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Fluorescence-based kinetic analysis of miniaturized protein microarrays

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Abstract

Ideal monitoring devices should enjoy a combination of characteristics, e.g. high sensitivity, multiplexing, portability, short time-to-result (TTR). Typically, no device meets all of these demands since some of them are contradictory, to some extent. Herein, we present a miniaturized platform based on fluorescent detection, which is sensitive, readily allows multiplexing, and allows real-time monitoring of the signal, thus allowing extraction of kinetic information as well as drastic reduction of TTR. This is achieved via miniaturization of active spots, integration with microfluidics, and algorithmic approaches. We validate its performance by comparing with evanescent field excitation, which obtains similar results, however without the addition of the necessary complex hardware.

Keywords: fluorescence detection; multiplexed detection; miniaturization; microfluidic; kinetic parameters; protein microarray

1 Introduction

Protein microarray technology was introduced by Roger Ekins in 1989 as a microspot assay (Ekins, 1989) and is used for studying protein-protein interaction (Bergsma et al., 2010) and clinical diagnosis (Soe et al., 2018). In typical protein microarray, different capture molecules (for example, antibodies) are immobilized in the form of arrayed spots (~100 μ m in diameter) on to a solid substrate and exposed to a sample containing (or not) corresponding labelled target molecules (protein or biomarker). The bound molecules are quantified using fluorescence intensity resulting from the spots. Effectively, each such spot serves as a biosensor for its respective target molecule, and thus the whole microarray can be thought of as a multiplexed biosensor.

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