BRUKER Optics

TENSOR series

thinkforward

TENSOR is designed to meet the demands of today's and tomorrow's analytical laboratories. It combines the highest performance and outstanding flexibility with an intuitive and easy to operate interface.

TENSOR series

- Easy operation
- Unmatched signal-to-noise performance, outstanding stability
- Excellent purge design
- Mid-IR, Far-IR and Near-IR capability
- Proprietary ultra-low noise electronics technology
- 21 CFR Part 11 compliant

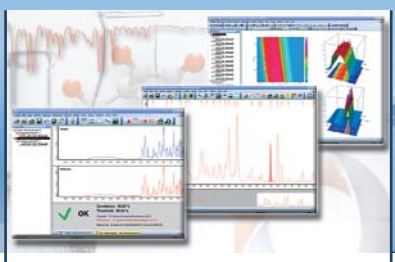
If you need a FT-IR spectrometer that can rapidly identify, quantify and verify your routine samples, *TENSOR* is the right tool for your laboratory. It combines the highest performance and outstanding flexibility with ease of use. A full line of FT-IR accessories enable the *TENSOR* series to be used for micro-analysis, thermogravimetric analysis, proteomics and more.

The Bruker Artificial Intelligence Network (BRAIN) is a network of intelligent features that make FT-IR spectroscopy easy, fast and reliable. Online PerformanceGuard™ insures for accurate results, allowing no room for error. All optical components, automation units and accessories in the *TENSOR* are monitored continuously.

All over the world, no matter where you are, plug in the power and Ethernet connections, and the *TENSOR* is ready for operation. *TENSOR* installation is easily finalized through the OPUS software, the most powerful software in the industry. Multimedia tutorials help train new users.

When a sampling accessory is placed in the sample compartment or a component (like source, detector, beamsplitter or a laser) is changed, it will be instantly and automatically recognized by *TENSOR* and all relevant parameters transferred to the software, so that you can immediately start your measurements.

TENSOR™ is an easy to use routine FT-IR spectrometer that can be configured to your needs.



Easy-to-use OPUS software with full 21 CFR Part 11 and GLP compliance.

TENSOR 37 offers easy beamsplitter exchange that enables range extension.

QuickLock™ base plate for easy exchange of sampling accessories.

FT-IR

www.brukeroptics.com/tensor

TENSOR 27 and ***HYPERION*** FT-IR microscope

TENSOR series

Easy operation

TENSOR is designed for today's laboratory requirements. It is based on the concept of combining easiest operation with highest instrument performance and flexibility. An extended network of functionality makes the *TENSOR* easy to operate:

- QuickLock™ accessories
- Automatic H_2O and CO_2 compensation
- Dedicated QA/QC software
- Self-optimizing evaluation methods
- Multimedia tutorials and help

Sensitivity

TENSOR provides highest sensitivity performance with the permanently aligned, high throughput patented RockSolid™ interferometer. The data acquisition is based on free running delta-sigma ADC's with true 24-bit dynamic range, which are integrated into the detector preamplifier electronics. The advanced DigiTect™ technology prevents external signal disturbance and guarantees the highest signal-to-noise ratio.

Flexibility

A full line of sampling accessories is available. A variety of external accessories complete the field of applications: *HYPERION* IR-microscope, TG- coupling and many others. Bruker Optics offers complete solutions for the life science applications.

Expandibility

Use the advantages of NIR for your quality control and assurance applications. The *TENSOR* 37 can easily be expanded to the NIR range, enabling you to use a full line of NIR sampling accessories.

Reliability

TENSOR is designed to deliver dependable and reliable results in every measurement. The internal validation unit (IVU) ensures correct measurement results. TENSOR integrates full 21 CFR part 11 and GLP compliance, support your IQ/OQ/PQ.

TENSOR series offer a large sampling compartment that can accomodate any FT-IR sampling accessory from any vendor.

External sampling accessories, such as the HTS-XT high throughput screening microplate reader (above) and other autosamplers for solids and liquids extent automation capabilities of the *TENSOR series.*

NIR fiber optic coupling unit enables the use of handheld fiber optic probes for easy sampling.

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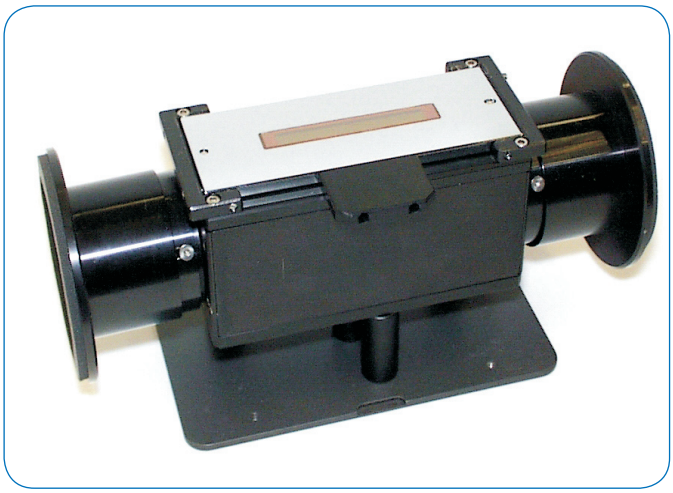
Product Note M50-07/07

A537-A/Q
Overhead ATR unit



Allows measurements of solids and liquids with attenuated total reflectance (ATR).

Features
- Prealigned
- Sealed purgeable housing
- Removable crystal for easy cleaning and replacement
- Different crystal materials
- Clamp device for solid samples (optional)
- Flatpat for solids, trough for liquids
- Heatable flow through crystal with optional top cover
- Mounted on QuickLock baseplate

Options
- A 537-L11 Trough for liquids, ZnSe crystal
- A 537-S 11 Flat plate for solids
- A 537-C Clamp for A 537
- A 537-FL Flow through crtystal
- A 537-FLT Flow through Crystal, temperature control capability
- Spare Crystals F 157:
  F157-10 Ge crystal for A 537-A
  F 157-11 ZnSe crystal for A 537-A

Applications
- Liquids
- Gels
- Pastes
- Solids
- Films
- Reaction monitoring

For more information, visit : www. brukeroptics.com

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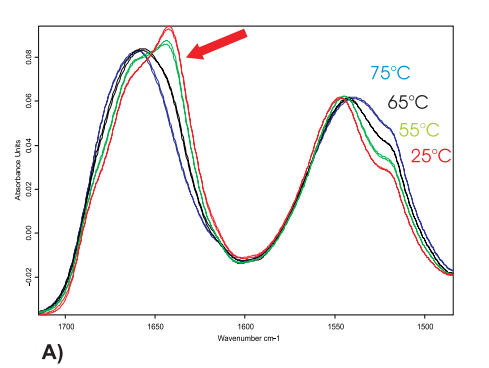
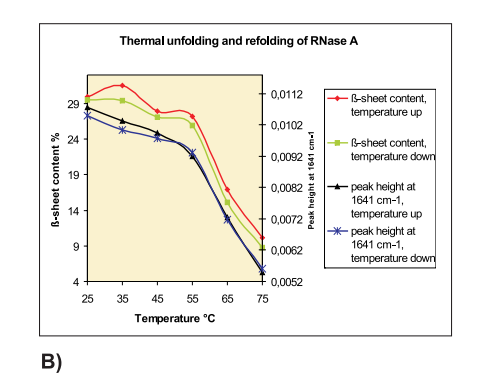
CONFOCHECK™

Infrared Protein Analysis

BRUKER Optics



A)



B)

Temperature induced unfolding/refolding of RNase A. In (A) the infrared spectra of RNase A for different temperatures (25-75 °C) are shown. It is obvious that during the heating the protein was unfolded and the ß-sheet content, indicated by the peak at 1641 cm^{-1} within the amide I band, was diminished. Each temperature is represented by two traces which were obtained during the heating and subsequent cooling cycle, respectively. The similarity between both traces at any temperature reveals that the heat induced unfolding process was completely reversible and the protein was renatured during the cooling procedure afterwards. The change in ß-sheet content during temperature modulation is plotted in (B). The ß-sheet content was calculated from the infrared spectra by PLS (partial least square) algorithmus using a database of proteins with known structure (X-ray). The red trace reflects the results during the heating process, the green trace during the subsequent cooling process. Alternatively, the signal intensity at 1641 cm^{-1} was used, representing ß-sheet structures. The black curve shows the peak height for the heating, and the blue curve for the cooling, respectively. Both approaches reveal again that the temperature induced unfolding of RNase A was completely reversible.

Infrared protein analysis

Infrared spectroscopy provides measurements of molecular vibrations due to the specific absorption of infrared radiation by chemical bonds. For more than 35 years, it is known that the form and frequency of the amide I band, which is assigned to the C=O stretching vibration within the peptide bonds, is very characteristic for the structure of the studied protein. From this band secondary structure elements (α-helix, ß-strand) can be derived, and the analysis of this single band allows elucidation of conformational changes with high sensitivity. However, the structural information from an infrared spectrum is not limited to the amide I band. Side chains of various amino acids can also be monitored and directly compared under varying environmental conditions (pH, salts, ligands), or in mutant proteins with altered primary sequence. Using infrared difference spectroscopy even single molecular bonds can be detected by differences in hydrogen bonding or other changes in their environment.

Why has infrared spectroscopy not been extensively used yet for protein structure investigations ?

Many scientists in protein research may have the impression that traditional infrared spectroscopy has limited capability to measure samples containing water and that available sampling technologies are difficult to perform requiring technical expertise and extensive experience in this field. In addition, the measured data may be considered insufficient with regards to reproducibility for the tasks of proteomics and there are the added perceived problems of data evaluation and interpretation. Finally, there is the misapprehension that rather large quantities of protein samples are required for a successful IR experiment.

Not one of these arguments is valid anymore because these issues have all been addressed when using the Bruker Optics CONFOCHECK as a dedicated infrared system for protein analysis.

Main applications for infrared spectroscopy in protein structure analysis

The advantages of infrared protein analysis are its ease of use and its relatively inexpensive capability of studying proteins in aqueous media at any buffer conditions. From the infrared spectrum, the secondary structure content of α-helix and ß-sheet can be determined directly. Moreover, the high specificity of the amide I band allows detection of conformational changes in proteins with high sensitivity. Any alterations in the structure regardless if they were induced by temperature or pH changes, ligand binding, mutations etc. are reflected by specific absorptive changes in the amide I region. Therefore, it is possible to isolate structural effects of a protein while binding to drugs, forming a multimeric structure or interacting with substrates and inhibitors. For drug discovery, the fundamental principle is utilized that similar compounds induce similar structural changes in the target protein, meaning similar absorptive changes in the amide I region. It should be stressed that these investigations are not restricted to water-soluble proteins, but can also be performed on membrane bound proteins. Furthermore, the formation of multimeric structures as protein aggregation, precipitation and/or crystallization can be monitored by infrared spectroscopy (IR), even in real time.

As IR follows the Lambert-Beer´s law, it also permits a very sensitive and accurate method for quantification of proteins in aqueous solution. Within seconds and without any bio- or immuno-chemical staining, protein concentrations as low at 0.03 mg/ml can be determined directly from solution.

IR is also very suitable for the study of enzymatic reaction kinetics. Transformation of substrates into products can be qualified and quantified based on specific infrared absorption features of these biomolecules. Here, it is advantageous that substrate and product molecules are measured in parallel and that no staining or labeling is required. By drying of the reaction mixtures before spectroscopic analysis, the sensitivity can even be increased from mM to μM concentrations. For this, samples are usually measured on IR transparent 96- or 384-wellplates. To accomplish high throughput screening (HTS), the analysis is performed on a fully automated IR microplate reader (HTS-XT).

Infrared Protein Analysis

***CONFOCHECK*™** *includes* ***TENSOR*™** *FT-IR spectrometer*

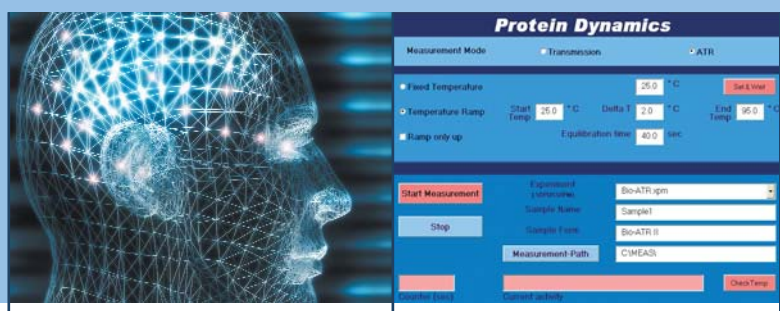
The advantages of infrared spectroscopy for protein structure analysis in comparison to other technologies

In contrast to X-ray crystallography (X-ray) and NMR spectroscopy (NMR), infrared spectroscopy (IR) can not reveal a 3D image of a protein structure, but is limited to the determination of the secondary structure. However, the restrictions of the former methods (the need for crystals or small water-soluble proteins) provide IR with a serious advantage. Using IR proteins can be studied in aqueous solution regardless of their molecular weight. Moreover, IR is very sensitive for detecting conformational changes from proteins. It is therefore a very suitable tool for the control of protein stability and/or the monitoring of induced unfolding/refolding processes. Protein concentrations can range from 0.03 - ∞ mg/ml and can be generated in any buffer system required for stability of the sample protein.

In comparison to circular dichroism spectroscopy (CD), which can also be applied for protein secondary structure analysis, the absorption features, reflecting conformational changes and secondary structure elements for IR are very specific and without any interference from aromatic amino acids, buffers and salts. As CD and differential scanning calorimetry (DSC), IR can monitor thermally and chemically induced alterations in proteins, but also provides detailed structural information about those processes. In contrast to CD, IR is not restricted to water-soluble proteins, and it is capable of investigating membrane proteins and following aggregation, precipitation and crystallization processes. According to the Lambert-Beer´s law, it also permits a very sensitive and accurate method for quantification of proteins in aqueous solution.

Suitability of infrared spectroscopy for tertiary structure analysis

To reveal its 3D structure, a protein has to be studied by X-ray crystallography (X-ray) or by NMR spectroscopy (NMR). Since X-ray analysis is restricted to protein crystals it is essential to know if the crystallized protein is still in the native state or if the structure was altered during the crystallization. This question can be answered very fast and easily by IR as any conformational changes are indicated by characteristic absorptive changes in the amide I region. Within a fraction of a minute the infrared spectrum of a protein sample under any buffer conditions is acquired and its secondary structure is derived from the obtained spectrum.



CONFOCHECK offers quick determination of secondary structure using its internal protein spectra library.

This software allows even inexperienced users to perform easily and successfully protein structure analysis by IR.

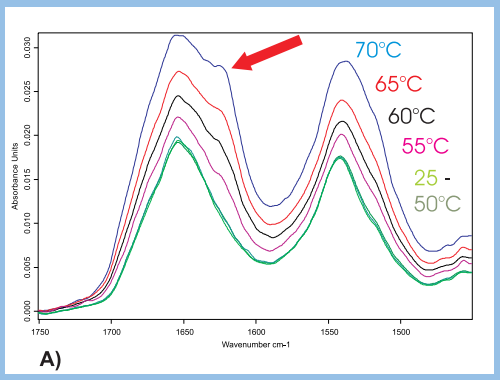
CONFOCHECK™

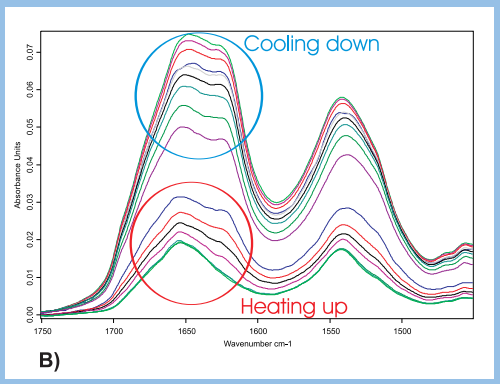
For measurements with NMR, highly concentrated samples of small proteins are required. IR can effectively be applied to check the suitability of a certain protein sample for the structural investigation, since it is capable of revealing structural alterations of the sample protein occurring during the concentration process. Any conformational changes or the formation of multimers would be recognized by infrared analysis. Moreover, IR would significantly decrease the effort of calculation in the data evaluation of NMR and/or X-ray, since the secondary structure (α-helix, ß-strand) of the sample protein is also derived from its infrared spectra.

CONFOCHECK a dedicated infrared system for protein analysis

CONFOCHECK is a dedicated and compact infrared system for protein analysis in aqueous solution. The main applications are the determination of secondary structure and the detection of conformational changes. Its specific concept facilitates a fast data acquisition with a high sample throughput controlled by a user friendly software interface.

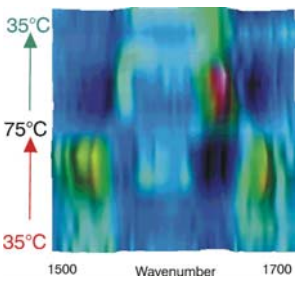
Typically, a volume of 5 µl from the aqueous protein sample (10 µg) is used to perform an experiment. Therefore, the sample is injected in the infrared sample cuvette which can be refilled in a flow-through manner. Within fractions of a minute, high quality spectra from proteins in solution are acquired. Afterwards, quantitative determination of secondary structure elements (α-helix, ß-strand) from the analyzed protein sample is accomplished within seconds via pattern recognition methods based on the internal protein spectra library. To follow temperature induced conformational changes, a cooling thermostat is integrated to accurately modulate the temperature of the sample accessories. Automatically, measurements of proteins in aqueous solution are recorded for the prespecified temperature range with a preselected increment. However, the applications are not limited to water-soluble proteins, but also membrane bound proteins can be studied by the CONFOCHECK. Moreover, this system is specifically designed to investigate protein stability and assembling processes such as aggregation, crystallization and precipitation. A dedicated dialysis accessory (Bio-ATR II) of the CONFOCHECK allows the real time study of protein ligand interactions and induced conformational changes in proteins. Since IR obeys the rules of Lambert-Beer´s law, the CONFOCHECK system is an ideal tool to determine protein concentrations in aqueous solution with high sensitivity and accuracy.





Temperature induced denaturation and precipitation of Lysozyme. In (A) the infrared spectra of Lysozyme for different temperatures (25-70 °C) are shown. The protein conformation changes clearly above 55°C, indicated by a strong rise of the absorption at 1630 cm^{-1}, representative for ß-sheet structures. In parallel to the conformational change, the protein starts to precipitate as is reflected by the increase of the overall protein signal. Though the denaturation seems to be completed above 55° (no further change in the amide I band), the precipitation grows constantly with time. Even during the subsequent cooling (B) the precipitation proceeds, and the unfolding was therefore found to be irreversible (no return of the amide I band).

innovative, inspired, intelligent



Temperature cycle of protein unfolding/refolding. Protein dynamics is a key application of the CONFOCHECK.

Infrared microplate reader HTS-XT, designed to fulfill HTS demands in enzyme kinetics and bio-catalysis.

CONFOCHECK can be equipped with three unique temperature controlled sampling accessories, to cover all applications of protein research.

AquaSpec cell

This flow through transmission cell is used to study water-soluble proteins. For biocompatibility, all tubes are made from PTFE and in-line filters are integrated to prevent contamination of the cell volume by aggregated and precipitated protein.

BioATR II

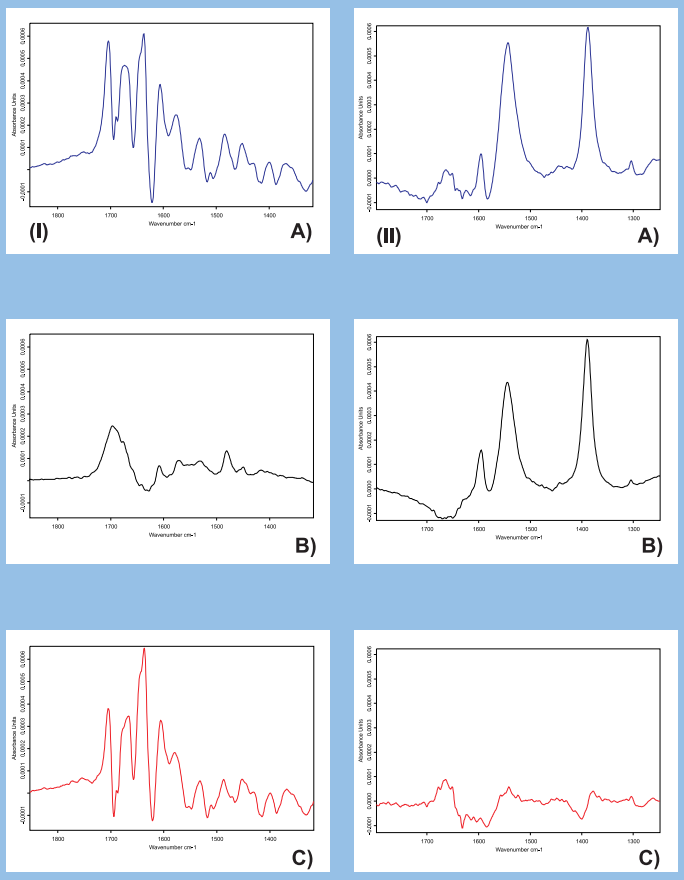
This sampling system is suitable for analyzing aggregation and precipitation processes of water-soluble proteins. Moreover, this accessory is applicable for the investigation of membrane proteins.

BioATR II with dialysis option

This ATR attachment is ideal to study real-time protein ligand binding and induced conformational changes. Low molecular weight compounds (ligands, salts, protons) can be applied to the protein of interest by dialysis in order to reveal structural alterations caused by the interaction.

Applications for CONFOCHECK

- Determination of secondary structure
- Detection of conformational changes in proteins
- Protein dynamics (temperature induced conformational changes)
- Protein quantification (down to 0.03 mg/ml in aqueous solution)
- Monitoring of protein aggregation, precipitation and crystallization
- Enzyme reaction kinetics



Protein ligand binding: (I) In (A), the difference infrared spectrum of Trypsin before and after application of benzamidine is shown. After subtraction of the pure ligand spectrum (B) the resulting difference spectrum (C) reflects the conformational change of the protein due to the ligand binding. The absorption change between 1700 and 1600 cm^{-1} (amide I band) is specific for changes of the secondary structure. (II) In (A), the difference spectrum of Trypsin before and after application of benzoic acid is shown. After subtraction of the pure ligand spectrum (B), the resulting difference spectrum (C) shows that no conformational change was induced by benzoic acid. The difference spectra from Trypsin after application of benzamidine (I C) and benzoic acid (II C) show representative curves for positive and negative binding results, respectively.