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Cover Story

Quantitative 3D mapping of drug absorption in skin

Mapping penetration of a given drug across the skin remains a key challenge to understand the molecular delivery pathways. Standard analytical techniques (including radio-labeled liquid scintillation counting, liquid chromatography, and mass spectrometry) used with skin samples mounted on Franz cells, or following sequential adhesive tape stripping, can provide an active molecule concentration profile along the skin depth. These techniques, however, are destructive and do not provide a 3D drug mapping across the skin depth. Overcoming these limitations requires a label free, high-resolution imaging technique capable of unbiased, quantitative chemical imaging as well as unambiguous skin morphological identification. Advanced optical techniques that make use of ultra-short laser pulses can overcome these challenges. Known as 'nonlinear imaging techniques', they rely on scanning a focused laser beam across the sample volume. Because the pulse is short (picosecond or femtosecond), the local electric field is strong enough to generate new frequencies that can be recorded with high sensitive detectors.

In two-photon auto-fluorescence, endogenous proteins, such as flavins and NADH, absorb two photons from the incoming infrared laser and generate visible light. Although not chemically specific, two-photon auto-fluorescence provides a 3D image of a tissue sample and reveals its structural morphology with a resolution of 0.3 μm. In the coherent Raman contrast imaging, two laser pulses of different frequency interact within the same sample. When the frequency difference between these two pulses equals the frequency of a specific molecular bond presents in the sample, a strong signal is generated that is compatible with video rate imaging. These contrasts, known as Coherent Anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS), are chemically specific, because they catch the intrinsic vibrations associated with specific chemical bonds. SRS was used to image ibuprofen in porcine skin [1], but the method was not capable of providing unbiased drug concentration. SRS signal was attenuated with increasing depth into the skin samples that precluded its linear relationship with molecular concentration.

The work by Dr. Rigneault and his colleagues described in this issue has pushed the coherent Raman technique a step further to image, in 3D, the unbiased drug concentrations in *ex-vivo* human skin [2]. Combining CARS imaging with two-photon auto-fluorescence, the Rigneault team describes 3D skin drug concentration profiles overlapped with detailed 3D skin morphology up to a depth of 100 μm. Pivotal to their approach is the use of deuterated drugs that show a carbon–deuterium vibration not found in endogenous skin. It is quite noticeable that their approach compares extremely well with an established technique using tape stripping followed by liquid chromatography/mass spectroscopy quantification. The 3D penetration pathways of various hydrophilic and hydrophobic cosmetic active molecules were investigated in excised human skin and artificial skin samples. A key finding in the study is the visualization of diffusion barriers that locate at the interfaces between skin compartments.

Although the approach presented by Dr. Rigneault and his team provides a new avenue for visualizing drug penetration in skin tissues, the technique needs to be improved further. First, the drug concentrations used in the study are still ten times higher than what is commonly used in the cosmetic industry. Second, a full 3D image (100 μm × 100 μm × 100 μm) is analyzed in 30 min, and clearly there is a room for improvement using faster CARS/SRS imaging modalities. Third, drug imaging is limited to a depth of 100 μm due to light scattering and absorption in skin. Imaging deeper depth faces the problems associated with focusing light in scattering tissues. For this, the most viable approach consists in developing coherent Raman endoscopes that could image the molecular vibrations associated with drugs at the tip of a fiber probe. The development of coherent Raman endoscopes is at an early stage as it requires delivering ultra-short pulses through optical fibers, a task made difficult by the spurious nonlinear interactions taking place in optical fibers [3]. Another potentially important application is in studying 3D drug distribution in complex tissues, including solid tumors. For this, polarization resolved coherent Raman techniques are expected to map the orientation of chemicals bonds in a complex sample [4]. As the advances in optical technology are moving forward fast, the tool for obtaining high resolution, 3D images of label free drugs in complex tissues will be just a matter of time.

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