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DEMYSTIFYING BACK SCATTER INTERFEROMETRY - A SENSITIVE REFRACTIVE INDEX DETECTOR

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BACKGROUND: Back Scatter Interferometry (BSI) is a sensitive method for detecting changes of the refractive index (RI) in small capillaries. The method was originally developed as an off-axial column detector for use in Liquid Chromatography or Capillary Electrophoresis systems, but it has been proposed that this method can also be used to detect molecular binding in a label-free manner. Recent work proposes BSI to be a unique sensor for detecting protein binding with various ligands and other protein interactions in order to obtain relevant binding kinetics. We hypothesize that BSI is actually acting like a common-path interferometer.

METHODS: A HeNe laser is directed at a glass capillary with inner diameter of 1.4 mm and reflected light from air/glass and liquid/glass interfaces interfere to form an RI dependent intensity fringe pattern at a CCD detector. The fringe shift relative to the change of RI of the sample; i.e. the sensitivity; is controlled by the physical interaction length between the interferometric sample beam and the sample itself. Using optical ray-tracing we calculate the sensitivity. We validate these theoretical findings by determining the RI increment (dn/dc) from a set of NaCl standard solutions.

RESULTS: Ray-tracing show that the basic interference pattern recorded with BSI can be fully described by two beams, one reflected from the surface of the capillary and a beam reflected from the back of the capillary wall. In accordance we find that the interferometric interaction length is given by twice the diameter of the capillary. Experimentally we find a sensitivity of 4700 rad/(g/ml) and estimate dn/dc for NaCl to be 0.169 ml/g, which is in accordance with literature. Furthermore we report a minimum detectability of $7x10^{-7}$ RI Units.

CONCLUSIONS: BSI works like a common-path interferometer. The sensitivity of the BSI system is given by twice the inner diameter of the capillary times the wavenumber of the light source. Our results suggest that Back Scatter Interferometry does not provide a unique measurement principle for sensing biochemical bindings compared to what should be possible using many commercial available refractometers.