TomoWave Laboratories

imaging sensing monitoring

Laser Optoacoustic BioSensor for NanoLISA Assay

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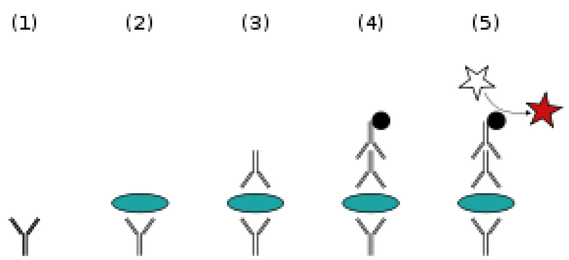
Optoacoustic Biosensor Technology

The need for fast and accurate identification of biological pathogens is fulfilled through optoacoustic detection of specifically bound contrast agents, in this case gold nanorods (GNR). TomoWave developed an optoacoustic biosensor based on its patented method and immunoassay termed NanoLISA [A.A. Oraevsky, P.M. Henrichs, US Patent 07,500,953, PCT 10/764,213]. This technique is exclusively dependent upon the presence of GNR, and the observed response is unaffected by the nature of the pathogen being studied. Optoacoustic detection enabled the design of a truly general biosensor, and permitted the direct quantification of pathogen populations within samples of blood and other biological fluids. Preliminary results confirmed the technique's sensitivity to be high enough to allow quantitative analysis without lengthy cultures [S.M. Maswadi, et al Proceedings SPIE 2008; vol. 6856, p. 151-158).

The prototype NanoLISA instrument demonstrated the optoacoustic biosensor has **limit of detection that is 5 times more sensitive than the gold-standard of ELISA**. High dynamic range is ensured through highly specific molecular targeting. To prevent matrix effects from affecting sensitivity (fluorophore quantum yield for ELISA), highly absorptive gold nanorods (GNR) are used as a contrast agent operating in the near-infrared spectral range where blood has minimum background absorption.

The technique can be adapted for detecting a vast array of common pathogens for which ELISA protocols exists, such as Malaria, HIV, Hepatitis (A, B, or C) and Herpes Simplex Virus. Through NanoLISA, measurements are performed without the need for lengthy multiplication process in culture. The instrument is designed to be portable, and is not limited to use in a lab setting. This makes NanoLISA biosensor an invaluable tool for emergency response field work and use in developing countries..

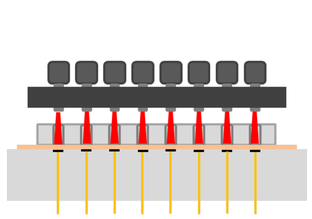
NanoLISA Assay simplified vs ELISA

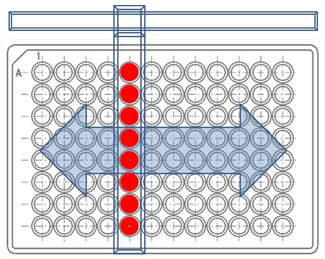


(1) Plate is coated with a capture antibody
(2) Sample is added: antigens present bind to capture antibody
(3) Detecting antibody binds to antigen
(4) Enzyme-linked secondary antibody is added, and binds to detecting antibody (MAB bound to GNR is the final step for NanoLISA)
(5) Substrate is added, and is converted by enzyme to detectable form Fluorophores for ELISA.

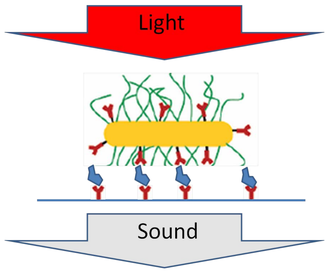
System Design

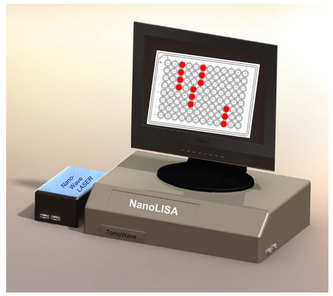
Optoacoustic biosensor features a simple illumination scheme as well as a reliably manufactured forward-mode detection system. The design can be scaled to any number of transducers within the detector: arrays can be built to accommodate standard ELISA microplates with various size wells.





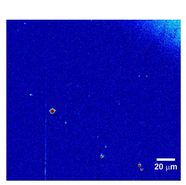
Laser Illumination in the near-infrared range is performed with low energy laser pulses of short duration (10 ns). Alternatively, quasi-CW diodes with very high repetition rates can be utilized using a homodyne detection scheme, well known for its sensitivity.



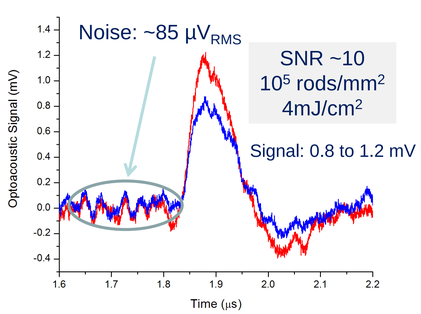


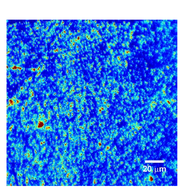
Validation of Performance

A 200 μL aliquot of a dilute GNR solution was deposited on an acrylic surface and spread over an area of several mm², and immediately washed off with copious amounts of deionized water.



Clean acrylic slide



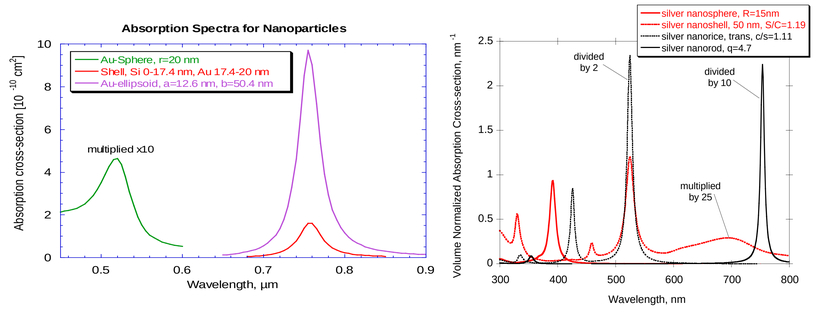


10^5 GNRs/mm^2

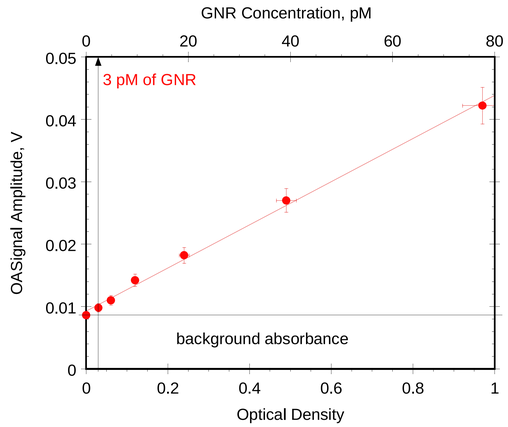
Monolayer of strongly diluted gold nanorods was well detectable by NanoLISA

Exceptional Sensitivity

Current methods of pathogen detection rely on ELISA , which has a limit of sensitivity about 10 pM of highly fluorescent agents, and often requires several days for culture. The present prototype of NanoLISA biosensor is 5 times more sensitive, and the next generation of the biosensor design employing silver nanorods, as well as the light delivery, can be tuned to further increase sensitivity by an order of magnitude [Conjusteau, A., et al Proc SPIE 2010; v. 7564, p. 75641L].



Optical absorption of GNRs is much stronger than that of nanoparticles made of organic dyes with equal volume. The optical absorption of silver nanorods (Ag-NR) is an order of magnitude stronger that that of GNR.



Noise limited detection threshold of the biosensor for GNR is 3 pmole/L.

Business Proposal for beta-testing

TomoWave developed, built and tested a single-transducer prototype of the optoacoustic NanoLISA biosensor. We offer our prospective customers to acquire prototypes of single transducer units and transducer array units for beta-testing. The advantage of NanoLISA biosensor for diagnostic laboratories is the significantly increased sensitivity (potentially over an order of magnitude better than current ELISA technology). NanoLISA offers a distinct advantage over ELISA technology by reducing the reliance upon time consuming culturing, highly specific targeting and possibility of measuring pathogen populations directly, rapidly, and reproducibly.

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