NRC Publications Archive Archives des publications du CNRC

Rapid detection of microorganisms with nanoparticles and electron microscopy

Naja, Ghinwa; Hrapovic, Sabahudin; Male, Keith; Bouvrette, Pierre; Luong, John H. T.

This publication could be one of several versions: author's original, accepted manuscript or the publisher's version. / La version de cette publication peut être l'une des suivantes : la version prépublication de l'auteur, la version acceptée du manuscrit ou la version de l'éditeur.

For the publisher's version, please access the DOI link below. / Pour consulter la version de l'éditeur, utilisez le lien DOI ci-dessous.

Publisher's version / Version de l'éditeur:

<https://doi.org/10.1002/jemt.20614>

Microscopy Research and Technique, 71, 10, pp. 742-748, 2008-06-20

NRC Publications Record / Notice d'Archives des publications de CNRC:

<https://nrc-publications.canada.ca/eng/view/object/?id=e7be1ee4-9ee2-4c03-9319-e6703fb218a9>

<https://publications-cnrc.canada.ca/fra/voir/objet/?id=e7be1ee4-9ee2-4c03-9319-e6703fb218a9>

Access and use of this website and the material on it are subject to the Terms and Conditions set forth at

<https://nrc-publications.canada.ca/eng/copyright>

READ THESE TERMS AND CONDITIONS CAREFULLY BEFORE USING THIS WEBSITE.

L'accès à ce site Web et l'utilisation de son contenu sont assujettis aux conditions présentées dans le site

<https://publications-cnrc.canada.ca/fra/droits>

LISEZ CES CONDITIONS ATTENTIVEMENT AVANT D'UTILISER CE SITE WEB.

Questions? Contact the NRC Publications Archive team at

PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca. If you wish to email the authors directly, please see the first page of the publication for their contact information.

Vous avez des questions? Nous pouvons vous aider. Pour communiquer directement avec un auteur, consultez la première page de la revue dans laquelle son article a été publié afin de trouver ses coordonnées. Si vous n'arrivez pas à les repérer, communiquez avec nous à PublicationsArchive-ArchivesPublications@nrc-cnrc.gc.ca.

Rapid Detection of Microorganisms With Nanoparticles and Electron Microscopy

GHINWA NAJA,^{1*} SABAHUDIN HRAPOVIC,² KEITH MALE,² PIERRE BOUVRETTE,² AND JOHN H.T. LUONG²

¹Department of Chemical Engineering, McGill University, 3610 University Street, H3A2B2 Montreal, Quebec, Canada

²Biotechnology Research Institute, National Research Council Canada, H4P 2R2, Montreal, Quebec, Canada

KEY WORDS *Escherichia coli*; *Rhodococcus rhodochrous*; *Candida* sp.; electrostatic interactions

ABSTRACT Rapid detection of microorganisms is highly desirable. A procedure has been developed based on interactions between gold nanoparticles and proteins of microorganisms (*Escherichia coli*, *Rhodococcus rhodochrous*, and *Candida* sp.) followed by scanning electron microscopy (SEM). The nanoparticle-cell interaction was confirmed by ultraviolet resonance Raman spectroscopy (UVRS) in the SEM focus. Cell suspensions in a buffer were interacted with gold nanoparticles (<10 nm in diameter) prepared from tetrachloroauric acid and sodium borohydride. Possible interference of elevated salt concentrations was eliminated by dialysis in deionized water. Small (10 μ L) aliquots of cell-nanoparticle suspensions were dried on a silicon wafer and photographed under an SEM. Characteristic bacterial or yeast cell images in the micrographs indicated the actual presence of microorganisms in the suspension examined. This was further confirmed by UV resonance Raman spectroscopy. *Microsc. Res. Tech.* 71:742–748, 2008. © 2008 Wiley-Liss, Inc.

INTRODUCTION

With high-resolution for surface imaging, scanning electron microscopy (SEM) is widely used for probing cell types and structures. Most often SEM requires the fixation of cells in suspension or even their attachment to surfaces. Aqueous chemical fixation and dehydration frequently cause solubilization or aggregation of cellular components, resulting in altered morphology (Little et al., 1991). The standard SEM protocol is also time-consuming and tedious (Amako and Umeda, 1977; Hernandez and Guillen, 2000), requiring at least 8 h to chemically stain cells for imaging. Therefore, alternative procedures have been developed to prepare bacteria for SEM studies. Crang and Pechak (1978) utilized osmium tetroxide vapor for fixation whereas the visualization of ultrastructural features of most bacteria could be improved by a nonchemical procedure, namely cryofixation followed by freeze-substitution. Graham and Beveridge (1990) further minimized specimen preparation using a variable pressure SEM to image hydrated microorganisms. Fox and Demaree (1999) introduced and compared the fixation process with a new protocol utilizing microwave irradiation to prepare *Enterococcus* for SEM. Seviour et al. (1984) described a rapid procedure for preparing both prokaryotic and eukaryotic microbial cells in suspension.

This study describes a simple and fast procedure using small amounts of chemicals for microorganisms imaging. This 30-min method is based on a fast interaction between gold nanoparticles and proteins of microorganisms and examination of the interaction products using scanning electron microscopy (SEM). A simple dialysis procedure would be an extra recommended step to enhance the quality of the SEM images. The technique was applied to different types of bacteria

and yeasts (*Escherichia coli*, *Rhodococcus rhodochrous*, and *Candida* sp.) with the resulting images compared with the conventional fixation procedure. Ultraviolet Resonance Raman spectra confirmed the presence of the microorganisms as in the SEM focus.

MATERIALS AND METHODS

Gold Nanoparticles Preparation

Gold nanoparticles (<10 nm diameter) were synthesized by reducing tetrachloroauric acid (HAuCl₄) with sodium borohydride (NaBH₄) in aqueous solution (Birrell et al., 1987). The glassware was cleaned in aqua regia (nitric acid/hydrochloric acid 1:3) and all solutions were prepared using Milli-Q (Millipore, Bedford, MA) A-10 gradient (18 M Ω cm) deionized water. In brief, 0.25 mM HAuCl₄ (8 mL) was prepared and then 200 μ L (10 $\times$ 20 μ L aliquots) of freshly prepared 0.1M NaBH₄ were added slowly with gentle mixing at 22°C to the gold solution. Resulting colloidal solutions exhibited a red-wine color within a few minutes. The gold nanoparticles were prepared and stored in the laboratory for the present study; however, these nanoparticles are readily available and could be purchased from several commercial suppliers.

Bacterial Cell Cultures

Escherichia coli ATCC 13529 and *Rhodococcus rhodochrous* ATCC 17895 (American Type Culture

*Correspondence to: G. Naja, Department of Chemical Engineering, McGill University, 3610 University Street, H3A2B2 Montreal, Quebec, Canada. E-mail: ghinwa.naja@mcgill.ca

Received 19 January 2008; accepted in revised form 21 April 2008

DOI 10.1002/jemt.20614

Published online 20 June 2008 in Wiley InterScience (www.interscience.wiley.com).

Collection, Manassas, VA), were cultivated on tryptic soy agar (TSA, Difco, Detroit, MI) and Difco nutrient agar solid media at 37°C and 26°C, respectively. The cultures were maintained at 4°C. Bacterial suspensions used in the SEM imaging were prepared by collecting colonies from solid cultures and suspending the cells in PBS, pH 7.4.

Yeast Cells Culture

Candida sp. (isolated and purified from a wastewater effluent sample) was maintained on tryptic soy agar at 4°C. The cells were grown on tryptic soy broth (TSB, Difco, Detroit, MI) at 22–24°C and 250 rpm. The culture was collected by centrifugation at 2,000 rpm for 5 min. The pellets were washed twice with PBS and collected by the same centrifugation procedure.

Simple Preparation Procedure for SEM

Three different microorganisms with various morphological features were chosen to test the method. Twenty microliter suspensions (10^7 cells/mL) of *R. rhodochrous*, *E. coli*, and *Candida* sp. were added separately to 2 mL of the gold nanoparticle solution. After dilution, each sample contained ~100 cells/mL. Following gentle shaking, the sample was dialyzed (Spectra/Por1, 6–8000 MWCO, Spectrum Laboratories, Rancho Dominguez, CA) against 4L of Milli-Q (Millipore, Bedford, MA) A-10 gradient (18 MΩ cm) deionized water at room temperature for 30 min. The dialysis membrane used is made from molecular porous regenerated cellulose containing a trace of heavy metals (Fe, Pb, and Zn) and sulfides. The dialysis step for salt removal is recommended; however, SEM imaging with good quality could still be obtained without this additional step. SEM imaging was performed on a 10 µL dehydrated drop of the obtained solutions on a diced silicon wafer (5 mm × 5 mm).

Three SEM controls were also performed to check the accuracy of the new technique by dehydrating a sample drop on a silicon wafer. The first control consisted of applying the new technique without adding microorganisms to the gold nanoparticles, while the second control used bacterial suspension without gold nanoparticles. The third control experiment was based on using the conventional staining procedure.

The procedure was repeated at least three times (with and without microorganisms) to ensure its reliability and reproducibility.

Scanning Electron Microscopy

SEM micrographs were obtained using a Hitachi scanning electron microscope (model S-2600 N, Tokyo, Japan) operating in the high-vacuum mode with an acceleration voltage of 12 kV. The technical information of the instrument is presented in Table 1.

Conventional Staining Procedure

Bacterial suspensions were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 h and washed overnight in phosphate buffer before being postfixated in phosphate buffered osmium tetroxide (1.0%) for 1 h (Glauert, 1992). The samples were dehy-

TABLE 1. Technical specifications of the scanning electron microscope instrument

Resolution at 25 kV	4 nm
Resolution at variable pressure mode	5 nm
Magnification	15× to 300,000×
Accelerating voltage	0.5 to 30 kV
Emission current	10^{-12} to 10^{-17} A
Vacuum range	1 to 270 Pa
Secondary electron image	High vacuum
Backscattered electron image	Semiconductor

drated in a graded series of aqueous ethanol solutions (30–100%). The samples were then mounted on aluminum stubs, sputtered with gold nanoparticles before SEM imaging.

Raman Spectroscopy

The same dehydrated samples were used to record the Ultraviolet resonance Raman spectra of *E. coli* by a laser Raman analyzer (LabRAM HR 800 by Horiba/Jobin Yvon, Longjumeau, France) equipped with a frequency-doubled Argon ion 229 nm laser (Lexel 95-SHG, Cambridge Lasers Laboratories, Fremont, CA). Although the Raman spectrometric analyzer is integrated with a microscope, the laser is operated at a power level less than 7 mW. The spectra were recorded with a resolution of 0.3 cm^{-1} /pixel of CCD due to the 800 mm focal length spectrometer of the LabRAM HR 800. A broadband antireflection coated UV objective (LMU UVB, 40×/0.50, WD 1 mm, OFR) was employed to focus the laser light on the sample. Nitrogen purging was required to minimize ozone absorption, to drive moisture out of the UV box and to keep the crystal dry. The purge pressure was 1 psi and the flow rate was around 1 L/min. Each spectrum took less than 30 s to acquire.

The instrument was wavelength calibrated with a Teflon wafer focused and collected as static spectra centered at 1300 cm^{-1} for UV-Raman Spectroscopy. The LabSpec software package (Horiba/Jobin Yvon) running under Windows XP was used for the instrument control and data capture. ASCII data were exported from the LabSpec software into Microcal™ Origin version 6.5 (Microcal Software) for further processing and reporting.

RESULTS AND DISCUSSION

The new and rapid procedure is based on the interaction between gold nanoparticles and proteins or DNA moieties of microbial cells (bacteria or fungi/yeasts) in suspensions. The interaction products are quickly examined by SEM for the presence of microorganisms. The SEM observations were confirmed by ultraviolet resonance Raman spectroscopy (UVRS) in the SEM focus.

Scanning Electron Microscopy

Images obtained for gold nanoparticles without microorganisms indicated that the droplet of aggregated nanoparticles on the silicon wafer had an outer ring consisting of gold nanoparticles layer (Fig. 1a). This ring was encircling gold nanoparticle aggregates which remained in the middle of the droplet. The control sample obviously contained no microorganism, confirming the accuracy of our procedure (Figs. 1b and 1c).

Fig. 1. SEM images of the dehydrated drop of gold nanoparticles after dialysis. (a) global view of the drop; (b) and (c) the outer ring.

Without gold particles, SEM imaging of *E. coli* shows micrographs of very poor quality (Figs. 2a and 2b), since no shapes consistent with bacterial cells could be distinguished. As samples are required to be imaged under vacuum, biological cells need to be either stained

or cryogenically frozen (McDonald, 2007; Refshauge et al., 2006). Stains are generally used to highlight cellular structures. Electron-dense compounds of heavy metals such as osmium or lead or uranium can be used to selectively deposit heavy atoms in the sample and enhance the structural detail (Ngwenya et al., 2005).

After applying the conventional staining procedure to the bacteria (*E. coli*), SEM of *E. coli* were recorded and presented in Figures 2c and 2d where bacterial cells ($1\ \mu\text{m} \times 0.1\ \mu\text{m}$) were clearly distinguished. The images in Figures 3a and 3b as well as in Figures 3c and 3d represent the SEM imaging of *E. coli* and *R. rhodochrous* before and after dialysis and interaction with gold nanoparticles. The SEM images of these microorganisms before and after 30 min of dialysis are very similar with the only difference being the presence of salt in the sample. Indeed, the dialysis procedure against deionized water is only recommended to improve the quality of imaging by removing the possibility of salt interference. However, this step could potentially cause some damage to the gold nanoparticle coated microorganisms exposed.

The reaction between the gold nanoparticles and the microorganisms could be attributed to electrostatic interaction between the charged nanoparticles and the bacterial or yeast surface containing charged chain molecules (Nakao et al., 2003). Gold nanoparticles are generally stabilized in solution by electrostatic repulsion having a negative shell (borohydride) around a positive core (gold). The formation of a gold layer onto bacteria or yeast required, in the present case, electrostatic interactions (Kumar et al., 2001; Warner and Hutchison, 2003). At the end of the process, the microorganisms were coated with a thin layer of gold nanoparticles and probably still alive as demonstrated by Berry and Saraf (2005). In their study, Berry and Saraf (2005) reported the importance of the teichoic acid tethered to the peptidoglycan molecules at the Gram-positive bacterial surface at one end which leaves the remainder of the chain in high mobility to “wrap” the gold nanoparticles. Teichoic acid contains phosphodiester contributing to the negative charge of Gram-positive bacteria and enabling the reaction with gold nanoparticles. On the other hand, one of the characteristics of Gram-negative bacteria is an outer membrane containing lipopolysaccharides and phospholipids with phosphonate groups creating a negative surface charge critical for the gold nanoparticle deposition (McLean and Beveridge, 1990). The deposited gold nanoparticles would partly function as markers for proteins and/or DNA of the microorganisms and probably make the cell structures more conductive for SEM imaging.

Notice that gold nanoparticles were prepared from gold salt in the presence of a large excess of the reducing agent sodium borohydride. Therefore, it was unlikely that a redox reaction could take place between the biomass and HAuCl_4 , since there would be no residual gold salt present. Gold nanoparticles have been reported to be negatively charged when produced by laser ablation (Sylvestre et al., 2004) due to hydroxylation. In addition, gold nanoparticles prepared from gold salt in the presence of a large excess of the reducing agent sodium citrate were also reported to exhibit a negative surface charge (Zhong et al., 2004). Because

Fig. 2. SEM images of *E. coli* (a) and (b) without any chemical or physical treatment; (c) and (d) using the conventional staining procedure (osmium staining).

of the negative charge of these nanoparticles, positively charged species such as amino groups of the biomass would be responsible for the biosorption event through electrostatic interactions. Self-assembled interactions between the gold nanoparticles and the thiol or -S-S- groups of the biomass proteins would also result in nanoparticle adherence. For amino acids of the biomass, the α -amine group has been reported to interact with colloidal gold (Zhong et al., 2004).

Figures 3a and 3b show *E. coli* imaging using gold nanoparticles. The rod shapes at the edge of the outer ring are individual bacterial cells. These Gram-negative bacteria were not uniformly distributed and their density changed, depending upon the location. The same cell dimensions were obtained as when the chemical staining procedure was applied. Figure 3b shows rod-shaped bacteria (1- to 2- μ m long and 0.1–0.2 μ m in diameter) with flagella anchored at the surface of the bacterial membrane (Sperandio et al., 2002). These rigid screw-like appendages had 1–5 μ m in length (Fig. 3b). These flagella are not observed on every bacterium, indicating that a high resolution SEM is needed to visualize this fine structural component. The cell surfaces appeared intact and smooth.

The Gram-positive *R. rhodochrous* was also tested, representing the cocci-*Bacillus* cellular shape with a diameter of $\sim$ 0.5 μ m (Figs. 3c and 3d). These cells were located and observed at the edge of the outer ring where gold nanoparticles formed a layer. The cells were well preserved with many cell-cell contacts. Figure 3d shows that the cell wall of *R. rhodochrous* was thicker and less uniform compared to *E. coli* (Fig. 3b). The Gram-positive bacteria (*R. rhodochrous*) cell wall features an about 20–30 nm thick layer of peptidoglycan into which teichoic acids are embedded. The total cell wall can be 50–150 nm thick (Beveridge, 1986). The Gram-negative bacteria (*E. coli*) have a much thinner peptidoglycan layer which makes 10% of the weight of the total cell wall that can be 30–80 nm (McLean and Beveridge, 1990).

The procedure using gold nanoparticles examined in this work was also tested for the SEM imaging of yeast cells. The images obtained (Figs. 4a–4c) show circular or egg-shaped yeast cells with a smooth surface whereas some cells appear to be in the process of budding (asexual reproduction, Fig. 4a). Some of the cells appeared to have a wrinkled membrane suggestive of morphological artifacts due to irregular

Fig. 3. SEM images of *E. coli* using the gold nanoparticle procedure before dialysis (a) and after dialysis (b). SEM images of *R. rhodochrous* using the gold nanoparticle procedure before dialysis (c) and after dialysis (d).

gold sorption layers. As can be seen from Figure 4a, the small bud, or daughter cell, is formed on the parental cell whereby the nucleus of the parental cell splits, forming a daughter nucleus that migrates into the budding daughter cell. The bud continues to grow until it separates from the parental cell, forming a new cell. The yeast cell dimension varies between 1 μm for daughter cells to 5 μm for parent cells, well within the range generally reported in the literature (Mendez-Vilas et al., 2001).

It is important to notice that all these images were obtained using a conventional SEM machine. The spatial resolution obtained using our SEM instrument did not exceed 0.1 μm and was far from reaching the specifications indicated by the manufacturer (Table 1). In addition to its dependence on the magnetic electron-optical system which produces the scanning beam, resolution is also limited by the size of the interaction volume, or by the extent to which the material examined interacts with the electron beam (Hermann and Muller, 1991). The SEM characteristics make the instrument best suitable for attaining the highest resolution when using metals (Postek and Vladar, 1998).

For withstanding high vacuum applied during the SEM observation, biological specimens are typically chemically stained (Bergmans et al., 2005). The development of the Environmental SEM (ESEM) in the late 1980s (Danilatos and Postle, 1982) allowed biological samples to be observed in low-pressure gaseous environments (10–50 Torr) and high humidity (up to 100%). This was made possible by the development of a secondary-electron detector capable of operating in the presence of water vapor and pressure-limiting apertures in the electron beam path to separate the vacuum region around the gun and lenses from the sample chamber. Resolution of the ESEM is currently adequate for the cellular scale; however, the design of this type of instrument makes it less accessible.

Artifacts in electron microscopy imaging could confuse the results and lead to wrong conclusions. Generally, controls containing no bacteria are not enough to confirm the reliability of the technique. To corroborate the presence of the bacteria surrounded by a gold nanoparticle layer as seen in the SEM focus, Ultraviolet Resonance Raman spectra were recorded focusing the laser on the same spot as observed by the SEM.

Fig. 4. SEM images of *Candida* yeast cells using the gold nanoparticle procedure after dialysis.

Ultraviolet Resonance Raman Spectroscopy (UVRRS)

Ultraviolet resonance Raman spectroscopy was used to confirm that there was an interaction between the gold nanoparticles (as observed by SEM) and proteins and/or DNA of the bacteria.

TABLE 2. Wave number assignment for the *E. coli* in the outer ring of the dehydrated drop of gold nanoparticles

Presence of bacteria in the outer ring	
Wavenumbers (cm ⁻¹)	Assignment
1690	Amide I (Xie et al., 2005)
1595	Tyr + Trp + Phe (small contribution) (Wu et al., 2000a)
1420	δ CH ₂ Scissoring (Xie et al., 2005)
1356	A + G (Manoharan et al., 1990; Wu et al., 2000b)
	A (Wu et al., 2000a,b)
	A + weak contributions of Tyr and Trp (Lopez-Diez and Goodacre, 2004)
1084	δ CH (Manoharan et al., 1990; Xie et al., 2005)
	Trp + Tyr (Fagnano and Fini, 1992)
770	Trp (Wu et al., 2000b)

Fig. 5. Raman spectra of the outer ring of the dehydrated drop containing (a) pristine gold nanoparticles; (b) gold nanoparticles with *E. coli*.

Indeed, UVR spectroscopy can successfully analyze proteins (Pimenov et al., 2005) and DNA (Wen and Thomas, 1998). By selectively exciting electrons of some functional groups, specific parts of macromolecules could be investigated by using different excitation wavelengths, and 229 nm has been chosen to provide excitation of resonance in the aromatic residues of proteins.

UVR was performed on the outer ring of the dehydrated drop of the mixture consisting of gold nanoparticles and *E. coli* and on the first control containing no

bacteria. The band attribution (Table 2) was based on several UVR studies in the literature (Fagnano and Fini, 1992; Lopez-Diez and Goodacre, 2004; Manoharan et al., 1990; Naja et al., 2007; Williams and Edwards, 1994; Wu et al., 2000a,b; Xie et al., 2005). Compared with the flat spectrum obtained for the first control without bacteria (Fig. 5a), the one obtained after dialyzing *E. coli* with gold nanoparticles exhibited a characteristic band at 1600 cm^{-1} that allows for the discrimination of *E. coli*. The overall bacterial Raman spectrum obtained indicated only a very slight contribution from nucleic acids and appeared to be dominated by protein amino acid peaks. In Figure 5b, the peaks at 1600 , 1084 , and 770 cm^{-1} correspond to the tyrosine and tryptophan aromatic amino acids. A small contribution of phenylalanine groups is seen in the 1600 cm^{-1} band whereas the 1680 cm^{-1} peak could be attributed mainly to the amide I structure. The peak around 1420 cm^{-1} could be attributed to the guanine and adenine compounds as well as to CH_2 deformation vibration. The band around 1356 cm^{-1} corresponds to the CH deformation vibration in proteins, to tyrosine amino acid as well as to the adenine nucleobase. Overall, the same peaks were obtained when studying pure *E. coli* (Naja et al., 2007) confirming the presence of these bacteria in the outer ring of the drop.

REFERENCES

- Amako K, Umeda A. 1977. Observation of bacterial flagella in SEM. *J Electron Microsc* 26:235–235.
- Bergmans L, Moisiadis P, Van Meerbeek B, Quirynen M, Lambrechts P. 2005. Microscopic observation of bacteria: Review highlighting the use of environmental SEM. *Int Endod J* 38:775–788.
- Berry V, Saraf RF. 2005. Self-assembly of nanoparticles on live bacterium: An avenue to fabricate electronic devices. *Angew Chem Int Ed* 44:6668–6673.
- Beveridge TJ. 1986. The immobilization of soluble metals by bacterial walls. In: Ehrlich HL, Holmes DS, editors. *Biotechnology and Bioengineering Symposium No. 16: Biotechnology for the Mining, Metal-Refining, and Fossil Fuel Processing Industries*. New York: Wiley Interscience. pp 127–140.
- Birrell GB, Hedberg KK, Griffith OH. 1987. Pitfalls of immunogold labeling: Analysis of light microscopy, transmission electron microscopy and photoelectron microscopy. *J Histochem Cytochem* 35:843–853.
- Crang RE, Pechak DG. 1978. Serial section reconstruction of black yeast. *Aureobasidium-Pullulans* by means of high voltage electron microscopy. *Protoplasma* 96(3/4):225–234.
- Danilatos GD, Postle R. 1982. The environmental scanning electron microscope and its applications. *Scan Electron Microsc Part* 1:1–16.
- Fagnano C, Fini G. 1992. Antibody Antigen interactions studied by means of Raman spectroscopy. *J Raman Spectrosc* 23:637–639.
- Fox NE, Demaree RS. 1999. Quick bacterial microwave fixation technique for scanning electron microscopy. *Microsc Res Tech* 46:338–339.
- Glauert AM. 1992. *Cytochemical Staining Methods for Electron Microscopy*. UK: Elsevier.
- Graham LL, Beveridge TJ. 1990. Evaluation of freeze substitution and conventional embedding protocols for routine electron microscopic processing of *Eubacteria*. *J Bacteriol* 172:2141–2149.
- Hermann R, Muller M. 1991. Prerequisites of high resolution scanning electron microscopy. *Scanning Microsc* 5:653–664.
- Hernandez F, Guillen R. 2000. Microwave processing for scanning electron microscopy. *Eur J Morphol* 38:109–111.
- Kumar A, Pattarkine M, Bhadbhade M, Mandale AB, Ganesh KN, Datar SS, Dharmadhikari CV, Sastry M. 2001. Linear superclusters of colloidal gold particles by electrostatic assembly on DNA templates. *Adv Mater* 13:341–344.
- Little B, Wagner P, Ray R, Pope R, Scheetz R. 1991. Biofilms—an ESEM evaluation of artifacts introduced during SEM preparation. *J Indust Microbiol* 8:213–221.
- Lopez-Diez EC, Goodacre R. 2004. Characterization of microorganisms using UV resonance Raman spectroscopy and chemometrics. *Anal Chem* 76:585–591.
- Manoharan R, Ghiamati E, Dalterio RA, Britton KA, Nelson WH, Sperry JF. 1990. UV Resonance Raman spectra of bacteria, bacterial spores, protoplasts and calcium dipicolinate. *J Microbiol Methods* 11:1–15.
- McDonald K. 2007. Cryopreparation methods for electron microscopy of selected model systems. *Method Cell Biol* 79:23–56.
- McLean RJC, Beveridge TJ. 1990. Metal-binding capacity of bacterial surfaces and their ability to form mineralized aggregates. In: Ehrlich HL, Brierley CL, editors. *Microbial Mineral Recovery*. New York: McGraw-Hill. pp 185–222.
- Mendez-Vilas A, Gallardo AM, Perez-Giraldo C, Gonzalez-Martin ML, Nuevo MJ. 2001. Surface morphological characterization of yeast cells by scanning force microscopy. *Surf Interface Anal* 31:1027–1030.
- Naja G, Bouvrette P, Hrapovich S, Luong JHT. 2007. Raman-based detection of bacteria using silver nanoparticles conjugated with antibodies. *Analyst* 132:679–686.
- Nakao H, Shiigi H, Yamamoto Y, Tokonami S, Nagaoka T, Sugiyama S, Ohtani T. 2003. Highly ordered assemblies of Au nanoparticles organized on DNA. *Nano Lett* 3:1391–1394.
- Ngwenya LB, Peters A, Rosene DL. 2005. Light and electron microscopic immunohistochemical detection of bromodeoxyuridine-labeled immunohistochemical detection cells in the brain: Different fixation and processing protocols. *J Histochem Cytochem* 53:821–832.
- Pimenov KV, Bykov SV, Mikhonin AV, Asher SA. 2005. UV Raman examination of alpha helical peptide water hydrogen bonding. *J Am Chem Soc* 127:2840–2841.
- Postek MT, Vadar AE. 1998. Image sharpness measurement in scanning electron microscopy—Part I. *Scanning* 20:1–9.
- Refshauge S, Watt M, McCully ME, Huang CX. 2006. Frozen in time: A new method using cryo-scanning electron microscopy to visualize root-fungal interactions. *New Phytol* 172:369–374.
- Seviour RJ, Pethica LM, McClure S. 1984. A simple modified procedure for preparing microbial-cells for scanning electron microscopy. *J Microbiol Methods* 3:1–5.
- Sperandio V, Torres AG, Kaper JB. 2002. Quorum sensing *Escherichia coli* regulators B and C (QseBC): A novel two-component regulatory system involved in the regulation of flagella and motility by quorum sensing in *E. coli*. *Mol Microbiol* 43:809–821.
- Sylvestre JP, Kabashin A, Sacher E, Meunier M, Luong JHT. 2004. Stabilization and size control of gold nanoparticles during laser ablation in aqueous cyclodextrins. *J Am Chem Soc* 126:7176–7177.
- Warner MG, Hutchison JE. 2003. Arrangement of functionalized gold nanoparticles using biopolymer scaffolds: Progress toward the development of new building blocks for nanoelectronic devices. *Abstr Pap Am Chem Soc* 225:U647–U647.
- Wen ZQ, Thomas GJ. 1998. UV resonance Raman spectroscopy of DNA and protein constituents of viruses: Assignments and cross sections for excitations at 257, 244, 238, and 229 nm. *Biopolymers* 45:247–256.
- Williams AC, Edwards HGM. 1994. Fourier transform Raman spectroscopy of bacterial cell walls. *J Raman Spectrosc* 25:673–677.
- Wu Q, Nelson WH, Elliot S, Sperry JF, Feld M, Dasari R, Manoharan R. 2000a. Intensities of *E. coli* nucleic acid Raman spectra excited selectively from whole cells with 251-nm light. *Anal Chem* 72:2981–2986.
- Wu Q, Nelson WH, Treubig JM, Brown PR, Hargraves P, Kirs M, Feld M, Desari R, Manoharan R, Hanlon EB. 2000b. UV resonance Raman detection and quantitation of domoic acid in phytoplankton. *Anal Chem* 72:1666–1671.
- Xie XS, Potma EO, Evans CL, Yang WY, Lin C. 2005. Coherent anti-Stokes Raman scattering (CARS) microscopy for biology and medicine. *Abstr Pap Am Chem Soc* 230:U197–U197.
- Zhong Z, Patskovsky S, Bouvrette P, Luong JHT, Gedanken A. 2004. The surface chemistry of Au colloids and their interactions with functional amino acids. *J Phys Chem B* 108:4046–4052.