**Comparison of SBSR and SB Techniques**

The SBSR attachment was developed to achieve two advantages: (i) better compensation of strong background absorption (e.g., water in biomembrane studies, organic solvent in heterogeneous catalysis, or a pink film in the case of polycarbonate surface modifications), and (ii) quasi-simultaneous comparison of sample and reference single-channel spectra. Most conventional FTIR spectrometers are single-beam (SB) instruments and require storage of a reference spectrum, e.g., from a biomembrane before the interaction with a substrate. Alterations of this membrane caused by external influence are then stored in a sequence of single-channel spectra, which is then converted into the mean of the stored reference spectrum. This procedure may lead to misinterpretations in cases where the reference system features some intrinsic instability. The detected spectral alterations may then be caused by the substrate and by the membrane itself.

On the other hand, the SBSR principle as depicted in this paper enables the measurement of conventional SB series as well as quasi-simultaneous sample (S) and reference (R) spectra, which may be converted into transmittance or absorbance spectra featuring predominantly the influence of an external perturbation (e.g., by a substance in the system under consideration). The corresponding ATR accessory which converts a single beam spectrometer into a pseudo-double-beam ATR or transmission spectrometer (SBSR: Single Beam Sample Reference) is most efficient in the so-called \textit{R} version (Fig. 1). Sample (S) and reference (R) are placed top of one another on the same internal reflection element (IRE). Both, S and R are accessible via independent flow-through cuvettes. The IR beam is now directed alternately through the S and R parts by computer-controlled vertical displacement of the IRE, thus enabling quasi-simultaneous collection of S and R single-channel spectra. The optical layout is designed for the use of high-resolution IR's with 45° angle of incidence. Recommended IRE size for thin layer spectroscopy are 50 mm × 20 mm x 1.0, 1.5 and 2.0 mm, resulting in a number of active internal reflections within 45° and 20°. The detector mount is also on the attachment to enable the adjustment of the focusing mirror in front of the detector.

Three applications are presented: (i) the interaction of the endotoxin LPS with a supported bilayer of positive surface charge studied by both, SB and SBSR spectroscopy (Fig. 2), (ii) the spectroscopic compensation of a supported bilayer as initial state of many experiments by SBSR spectroscopy (Fig. 3), and (iii) the interaction of creatin kinase with a negatively charged supported membrane studied by both, SBSR and SB (Fig. 4).

![Figure 1. Single Beam Sample Reference (SBSR) ATR attachment.](image1)

**Figure 1.** Single Beam Sample Reference (SBSR) ATR attachment. (A) The focus in the sample compartment is displaced to the position F by the planar mirrors M1 and M2. The off-axis parabolic mirror M3 produces a parallel beam with a diameter of one centimeter, i.e. half of the height of the IRE. The cylindrical mirror M4 focuses the light to the entrance face of the IRE. M5 has the same shape as M4 to correct for paraxial light along the path, with the detector POL (parallel) polarized IR ATR SBSR single channel spectra of a POPC:HDPyr (1:1)-monolayer adsorbed tail-to-tail to a DPPA Langmuir-Blodgett monolayer on the IRE. Both membranes exhibited the same age, since both were placed in the sample compartment of the SBSR cell. The black and red lines show the difference between the actual sample spectra and a stored (older) reference spectrum of the POPC:HDPyr (1:1)-monolayer adsorbed tail-to-tail to a DPPA Langmuir-Blodgett monolayer on the IRE. The bilayer in the sample and reference part of the SBSR cell seems to be quite identical. Buffer: 20 mM phosphate buffer pH 7, 100 mM NaCl. Angle of light incidence 46°–45°; number of active internal reflections 8–9; germanium ATR plate; temperature T = 25°C. For abbreviations see Fig. 2.

![Figure 2. IRR absobance spectra of a POPC:HDPyr (1:1)-DPPA-bilayer after treatment with different concentrations (1–1000 µM) of LPS from Pseudomonas aeruginosa (serotype 10).](image2)

**Figure 2.** IRR absobance spectra of a POPC:HDPyr (1:1)-DPPA-bilayer after treatment with different concentrations (1–1000 µM) of LPS from Pseudomonas aeruginosa (serotype 10). ATR parallel polarized light. Buffer: 20 mM phosphate buffer pH 7, 100 mM NaCl. Angle of light incidence 45°–45°; number of active internal reflections N = 35; germanium ATR plate; temperature T = 25°C. For abbreviations see Fig. 2.

![Figure 3. Spectroscopic compensation of a supported DPPA/POPC HDPyr (1:1)-bilayer by SBSR spectroscopy.](image3)

**Figure 3.** Spectroscopic compensation of a supported DPPA/POPC HDPyr (1:1)-bilayer by SBSR spectroscopy. (SCSA) and (SCRF). Parallel polarized IR ATR SBSR single channel spectra of a POPC:HDPyr (1:1)-monolayer adsorbed tail-to-tail to a DPPA Langmuir-Blodgett monolayer (SCSA) and a DPPA monolayer (SCRF) with buffer solution. Both spectra were taken from the sample compartment of the SBSR cell. (I) Parallel polarized IR ATR SBSR absorbance spectrum of the POPC:HDPyr (1:1)-bilayer on the IRE. (II) SBSR absorbance spectrum of the POPC:HDPyr (1:1)-bilayer on the IRE. The bilayer in the sample and reference part of the SBSR cell seems to be quite identical. Buffer: 20 mM phosphate buffer pH 7, 100 mM NaCl. Angle of light incidence 46°–45°; number of active internal reflections N = 35; germanium ATR plate; temperature T = 25°C. For abbreviations see Fig. 2.

![Figure 4. Comparison of Single Beam Sample Reference (SBSR) pseudo-double beam technique with conventional single beam (SB) technique. Very low energy in the 2500 cm⁻¹ and 1200 cm⁻¹ region resulted from subtracting with (+) and bending (90) O₃ absorptions of liquid D₂O, respectively, as shown by a single channel (SC) spectrum. The supported bilayers in the sample and reference cassettes consisted of a dipalmitoylphosphatidic acid (DPPA) bilayer and a cardiolipin (CL) adsorbed monolayer. Both membranes exhibited the same age, since the IRE layer covered the whole width of the IRE, and CL adsorption from vesicles occurred synchronously by two independent equal circuits. In a second step, a cardiolipin (CL) adsorbed monolayer was adsorbed from a circulating solution in the sample channel, ref. (1–5). Therefore, the absorbance spectra shown in the figure reflect a diluted CL, as well as any other differences between S and R channel. Obviously, there are more detectable differences in the SB mode than in the SBSR mode, because SB reflects the actual difference between S and R, while SB shows the difference between the actual sample spectrum and a stored (older) reference spectrum. In this case, the partly different results obtained in the SBSR and SB mode reflect primarily from a slow uptake of H₂O vapor by the circulating D₂O solutions. This leads to overlapping of the amide I (NH) and amide IV (CO) bands of CK, as well as an overcompensation of the D₂O absorption bands (2500 cm⁻¹ and 1200 cm⁻¹). It should be noted that the slight overcompensation of the D₂O absorption bands (2500 cm⁻¹ and 1200 cm⁻¹) is significant, since it reflects the reduced water content in the sample cuvette due to displacement by the CK layer. CK layer thickness 93 Å, Ge IRE, angle of incidence, 6°–51°, number of active internal reflections N = 35, the effective indices were: n₁ = 4.0, n₂ = 1.45, and n₃ = 1.30.

**Aknowledgements**

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**References**


