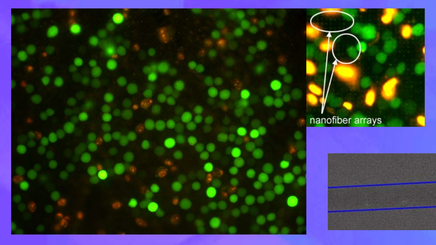
Characterization and Patterning Capabilities for Nano-Bio Research

Overview

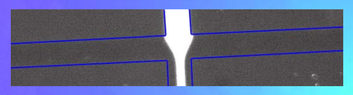
Nanobiotechnology research requires biologically compatible patterning and imaging techniques. Effective integration and characterization of biological and synthetic nanoscale systems requires multiple length scale patterning and tools for extraction of electrical, optical, and/or mechanical properties. Imaging systems must provide extensive information while leaving undisturbed fragile systems. Characterization often requires not only the handling of biological systems but also maintenance of appropriate environmental conditions. Further, gentle manipulation and/or delivery is required to preserve the function and integrity of organic materials. These stringent requirements significantly limit the patterning and imaging techniques available to process and characterize nanobio-based systems. A capability connected to The Center for Nanophase Materials Sciences (CNMS) is a research laboratory dedicated to nanobio related patterning and characterization. The laboratory includes confocal and epi-fluorescent microscopes, a combination scanning probe (SPM) and epifluorescence microscope system, piezo-based inkjet delivery, and high-throughput liquid handling and dispensing equipment. Further, facilities for conventional molecular biology, cell culture and microbiology are integrated into the laboratory. For further information, contact Mitch Doktycz (doktyczmj@ornl.gov).

Fluorescent Microcopy

The Zeiss Axioskop 2 FS plus fluorescence microscope is equipped with epifluorescent illumination using 100 W Hg arc lamp (from the top), Nomarski phase contrast optics (bottom illumination) and either top or bottom illumination using 12W halogens lamps. A 12 bit Retiga color CCD camera is mounted on the microsope for image collection. The system is mounted on a Burleigh Gibraltar™ Platform and X-Y stage, contains a Burleigh PCS-5000 Series Patch Clamp Micromanipulator and ceramic objectives for electrophysiology measurements. The entire system is mounted on a vibration isolation optical table.



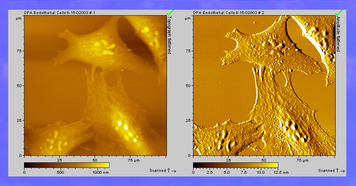
left : CHO cells expressing eCFP-N1 (*cyan fluorescent protein*). The CHO cells were impaled on vertically aligned carbon nanofiber arrays which temporarily compromises the membrane wall. Post-impalement fluorescence of the CHO cells indicates cell viability.



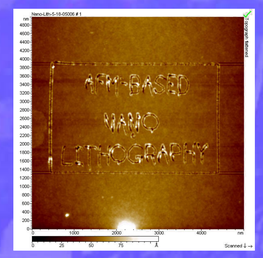
pinched fluorescin transport in a microfluidic channel by orthogonal cross-flows

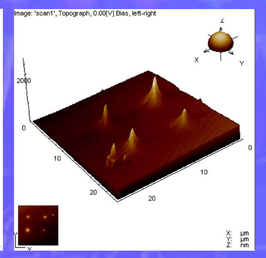
Combination SPM/Fluorescence Imaging System

A Molecular Imaging PicoPlus scanning probe microscope system is available. This system contains small (10 μm) and large (100 μm) closed-loop multipurpose scanners with low-coherence lasers and a Picoscan 3000 controller. The closed loop motion control allows for reproducible positioning and lithography on the nanometer scale. Liquid cells, flow cells and temperature control equipment are included. Additonally, the system contains magnetic (MacMode) and acoustical cantilever oscillation modes and the PicoTrec system that allows for simultaneous topography and chemical recognition. A video imaging system allows for sample viewing from above and through the scan head. Alternatively, the system is mountable onto a Zeiss Axiovert 135 epifluorescent microscope. The Zeiss contains a 100W Hg arc lamp source and a Princeton Instruments cooled CCD (RTE/CCD-1317-K/1 camera) imaging system. The entire system is mounted on a vibration isolation optical table.

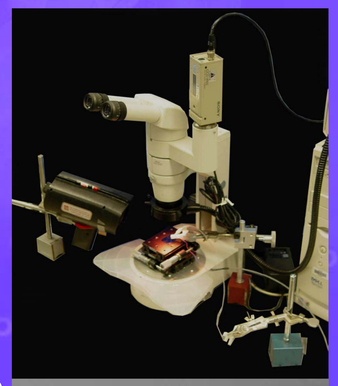


above: scanning probe microscopy images of mouse lung endothelial cells adhered to a rigid substrate





a) nanolithography by AFM tip force-contact through an gel-like resist layer supported on a rigid substrate. *b*) polypyrolle functionalized carbon nanofibers imaged using a high aspect ratio scanning probe tip



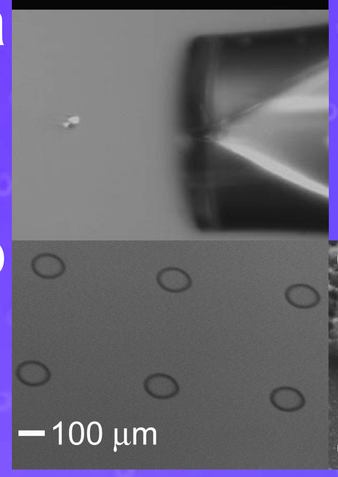
Piezo inkjet micro/nano fluidic delivery

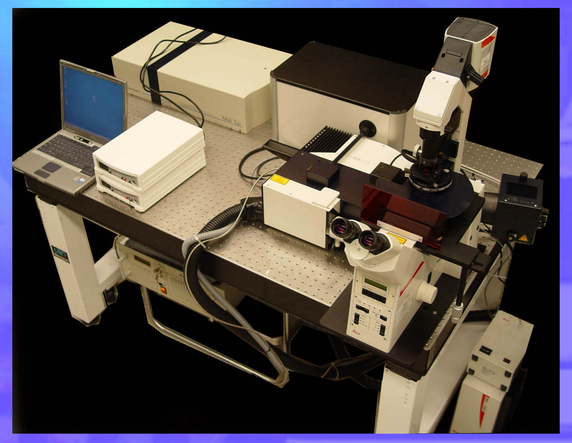
nL to pL volume dispensing and patterning is available using a piezo ink-jetting system. A MicroFab piezo controller is integrated with a customized Nikon 7000Z stereoscope with a strobe illumination and video imaging system.

below : *a*) an optical micrograph captured during a strobe pulse to capture picofluidic dispensing from a 20 μm diameter inkjet nozzle orifice.

b) poly-L-lysine spot array on Si by 30 μm diameter inkjet dispensing.

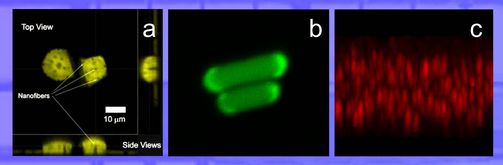
c) silicic acid polymerization to produce nano-textured silica at an individual poly-L-lysine droplet from the array displayed in b).





Confocal Fluorescence Microscopy

A Leica TCS SP2 MP scanning laser confocal microscopy system equipped with multiphoton excitation and Red (HeNe, 633 nm/10mW), Green (HeNe, 543/1.2mW) and Blue (Ar 458/5mW; 476nm/5 mW; 488nm/20mW; 514nm/20mW) laser systems and a 6-channel Acousto Optical Tunable Filter for laser line selection and attenuation. Further, the optical system is uv compatible. The inverted stage system is equipped with transmitted light detection for recording bright field images and a 50W mercury arc lamp for epifluorescent illumination. Heated sample holders and perfusion systems are in hand. The entire system is mounted on a vibration isolation optical table.



a) impaled cells by carbon nanofibers, *b*) E-coli expressing GFP, and c) covalently tethered DNA to carbon nanofibers

Sample Preparation/Liquid Handling and Patterning

Various instrumentation for automated sample preparation and handling are available. These include two Packard MultiPROBE II HT Ex automated liquid handling systems. One is a 4-tip system, the second is an 8-tip system. Both are equipped with vacuum manifolds and the 8-tip system possesses a plate-handling gantry. Additionally, a Hamilton 2200 liquid handling robot is used for nanoliter dispensing. This system is customized with high-resolution sub-stages and custom electronics for dispensing using solenoid-based ink-jet valves. Additionally, conventional microarray equipment including a Virtek Chipwriter Pro and a Perkin Elmer Scannarray 4000 confocal laser chip scanner are located in these laboratories.