ATOMIC FORCE MICROSCOPY

Believe in the force

The use of rigid copper oxide tips makes high-resolution molecular imaging by non-contact atomic force microscopy more reliable.

Ingmar Swart

Atomic force microscopy (AFM) with functionalized tips can be used to image the chemical structure of individual molecules with sub-molecular resolution[1]. Yet, the technique so far fails to unambiguously image intermolecular bonds, mainly because of the high flexibility of the commonly employed CO-terminated tips[2]. The ability to visualize hydrogen bonds, for instance, would strengthen our understanding of these interactions and could increase the capabilities of AFM imaging for studying (bio)molecular self-assembly and chemical reactions on surfaces. Writing in *Nature Nanotechnology*, Mönig et al. now report on AFM experiments on multiple molecular systems using a rigid copper-oxide passivated tip[3]. The spring constant of this tip is approximately one order of magnitude larger than that of a CO-terminated tip and artefacts induced by tip flexibility are avoided. Intermolecular contrast is observed in the images at locations where hydrogen bonds are present, suggesting the reliable imaging of hydrogen bonds. The contrast correlates with the strength of the hydrogen bond.

Nowadays, AFM is routinely used to image and structure matter with atomic scale precision. Under ultrahigh vacuum conditions, AFM can resolve not only the chemical structure[1], but also the bond order and the charge distribution of individual molecules[4–6]. Such high resolution is only possible when chemically passivated probes are used. Inert tips are necessary to prevent too strong a chemical attraction between the last tip atoms and the sample, which would otherwise lead to an accidental pick-up of the imaged molecule by the tip. The passivation can be achieved in different ways. Most often, a CO molecule is attached to the apex of the probe. The resulting CO-terminated tip has considerable flexibility, that is, the CO molecule can deflect when exposed to a lateral force (Fig. 1a). On the one hand, this flexibility can be exploited to determine the electrostatic force field above polar molecules[6], but it also introduces artefacts in AFM images: bonds can appear much longer than they actually are and bond-like contrast can appear between atoms where it is clear that there is no chemical bond[1,7,8]. This can be considered as the AFM analogue of an optical illusion. One intriguing question in the field is therefore whether hydrogen bonds between molecules in networks can be trustfully detected[2,9]. A model, which takes into account the flexibility of the CO-terminated tip but disregards the hydrogen bonds, can readily reproduce the experimentally observed intermolecular contrast[2,9]. This raised the question of whether AFM can image intermolecular bonds directly in real space, or if contrast features between neighbouring molecules are always caused by imaging artefacts.

Mönig and co-workers now circumvent the artefacts caused by the flexibility of the CO tip by employing copper oxide-terminated tips. While these tips are chemically inert — a prerequisite for bond imaging in the repulsive regime — their flexibility is strongly reduced compared to CO-terminated tips. To demonstrate that this can indeed prevent image distortion and spurious contrast between molecules, the researchers first image three types of molecule that were previously probed with flexible CO-terminated tips. In all cases, the bond lengths extracted from the new images are much closer to the values determined using other techniques. Crucially, contrast between atoms is only observed when there is a covalent bond between them.

Coming back to the outstanding question of hydrogen bond detection, the images of hydrogen-bonded molecular networks showed a marked intermolecular contrast at exactly those positions where a hydrogen bond is present. In this case, the contrast is certainly not an artefact of tip

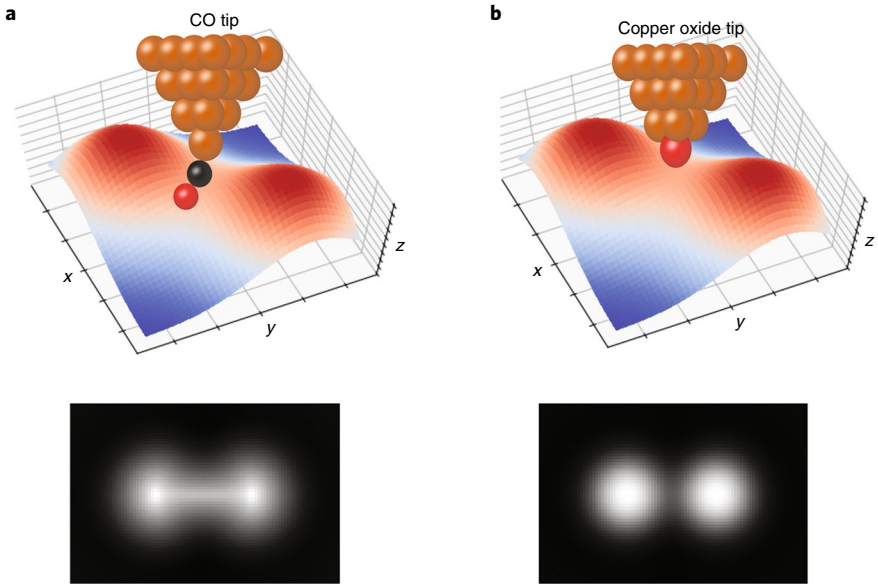

Fig. 1 | AFM with flexible and rigid tips. a, A CO-terminated tip will experience significant deflections when probing the potential energy surface generated by two non-bonded atoms (top). The bending reduces the repulsive force between tip and sample and leads to the formation of contrast between the two non-bonded atoms as shown in the simulated AFM image (bottom). **b**, Same as **a** but for a rigid tip. Note the absence of contrast between the two atoms in the simulated AFM image. The AFM images were simulated using the model described in ref. [2].

flexibility. Specifically, in one of the systems investigated, there are three areas with different hydrogen-bonded configurations. The intermolecular contrast correlates with the magnitude of the charge transfer upon network formation in all three cases. The larger the charge transfer, that is, the stronger the hydrogen bond, the more pronounced the image contrast.

Does this mean that AFM can be blindly used to prove the existence of hydrogen bonds in any molecule or molecular network? Unfortunately not, especially since the contrast formation mechanism is not yet fully understood; for example, there may be other imaging artefacts that result in contrast formation. It will be a key issue to elucidate why the contrast above inter- and intramolecular bonds is quite similar, despite the very different electron density between the atoms. A logical extension of the work presented by Mönig et al. would be to use rigid copper oxide tips to study molecular assemblies with van der Waals interactions only. These interactions are different from hydrogen bonds in the sense that they lack directionality and show much weaker charge redistribution. Such experiments could clarify the limits of atomically resolved AFM imaging and shed light on the physics underlying the contrast formation. In any case, the use of stiff and chemically passivated tips brings us a big step closer to reliable imaging of weak intermolecular interactions. It will be exciting to see how much further we can push the limits of AFM. ❐

Ingmar Swart
Debye Institute for Nanomaterials Science, Utrecht University, Utrecht, The Netherlands.
e-mail: i.swart@uu.nl

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BIOIMAGING

When radionuclides meet nanoparticles

Interaction between radionuclides and nanoparticles expands the in vivo imaging possibilities based on Cerenkov luminescence.

Gang Niu and Xiaoyuan Chen

Cerenkov luminescence (CL) is emitted when a charged particle passes through a dielectric medium at a greater speed than the phase velocity of light in that medium. In 1898, the blueish glow in the dark from CL led Marie Curie to discover radium. Though the Nobel Prize in Physics in 1958 was awarded for the discovery and interpretation of the Cerenkov effect, it was not until 2009 that the first in vivo imaging based on CL was achieved by Simon Cherry and Matthew Silva[1]. Since then, a series of preclinical studies[2–4] has been performed to advance Cerenkov-based optical imaging (CLI) into clinical trials[5,6]. By coupling the high resolution of optical imaging systems and the high avidity to tumour cells of ^{18}F-fluoro-2-deoxyglucose — the most popular imaging agent for positron emission tomography — CLI is able to provide intraoperative tumour margin assessment with a specialized camera[6]. However, the low quantum yield of emitting photons and the blue-weighted spectrum of CL seriously limit its biomedical applications. Now, writing in *Nature Nanotechnology*, Pratt and co-workers systematically investigate the interactions between radionuclides and nanoparticles (NPs) and reinvigorate the field of CL imaging by suggesting novel nanoparticle-based multimodal imaging methods using radionuclides previously considered unsuitable for the task[7].

After nearly two decades of extensive fundamental and preclinical studies, more and more nanoparticles have been applied to the clinic[8]. Meanwhile, radionuclides have enjoyed a rich history in diagnostic and therapeutic applications. The marriage of radionuclides and nanoparticles has emerged in numerous biomedical applications, especially for personalized medicine[8]. In diagnostics, the combination of radionuclide imaging, using gamma or positron emitters, with CL-based optical imaging permits whole-body disease mapping with high overall sensitivity and simultaneous high-resolution local scrutiny of the lesions for image-guided surgery[6]. In therapeutic applications, imaging of radionuclides such as beta or alpha emitters allows monitoring of the distribution and delivery of the therapeutic particles. The Cerenkov radiation from radionuclides has also been used to activate an oxygen-independent nanophotosensitizer, titanium dioxide (TiO_2), to overcome the shallow penetration limit of exogenous light[9].

In the study by Pratt et al., the authors report two seminal findings. First, their results explain the increase of luminescence photon flux that occurs when radionuclides are combined with nanoparticles (Fig. 1). Since the photon flux is paramount to boosting the sensitivity of optical imaging, a radiance increase of up to 3,000-fold well overcomes the limitations of CL. The authors observe that the increased photon flux comes from either β- or γ-scintillation, or from both, depending on the chosen route of radionuclide decay. Moreover, they also observe that on interaction with radionuclides, the emission peaks of several nanoparticles experience a red-shift. Because of their longer wavelengths, these excited protons penetrate tissues more easily and can be used for in vivo optical imaging. The second important finding described in the paper is that the interaction of radionuclides with nanoparticles containing heavy atoms generates X-ray emission, which can be exploited to perform single photon emission tomography (SPECT) for nanoparticle localization. Since the energy of the X-rays deriving from different materials varies, multiplex imaging can be achieved to further delineate the distribution of a single component in a mixture of nanoparticles.

To demonstrate clinical relevance, the authors mix the Food and Drug Administration-approved glass microspheres Theraspheres, which contain