Plasmonic Si@Au core-satellite nanoparticles prepared by laser-assisted synthesis for photothermal therapy

Ahmed Al-Kattan1, https://orcid.org/0000-0003-2454-8436, Gleb Tselikov1,2, https://orcid.org/0000-0002-6664-7881, Anton A. Popov1,3, https://orcid.org/0000-0001-8902-0371, Andrei V. Kabashin1,3, https://orcid.org/0000-0003-1549-7198

1 Aix Marseille Univ, CNRS, LP3, Campus de Luminy, Case 917, 13288, Marseille, France
2 Center for Photonics and 2D Materials, Moscow Institute of Physics and Technology, Dolgoprudny 141700, Russia
3 Institute for Engineering Physics of Biomedicine (PhysBio), Bio-nanophotonics Laboratory, 31 Kashirskoe sh., 115409 Moscow, Russia

Corresponding author: ahmed.al-kattan@univ-amu.fr

Abstract. We describe a laser-assisted methodology for the fabrication of Si@Au core-satellite nanostructures for photothermal therapy applications. The methodology consists in laser ablative synthesis of Si and Au NPs in water/ethanol solutions, followed by a chemical modification of the Si NPs by APTMS and their subsequent decoration by the Au NPs. We show that despite a relatively small size (< 40 nm) the formed core-satellites exhibit a strong plasmonic absorption peak centred around 610 nm and having a large tail over 700 nm overlapping with the first optical window of relative tissue transparency. Being relatively small and exempt of any toxic impurity due to ultraclean laser synthesis, the fabricated nanoparticles promise a major advancement of imaging and phototherapy modalities based on plasmonic properties of nanomatererials.

1. Introduction

Photothermal hyperthermia is one of exciting implementations of light-induced therapy, which consists in overheating of malignant tissues resulting in its destruction or activation of immune response against tumor. Plasmonic nanomaterials can efficiently sensitize external light stimulus very locally, thus drastically decreasing collateral damage to healthy tissues during a hyperthermic procedure. However, classical plasmonic nanomaterials based on simple spherical Au nanoparticles (NPs) are hardly useful for these tasks, as the plasmonic absorption band of these NPs is centered around 520-550 nm, which is far from the window of relative tissue transparency (650-1000 nm). This plasmonic mismatch problem can partly be solved by engineered Au-based nanoarchitectures such as Au nanorods [1] or dielectric-Au core-shell NPs [2,3]. Plasmonic absorption of such complex nanostructures can be tuned toward the transparency window by changing the aspect ratio of the nanorods or the ratio of the shell thickness to core diameter in core-shells. The engineered Au-based nanorods and core-shells have already demonstrated high efficiency as sensitizers of light-induced hyperthermia of cancer tissues, both in vitro and in vivo [1-3], but the employment of these nanostructures is not free of problems and challenges. The main problems of such nanostructures are their residual toxicity arising due to contamination by by-products during conventional chemical synthesis [2-5], elongated shape of the nanorods [4,6] and relatively large size of SiO2-Au core-shells [7], which can complicate their transport in the organism.
and excretion from it. The required red-shift of the plasmonic peak at smaller core-shell sizes could be achieved by using a core material with high refractive index such as silicon (Si) instead of silica (SiO₂) [8,9]. However, this is typically achieved by complicated chemical methods.

Alternative physical method based on pulsed laser ablation in liquids can offer a simple solution of the problem related to a residual contamination of nanomaterials [10,11] and complexity of their synthesis. This approach is based on natural formation of smallest clusters under irradiation of a solid target by laser beam. The clusters can then be deposited on a substrate to form a nanostructured layer [12,13] or form a colloidal NPs solution in a liquid medium [14-16]. This method does not require any chemicals and can be performed in an ultrapure liquid such deionized water excluding any residual contamination of the nanoparticle surface, which is critically important for projected biomedical applications [17,18]. As an example, our group recently elaborated the technique of femtosecond (fs) laser ablation in water and organic media for controllable synthesis of NPs from a variety of materials, including Au [19,20], Si [21-23], TiN [23,24] NPs. Laser ablation can also be used for the synthesis of complex structures such as core-shells, non-equilibrium alloys and mixed phase NPs. As an example, we recently successfully demonstrated fs laser ablative synthesis of contamination-free Si-Au core-shell nanostructures [25]. We found that such nanostructures, despite having a relatively small size (less than 50 nm), have plasmonic absorption feature in the window of relative tissue transparency.

In this paper we provide details about optimization of the synthesis protocol of such Si-Au core-shell NPs to achieve maximum red shift of the plasmonic feature. We believe that this nanostructures with exceptional purity could provide a major advancement in biomedical applications.

2. Experimental methods

Solutions of colloidal Si NPs were obtained from 0.5 µm Si micropowder, which was preliminary prepared by mechanical milling of a Si wafer as it was described in [21,22]. Si powder was dispersed in liquid by a ultrasonication for 15 minutes in a 4.5 mL vial at 0.4 mg/mL concentration. Three liquids were used: pure anhydrous ethanol (100% ethanol), 1:1 water/ethanol mixture (50% ethanol) and pure water (0% ethanol). Si micropowder solutions were then fragmented for 60 minutes using radiation from a Yb:KGW femtosecond laser (Amplitude systems, France, wavelength 1025 nm, repetition rate 10 kHz, pulse duration 480 fs). The laser beam was focused in the center of a vial filled by micropowder solutions using a 75 mm lens. The homogenization of the solution was done by a magnetic stirrer. Unfragmented Si powder was removed by the centrifugation at 3500 rpm/min for 20 minutes leaving Si NPs at ~0.1 mg/mL concentration. Au NPs were synthesized using the same laser in the ablation geometry, as previously described in [19,20,27]. For this, a gold target (99.99%, Sigma Aldrich) immersed in 7 mL of 0.1m aqueous NaCl solution was irradiated by a laser beam focused by the same 75 m lens. To ensure a homogenous ablation process, the ablation target was continuously moved with 2 mm/s speed using motorized linear translational stages (Newport).

The functionalization step was performed by adding a solution of 3-aminopropyltrimethoxysilane (APTMS) (Sigma Aldrich) to Si NPs solutions at ambient condition. The obtained mixture was stirred at room temperature for 24 hours. Then aqueous solutions of Au NPs were gently added to Si-APTMS NPs solutions and additionally stirred at ambient conditions for 24 hours.

A transmission electron microscopy (TEM) system (JEOL JEM 3010) operating at 300 kV was employed to perform structural characterizations (size distribution, crystalline structure, morphology) of Si-Au nanostructures. Samples for the electron imaging were prepared by placing a drop of a NPs solution on a carbon coated copper TEM grid (200 mesh, Oxford instruments) and its drying at ambient conditions. Statistical data were obtained based on analysis of size characteristics from several hundreds of NPs. ζ-potential measurements were performed using a Zetasure Nano ZS system (Malvern Instruments). Optical extinction spectra of the NPs were measured by a UV-Vis spectrophotometer (UV-2600, Shimadzu) using 10 mm optical path length quartz cuvettes.
3. Results and discussion
Laser ablation of a Si target resulted in crystalline Si NPs having spherical shape with lognormal size distribution and mode size of about $30 \pm 3$ nm as follows from TEM and SAED analysis (data not shown). At the same time, Au NPs prepared by laser ablation in 0.1 mM aqueous NaCl solutions also had a spherical shape with normal size distribution and mean size $4 \pm 0.8$ nm. As follows from the electron diffraction pattern (data not shown), the formed Au NPs had FCC lattice.

To overcome electrostatic repulsion of Si and Au NPs, which are both negatively charged ($-45 \pm 1.5$ mV and $-32 \pm 2.6$ mV, respectively), and to form core-shell structures we functionalized surface of Si NPs by an organosilane molecule 3-aminopropyliethoxysilane (APTMS), which is widely used to coat organic NPs and functionalize them with a variety of molecules. APTMS is a linear chain molecule with two alkoxy functional groups on one end and an amine functional group on the other end. Hydrolysis of methoxy groups of APTMS followed by their reaction with the hydroxyl Si-OH group on the oxidized Si NPs surface results in formation of Si-O-Si linkages and firm binding of APTMS molecules at the Si NPs surface. At neutral or acidic conditions amine group of the APTMS molecules is protonated, which renders positive surface charge of functionalized Si NPs. This was confirmed experimentally by measuring Zeta-potential. The potential of Si NPs increased from $-45 \pm 1.5$ mV to $2.58 \pm 0.20$ mV after functionalization by APTMS. Therefore, the functionalization makes possible the electrostatic coupling between positively charged functionalized Si NPs and negatively charged Au NPs [28].

The formed nanostructures had core-satellite geometry with a large Si core decorated by small Au NPs as shown in Figure 1. The Si cores of Si-Au core-shells were fully coated with a continuous shell of randomly distributed Au NPs, while separated Au NPs were rare in the solution. Statistical analysis of the size of nanostructures showed that the mean size of silicon cores was about 37 nm, while the thickness of Au NPs-based shell was about 4 nm, with the mean size of core-shells close to 40 nm, which matched the original size of Si and Au NPs.

![Figure 1. Statistical analysis of sizes of Si-Au core-shell nanostructures. Insert demonstrates typical TEM image of the core-shell NPs prepared by fs laser ablation in water.](image-url)
functionalized Si NPs and Au NPs in concentrations 0.1 mg/mL in different ratios, namely 1:1, 1:10, 1:30 (Si:Au). The 1:30 ratio provided the largest red shift of the plasmonic feature.

We finally studied dependence of plasmonic peak position on the liquid medium in which APTMS molecules were grafted to Si NPs surface. For this we separately functionalized Si NPs in pure anhydrous ethanol (100% ethanol), 1:1 water:ethanol mixture (50% ethanol) and in pure water (0% ethanol). The results are shown in Figure 2. As one can see, the increase of ethanol content resulted in increase of red shift of the plasmonic peak. In particular, at 0% ethanol (pure water) position of the plasmonic peak of the core-shell structures was almost identical to the position of the plasmonic peak of pure Au NPs. This mean that core-shell NPs did not form under such conditions most probably due to fast hydrolysis of methoxy functional groups of APTMS molecules and their self-polymerization before reaching surface of Si NPs. At the same time, Si-Au core-shell NPs prepared in 100% (anhydrous) ethanol had the largest red shift of the plasmonic peak, which was located close to 610 nm.

![Figure 2](image.jpg)

**Figure 2.** Extinction spectra of Si-Au core-satellite nanocomposites conjugated in different solvents: 100 % ethanol (black curve, anhydrous ethanol), 50% ethanol (red curve, 1:1 water: ethanol mixture) and 0% ethanol (blue curve, pure water).

Thus, by applying a chemical protocol on laser synthesized Si and Au nanomaterials, we managed to assemble core-satellite Si@Au nanostructures exhibiting a much red-shifted plasmonic extinction peak compared to spherical Au nanoparticles (610 nm compared to 520-550 nm). We suppose that the formed core-satellite structures are promising objects for photothermal therapy of cancer. As one of the main advantages for such applications, we see exceptional purity of formed nanoformulations due to complete avoidance of the chemical reduction step. Indeed, Si and Au NPs are produced separately by femtosecond laser ablation in deionized water, which leads to the formation of bare (ligand-free) surface in the absence of any contamination by toxic products. Laser-synthesized Au and Si NPs present highly safe objects for biological systems, as we showed in previous tests in vitro and in vivo [20-23]. Furthermore, laser-synthesized Si NPs are biodegradable as in biological environment they decay and excrete from the body with the urine within several days [22]. A subsequent application of chemical protocol on the basis on APTMS can not add any toxicity as APTMS is a highly biocompatible product demonstrated in various biomedical applications [28]. Another expectation is related to possible gradual...
decay of core-satellites under long-term exposition to biological environment and a complete excretion of decay products from the organism.

It should be noted that laser-ablation approached has already been applied for the synthesis of Au-based core-shells, using spontaneous formation of core-shells during the ablation or co-ablation of materials having appropriate physico-chemical properties [29-31], or by the combination of laser ablation with an additional chemical reduction step using HAuCl₄ solutions [32,33]. However, the first laser-ablative approach typically leads to a wide size and shape dispersion of formed composites, while the second combination approach still cannot avoid residual contamination by side products of the chemical reduction process. In this sense, the combined methodology proposed in our study is capable of avoiding problems of both approaches.

We believe that the core-satellite geometry of Si-Au nanocomposites can be advantageous for these applications compared to classical SiO₂-Au core-shell nanostructures [2,3,5] due to a much smaller size. Indeed, the diameter of core-satellites is less than 40 nm, which is in the range of relative safety of nanomaterials for biological systems and should favor transport of nanostructures in vivo to reach affected organs and their easier removal from the organism. Besides, the presence of laser-ablated Si nanostructures in the composition of core-satellites can enable a series of novel theranostic functionalities, including the generation of non-linear response for bioimaging [34], hyperthermia-based therapies under radiofrequency [35] and photon [36] excitation. As another potential application, we see the use of core-satellites as probes for Surface Enhanced Raman Scattering (SERS) for the identification of trace amounts of biological species. Inverted geometry of Au-Si core-shells, composed of Au core covered by Si shell, has already been reported in our previous study [37]. Exhibiting local electric field enhancement due to the excitation of plasmons around 520 nm, such core-shells demonstrated a high efficiency in the identification of bacteria cultures [37]. We expect that Si-Au core-satellites can provide similar enhancement and be used with red-shifted pumping wavelength (e.g., 630, 785 nm) for SERS excitation, which is preferable under some conditions. It should be finally noted that silicon/gold architecture also presents a very promising platform for surface plasmon resonance biosensing [38,39], which can have advantages over classical glass/gold platform [40-42] due to essentially different conditions of plasmon coupling. We believe that the proposed core-satellites can be also explored as nanoscale implementations of such biosensors.

4. Conclusions
In summary, we described a laser-assisted procedure for the fabrication of Si@Au core-satellite nanostructures, comprising of a Si core covered with small Au nanoparticles (NPs). The methodology employed laser ablative synthesis of Si and Au NPs in water/ethanol solutions, followed by their chemical modification and linking via organosilane APTMS molecules. The core-satellites had a strong plasmonic peak centered around 600-610 nm with a long tail over 700 nm, which overlaps with biological transparency window. Being relatively small and exempt of any toxic impurity due to ultraclean laser synthesis, the fabricated Si@Au core-satellites look as a promising object for cancer theranostics.

Acknowledgements
A.A.-K. thanks the International Associated Laboratory (LIA) MINOS project and GRAVITY project of the ITMO “Plan Cancer 2014–2019” INSERM program. The authors acknowledge contribution of the Russian Science Foundation (Project 19-72-30012) for the elaboration of protocols for the fabrication of highly calibrated nanoparticles.

Conflict of Interests
The authors declare no conflict of interest.

References
[1] Huang X, El-Sayed I H, Qian W, El-Sayed M A 2006 Cancer cell imaging and photothermal
therapy in the near-infrared region by using gold nanorods. *J. Am. Chem. Soc.* **128** 2115-2120.

[2] Hirsch L R, Stafford R J, Bankson J A, Sershen S R, Rivera B, Price R E, Hazle J D, Halas N J, West J L 2003 Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance *Proc. Natl. Acad. Sci.* **100** 13549-13554.

[3] Loo C, Lowery A, Halas N, West J, Drezer K 2005 Immunotargeted nanoshells for integrated cancer imaging and therapy. *Nano Lett.* **5** 709-711.

[4] Dasgupta S, Auth T, Gompper G 2014 Shape and orientation matter for the cellular uptake of nonspherical particles *Nano Lett.* **14** 687–693.

[5] Oldenburg S J, Averitt D, Westcott S L, Halas N J 1998 Nanoengineering of optical resonances. *Chem. Phys. Lett.* **288** 243-247.

[6] Yu M, Zheng J 2015 Clearance pathways and tumor targeting of imaging nanoparticles *ACS Nano* **9** 6655–6674.

[7] James W D, Hirsch L R, West J L, O’Neal P D, Payne J D 2007 Application of INAA to the build-up and clearance of gold nanoshells in clinical studies in mice *J. Radioanal. Nucl. Chem.* **271** 455–459.

[8] Lv W, Phelan P E, Swaminathan R, Otanicar T P, Taylor R A 2013 Multifunctional core-shell nanoparticle suspensions for efficient absorption *J. Sol. Energy Eng.* **135** 021004.

[9] Chaabani W, Chehaidar A, Plain J 2016 Comparative theoretical study of the optical properties of silicon/gold, silica/gold core/shell and gold spherical nanoparticles *Plasmonics* **11** 1525–1535.

[10] Kabashin A V, Delaporte P, Grodo J, Torres R, Sarnet T, Sentis M 2010 Nanofabrication with pulsed lasers *Nanoscale Res. Lett.* **5** 454–463.

[11] Zhang D, Gökce B, Barcikowski S 2017 Laser synthesis and processing of colloids: fundamentals and applications *Chemical Reviews* **117** 3990-4103.

[12] Kabashin A V, Meunier M 2003 Visible Photoluminescence from Nanostructured Si-Based Layers Produced by Air Optical Breakdown on Silicon *Appl. Phys. Lett.* **82** 1619–1621.

[13] Kabashin A V Meunier M 2003 Laser-induced treatment of silicon in air and formation of Si/SiOx photoluminescent nanostructured layers *Mater. Sci. Eng. B* **101** 60–64.

[14] Fojitk A, Henglein A 1993 Laser Ablation of Films and Suspended Particles in Solvent-Formation of Cluster and Colloid Solu-tions, *Ber. Bunsenges. Phys. Chem.* **97** 252-254.

[15] Dolgaev S I, Simakin A V, Vornov V V, Shafiev G A, Bozon-Verduraz F 2002 Nanoparticles Produced by Laser Ablation of Solids in Liquid Environment. *Appl. Surf. Sci.* **186** 546–551.

[16] Kabashin A V, Meunier M 2003 Synthesis of Colloidal Nanoparticles during Femtosecond Laser Ablation of Gold in Water *J. Appl. Phys.* **94** 7941.

[17] Kabashin A V, Timoshenko V Y 2016 What Theranostic applications could ultrapure laser-synthesized Si nanoparticles have in cancer? *Nanomedicine* **11** 2247–2250.

[18] Kabashin A V, Singh A, Swihart M T, Zavestovskaya I N, Prasad P N 2019 Laser-processed nanosilicon: a multifunctional nanomaterial for energy and healthcare. *ACS Nano* **13** 9841-9867.

[19] Maximova K, Aristov A, Sentis M, Kabashin A V 2015 Size-Controllable Synthesis of Bare Gold Nanoparticles by Femtosecond Laser Fragmentation in Water *Nanotechnology* **26** 65601.

[20] Bailly A-L, Correard F, Popov A, Tselikov G, Chaspol F, Appay R, Al-Kattan A, Kabashin A V, Bragger D, Esteve M-A 2019 In vivo evaluation of safety, biodistribution and pharmacokinetics of laser-synthesized gold nanoparticles *Sci. Rep.* **9** 12890.

[21] Baati T, Al-Kattan A, Esteve M A, Njim L, Ryabchikov Y, Chaspol F, Hammami M, Sentis M, Kabashin A V, Bragger D 2016 Ultrapure laser-synthesized Si-based nanomaterials for biomedical applications: In vivo assessment of safety and biodistribution. *Sci. Rep.* **6** 1–13.

[22] Al-Kattan A, Ryabchikov Y V, Baati T, Chirvony V, Sánchez-Royo J F, Sentis M, Bragger D, Timoshenko V Y, Estève M-A, Kabashin A V 2016 Ultrapure Laser-Synthesized Si Nanoparticles with Variable Oxidation States for Biomedical Applications *J. Mater. Chem. B* **4** 7852–7858.

[23] Petriev V M, Tischenko V K, Mikhailovskaya A A, Popov A A, Tselikov G, Zelepukin I, Deyev S M, Kaprin A D, Ivanov S, Timoshenko V Y, et al 2019 Nuclear nanomedicine using Si
nanoparticles as safe and effective carriers of 188Re radionuclide for cancer therapy Sci. Rep. 9 2017

[24] Popov A A, Tselikov G, Dumas N, Berard C, Metwally K, Jones N, Al-Kattan A, Larrat B, Braguer D, Mensah S, Da Silva A, Estève M-A, Kabashin A V 2019 Laser- synthesized TiN nanoparticles as promising plasmonic alternative for biomedical applications Sci. Rep. 9 1194.

[25] Zelepukin I V, Popov A A, Shipunova V O, Tikhonowski G V, Mirkasymov A B, Popova-Kuznetsova E A, Klimentov S M, Kabashin A V, Deyev S M 2020 Laser-synthesized TiN nanoparticles for biomedical applications: evaluation of safety, biodistribution and pharmacokinetics. Mater. Sci. Eng. C. 120 111717.

[26] Al-Kattan A, Tselikov G, Metwally K, Popov A A, Mensah S, Kabashin A V 2021 Laser Ablation-Assisted Synthesis of Plasmonic Si@Au Core-Satellite Nanocomposites for Biomedical Applications Nanomaterals 11 592.

[27] Sylvestre J, Poulin S, Kabashin A V Sacher E Meunier M Luong J H T 2004 Surface Chemistry of Gold Nanoparticles. Produced by Laser Ablation in Aqueous Media, J. Phys. Chem. B 108 16864–16869.

[28] Westcott S L, Oldenburg S J, Lee R, Halas N J 1998 Formation and adsorption of clusters of gold nanoparticles onto func-tionalized silica nanoparticles, Langmuir 14 5396–5401.

[29] Amendola V, Scaramuzza S, Litti L, Meneghetti M, Zuccolotto G, Rosato A, Nicolato E, Marzola P, Fracasso G, Anselmi C et al 2014 Magneto-plasmonic Au-Fe alloy nanoparticles designed for multimodal SERS-MRI-CT imaging Small 10 2476–2486.

[30] Lin F, Yang J, Lu S-H, Niu K-Y, Liu Y, Sun J, Du X-W 2010 Laser synthesis of gold/oxide nanocomposites. J. Mater. Chem. 20 1103–1106.

[31] Jo Y K, Wen S-B 2013 Formation of core–shell micro/nano particles through pulsed-laser deposition in liquid J Phys. D Appl. Phys. 46 035302.

[32] Jiménez E, Abderrafi K, Argabas R, Valdés J L, Martínez-Pastor J P 2010 Laser-ablation-induced synthesis of SiO2-capped noble metal nanoparticles in a single step Langmuir 26 7458–7463.

[33] Liu P, Chen H, Wang H, Yan J, Lin Z, Yang G 2015 Fabrication of Si/Au core/shell nanoplasmic structures with ultrasensitive surface-enhanced Raman scattering for monolayer molecule detection J Phys. Chem. C 119 1234–1246.

[34] Khairin A Y, Lysenko V V, Rogov A, Ryabchikov Y V, Geloen A, Tishchenko I, Marty O, Sennikov P G, Kornev R A, Zavestovskaya I N, Kabashin A V Timoshenko V Y 2019 Bi-modal nonlinear optical contrast from Si nanoparticles for cancer theranostics Adv. Opt. Mater. 7 1801728.

[35] Tamarov K P, Osminka L A, Zinovyev S V, Maximova K A, Kargina J V, Gongalsky M B, Ryabchikov Y, Al-Kattan A, Svirdov A P, Sentis M, Ivanov A V, Nikiforov V N, Kabashin A V, Timoshenko V Y Radio frequency radiation-induced hyperthermia using Si nanoparticle-based sensitizers for mild cancer therapy Sci. Rep. 4 7034.

[36] Oleschhenko V A, Yu. Khairin A, Alykova A F, Karpukhina O V, Karpov N V, Popov A A, Bezotosnyi V V, Klimentov S M, Zavestovskaya I N, Kabashin A V, et al 2020 Localized infrared radiation-induced hyperthermia sensitized by laser-ablated silicon nanoparticles for phototherapy applications Appl. Surf. Sci. 516 145661

[37] Köglr M, Ryabchikov Y V., Uusitalo S, Popov A, Popov A, Tselikov G, Vallimaa A-L, Al-Kattan A, Hiltunen J, Laitinen R, et al 2018 Bare laser-synthesized Au-based nanoparticles as nondisturbing surface-enhanced Raman scattering probes for bacteria identification J. Biophotonics 11 e201700225

[38] Patkovsky S, Kabashin A V, Meunier M, Luong J H T 2004 Near-infrared surface plasmon resonance sensing on a silicon platform, Sensor Actuat. B - Chem. 97 409-414.

[39] Patkovsky S, Kabashin A V, Meunier M, Luong J H T, 2003 Si-based surface plasmon resonance sensing with two surface plasmon polariton modes, Appl. Opt. 42 6905.

[40] Liedberg B, Nylander C, Lunström I 1983 Surface plasmon resonance for gas detection and
[41] Kabashin A V, Kochergin V E, Nikitin P I 1999 Surface plasmon resonance bio- and chemical sensors with phase-polarisation contrast, *Sensor Actuat. B - Chem*. **54** 51–56.

[42] Law W C, Markowicz P, Yong K T, Roy I, Baev A, Patskovsky S, Kabashin AV, Ho H P, Prasad P N 2007 Wide dynamic range phase-sensitive surface plasmon resonance biosensor based on measuring the modulation harmonics *Biosens. Bioelectron*. **23** 627–632.