

Combined Optical and Magnetic Resonance Microscope with Real-Time Performance

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The Challenge: Developing a new microscope control and acquisition system that can study two cells simultaneously with two different microscopic techniques.

The Solution: Using National Instruments ComponentWorks™ software (a component of Measurement Studio) and NI hardware to create a new confocal microscope with pulsed and steady-state lasers, varying control and acquisition frequencies, and remote access via the Web.

Battelle Memorial Institute is a company that serves both government and industrial clients in the development of new technologies and products. Battelle has operated PNNL since 1965. This project consisted of building a confocal optical microscope to use in conjunction with a nuclear magnetic resonance (NMR) microscope to simultaneously study live cells with two completely different microscopic techniques, magnetic resonance imaging (MRI), and confocal microscopy. By using two techniques to view the same two cells simultaneously, we can obtain different kinds of information about the sample. Receiving this information simultaneously is useful both operationally and scientifically for setting up lengthy NMR experiments. These experiments can help us to refine a diagnostic model for diseases.

Confocal Microscopy

Confocal microscopy derives structural information about translucent samples that fluoresce when excited by light of a given wavelength. We focus a laser to a point inside the sample; the resulting emission is captured on a photomultiplier tube (PMT) through an aperture. Moving the focus in a controlled fashion, you can map out features inside the sample and build a 3D picture from a series of X-Y planar images. The method is especially

useful in biological applications and is frequently used to image living cells. The laser can raise the energy levels of special dyes or stains and cause specific cell structures to emit photons.

Although there are commercial confocal microscopes on the market, our situation required that we use no magnetic parts in or near the magnetic chamber of the NMR microscope. With this requirement, we designed a microscope in-house that consisted of nonferrous components that were optically extended outside the magnetic chamber. We needed a driver and acquisition system for our microscope, so we turned to National Instruments Measurement Studio software, data acquisition (DAQ) boards, and motion control to provide high-speed data acquisition synchronized with laser scanning motion control.

Application Set-Up

We decided to build a data acquisition server application so users in different parts of the lab or in universities around the country could set up, acquire, and analyze images generated by the confocal microscope. The microscope server program was written in Visual Basic 6.0 to take advantage of the rapid application development environment, existing codes and tools, and to provide easy TCP/IP communication with clients. National Instruments ComponentWorks 3.0, a component of Measurement Studio, provided high-level hardware drivers to drive and synchronize output and input waveforms, as well as analysis tools to help post-process the signals acquired.

To drive the laser beam across the cell sample, we needed to control the motion of two mirrors – one that scans in the X-axis and one that scans in the Y-axis. Simultaneously, we needed to record laser-intensity signals on multiple channels. For Z-axis control, we used a specially designed ceramic stepper motor with a nonmagnetic encoder to move the laser focus to different planes in the cell.



The Confocal Microscope-NMR Spectrometer Hybrid Instrument



RasterScan configuration screen for a Pulsed Laser Acquiring One Analog and One Digital Signal

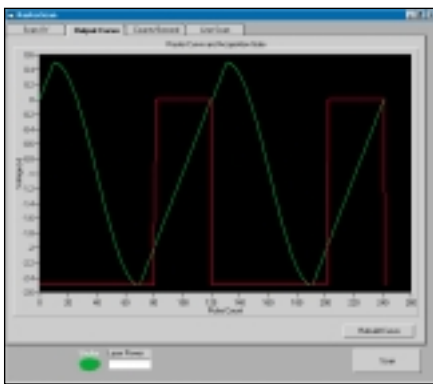
By acquiring an image slice at different planes, we can construct a 3D image of the cell.

We accomplished raster scanning in the X-axis by building a waveform that consists of a voltage ramp that scans a beam across the sample cells. We then used a modified sine curve to scan back across the sample to reduce jitter in the mechanics at both ends of the voltage ramp. A simple stepped-ramp waveform drives motion in the Y-axis. We controlled Y-axis motion synchronously with a PCI-MIO-16XE-10 DAQ board. We set the X-axis motion control waveform to repeat on one analog output channel of a National Instruments PCI-6110E multifunction I/O board; simultaneously we acquired two channels of data on the analog input channels.

To minimize post processing and conserve system memory, we gated data acquisition to occur only during the linear region of the ramp. The PCI-6110E multifunction onboard counter/timer circuits of the I/O boards generate the acquisition gate and clock.

We routed all output and input signals through a pair of National Instruments SCB-68 shielded I/O connector blocks enclosed in a CA-1000 configurable connector accessory enclosure. Measurement Studio provided the high-level device drivers for the DAQ boards in the form of ActiveX controls. These controls provided us with intuitive configuration and control of the hardware, which saved us development time. We controlled the ceramic-stepper motor with a NI PCI-Step-4CX motion control board and NI-Motion™ software, eliminating the need to write low-level motion control routines.

The capabilities of the motion and DAQ hardware, and high-level interface provided by Measurement Studio, along with good documentation and excellent service and support from National Instruments, helped us develop a solution for a relatively complicated problem, gave us great results, and reduced our development time.



RasterScan X-Axis Motion Profile and Acquisition Gate

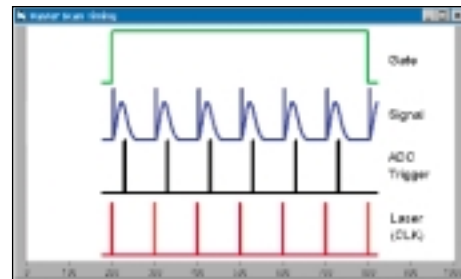
Conclusions

The overall goal of the project was to relate NMR and Confocal Microscopy information to produce chemical and physical information to underlying molecular biology. With this information, we can build a scientific bridge to whole-body magnetic resonance (MR) imaging, so we can refine the diagnostic model for diseases, particularly cancer recognition and response to therapy. This unique, combined microscope recently won a Discover award for scientific excellence and is an important part of a growing capability for biological imaging at PNNL.

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National Instruments software and hardware provide us with the flexibility to extend our control system. Currently, we drive five different laser spectroscopy experiments using the same software. Each of these experiments has a different speed requirement for motion and data acquisition. Some of our experiments read intensity (analog), while others count photons (digital). In other experiments, Z-axis motion is driven by a stepper motor or by an analog voltage. Even the laser type can differ,



RasterScan Timing for Pulsed Experiment

as we use both continuous and pulsed lasers. For example, with pulsed lasers we use the laser system as a timing clock to synchronize the data acquisition. By using Measurement Studio high-level drivers, we chose the appropriate multifunction DAQ or counter/timer boards for a particular system without changing the software.

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