

significant tightening of constraints on the new physics," says theorist Maxim Pospelov (University of Victoria, British Columbia). "It seems to disengage the anticipated CP -violating leptonic interactions from the electroweak scale. It pushes the new particles firmly into multi-TeV territory inaccessible to the next generation of sub-TeV electron-

positron colliders." Their discovery at CERN's Large Hadron Collider remains an open question.

Bertram Schwarzschild

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Nonlinear microscopy looks beneath the surface of historic artwork

For the first time, it's possible to create depth-resolved images of a painting without damaging it.

Fine paintings are more than skin deep: Human vision can penetrate tens to hundreds of microns, a depth that can include dozens of layers of paint. For centuries, painters have layered their paints for artistic effect, and conservators and art historians today are interested in probing those layers to learn about artists' materials and methods, undocumented restorations, and chemical changes in pigments over time.

But until now the only way to quantitatively analyze a painting's cross-sectional structure has been to slice away slivers of paint with a scalpel. Nondestructive techniques exist for studying the material composition of art and artifacts (see, for example, the Quick Study by Philippe Collon and Michael Wiescher, *PHYSICS TODAY*, January 2012, page 58), but none of them can image paintings with the necessary micron-scale depth resolution.

Now Duke University's Warren S. Warren, Tana Villafana, and Martin Fischer, in collaboration with conservation experts from the North Carolina Museum of Art in Raleigh and the National Gallery of Art in Washington, DC, have shown that femtosecond pump-probe microscopy of paintings can nondestructively create three-dimensional images that resolve layers of paint up to hundreds of microns deep and distinguish different pigments and materials.¹ As a proof of principle, the researchers have applied the technique, which was originally developed by Warren and others for biomedical imaging applications, to historic paintings that have already been extensively analyzed with the scalpel method.

Probing pigments

Warren's inspiration for the project came several years ago when he visited the National Gallery in London and

saw an exhibit on detecting art forgeries. "It was fascinating," he recalls, "but it was also clear that they were using technologies that would be decades out of date in the biomedical imaging community. So that set me to thinking about what we could do."

Pump-probe microscopy, which Warren and his group had been using to image pigments in skin tissue,² is a nonlinear optical technique that makes use of sample-mediated interactions between two pulsed laser beams. The first beam, or pump, induces molecular excitations, which affect how the second beam, the probe, interacts with the sample. Some molecular processes attenuate the probe—for example, molecules in the excited state might more readily absorb the probe wavelength than those in the ground state. Other processes, such as stimulated emission, enhance the probe's intensity.

Whatever the process, the magnitude of the pump-probe interaction depends on the product of the beam intensities, which is maximized in the small volume where the beams are focused most tightly. Measuring the intensity of the scattered probe light, therefore, gives information about what molecules are present in that focal volume, which can be positioned in 3D space as far beneath the surface of the painting as the lasers can penetrate.

But even in the focal volume, the beam intensities are tiny. To avoid damage to the sample, whether human skin or centuries-old artwork, Warren and colleagues limit themselves to an optical power of 5 mW, less than that of a laser pointer. As a result, the enhancement or attenuation of the probe is typically about 1 part in 10^6 . So feeble a signal is easily overwhelmed by laser intensity fluctuations and other sources of noise. To tease out the signal from the



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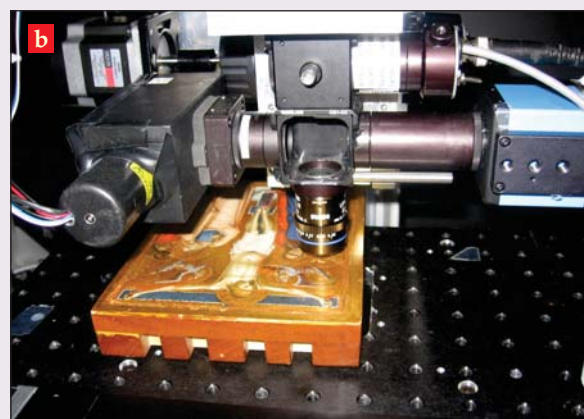
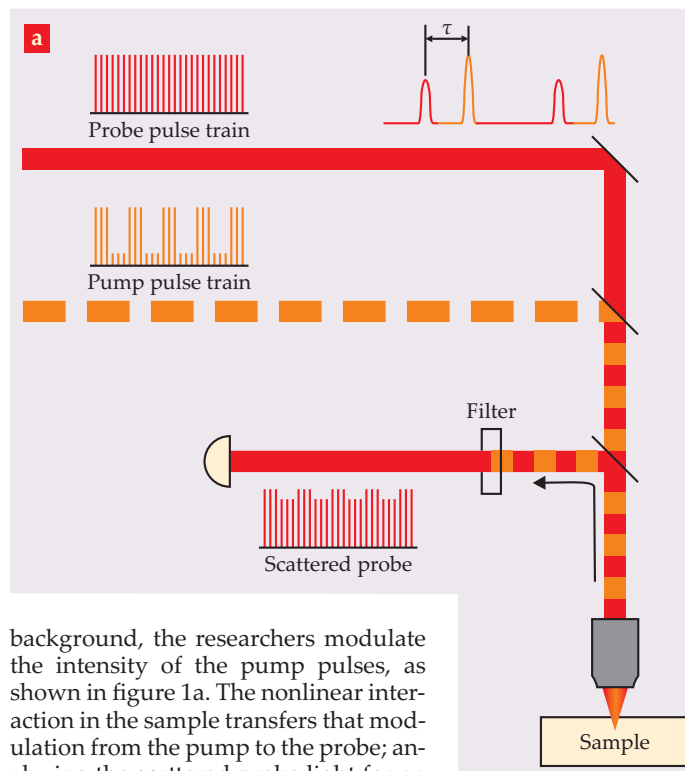


Figure 1. Pump-probe microscopy of historic artwork. **(a)** Two 80-MHz pulsed laser beams, the pump and the probe, are offset by a time delay τ and focused together in the sample. Nonlinear interactions between the two beams in the focal volume influence the intensity of the scattered probe light that reaches the detector. To help separate signal from noise, the pump intensity is modulated at 2 MHz; that modulation is transferred to the probe. (Adapted from ref. 1.) **(b)** The apparatus was used to analyze an early 14th-century painting, *The Crucifixion* by Puccio Capanna. The painting measures approximately 14 cm by 18 cm. (Courtesy of Martin Fischer.)

background, the researchers modulate the intensity of the pump pulses, as shown in figure 1a. The nonlinear interaction in the sample transfers that modulation from the pump to the probe; analyzing the scattered probe light for an oscillation at the modulation frequency thus gives the pump-probe signal.

The excitations generated by the pump laser have different lifetimes in different molecules. To discern the identity of the molecules in the focal volume, Warren and colleagues repeat the pump-probe measurement for different values of the time delay τ between the pump and probe pulse trains and compare the time-dependent signal with a library of known responses.

In skin imaging, that comparison is easy. Skin contains three main pigments—hemoglobin, eumelanin, and pheomelanin—that yield readily distinguishable signals when the pump and probe lasers are tuned to 720 nm and 810 nm, respectively. The range of pigments in an artist's palette is obviously much larger. But paintings produced prior to the advent of synthetic organic chemistry used just a few dozen main pigments, all based on minerals. Warren and colleagues are still working on cataloging all of them; so far, they've focused mostly on blue pigments, which give strong signals at the red and near-IR wavelengths the researchers are accustomed to using.

One such pigment is lapis lazuli (also known as ultramarine), a rich royal blue that was often used to mark the most important characters in religious paintings. During the Italian Renaissance, though, lapis lazuli was more costly than gold, so it wasn't unusual for artists to save money by painting a

thin layer of lapis lazuli on top of a thicker layer of a cheaper blue pigment. An exception to that cost cutting is found in Puccio Capanna's *The Crucifixion*, shown in figure 1b: The Virgin Mary's blue robe (partially obscured by the microscope objective in the figure) is known to have been painted with a 60- μm -thick layer of pure lapis lazuli. Warren and colleagues showed that pump-probe microscopy could obtain a clear lapis lazuli signal across the full thickness of the paint.

To test their method's ability to resolve layers of different materials, the researchers turned their attention to the light purple robe of the angel floating above Mary's head (directly under the microscope objective in figure 1b). The

robe was colored with lapis lazuli, red iron oxide, and metallic gold leaf, among other materials. The top left panel in figure 2, an optical image of a physical cross section removed from the painting, shows the known layering structure. The bottom left panel is the equivalent virtual cross section obtained from the intact painting using pump-probe microscopy.

A challenge in obtaining that image was to distinguish the gold and the iron oxide. As shown in the right panel, those two materials both have positive pump-probe signals (which, by convention, means that the pump-probe interaction increases the amount of probe light absorbed by the sample and decreases the amount scattered) with similar time de-

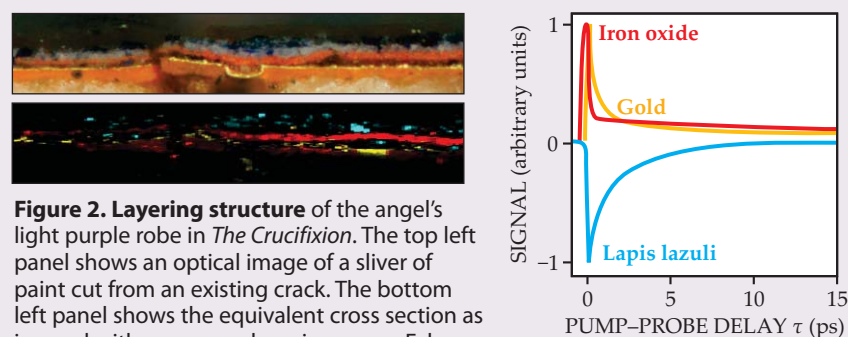


Figure 2. Layering structure of the angel's light purple robe in *The Crucifixion*. The top left panel shows an optical image of a sliver of paint cut from an existing crack. The bottom left panel shows the equivalent cross section as imaged with pump-probe microscopy. False colors represent lapis lazuli (light blue), iron oxide (red), and gold leaf (yellow). Both cross sections are 545 μm by 55 μm . The right panel shows the pump-probe responses used to distinguish the three materials. (Adapted from ref. 1.)

pendences. Telling them apart requires repeating the measurement for many values of the pump–probe delay τ . That limits the size of the area the researchers can image during the time they have access to a painting. They’re working on improving their detection sensitivity to make measurements more quickly.

So far, Warren and colleagues haven’t revealed anything about *The Crucifix-*

ion, or any other painting, that isn’t already known. Their focus has been on validating their method by comparing their images with those obtained by the scalpel method. But potential applications abound. Conservators are reluctant to take their scalpels to pristine regions of historic paintings, so their work has been concentrated on the paint adjacent to existing cracks.

Pump–probe microscopy, having no such limitation, offers the freedom to look beneath the surface over a painting’s entire area.

Johanna Miller

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A fruit-fly gene network may be tuned to a critical point

Precise measurements of fluctuations and correlations in gene expression levels reveal some striking behavior.

Every cell in a multicellular organism has the same DNA; cells in different organs acquire their different forms and functions by using different subsets of that DNA. It’s largely a mystery how an organism conveys to its constituent cells where they are in the body, and therefore which of their genes should be active, but some pieces of the puzzle are known.

For example, in developing fruit-fly embryos, a network of so-called gap genes is involved in telling cells where they’re positioned between the head and the tail. Each of the four main gap genes, first described¹ in 1980, gets its name from the consequences for a fly in which the gene is mutated: giant (Gt), hunchback (Hb), knirps (Kni, German for little person), and Krüppel (Kr, German for cripple). At a brief, specific point in development, when the embryo consists of some 6000 cells, the proteins expressed by the genes form a characteristic spatial distribution that

goes on to influence larger networks of genes that govern the fruit-fly body structure. The gap genes are known to be mutually repressive: High levels of the protein encoded by one inhibit the expression of the others. But the quantitative details of those interactions, and how they create the distinctive protein pattern, are unknown. Different interaction strengths with different functional forms create a many-dimensional parameter space of possibilities.

Now Princeton University theorists William Bialek and Dmitry Krotov argue that the gap-gene network may be operating at a critical point: a division in parameter space between regimes of qualitatively different behavior.² To reach that conclusion, they used experimental data collected by their colleagues Thomas Gregor and Julien Dubuis.³

Effective potentials

A network of two genes, A and B , has a two-dimensional space of outputs,

(g_A, g_B), where g is the concentration of the protein expressed by each gene. The output as a function of time depends on the rates of production and degradation of each protein, which in turn depend on a multitude of other factors, including the interaction between the genes. If the genes interact only weakly, the system has a single steady-state output: When, as often happens in real organisms, the system experiences a perturbation that pushes the protein concentrations away from their steady-state values, it responds with an effective “restoring force” back toward the steady state—much like a particle in a damped harmonic potential, as shown in figure 1. (Real gene-network dynamics aren’t necessarily captured by an effective potential, but many aspects of the network behavior can be qualitatively understood in those terms.)

When genes A and B are strongly mutually repressive, the system enters a bistable regime with two steady-state outputs: one in which A is expressed and B is suppressed, and one in which A is suppressed and B is expressed. To



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