

A Flash of Genius

Agilent Cary Spectroscopy Products



Agilent Technologies

Howard Cary



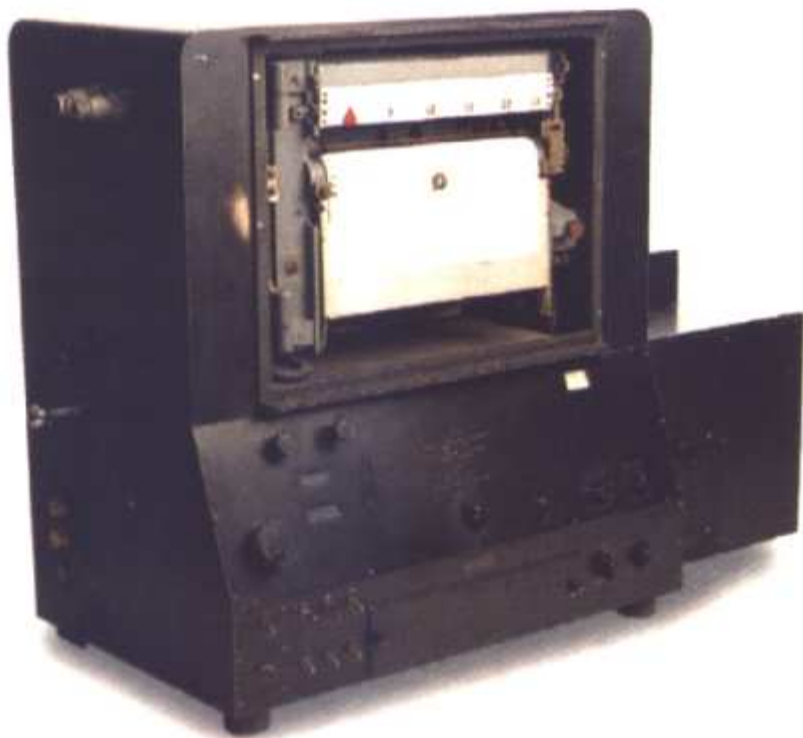
- 1941 Howard Cary worked with Arnold Beckman to produce the World's first UV/Visible spectrophotometer.
- 1946 Howard Cary founds Applied Physics Corporation, Monrovia, which later becomes Cary Instruments Inc.

Howard Cary

CARY



1947 - Introduction of the Cary 11



- Introduced in April 1947
- World's first recording Double-Beam UV-Vis spectrophotometer
- Prism monochromator
- First "High-Performance" spectrophotometer

Howard Cary



Howard Cary

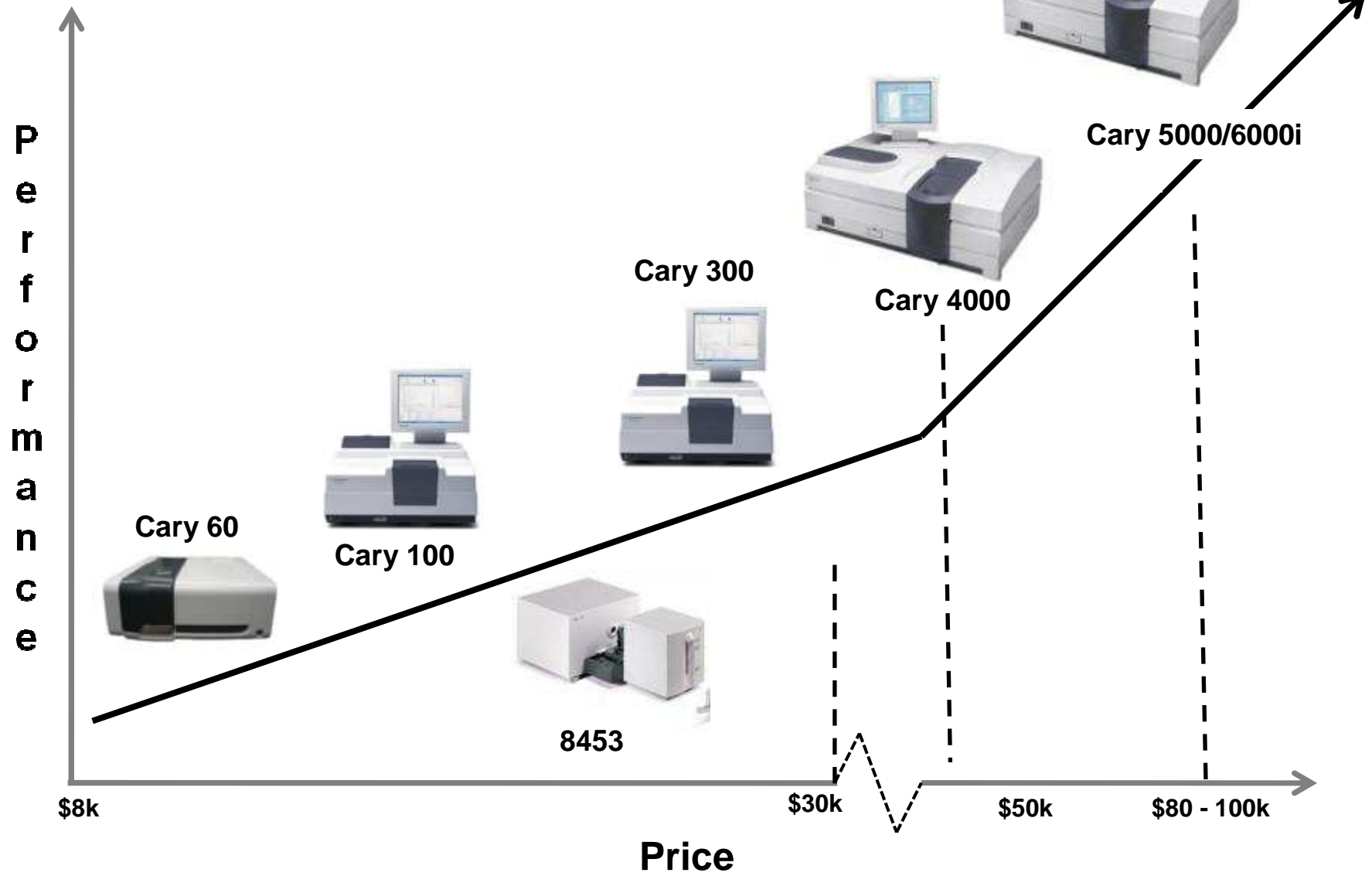
- 1966 Cary Instruments merged with Varian Instruments
- 1982 Cary Instruments moved to Melbourne, Australia
- 2010 Varian becomes part of Agilent Technologies and the Cary legacy continues...

CARY



Agilent Technologies

Agilent UV-Vis/NIR Portfolio



Performance

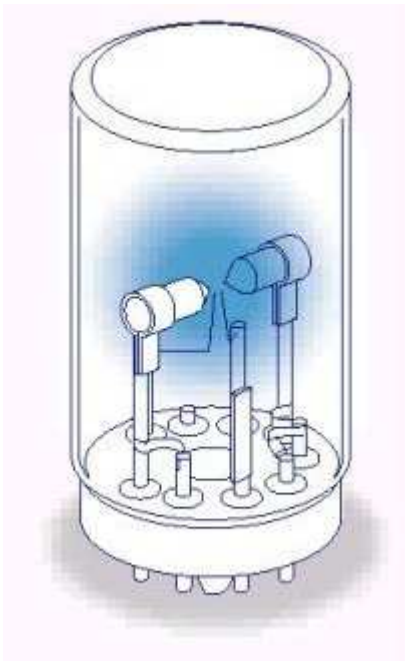
Abs	%T	Transmittance	Qty of Initial Light Measured	
0	100	1	All	
1	10	0.1	1/10 th	
2	1	0.01	1/100 th	8453
3	0.1	0.001	1/1000 th	
4	0.01	0.0001	1/10,000 th	Cary60
5	0.001	0.00001	1/100,000 th	Cary100
6	0.0001	0.000001	1/1,000,000 th	Cary300
7	0.00001	0.0000001	1/10,000,000 th	
8	0.000001	0.00000001	1/100,000,000 th	Cary4,5,
9	0.0000001	0.000000001	1/1,000,000,000 th	6000

Agilent Cary 60 Double Beam UV-Vis



Cary 60 - Key Features

- **Xenon Flash Lamp Source**



Application focus

- Chemical and Industrial
- Academic teaching
- Life Science

Xenon Flash Lamp Source - General

Broad excitation range with high efficiency so intensely bright - typically more than one order of magnitude brighter than D2 and Halogen lamps used in conventional double beam instruments.

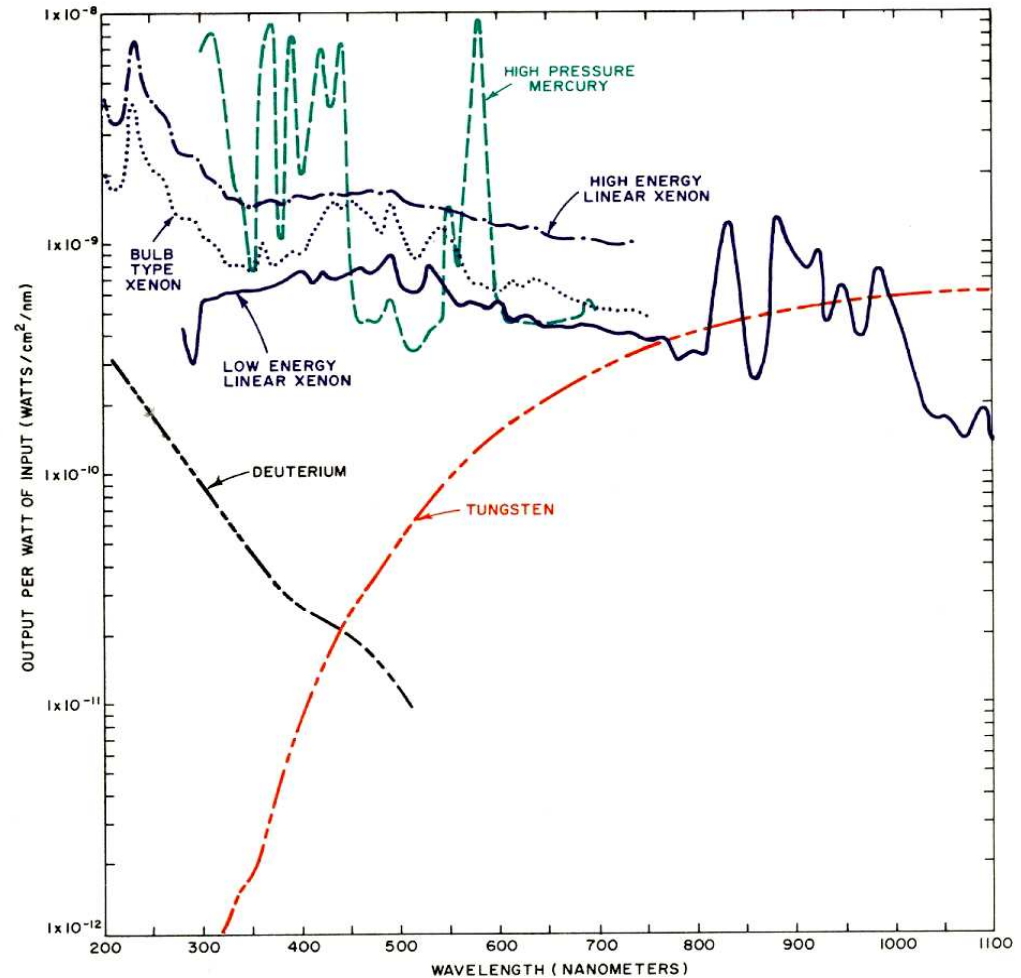
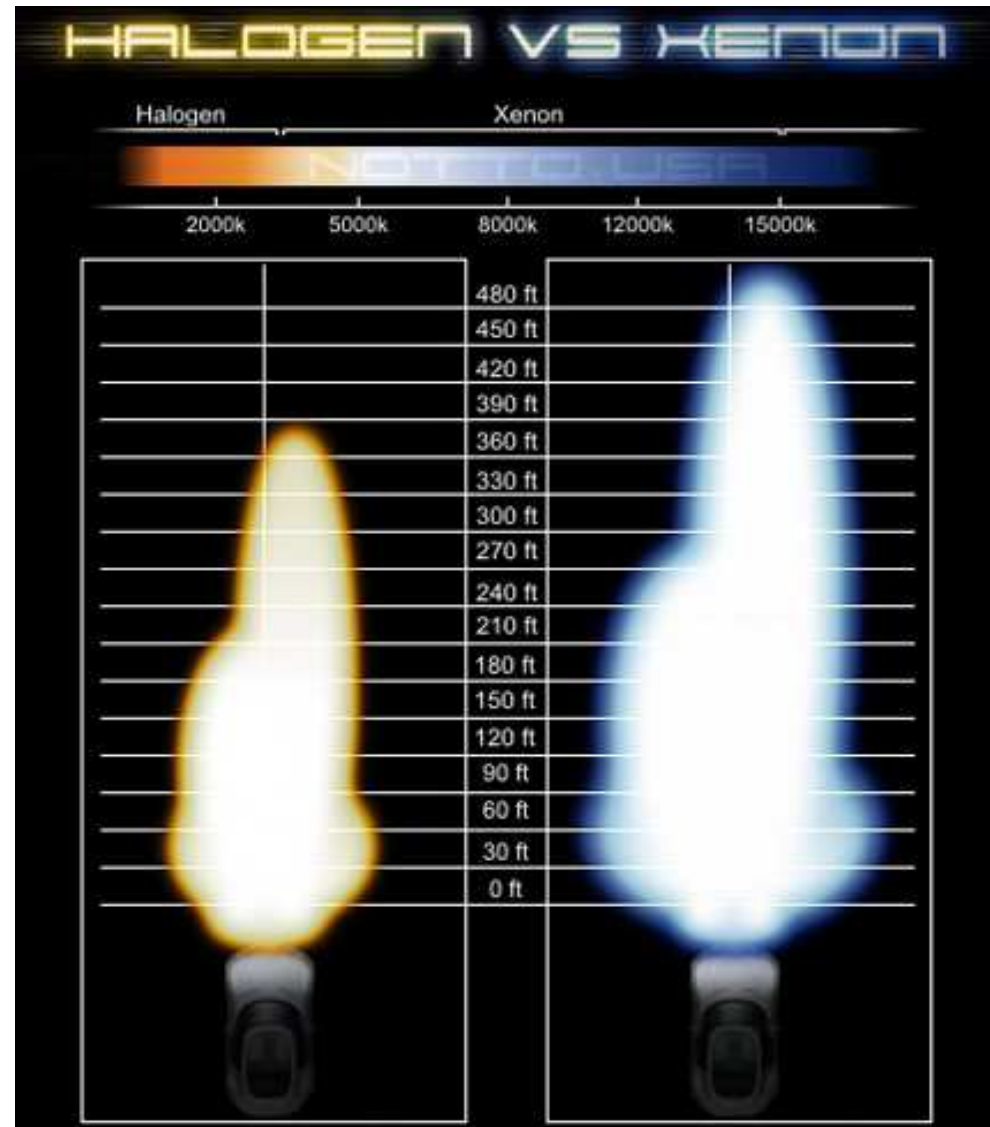


Figure 1. Xenon flashlamp spectrum versus other commonly used optical sources.

Xenon Flash Lamp Source - General

A good analogy is car headlights as shown here.



Xenon Flash Lamp Source - Lifetime

3 x 10⁹ flashes typical lifetime – 80 flashes/second

= 37,500,000 seconds

= 625,000 minutes

= 10,416 hours

= 434 days

= 62 weeks

HOWEVER.....The lamp needs NO WARMUP TIME and ONLY flashes during measurements so lamp lifetime can be considered to be well in excess of 10 years for most working labs, and we offer 3 years warranty on the lamp as standard!

Xenon Flash Lamp Source - Lifetime

By comparison....for a typical double beam instrument with D2 and Halogen sources:-

Typical Deuterium lamps are rated for 2000 hours of use

Typical Halogen lamps are rated for 1500 hours of use

These lamps are continuous sources and need at least 15 minutes warm-up time from instrument switch-on.

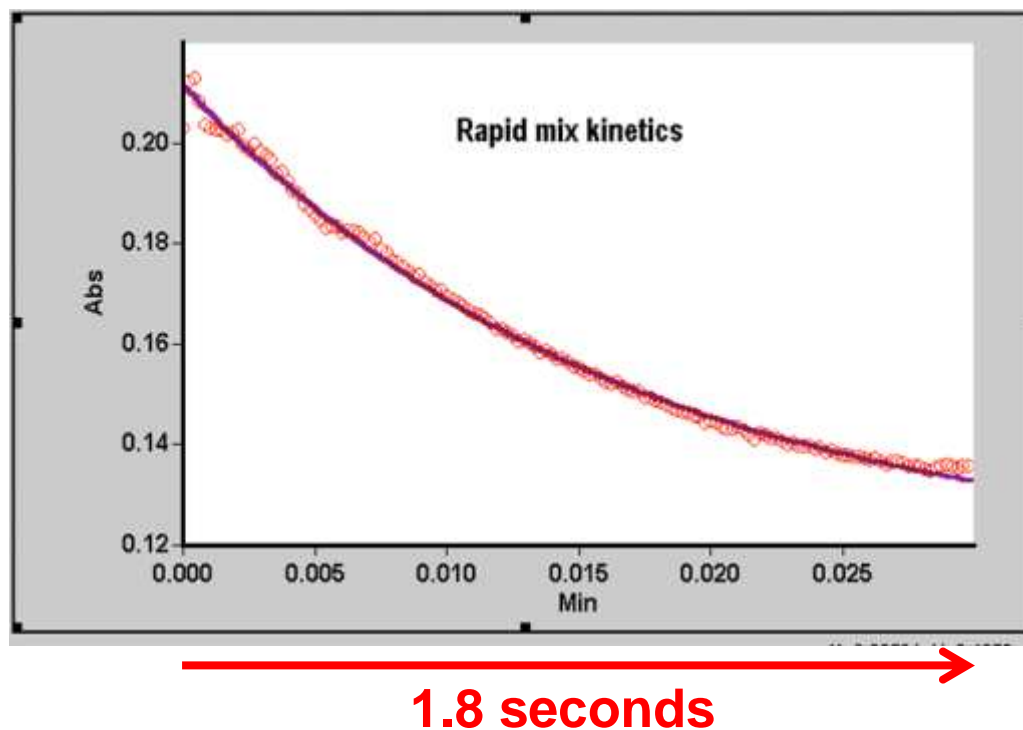
Assuming a standard 8 hour day, 5 days per week, and operation 48 weeks per year this equates to 1920 hours of system use, so typically both lamps would have to be replaced on an annual basis.

At a cost of around £350 for a D2 lamp and £50 for a Halogen, the typical “whole lifetime” cost of one of these systems would be around £4000!

Xenon Flash Lamp Source – Fast Data Collection

80 flashes/second means that we can collect 80 data points/seconds, and at maximum scan speed of 24,000nm/min. This allows us measure the kinetics of very fast reactions.

Complete spectral scan takes <3 secs.

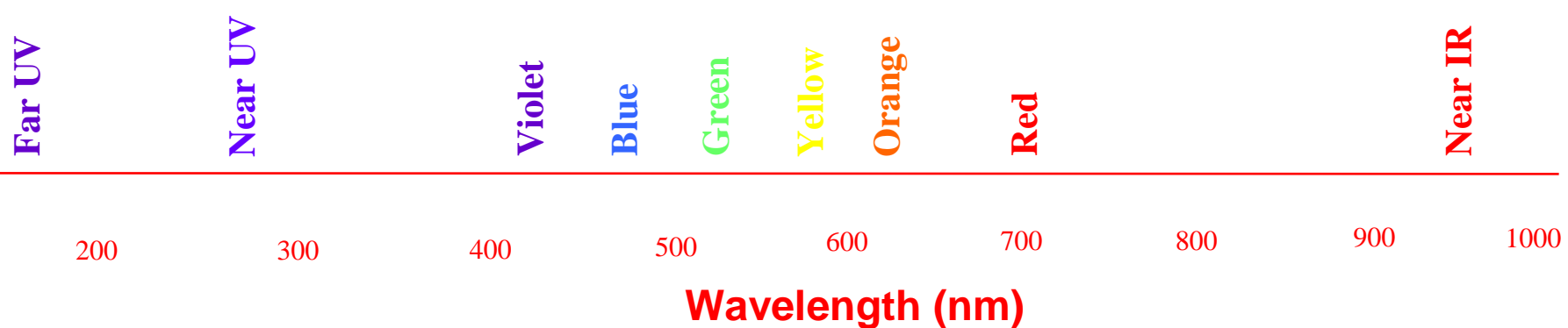
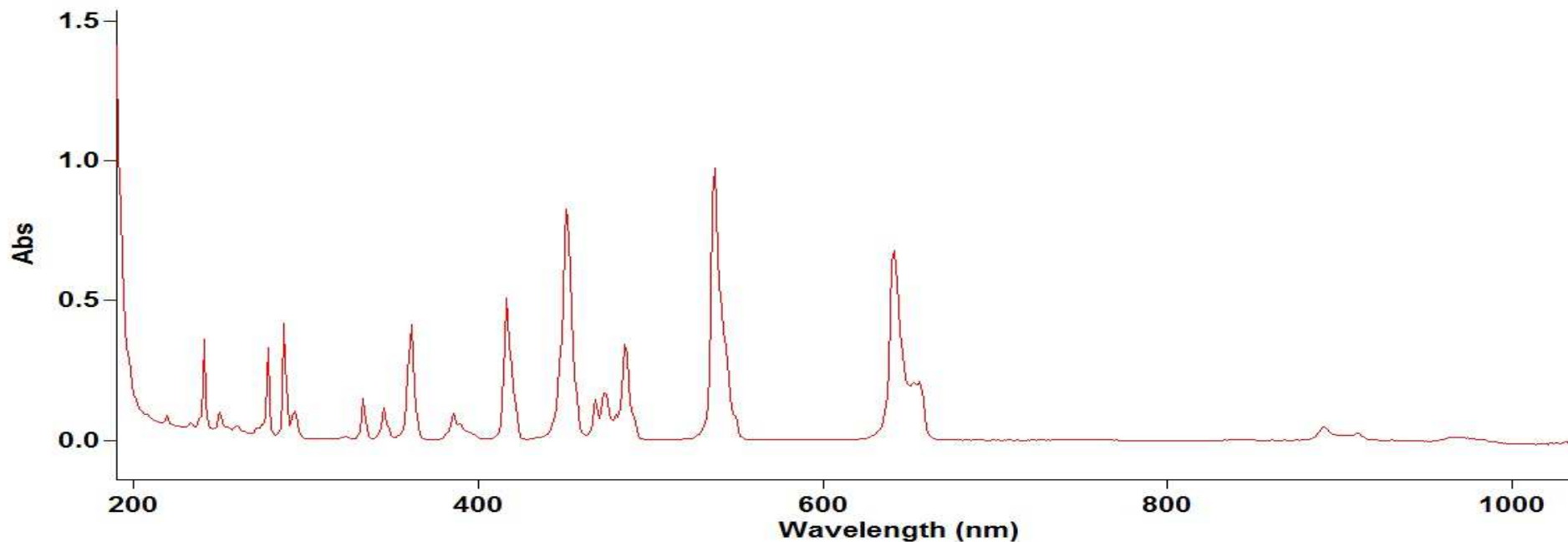


Key Features

- **Xenon Flash Lamp Source**
- **Wide Wavelength Range 190 – 1100nm**



Wide Wavelength Range



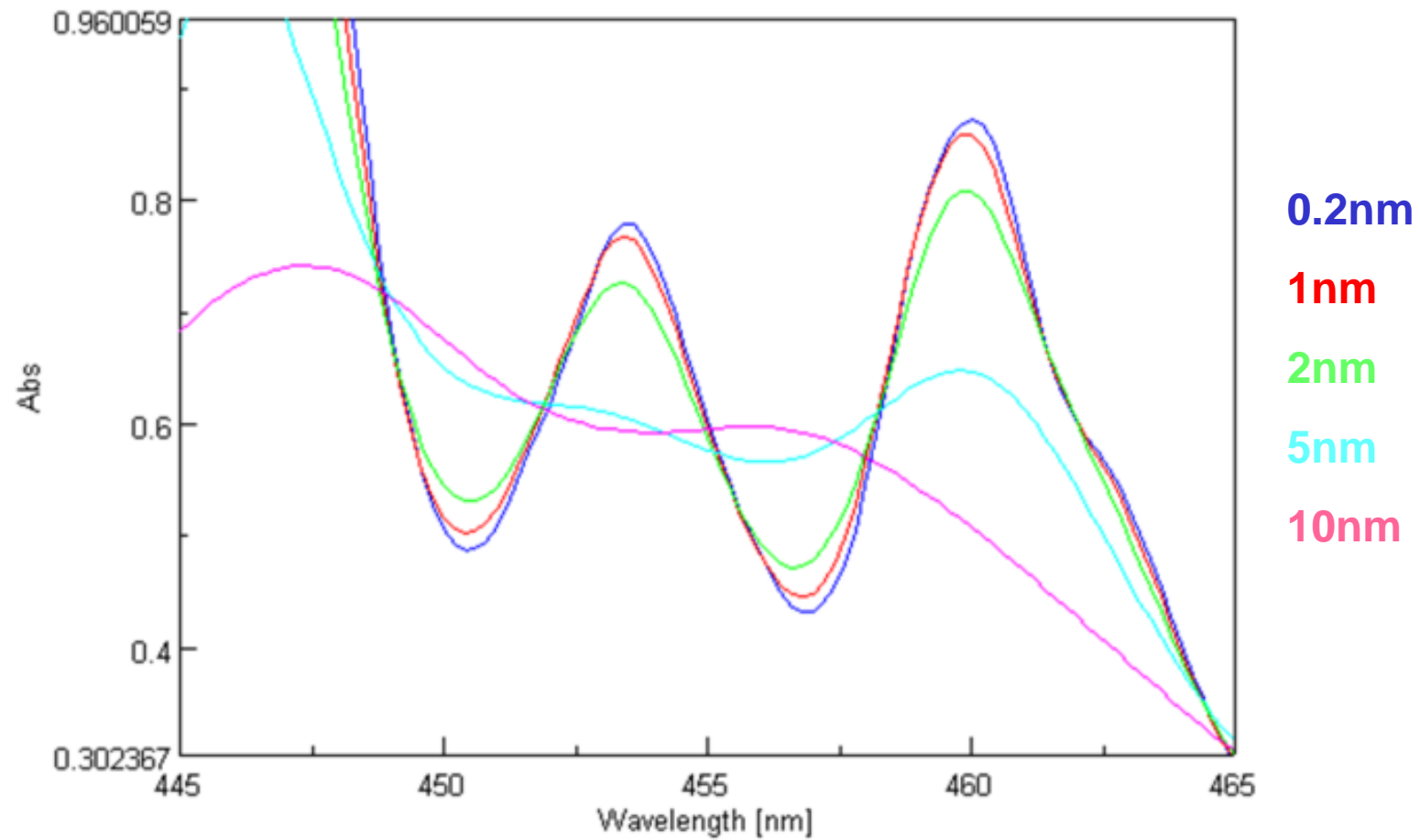
Key Features

- **Xenon Flash Lamp Source**
- **Wide Wavelength Range 190 – 1100nm**
- **Fixed 1.5nm Bandwidth**



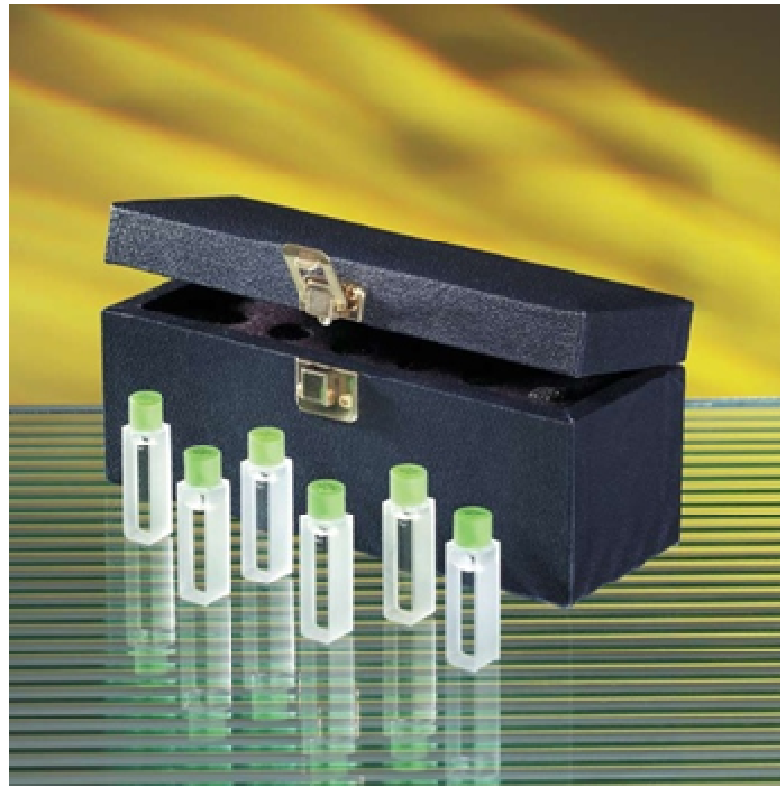
Fixed 1.5nm Bandwidth

Ideal bandwidth for liquid/solid measurement



Fixed 1.5nm Bandwidth

Ensures compliance with international pharmacopoeia requirements



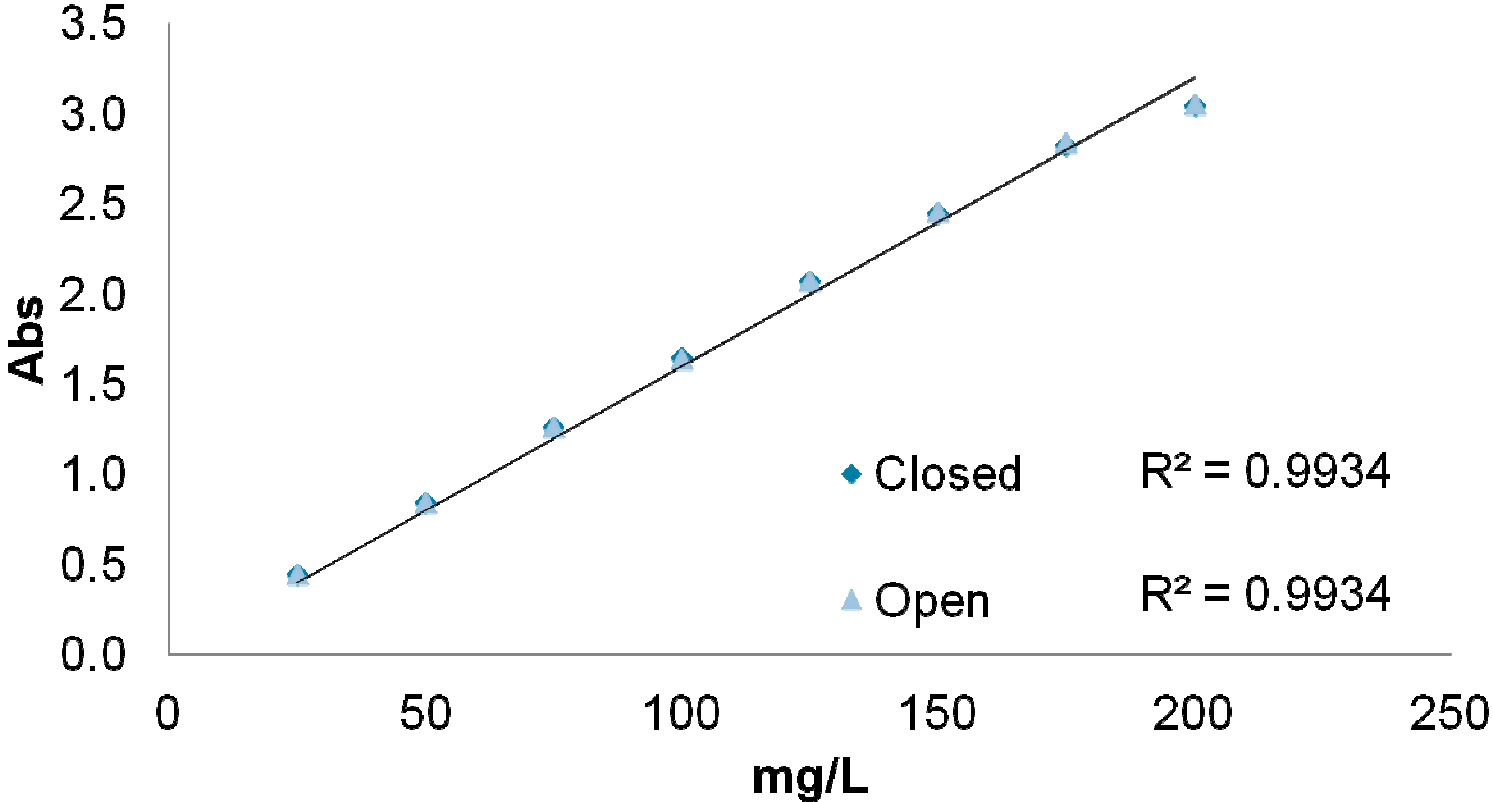
Key Features

- **Xenon Flash Lamp Source**
- **Wide Wavelength Range 190 – 1100nm**
- **Fixed 1.5nm Bandwidth.**
- **Room Light Immunity**



Room Light Immunity

Linearity even with sample compartment open



Measuring KMnO_4 in a cuvette with the sample compartment open and closed

Room Light Immunity

Assays can be performed with the spectrometer lid wide open, e.g. addition of enzyme cofactors, catalysts, titrations etc



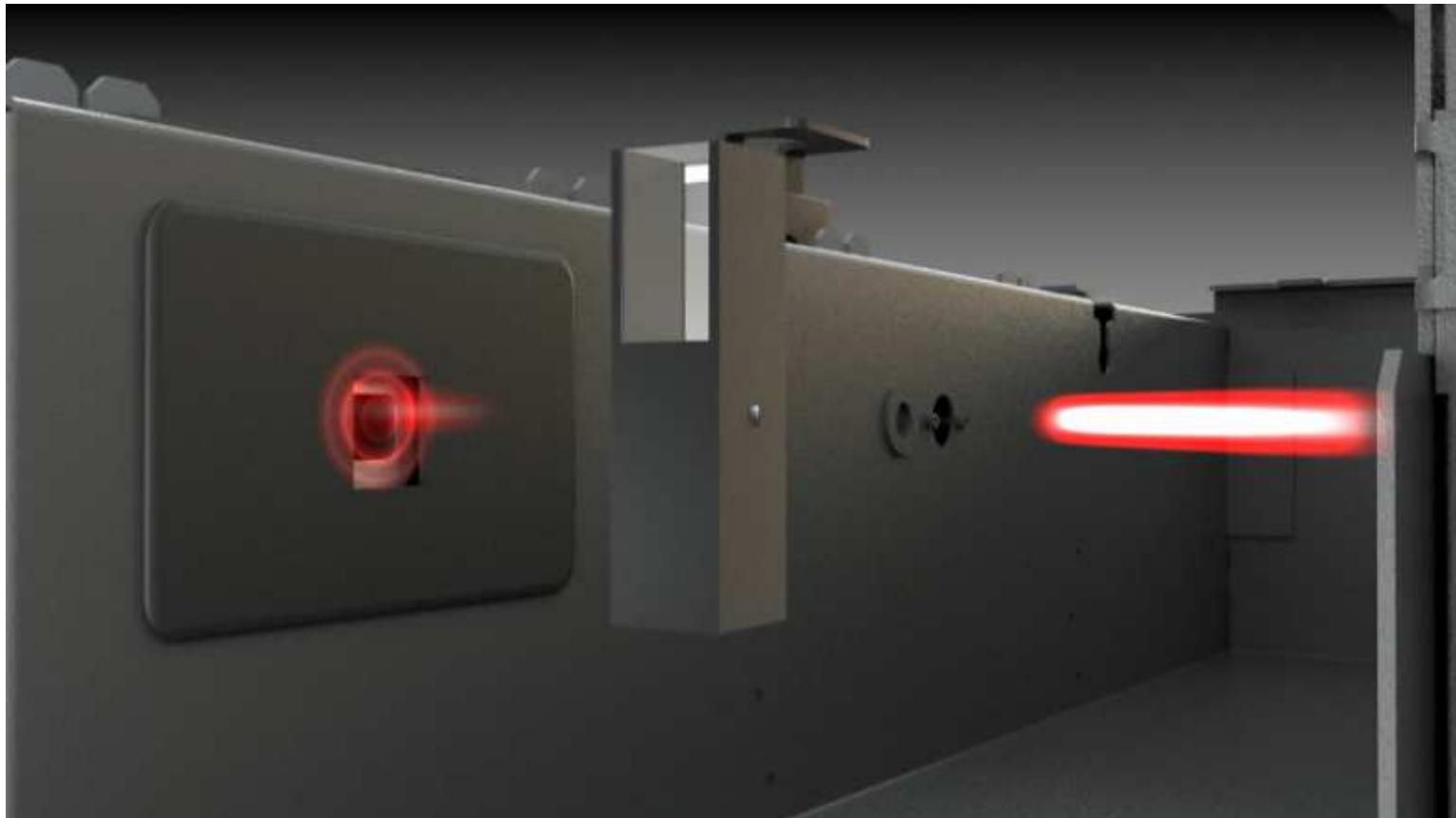
Key Features

- **Xenon Flash Lamp Source**
- **Wide Wavelength Range 190 – 1100nm**
- **Fixed 1.5nm Bandwidth.**
- **Room Light Immunity**
- **Focussed Beam**

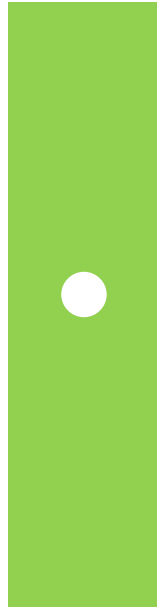


Focussed Beam

Small focused beam measuring just 1.5 x 1.0 mm
Perfect for use with low volume cuvettes



Focussed Beam



**Conventional
UV-Vis**

**Superior Fibre Optic
Coupling**



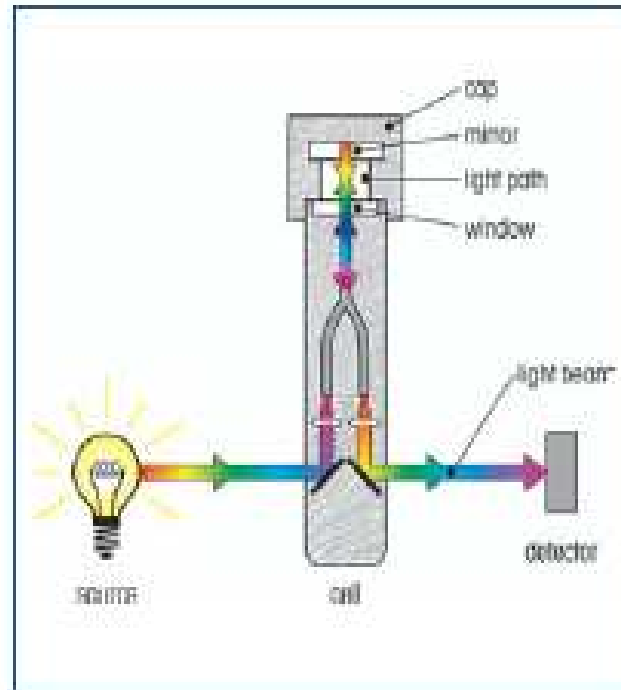
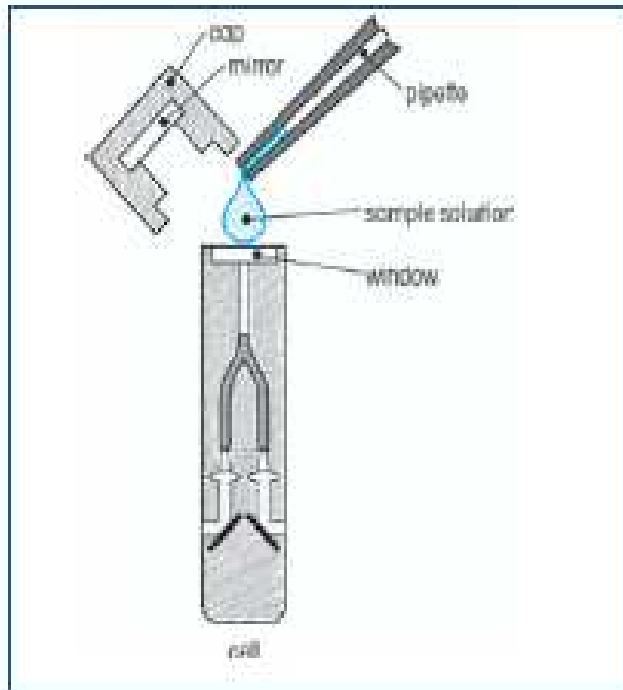
Cary 60



Focussed Beam

Hellma “Traycell” accessory is a special cuvette with integrated fibre-optics for low volume (sub-microlitre) assays.

Cary 60 can exploit the benefits of this accessory better than any other competitive instrument.



Key Features

- **Xenon Flash Lamp Source**
- **Wide Wavelength Range 190 – 1100nm**
- **Fixed 1.5nm Bandwidth.**
- **Room Light Immunity**
- **Focussed Beam**
- **Wide Range of Accessories**



Wide Range of Accessories



Self Installation



Customer “self-installation” protocol, including instrument validation, means that Cary 60 can be set up and operational within 1 hour without the intervention of a service engineer.

Child’s play!

Cary WinUV Software Control



Two (2) Software Products:-

- Cary WinUV Software
- Cary WinUV Pharma Software (21CFR11)

Complete qualification services (IQ/OQ) for the Cary 60 hardware, software and accessories

Cary WinUV Tutorial

Cary 60 Getting Started

Welcome Page Installing Accessories Application Tutorials

Welcome to the Cary 60 Tutorial

Now that you have completed installation and validation steps, it is time to install any accessories and then setup and run an experiment.

Click the button below to learn more about Cary WinUV. Otherwise, click the buttons above to learn how to install your accessories and how to set up experiments and collect data.



 **Cary WinUV overview**

 **Agilent Technologies**

Done

Markets and Applications



- Characterization of unknown or newly synthesized compounds
- Studying rates of reactions (kinetics) of chemical and biological systems
- Monitoring kinetics of chemical or biological reactions that occur at sub-second rate
- Measurement of thin films and optical components
- Analyzing photochemical reactions in-situ during sample irradiation



- DNA and protein quantification
- Measuring cold biological samples (4 °C) immediately after removal from the refrigerator
- Preparation of fluorescent liquid samples prior to emission measurements
- Analyzing small amounts of precious sample (3–40 µL)
- Study of turbid biological samples such as Cytochrome P450



- Quality control of raw materials and finished goods
- Color measurements and color matching
- Analysis of nutrients in water, food and agriculture
- Analysis of turbid solutions or relatively highly absorbing samples
- Analysis of surface coatings or bulk optics (e.g., sunglasses)
- Study of pigments in art conservation through reflectance measurements



Protein/Nucleic Acid (DNA/RNA) Purity

It is common for nucleic acid solutions to be contaminated with other molecules such as proteins, phenol etc

Proteins absorb at 280nm

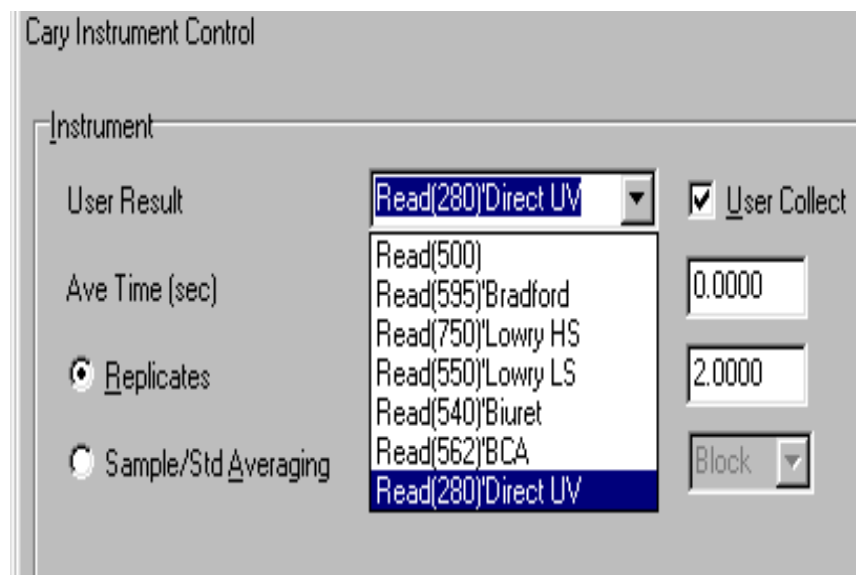
DNA/RNA absorbs at 260nm

To assess purity we use the ratio of 260:280nm

Pure DNA has a 260:280 ratio of ~1.8

Pure RNA has a 260:280 ratio of ~2.0

Pure proteins will give a value between 0.5 and 0.7



WinUV software has dedicated programs for this calculation.

APPLICATION NOTES AVAILABLE

SI-A-1219 Practical Limits of DNA Quantitation in Microliter samples

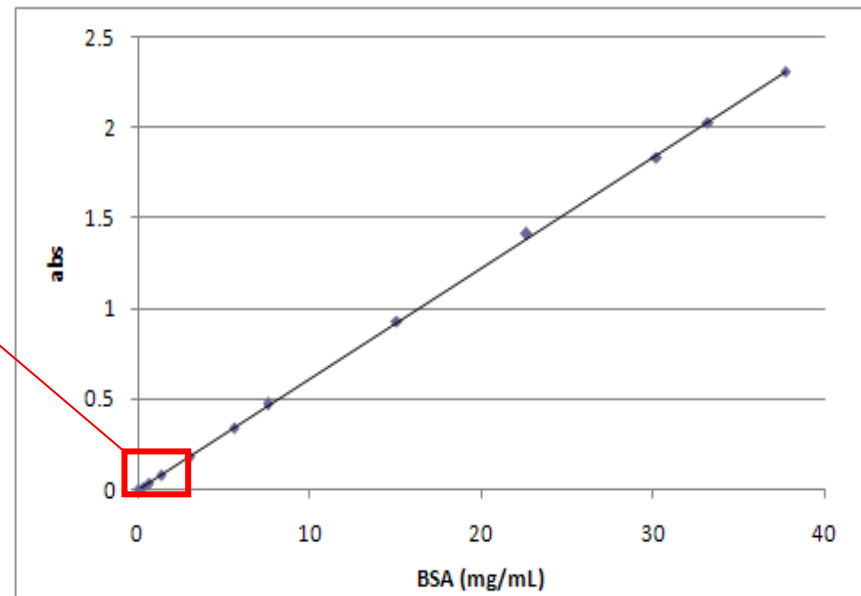
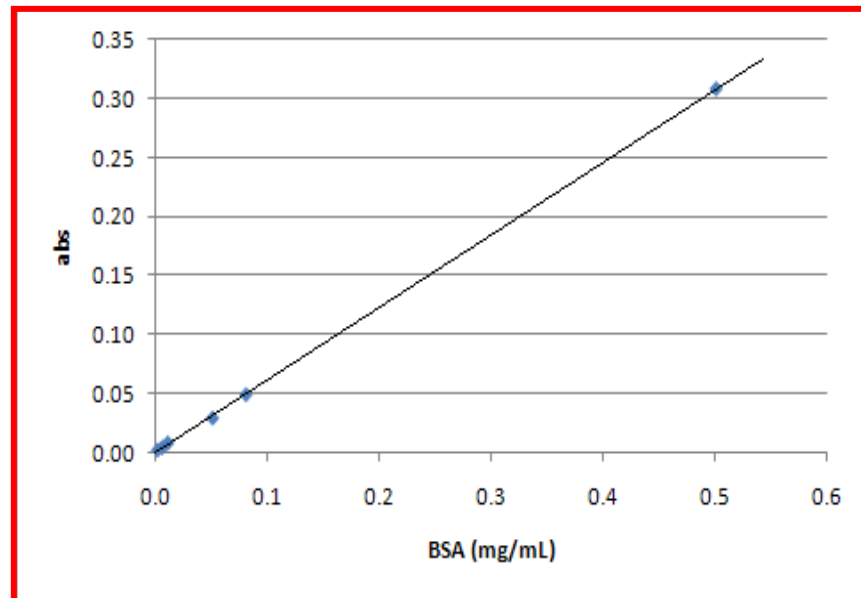
5990-7863EN Measuring the purity of low volumes of DNA at 4 °C with fiber optics microprobe

Protein/Nucleic Acid Conc. – Low Sample Volume

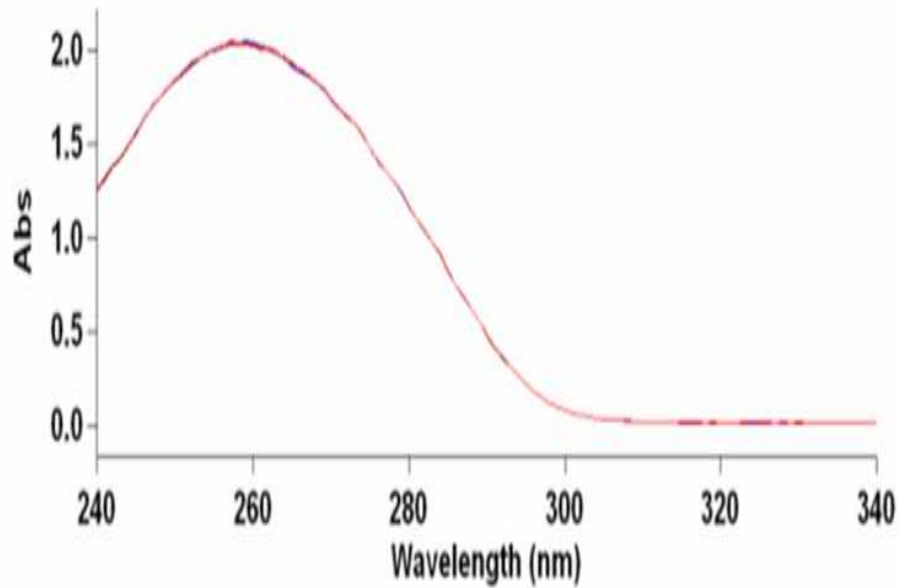
Challenges: ➤ Measure concentration accurately and reproducibility 4 μ L of sample

Solution: ➤ Cary 60 with ultra-low volume cuvette (Hellma Traycell)
➤ Direct measurement at 280nm
➤ Comparison against calibration produced from standards with known conc.

Benefits: ➤ Accurate and reproducible results
➤ Minimize dilutions and reduce sample preparation errors
➤ Preservation of precious/expensive samples



Protein Concentration – Low Sample Volume



Multiple wavelength scans of DNA demonstrate the superior reproducibility of the Cary 60 using only 4 μ L of DNA sample!



Cary 60 with Ultra Microvolume Cuvette



	Factor	2 mm Cap (ng/μL)	1 mm Cap (ng/μL)	0.2 mm Cap (ng/μL)	0.1 mm Cap (ng/μL)	Total Detection Range (ng/μL)
Factor		5	10	50	100	
dsDNA	50	6-425	*2-850	65-4250	125-8500	6-8500
ssDNA	37	5-315	10-630	50-3145	95-6290	5-6290
RNA	40	5-340	10-680	50-3400	100-6800	5-6800
Oligo	33	4-280	8-560	45-2800	85-5610	4-5610
Sample Volume		6 - 10 μL	3 - 5 μL	0.7 - 4 μL	0.5 - 3 μL	

Manufacturer recommendations

*tested on a Cary 60

Enzyme Kinetics

Enzymes are naturally produced proteins that act as catalysts to drive biological reactions, so the study of enzyme reactions is a key part in understanding the biochemistry of life.

In this type of study, the enzyme solution is usually held in a cuvette and the substrate solution is pipetted into the cuvette. The temperature of the solution is important, so these reactions are normally monitored using a thermostatted cell holder, and the solutions are stirred using a magnetic stirrer.



Using either fixed wavelengths, or a series of spectral scans, the reaction is monitored over time.

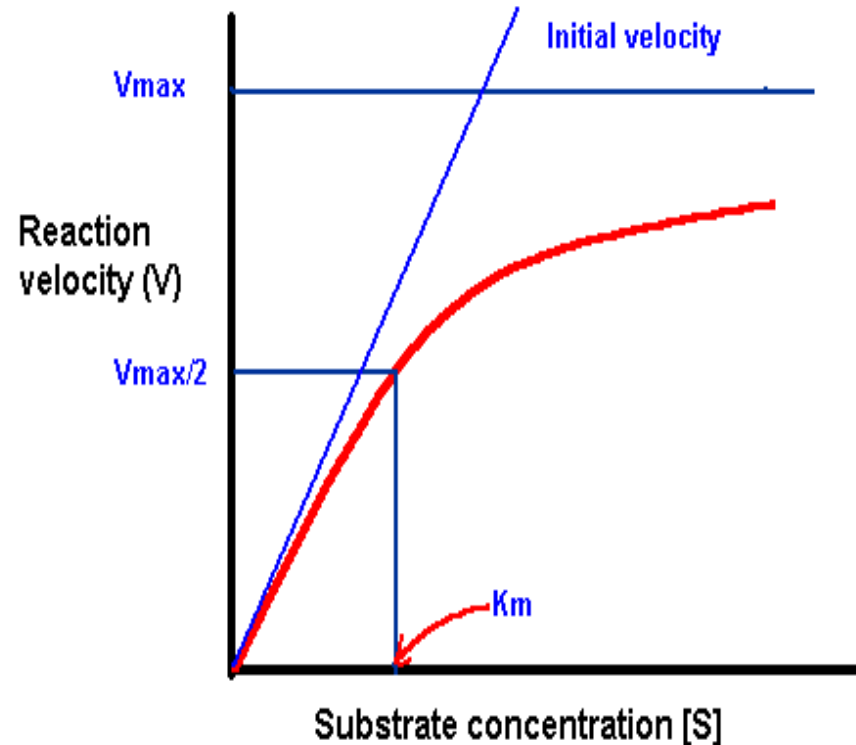


Enzyme Kinetics

A typical reaction is shown here in a Michaelis-Menten plot (red trace).

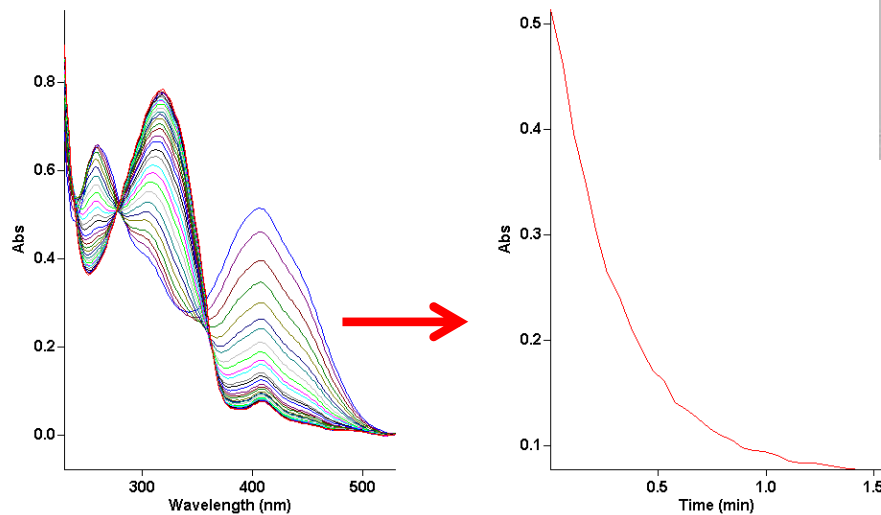
V_{max} : is the maximum rate of the reaction (i.e. rate of product formation) given by optimal concentration of substrate

K_m : the Michaelis constant; a measure of the “affinity” of enzymes for their substrates, i.e. how tightly they bind.

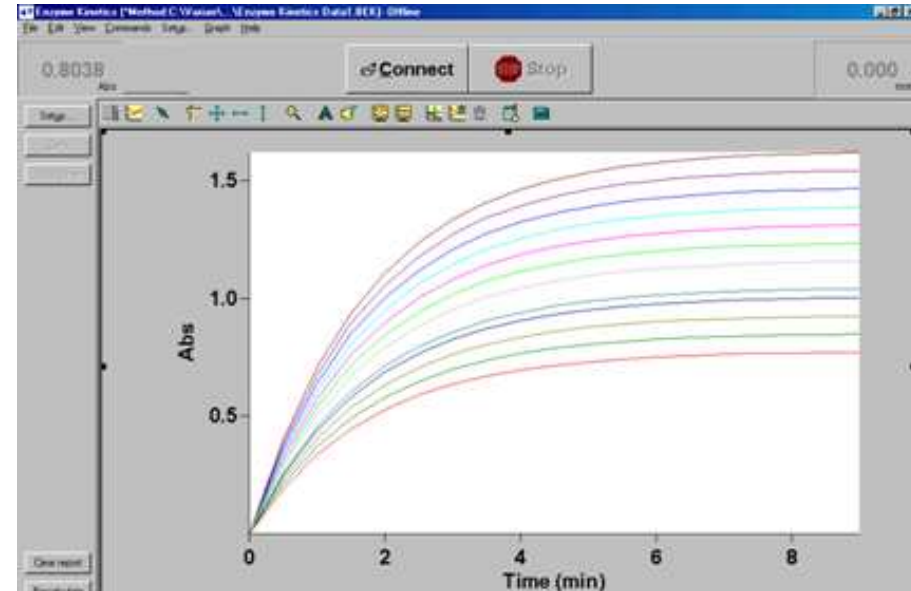


Enzyme Kinetics

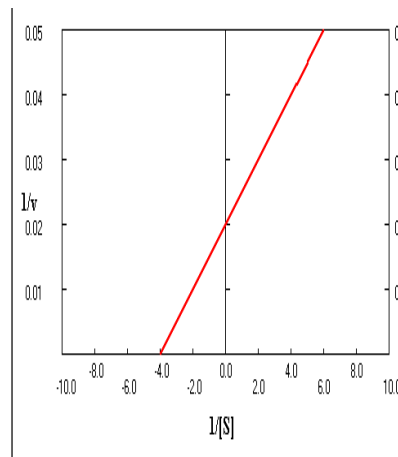
Cary 60 WinUV software offers dedicated programs for data collection, manipulation and calculations.



Reaction can be followed using complete spectral scans allowing kinetic data to be extracted for any wavelength of interest.



Fixed wavelength data collection from multiple cells.



Data can be plotted in many different formats, e.g. Lineweaver-Burk plot shown here.

Room Light Immunity Example

Application: Measuring cold samples (4 °C)

Chal

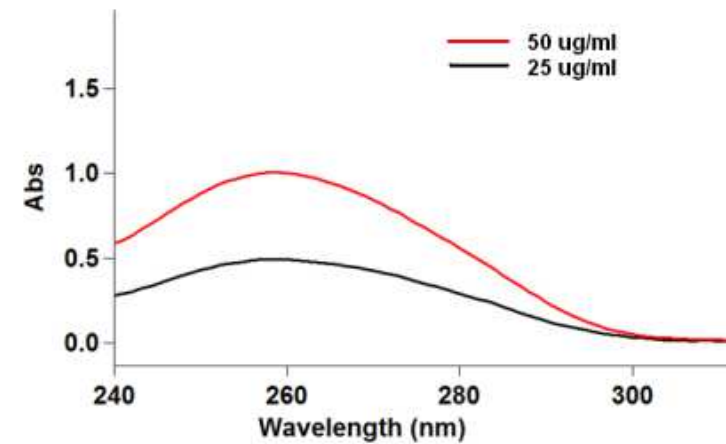
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Application Example for Solids

Application: Rapid Grading of Pearl Quality

Challenges:

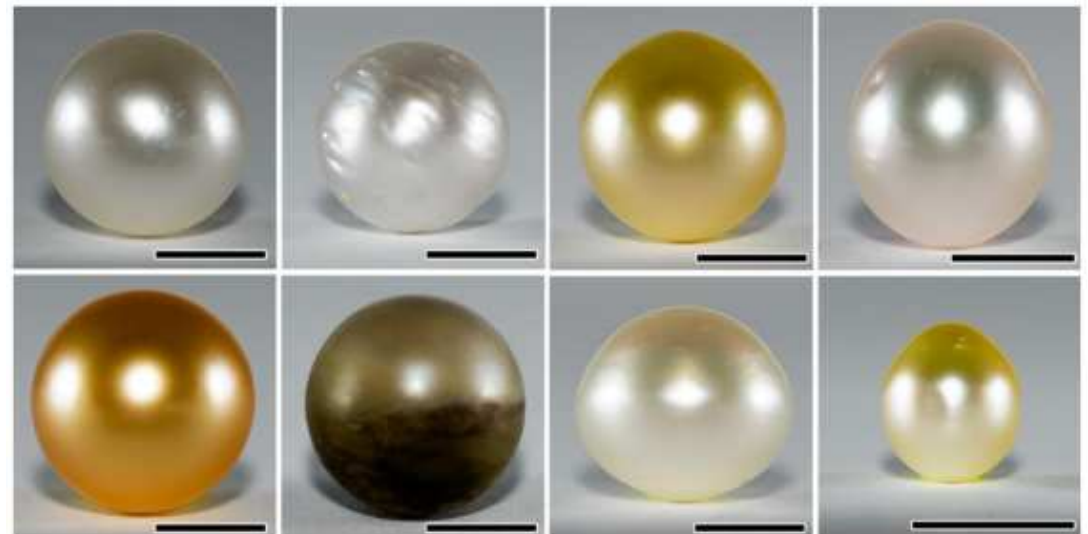
- To quickly and simply measure the “lustre” and “colour” of jewellery grade pearls

Solution:

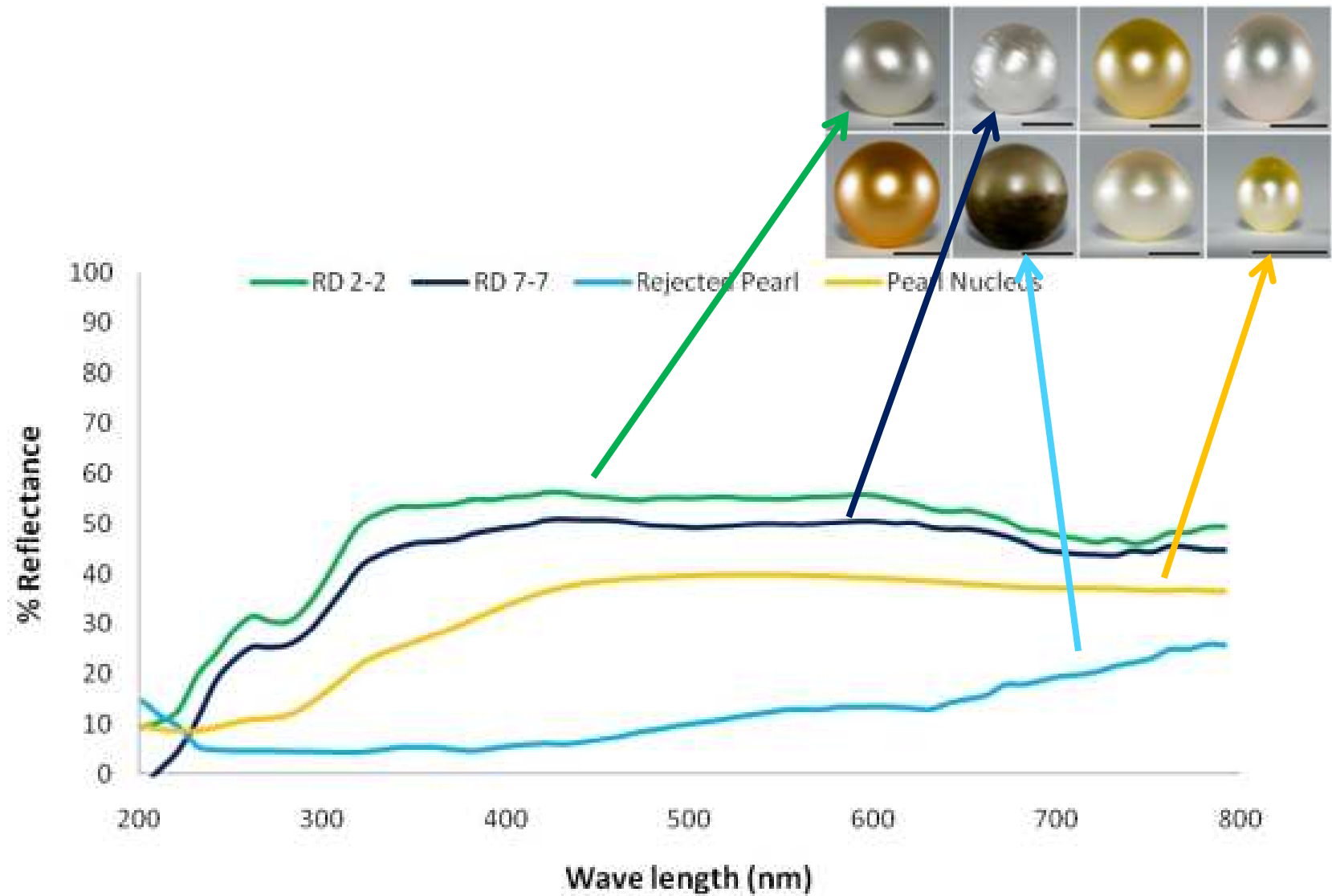
- Cary 60 with Barrelineo remote diffuse reflectance accessory

Benefits:

- Save time and money
- Reduce “subjective” nature of the tests
- Can be done by “unskilled” labour



Application Example for Solids



Thank You
Questions?



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