Miniaturized gel electrophoresis-thermal lens technique as a highly sensitive photothermal detection method

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Miniaturized Gel Electrophoresis Chip-Thermal Lens Microscopy (MGEC-TLM) can represent a powerful detection method based on photothermal phenomena for monitoring the electrophoretic mobility of biofunctionalized nanoparticles [1]. It is crucial to optimize the amount of DNA on the surface of biosensors-based DNA functionalized AuNPs [2-3]. Here, MGEC-TLM was used to detect the coverage density of thiol-functionalized oligonucleotides on the surface of gold nanoparticles (AuNPs).

TLM is a powerful photothermal technique that relies on the measurement of temperature changes, caused by the heat generated as a result of absorbed optical radiation. In other words, it is an indirect absorption spectroscopic detection method, which its sensitivity is at least a hundred-fold higher than that of optical absorbance spectrometry. The reason is that the sensitivity of the technique is proportional to the so-called enhancement factor ($E = [P(\partial n/\partial T)/\lambda k)]$ which depends on the thermo-optical properties of the sample such as the temperature coefficient of the refractive index ($\partial n/\partial T$), the wavelength of the probe beam (λ), the excitation power (P) and the thermal conductivity (k) [4]. Moreover, Thermal Lens Microscopy (TLM) offers high spatial resolution and can measure low volumes of nonfluorescence molecules or nanoparticles subjected to analysis in a microchannel.

In this system a 532 nm diode-pumped solid-state laser of maximum power of 100 mW is used as an excitation source (EL). A signal generator (SG) modulates the EL at a frequency of 118 Hz. A neutral density filter (NF) is placed after the EL to adjust its power to 15 mW. The excitation beam coming from the EL is first collimated by the lens L1 and L2. After that, the beam is focused onto the MGEC channel using a 0.25NA focusing objective. The probe laser (PL), a He-Ne laser, is collimated by a set of lenses and directed to the sample using the mirrors. Then the beam is directed to a Silicon detector (PD) across a 0.5 mm pinhole (P) and an interference filter (F2). The 532 nm filter (F1) was used for removing any residual emission of the fundamental wavelength 1064 nm. The analog signal from the photodiode is filtered and amplified using a lock-in amplifier and further digitalized at 1 k samples/s sampling rate. The data acquisition system mainly consists of a microcontroller based digitization Arduino board and a graphical user interface (GUI) running on a PC. The GUI was build using the LabVIEW graphical programming software to visualize the incoming data, capture the TLS signal

(peaks) and online recording of data. A power supply (EPS) is used to supply the desired voltage for electrophoretic nanoanalysis of NPs in the MGEC (Fig.1).

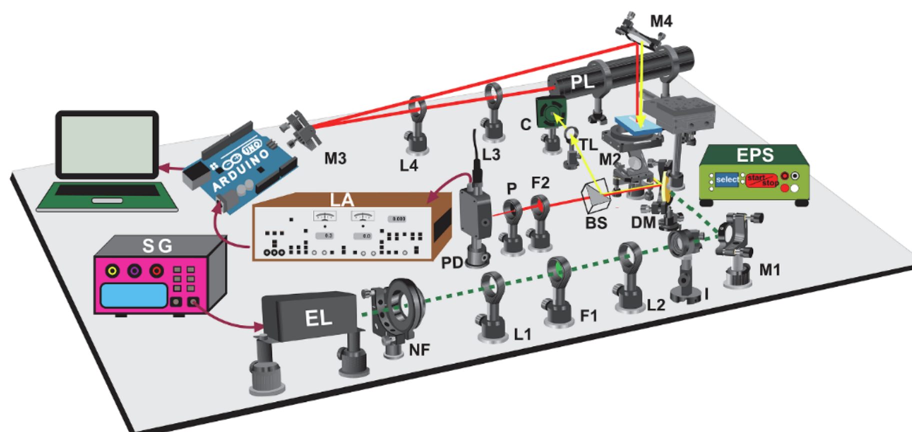

Fig. 1. Schematic illustration of MGEC-TLM, EL: excitation laser, PL: probe laser, C: CMOS camera, PD: photodiode, NF: neutral densityfilter, L1 to L4: lenses, M1 to M4: mirrors, DM: dichroic mirror, OL: focusing objective, P: pinhole, F1: 532 nm filter, F2: 632.8 nm filter, I: iris, BS: beam spliter, TL: tube lens, SG: signal generator, LA: lock-in amplifier, EPS: electrophoresis power supply

We developed a rapid yet sensitive approach for online monitoring the surface coverage of ssDNA loaded on AuNPs using MGEC coupled with TLS. Unlike conventional methods for determining the surface coverage, the design presented here does not require long signal acquisition times or tedious post-treatment steps including chemical modifications. With our implementation, we demonstrated an excellent electrophoretic analysis of DNA strands attached to AuNPs and a rapid nanoparticle separation in the gel. We showed that for 13 nm-AuNPs and short DNA strands (<30 bases), 300 DNA/AuNPs density can fully coat the particles, which represent a conjugate with high stability. The lowest detectable concentration of 10 nm AuNPs was found to be 23 pM. The use of TLS coupled with MC and MGEC holds great promise in biotechnology and nanotechnology fields, given its efficiency, speed, and throughput.

References

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