CHEM 524 -- Course Outline (Part 14) Absorption Spectroscopy — update 2011

For an html version of 2005 notes, click here

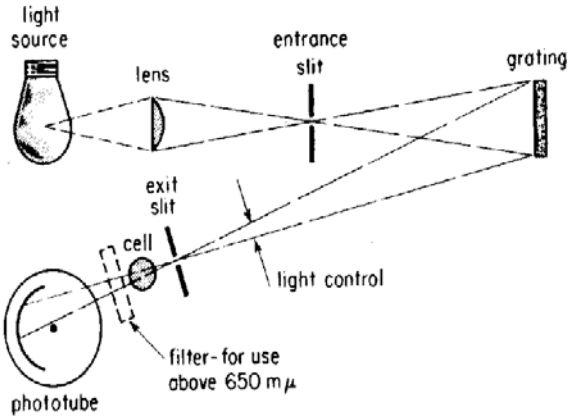
IX. Absorption Spectroscopy UV-VIS-near IR (Read Chap. 13)

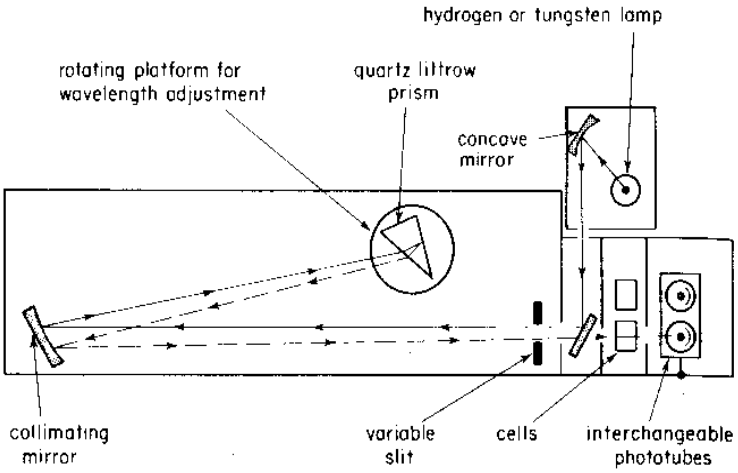
the most widely used analytical spectroscopic method

A. Example instruments--commonality--similar optics/detector/sources cover overlapping regions, make for multi-use instruments

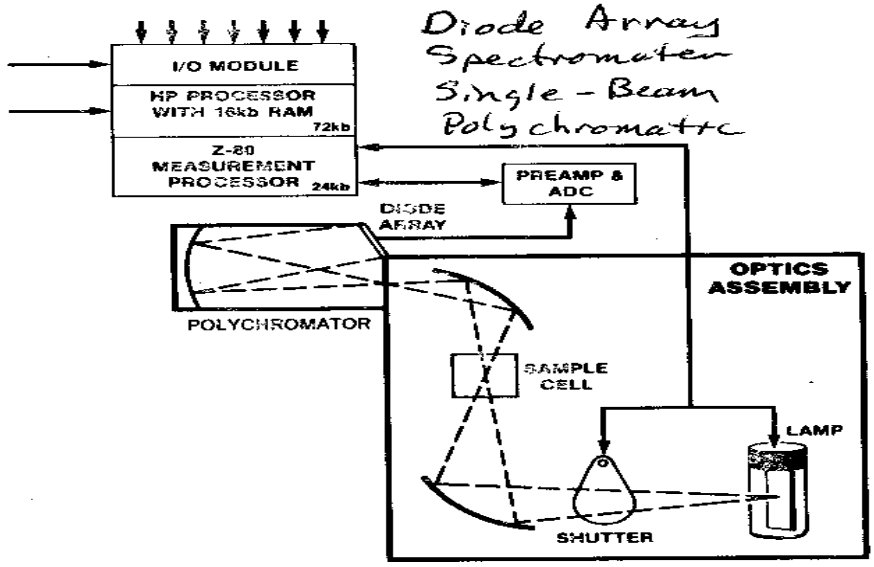
1. Single beam (very old designs) –

Spectronic 20, visible region, non-scan -- Beckman DU (prism) -- uv-vis (high throughput, glass optics, W-source, test tube cell prism), original non-scan - fix wavelength





2. Multichannel single beam (modern approach, fast, few moving parts, open sample area, low noise, get full spectrum, fixed resolution)-- diode array, HP5481 (original from H-P)

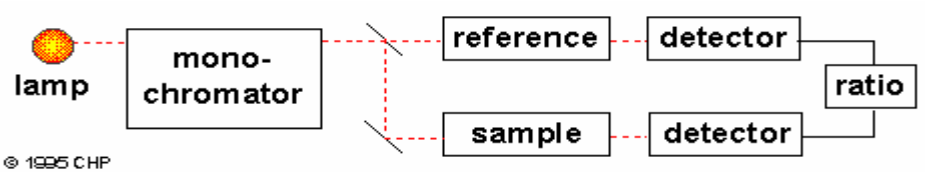


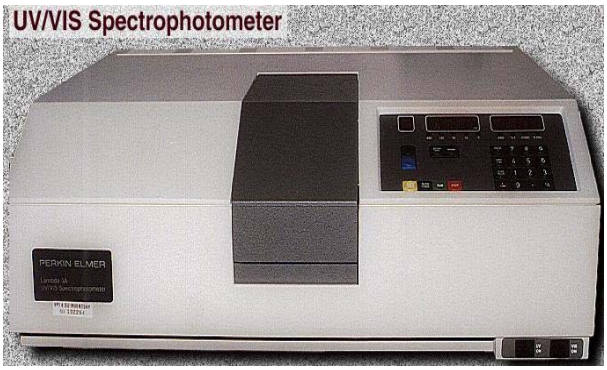
Now HP is Agilent, also has other spectroscopy lines (former Varian) and updated design

Full range 190 – 1100 nm, 1.5 sec scan - 1nm res.– two lamps, D_2 and W-I (qtz)

Agilent: HP 8453 ~$11,500 HP 8452 (older model)

3. Dual beam (auto. baseline corrected, abs. converted, dual in time)—

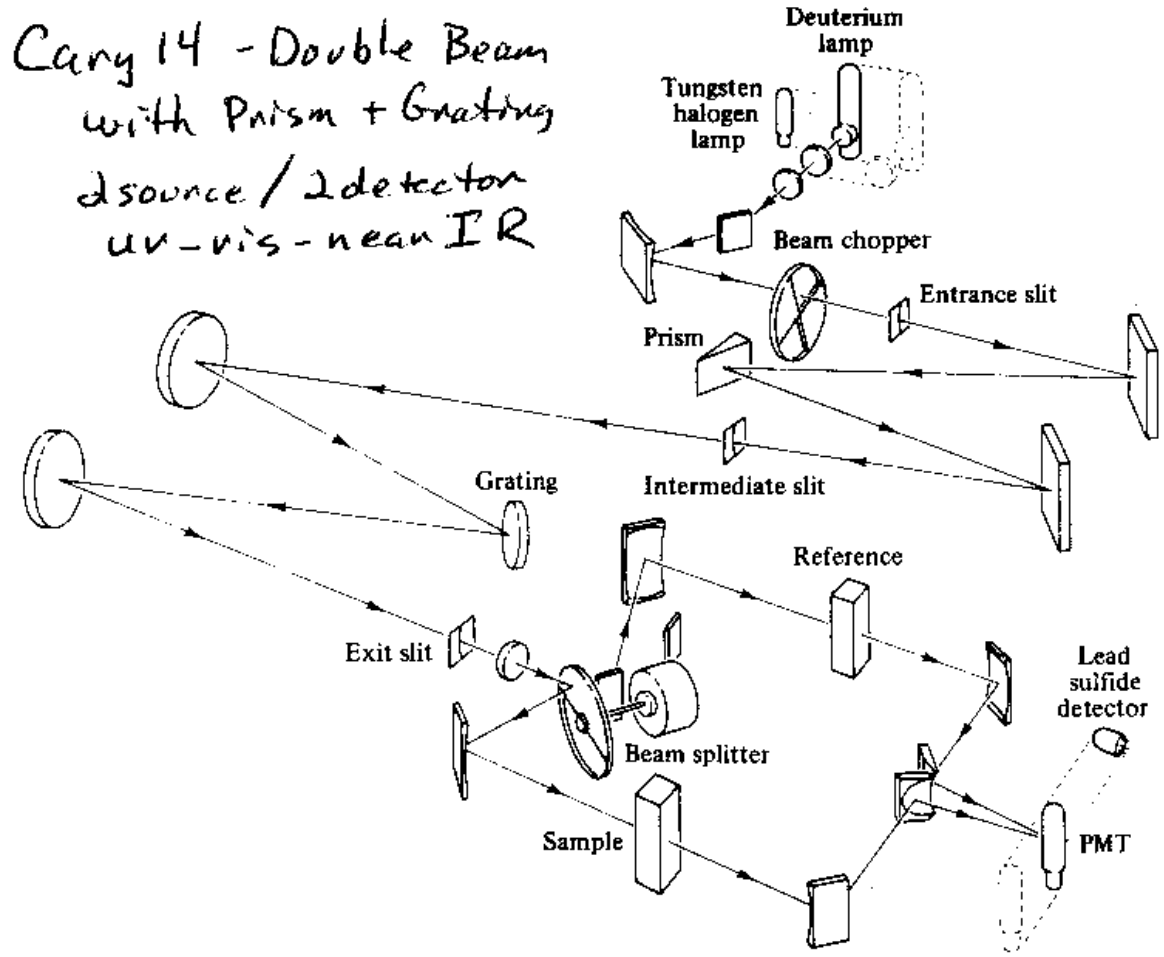




Various size and shape, generally simple, two detectors not required if beam split

Cary 14/17 – old design, most complex, precise – rotating sector wheel does beam split

Two source (UV & vis-NIR), two detector (UV-vis & NIR), two monochromator (prism &grating)

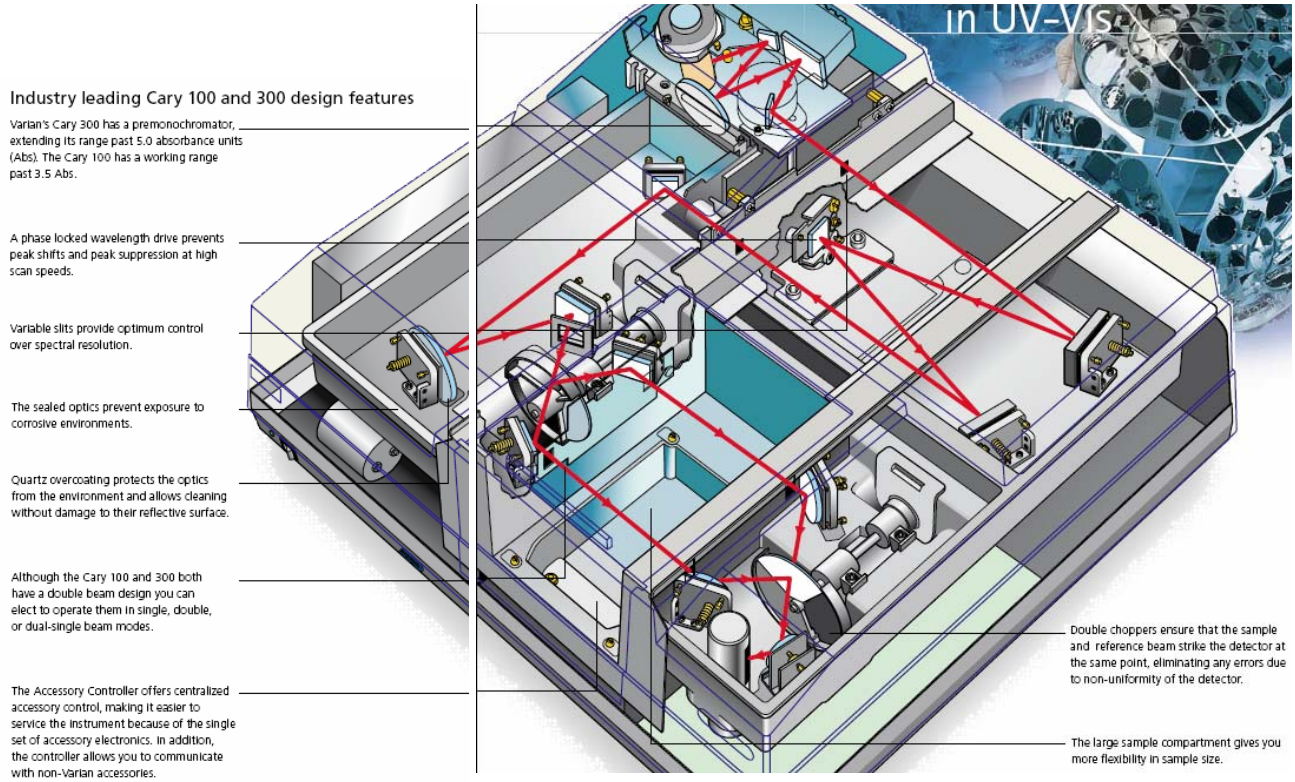


Black one is original, Cary 14 (or 11),

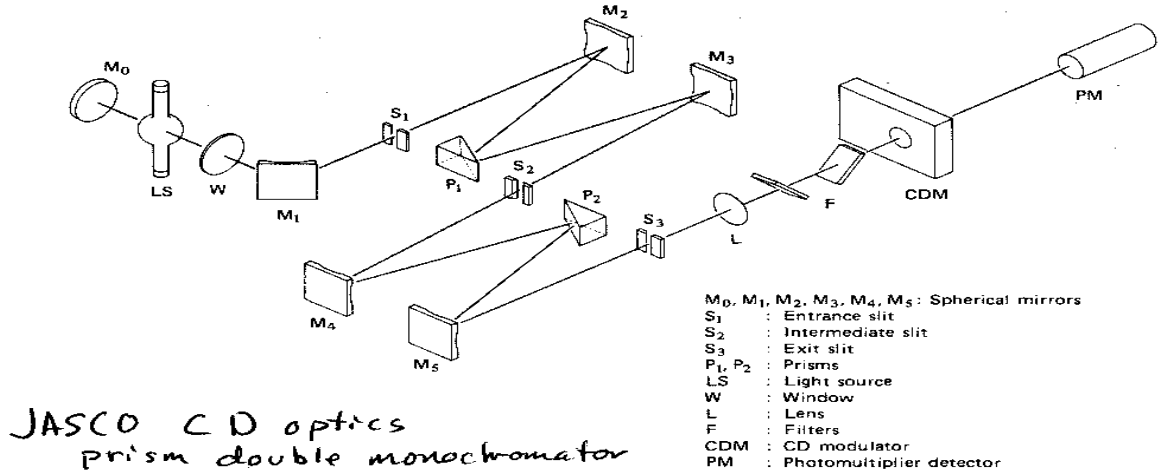
above - OLIS modified Cary 14 - add computer data acquisition

Modern Cary – line from Varian (now Agilent) - see below

- more compact – no prism
- many accessories, specialized versions

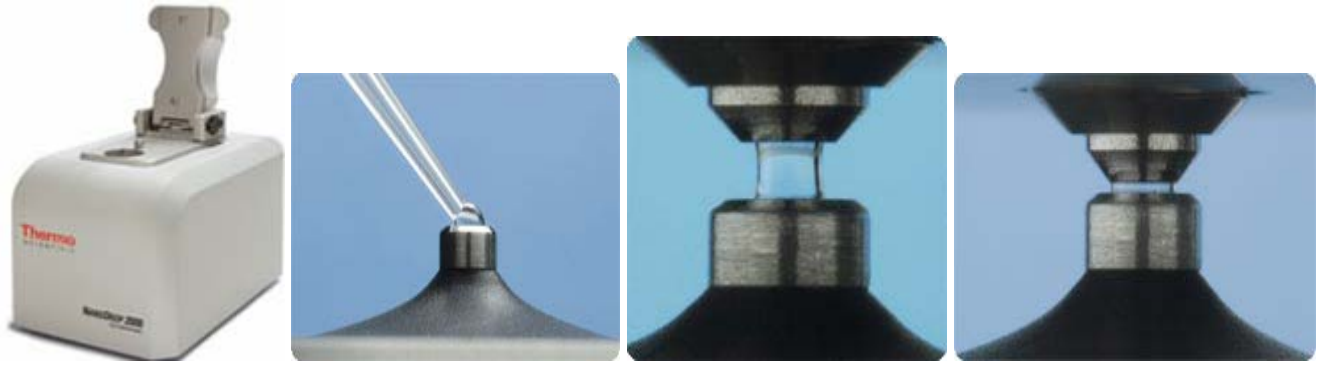


4.Differential (output ΔA, amplify the modulated spectrum)--



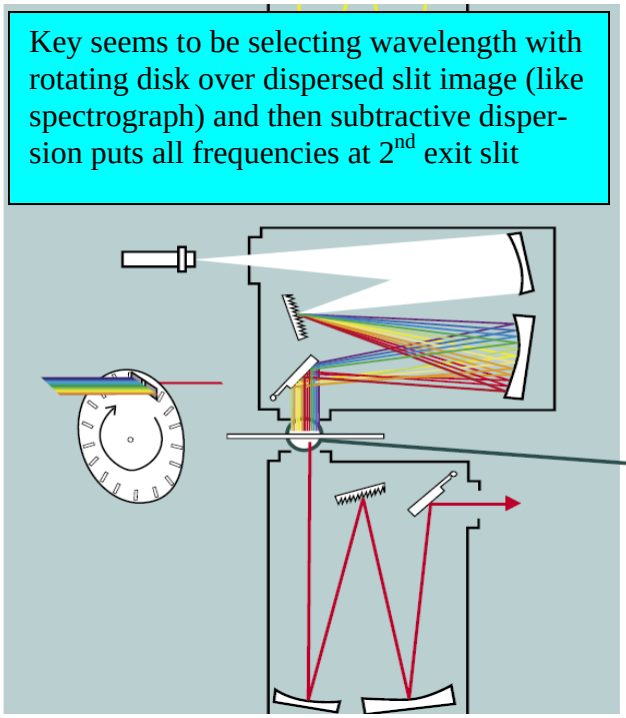
JASCO CD optics
prism double monochromator

Nano drop – no sample cell, 2 paths, new approach, uses CCD and low res monochromator, full spectra

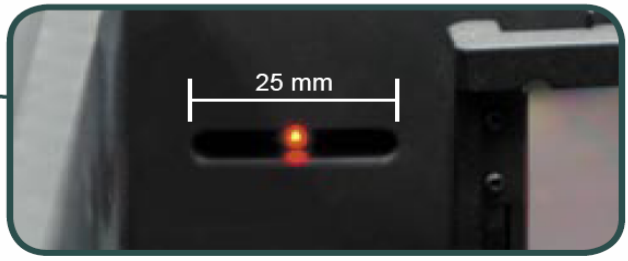


Time dependence, rapid scan – OLIS RSM – a unique approach

Key seems to be selecting wavelength with rotating disk over dispersed slit image (like spectrograph) and then subtractive dispersion puts all frequencies at 2nd exit slit

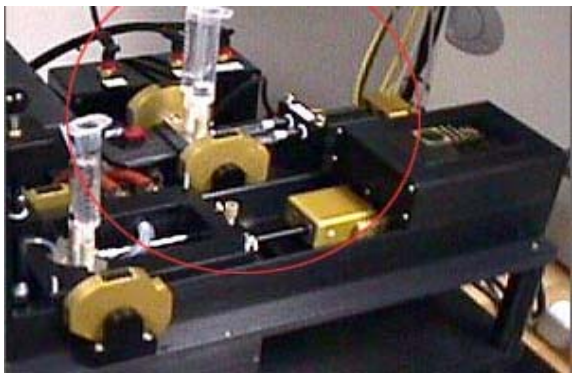


The ScanDisk has 16 slits and spins at 62.5 Hz to achieve the 1000 scans per second.
(16 * 62.5 = 1000)
Slits are spaced to allow a 50 microsecond dark period between each scan.

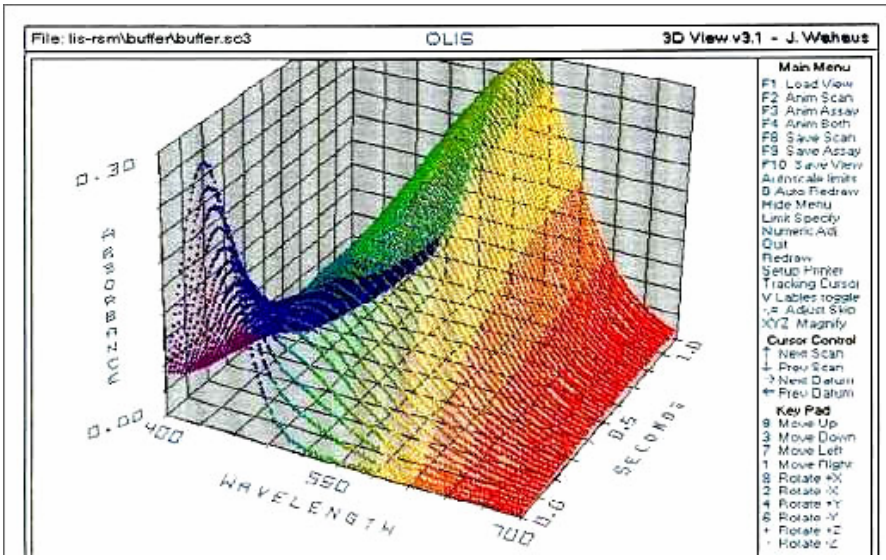


[1] US Patent 5,285,254, issued February 8, 1994.

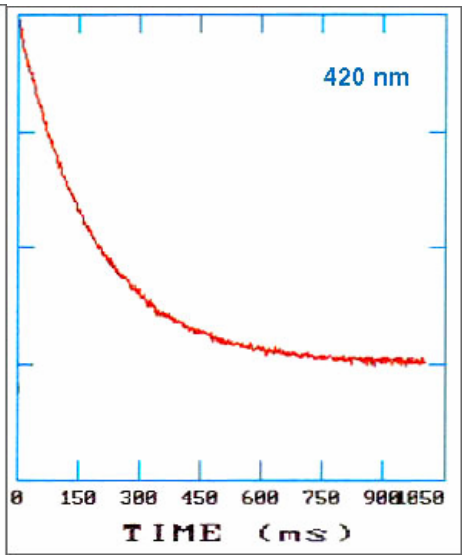
Setup of monochromators with readout/input



Stop-flow mixer sampling uses millisec response



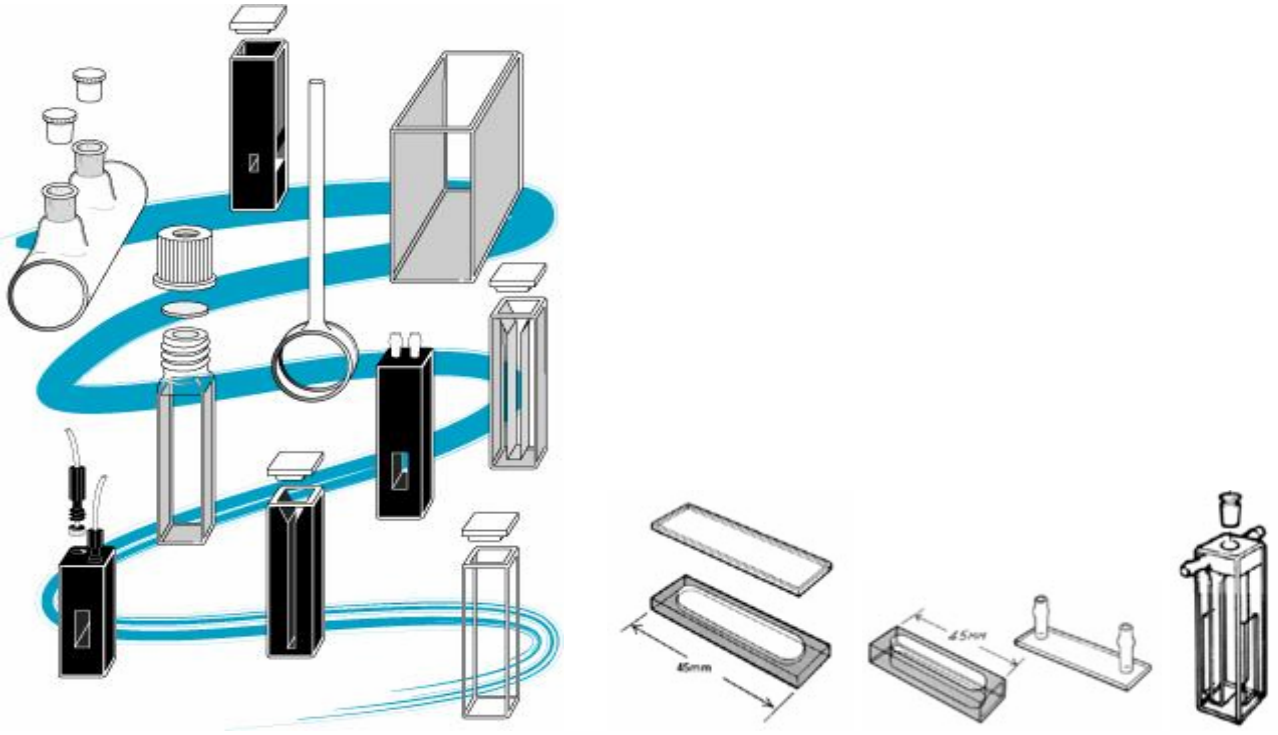
Data represented as 3-D plot, spectra vs. time – more information



Alternative, response at λ vs. time

B. UV-vis-NIR Sampling--quartz cuvettes/ also disposable/ many solvents/

i. DL limitation ($A > 10^{-5}$ possible), $A = \varepsilon bc$, so low ε or c, use longer path



lots of shapes and sizes, constrain volume, demountable for short path, flow thru, water jacket

C. Noise sources

--blank--shot noise unimportant, high light level, flicker due to sampling is a problem

--sample--low absorb--flicker, high absorb shot plus dark noise dominate

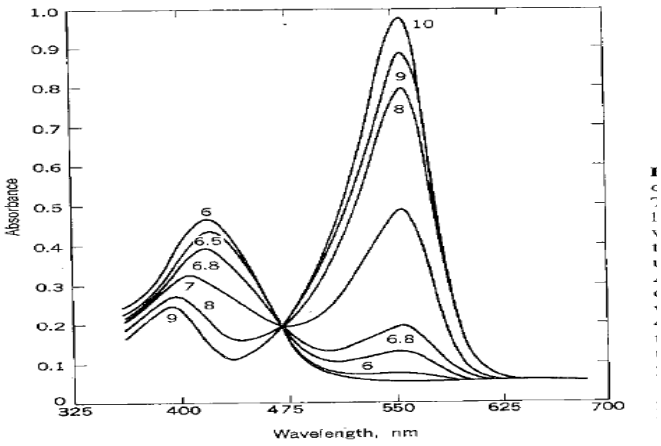
D. Lambert-Beer Law and deviation from linearity

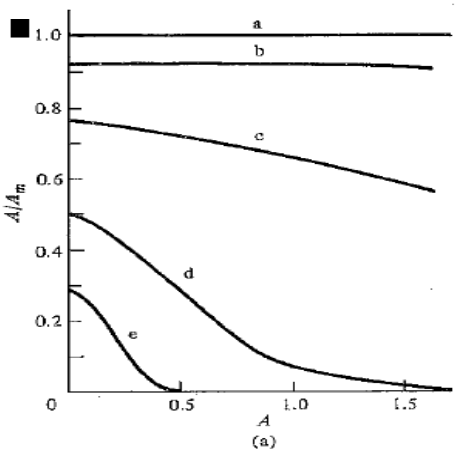
---non-zero intercept - blank off (cell or matrix match problem)

---Chem equilibrium, vary conc.

- Measure at isosbestic point - below

  or over region of ~constant absorbance for both species





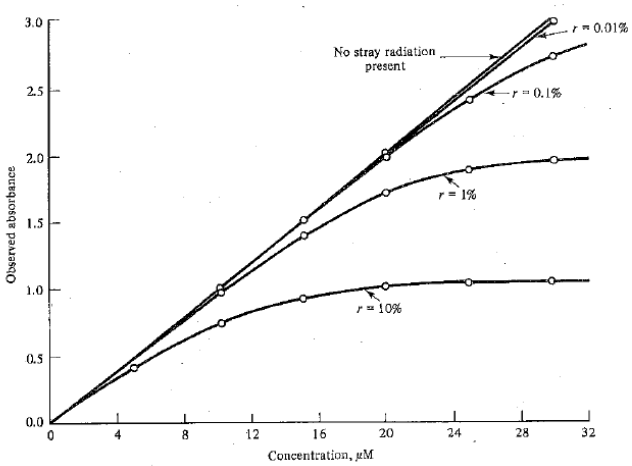
polychromatic radiation, slit width is non-zero, measure at top of band, A ~ const.

Slit effects: plot A/A_m vs. A at various $s/\Delta\lambda$ values (Fig. 13-14, above, right) general,

$s/\Delta\lambda$ = 0, 0.43, 0.85, 1.70, 2.55 for a,b,c,d,e – see broad band ($\Delta\lambda$) not issue

but Fig 13-15, ex. Pr sol'n—narrow bands biggest effect – $s/\Delta\lambda$ large

Minimize error: $s < 0.1\ \Delta\lambda$ at λ_m for A±0.5%



Stray radiation factor: $r = E_{SR}/E_R$

apparent transmittance increased,

absorbance reduced:

$T' = (T+r)/(1+r) \sim T+r$

Fig 13-16 demonstrates:

E. Sampling Problems

---Non parallel beam (path vary, effective ε OK if cell fixed), A too high

OK, if exact same for refer. and sample

---Multiple reflection, internal to cell, over est. A (effective longer path):

$\Delta A = 0.434\ F(1-T^2)$

F-fraction multiply reflected

-- Fluorescence, normally negligible unless very weak source or high A, like stray light

---Detection limit, re-express signal error in conc. $DL = ks_{bk}/\varepsilon b$

F. Applications Ch 13-5 -- read

Concentration determination using Beer-Lambert law, equilibrium standard

Multicomponent Quant. Anal.- if separate, easy, solve simultaneous equations

If overlap, use band shape and PCA or PLS – project out components

Methods – equilibrium analyses, determine ε for reagents and products,

then mix and measure reaction, ratioing components (overlap issue)

Stoichiometry, titrate "ligand" - measure A vs. c_L/c_M, curve breaks at ratio binding

Kinetics, direct -- follow absorbance vs. time, need to sort reagent from product

Chromatography – use filters or monochromator, need high extinction for short path

Reflectance –samples that do not transmit, convert reflect to absorb → Kubelka-Munk

Differential absorbance (dichroism) -- CD, LD – modulate polarization, detect difference

G. Reading Specification Sheets -- seek out important characteristics

--compare instruments on same basis, need to see how establish noise, dyn range etc.

Comparison of instruments, one company, Varian on Web just gives verbal discussions, need to contact directly to get real data for comparison:

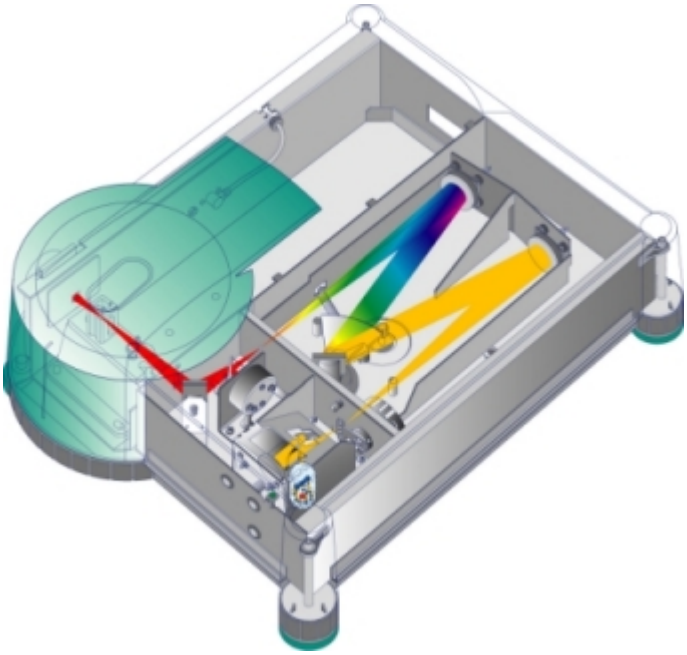
{note these are direct copies for you to compare, not my opinions!!}

Cary 50 UV-Vis spectrophotometer design *– good for kinetics*

The Cary 50 instrument relies upon a simple design with only 4 moving parts, a Xenon flash lamp and electronics contained in the PC.

The Xenon lamp has a long-life of the. And the Cary 50 is only on when the PC is switched on. Fixing electronics problems can be changing the board in the PC, controlled by the Cary WinUV software.

The Cary 50 is unaffected by room light. You can operate with the sample compartment open or closed,. So, if your sample won't quite fit in the sample compartment, you can leave the lid off. If you want to keep dust, dirt and room light out of the sample compartment, then you can use the lid.



By incorporating a Xenon flash lamp, Varian's Cary® 50* offers many key advantages over traditional UV-Vis spectrophotometers:

• The maximum scan rate is 24,000 nanometers (nm) per minute. That means you can scan the whole wavelength range of 190–1100 nm in less than 3 seconds.
• With a data collection rate of an impressive 80 points per second, you'll have all the information you need about your kinetics assay.
• The Varian Cary 50 can measure samples up to 3 Abs so you won't have to dilute as often.
• The Xenon lamp has a very long lifetime—3 x 10^9 flashes actually. So even if you measure continuously, seven days a week, the lamp will last at least a year. With less frequent use, you may never need to change it. That makes the Varian Cary 50 inexpensive to run.
• The Varian Cary 50's super-concentrated beam makes it ideal for fibre optic work, offering excellent coupling efficiency and light throughput. And, its room-light immunity makes shielding of a fibre optic probe unnecessary.

*US Patent 6,002,477

Cary 100 UV-Vis spectrophotometer – *basic instrument*-design shown before on p.4

The Cary 100 spectrophotometer is a mid-priced instrument, for routine laboratory work.
The Cary 100 is controlled by the Cary WinUV software. This Windows based software features a modular design which makes it easy to use. The instrument is shipped with liquid sample holders and of course it can be fitted with a wide range of accessories to provide extra capabilities.

Cary 100 design features include:

- Quartz overcoating - protects the optics from the environment and allows cleaning without damage to their reflective surface
- Sealed optics - prevents exposure to corrosive environments
- Variable slits - allow optimum control over data resolution. The spectral bandwidth can be set down to 0.2 nm. (Many instruments on the market are limited to a fixed SBW of 2 nm)
- A working range past 3.5 absorbance units - Dilution of a sample, to bring it into a range that the spectrophotometer can measure, is therefore not required
- A phase locked wavelength drive - prevents peak shifts and peak suppression at high scan speeds.
- Double choppers - ensure that the sample and reference beam strike the detector at the same point, removing any errors due to non uniformity of the detector
- The large sample compartment - gives you more flexibility in sample size
- Centralized accessory control - all accessories are centrally controlled by the Accessory Controller, built into the Cary instrument. Instead of each accessory having their own electronics, the Accessory Controller provides the interface between the Cary software and the accessories.

Cary 300 UV-Vis spectrophotometer *–upgrade of above, what do you get?* **~$20+K (?)**

The Cary 300 is controlled by the new Cary WinUV software. This Windows based software features a modular design which makes it easy to use. The instrument is shipped with liquid sample holders and of course it can be fitted with a wide range of accessories to provide extra capabilities.

Cary 300 design features include:

- Pre-monochromator - extends the working range of the Cary 300 past 5 absorbance units by lowering the stray light levels. Dilution of a sample, to bring it into a range that the spectrophotometer can measure, is therefore not required
- Quartz overcoating - protects the optics from the environment and allows cleaning without damage to their reflective surface
- Sealed optics - prevents exposure to corrosive environments
- Variable slits - allow optimum control over data resolution. The spectral bandwidth can be set down to 0.2 nm. (Many instruments on the market are limited to a fixed SBW of 2 nm)
- Phase locked wavelength drive - prevents peak shifts and peak suppression at high scan speeds.
- Double choppers - ensure that the sample and reference beam strike the detector at the same point, removing any errors due to non uniformity of the detector
- The large sample compartment - gives you more flexibility in sample size
- Centralized accessory control - all accessories are centrally controlled by the Accessory Controller, built into the Cary instrument.

Cary 5000 UV-Vis-NIR spectrophotometer –*lot more money!*— include NIR

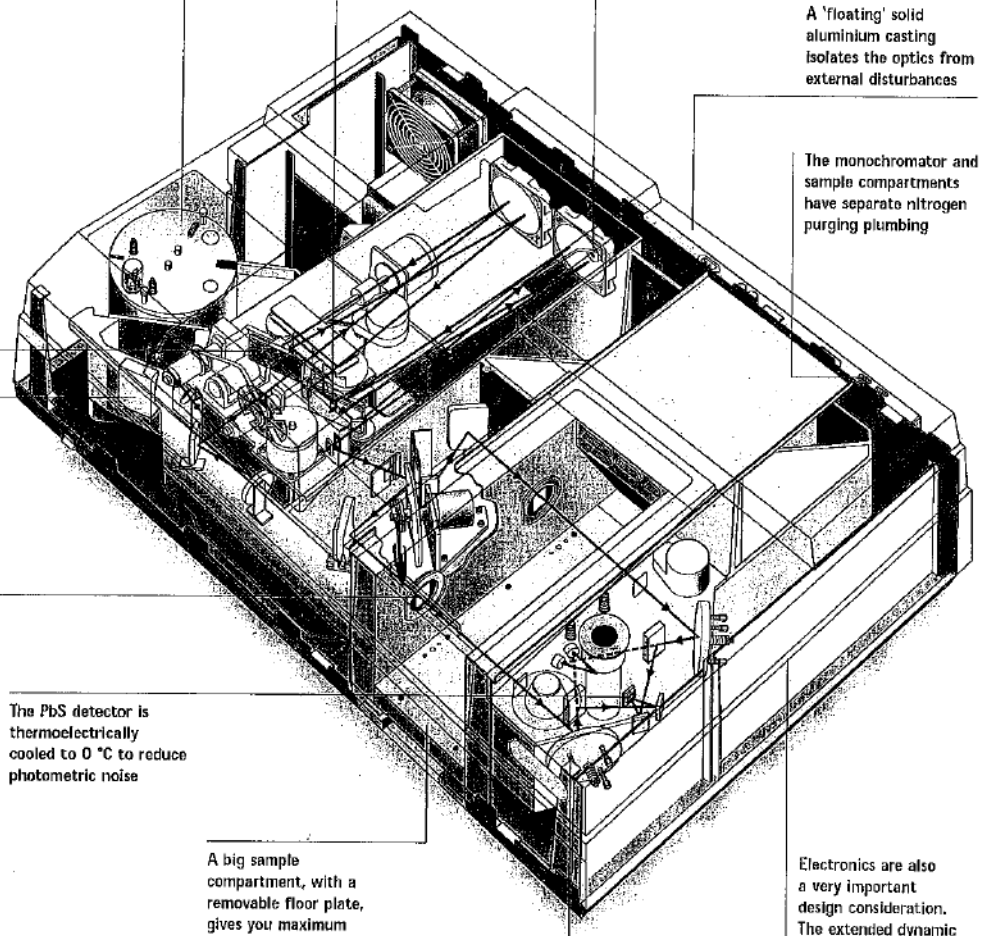
Modern replacement for old Cary 14/17 ~ $40K (guess!)

The Cary 5000 combines unparalleled Cary performance with Varian's innovative PbSmart™ technology, extending the wavelength range into the NIR to 3300 nm.

The instrument is shipped with solid sample holders and can be fitted with a wide range of accessories. It is controlled by the Cary WinUV software.

Cary 5000 design features include: ***-- design, see next page***

- Wavelength range - 175-3300 nm
- Optical Isolation System - incorporates a 'floating' solid aluminium casting that isolates the optics from external disturbances. You can be sure that your laboratory environment won't affect your instrument's performance.
- PbSmart™ technology - optimizes the performance of the PbS NIR detector in real time, providing noise and linearity performance never before achieved using this detector technology.
- Schwarzchild coupling optics - ensures the maximum level of light throughput. This produces more accurate measurements at low transmission levels -- means what??
- Silica overcoating - protects the optics from the environment and allows cleaning without damage to their reflective surface
- Variable and Fixed slits - allows optimum control over data resolution. The spectral bandwidth can be set down to 0.01 nm. The slits can be fixed in the NIR as well as the UV-Vis regions
- Out-of-plane double Littrow monochromator - minimizes photometric noise and stray light, providing excellent resolution – uses monochromator (grating) twice
- Advanced electronics design - The instruments can typically measure beyond 8 Abs with reference beam attenuation
- Nitrogen purging - The monochromator and sample compartments have separate nitrogen purging capabilities,
  allowing the sample compartment to be purged at a higher rate than the instrument
- 'Plug-and-Go'™ lamp management - lamps are now easily replaced and pre-aligned, requiring no adjustment. The lamp management electronics ensures that a wider range
  of lamp designs can be accommodated
- LockDown™ mechanism - you can mount accessories in the sample compartment quickly, easily and reproducibly. You'll spend less time on set up and more time on analysis!


The lamp turret has provision for a third lamp of your choice. The turret can be completely removed if you wish to install another light source
The slits can be fixed in the NIR as well as the UV-Vis
The out-of-plane design of the double Littrow monochromator reduces photometric noise and stray light, and produces excellent resolution
A 'floating' solid aluminium casting isolates the optics from external disturbances
The Cary 400/500 wavelength drive can change wavelengths at 16 000 nm/min in the UV-Vis and at 64 000 nm/min in the NIR
The monochromator and sample compartments have separate nitrogen purging plumbing
Schwarzchild coupling optics ensure a high level of light throughput. This produces more accurate measurements at low transmission levels
The sample compartment windows are tilted to prevent back-reflection and thus incorrect results
The PbS detector is thermoelectrically cooled to 0 °C to reduce photometric noise
A big sample compartment, with a removable floor plate, gives you maximum flexibility when mounting samples
The optics are silica overcoated, so that they can be cleaned without damage to the reflective surface
Electronics are also a very important design consideration. The extended dynamic range of the Cary instruments is a result of this. The Cary 400 and 500 instruments for example, are specified to measure up to 7 Abs with reference beam attenuation

LAMBDA 25, 35, & 45 UV/Vis Spectrophotometers – Perkin Elmer

These have real specifications to compare

PerkinElmer's LAMBDA 25/35/45 UV/Vis systems are easy to operate with the minimum of operator training. for regulated industries the Enhanced Security (ES) version of UV WinLab integrates seamless 21CFR part 11 compliance.

Click for data sheets, Compare to 650, to 950&1050

Below copied from Web site (not a recommendation)

LAMBDA 25 Choose the LAMBDA 25 for routine UV/Vis applications including liquids analysis, pharmacopeia and regulatory tests. • Range: 190 nm - 1100 nm • Bandwidth: 1 nm (fixed)	**LAMBDA 35** Choose the LAMBDA 35 for measurements on liquids, solids, pastes and powder samples and regulatory tests requiring variable bandwidths. • Range: 190 nm - 1100 nm • Bandwidth: 0.5 nm - 4 nm (variable) • Integrating sphere option	**LAMBDA 45** Choose the LAMBDA 45 for measurements on turbid and light-scattering liquid samples such as biological solutions and suspensions. • Range: 190 nm - 1100 nm • Bandwidth: 0.5 nm - 4 nm (variable) • Pre-monochromator for reduced stray light

Technical Specifications for the LAMBDA 25/35/45

Technical description and specifications

	LAMBDA™ 25	LAMBDA™ 35	LAMBDA™ 45
Part number* (Standard System)	L6020060	L6020064	L6020068
Part number* (Enhanced Security System)	L6020062	L6020066	L6020070
Wavelength range	190-1100 nm	190-1100 nm	190-1100 nm
Bandwidth variable variable	1 nm fixed	0.5, 1, 2, 4 nm	0.5, 1, 2, 4 nm
Stray light At 220 nm (NaI)	< 0.01%T	< 0.01%T	< 0.005%T
At 340 nm ($NaNO_2$)	< 0.01%T	< 0.01%T	< 0.005%T
At 370 nm ($NaNO_2$)	< 0.01%T	< 0.01%T	< 0.005%T
At 200 nm (KCl)	< 1%T	< 1%T	< 1%T
Wavelength accuracy At D_2 peak (656.1 nm)	±0.1 nm	±0.1 nm	±0.1 nm
Wavelength reproducibility 10 measurements at 656.1 nm	±0.05 nm	±0.05 nm	±0.05 nm
Photometric accuracy At 1 A using NIST 930D filter	±0.001 A	±0.001 A	±0.001 A
At 2 A using NIST 1930D filter	±0.005 A	±0.005 A	±0.005 A
Potassium dichromate	±0.010 A	±0.010A	±0.010 A
Photometric reproducibility Maximum deviation of 10 measurements at 1 A	< 0.001 A	< 0.001 A	< 0.001 A
Photometric stability Stability at 1 A, at 500 nm with 2-sec. response time	< 0.00015 A/hour	< 0.00015 A/hour	< 0.00015 A/hour
Photometric noise at 500 nm (RMS) Noise 500 nm/0 A RMS Slit 1 nm	< 0.00005 A	< 0.00005 A	< 0.00005 A
Baseline flatness Slit 1 nm	±0.001 A	±0.001 A	±0.001 A