Optimizing Your Absorption or Fluorescence Thermal Melt Run

CAG
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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

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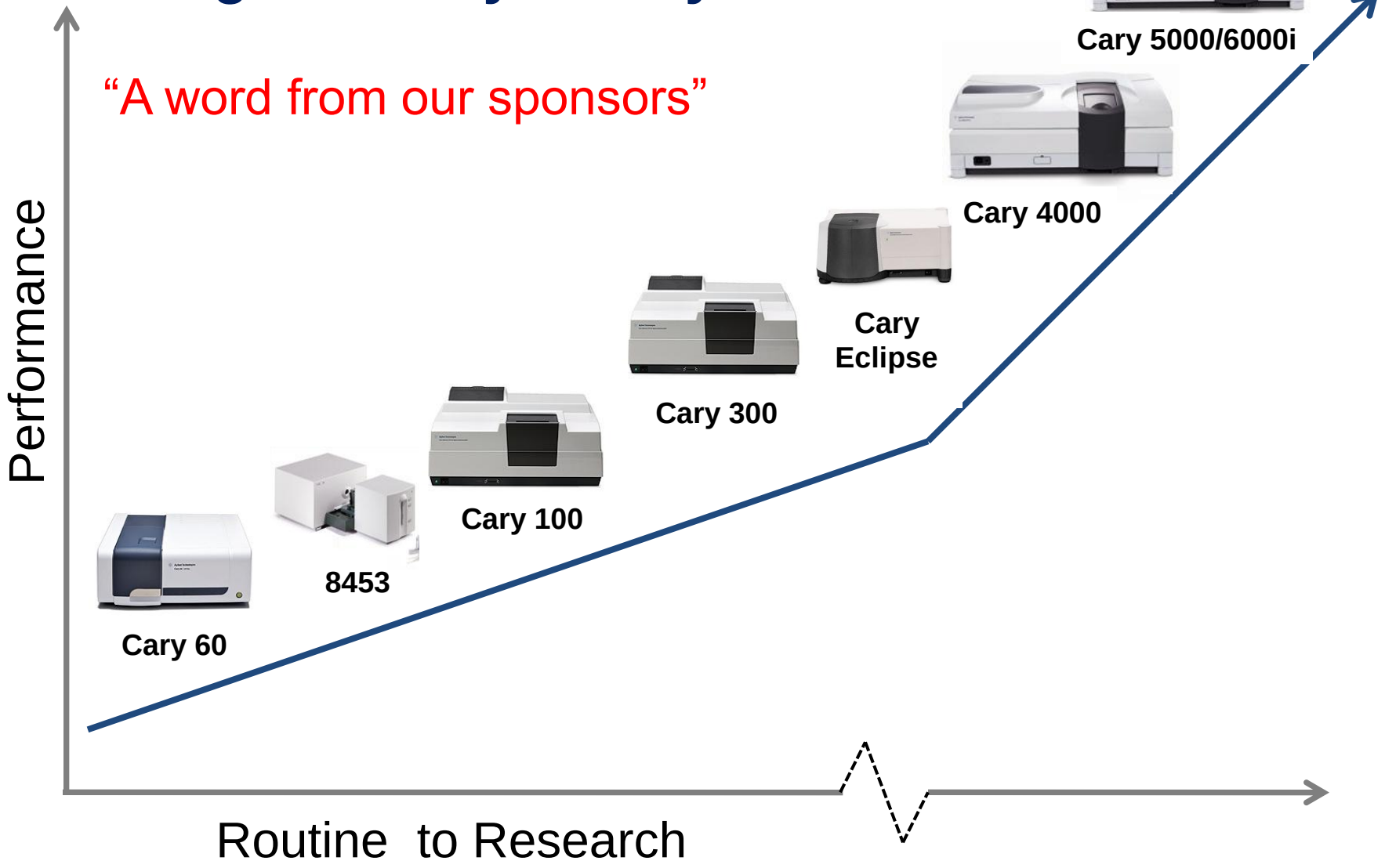
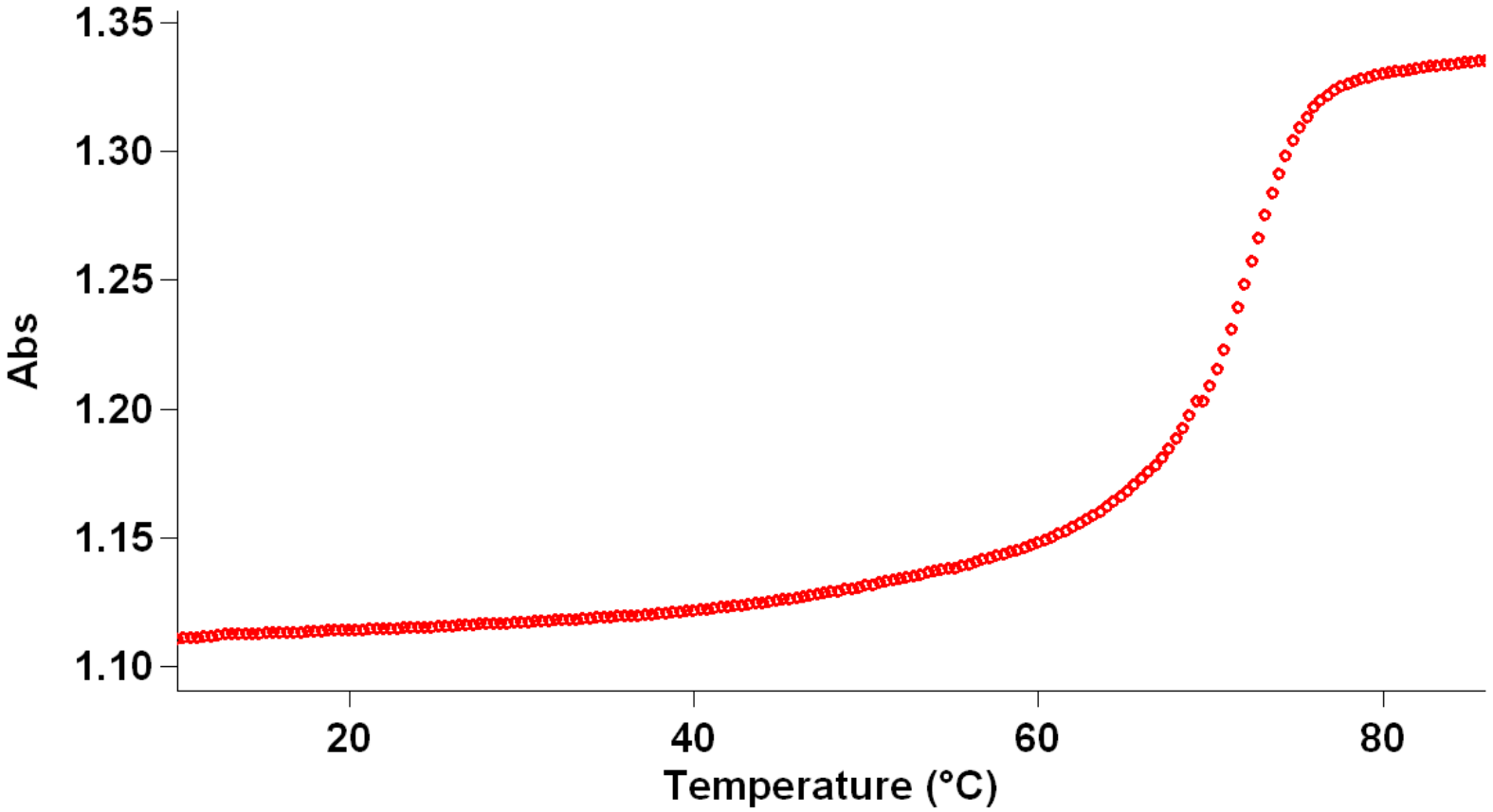


Thermal Melts using UV-Vis Absorption

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



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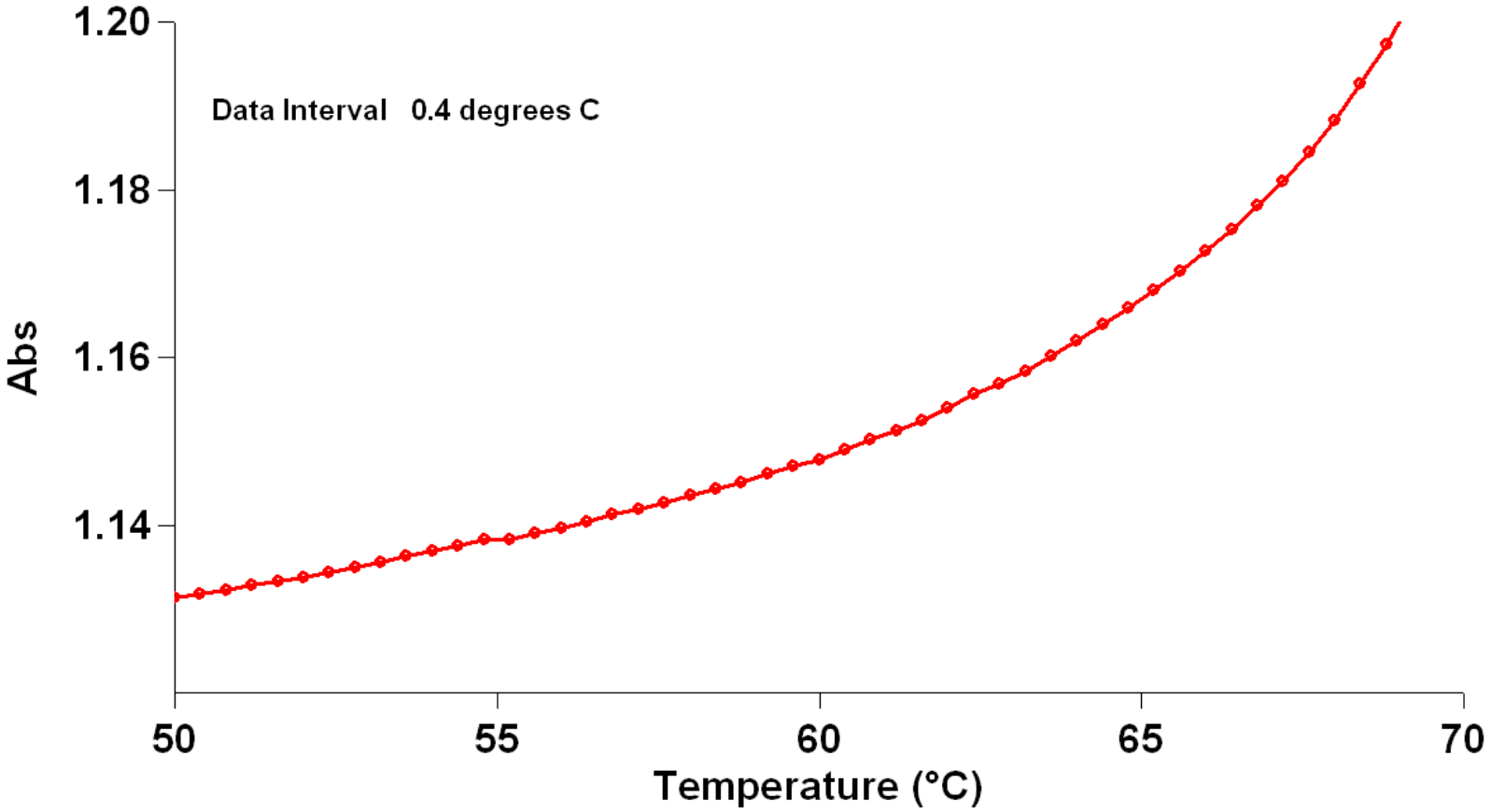
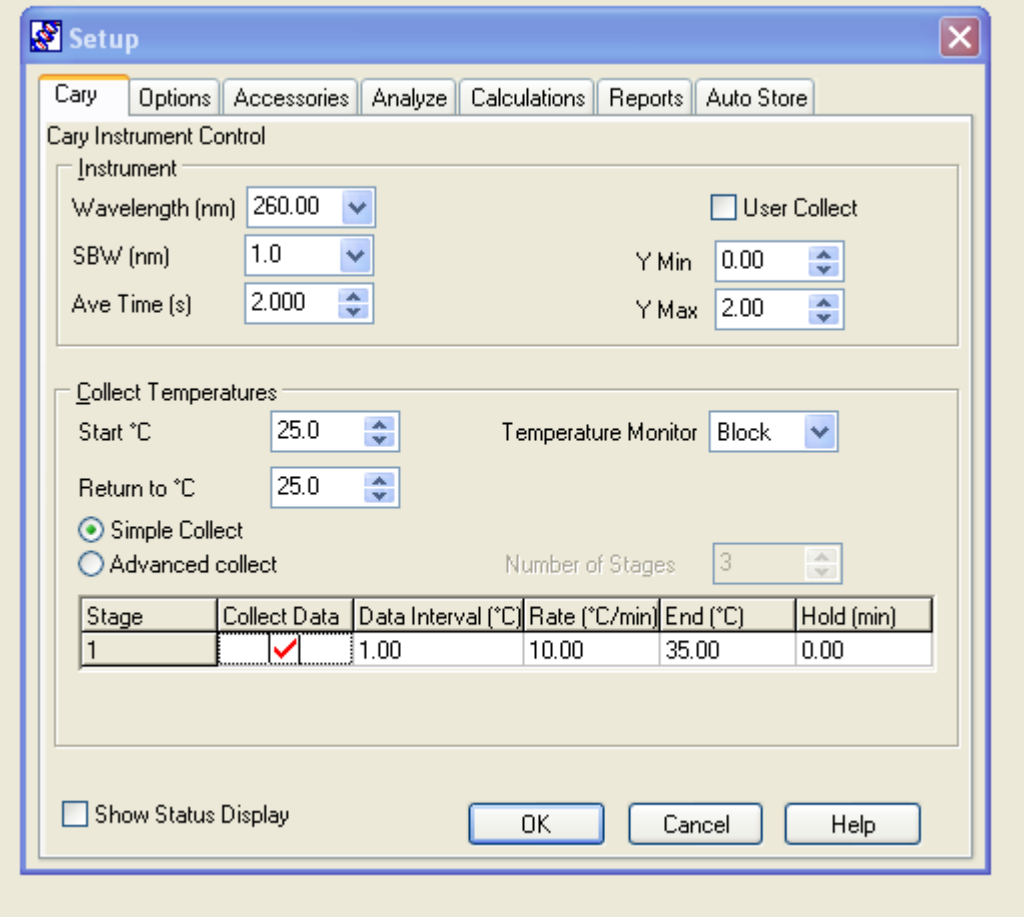
Thermal Melt Curve Expanded



SetUp Dialog Box and Setting Up Simple Collection



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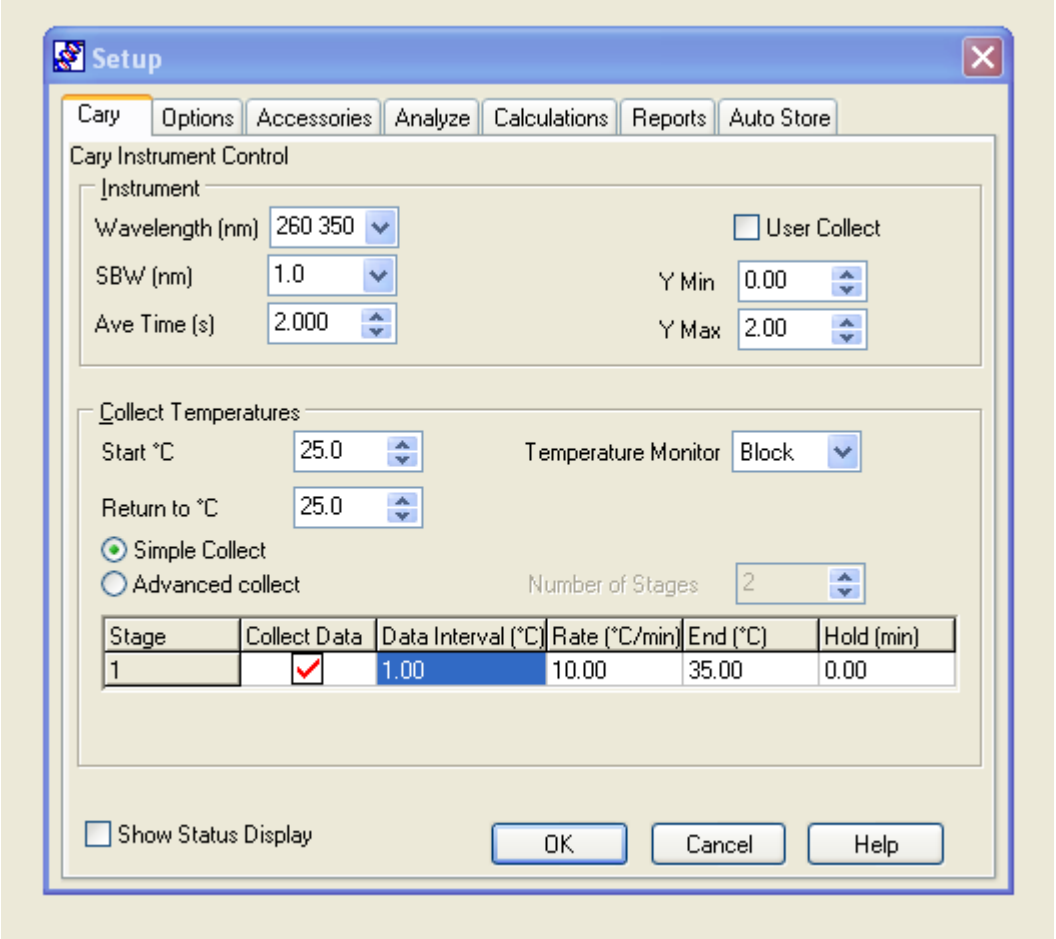
Using Multiple Wavelengths in a Single Melt



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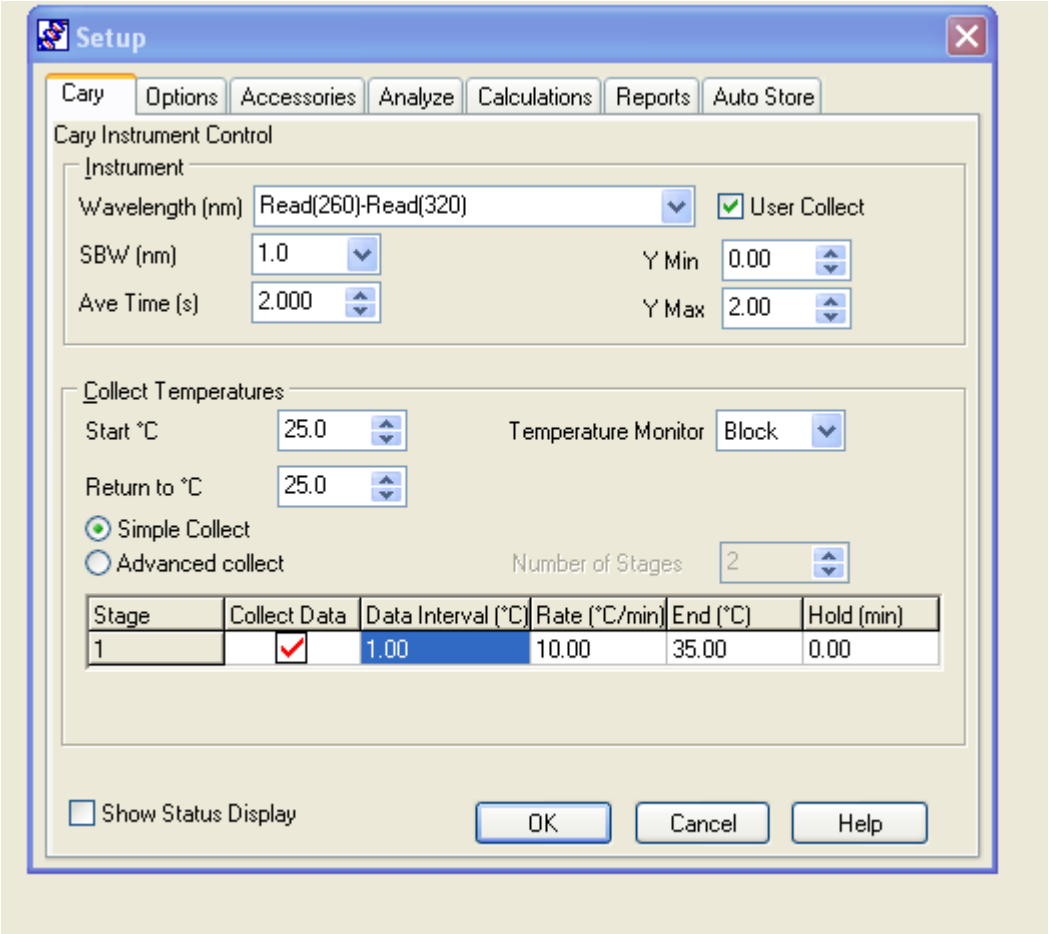
User Collect Function



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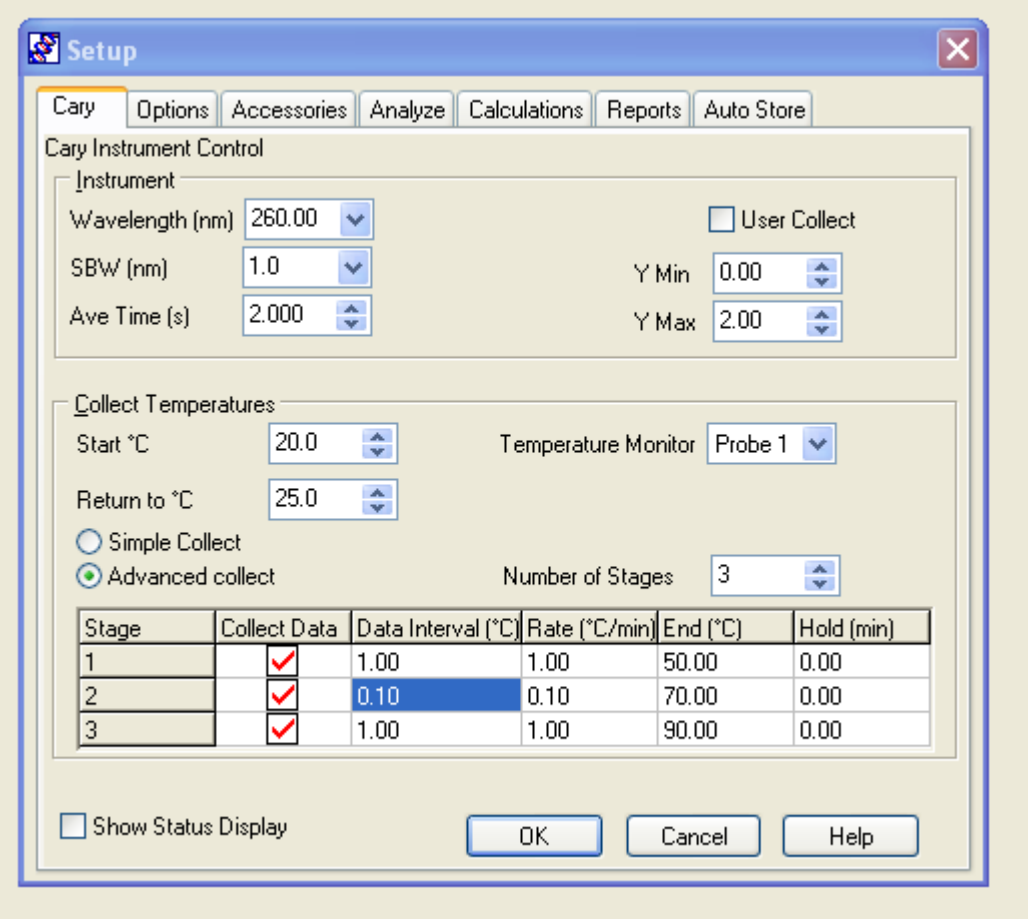
Typical Simple Single Ramp

- 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 deg / 0.1 deg per min = 700 minutes or 11 hours and 40 min

Advance Collect using Multiple Temperature Collection Stages



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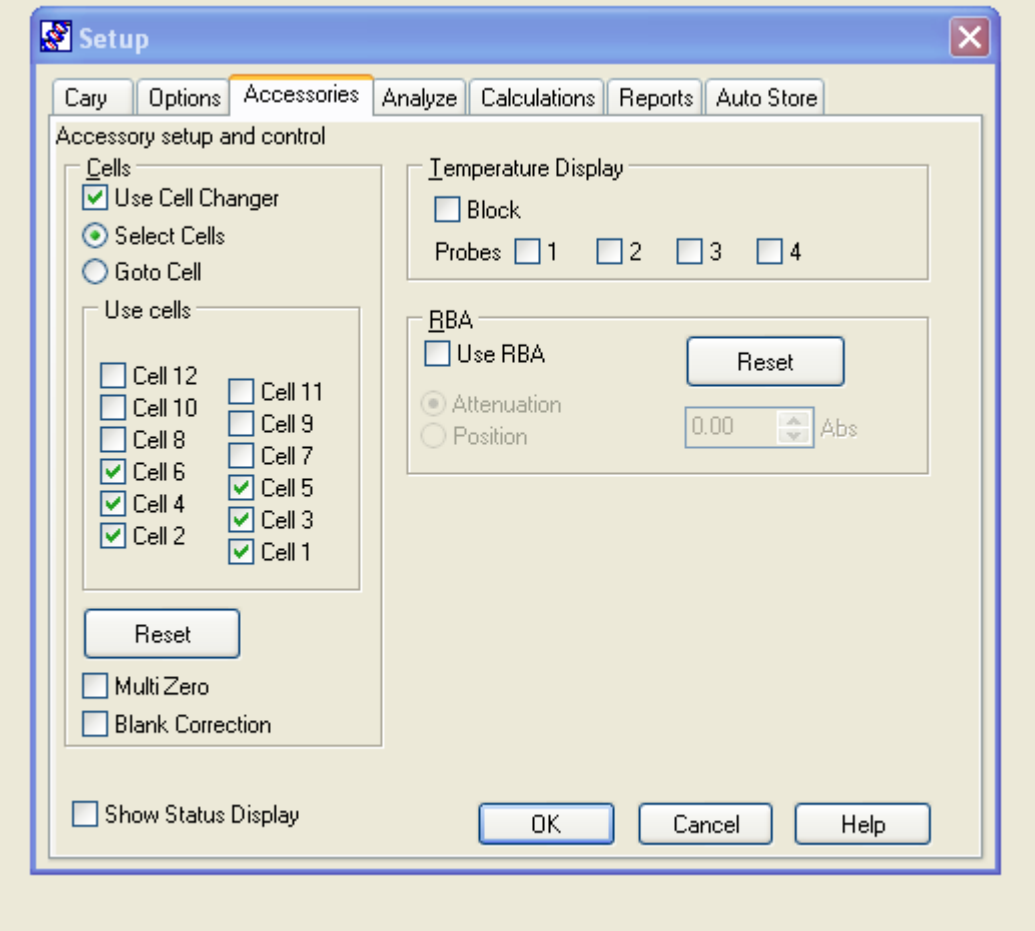
Optimizing Data Collection Using Multi-Stage Ramp

- 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

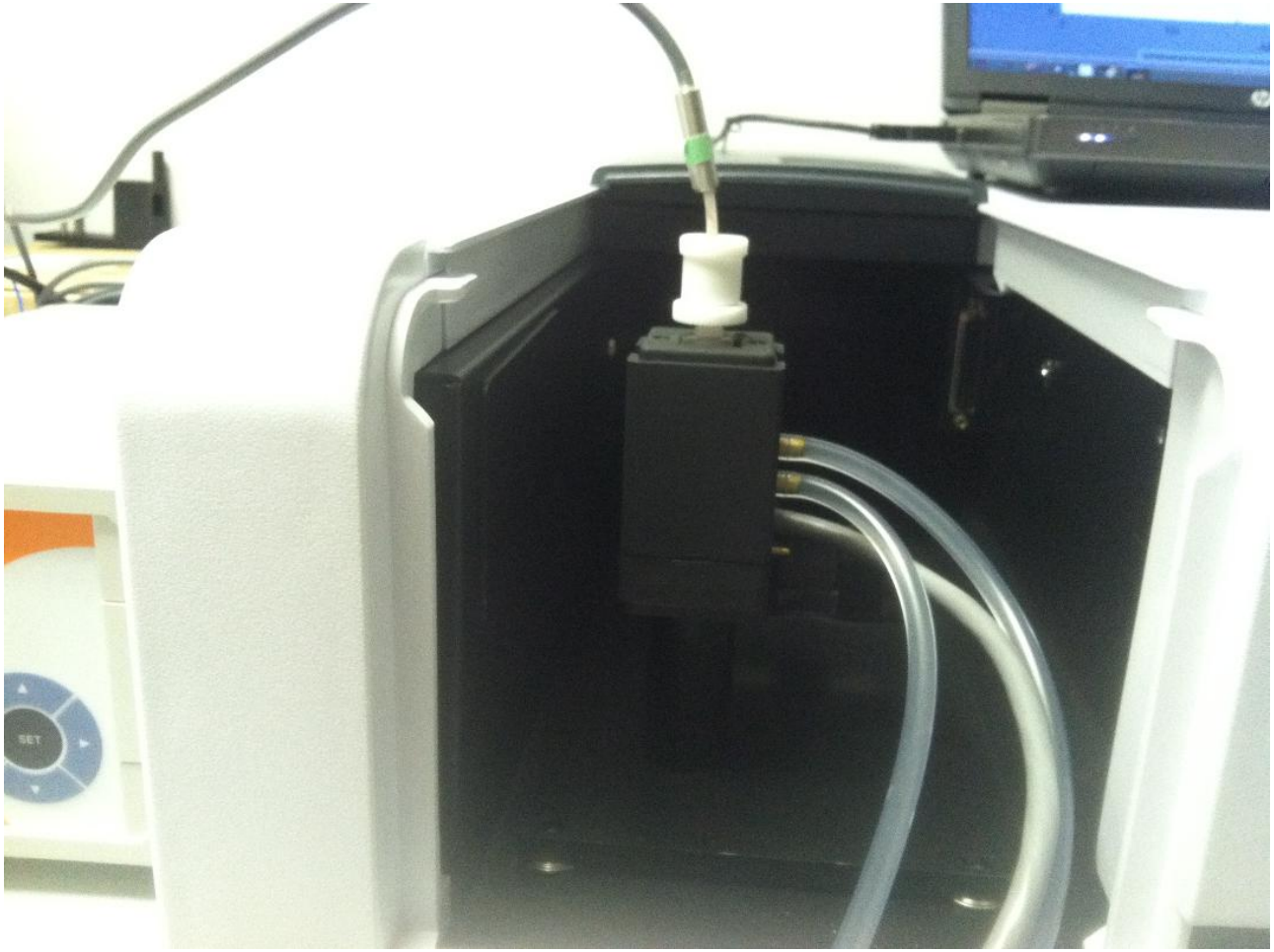
Double Beam SetUp



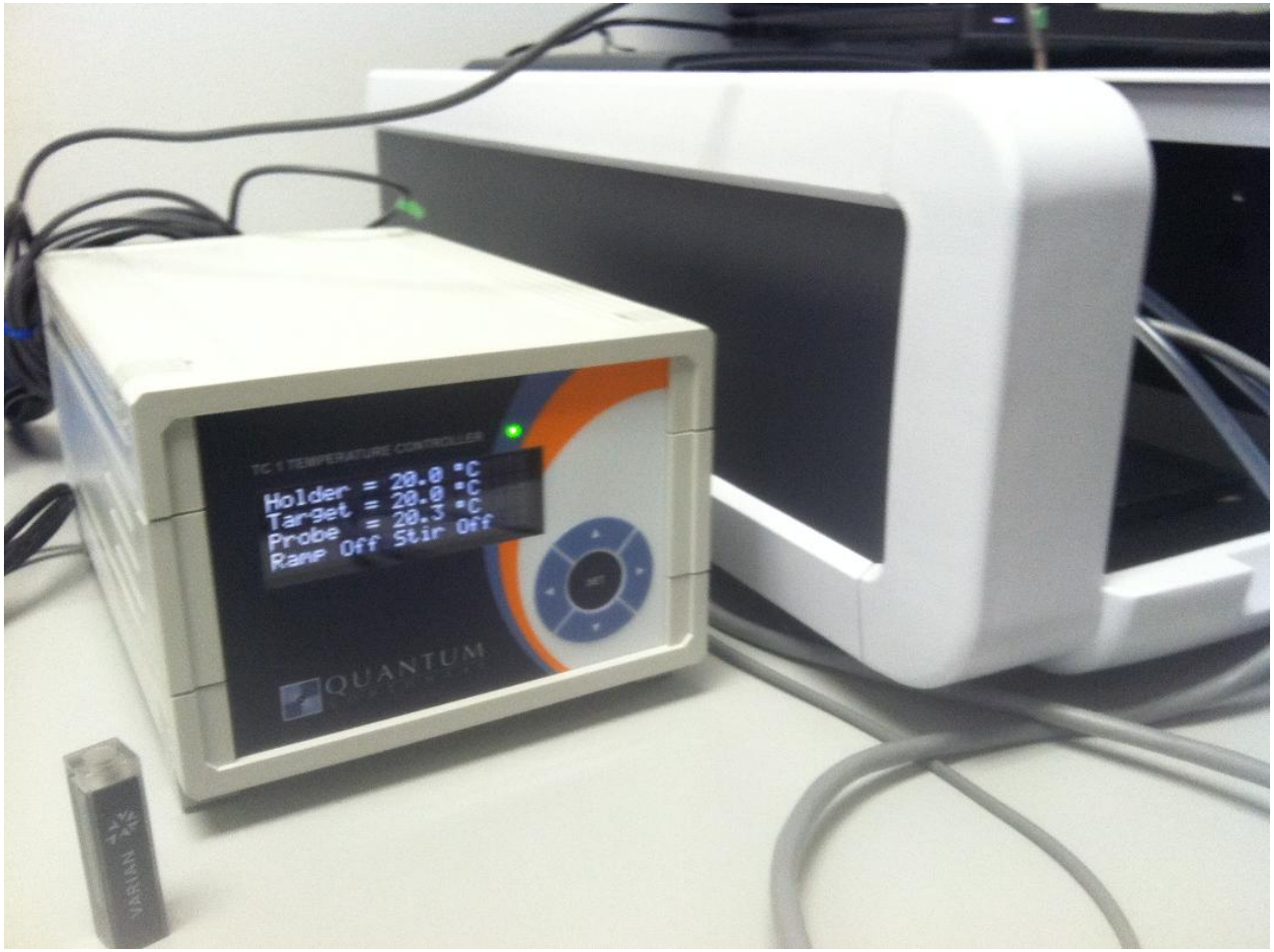
Where and When Should the Temperature be Measured

- 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



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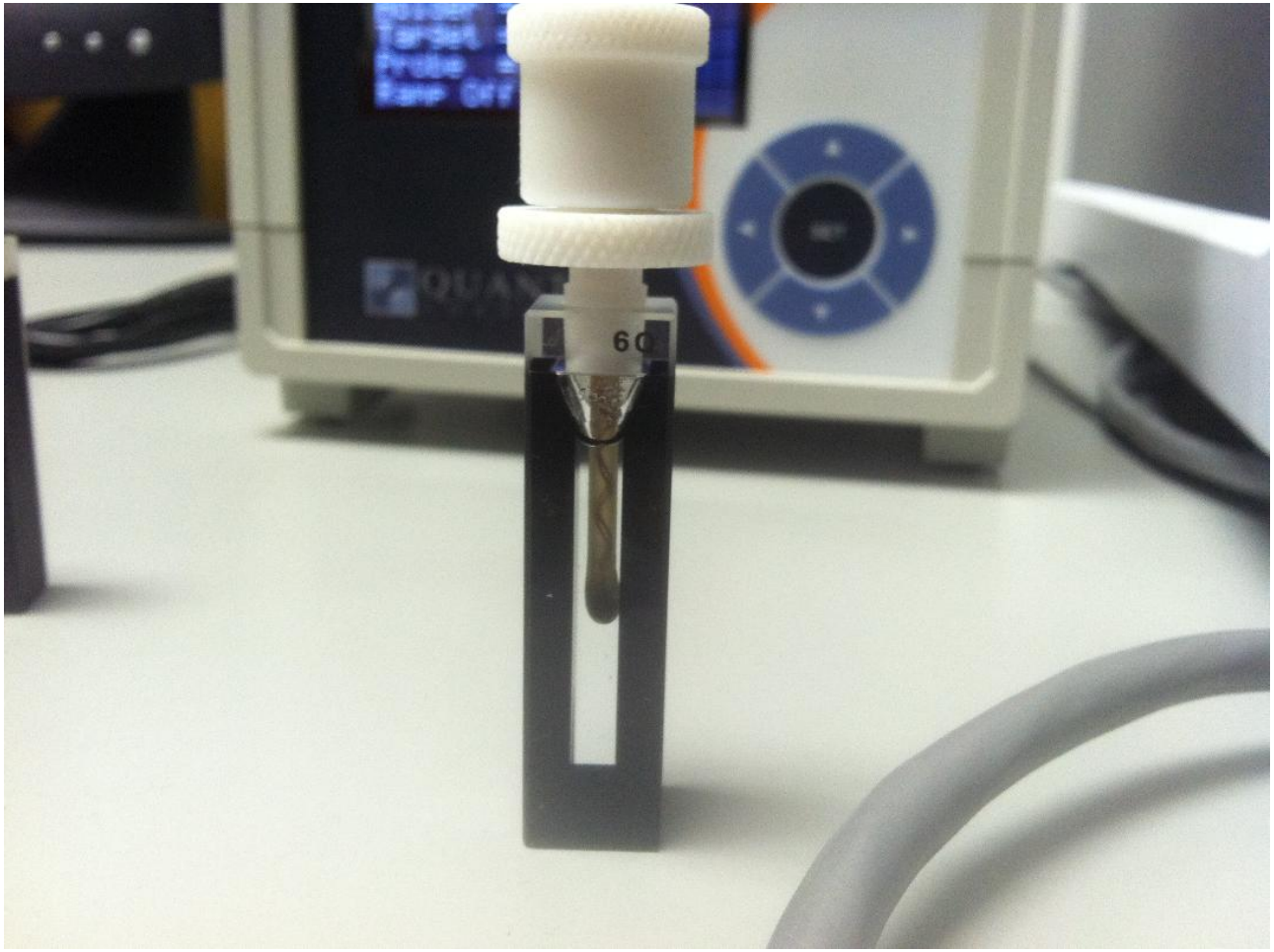
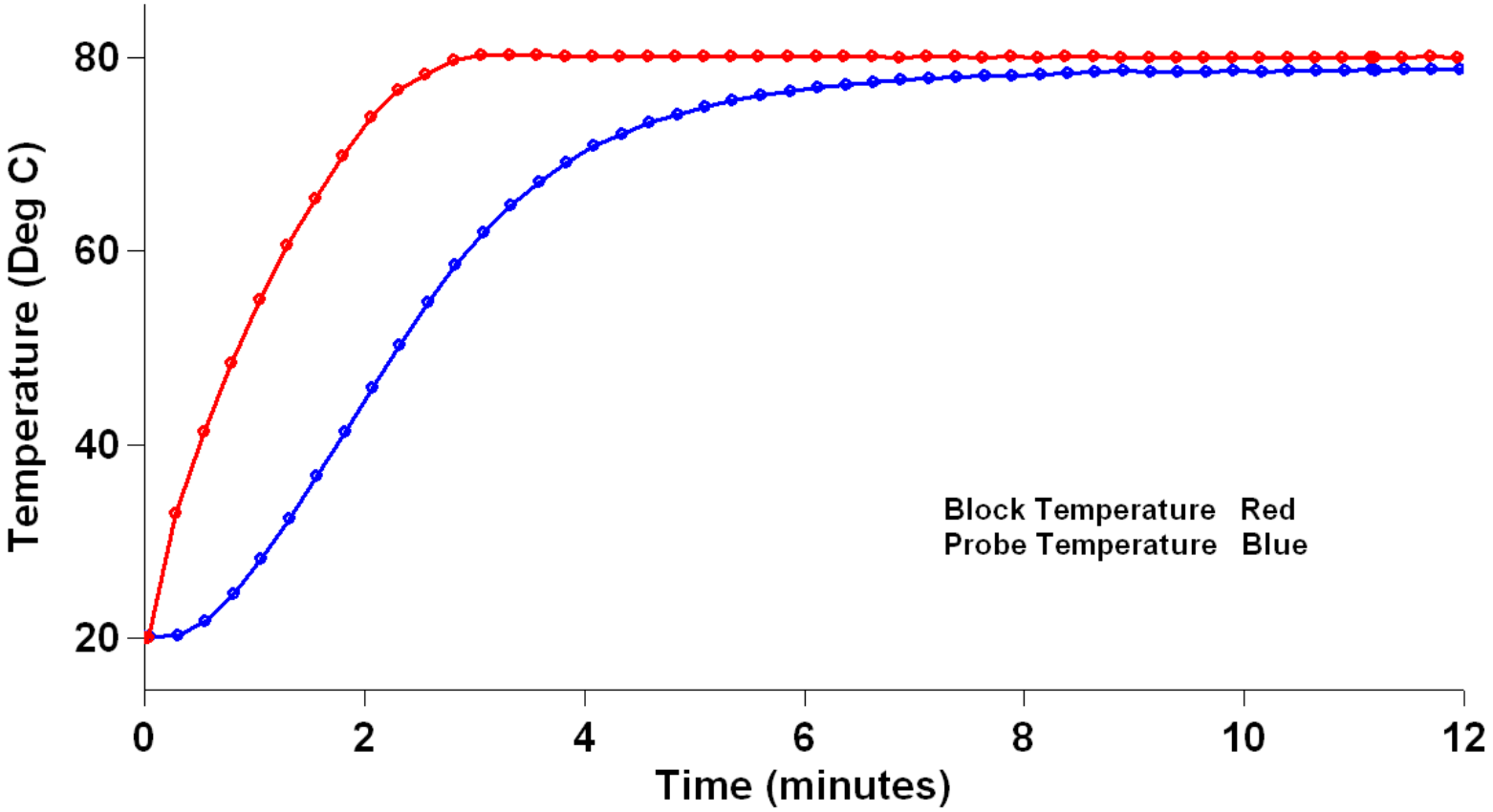
Temperature Probe In Semi-Micro Cell



Temperature Lag from Block to Solution Heating



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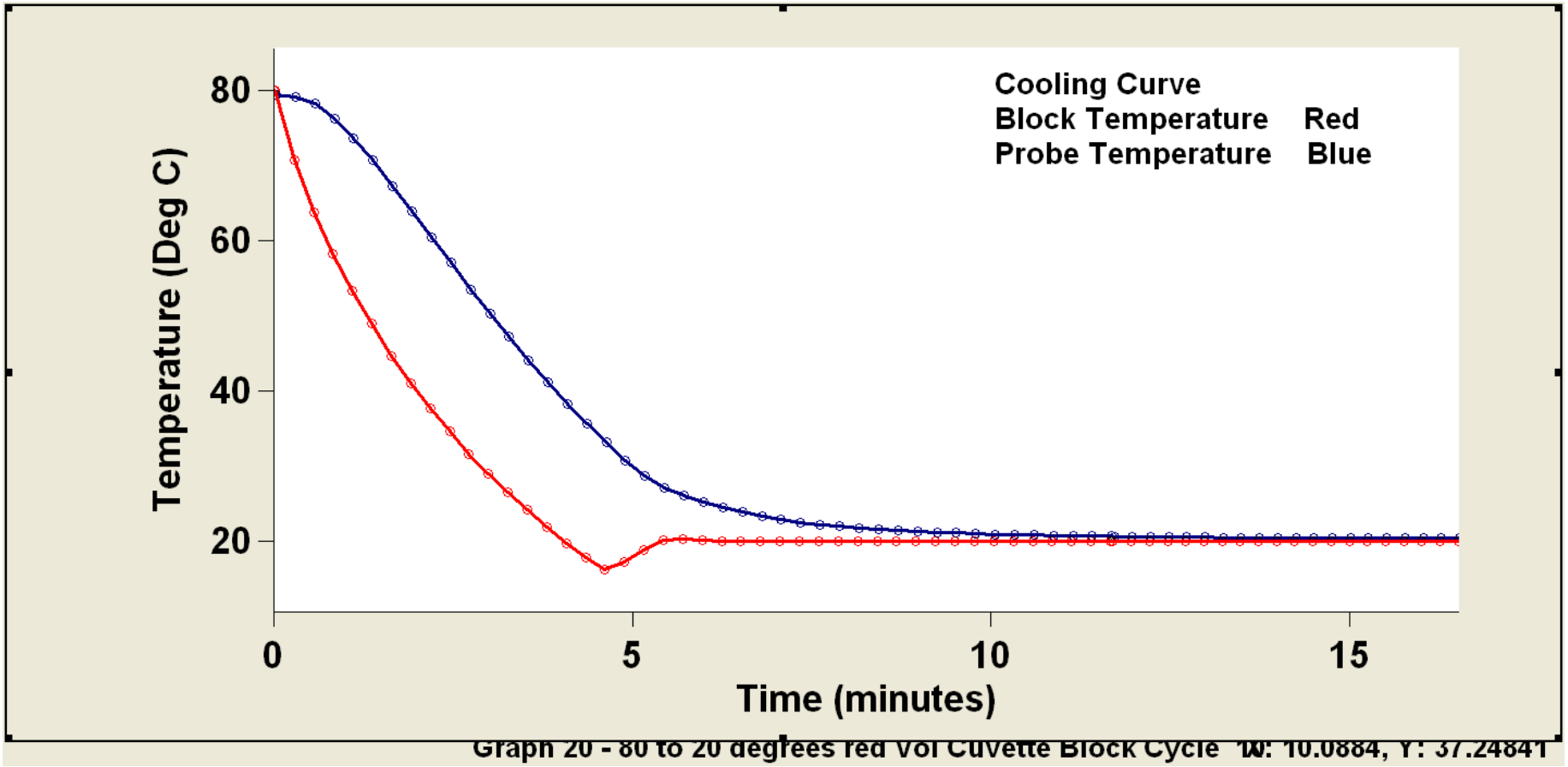
Temperature Lag from Block to Solution Cooling



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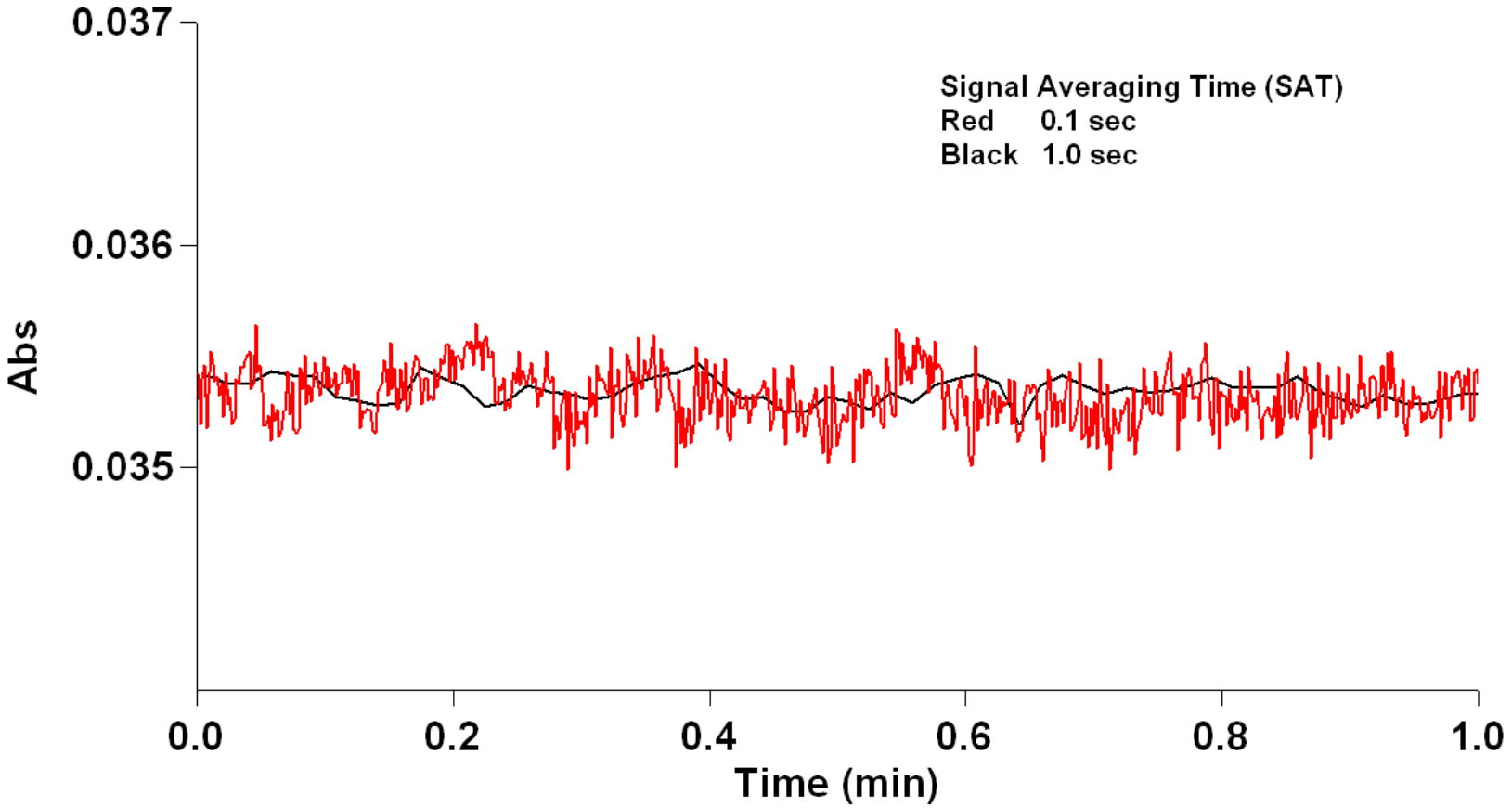
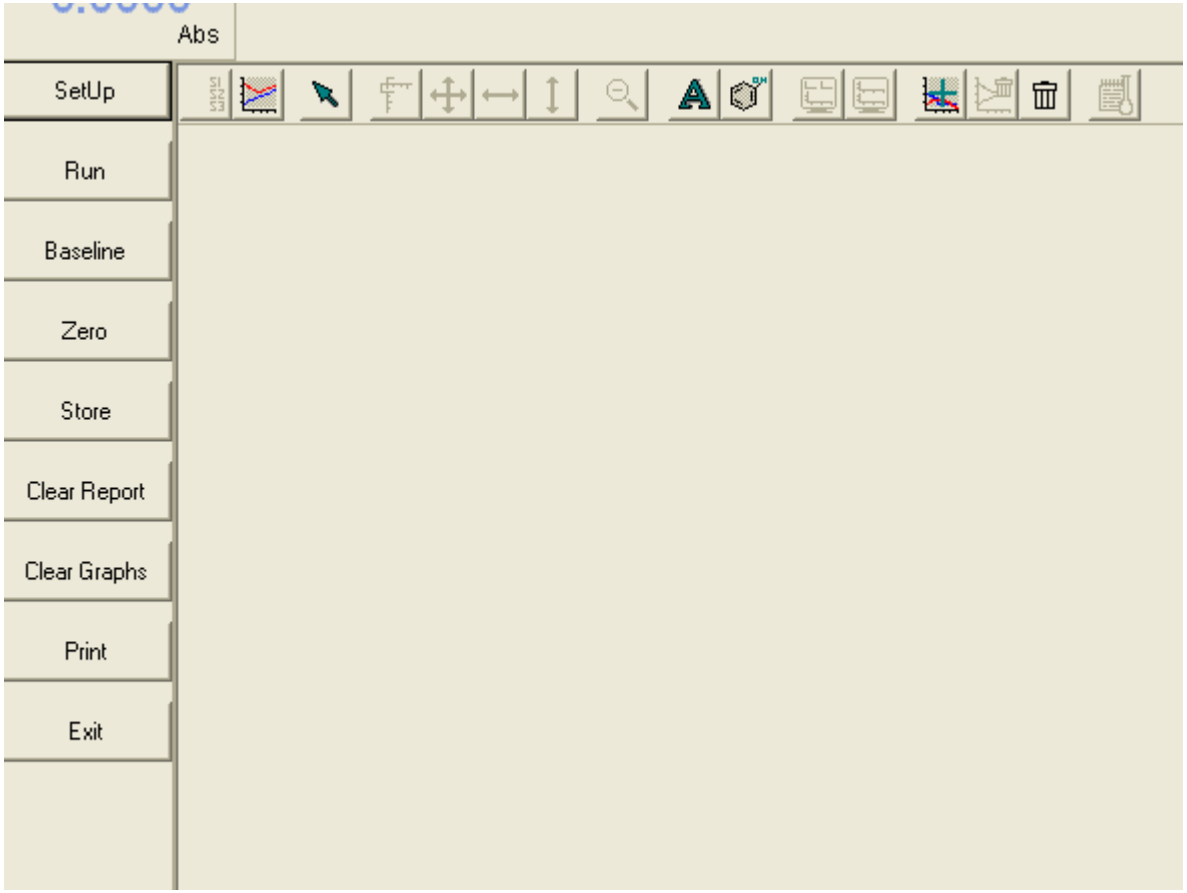
Effect of Signal Averaging Time on Noise



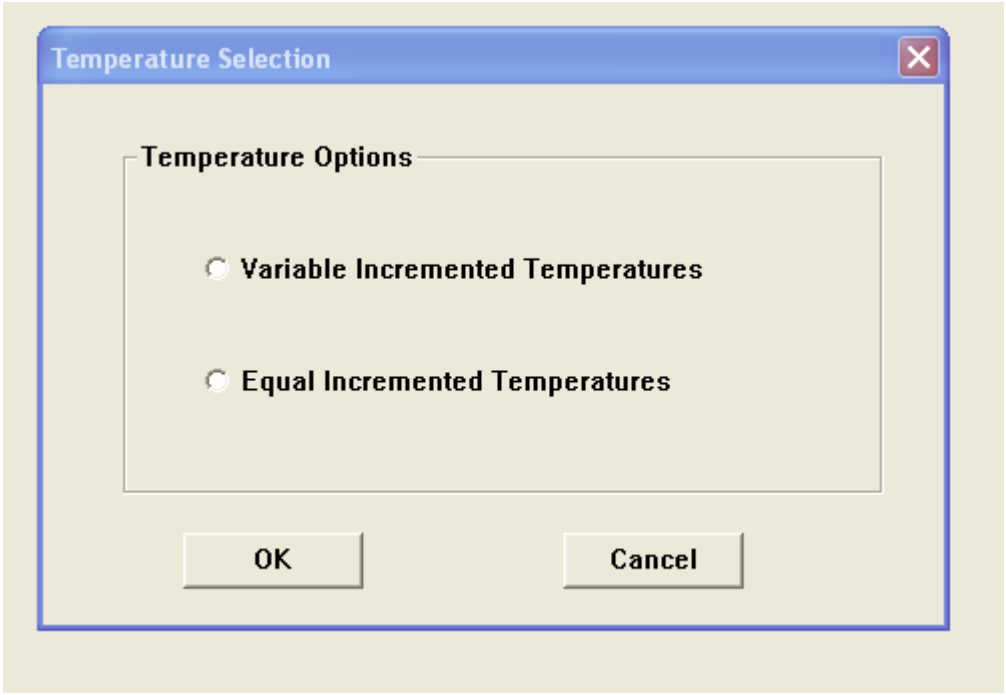
Additional Capabilities for Collecting Temperature Related Data

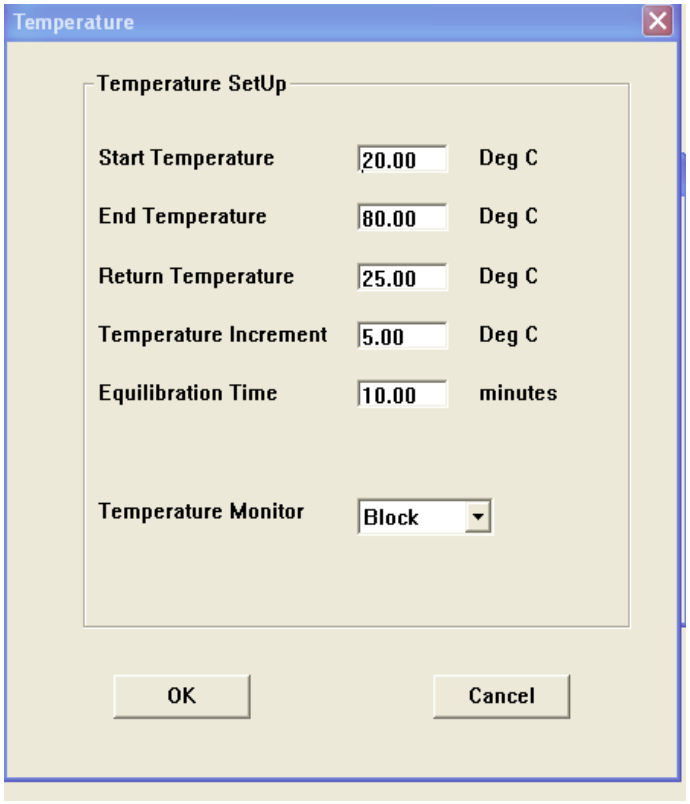
- Possible to Collect a Scan at User Define Temperatures
- While at each User Defined Temperature, it is possible to collect a number of scans at a user define time interval before going to the next temperature
- These capabilities are possible through ADL, which is the controlling language of the software.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

Initial Screen after Program Started



Temperature SetUp for Scans at Temperature





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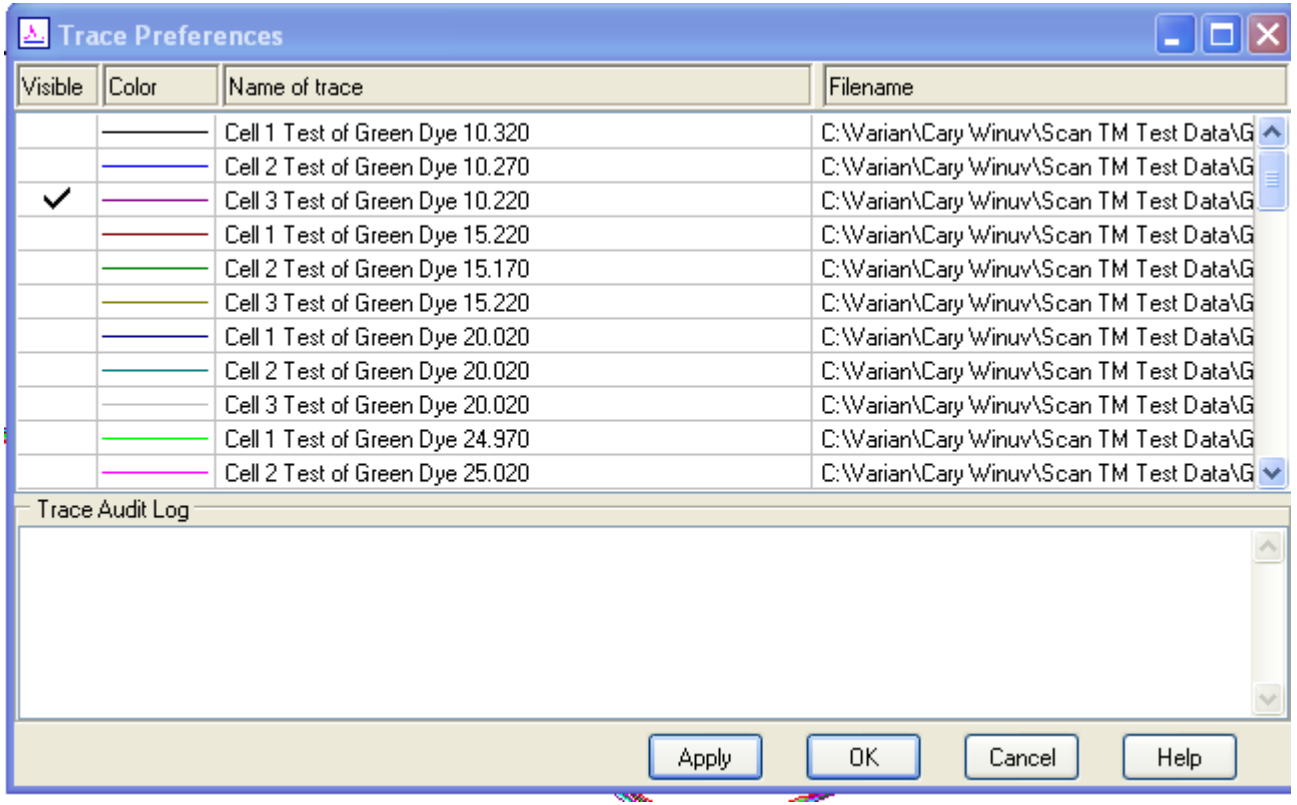
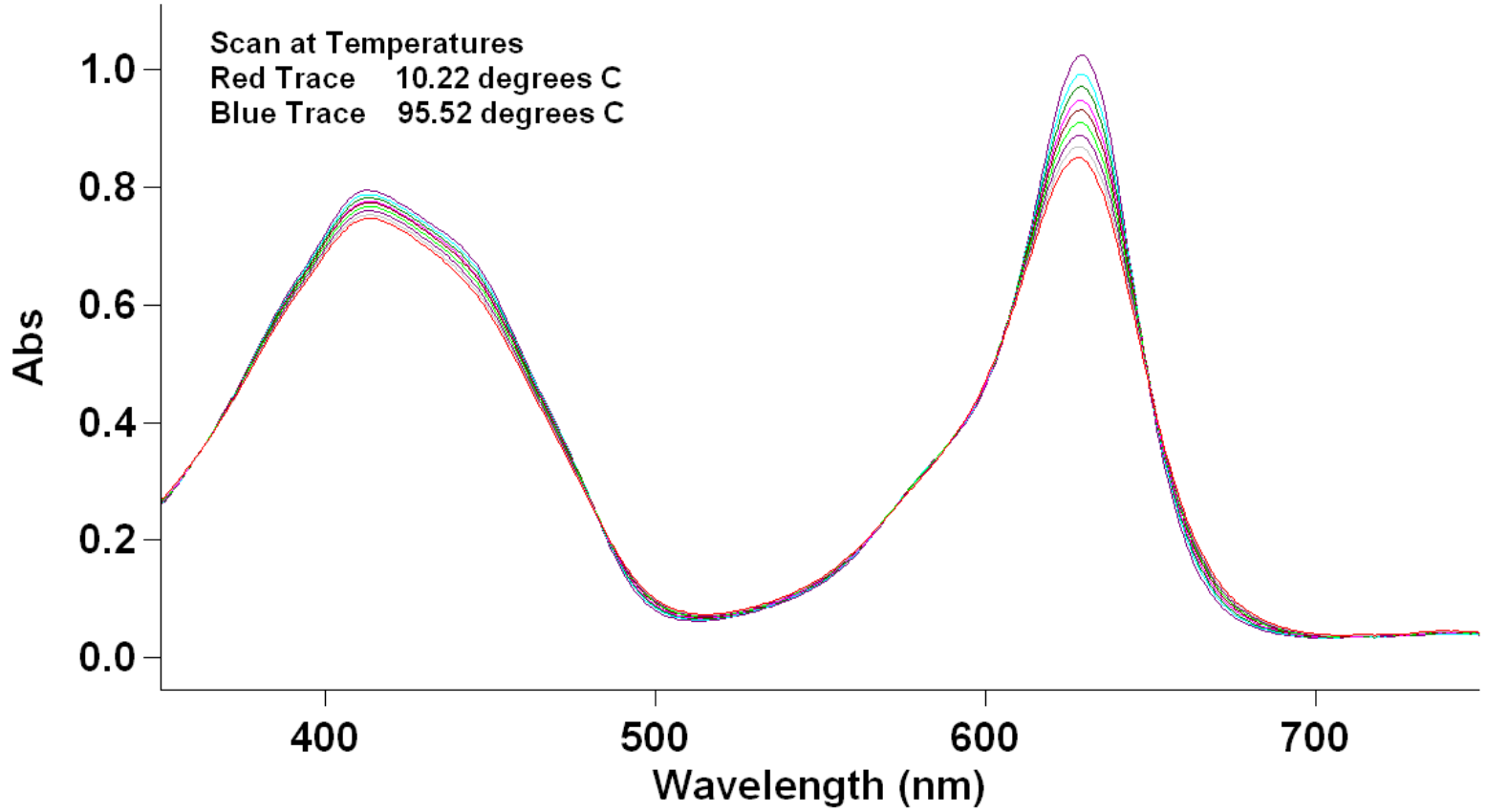
Trace Preferences Dialogbox Showing Trace Names



Scanning at Temperatures



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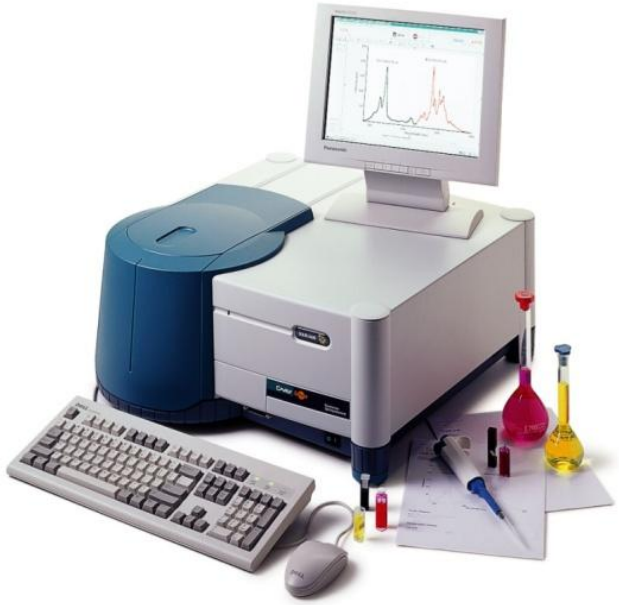
Thermal Melts using Fluorescence

Cary Eclipse Fluorescence Spectrometer

The Power of Xenon…

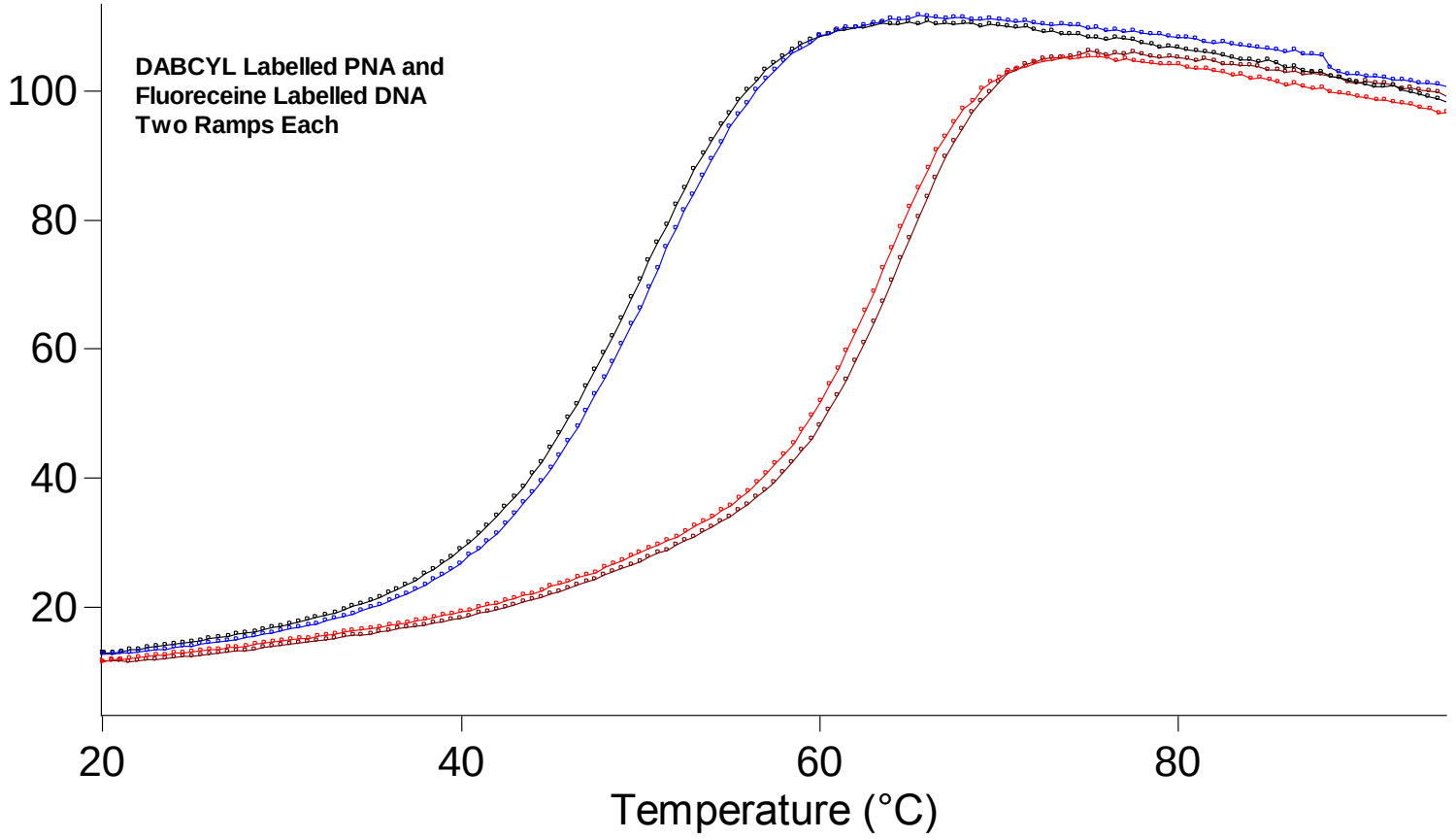
- Unique Xe flashlamp technology
- Measure small volume samples
- Fiber optics
- Room light immunity: unique, Varian patented technology
- Eliminates photo-degradation
- Long lamp lifetime



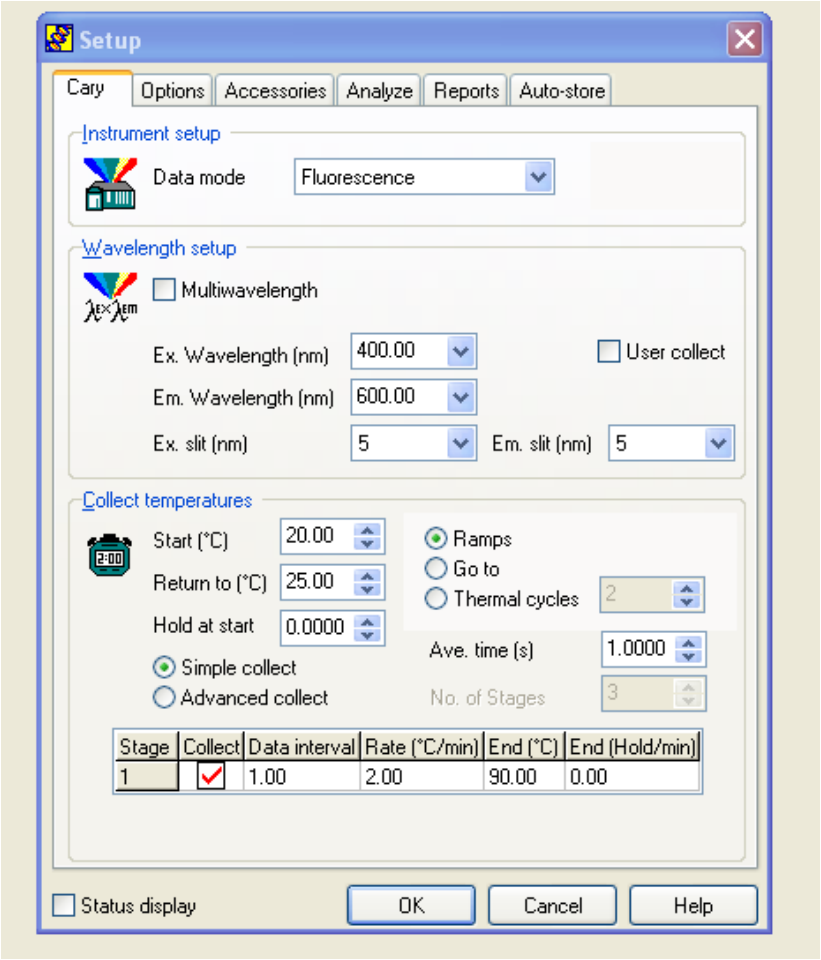
Application focus

- Biochemical applications
- Academia
- Industrial chemistry

Fluorescent Thermal Melt Curve



Simple Single Ramp Collection



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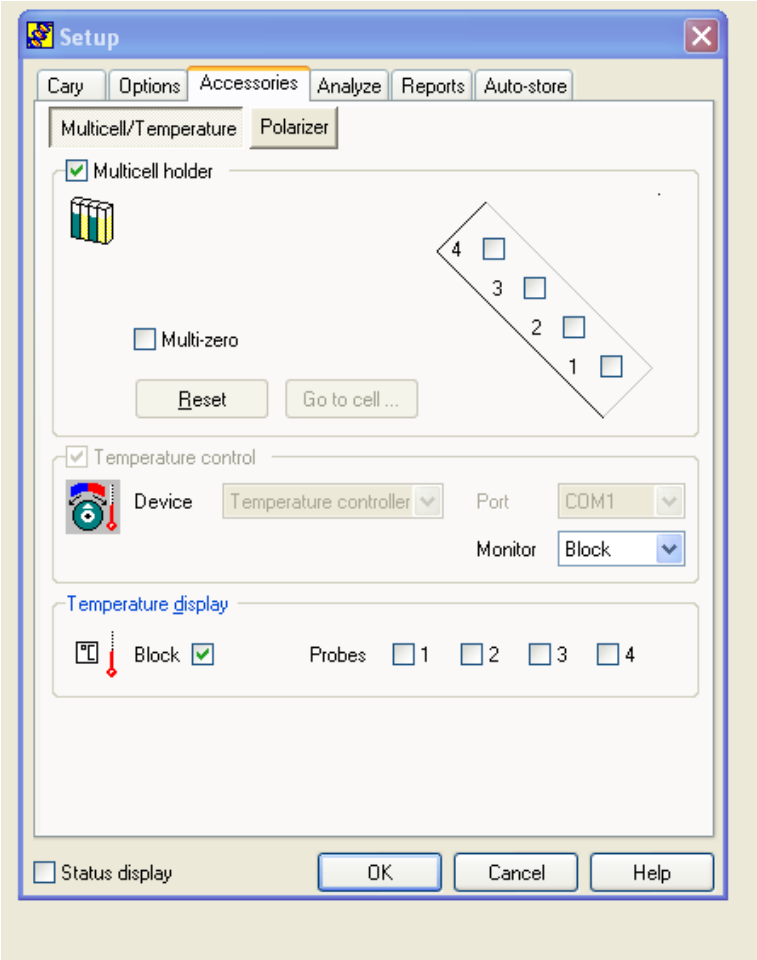
Sample Cell Selection and Temperature Monitoring Choice



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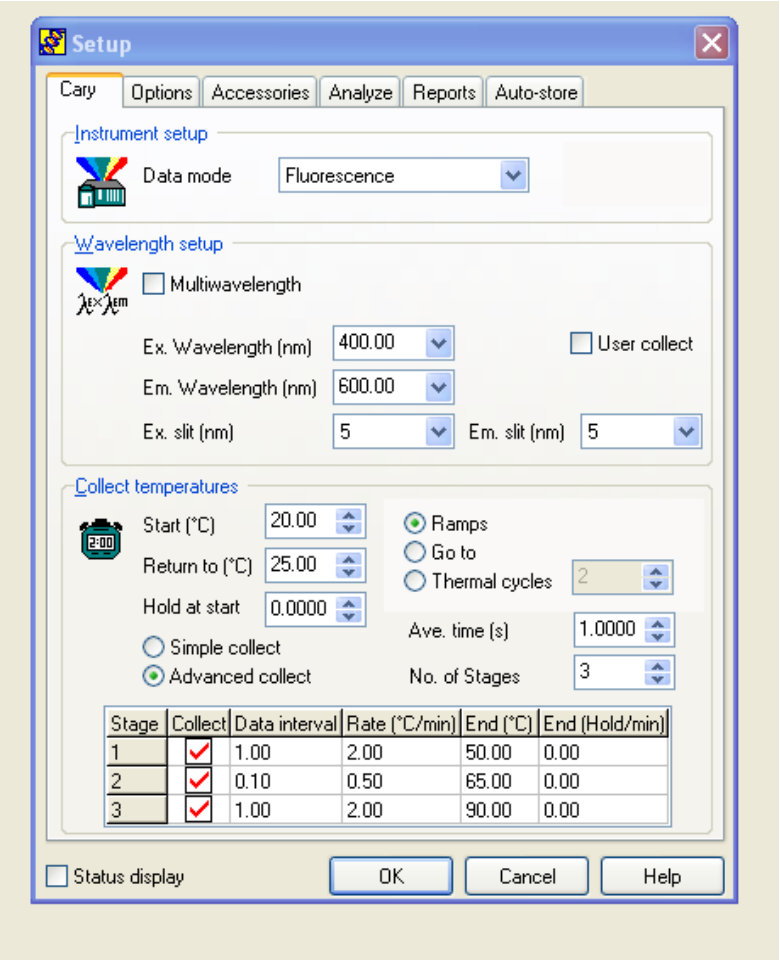
Advance Collection Choices



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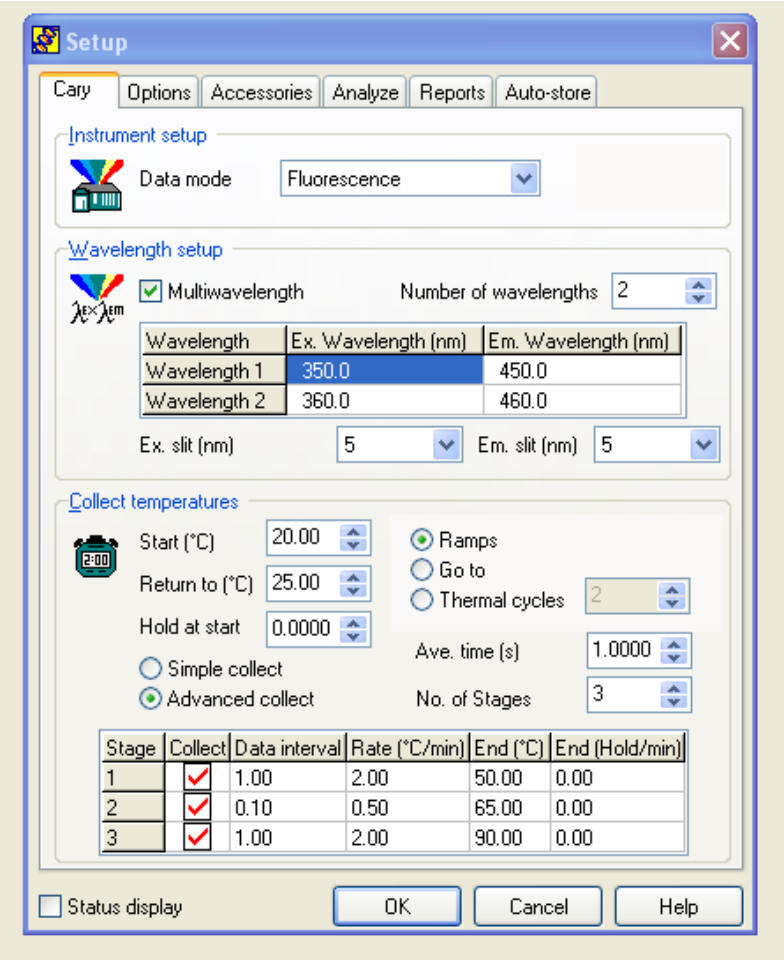
Multiple Wavelength Pairs



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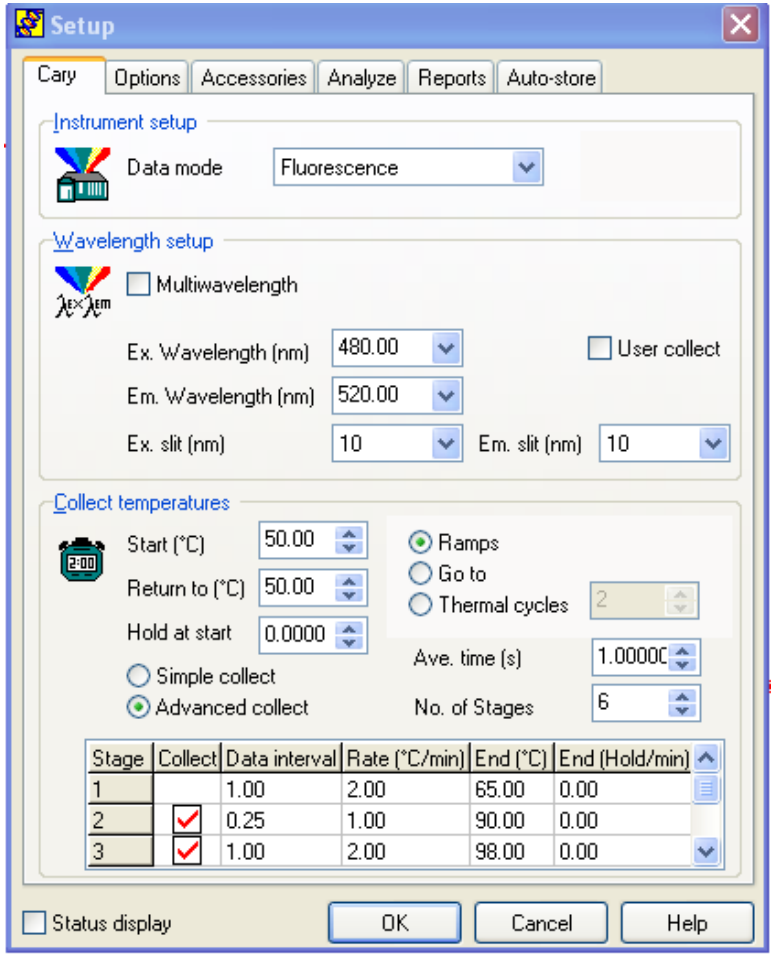
Advance Collection SetUp for Fluorescence Thermal going from High to Low Intensity



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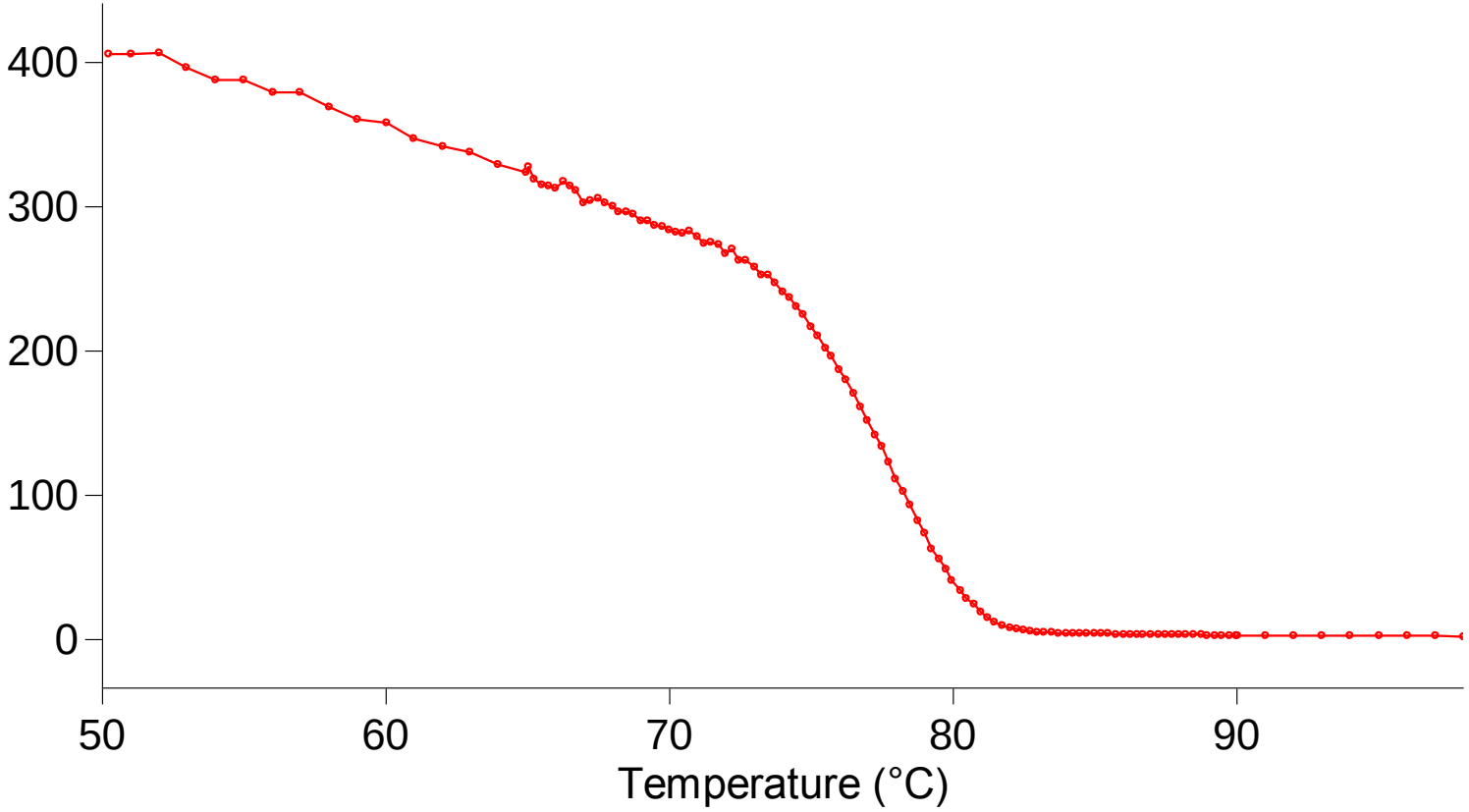
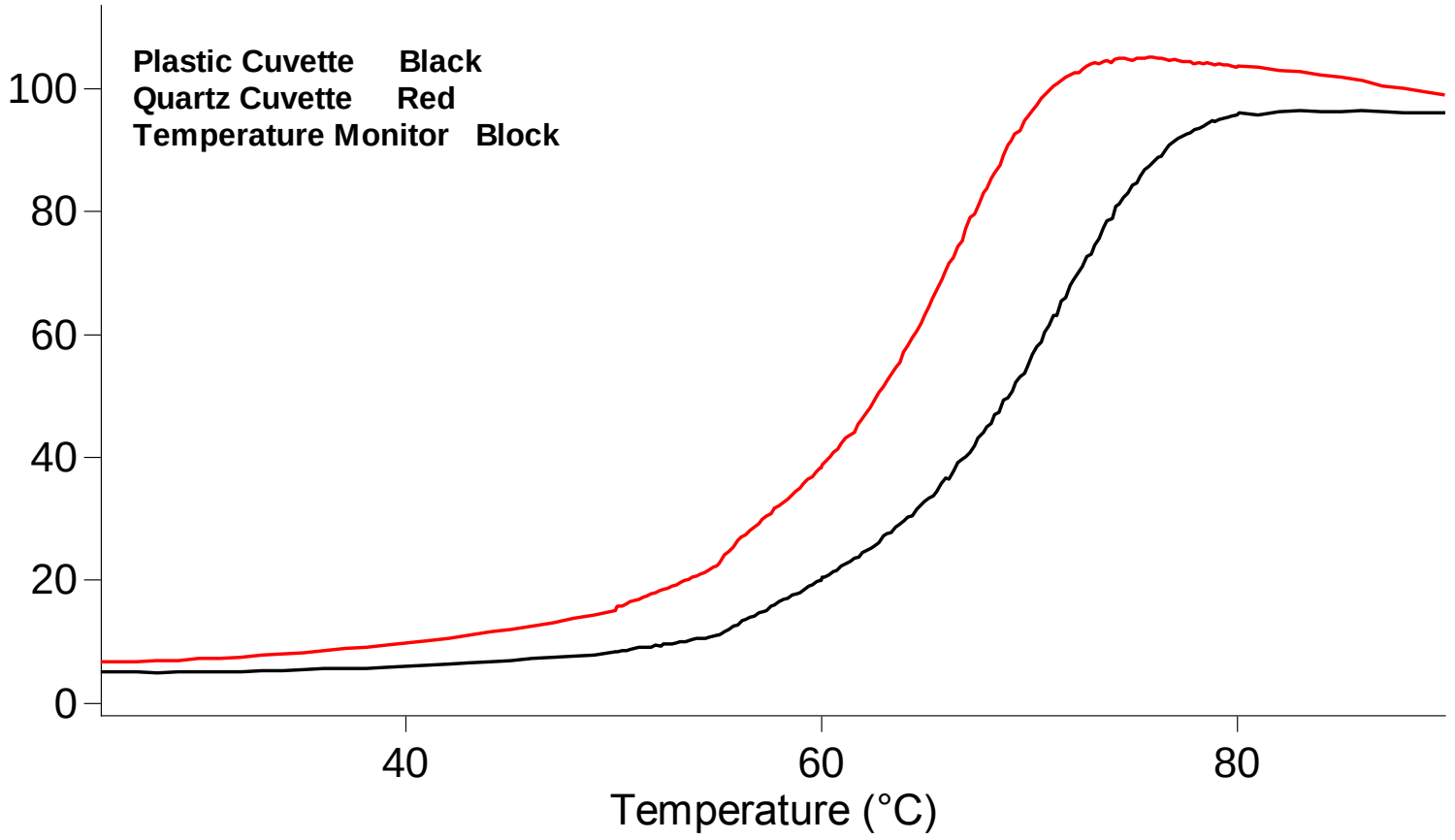
Example of Fluorescence Thermal Melt going from High to Low Intensity



Importance of Choice of Cuvette



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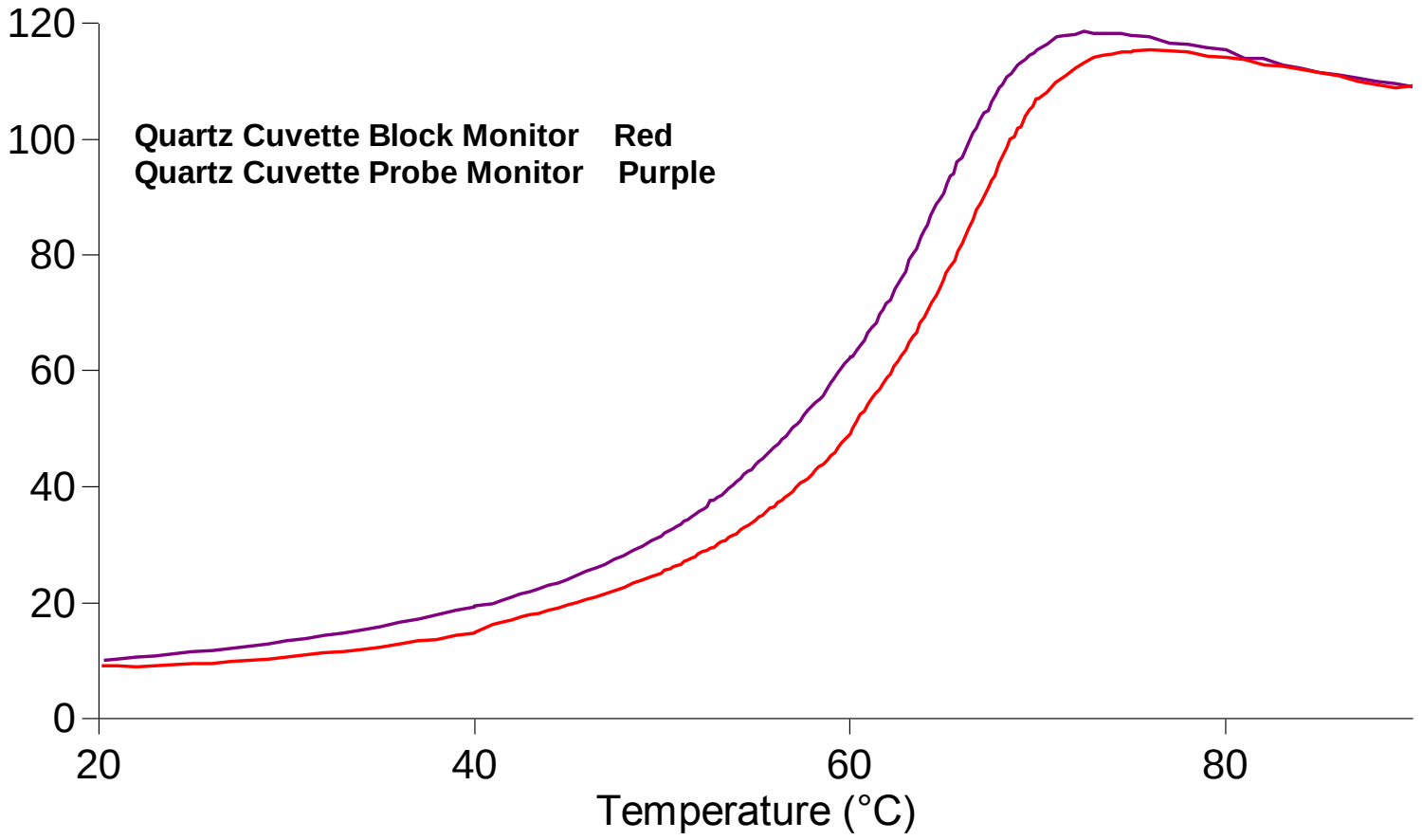
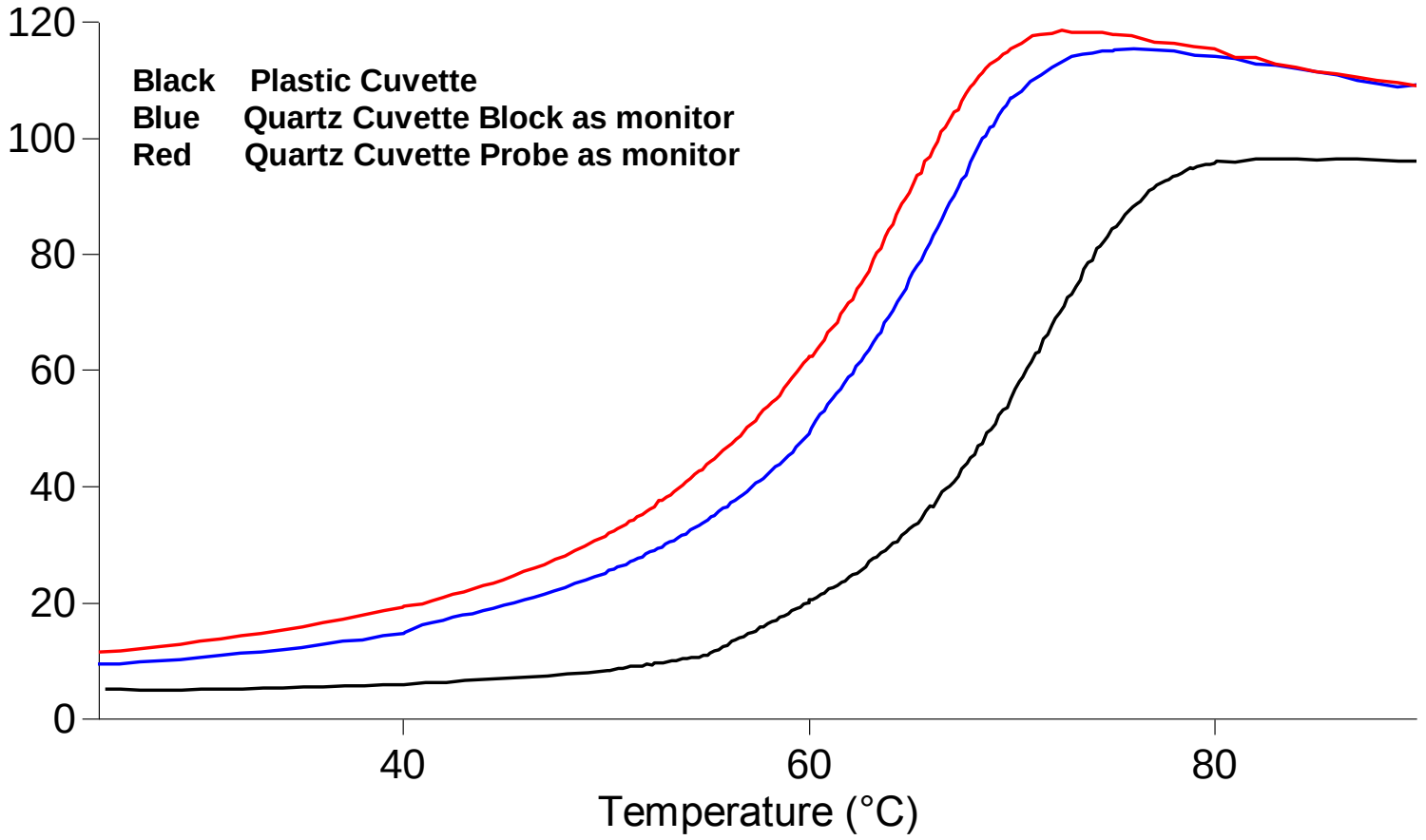
Importance of Choice of where Temperature is Monitored



Overlay of Cuvette and Temperature Monitor Choices



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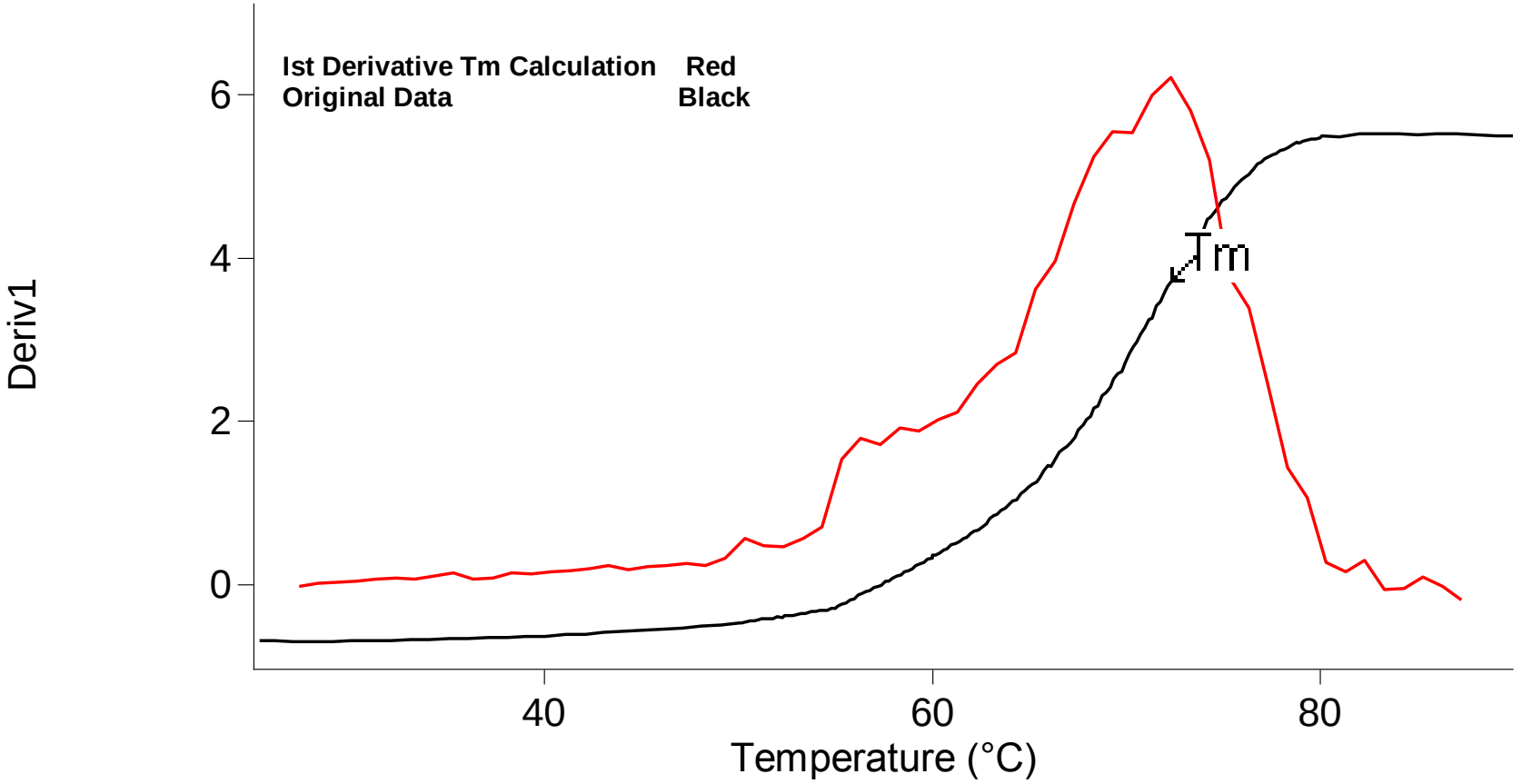
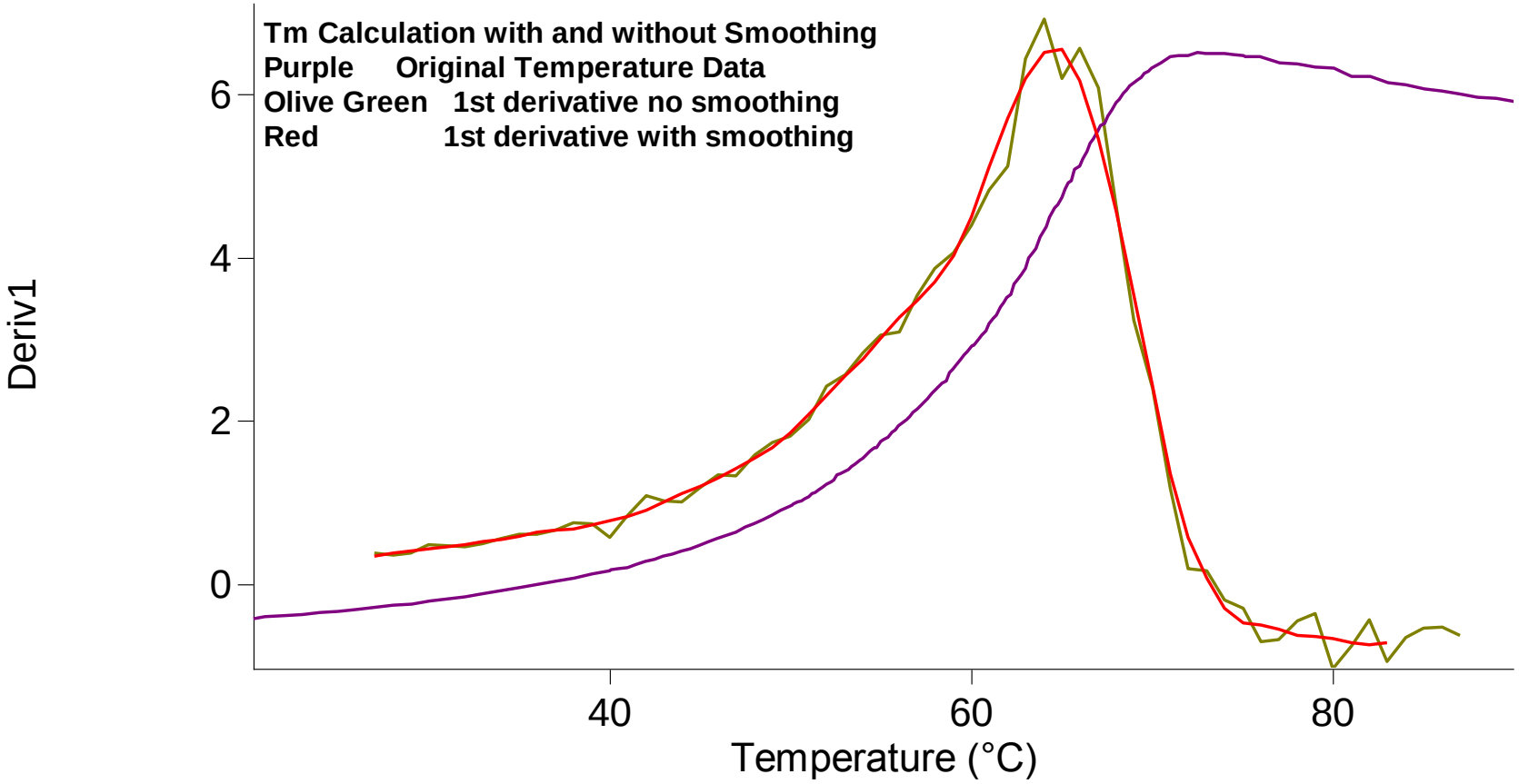
Determination of Tm



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

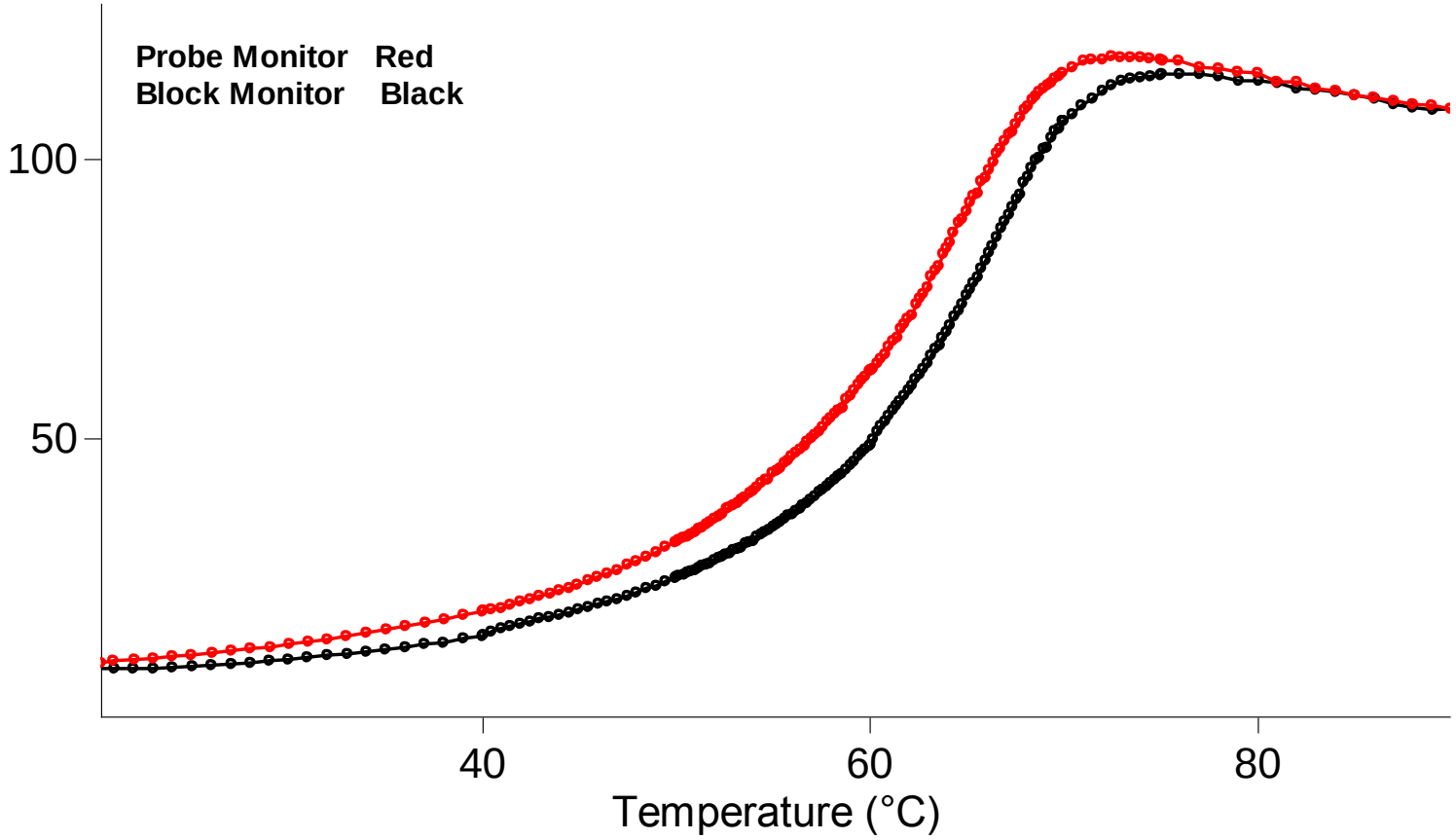
Effect of Noise on Tm Calculation



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