

## UCSD Laser Technique Sheds Light on Strokes

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A technique developed at the University of California, San Diego that precisely creates and images blood clots in the brain in real time could make it possible to understand the small strokes implicated in many forms of dementia, including Alzheimer's disease. The study, published this week in the early on-line edition of the journal *Public Library of Science Biology*, represents a collaboration between the research groups of David Kleinfeld, professor of physics at UCSD, and Patrick Lyden, professor of neurosciences at UCSD's School of Medicine. The paper will appear in the print edition of the journal in February.

Using a laser to trigger the formation of individual blood clots in tiny arteries of the brains of anesthetized rats, the researchers were able to monitor the resulting changes in blood flow. They say that their study provides a way to understand small strokes common in elderly humans. These strokes often cause no immediate symptoms, but they are thought to contribute to dementia and may ultimately cause larger strokes.

Images showing direction of blood flow (white and red arrows) before, during and after clot formation (red X )

**Credit:** *Chris Schaffer, Nozomi Nishimura and David Kleinfeld, UCSD, PLOS Biology*

"Our technique makes it possible, for the first time, to precisely target individual blood vessels to create a blood clot while causing very little collateral damage," explained Kleinfeld. "We can then follow, in real time, the changes in blood flow in surrounding vessels that occur as a result of the formation of a clot in one small artery of the brain."

"We know from MRI scans that small strokes are very common in the brains of elderly patients," added Lyden. "Such small strokes have been linked with dementia, and may also put patients at risk for a major stroke. The power of the technique we describe in the paper is that it allows us to study the response of the brain to stroke in a controlled way. By understanding what happens, we hope to learn how to prevent the major damage associated with stroke."

In the study, the team members used tightly focused laser light to excite a dye that they had injected into the bloodstream. The excited dye reacted with oxygen to form a free radical, which "nicked" the cells lining the blood vessel at the target location, and triggered the natural blood clotting cascade.

Using two-photon fluorescence microscopy—a powerful imaging tool that uses brief (less than one-trillionth of a second) laser pulses to peer below the surface of the brain, the researchers snapped frames every second before and after the formation of the blood clot. They also measured blood flow in the arteries upstream and downstream of the clot. Remarkably, immediately following the formation of the clot, blood flow downstream of the clot reversed itself.

"People tend to think of blood flow like a river," said Chris Schaffer, the lead author on the paper, who was an assistant project scientist working with Kleinfeld in physics at the time of the discovery. "If you dam one tributary, then everything downstream from there would be cut off. However, we've found that the more complicated topology of the blood vessels leads to the counterintuitive result that blood flow in some downstream vessels reverses direction to compensate for the blockage."

In the paper, the researchers discuss how this result can explain the observation, by clinicians, that certain regions of the brain seem to be protected from stroke. These protected regions of the brain have networks of vessels with extensive redundant connections. In the case of a blockage, these redundant connections permit blood to flow through alternate loops and be pushed in the opposite direction below the clot, as observed in this study. The reversal prevents downstream regions of the brain from being starved of oxygen.

In addition to what the researchers could observe in real time, the technique facilitates follow-up because the fluorescent molecules used to visualize blood flow bind to injured places in the artery.

"Rather than having to tediously search for the targeted vessels using brain sections, the fluorescence provides a kind of footprint that can be followed," said Beth Friedman, an associate project scientist working with Lyden in neurosciences and a contributing author on the paper. "Then you can look to see if there have been biochemical changes in the region of the clot, or changes in what genes are expressed, which is especially important to determine if an intervention protects against damage from stroke."

Kleinfeld and Lyden attributed the advance to collaboration across traditional disciplinary boundaries.

"Pat and I are coming from different worlds, but we had the same question at the back of our minds," said Kleinfeld.

"Joining forces allowed us to crack a puzzle that either one of us couldn't crack alone," added Lyden

Other contributors to the study were Nozomi Nishimura, Lee Schroeder and Philbert Tsai at UCSD and Ford Ebner at Vanderbilt University, Nashville. The research was supported by the David and Lucille Packard Foundation, the Veteran's Affairs Medical Research Department, the National Institutes of Health, the Burroughs Wellcome Fund and the National Science Foundation. Media Contact: Sherry Seethaler, (858) 534-4656.

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Movie shows formation of blood clot (dark, center). Fluorescent dye excluded from the clot forms a bright area just adjacent. Credit : *Chris Schaffer, Nozomi Nishimura and David Kleinfeld, UCSD, PLOS Biology*

