Novel Spectroscopic Sensors for Studies of Heterogeneous Biological and Environmental Samples

Research Vision: Many chemical sensors analyze samples at only one selected location; this severely limits the amount of chemical information that can be gained from heterogeneous samples. Heterogeneous samples, however, are very common in research ranging from the biomedical field on microscopic scales to industrial process monitoring and to remote sensing of environmental pollution. Novel analytical concepts are mandatory to investigate such complex chemical systems and processes. For this purpose our research combines experimental and more theoretical methodologies, i.e. analytical spectroscopy and optical sensor design is supported by developments of chemometric computations, pattern recognition/classification and image analyses.

The experimental approach of "spectroscopic imaging" is a first step to establish new perspectives in chemical sensing for heterogeneous samples. This is achieved by performing analytical spectroscopy utilizing 2-dimensional detector arrays. The basic idea as outlined in Fig. 1 (left) is to acquire a stack of images at different wavelengths; in this example, a tunable filter was placed in front of a thermal camera. Thus, in one wavelength scan an entire spectrum is acquired with every single pixel of the focal plane array's (FPA's) pixel. As 1,000’s or even 100,000’s of either infrared, Raman or fluorescence spectra are measured in parallel from different sample locations, fast analyses of heterogeneous samples become feasible. Conducting such experiments continuously enables studies of dynamic processes like deposition, etching or growth/decomposition of biological material.

In the mid-infrared approach discussed below spatial resolutions are feasible that range -in laboratory applications- from a few micrometers to a few millimeters and much larger scales in remote sensing. Such data cubes can be acquired in a few seconds; thus time dependent studies can be performed at time resolutions down to the range of seconds.

Fig. 1: (left) a data sets acquired by mid-infrared remote spectroscopic imaging: the sun heated up plastic furniture placed in a natural environment (distance: 50 meters) and the scene emits -spatially heterogeneous- material specific IR spectra. A sensor consisting of a thermal camera and a tunable optical filter placed in front of the camera acquired a stack of images at different wavelengths; (right) a principal component analysis extracted a set of basis vectors containing the spectroscopic information and spatial distributions of these portions of spectroscopic information. Three “distribution images” have been combined to form a red-green-blue image. Now spectroscopic information of different materials are coded in different colors (F. Vogt et al. J. Chemometrics (2004), 350-362)

In order to broaden our research horizon, we seek to establish interdisciplinary collaborations with scientists in related fields, i.e. physical, geological, biological and medical sciences, engineering (especially image analysis), mathematics, industrial partners etc. We want to bring together research in these fields and to apply it to biomedical (microscopic scale) and environmental research (remote sensing) as well as to innovative industrial process control.

# Chemometrics: the chemical discipline that uses mathematical and statistical methods to design or select optimal procedures and experiments, and to provide maximum chemical information by analyzing chemical data (definition by the journal Chemometrics & Intelligent Laboratory Systems).
Our goal is to find researchers who are interested in **collaborative research proposals** and **publications** by expanding the group’s main fields of study, i.e. analytical spectroscopy and chemometrics.

(A) Introducing the Analytical Concept “Spectroscopic Imaging” and Beyond

The first experimental concept is designed around a mid-infrared FTIR spectrometer, which will be equipped with an infrared microscope and a focal plane array detector (64 x 64 pixels). Thus, analyses of heterogeneous samples are feasible in biomedical, geological or pharmaceutical fields as well as for passive remote sensing of pollutant distributions. In laboratory applications, samples can be analyzed on a microscopic scale at a resolution of a few to a few 100 micrometers; much larger scales are studied in remote sensing.

**Fig. 3:** Schematics of our FTIR-based experimental setup (see ); it can use the internal light source for active measurements or switch to an input port though which infrared radiation emitted from a remote scene is captured in passive mode; this measurement mode will enable remote sensing of pollution or object recognition. The infrared focal plane detector (IR-FPA) can be moved back and forth from a sample chamber (millimeter sized objects) to the IR/VIS microscope; in both cases data cubes are acquired similar to the one shown in Fig. 1 (left) the microscope and the sample chamber feature an additional CCD camera for the visible wavelength range. In both setups (microscope and sample chamber) measurements can be done in reflection and transmission mode.

However, imaging techniques have one inherent drawback, i.e. depth information is lost during the measurement process. For instance, based on a picture of a sphere one cannot tell whether the object is indeed a sphere or a disc.

In order to overcome this drawback and in order to enable chemical sensing of 3D objects, the experimental setup shown in Fig. 3 and has been expanded by additional custom made optics. One approach, which is not discussed here, will be based on stereo-vision, i.e. capturing imaging data of the same object from two slightly different positions. We propose an approach that has been realized focusing on...
microscopy based studies. The setup is schematically shown in Fig. 5: Light emitted from an additional, external light source is collected and transformed into a parallel beam by means of an microscope objective. In this parallel beam a mesh is placed, which has a grid constant of ca. 100µm. This mesh superimposes a regular shaped shadow onto the light projected onto the microscopic sample by means of a second microscope objective, which faces in the opposite direction.

Fig. 4: This close-up (see ) shows the microscope stage and additional optics for sample illumination with visible light onto which a regular shaped shadow in superimposed by means of a mesh (see Fig. 5).

Fig. 5: A regular light pattern is generated by placing a mesh in the external, visible light beam (see ); the projection of this ‘imprinted’ light pattern onto a 3D sample shows distortions; surface structures are derived by analyzing the distortion of the known, original pattern (Fig. 6).

A typical measurement process consists of three consecutive steps.

Step 1: this setup is been used to project a mesh with a hole aperture of approx. 110µm x 110µm and a wire width of approx. 40µm onto an empty sample stage as well as onto two samples (Fig. 6). An image of the small sample stage area is captured with the microscope’s visible camera. In absence of a sample the regular shape of the mesh’s shadow is not disturbed (Fig. 6, top left). If, however a sample is present, clear distortions are recorded in the visible image (Fig. 6, right top and bottom). From this distortion the 3D surface
structure can be determined by means of a software package that is currently under development. The spatial resolution of the mesh’s shadow and thus the detectable surface structures (here: 110µm) can be decreased by placing two or more, slightly shifted meshes on top of each other or by scanning one mesh stepwise over the sample.

**Step 2:** Now an image cube similar to the one shown in Fig. 1 left is acquired. However, no tunable optical filter is used here but the FTIR spectrometer’s interferometer. Thus, a cube of interferograms is measured, which are then one by one FFTed. Since the interferometer triggers the capture of frames in the very same way it triggers the acquisition of an single point of an interferogram, the interferograms have the same quality as in conventional FTIR spectroscopy and require the same amount of time, i.e. seconds.

**Step 3:** For semi-transparent samples it might be advantageous to measure additionally an image cube in the microscope’s transmission mode. Based on the reflection and the transmission data the interior of sample can be analyzed at high spatial resolution. In case of non-transparent samples, time-resolved monitoring of deposition or etching processes can reveal –layer by layer- internal sample structures.

Future developments will expand these concepts to different spectroscopic techniques like 3D Raman or fluorescence imaging.

![Image](image1.png)

![Image](image2.png)

**Fig. 6:** Examples acquired with the setup shown in - ; *(top left)*: the mesh’s shadow projected onto an empty sample stage – no distortion is visible; *(top and bottom right)* a microscopic piece of a ibuprofen tablet has been placed onto the sample stage – no the projection of the mesh’s shadow gets distorted by the 3D sample; this sample has been selected to avoid challenging sample handling during design of the optical setup; *(bottom left)* a reflection spectrum acquired with the microscope single-pixel IR detector (the IR FPA will be delivered in June)); this example demonstrated how 3D surface structures and chemical (spectroscopic) information will gathered in one setup.

( B ) **Innovative Data Analyses for Evaluating Spectroscopic Image Cubes**

Experimental technique outline in the previous section require support from innovative data analysis methods in order to extract the relevant chemical information and to take full advantage of imaging techniques. Three main areas of research are discussed:

- Computation methods need to be developed for extracting the mesh’s shadow from images captures with the visible camera *(Fig. 6).* After extraction of the surface structure the mesh’s location measured by the pixel position must be
transformed into a $\mu$m reading; this can possibly be done by means of a calibration utilizing an object of known dimensions.

- Once the 3D surface structure has been determined, the system switches to the IR light source and acquires one or two data cubes in reflection and transmission mode (Fig. 5). The second topic in data evaluation deals with extracting qualitative and/or quantitative chemical information from these spectroscopic data. Sophisticated data analysis tools are needed if target analytes are embedded in largely unknown chemical matrices or when measurement conditions continuously change; this is commonly encountered e.g. in biological or environmental sensing. An additional challenge to keep in mind while analyzing IR spectra is that possibly unavoidable drifts cause drifts of the baseline; this must be taken into account during data evaluation. Our recently develop methodologies are a good starting point for robust and reliable in the presence of arbitrary disturbances and baseline drifts (Fig. 7).

![Graph](image1)

**Fig. 7:** (top left) Simulated spectra, which are disturbed by unknown interferents and random baseline drifts; (top right) our recent accomplishments in robust data evaluation correct for major concentration errors and gain additional information about (bottom left) spectral signatures of interfering analytes and (bottom right) baseline drifts (F. Vogt et al., J. Chemometrics (2003), 660-665)

- Another challenge that is often encounter in spectroscopic imaging is that data sets are so large that data evaluation becomes a very tedious process; especially in online monitoring it is highly unwanted that data evaluation reduces a sensors time resolution. In order to accelerate chemometric computations we currently develop compression methods, which reduce the computation times by significant factors without losing too much of the relevant chemical information. One example is shown in Fig. 8.

![Graph](image2)

**Fig. 8:** (left) From a mid-infrared image cube (Fig. 1) a red-green-blue scores image had been derived, which displays different spectroscopic information color-coded; (right) this slightly blurred image has been computed in the same way from a highly compressed cube, which contained <1% of the original amount of data (F. Vogt et al., J. Chemometrics (2004), 350-362; (2005), 510-520; (2005), 575-581).
In analytical chemistry often only spectroscopic information is considered and spatial information is neglected. However, there is strong potential to improve chemical analyses if spectroscopic and spatial information are analyzed. This is exemplified in Fig. 9 (left), where two different tissue types are shown which contain basically the same chemical information represented in pink and yellow areas. Due to this both tissue types are indistinguishable, if only color information were analyzed; however, one area shows a more homogeneous distribution than the other, which has a heterogeneous distribution of colors. Analyzing the different spatial patterns (here: regular/homogeneous or random) provides essential additional information for discrimination of here cancerous or healthy tissue areas. In this research area we will design chemometric algorithms, which not only evaluate spectroscopic information but also consider the distribution of spectral features for enhanced discrimination of spectroscopically very similar sample areas.

Fig. 9: (left) this stained microscopic tissue sample (J. Dubois et al., Anal. Chem.76(19), 360A-367A) demonstrates that texture represents additional information, which helps to discriminate different tissue types even when spectroscopy alone is deficient.; (right) analyzing the spatial distribution of spectroscopic properties (represented by hollow and solid circles) derives additional information for discrimination and characterization of both „samples“. If one would only look for hollow or solid circles, both „samples“ would be considered identical; however, analyzing the distribution of the circle types clearly discriminates them.

(C) Applications of Spectroscopic Imaging

Spectroscopic imagining will advance a broad variety of measurement tasks for which non-representative, local analyses are insufficient and sensing at high spatial resolution is essential. Prominent examples are the monitoring of environmental pollutant distributions and comprehensive quality control in pharmaceutical and food industry. Recently, conventional infrared spectroscopy has been applied to microscopic biomedical applications for discriminating between normal and diseased tissues. We will enhance such studies by developing fast, 3-dimensional spectroscopic imaging sensors. In order to support such analyzers we will also develop chemometric computation techniques, which are robust under changing and ill-defined measurement conditions. Further, pattern recognition techniques currently designed and applied in image analyses. These computation techniques are required in order to fully utilize the chemical information acquired from complex chemical samples and systems. All of the aforementioned applications will create new perspectives in high-resolution chemical sensing and will provide fundamental insights in chemical interactions and biological composition.
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Appointments
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7/2003 - 6/2005  Faculty Research Associate - Arizona State University, Department of Chemistry & Biochemistry
7/2001 - 6/2003  Post-doctoral Fellow - Georgia Institute of Technology, Department of Chemistry & Biochemistry
8/2000 - 7/2001  Research Scientist - Research Institute of Optronic and Pattern Recognition, Ettlingen, Germany
3/1997 & 7/2000  M.S. and Ph.D. in Physics (Technical University of Karlsruhe, Germany)

Representative Publications
M. Gilbert, C. Frick, A. Wodowski, F Vogt, Spectroscopic Imaging for Detection and Discrimination of Different E. Coli strains, Applied Spectroscopy (2008), accepted
M. Gilbert, F. Vogt, Augmenting Spectroscopic Imaging for Analyses of Samples with Complex Surface Topographies, Analytical Chemistry 79 (2007), 5424-5428
F. Vogt, B. Mizaikoff, Dynamic determination of the dimension of PCA calibration models using F-statistics J. Chemometrics (2003), 346-357
F. Vogt, B. Mizaikoff, Fault tolerant spectroscopic data evaluation based on extended Principal Component Regression correcting for spectral drifts and uncalibrated spectral features, J. Chemometrics (2003), 660-665
F. Vogt, M. Karlowatz, M. Jakusch, B. Mizaikoff, The automated sample preparation system MixMaster for investigation of volatile organic compounds with mid-infrared evanescent wave spectroscopy, Analyst 128 (2003), 397-403
F. Vogt, B. Mizaikoff, Secured PCR (sPCR) for detection and correction of PCR calibration model failures induced by uncalibrated spectral features, J. Chemometrics (2003), 225-236


