Quantitative Evaluation of Oligonucleotide Surface Concentrations Using Polymerization-Based Amplification

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Quantitative evaluation of biomolecule concentrations is becoming increasingly necessary due to the expanding discovery and characterization of potential disease markers and their corresponding applications in molecular diagnostics. Quantitative, sequence-specific analysis of polynucleotide biomarkers potentially allows for the monitoring of bacterial or viral loads, drug efficacy evaluation based on monitoring viral burden, elucidation of various disease states and progressions, and may provide clinical validation of a diagnosis. Specifically with application to human cancers, several types of nucleic acid biomarkers require quantitative analysis, such as evaluation of over or under expressed RNA transcripts, micro RNAs, or analysis of genomic amplifications or deletions. Mutation detection, traditionally performed on a qualitative basis, can also benefit from quantitative analysis through determination of wild type to mutant type ratios enabling distinction between stochastic and pathogenic mutations.

Polymerization-based amplification has been demonstrated to offer rapid, sensitive, and visual detection of the presence of labeled DNA in a multiplex biochip format. To access the feasibility of this method as a quantitative tool, the amount of polymer growth achieved from amplification of such Bio-recognition events as a function of surface-bound, labeled DNA concentration is evaluated. The approach taken involves fabricating aminosilated glass surfaces with oligonucleotide spots containing controlled concentrations of immobilized, biotinylated DNA targets capable of capturing amounts of Streptavidin (coupled to the initiator, Eosin) followed by contact with a monomer solution and a discrete light exposure. The thickness profile that results shows a dynamic (50 to 4800 biotin markers/micrometer^2) and a saturated region (6,000 to 15,000 biotin markers/micrometer^2). Efforts made to characterize the saturated region by shifting it to a dynamic and quantifiable response resulted in broadening of the dynamic range from 50 to 18,000 biotins/micrometer^2.

Eosin is a weak fluorophore and the fluorescent signal obtained after fabrication of slides and before polymerization-based amplification, can be used to quantify the binding events between the biotinylated oligonucleotide and the Streptavidin-initiator molecule. Incorporation of an amplified fluorescent, radiological, or chemiluminescent response with polymer film growth is desirable due to the numerous types of inexpensive detection instrumentation consistent with this method when signals are large. In an effort to develop this amplification method towards compatibility with fluorescent-based methods of quantification, incorporation of fluorescent nanoparticles into the polymer films is also evaluated. Polystyrene microspheres that encapsulate multiple hydrophobic fluorophores per particle, which prevent non specific initiation as a result of elimination of contact with the surrounding environment, are used. The resulting amplified fluorescent signal enables detection and quantification using inexpensive CMOS-based fluorescent readers as opposed to expensive laser-based microarray scanners employing PMT detectors.

As a concluding remark, the photoinitiator density is shown to be a crucial parameter in determining the amount of polymer growth observed from the surface, as this parameter can be manipulated depending on binding conditions to tune the amount of amplification generated from a given number of biotinylated targets localized on a biosensor surface. The visible light system is amenable to obtaining an amplified fluorescent response correlating with film thicknesses, enabling evaluation of biomarker quantities with inexpensive fluorescent instrumentation. The large, dynamic

variation in film thicknesses and amplified fluorescent signal obtained from polymerization-based amplification opens up many potential uses for this assay that were previously unachievable from only a positive/negative response. For example, background polymer growth arising from non-specific biomolecule interactions can be characterized such that only an increase over this background becomes significant. This gives polymerization-based amplification potential use in applications where both high specificity and sensitivity are critical, such as in a high-throughput SNP analysis. Similarly, markers that become significant only when deviating from characterized background levels can also be analyzed, which is the case for the majority of proteomic biomarkers.