RNA expression profiling at the single molecule level

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We present a microarray analysis platform, which enables detection of hybridized DNA sequences at the level of single molecules. Fluorescence detection is performed on an ultra-sensitive biochip reader. Oligonucleotide microarrays were printed on custom-made aldehyde-functionalized glass coverslips (UAR). The platform was evaluated by hybridizing fluorescent 60mer oligonucleotide to its complementary sequence covalently immobilized on the biochip surface. The System shows a linear dependence on the concentration over 7 orders of magnitude. The Dynamic Range, dependent on the unspecific binding of sequences on non-complementary spots, reaches 4.7 orders of magnitude. Furthermore we analyzed mRNA expression of HaCat cells by hybridization of reverse transcribed cDNA out of 200ng total RNA.

Such wide range in detection sensitivity needs reliable methods for exact data quantification. At low concentration the signal of each spot was quantified by counting the molecules; additionally the brightness of individual molecules was estimated by fitting a 2-dimensional Gaussian function. For high concentrations, the number of molecules per spot was inferred from the total signal per spot.

Good correlation with experiments on commercial microarrays using hundredfold higher sample amounts indicates the feasibility of this approach, which avoids application of error prone amplification methods.

This study was supported by the GEN-AU program of the Austrian Federal Ministry of Education, Science and Culture, by the state of Upper Austria and by the Research focus “Life Sciences and Health” of the University of Salzburg.

References

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