

MOLECULAR SPECTROSCOPY WORKBENCH

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Arterial Analysis with a Novel Near-IR Fiber-Optic Probe

This month, we provide a double-barrelled installment of the Molecular Spectroscopy Workbench. I have turned the first few pages over to **Robert A. Lodder** and **Lisa Cassis**, both of the University of Kentucky's Chandler Medical Center, Rose Street, Lexington, Kentucky 40536-0082. Lodder and Cassis have been developing a diffuse-reflectance fiber-optic probe to collect near-infrared spectra from the intimal surfaces of arteries. The colorful results of their summer experimentation are shown here for the first time. In Part 2 of this month's column, we present the findings of the instrument repair and maintenance survey I initiated in the February issue. This informal poll provides a glimpse at how spectroscopists view their instruments and the technicians they must call into action when things go wrong. Next month, I will review the highlights of the Third International Conference on Near-Infrared Spectroscopy, held last June in Brussels.

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Contributing Editor

Our recent work at the University of Kentucky Medical Center has been directed toward the development of a diffuse-reflectance fiber-optic probe to collect spectra from the intimal surfaces of arteries. This summer, a prototype fiber-optic probe was used in experiments that mapped the lipoprotein composition of the thoracic aorta in rats. The near-IR fiber-optic probe is intended for use in studies of the growth of atherosclerotic lesions and in the chemical examination of arterial endothelium (the portion of the artery now thought to be a major factor in the development of arterial blockages as well as in arterial blood-pressure control). Until now, it has not been possible to determine location and quantities of substances such as high-density lipoprotein (HDL), low-density lipoprotein (LDL), and apolipoproteins in living arterial tissue. The ability to perform such analyses opens up the possibility of studying the quantities of these materials over time in a lesion, both as the lesion

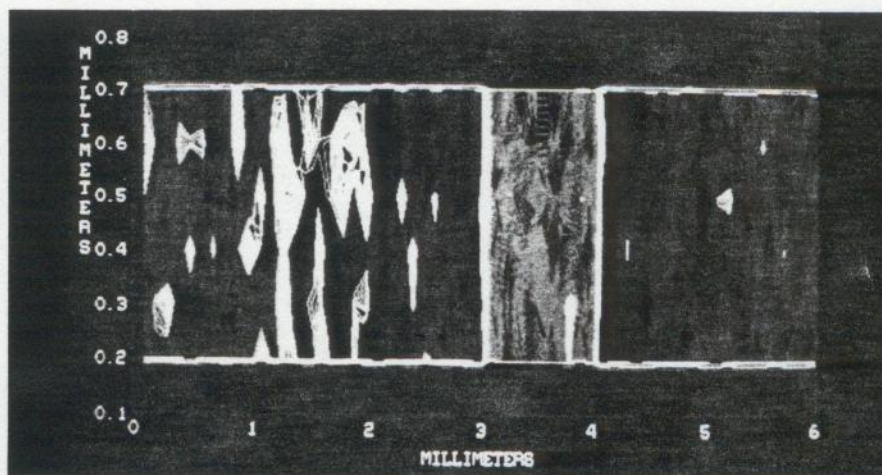


Figure 1. Aorta incubated without LDL. The "normal" aorta is shown in shades of blue.



Figure 2. Aorta incubated with LDL. Changes induced by LDL are shown in shades of red.

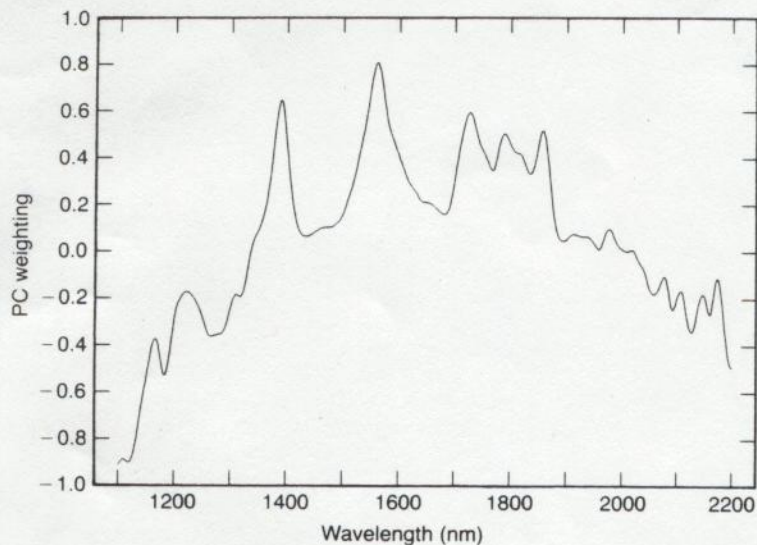


Figure 3. Principal-axis transformation weighting spectrum for LDL in aorta.

grows and as cholesterol-lowering drugs are used in attempts to shrink the lesion. Theories intended to describe what may cause lesion shrinkage, such as reverse cholesterol transport (a theory in which HDL acts to remove cholesterol from tissues to the liver, where it is excreted in the form of bile acids), will be tested with the fiber-optic device.

A compound parabolic concentrator (CPC), similar to those used for solar power concentration (1), was used to compress the beam from a transmitting fiber optic onto a small spot on the artery surface. The CPC

was molded from a polymer and had a polished aluminum lining. Nonimaging CPCs have been shown to improve light-gathering by a factor of four or more over lens-based focusing designs. The aperture at the distal end of the fiber-optic probe was $\sim 0.74 \text{ mm}^2$ and could be moved across the arterial surface in increments as small as $10 \text{ }\mu\text{m}$ with a micro-positioning stage. Near-IR light across a wavelength range from 1100 to 2500 nm was transmitted through the concentrator. Collimated source light was directed on the artery by the concentrator. Slightly skewed transmitted

rays were still focused into the aperture by the shape of the CPC. However, scattered light from the artery tends to travel in all directions from the point of scattering and therefore produced a substantial amount of scatter at the proximal end of the CPC. This scattered radiation was detected at the proximal end of the CPC by lead sulfide detectors located off the axis of the incident beam.

The false-color "maps" that appear in Figures 1 and 2 represent the lipoprotein composition of the thoracic aortas obtained from two laboratory rats. The aortas were each excised and partially denuded, removing the endothelium from a portion of the vessel wall of each rat. One of these aortas (Figure 1) was incubated for 2 h in Krebs's physiological salt solution. The other aorta (Figure 2) was incubated for 2 h in a Krebs's physiological salt solution that also contained LDL ($500 \text{ }\mu\text{g/mL}$).

The arteries were washed following incubation and passed beneath the concentrator while the concentrator was held fixed. The micro-positioning stage allowed spectra to be collected along the axis of the artery when the artery was opened to expose its intimal surface. Both segments of the thoracic aorta were scanned along a track $\sim 1 \times 6 \text{ mm}$ in dimension. Figure 3 depicts the spectral vector in the principal axis transformation matrix corresponding to the spectral change observed in the artery when the artery was incubated in LDL. The major spectral changes were observed near 1560 nm and between 1700 and 1870 nm.

Figure 4 summarizes the process by which colors were assigned to pixels in the arterial images. The "0"s represent sample spectra obtained from the aorta incubated without LDL, and the "+"s represent sample spectra obtained from the aorta incubated in the LDL. Distances are measured in standard deviations (SDs) in spectral hyperspace between the center of aorta spectral points incubated without LDL and each test-sample spectral point (which may or may not come from an aorta incubated in LDL). A parallel assimilation algorithm (2) was implemented on IBM 3090-600J supercomputers at the Cornell National Supercomputer Facility and the University of Kentucky Center for Computational Sciences to calculate the distances and to assemble the results into the final color images. The center of the aorta spectral points incubated without LDL are coded dark blue. Vertical motions along the solid lines in Figure 4 (representing base-line shifts that probably correlate to vessel wall thickness) are coded green. Horizontal motions along the dotted lines in Figure 4 (apparently representing uptake of LDL) are coded pink and red. Red represents a larger movement in SD than pink. The contour lines in the arterial images are drawn every 0.1 SD, and a color change occurs approximately every 3 SD.

The image in Figure 1 is predominantly blue and green, indicating that the aorta is similar to spectra obtained from such tissues incubated without LDL. The green portion is caused by fiber-optic scatter that may be the result of a thickening of the vessel wall that

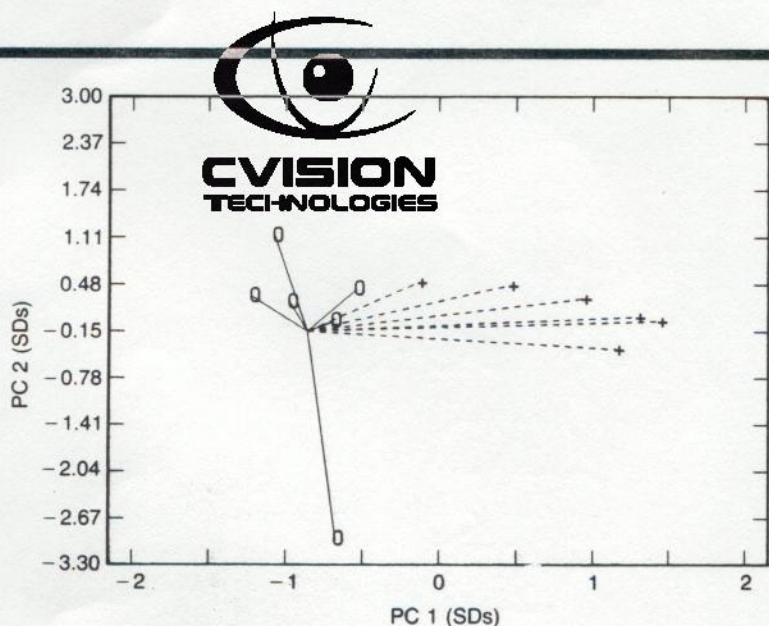


Figure 4. PC plot describing color assignment to pixels. 0's represent spectra from aorta incubated without LDL. +s represent spectra from aorta incubated with LDL.

brings the sample closer to the optical aperture. The image in Figure 2 shows nearly orthogonal changes that correspond to the uptake of LDL by the aorta wall. The red color represents regions of maximum uptake of LDL. The exact form in which this LDL resides in the arterial wall is still unknown.

In the future, we will be constructing an improved fiber-optic catheter that will be used to conduct in vivo imaging of arterial lesions. The system is expected to increase existing knowledge of arterial endothelium, permit kinetic studies to be performed on lesions both as they grow and as they shrink in response to drug therapies, and clarify the role of lipoproteins in the arteriosclerotic disease process.

REFERENCES

(1) P. Gleckman, J. O'Gallagher, and R. Winston, *Nature* **339**, 198-200 (1989).
 (2) R.A. Lodder and G.M. Hieftje, *Appl. Spectrosc.* **42**, 1351-1365 (1988).

PART 2: INSTRUMENT REPAIR SURVEY RESULTS

Well, here it is autumn again, and our thoughts now turn to school, raking leaves (for those of us with seasons), football, and new projects. *Now* we will learn the consequences of not performing preventive maintenance on our instruments last spring. Therefore, this seems like an appropriate time to look at the results of the instrument repair poll included in the February installment of this column (1).

I wouldn't want to color readers' interpretations of the numbers I am about to present, so I will withhold my comments on the results until the latter part of the column.

SERVICE NEEDS	
On average, how often must you replace expendable parts?	Are service calls usually necessary?
Every 0-6 months 32%	Yes 24%
Every 12 months 26%	No 76%
Every 1-2 years 15%	Was the operator's manual any help the last time you attempted to fix an instrument or did you ask someone else for help?
Every 2-3 years 21%	Manual 42%
Less often 5%	Other person 35%
Do replacement parts last as long as the originals?	Luck/skill 23%
Yes 82%	
No 12%	

THE PURCHASE	
During your last purchase, was the sales representative helpful in choosing the proper model and/or options?	
Yes 87%	
No 13%	
Were you ever sold an instrument that did not perform the task for which it was purchased?	
Yes 32%	
No 68%	
If yes, do you think it was because:	
The product was misrepresented . . . 43%	
I assumed I knew the capabilities of the instrument . . . 29%	
The rep didn't know how to answer my questions 14%	
The rep withheld information 14%	

QUALITY OF SERVICE	
What kind of training have you received from the supplier?	
Good 23%	
Fair 36%	
Poor 23%	
None 18%	
The last time you ordered a part, how long did it take to arrive?	
Overnight 14%	
<1 week 27%	
1 week 14%	
2-3 weeks 27%	
Don't know 9%	
When an instrument breaks down, what is the average response time before:	
(a) Your call is returned?	
No wait 4%	
1-2 hours 17%	
Same day 35%	
1-2 days 30%	
3-4 days 4%	
Never calls 4%	
(b) You receive the needed part?	
Overnight 38%	
~2-4 days 13%	
1 week 19%	
1-2 weeks 19%	
Never (used third party) 4%	
(c) A service rep calls?	
Overnight 6%	
1-2 days 33%	
<1 week 39%	
1-2 weeks 17%	
3 weeks 6%	
When a service technician calls on your facility, does he/she:	
Have all needed parts 19%	
Know the model in question 30%	
Have the knowledge to repair 30%	
Explain how to avoid problems 21%	
When the repair is made, does the instrument work as well as before the problem occurred?	
Yes 100%	

THE INSTRUMENTS	
What types of spectrometers do you use frequently?	In general, is durability or versatility the main characteristic you seek in a new instrument?
FT-IR 27%	Durability 59%
NMR 20%	Versatility 27%
Mass 18%	Both 14%
UV/Vis 16%	From your experience, is your most used instrument:
Grating near-IR 7%	Versatile 14%
Dispersive IR 4%	Durable 18%
Filter fluorometer 2%	Both 68%
Scanning fluorometer 2%	Overall, how would you rate your instruments in terms of dependability and serviceability?
Other 2%	Excellent 44%
Which suppliers do you patronize for these instruments?	Good 44%
Baird-Atkinson, Bio-Rad/Digilab, Bomem, Bruker, Coleman, Hitachi, Delsi-Nermag, Finnigan, MAT, General Electric, Guided Wave, Hewlett-Packard, Jasco, JEOL, Jobin-Yvon/Coherent, Kratos, Mattson, Nicolet, NIRSystech, PerkinElmer, Shimadzu, Varian	Fair 4%
	Poor 8%