More and more biosensor arrays for assaying gene expression are being developed, where mRNA and DNA samples are assayed by selective hybridization. The most widely used method for detecting the hybridization is by measuring the fluorescence signal of the fluorescently labeled target molecules. However, the sensitivity of fluorescence methods limits the sensitivity of the assays, therefore samples need to be amplified and purified in often very time consuming processes.

We propose a new hybridization detection method where single hybridization events can be detected using a simple setup compared to other single molecule methods [1]. Using darkfield microscopy, the consecutive positions of a small highly-scattering gold nanoparticle (40-100 nm) can be determined. This approach does not suffer from bleaching and sensitivity issues since it does not involve a fluorescent label. The nanoparticle is tethered to a glass surface using a DNA molecule. Due to the surrounding solution, it will exhibit (constrained) Brownian motion. By recording the position distribution of the nanoparticle over a certain time interval, the characteristic diffusion constant \( D \) can be obtained from:

\[
\langle x^2 \rangle = 2nD\Delta t
\]  

Where \( \langle x^2 \rangle \) is mean square displacement of the particle, \( n \) is the number of spatially (observed) dimensions and \( \Delta t \) is the elapsed time. After hybridization with a target molecule, the mechanical properties of the tether molecule will change, resulting in a change of the observed diffusion constant. With this method single hybridization events can be detected without time consuming preprocessing steps. Computer simulations help determine the statistical and systematic error of the estimation of the diffusion constant, such that the sensitivity of the detection can be evaluated [2].

In a subsequent step, multiplexing of the assay can be achieved by creating an array with nanoparticles tethered by DNA molecules with varying sequence. Furthermore, an ensemble of tether molecules can be used for measuring (low) concentrations of the target molecules.

REFERENCES