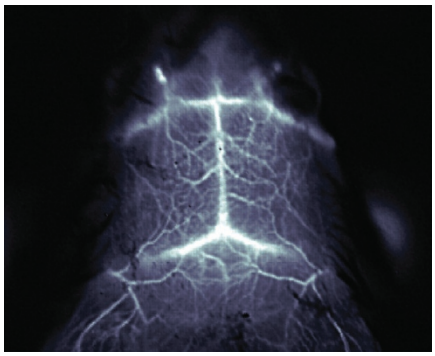
MICROSCOPY

A windowless peek into the brain

Imaging in a narrow region of near-infrared wavelengths reveals blood vessels at unprecedented depth and resolution without the need for a cranial window.



Non-invasive NIR-IIa fluorescence imaging of blood vessels in the mouse brain. Image reproduced from Hong *et al.*, Nature Publishing Group.

Typically, fluorescence imaging of the brain is performed in the visible or near-infrared regions from 400 to 900 nanometers (nm) and requires thinning or removal of the skull above the area of interest, which creates a cranial window. Nevertheless, the imaging depth is limited to around 1 mm because of light scattering. Hongjie Dai, Calvin Kuo and their colleagues at Stanford University show that they can improve penetration depth and resolution by imaging blood vessels using a narrow region in the near infrared that they call NIR-IIa and that ranges from 1,300 to 1,400 nm. This imaging technology does not require a cranial window. Dai says that the NIR-IIa wavelengths are the longest fluorescence wavelengths imaged so far.

Imaging in the NIR-IIa region has several benefits. Both light scattering and autofluorescence are reduced in this wavelength region. Therefore, higher penetration depths can be achieved compared to those of more traditional imaging with shorter wavelengths. In addition, light attenuation by water in the skin, skull and brain tissue is still low in this wavelength range, making it possible to image into the brain non-invasively through the intact skull.

GENOMICS

TRANSCRIPTION FACTORS WITHOUT FOOTPRINTS

A tool to find specific footprint signatures on DNA shows that regulatory proteins with short residency time do not leave footprints.

Gordon Hager and his colleagues at the US National Cancer Institute are interested in chromatin remodeling and, in particular, the role of nuclear receptors. Over the last few years they found the interaction between these receptors and chromatin to be unexpectedly dynamic, with the proteins only briefly binding to DNA. This finding led to what Hager describes as a conundrum because it contradicted established views on how transcription factors interact with DNA.

The established method to look for regulatory regions in DNA bound by proteins such as transcription factors is to search for open chromatin with a nuclease digest. The advent of high-throughput sequencing allowed researchers to identify DNase I–hypersensitive sites, regions of about 250 base pairs that yield more cleavage fragments because the nuclease has better access to the chromatin. Deeper sequencing showed smaller regions within the hypersensitive sites that were protected from digestion, termed transcription factor footprints. Zooming in on these footprints revealed cleavage signatures of ~10 base pairs. "That was assumed to be the protein sitting on the DNA, leaving a signature as a result of protecting specific nucleotides contacted by the transcription factor," explains Hager.

But this explanation is difficult to reconcile with Hager's finding of short residency times. "How can you have a footprint if the protein is only there for a few seconds?" he asks. "This has led to confusion and controversy. Many investigators have refused to buy the idea that proteins are moving so fast, because we have these footprints."

His team tackled the confusion by first focusing on footprint identification. Myong-Hee Sung and Songjoon Baek, staff scientists in the Hager group, developed an

To visualize blood vessels, Dai and his colleagues injected single-walled carbon nanotubes into the bloodstream of mice. "Carbon nanotubes can be tuned to different wavelengths by changing the nanotube diameter," says Dai, which makes them ideal for a variety of imaging conditions. To assess the performance of their imaging regimen, Dai and his team used nanotubes that were conjugated to a dye that fluoresces in the near-infrared region at around 800 nm, called NIR-I. When these nanotubes were imaged through the skull in the NIR-I region, major blood vessels were blurry, and capillaries could not be discerned. In contrast, even small capillaries could be observed when imaged in the NIR-IIa region. In fact, the researchers routinely imaged deeper than 2 mm, with a resolution better than 10 μm. They think that the image quality is improved because around 50% fewer photons are scattered by the skull and scalp in the NIR-IIa region than in the NIR-I region.

With this imaging technique, it is also possible to image blood flow at high temporal resolution. From the resulting movies, Dai and his team extracted parameters that allowed them to classify the imaged vessels as either arteries or veins. They applied the technology to visualize impaired blood flow in mouse models for stroke and observed severely reduced perfusion in the regions affected by arterial occlusion.

Dai thinks that the imaging technology can be applied to a number of different areas such as tumor, skin, eye and brain imaging. He is excited about the possibility of applying this technology to functional brain imaging. "It would be necessary to develop a fluorescent agent in the NIR-II region that responds to brain activity … like calcium sensor dyes," says Dai. Because of the low autofluorescence in the NIR-IIa window, Dai envisions that imaging in this region could also be suitable for single-cell or even single-molecule imaging *in vivo*.

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Hong, G. *et al.* Through-skull fluorescence imaging of the brain in a new near-infrared window. *Nat. Photonics* **8**, 723–730 (2014).

algorithm to detect footprints in DNase-seq data. Together with postdoctoral fellow Mike Guertin, they looked for footprints of steroid receptors, which reside on DNA for only ~6 seconds. They saw strong peaks with chromatin immunoprecipitation–sequencing, a technique that cross-links a protein to DNA and is thus independent of its dynamics—but they did not see footprints. Instead they saw only shorter signatures, similar to those one expects within a footprint.

Intriguingly, these signatures were present regardless of whether the receptor was bound to the DNA. "We were now very suspicious," recalls Hager, and the team decided to look at 'naked' DNA devoid of any protein. "And sure enough," he says, "the signatures are all there in the DNA." When they switched to a different nuclease, the signatures changed, confirming that the enzyme recognized the DNA. If a protein protected the signature, one would not expect to get different signatures with different nucleases. This led the researchers to conclude that the signatures do not result from protein protection of DNA and contain no information about which proteins bind.

Extending their study to more transcription factors, the team found a correlation between residency time and the appearance of a footprint. Proteins that remain on the DNA for longer—such as CTCF, which is bound for ~5 minutes—leave deep footprints. Hager's conclusion is that "the footprint is the aggregate signal of a protein's residency time."

To really understand how proteins regulate chromatin, protein dynamics must be taken into account. "We need much more biochemistry to understand what is going on. We need more reconstruction of remodeling systems *in vitro* to ferret out this incredibly complex sequence of events," says Hager. He adds, wistfully, "Unfortunately, few fellows are being trained with these skills."

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Sung, M.-H. *et al.* DNase footprint signatures are dictated by factor dynamics and DNA sequence. *Mol. Cell.* (doi:10.1016/j.molcel.2014.08.016) 18 September 2014.