SOME OPTICAL METHODS OF DETECTING PATHOGENIC NANO-BIO-PARTICLES

K. Kapanadze, G. Kakabadze, V. Kvintradze

Georgian Technical University
Tbilisi, Georgia
vakho710@gmail.com

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Abstract

For detecting and identification of nano-bio-particles (NBP) different optical methods of contact as well as non-contact types are considered. There was said that, contact systems would be suitable for collecting physical data (size, shape, mass, binding energy, electric magnetic and optical characteristics, etc.) of NBP for application them to stand-off detection systems. Both type of methods are very useful, they have their positive and negative sides, and in the case of acting together would better solve the problems of detection of pathogenic NBP.

1. Introduction

Rapid, early, and accurate detection and identification of pathogenic nano-bio-particles (NBP) is an important problem of healthcare. Detection and identification in general is complex processes and consists of several procedures (triggering, rapid identification, and laboratory confirmation). Applying the classical methods (to get final result) takes from several hours to several days. Rapid, early and accurate detection means to save time from propagation of illness. For the solution this tusk, the nanotechnologies are applied.

Particles that are smaller than the characteristic lengths associated with the specific phenomena often display new chemistry and new physics that lead to new properties that depend on size. When the size of the structure is decreased, surface to volume ratio increases considerably and the surface phenomena predominate over the chemistry and physics in the bulk. The reduction in the size of the sensing part and / or the transducer in a sensor is important in order to better miniaturize the devices. Science of nanomaterials deals with new phenomena, and new sensor devices are being built that take advantage of these phenomena. Sensitivity can increase due to better conduction properties, the limits of detection can be lower, very small quantities of samples can be analyzed, direct detection is possible without using labels, and some reagents can be eliminated [1].

A sensor is an instrument that responds to a physical stimulus (such as heat, light, sound, pressure, magnetism, or motion). It collects and measures data regarding some property of a phenomenon, object, or material. Sensors are an important part to any measurement and automation application. The sensor is responsible for converting some type of physical

phenomenon into a quantity measurable by a data acquisition (DAQ) system. Nano-sensors are any biological, chemical, or surgical sensory points used to convey information about nanoparticles to the macroscopic scale [1].

The methods for detection and identification of NBP can be based on different ways: physical, chemical, biological etc. All of them have as positive also negative (preparing procedures, separation pathogens from surrounding environment, i.e., sampling, too long time to get result, they can damage analyte and so on) sides. It is well-known that most appropriate (non-invasive, precise) way is optical method (contact or non-contact-remote) of detection and identification of NBP *in situ*, i.e., at given time and place. For execute this, is necessary to know physical characteristics (such as a size, shape, mass, density of particle it's frequency, index of refraction, dielectric constant etc.) of NBP.

2. Optical methods

The optical methods are of different kinds (flow cytometry, fluorescence, fiber optics, surface plasmon resonance, mass-spectrometry, Raman spectroscopy, etc.). In general, they are contact and non-contact types. For the contact (i.e. point) methods, is necessary the enough quantity of bio-particles (i.e., concentration), separated from environment for sensitive measurements. Below, contact type optical methods are considered.

2.1. Evanescent wave fiber optic bio-sensors (EWAB)

When light propagates through a fiber optic (**Figure 1**) on the basis of total internal reflection (TIR), a thin electromagnetic field (the "evanescent wave") generated decays exponentially with the distance from the interface with a typical penetration depth of up to several hundred nanometer [2]:

$$E(z) = E_0 \exp(^{\delta}/_{d_p}).$$

Here δ is the distance from the interface, the penetration depth (d_P) is given by the expression

$$d_p = \frac{\lambda_{ex}}{2\pi}[(n_2)^2 sin^2\alpha - (n_1)^2]^{-1/2},$$

where λ_{ex} is the wavelength of the light, n_1 is the refractive index of the cladding region and n_2 is the refractive index of the core, and α is the angle of incidence measured from the normal at the interface of the core and cladding.

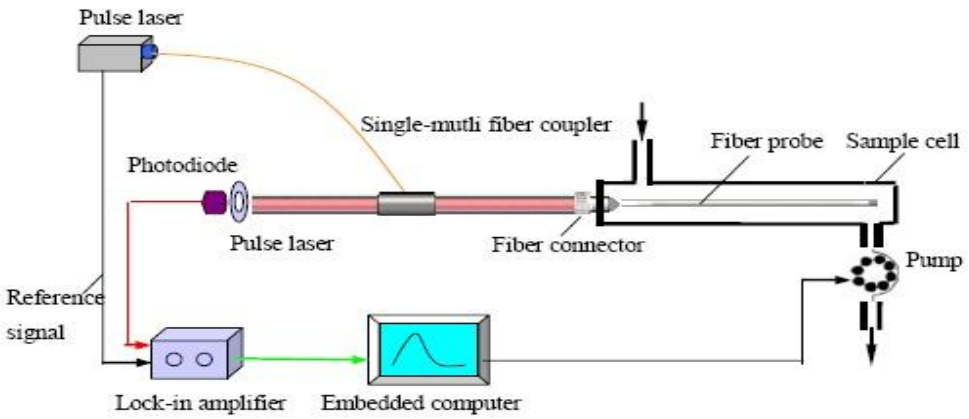

Figure 1. Principle scheme of the portable optical fiber biosensor [2].

This evanescent wave can excite fluorescence in the proximity of the sensing surface, e.g., in fluorescently labeled biomolecules bound to the optical sensor surface through affinity recognition interactions. The short range of the evanescent wave enables it to discriminate between unbound and bound fluorescent complexes, hence eliminating the normally required washing procedures. Moreover, evanescent field-based waveguides are well suited for study and detection of bio-molecular interaction. Ultrasensitive DNA detection was achieved by the EWAB based on quantum dots (QDs) [2].

2.2. SPR biosensors

Surface plasmon resonance (SPR) is a surface-sensitive optical technique that is associated with the evanescent electromagnetic field generated on the surface of a thin metal film when excited by an incident light under total internal reflection conditions. Due to the fact the evanescent field diminishes exponentially with increasing distance of penetration from the interface, SPR promotes monitoring of only surface-confined molecular interactions occurring on the transducer surface. SPR biosensors allow real-time detection of minute changes in the refractive index when bio-recognition molecules (e.g., antibodies) immobilized on a transducer surface bind with their bio-specific targets (e.g., analytes) in solution [2].

The use of SPR to detect environmental contaminants, including atrazine, dichloro-diphenyl-trichloroethane (DDT), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, carbaryl, 2,4-D, benzo[a] pyrene (BaP), biphenyl derivatives, and trinitrotoluene (TNT), has recently gained considerable interest {2].

2.3. Optical micro-resonators

Here, it has been developed [3] the highly sensitive, miniaturized, optical-sensing component that allows detection of single pathogens or disease biomarkers without having to label them.

This bio-sensing component derives its unprecedented sensitivity from the use of micro-sphere optical resonators. The optical resonance is created by launching and confining coherent light inside the microsphere, where it interferes constructively due to total-internal reflection.

Detection of single particles relies on the ability to discriminate the wavelength-shift signal against background noise, a task that requires narrow line-width, high-quality (Q) optical resonance and the use of nanoparticles that produce an observable resonance shift. Unfortunately, most important biological pathogens are virus nanoparticles in the 50 – 1000 nm size range, and discerning the resulting wavelength-shift signal is extremely challenging (its magnitude scales inversely with the virus size to the third power). Fortunately, the magnitude of the wavelength-shift signal can be sufficiently enhanced by making the microsphere resonator smaller. We confirmed a reactive sensing mechanism with inverse dependence on mode volume in experiments with virus-sized polystyrene Nano-particles. By comparing the electromagnetic theory for this reactive effect with experiments, we can determine the size (~ 100 nm) and mass (~ $5.2 \cdot 10^{-16}$ g) of a bound influenza A virion directly from the optimal resonance wavelength shift [3].

The non-contact (remote or stand-off) detection systems (LIDAR – Light detection and ranging) are not so precisely as point systems. They are long distance (1 – 5 km) operate systems,

they are used for atmospheric research and monitoring, they distinguish organic from nonorganic, but don't distinguish organic particles from each other, and they are used for early (alarm) warning, so they aren't discussed here.

Most interesting is detection of pathogenic nanoparticles indoor (for example: schools, cinemas, hospitals, etc.) for the short (1 – 50 m) distances. For this case, most valuable method is based on Raman scattering.

2.4. Raman spectroscopy

Raman spectroscopy has become a powerful instrument for study biological samples. This technique rapidly characterizes the tissue and bodily fluids in nondestructive and noninvasive fashion. Raman Spectroscopy used to identify different molecules and even functional groups within larger molecules. The bonds formed between atoms have specific vibrational frequencies that correspond to the atom's masses and the strength of the bond between them. Complex molecules therefore exhibit many peaks and can be readily identified by the pattern or "fingerprint" created by those peaks. Besides, the spectroscopy of Raman allows very sensitive for the discrimination of bacteria. Raman scattering of light has interacted with vibrational modes of the molecule, a vibrational spectrum may be obtained allowing for identification of molecules and their functional groups. Raman spectroscopy is sensitive to analyze molecular changes such as protein structures and concentrations. Raman spectroscopy has capability to detect molecules alternation and to analyze some difference at molecules level and its tool are possible to open many new factors in studying of viruses [4].

The idea of Raman spectroscopy stand-off detection is based on the features of Raman scattering. Raman scattering is a two-photon process that conveys information about the vibrational mode-structure of the scattering molecule. In normal Raman scattering, an incident photon of frequency ν_0 excites a molecule from its ground electronic level to a "virtual" energy level. If the energy of this virtual level is sufficiently different from that of the nearest real level, the molecule returns quickly back to its ground level; a second photon is emitted almost instantly. If the emitted photon has the same frequency as the incident one, the process is called Rayleigh scattering. However, interaction of the incident photons with the vibrations of a molecule can shift the frequencies of the scattered photons. The shifts are equal to the frequencies of the discrete vibrational modes of the molecule. This unique set of frequency-shifts produces a spectrum that is a vibrational "fingerprint" of the interacting molecule [5].

Raman scattering is strongest when vibrations cause a change in the polarizability of the electron cloud around the molecule. Therefore, the difference in energy between the incident and scattered photons is a characteristic of and provides structural information about the irradiated molecule [6]. The mathematical expression of Raman scattering is as following. When the light interacts with non-oscillating molecule it gives part of energy to molecule and therefore its frequency decreases (Stokes effect)

$$h\nu' = h\nu_0 - h\nu_m \quad or \quad \nu' = \nu_0 - \nu_m$$

Here ν_0 is the frequency of incident light and ν_m is the frequency of oscillating molecule. When the light interacts with oscillating molecule with energy of $h\nu_m$, it can take away energy of molecule and became of radiation of high frequency (anti-Stokes effect) [7]:

$$h\nu' = h\nu_0 + h\nu_m,$$

or

$\nu' = \nu_0 + \nu_m$.

Further, due to its narrow spectral lines and unique signatures, Raman spectroscopy enables selective identification of individual analytes in a complex, multicomponent mixture without the need for chemical separations. In addition, the technique requires little or no sample preparation, is nondestructive, and can use water as a solvent (since water is a poor Raman scaterer). The intensity of the scattering is related to the power of the laser used to excite the scattering, the square of the polarizability of the molecule, and the fourth power of the frequency of the exciting laser. Therefore, the most common choice for excitation is a visible laser [6].

Unfortunately, Raman scattering is an inherently weak process, precluding the possibility of remote trace analysis without some form of enhancement. However, surface-enhanced Raman scattering (SERS) can give an enhancement of up to about $10^6 – 10^7$ in scattering efficiency over normal Raman scattering. Even stronger enhancements of order of $10^{11} – 10^{13}$ come from sharp features or "hot spots", such as are found in nanostructures. Such extremely large enhancements can produce a total SERS cross-section comparable to that of fluorescence [6].

3. Conclusions

As it was mentioned above, the goal was to find proper characteristics among NBP and sensors to detect and identification of NBP, especially, remote, stand-off detection indoor pathogens. For this, are considered different types of optical methods (contact, non-contact) of detection of pathogen particles. It is seen, that some of them are well suited for study and detection of bio-molecular interaction, real-time detection changes in the refractive index when bio-recognition molecules (e.g., antibodies) bound on a transducer surface with their biospecific targets, allow detection of single pathogens or disease biomarkers without having to label them, detecting even virus nanoparticles.

All of these methods are contact methods (the pathogen particles must be separated from environment and concentrated in the small volume). They are applied mostly in the laboratory, but not *in situ*. These methods also are useful for collecting of experimental data about physical properties of bio-particles (size, shape, mass, binding energy, electric magnetic and optical characteristics, etc.). This data base will be fundament for the detection pathogens *in situ*.

One of the most suitable ways for remote (non-contact) detection of pathogens (*in situ.*) is Raman spectroscopy. This method identifies pathogens by their own pattern of "fingerprints". There is no need to separate and collect pathogens in the small volume. These "fingerprints" are independent of initial wavelength. It's truth, that Raman scattering is weak process, but it can be enhanced by SERS. So at the presence of high resolution advanced technique (or their combination) the problem of detecting and discrimination of NBP will be decided with high probability.

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