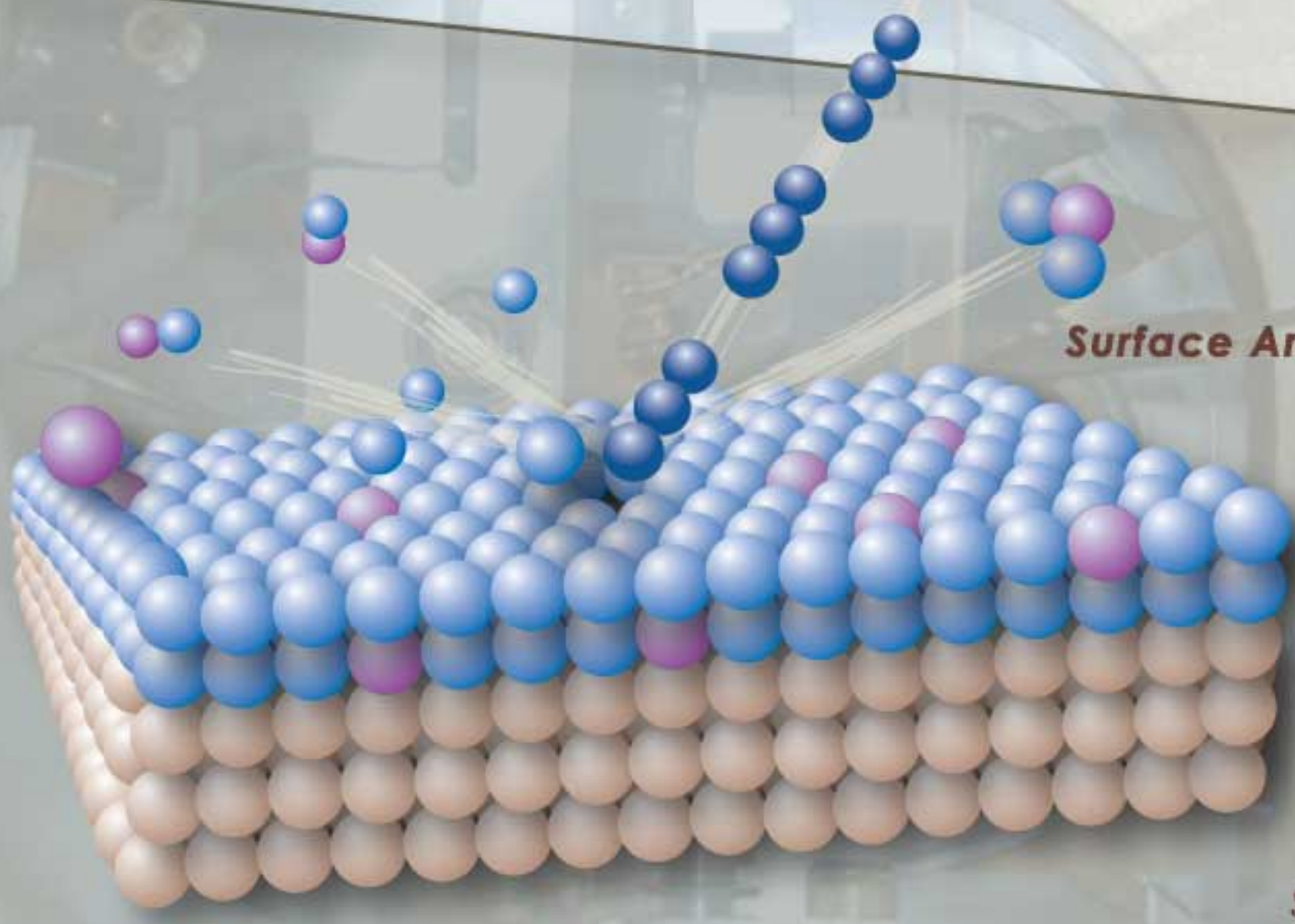
NESAC/BIO

National ESCA and Surface Analysis Center
for Biomedical Problems

"Frontiers in Biomedical Surface Analysis"



Surface Analysis Applied to:

- Biosensors
- Microarrays
- Cell Culture
- Biocompatibility
- Medical Devices
- Controlled Release
- Surface Modification
- Surface Biomolecules
- Biological Interactions

An Instrumentation Resource funded by:

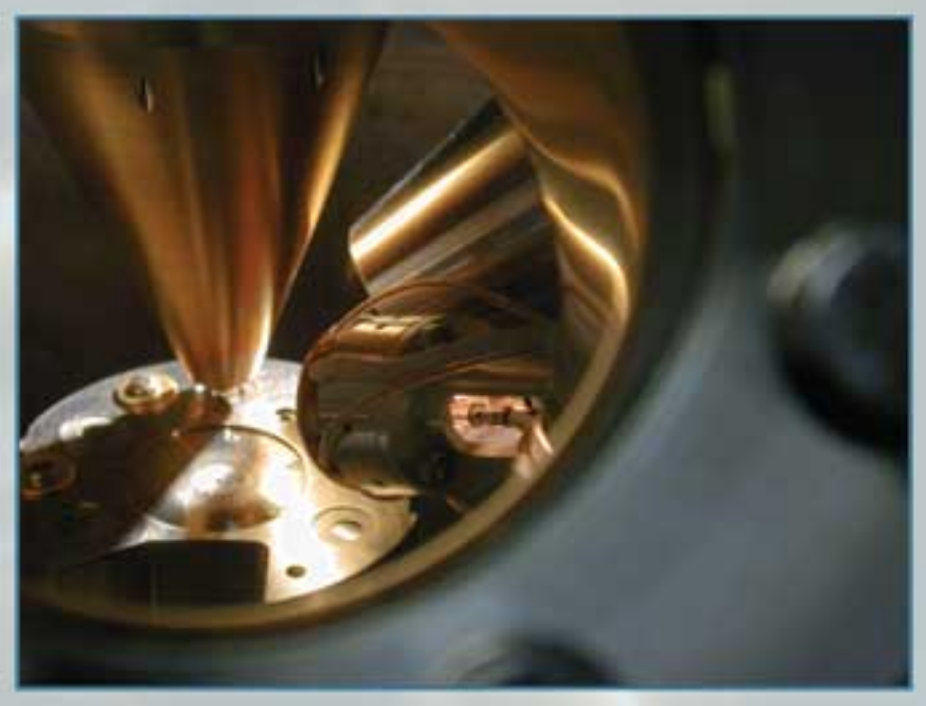
- The National Institute of Biomedical Imaging and Bioengineering
- The National Center for Research Resources
- The National Institutes of Health

About NESAC/BIO

NESAC/BIO's mission is to develop and apply new surface analysis technologies for biomedical research. Our surface analysis expertise combined with state-of-the-art instrumentation and data analysis methods allows us to expand the frontiers of biomedical surface analysis.

NESAC/BIO provides expert and detailed information about surface properties (composition, structure, spatial distribution, and molecular orientation) needed by biomedical researchers. State-of-the-art instrumentation is coupled with specialized experimental protocols and data analysis methods to address a wide range of biomedical research and development issues. Combining our surface analysis expertise with collaborators' biomedical expertise provides the information needed to develop new generations of biomaterials and biomedical devices.

New developments in materials science and biology that make it possible to control surface chemistry on an increasingly smaller scale are placing increased demands on surface analysis techniques. These developments include decreased lateral dimensions of chemical variations, increased complexity of molecules being introduced at the surface, and increased sophistication of surface manipulations. NESAC/BIO's continuing work at the frontiers of surface analysis allows us to meet the challenges and opportunities presented by these new developments.



NESAC/BIO continually evolves to meet new challenges and opportunities for biomedical surface analysis. Since starting as an ESCA center in 1983, NESAC/BIO has added the complementary techniques of ToF-SIMS, SPM, NEXAFS, SPR and SFG. Within each technique, the types of analyses have also evolved to provide a more comprehensive characterization of complex biomedical surfaces. The types of samples analyzed has also evolved over the years, from conventional polymers to self-assembled monolayers to biorecognition surfaces to microarray surfaces. To read more about the history, development and future opportunities for biomedical surface analysis see: Castner and Ratner, Surface Science, 500 (2002) 28-60.

Chemical State Imaging

As feature sizes continue to decrease imaging modalities must be developed to provide detailed, spatially resolved information about the chemical state of these features. NESAC/BIO has added new imaging instrumentation to complement our ongoing research in biorecognition AFM imaging. Imaging ESCA quantifies the surface chemical composition of biomaterials at spatial resolutions down to ~10 microns. Imaging ToF-SIMS provides molecular structure information at spatial resolutions below 1 micron. Combining C_{60} sputtering with ToF-SIMS imaging now permits 3-D images to be acquired for some biologic and organic materials. The adjacent figure shows 3-D images of a drug-loaded polyurethane coated onto a glass substrate.

NESAC/BIO Offers

NESAC/BIO interacts with the biomedical community through the five components of our program.

Core Research

The mission of NESAC/BIO's core research projects is to advance the state-of-the-art in biomedical surface analysis methods. This includes the development of new techniques, novel experimental protocols, and advanced data analysis methods. Current focus areas of NESAC/BIO's core research program include standards & model systems, chemical state imaging, analysis of complex biological surfaces, and development of new instrumentation and experimental protocols

Collaborative Research

NESAC/BIO collaborative research applies surface analysis methods developed in our core research to a wide range of challenging biomedical problems, including: adsorbed protein films, self-assembled films, cell growth surfaces, bacterial adhesion, implant corrosion, biomineralization, recognition biomaterials, and the characterization of oligonucleotide probes. Materials studied include immobilized biomolecules, ceramics, composites, fabrics, glasses, metals, polymers, tissues, microelectronics, and self-assembled films.

Analytical Service

NESAC/BIO can perform routine surface analysis on your biomedical samples. Service analyses performed include quantification of surface elemental and functional group composition, depth profiling, and surface imaging. Interpretive reports discussing the results of these analyses are provided to the users.

Dissemination

NESAC/BIO publishes research and review articles. We staff display booths and give presentations at major biomaterials and surface analysis scientific meetings. Our web site, which includes online standard spectra, is a resource for the biomedical surface analysis community.

Training

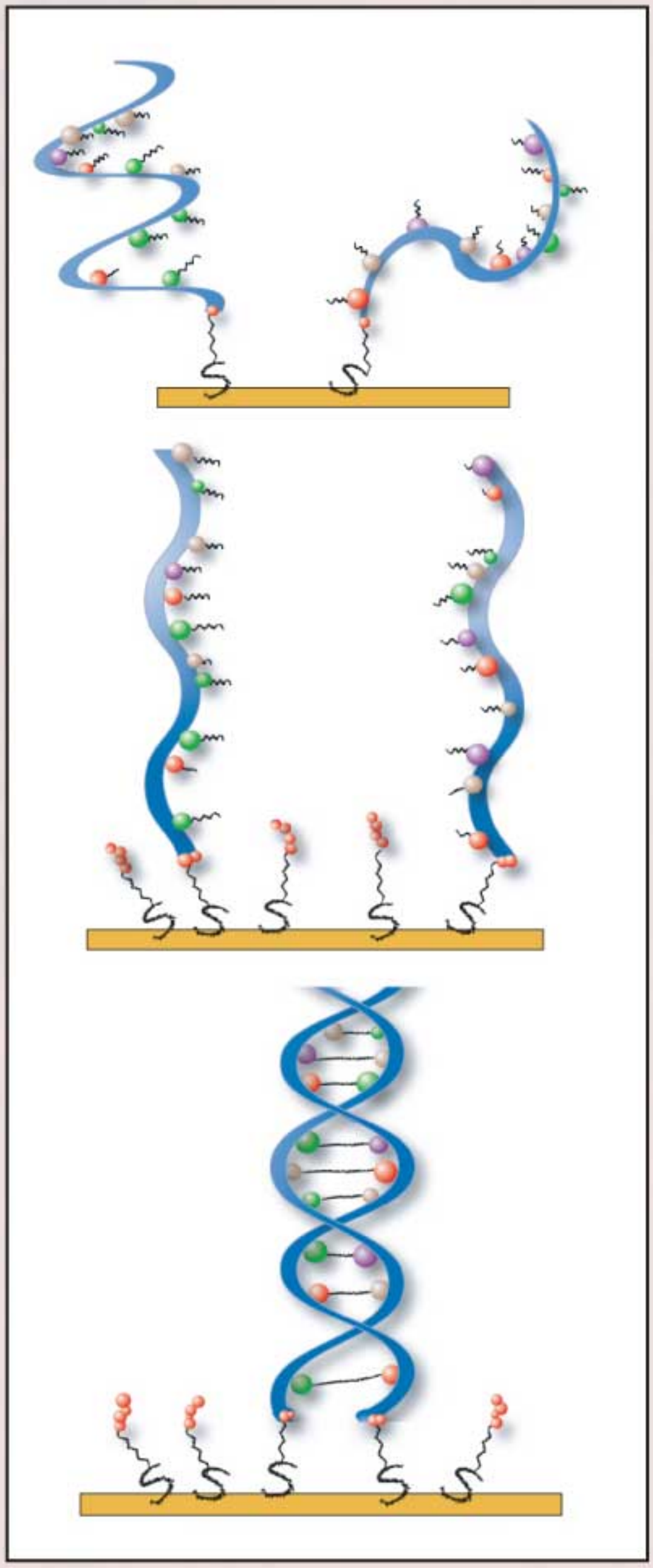
NESAC/BIO offers training in all aspects of surface analysis techniques, including theory, instrument operation, data acquisition, and data analysis. We offer courses, seminars, workshops, and one-on-one instruction. NESAC/BIO holds an annual training workshop each August, which includes lectures and surface analysis demonstrations in our laboratories. Attendees learn the capabilities of surface analysis methods and how to review data received from surfae analysis laboratories.

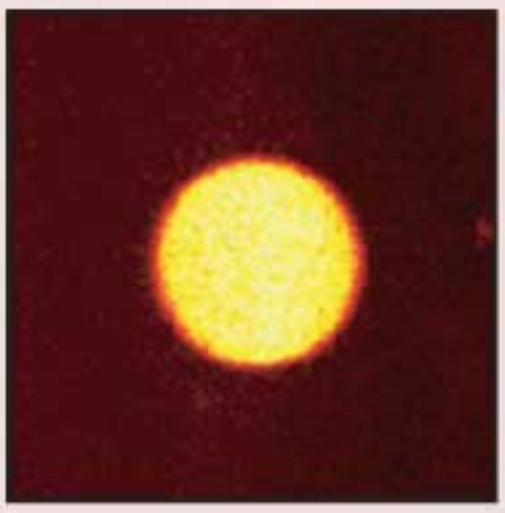
Characterization of DNA Surfaces

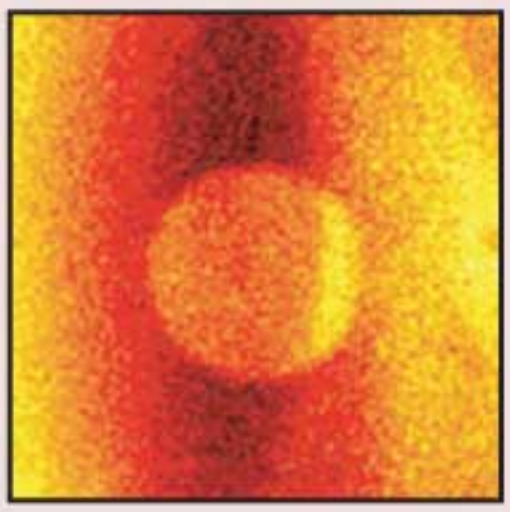
NESAC/BIO provides detailed, comprehensive characterization of complex surfaces.

Surface capture assays such as DNA microarrays are widely used biomedical devices, but the lack of quantitative information they currently provide has limited their impact in clinical applications. NESAC/BIO, in collaboration with Prof. David Grainger's research group at the University of Utah, has employed a comprehensive, multi-technique approach, including ESCA, ToF-SIMS, SPR, NEXAFS, ^{32}P radiolabeling and fluorescence measurements, to characterize the structure and composition of DNA surfaces and relate that information to their hybridization properties. Both model systems (i.e., thiolated DNA on gold surfaces) and microarrays are being investigated.

For thiolated DNA monolayers incorporation of short chain diluents causes a more upright orientation of the ssDNA oligomers and improved hybridization efficiency. By varying the type of diluent and monolayer architecture the hybridization performance in complex biological environments can be improved. For microarrays, imaging ESCA is used to determine hybridization efficiencies in individual microspots, while imaging ToF-SIMS is used to investigate the heterogeneities that exist within those microsposts. To read more about the surface analysis of DNA surfaces see Lee, et al., in JACS 129 (2007) 9429-9438; Anal. Chem. 79 (2007) 4390-4400; and Anal. Chem. 78 (2006) 3316-3325.







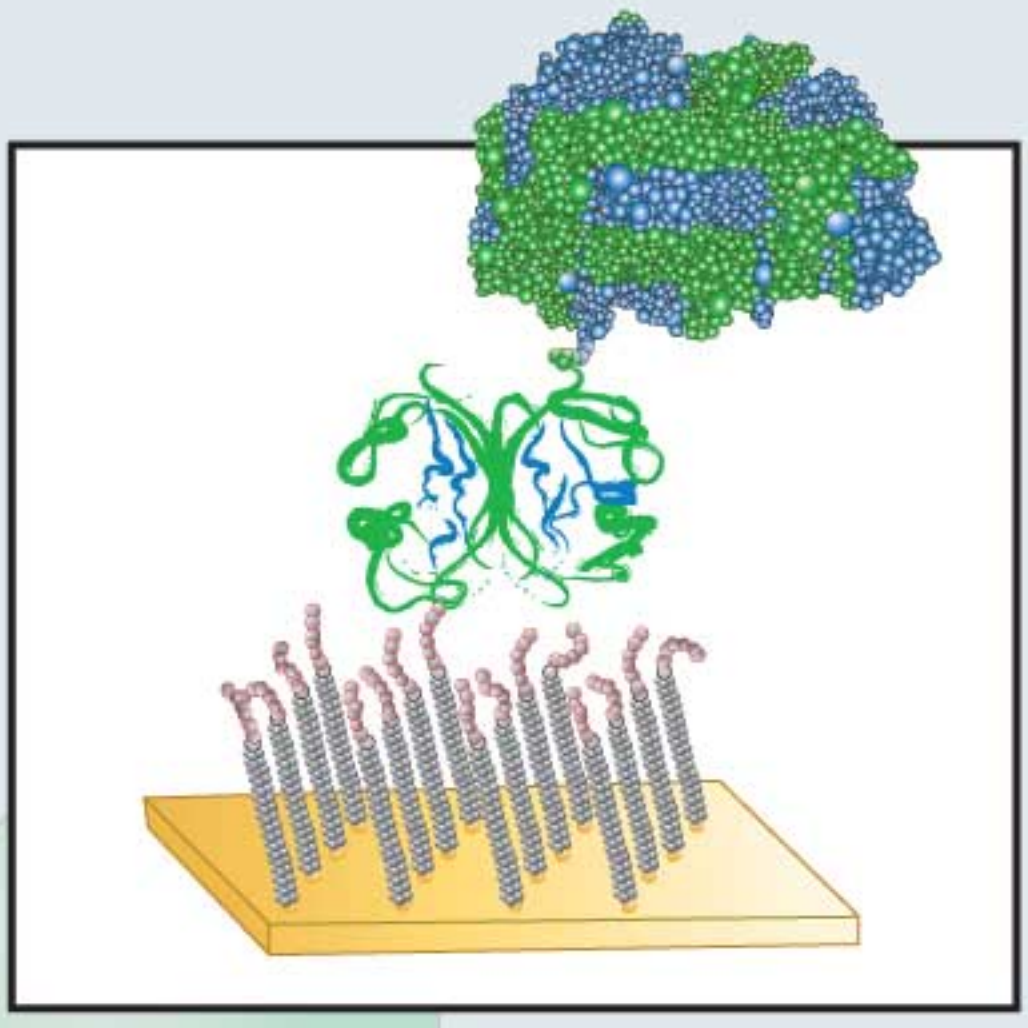
Characterization of Immobilized Proteins

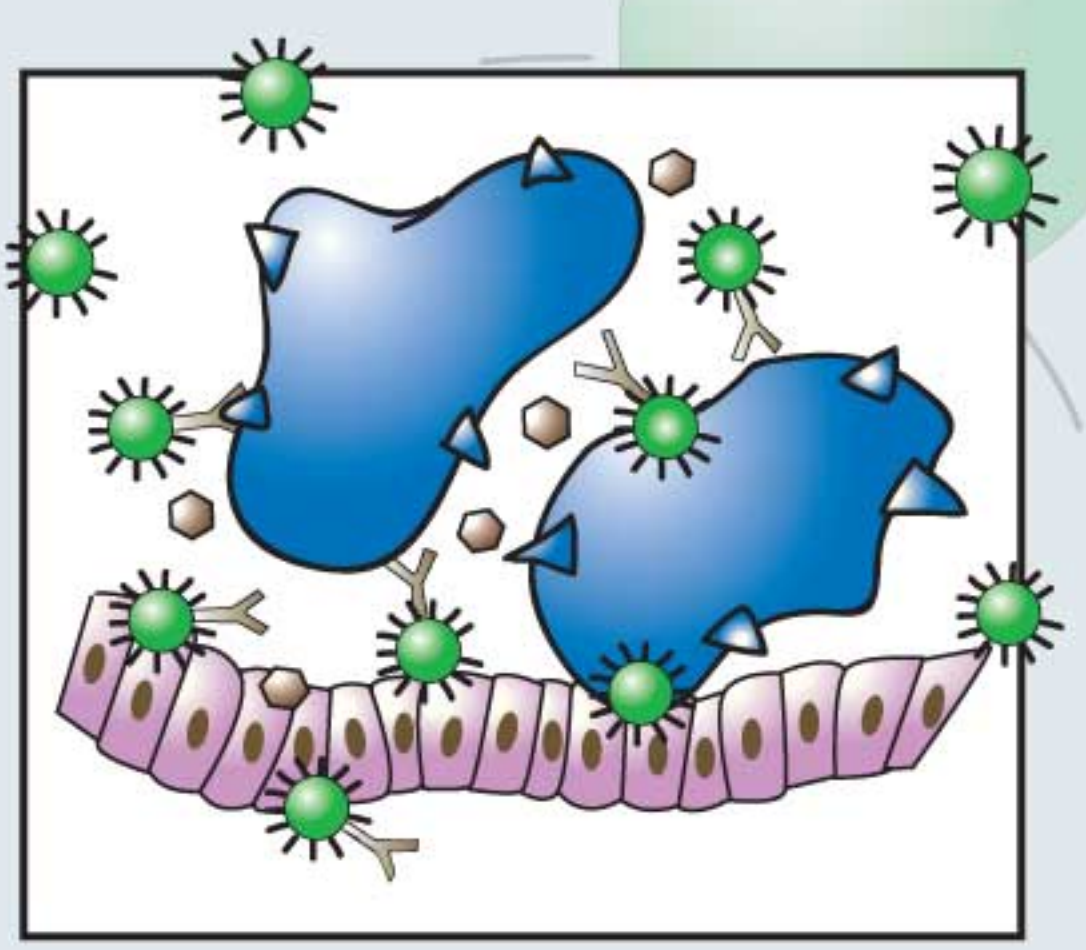
Techniques for determining the identity, concentration, orientation, spatial distribution and activity of immobilized proteins.

A few seconds after a biomaterial is implanted into the body it becomes covered with a layer of adsorbed proteins. The composition and structure of this protein film will determine the type of subsequent cellular interactions that occur on the implanted biomaterial. Thus, it is essential to characterize the identity, concentration, conformation, orientation spatial distribution and activity of surface immobilized proteins. Studies at NESAC/BIO using the complementary surface analysis techniques of ToF-SIMS, SPM, SPR, ESCA and multivariate analysis (MVA) are well suited for characterizing immobilized proteins.

ToF-SIMS protein data are complex, consisting of several fragments from each of the amino acids present in the protein, so the intensity pattern of the amino acid fragments must be used to provide information about the protein film. MVA combined with ToF-SIMS provides a powerful approach for determining information about the identity, concentration, conformation, orientation and spatial distribution of immobilized proteins. SPR is used to determine the activity of immobilized proteins (adsorption/desorption kinetics, binding affinities, etc.). AFM is used to visualize individual proteins and measure force interactions between the surface immobilized protein and a functionalized AFM tip. To read more about using surface analysis techniques to characterize immobilized proteins see:

Michel and Castner, SIA 38 (2006) 1386-1392; Hull, et al., Biophysical Journal 93 (2007) 2852 -2860; and Tyler, et al., Biomaterials 28 (2007) 2412-2423.





NESAC/BIO Instrumentation and Techniques

ESCA - Electron Spectroscopy for Chemical Analysis

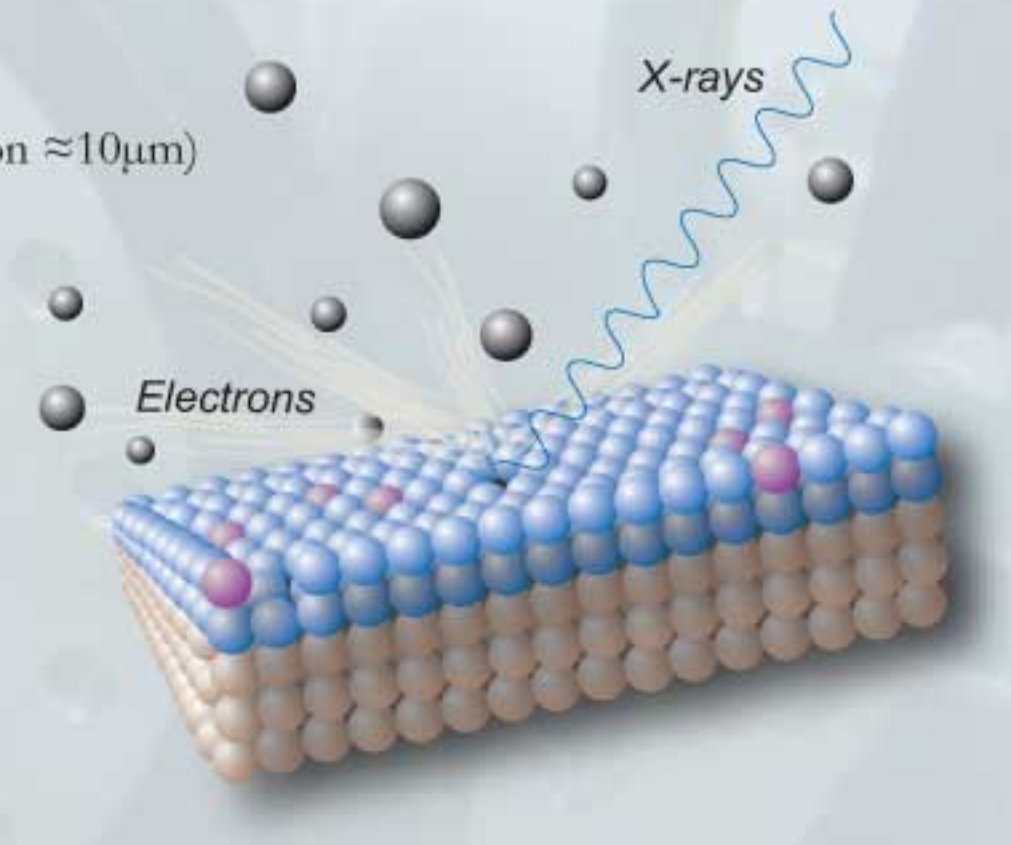
Monochromatic X-rays strike the surface. The energies of the ejected photoelectrons are used to determine which elements and functional groups are present in the surface region. The intensities of these photoelectrons are used to determine the concentration of the elements and functional groups.

ESCA Capabilities

- Identification of elements present in the outermost 10nm of a surface (concentrations > 0.1 atomic %)
- Determination of elemental surface composition (±10% or better)
- Information on lateral variation in composition (resolution ≈10μm)
- Information about the molecular environment (oxidation state, bonding atoms, etc.)
- Identification of organic groups using derivatization

Special NESAC/BIO ESCA capabilities

- Frozen-hydrated analysis ("cold stage")
- Multiple sample handling
- Non-destructive depth profiling (outer 10nm)

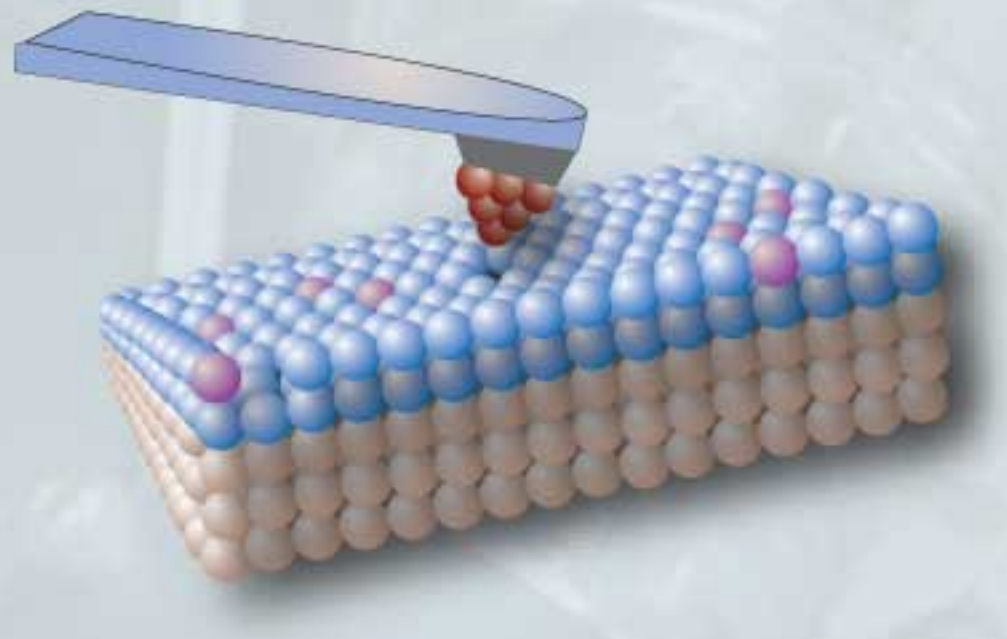


SPM - Scanning Probe Microscopy

A sharp probe tip is scanned across the surface using piezoelectrics. The response of the tip to the surface (change in height, frequency of oscillation, etc.) is used to generate a topographic image of the surface.

SPM Capabilities

- Imaging of surface atoms and molecules
- Analysis under water
- Atomic-scale surface manipulation and modification
- Quantitative measurement of surface roughness
- Force-distance curves
- Lateral and friction forces



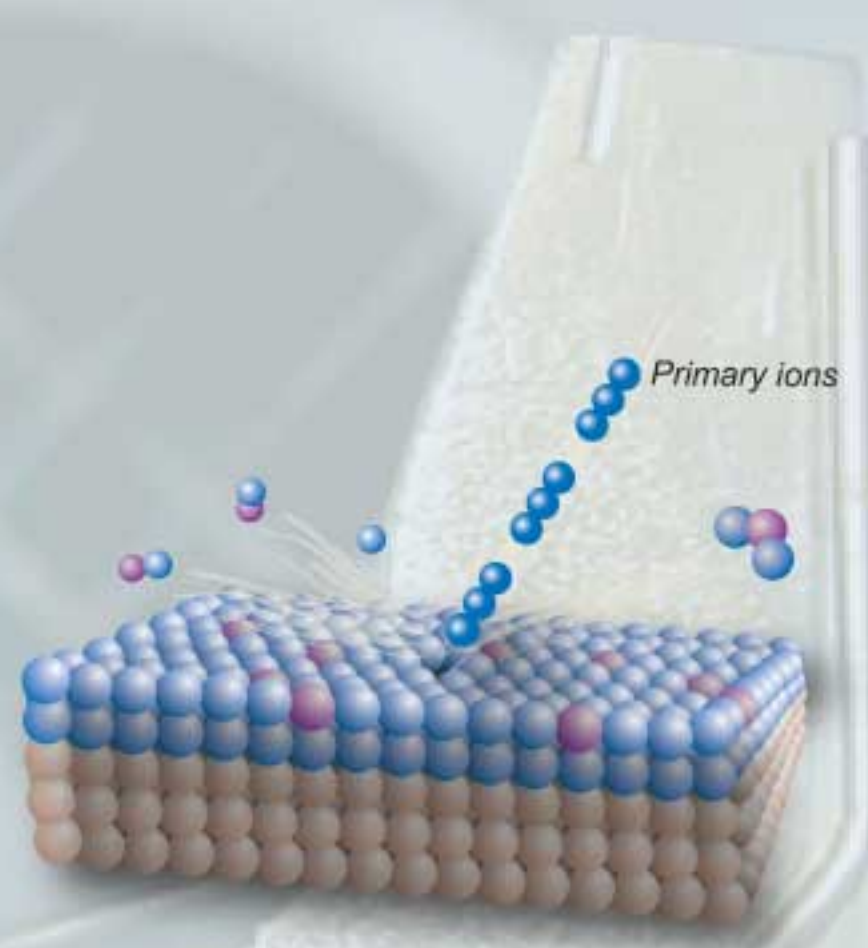
NESAC/BIO Instrumentation and Techniques

ToF-SIMS - Time-of-Flight Secondary Ion Mass Spectrometry

Primary ions strike the surface, sputtering off atomic and molecular fragments, a small fraction of which have a positive or negative charge. These secondary ions are accelerated to a constant energy and the time required for each ion to reach the detector is used to determine their mass.

ToF-SIMS Capabilities

- A mass spectrum of the outermost 1-2nm of a surface
- Identification of structural units present at the surface (e.g. monomeric components and repeat units)
- Fingerprint identification of polymers
- Information on surface degradation and contamination
- Spatial imaging of the surface chemistry
- Molecular depth profiling with C_{60} sputtering



Special NESAC/BIO ToF-SIMS capabilities

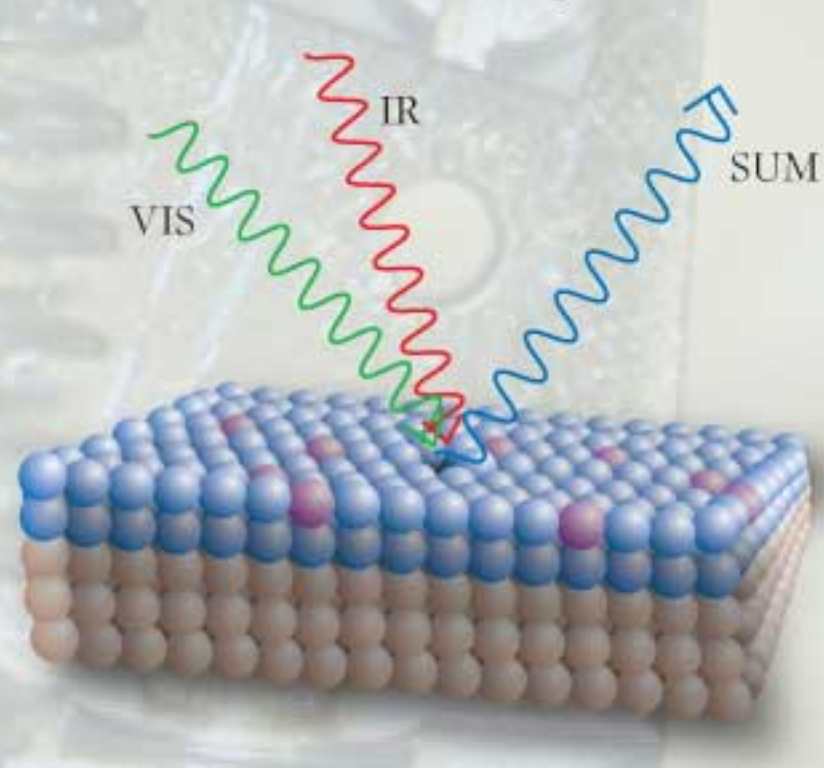
- High resolution mass spectra
- Detection of high mass species
- Imaging (lateral resolution ≈1 micron)
- Multivariate analysis of spectra and images

SFG - Sum Frequency Generation Vibrational Spectroscopy

A tunable IR beam is overlapped in time and space with a visible beam, producing a signal at the sum of the IR and visible frequencies when an interfacial species has vibrational modes that are both IR and Raman active. The intensity of the SFG peaks depends on the concentration, orientation and ordering of the interfacial species.

SFG Capabilities

- Vibrational spectra of species without inversion symmetry (i.e., interfacial species)
- Analysis at the liquid/solid, gas/solid, gas/liquid interface
- Real time analysis of interfacial reactions



Who's Who at NESAC/BIO

Core Personnel

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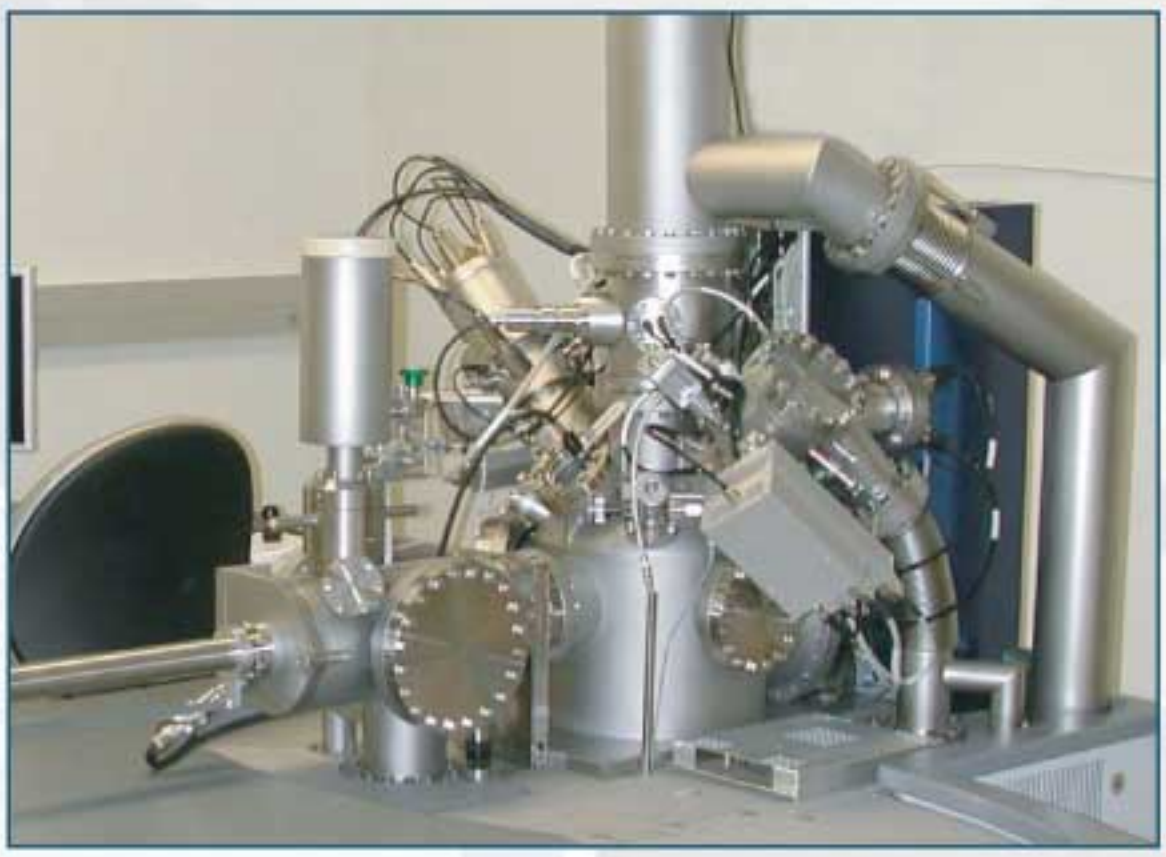
Instrumentation at NESAC/BIO



ESCA (Electron Spectroscopy for Chemical Analysis)

- Kratos AxisUltra DLD
- Surface Science Instruments S-Probe

ToF-SIMS (Time-of-flight Secondary Ion Mass Spectrometry)

- IONTOF 5-100

SPM (Scanning Probe Microscopy)

- Dimension Icon-PT AFM

SPR (Surface Plasmon Resonance)

- Plasmon II

SFG (Sum Frequency Generation)

- EKSPLA