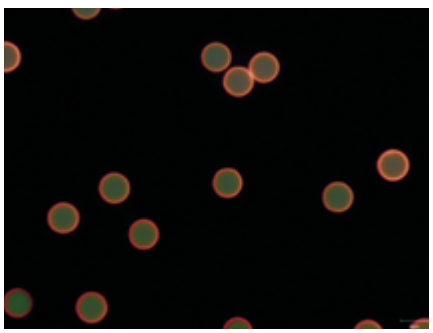


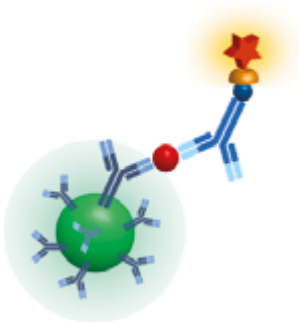
REM images showing different surface morphologies of carboxylated PMMA beads

PolyAn's high-performance polymer microparticles are functionalized with a 3D-surface chemistry comprised of a long-chain polymer with a defined number of reactive groups. In contrast to conventional coating procedures, the reactive polymer is covalently linked to the surface.

Schematic model of the 3D reactive matrix on the core polymer microparticle

Unspecific binding and aggregation of biomolecules is reduced by our low aggregation matrix. Our rigorous quality control procedures according to ISO 9001 ensure the constant loading and low batch-to-batch variation necessary for in-vitro diagnostics and biopharma applications.



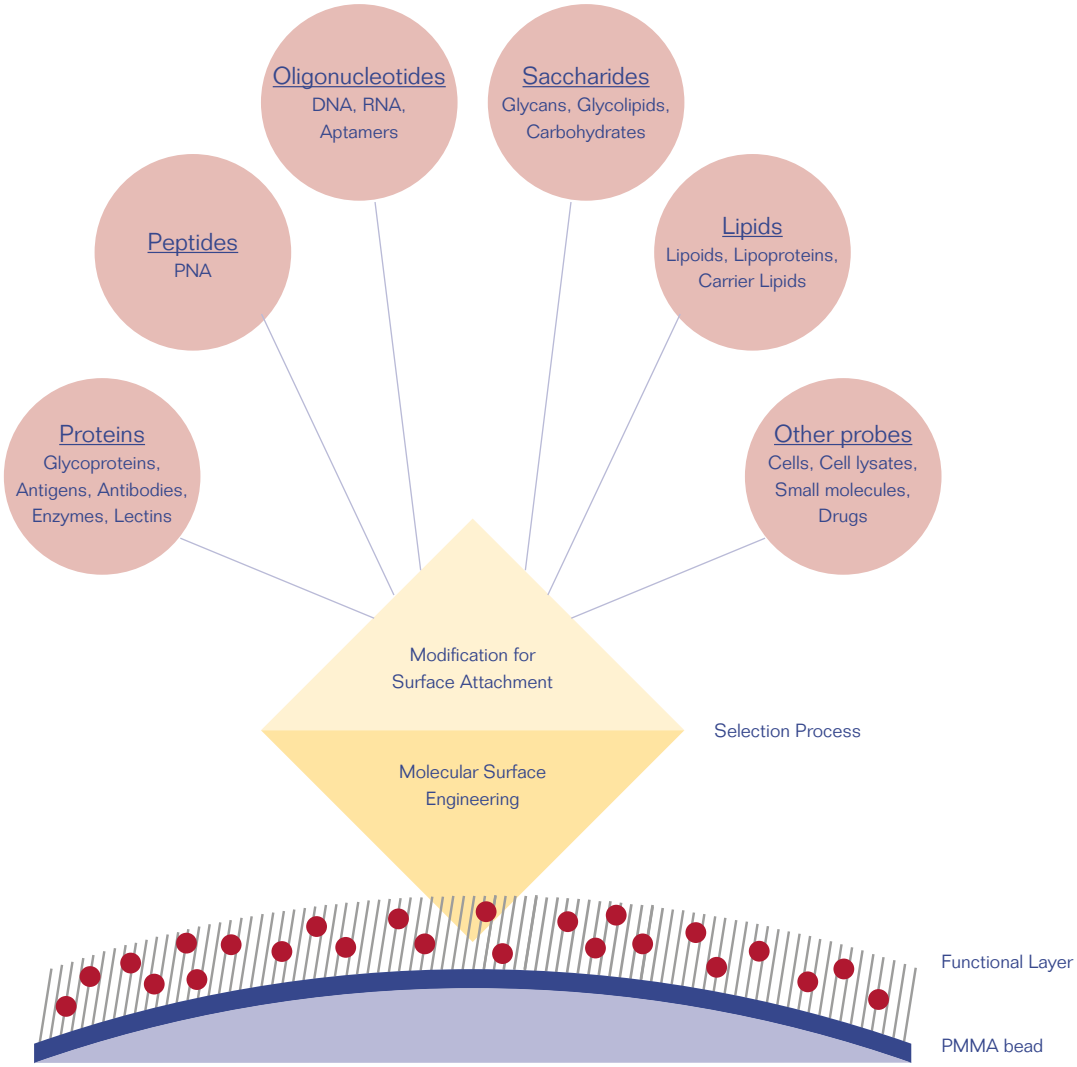


The fluorescence microscopy image above illustrates a successful binding event of dye-labelled biomolecules onto a fluorescent bead. The bead cores are encoded with a green-emitting dye. The successful immobilization of biomolecules is detected in a second channel using an orange emitting dye.

2. Molecular Surface Engineering for Beads

Immobilization of biochemical Species onto Surfaces

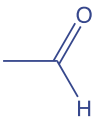
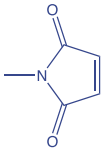
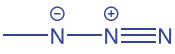
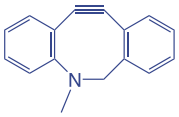
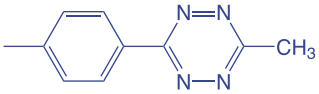
Selecting the optimal immobilization method for a probe is often an iterative process. For the immobilization of biochemical species various coupling techniques and approaches have been developed. PolyAn offers a very broad portfolio of bead surfaces to enable the selection of the optimal surface for each probe and application.



The strongest immobilization method in biochemistry is covalent attachment. A covalent bond is formed by sharing of electrons between two atoms. The dissociation energy for a typical covalent bond is 420 kJ/mol, and thus, far higher compared to the 130 kJ/mol of a typical electrostatic interaction. It can be distinguished between a covalent attachment of activated targets and a covalent attachment of biological species on activated surfaces.

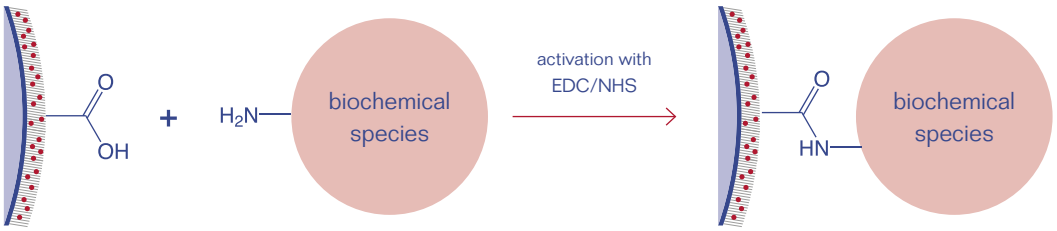
2.1 Overview of the Functional Bead Surfaces

PolyAn offers the following surfaces for immobilization of proteins, peptides, oligonucleotides, aptamers, glycans, and other probes.

Functional Group or Ligand	Structure	Application examples
3D-Carboxy		For EDC/NHS mediated coupling of Amine-terminated molecules
3D-Aldehyde		For Amine-containing molecules
3D-Maleimide		For binding of Thiol-containing molecules
3D-Azide		For binding of molecules via click chemistry
3D-Alkyne		For binding of Azide-modified molecules via copper-catalyzed click chemistry
3D-DBCO (Cyclooctine)		For binding of Azide-modified molecules via copper-free click chemistry
3D-MTZ (Methyltetrazine)		For fast ligation with TCO-modified molecules
Streptavidin or Neutravidin		For coupling of Biotin-functionalized molecules
Protein A, Protein G, or Protein A/G		For binding of IgG Antibodies
Customer-specific biolabeling		Custom bead labeling with Oligonucleotides, Peptides, Proteins/Antibodies, Biotin …
Low Aggregation/ Low Adsorption		Non-adsorbing matrix, for calibration and controls

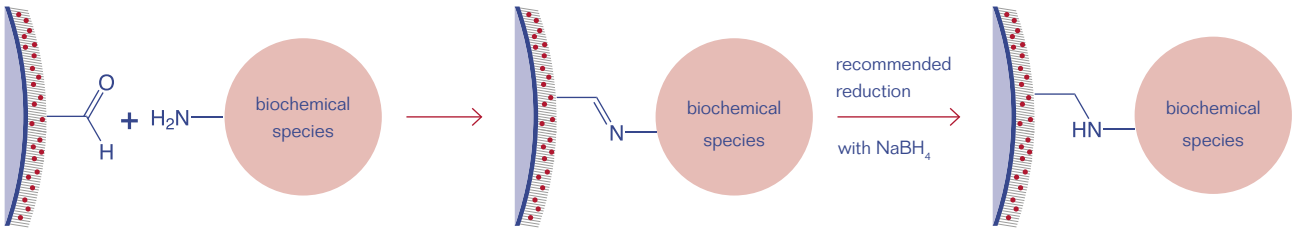
2.2 Bead Surfaces for Protein Chemistry

3D-Carboxy



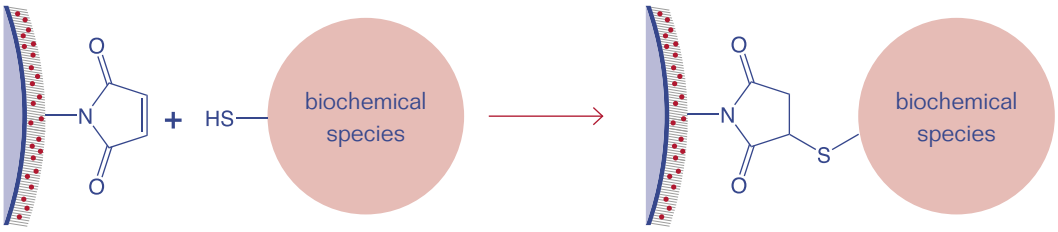
Carboxy groups can be activated with EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide) and NHS (N-hydroxysuccinimide) to form a highly reactive intermediate. This intermediate can be easily reacted with the Amine-groups of biochemical species like proteins or antibodies.

3D-Aldehyde



Aldehyde groups bind to Amine-containing molecules. In an intermediate state the Aldehydes form an Imine-group with the Amines (Schiff-base). In order to increase the bond strength it is also possible to reduce the Imines with e.g. $NaBH_4$ or TCEP (Tris(2-carboxyethyl)phosphine) to form stable Amines.

3D-Maleimide



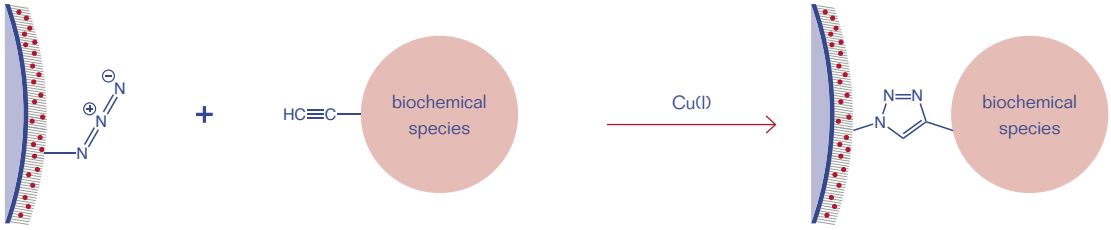
Maleimide-esters react with Thiol-groups of biochemical species. The Thiol-groups can be either natively present in the (bio)molecule, e.g. via the amino acid cysteine in proteins, produced via reductive cleavage of disulfide bonds with a reducing agent such as Dithiothreitol (DTT, Cleland's Reagent), or selectively introduced e.g. with 2-Iminothiolane (Traut's reagent) for amine-containing molecules.

2.3 Bead Surfaces for Click Chemistry

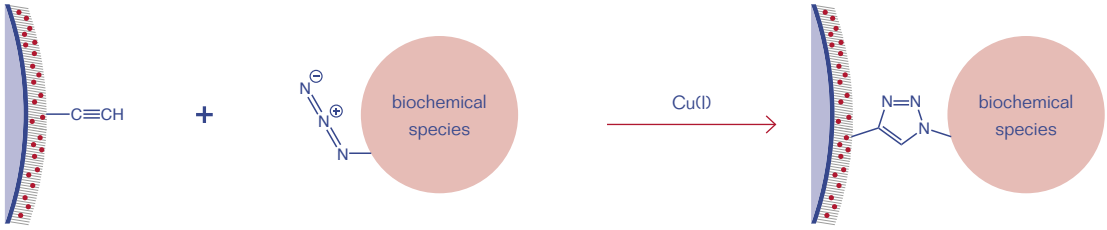
Click chemistry describes quick and irreversible one pot conjugation reactions that have a high reaction specificity, high yield of the desired product with only minimal byproducts. Bio-orthogonal reactions are conjugation reactions that do not interfere with biological processes. Such reactions are especially useful in biochemical applications, as they can be conducted under physiological conditions and address the need for highly specific and robust reactions in biological contexts.

PolyAn offers a variety of surfaces that are suitable for bio-orthogonal conjugation of biomolecules via click chemistry.

3D-Azide

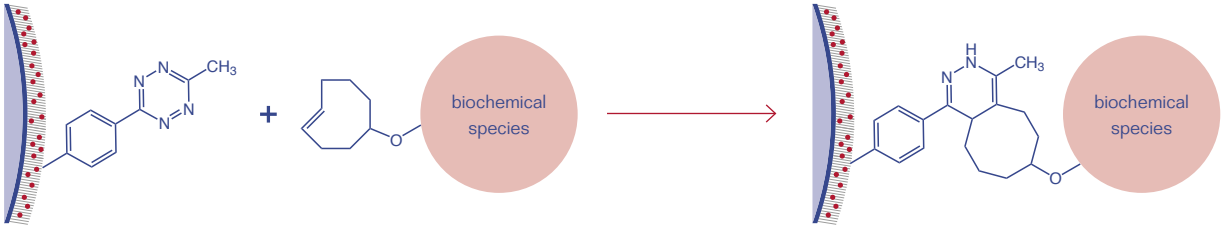


3D-Alkyne



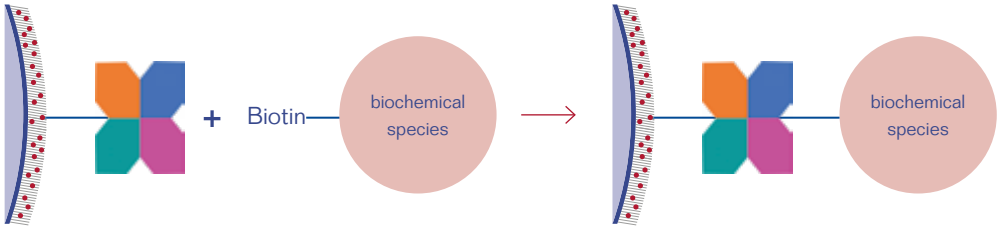
3D-DBCO

3D-MTZ



2.4 Streptavidin/Neutravidin-functionalized Beads

Streptavidin and Neutravidin are tetrameric proteins that can bind four Biotin molecules or any other Biotin-conjugated species with a very high specificity. The Streptavidin/Neutravidin-Biotin interaction is one of the strongest, non-covalent bonds known in biochemistry, having a dissociation constant of $K_D = 10^{-15}$ mol/L. Thus, Streptavidin and Neutravidin are versatilely applied in many bioanalytical applications.



PolyAn's Streptavidin or Neutravidin matrices are covalently attached to the bead surface, so that the molecules are less susceptible to desorption in the presence of surfactants, solutions of high ionic strength, or high temperatures, compared to adsorptive immobilization.

Advantages of Neutravidin

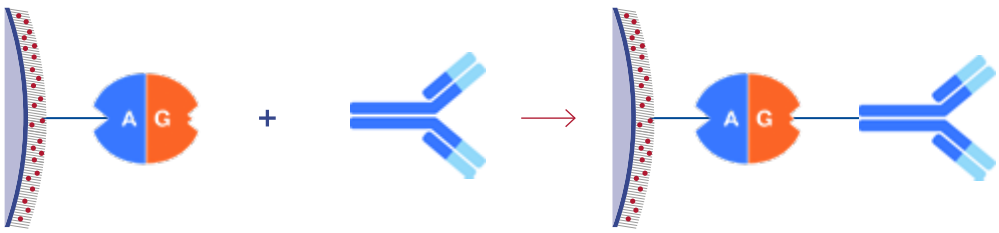
Although Streptavidin is still the most popular and widely used biotin-binding protein, Neutravidin offers several benefits:

— Near-neutral isoelectric point efficiently decreases non-specific interactions
— De-glycosylation prevents non-specific binding of lectins
— Less non-specific binding in cell assays

Its high biotin-binding affinity and low non-specific binding make Neutravidin the ideal biotin-binding protein!

2.5 Protein A/G-functionalized Beads

PolyAn´s Protein A, Protein G, and Protein A/G-functionalized beads are suitable for the non-covalent, oriented immobilization of IgG antibodies. Protein A, Protein G, and Protein A/G bind specifically to the heavy chain at the Fc-region of IgG, resulting in an oriented binding of the antibodies.

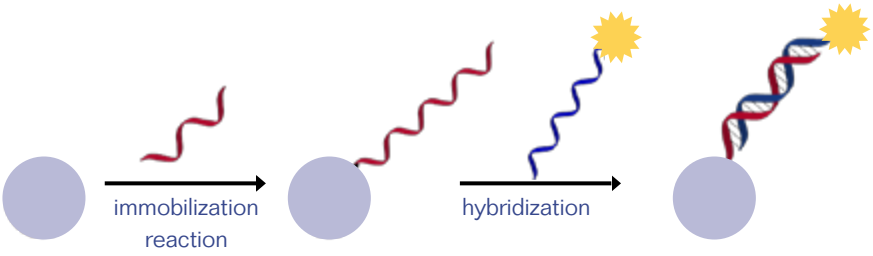


Protein A, Protein G, and Protein A/G possess different affinities towards antibodies of different species (Human, Mouse, Rat, Rabbit, Goat, …) as well as towards different immunoglobulin classes (IgG, IgA, IgM, …) and subclasses (IgG1, IgG2, IgG3, …). Thus, each specific antibody might require different immobilization conditions.

2.6 Custom-specific Biolabeling of Beads

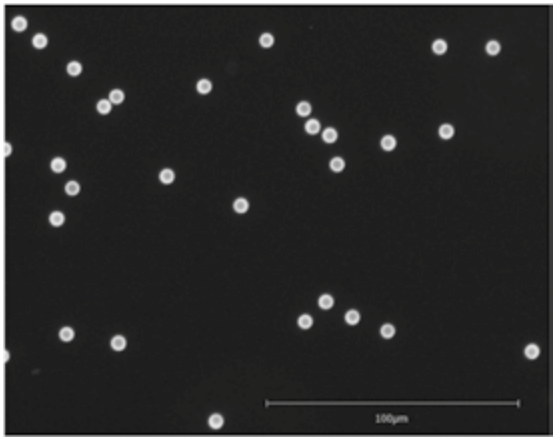
As part of our functionalization services PolyAn offers the custom modification of beads with various kinds of biomolecules such Proteins, Antibodies, Oligonucleotides (DNA/RNA), Peptides, or Aptamers.

Immobilization of Oligonucleotides via Click Chemistry



After immobilizing DNA-type oligonucleotides, hybridization efficiencies with complementary oligonucleotide strands are about 90%.

The successful immobilization and hybridization is also illustrated in the fluorescence image below. Multiple re-hybridizations were successful.



Olympus, 20x: Fluorescence image after hybridization with a dye-labeled anti-strand

Bead loading with oligonucleotides was determined by absorption spectroscopy using dye-labeled anti-strands. The oligonucleotide loading densities are 20 pmol/mg for 5 µm beads, 13 pmol/mg for 9 beads, and 6 pmol/mg for 20 µm beads.

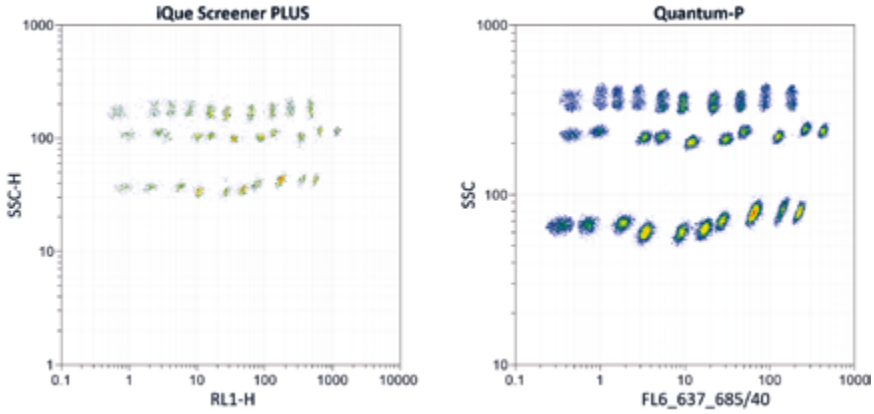
Our functionalization service includes the PolyAn PMMA beads and their custom modification. The final price depends on the oligonucleotide or peptide of choice.

3. Multiplex Beads

3.1 Multiplex Beads for Flow Cytometry

PolyAn Red4 Multiplex Beads (30-plex)

PolyAn Red4 Multiplex Beads provide a platform for the design of multiplexed assays that can be run on standard flow cytometers. PolyAn offers a set of 30 bead populations (30-plex) that can be distinguished both by the different fluorescence intensities of our PolyAn Red4 dye in the APC channel (Excitation 590–680 nm, Emission 660–780 nm) and three different bead sizes: 3.5 µm, 5.5 µm and 8.5 µm.



PolyAn Red4 30-plex Beads measured with different flow cytometers: Sartorius IntelliCyt iQue Screener Plus (left), Quantum Analysis Quantum-P (right)

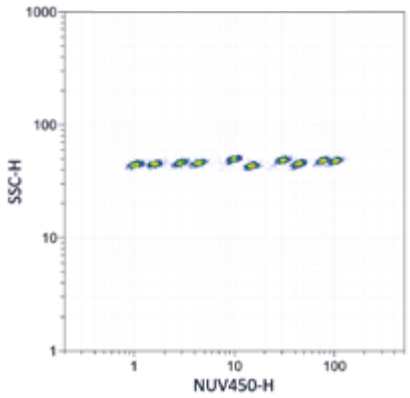
Products

Product-ID	Diameter	Surface	Color	Populations	Excitation/Emission
106 50 003 106 50 005 106 50 009	3.5 µm 5.5 µm 8.5 µm	3D-Carboxy 3D-Carboxy 3D-Carboxy	PolyAn Red4 PolyAn Red4 PolyAn Red4	10 peaks 10 peaks 10 peaks	590–680 nm/660–780 nm 590–680 nm/660–780 nm 590–680 nm/660–780 nm
106 52 003 106 52 005 106 52 009	3.5 µm 5.5 µm 8.5 µm	Streptavidin Streptavidin Streptavidin	PolyAn Red4 PolyAn Red4 PolyAn Red4	10 peaks 10 peaks 10 peaks	590–680 nm/660–780 nm 590–680 nm/660–780 nm 590–680 nm/660–780 nm

Our PolyAn Red4 Multiplex Beads are also available with Neutravidin and Protein A/G, as well as 3D-Aldehyde, 3D-Azide, 3D-Alkyne, 3D-DBCO, and 3D-MTZ surfaces. A custom modification with antibodies, peptides, or oligonucleotides is available upon request.

PolyAn Blue Multiplex Beads (10-plex)

PolyAn offers a set of 10 populations of 5.8 µm-sized beads (10-plex) that can be distinguished by the different fluorescence intensities of our PolyAn Blue dye in the DAPI channel (Excitation 350–400 nm, Emission 400–480 nm).



PolyAn Blue 10-plex Beads measured on a Beckvman Coulter CytoFlex-LX flow cytometer.

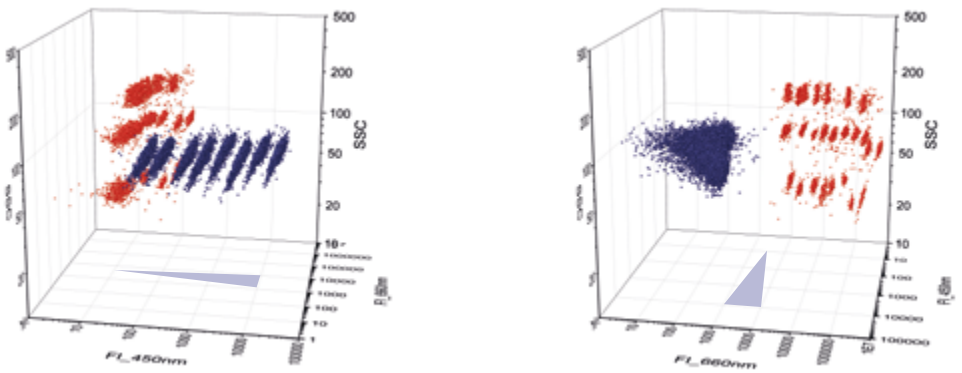
Products

Product-ID	Diameter	Surface	Color	Populations	Excitation/Emission
107 50 005	5.8 µm	3D-Carboxy	PolyAn Blue	10 peaks	350–400 nm/400–480 nm
107 52 005	5.8 µm	Streptavidin	PolyAn Blue	10 peaks	350–400 nm/400–480 nm

Our PolyAn Blue Multiplex Beads are also available with Neutravidin and Protein A/G, as well as 3D-Aldehyde, 3D-Azide, 3D-Alkyne, 3D-DBCO, and 3D-MTZ surfaces. A custom modification with antibodies, peptides, or oligonucleotides is available upon request.

Customized Multiplex Bead Mixtures

Each bead population of the PolyAn Red4 Multiplex Beads and PolyAn Blue Multiplex Beads can be combined independently to create a Multiplex Bead mixture that is most suitable for your application. The combination of Red4 and Blue Multiplex Beads leaves the PE channel free for the detection of binding events.



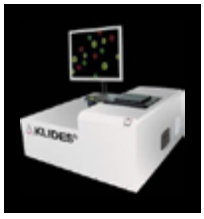
3D presentation for the simultaneous detection of 8 populations of the PolyAn Blue 10-plex Beads and 25 populations of the PolyAn Red4 30-plex Beads using a Beckman Coulter CytoFlex LX flow cytometer (same dataset, two different representation angles).

3.2 Beads for Scanning Cytometry and Microscopy

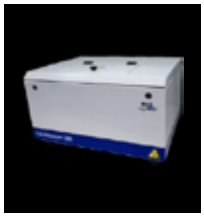
For fluorescence microscopy based detection systems PolyAn has developed several sets of multiplex beads that can be distinguished by both different sizes as well as different color encodings. In order to facilitate detection and reduce the requirements with regards to the optical system, these multiplex beads are larger and have a higher fluorescence intensity.

PolyAn offers several variants of bead coding in the field of scanning cytometry:

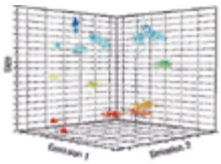
One bead size, one color, i.e. fluorescence coding by graduations in the emission intensity achieved by different fluorophore concentrations in the bead (e.g. akiron by Medipan GmbH).



Multiple bead sizes (at least two) in combination with multiple intensity gradations of a fluorophore embedded in the bead (e.g., AKLIDES by Medipan GmbH)

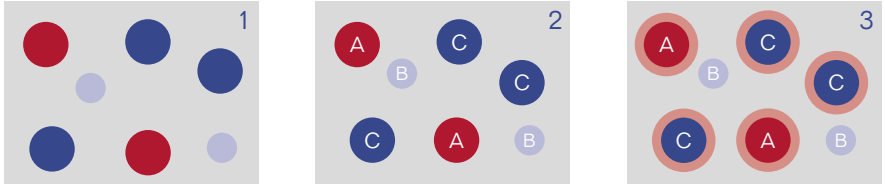


Two different fluorescence encodings, i.e. emission wavelengths, which can be realized by two different fluorophores in different concentrations in one bead size (e.g. Kaleidoscan by Attomol GmbH)



Two or more fluorescence encodings, i.e. emission wavelengths, that can be realized by two or more different fluorophores in distinct concentrations within various bead sizes. Here, the ratio of the fluorescence intensities can be determined as an encoding parameter (e.g. VideoScan*).

Imaging-based classification, assignment, and evaluation of multiplex beads
The images are detected by a CCD camera. The multi-color fluorescence image capture system uses pattern recognition algorithms for multiplex testing:

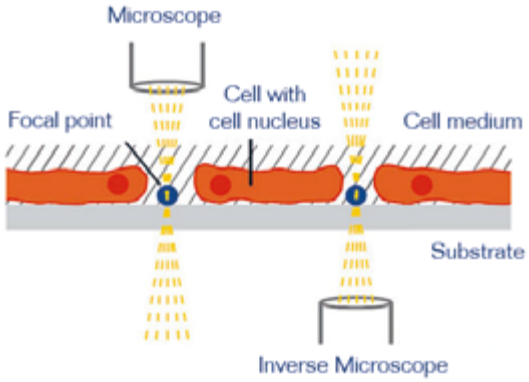


The bead populations are distinguished by their fluorescence and size. In the first step, the beads are focused by a dynamic autofocus (1). Subsequently, the beads are classified and assigned to their bead population (2). In the final step, the ligand fluorescence is detected using a fluorescence label illustrated by the red corona (3).

*S. Rödiger et al., A highly versatile microscope imaging technology platform for the multiplex real-time detection of biomolecules and autoimmune antibodies. Adv. Biochem. Eng. Biotechnol. 2013, 133. 35-74.

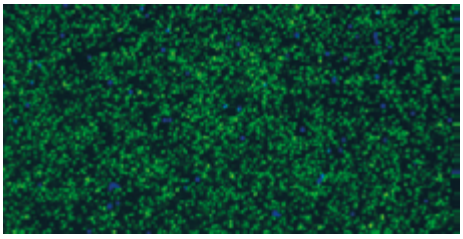
Focus Beads

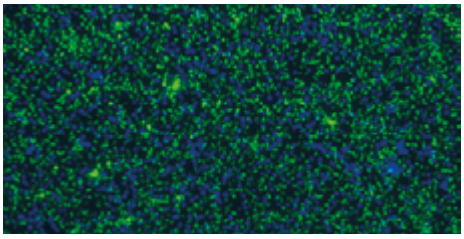
Quickly finding the correct focal plane is one of the key challenges for automated, microscope-based fluorescence imaging systems. PolyAn's Focus Beads address this problem: The 2 µm Focus Beads have a comparable size to cell nuclei and consist of a biocompatible PMMA-grade that is not cell-toxic and does not interfere with cell behavior. The Focus Beads are color-encoded with our PolyAn Blue dye, which emits in the DAPI channel, but is transparent in all other detection channels.



In most applications DAPI-focusing on cell nuclei is used to determine the bottom of slides, plates, or other substrates. However, this method can lead to errors when the cells detach from the bottom surface or are insufficiently stained.

PolyAn's Focus Beads have a well-defined fluorescence intensity, thus, avoiding problems caused by insufficient staining. The beads can be easily added to the cell suspension and quickly sink to the bottom to indicate the correct focal plane.





PolyAn's Focus Beads can be applied in a wide range of cell assays, e.g. in biofilms, adhesion assays, or for the detection of bacteria. Focus beads for other channels are available upon request.

Product-ID	Diameter	Dye
108 80 002	2 µm	PolyAn Blue

4. Bead-based Calibration Tools

4.1 Fluorescence Calibration Slides

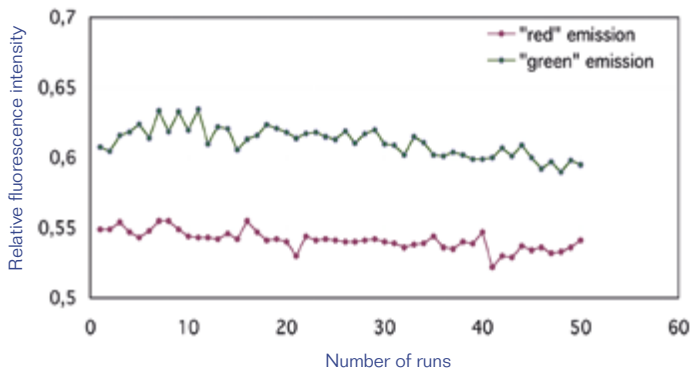
PolyAn's Fluorescence Calibration Slides are designed for the routine calibration of confocal fluorescence microscopes and other fluorescence imaging systems, e.g. for scanning cytometry.

The calibration slides are prepared by mounting statistically distributed monodisperse PMMA beads that contain ultra-stable fluorophores onto standard glass slides. The beads are protected with a coverslip from mechanical stress and show a homogeneous particle distribution without aggregates.

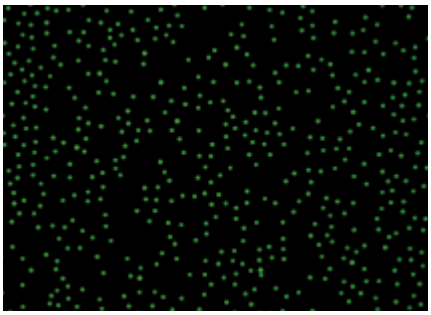
Product-ID	Detection channel
104 20 005	Blue emission (e.g. DAPI)
104 20 010	Green emission (e.g. FITC, Cy3)
104 20 020	Red emission (e.g. APC, Cy5)

Characteristics

— Monolayer of fluorescent beads on glass slides
— High photostability
— Homogeneous particle size and fluorescence intensity
— Single particles, no particle aggregates and homogeneous, statistical particle distribution
— Excellent slide-to-slide and batch-to-batch reproducibility, CV< 3%
— Long term stability: less than 0.5% decrease in fluorescence intensity after 1 month at 37°C
— Standard size: 75 x 25 x 1 mm glass slides, alternative formats are available upon request



Photostability: slides mounted with "Green" and "Red" emitting beads were measured multiple times over a period of 50 days. The fluorescence intensity after more than 50 measurements exceeded 97% of the initial intensity for both dyes, underlining their excellent photostability.

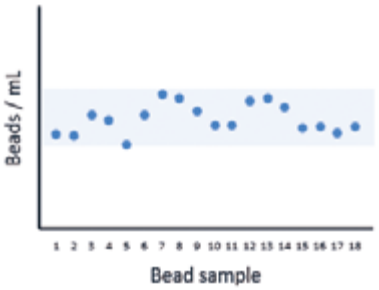


Fluorescence image of a calibration slide (green channel): homogeneous particle distribution, no aggregates.

* Cy® is a registered trademark of GE Healthcare.

4.2 Counting Beads

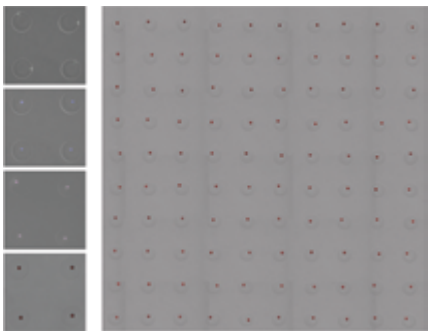
PolyAn's Counting Beads are highly monodisperse PMMA microparticles with a known particle number concentration, intended for use as counting standards for flow cytometers, particle and cell counters, as well as single-cell dispensing applications.



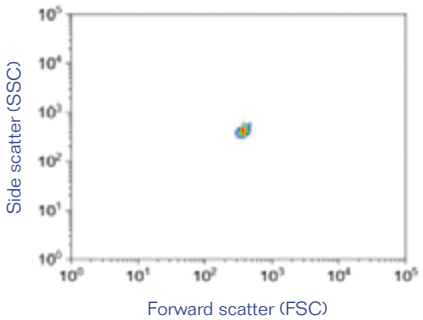
The Counting Beads can be tailored to your specific application: They are available in various sizes, ranging from 5–20 µm, as transparent beads, dye-colored (non-fluorescent) beads, or fluorescent beads encoded with either one fluorophore (single-channel) or multiple fluorophores (multi-channel) at emission wavelength (color) and emission intensities of your choice:

Mean diameter	5–20 µm, highly monodisperse
Number concentration	10^5–10^9 beads/mL
Optical properties	Transparent, Dye-colored, or Fluorescent
Buffer and surface	Customizable
Packaging options	Customized volume and packaging
Certification	PolyAn standard or external certification

PolyAn's Counting Beads can be used for instrument validation, quality control, and daily performance monitoring of particle counters and flow cytometers. They ensure comparability of results between different measurements, instruments, and laboratories. Counting beads can also be used as reference materials for individual cells or spheroids, e.g. in single-cell dispensers:



Mounted beads on an array slide

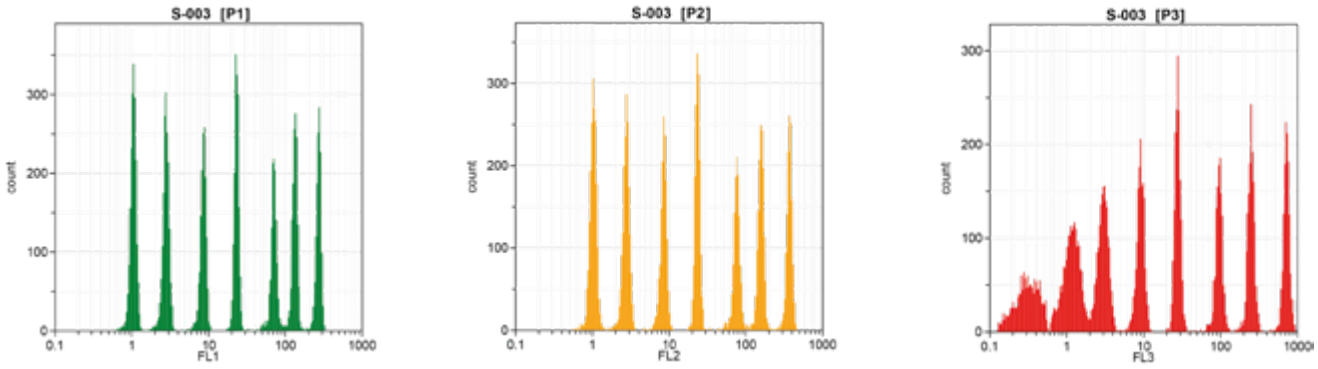


4.3 Spectrum Calibration Beads

PolyAn's Spectrum Calibration Beads (SCB) are monodisperse PMMA particles that contain a mixture of highly photostable fluorescent dyes, which are homogeneously encapsulated within the PMMA matrix. The Spectrum Calibration Nanobeads can be excited at any wavelength from 365–650 nm and emit at 400–750 nm, which enables their detection in all common fluorescence detection channels (DAPI, FITC, PE/TAMRA/Cy3®, APC/Cy5®, Cy7®) with only minimal photobleaching.

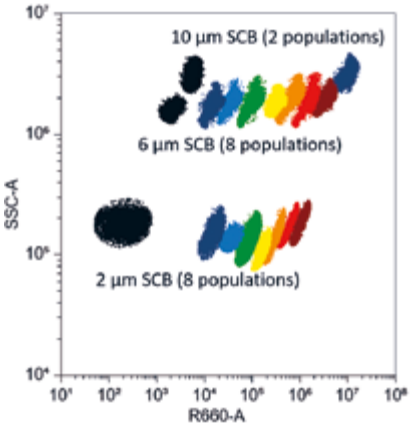
Spectrum Calibration Microbeads

PolyAn's Spectrum Calibration Microbeads are designed for the calibration of flow cytometers and other fluorescence imaging systems. Each color-encoded PMMA bead population (peak) contains a mixture of fluorophores that enables the Spectrum Calibration Beads to be excited at any wavelength from 365 nm to 650 nm.



8-peak Spectrum Calibration Beads with increasing fluorophore content for all detection channels, measured with Quantum Analysis Quantum-P flow cytometer (excitation at 488 nm, the one transparent population is only detectable in FL3).

Product-ID	Bead size	Populations
107 00 002	2 µm	8 peaks
107 00 006	6 µm	8 peaks
107 01 002	2 µm	5 peaks
107 01 006	6 µm	5 peaks
107 02 002	2 µm	1 peak
107 02 006	6 µm	1 peak
107 02 010	10 µm	1 peak
107 03 006	6 µm	1 peak (SCB 0.2.4)

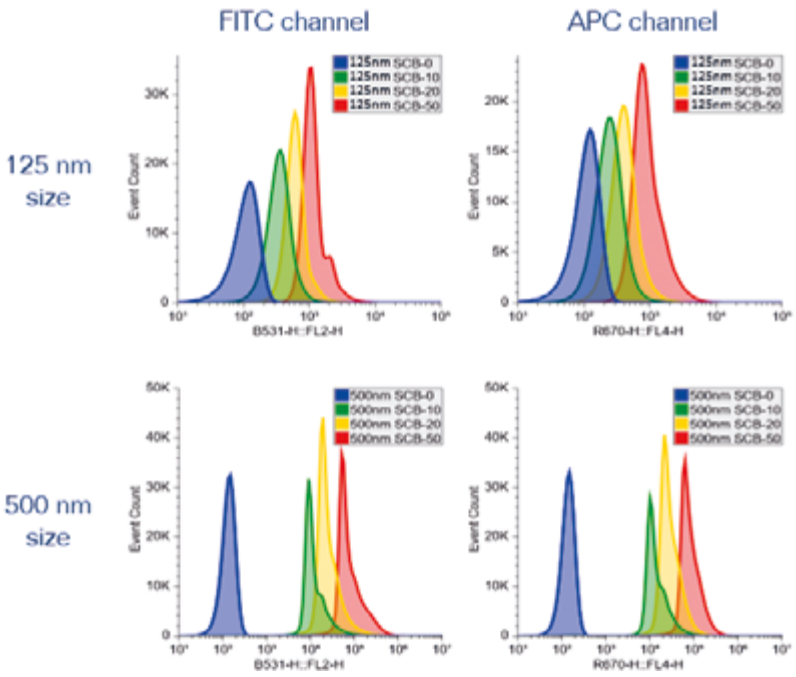


Spectrum Calibration Microbeads are available in all sizes between 2–20 µm, upon request. Please enquire regarding alternative fluorescence intensities and surface functionalities (e.g. Azide, DBCO, Protein A/G).

Spectrum Calibration Nanobeads

PolyAn's Spectrum Calibration Nanobeads can help to adjust fluorescence-based detection systems such as flow cytometers, conventional fluorescence microscopes, confocal laser scanning microscopes, and other image-processing systems.

Nanobeads for Flow Cytometers: As Spectrum Calibration Nanobeads resemble the size of extracellular vesicles (EV), they can also be used to adjust flow cytometers for EV detection. Here, PMMA-Nanobeads compare favorably with polystyrene particles due to their lower refractive index (RI), which is closer to the RI of extracellular vesicles.



Spectrum Calibration Nanobeads with 125 nm (top) and 500 nm (bottom) particle size measured in the FITC and APC detection channel of a CytoFLEX nano (Beckman Coulter Life Sciences) flow cytometer.*

Courtesy of Dr. Alfonso Blanco Fernández, Director of the Flow Cytometry Core Facility at University College Dublin (UCD) Conway Institute of Biomolecular & Biomedical Research

Nanobeads for Imaging Systems: PolyAn's Spectrum Calibration Nanobeads are particularly useful to calibrate imaging setups for multi-color applications and to check their capability to resolve and co-localize fluorescent objects of different color in the same optical plane.

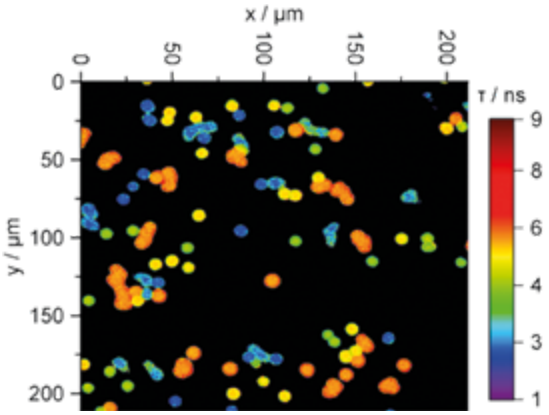
Product-ID	Bead size range	Populations	Surface
208 02 125	100–150 nm	4 peaks	3D-Carboxy
208 04 125	100–150 nm	4 peaks	Streptavidin
208 02 225	200–250 nm	4 peaks	3D-Carboxy
208 04 225	200–250 nm	4 peaks	Streptavidin
208 02 500	475–525 nm	4 peaks	3D-Carboxy
208 04 500	475–525 nm	4 peaks	Streptavidin

Spectrum Calibration Nanobeads are available in all sizes from 100–600 nm upon request. Please enquire regarding different fluorescence intensities and other surface functionalities.

5. Beads for Special Applications

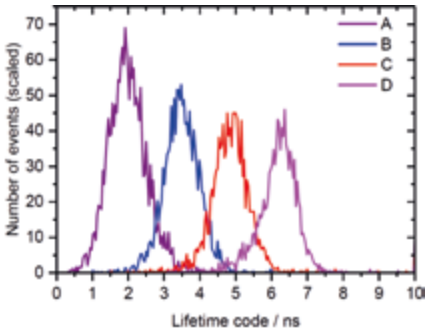
5.1 Fluorecence Lifetime Beads

Fluorescence lifetime (FLT) measurements can be used to discriminate fluorescent samples based on differences in their fluorescence decay rates. This approach can be applied for fluorescence lifetime imaging microscopy (FLIM), confocal microscopy, two-photon excitation microscopy, multiphoton tomography as well as for lifetime-based flow cytometry (FCM).

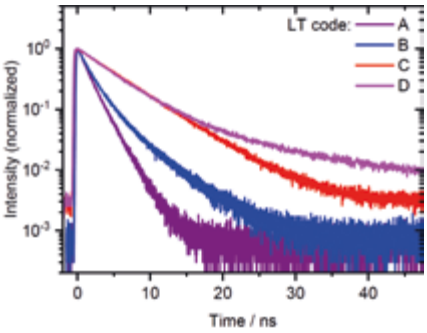


FLIM: FLT encoded beads with ligand fluorescence

PolyAn has developed fluorescence lifetime encoded beads (FLT beads) that can be distinguished according to their different fluorescence lifetimes.



Histogram: PolyAn FLT beads measured with a fluorescence lifetime flow cytometer



Decay curves of PolyAn FLT beads

Product-ID	Diameter	Surface	Fluorescence Lifetime
110 00 006	6.5 µm	3D-Carboxy LA	1.7 ns
110 10 006	6.5 µm	3D-Carboxy LA	2.7 ns
110 20 006	6.5 µm	3D-Carboxy LA	5.5 ns
110 30 006	6.5 µm	3D-Carboxy LA	7.9 ns

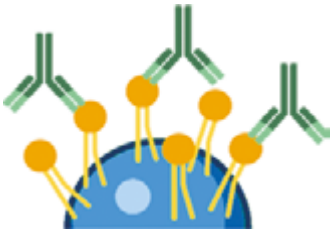
Our PolyAn FLT Beads are also available with 3D-Aldehyde, Streptavidin or Neutravidin, Protein A/G, 3D-Azide, 3D-Alkyne, 3D-DBCO, and 3D-MTZ surfaces. A custom modification with antibodies, peptides, or oligonucleotides is available upon request.

* Images courtesy of Bundesanstalt für Materialforschung und -prüfung (BAM), D. Kage et al., Luminescence lifetime encoding in time-domain flow cytometry. Scientific Reports (2018) 8: Article 16715.

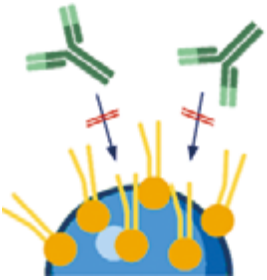
5.2 Hydrophobic Beads

Amphiphilic biomolecules like phospholipids, lipopolysaccharides, and lipoproteins play a key role in many biological processes, and can potentially be used as future biomarkers for the diagnosis of e.g. autoimmune and neurodegenerative diseases, atherosclerosis, diabetes, Alzheimer's disease, or cancer. PolyAn's hydrophobic beads enable the oriented immobilization of amphiphilic molecules via their lipophilic tails. This ensures that the hydrophilic, receptor-containing part of the biomolecule is presented optimally for binding of detector molecules (e.g. antibodies) within bioassays.

PolyAn hydrophobic beads are characterized by a high contact angle with water, and are stable in organic solvents such as methanol, acetone and even chloroform (up to 50% v/v). They allow a directed (oriented) immobilization of lipophilic/ amphiphilic biomolecules from organic solvents.



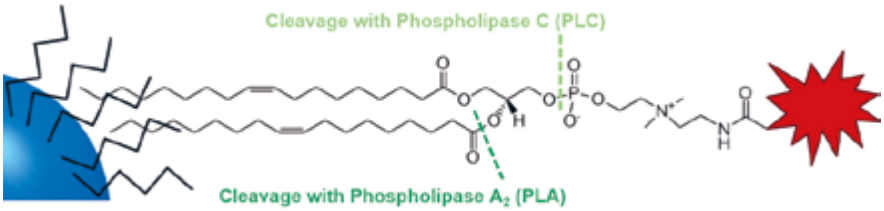
Oriented immobilization enables specific binding

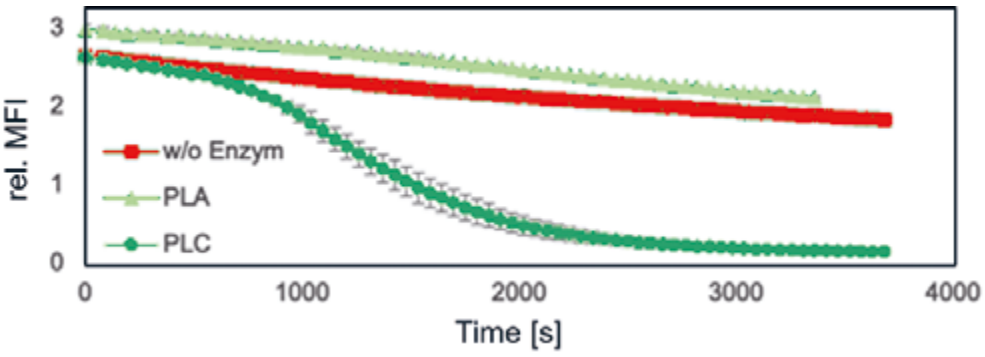


No binding possible due to wrong orientation

User case: Oriented Binding of Phospholipids

PolyAn's hydrophobic beads were applied for the directed binding of cardiolipin, phosphatidyl-ethanolamine, and phosphatidylcholine. Oriented immobilization was confirmed by enzymatic cleavage of the dye-labeled phospholipids: Dye-cleavage was only observed with Phospholipase C, but not with Phospholipase A, confirming oriented binding via the lipophilic tails:



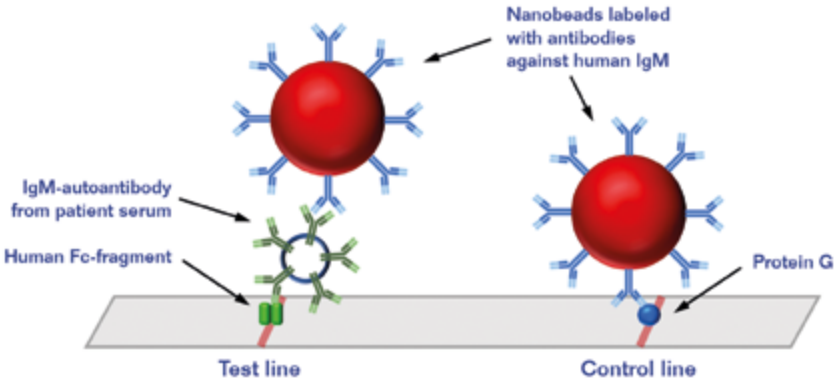


Figures adapted from F. Dinter et al., bioRxiv, 2023, DOI: 10.1101/2023.01.10.523433 (preprint).

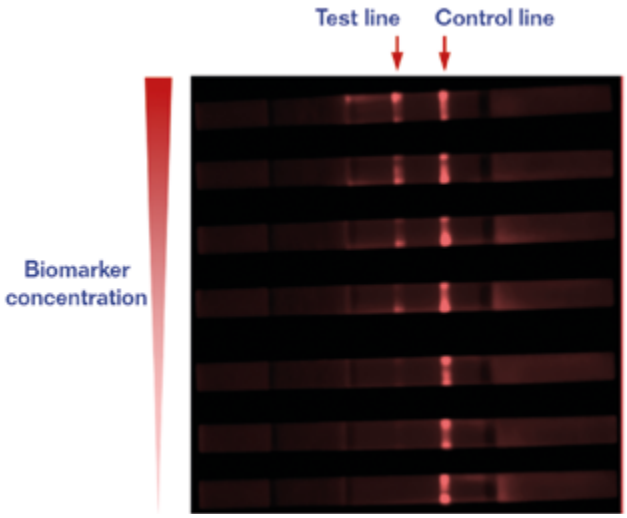
5.3 Lateral Flow Assays

PolyAn's fluorescent PMMA Nanobeads can be applied as reporters in a lateral flow assay (stripe tests) as an alternative to the conventional colorimetric read out using for example gold nanoparticles (colloidal gold).

Application example:
IgM autoantibodies with affinity for the Fc portion of IgG are well-accepted biomarkers for rheumatoid arthritis. Fluorescent PMMA Nanobeads have been used in autoimmune diagnostics to detect IgM autoantibodies in sera from patients with rheumatoid arthritis.



Principle of the Nanobead-based lateral flow assay. Test line: The fluorescence-encoded Nanobeads only accumulate at the test line, when IgM autoantibodies are present in a patient serum and bind to the immobilized human Fc fragment. Control line: The Nanobeads always accumulate at the control line, because of the binding of IgG antibodies to Protein G.



Nanobead-based lateral flow assay strips with different dilutions of serum from a patient with rheumatoid arthritis using a fluorescence scanner (excitation at 640 nm, detection at 700–750 nm).

*C. Schmidt et al., Fluorescence encoded poly(methyl methacrylate) nanoparticles for a lateral flow assay detecting IgM autoantibodies in rheumatoid arthritis. Anal. Biochem. 2021, 633, 114389.

5.4 Agglutination Assays

PolyAn offers functionalized Nanobeads and small Microparticles for agglutination assays.

Application example

Nanobead-based agglutination assay for the discrimination between different E. coli serotypes: 130 nm Nanobeads were coupled to a goat-antibody recognizing E. coli serotype O26. Subsequently, the Nanobead suspension was mixed with E. coli suspension containing serotype O157 (negative control) or O26. The suspensions were placed between a microscope slide and a cover slip. After a 5 min incubation, the suspension was analyzed with a fluorescence microscope at 488 nm.

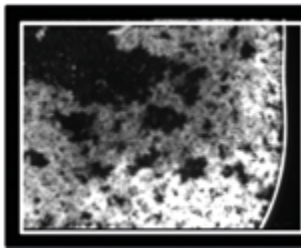
Overview

Nanobead suspension

Negative control

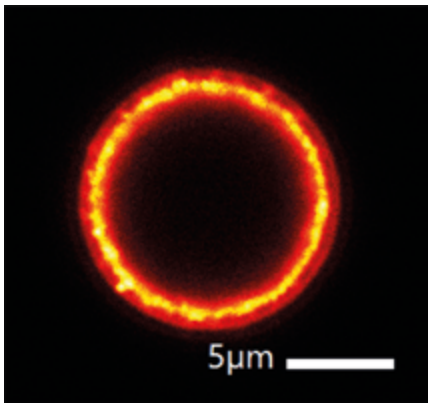
Antibody-labeled Nanobeads with negative control (10x magnification).

Agglutination

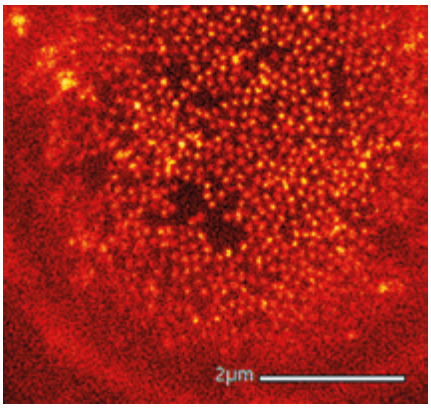


Detection of E. coli serotype O26 with antibody-labeled Nanobeads (10x magnification).

To verify the presence of antibodies on the Nanobead surface, the Nanobeads were mixed with Protein G-coupled microparticles. The binding of the fluorescently labelled Nanobeads on the microparticles was detected using fluorescence microscopy:



Confocal microscopy image of a fluorescent Nanobead corona on a microbead



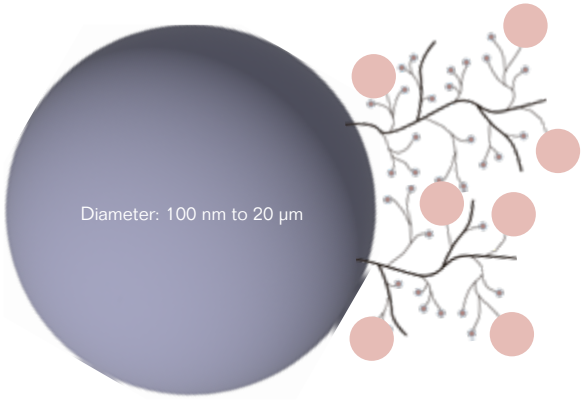
Super-resolution microscopy (STED) image of fluorescent Nanobeads on a microbead surface

*C. Liebsch et al., Solid-phase microbead array for multiplex O-serotyping of Escherichia coli. Microchim. Acta 2017, 184, 1405–1415.

6. Customer Information

6.1 Custom Bead Development

Customized Beads/Bead Populations with individual surface functionalization solutions.
As part of our Molecular Surface Engineering Services, we offer the individual development of beads for specific requirements..



Core/ Bead
— Transparent
— Fluorescent
  • Customized color, excitation/emission
  • Single dye or
  • Multiple dyes
  • Customized fluorescence intensity
  • Fluorescence lifetime
  • Multiplex encoding
  • Calibrated brightness

Shell/Surface
— Unmodified
— Low Aggregation
— Functionalized:
  • 3D-Carboxy
  • 3D-Aldehyde
  • 3D-Alkyne/3D-Azide
  • 3D-DBCO/3D-MTZ
  • 3D-Maleimide
  • Biotin/Streptavidin/Neutravidin
  • Protein A/G
  • Antibodies/Proteins
  • Peptides/Oligonucleotides
— Immobilized dyes

Custom product development is the cornerstone capability from which our products evolve. PolyAn has developed a broad repertoire of bead manufacturing capabilities that meet customer specifications with regards to tolerances, bio-compatibility, and assay conditions.
As a development partner, PolyAn facilitates efficiency and innovation to maximize your capacities in research and analysis. Let us know what you and your company are exploring and we can support you in making that a reality.

6.2 Ordering Information

We are looking forward to your telephone orders and technical enquiries at our Customer Service and Technical Service Department Monday–Friday. Office hours for telephone enquiries are 9:00 AM to 5:00 PM (Central European Time).

PolyAn GmbH
Schkopauer Ring 6
12681 Berlin
Germany

Tel +49 30 912 078 0
Fax +49 30 912 078 11
Email mail@poly-an.de
www.poly-an.de

Ordering Process

After placing your order you should receive an order acknowledgment via e-mail within 1–3 business days. When your products have been shipped, we will notify you via e-mail to provide you with the shipping information, e.g. tracking number.

Shipping and Handling

All prices are Ex-Works PolyAn, Berlin. The products can be shipped via FedEx, UPS, DHL Express or airmail. Please provide your account number, if available.

6.3 International Distributors

Canada, Mexico, USA	AutoMate Scientific, Inc. (USA) Tel: +1 510 845 6283 Email: info@autom8.com axiVEND (Microarray specialist) Tel: +1 833 294 8363 Email: info@axivend.com	Japan	Filgen, Inc. Tel: +81 52 624 4388 Email: support@filgen.jp Waki Company Japan Co., Ltd. Tel: +813 5876 4033 Email: sales@waki-bg.jp
China	APG Bio, LTD Tel: +86 21 545 835 65 Email: info@apgbio.com	Korea	Kyongshin Scientific Co., Ltd. Tel: +82 2 576 6303 Email: kss@kyongshin.co.kr
France	Proteigene SARL Tel: +33 (0)2 32 64 45 45 Email: dutriat@proteigene.com	Netherlands, Belgium, Luxemburg	Bio-Connect B.V. Tel: +31 (0)26 326 4450 Email: info@bio-connect.nl
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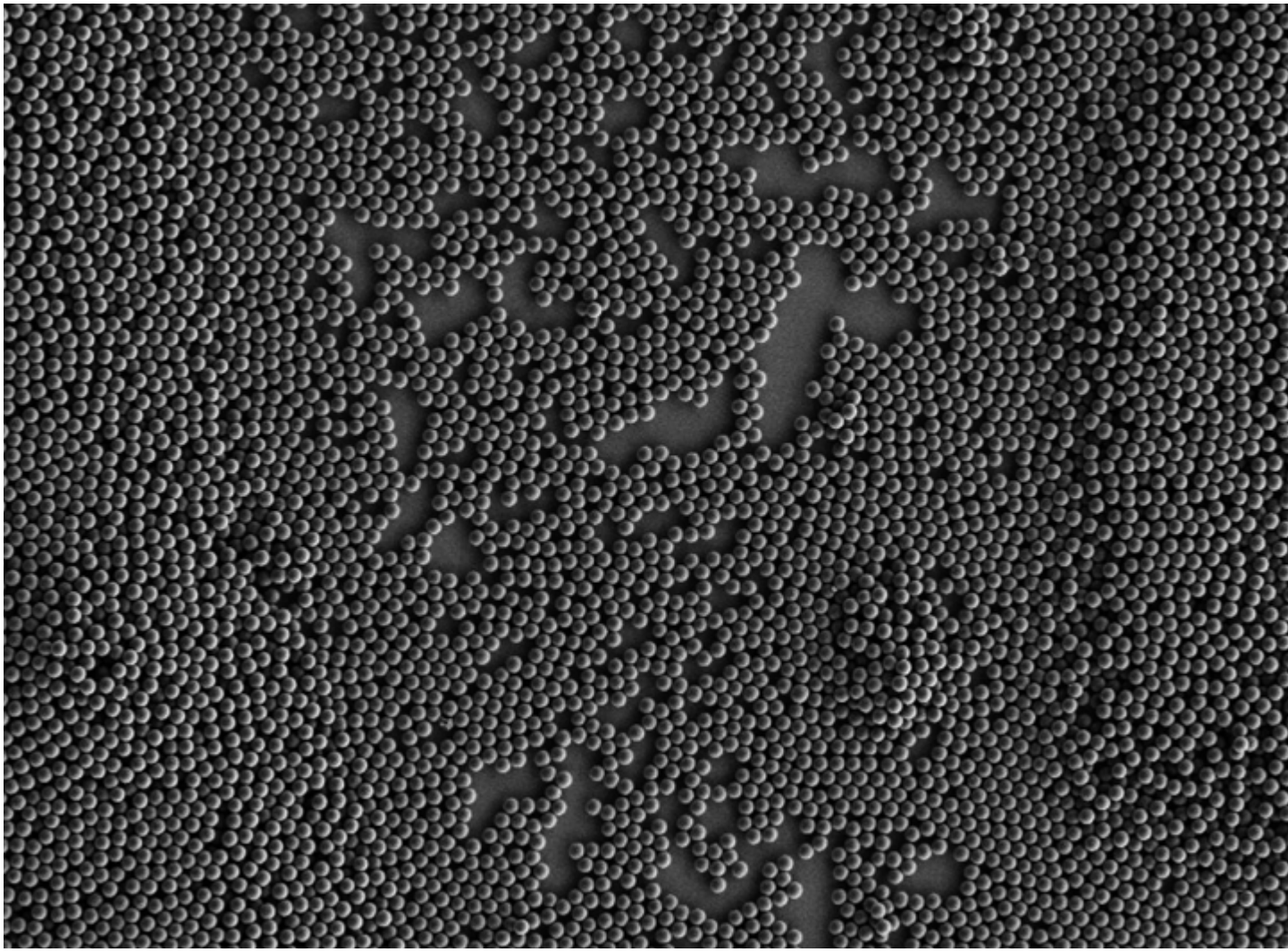

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