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Probing Biological Materials by Vibrational Analysis in the Electron Microscope

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Vibrational Analysis in the Scanning Transmission Electron Microscope (VASTEM), introduced in 2014 [1,2], has given rise to many new capabilities. E.g, it is now possible to distinguish different isotopes such as ^{13}C vs. ^{12}C in biological samples, and to map them with ~50 nm resolution [3]. It is also possible to detect the vibrational signature of a single atom of Si embedded in graphene [4].

When aiming for atomic resolution, the impact (non-dipole) vibrational signal and large illumination doses need to be used, which makes the technique unsuitable for biological analysis. But when the spatial resolution requirement is relaxed to about 30 nm, the delocalized vibrational dipole signal can be employed. Samples can then be probed by a beam parked outside the sample, in an "aloof" geometry, and radiation damage can be completely avoided [5]. Alternately, a narrow beam parked on a fragile sample can rapidly drill a hole in it, and when the hole is complete, further damage essentially stops, with undamaged regions away from the hole continuing to contribute to the signal [6]. These techniques may revolutionize biological analysis, and we are therefore exploring their potential.

The dipole signal yields spectra similar to infrared spectroscopy (IR), which has been used extensively in biology (e.g. [7]). The VASTEM energy resolution is not as good as for IR, but even at 3-10 meV (24-81 cm^{-1}) energy resolution that is presently available, and improving [8], vibrations of different molecular groups can be readily distinguished, and their energies assigned to specific modes [9]. Moreover, VASTEM covers a much greater energy range than individual optical spectroscopies, from about 10 meV to 1 eV (24-8100 cm^{-1}) and beyond, to optical, UV and X-ray energies.

Fig. 1 shows vibrational spectra from crystalline and amorphous ice. The combination band, arising from combined H-O-H bending and libration motion [10], is suppressed in amorphous ice, and the O-H stretch peak is reduced. The spectra preferentially probe vibrations that are aligned with the momentum exchange vector $\boldsymbol{q}$, and this needs to be taken into account for quantitative analysis.

Fig. 2 and 3 show the changes in vibrational spectra of guanine held at liquid N_2 temperature as a function of the electron dose. VASTEM can follow which bonds are destroyed during radiation damage, and what new bonds are made. The methodology we used exposed the sample to a well-defined dose, and acquired high-quality aloof spectra using an aloof beam, without exposing the sample further.

Vibrational analysis explores the local bonding only, and is not sensitive to long-range order. This means that a few broken bonds in the material do not cause major changes in the spectra. It makes

VASTEM less sensitive to radiation damage than diffraction and high resolution imaging, which depend on long-range order that is easily destroyed by irradiation. Among other potential applications, the technique promises to be a versatile tool for studying the detailed mechanisms of radiation damage. Its ability to trace the origin of atoms and molecules that come from different sources in the making of a complex sample, by using isotopic substitution, should be especially useful for such studies [11].

In summary, VASTEM has progressed greatly, and it endows science with many new capabilities. The full impact of the technique in biology is still to be felt, but the prospects appear very promising.

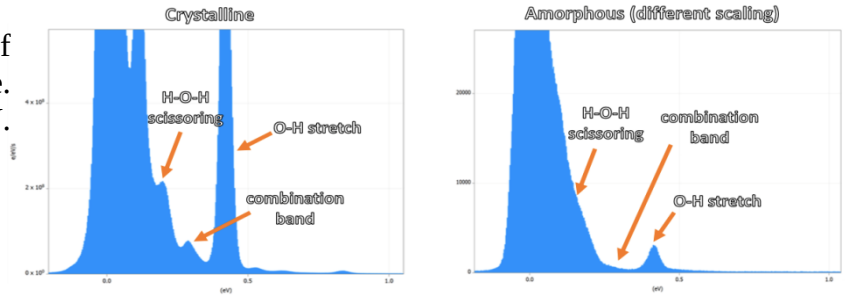

Figure 1. Vibrational spectra of crystalline and amorphous ice. Nion HERMES, 30 kV.

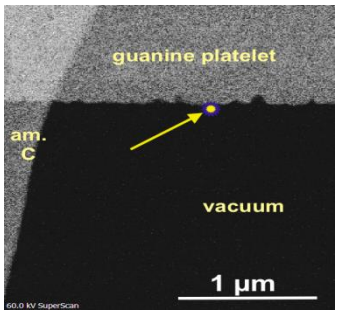

Figure 2. Examined guanine area. The aloof beam position is marked by small yellow "sun".

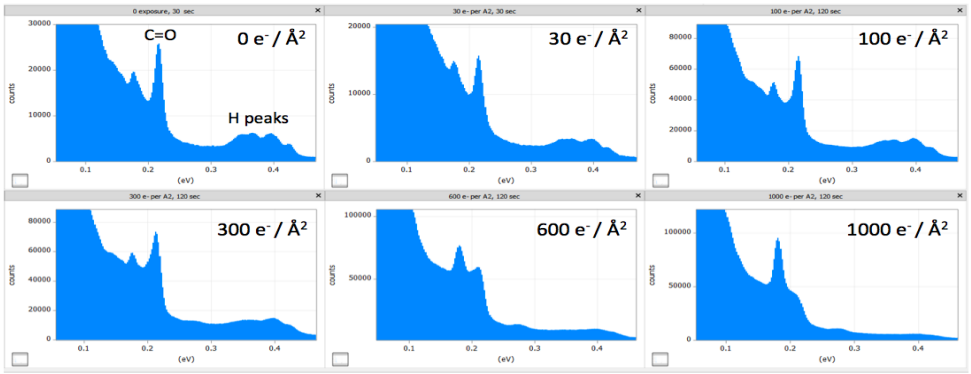

Figure 3. Radiation damage in guanine at liquid N_2 temperature. 60 kV, radiation doses are shown. Note the disappearance of the hydrogen vibrational peaks near 400 meV at high doses, the attenuation of the C=O stretch peak at 209 meV, and the strengthening and small energy shift of the peak at 170 meV.

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