

## PLASMON RESONANT PARTICLES, METHODS AND APPARATUS

This application claims priority under 35 U.S.C. §120 to Provisional Application Ser. No. 60/038,677, filed Feb. 20, 1997, entitled "Preparation and Use of Plasmon Resonant Particles", which is hereby incorporated by reference in its entirety.

### FIELD OF THE INVENTION

The present invention relates plasmon resonant entities (PREs), or particles, to methods of interrogating a field containing PREs, and to apparatus for carrying out the method, and to various applications of PREs.

### BACKGROUND OF THE INVENTION

There are a number of important commercial and scientific applications of interrogating a target for information about the target. For example, the aim of analyte diagnostic tests and methods is to detect the presence and/or amount of an analyte (the target). The target analyte may be detected by reacting the analyte with a detectable reporter that (i) can bind specifically to the analyte and (ii) is detectable with suitable detecting tools. The reporter may, for example, be a colored or fluorescence molecule, or a colloidal metal, or a reporter such as a radiolabel that requires special film or scintillation equipment for its detection.

In some diagnostic applications, it is desirable to detect proximity relationships in a target analyte, as evidenced by the interaction between two proximately located probes on the target analyte. This forms the basis of so-called homogeneous assays, where the presence of an analyte is determined by a detectable probe proximity effect observed when two distinct probes are brought together on closely spaced sites on the analyte. As an example, two fluorescent molecules, when brought together, may exhibit a detectable fluorescence quenching or a non-radiative energy transfer effect that acts to shift the Stokes radius between the excitation and emission peaks.

A chemical, biochemical, or biological target may be interrogated by a variety of chemical and spectrographic methods to determine chemical structure, the presence of certain chemical groups, or the environment of the chemical groups. Notable among these methods are magnetic resonance methods for determining chemical structure and chemical group environment, spectroscopic methods, such as UV, IR, Raman, ORD, and CD spectroscopy, for detecting specific chemical groups, and mass spectroscopy for determining structure by fragment molecular weight analysis.

Surface chemical analysis of a target sample may be carried out by bombarding the surface with high-energy particles, e.g., electrons, and detecting the energy of atoms that are ejected from the surface. Electron Spectroscopy for Chemical Analysis (ESCA) is an example of such an approach.

Often it is desirable to establish spatial and/or distance relationships in a target, generally requiring interrogation by microscopy. Light microscopy has the advantage of simplicity, ease of sample preparation, and the feature that the sample can be examined in a "wet" condition. Its disadvantage is the relatively low resolving power, directly related to the wavelength of the illumination source (in the 400–650 nm range) and inversely proportional to the numerical aperture of the lens systems (at best, about 1.4), limiting resolution to several hundred nm).

High-energy beam microscopes, such as the transmission electron microscope (TEM) and the scanning electron

microscope (SEM) can achieve resolution down to the low nm range, but require a high-vacuum environment of the target sample, limiting applications with biological samples. Atomic force microscopy (AFM) is useful for interrogating surface features of a target sample, also with a resolution in the low nm range. The method is limited to surface effects.

Radiographic and scintigraphic methods for detecting and/or localizing sites of high-energy emission are also widely used. These methods tend to be quite sensitive, being able to detect very low numbers of high-energy emission events, but suffer from relatively high-cost and poor resolution when target spatial information is desired.

Despite the variety of methods currently available, there are a number of target-interrogation tasks of commercial and scientific interest that are difficult or impossible with current methods. Among these are:

1. Detecting single (or only a few) molecular events, such as the presence of one or a few binding sites, or one or a few enzymic sites on a target. This capability would open up new diagnostic applications, e.g., related to the presence or absence of specific intracellular events, and reduce the amount of sample material needed for a reliable assay and allow miniaturization of the assay.
2. Resolving sub-wavelength distance relationships in a biological target in a natural hydrated state. As noted above, subwavelength resolution by high-energy beam microscopy requires the sample target to be in a desiccated state, precluding the observation of natural cellular processes, including subwavelength movement of cellular components, and allows the user to perturb the sample during observation.
3. Direct spatial mapping of selected target sites on a biological target, such as direct mapping of selected sequences in a chromosome for purposes of chromosome mapping. Currently, this type of information is either not practical, or in the case of chromosome mapping, is not possible at high resolution and precise localization of gene sequences.
4. Optical reading of microencoded information. The ability to detect unique patterns of individual reporter groups would have important applications in forensics, information storage, metrology, and security identification microcodes.

It would therefore be desirable to provide a method and apparatus for interrogating a field for the type of information outlined above that is impractical or impossible to obtain by prior art methods.

It would also be desirable to apply the method to various diagnostics applications, to achieve improved sensitivity, spatial and distance information, ease of sample preparation, and flexibility in the type of target sample that can be interrogated.

### SUMMARY OF THE INVENTION

In one aspect, the invention includes a method of interrogating a field having a plurality of PREs distributed therein. The method includes the steps of illuminating the field with an optical light source, and detecting a spectral emission characteristic for individual PREs and other light scattering entities in the field. From this information is constructed a computer image of the positions and values of the emission spectral characteristic of individual PREs and other light-scattering entities present in the field, as a basis for discriminating PREs with a selected spectral signature from other light-scattering entities in the field, to provide information about the field.