Published in final edited form as:

Phys Chem Chem Phys. 2013 April 28; 15(16): 6008–6015. doi:10.1039/c3cp00076a.

Molecular Sentinel-on-Chip for SERS-Based Biosensing†

Hsin-Neng Wang^{#a,b}, Anuj Dhawan^{*,c}, Yan Du^d, Dale Batchelor^e, Donovan N. Leonard^f,
Veena Misra^d, and Tuan Vo-Dinh^{*,a,b,g}

^aFitzpatrick Institute for Photonics, Duke University, Durham, NC, USA.

^bDepartment of Biomedical Engineering, Duke University, Durham, NC, USA.

^cDepartment of Electrical Engineering, Indian Institute of Technology Delhi, New Delhi, India.

^dDepartment of Electrical & Computer Engineering, NC State University, Raleigh, NC, USA.

^eDepartment of Materials Science and Engineering, NC State University, Raleigh, NC, USA.

^fDepartment of Physics and Astronomy, Appalachian State University, Boone, NC, USA.

^gDepartment of Chemistry, Duke University, Durham, NC, USA.

[#] These authors contributed equally to this work.

Abstract

The development of DNA detection techniques on large-area plasmonics-active platforms is critical for many medical applications such as high-throughput screening, medical diagnosis and systems biology research. Here, we report for the first time a unique “molecular sentinel-on-chip” (MSC) technology for surface-enhanced Raman scattering (SERS)-based DNA detection. This unique approach allows label-free detection of DNA molecules on chips developed on a wafer scale using large area nanofabrication methodologies. To develop plasmonics-active biosensing platforms in a repeatable and reproducible manner, we employed a combination of deep UV lithography, atomic layer deposition, and metal deposition to fabricate triangular-shaped nanowire (TSNW) arrays having controlled sub-10 nm gaps nanostructures over an entire 6-inch wafer. The detection of a DNA sequence of the Ki-67 gene, a critical breast cancer biomarker, on the TSNW substrate illustrates the usefulness and potential of the MSC technology as a novel SERS-based DNA detection method.

Introduction

Recently, there has been great interest in the development of surface-enhanced Raman scattering (SERS)-based analytical techniques for chemical and biomedical applications.^{1–6} By utilizing SERS-active metallic nanostructures or nanoparticles, a variety of chemicals and biomolecules have been successfully detected when adsorbed on or placed in close proximity to the metallic nanostructured surfaces.^{7–17} Raman scattering can be described as

†Electronic Supplementary Information (ESI) available: FDTD and RCWA calculations. See DOI: 10.1039/b000000x/

© The Royal Society of Chemistry

*Tel: 919 660 5598; tuan.vodinh@duke.edu Tel: +91 11 26591080; adhawan@ee.iitd.ac.in.

an inelastic light scattering process in which a target sample on which light is incident absorbs one photon and emits another photon at the same time, the second photon being either at a lower frequency (i.e. Stokes scattering) or at a higher frequency (i.e. Anti-Stokes scattering) than the incident light frequency. While Raman scattering cross-sections are extremely small - typically between 10^{-30} to 10^{-25} cm² per molecule - thereby limiting its ability to detect the analyte species at trace levels, the SERS effect increases the Raman scattering cross-section substantially enabling the application of this process for extremely sensitive detection of the analytes.¹⁸⁻²⁰ Reports on the large SERS enhancement factors of 10^{12} – 10^{15} have inspired the development of new sensing materials with highly sensitive detection levels.²¹⁻²⁴

The metallic nanostructures on a substrate or nanoparticles in solution are commonly used as signal enhancing platforms for the SERS signal associated with the analytes. In general two basic mechanisms of SERS have been discussed – the electromagnetic enhancement (EME) where the SERS signals from molecules are related to the electromagnetic fields in the vicinity of the molecules^{18-19,25-26} and chemical enhancement (CE), which is related to electronic coupling between the metallic substrate and the molecules thereby resulting in a change in the Raman cross-section of the coupled molecule-substrate complex.²⁷⁻²⁸ There are two main sources of electromagnetic enhancement of SERS¹⁸ – The first part of the enhancement is due to excitation of surface plasmons or electronic vibrations on the SERS substrate having a nanostructured metallic (e.g. Ag, Au, Cu) surface, which leads to significant enhancement in the localized electromagnetic fields in the vicinity of the substrate. Such an EM enhancement in the vicinity of the SERS substrates containing the Raman-active molecules leads to an increase in the Raman emission intensity, which is proportional to the square of the applied field at the molecule. Various models explaining important properties and characteristics of the surface plasmon effect have been reported.^{18-19,25-26} Another part of the EM enhancement originates from the plasmonic enhancement of the Raman signal emanating from the Raman-active analyte molecules, thereby leading to an EM enhancement factor that is proportional to the fourth power of the electric field around the nanostructures.

We have previously developed a novel SERS-based “molecular sentinel” (MS) nanoprobe to detect the presence of DNA sequences of interest in a homogeneous solution.²⁹ This molecular sentinel in solution (MSS) scheme approach incorporates the SERS effect modulation process associated with metal nanoparticles and hairpin DNA probes (30~40 nucleotides in length) tagged with SERS-active labels as the signal reporter. The metal nanoparticle is then used as a signal enhancing platform (nano-enhancer) for the SERS signal associated with the label. The specificity and selectivity of the MSS nanoprobe were first demonstrated by detecting *gag* gene sequence of the human immunodeficiency virus type 1 (HIV-1).²⁹ Recently, we have also demonstrated the multiplex capability of the MSS nanoprobe for the detection of multiple breast cancer biomarkers in one sample solution.³⁰

Although the Molecular Sentinel (MS) concept using molecular sentinels on metallic nanoparticles in solution has been demonstrated previously for detecting target nucleic acid molecules, this paper extends the MS approach into a unique “molecular sentinel-on-chip” (MSC) technology when the plasmonic nanostructures are developed at controlled locations

on the chips. In the case of molecular sentinels (MS) based on nanoparticles, one or more plasmonically active nanoparticles could come closer to each other in solution and this could change the coupling between the nanoparticles thereby affecting the SERS signals from the nanoparticles. On the other hand, in the Molecular Sentinel on Chip (MSC) concept, the plasmonic structures (such as metal-coated nanowires) of different sizes and spacings are developed on a fixed substrate and the spacing between the plasmonic nanostructures remains the same throughout the detection process, thereby ensuring that the reduction in SERS signals occurs only upon the specific binding of the target molecules to the probe molecules and not due to changes in spacings between the plasmonic nanostructures. This increases the reliability of the SERS measurements.

The application of the MSC system based on a SERS-active nanowire chips is investigated for the detection of DNA sequences. This approach involves using triangular-shaped nanowire (TSNW) arrays to enhance the SERS signals from the MSC hairpin probes located inside or near nanoscale gaps between plasmonically active metallic nanostructures.^{14-17,19} Fig. 1 schematically illustrates the operating principle of the MSC using a TSNW substrate. The sequence within the loop region of the hairpin probe is complementary to a specific target gene sequence of interest. The hairpin probe having a Raman label at one end is then immobilized onto a metallic TSNW substrate via a thiol group attached on the other end. The MSC detection strategy is based on the dependence of SERS enhancement on the distance between the metallic surface and the Raman label. In the absence of target DNA molecules, the hairpin configuration has the Raman label in close proximity to the metallic surface (closed state) and exhibits a high SERS signal as depicted in Fig. 1 (left). However, when complementary DNA targets are recognized by the MSC nanoprobe, hybridization occurs and the Raman label is separated away from the surface (open state) as depicted in Fig. 1 (right). As a result, the SERS signal of the Raman label is significantly reduced, indicating target recognition and capture. Moreover, we also employ the fact that a decrease of the overall SERS signal can also occur due to the moving away of some of the SERS-active dye molecules (attached to the probe molecules) from the inverted triangular SERS hotspots - due to opening of stem-loop structure of some of the probe DNA molecules upon hybridization with a complementary target molecule - formed at the bottom of the gap between the gold-coated triangular nanowires. Along with demonstrating the MSC technology, this paper also presents a relatively simple process of developing SERS substrates on a large area (6-inch wafers) in a reliable and controllable manner, such that these substrates contain plasmonic nanostructures with sub-10 nm gaps (that are potential 'SERS hotspots') between the nanostructures.

Experimental section

Fabrication of nanowire arrays

In this study, we employed a combination of deep UV lithography, atomic layer deposition, and metal deposition to fabricate the TSNW substrates having controlled sub-10 nm gap nanostructures over an entire 6-inch wafer. As shown in Fig. 2a, the fabrication of the TSNW array substrate involves the following steps: (A) development of silicon TSNWs on a 6-inch wafer, (B) coating with a spacer layer, and (C) deposition of gold film.

(A) Development of two-dimensional silicon nanowires on a 6-inch wafer—

Silicon substrates were first patterned using UV lithography at 193-nm with an ASML 5500/950B Scanner. Wet etching technique using Tetramethylammonium hydroxide (TMAH) was applied to form silicon nanowires of varying sizes and spacings (100 nm and 10 microns) between the wires over the entire 6-inch wafer (See Fig. 2b). To form two-dimensional nanowire arrays, parts of the silicon nanowires were periodically covered by SiO₂ using a second masking process and deep UV lithography was used to create silicon nanowires running in the direction perpendicular to the initial SiO₂ nanowire network.

(B) Coating with a spacer layer—Firstly, the silicon nanowires were coated with a layer of silicon germanium employing ultra high vacuum rapid thermal chemical vapor deposition as an optional step to reduce the gap between the nanowires before the metal deposition. This was followed by Atomic layer deposition (ALD) of hafnium oxide (See TEM Crosssections in Figs. 2d-e) or platinum for creating a conformal spacer layer to reduce the gap between the silicon nanowires before overcoating the spacer layer with a plasmonics-active metal such as gold or silver using electron beam evaporation. The ALD of hafnium oxide on the silicon nanowires was carried out at 200 °C while that of platinum was carried out at 300 °C.

(C) Deposition of gold film—In the final fabrication process, the SERS-active substrates were developed by over coating the TSNW structures with a 50-100 nm thick film of metal such as gold or silver using E-beam evaporation. Between two parallel TSNWs, there are small triangular sections and the minimum nanoscale gap between the gold film on the triangular shaped nanowires is less than 10 nm (Figs. 2c-e).

Although several techniques exist for developing SERS substrates,²¹⁻²⁴ employing TSNWs allows the development of plasmonic nanostructures with sub-10 nm gaps on a wafer-scale in a reliable and controllable manner, employing a relatively simple fabrication process.

Molecular sentinel oligonucleotide probes

In this study, the MSC hairpin and target oligonucleotides were synthesized by Integrated DNA Technologies (IDT, Coralville, IA). The oligonucleotide sequences used in this study are as follows:

KI67-MSC: 5'-SH-GCGTATTCTGCACACCTCTTGACACTC CGATACGC-TAMRA-3'.

Complementary target DNA: GCACTTTGGAGAGCAAATCT
GTGCAGAGAGTAACGCGGAGTGTCAAGAGGTGTGCA
GAAAATCCAAAGAAGGCTGAGGACAATG-3'.

Non-complementary DNA: 5'-GCCAGCGTCGAGTTGGTTTG CAGCTCCTGA-3'.

The underlined sequences indicate the complementary arms of the MSC hairpins, and the bold sequences represent the target sequences complementary to the loop regions of the MSC hairpins. The 3'-end of KI67-MSC hairpin probes was modified with 5-carboxytetramethylrhodamine (TAMRA). To effectively separate the Raman labels from the metallic surface upon hybridization to the target DNA, the length of the DNA hairpins was

designed to be 35 nucleotides. Conjugation of DNA hairpins onto nanowire arrays was achieved by using an alkyl thiol substituent at the 5'-end.

Functionalization of nanowire arrays with molecular sentinels

The MKI67-MSC nanoprobe were immobilized on a TSNW substrate by first incubating the substrate in a 1 μ M probe solution containing 1M NaCl and 10 mM sodium phosphate buffer (pH 7.0) for two hours at room temperature and then rinsing unattached DNA probes thoroughly with deionized water. The functionalized substrate was next exposed to 1-mM 6-Mercapto-1-hexanol (MCH) for 5 min followed by rinsing thoroughly with deionized water to displace non-specifically adsorbed DNA probes and to passivate the gold surface.³¹ The functionalized TSNW substrate was stored in 20 mM Tris-HCl buffer (pH 8.0) at 4 °C. To form the hairpin structure effectively, the MSC nanoprobe-coated TSNW substrate was incubated with 20 mM Tris-HCl buffer (pH 8.0) containing 10 mM MgCl₂ for at least 30 min at room temperature before SERS measurements.

SERS measurements

SERS measurements were performed using a Renishaw InVia confocal Raman microscope. A 50 mW HeNe laser (Coherent, model 106-1) emitting a 632.8 nm was used for excitation. The TSNW substrate was placed on a glass slide and mounted on an X-Y-Z translational stage. The light from the laser was passed through a laser line filter, and focused on the TSNW substrate with a 40X microscope objective. The Raman scattered light was collected by the same objective, and filtered with a holographic notch filter to block light due to Rayleigh scatter. An 1800 groove/mm grating was used to provide a spectral resolution of 1 cm⁻¹. Raman scattering was detected by a 1024 $\times$ 256 pixel RenCam CCD detector. The SERS spectra were acquired using a 10-sec integration time and processed with WiRE 2.0 software (Renishaw).

RCWA and FDTD Calculations

Finite Difference Time Domain (FDTD) calculations of electric fields in the spacing between the gold-coated triangular shaped silicon nanowires was carried out using an FDTD software called Fullwave 6.0 (extended Debye model for gold and silver). In the FDTD calculations, a continuous plane wave (633-nm) propagating in the z direction and having electric field (E-field) polarized in the x direction was incident on the gold nanowire structures. Enhancement in E-field as a function of the incident field (normalized to 1) was calculated. This software enables FDTD analysis of the metallic media to include Debye or Lorentz models of dispersion relations of the dielectric constants of the metals. In the simulations, we use the following dispersion model (an extended Debye model) for determining the dielectric constant for gold and silver. See schematic of simulated structure in Fig. 3a. The Rigorous Coupled Wave Analysis (RCWA) simulations were carried out using the software DiffractMOD 3.2, which also uses the extended Debye dispersion model.

TEM measurements

We obtained TEM cross-sectional images of the Au-coated nanowires by employing a Hitachi HF2000 is a 200kV Cold Field Emission Transmission Electron microscope (TEM).

The TEM cross-sections were prepared by employing Focused ion beam (FIB) milling (using FEI Quanta 3D FEG). More high resolution TEM images are provided in the Electronic Supplementary Material, Note 3).

Results and discussion

It has been previously demonstrated that single-molecule SERS can be achieved using metal nanoparticle arrays or aggregates when analytes are located in the “hot spot” (i.e. unusually intense electromagnetic field produced inside nanoscale gaps or nanoparticle junctions).²¹⁻²⁴ However, the main challenge lies in developing SERS-active substrates having controlled sizes, shapes and sub-10 nm gaps that can be fabricated in a reliable and reproducible manner.

The TEM cross-sections of the TSNWs, with the individual nanowires having a subwavelength length, are illustrated in Figs. 2c-e. Theoretical studies of rectangular grooves in zero-order silver gratings indicated substantial increase in the electric field intensity inside the grooves as the spacing between the metallic gratings decreased.³² The coupling of p-polarized incident radiation to surface plasmon polariton (SPP) modes in short-pitch metallic gratings have also been investigated.^{25,33-34} The narrow width of the grooves leads to strong coupling between surface charges on opposing walls of an individual grating groove, thereby leading to formation of standing SPP modes localized inside the groove. The coupling between the SPP modes inside the individual grooves increases with decreasing groove width. Figs. 2c-e show TEM cross-sections of triangular shaped silicon nanowires. Sub-10 nm gaps between the gold film deposited on top of the triangular-shaped silicon nanowires can be clearly observed, especially at the bottom region of the triangular-shaped nanowires – labeled as inverted triangular nanowire regions ‘G’ in Fig. 2a). As the Deep UV Lithography process used to develop the silicon nanowires can only allow a minimum gap of 100 nm between the nanowires to be achieved, we employ the growth or deposition of conformal thin film layers on top of the silicon nanowires to decrease the effective gaps between the nanowires. Subsequently, a plasmonics-active metal layer (e.g., Au, Ag) is deposited onto the nanowires. The TEM cross-sections (in Figs. 2d-e) also show the hafnium oxide spacer layer that was developed by employing atomic layer deposition (ALD) to conformally reduce the gap between the neighboring silicon nanowires before deposition of the gold film layer using E-Beam deposition. The silicon nanowires were coated with a layer of silicon germanium employing ultra high vacuum rapid thermal chemical vapor deposition as an optional step to reduce the gap between the nanowires before the metal deposition. The SERS substrates were evaluated for their uniformity for the different regions in which nanowires of different dimensions and spacings were developed (each region having nanowires of a certain dimension and periodicity). Upon taking four SERS measurements using a laser spot of $\sim 5 \mu\text{m}$, which ensures that several nanowires - on each $1 \text{ mm} \times 300 \mu\text{m}$ region in which nanowires of the same dimension and periodicity were developed - it was observed that there was less than 5% variation of the SERS signals from each region.

To investigate the plasmonics-active structures, numerical calculations - Rigorous Coupled Wave Analysis (RCWA) and Finite Difference Time Domain (FDTD) methods using the

software DiffractMOD 3.2 and Fullwave 6.2, respectively - were performed to determine the reflectance spectra from the plasmonics-active nanowires (TSNW) as well as to calculate the EM field enhancements in the spacings between the TSNW structures. In both the RCWA and FDTD calculations, the dispersion relation of plasmonics-active metals such as gold and silver was modeled using an extended Debye model.³⁵ A schematic of the simulated structures is shown in Fig. 3a. In the FDTD calculations (see details in Electronic Supplementary Material, Note 1), a continuous plane wave (633 nm) propagating in the z direction and having electric field (E-field) polarized in the x direction was incident on the gold nanowire structures. Enhancement in E-field intensity as a function of the incident radiation (normalized to 1) was calculated.

Fig. 3b shows concentration of the electromagnetic field intensity in certain regions in the gaps between the triangular gold-coated silicon nanowires. As the EM enhancement of SERS is approximately equal to the fourth power of the electric field enhancement,¹⁸ one can expect enhancement of the SERS signals in certain regions in between the gold-coated nanowires. High enhancement of SERS signals - at the top and the bottom regions in between the triangular shaped gold-coated nanowires - is essential for achieving high sensitivity of DNA (or RNA) detection as opening of the stem-loop of the MSC probe leads to the SERS-active dye attached to the ends of the molecules to move away from these plasmonics-active regions (i.e., regions having high enhancement of SERS signals). This leads to a decrease in the SERS signals from these molecules, thereby providing information about the specific detection of target DNA or RNA molecules. The RCWA calculations show the effect of varying the periodicity 'P' of the TSNWs on the plasmon resonance wavelength associated with reflection spectra of the nanowires (Fig. 3c). By selecting the correct dimensional parameters - the periodicity 'P' as well as the height 'H' of the TSNWs - one can match the plasmon resonance wavelengths in the reflection spectra of the nanowires with the wavelength (633 nm) of the incident laser employed (indicated by the dashed red line in Fig. 3c) in our SERS measurements. One can observe that as we change the periodicity 'P' from 240 nm to 260 nm, the plasmon resonance related dip in the reflection spectrum around 635 nm shifts slightly; although there are more significant shifts in the peak located around 780 nm. Hence, employing a periodicity in the range of 240-260 nm can allow us to couple the incident radiation of 633 nm (laser line wavelength) into plasmons. Moreover, the multiple dips in the reflection spectra indicate that the different structures (with different periodicities) can be employed for SERS measurements using lasers of different wavelengths. In both FDTD and RCWA simulations, the height 'H' of gold-coated inverted triangle-shaped nanowires was taken as ~ 475 nm. The effect of varying the refractive index of the medium surrounding the gold-coated nanowires is shown in Fig. 3d. The Rigorous Coupled Wave Analysis (RCWA) simulations in Fig. 3d show that the dips in the reflectance spectra have a red-shift on increasing the refractive index 'n' of the media surrounding the gold-coated inverted triangle-shaped nanowires, indicating that these dips in the reflectance spectra are due to excitation of surface plasmons in the gold-coated triangle-shaped nanowires (when TM polarized light is incident on the nanowires). Moreover, one can observe no dip in the reflectance spectra when TE polarized light is incident on the periodic gold-coated nanowires, for the different periodicities 'P' and refractive indices 'n' (See Figs. S1-S5 in the Electronic Supplementary Material, Note 2).

To demonstrate the applicability of the MS-based detection on the TSNW arrays, we chose a single transcript, the Ki-67 gene as the test system for use in the development of a nucleic acid detection platform.³⁶⁻³⁷ The Ki-67 gene encodes a cellular proliferation marker protein (Ki-67 antigen) and can potentially be used as an early predictor for cancer treatment efficacy.³⁶ The expression of the Ki-67 gene is well regulated throughout the cell cycle. In quiescent cells, the Ki-67 expression is very low. In breast cancer cells, the Ki-67 gene is significantly over-expressed.³⁷ This gene has been recognized as a critical breast cancer biomarker and has been adopted for diagnostic use in clinical practice.

The MSC hairpin probe (KI67-MSC) for the Ki-67 gene was carefully designed with a 6 base-pair stem sequence with the melting temperature (T_m) ≈ 46 °C allowing the formation of stable hairpin structure at room temperature in the absence of a complementary DNA target. The loop region of the hairpin probe was designed to incorporate a partial sequence with 23-mer complementary to the Ki-67 gene sequences. The 3'-end of the KI67-MSC nanoprobe was labeled with a SERS-active dye 5-carboxytetramethylrhodamine (TAMRA) as a signal reporter, and the 5'-end of the nanoprobe was modified with a thiol group to covalently attach the KI67-MSC nanoprobe to the gold-coated nanowire substrate. In the presence of a complementary DNA target, the T_m of the probe-target hybrid is higher than 65 °C, which would allow a more stable open-state configuration of the hairpin probe to form, resulting in the separation of the Raman label from the surface of the metal substrate and a decreased SERS signal. With the hairpin probe with 35-mer, the separation distance between the Raman label and the metal surface in the open state of the KI67-MSC nanoprobe was estimated to be over 10 nm. As the plasmonic enhancement of the incident EM fields is the highest at the surface of the gold-coated nanowires (TSNWs) and decreases as the distance away from the metal surface, this distance is large enough to significantly reduce the SERS signal.

The upper spectra (blank sample) in Fig. 4 shows the intense SERS signal from the KI67-MSC nanoprobe immobilized on a gold nanowire sample region (Fig. 3a). The strong SERS intensity of the major Raman peaks indicates that the MSC nanoprobe remains in the stem-loop configuration (closed state) on the TSNW substrate. Detection of unlabeled Ki-67 DNA target sequence was performed by incubating the functionalized TSNW substrate in a sample solution containing 1 μ M complementary DNA targets for one and a half hours to allow hybridization between the complementary 'probe' and Ki-67 'target' DNA. The SERS measurements were carried out (using a laser beam spot of ~ 5 μ m) in the same region (each region being 1 mm by 300 μ m and having nanowires of a certain dimension and periodicity) - and in $\sim$ the same section of the region - before and after the incubation. As the variation of the SERS signals in each region was observed to be less than 5%, carrying out measurements around the same section in each region before and after the incubation gives reliable SERS measurements values for the cases when the target DNA molecules are detected or not. The lower spectra (positive diagnostics) in Fig. 4 shows a decrease in SERS signal of the KI67-MSC nanoprobe after addition of the complementary Ki-67 target DNA (indicated by arrow signs). The decreased SERS signal indicates that hybridization with the complementary target DNA molecules causes opening of the stem-loop structure of the probe DNA molecules (open state), thereby separating the SERS dye, TAMRA, away from the plasmonics-active gold-coated nanowire surface, where the EM enhancement of the

incident optical fields is the highest. Moreover, following the opening of stem-loop structure of some of the probe DNA molecules upon hybridization with a complementary target molecule, the decrease of the overall SERS signal can also be attributed to the moving away of SERS dye molecules attached to some MSC probes from the SERS “hot spots” formed at the top and bottom regions in the gap between the gold-coated triangular nanowires. On the other hand, in the presence of non-complementary DNA (negative control: middle spectra in Fig. 4), the SERS intensity of the major Raman bands remains high, indicating that the MSC nanoprobe remains in the stem-loop configuration (closed state). According to the SERS intensity shown in Fig. 4, we estimated the SERS quenching efficiency to be 40~50 % of the original value upon hybridization of the KI67-MSC nanoprobe to the complementary target DNA. The remaining SERS intensity in the presence of the complementary target DNA may be contributed from those un-reacted nanoprobe located inside or near the high E-field regions on the TSNW substrate.

This paper first reports the proof of concept for the molecular sentinel-on-chip using a 1 μ M sample solution for the detection of Ki-67 DNA target. We delivered 150 μ L sample that spread over the entire chip area of $1.5 \times 1.5 \text{ cm}^2$. The laser beam spot of $\sim 5 \text{ }\mu\text{m}$ excited only a minute fraction (10^{-7}) of the sample (only 15 picoliter), which corresponds to 15 attomoles in the sample. Further work will be carried out to improve the detection sensitivity through the optimization of several fabrication parameters, such as probe surface density and chip size etc., in order to achieve a desired limit of detection. Moreover, the use of much smaller sample volumes to take advantage of the small spot size of the excitation laser beam will also be investigated. For practical clinical applications, it would be desirable to have a detection sensitivity in the range of 0.5-1.5 attomoles, which will allow detection of rare to moderately abundant mRNA transcripts (e.g. 10-100 copies/cell) from as little as 10,000-30,000 cells that can be harvested from clinical biopsies.³⁸⁻³⁹ Therefore, the sensitivity of the MSC will need to be improved by an order of 10-30 times for clinical applications using the proposed improvements. It is noteworthy that, in some cases, over 10^6 cells could be harvested from clinical biopsies³⁹⁻⁴⁰ making the MSC readily applicable for the detection of biomarkers in clinical samples.

Conclusions

We have demonstrated the feasibility of SERS-based DNA detection using the molecular sentinel-on-chip nanoprobe on a well-controlled and reproducible TSNW array with sub-10 nm metallic nanostructures over an entire 6-inch wafer, thus enabling tuning the localized surface plasmon resonance wavelength of the nanostructures with the incident light. This new approach utilizing a novel nano-platform could lead to the development of a reliable and useful tool for molecular diagnostics, potentially having multiplexing and high-throughput screening capabilities - application of the MSC technology for these capabilities will be evaluated in future studies. With the reproducible and controllable substrate fabrication process on a very large area (6-inch to 12-inch wafers), the array-based SERS molecular sentinel-on-chip technology could contribute to the development of novel DNA diagnostic tools. The label-free MSC approach of combining plasmonic modulation with DNA hairpin probes allows the SERS measurements to be performed immediately following

the hybridization reaction without washing steps, which greatly simplifies the assay procedures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank the sponsors of this work - National Institutes of Health of U.S.A. (Grants R01 EB006201 and R01 ES014774), the U. S. Defense Advanced Research Projects Agency (DARPA-N66001-09-C-2 0 8 2), and the Nanoscale Research Facility (NRF) at the Indian Institute of Technology-Delhi, India.

Notes and references

1. Fleischmann M, Hendra PJ, McQuillan AJ. Chem. Phys. Lett. 1974; 26:163.
2. Jeanmaire DL, Van Duyne RP. J. Electroanal. Chem. 1977; 84:1.
3. Albrecht MG, Creighton JA. J. Am. Soc. 1977; 99:5215.
4. Vo-Dinh T, Hiromoto MYK, Begun GM, Moody RL. Anal. Chem. 1984; 56:1667.
5. Vo-Dinh T, Houck K, Stokes DL. Anal. Chem. 1994; 66:3379. [PubMed: 7978314]
6. Moskovits M. Rev. Mod. Phys. 1985; 57:783.
7. Masson JF, Murray-Methot MP, Live LS. Analyst. 2010; 135:1483. [PubMed: 20358096]
8. Cao YC, Rongchao J, Mirkin CA. Science. 2002; 297:1536. [PubMed: 12202825]
9. Cui B, Clime L, Li K, Veres T. Nanotechnol. 2008; 19:145305.
10. Murphy CJ, Gole AM, Hunyadi SE, Stone JW, Sisco P, Alkilany A, Hankins PL, Kinard B. Chem. Comm. 2008; 5:544. [PubMed: 18209787]
11. Lee SJ, Morill AR, Moskovits M. J. Am. Chem. Soc. 2006; 128:2200. [PubMed: 16478159]
12. Qiu T, Wu XL, Shen JC, Ha PCT, Chu PK. Nanotechnol. 2006; 17:5769.
13. Khan MA, Hogan TP, Shaner B. J. Raman Spectrosc. 2008; 39:893.
14. Vo-Dinh T, Dhawan A, Khoury C, Norton S, Wang H-N, Misra V, Gerhold M. J. Phys. Chem. C. 2010; 114:7480–7488.
15. Isola NR, Stokes DL, Vo-Dinh T. Anal. Chem. 1998; 70:1352. [PubMed: 9553492]
16. Vo-Dinh T, Allain LR, Stokes DL. J. Raman Spectrosc. 2002; 33:511.
17. Wang H-N, Vo-Dinh T. Small. 2011; 7:3067. [PubMed: 21913327]
18. Otto A, Mrozek I, Grabhorn H, Akemann W. J. Phys. Condens. Matter. 1992; 4:1143.
19. Vo-Dinh T. Trends in Anal. Chem. 1998; 17:557.
20. Schatz GC. Acc. Chem. Res. 1984; 17:370.
21. Nie S, Emory SR. Science. 1997; 275:1102. [PubMed: 9027306]
22. Kneipp J, Kneipp H, Kneipp K. Chem. Soc. Rev. 2008; 37:1052. [PubMed: 18443689]
23. Otto A, Bruckbauer A, Chen YX. J. Mol. Struct. 2003; 661-662:501.
24. Xu H, Bjerneld EJ, Kall M, Borjesson L. Phys. Rev. Lett. 1999; 83:4357.
25. Garcia-Vidal FJ, Pendry JB. Phy. Rev. Lett. 1996; 77:1163.
26. Kottmann JP, Martin OJF, Smith DR, Schultz S. Phys. Rev. B. 2001; 64:235402.
27. Lombardi JR, Birke RL. J. Chem. Phys. 2007; 126:244709. [PubMed: 17614579]
28. Persson BNJ, Zhao K, Zhang Z. Phy. Rev. Lett. 2006; 96:207401.
29. Wabuyele MB, Vo-Dinh T. Anal. Chem. 2005; 77:7810. [PubMed: 16316192]
30. Wang H-N, Vo-Dinh T. Nanotechnol. 2009; 20:065101–1.
31. Herne TM, Tarlov MJ. J. Am. Chem. Soc. 1997; 119:8916.
32. Wirgin A, Lopez-Rios T. Opt. Comm. 1984; 48:416.
33. Garcia-Vidal FJ, Martin-Moreno L. Phys. Rev. B. 2002; 66:155412–1.

34. Dhawan A, Canva M, Vo-Dinh T. *Opt. Exp.* 2011; 19:787.
35. Dhawan A, Norton SJ, Gerhold MD, Vo-Dinh T. *Optics Express*. 2009; 17:9688. [PubMed: 19506618]
36. Urruticoechea A, Smith IE, Dowsett M. *Journal of Clinical Oncology*. 2005; 23:7212. [PubMed: 16192605]
37. Van Dierendonck JH, Keijzer R, Van de Velde CJH, Cornelisse CJ. *Cancer Research*. 1989; 49:2999. [PubMed: 2720660]
38. Stomper PC, Budnick R, Stewart CC. *Investigative Radiology*. 1998; 33:51. [PubMed: 9438510]
39. Duggan DJ, Bittner M, Chen Y, Meltzer P, Trent JM. *Nature Genetics*. 1999; 21:10. [PubMed: 9915494]
40. Ballet F, Bouma ME, Wang SR, Amit N, Marais J, Infante R. *Hepatology*. 2008; 4:849. [PubMed: 6090291]

Fig. 1.
The operating principle of the MS-based DNA detection on a TSNW substrate.

Fig. 2.

(a) Schematic showing the development steps of triangle-shaped nanowires (TSNW): Silicon nanowire by deep UV lithography and TMAH chemical etching, Coating with a spacer layer using atomic layer deposition, Deposition of a thin gold film layer using E-Beam deposition. (b) Picture showing half of a 6-inch SERS substrate wafer with gold nanowire structures. (c), (d) TEM cross-section of triangular silicon nanowires developed by TMAH chemical etching. (e) Enlarged view of the TEM cross-section region highlighted in Fig. 2d showing triangular silicon nanowires (in off-white color) over-coated with a silicon germanium layer (in light gray color) and a hafnium oxide layer (in dark gray color) and finally over-coated with a thin gold film layer (in black color). The TEM cross-sections were prepared by employing Focused ion beam (FIB) milling.

Fig. 3.

(a) Schematic of periodic gold-coated inverted triangle-shaped nanowire (TSNW) structures analyzed using FDTD and RCWA calculations. (b) Finite Difference Time Domain (FDTD) simulation showing enhancement of E-field (electric field) intensity in the gaps between the thin gold film layer deposited on top of hafnium oxide coated silicon nanowires, with the periodicity 'P' of the inverted triangle-shaped nanowires being 240 nm. (c) Rigorous Coupled Wave Analysis (RCWA) simulations showing the effect of varying the periodicity 'P' of the gold-coated inverted triangle-shaped nanowires on the plasmon resonance wavelengths in the reflection spectra of the nanowires. The refractive index 'n' of the surrounding media was taken as 1.33. The dashed vertical line indicates the incident laser line wavelength. (d) Rigorous Coupled Wave Analysis (RCWA) simulations showing the effect of varying the refractive index 'n' of the media surrounding the gold-coated inverted triangle-shaped nanowires. In both FDTD and RCWA simulations, the height 'H' of gold-coated inverted triangle-shaped nanowires was taken as $\sim 475 \text{ nm}$ and the incident radiation was TM polarized.

Fig. 4.

SERS spectra of the immobilized KI67-MSC nanoprobes in the presence or absence of complementary DNA targets. (a) Upper spectrum: blank (no target DNA present). (b) Middle spectrum: in the presence of 1 μ M non-complementary DNA (negative control). (c) Lower spectrum: in the presence of 1 μ M complementary target DNA (positive diagnostic). The arrow signs illustrate the decreased SERS intensity of the major Raman bands in the presence of complementary target DNA.