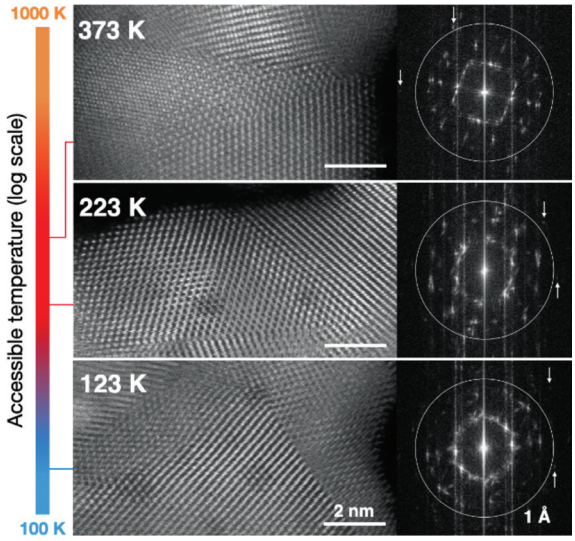
Highlights from Microscopy AND Microanalysis

Techniques Development

Atomic-Resolution Cryo-STEM Across Continuously Variable Temperature by BH Goodge, E Bianco, N Schnitzer, HW Zandbergen, and LF Kourkoutis, *Microsc Microanal* | doi:10.1017/S1431927620001427

Atomic-resolution cryogenic STEM provides a path to probe the microscopic nature of low-temperature phases in a wide variety of materials. Successful high-resolution cryo-experiments have been limited to certain fixed temperatures dictated by the cryogen, leaving most of a material's phase space unexplored. The novel side-entry continuously variable-temperature (CVT) liquid nitrogen cryo-holder specifically addresses this issue: the combination of liquid nitrogen cooling with local MEMS sample heating allows precise temperature control between ~100–1000 degrees K (Figure). Additional design considerations including a large-volume cryogen reservoir and active rod temperature compensation further mitigate many challenges of cryo-STEM experiments, dramatically reducing drift rates and enabling consistent sub-Å imaging resolution. These proof-of-concept experiments mark significant progress for the accessibility of variable-temperature cryo-electron microscopy, opening the doors to a new range of high-resolution *in situ* cryo-experiments including real-time observation of phase transitions, temperature cycling, and access to phases with narrow stable temperature windows.

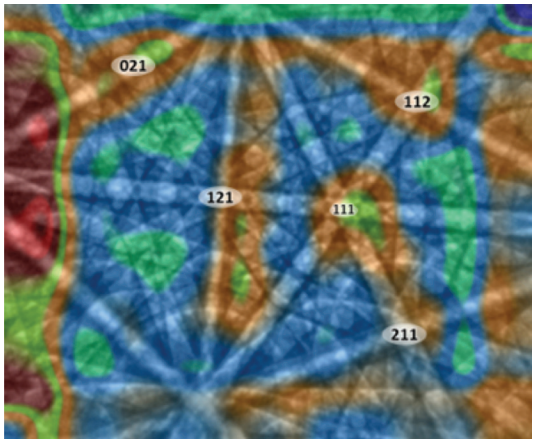


Single-frame HAADF-STEM images of a standard Au sample using the dual-tilt CVT liquid nitrogen cryo-holder show consistent sub-Å resolution across the ~100–1000 K temperature range.

Materials Applications

Deep Neural Network Enabled Space Group Identification in EBSD by K Kaufmann, C Zhu, AS Rosengarten, and KS Vecchio, *Microsc Microanal* | doi:10.1017/S1431927620001506

Electron backscatter diffraction (EBSD) is one of the primary tools in materials development and analysis. Phase identification and differentiation are necessary components of this technique for analysis of single- and multi-phase samples. After collection, current EBSD pattern indexing techniques can differentiate between a user-selected set of phases only if those contain sufficiently different crystal structures. To address these challenges, we report a machine learning-based technique for space group classification of diffraction patterns without user-selected phases or other inputs. Each diffraction pattern is individually analyzed in real-time by a deep neural network. Real-world performance of the deep neural network is gauged by presenting data from materials it has not encountered before and assessing its predictions. The ability to classify new data with exceptional accuracy was investigated through heatmaps of localized feature importance (Figure). This reveals the importance of features beyond Kikuchi lines and diffraction maxima and enables AI-assisted phase identification, enhanced phase differentiation, and autonomous phase mapping.



A heatmap highlighting the important regions for determining the correct space group (225) for the underlying NbC diffraction pattern.

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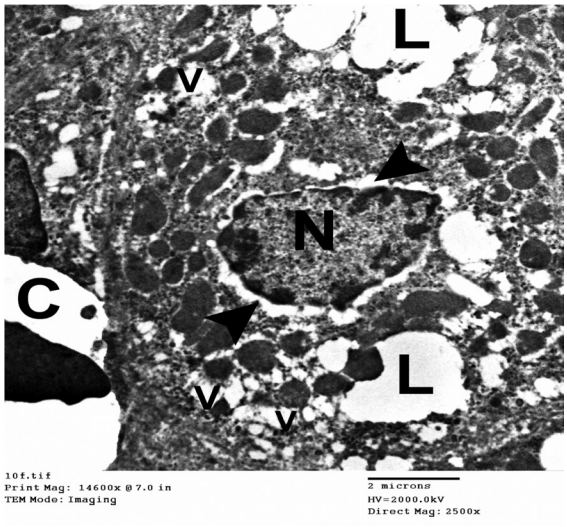
Biological Applications

Tramadol Promotes Oxidative Stress, Fibrosis, Apoptosis, Ultrastructural and Biochemical Alterations in the Adrenal Cortex of Adult Male Rats with Possible Reversibility After Withdrawal by AM Shalaby, AM Aboregela, MA Alabiad, and DF El Shaer, *Microsc Microanal* | doi:10.1017/S1431927620001397

Tramadol is a centrally acting analgesic drug used for the management of moderate to severe pain in a variety of diseases. Long-term use of tramadol can induce endocrinopathy. We evaluated the effect of tramadol dependence on the adrenal cortex and the effect of its withdrawal. Thirty adult male rats were divided into three groups: the control group, the tramadol dependent group, and the recovery group. Tramadol induced severe histopathological changes in the adrenal cortex in the form of disturbed architecture, swollen cells, and shrunken cells with pyknotic nuclei (Figure). Inflammatory cellular infiltration and affected areas of variable size were also detected. A significant increase in P53 and Bax immunoreaction was detected and confirmed by RT-qPCR. Ultrastructural examination showed irregular, shrunken adrenocorticocytes with dense nuclei. Dilated sER, mitochondria with disrupted cristae, and numerous coalesced lipid droplets were also demonstrated. All changes started to return to normal after the withdrawal of tramadol. Thus, it was confirmed that long-term use of tramadol can induce severe adrenal changes with subsequent recovery.



A fasciculata cell with an irregular nucleus (N) with dilated perinuclear space (arrowhead), confluent lipid droplets (L), and cytoplasmic vacuoles (v). Notice dilated blood capillary (C) X2500.