A micro volume bioaffinity DNA assay based on two-photon excited fluorescence

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Abstract

We have developed a bioaffinity assay concept based on the use of two-photon fluorescence excitation. In this new assay concept the solid phase microspheres can be observed individually directly from the reaction suspension without any separation step. Once a microsphere is identified in the focal volume of the two photon excitation by the optical system, the signal emitted from microsphere bound label molecules is measured. This signal is orders of magnitude stronger as compared to the signal emitted by the free fraction of the label in the surrounding solution.

Instrument and assay

The optical scheme of the instrument is shown in **Fig 1**. The beam of a pulsed Nd:YAG-*laser* is focused tightly into a *cuvette*. The laser generates two-photon excited fluorescence emission in the focus of the laser beam. The fluorescence emission is formed only in the clearly restricted 3-dimensional vicinity of the focal point. The fluorescence is measured with a photomultiplier tube (PMT).

When the assay is based on the use of microspheres, the fluorescence is measured in coincidence with back scattering signal of a microsphere in the focal point. The model microsphere assay is utilizing 3.2 µm streptavidin coated polystyrene microspheres as solid phase (**Fig 2**). Microspheres in $6*10^6$/ml concentration, biotin-oligo DNA dilution and 5 nM TAMRA-labeled DNA probe were mixed simultaneously and incubated at room temperature prior to measurement in the same reaction cuvette.

In liquid phase DNA measurements, we have used intercalating dyes for detection of double stranded DNA (dsDNA). These dyes show enhanced fluorescence emission when bound to dsDNA.

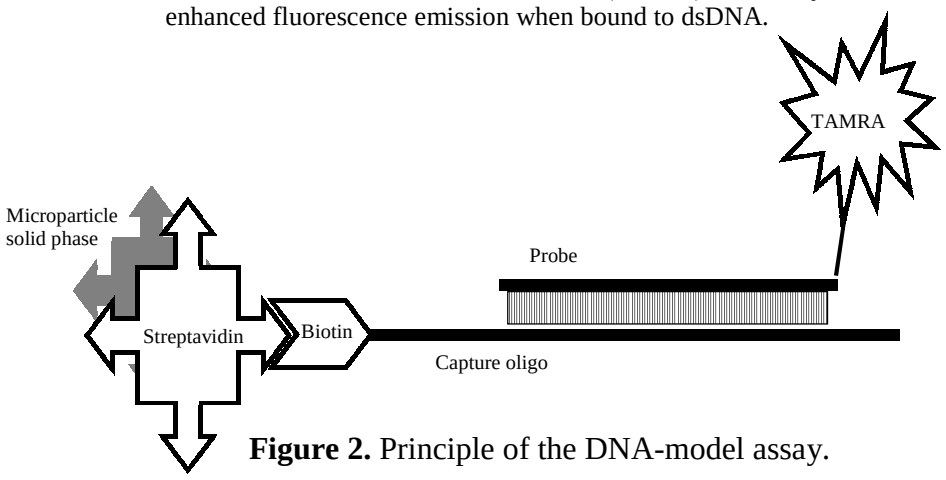

Figure 2. Principle of the DNA-model assay.

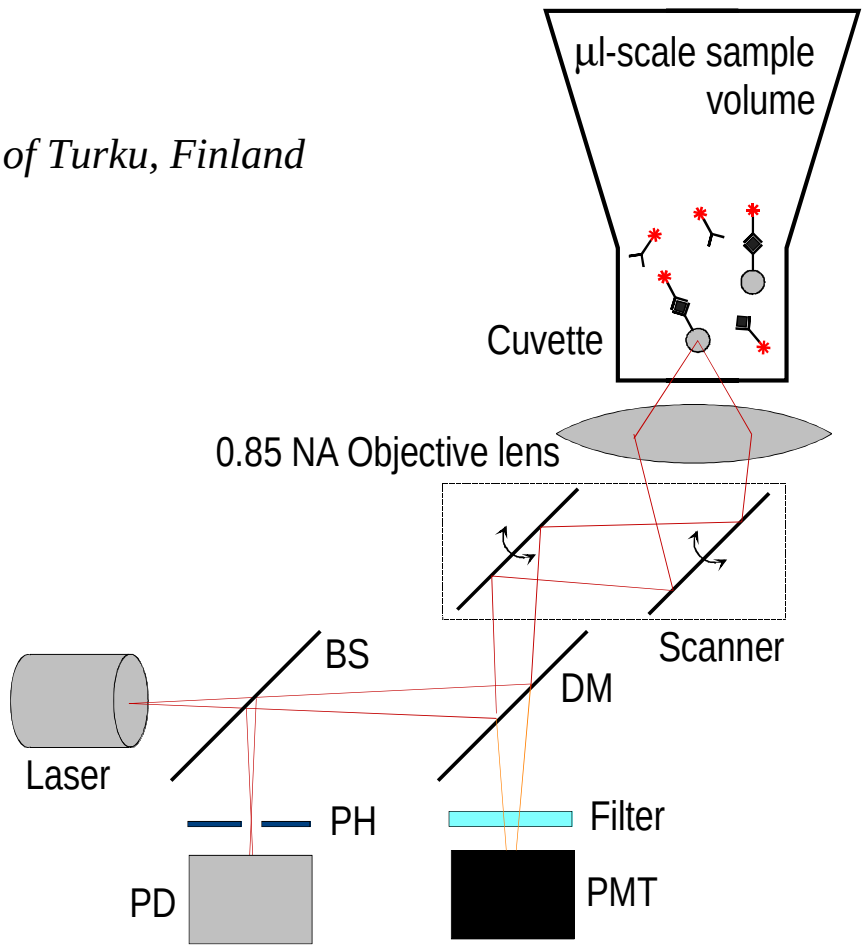

Figure 1. Optical schematics of the instrument

Results

A dilution series of dsDNA in the presence of SYBR Green I® is presented in **Fig 3**. The signal was not dependent on the measurement volume. With SYBR Green I®, the detection sensitivity was 10 ng dsDNA/ml, corresponding as little as 20 pg in 2 µl.

The standard curve of the microsphere DNA-assay is shown in **Fig 4**. Each point of the curve represents an average of around 100 microspheres. The detection principle enables direct scaling of the assay to sub-microliter volumes.

Conclusion

This concept makes possible to perform fast single step separation free bioaffinity assays in micro volumes, and is thus suitable for a vast number of applications involving real time monitoring of bioaffinity binding reactions. Consequently we believe that two-photon fluorescence excitation will make a remarkable impact as a research tool and a routine method in many fields of bioaffinity assays.

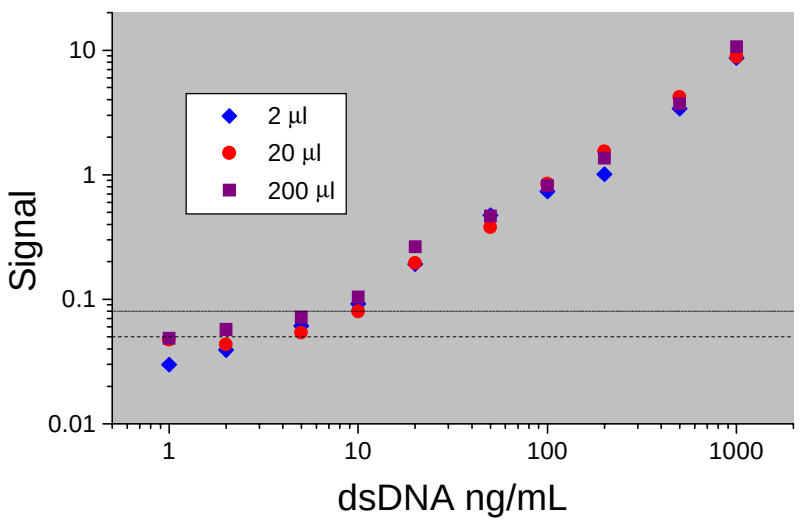

Figure 3. Measured DNA-assay standard curve.

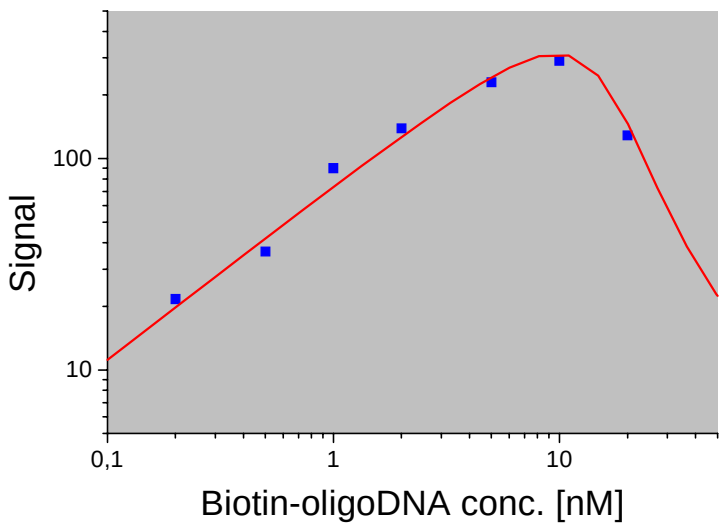

Figure 4. Measured DNA-assay standard curve.

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