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Improving immunoassays based on the analysis of scanning force microscopy images

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Abstract

Scanning force microscopy has been demonstrated to be an effective binding event detection step for immunoassays. In its simplest form—analysing small area images—the detection limit of the scanning force microscopic immunoassay (SFMIA) has been shown to be comparable to existing techniques. In the present work, we have examined how the performance of image analysis-based SFMIA can be improved. Firstly, we have used a surface analysis parameter that increases linearly with the concentration of binding events. This parameter—the surface area ratio—is the ratio of the surface area after antigen binding to the surface area of the original biospecific surface. With this parameter, SFMIA images can be rapidly analysed and converted into assay units. Secondly, we have demonstrated that by using silicon wafer supports that carry fiducial marks we can relocate to very high accuracy onto the biospecific surfaces and identify the changes due to antigen binding. By relocating in this manner the signal to noise ratio of the technique is enhanced. Thirdly, from simulations we have determined the SFM tip size and image area that optimizes the immunoassay sensitivity.

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1. Introduction

There are many issues driving the search for more sensitive immunoassays: the pre-clinical detection of infectious diseases in humans and animals; the identification of trace levels of illicit substances; enhanced safety assurance of foods

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and beverages; and increasingly rigorous environmental monitoring regimes. Leaving aside the differences in assay design, e.g. direct versus competitive, all solid phase immunoassays use antibodies[1] immobilised onto a solid support to bind their target antigens. The biospecific interaction between the immobilised antibodies and their antigens is followed by at least one transducing step where the biospecific interactions are converted into an output signal. For example, in the widely used enzyme-linked immunosorbent assay (ELISA) the bound antigens are subsequently labelled with fluorescent complexes that are then detected optoelectronically. Other methods involve the use of magnetic, physical and radioactive labels. Label-less methods are also in use, for example the BIACORE^{TM} instrument uses surface plasmon resonance to directly detect the adsorption of antigens onto antibody-coated gold surfaces.

Masai et al. proposed in 1990 [1] that scanning tunnelling microscopy (STM) could be used as the detection step in an assay format that they styled scanning tunnelling microscopic immunoassay (STMIA). In that paper, Masai et al. presented STM images of gold colloid particle-protein A complexes which had been previously immobilised onto either polycrystalline gold or highly oriented pyrolytic graphite (HOPG) supports. Subsequently, McDonnell et al. [2] proposed that the key disadvantage of STMIA, the requirement for a conducting sample and/or support could be overcome by using scanning force microscopy (SFM) as the binding event detection step. Using a simple model, McDonnell et al. predicted that scanning force microscopic immunoassay (SFMIA), using a single SFM cantilever to image a small area of immobilised antibodies (say $5\,\mu m \times 5\,\mu m$), would have detection limits comparable to methods such as the enzyme-linked immunosorbent assay (ELISA). They further predicted that considerably lower detection limits appeared attainable by using larger sensor surface areas, but with online detection of binding events rather than analysing conventional images, and foresaw a role for cantilever arrays in speeding up the assay and/or extending the detection limit.

Subsequently, within the frame of a European project,[2] Perrin et al. [3] demonstrated the feasibility of image analysis based SFMIA using the ferritin antigen. They obtained assay detection limits that were comparable to those for ELISA by quantifying $5\,\mu m \times 5\,\mu m$ area SFM images using a classical surface roughness parameter. In 1998, Perrin et al. [4] extended SFMIA to thyroid stimulating hormone (TSH). As TSH molecules are much smaller than ferritin molecules, SFM detection was enabled by using 15 nm-diameter gold particles conjugated to TSH. By using a sandwich assay format the sensitivity of SFMIA for TSH was found to be as good as the best commercial analysers. In 1999, Perrin et al. [5] extended the detection limit for TSH further still by conjugating TSH with magnetic particles and employing a novel magnetic field concentrator. More recently, this general method has been used to perform assays for viruses by counting bound virus particles in SFM images [6]. Johnson et al. [7] have used the method to screen anti-bacteriophage fd antibodies for optimal assay sensitivity.

In the present work, we have examined how the performance of image analysis based SFMIA can be improved. Firstly, we have used a global surface analysis parameter that increases linearly with the concentration of binding events. This parameter—the surface area ratio (SAR)—is the ratio of the surface area after antigen binding to the surface area of the original biospecific surface. With this parameter, SFMIA images can be rapidly analysed and converted into assay units. Importantly, particle threshold and counting is avoided. Whilst the latter works well when the assay target is large, e.g. conjugates [4,5] and virions [6], it can be very problematic when the assay target is small. Furthermore, the non-linearity [3] inherent in classical surface roughness parameters is avoided. Secondly, we have demonstrated that by using silicon wafer supports that carry fiducial marks we can relocate to very high accuracy onto the antibody surfaces and identify the changes due to

[1] In some assay formats, antigens rather than antibodies are immobilised.

[2] Contract MAT1-CT94-0013 of the Standards, Measurements and Testing Programme of the European Commission.