Abstract

Novel scientific instruments are required to apply post-genomic era data in medicine and biotechnology. Here we propose a high-sensitive nanosensor-based technique to quantify mRNA abundance form minute sample, even single cells. Quantification of multiple nucleic acid targets is used in many biological and biomedical applications, for example pathogen detection and identification as well as gene expression quantification in cancer diagnostics, tumor profiling and drug design. Currently quantitative Polymerase Chain Reaction (qPCR) is a "golden standard" for quantification of nucleic acids; however, its implementation to some biomedical application such as tumor profiling is limited due to insufficient level of multiplexing. In this project, we propose a technique which combines the powers of two widely used approaches came from molecular biology and nanotechnology: target-specific multiplex exponential amplification with 10-15 cycles of PCR followed by high-sensitive single-molecule detection with Atomic Force Microscopy (AFM). Note that amplicons are distinguished by their sizes so the level of multiplexing may be up to tens targets: as a single-molecule technique it has orders of magnitude higher sensitivity (1000x) compared to bulk fluorescent techniques such as microarray and capillary electrophoresis; no fluorescent dyes or any other types of labeling are used thus reducing the complexity and cost of the analysis. We demonstrated the application of our technique to the measurement of the expression level of 10 human genes in different samples. This technique can also be used in any case to quantify multiple nucleic acids targets such as copy number variation, splice, gene translocation and other genetic variations or a combination of them in a single tube at down to the single-cell level.