

Analytical Chemistry Cume Based on the Announced Publications:

1. "Single Silver Nanoparticles as Real-Time Optical Sensors with Zeptomole Sensitivity" A. D. McFarland, R. P. Van Duyne; *Nano Lett.* **2003**, 1057.
2. "Biomolecular Recognition Based on Single Gold Nanoparticle Light Scattering" G. Raschke, S. Kowarik, T. Franzl, C. Sönnichsen, T. A. Klar, J. Feldmann, A. Nichtl, K. Kürzinger; *Nano Lett.* **2003**, 3, 935.

10 1. What is a surface plasmon? What is the primary difference between SPR and LSPR?

A surface plasmon is a coherent oscillation of electrons within a thin conducting layer. When the film thickness (or more generally, dimension) of the film is comparable to the electron mean free path in the metal, the amplitude of the plasmon resonance is greatest. The primary difference between SPR and LSPR is the dimensionality of the material. SPR implies a semi-infinite thin film confined only in thickness but otherwise uniform. Localized surface plasmon resonance refers to the optical properties of a particle that is constrained in 3D.

10 2. What was the transduction mechanism in each experiment (i.e., what was the experimental observable and how was it correlated to surface binding)? How was it measured experimentally?

The raw signal in each case was the spectrum of the scattered light. The binding events were transduced by the measured red-shift in the individual particle scattering spectrum. In each case, the spectra were recorded by coupling a monochromator/CCD combination with a dark-field microscope.

10 3. The investigations by Rick Van Duyne's group (and presumably the Raschke work as well) utilized a single CCD camera for imaging AND localized optical spectroscopy. Describe how a single monochromator/CCD camera system can be used for either spatially resolved imaging or localized optical spectroscopy by adjusting only one setting in the monochromator. Can it do both simultaneously?

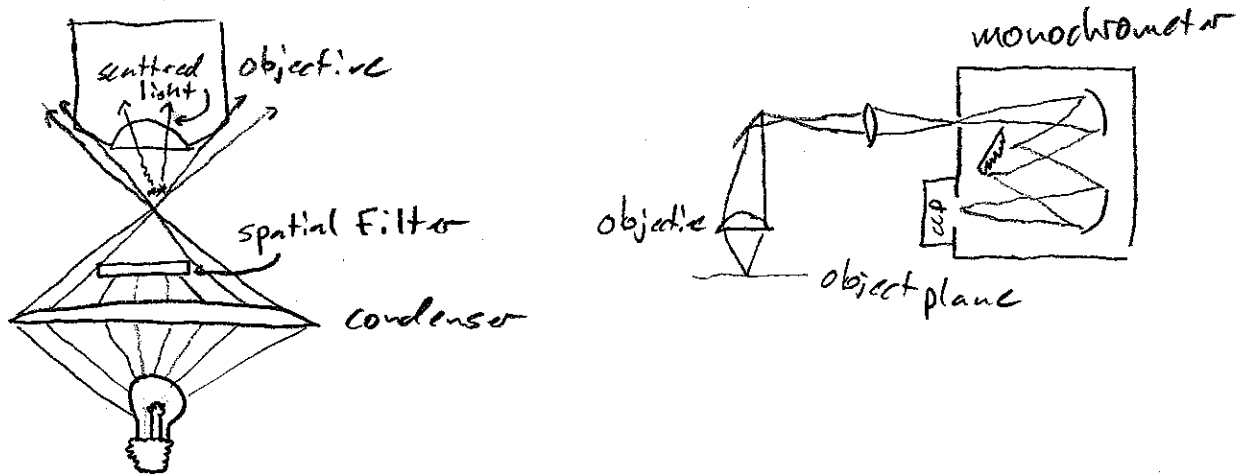
Yes - an imaging monochromator can be configured to simply reflect the raw image back onto the CCD array when positioned along the zero-order diffraction peak (i.e., the specular direction), or it can be used for spectroscopic measurements by repositioning to the first-order diffraction peak and adjustment of the slits/illumination area. In principle, both applications could be done simultaneously, but in practice it would require two CCD cameras.

Alternatively, repositioning the monochromator into the path of the light beam used to excite dark-field scattering would allow for simultaneous imaging and spectroscopy by scanning the monochromator while imaging.

15 4. Describe the fundamental principles and practical implementation of dark-field microscopy. Draw a detailed schematic of a dark field microscope coupled to a CCD camera for detection.

In dark-field microscopy, the center of the beam in the illumination source is removed using a spatial filter, and the sample is illuminated by oblique angles of incidence only. If configured

correctly, the illumination angle is large enough that none of it can be collected directly by the objective. Only the light scattered within the object plane can be efficiently detected, providing a relatively simple means to selectively probe the scattering spectra of individual particles.



10 5. Describe the principles and applications of nanosphere lithography. Describe the structures that result. What aspects of NSL are adjusted to control the height and length of the resulting nanostructures so precisely? Why could these structures not be generated using standard photolithography techniques?

In nanosphere lithography, one or two layers of polymer microspheres are assembled at a surface in a closed-packed array. A metal is then vapor-deposited on the surface. When the polymer microspheres are subsequently removed chemically, the metal overlayer is removed from everywhere but the interstitial regions between the spheres. In this way, hexagonal arrays of remarkably uniform (with precision of ~ 1 nm) particles can be relatively easily generated, and subsequently suspended into an aqueous medium. The particle sizes can be finely tuned by adjustment of the sphere dimensions, while the thickness can be controlled by the vapor deposition. Some of the particles analyzed in the Van Duyne paper were prepared in this manner. Highly uniform nanoparticles of comparable dimension could not be generated using existing photolithography techniques (or even electron lithography, for that matter) because of the small feature sizes.

10 6. In principle, the $\sim 10^8$ enhancement factors associated with LSPR could increase the efficiency of many optically driven processes, including fluorescence. In practice, the enhancements in fluorescence are often several orders of magnitude less than the enhancements in Raman when using a sufficiently intense excitation source, often saturating at $\sim 10^7 - 10^8$ fluorescence photons / second (compared to $\sim 10^6$ photons/second in the absence of enhancement). Why is the enhancement so much less than the theoretical maximum in fluorescence? Why does this same issue not affect the enhancements observed in Raman spectroscopic measurements?

There are several possible reasons (e.g., fluorescence quenching by the metal, increased rates of photobleaching, etc.), but the most likely explanation is kinetic in nature. The time-scale for

Raman scattering is on the order of femtoseconds because of the transient nature of the virtual state. By comparison, fluorescence requires absorption, nonradiative relaxation, emission, and nonradiative relaxation back down to the ground state before a molecule can undergo another cycle. Assuming an excited state lifetime of ~ 1 ns yields a theoretical maximum of 10^9 photons/second, no matter how strong the driving field.

10 7. What is the difference between extinction and absorption? Which one is measured by uv-vis spectrometry?

Extinction is the more general term, describing the total losses of the beam upon passage through a sample. It includes both absorption and scattering. A uv-vis spectrometer measures extinction, not absorption (i.e., I/I_0).

15 8. Let's take a step back to consider an established competing technology. Traditional SPR measurements can either be performed using a narrow-band fixed wavelength source or a white-light source. Draw a schematic for each instrument and describe the similarities and differences in their fundamental mechanisms of operation. In each case, what physical observable is measured and how is it affected by surface binding?

The resonance-condition in SPR is highly sensitive to the angle of incidence and the wavelength. Traditional SPR measurements with a narrow-band fixed wavelength source are performed by angle-tuning, in which a spectrally narrow beam is introduced to a sample at multiple angles of incidence and the angle-dependent reflectivity is measured using an array detector. By comparison, broad-band sources generally use a fixed angle of incidence for all wavelengths and record the loss of some spectral components upon reflection from a thin metal film. Changes in the refractive index of the medium adjacent to the thin film result in changes in the angle or wavelength corresponding to plasmon resonance.

10 9. One of the key claims of each of the articles was the possible extension of this sensing approach to massively parallel assays. With infinite development time and resources, propose an experiment and instrument to accomplish this objective. What are some practical complications you may encounter? What are some practical advantages and disadvantages of single nanoparticle assays compared to traditional surface assays (e.g., in traditional SPR or from nanoparticle arrays)?

Answers here will vary significantly for each person. Practical complications include things like fluid handling, surface cleanliness, signal transduction in massively parallel systems, etc. The primary advantages of single nanoparticle assays are arguably the sensitivity and detection limits. The total surface area of a nanoparticle is significantly less than that of a geometric surface, but the transduced signal can be recorded with comparable signal to noise.

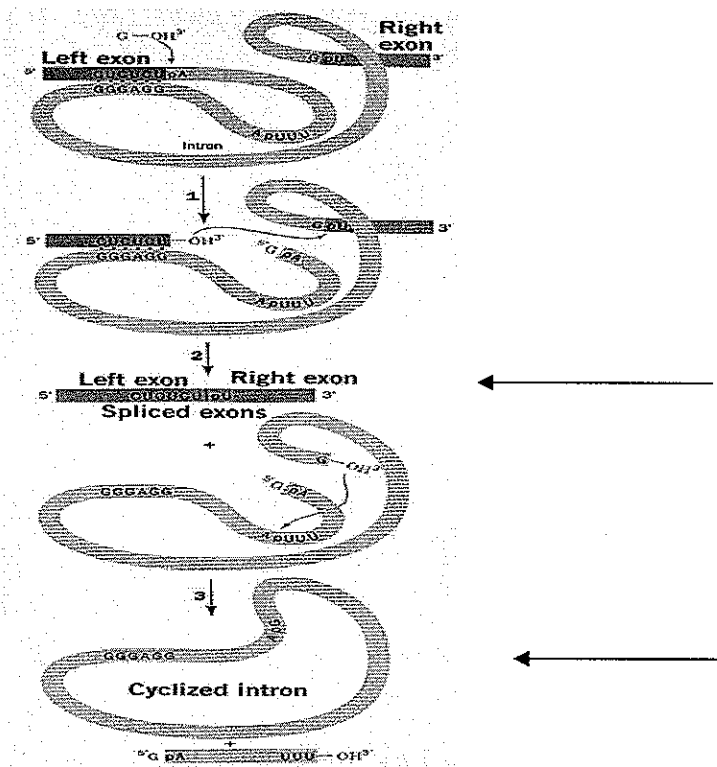
Crib to Biochemistry came, October 22, 2005

1A. RNA molecules that can act as enzymes to catalyze covalent changes in the structure of substrates (most of which are also RNA molecules). Catalytic RNA molecules are called ribozymes.

1B. It was long thought that all enzymes were proteins. Researchers in the laboratories of Cech and Altman found that in some cases, RNA molecules could act as an enzyme.

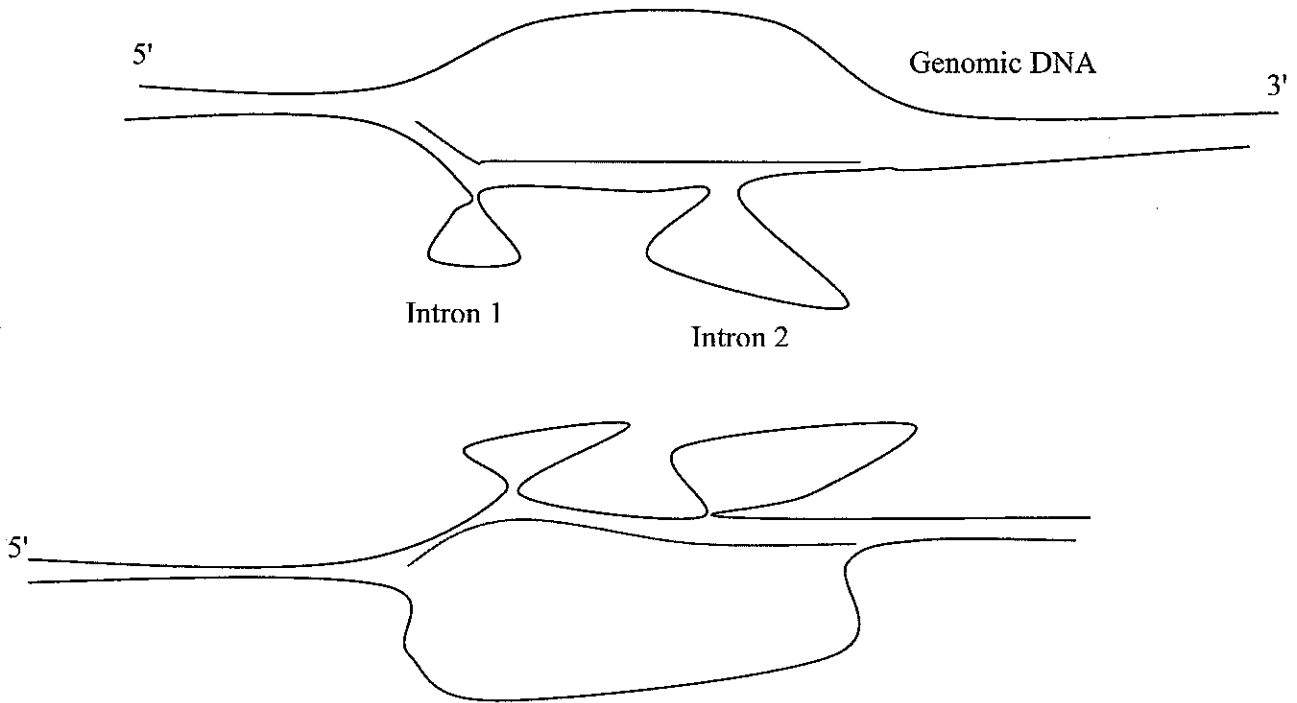
1C. The finding of catalysis by RNA implies that RNA is not solely a passive carrier of genetic information but can actively participate in directing cellular biochemistry. The discovery provides a possible solution to a long-standing question. That is, whether DNA or proteins were first formed in evolution. Since RNA can encode genetic information and serve as a catalyst in biochemical reactions, it can be deduced that life might have begun as RNA - the so-called "RNA World".

2. See the following figure, from Vogt and Vogt





Hybridization pattern of processed mRNA to genomic DNA



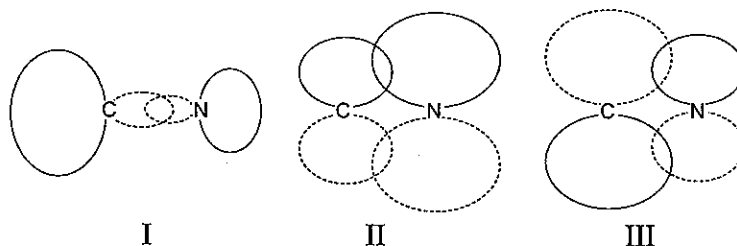
Hybridization pattern of cDNA to genomic DNA

4A. Hybridization of antisense RNA to mRNA will prevent protein synthesis by ribosomes. Examples include antisense RNA molecules designed to inhibit translation of HIV mRNA.

4B. RNA interference (RNAi) is a mechanism for posttranscriptional silencing of genes. An enzyme known as dicer cleaves double stranded RNA into sense and antisense RNA oligonucleotides 21 to 24 nucleotides (nt) in length. These small interfering RNAs (siRNA) guide the RNA interference-silencing complex (RISC) to messenger RNA transcribed from homologous genes. The complex cleaves and destroys the cognate RNA. This mechanism can be exploited to degrade mRNAs for proteins that may cause diseases such as cancer. The siRNA pathway is very specific. In principle, if designed correctly, double-stranded RNA molecules can be used to degrade specific mRNAs through the siRNA pathway.

Inorganic Cume October, 2005

1. The **correct** contours are below, with the carbon atom on the left in each case.



A. Contour I has sigma symmetry and is still weakly bonding even though admixture of 2s character suppresses overlap. This is contour W. 2s and 2p(z) orbital character from each atom

Contour II is π bonding and enriched in **nitrogen** 2p(y). There is a similar orbital built with the 2p(x) counterparts. This is from level X.

Contour III is a π -antibonding or π^* orbital, enriched in carbon. 2p(y) atomic orbital character. Also degenerate with a $\pi^*(x)$ counterpart.

B. The enhanced lobe at the carbon end provides for better overlap approaching from the C side.

C. The oxidation state is U(VI) so we have a d^0f^0 ion. The cyanide has to act as a π -donor so approach to contour II from the nitrogen side is favorable. Because of the error in the original drawing, we were generous in grading the connectivity aspect.

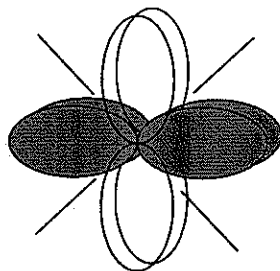
2. A. The $d(xy)$, $d(xz)$, and $d(yz)$ orbitals of the d^8 ion are partly filled in T_d symmetry and house 4 electrons between them.

B. The $d(x^2-y^2)$ and $d(z^2)$ orbitals are the LUMO's in this low-spin d^6 complex.

C. CO is isoelectronic with cyanide so contour III from above applies with O replacing N.

D. Ammonia is a Lewis base, and the HOMO is the lone-pair orbital, an sp^3 hybrid directed away from the hydrogens.

E. The following is an end-on view of the delta-bonding orbital. The convention is that the chlorides are along the x and y axes indicated by the lines. $d(xy)$ orbitals form the δ bond.



Organic Cumulative Examination

October 22, 2005

No Crib Available.

Written by Dr. Fuchs

Physical Cumulative Examination

October 22, 2005

No Crib Available.

Written by Dr. Szleifer