Quantitative methods for spatially resolved adsorption/desorption measurements in real time by surface plasmon resonance microscopy.

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A simple method for converting local reflectivity changes measured in surface plasmon resonance (SPR) microscopy to effective adlayer thicknesses and absolute surface coverages of adsorbed species is presented. For a range of high-contrast angles near the SPR resonance where the local metal surface's reflectivity changes linearly with angle, the change in reflectivity at fixed angle is proportional to the change in effective refractive index (eta(eff)) near the surface. This change in eta(eff) can be converted to absolute adsorbate coverage using methods developed for quantitative SPR spectroscopy. A measurement of the change in reflectivity due to changes in refractive index of bulk solutions, i.e., percent reflectivity change per refractive index unit (RIU), is the only calibration required. Application of this method is demonstrated for protein adsorption onto protein/DNA arrays on gold from aqueous solution using an SPR microscope operating at 633 nm. A detection limit of 0.072% change in absolute reflectivity is found for simultaneous measurements of all 200 microm x 200 microm areas within the 24-mm(2) light beam with 1-s time averaging. This corresponds to a change in effective refractive index of 1.8 x 10(-5) and a detection limit for protein adsorption of 1.2 ng/cm(2) (approximately 0.5 pg in a 200-microm spot). The linear dynamic range is Deltaeta(eff) = approximately 0.011 RIU or approximately 720 ng/cm(2) of adsorbed protein. Using a nearby spot as a reference channel, one can correct for instrumental drift and changes in refractive index of the solutions in the flow cell.

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