CLEM-SIMS as a new method for resin-embedded samples

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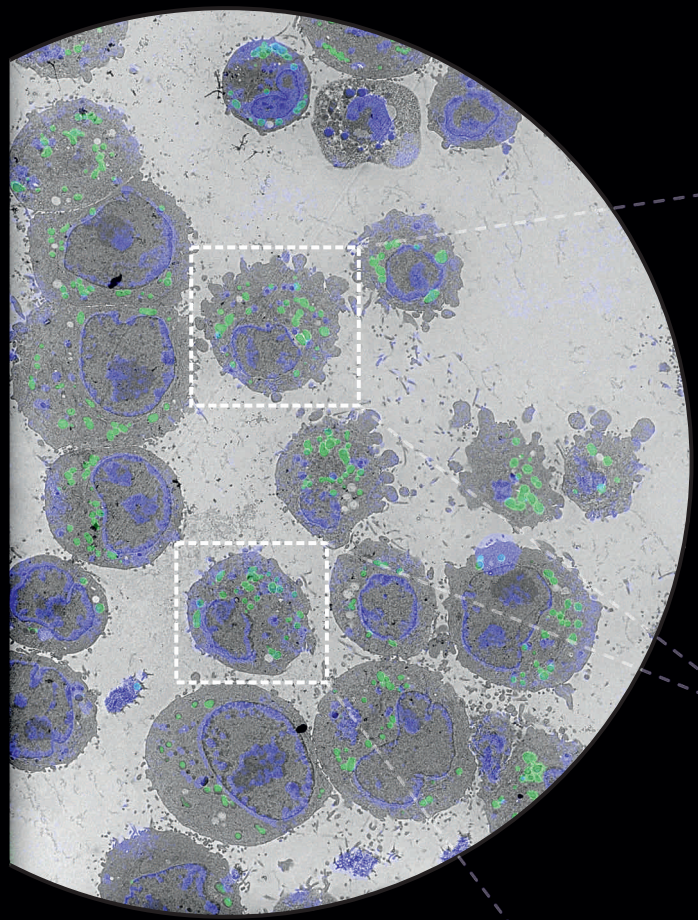
To date, cell biology relies on the strong analytical output provided by electron microscopy. However, electron microscopy exhibits limitations particulary with respect to functional labelling and the composition of cellular components. Here, we introduce a novel correlative approach which allows for extracting information on cellular morphology together with the precise localization of specific targets and elements from a single resin-embedded sample.



Labels used for LM: Mitotracker™ Deep Red FM - stains mitochondria (shown in green), Uranyl acetate as a dual purpose contrasting agent (shown in blue) (Tuijtel et al. 2017). No added heavy-isotope labels. $^{12}C^{15}N$ channel represent natural abundance of the isotope.

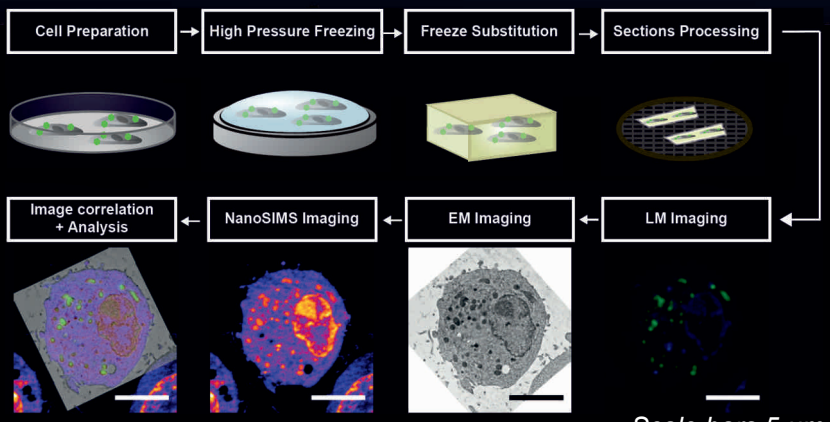
Brief description of the method:

1. Desired samples are prepared in a way that allows for the combination of all three imaging modalities on one single specimen with excellent structural preservation of the cellular details.

2. First the light microscopy data is obtained and serves as a roadmap to guide further investigations. Subsequent TEM reveals contextual ultra-structures (CLEM). NanoSIMS measurements provide the distribution of elements and potential heavy-isotope labels added to the samples prior to immobilization.

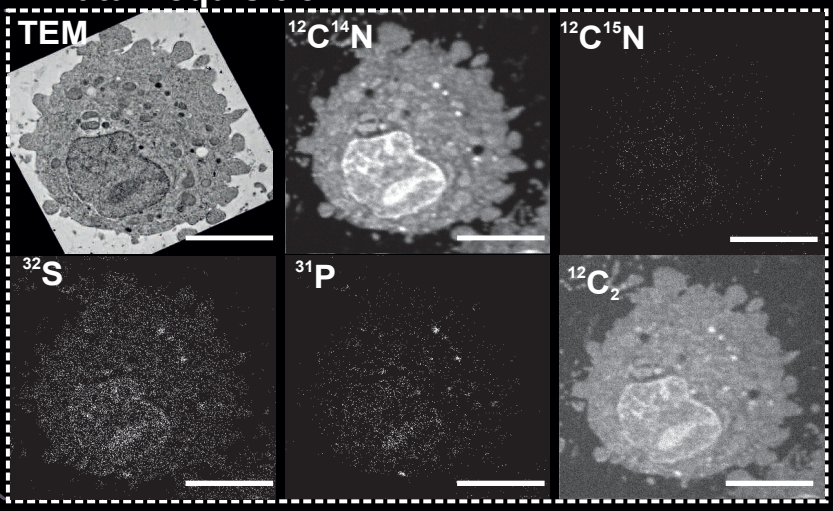
3. Image analysis and normalization obtains differences in potential label- and element-distributions. This very straight forward protocol allowed us to pin-point specific areas of interest and analyse functional, structural and turn-over information within one single sample. Taken together CLEM-SIMS provides hitherto inaccessible and novel insights into cellular function beyond the scope of conventional correlation approaches.

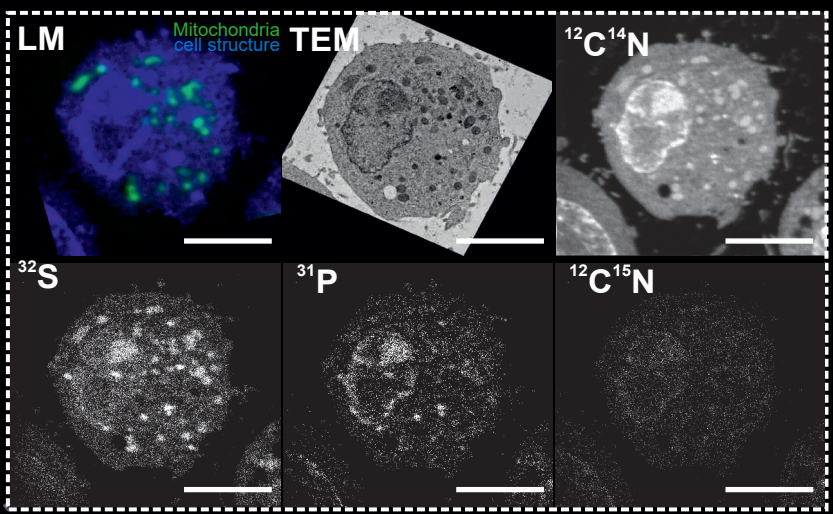
1. Workflow



2. Data Acquisition

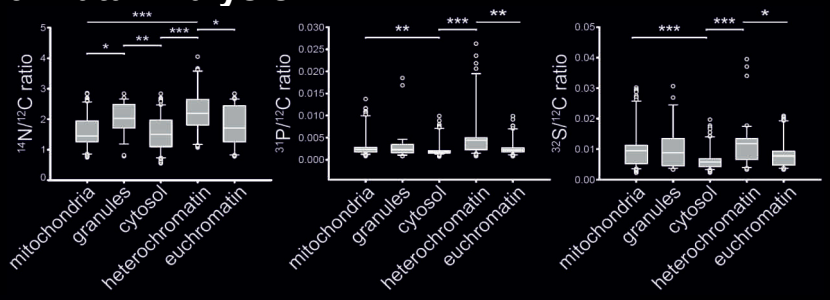
Scale bars 5 µm





3. Data Analysis

Scale bars 5 µm



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