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Correlative single cell Fourier transform infrared spectroscopy and super-resolution fluorescence microscopy reveals the effects of fixation on the biochemistry of mammalian cells.

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Synchrotron Fourier transform infrared (SFTIR) spectroscopy provides the best available signal-to-noise which allows for fast, sensitive detection of the holistic biochemistry of single live cells. Recently developed super-resolution fluorescence microscopy (SRFM) techniques based on single molecule emissions can yield an order of magnitude improvement in spatial resolution in the imaging of specifically targeted cellular structures. For the first time we have paired these two techniques to investigate the effects of various fixation parameters on the FTIR spectrum, the rendered super-resolution image, and the underlying biochemistry. To achieve this SFTIR spectra of single live COS7 cells were obtained; the cells were then fixed and a second spectrum of each hydrated correlated cell acquired. Finally, the cells were immunostained and the microtubule architecture visualized using direct stochastic optical reconstruction microscopy (dSTORM) with spatial resolutions of 20-30 nm achieved. The resulting FTIR spectra demonstrated that many of the spectral changes previously associated with cell fixation effects were due to dehydration, morphological variation and loss of cell constituents through washing. Key spectral changes that could be directly linked to the fixation parameters included changes in lipid content and ordering, DNA conformation, and glycogen and cytosolic protein concentration. Importantly, while FTIR spectroscopy was found to detect various subtle changes in the underlying biochemistry that SRFM could not, subdiffraction damage to the cell cytoskeleton could only be detected using SRFM. Not only does this work demonstrate the exquisite sensitivity of both techniques but also the future potential for correlative SFTIR/SRFM.

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