



HUMAN HEALTH

ENVIRONMENTAL HEALTH

Vol. 7 2009

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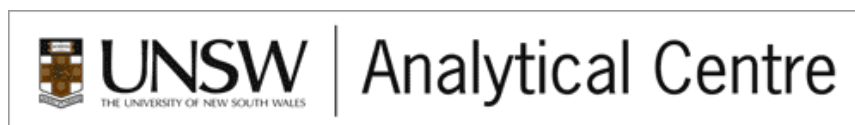
Dear Reader,

Welcome to the second edition of Material Vision for 2009.

This is an exciting time for Material Characterization Laboratories as PerkinElmer recently launched a new range of Single and Double Furnace DSC units, which are pushing the boundaries of science with heating and cooling rates up to 750 K/min. Some examples are described in this edition.

Also presented are techniques on how to measure Ultra Micro volumes using UV and how to setup FTIR and Raman libraries, search routines and interpretation.

The University of New South Wales Analytical Centre supports research and industry



The University of New South Wales has established a new Analytical Centre at its main Kensington campus in Sydney. The Analytical Centre opened 18 months ago and provides easy access and high level support for the key instrumentation used by researchers at The University of New South Wales, as well as other Universities in the region, commercial users and government organisations. The most recent development in the Centre is the "Spectroscopy Laboratory" dedicated to vibrational spectroscopy that serves the needs of a wide range of users from the materials science community. [Read More...](#)

Measurement of Ultra Micro Volumes of Nucleic Acids Using the LAMBDA Bio+ and the Hellma® TrayCell™

The need to measure ultra micro (<5 µL) volumes of nucleic acid is common in molecular biology. Typical applications include the quantitation of template prior to sequencing, PCR quantitation and purity analysis ("260/280 ratio"). While larger volumes can be readily measured in micro-, semi-micro or full size cuvettes, handling ultra micro amounts in very low volume ultra microcells has presented particular practical challenges. The difficulties presented with such small amounts of material include accuracy of pipetting, carryover and the issue of electrostatic interaction between droplets of the liquid and the plastic pipette tips. [Read More...](#)



Setting up Libraries

Using Search Plus

In previous editions of Material Vision we discussed the benefits of using Compare, Search and Interpretation to aid in identifying materials, components and functional groups. In this article it is described how libraries are created and the setup options available. Also described are the types of searches available, results and what they mean. [Read More...](#)



Single and Double Furnace DSC

Pushing technology and science to the limits

The Single and Double Furnace DSC systems provide a new design approach allowing upgradeability, enhanced software options and faster heating and cooling.



vitro Stem Cell Osteogenic
Differentiation with Raman
Spectroscopic
Mapping

UV Spectroscopy - Measuring the
daylight, solar energy and thermal
radiation properties of coated glazing

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TGA and STA Calibration
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- Contacts

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TALK TO US NOW

These enhancements provide greater reliability and robustness for OIT testing, modulated temperature DSC techniques and true isothermal kinetics. [Read More...](#)



TGA and STA Calibration

Guaranteeing reliable thermal analysis data

There are several calibration steps for the TGA and STA that need to be performed so that you can be confident that the system is giving the best and most reliable data. All calibrations should be performed upon installation of the instrument and adjustments made. Following this periodic calibration checks can be run to verify the reliability of the data. [Read More...](#)



Case Studies

If you have an interesting case study and would like to share it with others, we would be happy to feature it.

[Contact us here](#)

Edited by John K Arthur
PerkinElmer, Australia

[Download a complete version of this Material Vision Newsletter.](#)

Next Issue

Single/Double Furnace DSC

UV Small Spot Kit (SSK)
accessories for the 150mm
integrating spheres

Beer's Law
Calibration
Evaluation

DSC-Raman Hyphenation

21 CFR Part11



Author

Dr Chris Marjo

The University of New South Wales Analytical Centre Supports Research and Industry

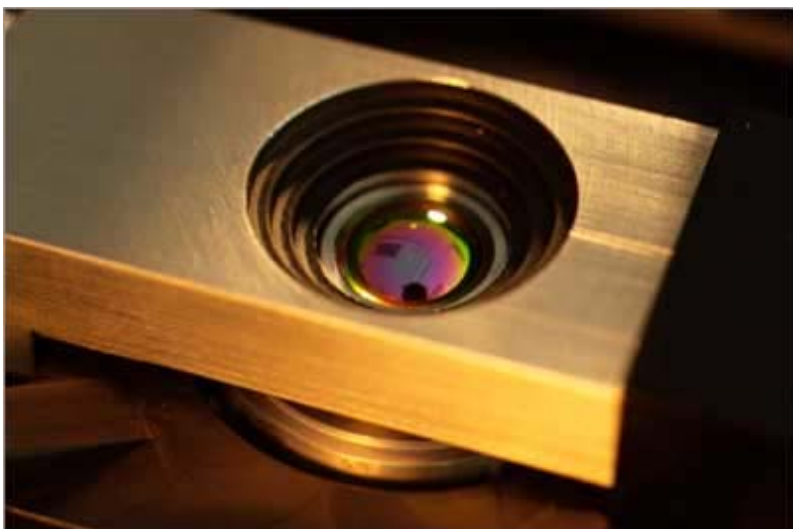
PerkinElmer has developed a partnership with the university that significantly augments the capabilities of the laboratory. The Analytical Centre is in an ideal location, providing clients' access for application development and workshops.

Dr Chris Marjo, the manager of the Solid State and Elemental Analysis Unit in the Analytical Centre, which encompasses the Spectroscopy Laboratory, says that, "instrument use has grown significantly, as more researchers become aware of the capabilities of vibrational spectroscopy and the speed that high resolution images can now be collected".

Access to the facilities in the laboratory is done through the on-line booking system or organized through the lab's scientific officer, Dr Anne Rich. Anne is responsible for basic competence training, overseeing research work and conducting workshops on the Raman spectrometers, FTIR Imaging and UV-Vis systems. In addition, Anne's experience of a wide range of materials and practical skills are valuable to the laboratory to aid in the development of instrumental capabilities and the scope of research that can be performed.



Dr Anne Rich using the Spotlight 400 at the Analytical Centre.



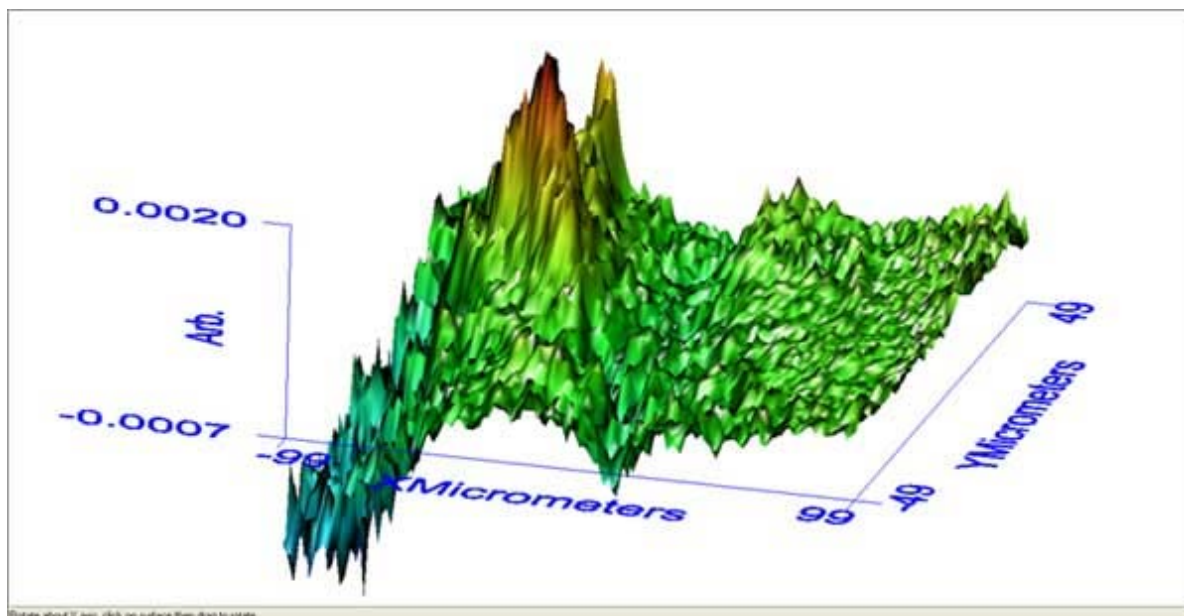
The Spotlight 400 Imaging Crystal used at the UNSW with 1.56 μm resolution capability.

Asked about the range of equipment in the laboratory, Chris points out that equipment was initially focussed on Raman spectroscopy to benefit the materials science community that existed before the opening of the laboratory: "From the beginning the laboratory has supported research into new materials or nanostructures on traditional materials. A classic example is the broadening of the Raman phonon peak found in crystalline silicon at 520cm^{-1} as a result of stress. Viewing changes in the silicon phonon peak provides critical information for our large photovoltaic community to understand how the surface structure of a solid influences its semiconductor behaviour. This also is a complementary technique to support data provided by our x-ray diffraction laboratory."

Increasingly the laboratory is seeing users from a wide range of research areas. Chris explained that, "The materials community is quite diverse and we are seeing more polymer and ceramic scientists. One area of importance is in biochars. These are charcoal materials that have been aged in the soil for decades that are found to significantly boost crop yields. Researchers in the area are trying to understand how to reproduce the aging process to create biochar that is ready to be used by cultivators immediately. Of course the low cost of biochar has huge implications for crop production in developing countries. The challenge is to understand how the carbonaceous material is altered over time in such a complex mixture like soil, and to look for the presence of new chemical bonds that develop at the char-mineral interface. The Spotlight 400 FTIR Imaging Microscope has been embraced by the biochar community for this purpose. The instrument provides fast, high spatial resolution chemical imaging using its ATR-imaging crystal. It is an impressive system and complements the Raman systems we also use".



The visible image of a biochar sample.



Chemical map measured on the Spotlight 400 showing the distribution of aromatic compounds over the biochar sample.

When asked about the training of an ever increasing number of users, Chris responded that, "One of the best decisions we made was to purchase the PerkinElmer RamanStation 400F spectrometer. The instrument is very simple to operate while providing excellent spectral performance. We have over sixty users of the laboratory with a wide range of abilities and experience. It has really helped that the RamanStation is so easy to demonstrate and use, and the functionality is robust enough so that everyone can experience this technique. It is a great introduction to the power of vibrational spectroscopy, and we are seeing many users who would never have used Raman spectroscopy adopt it enthusiastically for their research".

The Spotlight 400 FTIR and RamanStation 400F use the same processing software allowing researchers to process their IR or Raman data in exactly the same way using Principle Component Analysis (PCA), 2D and 3D layer managers, chemi-maps and band ratios.

A significant problem in Raman spectroscopy is when the Raman scattering is overwhelmed by fluorescence in the sample, particularly in materials such as organic polymers when they are measured using UV or green lasers. This can usually be overcome by using a longer wavelength excitation source, however the efficiency of the Raman scattering process drops as the wavelength of the excitation source increases. A 785nm laser provides a good compromise between minimising fluorescence and providing efficiency for Raman scattering, making the RamanStation their key instrument for analysis of polymers, organic compounds like pharmaceuticals, and novel organic nanomaterials such as graphene and carbon nanotubes.

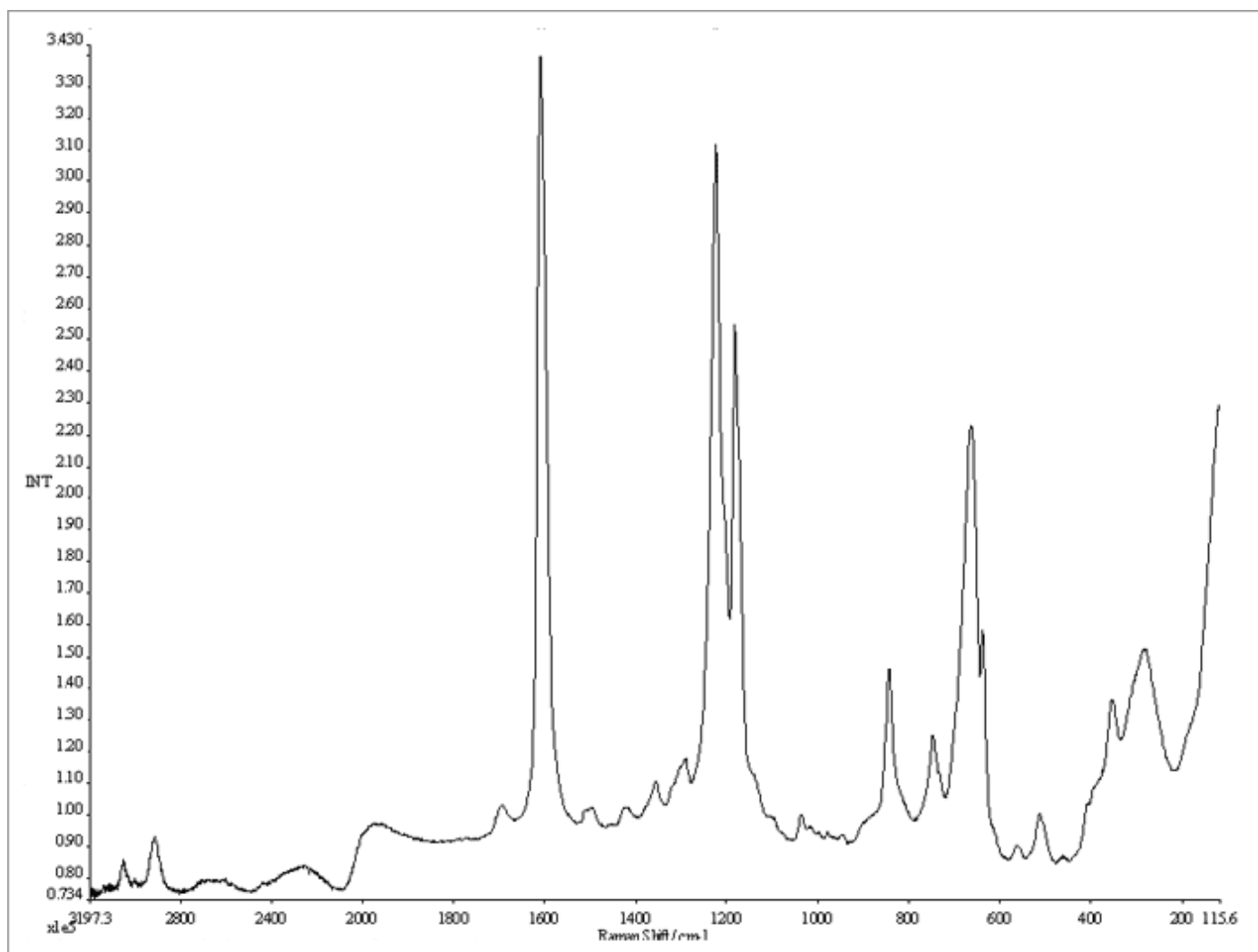
The range of measurement options on the RamanStation has also helped with the popularity of the instrument Chris pointed out that, "The Raman optic probe recently allowed us to safely and easily measure samples of ancient Roman glass from the Macquarie University. Also the mapping option is popular with samples that show chemical differences across millimetre to sub-millimetre length scales, such as polymer composites". Another group at UNSW is taking advantage of the RamanStation's versatility with "awkward" samples to measure the surface enhanced Raman scattering (SERS) on roughened gold electrodes to create new methods for trace-molecular analysis.



Danmar Gloria running SERS on the RamanStation 400F.



A roughened gold electrode that enables SERS measurements of organic species.



SERS spectrum of Danmar's test compound 1,4- Benzenedimethanethiol.

The future of the laboratory at the Analytical Centre will see a push for more researchers in the biological and chemical areas, and this has been helped by the Spotlight's Universal ATR system: "the UATR has been enthusiastically used by a diverse range of users who have previously had problems trying to get infrared spectra of their samples. The measurement is very simple and has been a popular tool to get rapid high-quality infrared spectra of previously difficult samples including artificial bone, ultrathin polymers, and tiny quantities of organic compounds. The ability to measure materials contaminated with water is a real advantage with this technique."

More information on access and the capabilities of this laboratory can be found at http://www.sseau.unsw.edu.au/spectroscopy_start.htm.

Measurement of Ultra Micro Volumes of Nucleic Acids

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Measurement of Ultra Micro Volumes of Nucleic Acids Using the PerkinElmer LAMBDA Bio+ and the Hellma® TrayCell™



Figure 1. LAMBDA Bio+ with Hellma® TrayCell™ with 600 ng/mL (approx.) sample.

Introduction

The need to measure ultra micro (<5 μ L) volumes of nucleic acid is common in molecular biology. Typical applications include the quantitation of template prior to sequencing, PCR quantitation and purity analysis ("260/280 ratio"). While larger volumes can be readily measured in micro-, semi-micro or full size cuvettes, handling ultra micro amounts in very low volume ultra microcells has presented particular practical challenges. The difficulties presented with such small amounts of material include accuracy of pipetting, carryover and the issue of electrostatic interaction between droplets of the liquid and the plastic pipette tips.

The PerkinElmer® LAMBDA™ Bio and LAMBDA Bio+ are designed for the measurement of nucleic acids and proteins (the LAMBDA Bio+ can also be used as a general purpose spectrophotometer). These are generally used with microcells (e.g. 50 μ L) or larger volumes but they give excellent results when used with the Hellma® Tray Cell. This combination results in a very flexible system which is able to measure sample amounts from 3 μ L to 3 mL. The TrayCell™ is extremely easy to use and carryover is eliminated due to its inherent design. The instrument is self-contained and so an external PC is not required. Data can, however, be transferred easily to a PC from the LAMBDA Bio+ via the USB link cable and software supplied as standard with the instrument.*

TrayCell™

The TrayCell™ is a 1 cm x 1 cm device that fits into the standard cuvette position on the spectrophotometer. The device is offered in a variety of beam heights but 15 mm should be used for all PerkinElmer UV/Vis spectrophotometers. Inside, the unit has a system of prisms and fiber optics which serve to periscope the light beam up to the sampling window and then back to the detector on the instrument (see Figure 2). The device is supplied with a cap which contains a small mirror. This cap ensures that the sample is measured at constant pathlength. The pathlength is achieved by a double pass through the sample as controlled by the cap height. For the examples described in this technical note, a pathlength of 0.02 mm was used. The instrument's internal software is able to adjust this value to the equivalent for a 1 cm cell by applying a factor of 50 to all measurements. There is no alignment procedure when the TrayCell™ is used with the LAMBDA Bio+

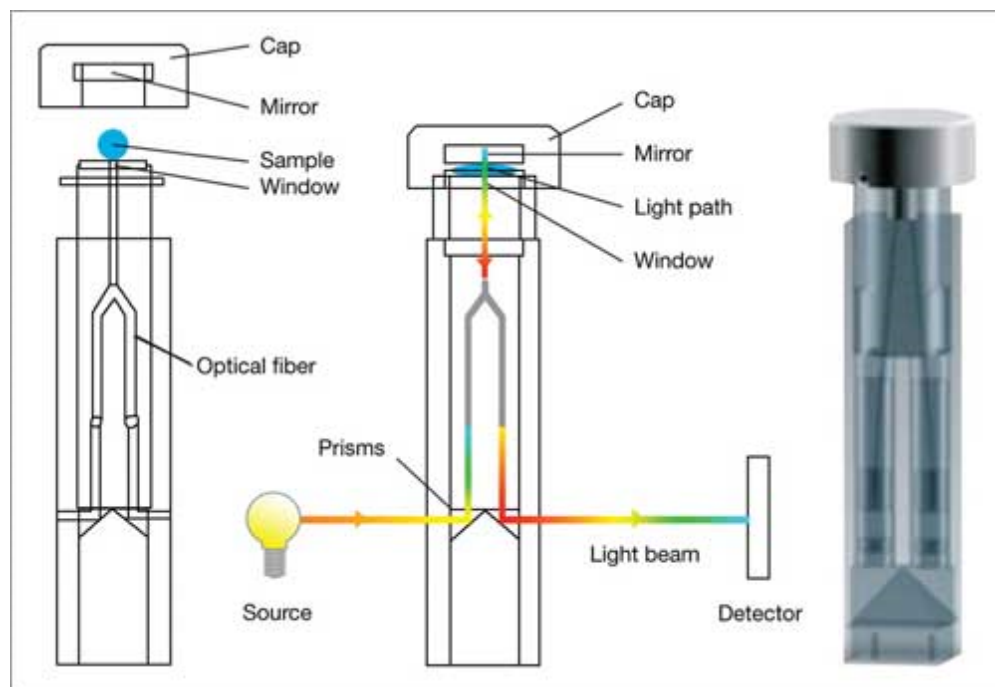


Figure 2. Configuration of the Hellma®.

and the device remains in the instrument during all phases of the measurement including filling (see Figure 3). The user simply pipettes about 3 μ L of liquid onto the window and then places the cap on top. The measurement can then be taken.

Experimental

Two samples of calf thymus DNA with respective concentrations of 600 and 1300 ng/ μ L (equivalent to mg/L) were prepared and measured using the LAMBDA Bio+ fitted with the Hellma® TrayCell™. A 3 μ L drop was deposited onto the quartz window on the TrayCell™ so as to cover it. The cap was then replaced and the measurement taken. All samples were blanked using deionized water.

Results and discussion

Samples were scanned and the data transferred to a PC using the software and USB cable supplied with the LAMBDA Bio+ (see Figure 4). The purity and concentration were also measured using the DNA measurement method, and a concentration factor of 50 was applied for double-stranded DNA (dsDNA). For single stranded DNA, this factor should be 33 and for RNA 40. The purity was measured at 260, 280 and 320 nm (Table 1). The 320 nm absorbance value was subtracted from the respective 260 nm and 280 nm measurements and then a ratio calculated. For the concentration measurement, the 320 nm absorbance value

was subtracted from the 260 nm reading and then multiplied by the concentration factor. The pathlength compensation factor (50) was applied in order to adjust values to a 1 cm cuvette. A purity ratio of over 1.7 indicates that the DNA is pure and not contaminated with protein.

The results were checked against a LAMBDA 25 dual beam UV/Vis spectrophotometer and found to be in good agreement.



Figure 3. Close-up TrayCell™ in the LAMBDA Bio+.

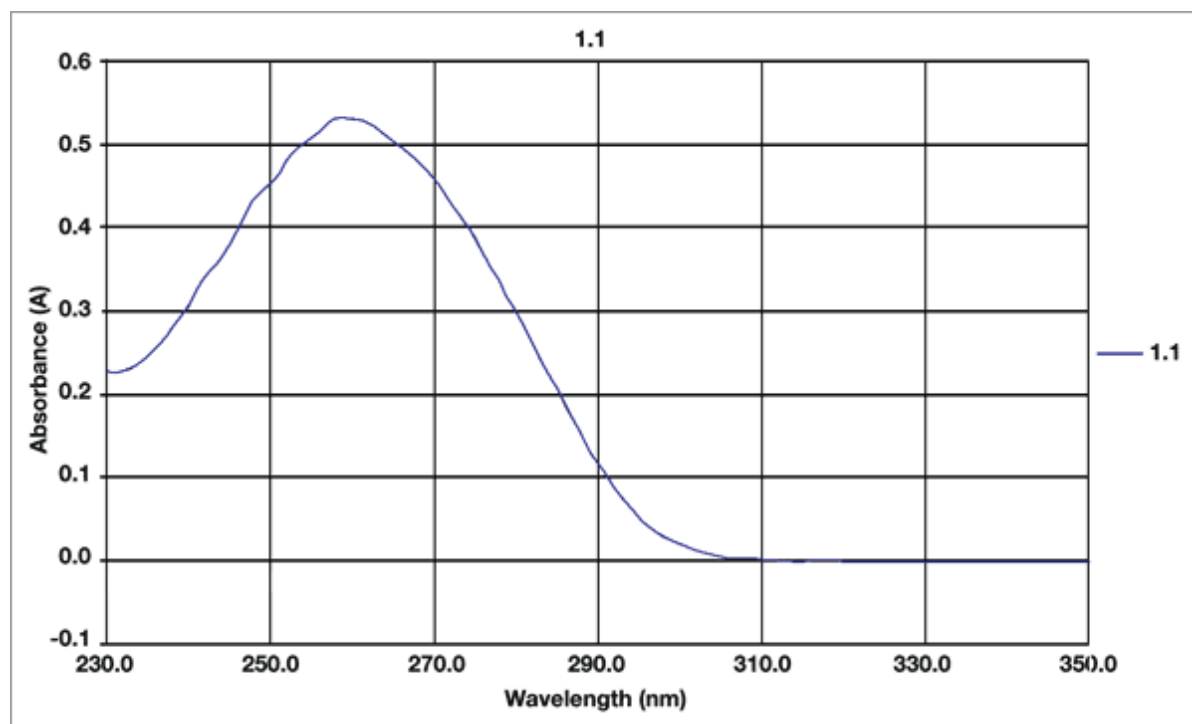


Figure 4. 500 ng/μL calf thymus DNA plotted in MS Excel® format.

Conclusion

The Hellma® TrayCell™ has been shown to be an ideal companion to the LAMBDA Bio/Bio+ spectrometer for the measurement of ultra low volumes of liquids such as Nucleic acids and proteins.

Table 1. Example of results output from LAMBDA Bio/Bio+.

Concentration: 610 ng/μL			
A230	A260	A280	A320

Table 1. Example of results output from LAMBDA Bio/Bio+.

0.105	0.246	0.141	0.002
A260/A280	A260/A230		
1.755	2.369		
Concentration: 1292 ng/μL			
A230	A260	A280	A320
0.212	0.515	0.289	-0.002
A260/A280	A260/A230		
1.777	2.416		

*Can be purchased separately for standard LAMBDA Bio.

Setting up Libraries Using Search Plus

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Setting up Libraries Using Search Plus

Creating a New Library

The **Create Library** command, in the Search Plus option, enables you to create a new library. You can then add entries to this library with the **Add to Library**, **Build Library**, and **Append Library** commands.

Creating the library file

1. Display the Library Utils menu and choose **Create Library**.
2. In the Create Library dialog, type the name of the new search library, including the full path, in the **Library Name** text box, or choose **Browse**, and use the file selector to find the required search library.
3. Choose the library size.
The numbers in the Select Size area specify the maximum number of entries that can be added to the library.
4. Choose a library **Type** and **Ordinate Type**.
If you choose **NIR**, you can perform only a Euclidean Search.
5. You can only perform an interpretative search on mid-infrared spectra with an ordinate type **%T %R** or **A**. You cannot include mid and near infrared spectra in the same library. Spectra of different ordinate types cannot be included in the same library.

6. For Raman libraries, choose Type **MIR** and Ordinate Type **INT**.
7. Enter a **Copyright Message of up to 60 characters** and choose **OK**.
The Create Library dialog closes.

Adding spectra to your new library

Now you will add some spectra to the library.

1. Display the Library Utils menu and choose **Build Library**.
The Build Library dialog is displayed. Note that the Library Name is the library that you have just created.
2. In the **Drive / Directory** text box choose select the directory that contains the spectra you want to add to the library.
3. Choose **Add All**.
All the Source Spectra are copied to the **Spectra to Add** text box.
4. Choose **OK**.
Spectrum Search Plus starts to build the library.

NOTE: If any of the spectra do not have valid spectrum ids, a message is displayed. You must enter a spectrum ID before you can continue. The spectrum ID must be of the format ABNNNB, where A is a letter, B is either a letter or a number, but not V through Z, and N is a number.

5. If you use files of peak tables then you can only build a search library. The **Build Library** command automatically generates the PSU data required for a library entry.

Adding the current sample to a library

The **Add to Library** command enables you to add the current sample to a library. It is active as soon as a sample has been specified. A maximum of 127 peaks is allowed for a library peak table.

1. Display the Library Utilities menu and choose Add to Library.
2. In the Add to Library dialog, enter a spectrum id for the entry.
The spectrum ID must be of the format ABNNNB, where A is a letter, B is either a letter or a number, but not V through Z, and N is a number.

3. Type a Spectrum Name of up to 60 characters following the naming convention.

NOTE: If the spectrum id and name are available in the peak table, the Spectrum id: and Spectrum Name: text boxes defaults to those values.

4. Type the name of the new search library, including the full path, in the Library Name text box, or choose Browse and use the file selector to find the required search library.
5. Choose OK.
The entries are added to the Search Library.

What can I use Search for?

IR Search helps you to identify an unknown material. It will search through a library or libraries to find the spectra that best match your unknown sample. It can also interpret your unknown spectrum directly by determining from the pattern of peaks (and absences of peaks)

which Possible Structural Units (functional groups) are likely to be present.

When should I use Compare and when should I use Search?

You should use IR Search when you do not know the identity of your sample, a search compares your sample spectrum with libraries that can contain many spectra, or compares the peaks in your spectrum with many Possible Structural Units to identify your sample.

Even if your sample is not in the libraries, an interpretation will give you information on what functional groups are present in your sample, and provide clues as to what it is.

If you know that your sample is one of a range of materials or if you want to measure how similar your spectrum is to another spectrum, you should use Compare; this compares your sample spectrum with one other spectrum or several spectra and reports the degree of similarity between the spectra.

What do the Different Search Types do?

There are three types of search available:

- ❖ **Interpretation** - an interpretation compares the peaks in your sample spectrum with the peaks that correspond to 896 PSUs (Possible Structural Units). A list of the PSUs in your sample spectrum is displayed. You do not need to have a search library to perform an interpretation.
- ❖ **Euclidean** - a Euclidean search compares every point in your sample spectrum with the points in spectra in a search library.
- ❖ **Library search** - a library search compares the position of the peaks in your sample spectrum with the position of peaks in the spectra in a search library.

What Type of Search should I use?

An **interpretation** is useful:

- ❖ If you think that your sample spectrum will not be present in a search library, the interpretative search will indicate what PSUs are present in the sample.

A **library** search is useful because:

- ❖ it is faster than an Euclidean search - it is comparing a relatively small number of peak positions whereas a Euclidean search compares a much larger number of points in the spectra;
- ❖ If you know that your sample contains a mixture of materials you can set up the search so that additional peaks from the other components are ignored.

A **Euclidean** search is useful where the search library spectra are not distinguishable from each other, for example:

- ❖ within classes of chemically-similar complex compounds where bands overlap strongly, the Euclidean search compares all the points in the spectrum and hence identifies peaks that are less-well separated;
- ❖ when you are searching for mixtures that are different in the proportions of their components, a Euclidean search takes account of peak intensity as well as position;
- ❖ When the signal-to-noise ratio of your spectrum is poor, a Euclidean search effectively ignores the noise in the spectrum.
- ❖ If you are searching for near infrared or Raman spectra, only a Euclidean search is available.
- ❖ A Euclidean search compares every point in a spectrum with spectra in a search library; the results from a Euclidean search are more reliable than a library search.

If you are using near infrared or Raman spectra only a Euclidean search is available.

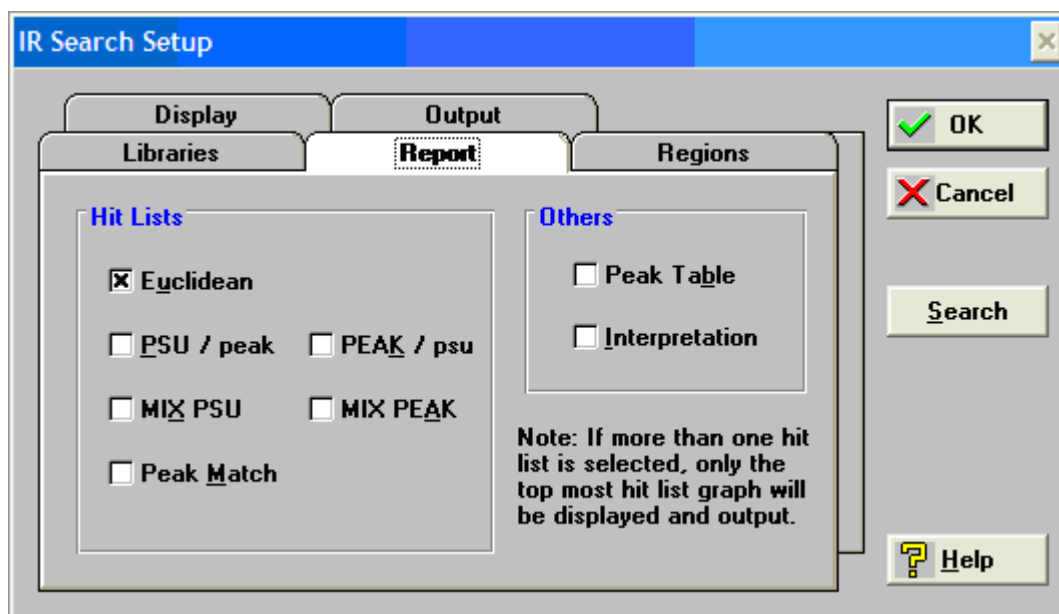
If your spectra does not have an ordinate axis in %T, %R or A only a Euclidean search is available.

What do the Search Results Mean?

When a search finishes the results are displayed as hit lists. If you have chosen **Display** in the **Graph Window** section of the Display tab, a graph window displaying your sample spectrum and spectra of the top items in the hit list are also displayed.

The hit lists give the compounds that most closely match your sample spectrum. There are six different hit lists that use different methods for scoring.

Euclidean	A hit list of the best spectrum shape matches.
PSU/peak	A hit list based primarily on the PSU score. The peak match score is used only to rank those hits with identical PSU scores.
PEAK/psu	A hit list based primarily on the peak match score. The weight given to PSU matches is lower for high peak match scores.
MIX PSU	A hit list based on the PSU score, but allowing for the spectrum being that of a mixture of compounds.
MIX PEAK	A hit list based on peak matches, but allowing for the spectrum being that of a mixture of compounds.
Peak Match	A hit list based on the peak match score only.



The hit lists are a useful aid to identification. However, we recommend that you make a visual comparison of the sample and library spectra before accepting the identification.

To display the sample spectrum and an item in the hit list:

- ◆ Double-click on an item in a hit list.

Starting a Search

1. Display and select the spectrum or spectra that you want to identify.
2. Display the Process menu and choose **IR Search**.

<u>U</u> ndo	Ctrl+Z
Data Tune-up	
<u>A</u> bsorbance <u>T</u> ransmittance Kubelka-Munk Convert <u>X</u> ...	
<u>D</u> ifference... Baseline Correction ▶ Smooth ▶ <u>D</u> econvolution... Spectral <u>C</u> alculator... Normalize... Abex... Derivative... Interpolate... <u>B</u> lank... <u>K</u> ramers-Kronig... ATR Correction...	
Compare IR <u>S</u> earch Interactive Interpretation... <u>Q</u> uant Prediction... Peak Area / Height... Peak Table...	

The hit lists are displayed.

We advise you **not** to select several spectra and several hit lists simultaneously, because the display can become overcrowded and confusing.

Displaying unknown and library spectra

You can see how well the unknown spectrum matches a library spectrum by double-clicking on the spectrum name in a hit list. The two spectra are displayed.

IR Search Setup - Regions

You may want to search for your spectra over a limited abscissa range or to exclude specific regions of your spectrum from the search; the Regions tab enables you to specify the abscissa range and to exclude regions.

Range

If you choose **Full Overlap**, the entire range that overlaps is used in the search. If you choose **Manual**, you can enter your own **Start** and **End** abscissa values for the region to be searched.

Blanks

Specifies regions to be excluded from the search.

IR Search Setup

Display

Output

Libraries

Report

Regions

Range

☒ Full Overlap

☐ Manual

Start: 4000.0 cm-1

End: 200.0 cm-1

Blanks

From: To:

Add...

Delete

OK

Cancel

Search

Help

Single and Double Furnace DSC

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Single and Double Furnace DSC - Pushing technology and science to the limits

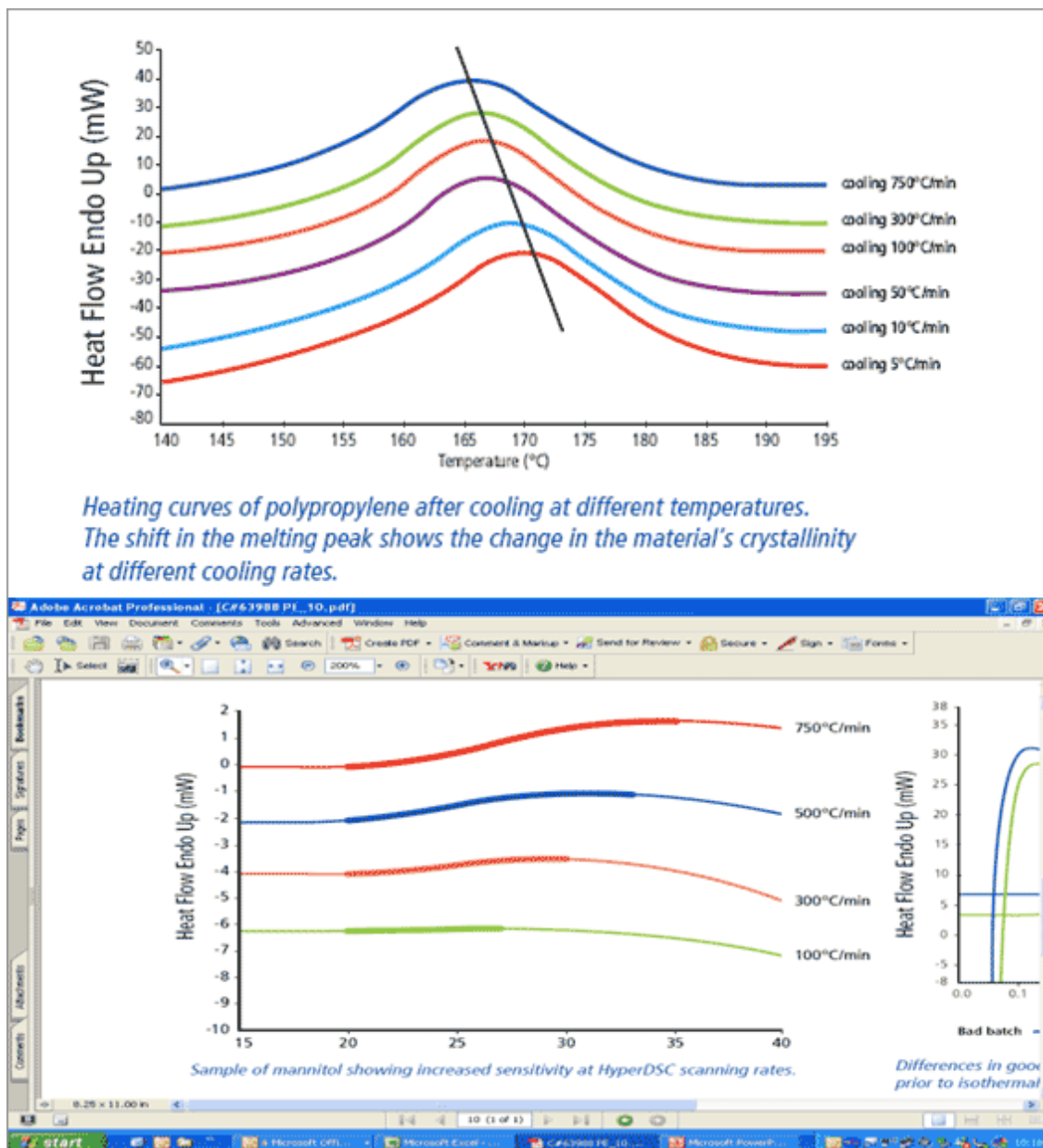
PerkinElmer have been involved with DSC technology for many years. Through research commitment and continual development, recent advances have seen the introduction of the new Single and Double Furnace DSC systems.

The Single Furnace DSC technology and user interface enhances the ease of use, robustness and reliability making it perfect for applications such as glass transition, crystallization and melting determinations as well as OIT and MT-DSC.



The Double Furnace DSC is pushing science to the limits with extremely fast heating and cooling under controlled conditions. A perfect example is the use of high heating and cooling rates to study polymorphism. Another example highlights how increased sensitivity can be gained from the higher heating rates.





The design of Double Furnace DSC also allows the capability of in-situ ballistic cooling to 1000°C/min, enabling you to perform experiments that mimic real-world processes. Experiments that require fast heating and cooling rates will require extremely fast data readout rates (100 points/second) with high data integrity.

A summary of what application areas best suit (but not limited to) the Single and Double Furnace DSC systems are listed below in the table for your review.

	Single Furnace DSC		Double Furnace DSC	
	DSC 4000	DSC 6000	DSC 8000	DSC 8500
QA/QC	ü			
Research & Development		ü	ü	ü
OIT		ü		ü
MT-DSC		ü	ü	ü
Photo-calorimetry		ü	ü	ü
Mimic process conditions		ü	üü	üüü

External high pressure cell			ü	ü
True Isothermal Kinetics			ü	ü
Cure Kinetics			ü	ü
Isothermal Crystallization			ü	ü
Ballistic Cooling				ü
HyperDSC				ü

TGA and STA Calibration

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TGA and STA Calibration - Guaranteeing Reliable Thermal Analysis Data

For the Pyris 1 TGA and STA 6000 the calibration should remain unchanged for some time, provided that there are no changes in the instrument's operating conditions. Even when the system is shut off, the calibration values are stored so that the next time the instrument is turned on, it will still be calibrated. Some of the conditions that could change either the temperature or the weight calibration and require recalibration are

- ❖ If the operating temperature range of your experiments changes, recalibration may be necessary. Check the temperature calibration in the range of interest to determine if the current calibration is still valid.
- ❖ If the purge gas type or purge gas flow is changed, the calibration should be checked for highest accuracy.
- ❖ If a new furnace is installed, the temperature calibration should be checked.
- ❖ If a new thermocouple is installed or if the position of the thermocouple changes, you should perform the temperature calibration again.
- ❖ If the analyzer is moved or leveled, you should perform the weight calibration again.
- ❖ If the hangdown wire is changed, you should perform a realignment of the gripper and recalibrate weight.

The calibration routines that need to be performed for the Pyris 1 TGA and STA 6000 are shown below in the table:

Calibration/Adjustment	Pyris1TGA	STA 6000
Weight	Yes	Yes
Sensor		Yes
Baseline		Yes
Temperature	Yes	Yes
Heat Flow		Yes
Furnace	Yes	

Temperature	<p>The temperature calibration is performed for a Pyris 1 TGA using magnetic standards of nickel and iron, which are supplied in the Spares kit. The runs should use the same conditions under which you would run your samples. The temperature calibration uses the Curie transition of the materials, i.e., the point at which the magnetic properties disappear.</p> <p>The STA 6000 utilizes the heat flow signal for the temperature calibration where the onset of fusion for metal standards is used to make adjustments.</p>
Weight	<p>The Spares kit provided includes a calibration weight that is used to verify and adjust the weight signal of the Pyris 1 TGA and STA 6000. The calibration weight is typically between 55mg and 100mg.</p>

Furnace	The Pyris 1 TGA furnace calibration takes approximately 1 hour. Enter the minimum and maximum temperatures between which the furnace will be calibrated. This will ensure that the sample temperature is under control over the whole range of the system.
STA	<p>Additional calibrations are required for the STA 6000. These involve the following steps:</p> <ul style="list-style-type: none"> ❖ Sensor Calibration – similar to Furnace Calibration. ❖ DTA Baseline Optimization – maintains the heat flow baseline as flat as possible over the required temperature range. ❖ Heat Flow Calibration – enthalpy determinations from chemical and physical changes are based on the melting of standards provided with the STA 6000.

Introduction to Dynamic Mechanical Analysis (DMA)



A Beginner's Guide

This booklet provides an introduction to the concepts of Dynamic Mechanical Analysis (DMA). It is written for the materials scientist unfamiliar with DMA.

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20 Basic Questions on Dynamic Mechanical Analysis

1. What is DMA?

Dynamic Mechanical Analysis, otherwise known as DMA, is a technique where a small deformation is applied to a sample in a cyclic manner. This allows the materials response to stress, temperature, frequency and other values to be studied. The term is also used to refer to the analyzer that performs the test. DMA is also called DMTA for Dynamic Mechanical Thermal Analysis.

2. How does DMA differ from Thermomechanical Analysis?

Thermomechanical Analysis, or TMA, applies a constant static force to a material and watches the material change as temperature or time varies. It reports dimensional changes. On the other hand, DMA applies an oscillatory force at a set frequency to the sample and reports changes in stiffness and damping. DMA data is used to obtain modulus information while TMA gives coefficient of thermal expansion, or CTE. Both detect transitions, but DMA is much more sensitive. Some TMAs can do limited DMA and the PerkinElmer® DMA 8000 is the only DMA that can do TMA.

3. How does a DMA work?

DMA works by applying a sinusoidal deformation to a sample of known geometry. The sample can be subjected by a controlled stress or a controlled strain. For a known stress, the sample will then deform a certain amount. In DMA this is done sinusoidally. How much it deforms is related to its stiffness. A force motor is used to generate the sinusoidal wave and this is transmitted to the sample via a drive shaft. One concern has always been the compliance of this drive shaft and the affect of any stabilizing bearing to hold it in position. A schematic of the analytic train of the DMA 8000, Figure 1, shows its innovative design that requires neither springs nor air-bearings to support the drive shaft.

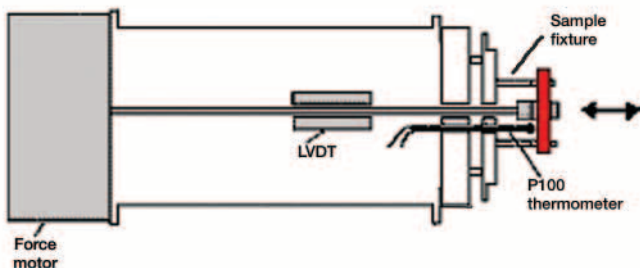


Figure 1. Schematic of the DMA 8000 analytic train.

4. What does DMA measure?

DMA measures stiffness and damping, these are reported as modulus and tan delta. Because we are applying a sinusoidal force, we can express the modulus as an in-phase component, the storage modulus, and an out of phase component, the loss modulus, see Figure 2. The storage modulus, either E' or G' , is the measure of the sample's elastic behavior. The ratio of the loss to the storage is the tan delta and is often called damping. It is a measure of the energy dissipation of a material.

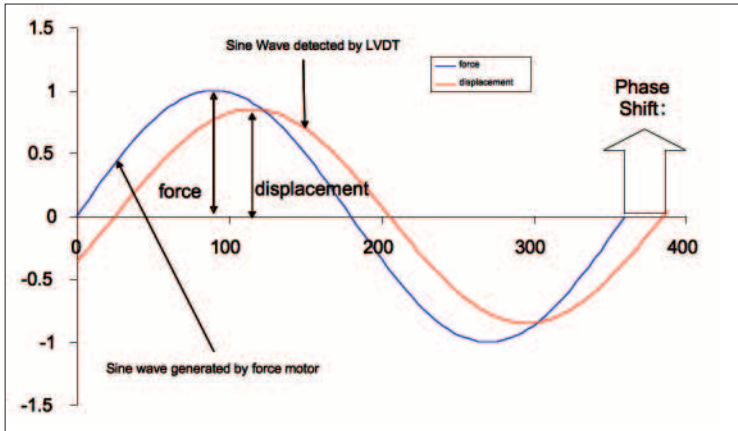


Figure 2. The relationship of the applied sinusoidal stress to strain is shown, with the resultant phase lag and deformation.

5. How does the storage modulus in a DMA run compare to Young's modulus?

While Young's modulus, which is calculated from the slope of the initial part of a stress-strain curve, is similar conceptually to the storage modulus, they are not the same. Just as shear, bulk and compressive moduli for a material will differ, Young's modulus will not have the same value as the storage modulus.

6. What is damping?

Damping is the dissipation of energy in a material under cyclic load. It is a measure of how well a material can get rid of energy and is reported as the tangent of the phase angle. It tells us how good a material will be at absorbing energy. It varies with the state of the material, its temperature, and with the frequency.

7. Why would I want to scan modulus as a function of temperature?

Modulus values change with temperature and transitions in materials can be seen as changes in the E' or $\tan \delta$ curves. This includes not only the glass transition and the melt, but also other transitions that occur in the glassy or rubbery plateau, shown in Figure 3. These transitions indicate subtler changes in the material. The DMA 8000's unique low starting temperature of -190°C in the standard furnace and of -196°C in the Fluid Bath let you easily look for these small molecular motions, Figure 3.

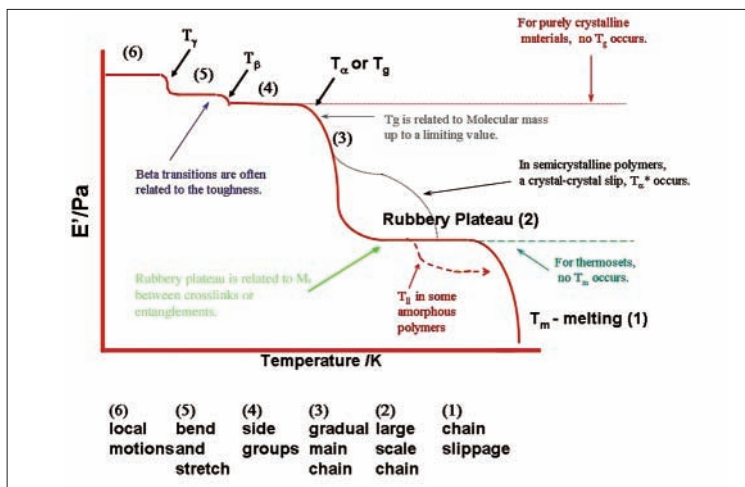


Figure 3. Modulus values change with temperature and transitions in materials can be seen as changes in the E' or $\tan \delta$ curves.

8. How do I get good data?

Good data requires several things: a properly calibrated instrument, a properly prepared specimen with a reasonable aspect ratio, using the right geometry, and applying both reasonable strains and heating rates. A properly calibrated instrument requires calibration for both temperature and force. A well prepared specimen should be of even thickness with parallel sides and right angle. Assuming the correct choice of geometry for the sample, a deformation of 50 microns and heating rates of $2\text{--}3^\circ\text{C}/\text{minute}$ normally work fine.

9. How do I know what geometry to use?

The choice of the geometry you run your sample in is dictated by the sample’s physical state at the beginning of the experiment, its difficulty in loading, and the experiment you want to run. For example, a stiff bar of polymer can be run in all of the flexure fixtures, but single cantilever is often used because it is simple to load and allows thermal expansion of the specimen, shown in Figure 4. Uncured thermosets are often run in shear. The DMA 8000 not only has the a full range of fixtures covering the normal 3-point bending, single cantilever, dual cantilever, tension, compression and shear fixtures, but also offers the novel Material Pocket for holding powders and soft samples that can not support their own weight. In addition, the design and flexible software make it possible to develop custom fixtures for your application.

Best Choice	Sample Modulus/Pa	Preferred Geometry (for indicated sample size)	Sample Thickness/mm	Free Length /mm	Ideal Heating/Cooling Rate/°C/min
	10 ¹⁰ to 10 ⁶	Tension	<0.02	2	5
X	10 ¹⁰ to 10 ⁵	Tension	0.02 to 1	2 to 10	5
X	10 ¹⁰ to 10 ⁶	Single cantilever	1 to 2	5 to 10	3
X	10 ¹⁰ to 10 ⁶	Single cantilever	2 to 4	10 to 15	2
	10 ¹⁰ to 10 ⁶	Single cantilever	>4	15 to 20	1
X*	10 ¹⁰ to 10 ⁶	Dual cantilever	2 to 4	10 to 15	2
		<i>*for highly orientated samples that are likely to retract above Tg.</i>			
X	10 ¹² to 10 ⁸	Three-point bending	1 to 3	10 to 20	3
	10 ¹¹ to 10 ⁷	Three-point bending	>4	15 to 20	2
X	10 ⁷ to 10 ²	Simple shear	0.5 to 2	5 to 10 (dia)	≤2
	10 ⁷ to 10 ²	Compression (good for irregularly shaped samples and any others that are difficult to mount)	0.5 to 10 (height or thickness)	5 to 10 (dia)	≤2

width Generally sample width is uncritical and 5 mm is recommended (a wider sample may not be held uniformly in the clamps).
A smaller value should be used for stiff sample in tension (1 to 2 mm).

Figure 4. Preferred geometry for indicated sample size.

10. How can I detect a Tg?

The glass transition (Tg) is seen as a large drop (a decade or more) in the storage modulus when viewed on a log scale against a linear temperature scale, shown in Figure 5. A concurrent peak in the tan delta is also seen. The value reported as the Tg varies with industry with the onset of the E’ drop, the peak of the tan delta, and the peak of the E’ curve being the most commonly used.

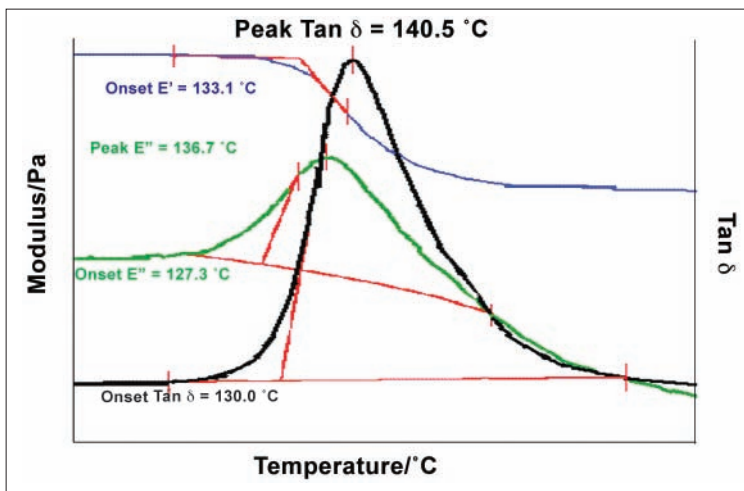


Figure 5. The glass transition (T_g) in the storage modulus and tan delta.

11. How do I know it's really a T_g ?

Running a multi-frequency scan and calculating the activation energy of the transition allows you to decide if the transition is really a T_g . The activation energy for a T_g is roughly 300-400 kJmol⁻¹. In comparison a T_b has an activation energy of about 30-50 kJmol⁻¹ and at the melt or T_m , the frequency dependency collapses.

12. Why does my T_g value sometimes not agree with my DSC value?

That's actually not surprising. The glass transition is really a range of behavior where scientist have agreed to accept a single temperature as the indicator per certain standards. Different industries have used different points from the same data set that can vary as much as 15 °C. DSC, TMA, and DMA measure different processes and therefore, the numbers vary a bit. You can see as much as a 25 degree difference in data from a DSC to DMA data reported as peak of tan delta. See Figure 5 for an example.

13. Can I do TMA in my DMA?

It depends on what you are looking for. Most tests like flexure, penetration, creep or a simple stress-strain run can be done. In the past, most dynamic mechanical analyzers have not been able to generate coefficient of thermal expansion (CTE) data, but the DMA 8000 can run TMA type experiments and obtain excellent CTE values for a wide range of samples run in extension. CTE tells you how your material will expand as a function of temperature. This information is vital for products where dissimilar materials will be heated together (for example motors and circuit boards) as well as curing systems where contraction on curing occurs.

14. Can I use DMA to study curing?

DMA is commonly used to study curing of materials as this process involves a dramatic increase in the modulus values. It is commonly used to get both the point of gelation and the point of vitrification for thermosetting materials. Cures can be studied with temperature ramps and isothermally at a fixed temperature. The DMA 8000 can be configured with optional quartz windows and special fixtures to allow the study of photo-curing systems.



Figure 6. DMA 8000 with special fixture to allow the study of photo-curing systems.

15. Why should I be concerned about frequency scans and multiple frequency runs?

Most materials can see many frequencies in their final product. An example is the rubber used in a windshield wiper which see a range of operating frequencies and temperatures in use. Modulus-frequency plots can tell you how your material will change as frequency changes. For viscous materials, this can give useful information about its flow. It is often advisable to not just look at modulus-frequency at one temperature, but to scan many frequencies as you heat a material. This allows you to see how transitions shift under the influence of frequency. For example, in some polymers a shift from 1 to 100 hertz will move a T_g by 14 degrees, which could cause a material to fail if the high frequency is not considered in its design.

16. What does Time-Temperature Superposition (TTS) tell me?

The Williams-Landel-Ferry model, or WLF, says that under certain conditions, time and temperature can be mathematically interchanged. A TTS, shown in Figure 7, lets you use data collected as frequency scans at a range of temperatures to predict behavior

at frequencies that are not directly measurable. The DMA 8000 has advanced software that makes this a fairly simple process. The data is often converted to time to predict lifetime performance. One should note that TTS calculations rest on some assumptions and is often invalid if these assumptions aren't met. One basic assumption is a single relaxation time and is tested by using a wicket or Cole-Cole plot.

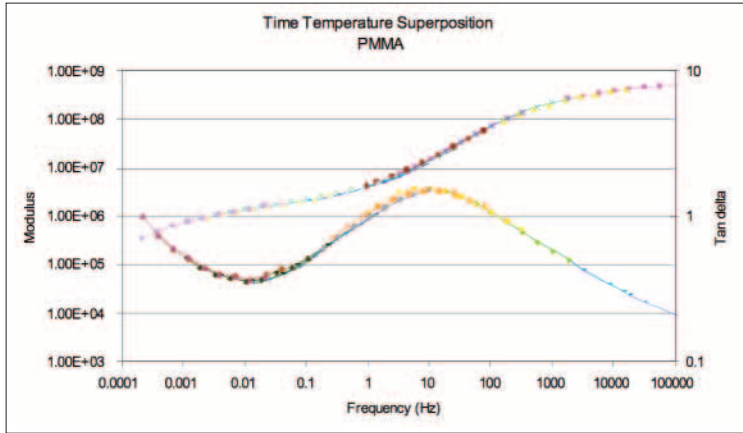


Figure 7. Time Temperature Superposition.

17. How can I tell if a TTS is valid?

You can tell if a TTS is valid by using the DMA 8000's software to generate a Cole-Cole or wicket plot. Plotting E' against either E'' or tan delta should give a nice half circle plot if the assumptions of the William Landel Ferry model are met. If they aren't, then the material is not rheologically simple and a WLF superposition will fail.

18. Why would I want to run my samples immersed in a fluid?

Certain solvents can cause a material to soften while they are exposed to them. Others can react with and harden the material. Both of these effects can cause failure in biomedical devices, coating, paints and gaskets to name only a few. In addition, the effect of a stress and a solvent often is different than just soaking a material in a solvent and then testing it in air. The DMA 8000's Fluid Bath allows collecting data of sample immersed in solutions under a variety of conditions.



Figure 8. DMA 8000 with Fluid Bath.

19. Can I use DMA to see if humidity affects my sample?

Humidity is known to have tremendous effects on the properties of materials from polymers to papers to natural products. The DMA 8000 has the option of being configured with an integrated humidity generator that allows precise control of the humidity in the furnace. This permits accurate and precise studies on how humidity affects the properties of your materials.

20. Is UV curing an important application for my DMA if I have a photo-calorimeter?

Yes, a photo-calorimeter only looks at the energy of photo-curing. Photo-curing in the DMA lets you see how the physical properties change and when the modulus of the curing material has reached acceptable limits in terms of strength and stiffness. This information is important for cost effective design of your cure cycle. Using a DMA with UV also allows you to investigate the degradation of materials and so evaluate additive packages, formulations, etc.

Common Symbols in the DMA Literature

A	Shift factor	How much a curve was moved to make it align with the reference curve.
D	Molecular weight distribution	The ratio of M_n and M_w (see molecular weight) types of molecular weights related to the horizontal movement of crossover point of frequency scan.
δ	delta	Phase angle or phase lag. See also $\tan \delta$.
E	Modulus	Modulus measured in flexure, tension or compression geometry.
E'	Storage modulus	A measure of the elastic response of a material but not the same as Young's modulus. Also called the in-phase component.
E''	Loss modulus	A measure of the viscous response of a material. Also called the imaginary modulus or out of phase component.
E*	Complex modulus	The sum of the in and out of phase components.
Ea	Activation Energy	Energy needed to cause a transition or reaction.
ϵ	Epsilon	Strain measured in flexure, tensile or compression geometry. Also used for dielectric properties in DETA as modulus is above.
G	Shear modulus	Modulus measured in shear geometry.
γ	Gamma	Strain measured in shear geometry.
J	Compliance	The ratio of sample strain to sample stress in the linear region of Creep Ramp.
Λ	Lambda	Modulus measured in torsion pendulum geometry, similar to E.
M	Molecular weight	The molecular mass or chain length of a material, important in polymers for determining the materials properties like the T_g . After monomer type, it is the single most important property of polymers. These are normally reported as number (M_n) and weight (M_w) averages. Related to the vertical movement of crossover point of frequency scan.
η	eta	Viscosity.

σ	sigma	Stress applied to a sample in flexure, tension, compression or shear.
T_α	Alpha transition	See T_g .
T_β	Beta Transition	Onset decrease in storage modulus during heating, accompanied by peak in tan delta, during a temperature scan. Found above the T_g , this is associated with localized backbone or side chain motions.
T_g	Glass Transition	The temperature indicating the relaxation in a polymer where a material changes from a glass to a rubber.
Tan δ	tan delta	Damping – the tangent of the phase angle and the ratio of E''/E' .
ν	Poisson's ratio	A ratio of the change in sample depth and width as the sample length is changed. Typically 0.5 for polymers.
ν_f	free volume	The space inside a polymer between molecules.
ω	radians	Frequency when reported in radians per second.

Regions of Viscoelastic Behavior

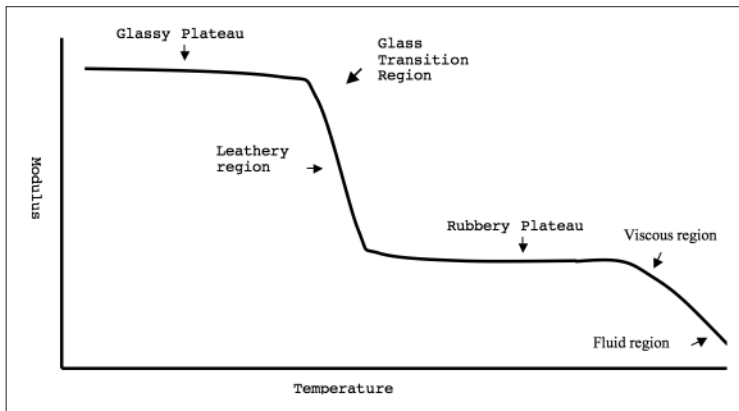


Figure 9. Regions of viscoelastic behavior.

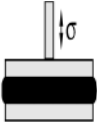
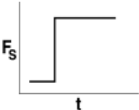
Glassy plateau	Sample is hard, springy or rock like. Bending and stretching of bonds is occurring. Tan delta is below 0.01. It is below the T_g .
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Glass transition region	Sample becomes less hard as storage modulus decreases and tan delta peaks. Tan delta peak height is typically between 0.1 and 1.2. The onset can be taken as the T _g .
Leathery region	Sample is tough but flexible. Side groups and cooperative segmental motion of the backbone occurs. Tan delta is below 1.0. This is slightly above the T _g .
Rubbery plateau	Sample is springy, putty like. Main backbone chain exhibits gradual slippage. Tan delta is near 1.0. This is above the T _g .
Viscous region	Sample is flowing, fluid as temperature increases. Large scale main chain mobility occurs. Tan delta is above 1.0. This is well above the T _g . Power law behavior is seen.
Fluid region	Sample is flowing, water like. Free chain movement and interchain slipping occurs. Tan delta is much higher than 1.0. This is above the T _m in crystalline materials. Often treated as part of viscous region.

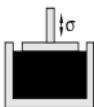
DMA Glossary

Activation Energy	Energy needed to cause a motion. Frequency dependent activation energy can be used to identify transition types.
Additives	Compare isothermal storage modulus.
Alloys	Mechanically blended combinations of polymers.
Alpha transition	The next transition in temperature below the melt. Normally the T _g .
Amorphous	Chemical structure that allows random folding and intertwining, not organized.
Amorphous content	Estimated by comparing the area of loss modulus peak at T _g .
Amorphous phase	Random portion chemical structure. Random orientation of the polymer backbone. Non-crystalline glass.
Amplitude	Height of sinusoidal displacement.



Anisotropy	Directionality of material properties within a sample.
Annealing temperature	The onset of the step decrease in the storage modulus, accompanied by a step increase in tan delta, during Temperature Scan heating. Just below the Tg. The temperature a polymer is heated to so that it relaxes from trapped stresses.
Backbone	The primary structure of a polymer. Typically a continuous chain of carbon atoms.
Beta transition	Onset decrease in storage modulus during heating, accompanied by peak in tan delta, during Temperature Scan heating.
Blending	Mixing of two polymers. A blend is a polymer mixture and can have one or more Tg depending on how it was mixed.
Branching	Estimated by comparing the storage modulus terminal zone in a frequency scan.
Cole-Cole Plot	Plot of E' versus E'' (or $\tan \delta$) used to check the validity of a TTS study. Also called a wicket plot.
Compatibility of polymer blends	How well two or more different polymers combine with each other.
Complex modulus	Bulk sample behavior. An indicator of viscoelasticity.
Compliance	The ratio of sample strain to sample stress in the linear region of Creep Ramp.
Compression	 <p>Disks or rectangles are deformed using cup and plate, plate and tray, sintered parallel plates, cone and plate measuring systems. This gives compressive modulus, an important value used in Finite Element Analysis (FEA).</p>
Copolymer	Compare isothermal modulus or slope of storage modulus decrease at Tg.
Creep	 <p>Observe sample strain as sample stress is increased and temperature is held constant. Gives time dependent behavior as a function of pressure (stress) or temperature.</p>

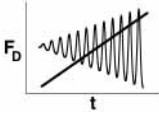
Cross link	Chemical bond between molecules.
Crossover point	Normally the crossover of storage modulus and complex viscosity on axes of the same scale on a log-log-log plot of a frequency scan. Also the crossover of E' and E'' (when $\tan \delta = 1$) in a cure study.
Crystalline	Chemical structure that is highly organized, compacted in very low energy states.
Crystalline phase	Highly ordered chemical structure. Closely packed and ordered orientation of a polymer backbone. This is typically the lowest energy state of a polymer.
CTE	Coefficient of thermal expansion. See dilatometry, LCTE, and TMA below.
Cure of thermosetts	Chemical connection between polymer backbones.
Cure rate (kinetics)	Compare slope of storage modulus increase during isothermal cure.
Damping	Dissipation (loss) of mechanical energy. Modeled by a dashpot.
Deborah number	The time of the experiment divided by the time of the property measured.
Degree of cure	How cured a material is. Normally estimated from either modulus values or the T_g in DMA.
Degree of polymerization	A measure of how far a polymerization reaction has gone. See degree of cure.
Dilatometer	Measures volumetric expansion by translating a three dimensional expansion into a deflection. This gives bulk (volumetric) expansion of irregularly shaped samples or the bulk modulus, which can be used with other values to calculate Poisson's ratio.
DMA	Dynamic Mechanical Analyzer. Applies a sinusoidal force and measures sample response at a given temperature.
DMA fingerprint	Frequency scan at a temperature above the T_g and below the T_m .



DMA modulus test

A quick 30 second test where you mount the sample and read the real time display for storage modulus. Used for QC.

Dynamic Scan



Observe sample strain as sample stress is increased while temperature and frequency are held constant. Slope gives DMA modulus which includes effect of damping.

Elastic



The ability of a material to return to its original shape. Modeled by a spring.

Elastomers

Materials that exhibit elastic properties, may be thermoplastic or thermosett.

End use properties

The properties (tests) used for deciding the fitness of a polymer for a task.

Entanglement

Physical (steric) interference of polymer movement.

Equilibrium modulus

Size of recovery portion of creep-recovery analysis or slope of creep strain back extrapolated to intercept the start of the creep cycle.

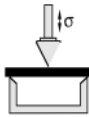
Equilibrium viscosity

Size of non-recovery portion of creep-recovery analysis or slope of creep strain.

Extensiometer

Large pull testing analyzers.

Flexural



Rods or cylinders are deformed using 3 point bending, 4 point bending, single and dual cantilever measuring systems. This is the easiest geometry to use.

Fluid

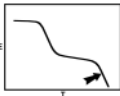


Flowing, water like.

Fluid bath

An alternative furnace that allows the sample to be immersed in a solution.

Fluid region



Sample is flowing, water like. Free chain movement and interchain slipping occurs. Tan delta is much higher than 1.0. This is above the T_m in crystalline materials.

Force

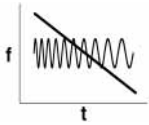


Application of mechanical energy.

Force motor

Applies a force to the sample that causes a sample deflection.

Frequency scan



Observes sample strain while increasing frequency of oscillation and holding stress and temperature constant. Used for fingerprint type identification of polymers and identifying the resonant and harmonic frequencies. Also used for calculating TTS and activation energy.

Friction

The force required to overcome surface molecular interaction between two materials.

Functional group

A chemical compound attached to a polymer backbone.

Furnace

Controls the sample temperature.

Gel point

Onset of the increase of storage modulus during Time Scan isothermal cure. The point where frequency dependence in E' disappears. The crossover point of E' and E'' in a cure.

Glass transition (T_g)

The temperature range where a material softens. The onset decrease in the storage modulus, accompanied by a peak in $\tan \delta$, during Temperature Scan heating. "Melting of the amorphous phase".

Glassy



Hard, springy, or rock like.

Heterogeneity

Inconsistent material content within a sample.

Homogeneity

Consistency of material content within a sample. A homogenous material is same in all directions.

Hooke's law $F = kx$

Describes the elastic behavior of springs.

Humidity generator

An alternative furnace that allows the sample to be studied in a controlled humidity environment.

Hysteresis

Energy stored after each cyclic deformation causes the difference between loadings.

Impact modifiers

Compare isothermal storage modulus.

Isotropy

Consistency of material properties within a sample.



Leathery



Tough, but flexible.

Linear coefficient of thermal expansion (LCTE)

Slope of sample height (TMA probe position) from beginning of run to just below T_g , during Temperature Scan heating.

Linear Variable Differential Transformer (LVDT)	An electronic ruler that measures the sample's response to the applied force.
Log scale	Used to display changes that occur over large ranges. Modulus is normally displayed on a log scale.
Mechanical properties	Physical characteristics of a sample. What a polymer does when physically disturbed or tested.
Melt viscosity	Compare complex viscosity of frequency scan at melt temperature.
Modifiers	Compare isothermal storage modulus.
Molecular weight	The molecular mass or chain length of a material, important in polymers for determining the materials properties like the Tg. After monomer type, it is the single most important property of polymers. These are normally reported as number (Mn) and weight (Mw) averages. Related to the vertical movement of crossover point of frequency scan.
Molecular weight distribution	The ratio of Mn and Mw (see molecular weight) types of molecular weights. Related to the horizontal movement of crossover point of frequency scan.
Modulus $E = \frac{\sigma}{\epsilon}$	Ratio of stress to strain.
Newton's law 	Describes the behavior of flowing materials.
Omega ω	Frequency in radians per second.
Orientation	Non-crystalline organization where polymer backbones become closely packed in a direction. Also seen with Polymer Liquid Crystal and nano-material fillers where the material is lying in a specific direction.
Period	The time it takes to complete one event.
Permanent set	Irrecoverable flow that exists after a material has been deformed.
Phase	Part of or "section of" a polymer. For example, many types of polyurethane have rubbery and crystalline phases.
Phase lag (δ) 	Delay between applied force and material response. Also called the phase angle. The delta (δ) in $\tan \delta$.

Plasticity	Exhibited by polymers that are stressed beyond the yield point. Deformation.
Plots of $\log f$ vs. $1/T \times 10^3$ ($^{\circ}\text{C}^{-1}$)	Used to show the frequency shift of phase transitions as the sample is cooled.
Poisson's ratio	A ratio of the change in sample depth and width as the sample length is changed. Typically 0.5 for polymers.
Polymer transitions	Changes in the physical appearance or behavior as a sample is heated.
Prediction of long term behavior	Often done by comparing recovery cycles of Recovery Analysis from the critical pressure (stress) and temperature, experiments with many creep-recovery or DMA cycles, and TTS.
Proportional limit	The greatest stress a material can withstand without permanently deforming.
Rate	How fast something happens, i.e. how fast you heat in degrees per minute.
Recovery	Observe sample strain as stress is increased and temperature is held constant. Gives time dependent relaxation behavior as a function of pressure (stress) or temperature.
Recovery time	The time when the sample stops changing after a recovery analysis.
Relaxation time	The time needed for molecules to relax after applying a stress to a material.
Resonance	The amplification of natural harmonics within a sample. A non-quantitative mechanical technique.
Resonant	A material that resonates; the frequency at which resonance occurs.
Resonant frequency	Amplification of natural harmonic oscillation.
Rheology	The study of flow and deformation of material.
Roller Kinetics	Method of using isothermal or temperature scanning cure studies to calculate the activation energy for curing.
Rubbery	Springy, putty-like.

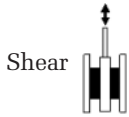


Rubbery plateau

Sample is springy, putty like. Main backbone chain exhibits gradual slippage. Tan delta is near 1.0. This is above the T_g.

Semi-crystalline polymers

Polymers that have some of their backbones organized into crystals and some of them random.



Sliding of one part of a material past another. A fixture that holds two samples so they exhibit shear behavior.

Side chain

A branch of the main polymer backbone.

Sinusoidal

A cyclic event, relating to the sine.

Solid-solid phase transition

Small transitions caused by changes in the solid state. Polymorphism in pharmaceutical or eutectic transitions in liquid crystals.

Spring constant

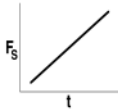


Description of force exerted by a material as it is deformed.

Static modulus

Slope of the stress vs. strain curve in the linear region of Creep Ramp.

Static Scan
(Creep Ramp)



Observed sample strain as sample stress is increased while temperature is held constant. Gives Young's modulus.

Steric effects

Effect of the physical position of atoms in a molecule relative to each other.

Storage modulus (E')

An indicator of elasticity. The in phase component of a DMA signal.

Strain

$$\epsilon = \frac{\Delta L}{L}$$

Ratio between the change in length and the original length of an extension sample.

Strain %

$$\epsilon\% = \frac{\epsilon}{100}$$

Strain multiplied by 100 to report conventional units.

Stress



The load applied to sample, adjusted for the geometry of the specimen.

Structure

Physical position of molecules relative to each other.

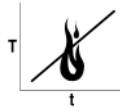
Structure-property relationships

How specific parts of the polymer contribute to specific physical behavior.

Tangent delta

The tangent of the phase angle. The ratio of loss to elasticity. An indicator of the viscoelasticity of a sample. Sometimes called damping.

Temperature Scan



Observing the sample strain while increasing sample temperature and holding frequency and stress constant. Used for characterizing thermally dependent behavior, like the Tg.

Tensile



Films or fibers are deformed using extension measuring systems. This is the best for films and fibers. TMA is often run in this geometry.

Terminal zone

Onset point in a frequency scan where storage modulus dramatically decreases.

Terpolymer

A copolymer with three repeating units.

Thermal expansion

The change in sample dimensions as it is subject to a controlled temperature program.

Thermal history

Previous thermal conditioning applied to the sample.

Thermoplastics

Materials that reversibly change when heated. i.e. ice-water-ice.

Thermosetts

Materials irreversibly change when heated. i.e. egg-hard boiled egg.

Thermosetting (crosslinked) polymers

Polymers that have their backbone chemically bonded to each other. This process is monitored in the DMA to develop cure cycles.

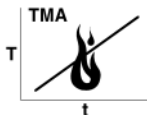
Time or isothermal scan

Observing the sample strain while holding temperature, frequency and stress constant. Used for curing, aging, and quick modulus reports. Often used with fluid baths, humidity generators, or UV accessories.


Time-dependent behavior

Sample behavior over time. An affect of the viscoelastic nature of polymers.

TMA



Thermomechanical Analyzer. Applying a weak static force and measuring sample response normally while changing temperature One observes sample height as temperature is increased. Gives coefficient of thermal expansion and identifies thermal transitions, like Tg.

Tougheners	Additives used to make a polymer less brittle. Often seen in the DMA as step changes in E' at low temperature.
Toughness	The ability of a material to absorb mechanical energy without fracturing or deforming.
Ultimate strength	The greatest stress a material can withstand without failing, breaking apart.
UV or Optical Furnace	A furnace that allows both visual monitoring of the sample as well as its irradiation to study photo-curing.
Viscoelasticity	The ability of a material to exhibit both elastic and viscous behavior.
Viscosity	The ability of a material to flow and deform.
Viscous 	Flowing, gooey.
Vitrification	Point where curing systems reach a viscosity so high as to limit further curing. Normally taken as the onset plateau of storage modulus during Time Scan isothermal cure.
Yield point	The stress that causes a material to permanently deform.
Yield strength	Stress that will cause a permanent physical change in the shape of a sample.
Young's modulus	The ratio of strain when an increasing stress is applied to a sample.

What do the changes in the data mean?

Technique	Data shows	This tells us
Thermomechanical Analysis	Changes in slope Slope of curve with temperature Change in volume (dilatometry)	Transition temperature Thermal expansivity or CTE Shrinkage on curing, volumetric expansion
Static Stress-Strain	Slope Yield Point, Yield Strength Proportional limit Ultimate Strength Elongation at break Area under curve	Young's Modulus Strength before distortion Load capacity End of linear region (max. F_T) Strength at breaking point Ductility Toughness

Dynamic Stress-Strain	Dynamic Proportional limit Storage, Loss Modulus Complex viscosity Tan δ Ultimate strength	End of linear region (max. F_D) Stiffness as function of load Flow under dynamic load Damping Strength at break by tugging
Creep-Recovery	Equilibrium Compliance, Modulus, Equilibrium Viscosity Creep Compliance Creep Rupture Relaxation spectra	Long-term behavior Extensional Viscosity Effect of load Strength Molecular Modeling
Stress Relaxation	Compliance and Modulus Retardation spectra Force as a function of temperature	Long-term behavior, MW, crosslinks, and entanglements Molecular modeling Shrinkage/expansion force
Dynamic Temperature/ Time Scan	Storage and Loss Modulus Complex Viscosity Tan δ Temperature of transitions as drops or peaks Modulus of rubbery plateau Crossover of E' and E'' on curing Shape of viscosity curve on curing	Change in stiffness. Mapping modulus Change in flow Damping, energy dissipation T_M T_g T_α T_β T_γ T_δ Molecular weight between crosslinks, entanglements Gel point Minimum viscosity, E_{act} Vitrification point
Dynamic Frequency Scan	Complex viscosity, loss modulus Storage modulus E'/E'' or n^* crossover Plateau regions Mastercurve	Flow as function of frequency Elasticity or stiffness as function of frequency Relative MW and D MW estimation MWD, long-term behavior, wide range behavior, molecular modeling

Further Readings

***Dynamic Mechanical Analysis: A Practical Introduction*, 2nd Edition, Kevin Menard, CRC Press, 2008.**

The basic book dealing with DMA as a tool in the modern laboratory

***Thermal Characterization of Polymeric Materials – 2nd Ed.*, Edith Turi, Editor, Academic Press, 1997.**

Complete text on Thermal Analysis. An excellent practical and theoretical reference for the thermal analyst.

Anelastic and Dielectric Effects in Polymer Solids, N. G. McCrum, B. E. Read and G. Williams, Dover, New York, 1967.

Review of basic polymer types and their behavior as measured by DMA and dielectric analysis. This text is an excellent tool for the interpretation of DMA results and still a useful reference.

Handbook of Plastics Testing Technology, Vishu Shah, Wiley, New York, 1984.

Overview of standard test methods used to characterize polymers

Introduction to Polymer Viscoelasticity, Montgomery Shaw and, William J. MacKnight, Wiley, New York, 2005.

A good first book before reading Ferry, it offers a good general introduction to the basic theory and equations of DMA. This text is an excellent resource for very simple descriptions of DMA theory

Mechanical Properties of Polymers and Composites 2nd Ed., Lawrence E. Nielsen and Robert Landel, Marcel Dekker, New York, 1994.

General text for understanding the mechanical properties of polymeric materials.

Handbook of Polymer Analysis, Hubert Lobo and Jose Bonilla, Editors, Dekker, 2003.

A survey of modern techniques in testing plastics and polymers.

Performance of Plastics, Witold Brostow, Editor, Hanser, 2001.

Overview of issues in how plastics perform with chapters on thermal analysis and rheology.

Rheology Principles, Christopher Mascosko, VCH, New York, 1994.

Excellent introduction to rheology. Very mathematical.

Viscoelastic Properties of Polymers, John D. Ferry, Wiley, New York, 1980.

Definitive text on rheology and DMA for the experienced. Provides in depth information about experimentally observed DMA and dielectric results.

Principles and Applications of Thermal Analysis, Paul Gabbott, Editor, Blackwell Publishing, Oxford, UK, 2008. *Overview of current applications in thermal analysis with a chapter focused on DMA.*

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Spectrum 100 Optica - Demonstrating the accuracy of transmittance measurements for high refractive index materials

Background

The Spectrum™ 100 Optica has been developed to provide accurate transmittance measurements on high refractive index materials.

There are several well known sources of error in standard FT-IR instruments that lead to spectral artifacts and inaccurate transmittance values for such samples¹. These have been addressed in the Spectrum 100 Optica. Interreflections involving the source, interferometer, sample and detector have all been eliminated. The use of delta-sigma analog-to-digital conversion avoids the need for gain switching and ensures excellent linearity². Although the linearity of DTGS detectors is widely thought to be well established, the presence of a sample changes the detector temperature, with a consequent change in responsivity. This was identified by NIST as a major source of error in FT-IR measurements of transmittance¹. For that reason, lithium tantalate is used as the standard detector in the Spectrum 100 Optica.

A further issue is the influence of the sample on the beam geometry, defocusing or displacing the beam at the detector. The Spectrum 100 Optica has two variable apertures in the beam path, allowing independent control of the size and the convergence of the beam at the sample. This reduces the sensitivity of the system to different sample thicknesses and to wedging.

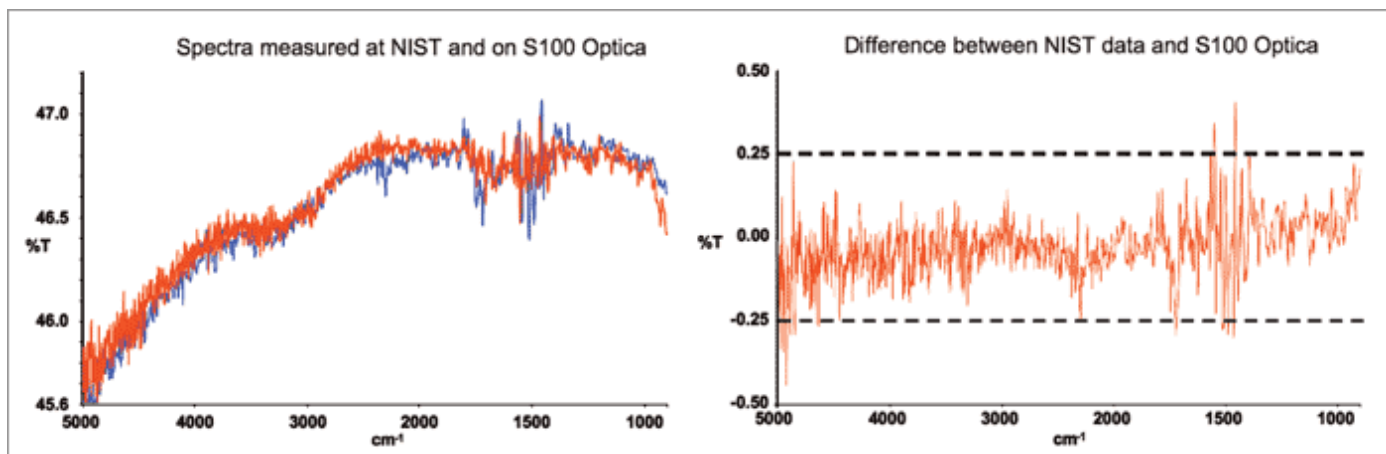
Testing transmittance accuracy

The time-honored approaches used to test the ordinate accuracy of dispersive spectrometers, rotating sector discs and doubleapertures, are not practicable for FT-IR spectrometers³. There are two alternative methods available. One is to rely on transmittance calculated from refractive index values that are often known to very high accuracy. The other is to use traceable standards from suppliers such as NIST or NPL, but such standards are not currently available for the mid-IR region.

Transmittance values derived from refractive index are subject to the uncertainty that the surface reflection may not be adequately described by the bulk refractive index. However, we have adopted this approach using germanium windows and have confirmed the transmittance values by having the samples measured at NIST.

Germanium

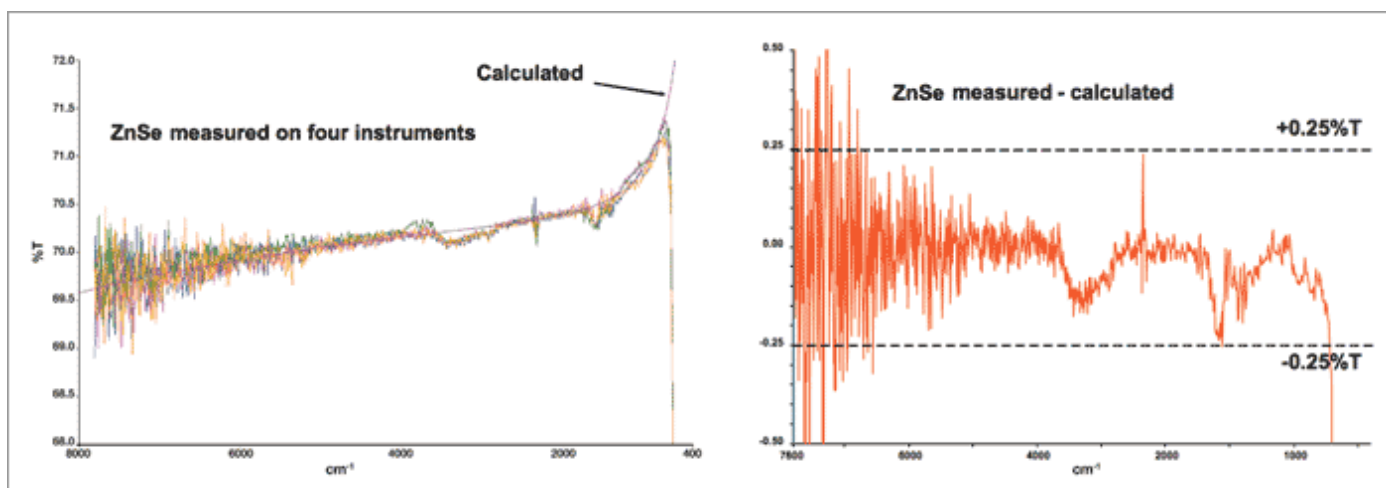
For 1 mm thick germanium measured on several instruments, the results agree well with refractive index calculations except between 4000 and 900 cm^{-1} (2.5 and 11 microns), but between 5000 and 4000 cm^{-1} (2 and 2.5 microns) the measured values are consistently lower than calculated by more than 0.1 %T. Measurements on the same samples at NIST are essentially identical with those on Spectrum 100 Optica, the differences being less than 0.1 %T over the range 5000 to 900 cm^{-1} (2 to 11 microns.) See Figure 1.



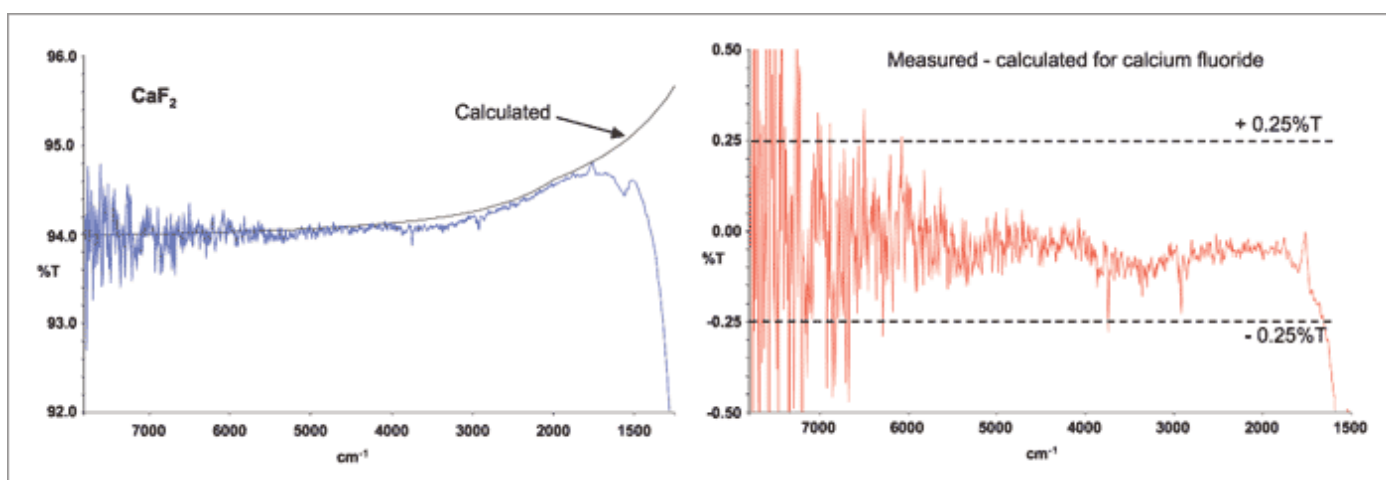
Figures 1a and 1b. Germanium measured on Spectrum 100 Optica and at NIST.

Other materials

To test performance at other transmittance values we have measured zinc selenide (70%T) and calcium fluoride (94 %T) and compared the results with calculations from the refractive indices. In both cases, the agreement between measured and calculated transmittance is within $\pm 0.1\%$ T in the regions where absorption is negligible. See Figures 2 and 3.



Figures 2a and 2b. Zinc selenide measured and calculated.



Figures 3a and 3b. Calcium fluoride measured and calculated.

Instrument to instrument variation

In the absence of standard sample with known transmittance values, it has been a common practice to compare values for the same sample on different instruments. When we have tried

this with Spectrum 100 Optica, the agreement is typically to better than $\pm 0.1\%T$ outside regions of atmospheric absorption. The example in Figure 4 is for germanium on three instruments.

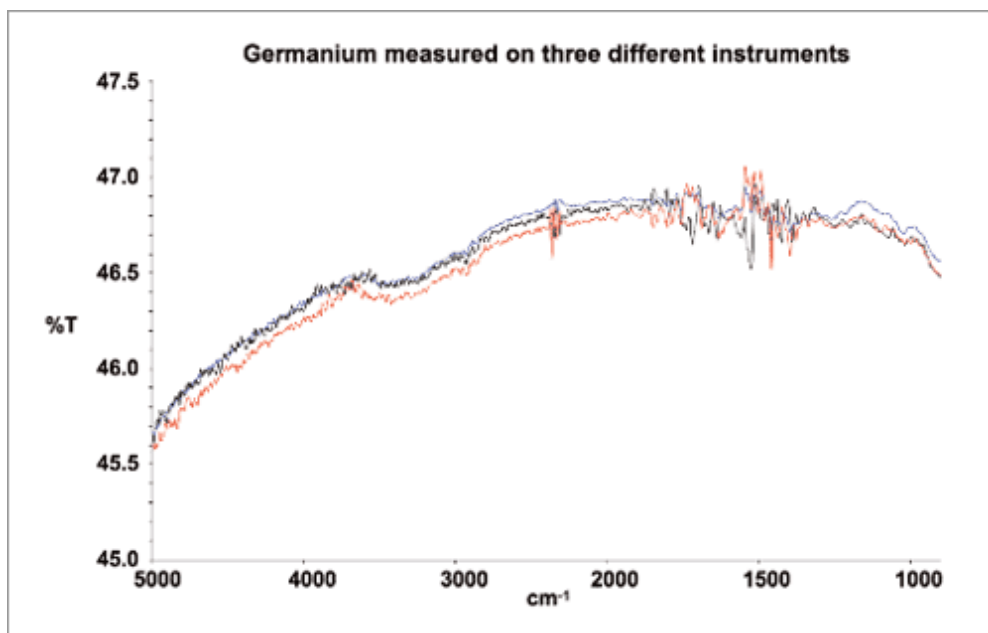


Figure 4. Germanium measured on three different Spectrum 100 Optica instruments.

Effects of sample thickness

A known problem is that optically thick samples change the focusing of the beam at the detector, with the potential effect of reducing the apparent transmittance. In the Spectrum 100 Optica, the magnitude of this effect is controlled by using the variable apertures to limit the convergence of the beam at the sample. This has been tested using germanium windows varying in thickness from 1 to 4 mm. For these thicknesses, the difference in transmittance is less than $0.2\%T$ above 1000 cm^{-1} (10 microns), where absorption is negligible. See Figure 5.

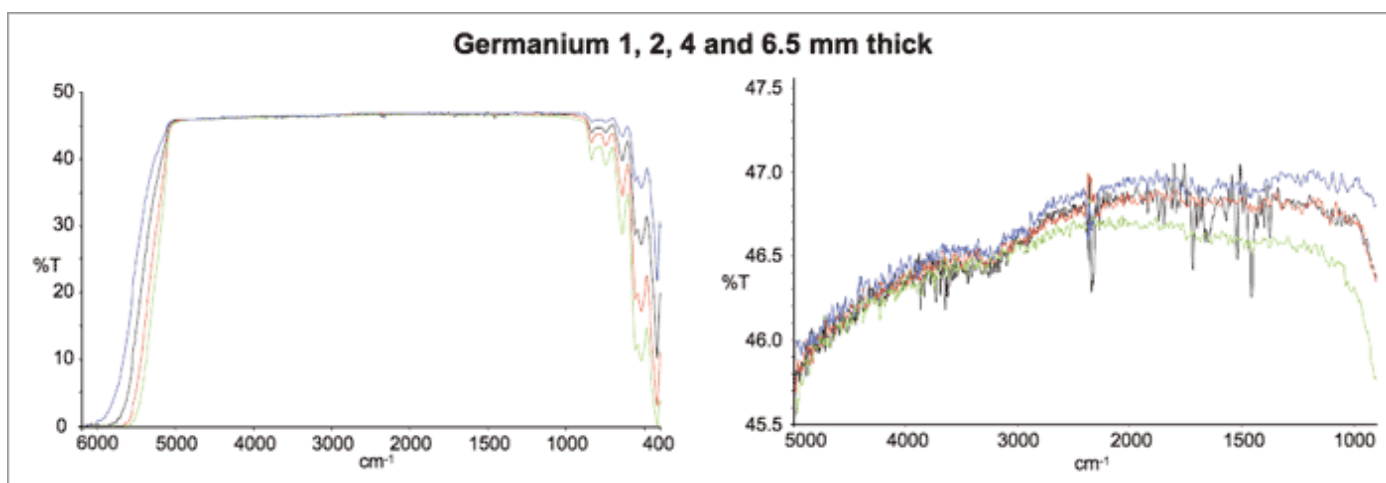


Figure 5. Transmittance of germanium windows of different thickness.

Wedged samples

Optically thick samples where the faces are not parallel deflect the beam and can therefore give incorrect transmittance values. In Spectrum 100 Optica, we have addressed this problem by a combination of conservative design and careful optical alignment. Each spectrometer is factory tested using a wedged germanium sample in different orientations to ensure correct optical alignment. Typical results for a sample with an 0.1 degree wedge are seen in Figure 6.

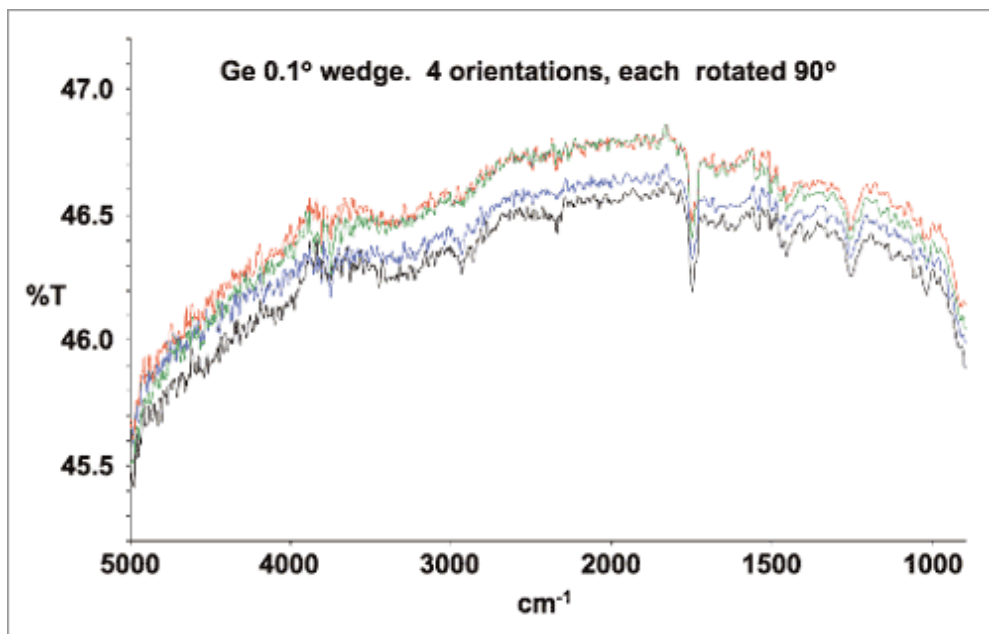


Figure 6. Measured transmission of wedged sample at different orientations.

Measurement of blocking regions

FT-IR spectrometers, unlike those based on monochromators, do not suffer from 'stray light'. However because all wavelengths are measured together, the dynamic range of the interferogram is a potential problem. Any non-linearities in the electronics or digital processing lead to artifacts at multiples of the true wavenumbers. The Spectrum100 Optica has a lower level of such artifacts than has been demonstrated on previous systems⁴. The transmission of the narrow band filter shown below is about 40% at 1596 cm⁻¹. With the Spectrum GX Optica there was an artifact at about 0.01%T at 3192 cm⁻¹, but with Spectrum 100 Optica any artifact is less than 0.005 %T. See Figure 7.

The freedom from artifacts means that measurements on blocking filters are limited only by resolution and noise level. A recommended test for FT-IR spectrometers is to measure totally absorbing bands in a film of polyethylene terephthalate⁵. At 4 cm⁻¹ resolution, the strong bands can be seen to have transmittance well below 0.01 %T, 4 absorbance. This can be contrasted with dispersive IR spectrometers such as the PerkinElmer 983 where stray light is typically around 0.1 %T. See Figure 8.

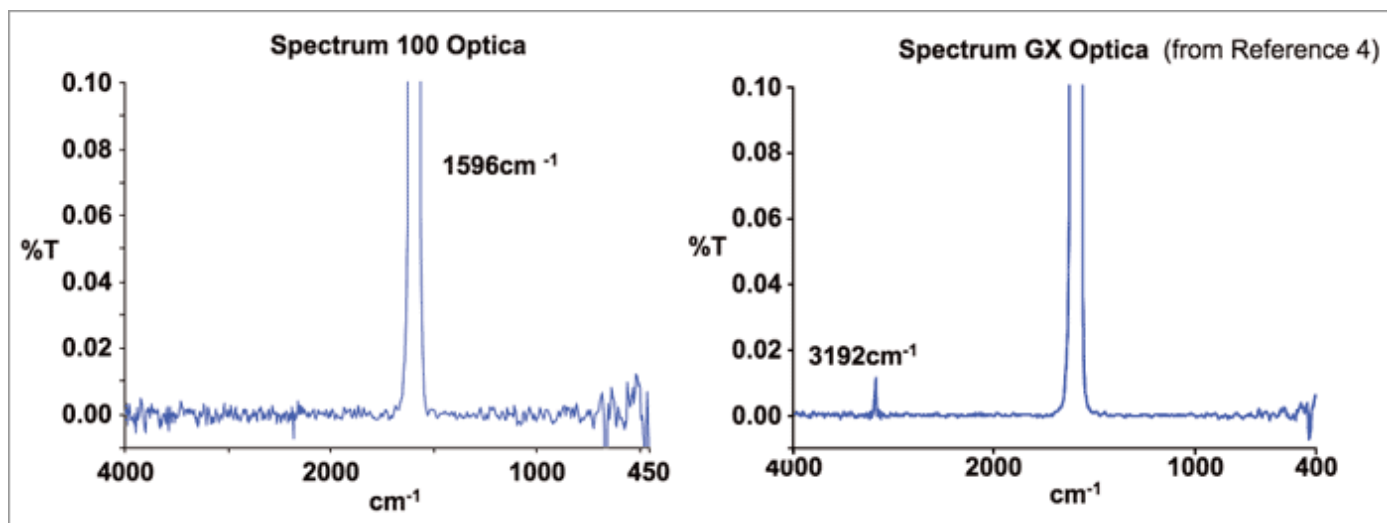


Figure 7. Measurement of a band pass filter with Spectrum GX Optica⁴ and with Spectrum 100 Optica.

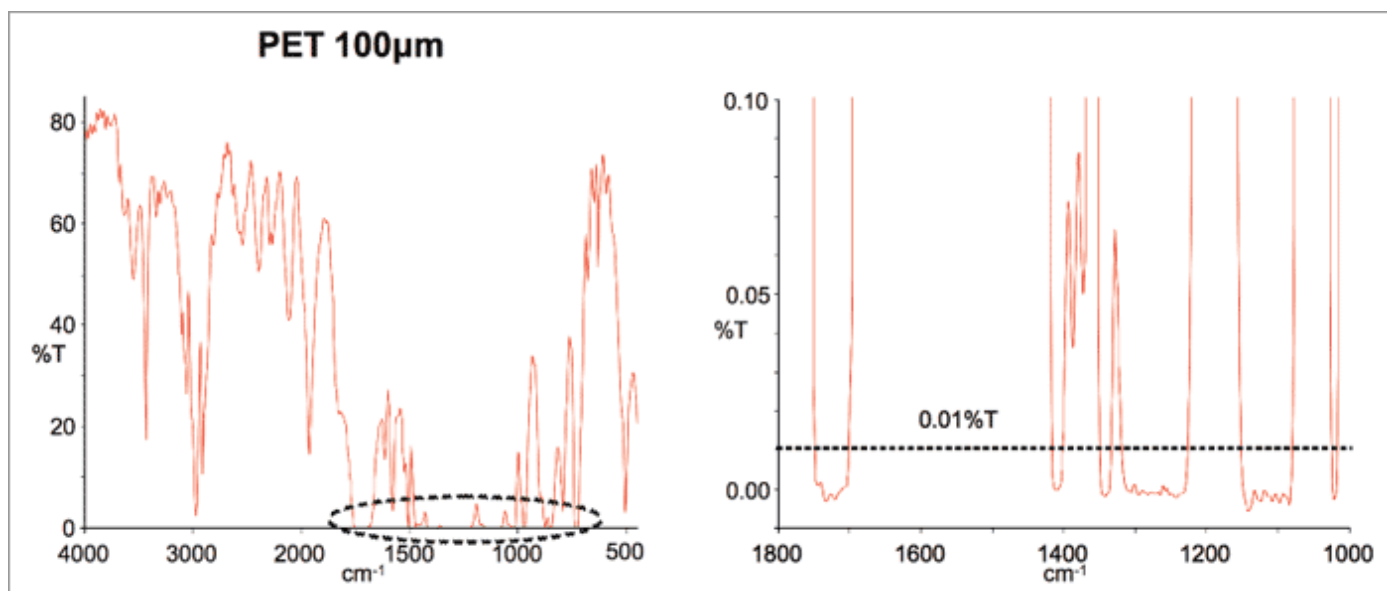


Figure 8. Totally absorbing bands of PET.

Spectral range

The accuracy specification is quoted for a range up to 5000 cm^{-1} ($2.0\text{ }\mu\text{m}$.) Spectra can be measured to 7800 cm^{-1} but there is a significant increase in noise at shorter wavelengths because of the low source output. The range extends well outside the traditional mid-IR region and overlaps with the PerkinElmer® LAMBDA™ 1050 UV/Vis/NIR spectrophotometer. This instrument is the established standard for optical measurements and so can be used to verify the performance of the Spectrum 100 Optica in the NIR. As an example of the agreement between the two systems, the spectra of germanium in Figure 9 agree to within 0.1 %T.

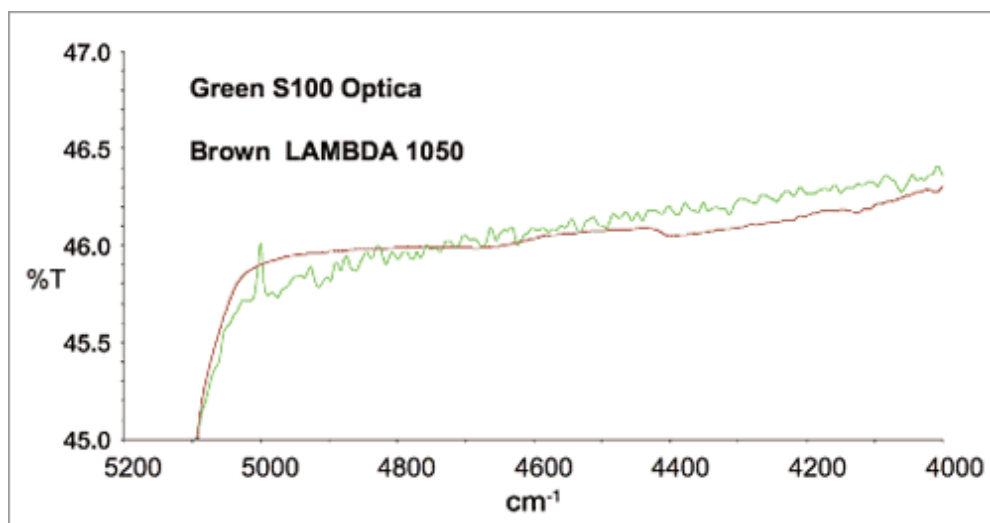


Figure 9. Comparison of Spectrum 100 Optica and LAMBDA 1050 measurements of the same sample.

Summary

In the development of the Spectrum 100 Optica, PerkinElmer has addressed the well known sources of error in the measurement of high refractive index materials with standard FT-IR instruments. In addition, a series of tests have proved that the highest levels of transmission accuracy are achievable with the Spectrum 100 Optica.

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2	J.W. Brault, Applied Optics 35 (16), 1996.
3	F.J.J. Clarke, Analytica Chimica Acta 380, 127-141 (1999).
4	R. Hunneman, R. Sherwood, C. Deeley and R. Spragg, Proceedings of the 11th International Conference on Fourier Transform Spectroscopy, 435-438, 1998.
5	P.R. Griffiths and J.A. de Haseth, Fourier Transform Infrared Spectroscopy (2nd edition), Wiley 2007.



Spotlight FT-IR Imaging System Helps Forensic Lab Win Triple Murder Conviction

Most criminals do not realize that a microscopic speck of paint could put them behind bars. For example, analyses using infrared microspectroscopy performed by the Michigan State Police Forensic Science Division (MSPFSD), Lansing Laboratory, helped to win a triple murder

conviction by matching paint from one victim's clothing to the murderer's car. MSPFSD forensic scientists found a microscopic paint chip on the hit-and-run victim's clothing, then associated each of its five layers to a chip taken from the van driven by the perpetrator. This evidence played a significant role in the killer's conviction for the third murder that he committed that day, a case that was otherwise puzzling because there was no known connection between killer and victim.

A 35-year-old Michigan resident had just stabbed his ex-girlfriend and her friend and was driving away from the murder scene at high speed in his van. According to witnesses, he then struck a jogger less than an hour later as the jogger crossed the street. The jogger was not injured and moved onto the sidewalk to dust himself off as the assailant sped away. A few seconds later, the assailant's van emerged again after driving around the block, jumped up onto the sidewalk and struck the jogger again, this time killing him.

Forensic evidence crucial to murder case

Prosecutors alleged that the assailant committed the first two murders because he was jealous that his ex-girlfriend was dating the other victim but had no idea of the motive in the jogger's death. The assailant pleaded no contest to the first two murders but said that he had no recollection of killing the jogger and pleaded innocent. Thus, forensic evidence was critical to the case against the assailant in the third murder.

The case was assigned to Christopher Bommarito, Forensic Scientist at the MSPFSD. The MSPFSD has approximately 150 scientists and their customers are the Michigan State Police as well as local and county police and fire departments throughout the state. Bommarito has extensive experience in the analysis of trace evidence including paint, glass, filaments, fibers, footwear and explosive residue. He has testified approximately 200 times in federal and state courts in six different states in relation to these analyses. He has also assisted and advised various federal and state authorities on clandestine drug laboratory investigations and other enforcement activities (Figure 1).



Figure 1. Fiber under microscope.

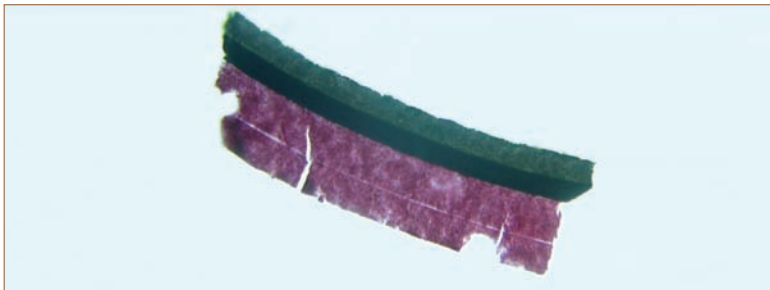


Figure 2. Cross-section of paint under microscope.

Finding a sample on the victim's clothing

Bommarito began his investigation by carefully examining the victim's clothing under a microscope, looking for a tiny paint chip. He was relying upon Locard's Exchange Principle, developed by Dr. Edmond Locard, which is a cornerstone of the forensic sciences. This principle states that when any person comes into contact with an object or another person, a cross-transfer of physical evidence occurs. Bommarito said that in a case where a vehicle strikes a person, he is nearly always able to retrieve paint evidence from the victim's clothing, as long as the victim came into contact with the painted portion of the vehicle (Figure 2).

Fourier Transform Infrared Spectroscopy (FT-IR) is by far the most popular instrument for analyzing paint evidence today. An FT-IR instrument measures the absorption of infrared (IR) energy, over a range of wavelengths, as different bonds in the molecule vibrate and move in characteristic fashions. This produces a "fingerprint" spectrum of a sample unique to that material. Coupling an FT-IR instrument to an IR microscope allows the IR light to be focused onto a small area allowing these high quality spectral "fingerprints" to be obtained from small samples or small areas of samples – as is often required in forensic analyses.

Challenges involved in paint evidence

"Obtaining an association between two paint chips using FT-IR is complicated by several factors," Bommarito said. "First of all, the particles transferred to the victim's clothing are typically extremely small, 10 microns by 10 microns is not unusual. Second, automotive paint chips typically consist of four or five layers of different materials and obtaining an association between the two chips requires separately analyzing each layer. There are several critical requirements in any instrument that we use for this analysis task. First of all, high spatial resolution is required in order to obtain spectra for the individual layers of the chip. Secondly, very low noise on the IR signal is required in order to obtain valid data from such small samples. The noise of an FT-IR instrument is usually defined by its signal-to-noise ratio – the higher this ratio the less noise will be seen in spectra. An FT-IR instrument's signal-to-noise can be improved by increasing data

acquisition times, however, this is undesirable as it has an obvious, direct affect on total analysis time and therefore reduces the sample throughput of the laboratory. Third, paint samples tend to have absorbencies in the wavenumber range from 550 to 650, so an FT-IR instrument should detect IR radiation with a high signal-to-noise ratio in that range (Figure 3).

The MSPFSD primarily uses the Spectrum™ Spotlight™ 300 FT-IR imaging systems from PerkinElmer®, Inc., Shelton, Connecticut. “We have used PerkinElmer instruments for seven or eight years but upgraded recently to the new Spotlight 300 because it offers a number of advantages,” Bommarito said. “First of all, the detection range of the medium range MCT has been improved to allow measurements to be made in the 550 to 650 wavenumber range which is helpful because there are pigments in paints that have absorbencies within that range. In addition, the spatial resolution and signal-to-noise ratio of the new instrument are both unusually high.”

The PerkinElmer Spectrum Spotlight 300 includes a revolutionary detector design that provides the MCT array detector and a single point MCT detector in the same dewar. The array technology allows the collection of high quality IR chemical images of large sample areas quickly and easily while for the most challenging samples, the single point detector provides the ultimate IR sensitivity and spectral range. The single element MCT detector in the Spectrum Spotlight has a smaller area than earlier FT-IR microscopes (100 μ compared with 250 μ). Background noise is generated from areas of the detector not illuminated by the sample signal – therefore the smaller the redundant area the lower the noise. This very high signal-to-noise is the primary reason why the Spectrum Spotlight can continue to make valid IR measurements in areas of the spectra below 650 cm^{-1} . In addition, the Spectrum Spotlight 300 is the first FT-IR microscope to use white light LED illumination with wide range brightness and contrast controls producing extremely high quality visible images enabling very small forensic samples and the thin layers within the paint-chip samples to be quickly located for IR sampling.

Confirming an association between the paint samples

After finding paint samples on the victim’s clothing, Bommarito visually compared them to samples from the assailant’s car and determined they were visually similar. He then mounted the chips in wax and cut cross-sections with a microtome.

He placed each cross-section on a potassium bromide slide and mounted them on the FT-IR instrument stage. He then generated a visible image on the screen and specified an area for each of the five layers from which infrared spectra were to be obtained. The instrument focused the infrared radiation through the sample and measured the absorbed and transmitted light for each frequency, plotting a graph of wavelength versus percent transmittance. The sample is then moved over the linear MCT array to generate, in real time, an IR image of the layer comprising hundreds of spectra as a false color visible image. Following this process for each layer in the two samples, Bommarito confirmed an association between each layer in both samples and testified to his conclusions in the assailant’s trial in the jogger’s murder.

The assailant was convicted of murder

In addition to the paint evidence, prosecutors in the assailant’s trial also introduced evidence from witnesses to the slaying and other forensic evidence, such as tissue found on the van. One of the victim’s relatives also testified that she visited the assailant in jail and asked him: “Why the jogger?” and that the assailant told her: “I thought it was my ex-wife’s boyfriend.” A jury found him guilty of the murder and he was sentenced to a third term of life in prison. “This was a tragedy, and we felt that our evidence was overwhelming and that it was premeditated and done during the commission of a serious felony. He is a dangerous, dangerous man,” said the prosecutor.

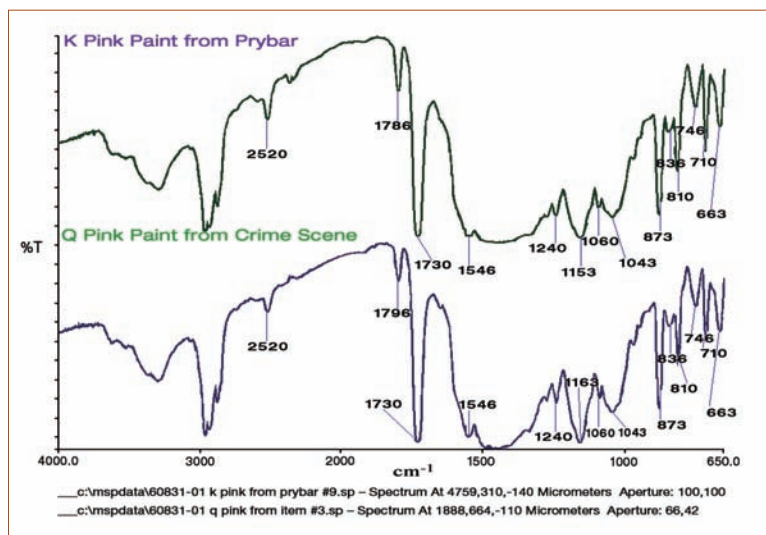


Figure 3. Comparison of FT-IR spectra from two paint samples.

Conclusion

Bommarito said that the MSPFSD has progressed beyond finding an association between a paint sample and suspect vehicle to identifying vehicle model, make, year and color even in cases where there is no suspect vehicle. The Royal Canadian Mounted Police (RCMP) and the Federal Bureau of Investigation (FBI) has recently created a database consisting of FT-IR results from paint samples taken from a wide range of different vehicles. The MSPFSD has integrated the Spectrum Spotlight 300 FT-IR with the library searching tool. This software takes the results of an FT-IR scan and finds matches within the RCMP's Paint Data Query System database. "This provides an example of the future of forensic science in my opinion," Bommarito said. "We are moving beyond simply providing evidence for or against a suspect to providing investigative leads. This technique has already led to the identification of suspect vehicles in several previously unsolved hit and run cases."

The Spectrum Spotlight 300 Imaging System described in this case study has been superceded by the Spotlight 400 FT-IR Imaging System.

Monitoring *in vitro* Stem Cell Osteogenic Differentiation

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A Nondestructive Method for Monitoring *in vitro* Stem Cell Osteogenic Differentiation with Raman Spectroscopic Mapping

Introduction

Over the past few years, the discovery that stem cells can differentiate into various cell types has driven an increase in stem cell research. In turn, this has opened up new possibilities for regenerative medicine and gene therapies.

Bone marrow derived stem cells have the capacity to differentiate into osteoblasts, chondroblasts, and adipocytes. These cell types are responsible for production of bone, cartilage and adipose tissue respectively. Monitoring the differentiation of cells *in vitro* is challenging. Common methods include cell staining and sorting, but these techniques can be tedious and error-prone, as well as damaging to the cells.

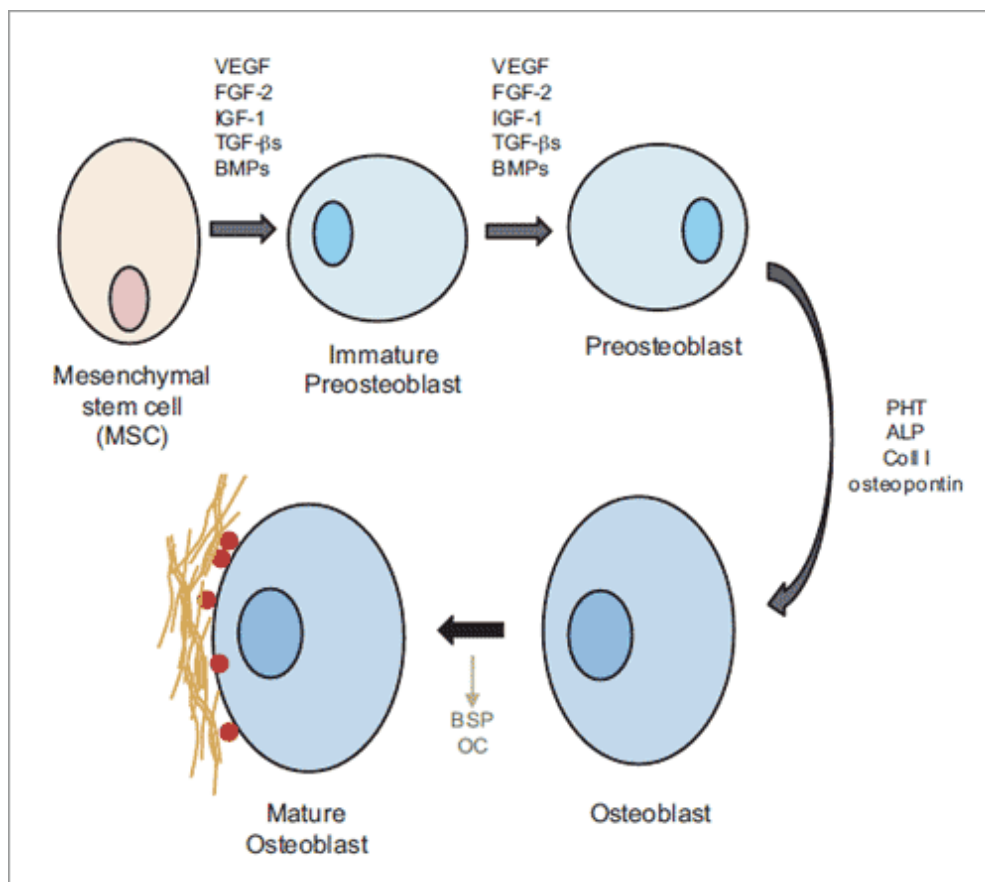


Figure 1. Bone marrow derived stem cell differentiation into the osteoblastic lineage. Growth factors are secreted by cells to promote differentiation at various stages. Differentiation into a mature osteoblast is demonstrated by secretion of mineral crystals onto a protein matrix.¹

Here, Raman spectroscopic mapping provides a nondestructive method for monitoring *in vitro* stem cell osteogenic differentiation (the formation of osteoblasts – bone forming cells – Figure 1). Osteogenic differentiation is a stepwise process greatly influenced by soluble

growth factors¹, and for this reason special osteogenic differentiation media are used to help mesenchymal stem cells (MSCs) differentiate into osteoblasts. First, the cells commit to the differentiation pathway and the first differentiation genes are induced. Next, the differentiation itself involves the induction of the entire differentiation gene set. Finally, the accumulation of gene-specific proteins marks cell maturation.² Figure 1 lists common gene expression profiles of the MSCs at various stages of osteogenic differentiation. Type I collagen expression begins as the cells differentiate from a preosteoblast into an osteoblast. Mature osteoblast differentiation, however, is denoted by mineral secretion onto a protein scaffold. Raman spectroscopy has the advantage of being able to detect not only changes in protein content and structure but also the deposition of mineral onto the protein matrix.

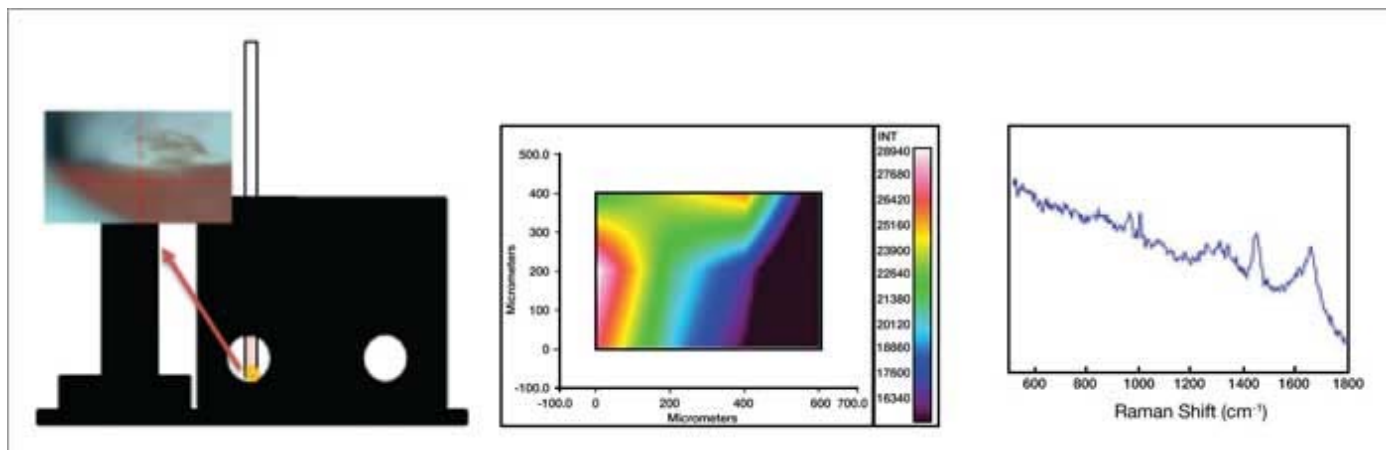


Figure 2. **(left)** Cell pellet in the bottom of the capillary. **(middle)** Raman image of cells in the capillary. **(right)** Raman spectrum representative of cells.

Raman monitoring of cell differentiation

To monitor the differentiation of the stem cells, approximately 5×10^5 human mesenchymal stem cells were pelleted at the bottom of a 2.5 mm diameter quartz capillary (Figure 2, left) and cultured in the capillary for 21 days. Cells were fed with osteogenic differentiation growth media containing dexamethasone and ascorbic acid and the capillaries were incubated at 37°C and 5% CO₂, in 15 mL conical vials. One hundred microliters of growth media was replaced in the capillaries every 3-4 days. Each capillary was examined at six time points: day 0, day 3 (36 hours after addition of osteogenic media), day 7, day 10, day 15, and day 21. Each Raman map contained 12-15 mapping points (Figure 2, center), depending on the shape of the cell pellet in the bottom of the capillary.

Studies were conducted on a PerkinElmer[®] RamanStation™ 400F with motorized stage using the following map- ping parameters: Wavelength range of 1800-500 cm⁻¹, 2 accumulations of 90 s acquisitions at each point in the map, 0.2 mm steps. Analysis time was kept short so as to minimize time samples were out of the incubator. Typical Raman bands observed in the spectra of the cells were 1004 cm⁻¹ (C-C ring breathing: phenylalanine/protein), 1305 cm⁻¹ (CH₂ deformation: protein), 1445 cm⁻¹ (CH₂ scissoring: protein), and 1660 cm⁻¹ (amide I: protein) in Figure 2 (right).³

When mineral deposition begins to occur, a Raman band at 960 cm⁻¹ (ν_1 P-O stretch: apatite-like mineral) can be observed. Once the mineral begins to mature, a Raman band at 1070 cm⁻¹ can also be observed (ν_3 PO₄³⁻ and ν_1 CO₃²⁻ stretch: apatite-like mineral). The mean spectra extracted from the Raman images at each time point are offset and overlaid in Figure 3, spectra are normalized using the 1550-1750 peak.

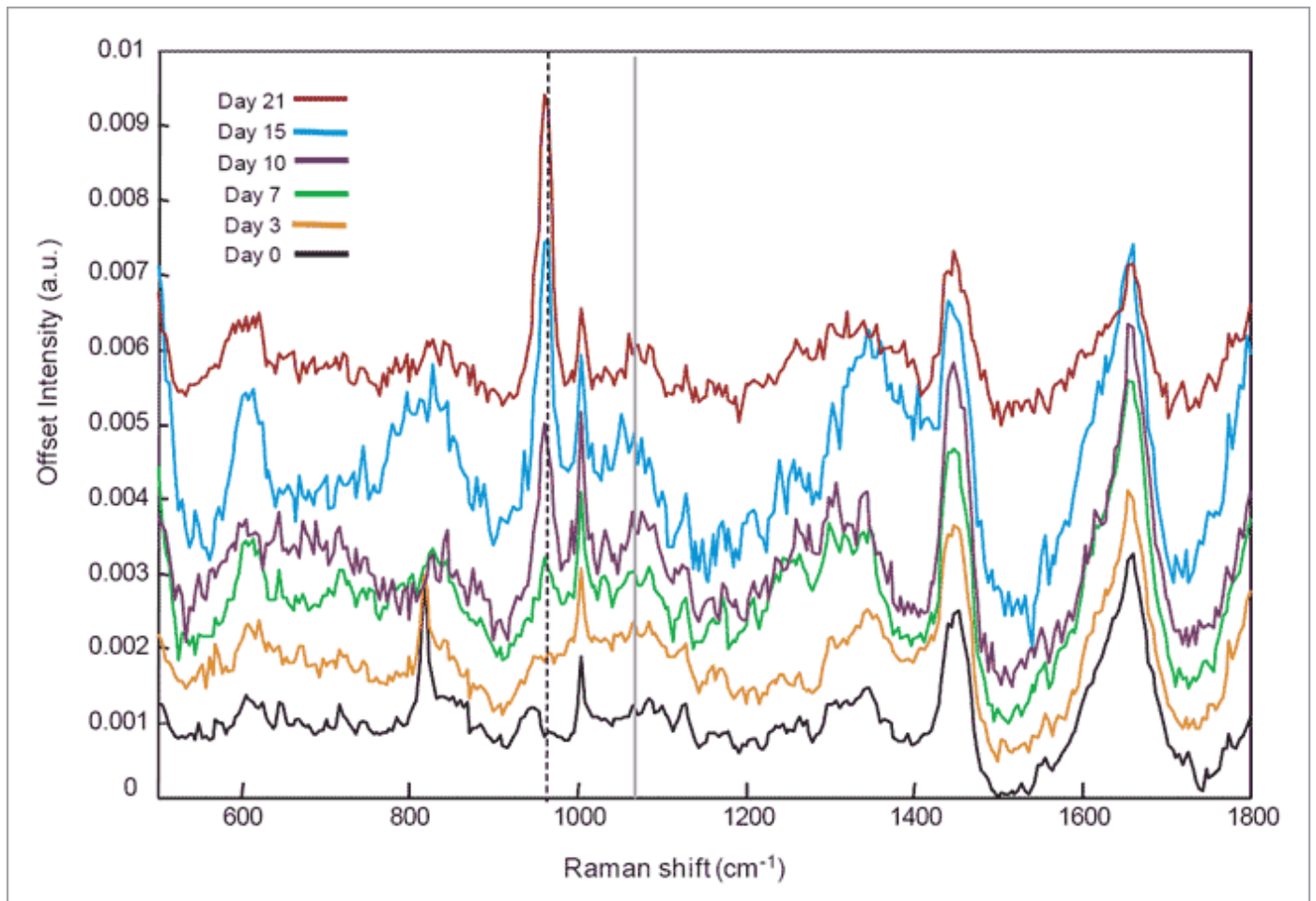


Figure 3. Mean Raman spectra of each observed time point during hMSC osteogenic differentiation. The black line indicates the 960 cm^{-1} mineral band and the gray line indicates the 1070 cm^{-1} mineral band.

The gradual appearance of the ν_1 P-O stretch at 960 cm^{-1} band begins at day 7 and increases in intensity through day 21. Using more conventional techniques for cell culturing (well plates), mineralization is generally observed after two weeks of culturing. Here, mineralization is detected as early as day 7. Mineralization can then be directly monitored by calculating the mineral-to-matrix ratio (MTMR) for each spectrum. The MTMRs of each spectrum were calculated by dividing the 960 cm^{-1} ν_1 P-O stretch vibrational band by the 1450 cm^{-1} CH_2 scissoring vibrational band (Figure 4). It is clear that the MTMR, indicative of total mineral content, increases over time, as expected. The increased intensity of the 1070 cm^{-1} band is indicative of carbonate ions being incorporated into the mineral crystal lattice. Carbonation of mineral tends to increase in normal bone as the mineral matures.⁴

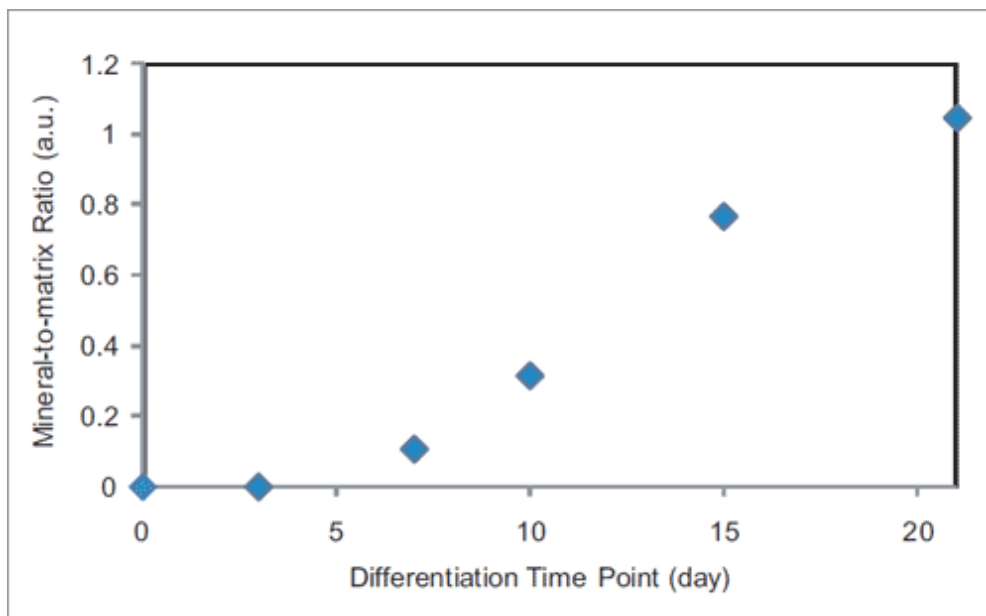


Figure 4. Mineralization is detected as early as day 7 and is then directly monitored by calculating mineral to matrix ratio for each spectrum.

Conclusion

Determining the extent of osteoblast mineralization is generally carried out using staining techniques such as Alizarin Red staining. Staining, however, destroys the cells, takes more than an hour to process for each time point and requires the preparation of complete matching sets of samples for parallel RNA studies. Thus to monitor osteogenic differentiation and mineralization at different time points requires multiple cell cultures, and does not enable the monitoring of the same cells over time. Here, we present Raman spectroscopic mapping as a technique to non-invasively monitor mesenchymal stem cell differentiation into an osteoblastic lineage in less than an hour.

Raman offers more rapid sample analysis without burden- some staining, and halves the number of cell lines that must be prepared and maintained. Furthermore, with this method, the RNA and mineralization data are correlated, significantly improving the robustness of the study.

Future studies might show whether a single Raman analysis could simultaneously provide information on osteoblast mineralization and cell lineage.

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Measuring the Properties of Coated Glazing

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Measuring the daylight, solar energy and thermal radiation properties of coated glazing

Abstract

This paper gives an update on state-of-the-art techniques for the optical characterization of architectural and automotive glazing products. It discusses the use of spectrophotometers and accessories for so-called routine measurements in quality control and for measuring glazing specifications according to international standards. We introduce a new improved 150 mm integrating sphere accessory for the solar energy range. We also present new accessories capable of measuring square metre sized samples. These unique tools provide a means for determining the daylight, solar energy and thermal radiation properties of tempered glass sheets.

Keywords: spectrophotometry, solar energy properties, thermal emissivity, coated glazing

1. Introduction

The measurement of optical properties of materials in the glazing industry is a key market area for manufacturers of spectrophotometers, covering a wide range of wavelengths. The basic optical properties for the calculation of solar energy transmittance and daylight properties of coated glazing according to EN410 and ISO9050 [1, 2] are spectral reflectance and transmittance. The standard procedure is to measure the transmittance and reflectance of small samples using a UV/Vis/NIR spectrophotometer equipped with an Integrating Sphere attachment. Using the procedures described in the standards [1,2], the measured spectra are combined to obtain the reflectance and transmittance spectra for an entire double or triple glazing unit. The visible daylight and solar energy properties of the glazing unit are then calculated from these spectra by taking a weighted average. In these calculations the solar energy distribution, the human eye sensitivity and other spectral distributions are used as weighing functions.

A key property in determining the energy-saving capability of coated glazing is the emissivity of the coating. European manufacturers determine the emissivity according to the standard EN 673 [3] using an IR spectrophotometer equipped with a specular reflectance accessory or other suitable reflectance attachment.

In this paper we discuss a new Integrating Sphere module for the UV/Vis/NIR range with enhanced sensitivity and other tools that were specially developed for measuring the daylight, solar energy and thermal radiation properties coated glass.

2. Available measurement tools

2.1 Standard measurements

Measurements of coated glazing in the UV/Vis/NIR range are generally performed in the wavelength range 250 - 2500 nm, which completely covers the UV, visible light and solar energy ranges. The transmittance measurements are performed under normal incidence. In the case of reflectance, a small angle close to the normal is being used (near-normal incidence).

The PerkinElmer LAMBDA 950 and 1050 UV/Vis/NIR spectrophotometers are currently the industry standard for ultra high performance, flexibility and convenience and are designed for analysis of coatings and component performance in both research and manufacturing of glazing products. The new LAMBDA 1050 is the highest performing UV/Vis/NIR spectrophotometer to date. With its enhanced performance in the NIR range it offers the highest sensitivity and resolution, ideal for the measurement of high performance glass and coatings. It features two large sampling compartments and a variety of snap-in modules and accessories.

Another new development is a 150 mm Integrating Sphere module (see Fig. 1) which uses an InGaAs detector for the NIR region. Like all our Integrating Sphere modules, once aligned it simply snaps into the spectrometer and is ready to be used without further adjustment.

The advantage of this new Integrating Sphere model is that its higher sensitivity results in faster measurements



Fig. 1. The L6020322 Integrating Sphere (InGaAs) snap-in Module for the LAMBDA 1050.

2.2 Measurements under oblique incidence

The standard measurement described in the previous section is not always sufficient and the need for reliable measurement techniques for measurements at oblique incidence is growing. In the past decade, considerable progress has been made in the development and of accurate tools for this purpose [4, 5]. An example of a tool that is now in use in over 35 labs in the glass industry is the Directional RT accessory shown in Fig. 2. Another tool that was recently developed for this purpose is the Automated Reflectance / Transmittance Analyser (ARTA) described in another paper in this conference[6].

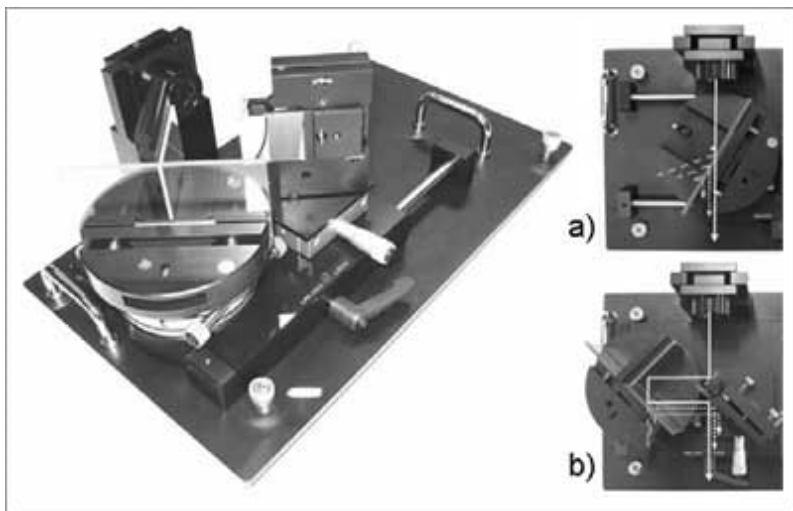


Fig. 2. The Directional Reflectance / Transmittance accessory (part nr. L631-0231) for the Lambda 800/900 and 850/950/1050 spectrophotometers: a) transmittance mode; b) reflectance mode.

2.3 Measurements on large samples

One of the problems that the glass industry is confronted with nowadays is the need to characterize large samples of tempered coated glazing. The tempering process of this glass occurs after the coating is applied and may affect the optical properties of the materials due to the high temperatures involved. Since tempering small samples is not possible and tempered samples cannot be cut without breaking into very small pieces, optical measurements on these samples require a special tool (see Fig. 3).

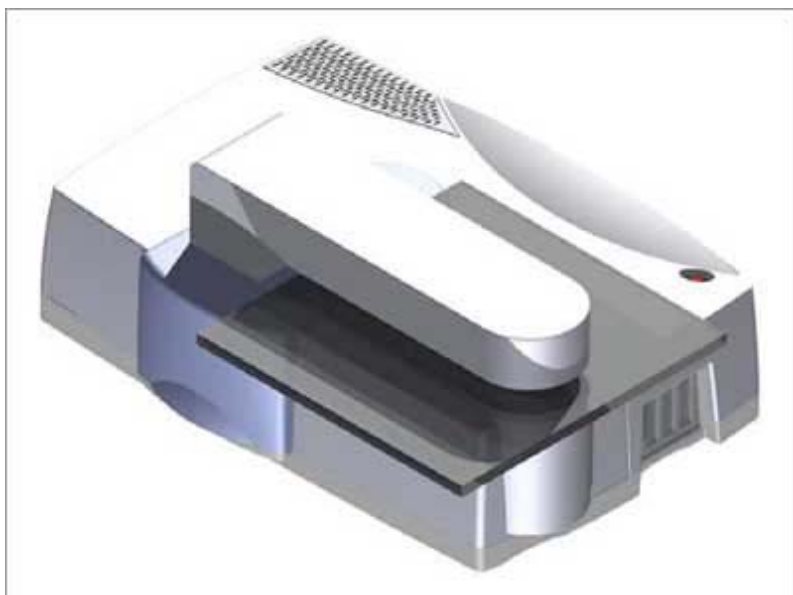


Fig. 3. Large Sample tool for measuring the UV/Vis/NIR characteristics of entire panes of coated glazing.

The set-up shown in Fig. 3 is capable of measuring both reflectance and transmittance of square metre size panes and even complete double and triple glazing units with thickness up to 45 mm. Switching between reflectance and transmittance modes is automated and both measurements are performed as a single task.

2.4 Measuring diffuse and patterned glazing

The optical properties of diffuse samples are probably the most difficult to measure with a spectrophotometer. One of the problems is that accurate measurements often require a very large integrating sphere with almost ideal properties. Since the detector area only occupies a few cm² of the sphere wall, a larger sphere means less energy to detect. For this reason, commercial integrating spheres for spectrophotometers are usually limited to a maximum size

of 150 mm (although some special customized spheres up to 230 mm diameter have also been produced).

When a diffuse sample is illuminated by the beam of the spectrophotometer, an area much larger than that of the beam diameter is transmitting due to internal scattering. A test with a large integrating sphere that has an adjustable sample port shows that the transmittance strongly depends on the port diameter when the port is too small [6]. Only for a large enough port all transmitted radiation can be collected.

Other problems occur in measurements on substrates with a 3D surface structure. With these samples, light is deflected rather than scattered. If the pattern is sufficiently smooth ("flat"), only a slight distortion of the transmitted light occurs and measuring the transmittance and reflectance with an integrating sphere produces accurate results. However, if the glass surface shows a distinctive pattern it is extremely difficult to get accurate measurement results.

We are currently working on solving these problems and the first tests with a 250 mm sphere with a large 85 mm diameter port (see Fig. 4) are quite promising.

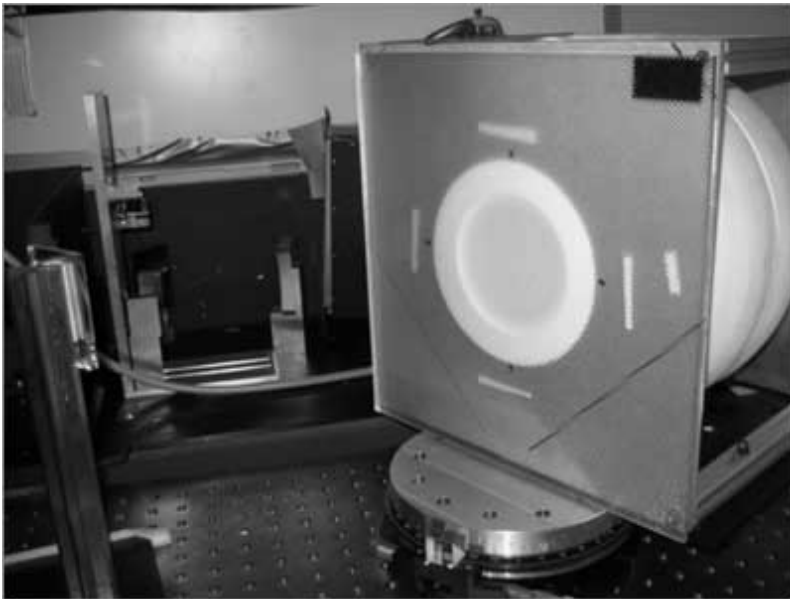


Fig. 4. Experimental set-up for testing a prototype Large Sphere tool for the Lambda 850/950/1050 systems. A patterned glass sample is positioned at the 85 mm entrance port.

We build the set-up with this sphere to measure directional transmittance of diffuse and patterned glazing. The reference beam was coupled into the sphere using a fiber bundle, similar to the ARTA [5]. A typical application for this set-up is the characterisation of greenhouse cover materials, where the transmittance for a wide range of incident angles is important (see Fig. 5). Another application is for Solar Panel cover glass.

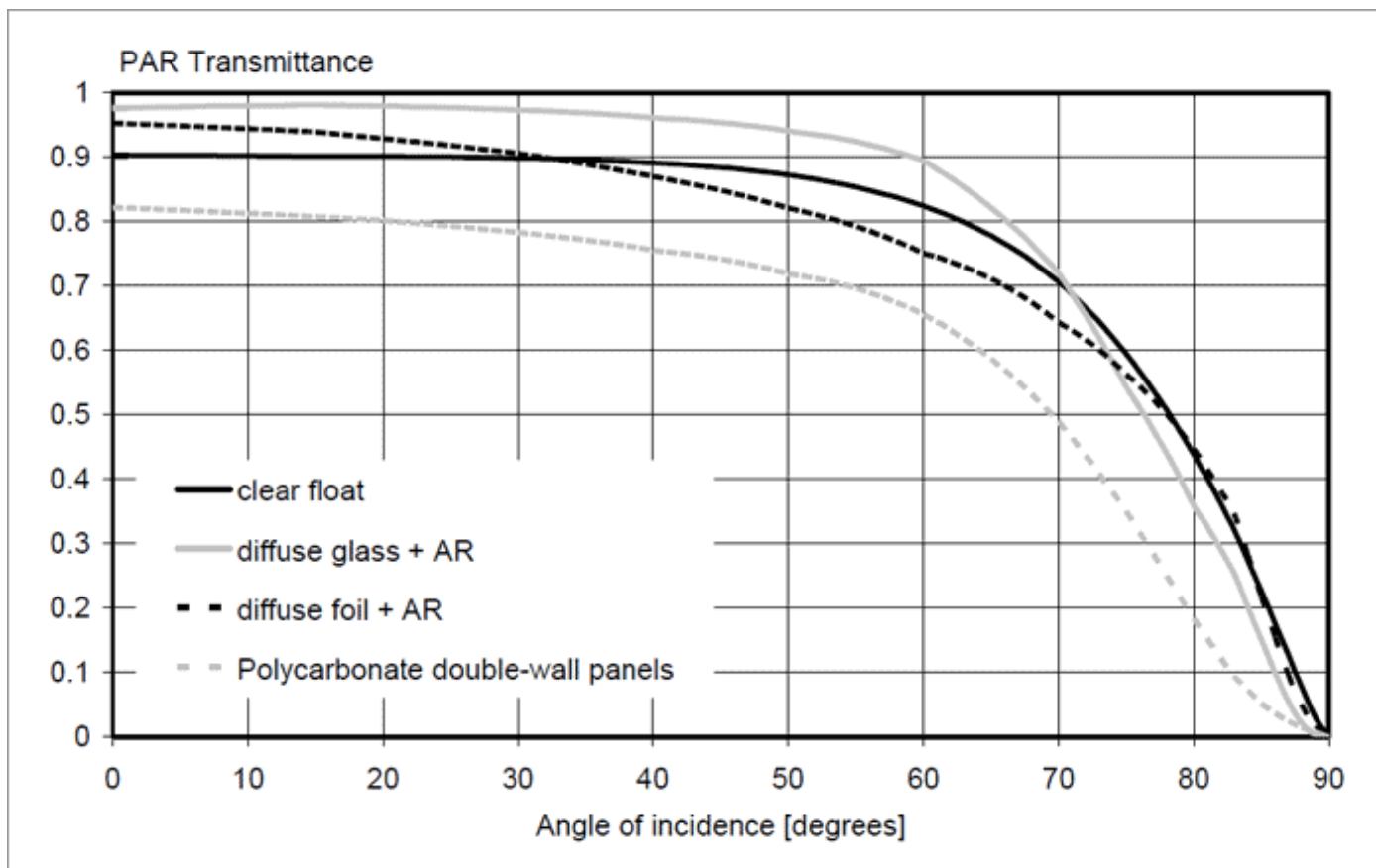


Fig. 5. Transmittance for Photosynthetically Active Radiation (PAR) measured on some greenhouse cover materials, measured with our 250 mm sphere directional transmittance set-up.

2.5 Measuring the emissivity of coated glazing

Based on the recommendations of the EU funded THERMES project [7], a tool was developed that provides a complete solution for State-of-the-Art measurement of the emissivity of coated glazing products according to EN673 and EN12898.

Basis of this tool is a unique reflectance accessory customized to fit into the accessory bay of the PerkinElmer Spectrum 100 FTIR Spectrophotometer (see Fig. 6.).



Fig. 6. Transmittance for Photosynthetically Active Radiation (PAR) measured on some greenhouse cover materials

The accessory is equipped with a 3-point sample support for maximum stability and accuracy. A stable horizontal sample positioning for large panes (> square meter size) is obtained with the help of two separate supports, height adjustable, to be positioned on the same table as the instrument. A laser alignment system is built in to enable checking and adjusting the alignment

of large panes. This makes it the only tool available on the market to do this type of measurements on coated toughened glass. Another advantage is that all optical components of the tool are protected against damage and contamination by a closed cover.

The tool is accompanied by a set of reference standards that guarantees traceability to international standards and all necessary procedures for measurement and calibration including software for calculations.

The usable wavelength range of the accessory is that of the Spectrum 100 configuration equipped with a KBr or CsI beamsplitter. The accessory is designed to measure direct reflectance of specular samples at an angle of incidence of 6 degrees (see picture below). The minimum sample size is 50 x 50 mm (with a special sample holder, this can be reduced to <15 mm). The maximum sample size is > 1 – 2 square metres (only limited by weight and handling capability).

3. Conclusion

Many of the new products of the glass and coating industry require dedicated measurement solutions. One of the current problems is how to perform the standardised measurements on large samples (tempered glass sheets). Another problem is imposed by the patterned and diffuse glazing products which are extremely difficult to measure and for which no standard measurement solution exists.

New tools have been described that provide adequate solutions for determining the daylight, solar energy and thermal radiation properties of these products.

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