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Applications of the Local Electrode Atom Probe in Biotechnology

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The Local Electrode Atom Probe (*LEAP*™) is an innovative three-dimensional atom probe microscope developed at Imago Scientific Instruments. An atom probe is a projection microscope coupled with a mass-spectrometer that provides quantum-level detection capability. The *LEAP*™ determines 3-D structure and composition by taking specimens apart piece-by-piece as single atoms, or as small molecular fragments. The position of each atom or molecular fragment within the specimen is imaged while elemental composition is simultaneously determined via time-of-flight measurement (see figure).

To date, atom probes have been almost exclusively used by a few dedicated research labs for the characterization of the atomic structure of metals, such as phase transformations, precipitates, grain boundaries, interfaces, and dislocations. Atom probes have not been more widely employed due to several limitations that include: i) Very significant constraints on specimen physical properties, namely the specimen must possess high strength in a needle-like configuration along with high levels of electrical conductivity (hence the historical analysis of metals); ii) The typical region of analysis in an atom probe is very small, typically on the order of 10 nm diameter and comprising only a few 100,000 atoms; iii) Analysis times are very long, typically requiring days to provide a circa 1 million atom image, and iv) Most atom probes are custom-built instruments and are complex and difficult to operate. None-the-less, atom probes can provide 3-D atomic-scale compositional and structural information that is unavailable by any other instrumentation.

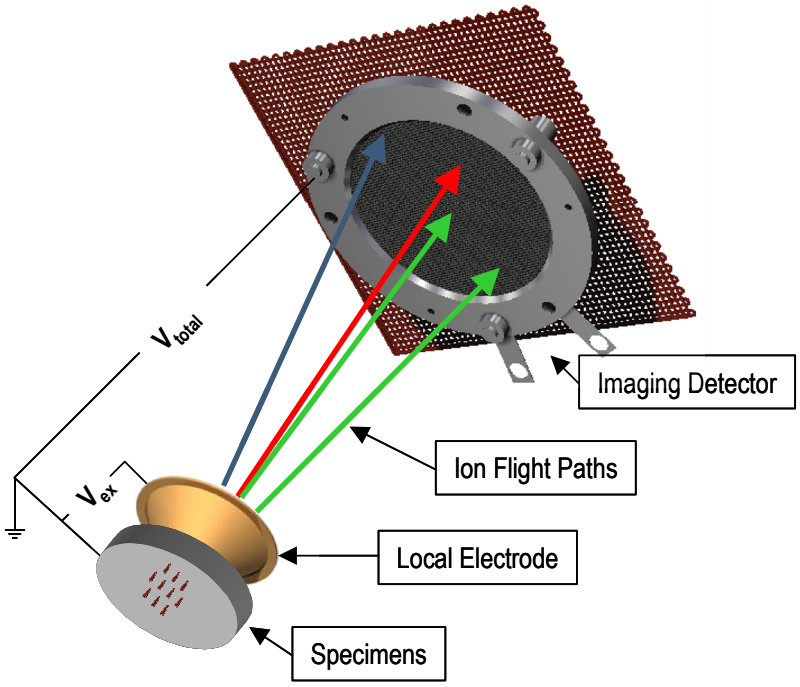
Imago Scientific Instruments' recently developed *LEAP*™ microscope removes these limitations and thereby vastly expands the range of application of the technique [1,2,3]. The *LEAP*™ enables 3-D atomic-scale compositional images to be obtained from specimens by non-experts with data collection rates over 100 times faster than current instruments, while also providing a 100 nm or larger field of view. These developments indicate that the *LEAP*™ microscope can be applied to a much wider range of materials [4].

Detailed knowledge of the 3-D atomic structure of devices and biological biomacromolecules (proteins, nucleic acids, organelles) is required to engineer the bio-nanotechnologies of the future, and to advance biomedical science. Current methods to determine the structure and composition of biological and biotechnology materials are slow, cumbersome, expensive, and still only provide limited and incomplete information. This is especially true for compositional analysis of biological materials since these intrinsically consist of low atom number elements that are difficult to analyze with electron microscopy and related methods. Imago's *LEAP*™ can uniquely address this need. Imago is developing methods to analyze the atomic structure of nano-biotechnology devices such as DNA chips, biosensors, and medical implants [5-6], as well as developing methods to determine the structure of biological macromolecules and complexes relevant to structural biology. Specimen preparation for *LEAP*™ analysis is similar to that used to preserve biological structure for other

vacuum instrumentation such as electron microscopes. Atom probe analysis also places several additional requirements and constraints on specimen preparation, including that the specimen area of interest must be shaped as a circa 50-100 nm radius sharp point [6].

The *LEAP*™ determines structure and composition by literally taking specimens apart atom-by-atom or as small molecular fragments, as described below and diagrammed in the following figure:

- An electric field is generated by an excitation voltage (V_{ex}) applied between a funnel-shaped local electrode and the sharp tip of a prepared specimen. An array of specimen tips is shown in the figure.
- V_{ex} is pulsed at tens of thousands of times per second causing specimen atoms or small molecular fragments to ionize (>10,000 ions/second).
- Ions are accelerated towards the local electrode, and then further accelerated to the position sensitive detector by a second voltage (V_{total}).
- Where the ion strikes the detector is a direct projection from its former position within the specimen, providing ~10^6 times magnification with <0.5 nm lateral resolution.
- Time-of-flight measurement determines each ion's elemental composition (including all elements/isotopes).
- One entire atomic layer is ionized before the second layer, thereby providing atomic layer axial resolution (~0.2 nm).



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