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Deeper Tissue Imaging with Total Detection
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variation in robustness, criticality, and other systems properties can be studied among individuals or populations, or in healthy versus disease states. Finally, computational models of underlying network architectures and properties can be developed to predict phenotypic outcome in response to different genetic backgrounds, environmental factors, or targeted perturbations aimed at reversing disease outcome. Systems genetics is now poised to address these and other fundamental questions in biology and medicine.

References and Notes

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APPLIED PHYSICS

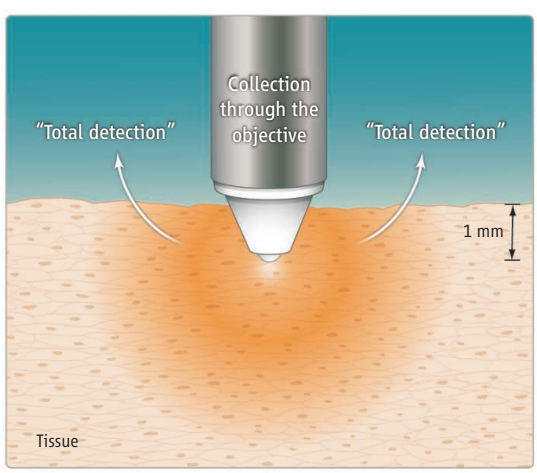
Deeper Tissue Imaging with Total Detection

Optical images of tissue can be obtained from greater depth when multiphoton-excited fluorescence is collected from all around a sample.

Enrico Gratton

If you simply held your finger in front of a strong source of light, you would see that visible light passes through centimeters of the tissue held in front of it. Even more light passes through tissue at near-infrared wavelengths, but even if you could see it, you would still not be able to distinguish between the bone and flesh, or other internal structures. The reason is that light scatters multiple times in the tissue, and the image blurs; resolution and contrast decrease as we try to look deeper into tissue. Other instrumental methods—such as x-ray tomography, ultrasound, and magnetic resonance imaging (MRI)—can "see through" nontransparent objects and have been revolutionary in medicine and material science, yet we are still unable to use visible light and its accompanying spectroscopic information to look inside tissues. Recently, Combs *et al*. (*1*, *2*) found a way to improve the amount of fluorescent light collected from highly scattering samples, thereby increasing the sharpness of images at depth that was never reached before. By constructing a light collector all around the surface being imaged, they increased the depth from which images could be obtained by about a factor of 2.

This approach represents a substantial improvement over current methods, and the technique can be applied to most instruments based on multiphoton excitation of the fluorescence. Until about 20 years ago, the maximum depth that an optical microscope could reach into an organ like the brain and still maintain the optical resolution was limited to about 100 μm. This depth limit was dramatically broken with the invention of the multiphoton microscope by Webb and co-workers at Cornell (*3*). The development of lasers that could deliver femtosecond pulses of near-infrared light with high peak power allowed fluorescence to be excited from molecules in a very small spot deep in the tissue. The molecules can absorb more than one photon, so the energy delivered is similar to that of an ultraviolet source. The location at which excitation occurs is determined by collecting the fluorescence at the surface of the sample, so it is possible to "image" its origin. A three-dimensional map is then constructed by scanning the focal point through the sample.



More than skin deep. Schematic representation of the imaging method used for fluorescence excitation and emission in multiple scattering tissues. Laser light is focused by an objective lens deep into tissue. As the excitation light travels through the medium, it loses intensity as it is scattered. In multiphoton excitation, only the light at the focal point can excite the tissue fluorescence, and fluorescence emission occurs in all directions. In the conventional microscope, only a small fraction of the emitted light can reach the detector because the light is conveyed by the same lens to the detector. In the instrument described by Combs *et al*., the amount of light that is collected with a parabolic mirror and conveyed to the detector is a much larger fraction of the total emission. The improved collection allows imaging deeper and with better contrast.

Even with the multiphoton microscope, there are limitations on how deep the fluorescence can originate and still be imaged. To produce the nonlinear effect that excites fluorescence, the instantaneous light intensity must be very high at a narrow spot. Even at a depth on the order of 1 mm, the formation of usable images is prevented by the scattering of the tissue, which defocuses and broadens the near-infrared excitation spot. Another problem is that the emitted light from the spot travels in all directions and only a small fraction is collected by the lens that is used to produce the excitation spot (see the figure). Combs *et al*. combined multiphoton fluorescence excitation with very high collection efficiency to increase the depth of imaging in scattering samples to about 2 mm.

Several approaches could further increase the imaging depth. Light shifted even further toward the infrared is scattered less by tissue and could be used in a multiphoton excitation scheme to image molecules that emit red light. Another improvement could come from the use of

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optical clearing agents (*4*); these hyperosmotic agents, such as dimethyl sulfoxide (DMSO), decrease the water content of tissue and thereby reduce optical scattering.

However, it appears that increasing imaging depth to the centimeter range will require the development of new approaches. Currently, technologies are available that allow us to image deep into tissues in the centimeter range, but at the expense of optical resolution and contrast (*5*). Still, there are several appealing aspects of using visible or near-infrared light to image deep into tissues. These wavelengths of light do not produce the ionizing effects of x-rays. The use of light is relatively low in cost compared with imaging techniques such as MRI and positron emission tomography. Optical imaging can achieve submicrometer spatial resolution, and contrast can be very high when imaging specific fluorescent molecules that are found naturally in tissue or genetically produced by the expression of fluorescence proteins. The ability to create optical images of the brain or other organs could help unravel molecular and cellular interactions in live animals.

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MOLECULAR BIOLOGY

Preference by Exclusion

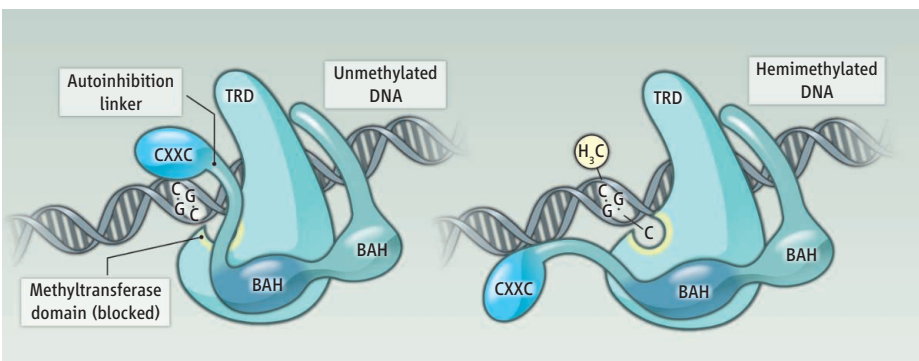
The structure of a methyltransferase indicates how the enzyme selects its DNA substrates.

Lucy A. Godley[1] and Alfonso Mondragón[2]

DNA methylation is a modification that controls gene expression and contributes to mammalian development, aging, and cancer cell biology (*1*, *2*). In mice and humans, the addition of a methyl group to a cytosine within a cytosine-guanine (CG) dinucleotide is catalyzed by DNA methyltransferase (DNMT) enzymes DNMT3A, DNMT3B, or DNMT1. The latter is the main "maintenance methylase" because it adds a methyl group primarily to double-strand DNA that is already methylated on one strand (hemimethylated). How DNMT1 prefers hemimethylated over unmethylated DNA, in contrast to DNMT3A and DNMT3B, has not been clear. On page 1036 of this issue, Song *et al*. (*3*) present the crystal structures of human and mouse DNMT1 in complex with unmethylated DNA, providing an explanation for the mechanism of substrate selection by this crucial enzyme.

DNMT1 is a modular protein with a carboxyl-terminal class I methyltransferase domain that is preceded by two bromo-adjacent homology (BAH) domains and a cysteine-rich CXXC domain (where C is cysteine and X is any amino acid). The methyltransferase domain is structurally related to that in bacterial methyltransferases such as M. *Hha*I (*4*) and M. *Hae*III (*5*), and also in human DNMT3A (*6*). The catalytic mechanism of DNMT1 involves entry of the target cytosine into the active site of the methyltransferase domain, where transfer of the methyl group from an *S*-adenosylmethionine molecule to the cytosine occurs. Surprisingly, in the structure of the complex of DNMT1 with unmethylated DNA, Song *et al*. find that although DNA is poised to enter the active site cleft, it is prevented from doing so by a long and highly acidic polypeptide loop called the autoinhibitory linker, which connects the CXXC domain and first BAH domain (see the figure). Song *et al*. observed the same overall arrangement in the mouse and human structures, indicating that the results are not an artifact of crystallization. Positioning of the loop is driven by interaction of the CXXC domain with the region of DNA containing the target CG dinucleotides. The CXXC domain makes specific interactions with the dinucleotides and also with the DNA backbone. Modeling of methylated cytosines shows that steric clashes would prevent these interactions and explains the preference of DNMT1 for hemimethylated over unmethylated DNA. The structures show that only unmethylated DNA can interact productively with the CXXC domain and that this interaction helps position the autoinhibitory linker correctly for blocking DNA methylation. Because hemimethylated DNA cannot interact favorably with the CXXC domain, it cannot drive the loop to enter the active site, and the methyltransferase domain is free to interact with the DNA. Thus, at least one crucial role of the CXXC domain is to discriminate between hemi- and unmethylated DNA and thereby control which molecules are modified.

However, the role of the BAH domains still remains unclear. Both are adjacent to the methyltransferase domain, but are directed away from the active site or DNA binding cleft. The second BAH domain has a long loop that extends to the other side of the methyltransferase domain and interacts with the target recognition domain (TRD), a region that contacts the CXXC domain. The TRDs



Substrate selectivity. (**Left**) In the presence of unmethylated DNA, the CXXC domain of DNMT1 interacts with DNA and drives the autoinhibitor linker to a position that precludes interaction of DNA with the methyltransferase catalytic domain. (**Right**) In the presence of hemimethylated DNA, the CXXC domain cannot interact with the DNA, and the autoinhibitor linker does not block the active site. This allows target DNA to be positioned properly in the active site for methylation to occur.

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