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.
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

- 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...
## Performance

<table>
<thead>
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<th>Abs</th>
<th>%T</th>
<th>Transmittance</th>
<th>Qty of Initial Light Measured</th>
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<td>100</td>
<td>1</td>
<td>All</td>
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<tr>
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<td>10</td>
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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.
A good analogy is car headlights as shown here.
Xenon Flash Lamp Source - Lifetime

$3 \times 10^9$ 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

Wavelength (nm)

Abs

Far UV  Near UV  Violet  Blue  Green  Yellow  Orange  Red  Near IR

200  300  400  500  600  700  800  900  1000

Agilent Technologies
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

![Graph showing wavelength vs absorption with different bandwidths: 0.2nm, 1nm, 2nm, 5nm, 10nm.](image)
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 KMnO₄ 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
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

Application focused, Windows 7 WinUV Software

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

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
Markets and Applications

ACADEMIA
- 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

BIOTECH & PHARMA
- 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

CHEMICALS
- 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 4μL 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 µL of DNA sample!
## Cary 60 with Ultra Microvolume Cuvette

<table>
<thead>
<tr>
<th>Factor</th>
<th>2 mm Cap (ng/µL)</th>
<th>1 mm Cap (ng/µL)</th>
<th>0.2 mm Cap (ng/µL)</th>
<th>0.1 mm Cap (ng/µL)</th>
<th>Total Detection Range (ng/µL)</th>
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<tbody>
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<td>dsDNA</td>
<td>50</td>
<td>10</td>
<td>50</td>
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<tr>
<td>Sample Volume</td>
<td>6 - 10 µL</td>
<td>3 - 5 µL</td>
<td>0.7 - 4 µL</td>
<td>0.5 - 3 µL</td>
<td></td>
</tr>
</tbody>
</table>

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).

\( V_{\text{max}} \): 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)

Challenge:
- Difficulty in measuring samples directly from storage (e.g., fridge) without compromising data quality

Solution:
- Using Cary 60 with Fiber Optics Microprobe
- Save time and money
- Improve workflow by minimizing sample handling

Benefit:
- No compromise in accuracy or reproducibility of data due to unique optical design

\[ \text{Abs} \]

\[ \text{Wavelength (nm)} \]

50 ug/ml
25 ug/ml
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 Barrelino 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?