

Nanoporous Anodic Alumina Barcodes: Toward Smart Optical Biosensors

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To design nanoporous structures with controlled geometrical features is an excellent strategy for developing such optical devices as microcavities.^[1–3] Among these nanoporous structures, porous silicon (PSi) and nanoporous anodic alumina (NAA) have demonstrated to be two outstanding platforms for fabricating devices with a unique set of optical properties (e.g. reflectance, transmittance, absorbance, photoluminescence and so forth).^[4–6] Amid these optical properties, the photoluminescence (PL) emission may be used as an active photonic mark for characterizing and classifying those nanoporous materials. In addition, the exclusive fingerprint is clearly useful for developing optical biosensors, in which a high degree of resolution, sensibility and biocompatibility are required.^[7,8]

Recently, porous silicon colloids with spherical shape have been successfully developed.^[9,10] It has been found out that these PSi colloids have a unique PL spectrum in the near IR region, which depends on the colloid size and its porosity. This characteristic feature opens up the control of the PL spectrum at will. For this reason, PSi colloids are considered as excellent candidates for producing future smart optical biosensors. Unfortunately, both the colloid size and its porosity are still rather uncontrollable for developing optical nanostructures on demand (i.e. with a controlled effective medium).^[10] In this scenario, NAA represents a promising alternative or complementation to the PSi colloids in the UV-Visible region. Previous studies have pointed out that the PL spectrum of NAA presents oscillations generated by the Fabry–Pérot effect.^[11] The number, intensity and position of those oscillations rely on the NAA thickness (i.e., the pore length). In preliminary experiments, we have observed that these oscillations are related to the pore diameter too. Therefore, the PL spectrum of NAA relies upon the pore length and its diameter. This exclusive characteristic offers us a great opportunity for designing NAA structures with tunable optical properties just by modifying the pore geometry. So far, several works have proposed a systematical and objective system for classifying some optical properties of several nanostructures (e.g., PSi colloids, PSi nanowires, PSi particles, silica nanotubes and so on).^[10,12–16] Herein, we put forward a barcode system in which a unique barcode is related to each NAA

geometry (i.e. the pore length and its diameter) by means of its PL spectrum. One of the foremost advantages of NAA is that the pore geometry can be exquisitely controlled. This makes it possible to switch the effective medium of NAA at will by geometry and generate a wide range of PL barcodes in the UV-Visible region. Furthermore, pores can be covered with a large number of materials (e.g. metals, polymers, oxides, etc.), what allows functionalizing NAA for fulfilling the requirements of later applications.

The preparation of NAA was carried out by the two-step anodization process described elsewhere (Experimental Section).^[17,18] In this procedure, the pore length was controlled by the anodization time and the pore diameter (i.e. the porosity) was enlarged by a wet chemical etching after anodizing. To cover entirely the range in which the PL spectrum oscillates, the pore length (L_p) and its diameter (d_p) were each set to three (i.e. 5.0, 8.7 and 12.4 μm) and four (i.e. 30, 41, 52 and 71 nm) levels, respectively. So, a total of twelve different NAA samples were analyzed. The fabrication conditions and the geometric characteristics of each NAA sample are summarized in Tables 1S and 2S (Supporting Information), respectively. From preliminary experiments, we established that the PL oscillations disappear from the PL spectrum when the pore length is longer than 25 μm (i.e. about after 5 h of anodization). Moreover, we verified that the NAA structure collapses after 35 min of pore widening. Another question that is worth noting is that, for the same pore length, the intensity of each oscillation in the PL spectrum is noticeably lower for those NAA samples fabricated by a one-step than by a two-step anodization process (Supporting Information). **Figure 1a–d** shows a set of top view scanning electron microscopy (SEM) images of four NAA samples with different pore diameters and the same length. **Figure 2** summarizes the PL spectra of all the NAA samples analyzed in this study as well as the barcodes generated from them. At first glance, it is observed that each NAA sample is characterized by an exclusive barcode. Notice that, for a given pore length, the number, intensity and position of the PL oscillations can be adjusted by increasing the porosity (i.e. by enlarging the pore diameter). Concretely, when the pore length is 5.0, 8.7 and 12.4 μm , the PL spectrum shows a relative low, medium and high number of oscillations, respectively (Figure 2a–c and Table S2). It is confirmed that, as the pore diameter is enlarged, the intensity and the number of these oscillations increases and decreases, respectively. Furthermore, there is a slight shift in the positions of the PL oscillations as a result of the porosity increase. All this certifies the sensing ability of NAA to small changes in the effective medium, which may be produced by changing the pore

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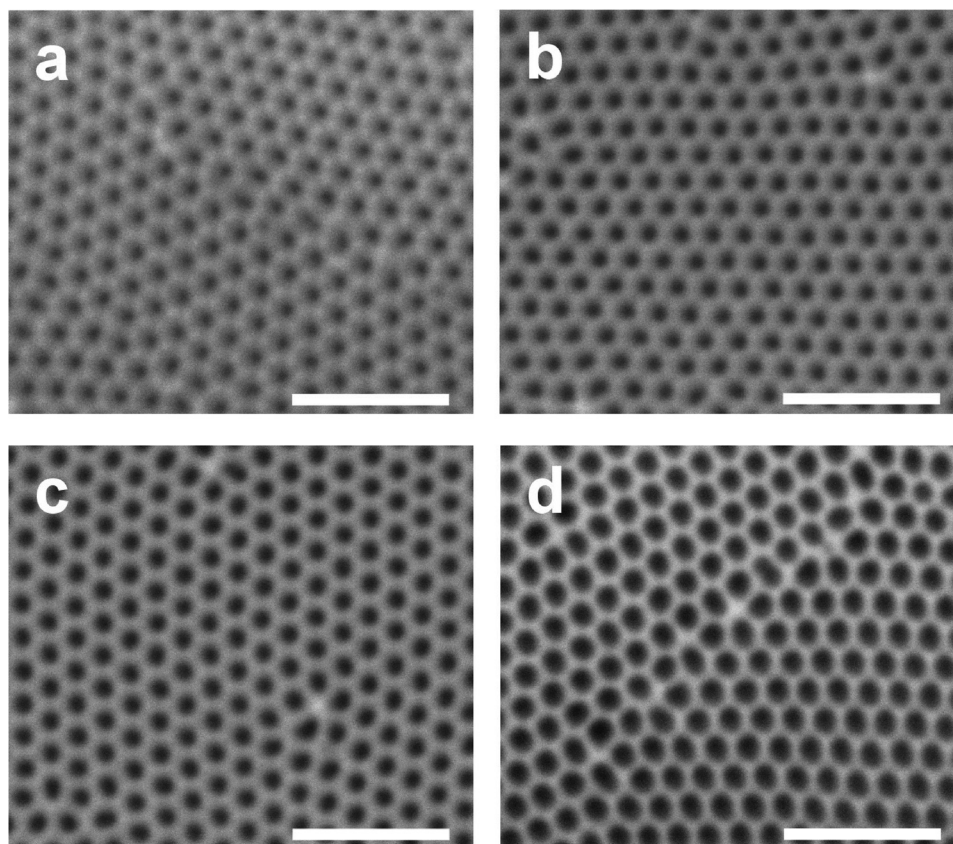


Figure 1. Set of top view SEM images of four NAA samples with different pore diameters (scale bar = 500 nm). a) PL-OSC(1) $d_p = 30 \pm 3$ nm. b) PL-OSC(2) $d_p = 41 \pm 3$ nm. c) PL-OSC(3) $d_p = 52 \pm 3$ nm. d) PL-OSC(4) $d_p = 71 \pm 2$ nm.

length and its diameter or by an external substance. Point that, when the pore length is longer than $25 \mu\text{m}$ and the porosity is higher than 40%, the Fabry–Pérot effect disappears and the oscillations in the PL spectrum of NAA almost vanish. This phenomenon can be explained in structural terms. Regarding the effect of the pore length, we have to consider that the coherence length of the light emitted from the NAA is limited and for longer pores (i.e. $25 \mu\text{m}$) the interferences generated by multiple reflections disappear from the PL spectrum. As for the porosity effect, the pore wall structure in NAA can be divided into three layers: namely, I) outer layer (F -PL centres from electrolyte impurities), II) middle layer (F^+ -PL centres from oxygen vacancies), and III) inner layer (pure alumina (Al_2O_3) without PL centres). The outer layer disturbs the transmittance of the light from the excitation source and the emission of the light from the middle layer.^[19] For this reason, when the pore diameter is enlarged, the thickness of the outer layer is reduced and, thus, the intensity of the PL oscillations increases. However, for longer etching times (i.e. larger pore diameters), the middle layer is partially dissolved, what is reflected in a noticeable decrease in the PL intensity. The amplification of the PL oscillations in NAA is associated with a strong enhancement of the photoluminescence at wavelengths corresponding to the optical modes of the Fabry–Pérot cavity constituted by the system Air–NAA–Aluminum. The result is

a PL spectrum with an abundance of well-resolved and narrow oscillations. In this optical cavity, the number, intensity and position of the oscillations not only can be tuned by increasing the NAA thickness but also by modifying its porosity (i.e. the effective medium).

The optical thickness of the NAA (nL_p) can be estimated from the PL oscillations by Equation 1:

$$nL_p = (hc)/(2\Delta E) \quad (1)$$

where n is the refractive index of the NAA, h and c are the Planck's constant ($6.626 \cdot 10^{-34}$ J s) and the speed of light ($2.998 \cdot 10^8$ m s⁻¹), respectively and ΔE is the mode spacing.^[20]

This approximation is supported by two assumptions: namely, A) the band gap of NAA (4.2 eV) is approximately four-fold the photon energy of the wavelength range in which the PL oscillations are analyzed ($375\text{--}575$ nm $\rightarrow$ 1.15 eV).^[21] Therefore, the refractive index of NAA can be considered constant over this wavelength region. B) the extinction coefficient of NAA at 475 nm is rather low ($1 \cdot 10^{-4}$).^[4]

The results obtained from Figure 2 can be verified in a clearer way in Figure 3, which shows that both the number of oscillations and the optical thickness increase with the aspect ratio (i.e. $L_p \cdot d_p^{-1}$) at constant pore length (Figure 3 a). Moreover, the effective refractive index of the NAA (n_{eff}) can be calculated from the Bruggeman's equation (Equation 2):

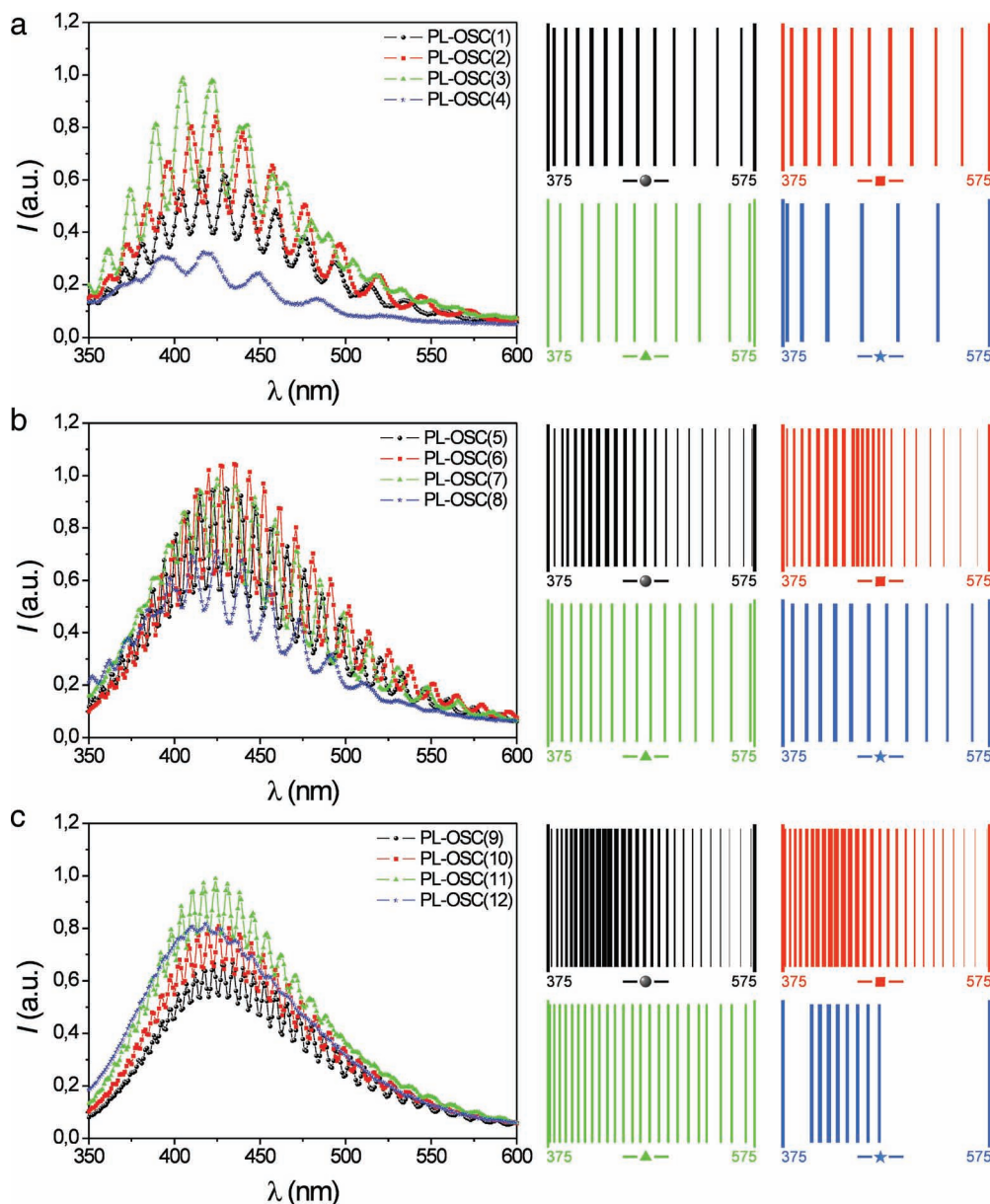


Figure 2. Photoluminescence spectra of NAA as a function of the pore length and its diameter and the resulting barcodes after encoding the PL oscillations ($\lambda_{ex} = 320$ nm). a) PL-OSC(1-4) $L_p = 5.0 \pm 0.1$ μm . b) PL-OSC(5-8) $L_p = 8.7 \pm 0.1$ μm . c) PL-OSC(9-12) $L_p = 12.4 \pm 0.2$ μm .

$$(1 - P) \frac{n_{\text{Alumina}}^2 - n_{\text{eff}}^2}{n_{\text{Alumina}}^2 + 2n_{\text{eff}}^2} + P \frac{1 - n_{\text{eff}}^2}{1 + 2n_{\text{eff}}^2} = 0 \quad (2)$$

where n_{Alumina} is the refractive index of pure alumina (1.67) and P the NAA porosity.^[22]

From this, it is calculated that the effective refractive index of NAA can be tuned from 1.61 up to 1.36 by increasing the NAA porosity from 8.2 up to 44.4% (Figure 3 b). Therefore, we are able to change accurately the effective medium at will by increasing the NAA porosity.

The PL barcode system that we propose is based on the universal product code (UPC).^[23] In this system, each bar position corresponds to the wavelength of each oscillation in the PL

spectrum and the higher the oscillation intensity the wider the bar. So far, some optical nanostructures have been successfully used as a base for developing similar barcode systems.^[10,12–16] Nevertheless, the optical encoding procedure based on NAA has some advantages over those systems:

- I) Certain information about some processes which take place in live cells can be extracted by techniques based on spectroscopic labels. Current biomedical research needs to develop improved optical labels regarding sensitivity, specificity and biocompatibility.^[24] In that regard, the multiple PL spectra generated from NAA yield a wide range of different barcodes for producing exclusive spectroscopic signatures in the UV-Visible region.

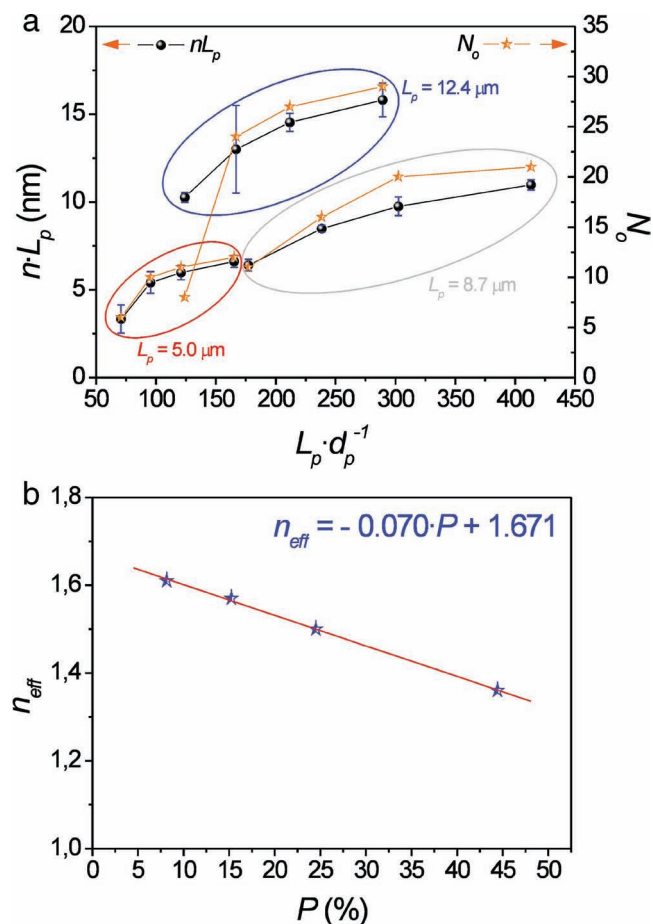


Figure 3. Optical properties of NAA as a function of its geometrical features. a) Optical thickness (nL_p) and number of oscillations (N_o) in the PL spectrum versus aspect ratio ($L_p d_p^{-1}$). b) Effective refractive index (n_{eff}) versus porosity (P).

II) The pore length and its diameter in NAA can be exquisitely controlled by the anodization parameters and the pore widening stage, respectively. Given that the PL spectrum relies on the pore geometry, the effective medium can be tuned *ad libitum* for later applications. As Figure 2 shows, the intensity, position and number of oscillations in the PL spectrum can be previously designed for sensing purposes in the UV-Visible region. For instance, those barcodes with a high number of bars (i.e. oscillations in the PL spectrum) are envisaged for developing optical biosensors in which a high sensibility to small external changes is required. Otherwise, barcodes with a low number of bars would be more suitable when a high specificity (i.e. few intensive oscillations at specified positions) is needed. To demonstrate the sensing responsiveness of NAA to external substances, we studied the PL spectrum of two NAA samples after infiltrating with two different substances (Supporting Information). The first one was an oxazine dye. Oxazines are sensitive chemicals to their surrounding environment since they exhibit solvatochromism (i.e. the change in color of a chemical substance as a result of a change in the solvent polarity). Hence, they are commonly utilized in various applications as molecular

probes.^[25] The second one was glucose, which is a monosaccharide and the primary source of energy in live cells. Additionally, glucose is a usual medical analyte quantified in blood analysis since higher levels of it can be a sign of diabetes. The results obtained from these experiments validate the use of NAA as a platform for producing optical biosensors.

- III) Another advantage of using NAA is that the PL spectrum remains stable throughout. Therefore, in contrast to other materials, it is not necessary to passivate them for avoiding shifts in the PL spectrum in the course of time.^[10,26] To confirm this, the PL spectrum of several types of NAA samples was measured after several weeks and no significant changes in the number, intensity and position of the oscillations were observed (Supporting Information).
- IV) The well-defined geometry of pores in NAA (i.e. cylindrical geometry) allows modifying the effective medium with functional materials in a controlled manner. As for this, such techniques as atomic layer deposition, dip coating and layer-by-layer deposition are envisaged for being suitable methods in order to functionalize the NAA pores.^[27–30]
- V) NAA is a biocompatible, thermally stable, environment-resisting and biodegradable material. Recently, NAA has been tested as a drug delivery system in medicine and label-free optical biosensors for recognizing specific elements (e.g. antibodies, DNA molecules and so on). For these reasons, the proposed barcode system is expected to be used to develop smart optical sensors in such research fields as biotechnology and medicine.^[31–33]

In conclusion, we put forward a new encoding system for developing smart optical biosensors based on the PL spectrum of NAA in the UV-Visible range. In this system, each bar corresponds to an oscillation in the PL spectrum. The width and position of each bar are related to the intensity and position of each oscillation, respectively. This makes it possible to produce a large number of distinct barcodes. Another feature of the proposed system is that the effective medium of NAA can be tuned at will by structural tuning. Furthermore, the exceptional properties of NAA (e.g. biocompatibility, stability, etc.) make NAA an excellent platform for developing handy nanodevices in biology and medicine. Additionally, the NAA pores can be covered with many functional substances, what opens up a new window for latter applications in many other research fields.

Experimental Section

NAA Fabrication: The NAA samples were fabricated by the two-step anodization process. Before anodizing, aluminum (Al) substrates were electropolished in a mixture of ethanol (EtOH) and perchloric acid (HClO_4) 4:1 (v:v) at 20 V for 4 min. After this, the first anodization step was performed in an aqueous solution of oxalic acid ($\text{H}_2\text{C}_2\text{O}_4$) 0.3 M at 40 V and 6 °C for 20 h. Subsequently, the alumina film was selectively dissolved by wet chemical etching in a mixture of phosphoric acid (H_3PO_4) 0.4 M and chromic acid (H_2CrO_7) 0.2 M at 70 °C. Then, the second anodization step was conducted under the same anodization conditions as the first step. The anodization time during this step was adjusted in order to modify the pore length (i.e., 60, 105, and 150 min). Finally, the pore diameter was enlarged by a wet chemical etching in an aqueous solution of H_3PO_4 5 wt%.

NAA Characterization: The NAA samples were characterized by environmental scanning electron microscopy (ESEM FEI Quanta 600). The photoluminescence measurements were performed in a fluorescence spectrophotometer from Photon Technology International Inc. with a Xe lamp used as the excitation light source at room temperature, an excitation wavelength (λ_{ex}) of 320 nm and an emission angle of 20°. The standard image processing package (Image), public domain program developed at the RSB of the NIH, USA) was used to carry out the ESEM image analysis.^[34]

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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- [1] T. Stöferle, N. Moll, T. Wahlbrink, J. Bolten, T. Mollenhauer, U. Scherf, R. F. Mahrt, *Nano Lett.* **2010**, *10*, 3675.
- [2] L. A. DeLouise, P. M. Kou, B. L. Miller, *Anal. Chem.* **2005**, *77*, 3222.
- [3] D. Xia, Z. Ku, S. C. Lee, S. R. J. Brueck, *Adv. Mater.* **2011**, *23*, 147.
- [4] L. F. Marsal, L. Vojkuvka, P. Formentín, J. Pallarès, J. Ferré-Borrull, *Opt. Mater.* **2009**, *31*, 860.
- [5] E. Xifré-Pérez, L. F. Marsal, J. Pallarès, J. Ferré-Borrull, *J. Appl. Phys.* **2005**, *97*, 064503.
- [6] E. Xifré-Pérez, L. F. Marsal, J. Ferré-Borrull, J. Pallarès, *J. Luminescence* **2006**, *121*, 301.
- [7] J. Pepper, R. Noring, M. Klempner, B. Cunningham, A. Petrovich, R. Bousquet, C. Clapp, J. Brady, B. Hugh, *Sens. Actuators B* **2003**, *96*, 565.
- [8] A. Yamaguchi, K. Hotta, N. Teramae, *Anal. Chem.* **2009**, *81*, 105.
- [9] R. Fenollosa, F. Ramiro-Manzano, M. Tymczenko, F. Meseguer, *J. Mater. Chem.* **2010**, *20*, 5210.
- [10] F. Ramiro-Manzano, R. Fenollosa, E. Xifré-Pérez, M. Garín, F. Meseguer, *Adv. Mater.* **2011**, *23*, 3022.
- [11] K. Huang, L. Pu, Y. Shi, P. Han, R. Zhang, Y. D. Zheng, *Appl. Phys. Lett.* **2006**, *89*, 201118.
- [12] F. Cunin, T. A. Schmedake, J. R. Link, Y. Y. Li, J. Koh, S. N. Bhatia, M. J. Sailor, *Nat. Mater.* **2002**, *1*, 39.
- [13] J. H. Park, L. Gu, G. Von Maltzahn, E. Ruoslahti, S. N. Bhatia, M. J. Sailor, *Nat. Mater.* **2009**, *8*, 331.
- [14] C. Chiappini, X. Liu, J. R. Fakhoury, M. Ferrari, *Adv. Funct. Mater.* **2010**, *20*, 2231.
- [15] S. O. Meade, M. J. Sailor, *Phys. Status Solidi RRL* **2007**, *1*, R71.
- [16] B. He, S. J. Son, S. B. Lee, *Anal. Chem.* **2007**, *79*, 5257.
- [17] H. Masuda, K. Fukuda, *Science* **1995**, *268*, 1466.
- [18] K. Nielsch, J. Choi, K. Schwirn, R. B. Wehrspohn, U. Gösele, *Nano Lett.* **2002**, *2*, 677.
- [19] Y. Yamamoto, N. Baba, S. Tajima, *Nature* **1981**, *289*, 572.
- [20] M. I. Nathan, A. B. Fowler, G. Burns, *Phys. Rev. Lett.* **1963**, *11*, 152.
- [21] W. L. Xu, H. Chen, M. J. Zheng, G. Q. Ding, W. Z. Shen, *Opt. Mater.* **2006**, *28*, 1160.
- [22] A. M. Jayannavar, N. Kumar, *Phys. Rev. B* **1991**, *44*, 14.
- [23] R. C. Palmer, in *The Bar Code Book: Reading, Printing, and Specification of Bar Code Symbols*, Bloomington, USA **2007**.
- [24] J. Kneipp, H. Kneipp, B. Witting, K. Kneipp, *Nanomedicine* **2010**, *6*, 214.
- [25] S. Fleming, A. Mills, T. Tuttle, *Beilstein J. Org. Chem.* **2011**, *7*, 432.
- [26] L. T. Canham, C. L. Reeves, J. P. Newey, M. R. Houlton, T. I. Cox, J. M. Buriak, M. P. Stewart, *Adv. Mater.* **1999**, *11*, 1505.
- [27] J. Bachmann, J. M. Knez, S. Barth, H. Shen, S. Mathur, U. Gösele, K. Nielsch, *J. Am. Chem. Soc.* **2008**, *129*, 9554.
- [28] K. Pitzschel, J. M. Montero-Moreno, J. Escrig, O. Albrecht, K. Nielsch, J. Bachmann, *ACS Nano* **2009**, *3*, 3463.
- [29] I. Seo, C. W. Kwon, H. H. Lee, Y. S. Kim, K. B. Kim, T. S. Yoon, *IEEE Trans. Nanotechnol.* **2009**, *8*, 707.
- [30] F. Trivinho-Strixino, H. A. Guerreiro, C. S. Gomes, E. C. Pereira, F. E. G. Guimaraes, *Appl. Phys. Lett.* **2010**, *97*, 011902.
- [31] S. D. Alvarez, C. P. Li, C. E. Chiang, I. K. Schuller, M. J. Sailor, *ACS Nano* **2009**, *3*, 3301.
- [32] S. Simovic, D. Losic, K. Vasilev, *Chem. Commun.* **2010**, *46*, 1317.
- [33] J. E. N. Dolatabadi, O. Mashinchian, B. Ayoubi, A. A. Jamali, A. Mobed, D. Losic, Y. Omidi, M. de la Guardia, *Trends Anal. Chem.* **2011**, *30*, 459.
- [34] M. D. Abramoff, P. J. Magalhaes, S. J. Ram, *Biophoton. Int.* **2004**, *11*, 36.