

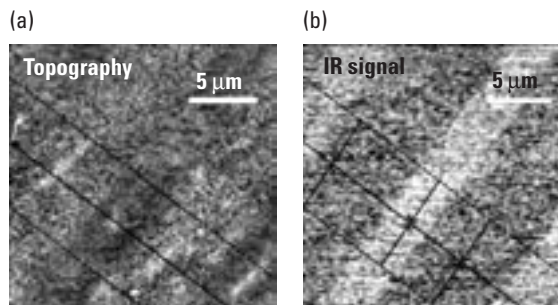
## Near-field IR microscopy

Imagine combining the nanometer-scale resolution of near-field microscopy with the added information of chemical imaging. Gilbert Walker and co-workers at the University of Pittsburgh do just that in what they say is the first reported use of near-field scanning IR microscopy to image a molecular monolayer. In this example, the researchers resolved samples patterned with alternating stripes of DNA and hexadecanethiol by focusing on the phosphate absorption band.

Their near-field microscope is a combination of commercial and homebuilt components, with a tunable CO<sub>2</sub> laser delivering 20–100 mW of power and illuminating a 50- $\mu$ m spot. The scattered IR radiation is modulated by an oscillating metallic probe, and the background

signal is reduced by demodulating at twice the probe's oscillation frequency. To home in on the DNA phosphate signal, only radiation at 980 cm<sup>-1</sup> was acquired.

The images obtained by this approach show strands of DNA spread over the surface, with individual molecules extending from the surface by approximately half of their backbone length. Moreover, the images are free of surface artifacts. However, the lateral resolution is only ~200 nm, as compared with a limit of ~15–30 nm for apertureless near-field IR microscopy. With further improvements, the



A chemically informed image. (a) The left image shows the topography of the striped DNA-hexadecanethiol surface, but when viewed as (b) an IR signal image, the DNA regions clearly stand out as the darker areas.

researchers think that this number may approach the probe size of ~50 nm. (*Langmuir* 2002, 18, 5325–5328)

## MALDI monitors recombinant proteins

Although the use of recombinant proteins is widespread, researchers have still not found good ways to monitor their expression over time. Now, Peter Roepstorff and colleagues at the University of Southern Denmark demonstrate that MALDI MS can monitor induced and constitutively expressed intracellular and secreted recombinant proteins in whole cells or cell supernatants. The researchers also suggest that MALDI MS can be used for preliminary studies of differential protein expression.

The researchers collected linear, posi-

tive-ion MALDI spectra using delayed extraction from whole bacterial and yeast cells, from proteins secreted into a supernatant by human cells, and intracellularly from insect cells. Sample preparation was minimized in all protocols; despite the complexity of the spectra from these crude preparations, the researchers report that they obtained useful data.

To use MALDI MS for differential profiling on whole *E. coli* cells, the researchers identified endogenous proteins, such as the 50S ribosomal protein,

which could be used as internal calibrants. Other peaks clearly differed between induced and uninduced samples, yielding putative profiles of the two cellular states. Although MALDI spectra are not quantitative, the researchers say that knowing the mass of the recombinant protein and the increase in signal abundance and comparing them with the endogenous signals can provide differential expression data that is complementary to gel electrophoresis. (*Anal. Biochem.* 2002, 305, 242–250)

Image unavailable for use on the Web.

Differences between induced and uninduced whole *E. coli* cells in molecular weight ranges (a) 10–30 kDa and (b) 30–60 kDa. Asterisks indicate singly and doubly charged ion signals. (c) Spectra of whole *E. coli* cells acquired using bovine serum albumin as an internal molecular weight standard. (Adapted with permission. Copyright 2002 Elsevier Science USA.)