"New" Developments in Infrared Spectroscopy

Chicago SAS Workshop

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Commercial! Much of this is from SAS short course:

BIRS*- *Biological Infrared and Raman Spectroscopy

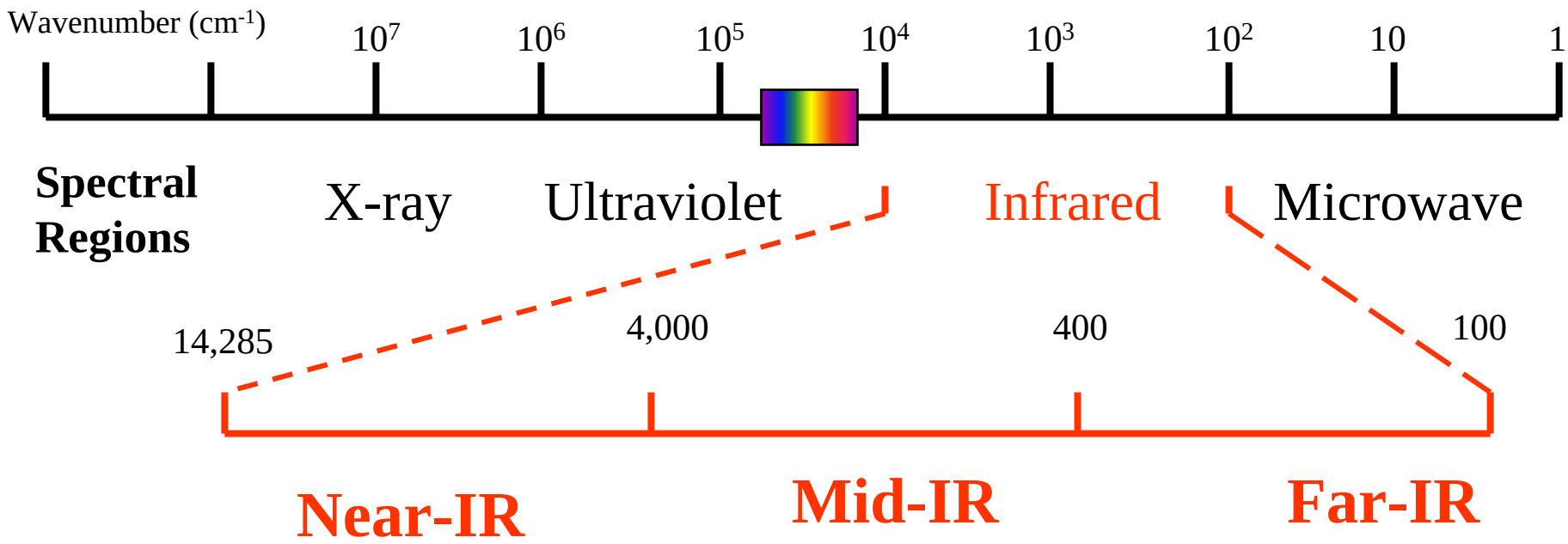
SAS Short Course presented by the Society for Applied Spectroscopy

At a meeting near you!

Originally Developed by Rina K. Dukor, Tim Keiderling and later by Brian Smith

(Explains some of the bias in examples)

Electro-Magnetic Spectrum



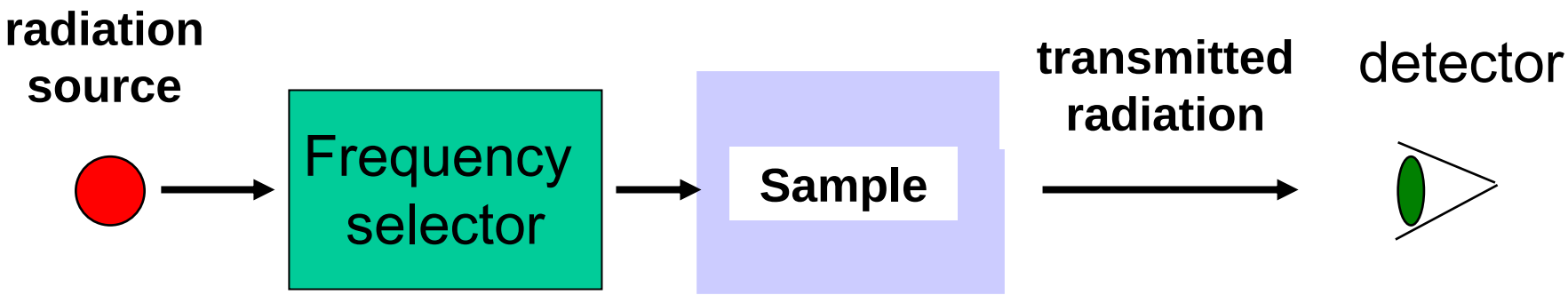
We have worked down in λ to here during the day
—is there any "new"?

Vibrational Spectroscopy

- Basic techniques now old, established, still quite useful
  - IR (absorption, dipole dependent)
  - Raman scattering complement
- Many variants advanced techniques
  - Modulation – Polarization (LD) and surfaces
  - Multichannel dispersive spectrometer
  - VCD (Vibrational Circular Dichroism) Chirality
  - Microscopy and Imaging
  - Time dependence – T-jump, Stop flow
  - Ultra fast (2-D, pump- probe)—laser based
  - Theory, explain what you observe

Techniques of Infrared Spectroscopy

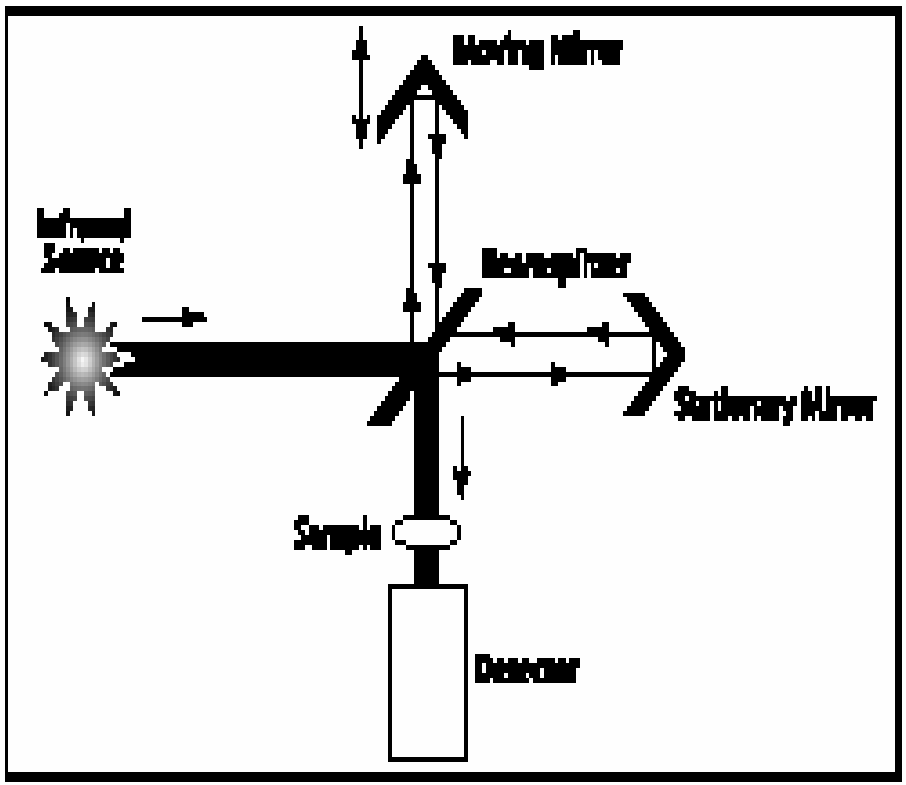
Infrared spectroscopy deals with absorption of radiation--detect attenuation of beam by sample at detector



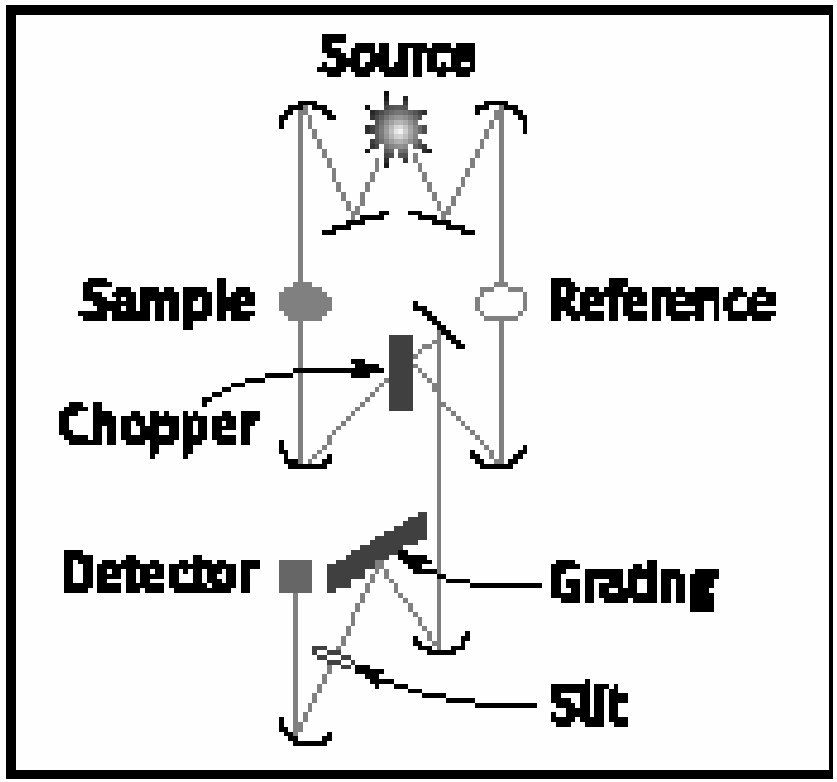
Dispersive spectrometers (old) measure transmission as a function of frequency (wavelength) - sequentially--same as typical UV-vis

Interferometric spectrometers measure intensity as a function of mirror position, all frequencies simultaneously--Multiplex advantage

Comparison of IR Methods – Dispersive & Fourier Transform



Interferometer Diagram



Dispersive spectrometer diagram

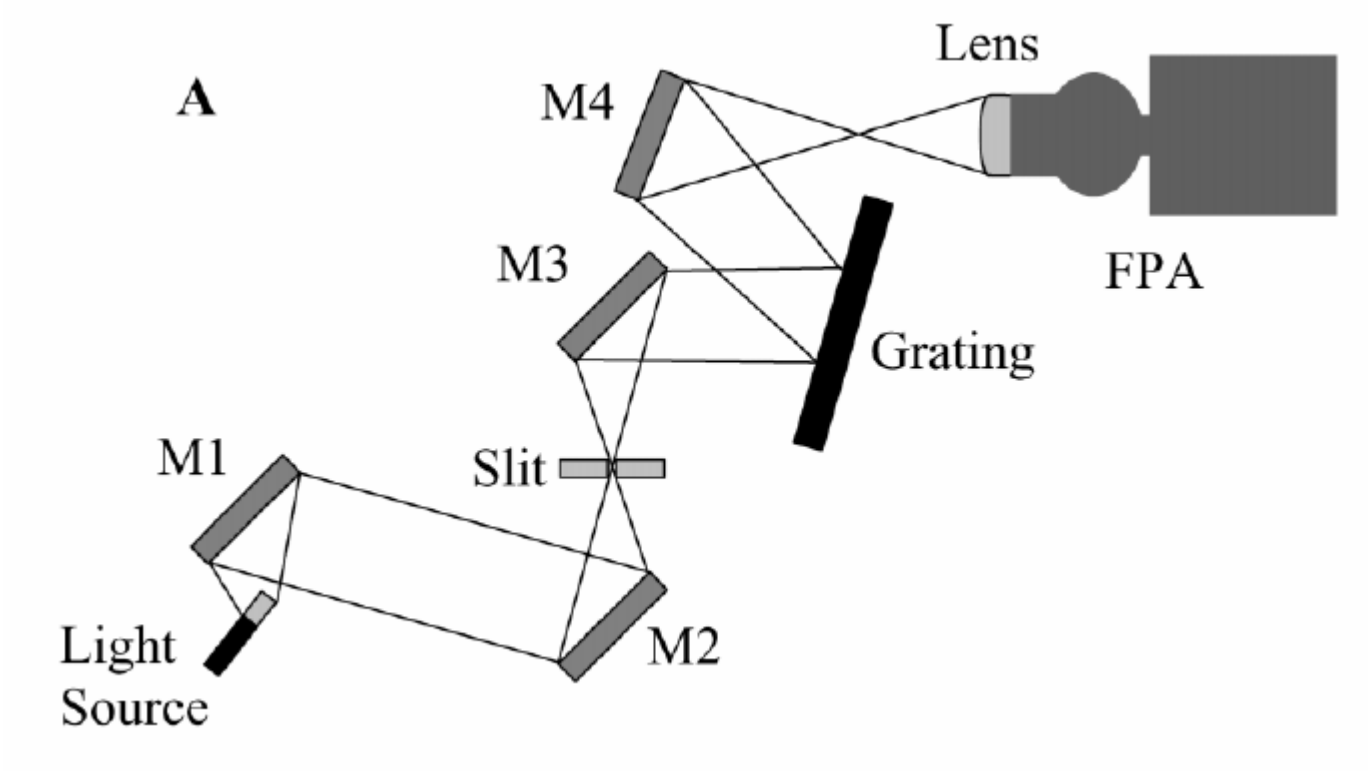
But add to this now many laser-based technologies!

Nicolet/Thermo drawings

New Developments in Planar Array Infrared Spectroscopy

I. Pelletier, C. Pellerin, D. Bruce Chase, John F. Rabolt

APPLIED SPECTROSCOPY **59, 156**, 2005



This approach opens up new ways to get either spatial or time dependent information

Dispersive revival

Going back to dispersive - new detection strategies

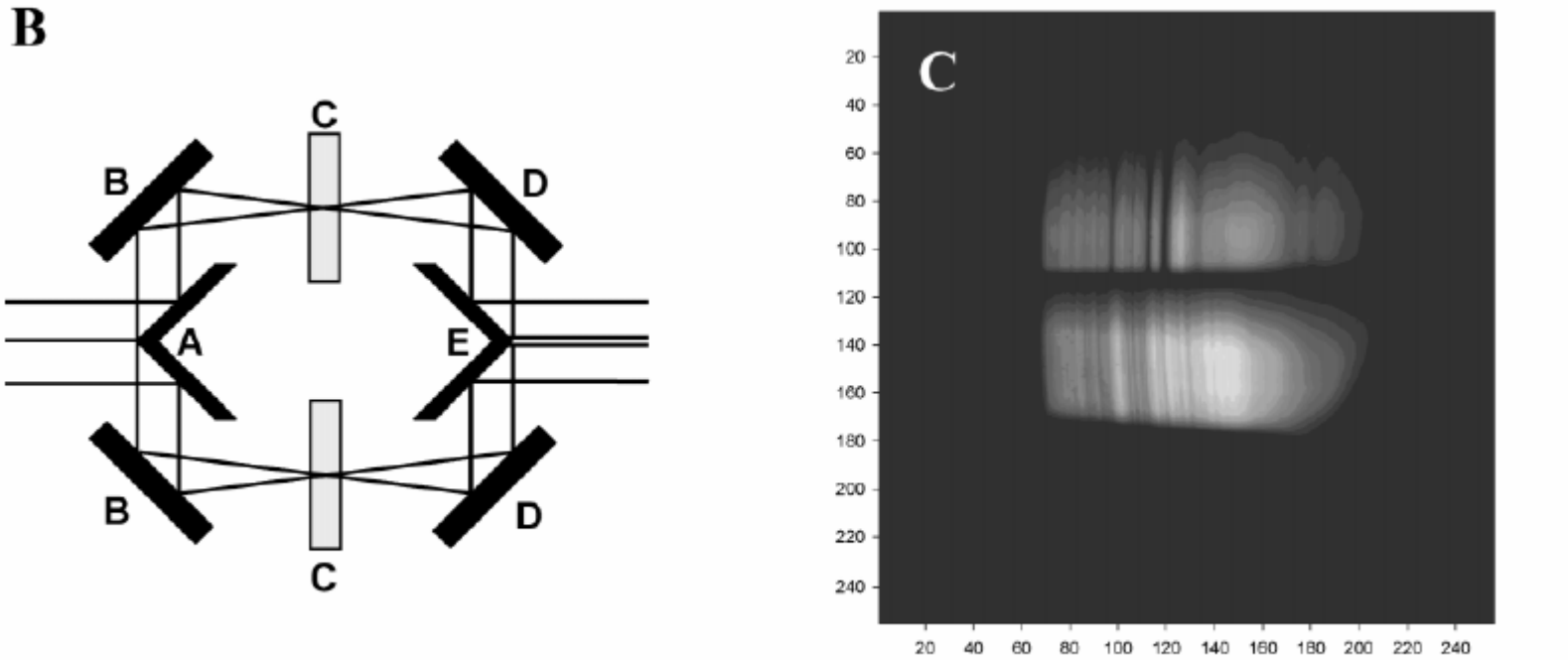


FIG. 1. (**A**) Scheme of the PA-IR spectrograph. (**B**) Scheme of the dual-beam accessory. (**C**) Spectral image recorded with a polystyrene film placed in the top portion of this accessory and air in the bottom portion. On this image, bright areas represent high single beam intensity regions.

Double beam is possible, use the detector spatial variation

No chopper, exactly coincident in time

Time dependence tradeoff with S/N

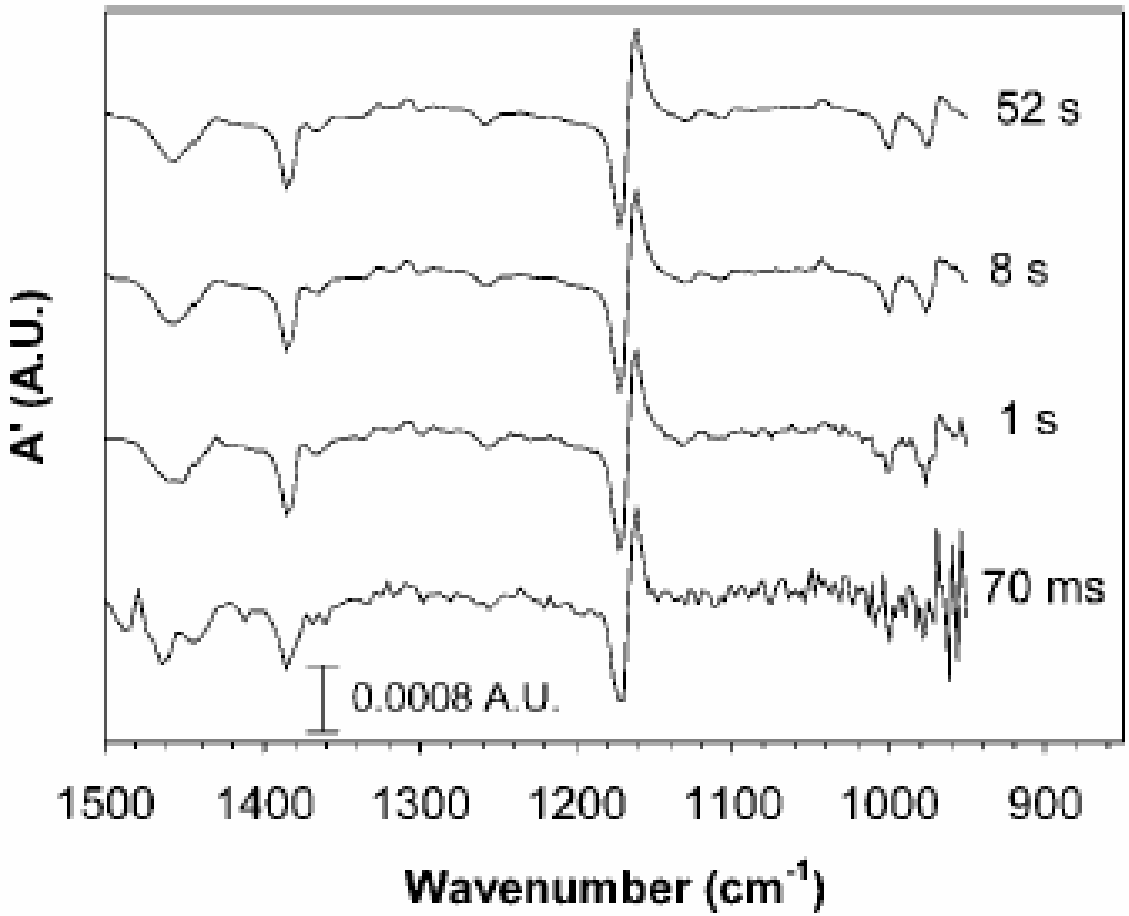


FIG. 4. In-phase PA-IR spectra of the isotactic polypropylene sample deformed at 14.36 Hz recorded in a total acquisition time of 52 s, 8 s, 1 s, and 70 ms.

Polymer stretching spectra--Faster response of course costs S/N

Acquisition of Mid-Infrared Spectra from Nonrepeatable Events with Sub-100-*ìs* Temporal Resolution Using Planar Array Infrared Spectroscopy

C. M. Snively, C. Pellerin, John F. Rabolt, D. Bruce Chase

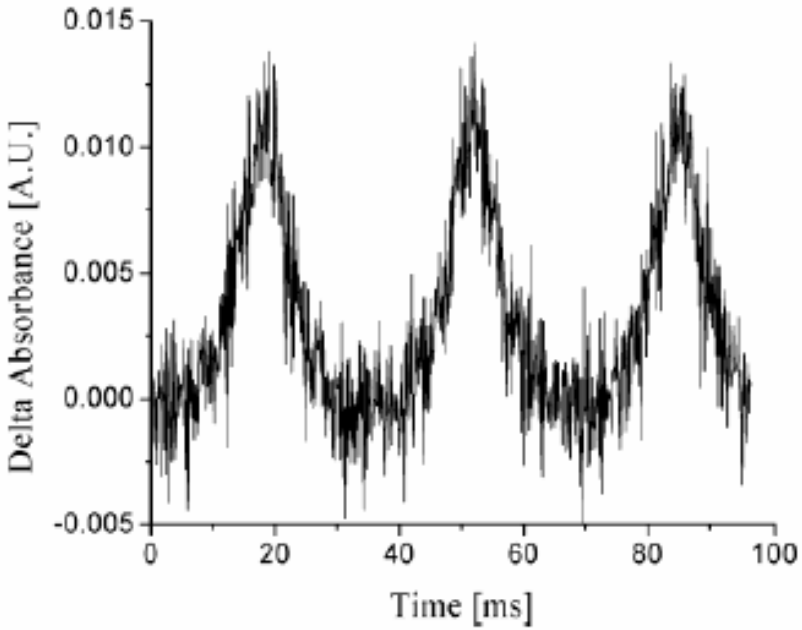


Figure 4. Traces generated by plotting the 1606-cm^{-1} C=C stretching mode of 5CB as a function of time during the application of a 15-Hz, 10-V_{p-p} electric field. (top) Data processed with the steps shown in Figure 1. (bottom) Data from the top trace processed with an additional subtractive normalization (see text).

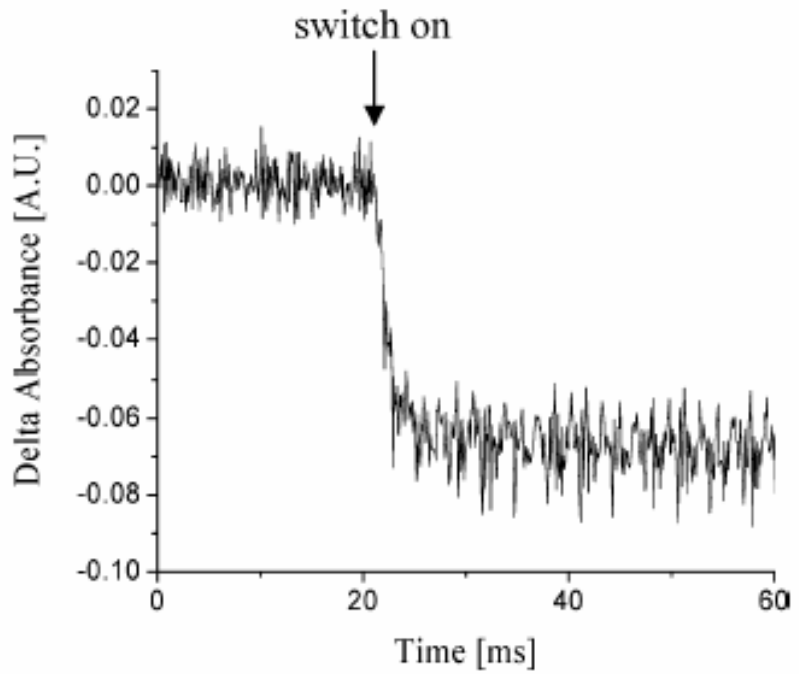
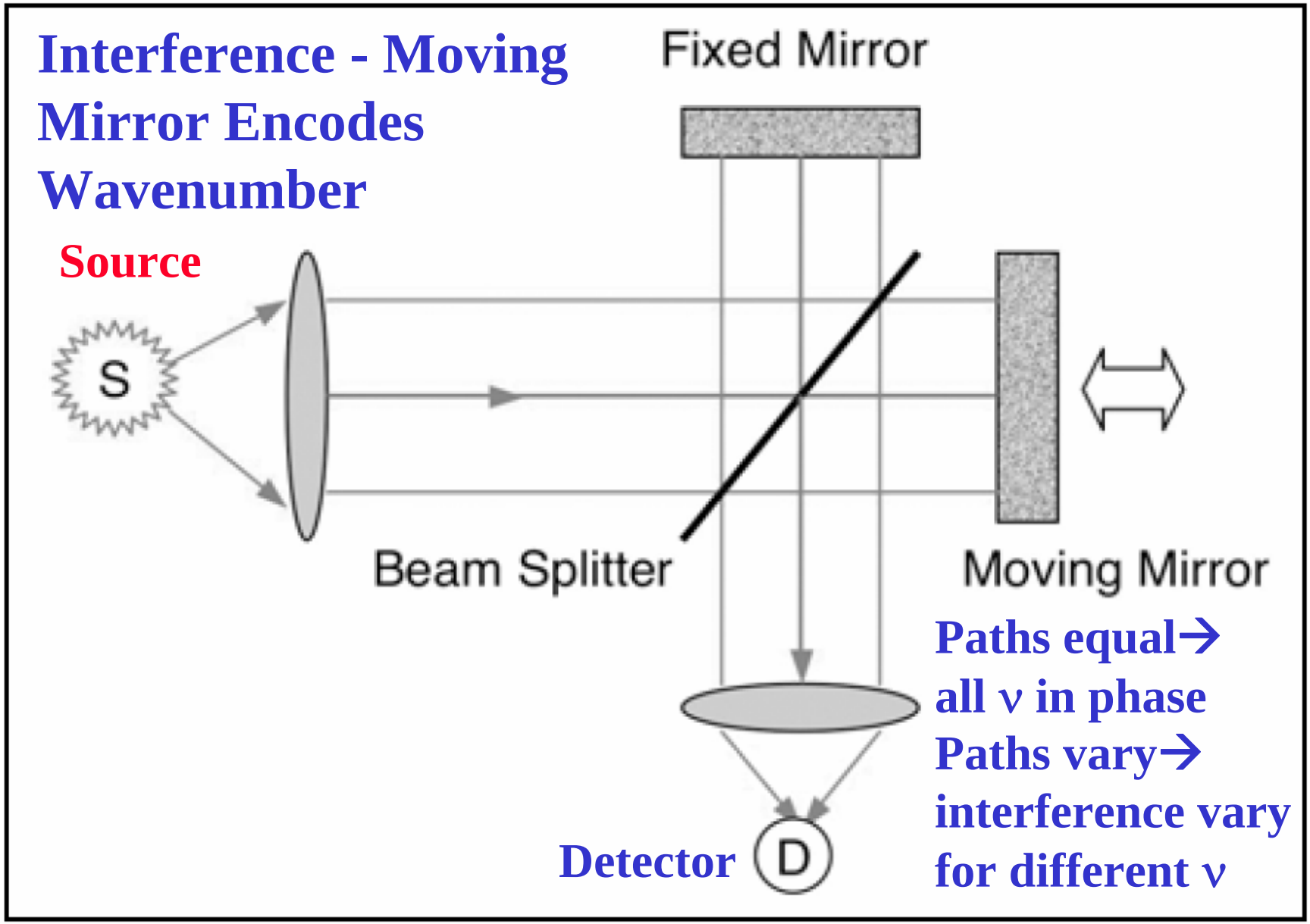


Figure 6. Switch-on region of the same data set used to generate Figure 5, with the data plotted at the maximum temporal resolution of 99.17 μs. These data were plotted using the subtractive normalization illustrated in Figure 4.

Fast response to follow sudden or repeating processes (sub ms)

FTIR – the dominant IR method now for >20 years



Major Fourier Transform Advantages

- Multiplex Advantage
  - All spectral elements are measured at the same time, simultaneous data aquisition. Felgett's advantage.
- Throughput Advantage
  - Circular aperture typically large area compared to dispersive spectrometer slit for same resolution, increases throughput. Jacquinot advantage
- Wavenumber Precision
  - The wavenumber scale is locked to the frequency of an internal He-Ne reference laser, +/- 0.1 cm^{-1}. Conne's advantage

These are terrific, ***if*** you can use them to improve your experiment

Is anything new in FTIR??

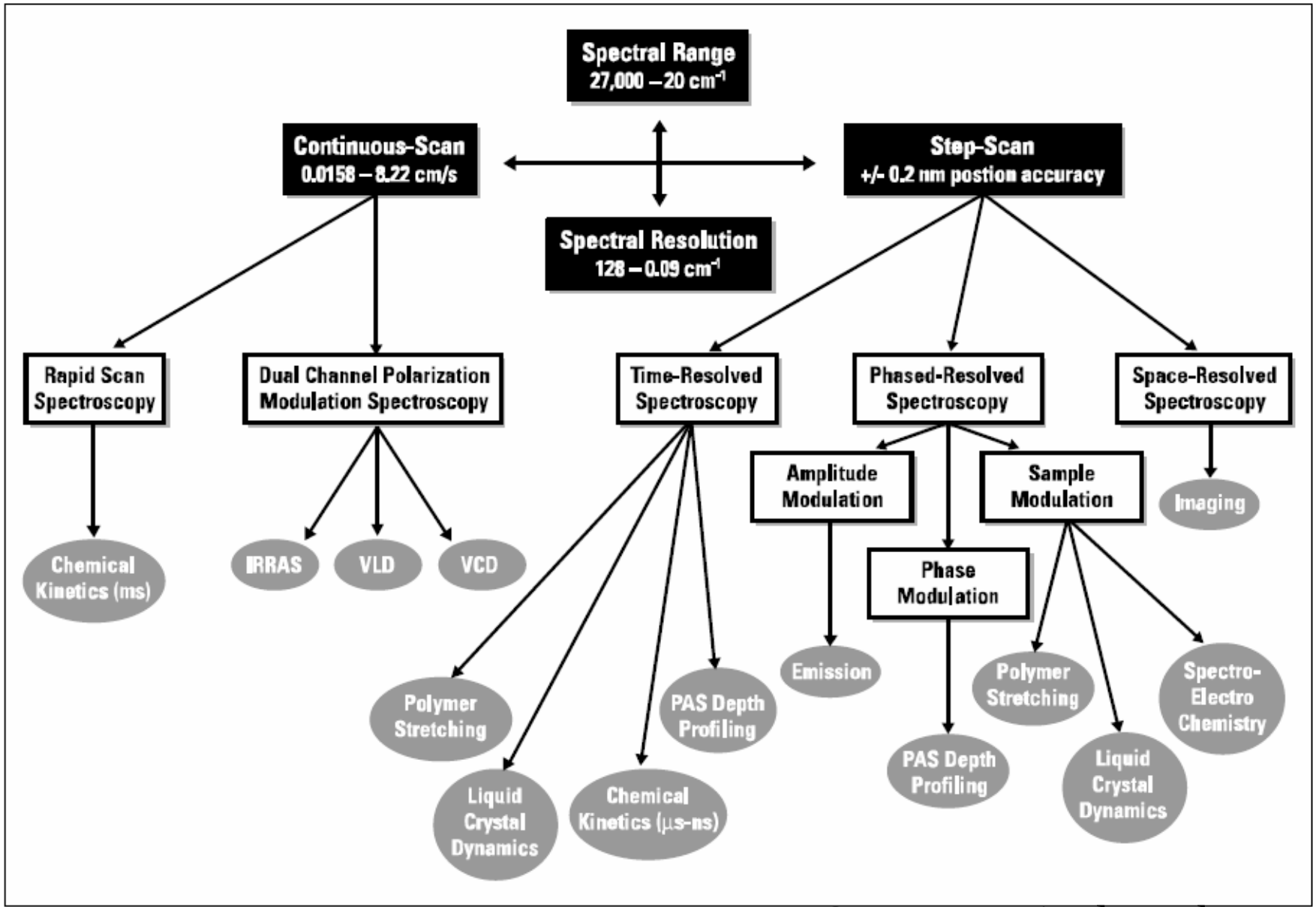
Routine instruments

Smaller, sealed, stable due to small size and corner cubes
—not a big deal anymore, available from many vendors
No air—mechanical bearings
Software—originally manufacturer— then Galactic plus PC
—now improved variety Vendor and manufacturer
Feedback to alignment—ultra stability on larger instruments
Kinetic collects, DMA/ DSP processing
Step-scan – bring back the original,
permit more modulation faster time response

Ultra fast scan —Manning spectrometer

Fast response – kinetic spectroscopy –the big “new” advance

Going beyond normal IR spectra – FT variations

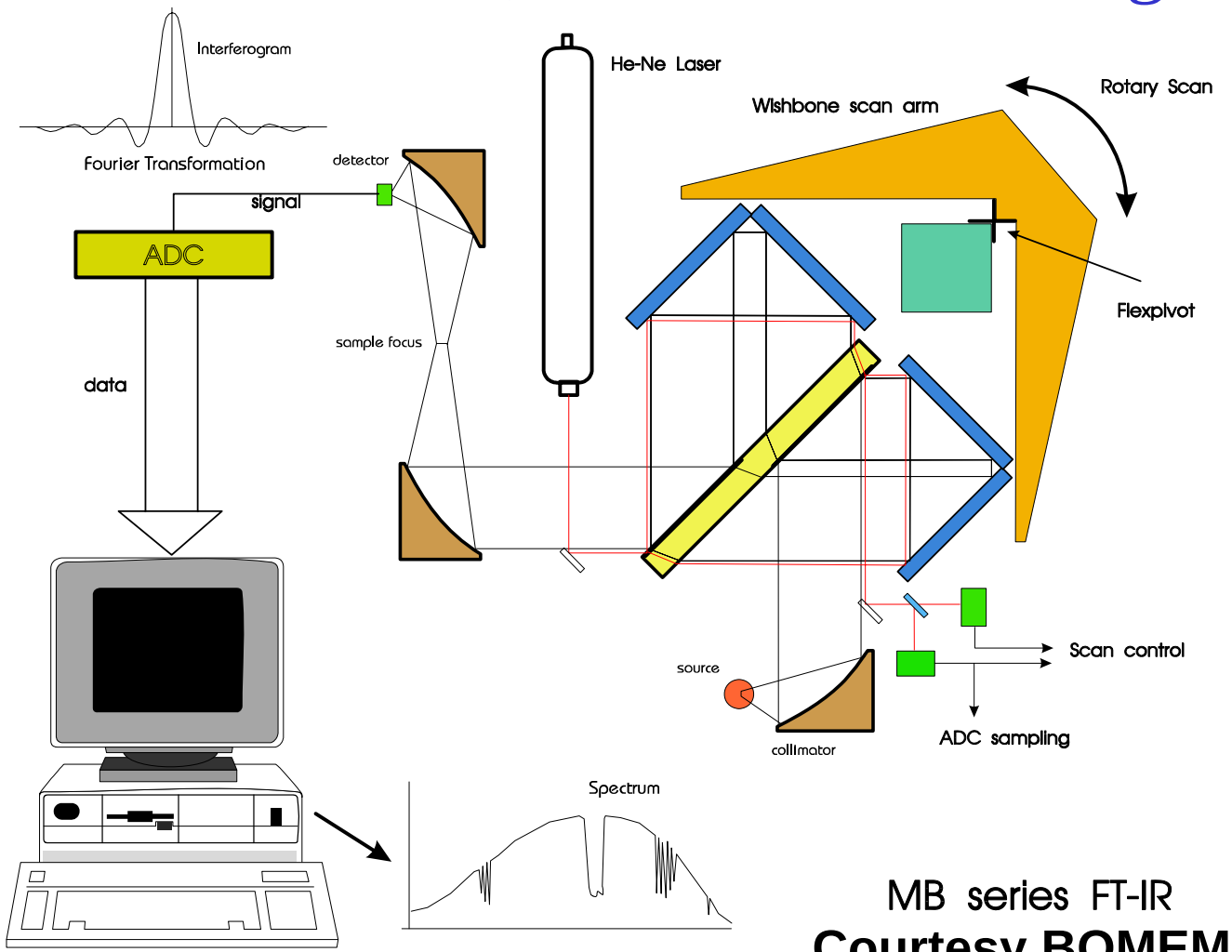


Source—Nicolet, Thermo

Now old, alternate, simpler FT-IR :

Corner cube mirrors, mechanical bearings

Example

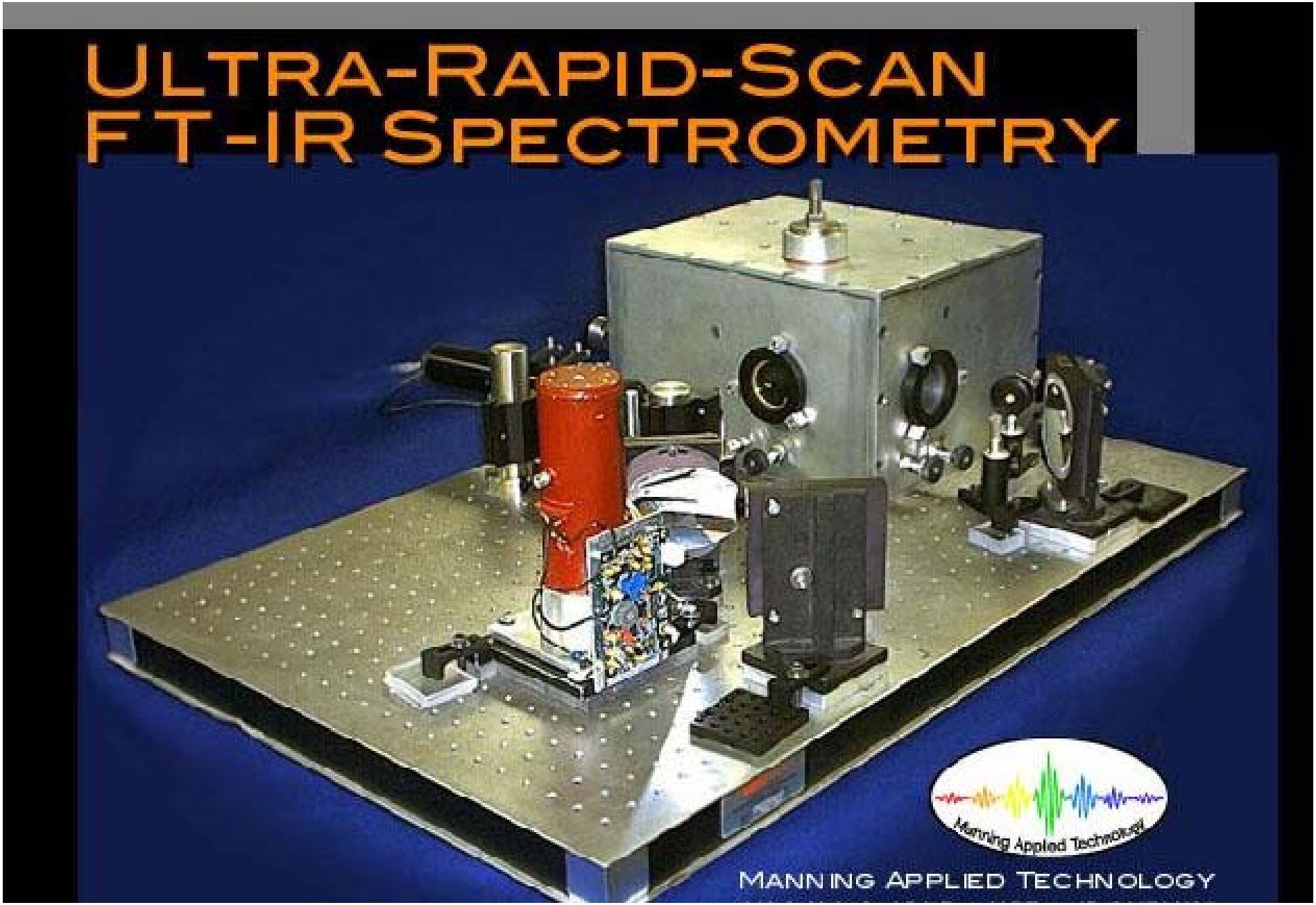


MB series FT-IR

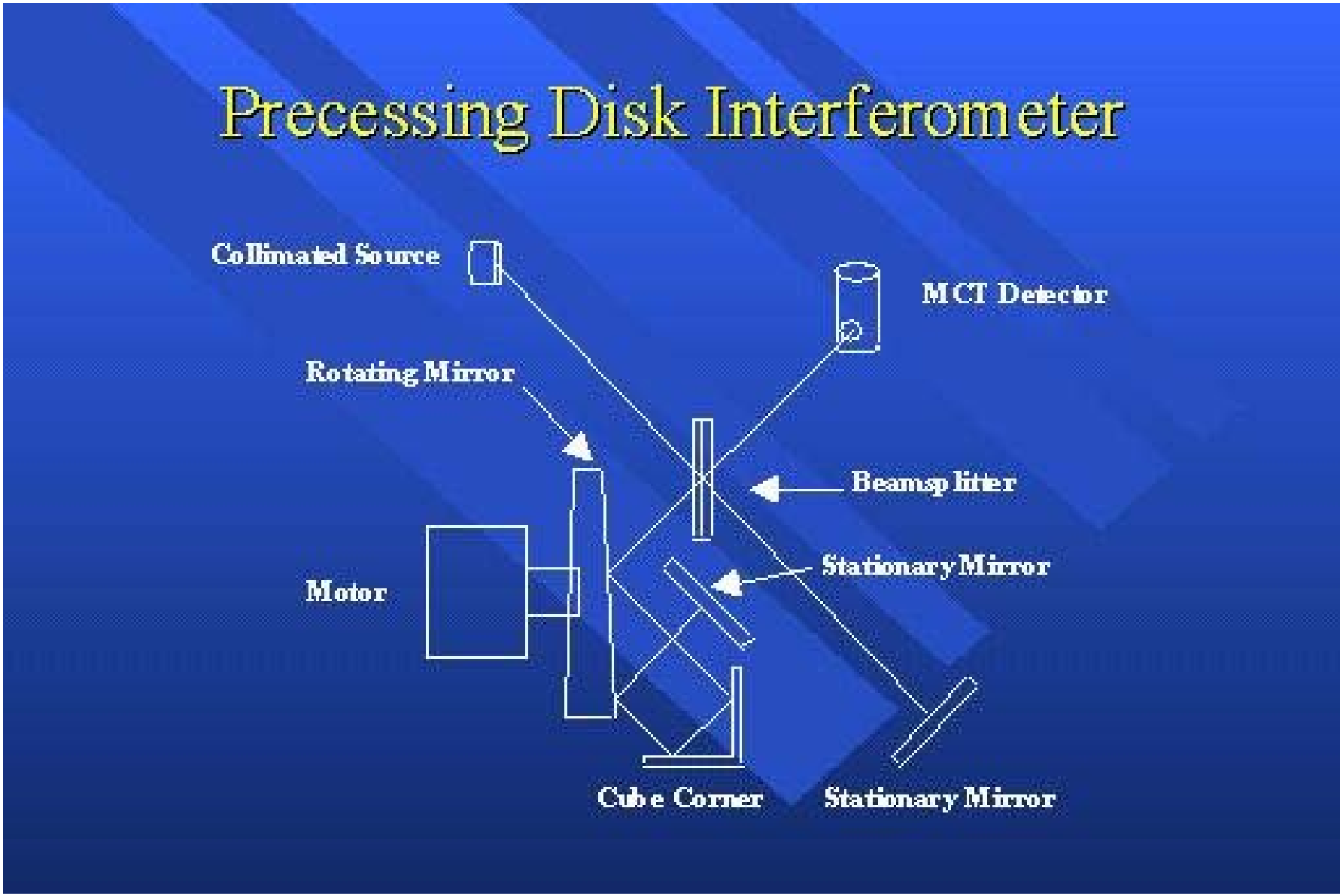
Courtesy BOMEM

Design often found in routine instruments, no air needed, highly stable

Ultra-Rapid-Scan FT-IR Spectrometry



From Chris Manning, Manning Technologies



Vary the path in one beam with rotating disk – potential msec response

From Chris Manning, Manning Technologies

Two types of time-varying systems

- **repeatable**

-synchronously stretched polymer

-photocycle of bacteriorhodopsin

-reversible electrochemical reaction

-photoacoustic response

- **non-repeatable**

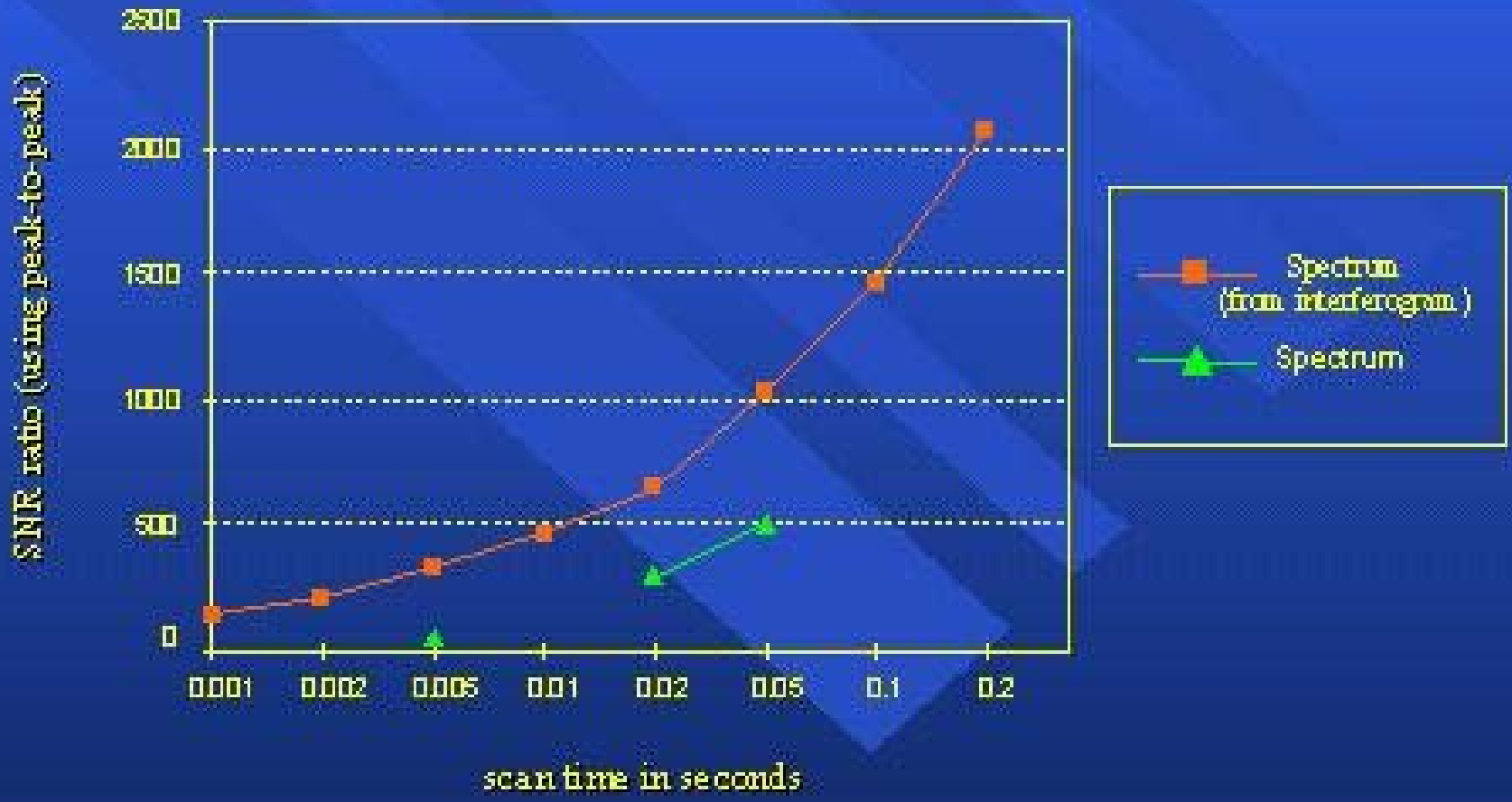
-irreversible photochemical reaction

-explosion

-exhaust stream of passing vehicle

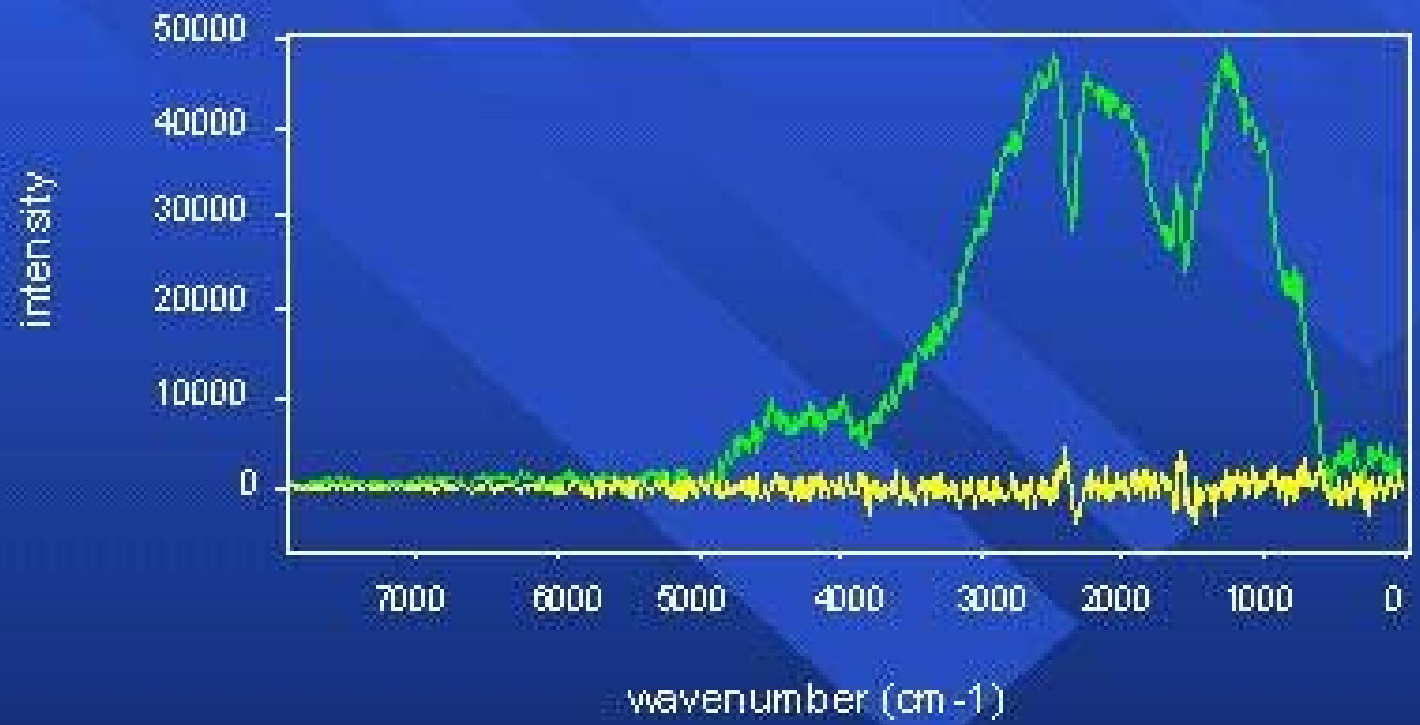
From Chris Manning, Manning Technologies

SNR performance



From Chris Manning, Manning Technologies

Spectrum, 1.5 milliseconds, 8 cm^{-1}



From Chris Manning, Manning Technologies

Typical Elements of FT-IR

IR Source (with input collimator)

- **Old:** Mid-IR: Silicon Carbide glowbar, Near IR: Tungsten Quartz Halogen lamp,
- **New:** variants on above and truly exotics: Synchrotron, lasers

IR Detectors:

- **Old:** DTGS (*Slower broad response)*,

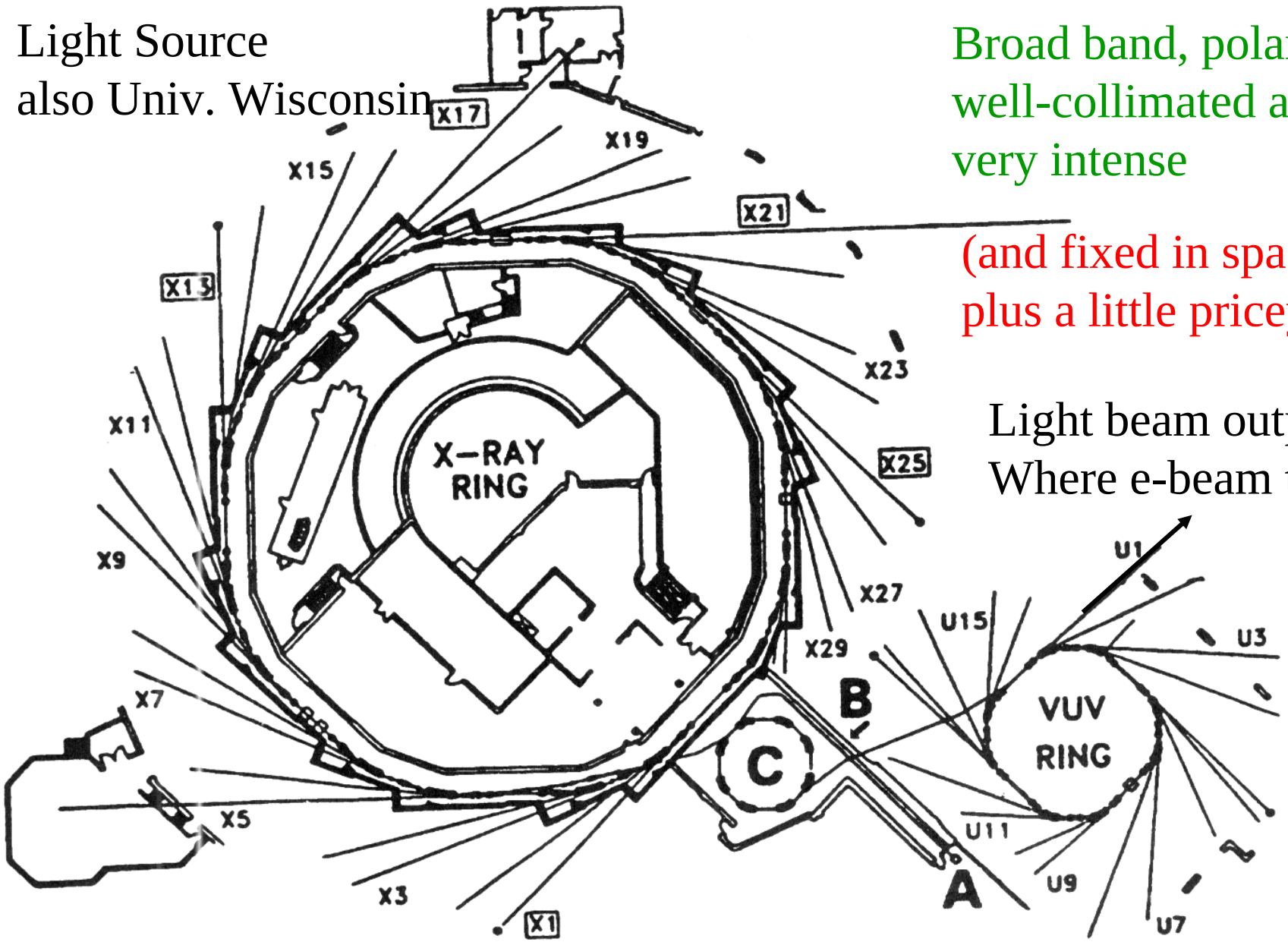
  MCT - *photo conductor (need liquid N_2, faster mirror velocity)*
- **New:** array detectors (image), fast photovoltaics (kinetics)

Sample Compartment

- **Old:** IR beam focused (< 6 mm), sample accessories from history
- **New:** virtually anything you want, plus plug and play, reflection, ATR, microscopy, remote sensing, the works

Synchrotron Light Sources – the next *big* thing

Brookhaven National
Light Source
also Univ. Wisconsin

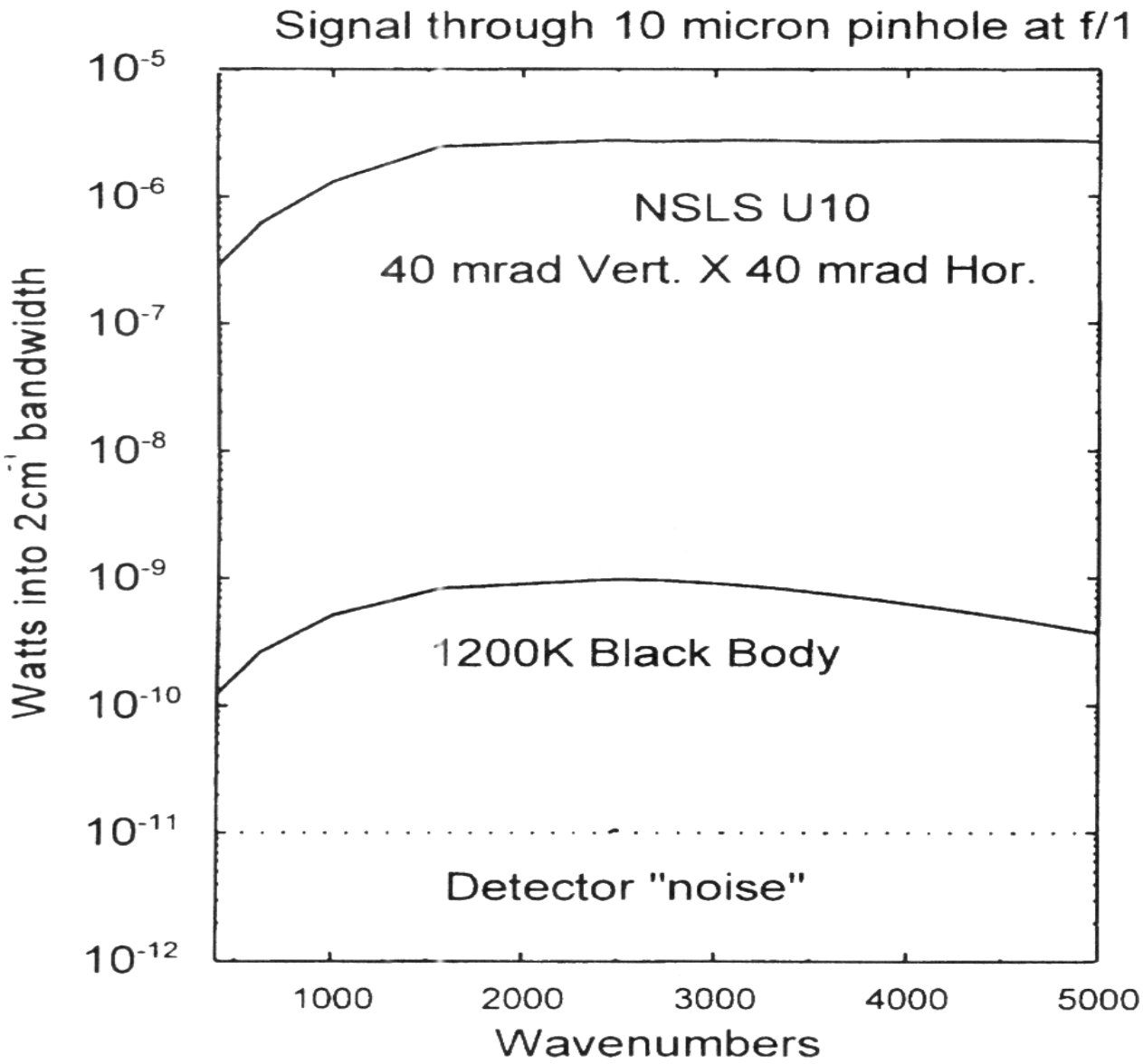


Broad band, polarized
well-collimated and
very intense

(and fixed in space!)
plus a little pricey!

Light beam output
Where e-beam turns

Synchrotron advantage – high *brightness*



Couple synchrotron to FTIR and to microscope/beam condenser

small spot size **high brightness**

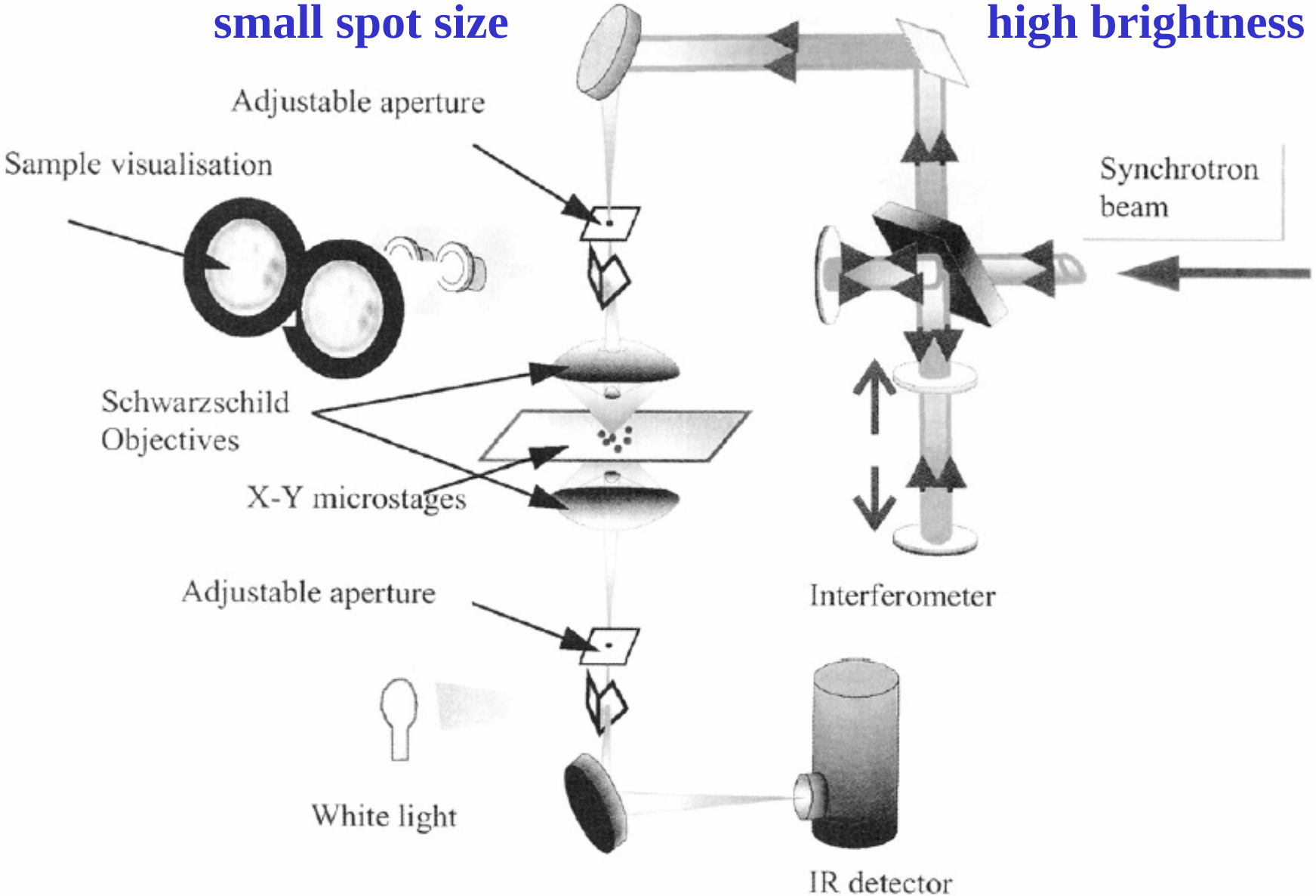
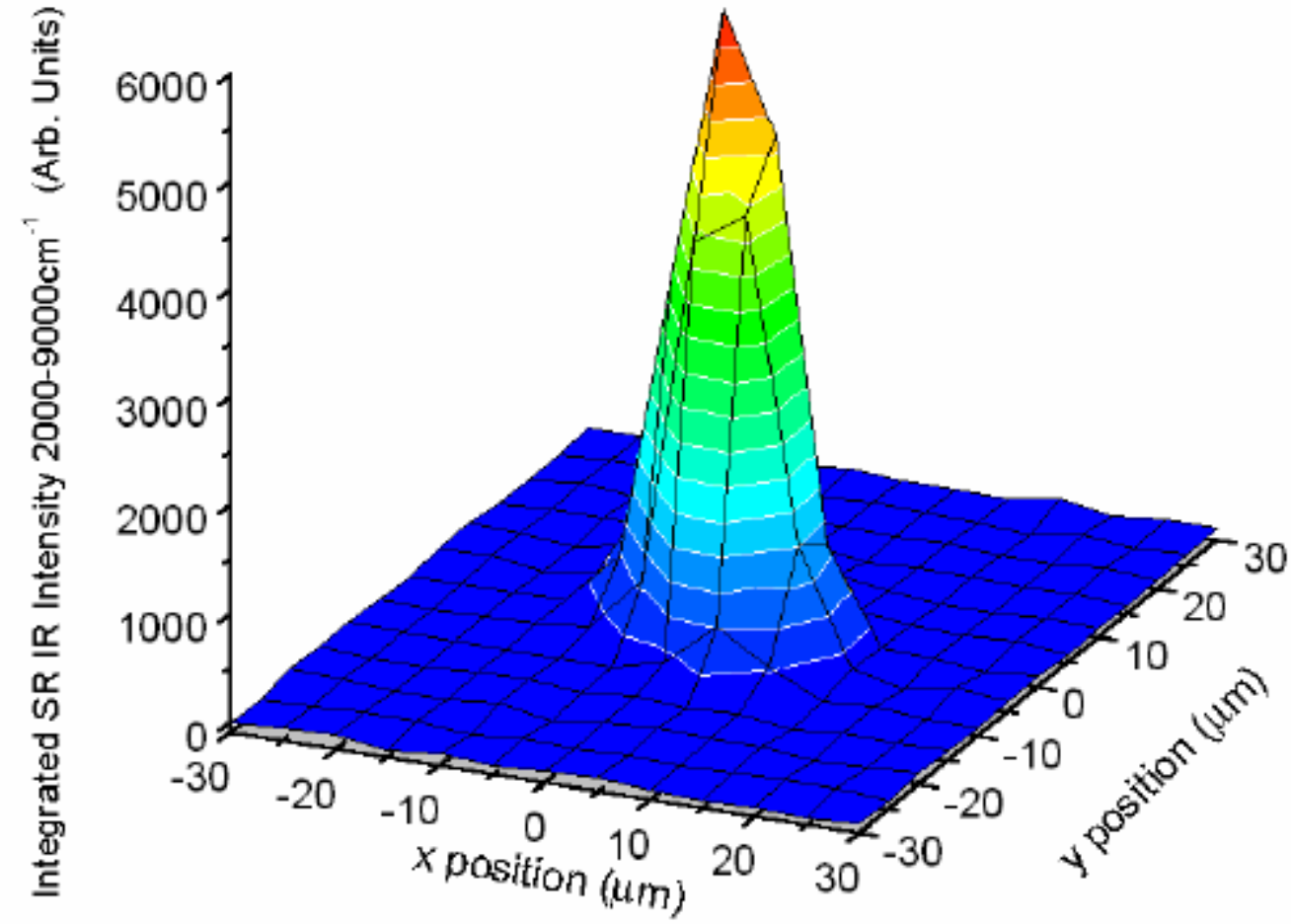


FIG. 1. Experimental arrangement for *in situ* synchrotron reflectance infrared microspectroscopy studies of ink on paper.

Wilkinson et al., Appl. Spectr.2002—Laurence Berkeley - US Treasury

10-micron spot size achieved for
high spatial resolution FTIR spectromicroscopy

Michael C. Martin and Wayne R. McKinney – LBL report



Example of small spot size for microscopy

Glowbar ~100 μ result in much less power through pinhole

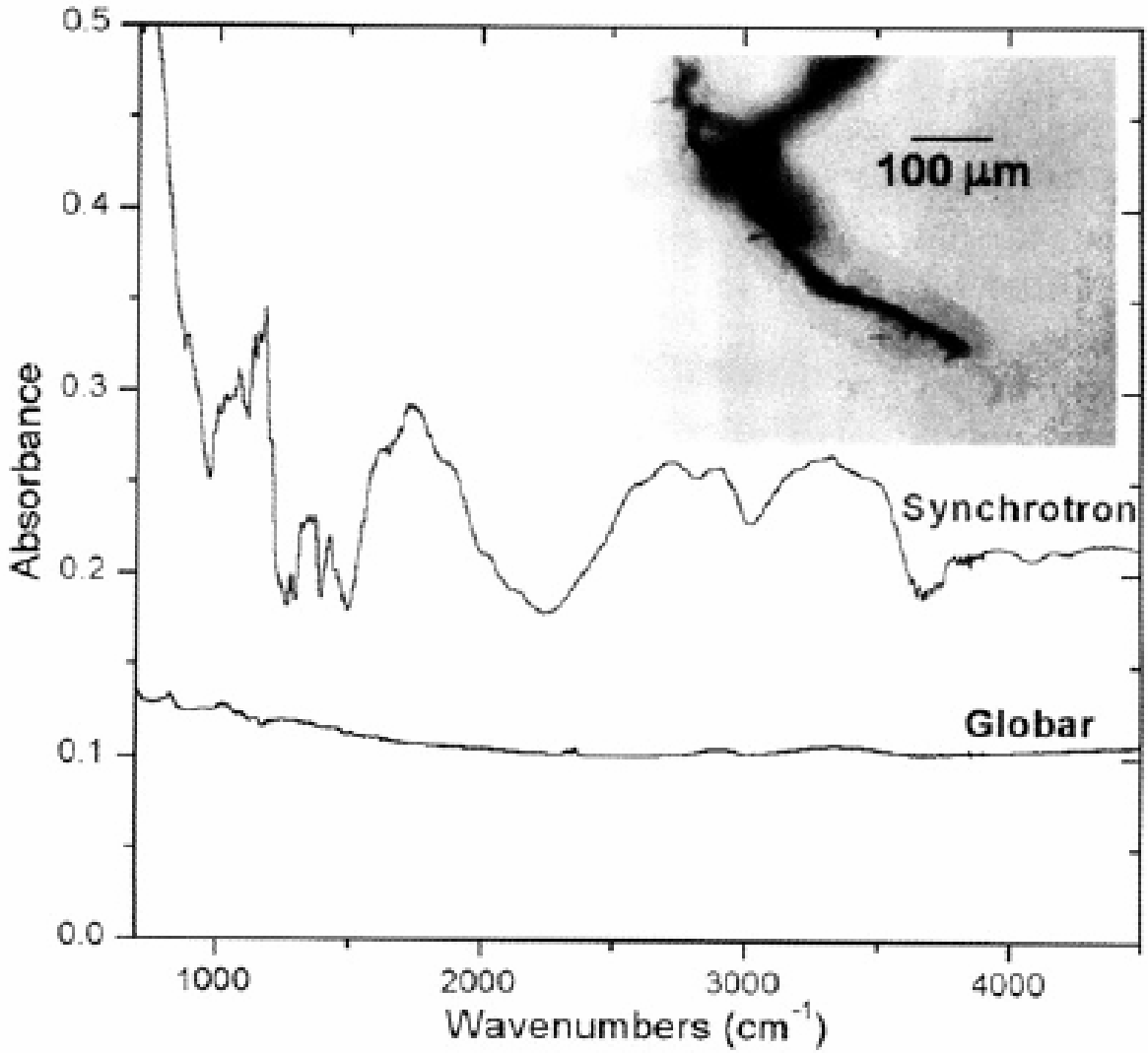


FIG. 3. Infrared spectra of ink on a paper fiber using both synchrotron and Globar® infrared sources. The difference in the two spectra is due to the much greater brightness of the synchrotron impinging on the fiber.

Ink on paper, synchrotron brightness builds stronger spectrum - focus

LBL-Treasury - Wilkinson et al., Appl. Spectr.2002

Mid-IR Sampling Techniques

- **LIQUIDS**
  - Transmission—really little change for decades
  - ATR (Attenuated Total Reflectance) – flow
  - Hyphenated technologies, e.g. GC-FTIR, HPLC-FTIR

Separation/Selectivity strength for combine chromatography-spectra, but sample small so use of FTIR as detector not so sensitive

GC-FTIR - Not much activity recently—

HPLC - FTIR - Lendl, J Chromatography A 1080 (2005) 132 – reviews difficulties - Solvent absorbance
- Lack of sensitivity

Benefit—spectral library enhance recognition of analytes

Sampling from an HPLC onto FTIR-ATR detection system

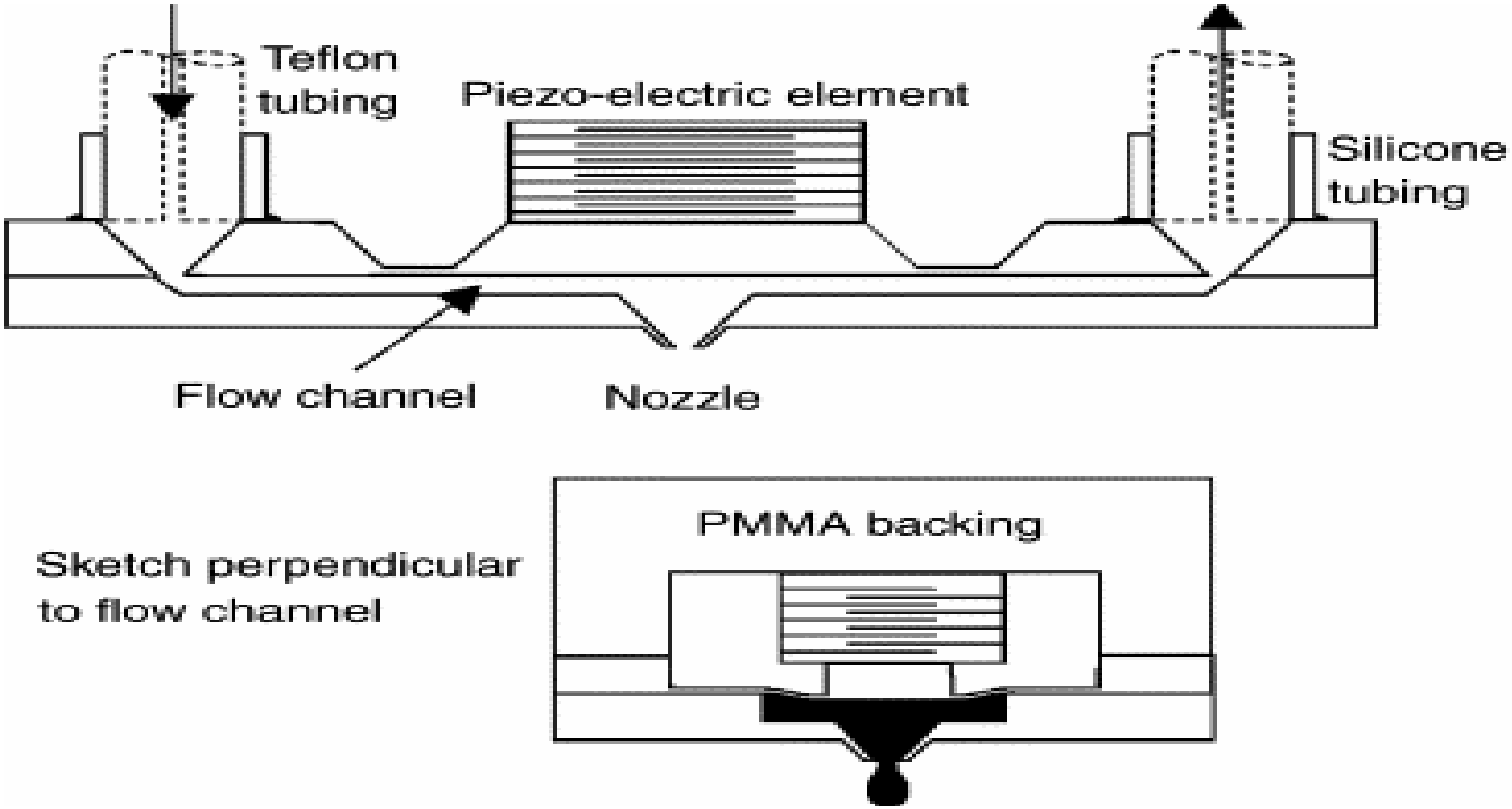
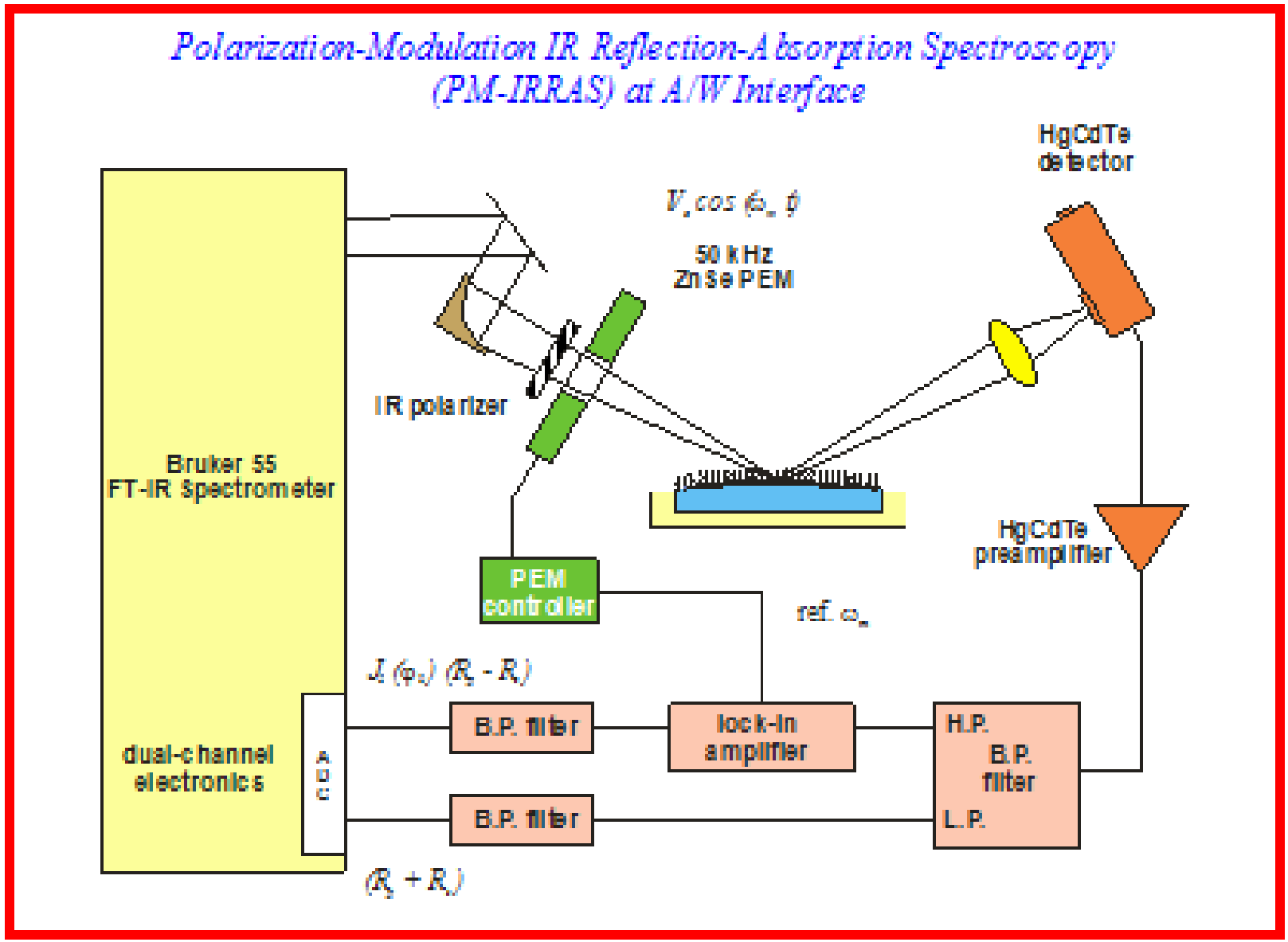


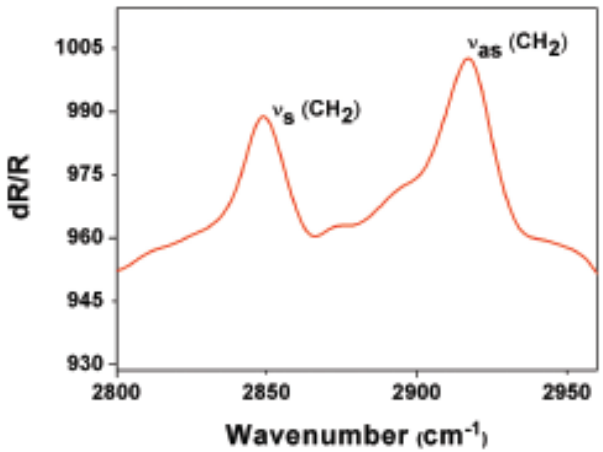
Fig. 1. The principle design of the flow-through microdispenser. Bottom: electrically induced deformation of piezo-element causes droplet ejection.

Izabella Surowiec, Josefa R. Baena, Johannes Frank, Thomas Laurell, Johan Nilsson, Marek Trojanowicz, Bernhard Lendl: *Journal of Chromatography A*, 1080 (2005) 132-139

IRRAS- Looking at the liquid surface, focus on interface



From R. Dluhy website U.GA



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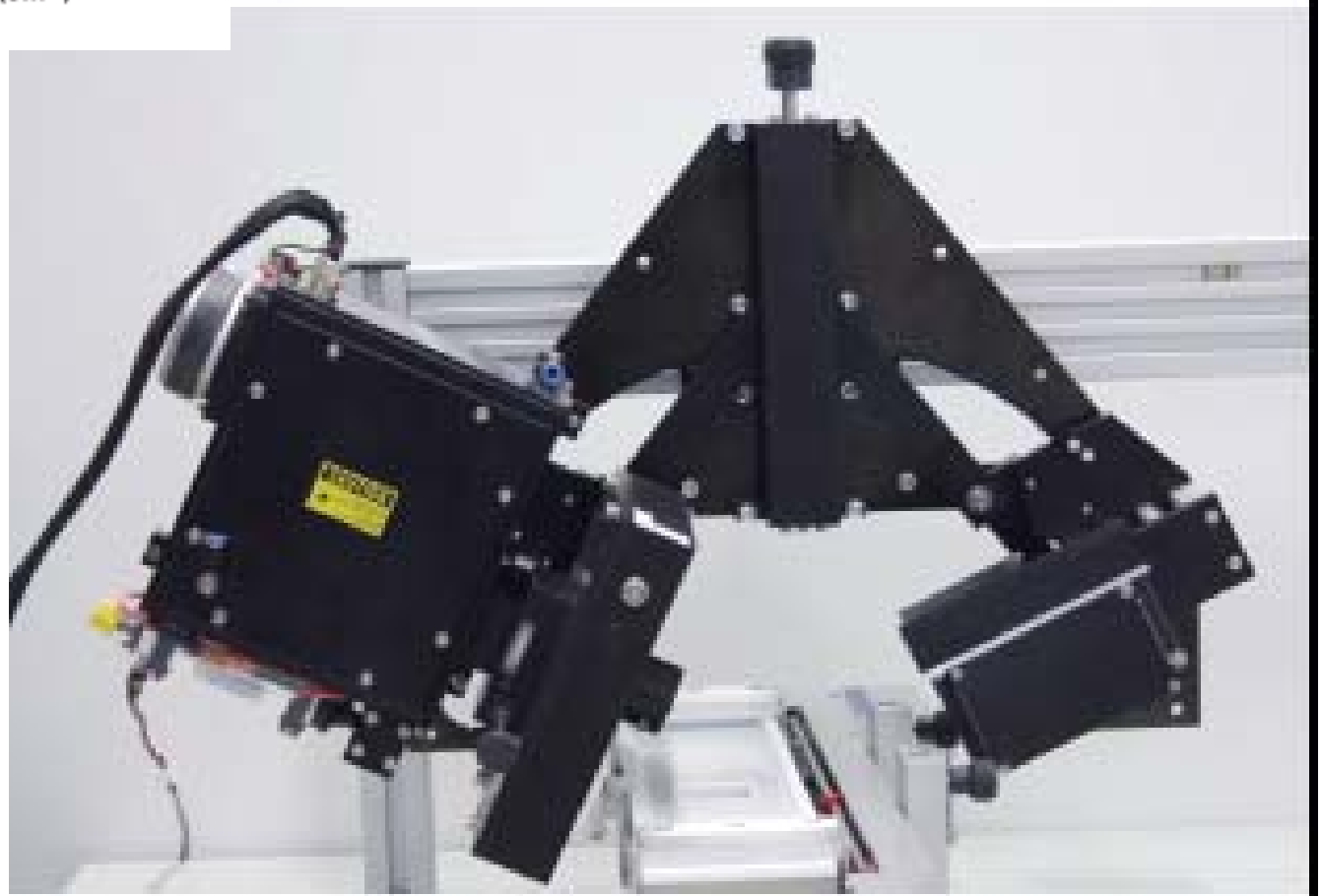
KSV PMI 550 – PM-IRRAS

This one goes right on to the LB trough

Mini FTIR mount on the goniometer arm



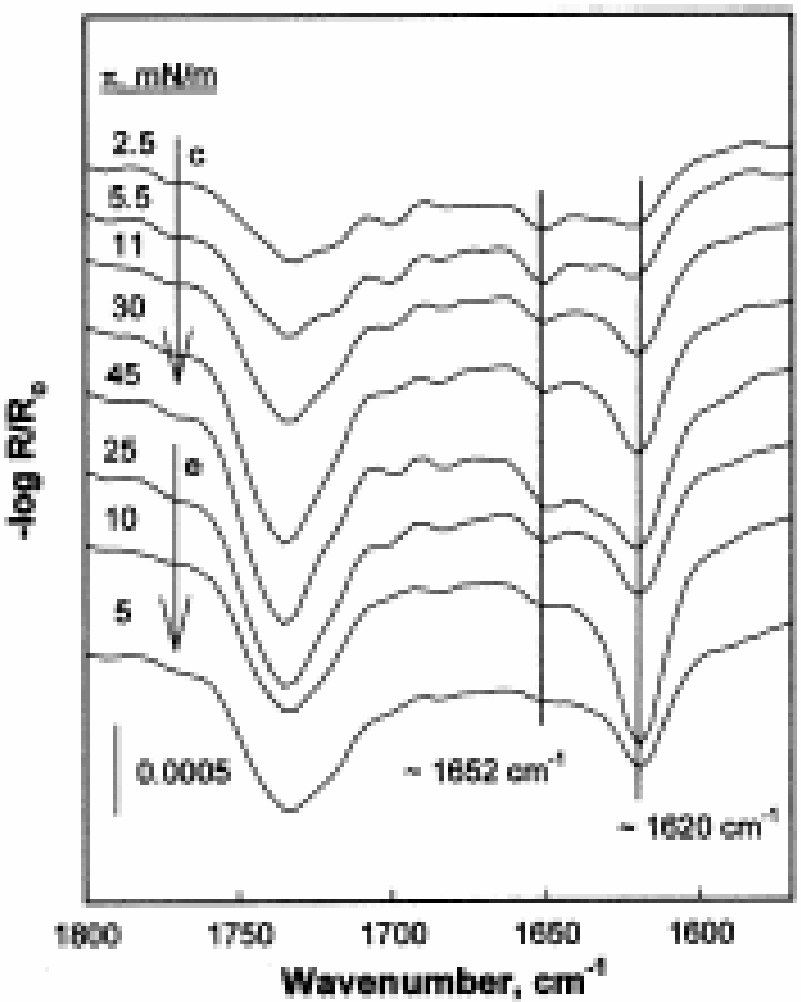


FIGURE 2: IRRAS spectra of the lipid carbonyl and peptide amide I region (1550−1800 cm^{-1}) for a mixed film of DPPC with 5 mol % KL_4 on a D_2O subphase. Spectra were acquired using s-polarization, and surface pressure values are noted from top to bottom during compression (c) and expansion (e) of the film. The angle of incidence was 50°.

IRAS of $(KL_4)_4K$ on an aqueous phospholipid DPPC monolayer

Cai, Flach, Mendelsohn

Increase surface pressure and sheet component appears
Helical component less variation

An Infrared Reflection−Absorption Spectroscopy Study of the Secondary Structure in $(KL_4)_4K$, a Therapeutic Agent for Respiratory Distress Syndrome, in Aqueous Monolayers with Phospholipids†

Lipid-gramicidin at air-water interface

-polarized IRRAS indicate orientation

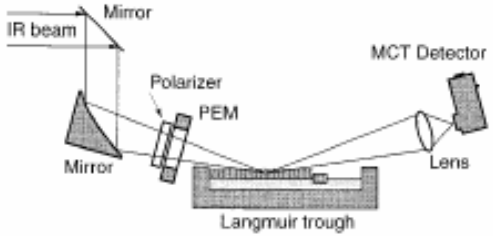
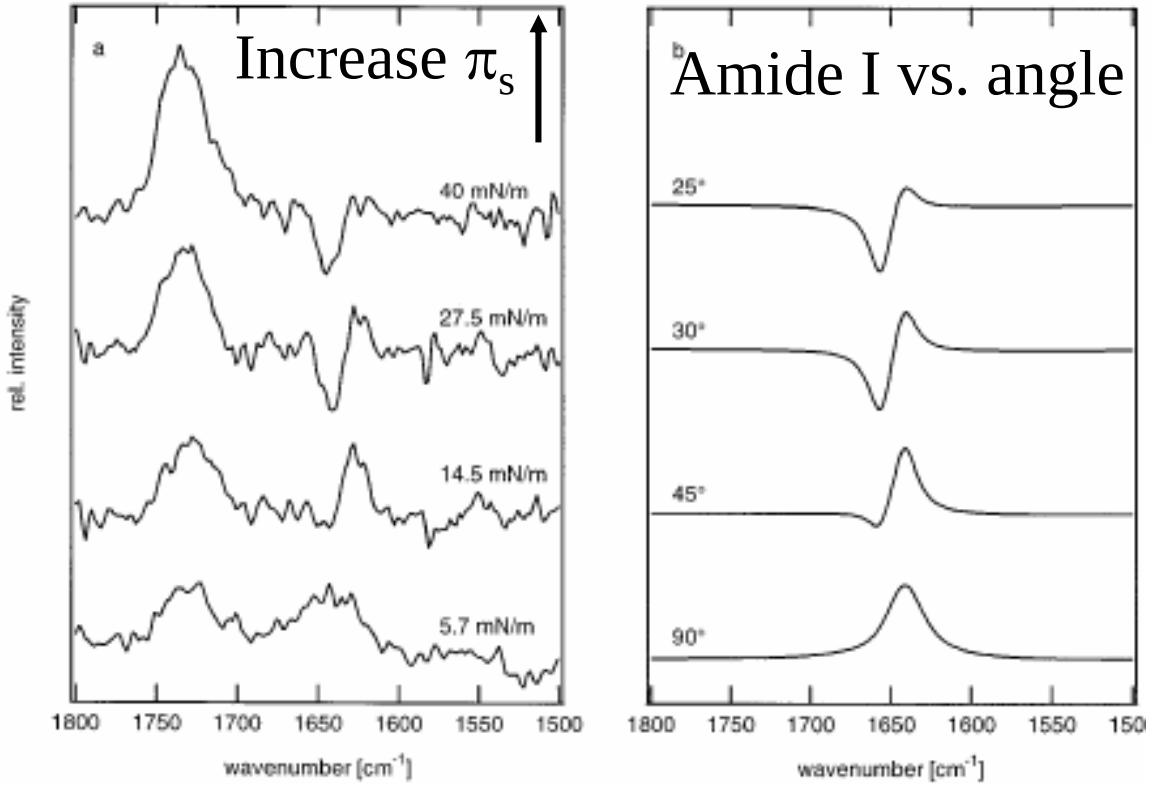
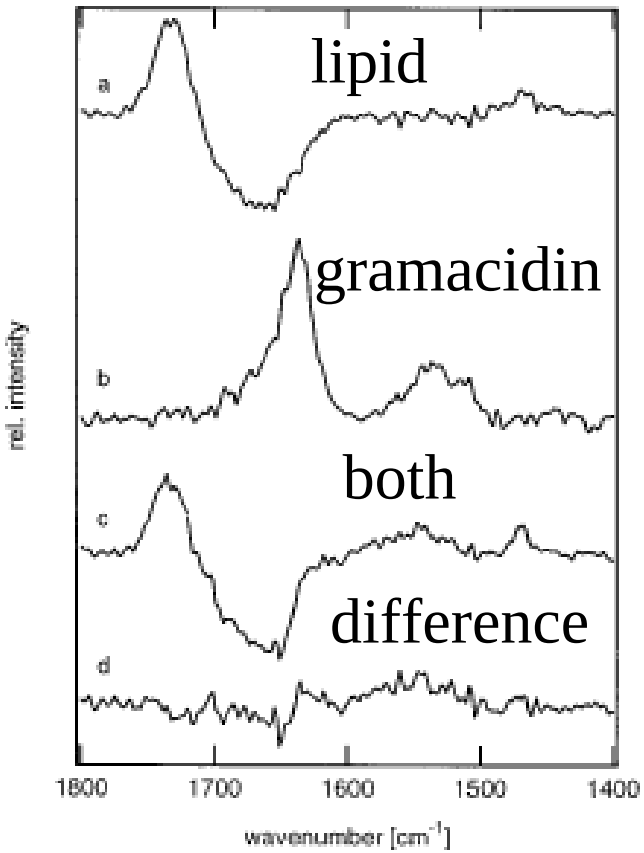


FIGURE 1 Scheme of the PM-IRRAS set-up. The optical pathway encloses several plane mirrors, one off-axis parabolic mirror, a BaF_2 wire grid polarizer, a photoelastic modulator, a ZnSe lens, and a MCT detector.



7 (*a*) PM-IRRAS spectra of a DMPC/gramicidin A layer (8:1 molar ratio) at the indicated film pressures on a D_2O subphase. AS spectra of the amide I band of a DMPC/gramicidin A layer (8:1 molar ratio) at the indicated tilt angles, θ, on a D_2O subphas



Polarization-Modulated FTIR Spectroscopy of Lipid/Gram Monolayers at the Air/Water Interface

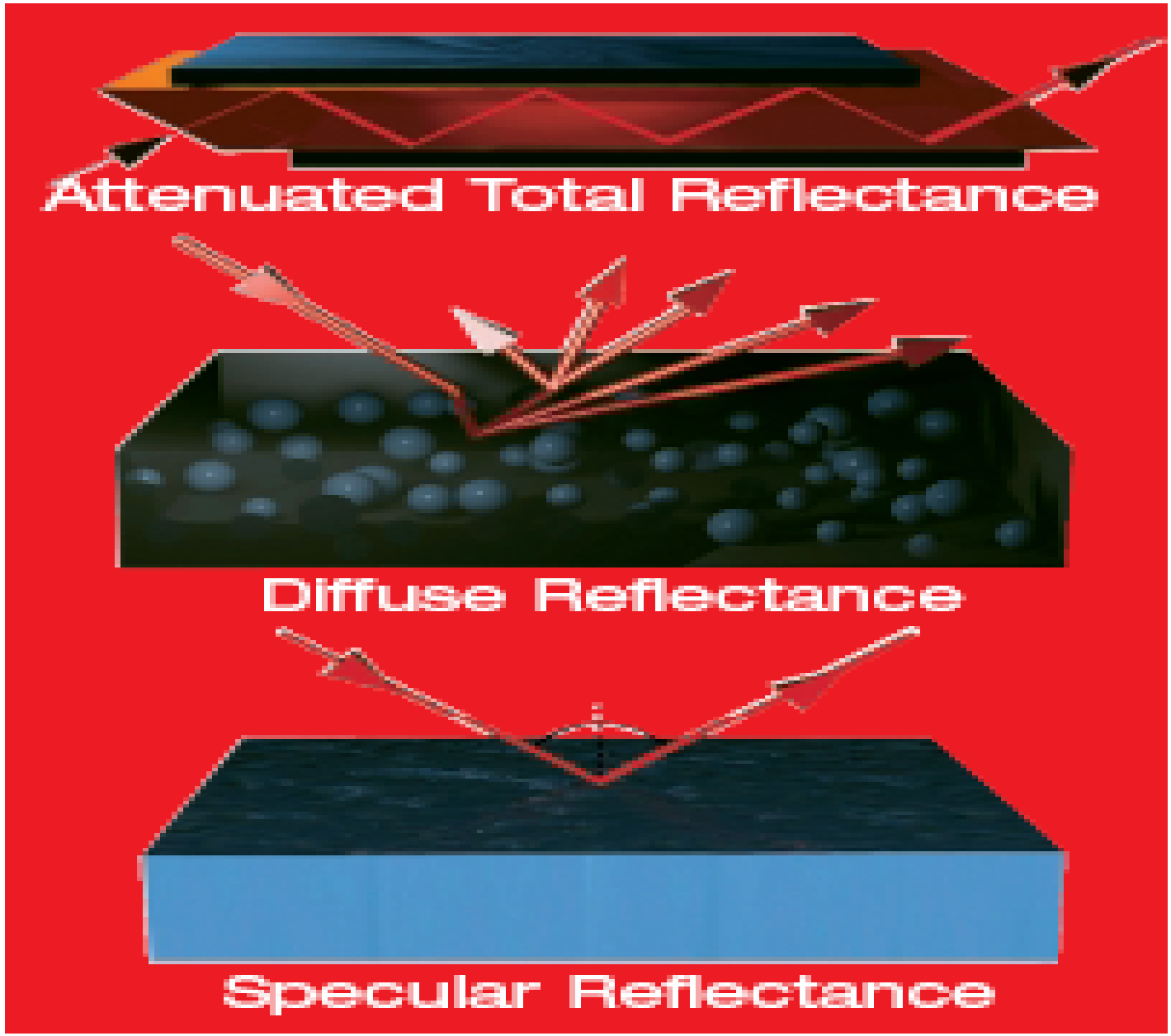
Wolf-Peter Ulrich and Horst Vogel

IGURE 5 PM-IRRAS spectra taken at the air/H_2O interface of (*a*) ure DMPC monolayer at 30 mN/m, (*b*) a pure gramicidin layer at N/m, (*c*) a DMPC/gramicidin A layer (8:1 molar ratio), and (*d*) weight btraction of spectrum *a* from spectrum *c* to eliminate $\delta(H_2O)$.

SOLID STATE IR Sampling:

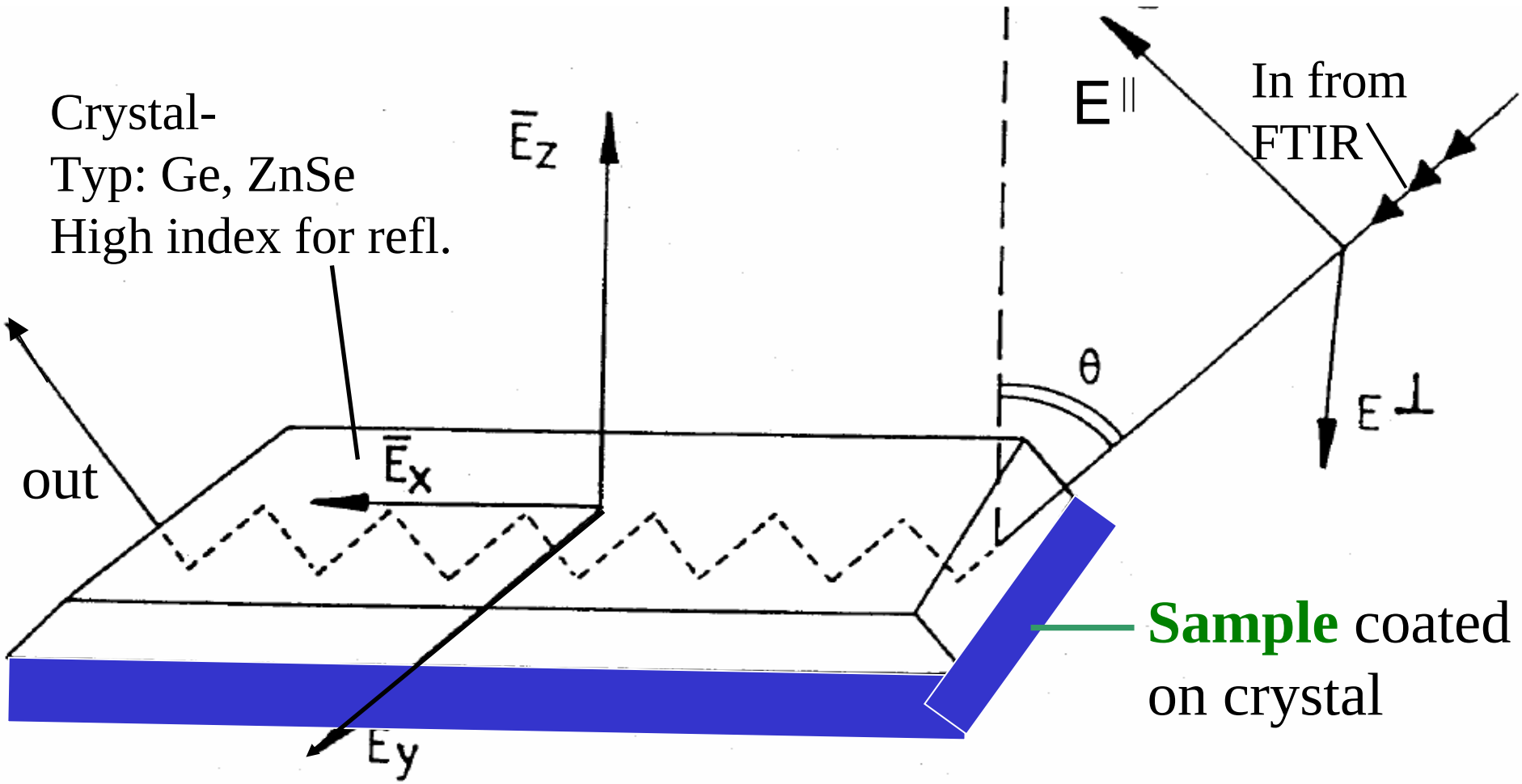
- IR spectroscopy is the most flexible method capable of studying analytes in the solid state
- Solid state spectra often means little sample prep, data can be collected in several ways:
  - KBr pellets
  - Deposited film in transmission
  - Diffuse reflectance
  - Attenuated Total Reflectance (films and solution)
  - Film studies can encompass membranes and mixed systems, membrane – peptide interactions and orientations
  - In Biology, can even study tissue and cells by using microscopy for imaging

Reflectance methods



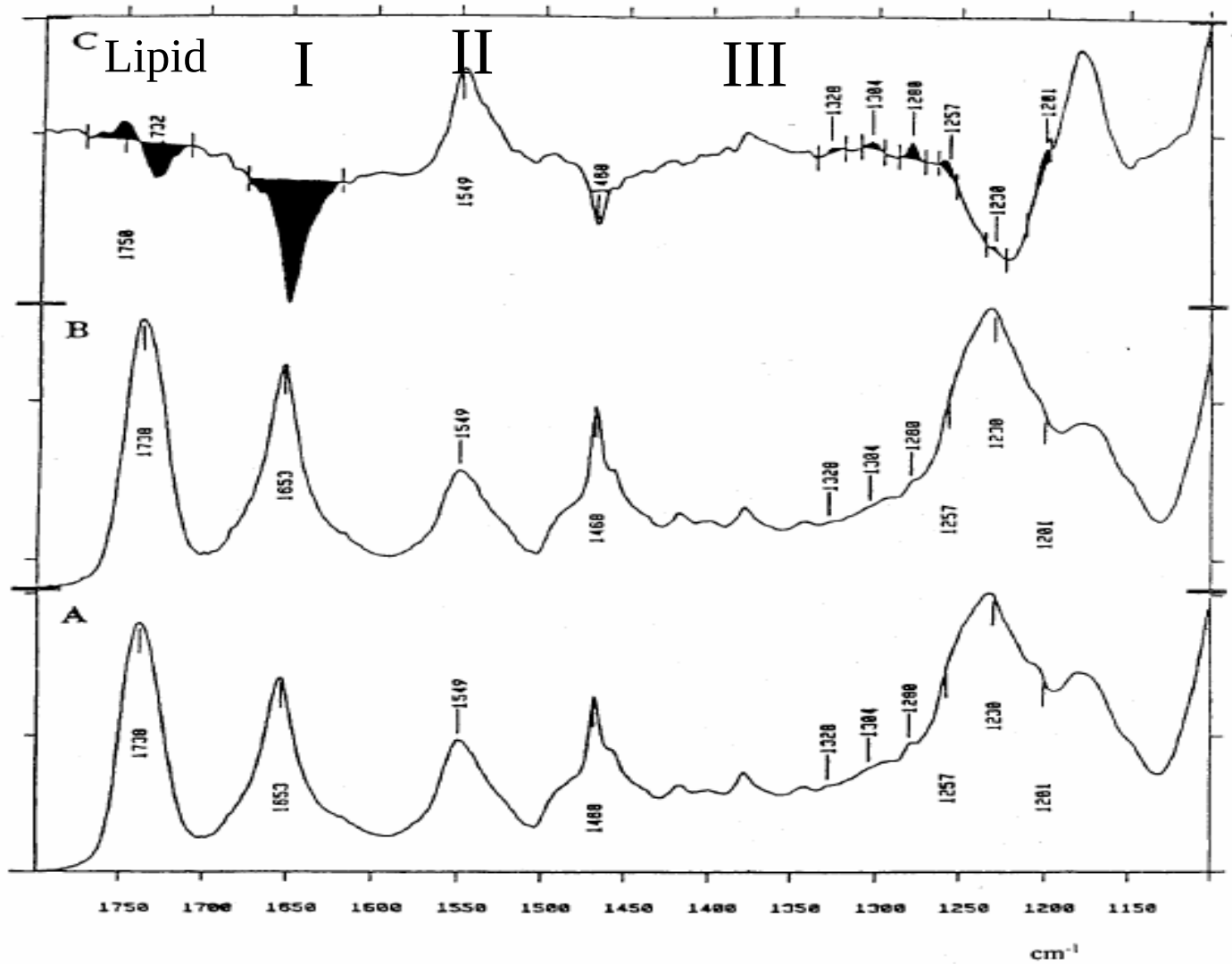
ATR Polarization Measurements

IR beam multiply reflects *inside crystal -- penetrates surface keeps polarizations: E_{perp} in surface, E_{para} partially out*



Internal reflection element for ATR

Linear dichroism of a protein in membrane



$A^{\parallel} - A^{\perp}$

$A^{\perp}$

$A^{\parallel}$

Figure 4 : IR spectra of the apoLp-III-DMPC complex. Spectrum A was obtained with 90° polarized light and spectrum B with 0° polarized light,

ApoL-p-III-DMPC complex – Goormaghtigh and co-workers, ACS Symp. 2000

Possible orientation of a helical peptide in a membrane

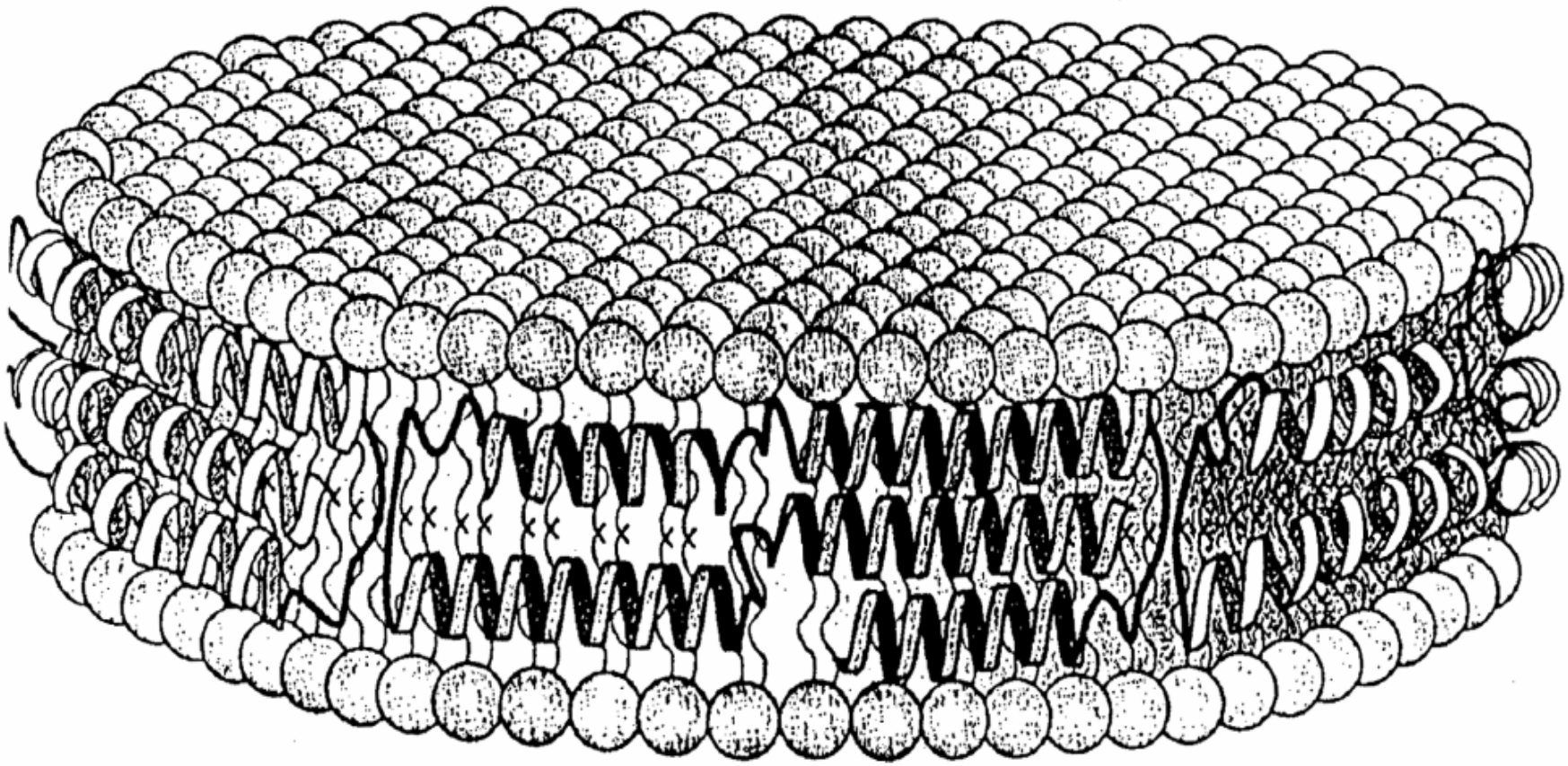


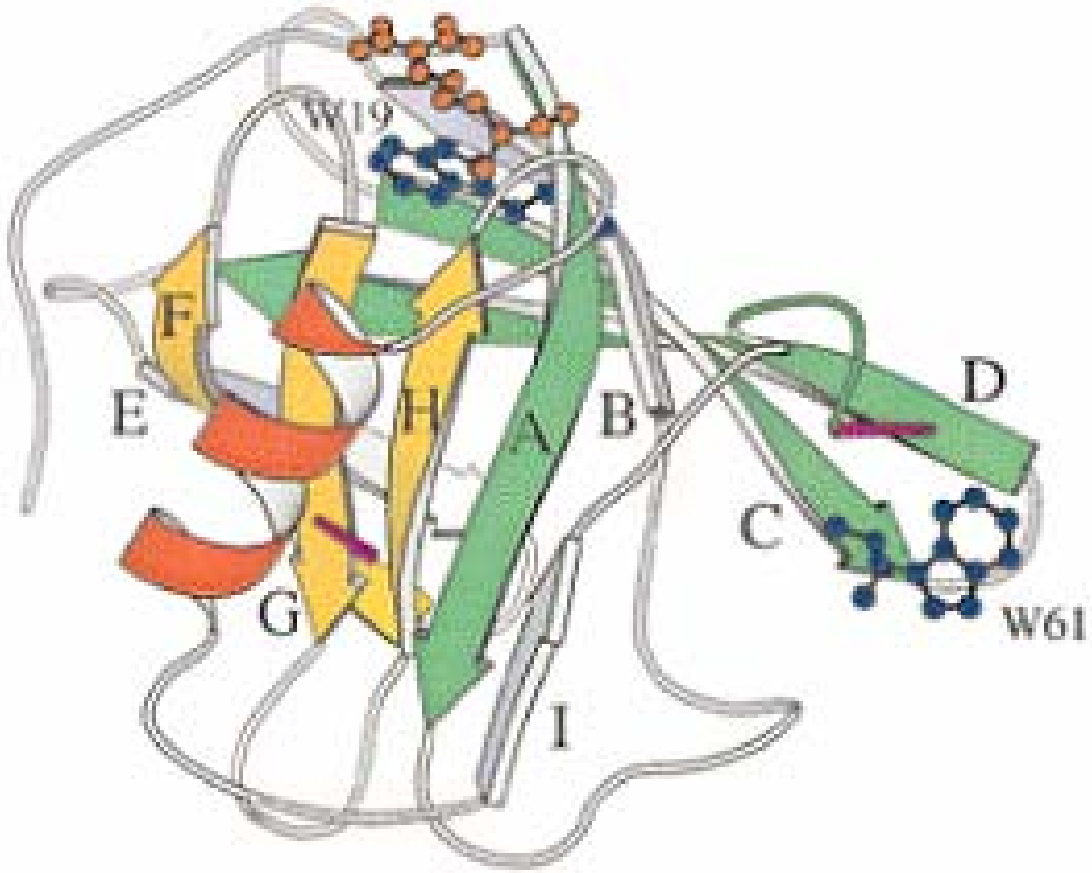
Figure 3 : Hypothetical representation of apoLp-III:DMPC complex

In this situation, helix dipole (and amide I) polarized parallel to surface

– Goormaghtigh and co-workers, ACS Symp. 2000

β-lactoglobulin: a protein that goes both ways!

Red, α-helix;
green, regions to have helical propensities;
purple, disulfide bonds
Trp19 & 61 are indicated



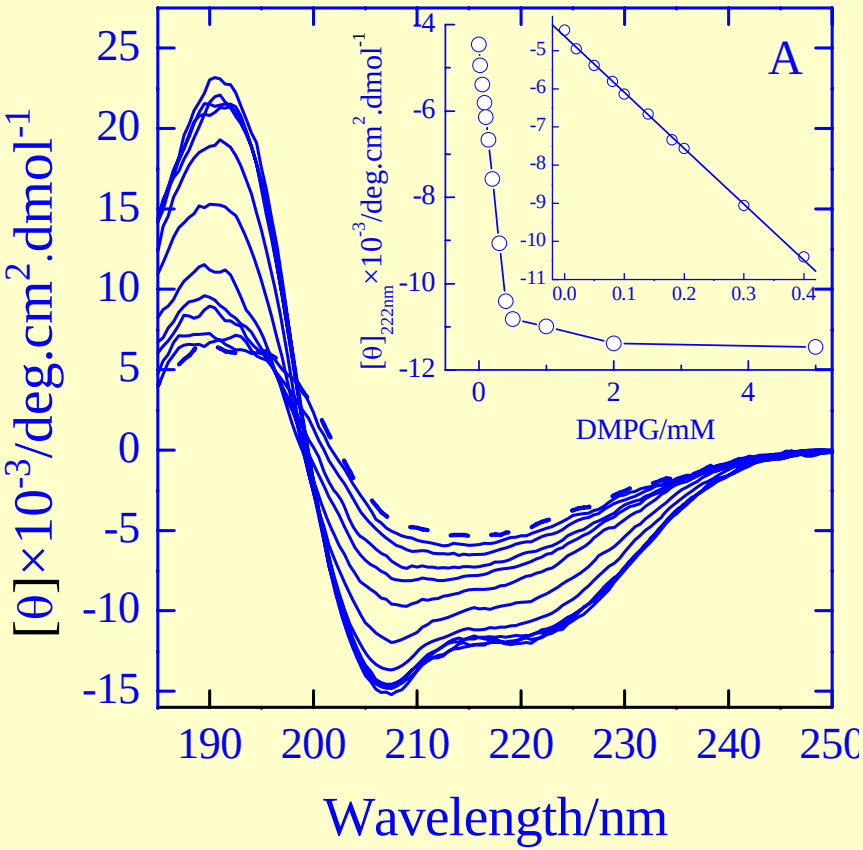
Native state: β-sheet dominant, but high helical propensity.

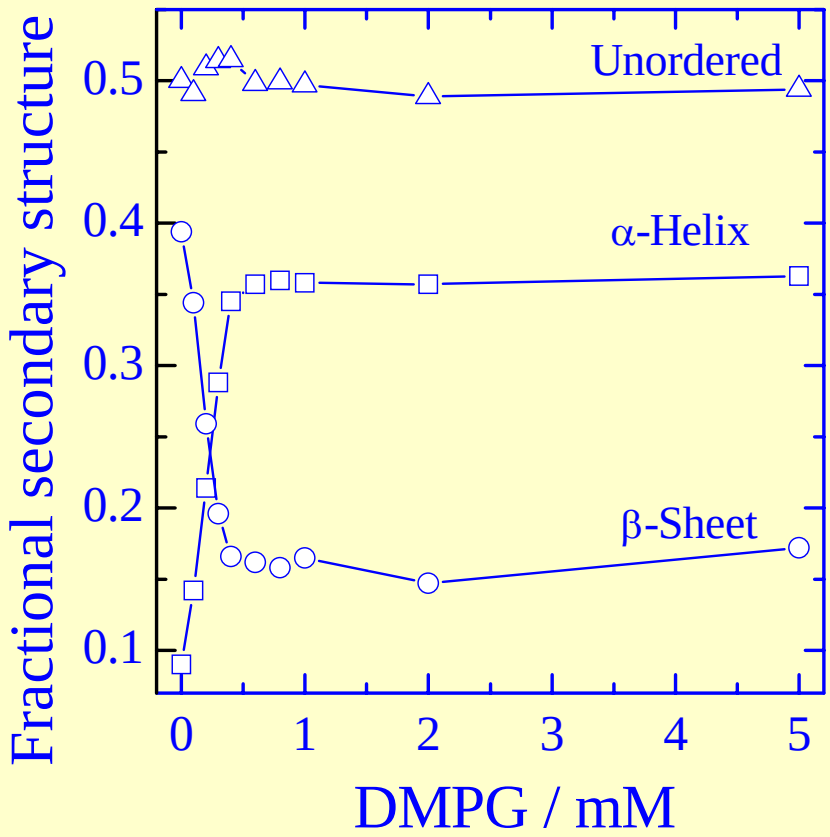
Model: intramolecular β↔α transition pathway as opposed to folding pathways from a denatured state.

Zhang & Keiderling, Biochemistry 2006

Lipid-induced Conformational Transition β-Lactoglobulin

1. DMPG-dependent β→α transition at pH 6.8

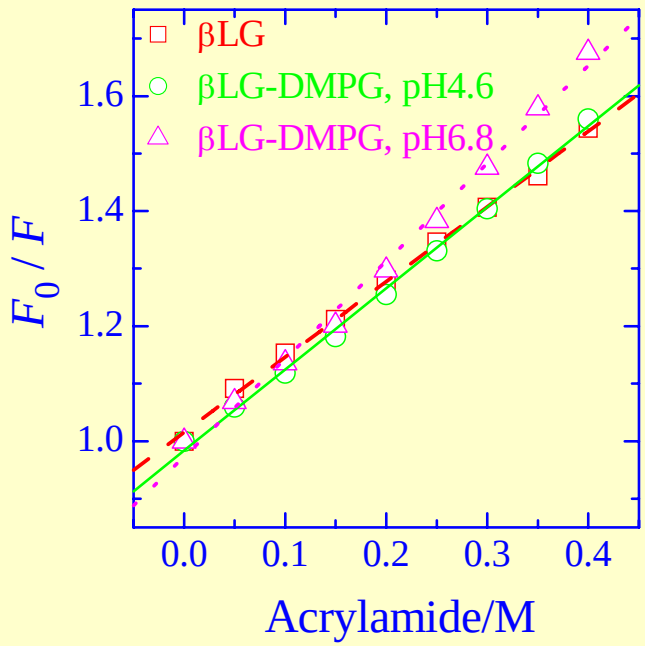




Zhang & Keiderling, Biochemistry 2006

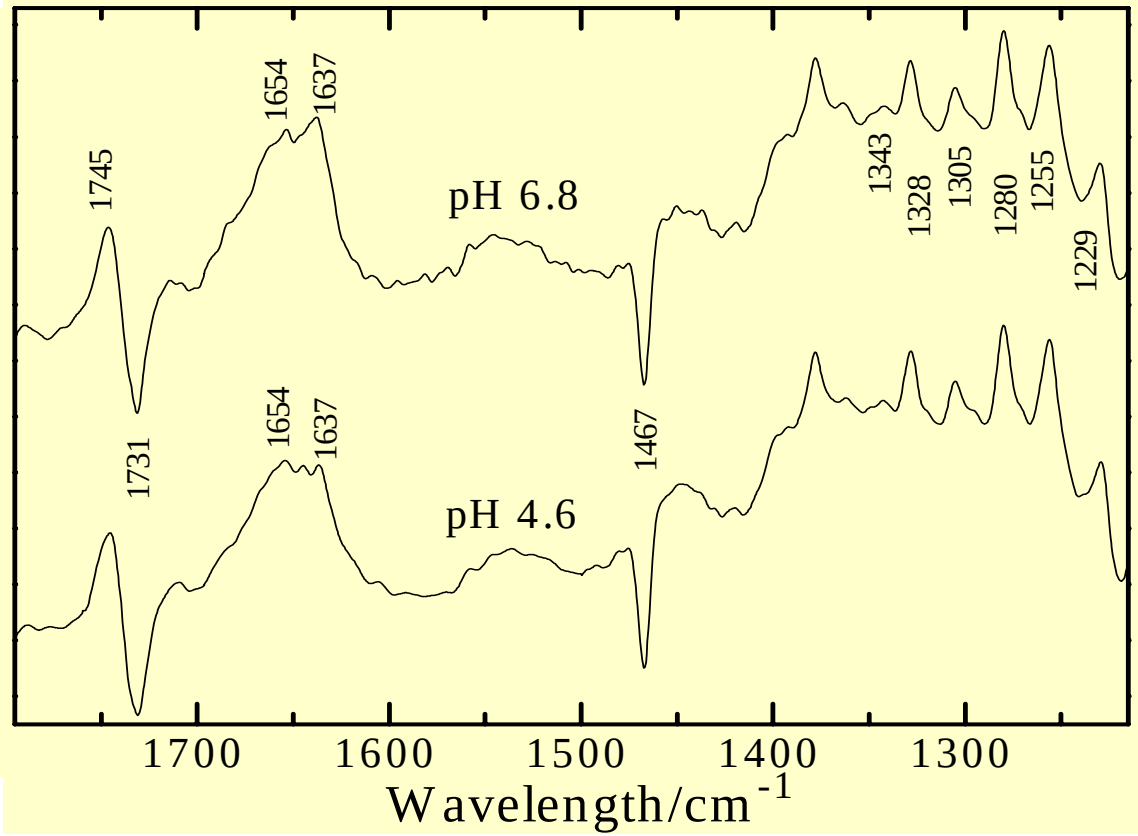
Lipid bilayer insertion of β-Lactoglobulin

Fluorescence quenching



At pH 6.8 & 4.6, 4 & 6 nm blue shift in λ_{max}.

ATR-FTIR orientation



α-helix ⊥Membrane surface

Zhang & Keiderling, Biochemistry 2006