



A multiparametric separation free bioaffinity assay method based on two-photon fluorescence excitation

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Abstract

We present a concept for multiparameter separation free microvolume bioaffinity assay. In this study a two-parameter immunoassay is shown. The concept is based on the use of two-photon excited fluorescence and several categories of polystyrene microspheres as a solid phase for bioaffinity binding reaction.

Measurements are performed by observing individual microspheres in the diffraction limited focal volume of two-photon excitation. The method is suitable for measuring bioaffinity binding assays directly from the reaction volume without separation and washing steps.

Instrument and immunoassay

The optical schematic of the instrument, TopDoc, is shown in Fig 1. The two-photon excited fluorescence is generated using a passively q-switched microchip Nd:YAG-laser. The laser is producing <1 ns pulses at 1064 nm with ~20 kHz repetition rate. The laser beam is focused into a cuvette with a microscope objective lens of N.A. 0.65. The fluorescence in range 540-750 nm is divided into three channels and measured in coincidence with back scattering signal of the microspheres. One fluorescence channel is used for measurement of bioaffinity signal and the other channels for microsphere identification signal. As the scattering signal appears, the scanner unit is stopped, and the microspheres are actively trapped into the focus of laser beam.

For two-parameter immunoassay measurement, a combination of alpha-feto-protein (AFP) immunoassay and 0-control was chosen. AFP assay is utilising 3.1 µm carboxy-modified polystyrene microspheres as solid phase carriers. Monoclonal mouse anti-AFP IgG molecules are covalently coupled to the microspheres. As a tracer the same IgG molecules are labelled with dipyrrometheneboron difluoride dye having single photon emission maximum at 580 nm. 0-control microspheres are passively coated with albumin. Analyte, reagent, 0-control and anti-AFP-IgG coated microspheres are mixed simultaneously and incubated in prior to measurement in the same reaction cuvette. The 0-control indicates the unwanted non-specific binding of AFP and tracer molecules. The standard curve of the AFP-assay is shown in Fig 2.

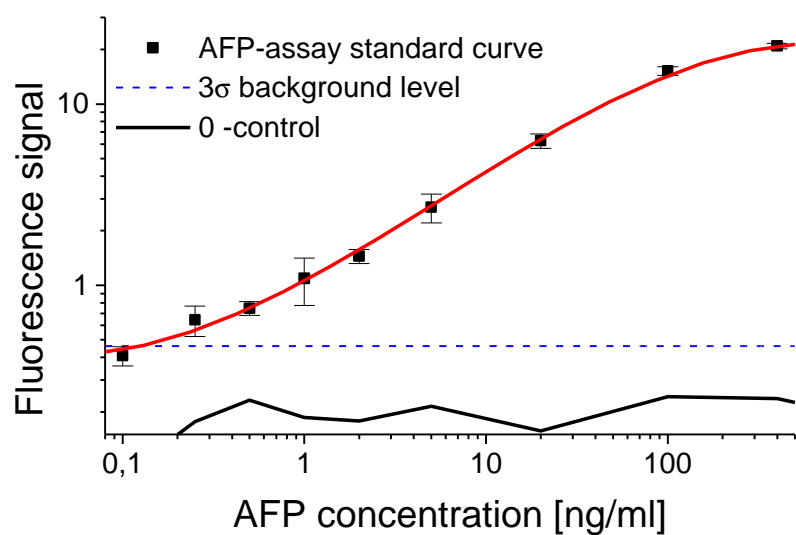


Fig 2. Standard curve of AFP-assay

The anti-AFP-IgG coated microspheres are doped with an identification fluorochrome having emission maximum at 650 nm, whereas the 0-control microspheres do not contain any identification fluorochrome. During the measurement, both the tracer fluorochrome and the identification fluorochrome are simultaneously excited using the same laser. Measurement results, shown in Fig 3 as a dot plot, demonstrates the capability of the system to measure signals from two different microsphere categories simultaneously.

References:

Pekka Hänninen, Alekski Soini, Niko Meltola, Juhani Soini, Jori Soukka and Erkki Soini, *A new microvolume technique for bioaffinity assays using two-photon excitation*, 2000, *Nature Biotechnology*, 18, 548-550

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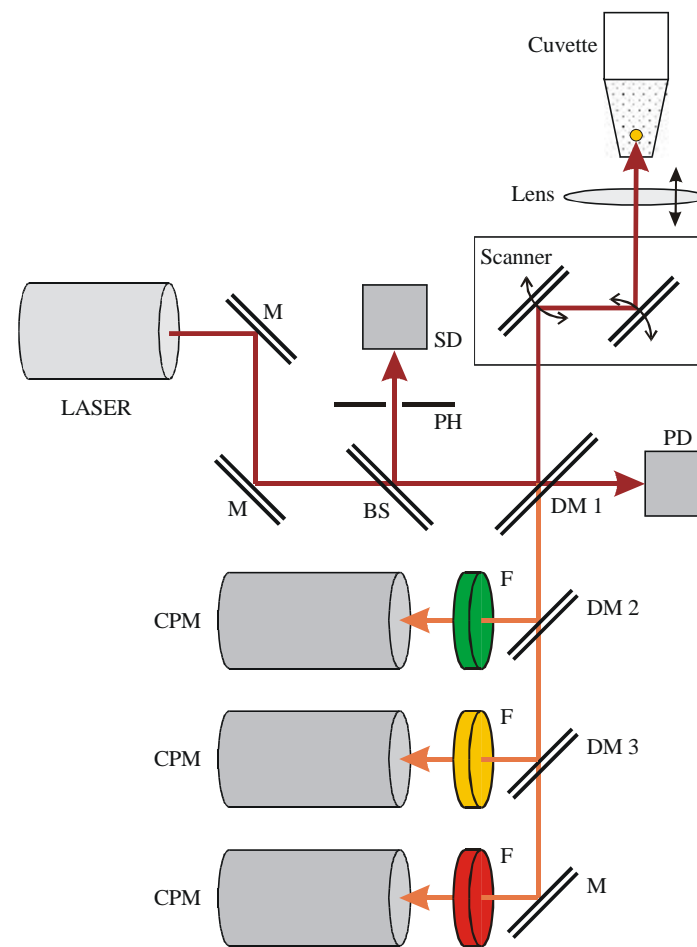


Fig 1. Optical schematic of the instrument

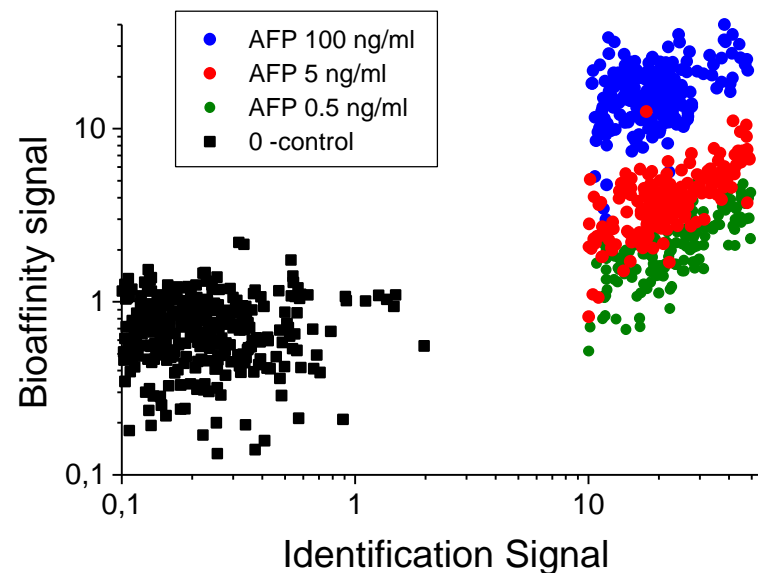


Fig 3. Overlaid data of 3 different AFP immunoassays and corresponding 0-controls.

Conclusion

The method based on two-photon fluorescence excitation makes possible fast single step, separation free two-parameter immunoassays. The method allows a separation free assay in very small volumes, i.e. less than 1 µl, can be extended to measure three or more parameters simultaneously. The method is very useful also for high throughput screening assays.