X-ray Imaging from Tissues to Cells to Subcellular Structures

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Exploration of biological samples with the aid of X-ray imaging has developed rapidly over the past 20 years. From the early days of Advanced Photon Source, in late 1990's my laboratory started to use hard X-ray fluorescence microscopy (XFM) to evaluate concentration and distribution of native and artificially introduced elements in biological samples. With time, we learned that XFM is equally important in evaluation of "large tissue features" that require scanning of hundreds or even thousands of microns as well as in tomographic imaging of single cells at submicron resolution. We have also affirmed that even though cryogenic sample preparation and imaging at cryogenic temperatures provide the best "snapshots" of specific cellular events, there is merit in imaging of formalin-fixed, paraffin-embedded (FFPE) tissue samples. Others have referred to development of other types of biomedical analysis of FFPE as "The Holy Grail for molecular diagnostics" (Donczo and Guttman, 2018); we have found that many chemical elements that make cells do not alter in the course of FFPE preparation to allow us to recognize different makeup of different cell types within a tissue. Working with FFPE patient samples from different repositories, as well as archival FFPE processed animal tissues we found that, for example, high zinc signal may be expected in cell nuclei from cells harboring human papilloma virus (HPV), as well as in phagocytic cells, or, at the level of large tissue areas – in healthy prostate tissues but not prostate cancer or breast ductal carcinoma in situ (Poropatich et al., in press; Refaat et al 2016; Paunesku et al., 2012; Haley 2011; Barrea et al., 2010; Jansen et al., 2009). Working with cryogenically or room temperature prepared cell monolayer samples, we were able to find that the complete native elemental concentrations are best preserved through cryogenic preparation, but that, at the same time, nanoparticle distribution in cells remains stable regardless of the preparation approach (Popović et al., 2019; Brown et al., 2018; Dučić et al 2017; Jin et al., 2017; Yuan et al., 2013; Arora et al., 2012; Thurn et al., 2009; Paunesku et al., 2008, 2007, 2006, 2003). While sample preparation and the exact approach to imaging modulate what exact elemental data will be acquired most acurately, XFM always permits broader and more robust evaluation of sample content than, for example, immunocytochemistry which is one of the most frequent approaches for cell imaging. With the vast resources provided through numerous FFPE tissue archives, we hope that XFM will evolve new means to maximize information extraction from such samples, just as the development of approaches for cryogenic sample preparation grows to become more robust and reliable.