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OPEN ACCESS
IOP Publishing
Journal of Physics: Condensed Matter
J. Phys.: Condens. Matter **32** (2020) 393002 (16pp)
https://doi.org/10.1088/1361-648X/ab8523

Topical Review

Sensing and structure analysis by *in situ* IR spectroscopy: from mL flow cells to microfluidic applications

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Received 23 December 2019, revised 18 March 2020
Accepted for publication 31 March 2020
Published 23 June 2020

Abstract

In situ mid-infrared (MIR) spectroscopy in liquids is an emerging field for the analysis of functional surfaces and chemical reactions. Different basic geometries exist for *in situ* MIR spectroscopy in milliliter (mL) and microfluidic flow cells, such as attenuated total reflection (ATR), simple reflection, transmission and fiber waveguides. After a general introduction of linear optical *in situ* MIR techniques, the methodology of ATR, ellipsometric and microfluidic applications in single-reflection geometries is presented. Selected examples focusing on thin layers relevant to optical, electronical, polymer, biomedical, sensing and silicon technology are discussed. The development of an optofluidic platform translates IR spectroscopy to the world of micro- and nanofluidics. With the implementation of SEIRA (surface enhanced infrared absorption) interfaces, the sensitivity of optofluidic analyses of biomolecules can be improved significantly. A large variety of enhancement surfaces ranging from tailored nanostructures to metal-island film substrates are promising for this purpose. Meanwhile, time-resolved studies, such as sub-monolayer formation of organic molecules in nL volumes, become available in microscopic or laser-based set-ups. With the adaption of modern brilliant IR sources, such as tunable and broadband IR lasers as well as frequency comb sources, possible applications of far-field IR spectroscopy in *in situ* sensing with high lateral (sub-mm) and time (sub-s) resolution are considerably extended.

Keywords: *in situ*, infrared spectroscopy, ATR, solid–liquid interface, enhancement interface, microfluidic, biosensing

(Some figures may appear in colour only in the online journal)

1. Introduction

Many optical spectroscopic techniques have the advantage of being non-invasive, destruction-free and non-contact methods that can be operated under various environmental conditions, specifically for the analysis of, or in, liquids. Linear optical methods such as classical infrared (IR) reflection/transmission spectroscopies [1] and ellipsometry [2], as well as plasmon resonance [3], Raman [1] and fluorescence spectroscopies [4], come with a different set of merits and constraints for the study of interfaces and sensing platforms in liquid environments resulting from the different light–matter interaction mechanisms.

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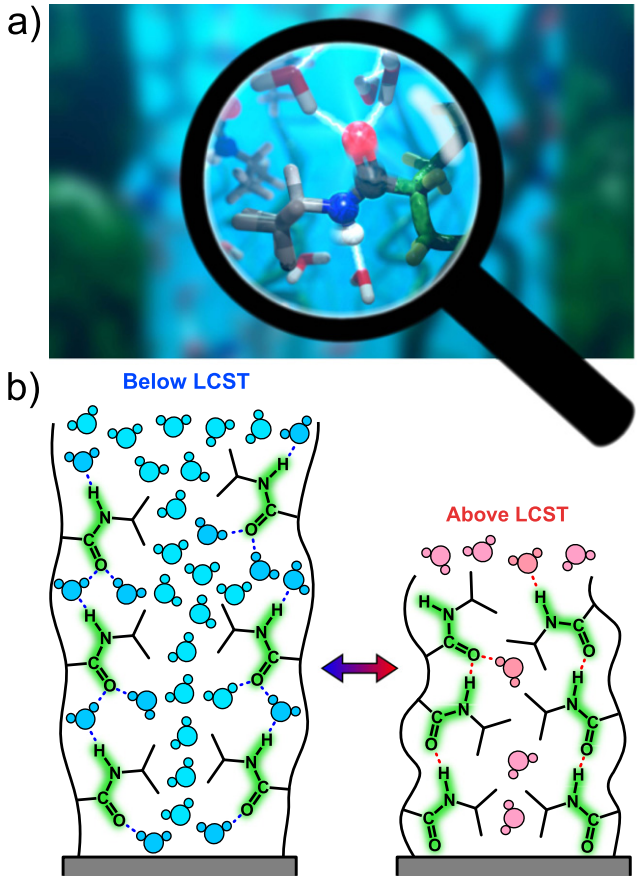

Figure 1. (a) Artist's illustration of an IR ellipsometric study, which uncovers chemical, structural and interaction properties of a thin polymer brush film. (b) Schematic of the swelling–deswelling transition of a thermoresponsive brush below and above its lower critical solution temperature (LCST), with characteristic changes in molecular interactions. Reprinted with permission from [31]. Copyright (2018) American Chemical Society.

Vibrational spectroscopy techniques such as Raman and IR are of particular interest, as they enable label-free studies of specific chemical, structural and functional sample properties (see figure 1) under on-line or *in situ* conditions. The range of applications for *in situ* Raman and IR vibrational spectroscopy significantly broadened in consequence of recent technological developments with respect to the specific measurement schemes, the optical equipment, and the implementation of functional or enhancement substrates. Key goals of these developments were to reduce measurement times, to advance hyperspectral imaging, to improve measurement sensitivity, and to downscale sample volumes required for the analysis. Furthermore, optical modeling and multivariate analysis were steadily developed for the interpretation of (multi-modal) experimental data.

The application of optical methods in the spectral range from near-ultraviolet to near-IR is very common because a variety of transparent materials are readily available for the development of optics and liquid cells (e.g., polymers or glasses). In addition, aqueous solutions often exhibit sufficient transparency in this spectral range. Therefore, the optical beam can pass through the liquid reservoir or probe an interface even through a thick liquid layer. However, there is no principle limitation to extend the accessible spectral range to the mid-IR (MIR) if materials with sufficiently high MIR transparency are used [5–93]. Even a further extension down to the THz range [63] is feasible.

Classical *in situ* IR techniques such as attenuated total reflection (ATR) or external reflection spectroscopies like reflection absorption IR spectroscopy (RAIRS) have been established over decades. *In situ* infrared spectroscopic ellipsometry (IRSE) was introduced in recent years allowing for comprehensive quantitative IR studies in milliliter (mL) flow cells when combined with optical modeling.

Microfluidic concepts [5–19] and the combination of ATR with fiber/waveguide geometries [19–25] enable the sensitive analysis of micro- and nanoliter volumes. So-called optofluidic approaches could push multiple novel chemical and bioanalytical sensor concepts [94–97], also potentially combining different nonlinear and linear optical spectroscopic methods with microfluidic techniques. Important applications of such optofluidic platforms are biosensing, monitoring of catalytic processes, lab-on-a-chip devices, drug development, and biochemical analyses [5–19, 94–97].

An interesting and rapidly emerging technical development is the application of plasmonic enhancement substrates. The consideration of enhancement particles [98, 99], structures [100–105] and interfaces to exploit the effect of surface enhanced infrared absorption (SEIRA) [98, 99] can significantly increase the sensitivity of IR spectroscopy and ellipsometry in liquid environments [12, 48, 88, 106]. Moreover, the combined platforms for multimodal detection, such as simultaneous surface enhanced Raman scattering (SERS) and SEIRA measurements, could significantly expand the area of bioanalytical, electrochemical and sensing applications [107 and references therein]. Compared with Raman [108], IR spectroscopy may offer the possibility of a more straightforward identification and quantification in applications where thin films down to the sub-monolayer level are investigated [31, 50]. Single-molecule detection levels as in SERS are in reach for IR-spectroscopic techniques [109], especially for large molecules.

Most of the recent developments in the field of *in situ* IR techniques for solid–liquid interface characterization were reported on set-ups that still operate with classical FTIR (Fourier-transform IR) technology. However, there is a fast-growing field of IR analytics that combines *in situ* measurement methods with brilliant infrared sources, in particular quantum cascade lasers (QCLs). These new developments enable a more in-depth analysis of functional thin films and surfaces for multiple technological applications. For the presented examples of *in situ* IR spectroscopy, this review will focus on nm-thin films and monolayers on silicon for optoelectronic, biosensing, and catalytic interfaces, highlighting the application potential of the techniques to broad areas in science, metrology, and industry.

2. Technical aspects of *in situ* IR spectroscopy

This section starts with a review of the most commonly used *in situ* measurement geometries. In the second part, an introduction to common measurement techniques referring to IR spectroscopy in linear optics is given. Several possible variations of substrate interfaces to enhance the measured signal are discussed in the last part.

2.1. In situ IR geometries

Figure 2 summarizes selected possible *in situ* measurement geometries for MIR analytics of liquid samples or in liquid environments, in particular for the analysis of thin films at the solid–liquid interface. Figure 2(a) refers to typical geometries either in ATR or simple reflection, where the solid–liquid interface is probed through an ATR crystal or an optical window. For an ATR crystal or sphere in a single- or multiple-reflection geometry, the accessible spectral range depends on the optical path length through, and residual absorption of, the used materials. Typically, the path lengths through the IR-transparent materials of such cells are larger than for those with a microstructured surface or a simple reflection geometry. A multiple-reflection ATR geometry enhances the measured signal and provides high detection sensitivities. Larger incidence angles, and thus ATR conditions, for probing a solid–liquid interface can be achieved with a macroscopically planar material by using a microstructured surface [110]. However, the structure of the window interface might contribute to a depolarization of the incident radiation.

On the contrary, a simple reflection geometry (figure 2(a) on the right) allows for more defined measurements of reflected amplitudes and phases, as well as a high sensitivity to thin-film optical effects. Figure 2(b) shows a reflection geometry for direct measurements on the liquid surface. Figure 2(c) depicts variants in which the optical beam passes through the liquid sample, either in a reflection or a transmission geometry. Figure 2(d) sketches the application of a fiber geometry where the ATR conditions are met at the interfaces of the waveguide. Reflection and transmission geometries can also be combined requiring the specific modification of the used prisms or spheres [85, 86].

The IR transparency of solvent and substrate material imposes several restrictions for the realizations of flow cells for *in situ* IR spectroscopic measurements. Hence, reflection (figure 2(b)) and transmission (figure 2(c)) measurements [84] where the IR beam has to pass through the liquid volume can only be realized for sufficiently thin layers or channels (e.g., for water and blood below about 15 μm penetration depths [32] in the MIR). These obstacles can be overcome when MIR-transparent materials are chosen for the substrates or top windows of the cell, and the liquid reservoir or interface of interest is studied directly through this transparent material.

2.2. In situ IR techniques

In the following subsections, common *in situ* measurement techniques are introduced. ATR-IR spectroscopy is probably among the most widely used IR spectroscopic techniques. Both ATR and specular reflection techniques are sensitive methods to characterize thin films. For both methods, additional polarization dependent measurements can give access to anisotropic sample properties. Advanced IR ellipsometric measurement schemes, such as generalized or Mueller-matrix (MM) ellipsometry [111], can also facilitate the study of complex biaxial or bi-isotropic films and enable one to include the depolarization properties of the sample (and set-up) in the measurement and optical interpretation [93, 112].

The time resolution of most classical far-field *in situ* FTIR techniques is in the range of several seconds to 10 s. For dynamic studies of non-cyclic processes with higher time resolutions, rapid-scan interferometers [113] can be employed. IR methods can also be coupled with a brilliant light source and with a single-shot detection scheme to achieve even sub-second time resolutions [114–116].

2.2.1. Reflection absorption IR spectroscopy (RAIRS). RAIRS spectra are widely used for the analysis of characteristic thin-film fingerprints, structure of adsorbed molecules, and layer properties. Typically for quantification, the measured signals are compared to those of a well-known reference sample, the empty channel or a spectrum of the initial surface during an *in situ* experiment. Therefore, spectral RAIRS signals are often displayed as referenced differences

$$\frac{R_{\text{sample}} - R_0}{R_0}$$

with the sample reflectance (or reflectivities) R_{sample} and reference reflectance R_0, or (in analogy to transmission spectroscopy) as absorbance [117]:

$$A = -\log\left(\frac{R_{\text{sample}} - R_0}{R_0}\right)$$

Beside the name RAIRS, the specular reflection IR method is also known under several acronyms including ERIRS (external reflection infrared spectroscopy), IR-ERS, [118] and IRRAS. In a typical reflection absorption geometry, a single external reflection of the IR radiation at a sample-covered substrate is probed. By adding polarizers, s- and p-polarization dependent reflectance spectra can be obtained. *In situ* IRRAS has been applied in several works either at the air–liquid or the solid–liquid interface, e.g., for the studies of antimicrobial surfaces [119], soluble surfactants [120], organic or bio-related monolayers [121–124], or in a liquid cell for, e.g., spectroelectrochemistry [125] and the study of adsorption/desorption processes of molecules [126, 127].

2.2.2. IR spectroscopic ellipsometry (IRSE). IRSE allows for more detailed measurements compared to RAIRS. It can measure s- and p-polarized reflectance spectra, and additionally probe relative phase and amplitude information of the elliptically polarized reflected or transmitted IR beam. IRSE measures the quantity ρ, which is the ratio of the complex reflection coefficients of p- and s-polarized light, defining the basic ellipsometric parameters tan Ψ (relative amplitude ratio)

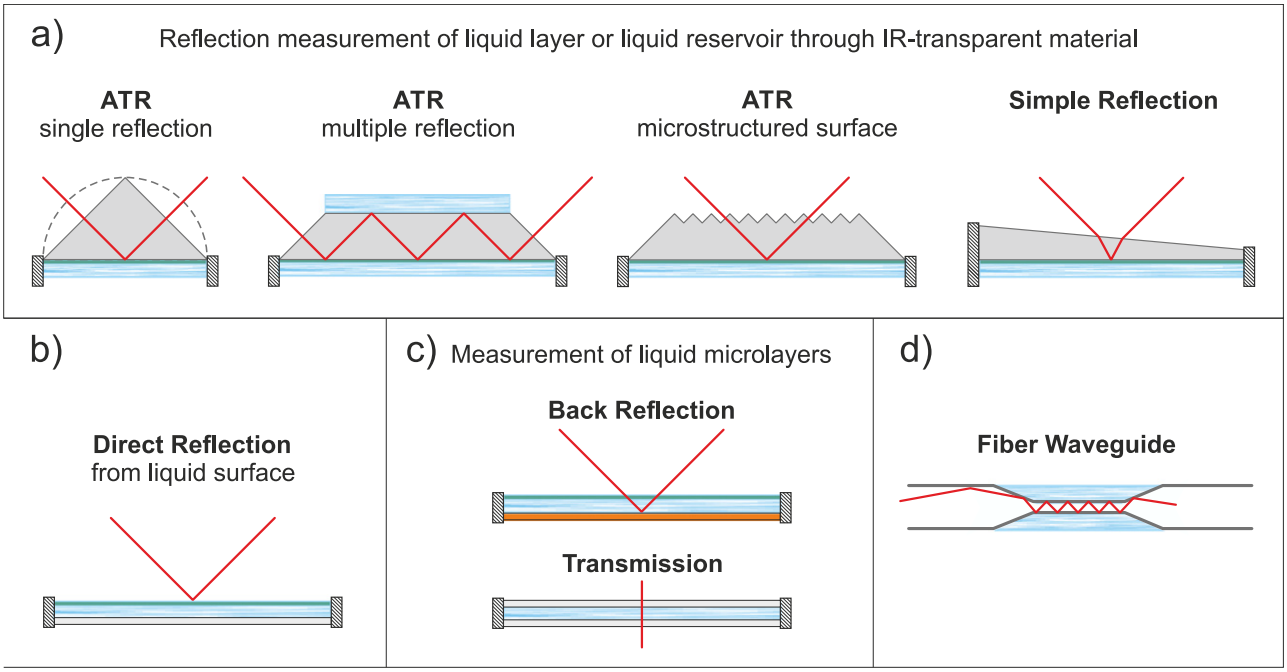

Figure 2. *In situ* measurement geometries: (a) ATR in single reflection, multiple reflection, through a microstructured surface, and a simple reflection geometry at a lower angle of incidence at the solid–liquid interface; (b) direct reflection from the liquid surface; (c) reflection from the backside, transmission through a liquid micro layer; (d) fiber waveguide according to reference [21, 24].

and Δ (relative phase shift),

$$\rho = \frac{r_p}{r_s} = \tan\,\Psi \cdot e^{i\Delta}$$

where $\tan\Psi$ can be calculated from the square-root of the ratio of p- and s-polarized reflectances:

$$\tan\,\Psi = \sqrt{\frac{R_p}{R_s}}$$

The ellipsometric parameters are absolute, self-referenced measurement values. Therefore, this measurement scheme does not require the use of a reference sample. Advanced ellipsometric measurements also allow for the determination of polarization degrees and Mueller-matrix elements, which are related to the depolarizing and anisotropic sample properties [2, 93, 111, 128, 129].

Flow cells for *in situ* measurements of solid–liquid interfaces are only commercially available as accessories for VIS ellipsometers. Such cells are not yet on the market for IR-spectroscopic ellipsometry. However, since the introduction of IRSE with liquid flow cells in 2007 [33], the technique has been widely applied to numerous thin-film studies.

2.2.3. ATR. The method relies on an optical total-reflection geometry for sensitively probing up to several micrometers beyond the ATR-material–sample interface. Similar to RAIRS, ATR signals are often displayed as referenced differences or absorbances. A number of commercial devices exist that are adaptable to sample compartments or external reflection optics of IR-spectroscopic devices with fiber, macro or micro optics. Detailed theoretical and experimental descriptions of the ATR technique, which is also called internal reflection spectroscopy because it uses an IR-transparent internal reflection element, can be found in references [41–45]. Suitable materials with chemical compatibility and optical transparency in the IR spectral range need to be chosen for fluidic cells [9]. These constraints and the required geometry for the total reflection condition have to be considered for the optical design of an *in situ* measurement cell or platform.

2.3. In situ enhancement interfaces

Recent advancements of plasmonic and porous enhancement surfaces are addressed in this section. Beside the possibility of increasing the measured signal by (i) the use of brilliant light sources with high spectral and lateral power densities, (ii) the use of optimized cell geometries based on multiple reflections or waveguides, also (iii) the sensing interface could be modified to achieve signal enhancement. For the latter, this subsection discusses technical possibilities to increase the measured signal from a surface in an *in situ* IR-spectroscopic experiment by making use of larger surface areas and electric-field enhancements.

2.3.1. Interfaces with larger surface area. Compared to the monolayer adsorption on a planar substrate, porous or fiber-covered surfaces can provide a significantly larger surface area and thereby lead to increased IR signals due to a higher amount of adsorbed material.

In situ ATR spectroscopy of mesoporous silica films can be used for the monitoring of adsorption processes and trace analysis [29, 30]. Specifically, an ATR silicon crystal covered with such a film can serve as a large-surface-area layer exhibiting

material enrichment factors for adsorbed molecules of up to 210 [29, 30].

2.3.2. Plasmonic chip-based technology. Detection surfaces integrated in *in situ* cells can be modified with metallic films or nanostructures. These modifications provide properties to exploit both signal enhancement due to a larger surface area and due to the plasmonic properties by surface enhanced infrared absorption (SEIRA) [98, 99]. Metallic island films can provide average enhancement factors 'usually found to be in the 10–100 region' [99] over the full MIR range and the complete probed spot [98, 99]. They are used for simple reflection [98, 99] (see also section 3.3) as well as ATR geometries [40, 48] (see also section 3.1). Special types of substrates, such as metallic island gradient substrates, offer continuously varying plasmonic properties with respect to the island density. This feature can be exploited for optimization and a detailed study of the effect of the enhancement on the obtained signal with only a single solid–liquid interface [12, 105]. The integration of a gold-island film enhancement substrate in a microfluidic cell allows classical IR-microscopic measurements in a single-reflection geometry [12, 105], reaching high sensitivities down to sub-monolayer level, which corresponds to detection surface densities of ng cm^{-2}.

A different type of enhancement substrates employs plasmonic nanoantennas whose properties can be precisely tailored to provide a high field enhancement over a selectively chosen narrow spectral range and at highly localized hot-spots such as the apex of nanoantennas. These substrates can deliver enhancement factors of up to 10^7 at specific frequencies and spatially highly localized spots [100–105]. This type of substrates has been used successfully for studies of the secondary structure of proteins under *in situ* conditions [88].

3. Applications

In this chapter, applications of thin-film sensitive methods such as ATR, IR ellipsometry and SEIRA microfluidics are reviewed. A short collection of different applications is listed at the beginning of each subsection. Selected examples of thin-film investigations are given to highlight possible applications.

3.1. In situ ATR

In situ ATR has a wide range of applications that require a dedicated review article on their own [e.g. 57, 70]. In the following, we therefore provide only a brief survey of the wide field of applications, and show selected examples related to studies of thin films in more detail.

Numerous *in situ* ATR applications in liquid or wet environments have been reported either using single- or multiple-reflection geometries [9–25, 42, 47, 49–60, 70–73]. Examples are catalytic [47], voltammatric [48], biofilm [7, 21], cell [11, 21, 49, 57, 73], and titration studies [50], investigations of superhydrophobic surfaces [51], adsorption and complexation of biomolecules [52, 71], sulfate bonding mechanisms on Goethite [53], cement on barnacles [55], ionic liquids and supercritical fluids [56], tissues [57], imaging of drug diffusion [42], polymers [58, 70, 72], electro-oxidation [59], protein adsorption [21, 60], proteins and peptides in lipid bilayers [129] and powder–liquid interfaces [43].

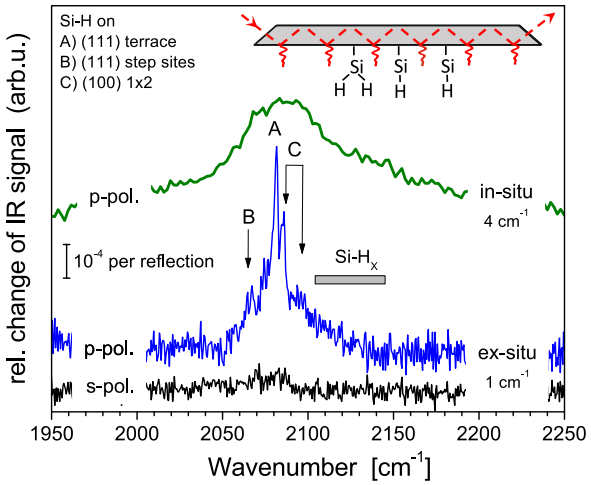

Figure 3. ATR-IR spectra of a hydrogen-passivated silicon surface under Argon atmosphere and in contact with an aqueous electrolyte. Reprinted from [77], Copyright (2002), with permission from Elsevier.

Beside these technical applications, *in situ* ATR can be combined with IR ellipsometric measurements [74, 76], and is therefore also relevant for the determination of refractive and absorption indices of solutions [32, 74–76].

In the following, two examples of interface studies with sub-monolayer sensitivity are presented.

Hydrogen-passivated silicon can be used as a starting surface for radical reaction molecular depositions. *In situ* ATR with multiple reflections allows the sensitive evaluation of such an initial surface and the monitoring of the surface with respect to chemical changes in the sub-monolayer regime. Figure 3 compares IR spectra of a flat and hydrogenated Si surface in Argon atmosphere with the spectrum of the same surface in contact with an aqueous electrolyte. The spectrum measured in p-polarization in Argon shows a strong and narrow Si–H band with a broader background due to different Si–H, $Si–H_2$ and $Si–H_3$ contributions. The spectrum of the Si–H surface measured in s-polarization shows only a minor contribution of Si–H, indicating that Si–H bonds are oriented mainly perpendicular to the Si surface. The spectrum measured in electrolyte exhibits an overall broadened, slightly less intense Si–H related absorption band with slightly lower intensity.

The second example is an *in situ* monolayer study [50] in which an ATR set-up with about 30 reflections (see figures 4(a) and (b)) was employed. The authors have prepared well-defined mixed decyl/10-carboxydecyl monolayers on hydrogen-terminated Si(111) surfaces via direct photochemical hydrosilylation and studied their behavior in dependence of a varying pH value of the electrolyte solution. *In situ* spectra (see figure 4(c)) were analyzed quantitatively with respect to the pH-dependent dissociation of carboxyl groups into carboxylate ions in the monolayer. A conversion takes place of the ν(C=O) band (related to COOH group)

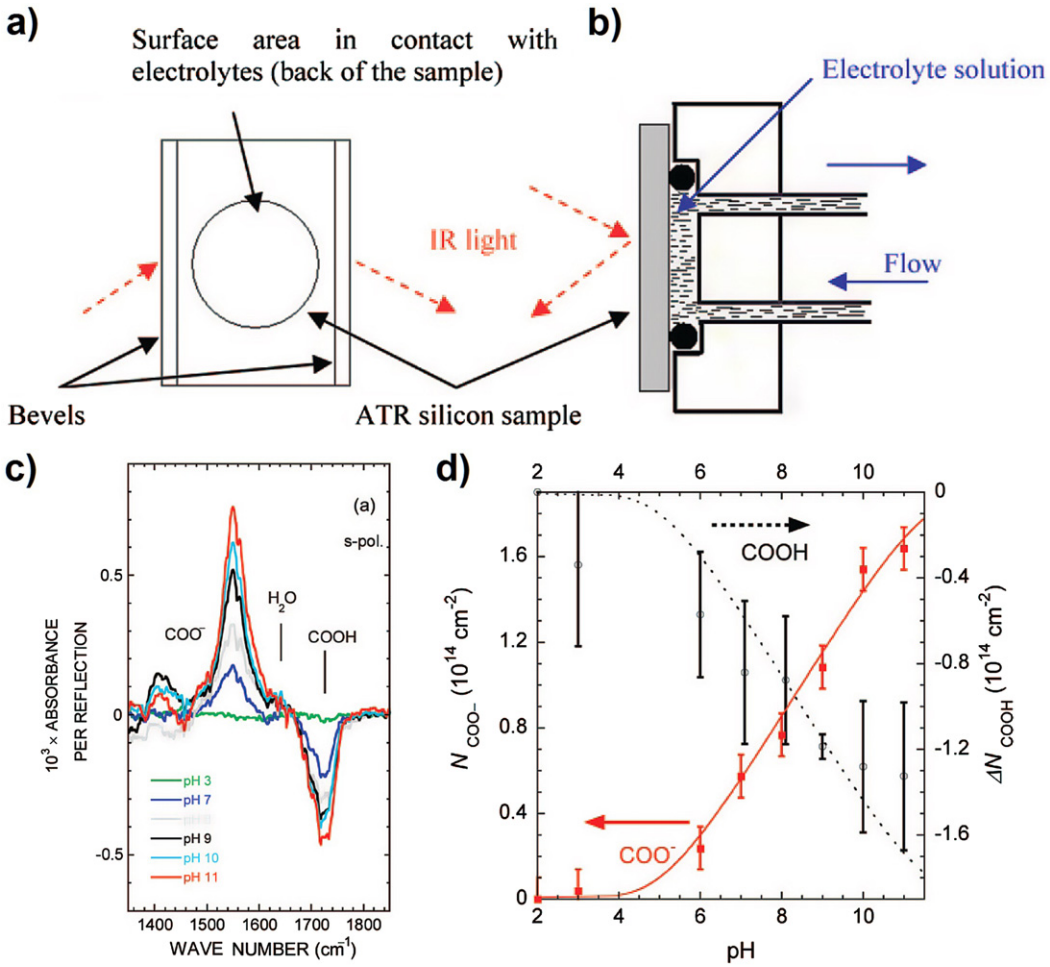

Figure 4. (a) *In situ* ATR set-up from reference [50]; (b) 100 μL flow cell; (c) pH dependence of the stretching vibrations of COOH and COO^- groups in s-polarized *in situ* ATR spectra with respect to an initial spectrum at pH 2; (d) Calculated surface concentration of COOH and COO^- groups. Reprinted with permission from [50]. Copyright (2008) American Chemical Society.

around 1720 cm^{-1} into the $\nu_s(COO^-)$ and $\nu_{as}(COO^-)$ bands at 1400 cm^{-1} and 1550 cm^{-1}, respectively. The surface concentration of COOH and COO^- groups (see figure 4(d)) was calculated from the respective band amplitudes, showing approximately 10% ionized groups at pH 6 with a progressive trend towards a more, but not fully, ionized layer at pH 11. This dissociation behavior differs from the one the authors observed in bulk solutions (3D) and can be explained by solvation constraints and different electrostatic interactions between the molecules, which are ordered in a 2D layer at the surface [50]. This effect is of general importance for many surface reactions, and, as stated in reference [50], may markedly affect the kinetics of immobilization reactions important for the formation of functional surfaces in general.

3.1.1. Current in situ ATR improvements. The implementation of a brilliant light source can improve the ATR technique with regard to sensitivity as well as lateral and time resolution (see also section 2.2.3). In order to measure minute amounts of analyte, a wide range of micro- and nanofluidic approaches were introduced in recent years [5–19]. Different concepts for fiber-based ATR spectroscopy have been developed. For example, IR-transparent fibers can be coupled to internal single- or multiple-reflection elements or to fiber optic waveguides [21–25]. The latter approach formed the new field of fiber evanescent wave spectroscopy (FEWS) [21]. Fiber-based waveguide sensing can also be coupled to QCL technology [20, 90], and has many applications ranging from chemical and biosensing to environmental monitoring [21–25].

3.2. *In situ IR ellipsometry*

In situ IR ellipsometry has been established for various applications of thin films and interfaces [26–28, 31, 33–39, 67–69, 78, 79]. The method is able to probe both material-specific vibrational bands and structure-related baselines, enabling the detailed analysis of molecular and functional thin-film properties, in particular when combined with optical simulations for quantitative spectra evaluation.

Quantitative IRSE approaches were introduced for modeling the electrochemical grafting of ultrathin nitrobenzene [34] and maleimido-phenyl multilayers [36], the growth process of PSS-doped polyaniline films [28], PDA growth processes [37], as well as the swelling–deswelling transition of polyacrylamide brushes and thin films [31, 67]. Recent works showed the high application potential of *in situ* IRSE for the

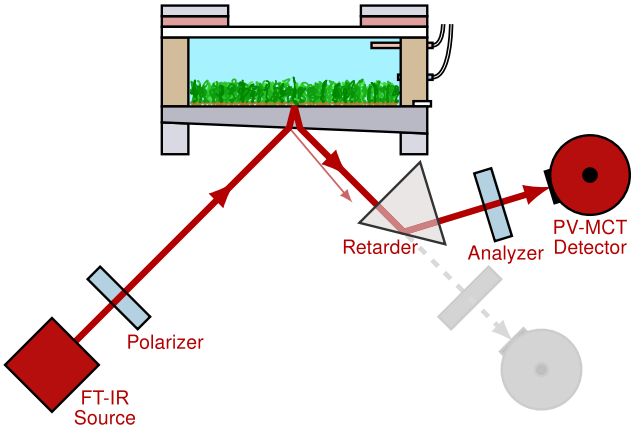

Figure 5. Schematic of an IR ellipsometer [2] coupled with an *in situ* flow cell to monitor functional polymer thin films in liquid environments. Reprinted by permission from Springer Nature Customer Service Centre GmbH: Springer Nature, Ellipsometry of Functional Organic Surfaces and Films, copyright Springer Nature 2018.

analysis of sensor and biofunctional surfaces [31, 36, 39, 78, 79].

In this section, key applications of *in situ* IR ellipsometry for investigations of functional polymer surfaces [26, 31, 33, 35, 64–69] and electrochemical preparations [27, 28, 34, 36–39, 62, 78, 79] are discussed.

3.2.1. Biofunctional polymer surfaces. Functional organic thin films such as polymer brushes are of high technological interest in various bioanalytical applications, e.g., in antimicrobial surfaces, cell templates, drug delivery and biosensing. Figure 5 shows a schematic of a polymer brush investigated in an *in situ* IR ellipsometric set-up. The *in situ* flow cell is coupled to the IR ellipsometer, which employs a retarder prism for accurate phase measurements. The brush itself is prepared on, and probed through, an IR-transparent silicon substrate. The wedge shape of the substrate ensures that measured spectra originate from a single reflection undisturbed by window oscillations. The small wedge angle of 1.5° enables straightforward sample handling and facilitates, e.g., spin-coating and other preparation steps required for interface formation.

In situ IRSE was extensively applied for investigating the properties of various types of mono, binary and mixed polymer thin films, as well as their use for controlling protein adsorption and desorption via external stimuli like temperature or pH [21, 33, 35, 88–90, 92]. Topical applications of such films are biomedical surfaces such as antifouling coatings and tunable bioactive surfaces for drug delivery or controlled cell growth [35].

For example, *in situ* IRSE provided detailed insights into the protein adsorption properties of functional polymer films made from PAA, PNIPAAm, PGMA, and their block-copolymers PNIPAAm-*co*-PGMA [88]. As shown in figure 6, proteins adsorbed to these surfaces give rise to characteristic amide I and II bands, which are associated with the protein secondary structure. An analysis of these bands revealed distinct stimuli-dependent ad- and desorption behaviors (and kinetics) of human serum albumin (HSA) and fibrinogen, depending on film architecture, composition, and tunable hydration state. In the case of HSA adsorption on PAA, partial desorption was observed when increasing the pH of the aqueous environment. It was found that the protein structure of HSA bound within the polymer film remains intact, which is crucial information for bioapplications like drug release. The high chemical contrast of IRSE also made it possible to distinguish whether or not a functional surface could be regarded as protein repellent.

In situ IR ellipsometry was employed to study different functional thin polymer films with respect to structure, hydration, chemical properties, and molecular interactions [26, 31, 33, 65, 66, 69]. The method revealed pH-responsive, reversible swelling and complex formation of mixed PAA-*mix*-P2VP polyelectrolyte brushes [26, 33]; pronounced hysteresis effects in PAA mono brushes regarding swelling–deswelling, carboxyl dissociation and reprotonation, and ion/counterion distribution [65]; as well as different strong and weak carbonyl-water hydrogen-bond interactions contributing to swelling and hydration of oxazoline-based polymer brushes in H_2O and D_2O [66].

By analyzing position and shape of vibrational markers like ν(C=N) and ν(C=O) bands, *in situ* IRSE is particularly suited for investigating solvatochromic effects as well as specific molecular interactions, also in solvents other than water [69].

Concerning molecular interactions in particular, and the study of biofunctional polymer thin films in general, the full potential of *in situ* IRSE as a quantitative analytical technique was demonstrated in [31] by combining ultrasensitive measurements with theoretical calculations based upon detailed optical models. The approach delivered simultaneous quantitative information on film hydration, structural and chemical properties [31] of thin PNIPAAm layers and brushes in dependence of external stimuli like ambient water temperature or humidity of air.

PNIPAAm is a secondary amide with hydrophilic amide (HNCO) and hydrophobic isopropyl groups. Figure 7 gives an overview of typical *in situ* IRSE spectra of PNIPAAm films measured in different environments. Similar to the proteins discussed before, the spectra show complicated band compositions of the polymer's amide I and II bands. Moreover, there are strong overlaps of the polymer-related bands with those of water. In traditional spectroscopy, such superimpositions usually cause tremendous problems for quantitative analyses.

PNIPAAm's amide I band, which is mainly associated with C=O stretching modes, is comprised of at least five major components. Their vibrational frequencies depend on short-range hydrogen-bond interactions. Hydrogen bonding with water molecules or neighboring amide segments of the polymer chain leads to distinct oscillator redshifts. These shifts can be interpreted in combination with DFT (density functional theory) calculations in order to identify and assign the various types of intra- and intermolecular interactions (see figure 7 bottom).

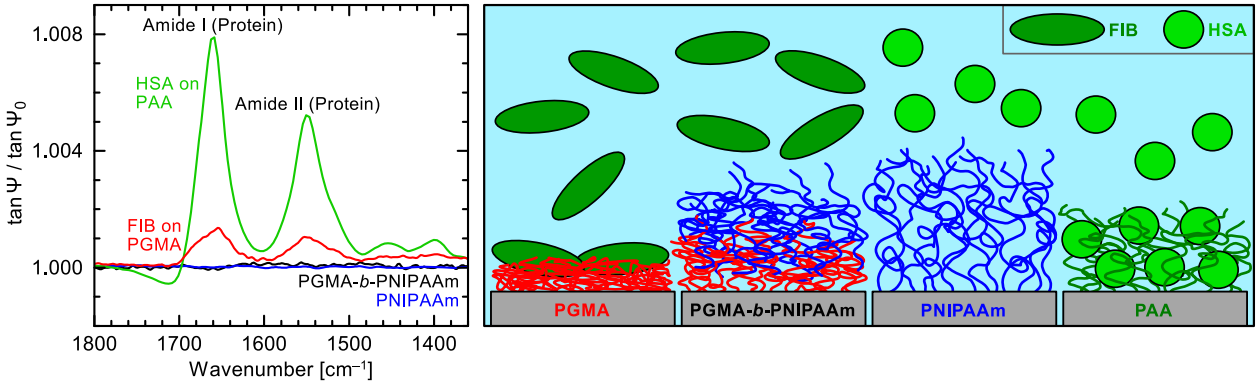

Figure 6. Protein adsorption and repellence observed with *in situ* IRSE for different types of functional thin polymer films. Reprinted with permission from [64]. Copyright (2015) American Chemical Society.

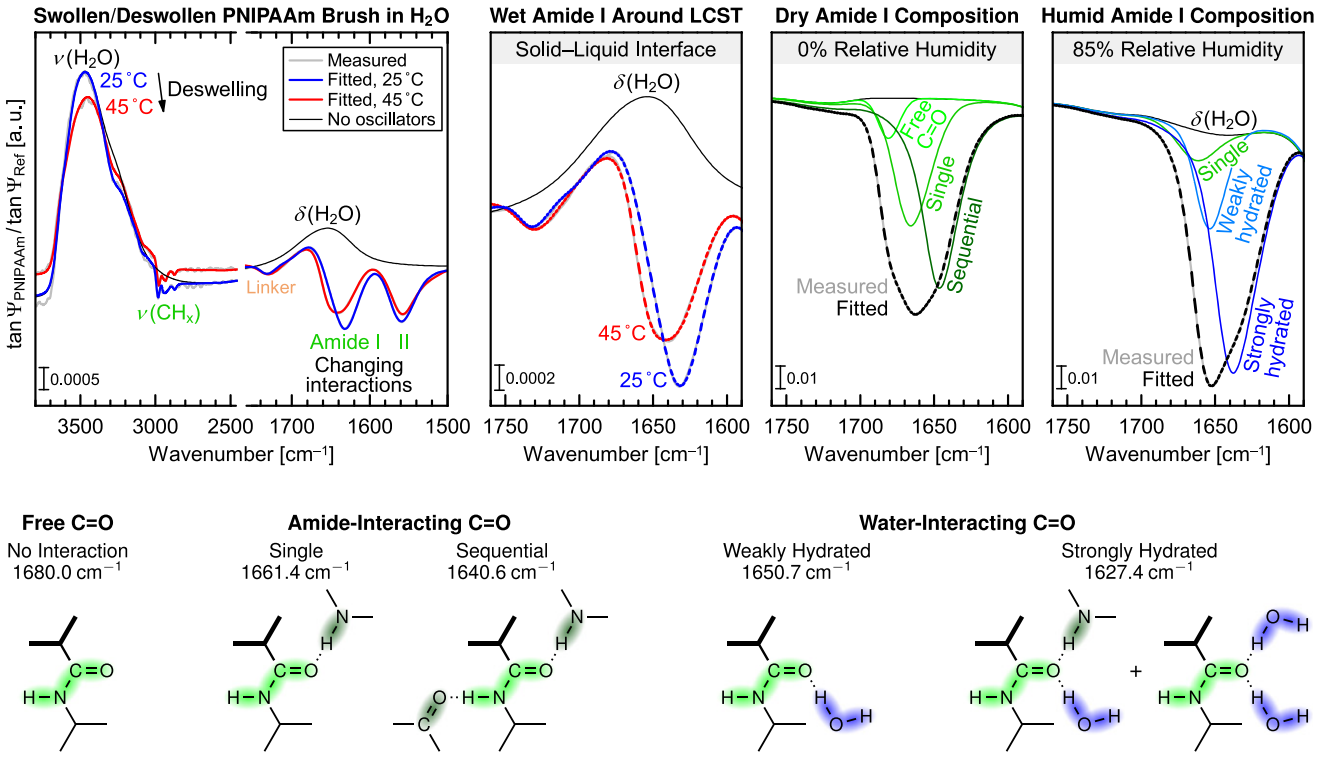

Figure 7. Top: measured and fitted referenced tan Ψ *in situ* IR ellipsometry spectra and band compositions of swollen and collapsed PNIPAAm films in various environments (left: aqueous solution below and above the LCST; right: dry and humid air). Bottom: characteristic inter- and intra molecular interactions assigned to C=O-stretching amide I resonances. The oscillator frequency progressively shift to lower wavenumbers with increasing number and strength of carbonyl hydrogen bonds. Reprinted with permission from [31]. Copyright (2017) American Chemical Society.

The spectra are quantitatively analyzed by optical modeling based upon an effective-medium approach. The polymer's dielectric function is built from a sum of oscillators (describing the various vibrational modes) and embedded in an effective dielectric medium of water and polymer. As seen in figure 7, the IRSE optical model is able to correctly describe the observed *in situ* spectra and to automatically account for the spectral overlap between polymer and solvent signatures. Moreover, structural sample information, such as swelling degree and water content of the brushes, can be extracted from the fit via an analysis of the water vibrational bands. Molecular interactions are quantified by the amplitudes of the respective oscillators associated with the different molecularly interacting groups.

The fits reveal remarkable details regarding, e.g., thermoresponsive intra- and intermolecular interactions of the polymer's amide groups, film swelling, hydration of the hydrophilic amide and hydrophobic isopropyl groups, as well as the number of water molecules per monomer and their individual contribution to overall film hydration and specific interactions (strong, weak, or no hydration) [31].

The above example highlights the wealth of information accessible with *in situ* IRSE. The ability to investigate and quantify molecular interactions and structural properties of

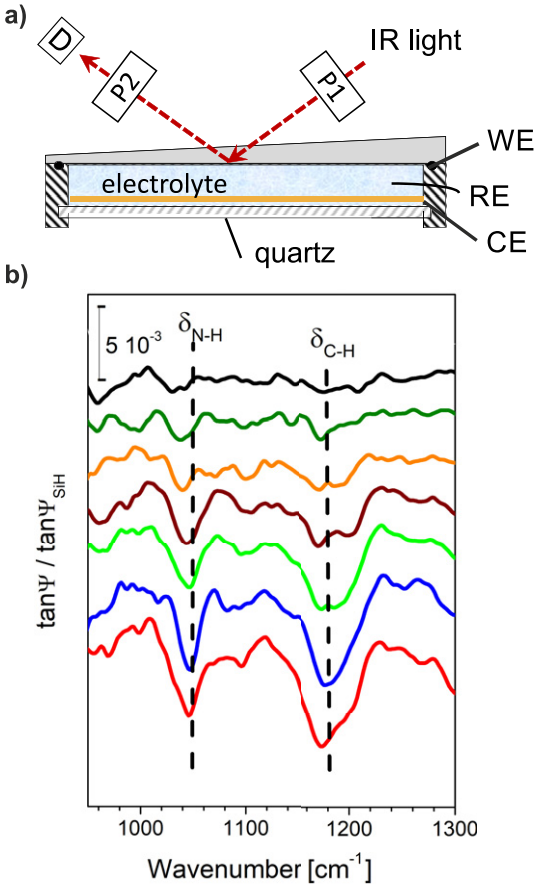

Figure 8. (a) Schematic of an electrochemical *in situ* cell for IRSE measurements. (b) IR ellipsometric monitoring of the electrochemical deposition of polypyrrole (PPy). Smoothed tan Ψ spectra (normalized to tan Ψ of the H-passivated Si(111) surface before the preparation) with increasing number of anodic potential pulses are shown from top to bottom. Reprinted with permission from [12]. Copyright (2018) American Chemical Society.

ultrathin films is an important building block for miniaturization toward microfluidic concepts.

3.2.2. Electrochemical applications. *In situ* IR ellipsometry has been applied for monitoring various electrochemical preparations, among them functional interfaces of oligomers of small molecules like nitrobenzene [34, 38], maleimidophenyl [36], functionalized graphene [39, 78, 79], as well as polymers for hybrid organic solar cells [27, 28, 61, 62] and biofunctional applications [37, 39, 79].

Figure 8(a) shows an electrochemical *in situ* cell for IR ellipsometric measurements. The cell comprises a silicon top window as substrate (WE: working electrode) and is equipped with a contact to a potentiostat for electrochemical deposition (CE: counter electrode, RE: reference electrode). A quartz window is mounted on the opposite side of the liquid reservoir, allowing for complementary measurements by, e.g., reflectance anisotropy spectroscopy (RAS) [27] or Raman back-scattering spectroscopy.

As an example for the *in situ* monitoring of an electrochemical deposition, figure 8(b) displays the temporal development of IR spectra of a polypyrrole (PPy) film grown by pulsed deposition from an aqueous solution containing pyrrole molecules. The amount of deposited PPy scales with the number of potential pulses applied in the electrochemical deposition process. From lower to higher number of deposition pulses (top to bottom spectra), the band amplitudes due to C–H and N–H vibrations at about 1180 cm^{-1} and 1050 cm^{-1}, respectively, are increasing [107, 108]. The N–H vibrational band might overlap with weak bands related to SiO_x vibrations. However, a strong oxidation of the interface can be ruled out because no significant band associated with the Berreman mode of the silicon oxide is found around 1220 cm^{-1}.

3.2.3. Current in situ IRSE improvements. Important novel developments of *in situ* IRSE are the extension of polarimetric set-ups by brilliant light sources such as QCLs [92, 114–116, 130], and the implementation of more advanced measurement schemes such as Mueller-matrix ellipsometry [93, 112].

3.2.3.1. Mueller-matrix ellipsometry. Recent technical advancements showed that IR Mueller-matrix (MM) ellipsometry can be used to study complex thin films [69, 93, 112]. This extended ellipsometric technique measures not only changes in co-polarization (pp, ss) but also cross-polarization (ps, sp) and depolarization. MM ellipsometry is therefore of high interest for investigations of anisotropic and/or depolarizing thin films, in particular of adsorption processes, which are anisotropic processes by nature. A first study [93] proved it feasible to measure the partial 4 × 3 MM of a 68 nm thin PGMA film at the polymer–water interface. Sub-minute time resolutions were reported for monitoring the relaxation of an anisotropic, stretched polyethylene foil [93]. Highly sensitive full 4 × 4 IR MM measurements were also demonstrated recently [112], opening the door for in-depth *in situ* investigations of thin films.

3.2.3.2. Brilliant light sources. Another interesting upgrade for *in situ* IRSE are brilliant light sources, as they enable spatially and temporally higher-resolved measurements. Employing such sources allows the study of homogeneity effects, the investigation of smaller volumes, and the analysis of non-cyclic, time-dependent processes.

A decade ago, using the *in situ* IRSE set-up at the BESSY II synchrotron facility, the chemical and structural homogeneity of a binary polyelectrolyte brush, consisting of PEG and PAA-*b*-PSS, could be investigated with a spot size of about 1 mm^2 [68].

Recent developments [114–116] demonstrate that such studies can also be performed in standard laboratory environments. For example, the combination of a QCL with a single-shot ellipsometer design based upon four parallel polarization-state detection channels was able to translate many advantages of IR synchrotron ellipsometry to the lab. In references [124, 125], the world's first IR laser-based polarimeter for measurements in a reflection geometry with high spectral (<0.5 cm^{-1}), sub-millimeter (125 μm) spatial, and sub-decisecond time resolution was presented. These new possibilities have high potential for *in situ* studies of small

volumes and the investigation of time-dependent processes in the ms and even µs range.

3.3. SEIRA microfluidics

Methods for destruction-free, label-free analysis of μL and nL liquid volumes are important for multiple bioanalytical applications and could be a key component in the realization of novel concepts, e.g., for personalized medicine or in biosensing. Integrating these methods with microfluidics enables one to apply the wide variety of established microfluidic techniques for reliable and reproducible sample manipulation, providing a high level of control over the fluidic environment and other sample-related process and preparation parameters. With these capabilities, IR microfluidic sensing can be important in molecule-specific chemical and structural detection, which is relevant for drug development and down-stream bioanalytics. Promising developments in this direction are approaches involving SEIRA interfaces for microfluidic chips that enable studies of liquids with low analyte concentrations [12, 88]. Furthermore, the ability to work with small volumes on the scale of μL to nL opens up the technology to any application where the sample amount is strongly limited, as it is often the case in biosensing and other biomedical applications.

Combining microfluidics for sample handling with an IR microscopic set-up in an internal reflection geometry (see section 2.1) or an external reflection geometry (such as it is also used in IRRAS, see section 2.2.1) is a powerful approach to conduct investigations using minute sample volumes down to a few nL. The utilization of plasmonic enhancement substrates is required for signal enhancement (see section 2.3.2) in order to analyze such small volumes within reasonable measurement times and with sufficient sensitivity in measurements where sampling areas are only a few hundreds of μm^2. Using these types of substrates increases sensitivity by several orders of magnitude.

Various types of plasmonic films and nanostructures have been developed and applied for *ex situ* applications [98–105]. Roughened gold electrodes and metal-island films [98, 99] provide broadband enhancement over the whole surface and the full MIR region at moderate enhancement factors ('usually found to be in the 10–100 region' [99]). Larger, but spatially and spectrally highly confined, enhancement (up to 10^7) can be achieved by tailored nanoantennas and related nanostructures [100–104]. The potential of integrating enhancement substrates with microfluidics for *in situ* applications has already been demonstrated regarding the analysis of protein secondary structures [88].

In a recent publication [12], an optofluidic cell [130] was presented that integrates gold-island film substrates fabricated on an IR transparent silicon substrate for signal enhancement for *in situ* SEIRA sensing with sub-monolayer sensitivity in nL volumes. Figure 9(a) schematically shows the SEIRA optofluidic cell (in a simple reflection geometry), which can incorporate measurement windows made from different IR-transparent materials functionalized with a gold-island film for signal enhancement. The cell was designed

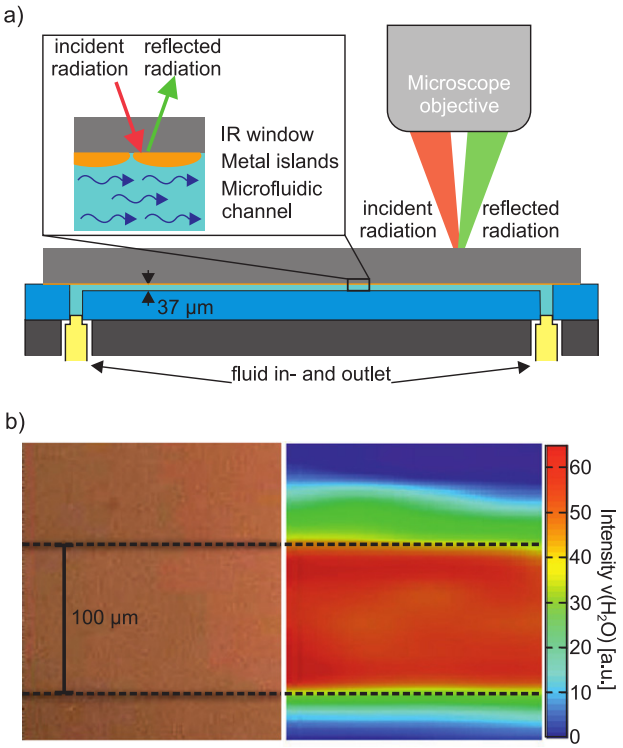

Figure 9. *In situ* SEIRA optofluidic cell [12] for *in situ* nL IR sensing. (a) Schematic of the optofluidic cell. Adapted with permission from reference [12]. Copyright (2018) American Chemical Society. (b) Chemical imaging (10 μm step size) of a 100 μm wide microfluidic channel filled with water; color coding refers to the intensity of the water stretching vibrational mode.

to be used in conventional and commercially available IR microscopes, but can also be adapted for applications in other IR and IRSE set-ups. The microfluidic chip can be fabricated from different materials commonly used in microfluidics, such as glass or polymers. Various microfluidic chip designs can be realized. The optofluidic platform's large accessible measurement area enables chemical mapping on different domains of the microfluidic chip. Beside point-by-point mapping, a focal plane array can also be used for channel imaging [14–17, 87].

Figure 9(b) shows an image of a 2D map (10 μm steps in x and y direction, using an IR microscope at the BESSY II synchrotron) along a microfluidic channel, with color-coded intensity of the water stretching vibration. This particular microfluidic chip comprised four long straight channels (100 μm width, 37 μm depth, 42 mm length) with a total volume of approximately 155 nL each. In this configuration, the effective measurement volume and area were about 1.1 nL and 3×10^{-4} cm^2, respectively.

By employing an enhancement substrate, high sensitivities with a limit of detection (LOD) in the range of a few ng cm^{-2} are achieved. With such LOD, investigations of the kinetics of adsorption/desorption processes and monolayer formation at the solid–liquid interface become feasible.

In the following, two examples are presented that highlight the potential of the optofluidic platform for diverse *in situ* applications.

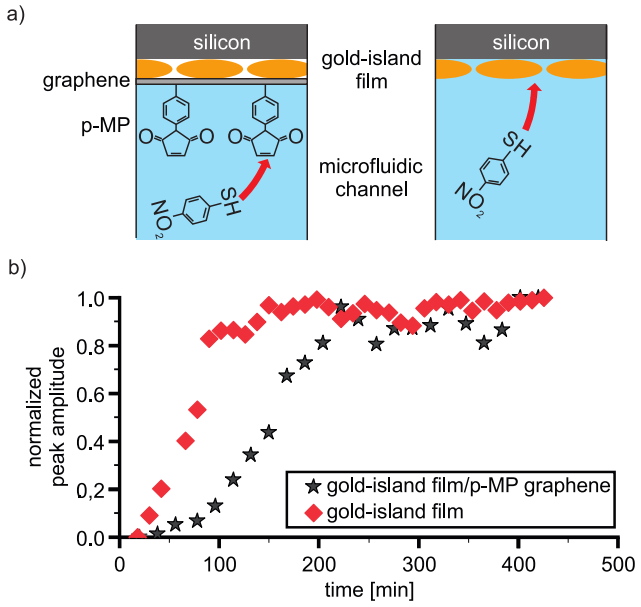

Figure 10. *In situ* SEIRA optofluidic study of the adsorption kinetics of 4-NBM on functionalized and bare gold-island film substrates. (a) Schematic of the investigated interfaces. (b) Time dependent development of the normalized peak amplitude of the NO_2 vibrational band of 4-NBM on the different interfaces. Reprinted from [39], Copyright (2018), with permission from Elsevier.

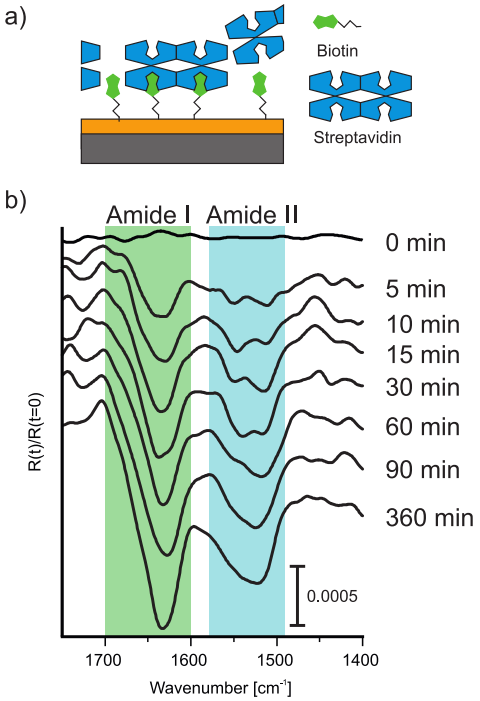

Figure 11. Study of the binding of streptavidin on a biotinylated enhancement substrate. (a) Schematic of process in which streptavidin binds one biotin molecule per binding pocket. (b) *In situ* microscopic IR spectra obtained at different time points. Spectra are referenced to an initial spectrum of the channel filled with PBS buffer. Reprinted with permission from [12]. Copyright (2018) American Chemical Society.

In the first example, the platform was applied to investigate the adsorption kinetics of (4-nitrobenzyl) mercaptane (4-NBM) on an enhancement substrate functionalized with p-maleimido-phenyl (p-MP) modified graphene [113, 114] (see figure 10). The binding process between the 4-NBM and the p-MP residues of the functionalized enhancement substrate [39] is depicted in figure 10(a). In order to gain evidence on the nature of the binding process, the adsorption kinetics have been compared to the one observed on an unmodified enhancement substrate (see figure 10(b)).

The presented time-dependent adsorption curves show the normalized peak amplitude reflectivity of 4-NBM's nitro-group vibrational band around 1522 cm^{-1} measured for the graphene-functionalized (black stars) and unmodified enhancement substrate (red rectangles). Distinct differences in the adsorption kinetics are observed for the different interfaces. In the case of 4-NBM adsorption on the unmodified enhancement substrate, the exponential increase in the band amplitude is indicative of a zero-order reaction, as is typically found in the adsorption of thiolated molecules on gold interfaces. In the case of adsorption on the functionalized enhancement substrate, the kinetics show a higher-order polynomial progression. This kind of kinetics is indicative of a higher-order reaction process, e.g., a Michael addition reaction creating a bond between the thiol group of 4-NBM and the imide group of p-MP. A further indicator for a more complex binding reaction is the increased time it takes to reach a saturation in the adsorption curve. These findings supported the assumption of a chemisorption of 4-NBM on the p-MP functionalized surface.

The second example investigates the adsorption process of streptavidin on a biotinylated enhancement substrate (see figure 11). This example demonstrates the possibility given by the optofluidic cell to monitor molecular deposition also on a previously functionalized enhancement substrate, which is of high relevance for investigating the formation of functional surfaces in biosensing applications and molecular sensing. Furthermore, the capability to measure with sub-monolayer sensitivity can be exploited to elucidate structural changes of proteins during an adsorption/binding process. This is highly relevant for the investigation of biosensing interfaces but also for studies on the interaction between molecules and proteins in biomedical and pharmaceutical research.

Figure 11(b) shows exemplary *in situ* IR spectra at selected time points of the adsorption of streptavidin on the biotinylated enhancement. Characteristic amide I and II vibrational bands related to the protein secondary structure can be identified. The amide I band is centered around 1637 cm^{-1}, which is commonly associated with a high content of β-sheet structure elements in a protein [80], and correlates well with the known β-barrel structure of streptavidin's native state [40]. The dynamic changes in the shape of the amide I and II band are indicative of changes in the protein secondary structure as a consequence of the binding of streptavidin molecules on the biotinylated surface [131].

Further studies are required to unravel the details of the underlying processes. However, with carefully performed

complementary measurements and/or optical simulations, a detailed analysis of the structural properties could be performed. Notably, only the last spectrum of the series corresponds to an adsorbed protein monolayer, i.e., to the maximum amount of streptavidin that can be bound to the interface. All other spectra show sub-monolayer protein coverages. Using literature values for the surface density of a streptavidin layer on a biotinylated interface [132], an LOD can be calculated from the amide I band area of the saturated monolayer. Taking into account the slightly increased surface area due to the roughness of the gold-island film, an effective surface density of (4.16 ± 0.16) pmol cm^{-2} is found for the monolayer. The noise level in the measurement corresponds to an estimated LOD of (0.12 ± 0.01) pmol cm^{-2} or (7.1 ± 0.3) ng cm^{-2}.

3.3.1. Current SEIRA microfluidics improvements.

3.3.1.1. Optofluidic platform. The development of an optofluidic platform opens up the possibility to use minute amounts of samples for the investigation by SEIRA spectroscopy. Its exceptional sensitivity and the possibility to gain information on the chemical structure of the molecule renders the presented system highly interesting for a variety of applications, in particular in the fields of environmental and biosensing. The additional ability to study inter- and intramolecular interactions gives an interesting lead into developing novel strategies for *ex vivo* studies to analyze interactions between proteins and molecules, e.g., in biomedical research. Access to information on molecule- and environment-specific interactions are of particular interest, as they may provide information on disease-related mechanisms, which might help to identify new targets for treatments, or aid in the evaluation of the mode of action of potential novel drugs.

Further improvements and multi-modal usage of the optofluidic platform are possible. In the case of the enhancement substrates, using metals other than gold for the plasmonic nanostructure, such as copper or other catalytically active metals, could enable *in situ* studies of catalytic processes at such interfaces. Moreover, the possibilities arising from the integration of other microfluidic concepts could allow a translation of the method, e.g., to electrochemical studies or to the investigation of cell cultures, potentially extending the range towards organ-on-chip systems or other highly integrated lab-on-chip platforms. Also, the combination with downstream analytics such as mass spectrometry is of high interest for future applications.

3.3.1.2. Brilliant light sources. As shown in figure 9, the combination of the discussed optofluidic concept with a brilliant light source facilitates laterally highly resolved mapping of a microfluidic channel.

Currently, we are working on the adaption and incorporation of the optofluidic cell into a new QCL-based single-shot ellipsometer (see also section 3.2.3). For individual spots in the optofluidic cells, the use of the QCL provides time resolutions on the order of milli- to microseconds. In combination with multi-method investigations that integrate, e.g., SERS in addition to SEIRA [107, 130], these developments will be of high interest to study numerous novel sensing applications. Prominent examples are identification and structure analysis, as well as gaining a better understanding of the dynamics of molecular interactions and processes at solid–liquid interfaces.

4. Summary

ATR, IR ellipsometry and SEIRA microfluidics have been presented as methods for destruction-free and label-free *in situ* IR spectroscopy in mL to nL flow cells. Multiple technological developments render *in situ* IR spectroscopy a rapidly developing field for analytics of lowest liquid volumes and sub-monolayer coverages with high sensitivity for chemical, structural and time-dependent properties. Recent advancements in the coupling of fibers and waveguides, the use of porous and versatile enhancement substrates, and the involvement of laboratory IR laser sources have significantly broadened the area of applications and proved *in situ* IR spectroscopy to be a vital and fast developing research and technological field.

Particularly the technological developments allowing miniaturization and variable operational areas are of high importance for bio- and environmental analytics. Here the use of enhancement interfaces or fiber optics leads to increases of detection limits even when studying smallest liquid volumes. The incorporation of (pulsed) brilliant IR sources in IR methods helps to realize optical set-ups with high optical throughputs, faster and spatially higher resolved measurements with significant improvements regarding hyperspectral imaging and time-resolved measurements in the μs to ms range. New laser-based MIR technological developments exhibit a high potential for biomedical and electrochemical *in situ* applications. Different methodical concepts for the MIR already exist and are being transferred for *in situ* studies, e.g. (i) measuring optically [133], (ii) detecting the near-field scattered light [135], (iii) and detection of the photothermal expansion [134, 136]. In addition, methods based on frequency comb techniques [137, 138] further broaden the range of future brilliant IR spectroscopic developments. Beside the experimental advancements, also the optical interpretation of measured spectra was further refined by applying numerical and analytical optical simulations.

In summary, the recent technological advances of *in situ* IR spectroscopy have, to our opinion, pushed new frontiers in studying thin films at solid–liquid interfaces. These advances make it possible to address important questions and challenges in the research of catalysis [e.g. 139], chemical processes [e.g. 9], batteries [e.g. 82], protein analytics [e.g. 89], drug testing [e.g. 90], clinical applications [e.g. 140], point-of-care medical diagnosis [e.g. 81, 83, 90] and environmental analytics [e.g. 91]. Investigations by *in situ* IR spectroscopy yield complementary information in these applications and offer the advantage of providing insights on the chemical and structural properties of molecules and molecular interactions.

Acknowledgments

We are indebted to T Oates and D Gkogkou for cooperation in the field of enhancement substrates and *in situ* vibrational sensing, D Janasek in the field of microfluidics, and U Schade and M Gensch in the field of synchrotron IR spectroscopy. We acknowledge technical support by I Engler and Ö Savas, and financial support by the European Union through EFRE 1.8/13, as well as financial support by the Ministerium für Kultur und Wissenschaft des Landes Nordrhein-Westfalen, Der Regierende Bürgermeister von Berlin-Senatskanzlei Wissenschaft und Forschung, and the Bundesministerium für Bildung und Forschung.

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References

[1] Griffiths P R and Chalmers J 2006 *Handbook of Vibrational Spectroscopy* vol 2 (Chichester: Wiley)
[2] Hinrichs K and Eichhorn K-J 2018 *Ellipsometry of Functional Organic Surfaces and Films* (Springer Series in Surface Sciences) 2nd edn (Heidelberg: Springer)
[3] Schasfoort R B M (ed) 2017 *Handbook of Surface Plasmon Resonance* 2nd edn (Croydon: The Royal Society of Chemistry)
[4] Sauer M, Hofkens J and Enderlein J 2011 *Handbook of Fluorescence Spectroscopy and Imaging: From Single Molecules to Ensembles* (Weinheim: Wiley)
[5] Chan K L A, Gulati S, Edel J B, de Mello and A J and Kazarian S G 2009 Chemical imaging of microfluidic flows using ATR-FTIR spectroscopy *Lab Chip* **9** 2909–13
[6] Chan K L A, Niu X, de Mello A J and Kazarian S G 2010 Rapid prototyping of microfluidic devices for integrating with FT-IR spectroscopic imaging *Lab Chip* **10** 2170–4
[7] Holman H Y N, Miles R, Hao Z, Wozei E, Anderson L M and Yang H 2009 Real-time chemical imaging of bacterial activity in biofilms using open-channel microfluidics and synchrotron FTIR spectromicroscopy *Anal. Chem.* **81** 8564–70
[8] Karabudak E 2014 Micromachined silicon attenuated total reflectance infrared spectroscopy: an emerging detection method in micro/nanofluidics *Electrophoresis* **35** 236–44
[9] Perro A, Lebourdon G, Henry S, Lecomte S, Servant L and Marre S 2016 Combining microfluidics and FT-IR spectroscopy: towards spatially resolved information on chemical processes *React. Chem. Eng.* **1** 577–94
[10] Greener J, Abbasi B and Kumacheva E 2010 Attenuated total reflection Fourier transform spectroscopy for on-chip monitoring of solute concentrations *Lab Chip* **10** 1561–6
[11] Chan K L A and Kazarian S G 2013 Aberration-free FTIR spectroscopic imaging of live cells in microfluidic devices *Analyst* **138** 4040–7
[12] Kratz C, Furchner A, Oates T W H, Janasek D and Hinrichs K 2018 Nanoliter sensing for infrared bioanalytics *ACS Sens.* **3** 299–303
[13] Lehmkuhl B, Noblitt S D, Krummel A D and Henry C S 2015 Fabrication of IR-transparent microfluidic devices by anisotropic etching of channels in CaF_2 *Lab Chip* **15** 4364–8
[14] Kaun N, Vellekoop M J and Lendl B 2006 Time-resolved Fourier transform infrared spectroscopy of chemical reactions in solution using a focal plane array detector *Appl. Spectrosc.* **60** 1273–8
[15] Hinsmann P, Haberkorn M, Frank J, Svasek P, Harasek M and Lendl B 2001 Time-resolved FT-IR spectroscopy of chemical reactions in solution by fast diffusion-based mixing in a micromachined flow cell *Appl. Spectrosc.* **55** 241–51
[16] Kakuta M, Hinsmann P, Manz A and Lendl B 2003 Time-resolved Fourier transform infrared spectrometry using a micro fabricated continuous flow mixer: application to protein conformation study using the example of ubiquitin *Lab Chip* **3** 82–5
[17] Baena J R, Kulka S, Kaun N, Frank J, Svasek P, Moss D, Vellekoop M J and Lendl B 2004 Mid-IR synchrotron radiation for molecular specific detection in microchip-based analysis systems *Anal. Bioanal. Chem.* **378** 1735–40
[18] Ewing A V, Clarke G S and Kazarian S G 2016 Attenuated total reflection-Fourier transform infrared spectroscopic imaging of pharmaceuticals in microfluidic devices *Biomicrofluidics* **10** 024125
[19] Chang Y-C, Wägli P, Paeder V, Homsy A, Hvozdara L, van der Wal P, Francesco J D, de Rooij N F and Herziga H P 2012 Cocaine detection by a mid-infrared waveguide integrated with a microfluidic chip *Lab Chip* **12** 3020–3
[20] Jouy P *et al* 2014 Mid-infrared spectroscopy for gases and liquids based on quantum cascade technologies *Analyst* **139** 2039–46
[21] Lucas P and Bureau B 2011 *Advanced Infrared Glasses for Biochemical Sensing* (Biointerface Characterization by Advanced IR Spectroscopy) ed Pradier C M and Chabal Y J (Amsterdam: Elsevier) ch 8
[22] Eccleston R, Wolf C, Balsam M, Schulte F, Bongards M and Rehorek A 2016 Mid-infrared spectroscopy for monitoring of anaerobic digestion processes–prospects and challenges *Chem. Eng. Technol.* **39** 627–36
[23] Minnich C B, Buskens P, Steffens H C, Bäuerlein P S, Butvina L N, Küpper L, Leitner W, Liauw M A and Greiner L 2007 Highly flexible fibre-optic ATR-IR probe for inline reaction monitoring *Org. Process Res. Dev.* **11** 94–7
[24] Schädle T and Mizaikoff B 2016 Mid-infrared waveguides: a perspective *Appl. Spectrosc.* **70** 1625–38
[25] Steiner H *et al* 2003 *In Situ* sensing of volatile organic compounds in groundwater: first field tests of a mid-infrared fiber-optic sensing system *Appl. Spectrosc.* **57** 607–13
[26] Hinrichs K, Aulich D, Ionov L, Esser N, Eichhorn K-J, Motornov M, Stamm M and Minko S 2009 Chemical and structural changes in a pH-responsive mixed polyelectrolyte brush studied by infrared ellipsometry *Langmuir* **25** 10987–91
[27] Sun G, Zhang X, Kaspari C, Haberland K, Rappich J and Hinrichs K 2012 *In Situ* monitoring the growth of polypyrrole films at liquid/solid interface using a combination of polarized infrared spectroscopy and reflectance anisotropy spectroscopy *J. Electrochem. Soc.* **159** H811–5
[28] Sun G, Zhang X, Rappich J and Hinrichs K 2015 *In situ* infrared ellipsometric monitoring of the growth process of polyaniline thin films and doping with poly(4-styrenesulfonate) *Appl. Surf. Sci.* **344** 181–7
[29] Baumgartner B, Hayden J, Schwaighofer A and Lendl B 2018 *In Situ* IR spectroscopy of mesoporous silica films for monitoring adsorption processes and trace analysis *ACS Appl. Nano Mater.* **1** 7083–91
[30] Baumgartner B, Hayden J, Loizillon J, Steinbacher S, Grosso D and Lendl B 2019 Pore size-dependent structure of confined water in mesoporous silica films from water adsorption/desorption using ATR–FTIR spectroscopy *Langmuir* **35** 11986–94

[31] Furchner A, Kroning A, Rauch S, Uhlmann P, Eichhorn K-J and Hinrichs K 2017 Molecular interactions and hydration states of ultrathin functional films at the solid–liquid interface *Anal. Chem.* **89** 3240–4

[32] Rowe D J, Smith D and Wilkinson J S 2017 Complex refractive index spectra of whole blood and aqueous solutions of anticoagulants, analgesics and buffers in the mid-infrared *Sci. Rep.* **7** 7356

[33] Mikhaylova Y, Ionov L, Rappich J, Gensch M, Esser N, Minko S, Eichhorn K-J, Stamm M and Hinrichs K 2007 *In situ* infrared ellipsometric study of stimuli-responsive mixed polyelectrolyte brushes *Anal. Chem.* **79** 7676–82

[34] Rappich J and Hinrichs K 2009 *In situ* study of nitrobenzene grafting on Si(111)-H surfaces by infrared spectroscopic ellipsometry *Electrochem. Commun.* **11** 2316–9

[35] Hoy O *et al* 2010 Synthetic hydrophilic materials with tunable strength and a range of hydrophobic interactions *Adv. Funct. Mater.* **20** 2240–7

[36] Kanyong P, Sun G, Rösicke F, Syritski V, Panne U, Hinrichs K and Rappich J 2015 Maleimide functionalized silicon surfaces for biosensing investigated by *in situ* IRSE and EQCM *Electrochem. Commun.* **512** 103–7

[37] Sun G, Zu F, Koch N and Rappich H K 2019 In *s*itu infrared spectroscopic monitoring and characterization of the growth of polydopamine (PDA) films *Phys. Status Solidi* B **256** 1800308

[38] Hinrichs K, Roodenko K, Rappich J, Chehimi M M and Pinson J 2012 *Analytical Methods for the Characterization of Aryl Layers* (Aryl Diazonium Salts) ed Chehimi M M (Weinheim: Wiley)

[39] Hinrichs K, Shaykhutdinov T, Kratz C, Rösicke F, Schöniger C, Arenz C, Nickel N H and Rappich J 2018 Electrochemical modification of large area graphene and characterization by vibrational spectroscopy *Encyclopedia of Interfacial Chemistry* ed Wandelt K (Oxford: Elsevier) pp 80–94

[40] Jiang X, Zuber A, Heberle J and Ataka K 2008 *In situ* monitoring of the orientated assembly of strep-tagged membrane proteins on the gold surface by surface enhanced infrared absorption spectroscopy *Phys. Chem. Chem. Phys.* **10** 6381–7

[41] Harrick N J 1967 *Internal Reflection Spectroscopy* (Hoboken, NJ: Wiley)

[42] Dougan J A, Chan K L and Kazarian S G 2014 FT-IR imaging in ATR and transmission modes: practical considerations and emerging applications *Infrared and Raman Spectroscopic Imaging* ed Salzer R and Siesler H W (Weinheim: Wiley)

[43] Tolstoy V P, Chernyshova I and Skryshevsky V A 2003 *Handbook of Infrared Spectroscopy of Ultrathin Films* (Hoboken New Jersey: Wiley)

[44] Ramer G and Lendl B 2013 Attenuated total reflection Fourier transform infrared spectroscopy *Encyclopedia of Analytical Chemistry* ed Meyers R A (Hoboken, NJ: Wiley)

[45] Bürgi T 2011 Attenuated total reflection infrared (ATR-IR) spectroscopy, modulation excitation spectroscopy (MES), and vibrational circular dichroism (VCD) *BioInterface Characterization by Advanced IR Spectroscopy* ed Pradier C M and Chabal Y J (Amsterdam: Elsevier) pp 115–44 ch 5

[46] McQuillan A J 2001 Probing solid/solution interfacial chemistry with ATR-IR spectroscopy *Adv. Mater.* **13** 1034–8

[47] Andanson J-M and Baiker A 2010 Exploring catalytic solid/liquid interfaces by *in situ* attenuated total reflection infrared spectroscopy *Chem. Soc. Rev.* **39** 4571–84

[48] Shao M H and Adzic R R 2005 Electro oxidation of ethanol on a Pt electrode in acid solutions: *in situ* ATR-SEIRAS study *Electrochim. Acta* **50** 2415–22

[49] Kuimova M K, Chan K L A and Kazarian S G 2009 Chemical imaging of live cancer cells in the natural aqueous environment *Appl. Spectrosc.* **63** 164–71

[50] Aureau D, Ozanam F, Allongue P and Chazalviel J-N 2008 The titration of carboxyl-terminated monolayers revisited: *in situ* calibrated fourier transform infrared study of well-defined monolayers on silicon *Langmuir* **24** 9440–8

[51] Vrancken N, Li J, Sergeant S, Vereecke G, Doumen G, Holsteyns F, Chen C, Terryn H, De Gendt S and Xu X M 2018 *In situ* ATR-FTIR for dynamic analysis of super hydrophobic breakdown on nanostructured silicon surfaces *Sci. Rep.* **8** 11637

[52] Li B, Barkleit A, Raff J, Bernhard G and Foerstendorf H 2010 Complexation of U(VI) with highly phosphorylated protein, phosvitin. A vibrational spectroscopic approach *J. Inorg. Biochem.* **104** 718–25

[53] Peak D, Ford R G and Sparks D L 1999 An *in Situ* ATR-FTIR investigation of sulfate bonding mechanisms on goethite *J. Colloid Interface Sci.* **218** 289–99

[54] Schmitt J and Flemming H-C 1998 FTIR-spectroscopy in microbial and material analysis *Int. Biodeterior. Biodegrad.* **41** 1–11

[55] Barlow D E, Dickinson G H, Orihuela B, Rittschof D and Wahl K J 2009 *In situ* ATR–FTIR characterization of primary cement interfaces of the barnacle Balanus amphitrite *Biofouling* **25** 359–66

[56] Kazarian S G, Briscoea B J and Welton T 2000 Combining ionic liquids and supercritical fluids: *in situ* ATR-IR study of CO2 dissolved in two ionic liquids at high pressures *Chem. Commun.* **20** 2047–8

[57] Chan K L A and Kazarian S G 2016 Attenuated total reflection Fourier-transform infrared (ATR-FTIR) imaging of tissues and live cells *Chem. Soc. Rev.* **45** 1850–64

[58] Everall N J, Chalmers J M and Griffiths P R 2007 *Vibrational Spectroscopy of Polymers: Principles and Practice* (Chichester: Wiley)

[59] Yajima T, Uchida H and Watanabe M 2004 *In situ* ATR-FTIR spectroscopic study of electro-oxidation of methanol and adsorbed CO at Pt−Ru Alloy *J. Phys. Chem.* B **108** 2654–9

[60] Tsai D-H, DelRio F W, Keene A M, Tyner K M, MacCuspie R I, Cho T J, Zachariah M R and Hackley V A 2011 Adsorption and conformation of serum albumin protein on gold nanoparticles investigated using dimensional measurements and *in situ* spectroscopic methods *Langmuir* **27** 2464–77

[61] Intelmann C M, Hinrichs K, Syritski V, Yang F and Rappich J 2008 Recombination behaviour at the Ultrathin polypyrrole film/silicon interface investigated by *in situ* pulsed photo luminescence *Jpn. J. Appl. Phys.* **47** 554–7

[62] Rappich J, Sun G and Hinrichs K 2018 *In situ* monitoring of the electrochemical surface modification by thin organic layers *Advanced Green Chemistry* (Series on Chemistry, Energy and the Environment) ed Horváth I T and Malacria M (Singapore: World Scientific) pp 205–55

[63] Thrane L, Jacobsen R H, Jepsen P U and Keiding S R 1995 THz reflection spectroscopy of liquid water *Chem. Phys. Lett.* **240** 330–3

[64] Kroning A *et al* 2015 *In situ* infrared ellipsometry for protein adsorption studies on ultrathin smart polymer brushes in aqueous environment *ACS Appl. Mater. Interfaces* **7** 12430–9

[65] Aulich D, Hoy O, Luzinov I, Brücher M, Hergenröder R, Bittrich E, Eichhorn K-J, Uhlmann P, Stamm M, Esser N and Hinrichs K 2010 *In situ* studies on the switching behavior of ultrathin poly(acrylic acid) polyelectrolyte brushes in different aqueous environments *Langmuir* **26** 12926–32

[66] Kroning A, Furchner A, Adam S, Uhlmann P and Hinrichs K 2016 Probing carbonyl–water hydrogen-bond interactions in thin polyoxazoline brushes *Biointerphases* **11** 019005

[67] Furchner A, Bittrich E, Uhlmann P, Eichhorn K-J and Hinrichs K 2013 *In situ* characterization of the temperature-sensitive swelling behavior of poly(*N*-isopropylacrylamide) brushes

by infrared and visible ellipsometry *Thin Solid Films* **541** 41–5
[68] Aulich D, Hoy O, Luzinov I, Eichhorn K-J, Stamm M, Gensch M, Schade U, Esser N and Hinrichs K 2010 In-situ IR synchrotron mapping ellipsometry on stimuli-responsive PAA-b-PS/PEG mixed polymer brushes *Phys. Status Solidi* C **7** 197–9
[69] Ogieglo W, Furchner A, Ghanem B, Ma X, Pinnau I and Wessling M 2017 Mixed-penetrant sorption in ultrathin films of polymer of intrinsic microporosity PIM-1 *J. Phys. Chem.* B **121** 10190–7
[70] Müller M, Torger B, Bittrich E, Kaul E, Ionov L, Uhlmann P and Stamm M 2014 *In situ* ATR-FTIR for characterization of thin biorelated polymer films *Thin Solid Films* **556** 1–8
[71] Bauer H H, Mueller M, Goette J, Merkle H P and Fringeli U P 1994 Interfacial adsorption and aggregation associated changes in secondary structure of human calcitonin monitored by ATR-FTIR Spectroscopy *Biochem.* **33** 12276–82
[72] Müller M, Kessler B and Lunkwitz K 2003 Induced orientation of α-helical polypeptides in polyelectrolyte multilayers *J. Phys. Chem.* B **107** 8189–97
[73] Gelfand P, Smith R J, Stavitski E, Borchelt D R and Miller L M 2015 Characterization of protein structural changes in living cells using time-lapsed FTIR imaging *Anal. Chem.* **87** 6025–31
[74] Tiwald T E, Thompson D W, Woollam J A and Pepper S V 1998 Determination of the mid-IR optical constants of water and lubricants using IR ellipsometry combined with an ATR cell *Thin Solid Films* **313** 718–21
[75] Wang C C, Tan J Y, Ma Y Q and Liu L H 2017 Infrared optical constants of liquid palm oil and palm oil biodiesel determined by the combined ellipsometry-transmission method *Appl. Optic.* **56** 5156–63
[76] Röseler A and Korte E H 1995 Ellipsometric ATR spectroscopy for measuring the infrared refractive index *J. Mol. Struct.* **349** 321–4
[77] Rappich J and Dittrich T 2002 *Electrochemical Passivation of Si and SiGe Surfaces* (Thin Films vol 29) ed Francombe M H (Amsterdam: Elsevier) pp 135–259
[78] Rösicke F, Gluba M A, Hinrichs K, Sun G, Nickel N H and Rappich J 2015 Quantifying the electrochemical maleimidation of large area graphene *Electrochem. Commun.* **57** 52–5
[79] Rösicke F, Gluba M A, Shaykhutdinov T, Sun G, Kratz C, Rappich J, Hinrichs K and Nickel N H 2017 Functionalization of any substrate using covalently modified large area CVD graphene *Chem. Commun.* **53** 9308
[80] Barth A 2007 Infrared spectroscopy of proteins *BBA-Bioenergetics* **1767** 1073–1101
[81] Pahlow S, Weber K, Popp J, Wood B R, Kochan K, Rüther A, Perez-Guaita D, Heraud P, Stone N, Dudgeon A, Gardner B, Reddy R, Mayerich D and Bhargava R 2018 Application of vibrational spectroscopy and imaging to point-of-care medicine: a review *Appl. Spectrosc.* **72** 52–84
[82] Corte D A D, Caillon G, Jordy C, Chazalviel J-N, Rosso M and Ozanam F 2016 Spectroscopic insight into Li-ion batteries during operation: an alternative infrared approach *Adv. Energy Mater.* **6** 1501768
[83] Haas J and Mizaikoff B 2016 Advances in mid-infrared spectroscopy for chemical analysis *Annu. Rev. Anal. Chem.* **9** 45–68
[84] Fabian H, Lasch P and Naumann D 2005 Analysis of biofluids in aqueous environment based on mid-infrared spectroscopy *J. Biomed. Optic.* **10** 031103
[85] Klimov E, Fuelleborn M and Siesler H W 2003 Electric-field-induced reorientation of liquid crystalline p-cyanophenyl-p-n-alkylbenzoates: a time-resolved study by fourier transform infrared transmission and attenuated total reflection spectroscopy *Appl. Spectrosc.* **57** 499–505
[86] Klimov E and Siesler H W 2004 Fourier transform infrared spectroscopic investigation of the electric-field-induced reorientation of the nematic 7CPB with different prealignment *Appl. Spectrosc.* **58** 952–7
[87] Jang H, Pawate A S, Bhargava R and Kenis P J A 2019 Polymeric microfluidic continuous flow mixer combined with hyper spectral FT-IR imaging for studying rapid biomolecular events *Lab Chip* **19** 2598–609
[88] Etezadi D, Warner IV J, Ruggeri F, Lashuel H A and Altug H 2017 Nanoplasmonic mid-infrared biosensor for *in vitro* protein secondary structure detection *Light Sci. Appl.* **6** e17029
[89] Schwaighofer A, Montemurro M, Freitag S, Kristament C, Culzoni M J and Lendl B 2018 Beyond Fourier transform infrared spectroscopy: external cavity quantum cascade laser-based mid-infrared transmission spectroscopy of proteins in the amide I and amide II region *Appl. Chem.* **90** 7072–9
[90] Schwaighofer A, Brandstetter M and Lendl B 2017 Quantum cascade lasers (QCLs) in biomedical spectroscopy *Chem. Soc. Rev.* **46** 5903–24
[91] Zheng B, Li W, Li H, Liu L, Lei P, Ge X, Yu Z and Zhou Y 2016 Separation and determination of estrogen in the water environment by high performance liquid chromatography-fourier transform infrared spectroscopy *Sci. Rep.* **6** 32264
[92] Ebner A, Zimmerleiter R, Cobet C, Hingerl K, Brandstetter M and Kilgus J 2019 Sub-second quantum cascade laser based infrared spectroscopic ellipsometry *Opt. Lett.* **44** 3426–9
[93] Furchner A, Walder C, Zellmeier M, Rappich J and Hinrichs K 2018 Broadband infrared Mueller-matrix ellipsometry for studies of structured surfaces and thin films *Appl. Optic.* **57** 7895–904
[94] Psaltis D, Quake S R and Yang C 2006 Developing optofluidic technology through the fusion of microfluidics and optics *Nature* **442** 381–6
[95] Wu A and Gu M 2011 Microfluidic sensing: state of the art fabrication and detection techniques *J. Biomed. Optic.* **16** 080901
[96] Fan X and White I M 2011 Optofluidic microsystems for chemical and biological analysis *Nat. Photon.* **5** 591–7
[97] TermehYousefi A, Bagheri S and Adib N 2015 Integration of biosensors based on microfluidic: a review *Sens. Rev.* **35** 190–9
[98] Osawa M 2001 Surface-enhanced infrared absorption *Near-Field and Surface Plasmon Polaritons* (Topics in Applied Physics vol 81) (Berlin: Springer) pp 163–87
[99] Aroca R F, Ross D J and Domingo C 2004 Surface-enhanced infrared spectroscopy *Appl. Spectrosc.* **58** 324A–38A
[100] Adato R and Altug H 2013 *In situ* ultra-sensitive infrared absorption spectroscopy of biomolecule interactions in real time with plasmonic nanoantennas *Nat. Commun.* **4** 2154
[101] Neubrech F, Pucci A, Cornelius T W, Karim S, García-Etxarri A and Aizpurua J 2008 Resonant plasmonic and vibrational coupling in a tailored nanoantenna for infrared detection *Phys. Rev. Lett.* **101** 157403
[102] Dong L, Yang X, Zhang C, Cerjan B, Zhou L, Tseng M L, Zhang Y, Alabastri A, Nordlander P and Halas N J 2017 Nanogapped Au antennas for ultrasensitive surface-enhanced infrared absorption spectroscopy *Nano Lett.* **17** 5768–74
[103] Neuman T, Huck C, Vogt J, Neubrech F, Hillenbrand R, Aizpurua J and Pucci A 2015 Importance of plasmonic scattering for an optimal enhancement of vibrational absorption in SEIRA with linear metallic antennas *J. Phys. Chem.* C **119** 26652–62
[104] Adato R, Aksu S and Altug H 2015 Engineering mid-infrared nanoantennas for surface enhanced infrared absorption spectroscopy *Mater. Today* **18** 436–46

[105] Kratz C, Oates T and Hinrichs K 2016 Optimization and quantification of surface enhanced infrared absorption using gradient gold island films *Thin Solid Films* **617** 33–7

[106] Osawa M, Yoshii K, Ataka K-I and Yotsuyanagi T 1994 Real-time monitoring of electrochemical dynamics by submillisecond time-resolved surface-enhanced infrared attenuated-total-reflection spectroscopy *Langmuir* **10** 640–2

[107] Gkogkou D, Shaykhutdinov T, Kratz C, Oates T W H, Hildebrandt P, Weidinger I M, Ly K H, Esser N and Hinrichs K 2019 Gradient metal nanoislands as a unified surface enhanced Raman scattering and surface enhanced infrared absorption platform for analytics *Analyst* **144** 5271–6

[108] Le Ru E C and Etchegoin P G 2012 Single-molecule surface-enhanced Raman spectroscopy *Annu. Rev. Phys. Chem.* **63** 65–87

[109] Kottke T, Lórenz-Fonfría V A and Heberle J 2017 The grateful infrared: sequential protein structural changes resolved by infrared difference spectroscopy *J. Phys. Chem.* B **121** 335–50

[110] Schumacher H, Künzelmann U, Vasilev B, Eichhorn K-J and Bartha J W 2010 Applications of microstructured silicon wafers as internal reflection elements in attenuated total reflection Fourier transform infrared spectroscopy *Appl. Spectrosc.* **64** 1022–7

[111] Pérez J J G and Ossikovski R 2016 *Polarized Light and the Mueller Matrix Approach* (Boca Raton: CRC Press)

[112] Furchner A, Kratz C, Ogieglo W, Pinnau I, Rappich J and Hinrichs K 2020 Ultrasensitive broadband infrared 4 × 4 Mueller-matrix ellipsometry for studies of depolarizing and anisotropic thin films *J. Vac. Sci. Technol.* B **38** 014003

[113] Shilov S V, Okretic S, Siesler H W and Czarnecki M A 1996 Segmental mobility of liquid crystals and liquid crystalline polymers in an electric field: a study by time-resolved rapid-scan and step-scan FTIR spectroscopy *Appl. Spectrosc. Rev.* **31** 125–65

[114] Furchner A, Kratz C and Hinrichs K 2019 Sub-second infrared broadband-laser single-shot phase–amplitude polarimetry of thin films *Opt. Lett.* **44** 4387–90

[115] Furchner A, Kratz C, Rappich J and Hinrichs K 2019 Hyperspectral infrared laser polarimetry for single-shot phase–amplitude imaging of thin films *Opt. Lett.* **44** 4893–6

[116] Hinrichs K, Shaykhutdinov T, Kratz C and Furchner A 2019 Brilliant mid-infrared ellipsometry and polarimetry of thin films: toward laboratory applications with laser based techniques *J. Vac. Sci. Technol.* B **37** 060801

[117] Griffiths P R and de Haseth J A 2007 *Fourier Transform Infrared Spectrometry* 2nd edn (Hoboken, NJ: Wiley)

[118] Porter M D 1988 IR external reflection spectroscopy: a probe for chemically modified surfaces *Anal. Chem.* **60** 1143A–55A

[119] Bringezu F, Majerowicz M, Maltseva E, Wen S, Brezesinski G and Waring A J 2007 Penetration of the antimicrobial peptide dicynthaurin into phospholipid monolayers at the liquid–air interface *ChemBioChem* **8** 1038–47

[120] Walsh C B, Wen X and Franses E I 2001 Ellipsometry and infrared reflection absorption spectroscopy of adsorbed layers of soluble surfactants at the air–water interface *J. Colloid Interface Sci.* **233** 295–305

[121] Mendelsohn R, Brauner J W and Gericke A 1995 External infrared reflection absorption spectrometry of monolayer films at the air–water interface *Annu. Rev. Phys. Chem.* **46** 305–34

[122] Mendelsohn R, Mao G and Flach C R 2010 Infrared reflection–absorption spectroscopy: principles and applications to lipid–protein interaction in Langmuir films *BBA-Biomembranes* **1798** 788–800

[123] Stole S M and Porter M D 1990 *In situ* infrared external reflection spectroscopy as a probe of the interactions at the liquid-solid interface of long-chain alkanethiol monolayers at gold *Langmuir* **6** 1199–202

[124] Pastrana-Rios B, Taneva S, Keough K M, Mautone A J and Mendelsohn R 1995 External reflection absorption infrared spectroscopy study of lung surfactant proteins SP-B and SP-C in phospholipid monolayers at the air/water interface *Biophys. J.* **69** 2531–40

[125] Meier D M, Urakawaa A, Mäder R and Baikera A 2009 Design and performance of a flow-through polarization-modulation infrared reflection-absorption spectroscopy cell for time-resolved simultaneous surface and liquid phase detection under concentration and temperature perturbations *Rev. Sci. Instrum.* **80** 094101

[126] Meier D M, Urakawa A and Baiker A 2009 Adsorption behavior of salicylic, benzoic, and 2-methyl-2-hexenoic acid on alumina: an *in situ* modulation excitation PM-IRRAS study Phys. *Chem. Chem. Phys.* **11** 10132–9

[127] Mendoza Galván A, Järrendahl K and Arwin H 2019 Muller-matrix modeling of the architecture in the cuticle of the beetle Chrysina resplendens *J. Vac. Sci. Technol.* B **37** 062904

[128] Arteaga O 2014 Useful Mueller matrix symmetries for ellipsometry *Thin Solid Films* **571** 584–8

[129] Tamm L K and Tatulian S A 1997 Infrared spectroscopy of proteins and peptides in lipid bilayers *Q. Rev. Biophys.* **30** 365–429

[130] Janasek D, Hinrichs K, Kratz C and Oates T W H 2016 Vorrichtung zur Detektion und Charakterisierung von organischen Molekülen in einem flüssigen Probenvolumen *German Patent*: 10 2016 101 001.4

[131] Weber P C, Ohlendorf D, Wendoloski J and Salemme F 1989 Structural origins of high-affinity biotin binding to streptavidin *Science* **243** 85–8

[132] Peluso P, Wilson D S, Do D, Tran H, Venkatasubbaiah M, Quincy D, Heidecker B, Poindexter K, Tolani N and Phelan M 2003 Optimizing antibody immobilization strategies for the construction of protein microarrays *Anal. Biochem.* **312** 113–24

[133] Hughes C, Clemens G, Bird B *et al* 2006 Introducing Discrete Frequency Infrared Technology for High-Throughput Biofluid Screening Sci Rep **6** 20173

[134] Zhang D, Li C, Zhang C, Slipchenko M N, Eakins G and Cheng J-X 2016 Depth-resolved mid-infrared photo thermal imaging of living cells and organisms with sub micrometer spatial resolution *Sci. Adv.* **2** e1600521

[135] Lu Y H, Larson J M, Baskin A, Zhao X, Ashby P D, Prendergast D, Bechtel H A, Kostecki R and Salmeron M 2019 Infrared nano spectroscopy at the Graphene–electrolyte interface *Nano Lett.* **19** 5388–93

[136] Jin M, Lu F and Belkin M 2017 High-sensitivity infrared vibrational nanospectroscopy in water *Light Sci. Appl.* **6** e17096

[137] Vodopyanov K L, Sorokin E, Sorokina I T and Schunemann P G 2011 Mid-IR frequency comb source spanning 4.4–5.4 μm based on subharmonic GaAs optical parametric oscillator *Opt. Lett.* **36** 2275–7

[138] Klocke J L, Mangold M, Allmendinger P, Hugi A, Geiser M, Jouy P, Faist J and Kottke T 2018 Single-shot sub-microsecond mid-infrared spectroscopy on protein reactions with quantum cascade laser frequency combs *Anal. Chem.* **90** 10494–500

[139] Dreimann J M, Kohls E, Warmeling H F W, Stein M, Guo L F, Garland M, Dinh T N and Vorholt A J 2019 In *s*itu infrared spectroscopy as a tool for monitoring molecular catalyst for hydroformylation in continuous processes *ACS Catal.* **9** 4308–19

[140] Finlayson D, Rinaldi C and Baker M J 2019 Is infrared spectroscopy ready for the clinic? *Anal. Chem.* **91** 12117–28