

A collection of red, textured spheres of various sizes scattered across the top half of the page. Some spheres are in sharp focus, while others are blurred, creating a sense of depth. They appear to be floating or falling against a light gray background.

Multiplex Analysis with Bead-based Assays

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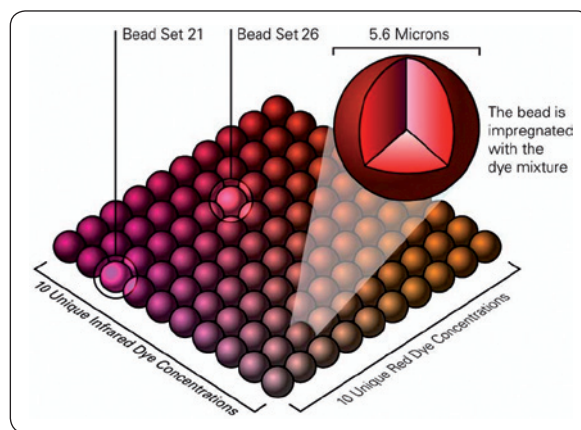
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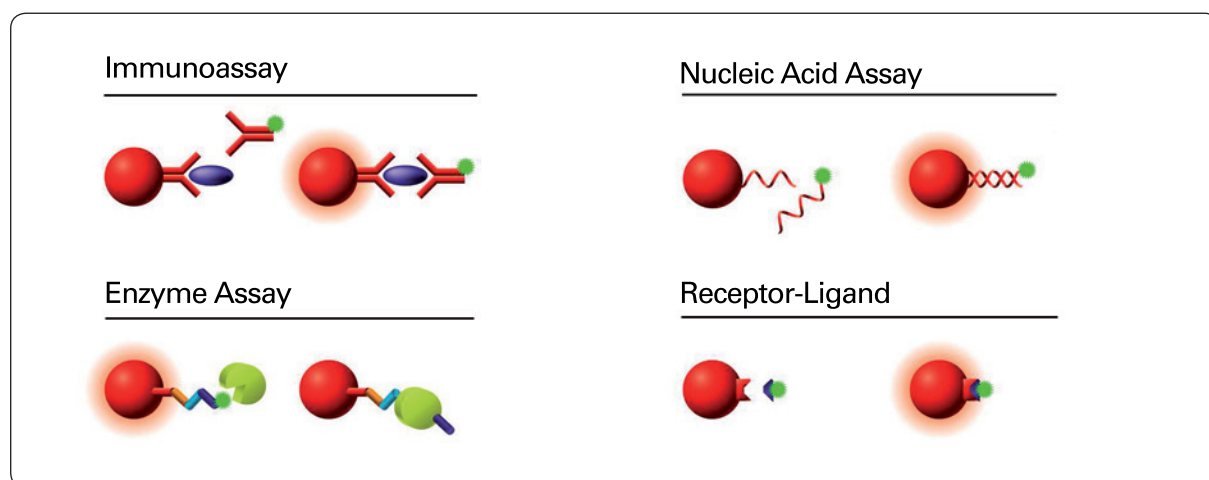
The Principle



Multiparameter assay systems from Multimatrix are based on Fluorescent Bead Arrays (FBA) and allow the simultaneous and quantitative analysis of up to one hundred different parameters in a single sample. Multiplex analyses result in multiparameter profiles with increased analytic significance as compared to conventional test systems. Due to the fact that Fluorescent Bead Arrays from Multimatrix are easy to handle and fully automated, they save time and money even when compared to a single parameter measurement using traditional methods. The technology is based on microscopic spherical polystyrol particles, the so-called microspheres, that serve as a solid phase for molecular detection reactions. Thus, the basic principle of FBAs is similar to ELISA and Western blot. The microspheres are currently available in one hundred different fluorescent color tones of red and infrared, each carrying its own specific detection reagent on the surface. As a result, up to one hundred different detection reactions can be carried out simultaneously in a very small sample volume. Fluorescent Bead Arrays are based on the LabMAP technology of



Luminex Corporation. Due to their high flexibility, Multiplex Bead Arrays can be used to measure all kinds of different analytic parameters that are characterized by specific binding of two defined molecular interaction partners:





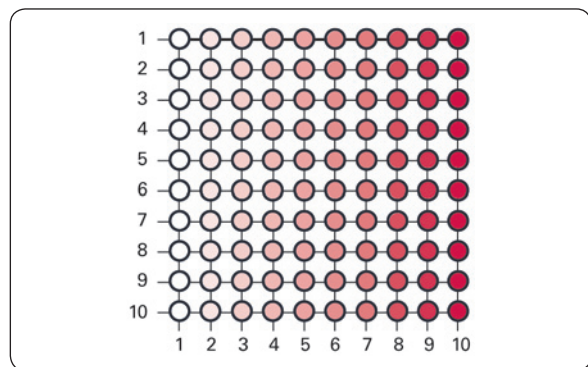
The Technology

Classification – Binding of analytes – Detection of bound analytes



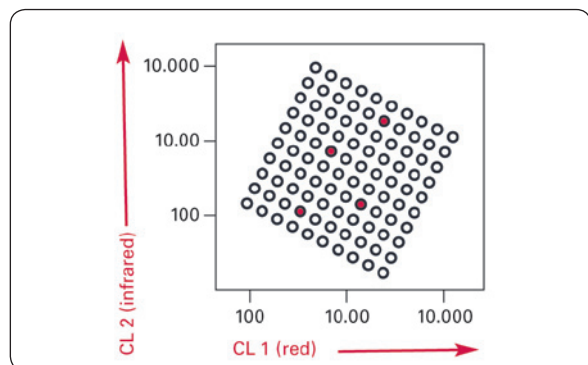
Classification

The microspheres are labeled with two distinct fluorescent dyes (red and infrared) which emit light in different regions of the optical spectrum. The combination of these dyes in ten different concentration steps results in one hundred fluorescent color tones of red and infrared that can be discriminated by their specific spectral properties. Each of the resulting fluorescence intensities uniquely defines a population of microspheres (bead class or „region“). These populations are the basis for a multiplex assay format where each bead class represents a defined test. Fluorescent color coding of the microspheres is the basis for their identification by the analysis system and their precise correlation with their respective population.



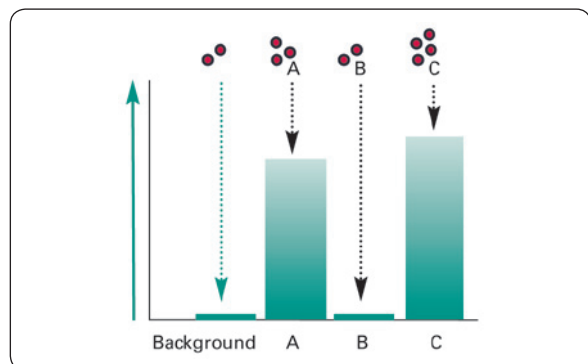
Binding of analytes

Each Multimatrix test kit contains a so-called Bead Mix (a mixture of differently coated microsphere populations). Upon incubation of the Bead Mix with the sample, complementary reaction partners (analytes) in the sample bind to the microspheres in a specific and concentration-dependent manner. As the microspheres can be unequivocally classified by their specific fluorescent color codes it is possible to measure up to one hundred different analytes simultaneously and quantitatively.



Detection of bound analytes

A specific detection molecule (reporter) is used to label analytes that are bound to the microspheres. This reporter molecule shows a high specific affinity to its respective analyte target and carries an additional fluorescent dye that emits in the green region of the spectrum. Since this spectral region is different from the regions of the internal dyes it is possible to perform the classification of the microspheres and the quantitation of bound analytes at the same time.





The Technology

Sample absorption and hydrodynamic focussing – Optics platform

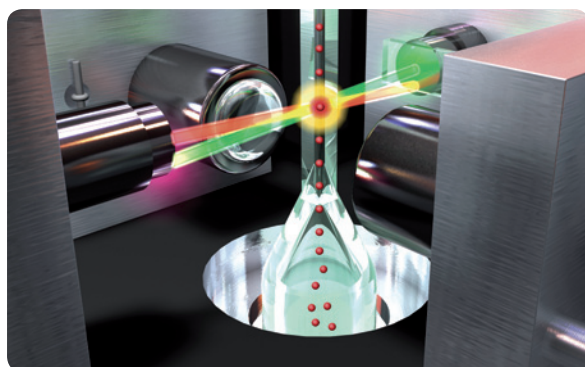


Analysis and evaluation of Fluorescent Bead Arrays is performed with the Luminex analysis system. This system is based on the technology of flow cytometry using two different lasers. Application of the so-called XY-platform allows the fully automated analysis of samples in a 96- well format.



Sample absorption and hydrodynamic focussing

The sample is drawn from a microtiter plate by a syringe pump and transported to the cuvette. During the injection of the sample into the cuvette the microspheres are surrounded by sheath fluid in a way that ensures a laminar flow between the focussed sample stream and the surrounding sheath fluid tunnel. By this process of hydrodynamic focussing the microspheres are singularized and precisely directed through the focus of the laser-based detection system.



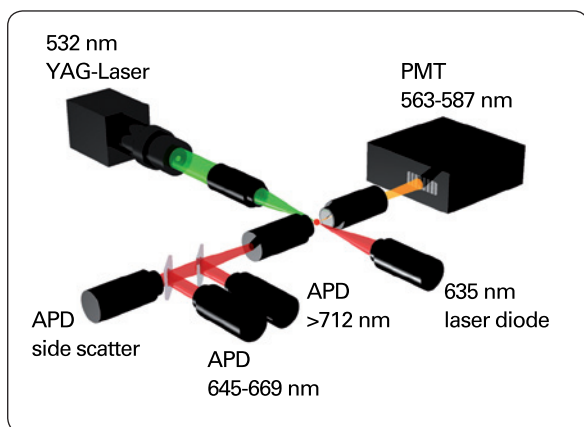
Optics platform

For both the classification of the microspheres and the quantitative detection of bound reporter molecules there are two solid-state lasers installed on the optics platform of the analysis system. Both laser beams are aligned to the center of the focussed particle stream inside the cuvette. The fluorescence signals that are emitted in a direction perpendicular to the two laser beams are directed to the respective detectors via mirrors and filters.



The Optics

Optics platform – Quantitation – Signal processing



Optics platform

For both the classification of the microspheres and the quantitative detection of bound reporter molecules there are two solid-state lasers installed on the optics platform of the analysis system. Both laser beams are aligned to the center of the focussed particle stream inside the cuvette. The fluorescence signals that are emitted in a direction perpendicular to the two laser beams are directed to the respective detectors via mirrors and filters. The classification of the microspheres results from the signal ratio of two photodiodes. In addition to the bead classification the laser diode is used for determination of the side

scatter signals of the particles which correlate to the particle size. The side scatter signal is also detected by an avalanche photodiode. Taking the side scatter into account prevents signals from aggregated microspheres (e.g. doublets) or fluorescent contaminants from being included in the results.

Quantitation

An Yttrium-Argon-Germanium solid-state laser (532 nm) which excites the fluorescent dye of the reporter molecules is used for quantitation of the analytes. The intensity of the emitted light correlates with the amount of analyte that is bound to a particular microsphere. A photomultiplier tube (PMT) is used for signal detection in order to allow a high sensitivity. According to the detection window of the PMT fluorescent dyes with emission maxima between 563 and 587 nm are used for labeling of reporter molecules.

Signal processing

Fluorescence signals from the microspheres are detected both by photodiodes (classification, size exclusion) and the photomultiplier tube (quantitation). Within the analysis system the signals are digitized and transmitted to the signal processor where the digital signals are amplified and processed. The Luminex analysis system is able to process and record up to 20,000 events per second.



Multiplex Detection Reactions



Test reagents from Multimetrix contain a mixture of different microsphere populations (Bead Mix). Each of these populations is characterized by a specific red fluorescent color tone and a capture reagent (A, B, C) on the surface.

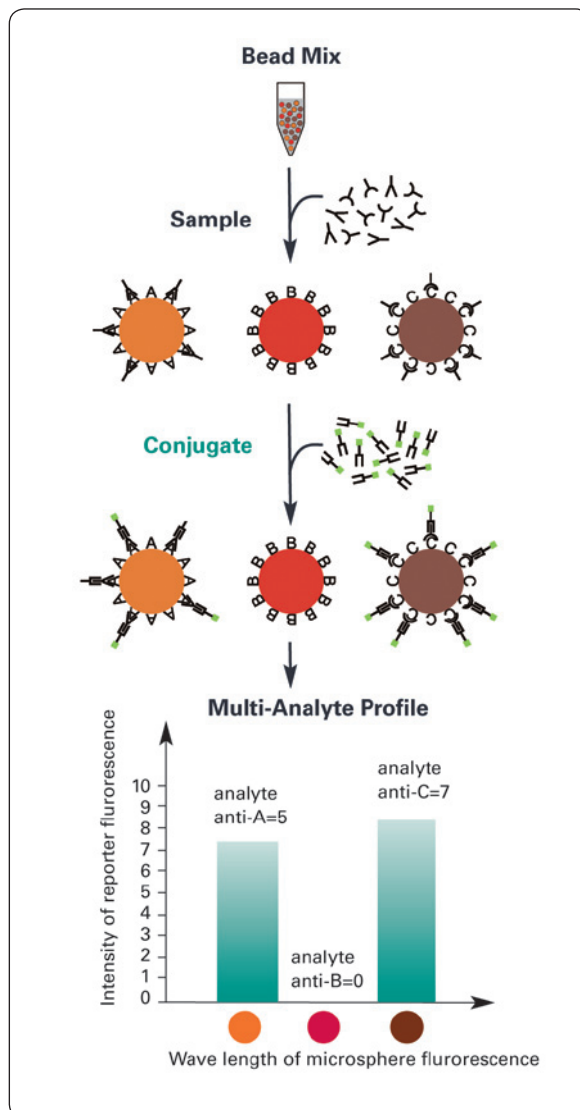
When incubated with a few microliters of a patient sample each microsphere population captures its specific target molecules (analyte) from the liquid phase. The analytes that are bound to the surface of the microspheres can be labeled using a green fluorescent labeling reagent (conjugate).

Within seconds thousands of microspheres are analyzed individually for their red (analyte classification) and green (reporter quantitation) fluorescence using a Luminex analysis system. The data resulting from multiplex detection reactions are automatically processed and evaluated by the analysis system.

The results are represented by multianalyte profiles that provide an analytic value which is superior to any single parameter measurement.

Bead-based Assays are particularly suitable for high-through-put applications in the fields of

- Laboratory diagnostics
- Pharmacological Screening
- Quality control
- Expression profiling
- SNP analysis





Advantages of the Luminex Technology



The technology of bead-based assays combines the sensitivity of the ELISA with the specificity of the Western blot. In addition, such assays allow exact quantitation and fully automated analysis of large numbers of samples.

That's new!

- The application of fluorescence-based analysis combined with the multiplex format allows highly sensitive quantitation of various analytes in very small sample volumes.
- The combination of different measurement ranges and detection principles for simultaneous quantitation of different analytes in a single test demonstrates the enormous flexibility of bead-based assays.
- The hydrophilic properties of the microspheres prevent unspecific binding of proteins and thus allow immunological tests with excellent signal-to-noise ratios and very low detection limits.
- Multiparameter assays based on the Luminex technology allow the fully automated test performance and data analysis.

- 1. Exacte Quantication**
- 2. Sensitivity of ELISA**
- 3. Specifity of Western Blot**
- 4. Automated Analysis**

The combination of these characteristic features results in bead-based assays that provide you with an unprecedented analytical accuracy and efficiency and at the same time save your money!



Products and Services



We develop multiplex reagent kits for diagnostic and bioanalytic applications.

The Luminex analysis system and our product portfolio for use in diagnostic and research is available through our competent distribution partner PROGEN Biotechnik GmbH.

We develop and produce customized solutions for detection of analytes according to your specifications. Furthermore we offers a testing service on customer demand.

- Detection of infectious diseases
- Detection of cytokines, chemokines and growth factors
- Quantitative gene expression analysis
- HPV genotyping

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