AUTOLAB APPLICATION NOTE

Autolab SPR systems with a flexible range in refractive index to broaden the application range.

The Autolab SPR instruments can monitor binding events in real time without labelling and the system can therefore be used to determine both affinity and rate constants of interactions between any molecules.

The analytical technique used is called Surface Plasmon Resonance, this is a physical phenomena that occurs when p-polarized light hits a thin metal layer under a certain angle, during total internal reflection conditions.

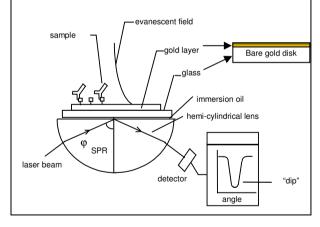


Figure 1. SPR setup

In our SPR setup, we have a replaceable gold coated glass disk on a hemi-cylindrical lens (see figure 1). Immersion oil, with matching refractive

index, i.e. identical to the refractive index of the hemi-cylinder and the glass of the substrate, is placed between the disk and the hemi-cylinder.

The optical path setup

Figure 2 shows the design of the optical path. The coarse angle of incidence is manually set at a certain position with a spindle. The 'dip' position, or the SPR angle, depends on the refractive index of the solution on top of the gold surface.

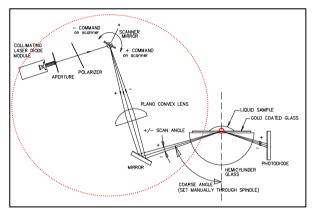


Figure 2. Schematic picture of the optical path.

The coarse angle can be set between 62° and 78° degrees, by turning the spindle from position 10 to 25 respectively. Turning the spindle will change the angle of incidence by rotating the complete optical setup (dashed red circle, figure 2) around the optical centre (small red circle) on the gold surface. The extreme positions of the spindle, limit the dynamic range of the refractive index of the sample under study.

The refractive index range

A refractive index change near the surface leads to a changed SPR angle position at which light will be adsorbed by the gold, this changing position is measured at the detector. Therefore, it is the refractive index range that determines the application range. To increase that refractive index range we can supply hemi-cylinders and substrates with different glass specifications. To show the refractive index range differences between the different glass specifications, we used solutions of different refractive indices; like Methanol (RI=1.326), 2-propanol (RI=1.375) and Cyclohexane (RI=1.424).

Refractive index range of the N-BK 7 glass

The standard supplied glass type is the N-BK 7 glass with a refractive index of 1.518. At spindle position 21.5 the outer limit of the 4000 m^{\circ} is reached, but with turning the spindle (to spindle position 17.5) it is clear that the refractive index minimum is lower than the shown 1.326. In theory it is 1.26.

Measurement data (See diagram in figure 3.) Channel 1 A1.) Methanol, RI=1.326, SPR angle = -1732m° Spindle position = 23.0 B1.) 2-Propanol, RI=1.375, SPR angle = 1269m° Spindle position = 18.0 C1.) Methanol, RI= 1.326, SPR angle = 2022m° Spindle position = 21.5

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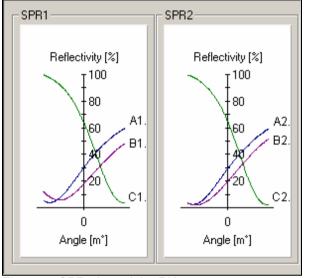


Figure 3. SPR plots of the BK 7 measurements.

It is clear that the 2-Propanol solution is easily within the SPR angle range. The minimum spindle position possible is 10 and therefore the maximum refractive index of the SPR system will be 1.38.

Refractive index range of the N-BAF 3 glass

This type of glass has a refractive index of 1.583. The methanol is measured at the limits of the refractive index range. The data clearly show that cyclochexane can be easily detected by the system. Taken the systems minimum spindle position into account, it is clear this type of glass can handle a refractive index of 1.44.

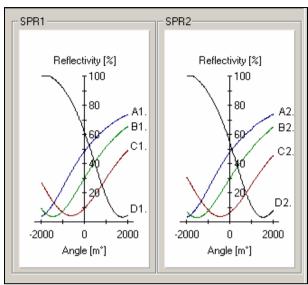


Figure 4. SPR plots of the BAF 3 measurements.

Measurement data (See diagram in figure 4.) Channel 1 **A1.) Methanol, RI=1.326**, SPR angle = - 2140 m° Spindle position = 23.0 **B1.) 2-Propanol, RI=1.375**, SPR angle = -1437 m° Spindle position = 18.0 **C1.)Cyclohexane, RI=1.424**, SPR angle = 675 m° Spindle position = 12.0 **D1.) 2-Propanol, RI= 1.375**, SPR angle = 1792 m° Spindle position = 21.5

Conclusion

In this application note, the results are shown of the increased refractive index range of the SPR system due to changing the hemi-cylinder and sensor disk glass. The increased refractive index range is especially useful in the field of 'thick' films, like multiple layers of polymers or organic solutions reactions.

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