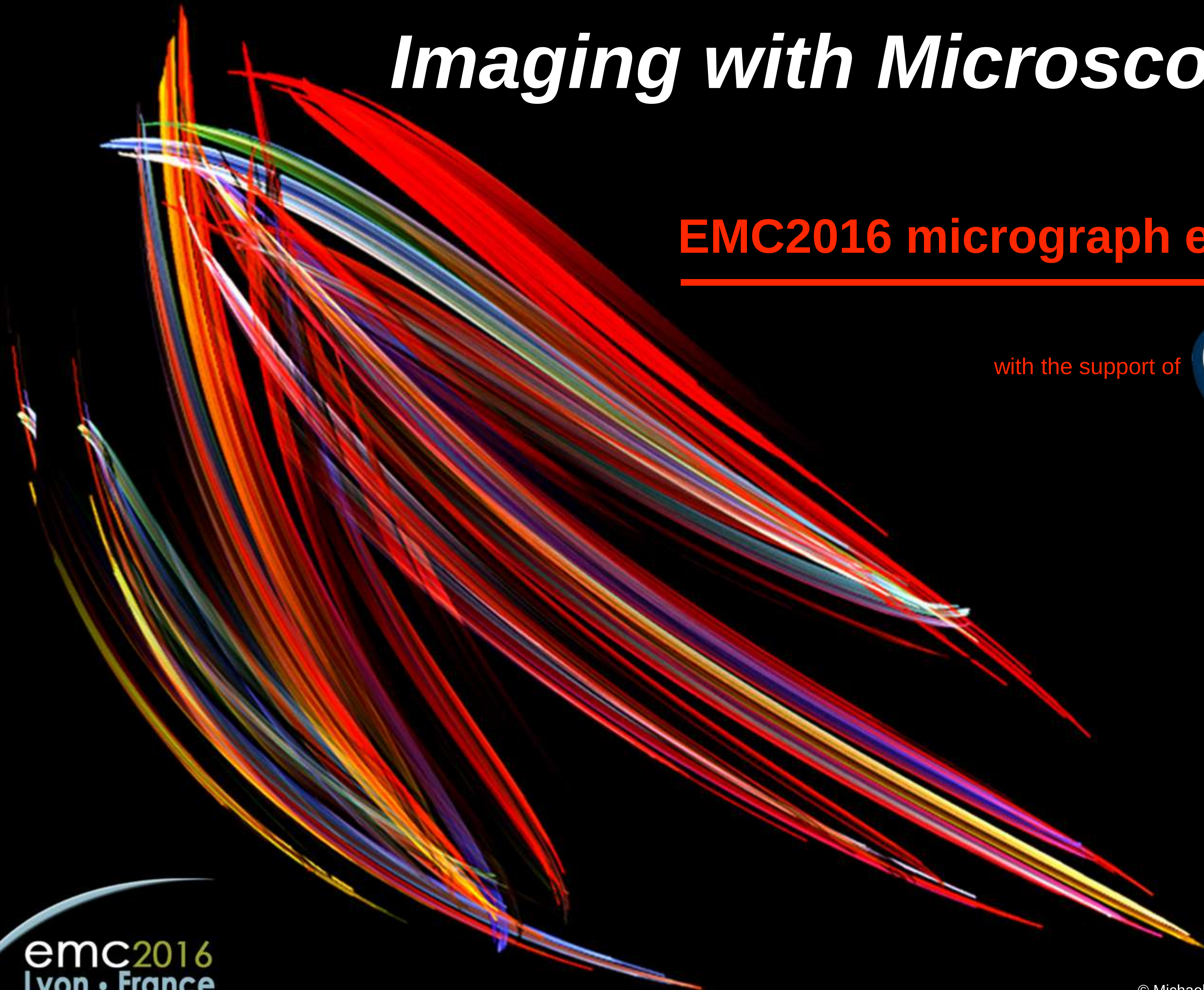
Imaging with Microscopes

EMC2016 micrograph exhibit

with the support of CNRS



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Pleurosigma diatoms (200x. Darkfield, polarized Light)

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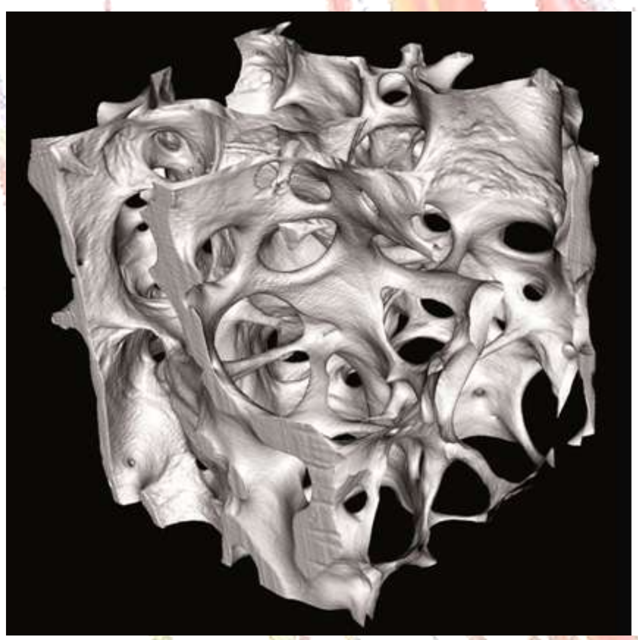
Bones in 3D – X-Ray µ/nanoCT

Françoise Peyrin and coll., Université de Lyon / ESRF, F

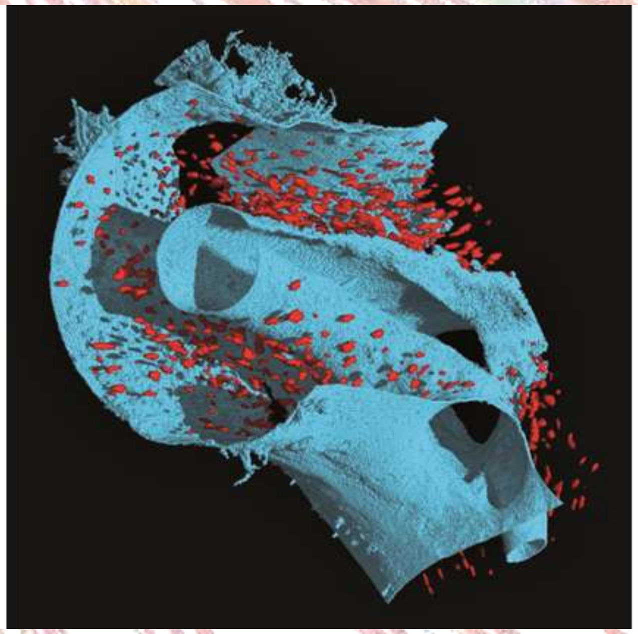
Françoise Peyrin (DR) is leading the Tomographic Imaging and Radiotherapy team in the CREATIS Laboratory (Univ. Lyon, INSA-CNRS-INSERM) specialized in medical imaging. Her research interest is in 3D biomedical imaging particularly in X-ray tomography, tomographic image reconstruction and 3D image analysis.
She is particularly developing new imaging methods for studying the mechanisms implied in bone fragility diseases such as osteoporosis. Bone strength depends on the organization of bone at different scales. 3D Synchrotron Radiation (SR) Computer Tomography (CT) has been developed to characterize the microscopic organization of bone tissue from the micro to the nano scale. These techniques take advantages of the properties of the X-ray beam extracted from synchrotron radiation (monochromaticity, high flux, coherence). Different setups allowing to implement 3D parallel beam absorption or phase CT, as well as magnified phase nano CT have been developed at the ESRF, Grenoble, France. By coupling 3D SR micro/nano CT image acquisition to dedicated 3D image analysis techniques, it is possible to obtain new quantitative information about the organization of bone from the microscopic scale to the cellular scale.



Trabecular Bone (voxel 7µm)

This image shows the complex architecture of the trabecular bone network. Trabecular bone is the inner part of our bones. Its organization can be altered in bone diseases such as osteoporosis This image is one of the first 3D images obtained on the synchrotron radiation micro-Computerized Tomography (CT) device developed at the ESRF (beamline ID19). The voxel size of 7 µm provides a fine rendering of the trabecular surfaces in a human vertebrae.

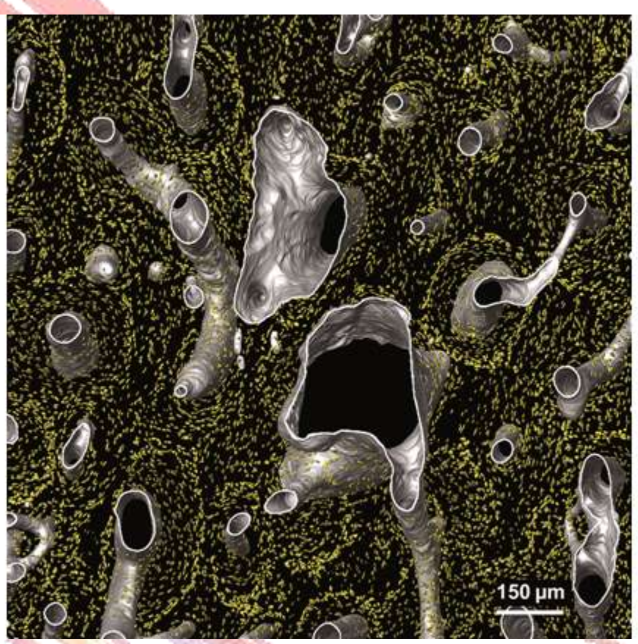
Credits: M. SALOMÉ, F. PEYRIN, P. CLOETENS, C. ODET, A.-M. LAVAL-JEANTET, J. BARUCHEL, P. SPANNE, *Medical Physics* **26** (1999) 2194.



Bone microvascularisation (voxel 5 µm)

Bone micro vascularisation plays a major role in physiological events such as fracture healing, bone growth and pathological processes such as metastasis. This image represents the microvascular structure (in yellow and red) together with the trabecular and cortical bone (beige) in a rat femur. The image was obtained by using SR micro-CT (ESRF) with a voxel size of 5µm, after injection of a barium sulfate contrast agent (coll MH Lafage-Proust, LBTO, Saint-Etienne, France). An image processing workflow was developed to segment the different structures. The method was applied to investigate the effect of intermittent Parathyroidhormone (PTH) administration on angiogenesis and osteogenesis in rats.

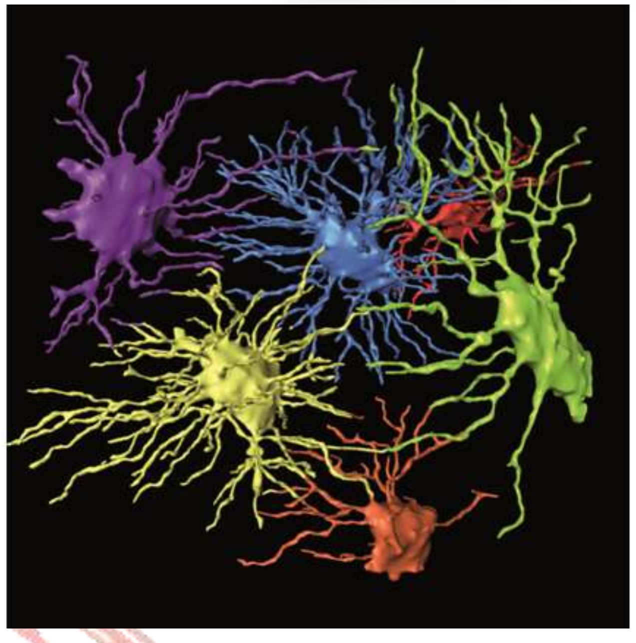
Credits: M. LANGER, R. PRISBY, Z. PETER, A. GUIGNANDON, M.H. LAFAGE-PROUST, F. PEYRIN, *IEEE Trans. on Nuclear Sci.* **8** (2011) 139-145.



Bone cells in cortical bone (voxel 1.4 µm)

Cortical bone, found at the periphery of our bones, has a major role in bone fragility. Compared to trabecular bone, it is a dense structure. This image illustrates the micro organization of human Haversian cortical bone, where the large pores (grey) correspond to the Havers canals and the small pores (yellow) to osteocyte lacunae (bone cells). The image was obtained with SR micro-CT (ESRF) with a voxel size of 1.4 µm. An automatic image analysis procedure was developed to extract quantitative parameters on osteocyte lacunae (density, length, width, height, shape…). It allowed to obtain statistics on large population of cells (>1000 per image).

Credits: P. DONG, S. HAUPERT, B. HESSE, M. LANGER, P.-J. GOUTTENOIRE, V. BOUSSON, F. PEYRIN, *Bone* **60** (2014) 172-185.



Bone cell network (voxel 300 nm)

The osteocytes are the most abundant bone cells (about 20000/mm^3). Thanks to connections through thin channels called canaliculi (diameter 100-500 nm), they can communicate and acts on bone remodeling. The investigation of this network is challenging since it is deeply embedded in the bone tissue. We developed a method based on SR micro-CT at a sub-micrometric scale (voxel 300 nm) to image in 3D the cells and their canaliculi. In addition, a specific method based on geodesic voting was proposed to segment the cells and their connections. This technique will allow new insight in the 3D organization of the cell network.

Credits: M.A. ZULUAGA, M. ORKISZ, P. DONG, A. PACUREANU, P.-J. GOUTTENOIRE, F. PEYRIN, *Phys. Med. Biol.* **59** (2014) 2155-2171.

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Microscopic Monsters – (E)SEM

Thierry Berrod, MonaLisa Prod., Lyon, F

Thierry Berrod is a film-maker who founded the company MonaLisa Production aiming at producing documentaries and cultural broadcasts for TV channels. In the 2000's, he produced a TV series, "Squatters", based on images taken of insects, bugs, mites, ticks and other termites in an Environmental Scanning Electron Microscope. His movies have been distributed worldwide and won numerous prizes. In "acarid cannibals" for instance, cannibal mites, or "invisible invaders" as he calls them, fight and eat each other, go about their business and even copulate under the electron beam. Thierry Berrod is fascinated by insects or "invisible invaders" as he calls them; he produces sumptuous images of these microscopic dinosaurs, arranging characters attitude and scenes and decorations like any director of actors in a traditional movie.

Bee

Domestic bee (Apis), sticking out its tongue to collect the nectar.

Tse-Tse Fly

a close-up shot of its stinging rostruzum: a lethal weapon that transmits to humans the sadly famous "sleeping sickness"

Camponotus Ant

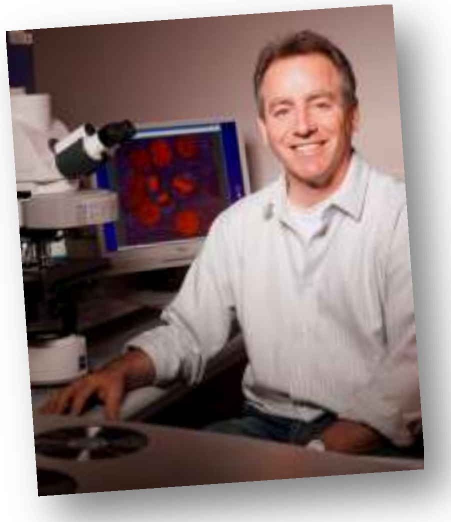
Camponotus Ant spreading its antennae to sense the surroundings.

African termite

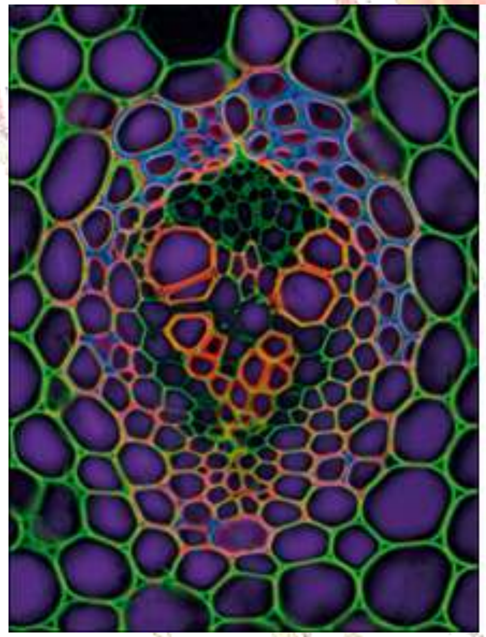
African termite in defense position.

A biologist Micro-World – Optical Microscopy

John Runions, Oxford University, UK

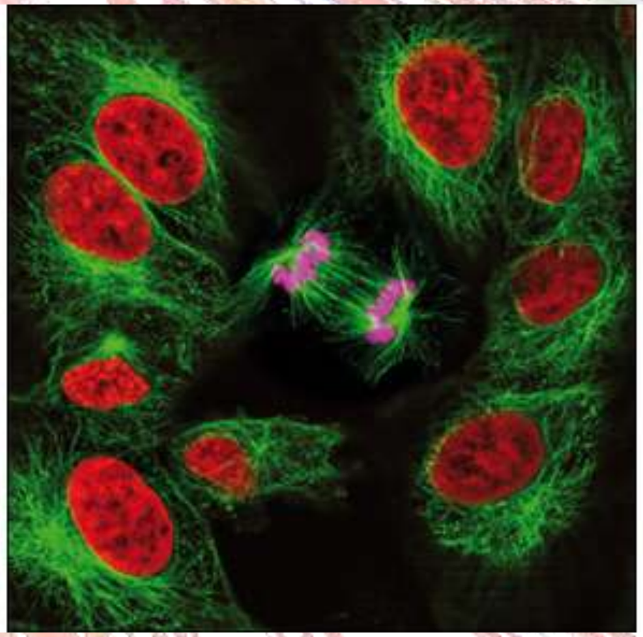


John is a cell and molecular biologist who uses confocal microscopy to study living cells. His latest work uses experimental approaches such as FRAP, FRET, and TIRF to measure dynamic processes such as membrane protein diffusion. The study organism is usually *Arabidopsis* but he has made forays into imaging development of *Drosophila*, and cancer cell biology. Experimental imaging of molecular processes generally yields low-resolution images but John tries to take the time to compose artful higher-resolution images to illustrate the beauty of the micro-world. All images were made by confocal microscopy with post-processing in Adobe Photoshop™.



A vascular tissue bundle in the stem of *Dracenea marginata* (Dragon tree)

Several tissues are visible here, including parenchyma cells which store water (large cells with thin cell walls around periphery, green), xylem which transports water (thick cell walls, red), fibres which provide support and stiffness (very thick cell walls, blue), and phloem which transports nutrients (very thin cell-walled cluster between xylem and fibres, green). This micrograph was produced by a fun and interesting technique. An old teaching slide of a permanently mounted stem section was imaged with different laser wavelengths from 405 to 633 nm. The fluorescence was produced as emission from the original stains and as autofluorescence from cell-wall components. Different cell types contain cell wall chemistry that emits fluorescence in different detection regions. The final image is a composite of the fluorescence channels with the transmitted light image (purple). Zeiss LSM 510 META confocal microscope.

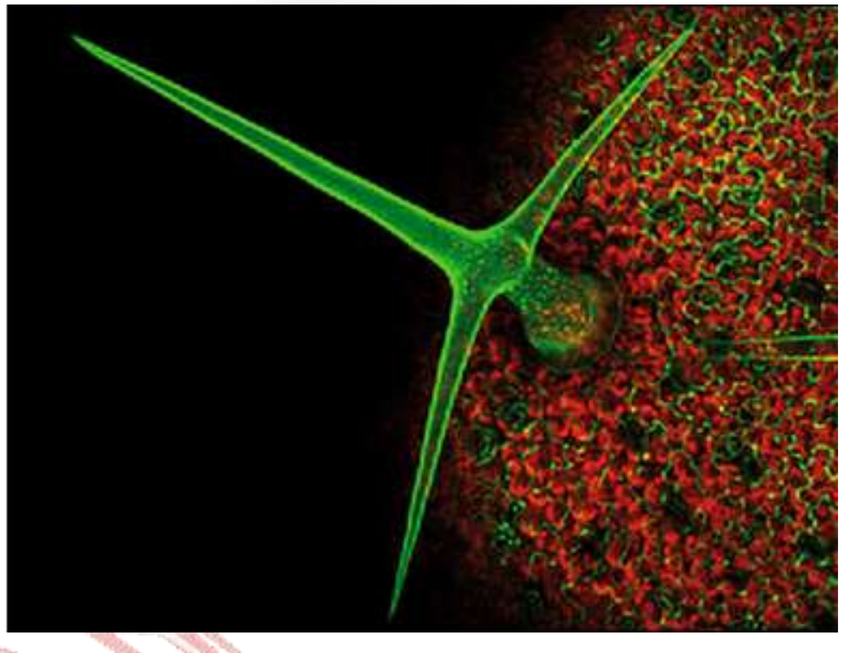


HeLa cells in anaphase

Cell division in living HeLa cells. Microtubules are immunolabelled with GFP (green). The cell in the centre is in early telophase of mitosis. The spindle apparatus which is made of microtubules is clearly visible and chromosomes can be seen condensed in the daughter nuclei which are marked with histone H2B labelled with mRFP (magenta). Most other cells are in interphase (large nuclei, red) except for the two towards the lower left which have smaller nuclei in which chromosomes are starting to condense in early prophase. This is a maximum projection of 10 images from an axial series. Zeiss LSM 510 META confocal microscope.

Drosophila head

Head and thorax of *Drosophila melonagaster*. Details of the insect's compound eye, mouth parts, and musculature are visible. This image was made from an old slide of a specimen embedded in permanent mounting medium. The specimen was scanned using several laser wavelengths (405, 488, 543, 633 nm) and the resulting images were combined and colour-contrasted in Adobe Photoshop. The original specimen was unstained so all emission resulted from autofluorescence of different structures within the fly. Even the mounting medium had aged and turned amber and this produced the lovely background. Try this technique with slides from your own collection! This is a maximum projection of 20 images in an axial series. Zeiss LSM 510 META confocal microscope..



Arabidopsis trichome

A trichome (large green structure) on the surface of a living Arabidopsis thaliana leaf. Trichomes are large projecting cells on the surface of leaves which probably afford protection against feeding insects, particularly, as here, at very early stages of leaf development. All cell walls have been marked with CFP (coloured green) so besides the trichome and its basal cells, epidermal cells and stomatal guard cells of the leaf are outlined. Autofluorescence of chlorophyll (red) in chloroplasts of cells underlying the leaf epidermis results naturally from blue-light excitation during imaging. This image is a maximum projection of 150 images from an axial series. Leica SP2 confocal microscope.

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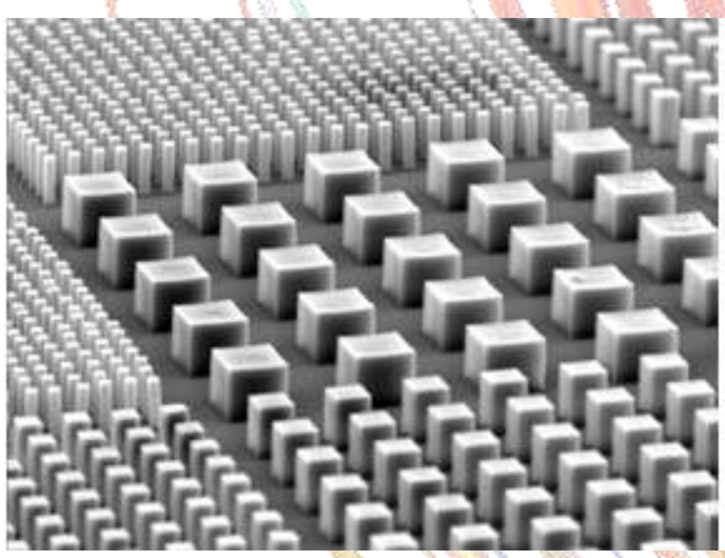
Small Scale Structures – SEM

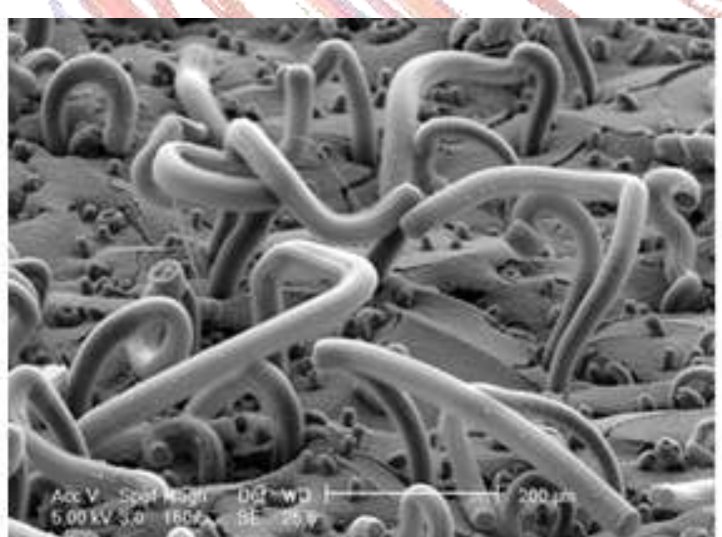
John Hart, MIT, Cambridge, USA

Professor Anastasios John Hart leads the Mechanosynthesis Group which seeks to discover and exploit micro- and nanoscale phenomena toward new and improved energy storage materials, electronic devices, composite structures, engineered surfaces, medical diagnostics, and consumer products.. John's research currently focuses on synthesis and applications of nanostructured materials and machine or instrument design. An other activity concerns scientific visualizations of small-scale structures ranging from nanometers to millimeters. For promoting popular awareness and education about nanomaterials and related technologies, SEM images are visible on the 'nanobliss' web gallery which associates science, art and architecture. Many of these micrographs have been featured in scientific and popular literature: scientific journals and also trade magazines, blogs, and other media outlets worldwide..



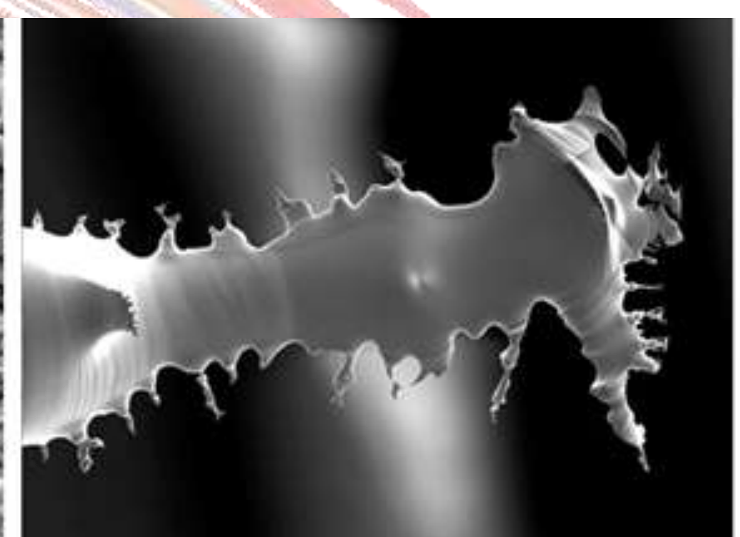
'Metropolis'

Self-organized and lithographically-patterned architectures made of self-organized grown carbon nanotubes: patterning consists in spreading a catalyst layer only over certain areas of the silicon substrate, leading to well-controlled geometries at the nanoscale.



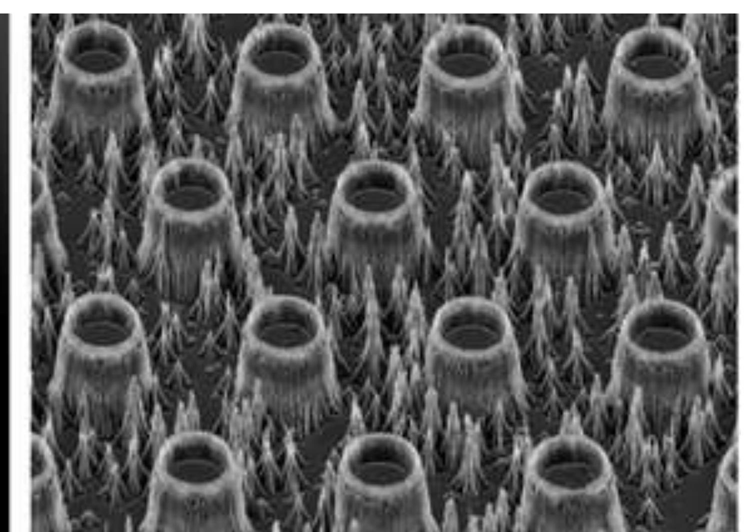
Disordered Carbon NanoTubes entanglements

Remarkable architectures form by self-organization of carbon nanotubes as they grow upward from the silicon substrate from the catalyst layer. If the catalyst is uniformly distributed, nanotubes grow everywhere on the substrate and how the nanotubes organize is defined by how they push and pull each other to produce the architectures shown.



'Sawtooth' (frozen structure)

Intricate shapes formed upon re-solidification of a silicon wafer which was first melted by resistive heating.



Silicon 'Micrograss'

Surprising Si "micrograss" structures are formed by plasma etching of silicon in an atmosphere which causes small pieces of silicon dioxide to sputter and then block (mask) etching where they land.

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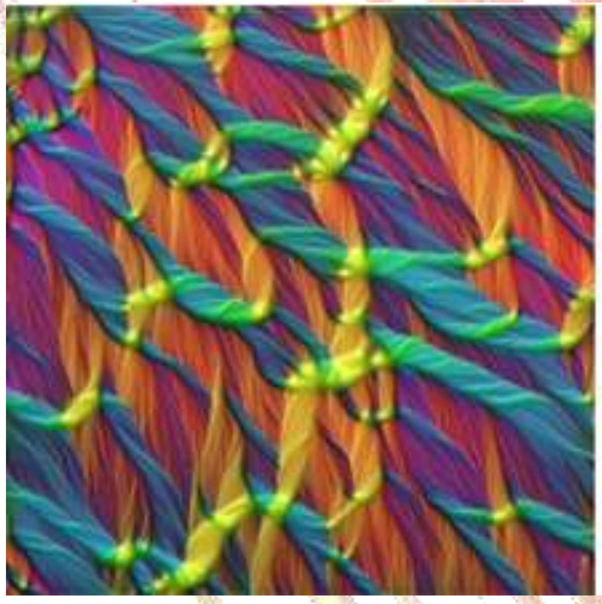
Nanoscale Electromagnetic Fields in the TEM

Rafal Dunin-Borkowski and coll., ER Centre, Jülich, DE

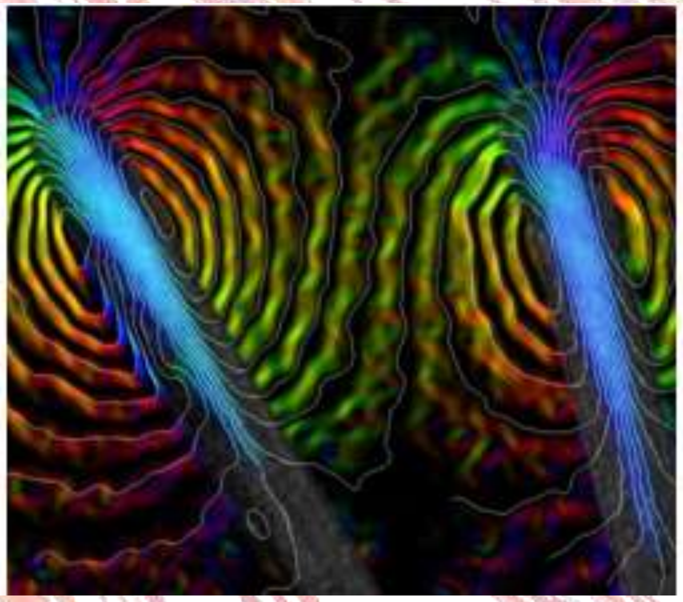
Professor Rafal Dunin-Borkowski is Director of the Ernst Ruska-Centre for Microscopy and Spectroscopy with Electrons in Jülich, Germany. His research activities are centred on the development and application of a wide range of techniques and approaches in transmission electron microscopy (TEM), including off-axis electron holography, electron tomography, quantitative high-resolution TEM, aberration-corrected imaging, image simulation and processing, instrumentation development and in situ TEM. Here, he presents a series of images of nanoscale electromagnetic fields in materials imaged using off-axis electron holography and Lorentz TEM. Such fields influence the phase of the electron wave incident on a specimen in the TEM. Their measurement is important across a wide variety of subjects and disciplines, ranging from magnetic recording, biomagnetism and paleomagnetism to semiconductor devices and the fundamental physics of processes such as field emission.



Magnetic domains in a thin cobalt film

The colors in the image show the different directions of the magnetic field in a layer of polycrystalline cobalt that has a thickness of only 20 nm. The direction of the magnetic field in the film changes at the positions of domain walls. The field of view is approximately 200 µm. The image was acquired using the Fresnel mode of Lorentz microscopy in a field emission gun transmission electron microscope. It was recorded out of focus to enhance the contrast of the domain walls, and then converted to a color induction map by applying the Transport of Intensity Equation to the image intensity.

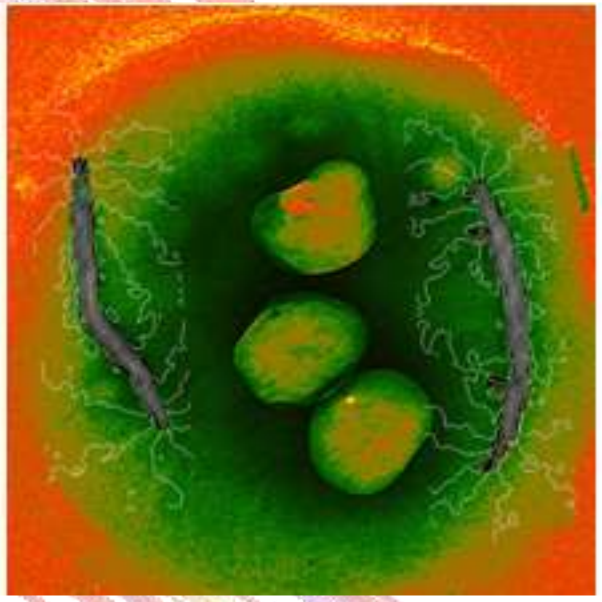
Credits: Anke HUSMANN, Martha MCCARTNEY, Chris BOOTHROYD, Rafal DUNIN-BORKOWSKI.



Magnetic nanotubes

Magnetic field lines recorded using off-axis electron holography showing the ends of two carbon nanotubes that contain catalyst particles formed from a magnetic cobalt-palladium alloy. The nanotubes have diameters of 70-100 nm. The colors represent the direction and intensity of the measured magnetic field.

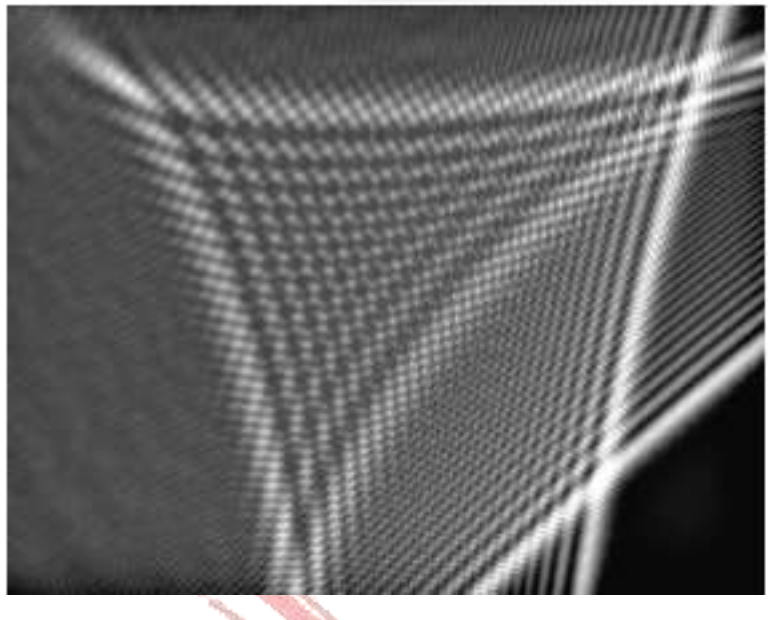
Credits: Ed SIMPSON, Yasuhiko HAYASHI, Takeshi KASAMA, Rafal DUNIN-BORKOWSKI.



Magnetic field lines in a bacterial cell

Magnetic field lines in a single bacterial cell. The fine white lines are the magnetic field lines in the cell, which were measured using off-axis electron holography. Such bacteria live in sediments and bodies of water and move parallel to geomagnetic field lines as a result of the torque exerted on their magnetosome chains by the earth's magnetic field.

Credits: Richard FRANKEL, Mihaly POSFAI, Peter BUSECK, Rafal DUNIN-BORKOWSKI.



Caustic pattern formed from an electron wavefield

Caustic pattern formed using electrons in a defocused bright-field TEM image of two approximately collinear metallic tips, which have a voltage applied between them in a transmission electron microscope. Such patterns depend sensitively on defocus, on the applied voltage between the tips and on their separation and lateral offset. Their features include fold, butterfly and elliptic umbilic catastrophes and can be interpreted on the basis of a projected electrostatic potential model for the electron-optical phase shift.

Credits: Amir H. TAVABI, Vadim MIGUNOV, Christian DWYER, Rafal DUNIN-BORKOWSKI, Giulio POZZI.

Atoms in Crystals – HR (S)TEM

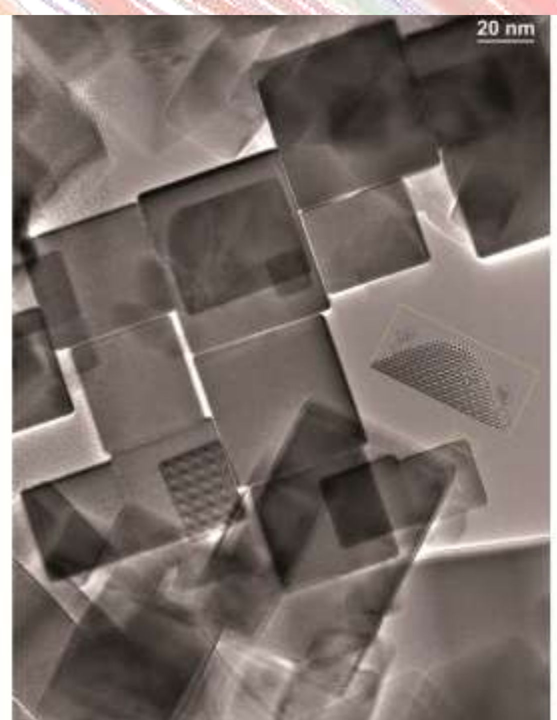
Thierry Epicier et coll., University of Lyon, F

Thierry EPICIER is a Research Director within the CNRS (French 'National Centre for Scientific Research') in the field "Chemistry of Materials, Nanomaterials and Processes". He works at the MATEIS laboratory (mateis.insa-lyon.fr/) at INSA de Lyon (National Institute for Applied Sciences).
His activities deal with 'all-purposes' electron microscopy (SEM, TEM, FIB), with the aim of establishing correlation between structure, nano- and microstructures, and macroscopic and functional properties of (multi-)materials and nanomaterials. Taking advantage of resolution performances of modern microscopes, he pays much attention to produce images where atomically resolved micrographs really reflecting the atomic structure, especially in the case of High Resolution TEM observations.

Precipitation in an industrial Al-alloy

HRTEM image of a metastable 'Q' AlMgSiCu phase precipitated in a Al 6061 alloy (Scherzer structure image in the [0001]-Q // [001] Al orientation; inset is the equivalent image in STEM; Cs-corrected TEM 300 kV).

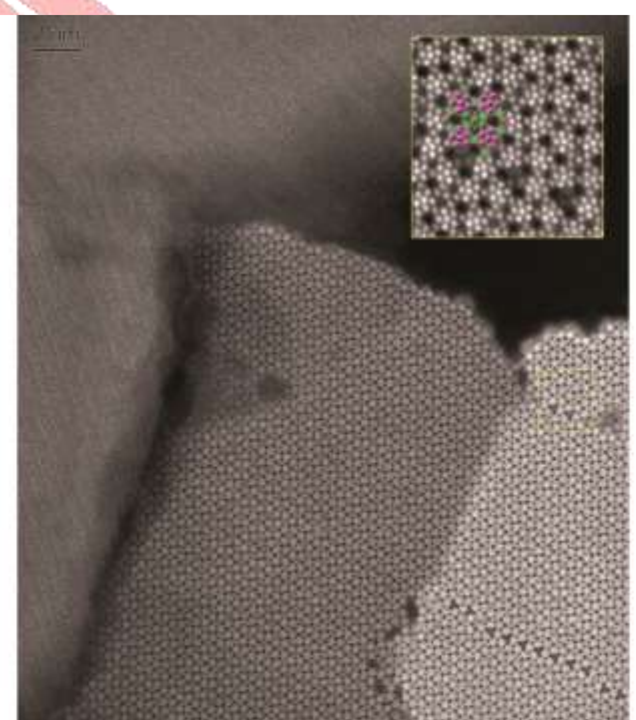
Credits: T. EPICIER, C. CAYRON, *Metallurgical Research and Technology* **109** (2012) 393-407.



Ceria nanocubes

Stacking of nanocubes of cubic cerium oxide CeO_2; inset is an enlarged detail showing the atomic structure of {001} (Cs-corrected TEM 300 kV).

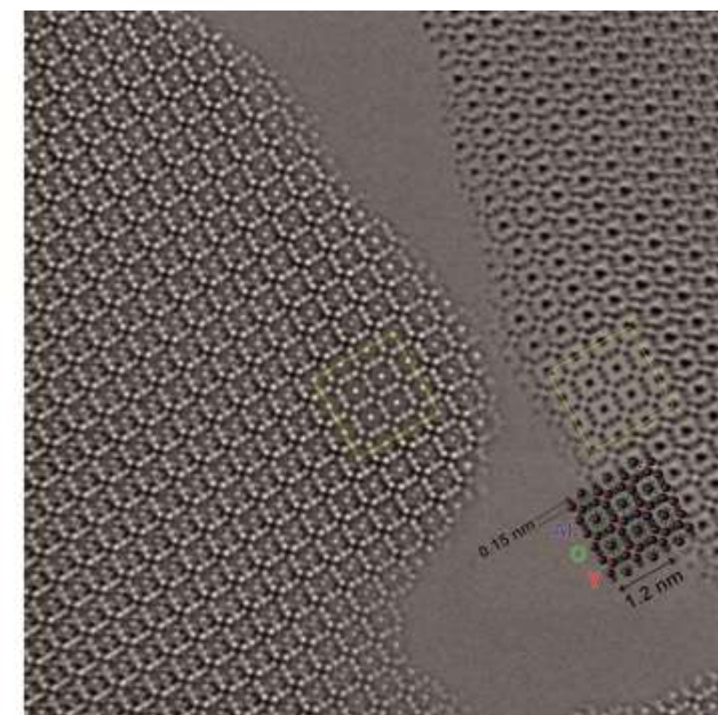
Credits: sample courtesy Amanda K.P. MANN, Zili WU, Steven H. OVERBURY, ORNL, USA; micrograph Thierry EPICIER.



MoVTe oxide M1 phase

Cationic structure of the light alkane mild oxidation catalyst MoVTeO (inset: atomic projection in the [001] orientation; STEM 300 kV).

Credits: M. AOUINE, T. EPICIER, J.M. MILLET, *ACS Catal.* **6** (2016) 4775-4781.



Yttrium Aluminium Garnet

In situ etching in an Environmental TEM has allowed a more or less controlled thinning of a YAG crystal, down to about 2-3 nm as estimated from simulations. Both sides shown here are 'white' and 'black' atoms (left and right respectively); yellow frames are [001] simulated images form the structural model shown as insert (Cs-corrected TEM 300 kV).

Credits: J. HOSTASA, L. ESPOSITO, A. MALCHERE, T. EPICIER, A. PIRRI, M. VANNINI, G. TOCI, E. CAVALLI, A. YOSHIKAWA, M. GUZIK, G. ALOMBERT-GOGET, Y. GUYOT, G. BOULON, *J. Mater. Res.* **29** 19 (2014) 2288-2296.