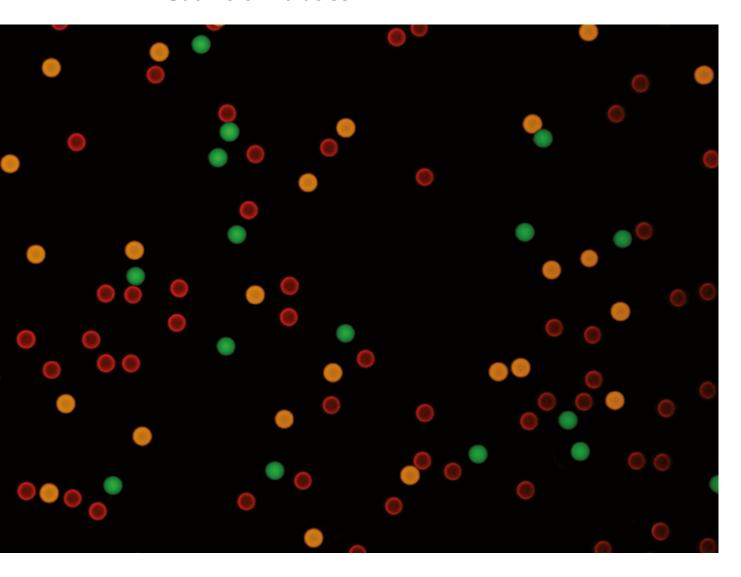


# Polymer Microparticles & Submicron Particles



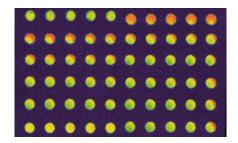


# About PolyAn

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.

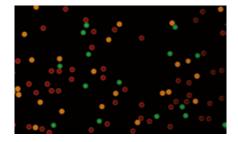
### Functionalized Surfaces for Microarrays

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



# Microparticles & Submicron Particles

PolyAn is offering a portfolio of monodisperse polymethyl methacrylate (PMMA) microparticles (beads) for multiplex bead assays, calibration of flow cytometers, and calibration of fluorescence imaging systems. PolyAn's microparticles 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/conjugation of biomolecules that cannot be immobilized efficiently by passive adsorption. PolyAn offers Amine-binding, 3D-Azide and Streptavidin-coated 96-well plates for challenging ELISA applications.



### Calibration Tools

Re-usable calibration tools for fluorescence based detection systems. PolyAn's calibration slides for cell assays can be used as quality controls in a number of in vitro diagnostics (IVD) systems for immunology applications.



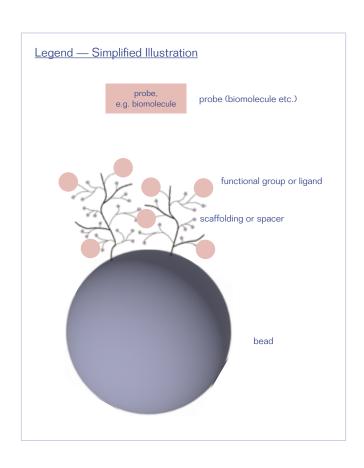
# Molecular Surface Engineering Services

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.



# Index

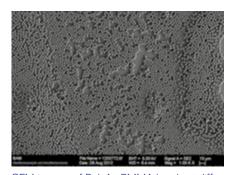
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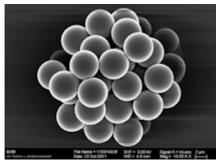


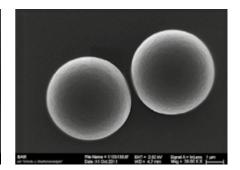
# 1. Microparticles (Beads)

Polymer Microparticle Characteristics

Our polymer microparticles are based on a polymethyl methacrylate (PMMA) core with nanoscale 3D-surface modification.

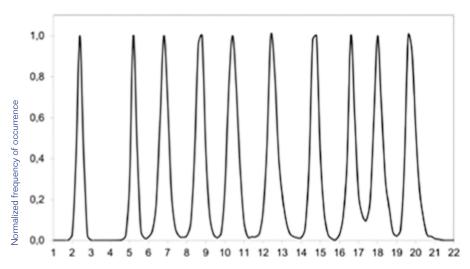






SEM images of PolyAn PMMA beads at different magnifications

Using PMMA ensures an excellent optical brilliance and a low autofluorescence compared to other microparticle materials. The refractive index of 1.48 is close to the refractive index of cells (ca. 1.38). Our microparticles have a density of 1.19 g/cm³ and a glass transition temperature (Tg) of about 110°C. PolyAn uses a biocompatible grade of PMMA.



Particle size distribution [μm]



Escherichia Coli, 1–2 µm\*



HEp2-Cell, 10 μm\*\*

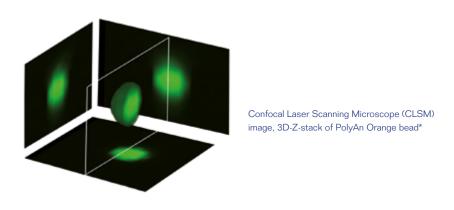


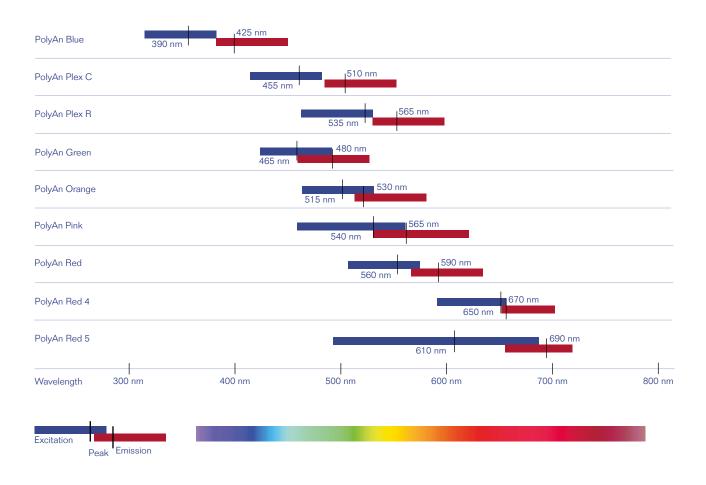
Macrophage, 20 μm\*\*\*

# Fluorescence Encoding Tool Box

With PolyAn's production process the fluorophores are incorporated into the beads during their formation. This ensures a much more homogeneous distribution of the dyes 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.

# Homogeneous Distribution of Fluorophores



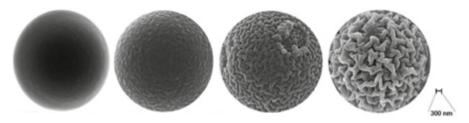


<sup>\*</sup> Image courtesy of Bundesanstalt für Materialforschung und -prüfung (BAM)

# 2. Molecular Surface Engineering for Beads

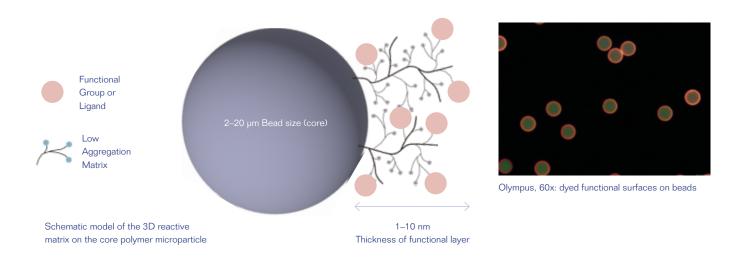
PolyAn's high-performance polymer microparticles are functionalized with a 3D-surface chemistry comprised of a long-chain polymer with a defined number of reactive groups. In contrast to conventional coating procedures, the reactive polymer is covalently linked to the surface.

### 2.1 PMMA Microparticles (Beads)



REM images showing different surface morphologies of carboxylated PMMA beads

By using MSE-technology, a thin polymer shell of a few nanometers, consisting of functional groups, is anchored on the bead surface. This occurs without changing the physical and optical properties of the PMMA core. Our 3D-functionalized particles are suitable for covalent coupling of molecules or for ionic interaction.



PolyAn offers the following surfaces for immobilization of biomolecules and other probes:

- 3D-Carboxy for EDC/NHS mediated coupling
- 3D-Aldehyde for one-step binding of biomolecules
- 3D-Alkyne, 3D-Azide, 3D-DBCO, and 3D-MTZ for click chemistry
- Streptavidin and Neutravidin for coupling of biotinylated biomolecules
- Antibody coupling via direct bioconjugation or via Protein A/G

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 molecular diagnostics and pharma screening.

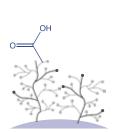
# 2.2 Functional Surfaces on Beads

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

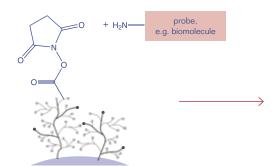
Functional Group or Ligand	<u>Structure</u>	Application examples
3D-Carboxy	ОН	For EDC/NHS mediated coupling of Amine-terminated molecules
3D-Aldehyde	——(N	For Amines, Hydrazines, and Aminoalkoxyacetyl-modified molecules
3D-Alkyne	—с≡сн	For binding of Azide-modified molecules via copper-catalyzed click chemistry
3D-Azide	——N——N <u>===</u> N	For binding of molecules via click chemistry
3D-Cyclooctine (DBCO)		For binding of Azide-modified molecules via copper-free click chemistry
3D-Maleimide		For binding of Thiol-containing molecules
PEG-Biotin	HN NH	For coupling of Streptavidin or Neutravidin conjugated molecules
Methyltetrazine (MTZ)	N=N $N=N$ $N=N$ $N=N$	For fast ligation with TCO-modified molecules
Streptavidin or Neutravidin	×	For coupling of Biotin-functionalized molecules
Antibody		For Antigen binding and detection
Protein A, Protein G, or Protein A/G	A G 3	For binding of IgG Antibodies
Low Aggregation	7	Non-adsorbing matrix, for calibration and controls

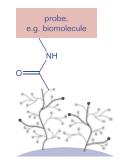
# 3D Carboxy Surfaces for EDC/NHS mediated Coupling

PolyAn's 3D Carboxy microparticles with antifouling behavior are suitable for coupling of proteins, antibodies, and other amine-terminated molecules.









The activation of Carboxyl groups with EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide) and NHS (N-hydroxysuccinimide) leads to highly reactive esters, which can be easily reacted with nucleophiles, e.g. Amines or Hydrazines. The NHS-ester reacts with the  $\rm NH_2$ -groups of biochemical species to form a covalent bond with the surface.\*

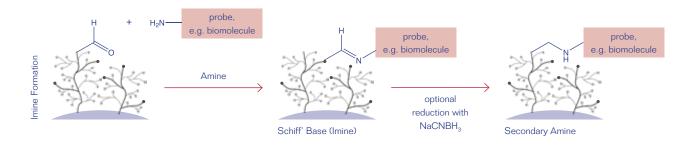
### Key features

- Easy activation using carbodiimide EDC
- Generating NHS surface as an intermediate step, sequential coupling is possible
- Amine-containing biomolecules covalently bind to activated Carboxy surface
- Low concentration of Amine-containing biomolecules is possible
- A stable covalent bond between the surface and the biomolecules is formed, i.e. no leaching can occur

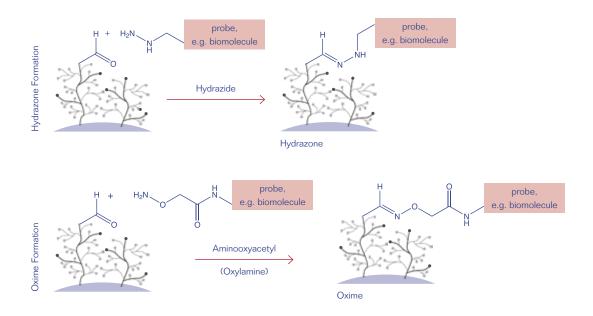
<sup>\*</sup>A. Hennig et al., Scope and limitations of surface functional group quantification: Exploratory study with poly(acrylic acid) grafted micro- and nanoparticles. J. Amer. Chem. Soc. 2012, 134, 8268–8276.

# 3D-Aldehyde functionalized Beads for direct Coupling

Aldehyde groups react immediately with  $\mathrm{NH_2}$ -groups or other suitable functional groups of the probe to form a covalent bond with the surface. Thus, no activation of the bead surface is necessary prior to binding of the probe. The 3D-Aldehyde matrix has an integrated spacer structure to ensure optimal binding conditions. If necessary, the loading with Aldehyde groups can be adapted to the specific application.



### Some Alternative Reactions



### Key features

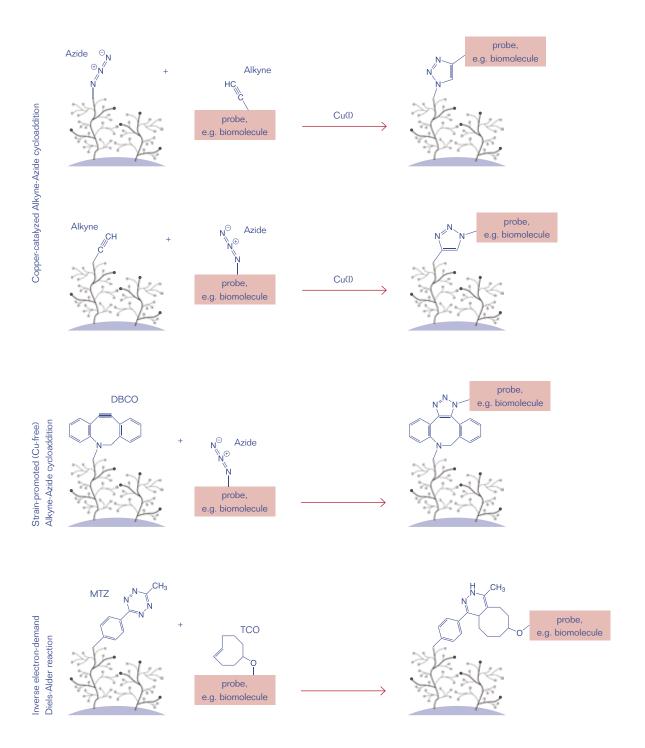
- Aldehyde groups bind to Amines, Hydrazines, and Aminoalkoxyacetyl-modified molecules\*
- No activation of surface with e.g. glutaraldehyde necessary
- Suitable for one-step coupling of biomolecules
- Gentle immobilization without cross-linking of biomolecules
- Attached biomolecules can be stabilized by reducing the imines with sodium cyanoborohydride

<sup>\*</sup>A. Roloff et al., Quantification of aldehydes on polymeric microbead surfaces via catch and release of reporter chromophores. Anal. Chem. 2019, 91, 8827 8834.

# 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, and only minimal and inoffensive byproducts. Bio-orthogonal reactions are conjugation reactions that do not interfere with biological processes. Such reactions are especially useful in chemical biology, 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.

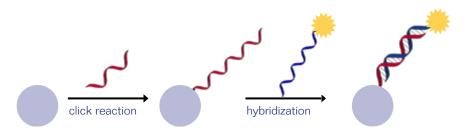


### Key features

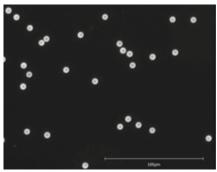
- Alternative to conventional Streptavidin-Biotin coupling
- Less unspecific interactions compared to Streptavidin
- No reactions of Alkynes, Azides, DBCO, or MTZ with regular biomolecule functionalities, e.g. Hydroxy, Amino, and Carboxy groups

# Directed coupling of Oligonucleotides and Peptides

As part of our functionalization services PolyAn is now offering the custom modification of beads with oligonucleotides or peptides.



Fluorescent PMMA beads with capture oligonucleotide immobilized via click chemistry, and hybridization e.g. with a dye-labeled anti-strand



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

 $\frac{Immobilization\ of\ Oligonucleotides\ via\ Click\ Chemistry}{After\ immobilizing\ DNA-type\ oligonucleotides,\ hybridization\ efficiencies\ with\ complementary\ oligonucleotide\ strands\ are\ about\ 90\ \%.}$ 

Bead loading with oligonucleotides was determined by absorption spectroscopy using dye-labeled anti-strands. The successful immobilization and hybridization is also illustrated in the fluorescence image above. Multiple re-hybridizations were successful.

Product ID	Diameter	Color	Surface	Solids Content	Loading
105 27 005	5 μm	transparent	oligonucleotide	0.1 %	20 pmol/mg
105 27 009	9 µm	transparent	oligonucleotide	0.1 %	13 pmol/mg
105 27 020	20 μm	transparent	oligonucleotide	0.1 %	6 pmol/mg

Fluorescence encoded beads with oligonucleotides are also available upon request.

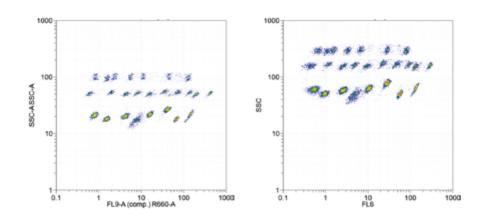
Our functionalization service includes the PolyAn PMMA beads and their custom modification. The final price depends on the oligonucleotide or peptide of choice.

# 3. Multiplex Beads

### 3.1 Multiplex Beads for Suspension Assays

### PolyAn Red4 encoded Multiplex Beads (25-plex)

PolyAn Multiplex Beads provide a platform for the design of multiplexed assays that can be run on standard flow cytometers. PolyAn offers a set of 25 bead populations (25-plex) that can be distinguished both by 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:  $3.5~\mu m$ ,  $5.5~\mu m$  and  $8.5~\mu m$ .



PolyAn Red4 Multiplex Beads: Detection of the Red4 25-plex with different flow cytometers (left: Beckman Coulter CytoFlex-LX, right: Quantum Analysis Quantum-P)

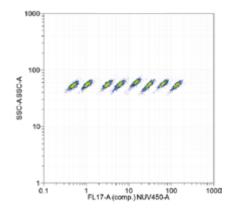
### **Products**

Product-ID	Diameter	Surface	Color	Peaks	Excitation/Emission
106 50 003	3.5 µm	3D-Carboxy LA	PolyAn Red4	8	590–680 nm/660–780 nm
106 50 005	5.5 µm	3D-Carboxy LA	PolyAn Red4	10	590–680 nm/660–780 nm
106 50 009	8.5 µm	3D-Carboxy LA	PolyAn Red4	7	590–680 nm/660–780 nm
106 52 003	3.5 µm	Streptavidin	PolyAn Red4	8	590–680 nm/660–780 nm
106 52 005	5.5 µm	Streptavidin	PolyAn Red4	10	590–680 nm/660–780 nm
106 52 009	8.5 µm	Streptavidin	PolyAn Red4	7	590–680 nm/660–780 nm

Our PolyAn Multiplex Beads are also available with Neutravidin and Protein A/G, as well as with 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 encoded Multiplex Beads (8-plex)

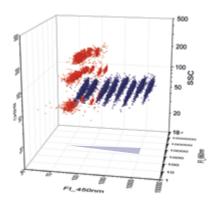
PolyAn offers a set of 8 populations of 5.8  $\mu$ m-sized beads (8-plex) that can be distinguished by the different fluorescence intensities of our PolyAn Blue dye in the DAPI channel (Excitation 350–400 nm, Emission 400–480 nm).

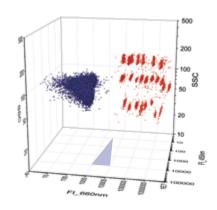


PolyAn Blue 8-plex Beads measured on a BC CytoFlex-LX

### 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 flow cytometric detection of a mixture of PolyAn Blue 8-plex and PolyAn Red4 25-plex Beads using a BC CytoFlex LX (same dataset, two different viewing angles).

### **Products**

Product-ID	Diameter	Surface	Color	Peaks	Excitation/Emission
107 50 005	5.8 μm	3D-Carboxy	PolyAn Blue	8	350–400 nm/ 400–480 nm
107 52 005	5.8 µm	Streptavidin	PolyAn Blue	8	350–400 nm/ 400–480 nm

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

### 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.

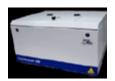
PolyAn offers several variants of bead coding in the field of scanning cytometry:



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).



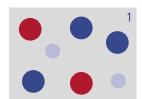
Multiple bead sizes (at least two) in combination with multiple intensity gradations of a fluorophore embedded in the bead (e.g., AKLIDES by Medipan GmbH)

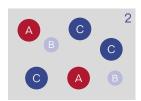


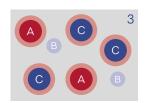
Two different fluorescence encodings, i.e. emission wavelengths, which can be realized by two different fluorophores in different concentrations in one bead size (e.g. Kaleidoscan by Attomol GmbH)

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\*).

# <u>Classification, assignment, and evaluation of multiplex beads</u> 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).

<sup>\*</sup>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.

## 4. Fluorescence Lifetime Beads

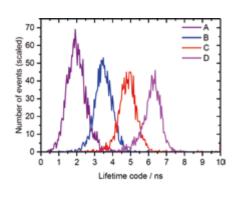
# x/µm 150 50 50 150 150 200

FLIM: FLT encoded beads with ligand fluorescence

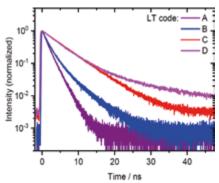
# Microparticles for Fluorescence Lifetime Applications

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

PolyAn FLT Beads







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.

<sup>\*</sup> 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. Calibration Tools

### 5.1 Fluorescence Calibration Slides

- For the routine calibration of fluorescence microscopes
- For automated fluorescence imaging systems, e.g. scanning cytometry

PolyAn's calibration slides are designed for the routine calibration of confocal fluorescence microscopes and other fluorescence imaging systems. They are prepared by mounting statistically distributed monodisperse PMMA beads that contain ultra-stable fluorophores onto standard  $75 \times 25 \times 1$  mm glass slides. The beads are protected from mechanical stress with a coverslip.

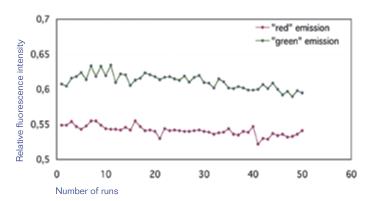
### Available for three different emission wavelengths

- Blue emission channel e.g. DAPI
- Green emission channel e.g. FITC, Cy3®
- Red emission channel 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%</li>
- 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



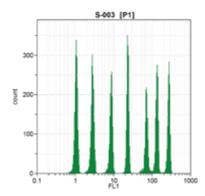
Photostability: slides mounted with "Green" and "Red" emitting beads were measured multiple times over a period of 50 days. The fluorescence intensity after more than 50 measurements exceeded 97% of the initial intensity for both dyes, underlining their excellent photostability.

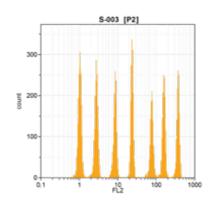
Fluorescence image of a calibration slide (green channel): homogeneous particle distribution, no aggregates.

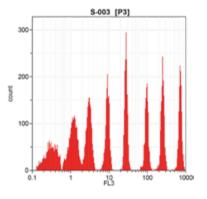
<sup>\*</sup> Cy® is a registered trademark of GE Healthcare.

# 5.2 Spectrum Calibration Beads

PolyAn's Spectrum Calibration Beads are designed for calibration of flow cytometers and other fluorescence imaging systems. Each color-encoded PMMA bead population (peak) contains a mixture of fluorophores that allows performance validation at all wavelengths.

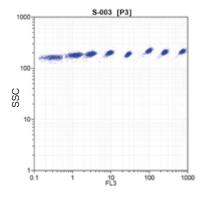






8-peak Spectrum Calibration Beads with increasing fluorophore content for all channels. One transparent population (only in FL3 detectable) and 7 fluorescence encoded populations.

Measurement with QA Quantum P flow cytometer. Excitation laser line at 488 nm.



### Key features

- Contains a mixture of fluorophores that enables the Spectrum Calibration Beads to be excited at any wavelength from 365 nm to 650 nm.
- Fluorophores are homogeneously encapsulated in the PMMA matrix. Special shell prevents leaching of fluorophores.
- Allows the calibration of the FITC, PE, PE-TR, PE-Cy5, and APC channels with the same set of particles.
- Set of up to 8 similar size microparticle populations (peaks) with different fluorescence intensities. The fluorescence intensities in separate channels can be tailored to specific requirements, if needed.

Product-ID	Description
107 02 006	Spectrum Flow Cytometer Calibration Beads, 1 population (peak)
107 01 006	Spectrum Flow Cytometer Calibration Beads, 5 populations (peaks)
107 00 006	Spectrum Flow Cytometer Calibration Beads, 8 populations (peaks)
107 02 010	10 μm, Spectrum Flow Cytometer Calibration Beads, 1 population (peak)

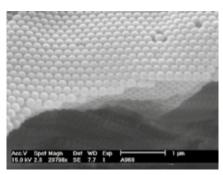
Individual packaging, other sizes and alternative fluorescence intensities (peaks) are available upon request. Please contact our customer service for a custom development.

### 6. Submicron Particles (Nanobeads)

### 6.1 Fluorescent PMMA Nanobeads

PolyAn's PMMA Nanobeads are available in different sizes between 100 nm and 500 nm. They are characterized by a narrow particle size distribution (see SEM image below) and an excellent batch-to-batch reproducibility. PMMA Nanobeads are non-toxic, and thus, suited for live cell applications.

PMMA Nanobeads are available with Carboxy and Aldehyde surface functionalization for immobilization of biomolecules (proteins, antibodies, peptides, oligonucleotides, etc.). PolyAn can also offer plain (unmodified) beads as well as coating services with Streptavidin, Neutravidin, antibodies, and a wide range of other biomolecules.



SEM image of 170 nm PMMA Nanobeads beads forming a photonic crystal

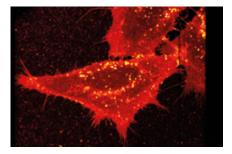


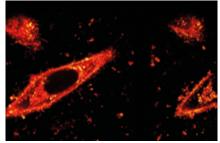
PMMA Nanobeads beads with tunable spectral properties and high fluorescence intensity

#### **Applications**

We offer a large range of different PMMA Nanobeads suitable for particle-based technologies, including:

- Immunoturbidimetric assays
- Nephelometric assays
- Cell staining/compartment staining
- Solid phase immunoassays
- Calibration (e.g. flow cytometry, fluorescence microscopy)





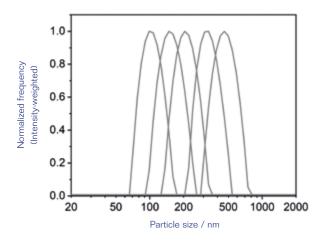
High resolution fluorescence images (STED)\* of cell staining using fluorescent PMMA Nanobeads

<sup>\*</sup> Data and images courtesy of BTU Cottbus-Senftenberg

### Size and polymer

All Nanobeads are comprised of polymethyl methacrylate (PMMA). Using PMMA ensures an excellent optical brilliance and low autofluorescence compared to other microparticle materials. The refractive index of 1.48 is close to the refractive index of cells (ca. 1.38). Our microparticles have a density of 1.19 g/cm³ and a glass transition temperature (Tg) of about 110°C. PolyAn uses a biocompatible grade of PMMA.

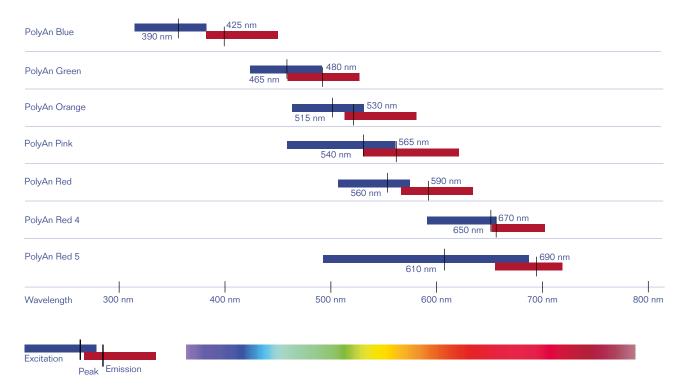
PMMA Nanobeads are available in distinct, monodisperse populations with (mean) diameters between 100 nm to 500 nm as illustrated below.



DLS measurement of differently sized PMMA Nanobeads.

### Fluorescence Encoding Tool Box

Fluorescence encoded Nanobeads are available with a wide spectrum of different fluorophores. Different fluorescence intensities and also combinations of multiple fluorophores in one bead are possible.



### Non-Fluorescent Nanobeads

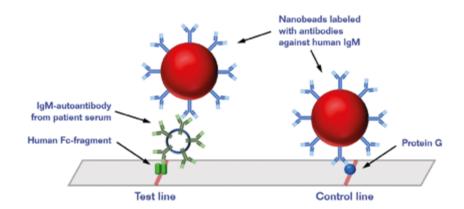
Transparent Nanobeads (without fluorophores) as well as non-fluorescent Nanobeads of different colors for colorimetric applications are available upon request.

### 6.2 Lateral Flow Assays

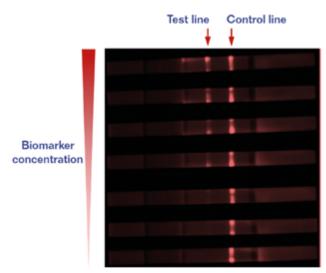
PolyAn's fluorescent PMMA Nanobeads can be applied as reporters in a lateral flow assay (stripe tests) as an alternative to the conventional colorimetric read out using for example gold nanoparticles (colloidal gold).

#### 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).

<sup>\*</sup>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.

# 6.3 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

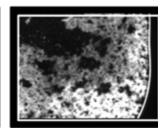
Negative control

Agglutination



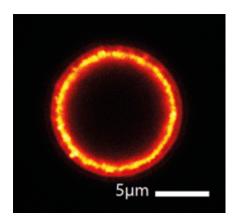


Antibody-labeled Nanobeads with negative control (10x magnification).

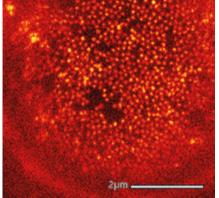


Detection of E. coli serotype O26 with antibody-labeled Nanobeads (10x magnification).

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:







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

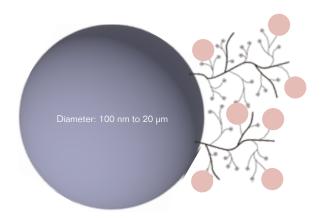
<sup>\*</sup>C. Liebsch et al., Solid-phase microbead array for multiplex O-serotyping of Escherichia coli. Microchim. Acta 2017, 184, 1405-1415.

# 7. Molecular Surface Engineering Services

#### Our service

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

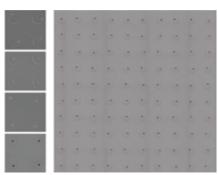
- Transparent
- Fluorescent
  - Customized color, excitation/emission
  - Single dye or
  - Multiple dyes
  - Customized fluorescence intensity
  - Fluorescence lifetime
  - Multiplex encoding
  - Calibrated brightness

#### 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.



Mounted beads on an array slide

# 8. 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 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

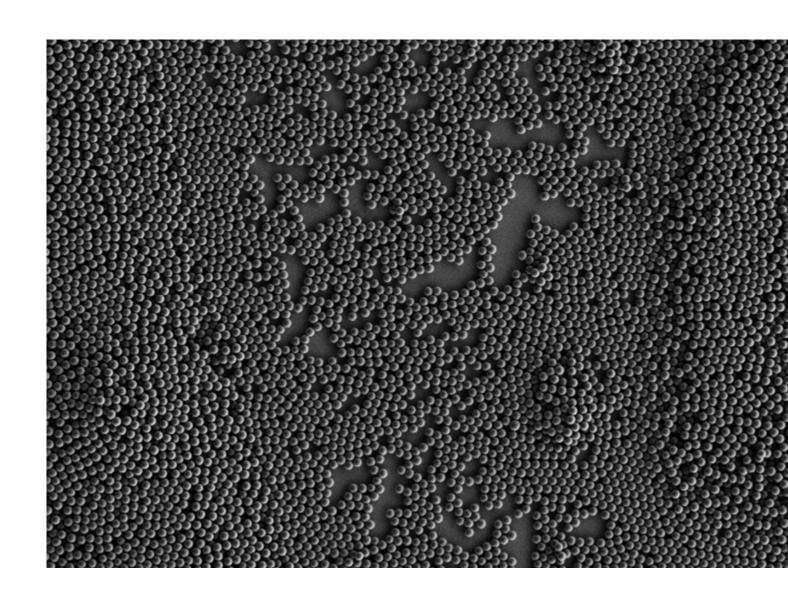
All prices are Ex-Works PolyAn, Berlin. The products can be shipped via FedEx, UPS, DHL Express or airmail. Please provide your account number, if available.



# 8. Distributors

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