

# Machine-learning-based Single-molecule Quantification of Circulating MicroRNA Mixtures

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MicroRNA (miR) are small non-coding RNAs that regulate gene expression and are emerging as powerful indicators of diseases. MiRs are secreted to blood plasma and thus may report on systemic aberrations at an early stage via liquid biopsy analysis. We present a method for multiplexed single-molecule detection and quantification of a selected panel of miRs. The proposed assay does not depend on sequencing, requires less than one ml of blood and provides fast results by direct analysis of native, unamplified miRs. This is enabled by a novel combination of compact spectral imaging together with a machine learning based detection scheme that allows simultaneous multiplexed classification of multiple miR targets per sample. The proposed end-to-end pipeline is extremely time efficient and cost-effective. We benchmark our method with synthetic mixtures of three target miRs, showcasing the ability to quantify and distinguish subtle ratio changes between miR targets.