This short presentation shows the results of three unprecedented experiments possible only with the Olis CLARiTY 1000.

The photo shows the novel DSPC within the dual beam sample compartment.

An entirely new era
of studying scattering biological suspensions
has been entered.

You have gone full circle, from the reversion spectroscope at the start of the 20th century to the detector that we all want in the 21st century.

That is truly cool. Best Wishes – John S. Olson

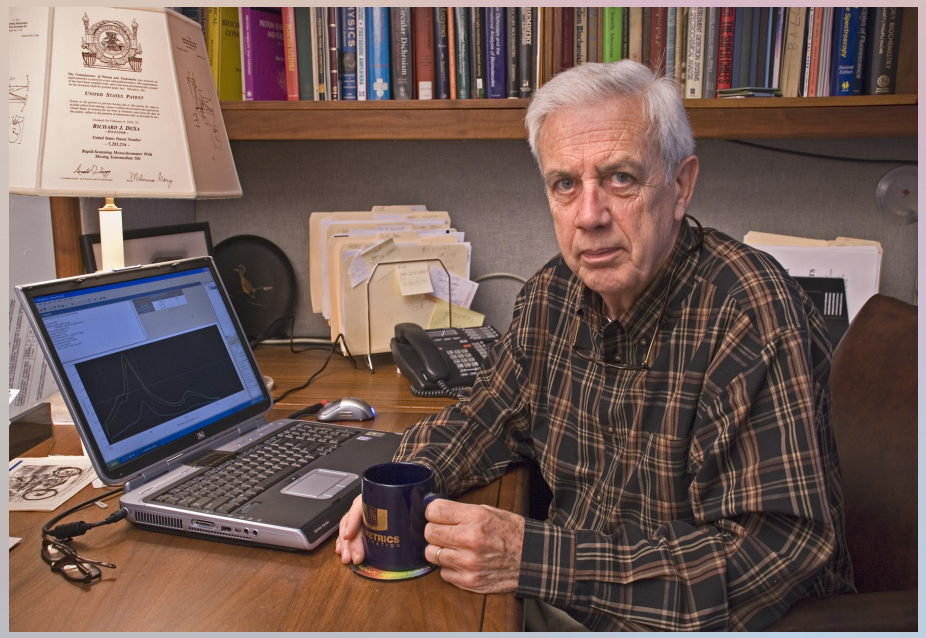
Ralph and Dorothy Looney Professor of Biochemistry and Cell Biology
Rice University

Personal correspondence, July 2010

The three experiments were conducted by Dr. Richard DeSa –

the inventor of both the Rapid-Scanning Monochromator with Moving Intermediate Slit
(aka, Olis RSM 1000) that he used to collect
the following 300 nm width scans at a rate of 2 per second
and
the DeSa Suspension Presentation Cavity (DSPC, seen on the first page
and key to the CLARiTY performance) –

using an exceedingly dilute suspension of intact human red blood cells.



Experiment 1:

Watching as intact live red blood cells change
from oxygenated to deoxygenated
as the environmental O_2 is consumed.

Figure 1: The spectrophotometric recording of the release of O_2 from HbO_2 in human red blood cells (RBC).

300 nm span scans, collected at a rate of 2/second for 220 seconds.

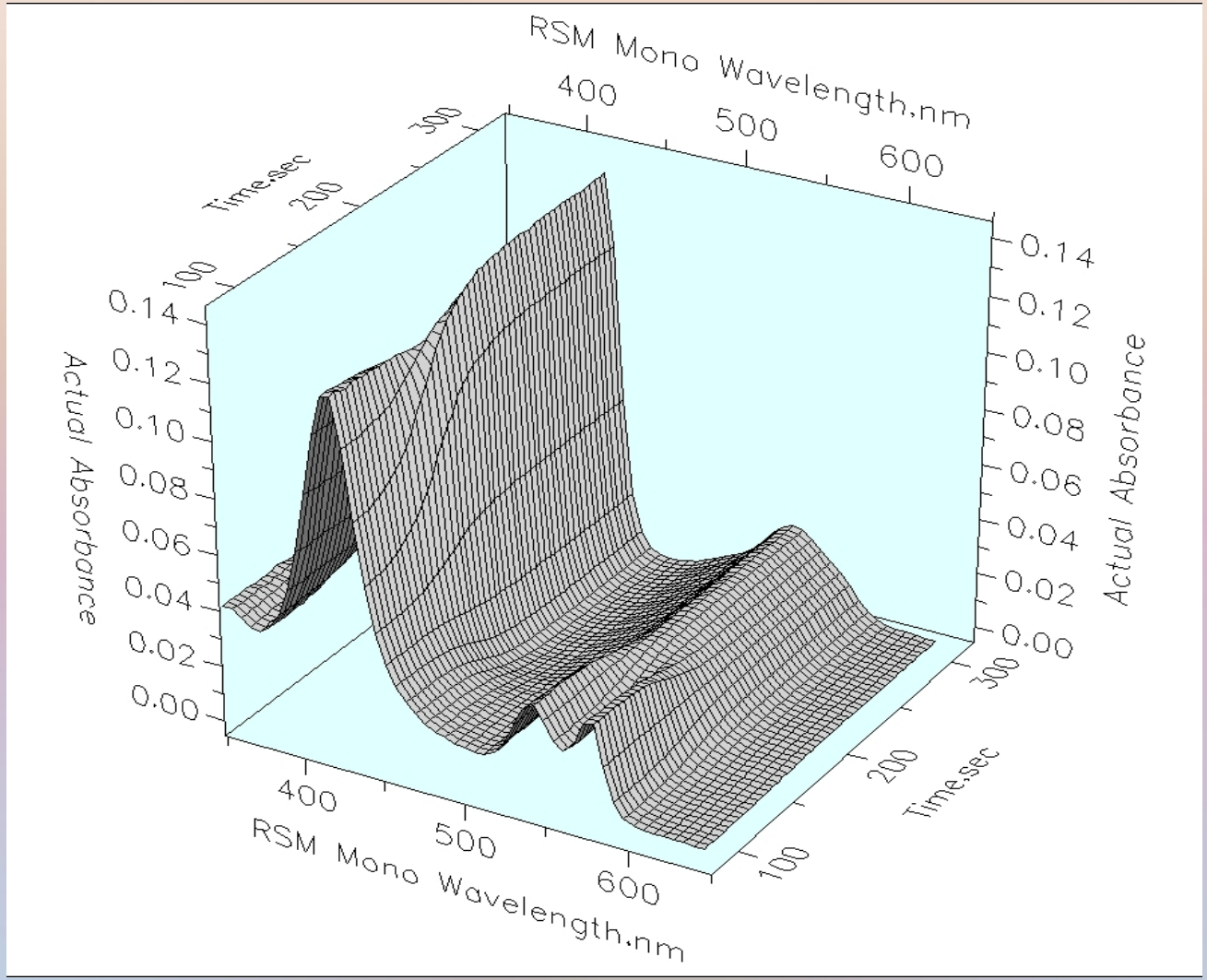
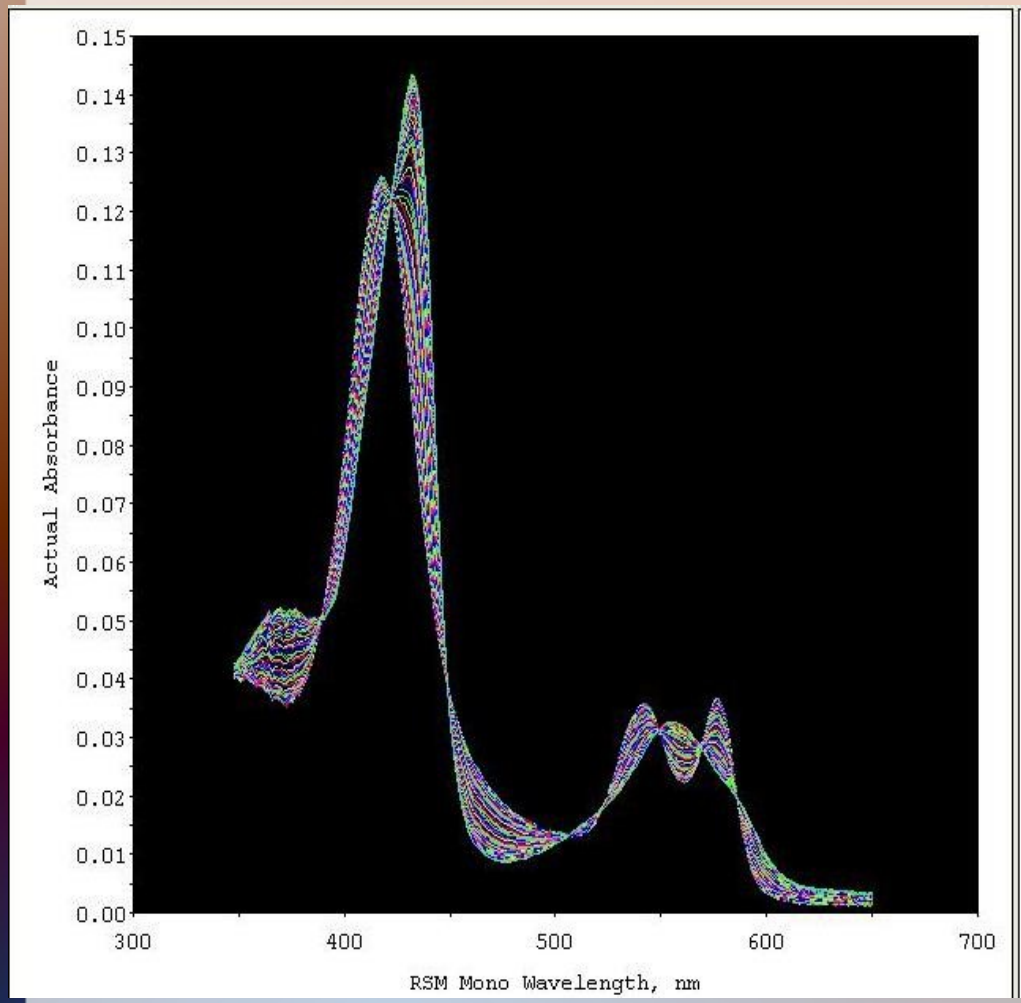


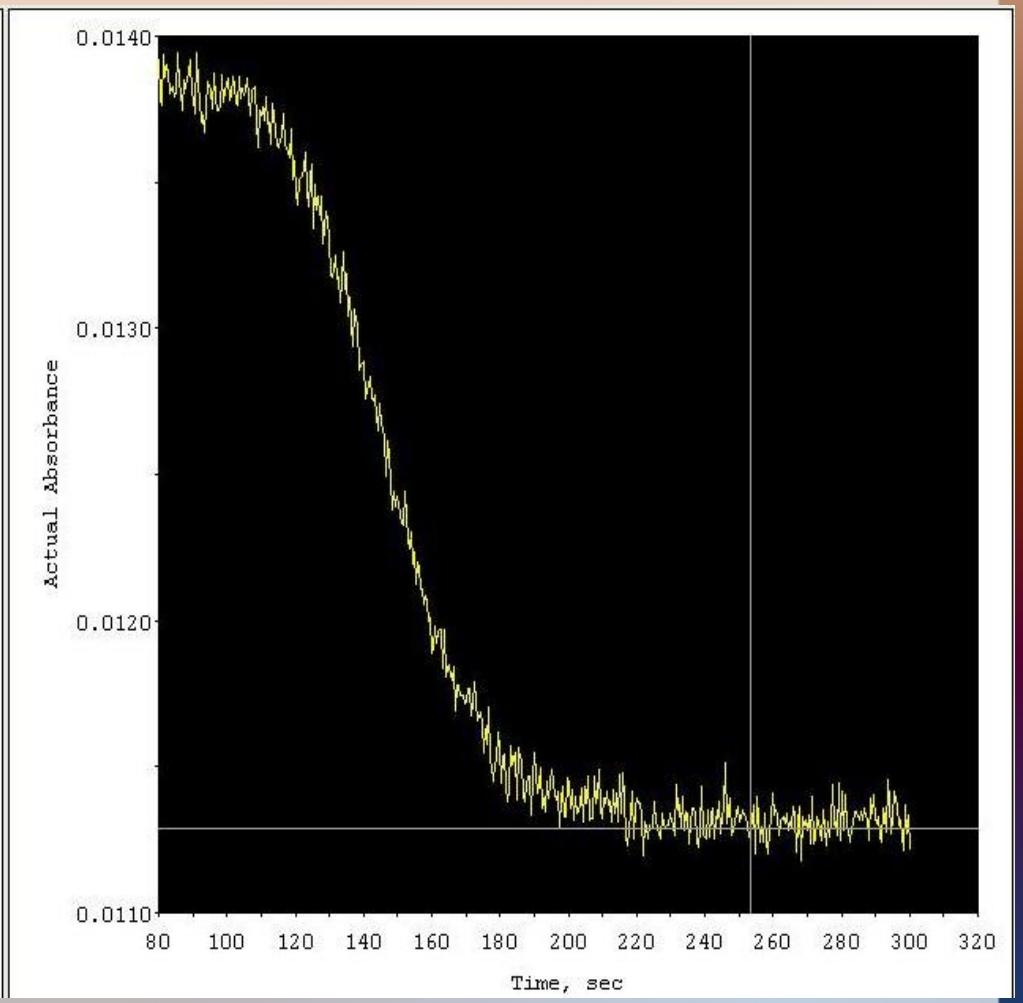
Figure 2 Clarifies what was shown in the 3D image. The graph to the right shows the kinetics.

The cells started oxygenated, the downward sloping line is the absorbance at 500 nm as the oxygen decreases, and the flat line at the bottom tells us that all of the HbO2 has released its oxygen.

Spectra (300 nm width @ 2 scans/ second)



Abs change at 500 nm (notice Y axis!)



***No* other spectrophotometer has ever collected spectra of such quality from intact RBCs in suspension.**

Because ...

RBC suspensions scatter the light so much that conventional spectrophotometers are unable to collect accurate spectra at any rate (let alone at 2/sec).

***The total immunity of the CLARiTY to scatter*,**

its high photometric accuracy,
and
the high speed wavelength scanning of the RSM
combine
to allow the study and precise mathematical analysis
of real-time high speed functioning of
the HbO_2 system.

This instrument is ideal for advancing our understanding of this centrally important function in animal life.

It is ***only*** with this technology that one can truly answer the question "How does Hb function in real life?"

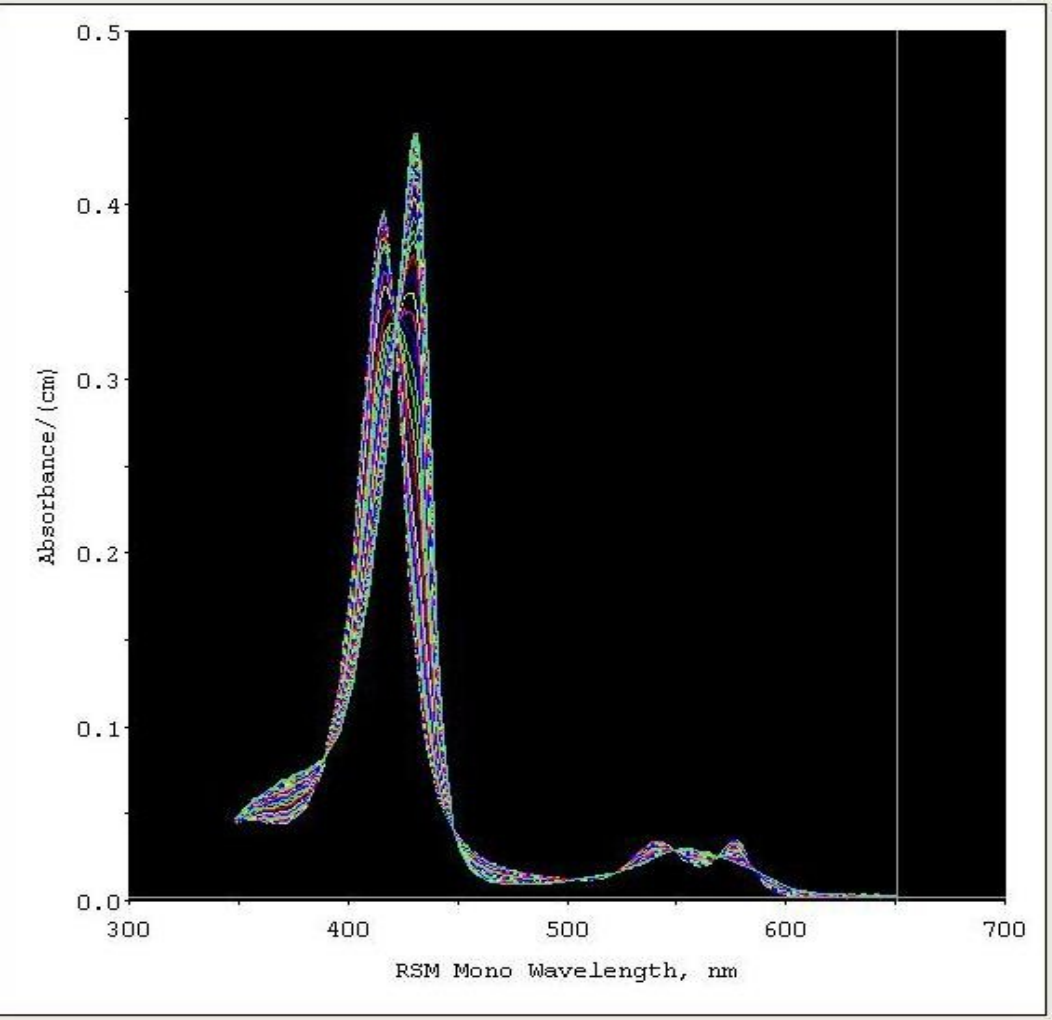
Experiment 2: Deoxygenation of Hb released from lyzed RBCs.

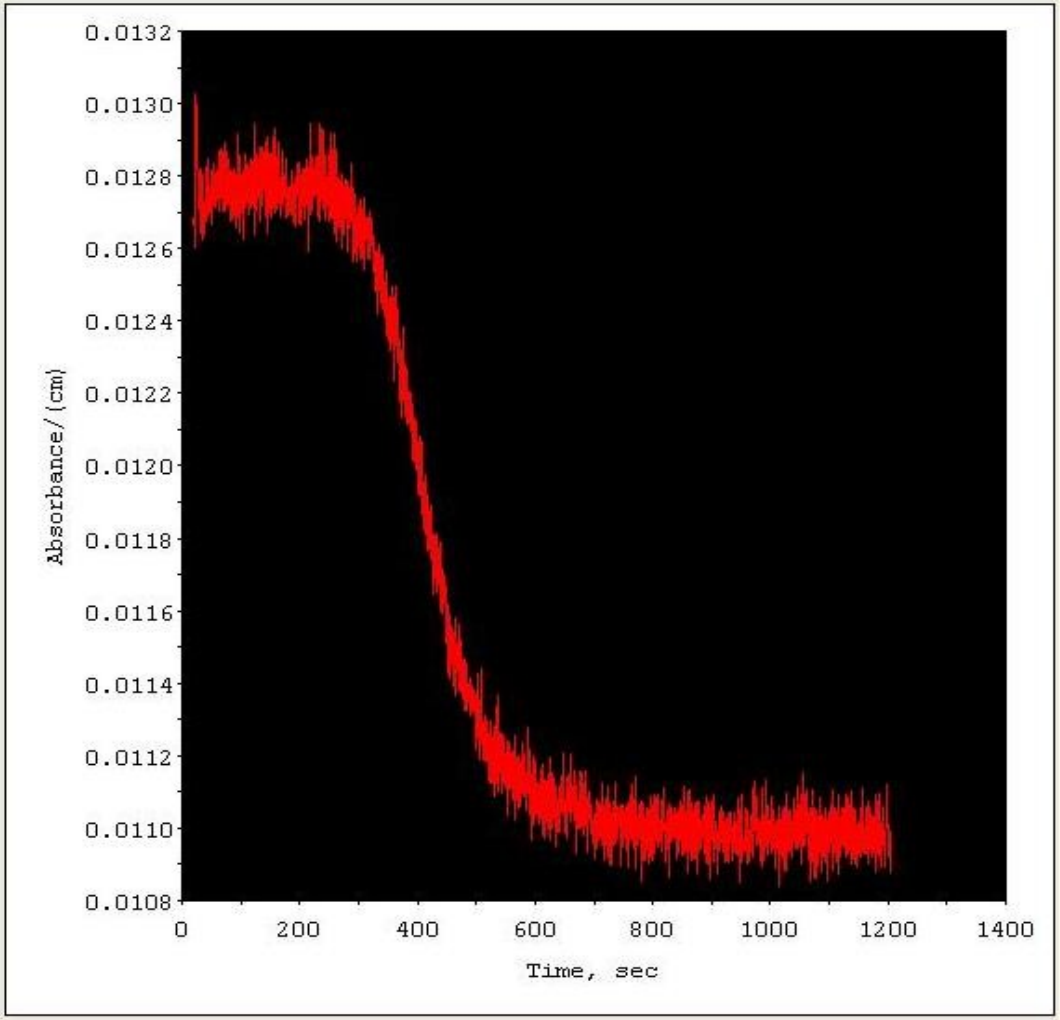
This is the condition under which most experiments of HbO2 are done, *because intact RBC cannot be studied in conventional spectrophotometers (of the last 150 years)*

- RBCs were put in the DSPC in phosphate buffer without NaCl.
- The cells lysed in less than 20 seconds and the data shown were collected.
- There is a substantial and meaningful increase in the absorbance of the Soret region since the packing effect (hypochromism) seen with intact RBCs no longer is present.
- The deoxygenation occurs with no apparent shift in the isobestic point as the HbO_2 changes to Hb.

This is similar to the result obtained when intact RBCs are examined

(i.e, data shown in figures 1 and 2).





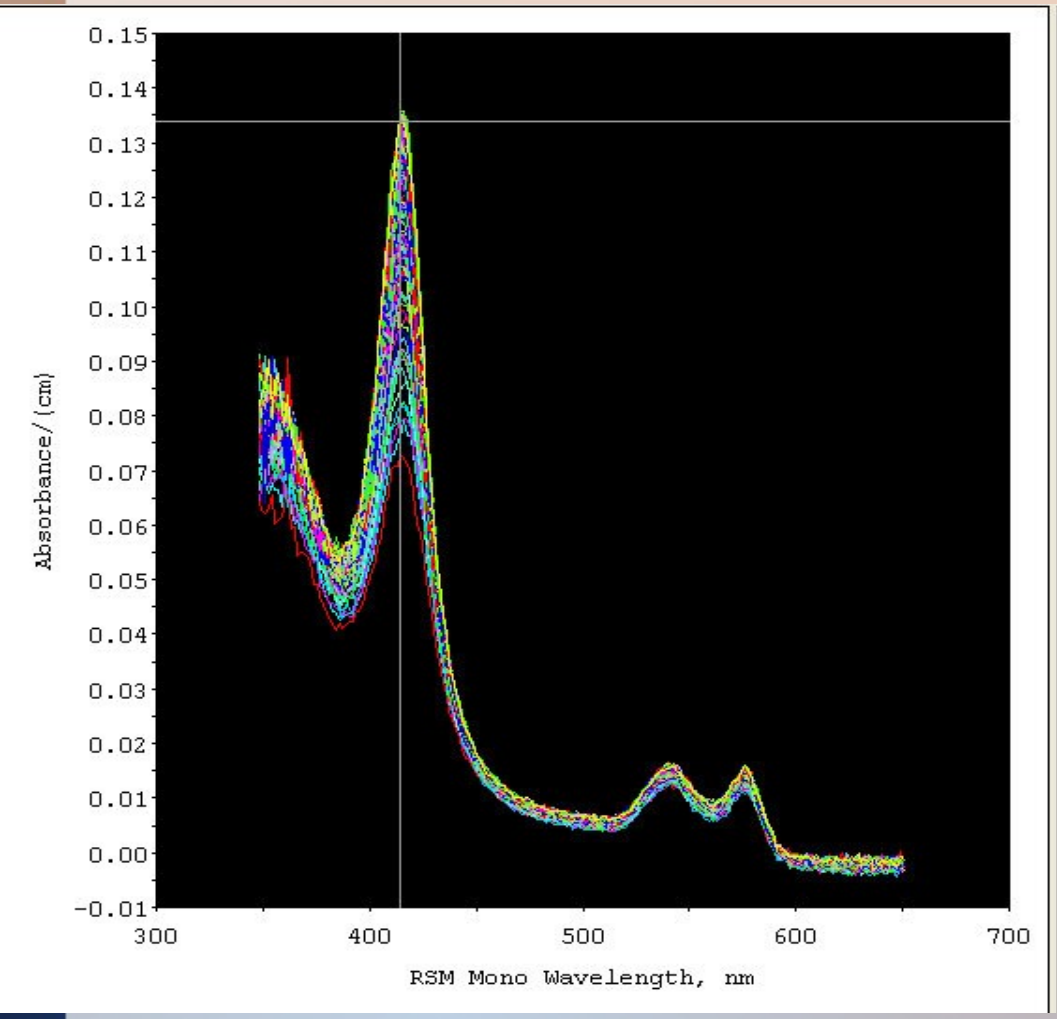
“The Olis CLARiTY offers the simplest and most direct way of studying a bio-molecule under these two conditions, oxygenated hemoglobin tightly packed in the RBC or free in solution.”

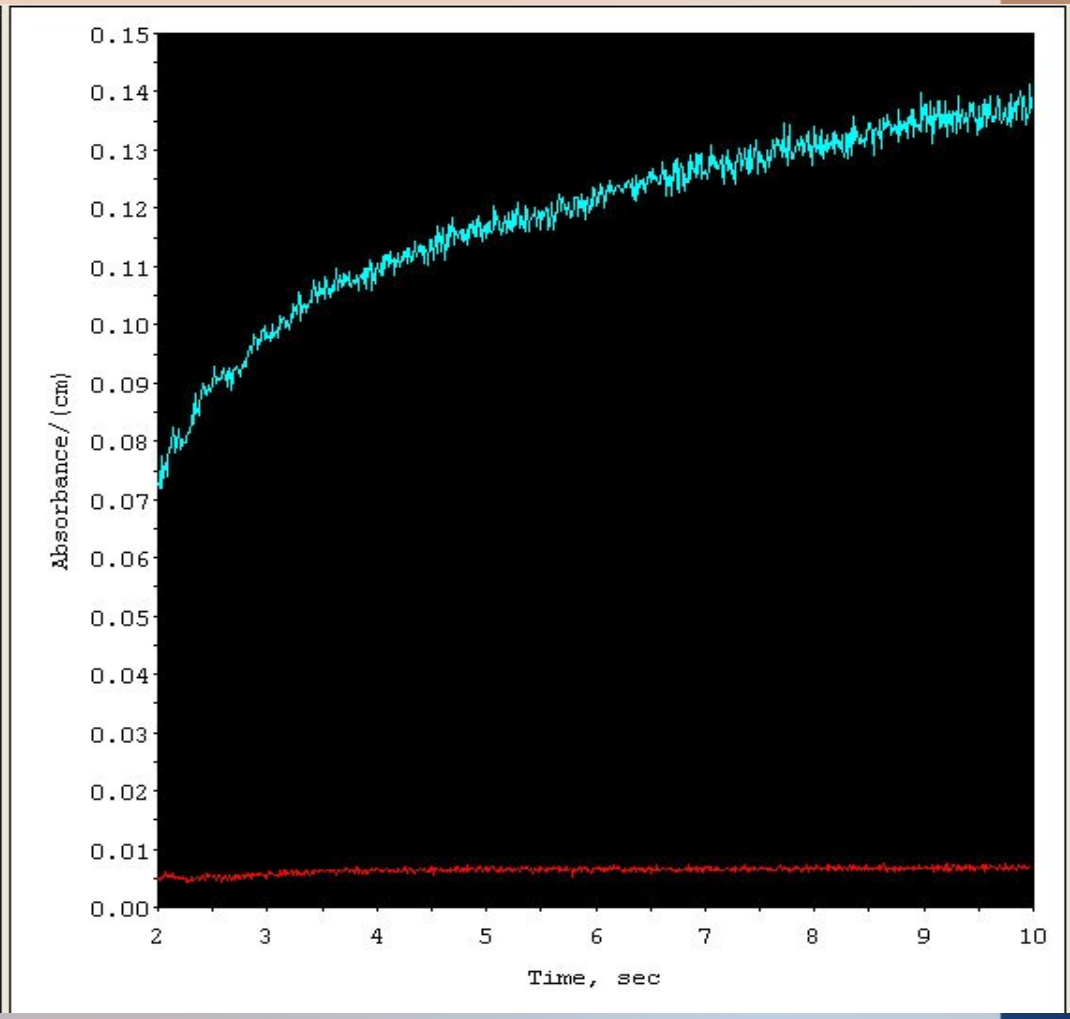
Lawrence J. Parkhurst, Ph.D.
Hewett University Professor, UN Lincoln

(whose blood cells were used for the experiments!)

Experiment 3: *The process of the HbO2 releasing from the RBCs as they lysed during a 10 second time period.*

The data transition from very good spectra of HbO_2 in intact RBC (Figure 1) to very good spectra of free HbO_2 in a clear buffer solution (Figure 3).





Today's three experiments prove that you can now do spectrophotometry on highly scattering suspensions.

Imagine loading your Olis CLARiTY's DSPC

with suspensions

(mitochondria, yeast, bacteria, algae, nanoparticles ...)

and getting accurate absorbance spectra,*

(*And, with the right additional hardware, fluorescence spectra, too!)

The Olis CLARiTY

- **Total immunity to the effects of scatter**
- Cuvettes with effective pathlengths exceeding 20 cm, providing dramatic sensitivity enhancement
- High speed wavelength scanning (100 scans/ second), so that full spectra are available to follow kinetics
- Dual beam optical system with photomultiplier tube detectors for best photometric precision and accuracy

“Not a comment but an opinion -

This system is an almost incredible advance on the best Britton Chance was ever able to do for turbid suspensions. I do hope OLIS is able to sell a lot of them.”

Quentin H. Gibson
Emeritus Prof, Cornell Univ
Personal correspondence, July 2010

The spectra were really beautiful and impressive . . . Very exciting, and beautiful results!

Larry J. Parkhurst

The CLARiTY changes everything with respect to thinking in spectrophotometry. Awesome! I just have to have one in my lab. Will be writing grants later this year to include a purchase.

Paul S. Hoffman
Professor, University of Virginia School of Medicine

Clear, unambiguous spectra
at rates adjustable to the system under study.

What research projects can you pursue now since

Scatter Does Not Matter?

Get more information on the Olis CLARiTY spectrophotomters
by calling 1.706.353.6547 or
writing to sales@olisweb.com

If he's available, Dr. DeSa himself will be happy to speak with you ,too!