Biomedical Applications of Microprobe Analysis

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The general trend of microscopical investigation in biology from the 1950's to the early 1970's was towards obtaining structural information. This goal initially was met using heavy metal and/or aldehyde fixatives, room temperature dehydration with polar organic liquids, embedding with epoxy and acrylate resins, and thin sectioning at room temperature. By the mid 1970's, a perceptible change occurred in the direction of both light and electron microscopy towards investigation of the chemical reactivity and composition of structures made visible with increasingly better spatial resolution for light and electron microscopes (< 0.1 nm). By the mid 1980's and 1990's innovations in microanalytical techniques, including x-ray microscopy and microanalysis, secondary ion mass spectrometry, laser microprobe mass analysis, the scanning probe microscopies, and confocal/multi-photon microscopy, and development of compounds for visualization of molecular and ionic sites within individual living cells as well as membranes, redefined the goal of microscopical preservation: to stabilize cell *structure and composition* as they exist in the living state.

Microprobe analysis in biology is used here mainly in the context of acquisition and analysis of compositional data at cellular and subcellular spatial resolutions using microbeams on the nanometer scale. Clearly this is only one aspect of a very broad field of scientific inquiry that generally falls under the rubric of Compositional Imaging that can involve many different sources of radiation including electrons, ions and photons. Indeed there are often subtle distinctions in biological microscopy between "structural microanatomy" and "chemical microanatomy" - they obviously can be significantly different, for example during the early stages of cell injury when changes in the elemental distribution within a cell can become readily apparent before any observable changes in morphology via "conventional" microscopy.

A variety of frequently encountered biomedical problems lend themselves readily to investigation by correlative microprobe analysis. e.g., a combination of scanning or transmission electron microscopy and energy dispersive x-ray microanalysis with x-ray microscopy. In a clinical setting, the most common application is identification of xenobiotics or exogenous substances, such as localization and quantitation of inorganic particulates in lung tissues in patients with pneumoconiosis; identification of foreign materials within granulomas; and analysis of foreign bodies. A variety of metals and other elements may be detected with energy dispersive X-ray (EDX) microanalysis, including copper in tissues of patients with Wilson's disease, thorium and gadolinium in patients injected with radiographic contrast agents, or gold in patients treated with long-term chrysotherapy. Endogenous particulates such as urinary calculi, gallstones, intraarticular and periarticular crystalline deposits in patients with rheumatic disease, dystrophic or metastatic calcifications, and hemosiderin may be analyzed rapidly and efficiently by means of EDX. Certain organometallic drugs such as amiodarone (iodine)

or sodium stibogluconate (antimony) may also be detected in prokaryotes and eukaryotic cells and tissues. Analytical electron microscopy has been a useful adjunct to forensic pathology for many years in diverse areas such as identification of trace evidence constituents or detection of arsenic or lead in victims with heavy metal poisoning. The detailed elucidation of anatomic, physiologic, and pathologic conditions provided by microprobe analysis is a useful diagnostic and investigative approach in clinical medicine; the analytical results often have diagnostic, therapeutic, and/or medicolegal implications. This technology is growing rapidly as it is complemented by other techniques such as mass spectrometry, and laser Raman and infrared microspectroscopy.

Insoluble crystalline deposits and covalently bound elements, such as those found in many diagnostic pathology specimens, may be qualitatively preserved by routine chemical methods and examined by these microprobe techniques. However, experimental approaches in basic research in biology and in the environmental arena - for example, quantitation of free and bound ions within subcellular compartments, the identification and localization of trace elements in metalloproteins - that are concerned with unambiguously measuring potentially diffusible substances, require the application of **cryopreparative** techniques. By definition, cryofixation or instantaneous lowering of cell temperature stops all metabolic and diffusional processes, and significantly reduces molecular transformational and vibrational movements. No chemical fixatives, solvents or embedding media can be employed at any stage of preparation.

In summary, different specific tactical approaches, tailored for the application in question, can be adopted to successfully convert the molecular, ionic and elemental constituents of cells and tissues into a state suitable for microprobe analysis and imaging.