

CHEM 524 -- Course Outline (Part 14) Absorption Spectroscopy —2013

For an html version of 2005 notes, [click here](#)

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

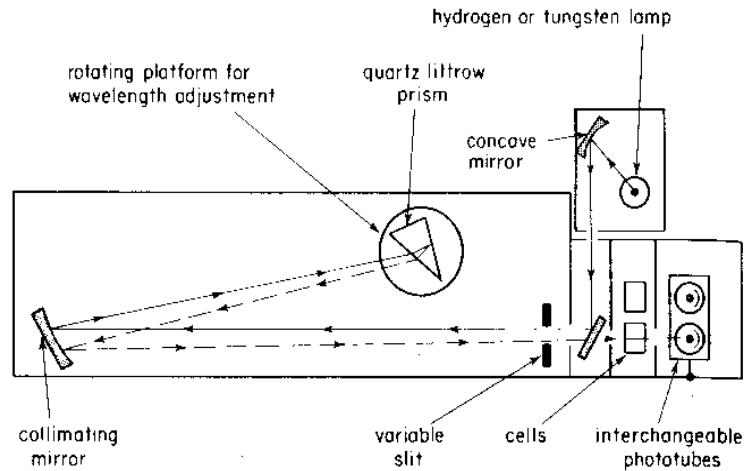
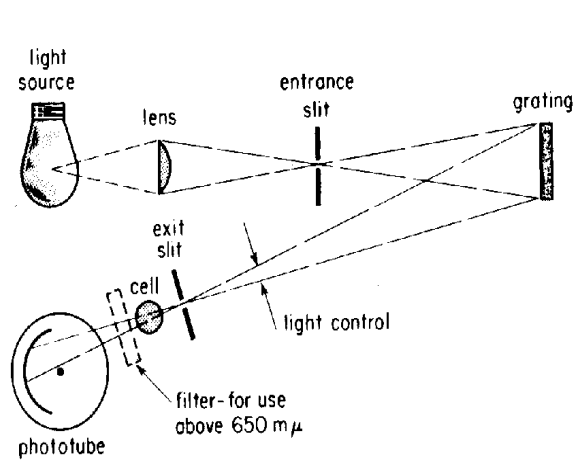
the most widely used analytical spectroscopic method

A. [Example instruments](#)--commonality--similar optics/detector/sources cover overlapping regions, results in multi-use instruments

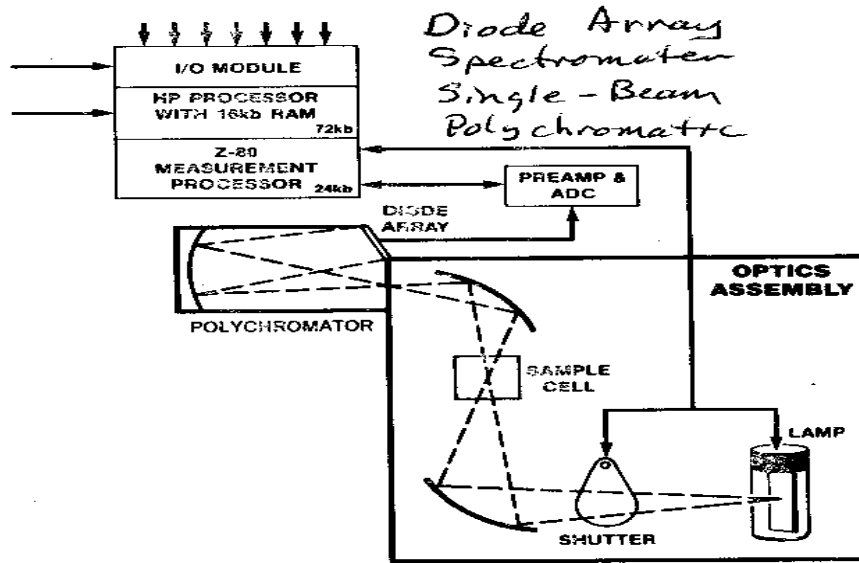
1. Single beam (very old designs) –

[Spectronic 20](#), visible region, non-scan glass optics, W-source, test tube cell primary use –education, single I absorp.

-- [Beckman DU](#) (prism) -- uv-vis (high throughput, prism), original non-scan - fix wavelength Littrow optics, displace in/out beam vertically



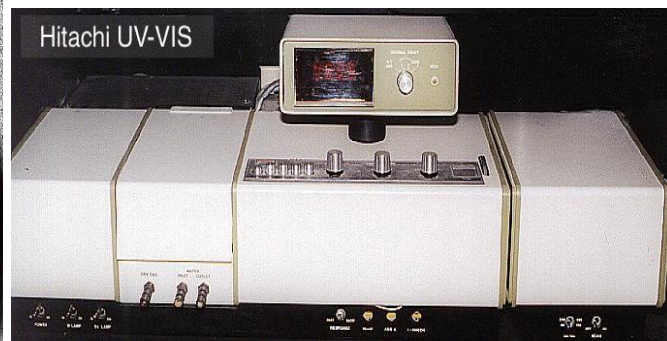
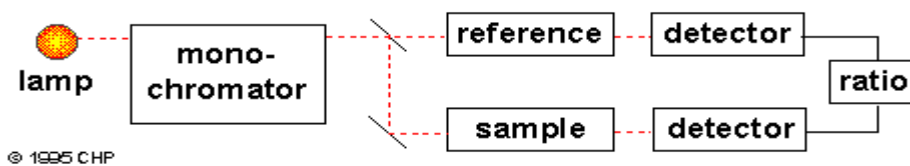
2. **Multichannel single beam** (modern approach, fast, few moving parts, open sample area, low noise, get full spectrum, fixed resolution)-- [diode array, HP5481](#) (original from H-P) – since absorbance, detector can be used without intensifier and cooling not as critical



Now HP is Agilent, also has other spectroscopy lines (former Varian) and updated design
 Can get Full range 190 – 1100 nm, 1.5 sec scan - 1nm res.– two lamps, D₂ and W-I (qtz)
 Agilent: HP 8453 ~\$11,500 HP 8452 (older model)



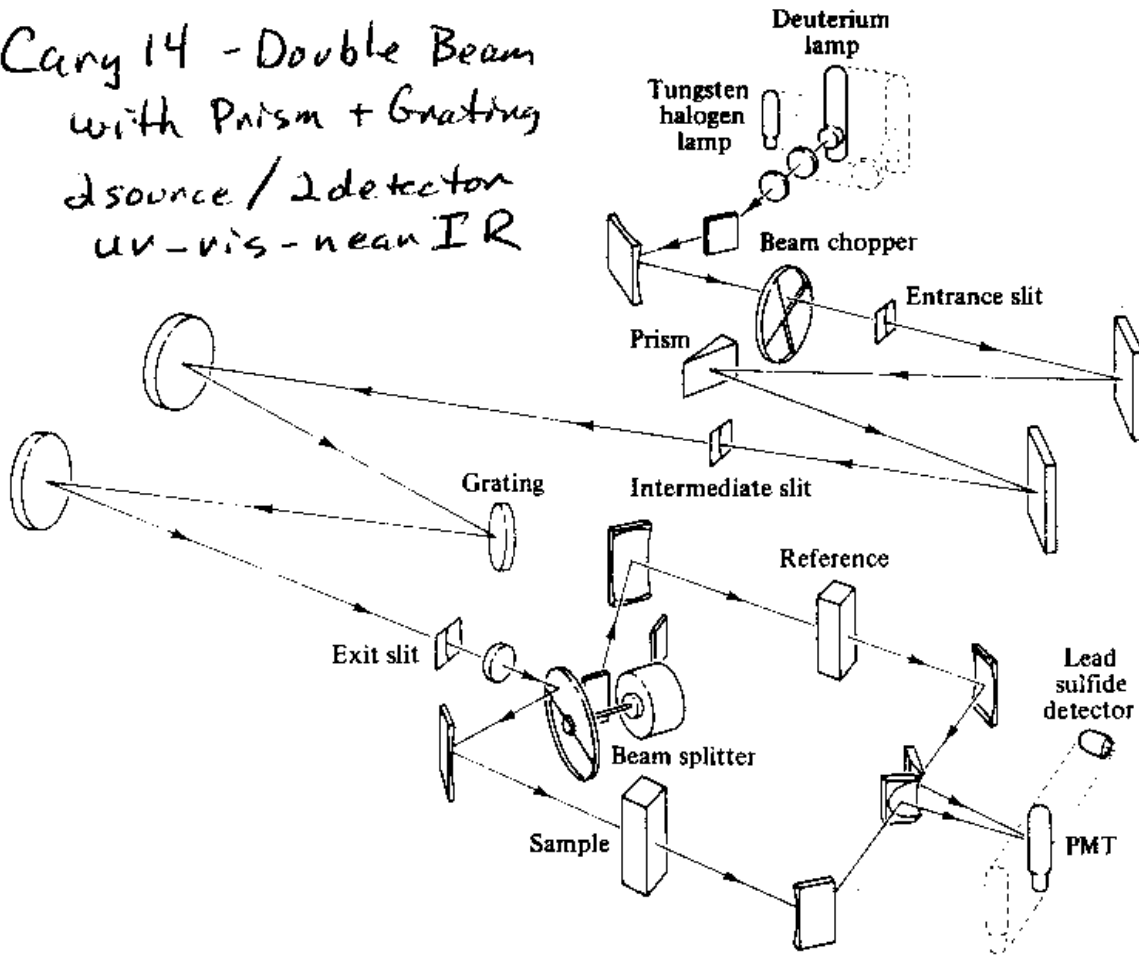
3. **Dual beam** (auto. baseline corrected, abs. converted, [dual in time](#))—



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)

*Cary 14 - Double Beam
 with Prism + Grating
 2 source / 2 detector
 UV-vis - near IR*



Black one (left) is original, Cary 14 (or 11),
 above - OLIS modified Cary 14 (Dept. owns one)
 - add computer data acquisition
 Modern Cary – line from Varian (now Agilent) - see below
 - more compact – no prism
 - many accessories, specialized versions
 - Modern software data acquisition, Temp control

Industry leading Cary 100 and 300 design features

Varian's Cary 300 has a premonochromator, extending its range past 5.0 absorbance units (Abs). The Cary 100 has a working range past 3.5 Abs.

A phase locked wavelength drive prevents peak shifts and peak suppression at high scan speeds.

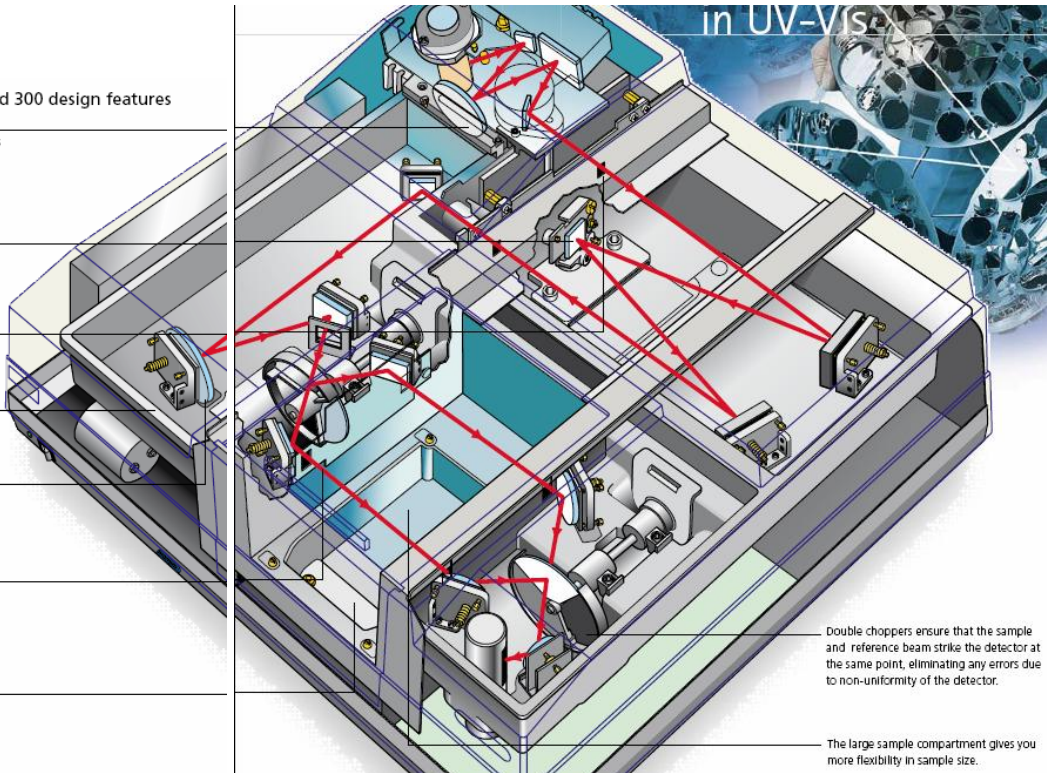
Variable slits provide optimum control over spectral resolution.

The sealed optics prevent exposure to corrosive environments.

Quartz overcoating protects the optics from the environment and allows cleaning without damage to their reflective surface.

Although the Cary 100 and 300 both have a double beam design you can elect to operate them in single, double, or dual-single beam modes.

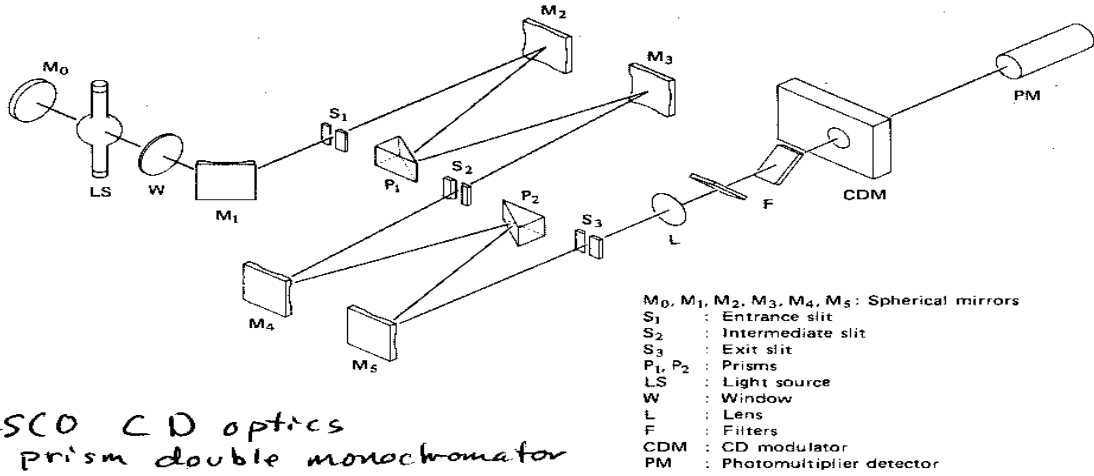
The Accessory Controller offers centralized accessory control, making it easier to service the instrument because of the single set of accessory electronics. In addition, the controller allows you to communicate with non-Varian accessories.



Double choppers ensure that the sample and reference beam strike the detector at the same point, eliminating any errors due to non-uniformity of the detector.

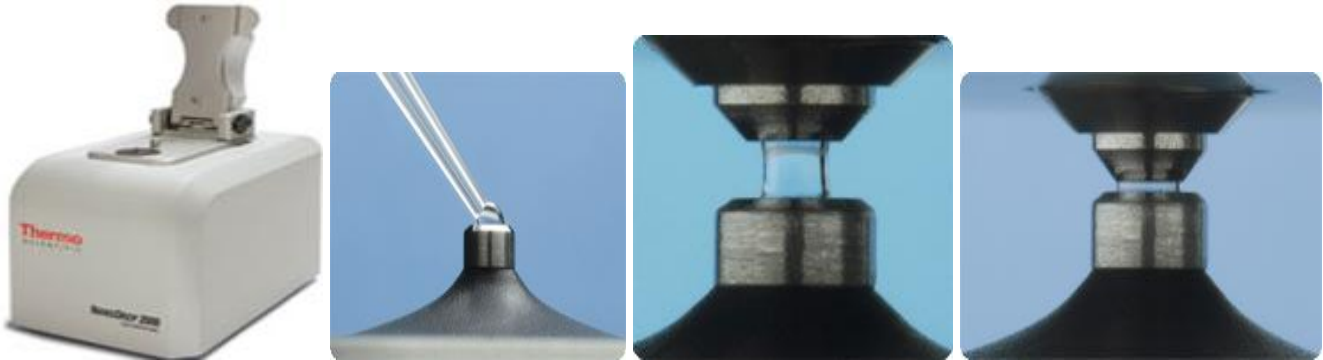
The large sample compartment gives you more flexibility in sample size.

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



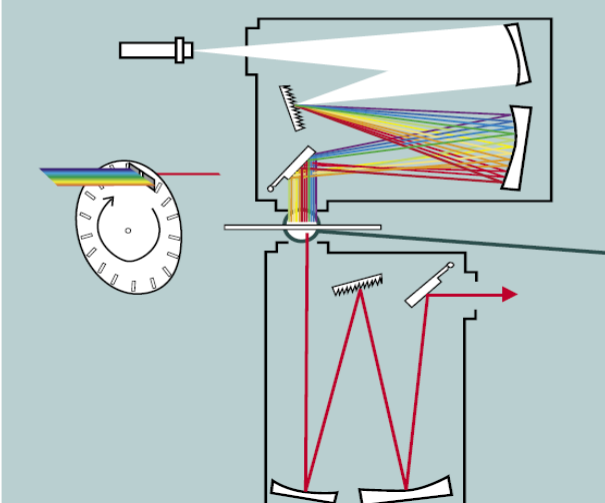
JASCO CD optics
prism double monochromator

Nano drop – new approach, no sample cell, drop $1 \mu\text{l}$ on “spot”, surface tension keeps there
 automated spectral collection: uses pulse Xe source, fiber optic, “linear CCD”, low res monochromator \rightarrow full 250-750 nm spectra, 2 path lengths, 1 and 0.1 mm

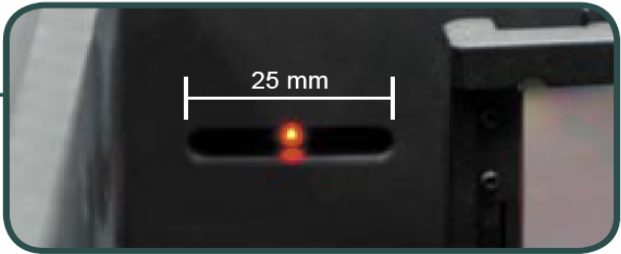


Time dependence, rapid scan – OLIS RSM – a unique approach

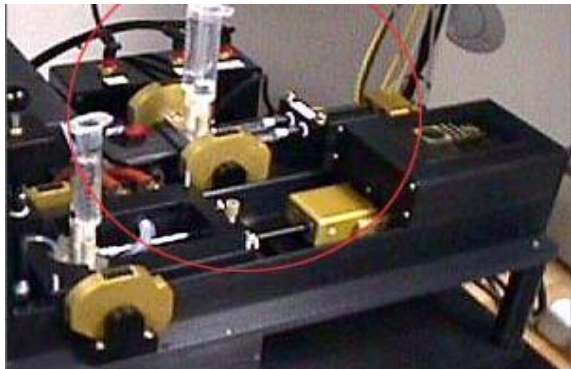
Key seems to be selecting wavelength with rotating disk over dispersed slit image (like spectrograph) and then subtractive dispersion puts all frequencies at 2nd exit slit



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.

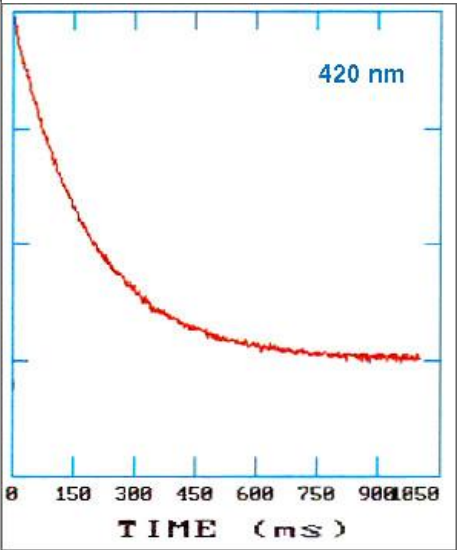
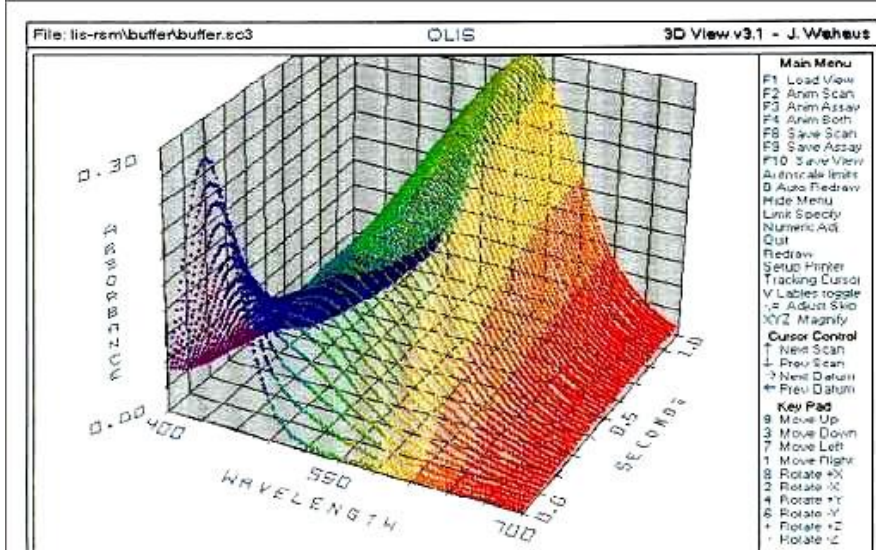


¹ US Patent 5,285,254, issued February 8, 1994.
² See the patent by Dr. C. E. Sorensen for the fourth version of Dr. Richard L. DeGroot



Setup of monochromators with readout/input

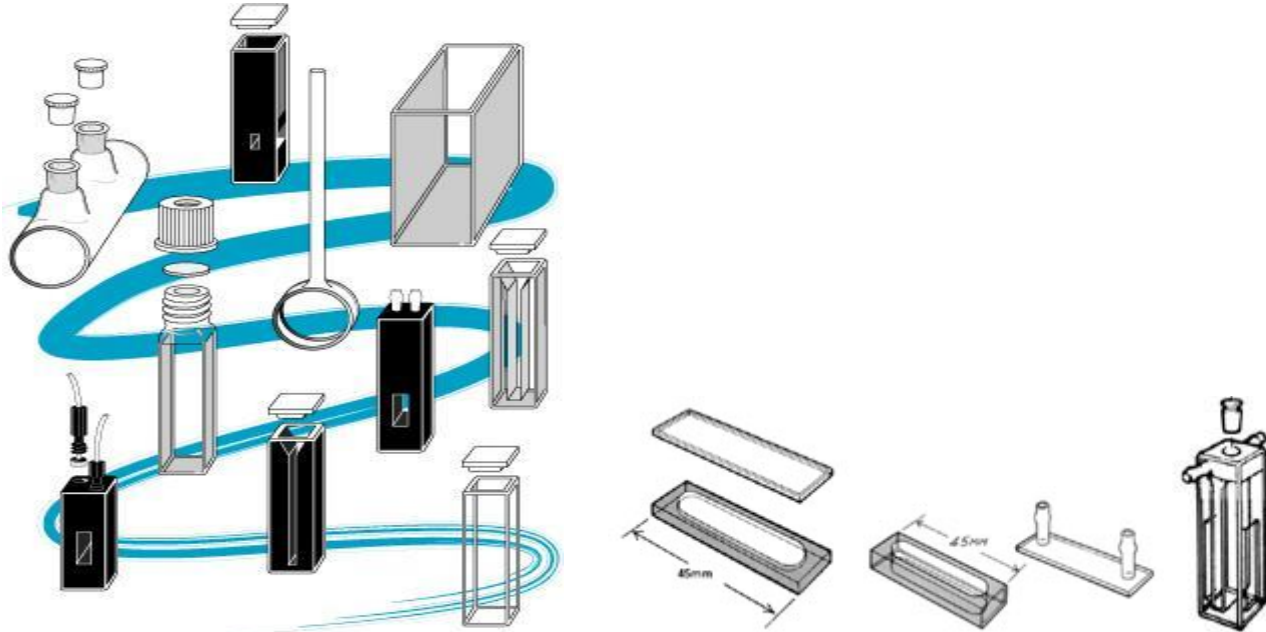
Stop-flow mixer sampling uses mec response



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), $A = \epsilon bc$, 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

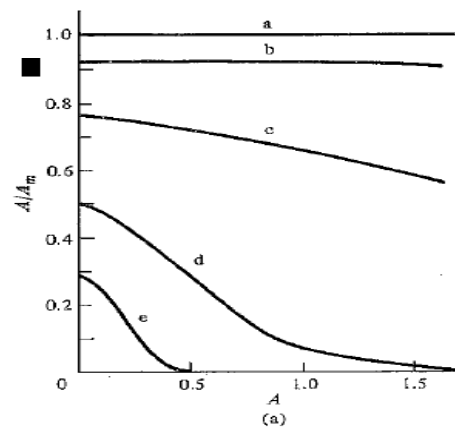
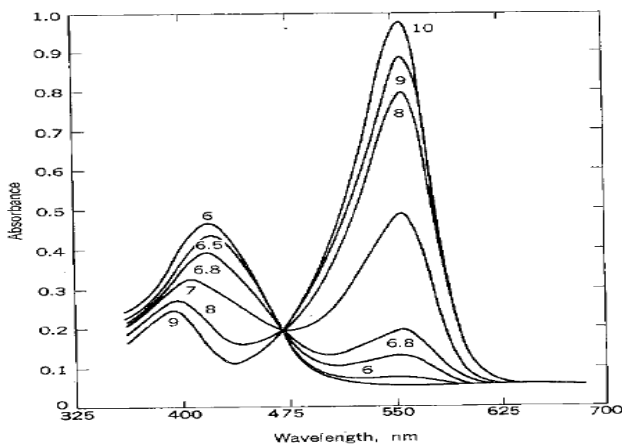
D. **Lambert-Beer Law and deviation from linearity**

---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



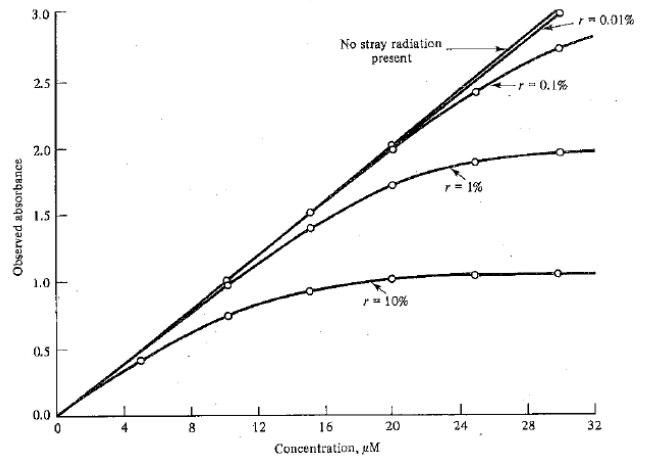
polychromatic radiation, slit width is non-zero, measure at top of band, $A \sim \text{const.}$

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

Minimize error: $s < 0.1 \Delta\lambda$ at λ_m for $A \pm 0.5\%$ → narrow bands biggest effect – $s/\Delta\lambda$ large

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 (pathlength vary for different rays, -- effective ϵ), A too high

OK, if exact same orient. for refer. and sample

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

$$\Delta A = 0.434 F(1-T^2) \quad \text{-- F-fraction multiply reflected}$$

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

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

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 \rightarrow Kubelka-Munk

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

G. Near IR spectroscopy – a sort of hybrid, uses technology with much in common with UV-vis, but has two applications, one electronic (spectroscopic), one vibrational (analysis)

a. **Transition metal complex** spectra often absorb in near IR, d-d and f-f transitions, these are forbidden ($\Delta \ell = 0$) but by mixing with ligand orbitals get intensity (or by reduction in symmetry) normally use **uv-vis-nir spectrometer** (eg. Cary 14, Cary 5000, PE 1050, etc.) as above Interested in resolution, spectra accuracy, spectral coverage (incl. vis), interpret spectra

b. **NIR vibrational spectra**, used for analyses of bulk samples, minimal prep, typically identify components & content (amount), use pattern recognition: PCA, partial least squ., neural net \rightarrow 2500-800 nm, overtone & combination bands – weak, can do large path/no prep. often use **dedicated instruments**, many are dispersive, technology like vis, but diode det. Some based on FTIR, but quartz B/S and optics, W-halogen source, low resolution Ease of use, low training, **dedicated analytical applications** are usual goals Data processing is major issue, statistical pattern analyses and library matching

Near-IR medical application and imaging. → issue is relative transparency of tissue in near IR and the possibility of monitoring **Hemoglobin content**. 800-1000 nm, can use CCD
Can monitor degree of O₂ in blood, location and change of blood flow in brain (cortex) termed: diffuse optical tomography (DOT), near IR imaging (NIRI), functional NIRS (fNIR).
Alternate method, optical coherence tomography (OCT), 3-D image of tissue, 1-2 mm deep

H. *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 – *simple, bottom line, 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.

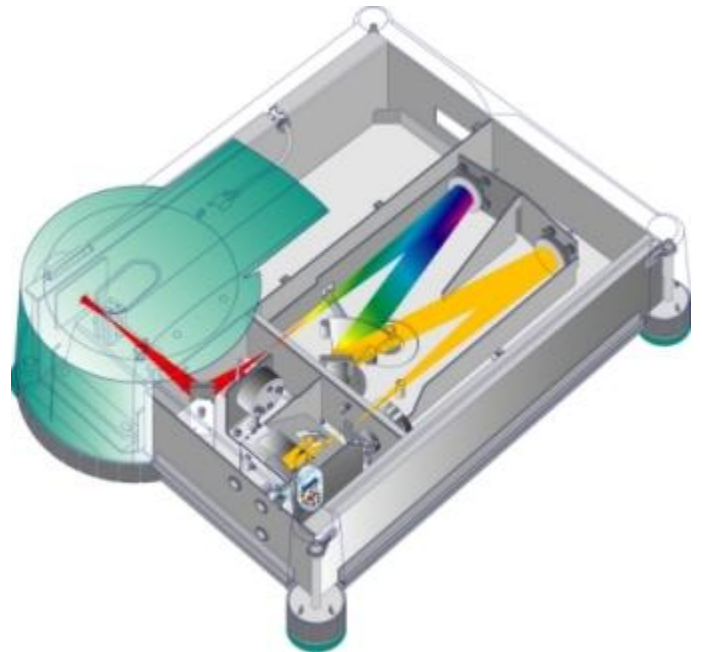
The Xenon lamp has a long-life. And the Cary 50 is only on when the PC is switched on. Fixing electronics problems can be changing the board in the PC, controlled by the Cary WinUV software.

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— 3×10^9 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 100, 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

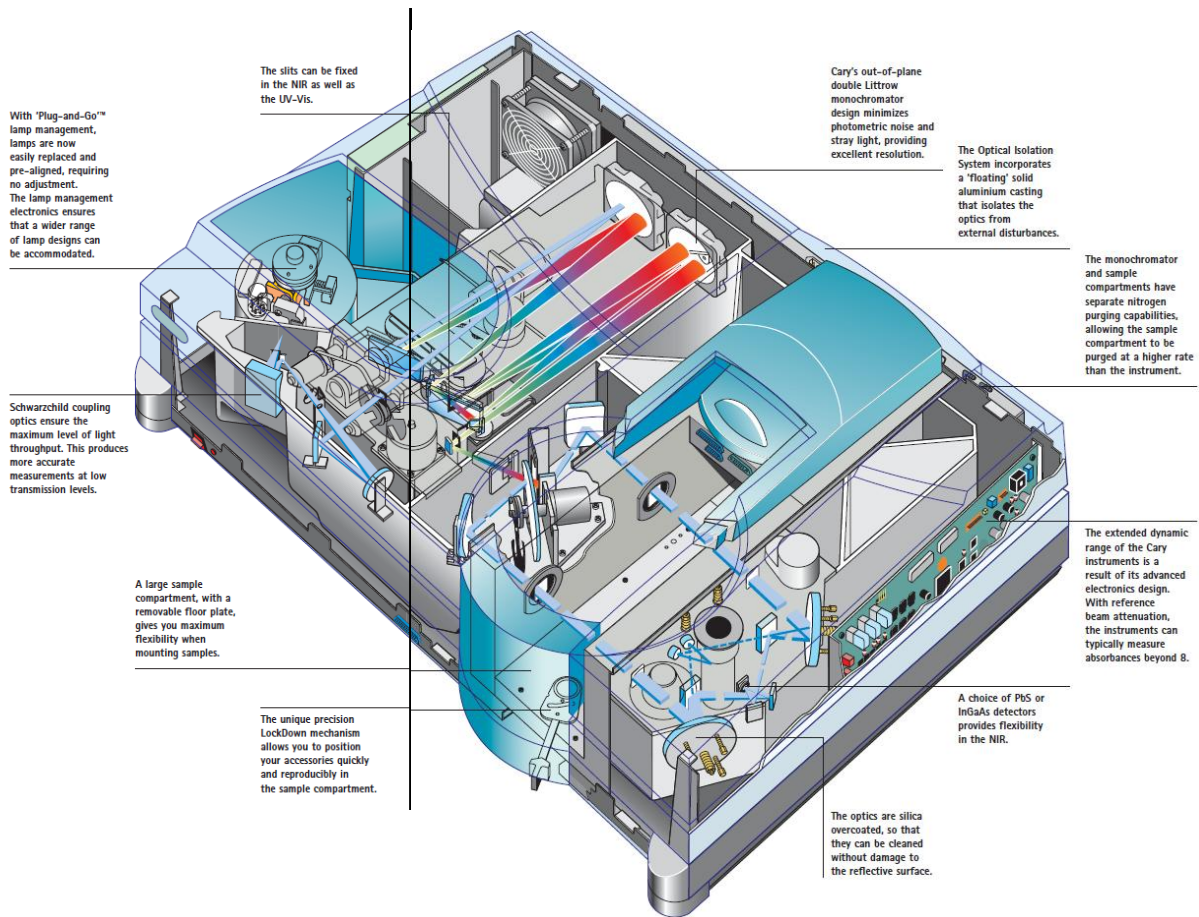
Modern replacement for old Cary 14/17 ~ \$40K (guess!)

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!



Accessories are available to enable different detection/sampling schemes

For this line and other manufacturers, such accessories plug in and are recognized by computer software – usually have preprogrammed routines for use



UV-Vis & UV-Vis-NIR Accessories	Cary 60	8453	Cary 100	Cary 300	Cary 4000	Cary 5000	Cary 6000i
Solid Samples							
Brewster angle					•	•	•
Diffuse reflectance accessory (DRA) - External			•	•	•	•	•
Diffuse reflectance accessory (DRA) - Internal			•	•	•	•	•
Diffuse reflectance aperture kit (External DRA) for small samples					•	•	•
Diffuse reflectance - praying mantis					•	•	•
Diffuse reflectance powder cell kit (all DRAs)			•	•	•	•	•
Diffuse reflectance small spot kit (External DRA)			•	•	•	•	•
Fiber optic probes - solids	•		•	•			
Film holder			•	•	•	•	•
Powder cell kit					•	•	•
Polarizer/Depolarizer			•	•	•	•	•
Solid sample holder	•		•	•	•	•	•
Specular reflectance - fixed angle (floor)			•	•	•	•	•
Specular reflectance - fixed angle (side)	•		•	•	•	•	•
Specular reflectance - variable angle					•	•	•
Specular reflectance - VW absolute			•	•	•	•	•
Sunglass holder	•		•	•			
Liquid Samples							
Autosampler		•	•	•	•	•	•
Cell holders - liquid	•	•	•	•	•	•	•
Circulating water bath	•		•	•	•	•	•
Dissolution manifold			•	•			
Fiber optic dip probes - liquids	•		•	•			
Fluorescence			•	•	•	•	•
Gel scanning sample transport			•	•	•	•	•
Microvolume Traycell	•						
Multicell holder (including water thermostatable)	•	•	•	•	•	•	•
Peltier 1x1 cell holder			•	•	•	•	•
Peltier multicell holder (6x6)			•	•	•	•	•
Peltier single cell holder	•	•					
Peltier thermostatable pump			•	•			
Routine sampler pump (internal)			•	•			
Sipper pump (external)		•					
Stopped flow/Rapid mix	•	•	•	•	•	•	•
Temperature controller		•	•	•	•	•	•
Temperature probe	•	•	•	•	•	•	•
Test tube holder	•						
Other							
Accessory controller (built into 4000/5000/6000i)			•	•	•	•	•
Extended compartment for large samples			•	•	•	•	•
Photometric accuracy test kit			•	•	•	•	•
Linearity test kit			•	•	•	•	•
Rear beam attenuator for additional sensitivity			•	•	•	•	•
Thermostatted accessories					•	•	•

Perkin Elmer (and other companies) have directly competing lines
 (low grade, UV-Vis plus access, top grade and NIR) – mid grade:
 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)

<p>LAMBDA 25 Choose the LAMBDA 25 for routine UV/Vis applications including liquids analysis, pharmacopeia and regulatory tests.</p> <ul style="list-style-type: none"> • Range: 190 nm - 1100 nm • Bandwidth: 1 nm (fixed) 	<p>LAMBDA 35 Choose the LAMBDA 35 for measurements on liquids, solids, pastes and powder samples and regulatory tests requiring variable bandwidths.</p> <ul style="list-style-type: none"> • Range: 190 nm - 1100 nm • Bandwidth: 0.5 nm - 4 nm (variable) • Integrating sphere option 	<p>LAMBDA 45 Choose the LAMBDA 45 for measurements on turbid and light-scattering liquid samples such as biological solutions and suspensions.</p> <ul style="list-style-type: none"> • Range: 190 nm - 1100 nm • Bandwidth: 0.5 nm - 4 nm (variable) • Pre-monochromator for reduced stray light
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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	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
Prices	\$10K +	? \$15K	? >\$20K

Teaching version lowest, accessories build price quickly!