

Optimizing Your Absorption or Fluorescence Thermal Melt Run

CAG

Presented by Mark Fisher

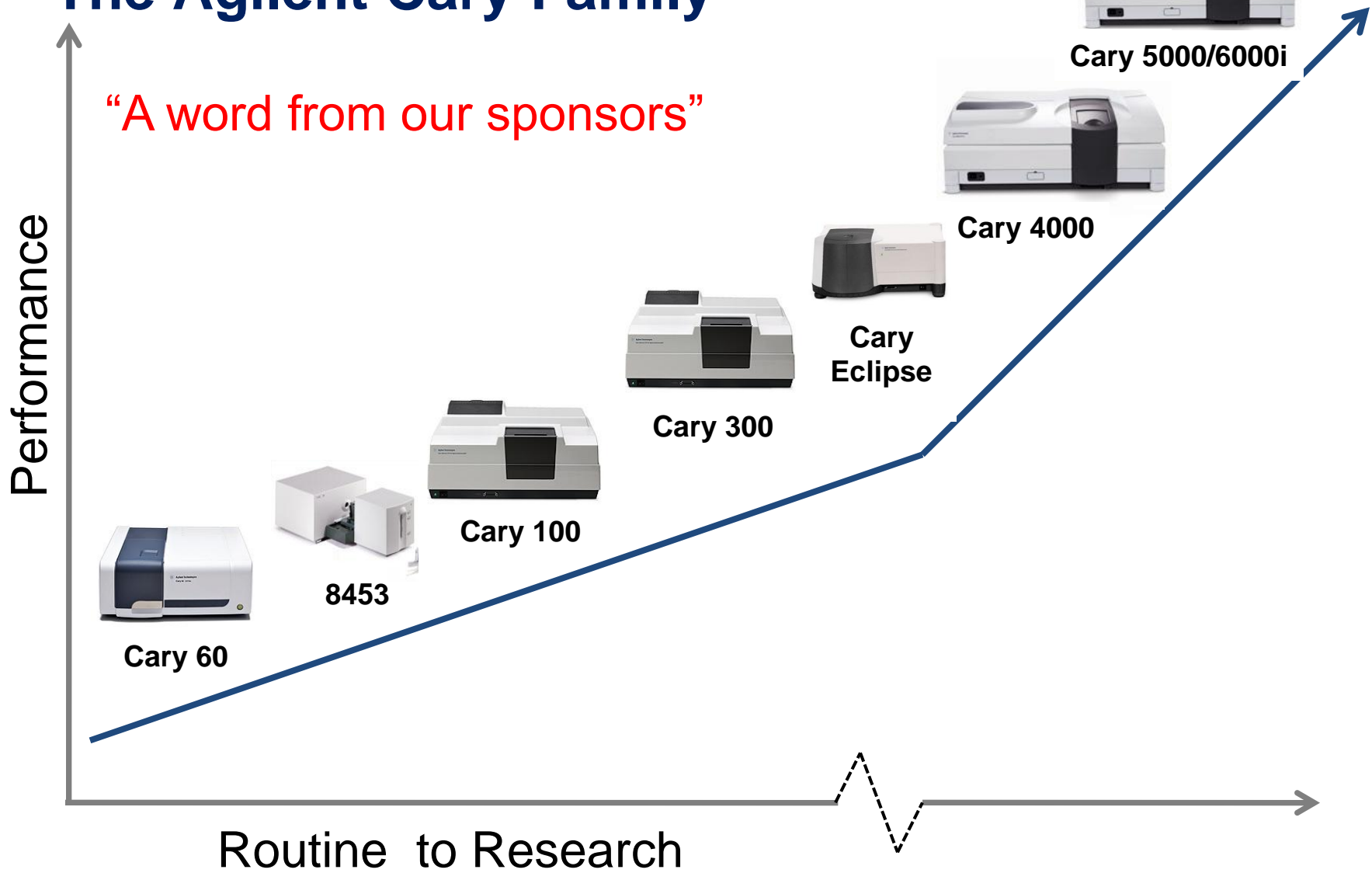
Applications Engineer

November 6, 2012

Thermal Melt Application

- Typically used to follow a change in signal as temperature is changed
- Absorption or Fluorescent Intensity can be used to monitor system as temperature is ramped
- Single Wavelength monitoring is generally done
- Scan of a Spectral Region may be desired
- How can you increase Sample throughput ?

UV-Vis NIR Spectroscopy Solutions The Agilent Cary Family

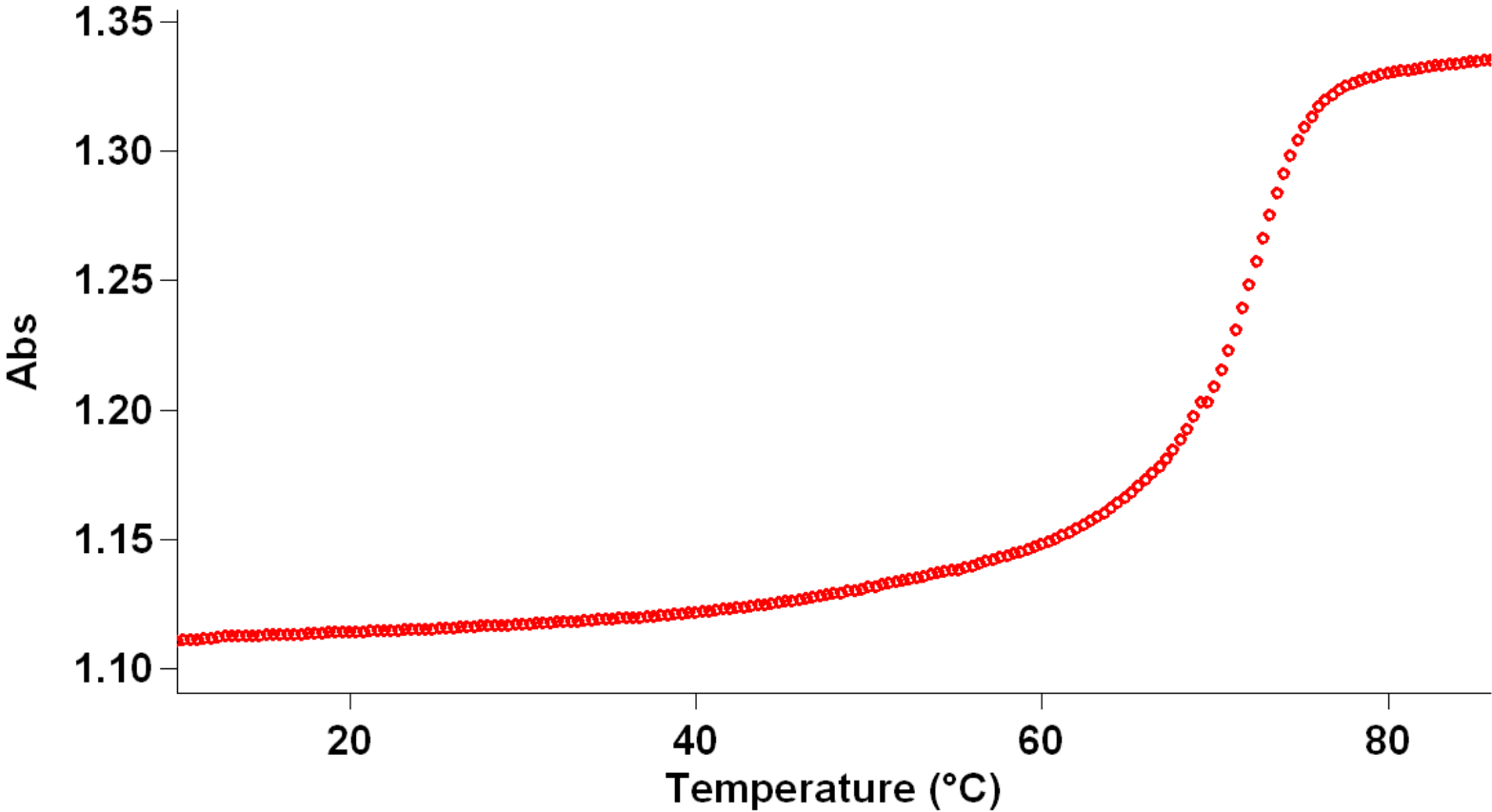


Thermal Melts using UV-Vis Absorption

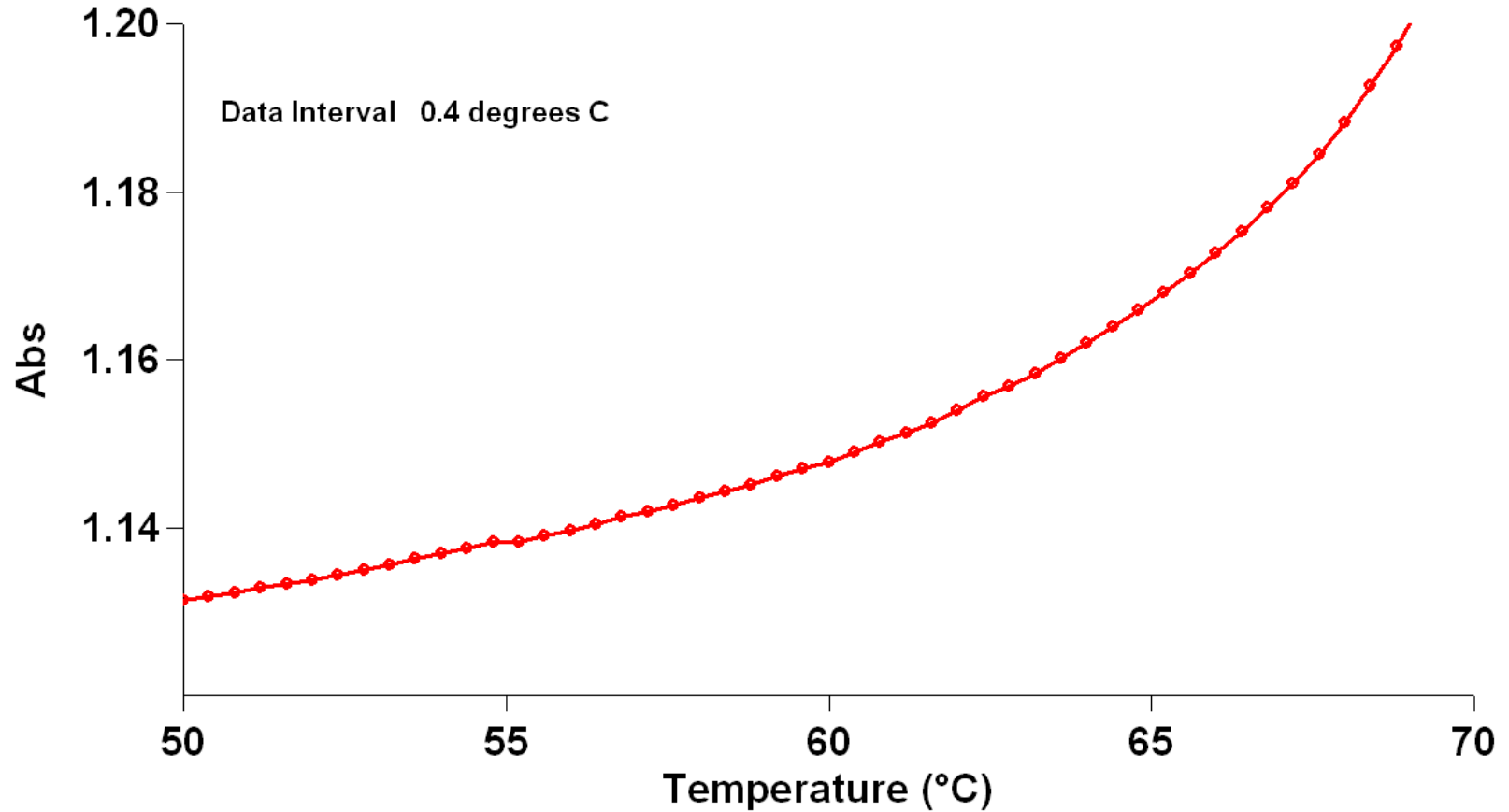
Instrumentation Needed for a Thermal Melt Collection

- Instrument typically Cary 100 or 300 (8453 is a possibility as well)
- Multi-cell Changer with Temperature Control
- Temperature Probe
- Purge Gas if Initial or Final Temperature 15 deg C or less

Typical Thermal Melt Curve



Thermal Melt Curve Expanded



Setup Dialog Box and Setting Up Simple Collection

The screenshot shows the 'Setup' dialog box with the 'Cary' tab selected. The 'Cary Instrument Control' section includes settings for Wavelength (260.00 nm), SBW (1.0 nm), Ave Time (2.000 s), and Y Min (0.00). The 'Collect Temperatures' section shows Start °C (25.0), Return to °C (25.0), and Temperature Monitor (Block). The 'Simple Collect' radio button is selected, and the Number of Stages is set to 3. A table below shows the collection stages.

Stage	Collect Data	Data Interval (°C)	Rate (°C/min)	End (°C)	Hold (min)
1	<input checked="" type="checkbox"/>	1.00	10.00	35.00	0.00

Using Multiple Wavelengths in a Single Melt

Setup

Cary Options Accessories Analyze Calculations Reports Auto Store

Cary Instrument Control

Instrument

Wavelength (nm) 260.350 User Collect

SBW (nm) 1.0 Y Min 0.00

Ave Time (s) 2.000 Y Max 2.00

Collect Temperatures

Start °C 25.0 Temperature Monitor Block

Return to °C 25.0

Simple Collect Number of Stages 2

Advanced collect

Stage	Collect Data	Data Interval (°C)	Rate (°C/min)	End (°C)	Hold (min)
1	<input checked="" type="checkbox"/>	1.00	10.00	35.00	0.00

Show Status Display OK Cancel Help

User Collect Function

Setup

Cary Options Accessories Analyze Calculations Reports Auto Store

Cary Instrument Control

Instrument

Wavelength (nm) Read(260)-Read(320) User Collect

SBW (nm) 1.0 Y Min 0.00

Ave Time (s) 2.000 Y Max 2.00

Collect Temperatures

Start °C 25.0 Temperature Monitor Block

Return to °C 25.0

Simple Collect Advanced collect Number of Stages 2

Stage	Collect Data	Data Interval (°C)	Rate (°C/min)	End (°C)	Hold (min)
1	<input checked="" type="checkbox"/>	1.00	10.00	35.00	0.00

Show Status Display

OK Cancel Help

Typical Simple Single Ramp

- 0.1 degrees/minute
- Collect data every 0.1 degrees or 0.5 degrees
- 20-90 degrees C

- Time Required to Finish Data Collection on a Sample
 $70 \text{ deg} / 0.1 \text{ deg per min} = 700 \text{ minutes}$ or 11 hours and 40 min

Advance Collect using Multiple Temperature Collection Stages

The screenshot shows a 'Setup' dialog box with the following configuration:

- Cary Instrument Control:**
 - Instrument: Wavelength (nm) 260.00, SBW (nm) 1.0, Ave Time (s) 2.000
 - User Collect
 - Y Min: 0.00, Y Max: 2.00
- Collect Temperatures:**
 - Start °C: 20.0, Return to °C: 25.0, Temperature Monitor: Probe 1
 - Simple Collect, Advanced collect
 - Number of Stages: 3
- Temperature Collection Stages Table:**

Stage	Collect Data	Data Interval (°C)	Rate (°C/min)	End (°C)	Hold (min)
1	<input checked="" type="checkbox"/>	1.00	1.00	50.00	0.00
2	<input checked="" type="checkbox"/>	0.10	0.10	70.00	0.00
3	<input checked="" type="checkbox"/>	1.00	1.00	90.00	0.00

At the bottom, there is a checkbox for 'Show Status Display' and buttons for 'OK', 'Cancel', and 'Help'.

Optimizing Data Collection Using Multi-Stage Ramp

- 20 to 50 degrees Ramp at 1.0 deg/min
- 50 to 70 degrees Ramp at 0.1 deg/min
- 70 to 90 degrees Ramp at 1.0 deg/min
- Collect Data every 0.1 deg or 0.5 deg
- Time Required to Finish Data Collection

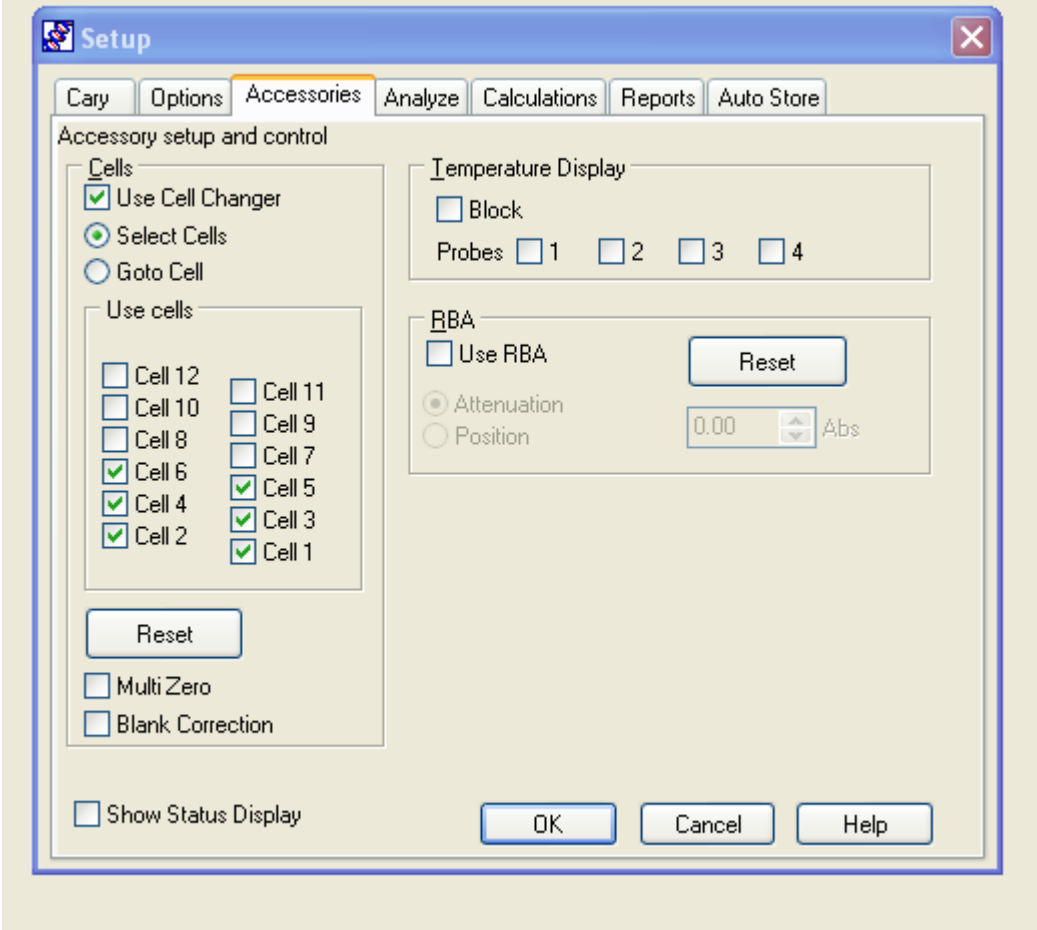
20 to 50 deg 30 deg/ 1 deg per min = 30 min

50 to 70 deg 20 deg/ 0.1 deg per min = 200 min

70 to 90 deg 20 deg/ 1 deg per min = 20 min

Total Time 250 min or 4 hours and 10 min

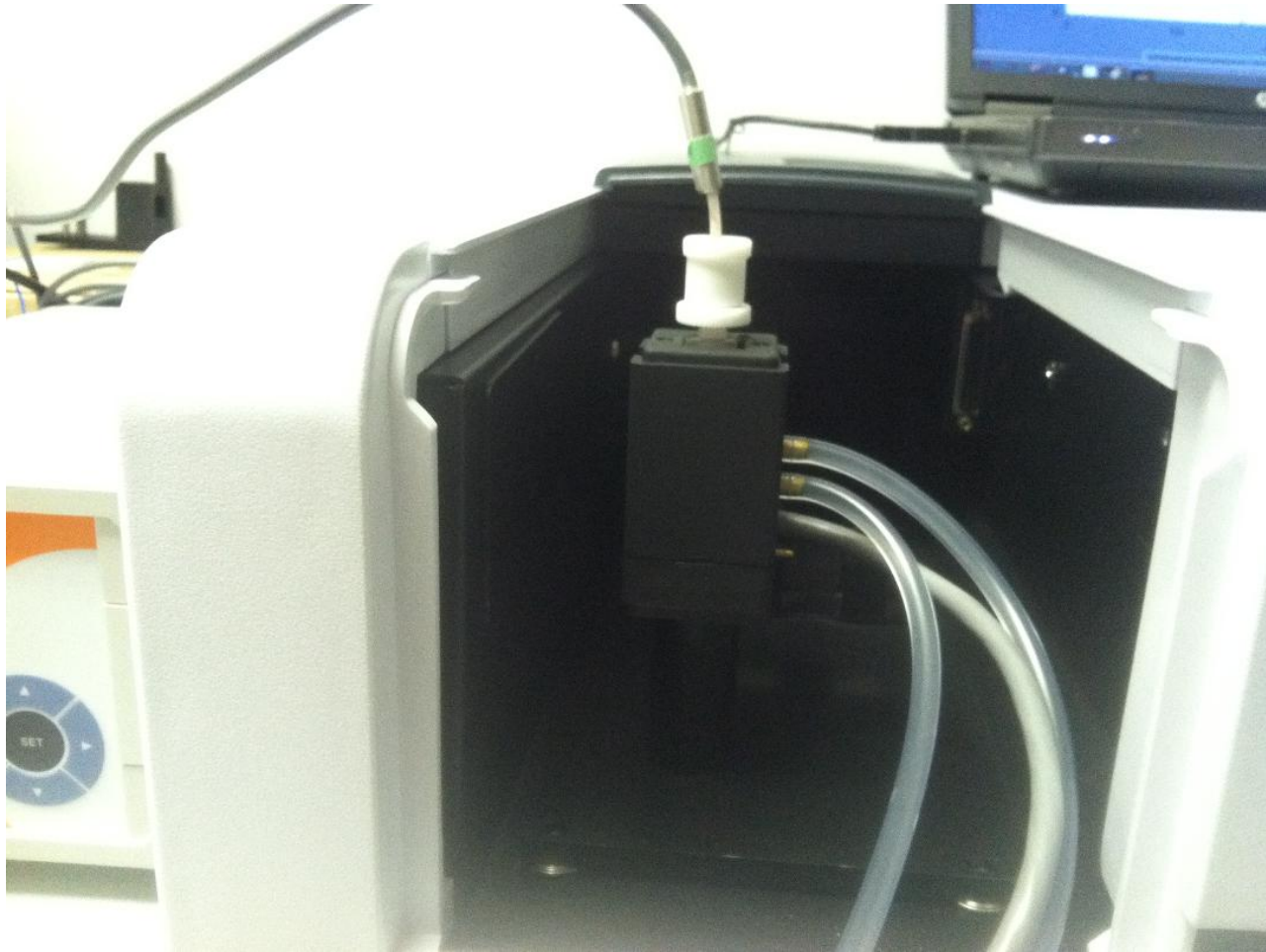
Double Beam SetUp



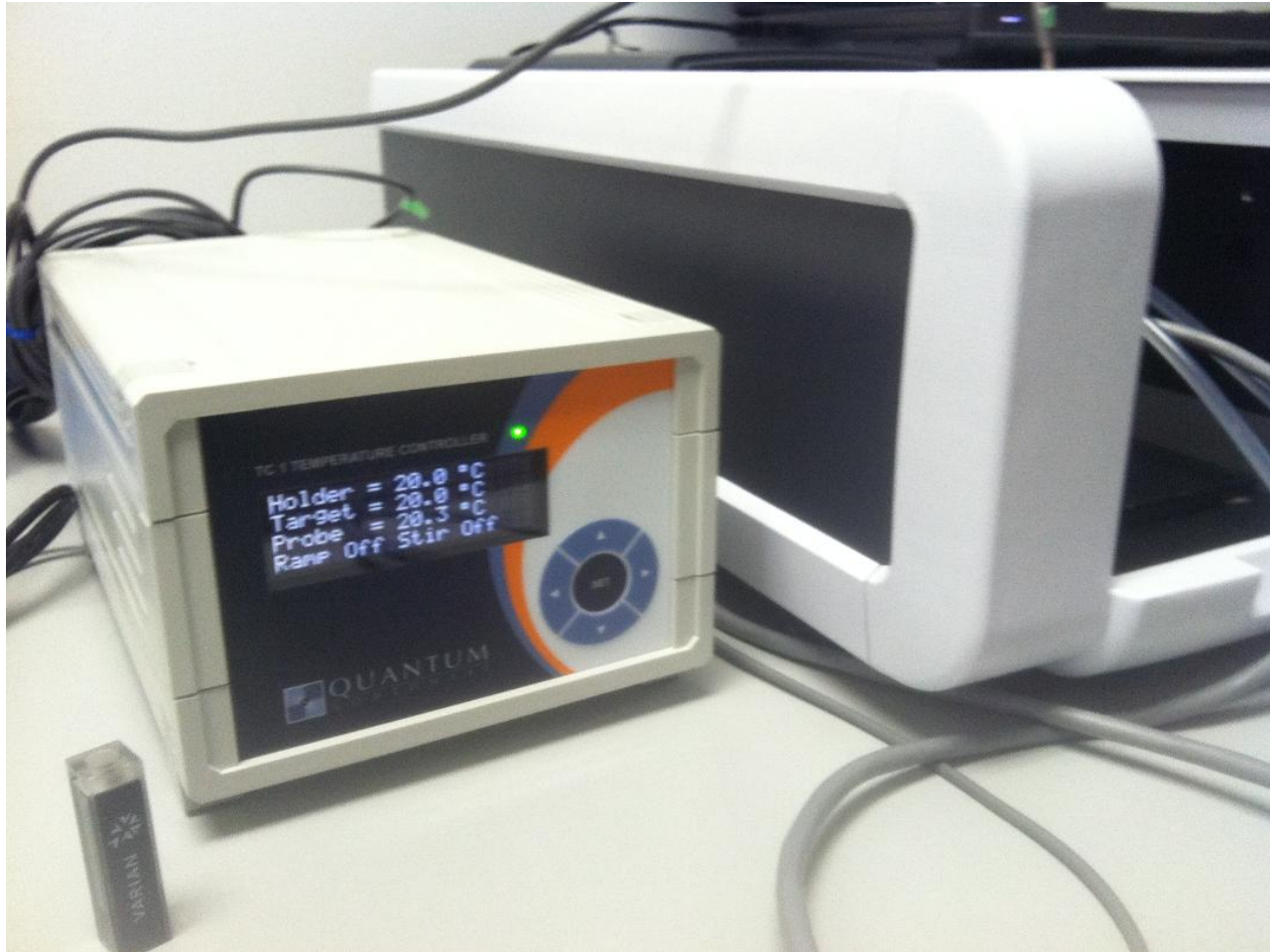
Where and When Should the Temperature be Measured

- Choices for Monitoring Temperature
 - Block
 - Probe inserted into Cuvette
 - Can Sacrifice one cuvette for data collection if you do not want sample in contact with probe
- Temperature measured before each measurement

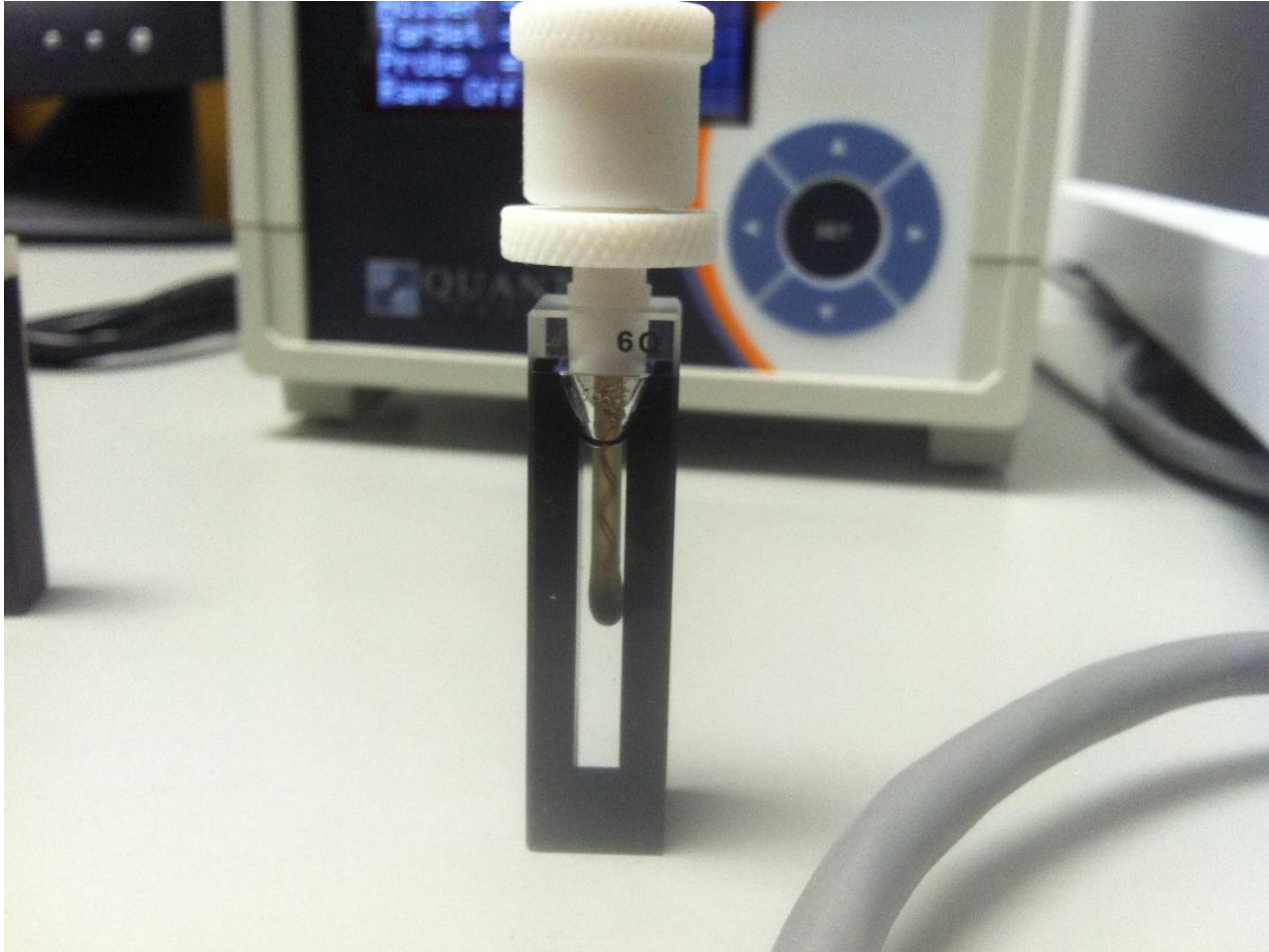
QNW T2 Single Cell Peltier Accessory



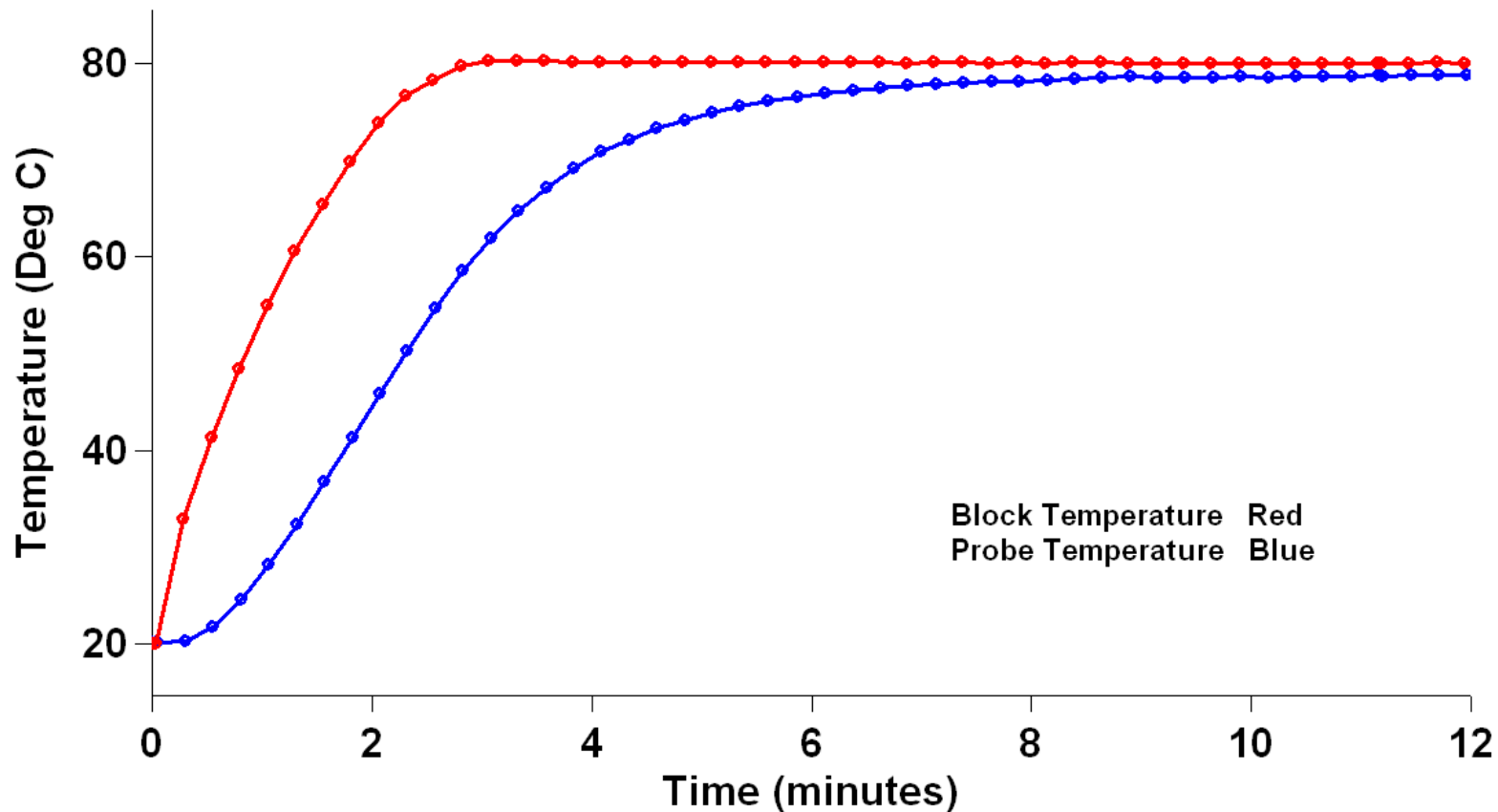
QNW T2 Temperature Controller



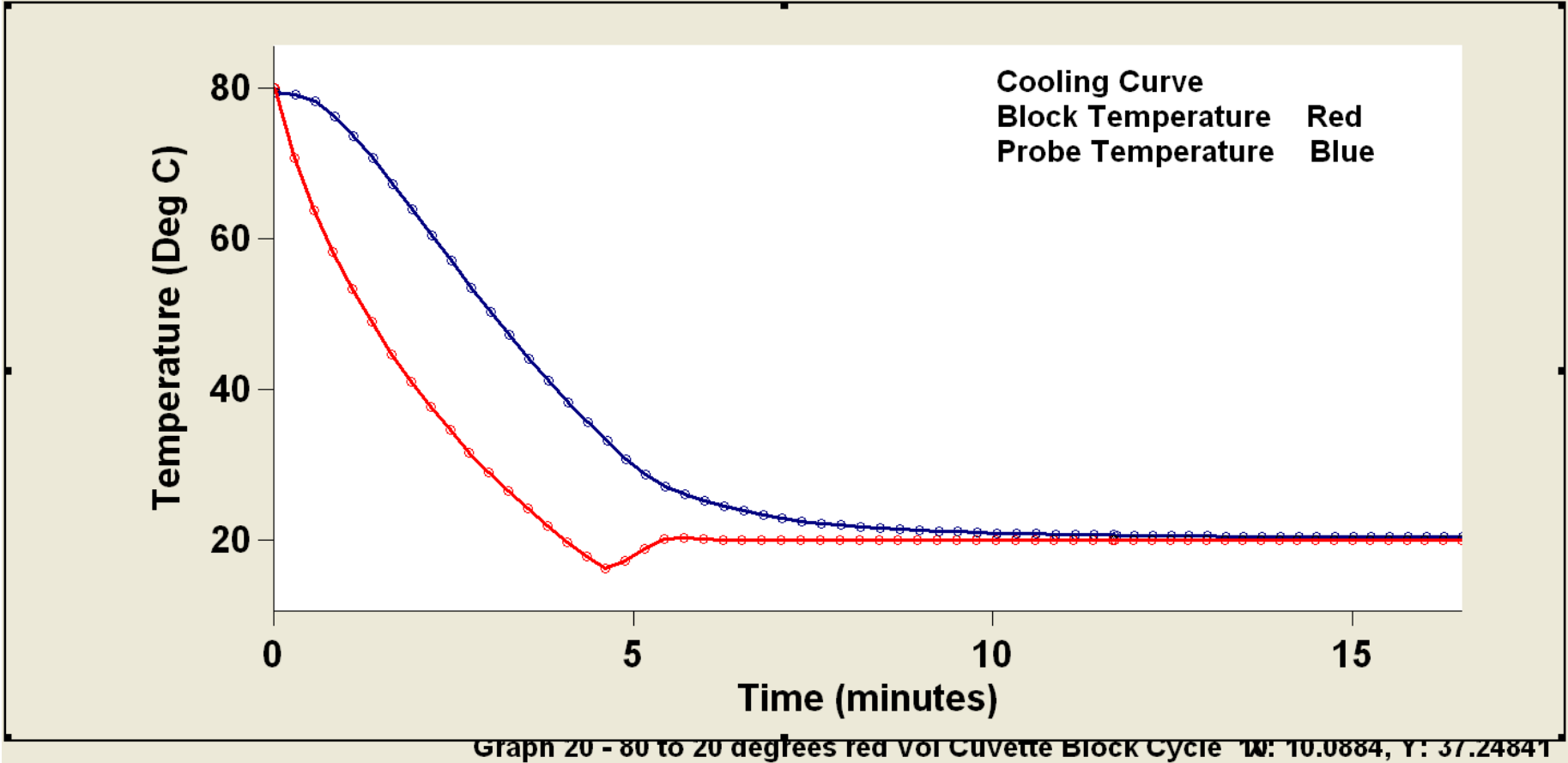
Temperature Probe In Semi-Micro Cell



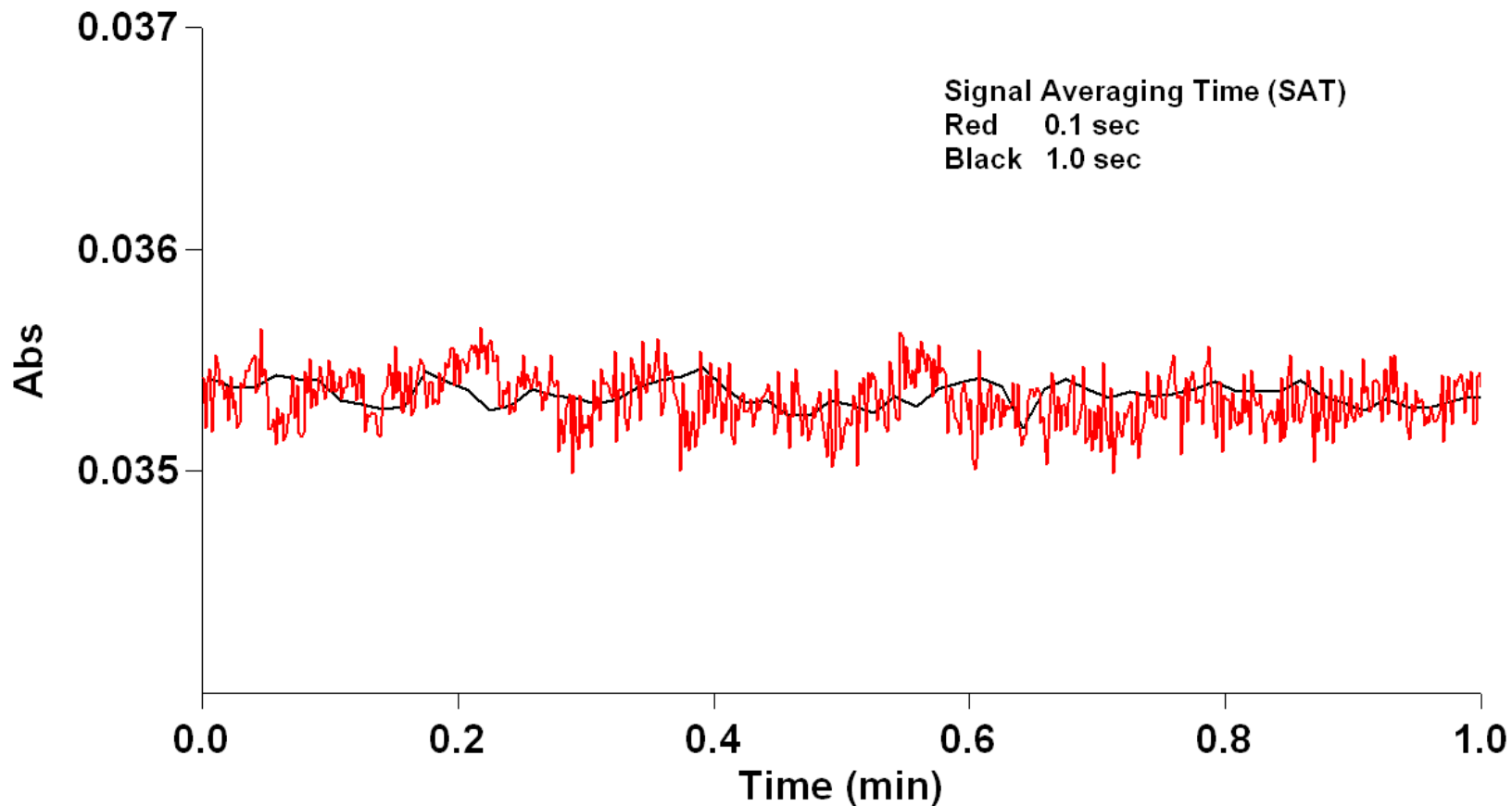
Temperature Lag from Block to Solution Heating



Temperature Lag from Block to Solution Cooling



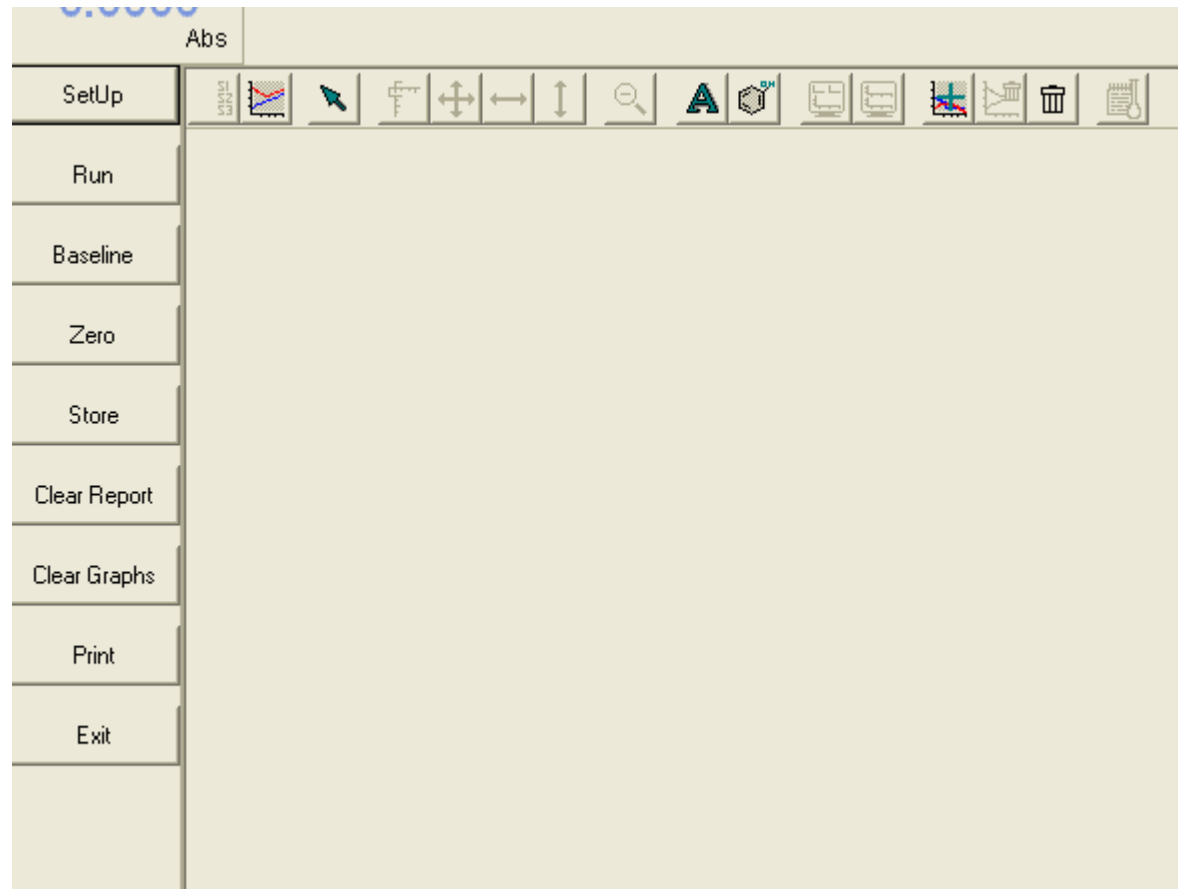
Effect of Signal Averaging Time on Noise



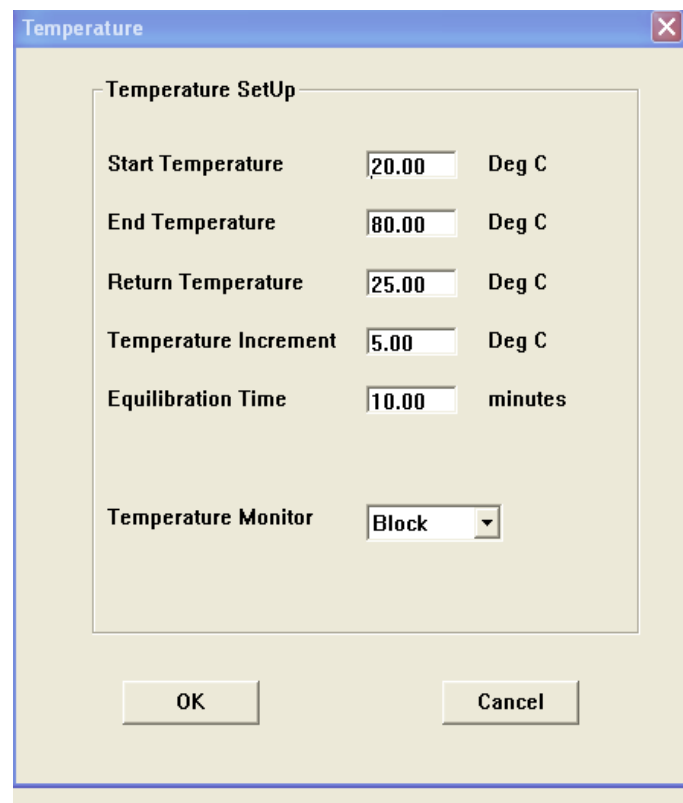
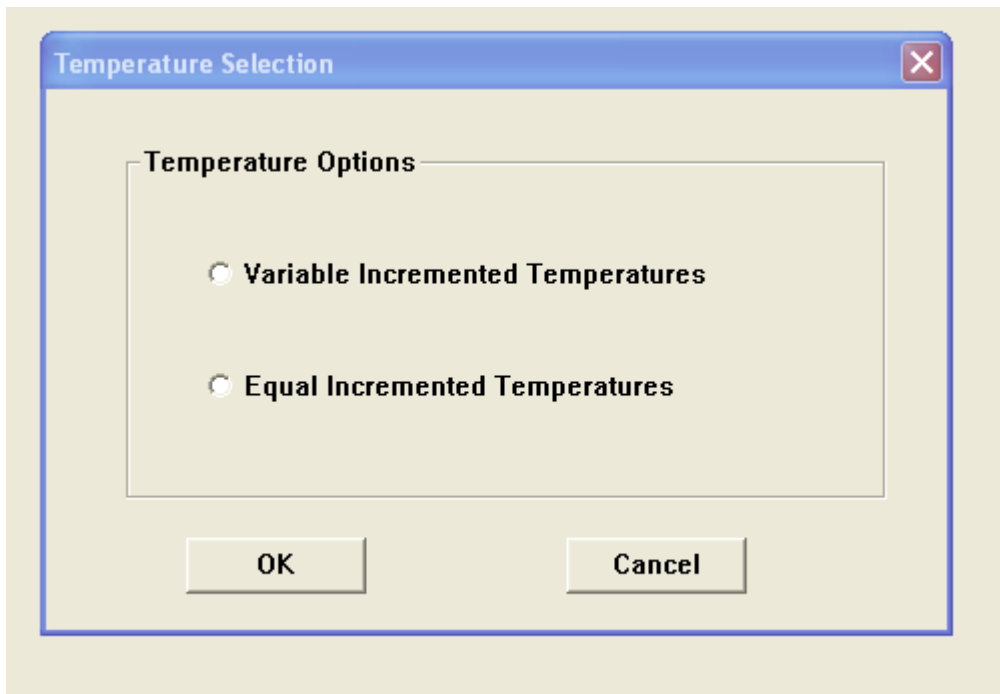
Additional Capabilities for Collecting Temperature Related Data

- Possible to Collect a Scan at User Define Temperatures
- While at each User Defined Temperature, it is possible to collect a number of scans at a user define time interval before going to the next temperature
- These capabilities are possible through ADL, which is the controlling language of the software. User have access to an editor to able to write/edit macros

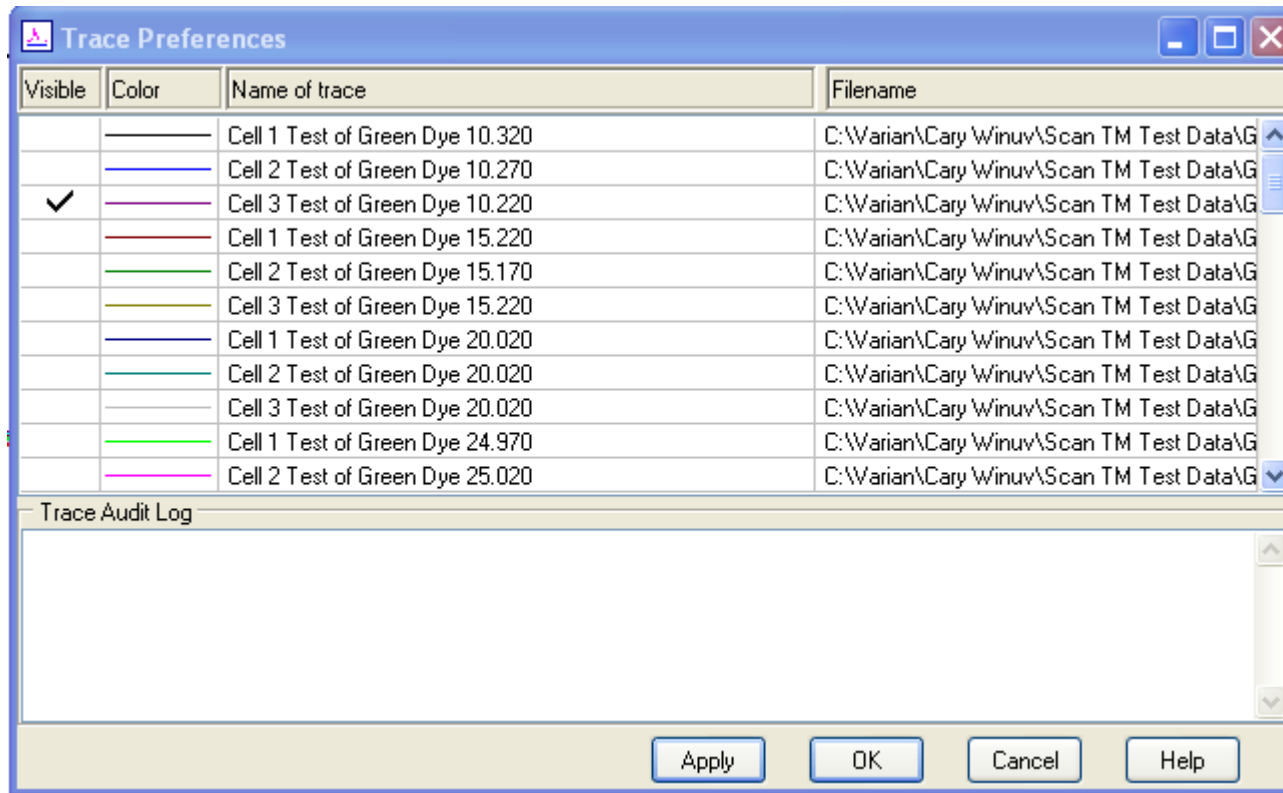
Initial Screen after Program Started



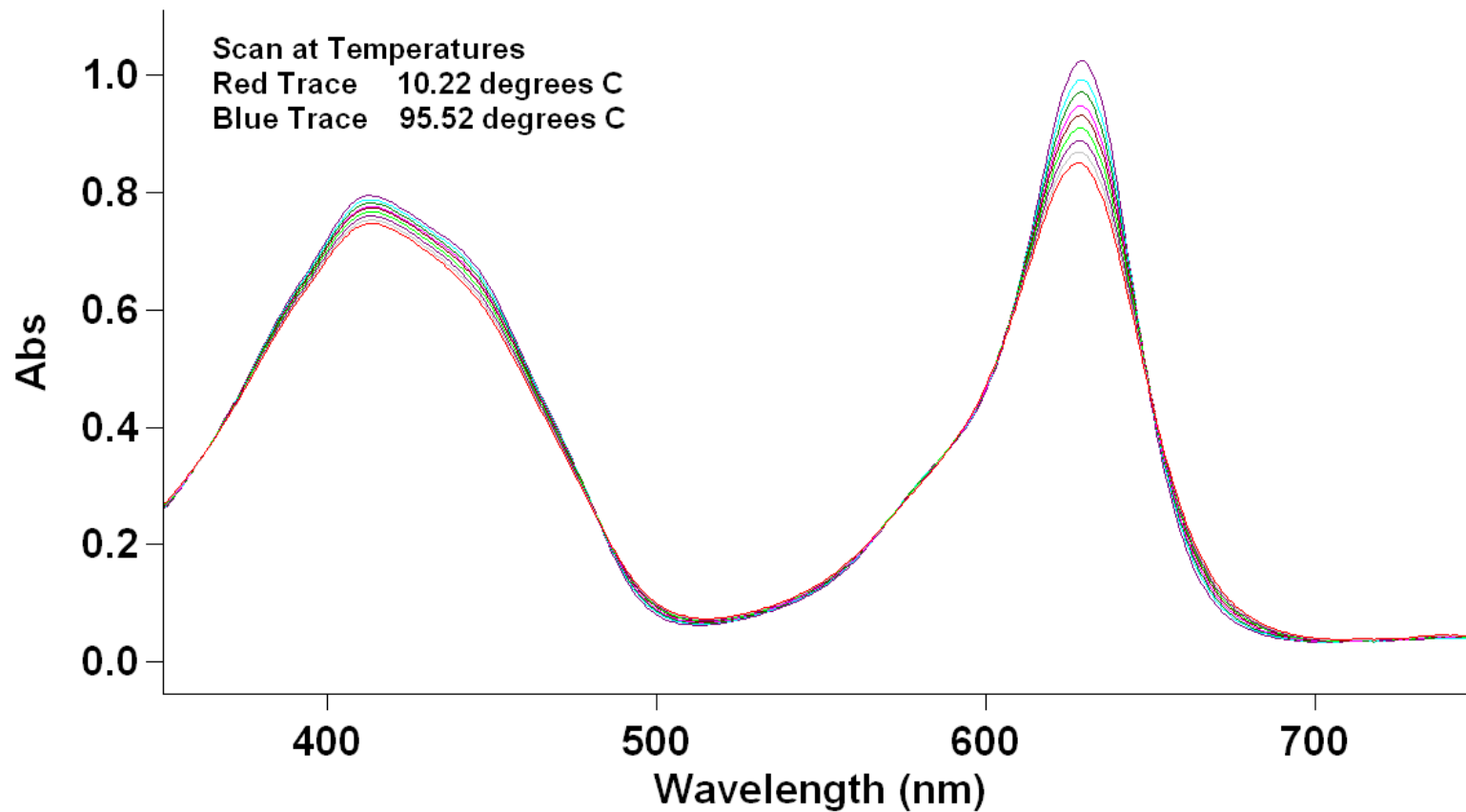
Temperature SetUp for Scans at Temperature



Trace Preferences Dialogbox Showing Trace Names



Scanning at Temperatures

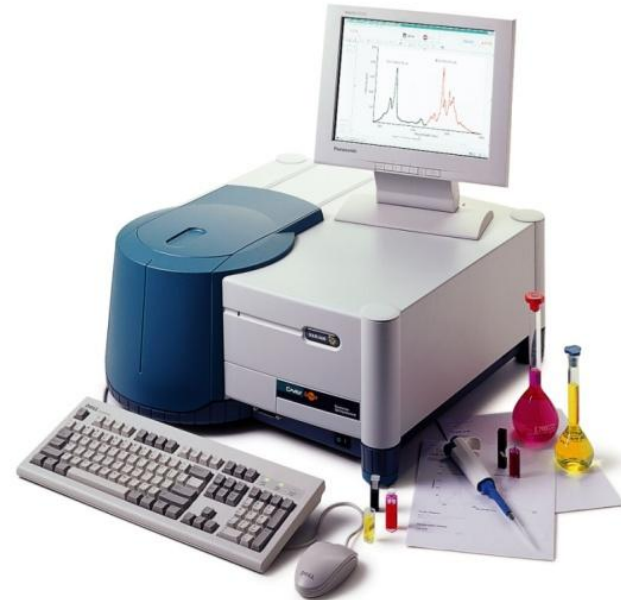


Thermal Melts using Fluorescence

Cary Eclipse Fluorescence Spectrometer

The Power of Xenon...

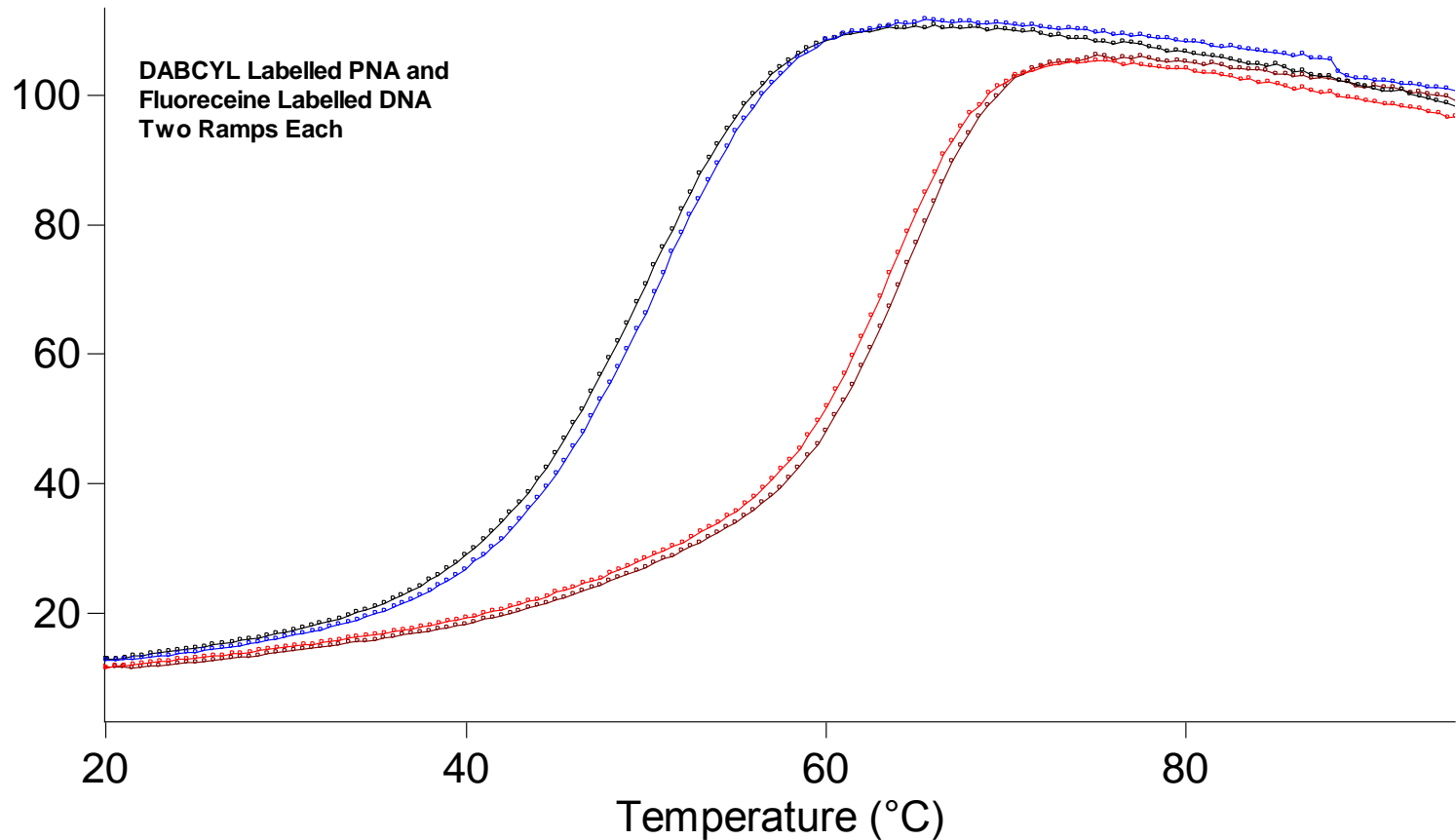
- Unique Xe flashlamp technology
- Measure small volume samples
- Fiber optics
- Room light immunity: unique, Varian patented technology
- Eliminates photo-degradation
- Long lamp lifetime



Application focus

- Biochemical applications
- Academia
- Industrial chemistry

Fluorescent Thermal Melt Curve



Simple Single Ramp Collection

Setup

Cary Options Accessories Analyze Reports Auto-store

Instrument setup

Data mode Fluorescence

Wavelength setup

Multiwavelength

Ex. Wavelength (nm) 400.00 User collect

Em. Wavelength (nm) 600.00

Ex. slit (nm) 5 Em. slit (nm) 5

Collect temperatures

Start (°C) 20.00 Ramps

Return to (°C) 25.00 Go to

Hold at start 0.0000 Thermal cycles 2

Simple collect Ave. time (s) 1.0000

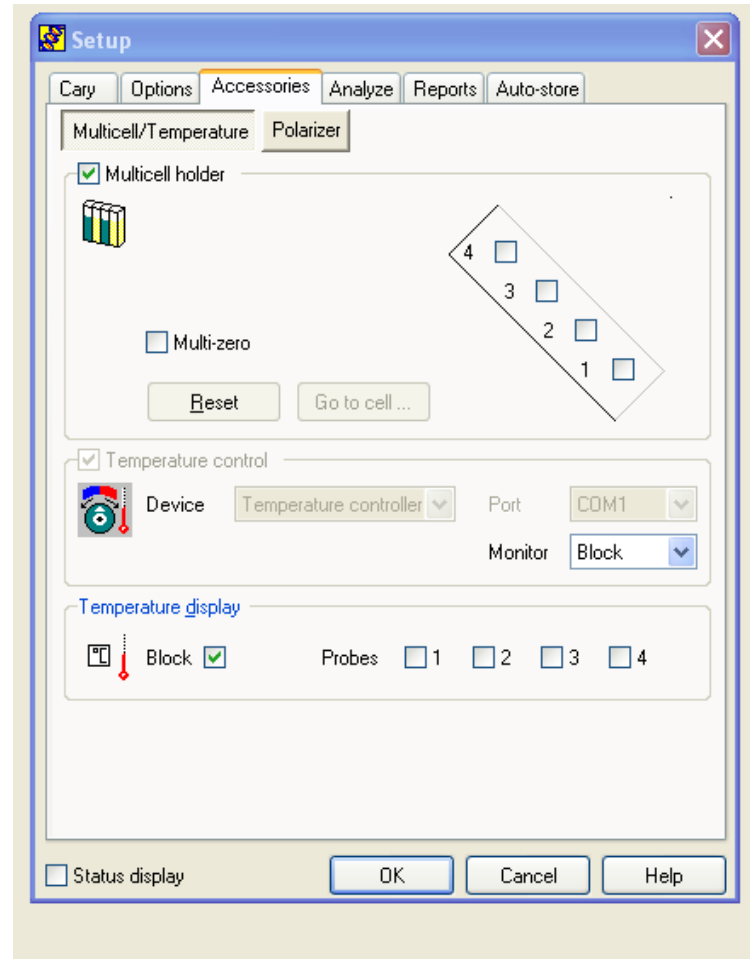
Advanced collect No. of Stages 3

Stage	Collect	Data interval	Rate (°C/min)	End (°C)	End (Hold/min)
1	<input checked="" type="checkbox"/>	1.00	2.00	90.00	0.00

Status display

OK Cancel Help

Sample Cell Selection and Temperature Monitoring Choice



Advance Collection Choices

Setup

Cary Options Accessories Analyze Reports Auto-store

Instrument setup

Data mode: Fluorescence

Wavelength setup

Multiwavelength

Ex. Wavelength (nm): 400.00 User collect

Em. Wavelength (nm): 600.00

Ex. slit (nm): 5 Em. slit (nm): 5

Collect temperatures

Start (°C): 20.00 Ramps

Return to (°C): 25.00 Go to

Hold at start: 0.0000 Thermal cycles: 2

Simple collect Ave. time (s): 1.0000

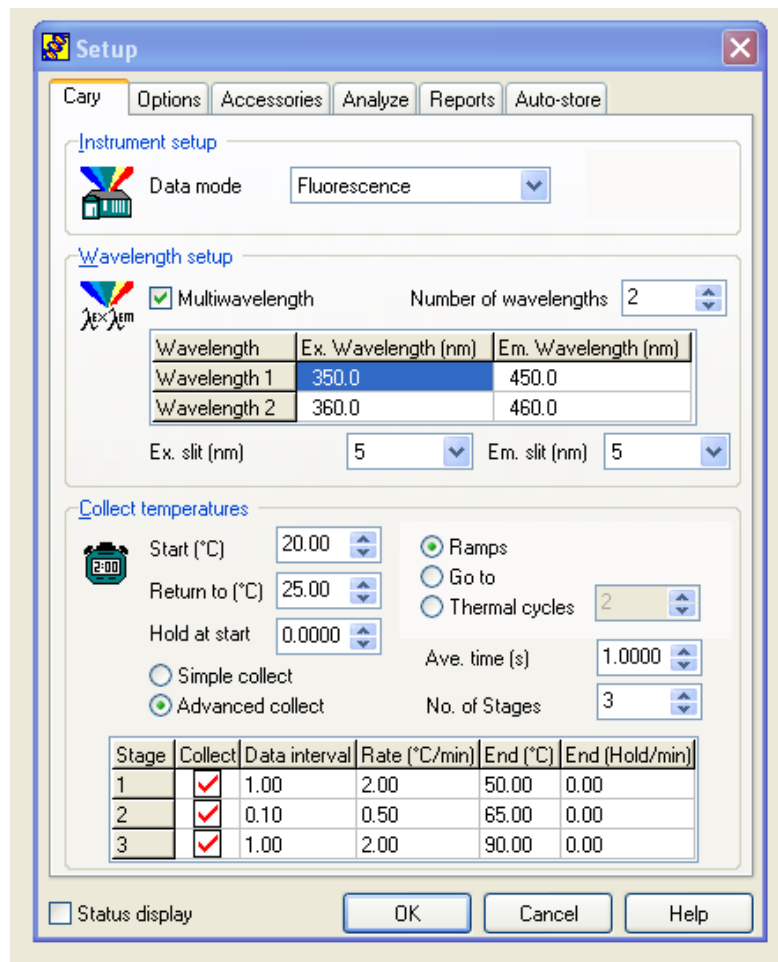
Advanced collect No. of Stages: 3

Stage	Collect	Data interval	Rate (°C/min)	End (°C)	End (Hold/min)
1	<input checked="" type="checkbox"/>	1.00	2.00	50.00	0.00
2	<input checked="" type="checkbox"/>	0.10	0.50	65.00	0.00
3	<input checked="" type="checkbox"/>	1.00	2.00	90.00	0.00

Status display

OK Cancel Help

Multiple Wavelength Pairs



Advance Collection SetUp for Fluorescence Thermal going from High to Low Intensity

Setup

Cary Options Accessories Analyze Reports Auto-store

Instrument setup

Data mode: Fluorescence

Wavelength setup

Multiwavelength

Ex. Wavelength (nm): 480.00 User collect

Em. Wavelength (nm): 520.00

Ex. slit (nm): 10 Em. slit (nm): 10

Collect temperatures

Start (°C): 50.00 Ramps

Return to (°C): 50.00 Go to

Hold at start: 0.0000 Thermal cycles: 2

Simple collect Ave. time (s): 1.00000

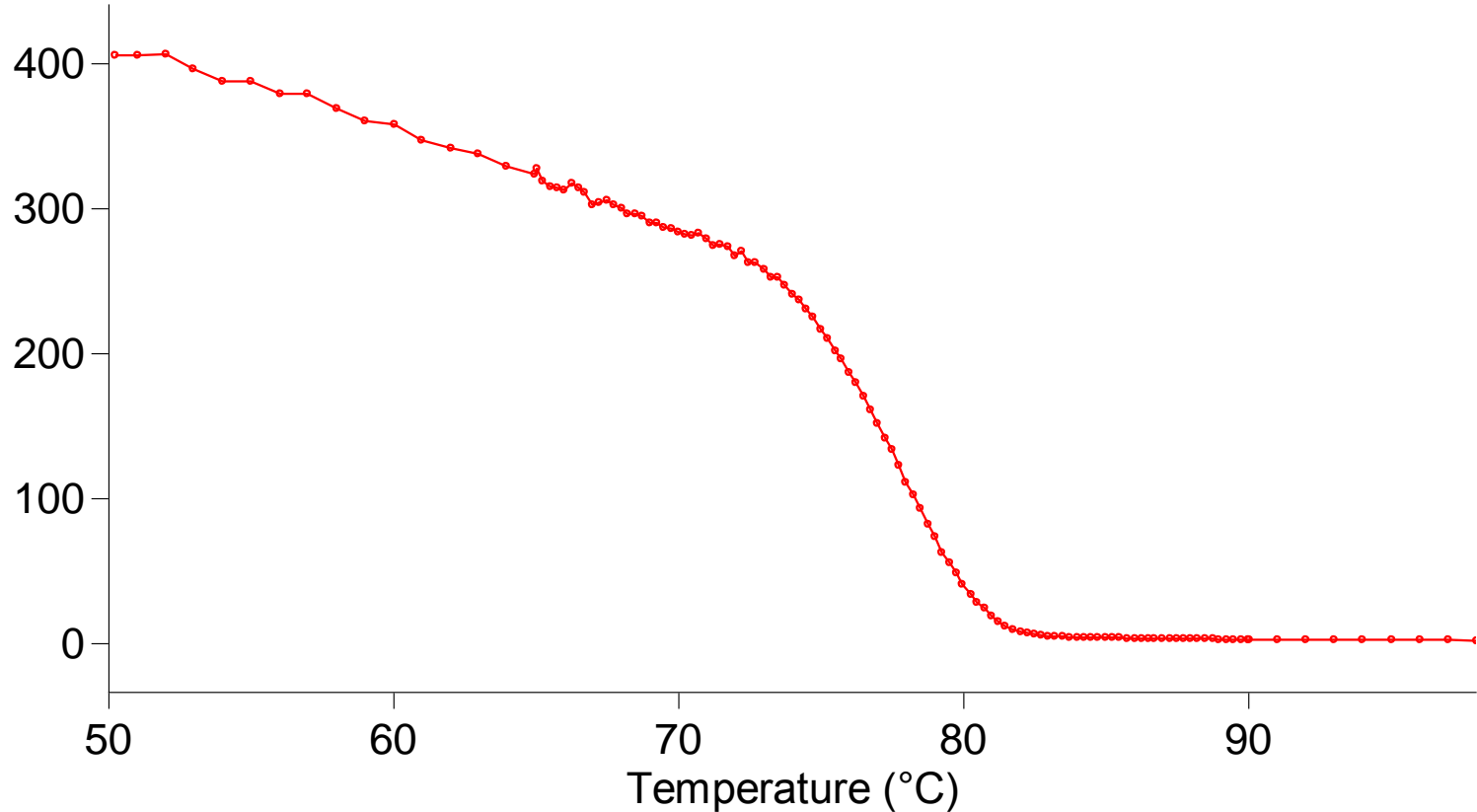
Advanced collect No. of Stages: 6

Stage	Collect	Data interval	Rate (°C/min)	End (°C)	End (Hold/min)
1		1.00	2.00	65.00	0.00
2	<input checked="" type="checkbox"/>	0.25	1.00	90.00	0.00
3	<input checked="" type="checkbox"/>	1.00	2.00	98.00	0.00

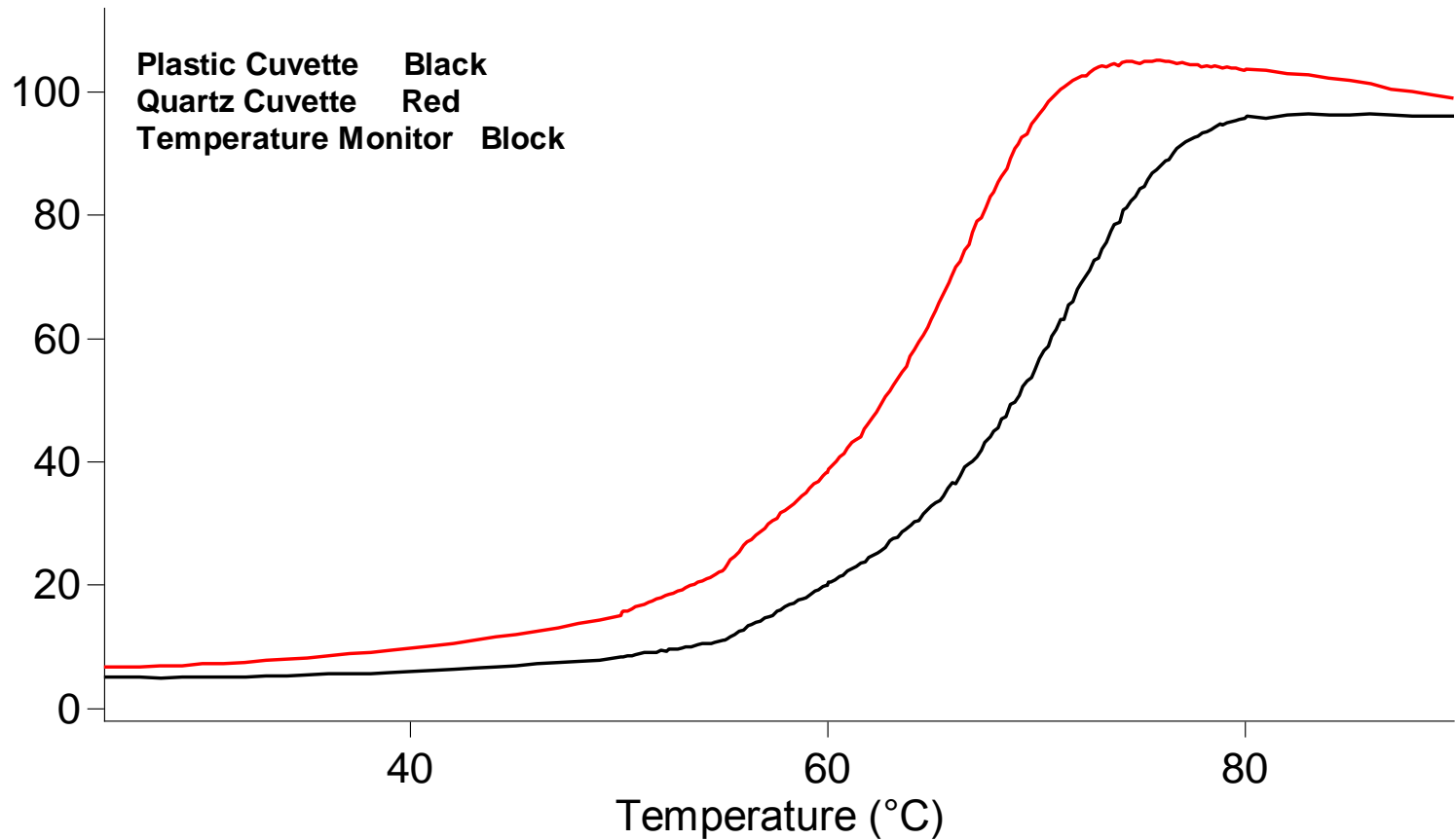
Status display

OK Cancel Help

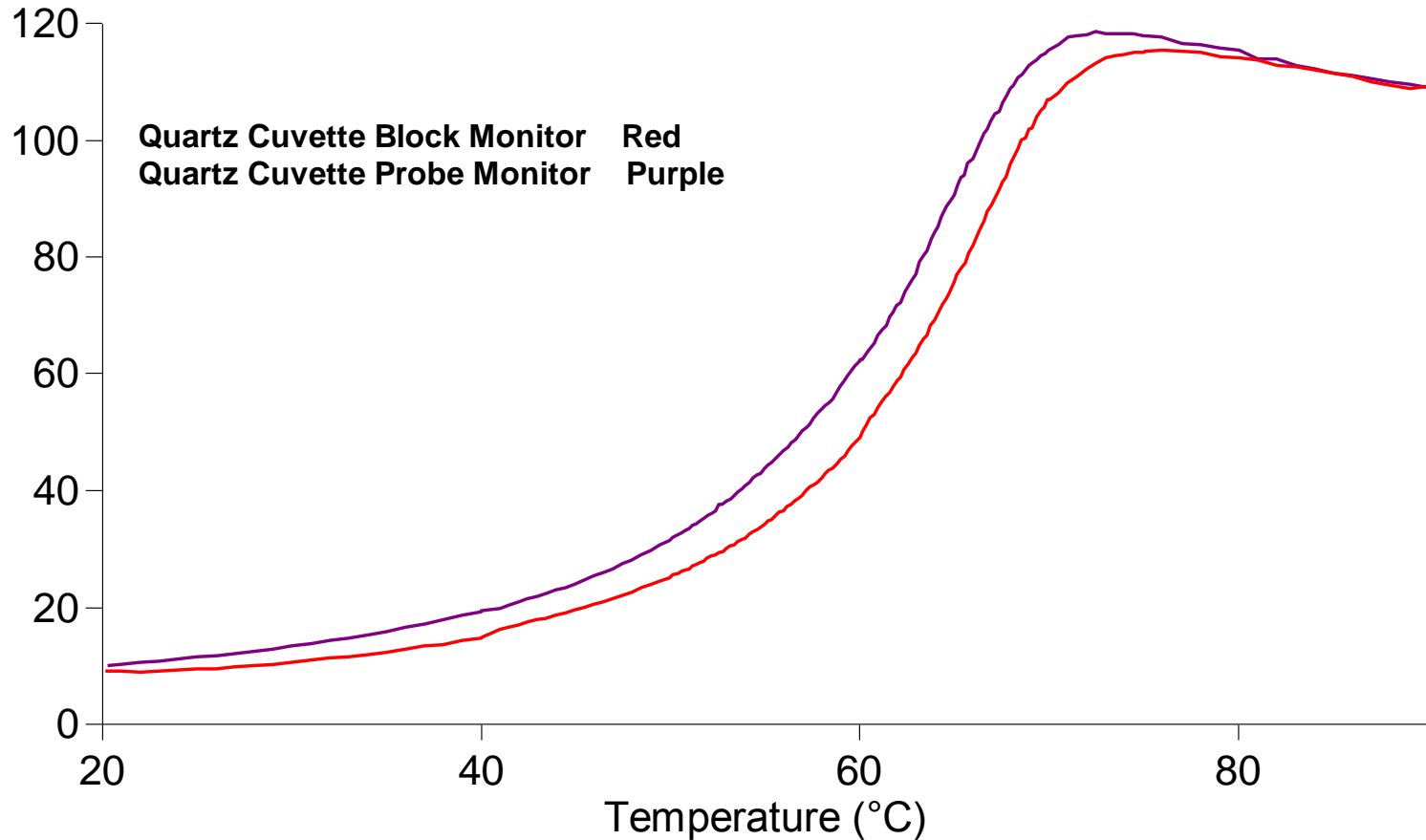
Example of Fluorescence Thermal Melt going from High to Low Intensity



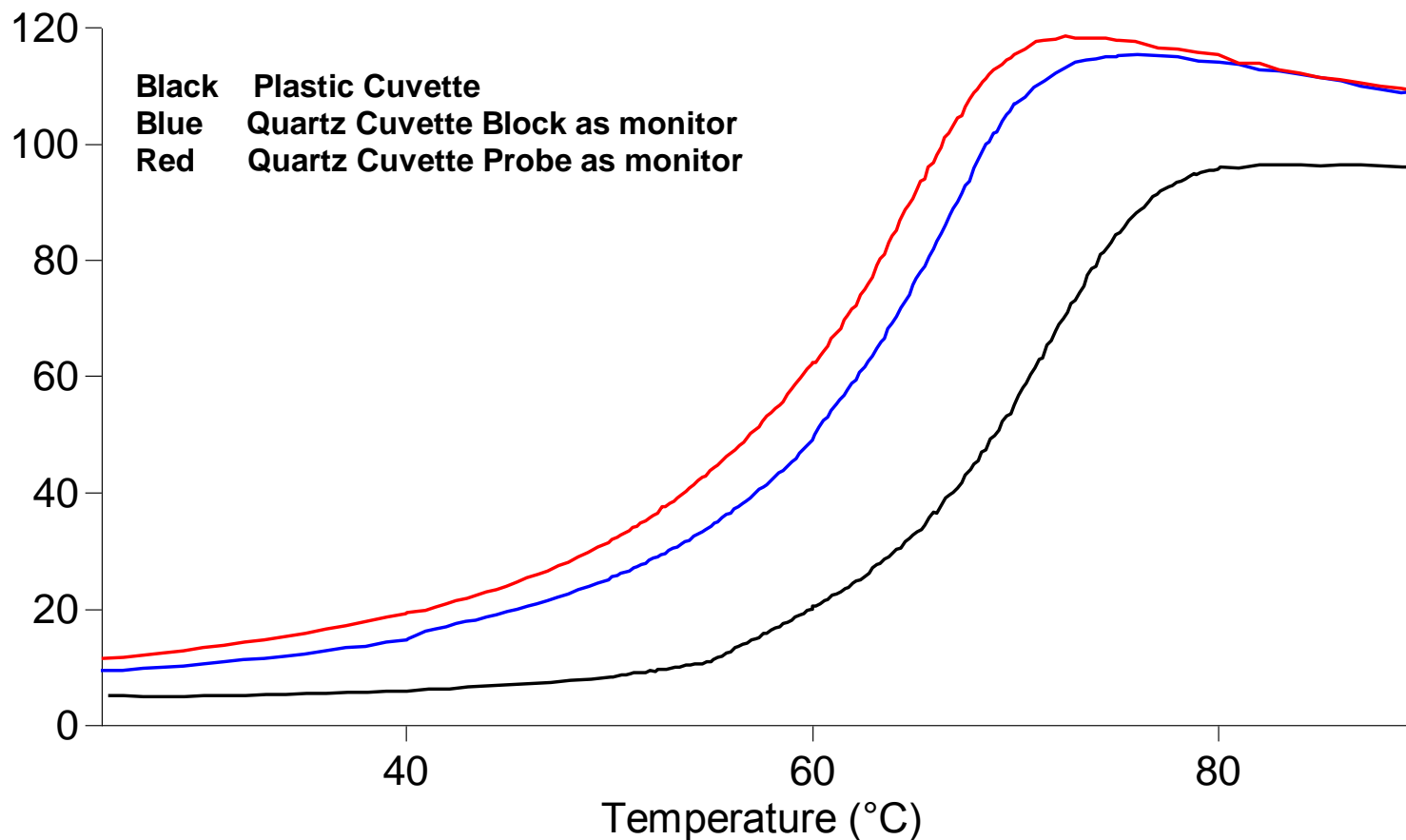
Importance of Choice of Cuvette



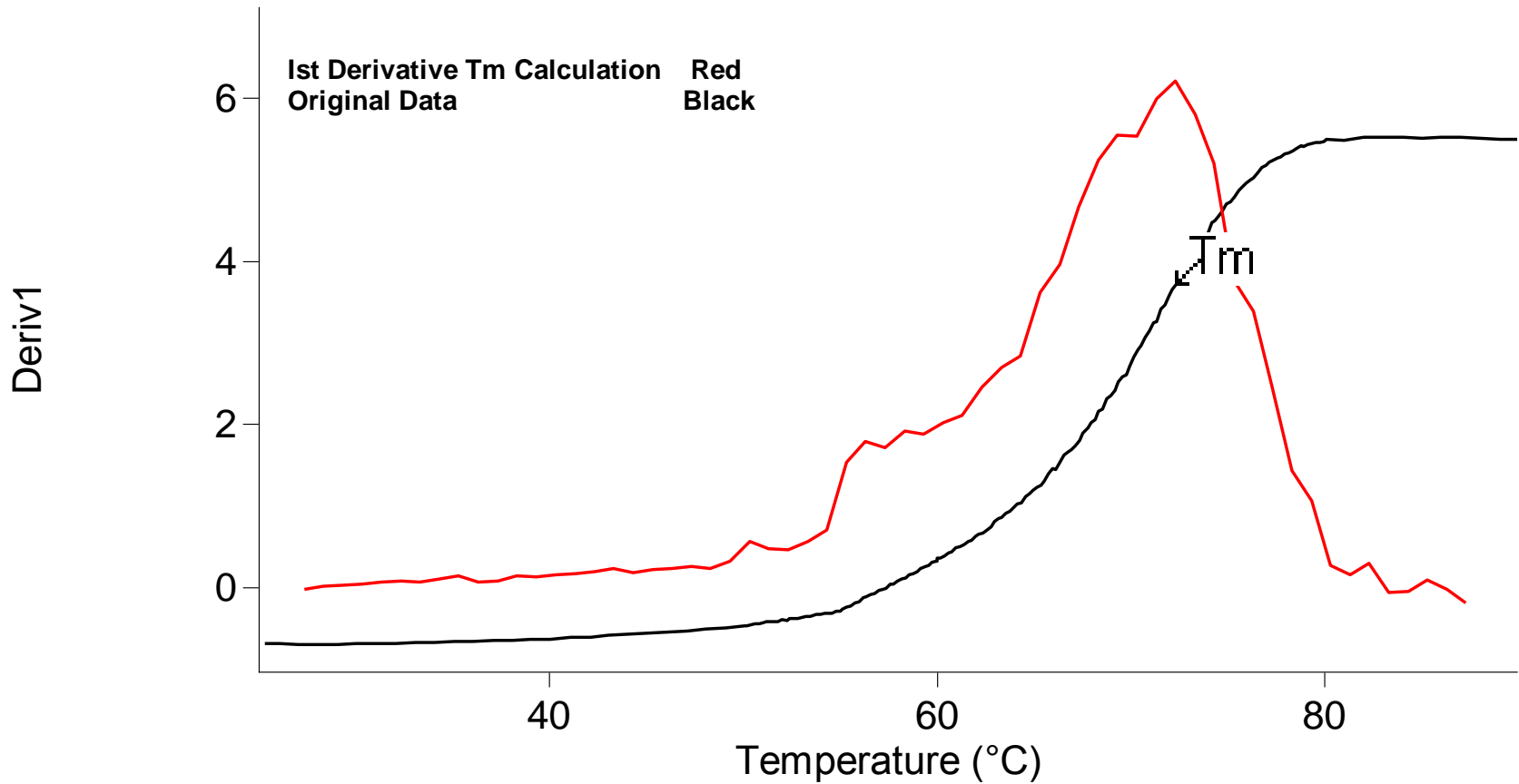
Importance of Choice of where Temperature is Monitored



Overlay of Cuvette and Temperature Monitor Choices



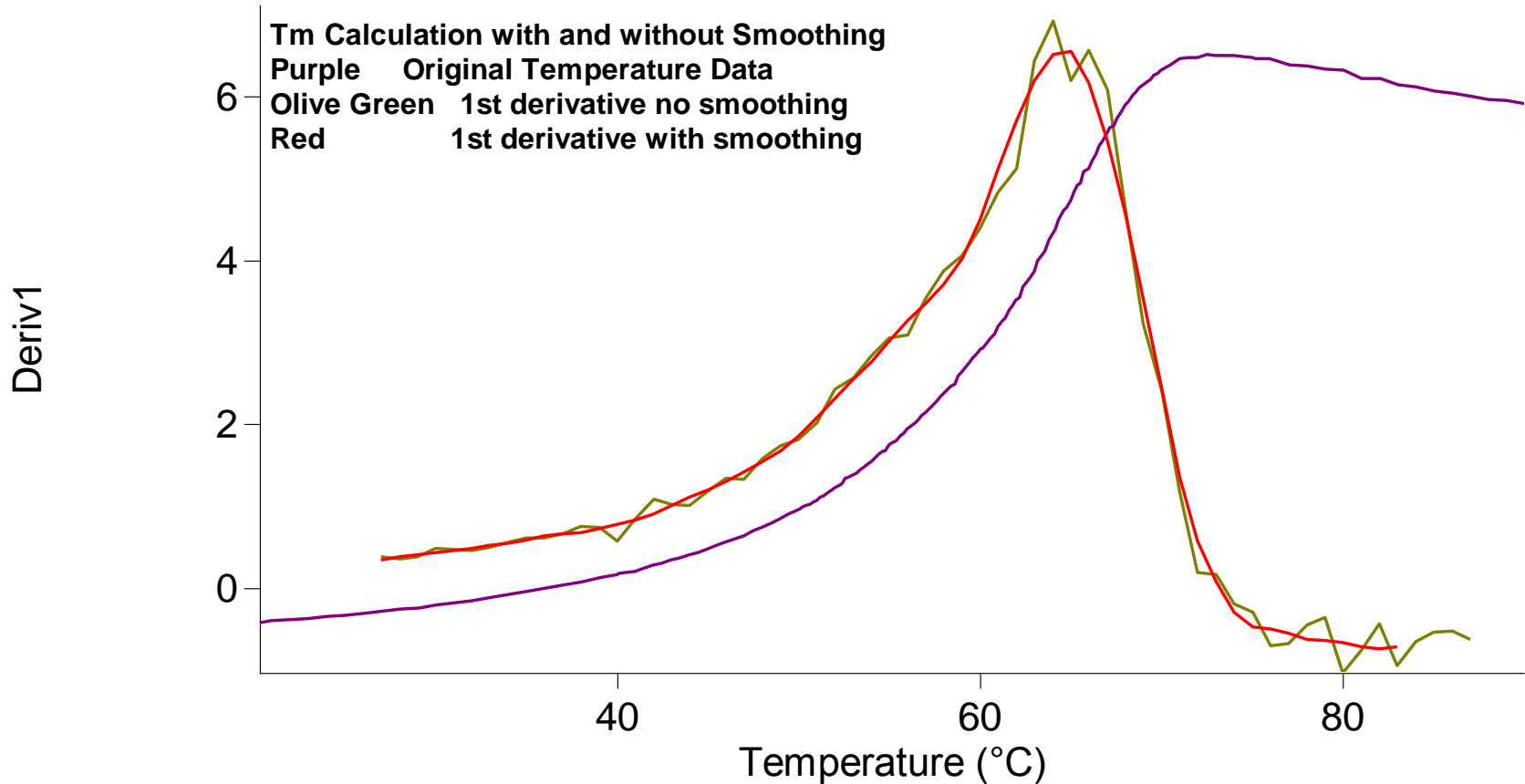
Determination of Tm



Calculated Tm's

Cuvette Type	Calculated Tm (deg C)
Plastic	72.34
Quartz (Block used as Temperature Monitor)	67.00
Quartz (Probe used as Temperature Monitor)	64.00

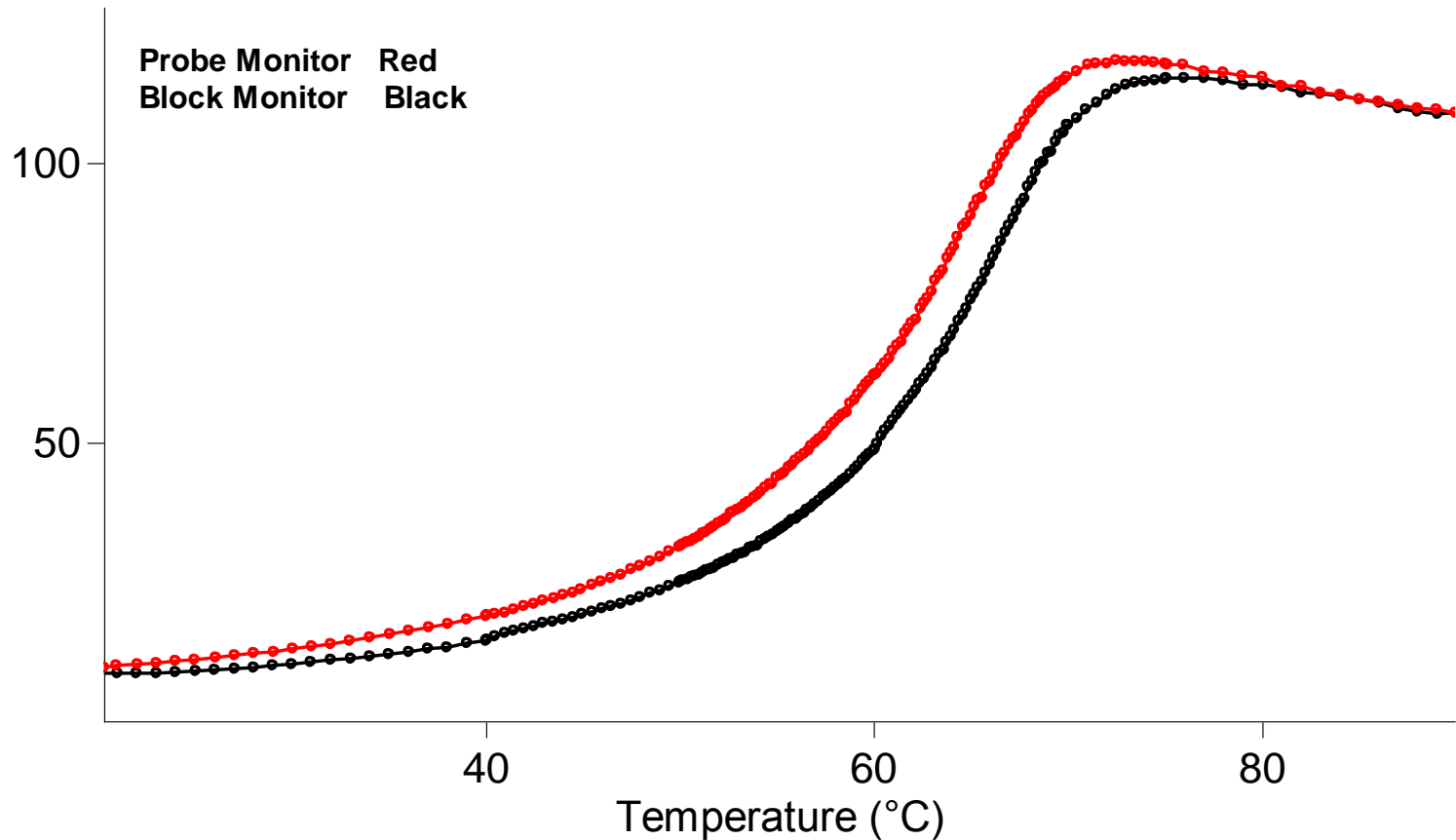
Effect of Noise on Tm Calculation



Effect of Noise on Tm Calculation

Cuvette Type and Calculation Conditions	Calculated Tm (deg C)
Quartz Cuvette using Probe as monitor no smoothing	64.00
Quartz Cuvette using Probe as monitor with smoothing	65.00

Example of Optimization of Temperature Ramp and Data Interval



Additional Capabilities in Collecting Temperature Related Data

- Like UV-Vis, it is possible to Collect a Scan at a series of user define temperatures with a user define equilibration time
- While not yet available, it is possible to control the autopolarizers in addition to the temperature to automatically collect data for anisotropy calculations for a set of user define temperatures

Questions

Thanks For Your Patience And Good Humor

Shortly You Will Receive A Link
To Down Load The Presentation

My Contact is Mark.Fisher@Agilent.com

Thanks Again