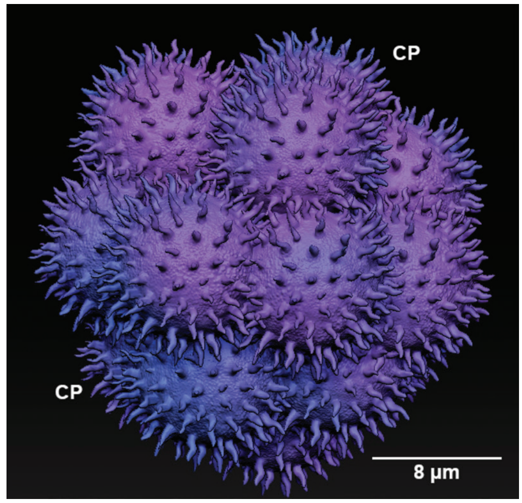
Highlights from Microscopy AND Microanalysis

Biological Applications

The Relevance of Ultrastructural Studies of Metastatic Cells from Women with Breast Cancer History by ID Moreira, A Peres, A Campos, CG Bica, GT dos Santos, JC Prolla, LA Carús, MS Ferreira, MC Fernandes, NC Bassani, and GO Introíni, *Microsc Microanal* | https://doi.org/10.1017/S1431927621013581.

In malignant breast cancer pleural effusions (MPE) single cells and cell clusters are observed. MPEs consisting of cell clusters have a better prognosis when compared to cases in which an isolated cell pattern is found. We investigated the structure of cells in MPE by light and transmission electron microscopy (LM/TEM), and the creation of a digital sculpture using ZBrush® software. In our findings, clusters exhibited structural stability, vesicles allowing exocytosis of electron-dense fibrous elements, and cytoplasmic protrusions contributing to migratory and invasive behavior. They acquired a spheroid conformation, modifying the supply exchange dynamics. This cooperative relationship seems to be related to a long-term permanence in MPE. Single-cell MPE presented different necrotic phenotypes, and many displayed leukocyte-like characteristics. The absence of a collaborative network presumably triggers their more aggressive behavior. Its putative fusion with leukocytes can maximize efficiency for transendothelial migration, increasing the chances of metastatic success and reducing the survival of women with recidivism.

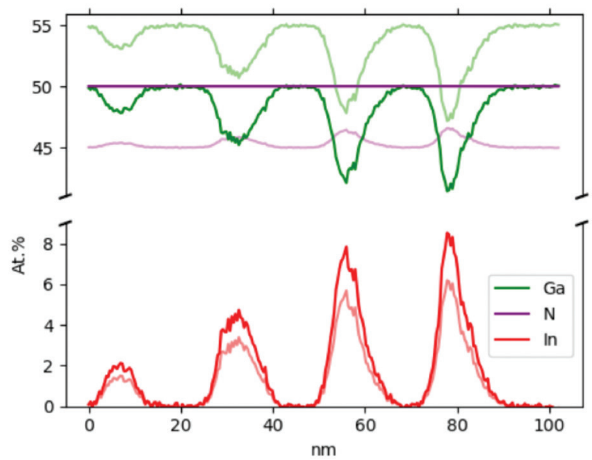


3D berry-like cluster model. Digital sculpture and painting using ZBrush® software. CP = cytoplasmic protrusions.

Materials Applications

Composition Analysis by STEM-EDX of Ternary Semiconductors by Internal References by JS Nilsen and ATJ van Helvoort, *Microsc Microanal* | https://doi.org/10.1017/S1431927621013672.

Energy dispersive X-ray spectroscopy (EDX) is the most widespread method for compositional analysis in transmission electron microscopy. To date, quantification is mainly based on the Cliff-Lorimer (k-factor) method. Although easy to perform, the quantification accuracy is limited because of the use of calculated k-factors and because compensating for absorption effects can be challenging. Here, a practical method to determine the composition within ternary heterostructured semiconductor compounds using EDX in scanning transmission electron microscopy is developed. The method requires minimal external input factors, such as user-determined or calculated sensitivity factors, by incorporating the analysis of a known compositional relationship for a reference area, in the present case a fixed stoichiometric III–V ratio. The method accounts for absorption effects influencing the quantification without the need for absorption corrections, which require additional input parameters. Along with the open-access paper, a tutorial Jupyter notebook with the open-source code, as well as example data, are made available.



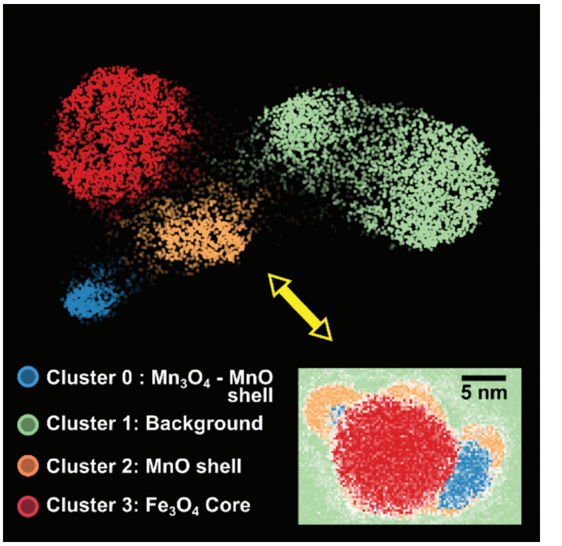
Example of internal determination of composition compared to quantification with calculated k-factors (lighter color) for a tapered GaN nanowire with $In_xGa_{1-x}N$ quantum wells.

Microscopy AND Microanalysis

Techniques

Strategies for EELS Data Analysis. Introducing UMAP and HDBSCAN for Dimensionality Reduction and Clustering by J Blanco-Portals, F Peiró, and S Estradé, *Microsc Microanal* | https://doi.org/10.1017/S1431927621013696.

A variety of machine learning algorithms are often included in the analysis of TEM spectral datasets. For instance, the use of linear matrix factorization dimensionality reduction techniques (for example, principal component analysis and non-negative matrix factorization) are regarded as standard procedure in electron energy loss spectroscopy (EELS) analysis for spectral unmixing and noise cleansing. Furthermore, spectrum image segmentation routines based on clustering analysis (for example, K-Means) have gained traction among the EELS community, given their ability to retrieve an unsupervised classification of pixels attending to the spectral features of the dataset. Here, we make a systematic revision of some of the commonly used dimensionality reduction and clustering algorithms for the analysis of EELS datasets. We also introduce the state-of-the-art algorithms of UMAP (a non-linear dimensionality reduction algorithm) and HDBSCAN (a density-based clustering algorithm) for analysis of EELS spectrum images (Figure). We show how their combined use conforms a fully data-driven methodology that clearly outperforms any of the other possible combinations of algorithms tested.

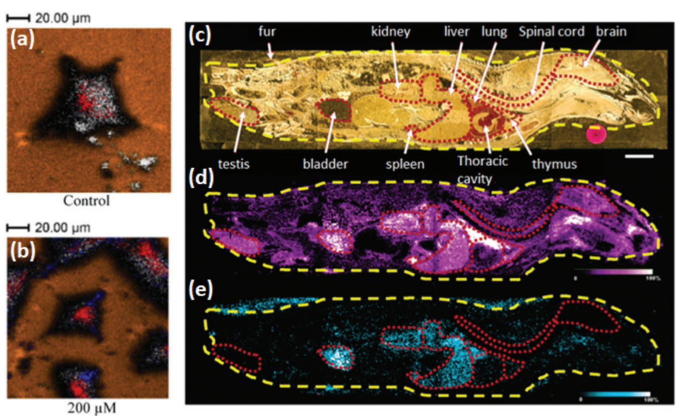


UMAP embedding for an EELS spectrum image of an iron and manganese oxide core-shell NP, colored according to the clustering classification resolved by HDBSCAN.

Review Article

Cells and Tissue Imaging by TOF-SIMS and MALDI-TOF: An Overview for Biological and Pharmaceutical Analysis by M Noun, R Akoumeh, and I Abbas, *Microsc Microanal* | https://doi.org/10.1017/S1431927621013593.

The potential of mass spectrometry imaging (MSI) has been demonstrated in cell and tissue research since 1970. MSI can reveal the spatial distribution of a wide range of atomic and molecular ions, such as drugs, lipids, proteins, and DNA. MSI techniques, notably matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) and time of flight secondary ion mass spectrometry (TOF-SIMS), witnessed a dramatic upsurge in studying and investigating biological samples. This advancement is attributed to submicron lateral resolution, high sensitivity, good precision, and accurate chemical specificity, which make these techniques suitable for decoding and understanding complex mechanisms of certain diseases, as well as monitoring the spatial distribution of specific elements and compounds. In this paper, the application of both techniques for cell and tissue analyses and adequate sampling before analysis are covered. In brief, the importance of these techniques as diagnostic tools and robust analytical techniques in the medicinal, pharmaceutical, and toxicology fields is highlighted through representative published studies.



Examples of MSI analyses. Left (a): 2D TOF-SIMS chemical map of A-172 cells in control and (b) in cells treated with 200 µM concentration of the ABT-747. The images represent the secondary emission of $HP_2O_6^-$ in red, sum of fatty acids in gray, Au_3^- in orange, and ABT-737 ($C_{42}H_{44}ClN_6O_5S_2^-$) in blue. Right (c): whole rat sagittal tissue given olanzapine across 4 gold MALDI plates, MALDI imaging of (d): olanzapine, and (e) N-desmethyl metabolite (*m/z* 256). (Vanbellingen et al. (2016), Khatib-shahidi et al. (2006)).