Exobiology Branch

Overview

The Branch's research focuses on the advancement of the scientific understanding of the origin and distribution of life by conducting research on the cosmic history of biogenic compounds, prebiotic evolution, and the early evolution of life. This is accomplished via laboratory experiments, theoretical studies/computational modeling, and field investigations. Branch personnel are also involved in the development of flight instruments, experiments, and small mission definition with particular emphasis being placed on studies of Mars and the development of instrumentation for martian flight missions. Several Branch scientists are part of a task module that is a component of the Ames membership in the Astrobiology Institute. Branch scientists provide expertise in exobiology, astrobiology, planetary protec-tion, and other areas of planetary science to NASA Headquarters and external review and advisory panels, and some serve as editors and associate editors of scientific journals.

Exobiology studies includes the history, distribution, and chemistry of biogenic elements in the solar system; prebiotic chemical evolution and the origin of life; and the history of Earth's early biosphere as recorded in microorganisms and ancient rocks. The research is conducted both on Earth and in space. The Branch also serves as the center of expertise within the agency for issues of planetary protection. As the agency lead center in exobiology, Branch exobiologists exercise a leadership role in NASA's Exobiology Program through program planning, performance reviews, advisory services to related NASA programs, and external relations.

David F. Blake

Chief, Exobiology Branch

DEFINITIVE MINERALOGICAL ANALYSIS ON MARS

David Blake and Philippe Sarrazin

The search for evidence of life, prebiotic chemistry or volatiles on Mars requires the identification of rock types that could have preserved these. Anything older than a few tens of thousands of years will either be a rock, or will only be interpretable in the context of the rocks that contain it.

The key role that definitive mineralogy plays is a consequence of the fact that minerals are thermodynamic phases, having known and specific ranges of temperature, pressure and composition within which they are stable. More than simple compositional analysis, definitive mineralogical analysis can provide information about pressure/temperature conditions of formation, past climate, water activity, the presence of biologically significant gases and the like.

Mineralogical identification—the determination of *crystal structure*—is a critical component of Mars Astrobiological missions. Definitive mineralogical instruments have never been deployed on Mars, and as a result, not a single rock type or mineral has been identified with certainty.

Minerals are defined as unique structural and compositional phases that occur naturally. There are about 15,000 minerals that have been described on Earth. There are likely many minerals yet undiscovered on Earth, and likewise on Mars. If an unknown phase is identified on Mars, it can be fully characterized by structural (X-ray Diffraction, XRD) and elemental (X-ray Fluorescence, XRF) analysis without recourse to other data because XRD relies on the first principles of atomic arrangement for its determinations. Diffraction is the principal means of identification and characterization of minerals on Earth.

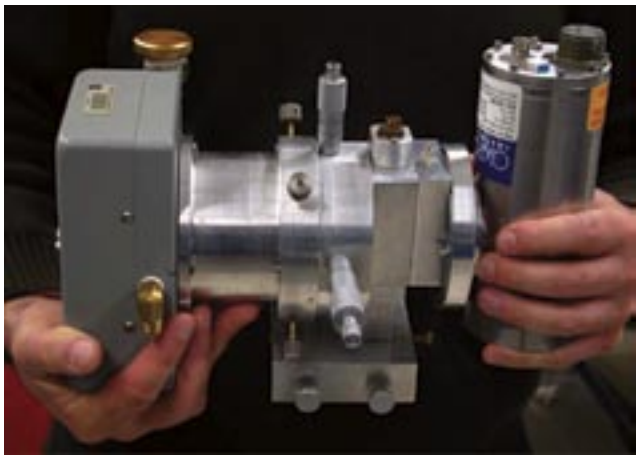

Figure 1: CheMin II instrument

The CheMin II XRD/XRF instrument (so called because it is capable of CHEmical and MINeralogical analysis) is capable of quantitative mineralogical analysis. The original prototype has been modified (and made portable) by replacing the Philips-Norelco tube tower with an Oxford Instruments small-focus X-ray source (figure 1). In the current version, the small-focus source (70 µm diameter) and a 30 µm final aperture yield a beam diameter at the sample of ~100µm. A wide variety of minerals and rocks has been analyzed utilizing 40 KV accelerating voltage and 0.25 microamps beam current (10 watts). Interpretable patterns of single minerals can be obtained in less than an hour and quantifiable patterns of complex rocks can be obtained in a few hours.

Quantitative mineralogical analyses have been obtained for a variety of minerals using the CheMin II prototype. Refinements have been made of apophylite (a zeolite), limestone, limestone-evaporite, San Carlos olivine, the Mars meteorite Zagami and many others. Calculated cell parameters for the San Carlos olivine from CheMin data are 4.76, 10.24, and 5.99 Å, yielding a composition of Fo90 - Fo95 (figure 2).

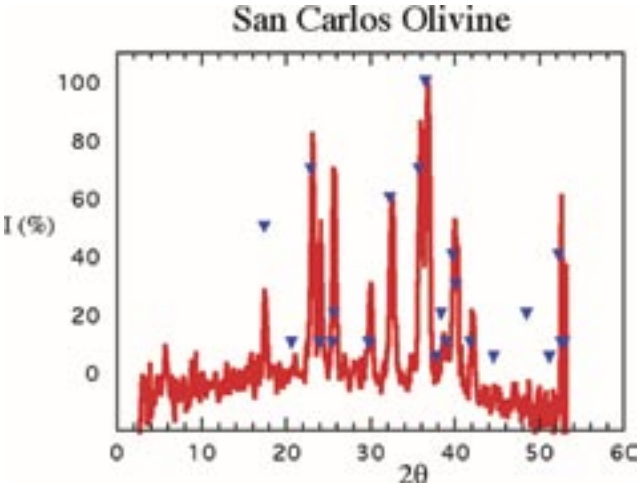

Figure 2: Diffractogram of CheMin olivine data (red) vs. positions & intensities of forsterite standard (blue triangles)

A third prototype (CheMin III) is under construction that will utilize a small-focus (45 µm) Co X-ray source, an ambient pressure sample insertion mechanism, and an air-cooled, vacuum sealed, 1K X 1K deep-depleted CCD. Deep-depletion will increase the quantum efficiency (QE) for diffracted X-ray detection from 0.05 to nearly 0.50, yielding a 10-fold increase in count rate. The instrument will be operable from a laptop computer running Labview™ software.

CARBON ISOTOPIC FRACTIONATION ASSOCIATED WITH CYANOBACTERIAL BIOMARKERS: 2-METHYLHOPANOIDS AND METHYL-BRANCHED ALKANES.

Linda L. Jahnke, Tsegereda Embaye and Roger E. Summons

Biomarker analysis of ancient organic sediments has demonstrated the dominance of cyanobacterial ecosystems going back in geological time to 2700 Ma. The presence of 2-methyl-hopanoids and methyl-branched alkanes serve as biomarkers for this important group of oxygenic photosynthetic bacteria both in geological samples and in contemporary environments. Knowledge of the molecular structures and the carbon isotopic compositions of individual biomarkers might allow recognition of source organisms and environmental conditions. Cyanobacteria have been the significant primary producers throughout most of Earth's history, but little is known about the molecular diversity of their lipid biomarkers or the effects of carbon isotopic fractionations associated with the biosynthesis. We have focussed our study on several pure cultures which synthesize a variety of branched alkanes, and 2-methylhopanoids. Two of these cyanobacterial cultures, *Chlorogloeopsis fritschii* and *Phormidium luridum*, have been obtained from culture collections. Both organisms contain several distinct cyanobacterial biomarker lipids (Fig. 1).

Our work has also involved analysis of a collection of natural microbial mats constructed by fine filamentous cyanobacteria, the coniform mats found in the Midway Geyser Basin of Yellowstone National Park. These mats are considered the best analog for the fossil conophytons, a type of stromatolite dating back 3450 Ma. From these mats, other *Phormidium* cyanobacteria have been isolated, and their phylogenetic relatedness and lipid biomarkers characterized. It is our hope that this study will elaborate links between Phormidium and conophyton stromatolites.

Pure culture studies: Cyanobacteria were grown in feed-batch cultures with a constant gas flow, either high CO_2 (generally 1% v/v) or atmospheric air. Little difference was observed for ^{13}C-discrimination associated with growth on 1% CO_2 for three individual cyanobacteria, with biomass ranging from 21.6 to 22.4‰. The bacteriohopanepolyol (BHP) was generally depleted relative to biomass by 4.0 to 7.4‰ with 2-methyl-BHP often somewhat heavier than non-methylated BHP by 2 to 3‰. This relationship did not appear to depend on the level of CO_2 provided to the culture for growth.

The isotopic composition of alkanes was more complex and depended on carbon chain length and methyl-branching. Generally for each individual cyanobacterium, longer chain and methylated alkanes were heavier

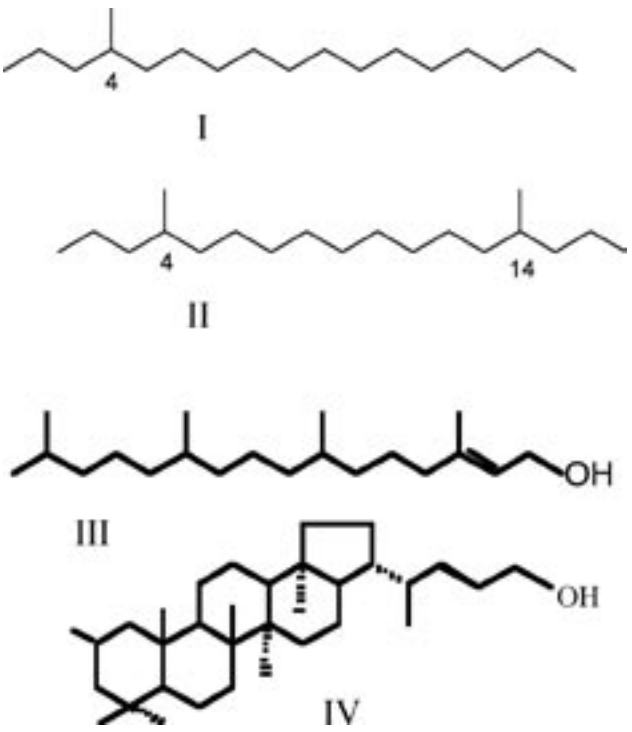

Fig. 1. Lipid biomarkers isolated from Chlorogloeopsis fritschii. *Branched alkanes 4-methyl-octadecane (I) and 4,14-dimethyloctadecane (II). Isoprenoid lipids represented by chlorophyll derived phytol (III) and BHP derived 2-methylhopanol (IV).*

than their shorter, normal chain counterparts. In *C. fritschii* grown with 1% CO_2, n-C_{17} was depleted by 12.1‰ relative to biomass, while the 4-methyl- and 3-methylheptadecanes were 8.7 and 8.0‰, and the 4,14-dimethyl- and 3,14-dimethylheptadecanes were 2.4 and 2.9‰, respectively. A 5-methyl-octadecane present in low abundance (~3% of alkanes) was also relatively depleted, particularly in relation to monomethyl heptadecanes (Fig. 2). Similarly, in an air sparged P. luridum, n-C_{17} was depleted by 10.5‰ relative to biomass, and the 7-methyl- and 7,11-dimethylheptadecanes by 5.6 and 4.5‰, respectively.

The biomarker composition of the cyanobacteria isolated from Yellowstone coniform mats varied considerably. These cyanobacteria were predominately of the *Phormidium*-type and formed three distinct groups based both on lipid biomarker composition and 16S rRNA sequence similarities. 16S rRNA gene sequence analysis indicated that the three groups were closely related to one another and to *P. luridum*. Although these *Phormidium* groups were closely related, lipid composition varied widely. The group represented by *Phormidium* RCO synthesized only straight chain alkanes, primarily n-C_{18} and n-C_{19}, and no hopanoids. *Phormidium* RCG, which represented the second group of isolates, contained n-C_{17} and large amounts of methyl-branched alkanes (7-methyl- and 7,11-dimethylheptadecanes) similar to those found in *P. luridum*, but only a C_{32} BHP (as IV° Fig 1). The biomarker composition of the last group, represented by Phormidium OSS4, was the most complex. The major BHPs were a 2-methyl-C_{31} and a desmethyl-C_{31} with lesser amounts of the C_{32} homologs. The alkanes extended from n-C_{16} to n-C_{22} with a variety of methyl-branched alkanes. The alkane composition of *Phormidium* OSS4 was also affected by growth temperature. In the 30 to 45°C range, mid-chain methyl-alkanes were the major constituents of the hydrocarbon fraction. A small amount of dimethyl-alkane was also present, primarily in a culture grown at 30°C. The straight chain and 2-methylalkanes increased with higher growth temperatures and were dominant in cells grown in the 50 and 55°C range. The isotopic relationships among biomarkers were generally similar to those described above. In one culture grown at 55°C, the fractionation factor ($\varepsilon_{biomarker}$) for individual alkanes

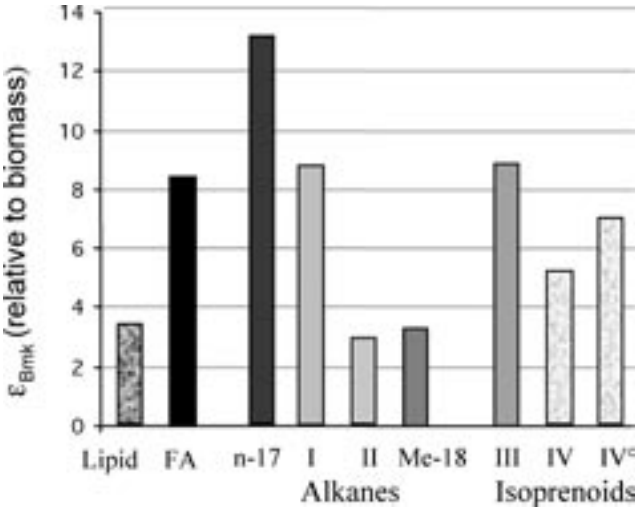

Fig. 2. Carbon isotopic composition of lipid biomarkers isolated from C. fritschii *grown with 1% CO_2 where the fractionation factor, $\varepsilon_{biomarker}$, is calculated for the lipid components relative to biomass and = (a-1)1000. Normal (n-17) heptadecane and 5-methyloctadecane (Me-18). Numerals refer to structures in Fig. 1, IV° is the desmethyl equivalent of IV, a C_{32} hopanol.*

relative to biomass ranged from 8.1‰ for n-C_{17} to -0.6‰ for n-C_{21} and 2.1‰ for 2-methyloctadecane to –0.6‰ for 2-methylicosane. There was also a suggestion of increased fractionation associated with alkane synthesis as the growth temperature was increased with an overall e value obtained by mass balance of 5.5‰ for a 30°C culture and 2.1‰ for a 55°C culture.

Environmental studies: The biomarker composition of the coniform mats varied considerably depending on the environmental setting, but generally contained both 2-methyl-BHP, C_{31} and C_{32} types, and methyl-branched alkanes, primarily the 7-methyl-heptadecane and 7,11-dimethylheptadecane. As with pure culture studies, the monomethyls- were somewhat more depleted (ε = ~11‰) relative to total organic carbon (TOC) than the dimethyls (ε = ~9‰). Isoprenoid lipids were generally more enriched in ^{13}C than alkanes. Values for chlorophyll associated phytols and the desmethylhopanols (C_{31} and C_{32}) were both generally in the 5 to 6‰ range. As with pure cultures, the 2-methylhopanols were 1 to 2‰ heavier than their desmethyl homologues.

PENNING IONIZATION ELECTRON SPECTROSCOPY (PIES)

Daniel R. Kojiro, Valery A. Sheverev, Nikolai A. Khromov, and Norishige Takeuchi

Exobiology flight experiments require highly sensitive instrumentation for the in situ analyses of volatile chemical species that occur in the atmospheres and surfaces of various bodies within the solar system. The complex mixtures encountered place a heavy burden on the analytical instrumentation to detect and identify all species present. The minimal resources available onboard for such missions mandate that the instruments provide maximum analytical capabilities with minimal requirements of volume, weight and consumables. The objective of this research is to develop analytical technologies for the analysis of complex extraterrestrial mixtures of interest to Astrobiology. These are often complex mixtures with many components ranging in concentration from a few parts-per-billion to a few per cent. Typical analytical requirements are:

- *Universal response*
- *Part per billion sensitivity*
- *Response range of over 106*
- *Instantaneous recovery time (quick analyses).*

Ideally, the instrument should be able to meet these analytical requirements while operating under severely restricted conditions. It should be:

- *Rugged*
- *Tiny*
- *Use little or no power for operation or for maintenance*
- *Require few consumables (carrier gas).*

A new technique being investigated is Penning Ionization Electron Spectroscopy (PIES). PIES measures the energy of electrons released from sample molecules ionized by collisions with metastable helium (Penning Ionization). From that measurement, the ionization potential of the sample molecule is determined and is used to identify the molecule. PIES has the potential of providing both sample detection and direct molecular identification of a gaseous species.

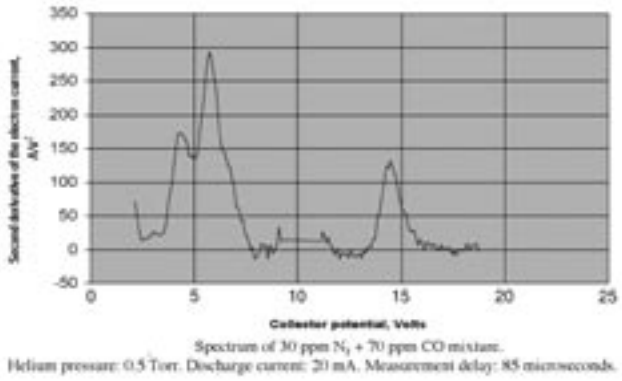

Figure 1 is a PIES spectrum of a mixture of N_2 and CO, (both molecular weight 28). The peak at 14.4 electron volts is from ionization from two metastable helium atoms. The primary N_2 peak is at 4.2 electron volts and for CO the primary peak is at 5.8 electron volts. Although both gases have the same molecular weight, they produce separate identifying peaks in the PIES spectrum.

THE ORIGIN AND EARLY EVOLUTION OF MEMBRANE PROTEINS

Andrew Pohorille and Michael Wilson

The formation of protocells—membrane enclosed structures endowed with ubiquitous cellular functions—was a central step in evolution from inanimate to animate matter. Many essential cellular functions are performed by proteins embedded in membranes. These proteins or protein complexes are among the largest macromolecular structures found in cells and their mode of action is often complicated and subtle. This creates a difficulty for explaining the origin of cells. If functions of membrane proteins were essential to the existence of even the simplest cell it must be explained how they could have been performed, even less efficiently or selectively, by simple precursors of proteins - peptides.

On the basis of a series of detailed molecular dynamics computer simulations it was demonstrated that the emergence of membrane proteins might have been quite feasible. Specifically, the stability of monomers and dimers of a peptide built of leucine (L) and serine (S) amino acids in membrane-mimetic system was studied. The sequence of this peptide was $(LSLLLSL)_3$. Also the transmembrane aggregate of four identical a-

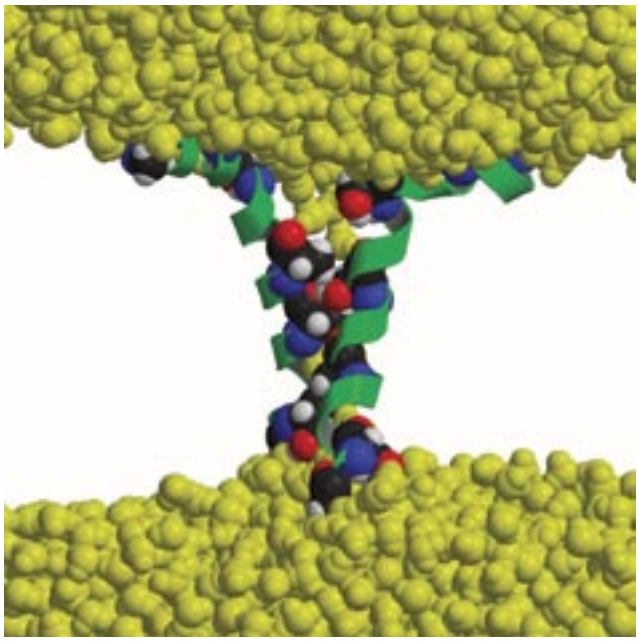

Figure – the $(LSLLLSL)_3$ peptide in the transmembrane orientation. The membrane-forming molecules located between two lamellae of water (in yellow) was removed for clarity.

helices that forms an efficient and selective voltage-gated proton channel was investigated. Finally, a peptide that forms sequence-specific dimers was studied.

Many peptides are attracted to water-membrane interfaces. Once at the interface, most nonpolar peptides spontaneously fold to (-helices. Whenever the sequence permits, peptides that contain both polar and nonpolar amino also adopt helical structures, in which polar and nonpolar amino acid side chains are immersed in water and membrane, respectively. The formation of such helices is primarily governed by the sequence of polar and nonpolar amino acids. Considering that specific identity of side chains is less important, the existence of helical peptides at interfaces of protocells should not have been rare.

Helical peptides located parallel to the interface could insert into the membrane and adopt a transmembrane conformation. However, insertion of a single helix is associated with a positive (unfavorable) free energy change. This is because polar groups in the peptide, which remain partially immersed in water at the interface, become completely dehydrated. However, the loss of free energy is smaller for helices than for other structures because polar groups in the peptide backbone are involved in intramolecular hydrogen bonding.

The unfavorable free energy of association can be regained by spontaneous association of peptides in the membrane. The first step in this process is the formation of dimers, although the most common structures involve aggregates of 4–7 helices. The helices could readily arrange themselves such that they formed pores capable of transporting ions and small molecules across membranes. Stability of transmembrane aggregates of simple proteins is often only marginal and, therefore, it can be regulated by environmental conditions, such as external electric fields, specific nature of membrane-forming molecules or small changes in the sequence of amino acids. This ability to respond to environmental signals might have led to the earliest, although quite imprecise, regulation of transmembrane functions.

A key step in the earliest evolution of membrane proteins was the emergence of selectivity for specific substrates. Many simple channels could achieve selectivity through placing one or only a few properly chosen amino acids in certain positions along the channel, which acted as filters or gates. From the evolutionary standpoint it is a convenient solution because it does not require imposing conditions on the whole sequence.

Many further steps were required before the simple aggregates of transmembrane peptides reached the structural and functional complexity, diversity and refinement of contemporary membrane proteins. The helices became connected by extra-membrane linkers to stabilize them inside the membrane. The resulting proteins aggregated to larger, higher-order structures. Protein sequences became optimized for highly specific functions. Finally, membrane proteins acquired large, water-soluble domains, which play regulatory role or help to supply energy for active transport. These evolutionary advancements opened the doors for the emergence of multicellular organisms.

CARBON NANOTUBE FIELD EMISSION X-RAY TUBE

Philippe Sarrazin, Lance Delzeit, David Blake

ARC is developing an X-ray tube for CheMin, a mineralogical instrument for planetary exploration. This instrument combines X-ray diffraction and X-ray fluorescence techniques to provide definitive mineralogical analyses onboard a lander or a rover. Space deployment of this instrument requires an X-ray tube that is miniature in size, low-power, and microfocused, meaning that the X-rays are generated from a very small spot (10–50 µm in size). Such an X-ray tube is not readily available.

An X-ray tube is composed of an electron-source facing a metallic target inside a vacuum enclosure. Electrons emitted by the source are accelerated towards the target by high-voltage. The collision of high-speed electrons with the target leads to the emission of X-ray radiation characteristic of the target material. The thermionic sources (hot filament) commonly used as electron sources (in conventional X-ray tubes) cause major problems for the deployment of a miniature X-ray tube in space: poor efficiency, heat generation, limited focusing capability. An alternative method for emitting electrons is field emission which is based on the extraction of electrons from sharp tips by an electric field. Field emitters can potentially improve efficiency, stability and reliability of miniature X-ray sources, however, until recently, no field emitter have shown the appropriate characteristics for X-ray tube application. Miniature microfocused x-ray tubes require very small emitters (10–100 µm in diameter) that are yet capable of delivering sustainable currents of about 100 µA. The work presented here is the development of a new type of electron-source for miniature X-ray tubes using field emission from carbon nanotubes (CNTs).

CNTs are the sharpest objects known, are very good conductors of electricity and are mechanically and chemically extremely robust. This combination of properties makes them very good candidates for field emission. CNT emitters were fabricated using thermal Chemical Vapor Deposition (CVD) techniques developed by Ames (figure 1). A major effort was dedicated to the adaptation of the CNT growth processes to different types of substrate materials. Electron emission properties of multiwall nanotube films of various densities were characterized using an instrument specifically developed for this project. Very good emission characteristics were measured with turn-on fields of 1.5 to 2 V μm^{-1}, and high current densities (figure 2). With optimized CNT density and very small emitters, outstanding sustainable current densities above 1 $A.cm^{-2}$ under moderate electric field (7–10 V μm^{-1}) have been measured. An industrial partner, Oxford XRT Inc., has implemented these cathodes in miniature X-ray tubes and is conducting performance tests. An X-ray tube is being submitted to a life-time test and has been oper-

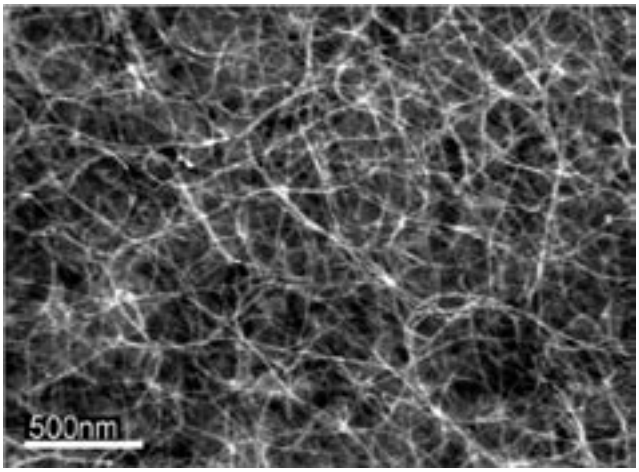

Figure 1: Carbon nanotube film obtained by thermal CVD.

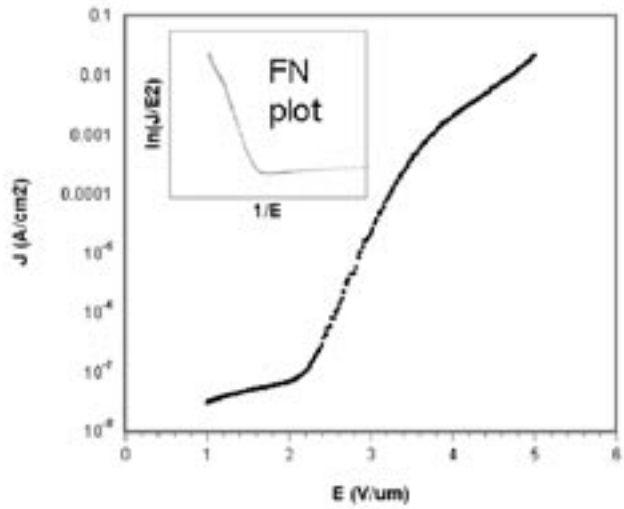

Figure 2: Example of field emission from a CNT film; main: applied electric field vs current density; insert: same data in a Fowler-Nordheim plot characteristic of field emission.

ated continuously for several months without any sign of deterioration. Current efforts are oriented towards the optimization of the emitter fabrication to produce even smaller electron-source with improved current density and stability.

Figure 3: CNT based miniature X-ray tube built by Oxford XRT Inc.