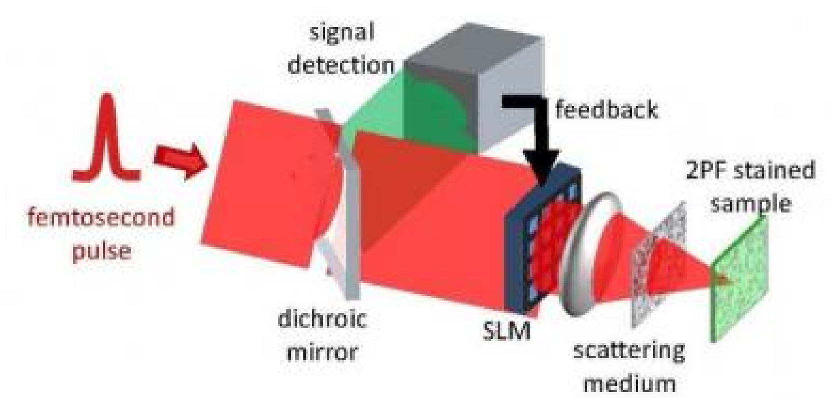
Perfect focus through thick layers may bring better vision to medicine

September 11 2014



How do you see through a wall of fog and still make a clear image? Researchers using a specialized version of an adaptive optics microscope have seen through an obscuring "scattering medium" to focus in on incredibly small details -- a first without using an invasive "guide star" for reference. This graphic illustrates the experimental setup in which incredibly brief laser pulses are monitored and refocused by a Spatial Light Modulator (SLM) so they can pass through the scattering medium to image otherwise hidden details on a target sample. Credit: *Optica*

Zooming in on diseased tissue or scanning fragile biological samples are essential tools in medicine and biological research, but this often requires peering through layers of tissue and other materials that can blur and distort the image. Certain modern microscopes can compensate for this, but only for weak aberrations or by using invasive "guide stars," imaging aids that provide a stable reference point.

In a first-of-its-kind demonstration, published today in The Optical Society's (OSA) new high-impact journal *Optica*, a team of researchers has developed a powerful technique to focus laser light through even the murkiest of surroundings without the need for a guide star. This innovation, a specialized version of an adaptive optics microscope, can resolve a point less than one thousandth of a millimeter across.

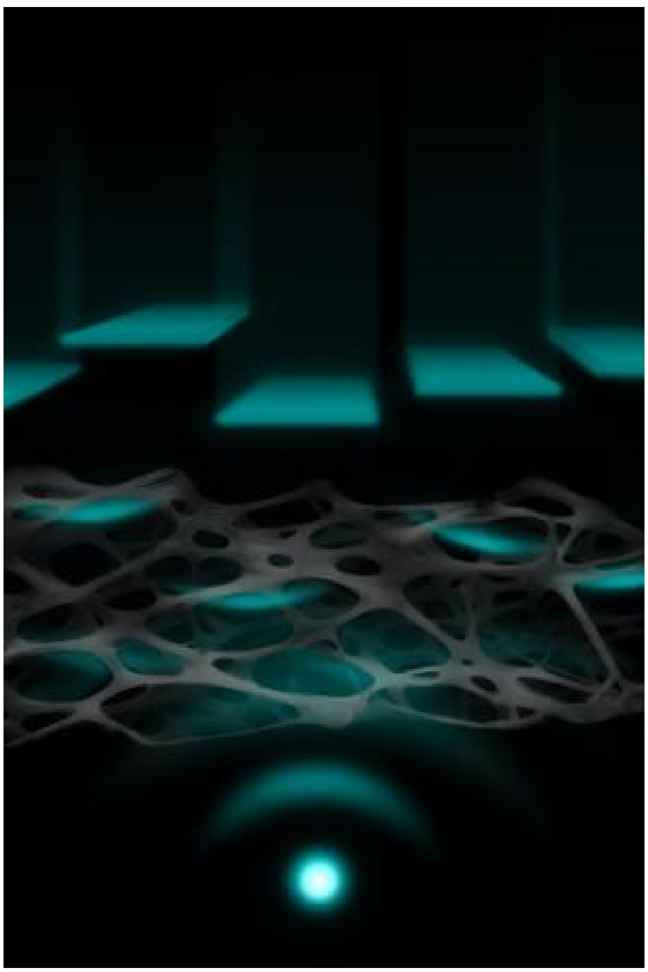
"Imagine shining a flashlight through a thick fogbank to try to see a single dot," said Yaron Silberberg, a researcher at the Weizmann Institute of Science in Israel and co-author on the Optica paper. "The light would become so scattered as it traveled through the fog that you wouldn't be able to make out what was hidden inside. By carefully shaping the light going in, however, it would be possible to home in on your target. That is exactly what the researchers were able to achieve in a way no one has ever done before."

The team modified a standard two-photon scanning microscope—one that uses bursts of laser light to build up a picture point-by-point and then line-by-line—by incorporating a high-resolution wavefront shaping device. Doing so enables the microscope to peer through a visually opaque obscuring layer that would otherwise produce a highly blurred, foggy image.

Wavefront shaping sees through the murk

The wavefront shaping approach is based on "adaptive optics," which is used in both science and medicine, to correct for the blurring of an image by analyzing the way light waves are distorted as they pass through different materials. In astronomy, for example, telescopes with adaptive optics remove the twinkling from starlight to see distant objects as clearly as if the telescope were in space. While adaptive optics works wonders to correct slightly distorted images, such as by atmospheric turbulence, it cannot compensate for severe distortions, such as the scattering by fog or imaging through the shell of an egg.

Yet, recently, this new variation on the adaptive optics approach was shown to handle such tasks. Even when a perfect correction of the distortions is impossible, wavefront shaping can produce a crisp high-contrast image, even through visually opaque barriers.



An artist's rendering of a shaped femtosecond pulse wavefront focusing through a scattering tissue. By pre-shaping the wave going into a complex scattering medium, it is possible to bring it to a sharp focus, enabling microscopy and other applications. Credit: Meital Covo

Breaking free from the guide star

However, wavefront shaping, like adaptive optics, requires a reference point to bring targets into sharp focus. This point—known as a guide star due to its first use in astronomy—must be placed in relatively the same area or field of view as the object being studied. In astronomy, bright nearby stars or powerful lasers are used to adjust the optics of the telescope to produce a nearly perfect image.

In biology and medicine, however, guide stars—for example, an implanted fluorescent particle—need to be physically inserted in to the imaged specimen. This process can interfere with the sample being studied or damage delicate tissue. This is particularly problematic, for example, when trying to study a living embryo inside a shell.

"Until today, all-optical focusing through scattering media required invasively implanting a point-like guide star," said Ori Katz, a scientist at the Langevin Institute in Paris, France, and co-author on the Optica paper. "For the first time, we have shown that it is possible to focus light through visually opaque barriers without using such a guide star."

To achieve this guide-star free imaging, Silberberg and his colleagues used a standard laser scanning two-photon microscope to focus in on a single point behind an obscuring scattering layer.

The laser emits light pulses lasting approximately 100 femtoseconds (a femtosecond is one millionth of a billionth of second), which are directed through the obscuring layer and onto a target. The microscope was able focus in on a point about one thousandth of a millimeter across.

As the light passes through the intervening layer, it becomes highly scattered. In conventional adaptive optics, the returning signal from the guide star would have been measured and then corrected or reshaped, into its original form. With no clear reference point or guide star, however, there would normally be no way of correcting such a highly scattered focus.

The researchers found the answer in the scattered light itself. By using the fact that two-photon fluorescence responds in a so-called nonlinear manner to the intensity of the excitation light, they were able to glean important information about the wavefront required to compensate for the scattering. Rather than the conventional adaptive optics approach, they altered the original pulsed light going in to form a focused beam that they later scanned to generate an image of the fluorescent object hidden behind the obscuring layer.

These are two different views of the same fluorescent target image. The first (a) is made using the conventional microscopy technique known as Two Photon Fluorescence. The second (b) is the highly resolved image made with the new waveform shaping, guide star-free technique developed by the researchers (an SLM optimized scan of the target). The third image (c) is a direct image of the target sample used to verify the accuracy of the new guide-star free technique. The nearly identical image confirmed that waveform shaping was able to compensate for the obscuring layer that thwarted conventional microscopy. Credit: *Optica*

"What we have discovered is that it's possible to efficiently 'pre-correct' the laser beam using the nonlinear fluorescence signal," noted Katz. "The end result is that instead of having a distorted, blurred light source on the object to be imaged, we have a tightly focused, or in this case, refocused beam of light."

Very recently, other groups have shown that similar focusing is also possible using an acoustic-based guide star. But, according to the researchers, this combined optical/acoustical system is substantially more complicated and the focus is not nearly as sharp.

Future applications in imaging, surgery

The researchers also clarify that their technique is only a basic demonstration of the principle and more work is needed to put it to practical use. "We hope that it can help in microscopic imaging, such as in the direct imaging of embryonic development," said Silberberg. "It may also help in guiding laser surgery."

The next step in developing this technology is to shorten the amount of time it takes to achieve the necessary focus.

"We are excited about this project because it has produced new understanding and a new way of seeing through visually opaque samples," concluded Katz.

More information: O. Katz, E. Small, Y. Guan, Y. Silberberg, "Noninvasive nonlinear imaging through strongly-scattering turbid layers," *Optica* 3, 170-174 (2014).

Provided by Optical Society of America