Dispersions of alkyl-capped silicon nanocrystals in dilute organic solvent/water mixtures: steady-state photoluminescence and ageing studies

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Abstract

Alkyl-capped silicon nanocrystals can be dispersed in aqueous media by shaking or stirring their solutions in organic solvents (DMSO, ether, THF) with excess water. THF is the most straightforward choice with which to prepare stable aqueous dispersions, because the nanocrystals are very soluble in THF and it is also miscible with water. As little as 0.01 % v/v tetrahydrofuran is sufficient. DMSO and ether were the preferred choices for subsequent staining of live cells because THF shows some acute toxicity even when very dilute. The luminescence intensity of the aqueous dispersions is linear in particle concentration and independent of pH over the range 5-9. The sols retain their photoluminescence and are stable against flocculation for at least 6 months.

Introduction

Alkylated silicon nanocrystals (alkyl-SiNCs) are currently being investigated for possible applications as luminescent labels in biological applications.[1, 2, 3, 4, 5, 6] This interest derives, in part, from the bright luminescence of SiNCs at long wavelengths where biological systems do not absorb strongly. Depending on their surface termination,[7, 8, 9] SiNCs emit red light even at diameters as small as 2.5 nm. More generally, semiconductor nanocrystals are of interest as luminescent tracers because of their superior photostability compared to molecular dyes.[10, 11, 12, 13, 14, 15] When the distribution of a luminescent organic dye is mapped by confocal techniques, the high excitation light intensity often results in undesirable photochemical side-reactions and bleaching of the luminescence within minutes. Such irreversible photobleaching of inorganic semiconductor nanocrystals is usually much slower: this allows imaging of a system over long periods of time or long integration times in cases where the emission is weak on grounds of concentration.[15]

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The best understood and most commonly employed quantum dot labels are based on CdSe,[16, 17, 18, 19] but other materials, e.g., InAs cores with ZnSe shells which emit red and NIR light at core diameters < 2 nm,[20] are being investigated. The absence of leachable metals ions in silicon nanoparticles could be a significant advantage, because reports of toxicity of heavy metal-based binary semiconductor quantum dots have appeared.[21, 22, 23] However, in the light of reports of DNA damage from reactive oxygen species produced at CdSe nanoparticles[24] and the known toxicity of other small particles, e.g., α-quartz for similar reasons,[25, 26] it is perhaps likely that all semiconductor nanoparticles will show some toxicity.

Substantial quantities of alkyl-SiNCs can be prepared by a variety of chemical methods including: (i) disruption of porous silicon;[27, 28, 29, 30, 31] (ii) decomposition of molecular precursors[5, 32, 33, 34, 35, 36] and (iii) direct chemical synthesis, [2, 37, 38, 39, 40] e.g., by reactions of silicides or $SiCl_4$, or in micellar media.[41, 42, 43] In order for silicon nanoparticles to be useful as quantifiable luminescent labels in biological applications, the particles should be dispersed in aqueous media, their luminescence should be stable over long periods of time, the luminescence intensity should be pH-independent in a range bounding physiological pH, and the luminescence should be linear in nanoparticle concentration. Molecular dyes often have pH-dependent luminescence arising from protonation equilibria that can make quantitation uncertain and they also show self-quenching effects due to aggregation that result in nonlinear calibration plots. Although molecular dyes are susceptible to photobleaching and quantum dots are usually robust, bare hydrogen-terminated silicon nanoparticles and porous silicon do undergo chemical oxidation by water and air. [44, 45, 46, 47, 48]

Alkylated silicon quantum dots prepared by our method using undecene have a silicon core of diameter $\simeq$2.5 nm and are surrounded by an 11-carbon-thick organic monolayer which stabilises the particles against corrosion under ambient conditions,[27, 28] but makes the particles hydrophobic and totally insoluble in water. Other workers have sought to make silicon and germanium nanoparticles water soluble by coating with polymers[1, 49], surfactants/phospholipids,[50] or by using a monolayer that presents amine or acid functional groups to the solution.[2, 51, 52] However, in some applications, e.g., intracellular investigations, the hydrophobic capping layer may be an advantage because it is likely to facilitate the transport of the particles across the cell membrane.

It is known that hydrophobic particles can be dispersed in aqueous media by shaking and that degassing increases the stability of the sol to flocculation.[53, 54, 55] In this report we show that hydrophobic alkyl-silicon quantum dots can be dispersed in water by first dissolving the particles in an organic solvent, e.g., tetrahydrofuran (THF), DMSO or ether, and then shaking with excess water: the lyophobic sols that form are stable against flocculation for at least 6 months. In order to determine the suitability of these sols for quantitative luminescence imaging work, we have investigated the concentration and pH dependence of their photoluminescence, studied the ageing effect on the luminescence spectra and demonstrated their internalization by cultured human cells. Owing to its miscibility with water, THF is the most suitable choice of solvent to prepare aqueous dispersions for quantitative spectroscopic work; however, DMSO and ether are better choices for biological experiments because they show no acute cytotoxicity at the dilutions employed. A full discussion of the toxicological studies and the kinetics and mechanism of intracellular uptake of alkyl-SiNCs will be reported elsewhere: this manuscript is concerned with the aspects relevant to the analytical use of alkyl-SiNCs in aqueous suspension.

Experimental

Preparation of alkylated silicon nanoparticles.

Photoluminescent porous silicon layers were formed by galvanostatic anodization (10 min at 130 mA cm^{-2}) of $\simeq$1 cm^2 chips of boron-doped p-Si(100) oriented wafer (1-10 Ω cm resistivity, Compart Technology, Peterborough, UK) in a 1:1 v/v solution of 48% aqueous HF (VWR) and absolute ethanol (Aldrich). The electrochemical cell was machined from PTFE and circular in cross-section (1 cm diameter). The silicon wafer was sealed to the base using a Teflon-coated Viton^{TM} O-ring. The counter electrode was a piece of tungsten wire coiled into a loop to improve the uniformity of the current distribution and the etching was carried out using a benchtop power supply (Thurlby-Thandar, TS3021S or Keithley 2601) to provide a constant etching current.

The chip was then transferred into a Schlenk flask on a grease-free vacuum line (employing Young's taps) and dried under vacuum (rotary oil pump) for 15 min. The chips were then refluxed for 2 hours in 5 mL toluene solution (Merck, distilled over Na) containing 0.1 mL of 1-undecene (Aldrich). A pale yellow, luminescent solution was formed and undissolved porous silicon particles were filtered off (Whatman No 1 filter paper) before all solvent and unreacted alkene were removed under reduced pressure and trapped in a liquid nitrogen-cooled flask. Residual alkene/solvent was removed by coevaporation with 5 mL dichloromethane (DCM) (x3) and then 5 mL methanol, if necessary, until a waxy yellow quantum dot solid remained. This solid was soluble in non-polar organic solvents, e.g., THF, DCM and toluene. Of the order of 100 μg of alkyl-SiNCs are produced per Si chip.

Dispersion of alkylated silicon nanoparticles in water and organic solvents

The yellow solid obtained from a single 1 cm^2 chip was divided into 2-10 equal portions and the alkyl-SiNCs were re-suspended in a small volume of organic solvent (0.1 mL; THF, ether or DMSO). De-ionized water (Milli-Q, nominal 18 MΩ cm, Millipore, UK) was added with shaking or vigorous stirring to produce clear, pale yellow suspensions. In the text we report the composition of the medium as a percentage by volume of the solvent in water. For most experiments, and unless otherwise indicated, the composition was 1% THF v/v. In order to investigate the pH dependence of the luminescence, some aqueous buffers were used in place of pure water for the experiments in figures 4-7. For pHs 4-6, we used 0.1 M sodium ethanoate and adjusted the pH with HCl_{aq}. We prepared pH 7 and pH 8 buffers from 0.1 M Na_2HPO_4 using HCl_{aq} and $NaOH_{aq}$ to adjust the pH, and for pH 9-11, we used 0.1 M $NaHCO_3$ and adjusted the pH with $NaOH_{aq}$.

Luminescence and absorption spectroscopy

Absorption spectra (Cary model 100 spectrometer) and fluorescence spectra (Spex FluoroMax/GRAMS 32) of the dispersions of SiNCs were measured in the 200-800 nm and 335-850 nm regions, respectively, using quartz cuvettes of 1 cm pathlength. Emission spectra (detection at 90^o to excitation) were measured as the sample was excited with light of wavelength = 330 nm. A long-pass filter was placed between the sample and detector to reject scattered light of wavelength < 385 nm.

For the relative quantum yield measurements both excitation and emission slits were set to 1.5 nm. These slit widths were chosen to keep the uncorrected signal of the most emitting sample below 1 x 10^6 cps (i.e. all samples had signals in the linear response region of the detector). Excitation was at every 25 nm from 250 nm to 500 nm; however, because of the difficulties of matching the absorbances of the standards and the samples at the excitation

wavelength, we were only able to obtain quantitative data in a range of about 350 to 475 nm. The quantum yield ϕ_X of the sample relative to that, ϕ_R, of the reference compound was calculated using,

$$\phi_X = \phi_R \frac{\int E_X d\bar{\nu}}{\int E_R d\bar{\nu}} \frac{A_R}{A_X} \frac{n_X^2}{n_R^2}, \tag{1}$$

where A is absorbance at the excitation wavelength, n, is the refractive index (assumed equal to that of the solvent on grounds of concentration) and the emission intensity, E, is integrated over the wavenumber range of the emission peak. The subscripts R and X refer to the fluorescent standard and the sample. Since these SiNCs have a large apparent Stokes shift, which is unusual for organic dyes, and emit in a spectral region where there are very few suitable fluorescent standards, we used two very well-characterised standards; fluorescein dianion and rhodamine 6G in ethanol. The emission of these compounds is at shorter wavelength (fluorescein @520 nm and rhodamine @550 nm) than the SiNCs and therefore, to count quanta correctly, it is important to integrate the emission over a range proportional to photon energy, i.e., wavenumber rather than wavelength. There is some variation in the literature for the absolute values of quantum yields for the standards used. The values used in this work were taken from a recent careful study and were $\phi_R = 0.925$ for fluorescein in 0.1 N NaOH(aq) and $\phi_R = 0.95$ for rhodamine 6G in ethanol.[56] The absorption and emission spectra were also corrected for scattering and the inner filter effect in our quantum yield calculations.

Luminescence staining of HeLa cells with alkyl-SiNCs

HeLa cells (immortalized epithelial cervical carcinoma) were cultured on 22 mm diameter cover slips in either DMEM+10%FCS (foetal calf serum) or MEM with 15% FCS. In either case, the culture medium contained 1% streptomycin-penicillin-glutamine (Sigma-Aldrich) and was maintained at 37^{o}C in 5% CO_2 until the cells were approximately 60% to 80% confluent. In order to demonstrate the utility of alkylated silicon nanoparticles as a luminescent stain, we exposed HeLa cells to suspensions of alkyl-SiNCs in DMSO, THF and ether/aqueous culture medium mixtures for various times (30, 60, 120, 240 min). Typically 2 μL of solvent containing about 5 pmol of alkyl-SiNCs was added to 1 mL of aqueous medium (0.2% v/v). The amount of alkyl-SiNCs was determined by weighing the freshly-prepared sample and using a molecular mass estimated from previously reported measurements of particle size.[57]

The cells were washed with phosphate-buffered saline, and fixed with a solution of 4% citrate in 60% acetone for 30s. After washing the cover slip with PBS, a drop of mounting medium (Vectashield, Vector, Sigma-Aldrich) was added on the top of the cover slip before fixing it upside down on a microscope slide and sealing the edges with nail varnish. The fixed cells were examined under a confocal fluorescence microscope (Leica TCS SP2, Spectral Confocal and Multiphoton Microscope with Argon/Krypton Laser, Leica Microsystems Ltd, Milton Keynes, UK). The excitation light was the 488 nm line of an Ar ion laser and the emitted light in the range $550 < \lambda < 650$ nm was collected.

Toluene and THF, whilst effective as solvents for the dots, showed toxicity towards the cells. Although no evidence of toxicity was observed with DMSO, the solubility of the dots was rather reduced in DMSO when compared with other solvents. Ether was found to be optimum solvent in terms of solubility and lack of cell toxicity when used at 0.2% v/v.

Results and Discussion

Alkylated silicon nanocrystals (alkyl-SiNCs) were made by refluxing hydrogen-terminated porous silicon in 1 mol dm^{-3} solutions of undec-1-ene in toluene. The details of the preparation of such C_{11}-SiNCs have been reported before.[27] In this report we used essentially the same procedure, but with a simplification in technique and a lower current density - the absorption and emission spectra are, however, similar. The mechanical stress of bubble formation breaks up the porous silicon and the hydrogen-terminated SiNCs react under these conditions with the undec-1-ene via a hydrosilation reaction [58] that forms a robust Si-C bonded monolayer coating the particles. We have previously characterised the structure and composition of these particles by a range of spectroscopic and microscopy techniques. The particle diameter (Si core) produced by this preparation was determined by a combination of STM, photoluminescence and Raman spectroscopy to be about 2.5 nm.[28, 27, 59] Additional studies using atomic force microscopy (AFM), X-ray diffraction and transmission electron microscopy have confirmed these measurements (supporting information). Powder X-ray diffraction showed the crystalline nature of the SiNCs and the particle diameter obtained from the peak widths using the Scherrer formula, 2.6 nm, was consistent with the microscopy data within the accuracy of the measurement.[57] Including the organic monolayer, the total particle diameter is about 5 nm. We confirmed the particle size of some samples in the present study using tapping mode^{TM} AFM[1] to determine the height of the alkyl-SiNCs deposited on a Si(111)-H surface, which had also been alkylated with undecene to form an equivalent C_{11}-monolayer on atomically-flat Si(111).[60] By ensuring the particles and the surface have the same monolayer chemistry, the errors that can occur in AFM determinations of particle diameter are minimised.[61]

The C_{11}-monolayer of the alkyl-SiNCs also solubilises the particles in the toluene and we have previously reported spectroscopic studies of these transparent, stable sols.[27] The particles may be dried under vacuum to form a waxy, pale yellow quantum dot solid which has been characterised by FTIR, photoemission spectroscopy and X-ray excited optical luminescence.[59] These spectroscopies show that the particles consist of an Si core surrounded by an alkyl monolayer with small amounts of suboxide. The particles can be further oxidised to produce significant amounts of surface Si(+4) species associated with an additional blue luminescence band.[59, 62]

The solid is soluble in many organic solvents (THF, DCM, toluene), but insoluble in water even under sonication. The alkyl layer renders the particles strongly hydrophobic and protects the Si core from corrosion by water or strong acid (1 M HCl_{aq}), in which they show unchanged orange-red luminescence, although they are destroyed by strong alkali (1 M $NaOH_{aq}$).[28] However, we found that the lyophilic sol formed by dispersing the particles in THF can be rapidly diluted with water to form lyophobic sols containing as little as 0.01% THF by volume (figure 1). These dispersions retain their bright orange emission under a hand-held UV lamp ($\lambda = 365$nm) even after storage for 6 months in the dark under ambient conditions. The sample shown in figure 1 was still luminescent after 6 months, though a few particles - also luminescing orange - were observed adhering to the walls of the glass flask after 4 months. Luminescence spectra do show some changes in a period of a few days and we discuss the ageing effects on the spectra below. It is worth noting that to form these aqueous sols, the particles must be dissolved in pure solvent first and then mixed with water: the dry alkyl-SiNC solid does not disperse directly in 1% THF/water. This shows that the stability of the sol in figure 1 against flocculation, and those discussed below, is a kinetic effect.

We measured the quantum yield of the luminescence of alkyl-SiNCs dissolved in dichloromethane, ether, toluene, tetrahydrofuran (THF) and THF/water mixtures using fluorescein dianion in basic ethanol and rhodamine 6G in ethanol as reference standards. The quantum yield in DMSO could not be determined accurately because of the

[1] tapping mode^{TM} is a trademark of Digital Instruments, CA, USA

Figure 1: A 1L flask containing 0.01% v/v THF/water in which alkylated silicon quantum dots have been dispersed. The orange luminescence from the silicon was excited by a hand-held UV lamp (λ = 365 nm). The contrast and brightness of the photograph has been enhanced for printing, but the image has not otherwise been processed.

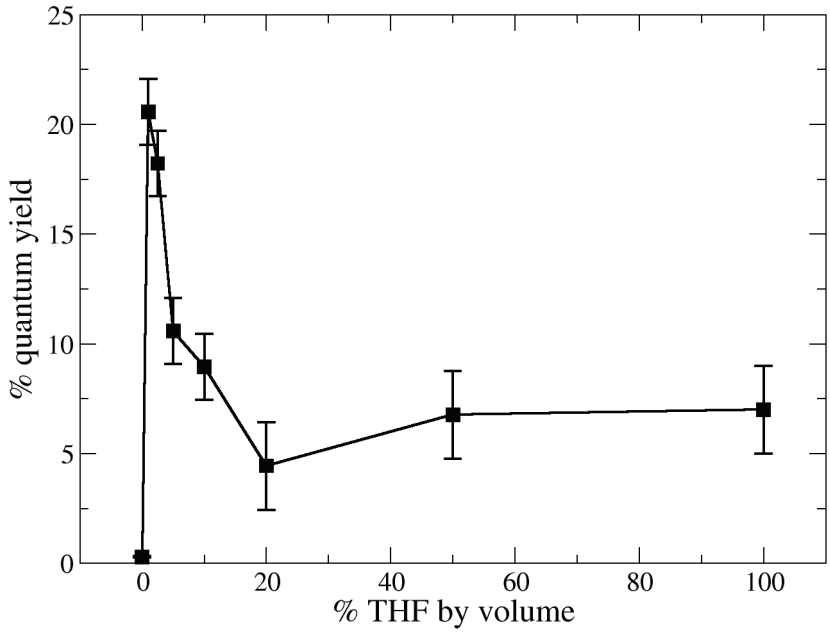

Figure 2: Luminescence quantum yield of alkylated silicon quantum dots against % THF by volume in THF/water mixtures. The amount of silicon quantum dots is the same in each sample. The excitation wavelength was 330 nm and the intensity given is that at the peak of the PL spectrum at 665 nm.

poor solubility of the particles in this solvent. The quantum yields, expressed as the percentage of photons emitted per photon absorbed, were the same using either standard. The variation of quantum yield with wavelength was less than the uncertainty and therefore mean values of the luminescence quantum yields in the pure solvents, dichloromethane, toluene and THF, are given in table 1. Figure 2 shows a graph of luminescence quantum yield against the volume fraction of THF in the final dispersion. This dataset was obtained from a single preparation of alkylated silicon quantum dots which was divided into 8 equal portions. Therefore, although we do not know the absolute value of the concentration of the samples, we do know that the variation of intensity is not a concentration effect.

It is well known that the luminescence of porous silicon can be quenched by organic solvents and added quenchers in a manner that correlates with polarity as well as basicity and steric factors.[63, 64, 65, 66, 67] THF solutions show the lowest quantum yield amongst the solvents tested, therefore it is not surprising that the luminescence of the particles is weaker in those water/THF mixtures containing the most THF, which is also known to be a strong quencher of porous silicon luminescence.[63] The pure water sample shows no luminescence: the luminescence of these particles is not quenched, but is not observed simply because the particles could not be dispersed in pure water and remain on the bottom of the sample vial in which they are prepared. The maximum luminescence quantum yield was observed for the sample with the least THF (1% v/v) and a sharp fall-off in intensity at higher volume fractions of THF was observed due to quenching by the solvent. We focused on the 1% v/v THF/water samples for more detailed spectroanalytical measurements.

Figure 3 shows the luminescence intensity of samples of alkylated silicon quantum dots as a function of their concentration (relative to the weakest suspension) in a mixed solvent comprising 1% v/v THF/water. The samples were prepared by serial dilution with 1% v/v THF/water from the most concentrated suspension in order to determine the extent of self-quenching in this system analogous to that observed for molecular dyes where aggregation is common. Although the hydrophobic particles might be expected to aggregate readily in a solvent

Table 1: Quantum yields of SiNCs in different solvents expressed as the percentage of photons emitted per photon absorbed. The quantum yields were roughly excitation wavelength independent over a range 350 - 475 nm and the values in the table are the mean values obtained from measurements between 350 and 475 nm at 25 nm intervals using both fluorescein and rhodamine standards.

solvent	quantum yield (%)
dichloromethane	14 ± 3
toluene	12 ± 3
tetrahydrofuran	7 ± 2
diethyl ether	19 ± 5

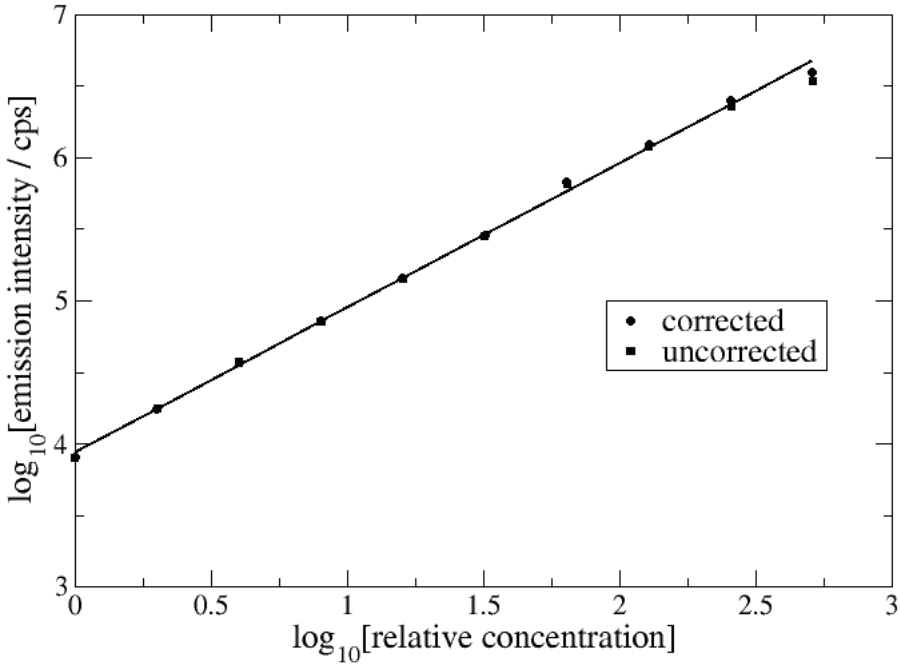

Figure 3: Peak luminescence intensity of alkylated silicon quantum dots against the concentration of quantum dots in 1% v/v THF/water. The concentration is relative to the most dilute suspension. The solid line was obtained by linear least-squares regression and the slope is 1.01. The excitation wavelength was 330 nm and the emission peak was observed at 665 nm. The luminescence intensity was corrected for the inner filter effect according to the procedure of Kubista et al. [68] using the absorption spectra (filled circles).

mixture which is 99% water, the particles would not be expected to self-quench in the same manner; after all, the solid is brightly luminescent. The linearity of the plot confirms that no self-quenching takes place over almost 3 orders of magnitude in concentration. The upper limit of concentration reflects the point at which the correction for the inner filter effect, arising from strong absorption of the excitation light, becomes pronounced. Above this concentration, the correction increases and quantitation on the basis of luminescence becomes less reliable.

An aspect of interest to the cell biologist attempting to quantify the luminescence of quantum dot labels is the effect of pH on the luminescence intensity. Figure 4 shows photoluminescence spectra of equal aliquots taken from a single preparation and dispersed in 1% v/v THF/water, but using a series of different buffers to vary the pH. The most intense luminescence is at pH 7, but over the range 4-9 the luminescence intensity varies by only about 15%. Between pH 9 and pH 10 a much larger decrease in luminescence is observed: this is related to a slow etching of the particles rather than their agglomeration and we present some evidence for this from studies of the ageing of the sols below. The data in figure 4 were obtained <1 hour after dispersing the sols in water and does show that in the physiologically relevant pH range, the luminescence is almost pH independent.

In Fig. 4, a smaller luminescence peak at about 430 nm is observed in addition to the orange luminescence at 665

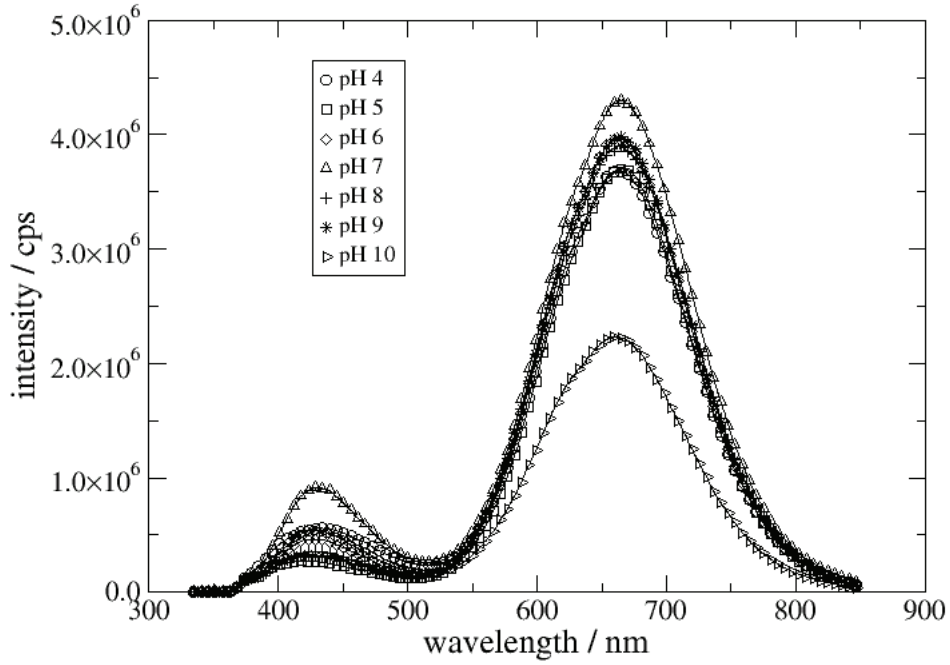

Figure 4: Luminescence spectra in buffers of different pH for alkylated silicon quantum dots in 1% v/v THF/water. The excitation wavelength was 330 nm.

nm; the latter is characteristic of alkylated silicon quantum dots prepared by our method and dispersed in nonpolar solvents.[27] Both blue and orange luminescence have been observed in SiNCs and, in general, particles derived from fluoride-etched silicon show orange luminescence [69] whereas those prepared by chemical synthesis tend to emit blue/UV light,[37, 2] even in the presence of a little oxide.[40] The blue peak in our samples could therefore originate from the Si core of a fraction of smaller particles or, bearing in mind that SiO_2 shows blue photoluminescence at about 440 nm,[70] from trace surface oxide formed after dispersion in the aqueous sol. Assignment of this blue feature in the PL spectrum is not straightforward because the effect of oxygen on the PL spectra of SiNCs is complex. At least three types of phenomena have been reported: (i) blue-shifts of the PL peak because of increasing the quantum confinement upon reduction in the size of the Si core; (ii) red-shifts of the PL peak because of mid-gap states associated with oxygen atoms,[47, 37] and (iii) appearance of additional blue peaks in originally red-emitting particles. [69, 59, 62, 46] In porous silicon, the blue-shift of the PL at small crystallite sizes only occurs in the rigorous absence of oxygen because oxygen-related surface states are expected to limit the maximum PL energy to a value in the orange part of the spectrum.[47] Theoretical studies on SiNCs suggest that the HOMO-LUMO gap of O-terminated particles of diameter 1.0-1.4 nm corresponds to orange-red luminescence and that hydrogen-termination is required for blue emission to be observed from the Si core of small particles.[9, 36] FTIR and XPS show we have a little sub-oxide on the surface of our particles.[27, 57] Using X-ray-excited optical luminescence (XEOL)[59] and vacuum ultraviolet-excited optical luminescence [62] we have recently presented evidence that this blue peak in *our* preparations originates from states associated with surface oxide. The argument runs as follows: as the particles oxidise during exposure to X-rays *and* water,[59] a new peak at higher binding energy appears in the Si2p photoemission spectra corresponding to oxide and we simultaneously observe blue emission when the X-ray photon energy is tuned to the binding energy for Si2p electrons in the oxide. We can rule out the assignment of the blue XEOL peak to emission from a small Si core, because orange XEOL is observed simultaneously and also at photon energies corresponding to the Si2p level of unoxidised Si atoms. Further, using vacuum ultraviolet photons in the range 5-23 eV,[62] we have observed that the excitation spectrum of the blue luminescence has characteristics of silicon oxide.

Since the drop in luminescence intensity at high pH suggests an etching process, we have investigated how the

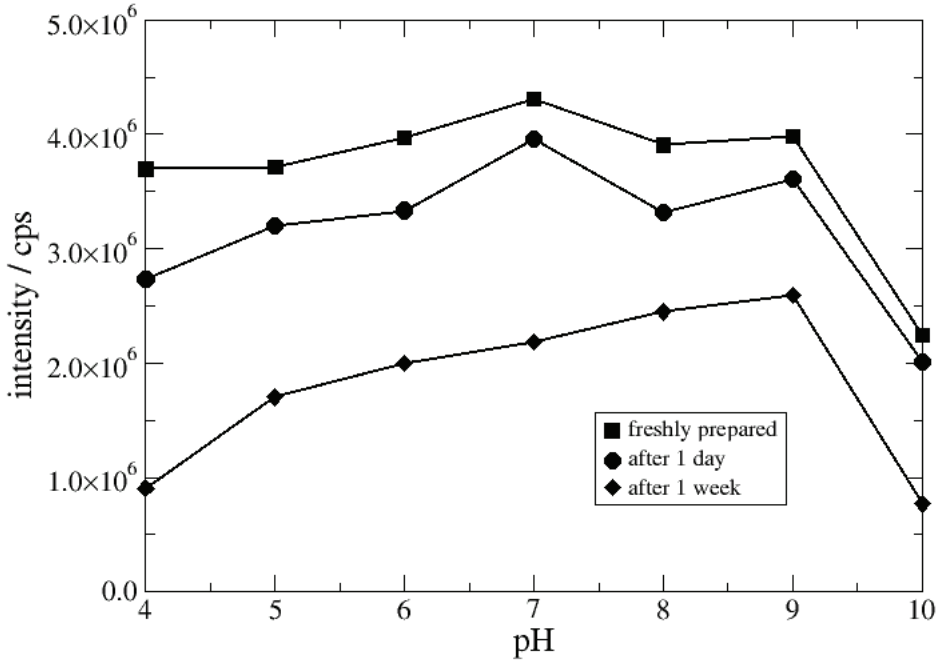

Figure 5: Luminescence intensity ($\lambda_{max}$is given in figure 6) of alkylated silicon quantum dots in 1% v/v THF/water at different times after preparation of the sols (excitation wavelength = 330 nm). The samples were stored in glass vials in the dark under ambient conditions and no attempt was made to purge the suspensions of oxygen.

alkylated silicon quantum dots age in 1% v/v THF/water. Over the pH range 5-9 we find that after 1 day the luminescence retains about 80-90% of the initial value, although after 1 week the intensity has dropped to 50% for pH 7 and about 60% for pH 8-9. (figure 5) In the most acidic (pH 4) suspension, the sample is much less stable and has lost about 75% of its initial intensity after 1 week. The most alkaline suspension is actually quite stable over the first day, but then loses about 65% of its intensity after 1 week. The neutral sols retain enough luminescence to be easily visible to the eye over long periods of time (6 months, figure 1). Some agglomeration is observed over these long periods, but not enough to explain a drop in intensity of 50% after one week and, anyway, the agglomerated particles still emit strong orange luminescence. These changes most likely reflect slow chemical reactions of the particles with water which introduce defects that act as nonradiative recombination centres.

The wavelength of the orange/red luminescence peak decreases with ageing for all the pHs studied (figure 6). A shoulder also appears at the high energy side (figure 7). This is consistent with a slow corrosion reaction of the alkyl-SiNCs in the aqueous medium, the formation of oxide and the reduction in the diameter of the Si core which would cause a shift to higher energy of the orange photoluminescence maximum according to the quantum confinement model. The blue emission band (ca. 430 nm, see figure 7) actually increases slightly with ageing of the sol, but we can interpret this as an increase in the amount of luminescent silicon oxide species which we have previously shown to be the origin of blue luminescence in our preparations of alkyl-SiNCs.[59] Although the monolayers formed on single crystal silicon surfaces by the hydrosilation route are known to be extremely robust towards oxidation over long periods,[72, 60, 73] it is likely that the monolayers on these small particles contain more defects or are less ordered and therefore water can penetrate to the underlying Si atoms. The high pH suspensions show a significantly larger blue-shift: this suggests that more rapid corrosion of the particles occurs at pH > 9. Although a large drop in luminescence intensity occurred for the most acidic suspension, the peak wavelength does not change dramatically. This was not due to flocculation of the particles or adsorption on walls of the glass vial and again we interpret it as a quenching effect brought about by defect states related to slow

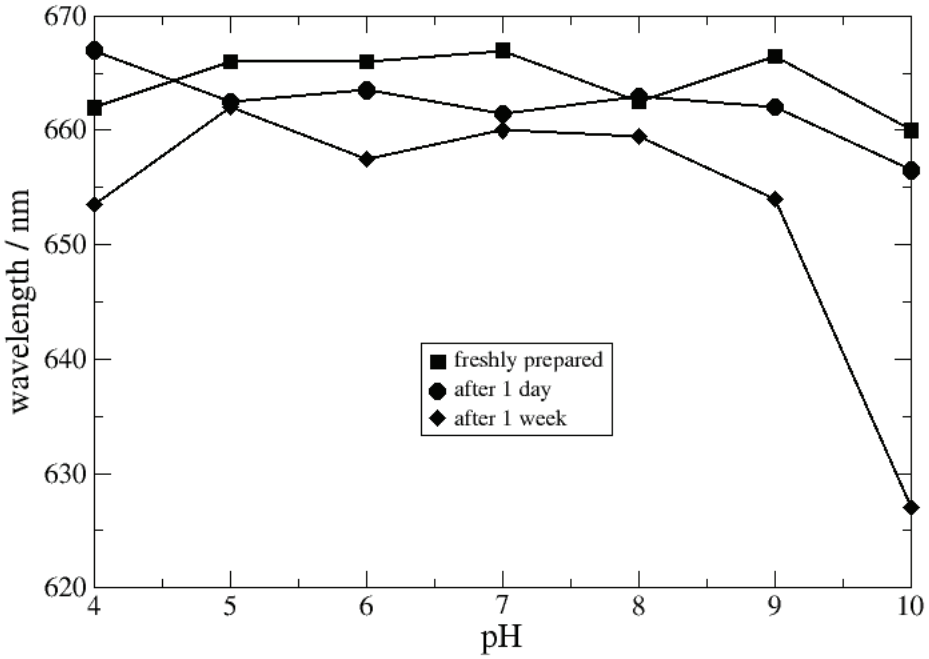

Figure 6: Wavelength of the orange peak in the luminescence spectra of alkylated silicon quantum dots in 1% v/v THF/water at different times after preparation of the sols (excitation wavelength = 330 nm). The samples were stored in glass vials in the dark under ambient conditions and no attempt was made to purge the suspensions of oxygen.

reaction of the particles with water.

A detailed study of the kinetics of uptake of $C_{11}-$SiNCs by various cell lines and an assessment of the toxicity of the particles will be published elsewhere. However, here we present data which illustrates the use of lyophobic aqueous dispersions of C_{11}-SiNCs for staining of cells by confocal fluorescence microscopy. THF, DMSO and ether were all tried as vehicles to disperse the C_{11}-SiNCs in cell culture medium. Figure 8 shows confocal fluorescence micrographs of HeLa cells after 1h exposure to medium containing 5 pmol C_{11}-SiNCs. Two cells selected from a larger field are shown; the left-hand image was obtained after incubating the cells in medium that had been shaken with a solution of C_{11}-SiNCs in ether and the right-hand image is from cells incubated in medium that had been shaken with a solution of C_{11}-SiNCs in THF. The images show clearly the morphology of the cells and the luminescence of the C_{11}-SiNCs is easily detected. The nanocrystals have a tendency to adhere to the cell membrane, presumably because of the hydrophobic capping undecyl monolayer, but some penetrate the membrane and luminescence from some internal membrane structures is bright. There is also some, weaker, luminescence from the cytosol. Although THF is the simplest choice of organic solvent to use to prepare an aqueous dispersion of C_{11}-SiNCs, because it is water-miscible, we found that even small traces of THF result in necrosis in a significant percentage of cells ($\simeq 30\%$ in the micrographs of larger fields of cells exposed to THF). DMSO is widely used in cell biology and we found no necrosis with this solvent, however, the C_{11}-SiNCs are sparingly soluble in DMSO. The best compromise choice of solvent was ether, in which the C_{11}-SiNCs are highly soluble, and which evaporates very rapidly to leave no trace in the medium. No evidence of toxicity was observed with ether; the main difficulty is its immiscibility with water and therefore the ether/water mixture must be shaken vigorously to produce a suitable dispersion.

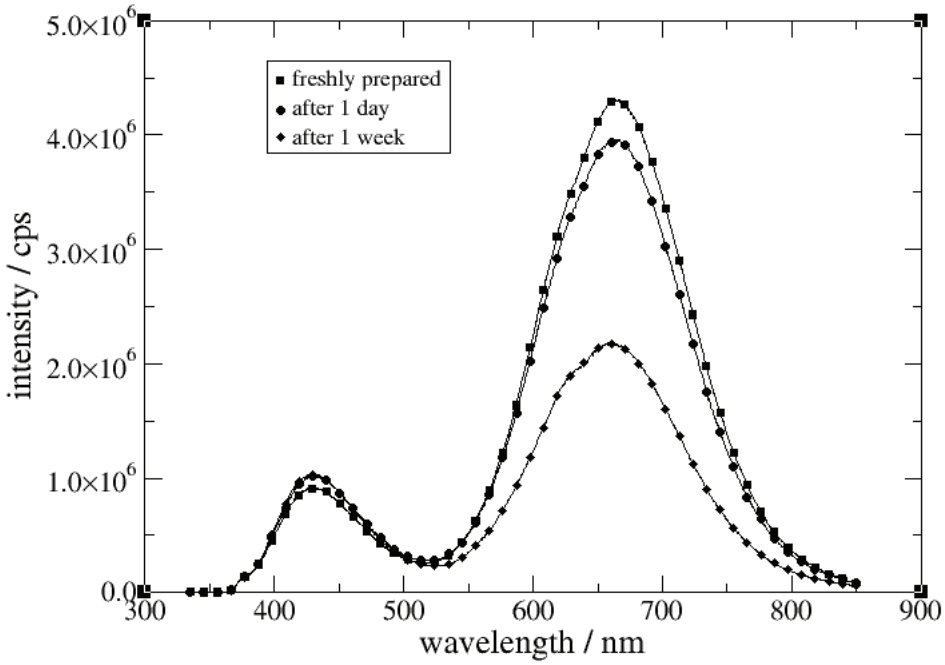

Figure 7: Ageing effect on luminescence spectra in pH 7 buffer for alkylated silicon quantum dots in 1% v/v THF/water (excitation wavelength = 330 nm). The samples were stored in glass vials in the dark under ambient conditions and no attempt was made to purge the suspensions of oxygen.

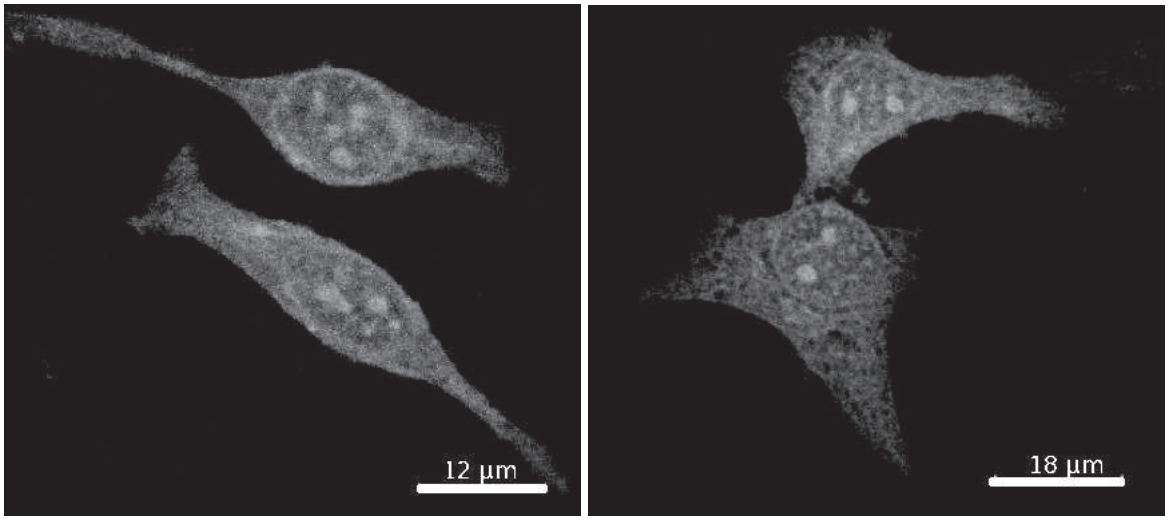

Figure 8: HeLa cells observed under a confocal fluorescence microscope (excitation =Ar ion line at 488 nm) after 1h incubation in culture medium containing C_{11}-SiNCs. The aqueous dispersion of SiNCs in the medium was prepared by shaking 1 mL medium with 0.2% v/v of an organic solvent containing C_{11}-SiNCs. Left: solvent=ether; Right: solvent = THF

Conclusions

Lyophobic dispersions of alkylated silicon quantum dots in THF/water mixtures containing 1% THF by volume are transparent and strongly luminescent. The photoluminescence spectra of the aqueous suspensions of our (Si core diameter ca. 2.5 nm) particles show a major orange-red emission band at about 665 nm and a minor, blue emission band at about 430 nm. Half the orange luminescence intensity is retained after 1 week, the luminescence is independent of pH over the range 5-9 and the luminescence intensity is strictly linear in particle concentration over at least 3 orders of magnitude: these properties make alkyl-SiNCs promising as red fluorophores for quan-

titative labelling work in biological systems. Although red-emitting SiNCs generally show longer luminecsence lifetimes than blue-emitting SiNCs,[2] the red-emitting particles nevertheless can be used as a luminescent label in confocal fluorescence microscopy.

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Dispersions of alkyl-capped silicon nanocrystals in dilute organic solvent/water mixtures: steady-state photoluminescence and ageing studies

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Supporting Information

Contents

This supporting information file provides microscopic and spectroscopic characterisation of the alkyl-capped silicon nanocrystals (alkyl-SiNCs) demonstrating the crystallinity of the Si core and the presence of the alkyl capping monolayer. According to these measurements, the particles have about the same size and structure as those reported in ref. [1].

Finally, additional data showing the uptake of alkyl-SiNCs by HeLa cells from THF/water dispersions is presented. This data also shows the toxicity of THF towards the cells, which is absent in similar images obtained using ether as the vehicle to disperse alkyl-SiNCs in the medium.

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1 Methods

1.1 Photoemission spectroscopy

Photoemission spectra of C_{11}-SiNCs were taken using synchrotron radiation at beamline I511 of MAX-Lab, Lund, Sweden. 140 eV photons were used in the Si2p spectra and 354 eV photons were used in the C1s region. The sample was prepared by evaporation of a thick film of SiNCs from a solution in dichloromethane onto a gold nitride foil. Detailed photoemission studies of our SiNCs have been published elsewhere.[1, 2]

1.2 Raman and Luminescence Spectroscopy

A CRM200 confocal Raman microscope (Witec GmbH, Ulm, Germany) was used to capture Raman and luminescence spectra. The 488 nm line of an argon ion laser provided the excitation light and the emitted and/or scattered light passed through a Raman edge filter to remove elastically scattered light. The filtered light was collected by a multimode optical fiber which served also as the confocal pinhole. The collected light was analysed by a spectrograph containing a cooled CCD with typical settings for luminescence experiments of: 150 lines/mm (grating) and an integration time of 1 s, or 1800 lines/mm (grating) and 10 s of integration. Gold nitride films were used, rather than pure gold, as substrates for Raman spectroscopy because the morphology of these films produces a significant SERS enhancement: the preparation and characterization of these films has been reported elsewhere.[3]

1.3 Infrared Spectroscopy

C_{11}-SiNCs were dried on an Si(100) chip and the infrared spectrum was measured in normal transmission mode. The clean Si(100) chip was used to obtain the background. The instrument was a Bio-Rad Excalibur with an MCT detector and 32 scans at 4 cm^{-1} resolution were co-added and averaged.

2 Additional Supporting Data

The chemical composition of the SiNCs was confirmed by photoemission and infrared spectroscopy to consist of a silicon core covalently bound to saturated C_{11} alkyl chains. The crystalline nature of the silicon core was confirmed by Raman spectroscopy, X-ray diffraction (powder patterns) and high resolution scanning transmission electron microscopy. The Scherrer equation was used to extract the diameter of the Si core from the XRD powder pattern linewidths and the value of 2.6 nm was broadly consistent with the electron microscopy and previous estimates from probe microscopy.[2]

2.1 Photoemission spectroscopy

Figures 1 shows Si 2p and C 1s spectra of SiNCs deposited as a thick film on a gold nitride foil. As we have discussed previously, such samples are very insulating and the binding energies and peak widths are strongly affected by

changes in screening effects as the film thickness varies. The peak position of ca 103 eV is, in fact, due to unoxidised Si despite the binding energy being higher than for single crystal Si.[2] The presence of oxide, Si(+4), would be signalled by the appearance of a second peak shifted positive of the Si(0) peak by about 3.3 eV. The breadth of the Si 2p peak does however mean that we cannot rule out sub stoichiometric quantities of oxide - this is indeed observed in the FTIR data below.

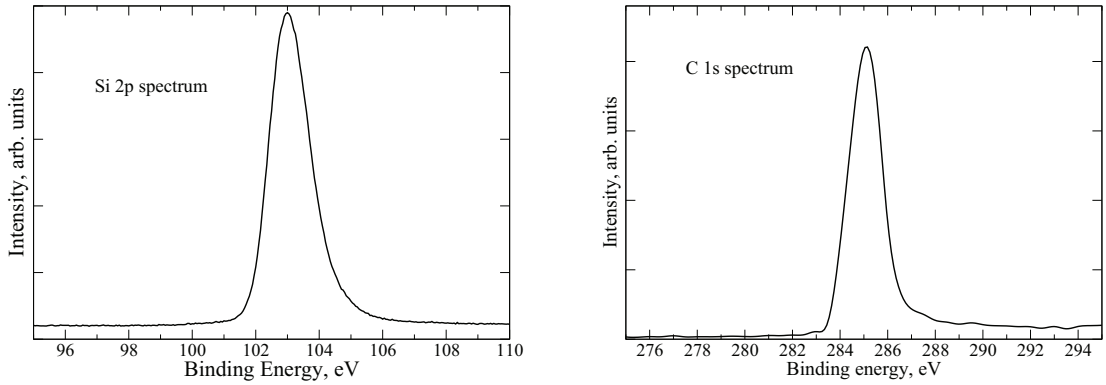


Figure 1: Photoemission spectra of C11- SiNCs on a gold nitride foil. Left: Si 2p spectrum obtained in normal emission using 140 eV photons; Right: C 1s spectrum obtained in normal emission using 354 eV photons

2.2 Infrared spectroscopy

Figure 2 shows an infrared spectrum of a dry film of alkyl-SiNCs.

Features characteristic of the saturated alkyl capping layer:

(1) absence of vinylic C-H stretches at 3080 cm^{-1} and the sharp C=C stretch at 1640 cm^{-1} indicates there are no sp^2 carbon atoms;

(2) aliphatic C-H stretches in the range 2960 - 2850 cm^{-1} (including the feature due to methyl groups at 2960 cm^{-1}) and the methylene scissor mode at ca. 1470 $cm^{-1}$are characteristic of an n-alkyl layer;

(3) broadened Si-H stretching feature at ca 2100 cm^{-1}which is characteristic of residual Si-H on alkylated silicon [4]

and (4) the broad feature at ca. 1050 cm^{-1} and the small feature at ca. 2250 cm^{-1} confirms the presence of a small amount of silicon oxide. The coverage of oxide is much less than that of alkyl chains based on the relative intensities of the features due to O_nSi-H and Si-H stretching modes (which have similar oscillator strengths [5]) as well as the Si-O and C-H features (the Si-O str has a very large oscillator strength).

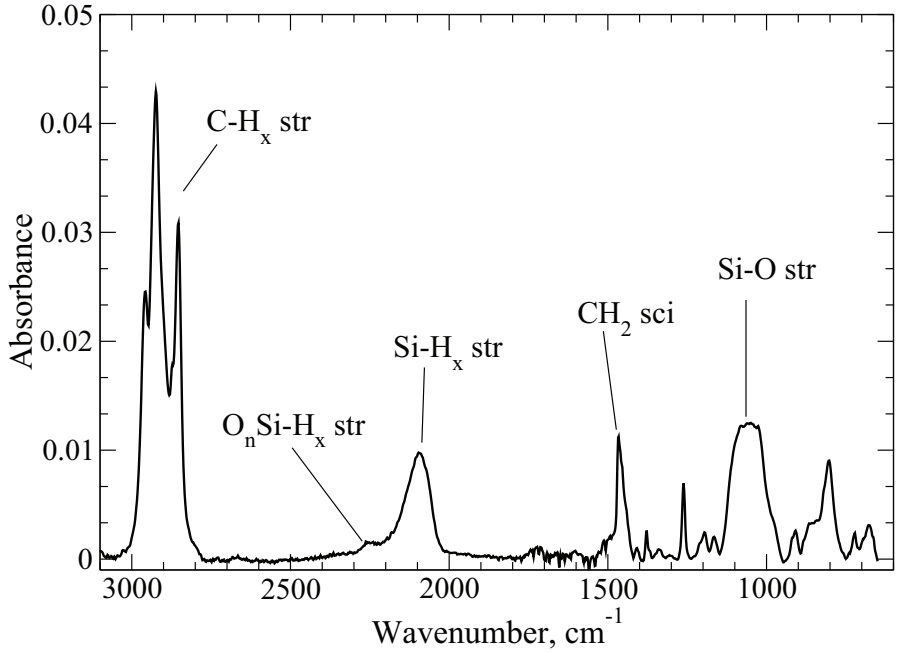


Figure 2: FTIR spectrum in normal transmission of C_{11}-SiNCs deposited from dichloromethane solution on a 1 cm^2 Si(100) chip. The background was the same chip before deposition of the alkyl-SiNCs.

2.3 Raman - Luminescence Microscopy

Figure 3 shows a luminescence and Raman spectrum of C_{11}-SiNCs deposited on a gold nitride foil from dichloromethane solution. An Ar-ion laser (488 nm) line was used to excite the luminescence and two spectra are shown: one was obtained using a 150 line / mm grating which allows collection of both Raman and luminescence from the particles; the other employed a 1800 line / mm grating to show the Raman peak due to the Si at a Raman shift of 515 cm^{-1} in greater detail (figure 4). The dark count readings of the CCD have been subtracted from both spectra.

The Raman feature at 515 cm^{-1}is characteristic of crystalline silicon and the shift to lower wavenumber than the bulk value is typical of quantum-confined silicon nanocrystals.[6, 7, 8] The luminescence peak position is also consistent with the Si core diameter (ca. 2.5-2.6 nm) determined by XRD linewidth analysis and electron microscopy in the sections below.

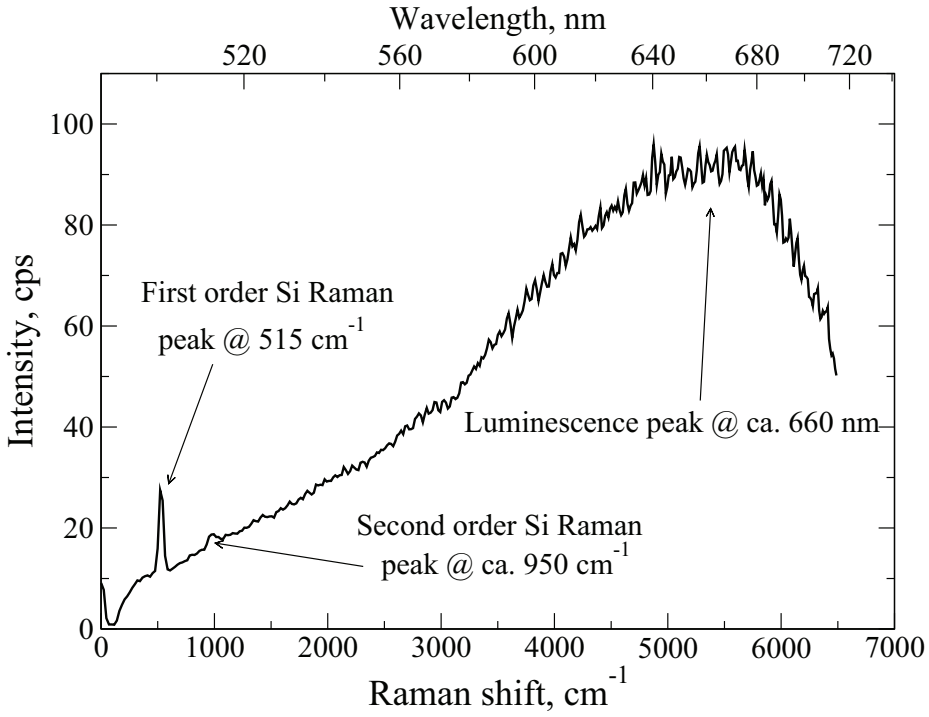


Figure 3: Raman and luminescence spectrum of SiNCs deposited as a film on gold nitride from a solution in dichloromethane. Excitation wavelength = 488 nm, grating = 150 lines / mm.

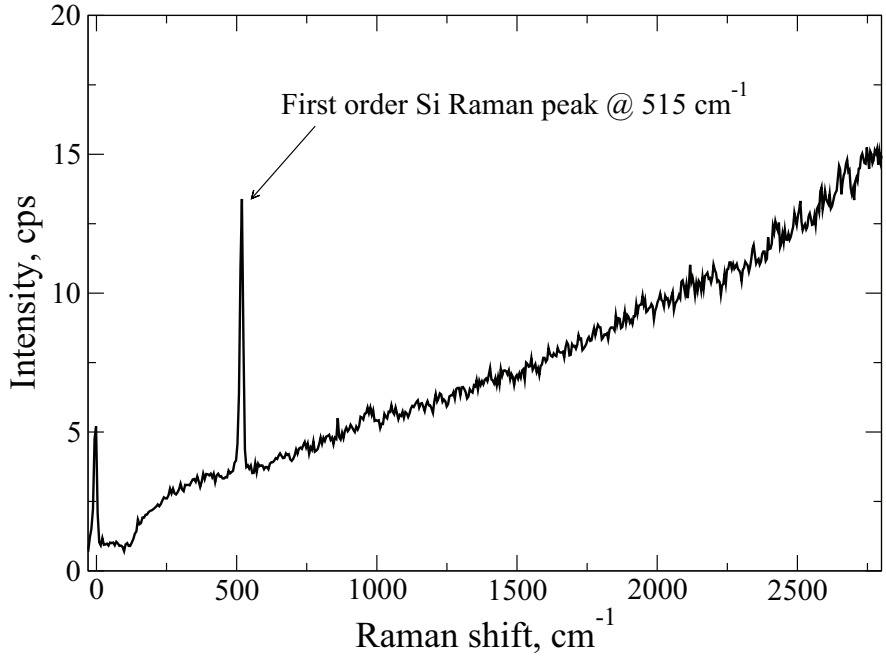


Figure 4: Raman and luminescence spectrum of SiNCs deposited as a film on gold nitride from a solution in dichloromethane. Excitation wavelength = 488 nm, grating = 1800 lines / mm.

2.4 X-ray Diffraction (XRD)

XRD pattern for C_{11}-SiNCs cast as a film from solution in dichloromethane (figure 5). The peak positions were assigned by comparison with a crystalline Si primary reference: Natl. Bur. Stand. (U.S.) Monogr. 25, 13 , 35, (1976).

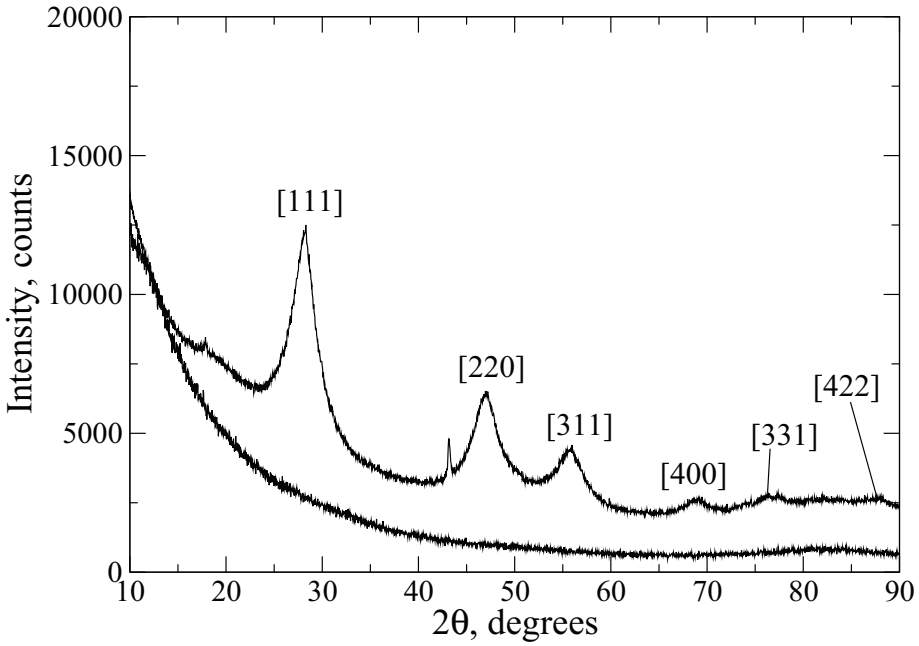


Figure 5: XRD pattern of a film of C_{11}-NCs cast from dichloromethane solution. The peaks are assigned to the indicated lattice planes of crystalline silicon. The lower curve is the background scattering from the blank.

Figure 5 shows the expected peaks corresponding to crystalline silicon[2]. The feature at $2\theta \simeq$ 28 degrees due to [111] planes was fitted with a pseudo-Voigt function (figure 6). Using the Scherrer formula and the peak width from the fit, the Si particle diameter (assumed equal to that of the crystallite) is 2.6 nm. The value deduced from the feature at 47 degrees was the same.

[2]The small feature at 2θ =43 degrees is due to a trace of Cu on the sample stage.

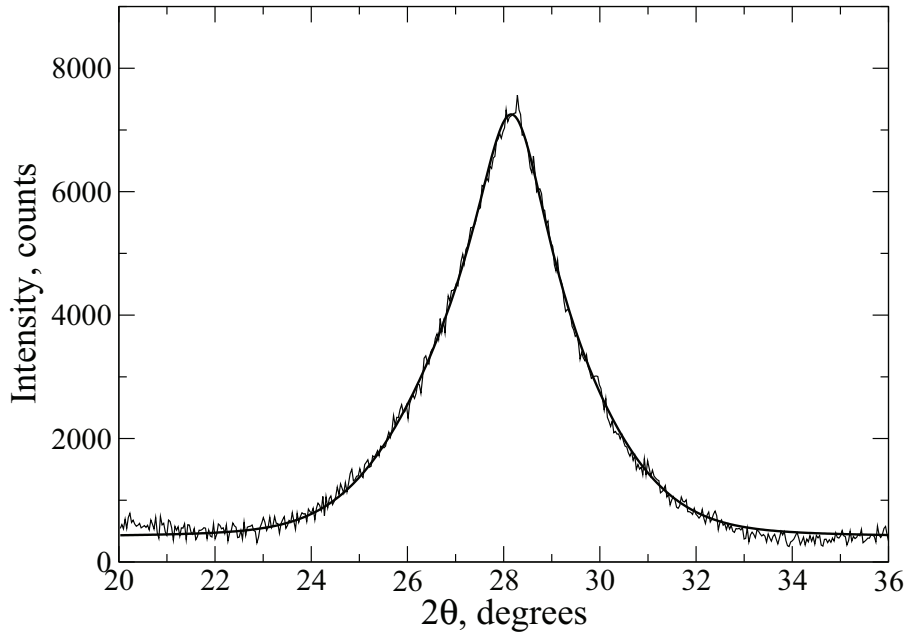


Figure 6: Pseudo-Voigt fit to the (111) peak of the XRD pattern for C_{11}-NCs after subtraction of a linear baseline.

2.5 Scanning transmission electron microscopy (STEM)

Further support for the particle size measurements and the crystallinity of the SiNCs was obtained from scanning transmission electron microscopy. The experiments were carried out in the aberration-corrected Daresbury Super-STEM (Daresbury Laboratory, CCLRC, Daresbury, UK). High resolution bright field and high angle annular dark field (HAADF) images, the latter revealing atomic Z-contrast, were taken simultaneously.

Figure 7 (left) shows a typical high resolution STEM HAADF (aberration-corrected) image of a single C_{11}-SiNC on a carbon grid. The sample was prepared by placing a drop of dichloromethane solution of C_{11}-SiNCs on a standard carbon grid. The particle is roughly spherical and the diameter is about 2.5 nm. Electron energy loss spectra (Si L edge) were used to confirm that the white area in the image corresponds to the Si core. A detailed STEM and EEL study will be published elsewhere.

We also obtained high resolution bright-field STEM images of C_{11}-SiNCs (figure 7, right) that were evaporated at 200^0C in UHV and collected on a carbon grid.[1] The presence of Si was confirmed by electron energy loss sp ectra (Si L edge) and the direct observation of lattice fringes of the appropriate spacing ([100] planes). The image quality is slightly better than for the material deposited from solution; this is probably due to the removal of trace solvent - previously reported to affect the imaging of SiNCs [9] - as the UHV chamber is pumped down.

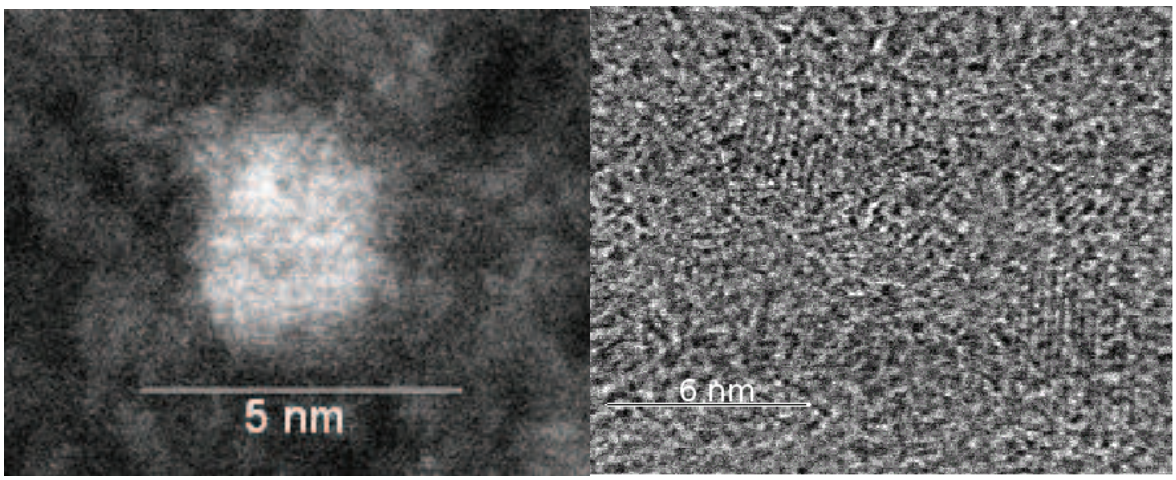
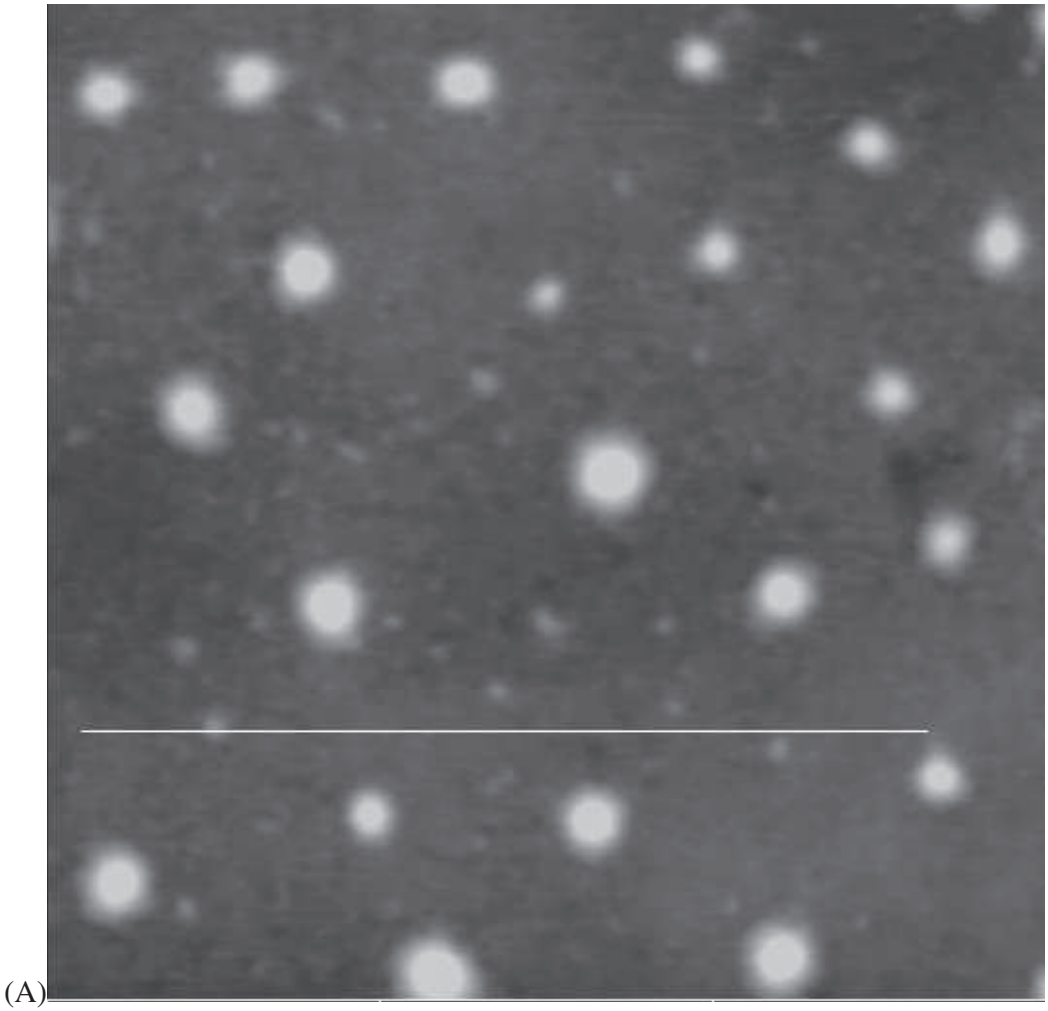


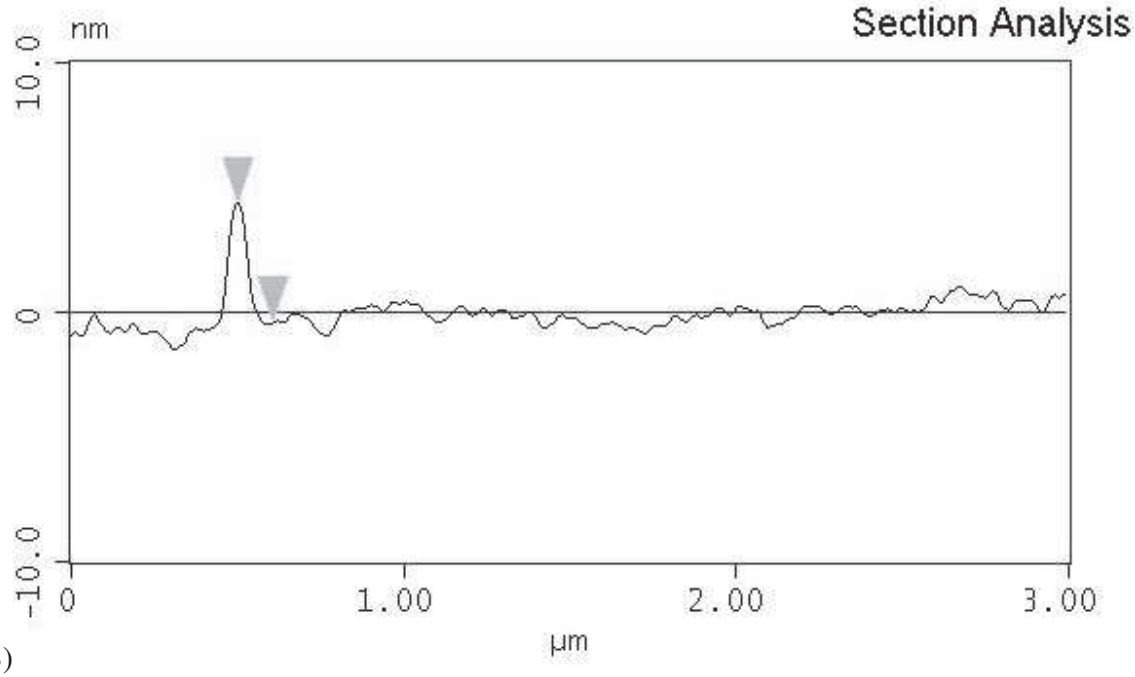
Figure 7: Left: STEM HAADF (aberration-corrected) images of a single C_{11}-SiNC particle; the white region corresponds to the Si core. Right: bright field image showing several particles in which the lattice fringes due to [001] planes are visble. In both cases, the beam energy was 100 keV and the resolution was about 0.1 nm.

2.6 Atomic force microscopy

Figure 8 shows tapping mode AFM images of alkyl-SiNCs deposited on undecyl-capped Si(111) surfaces. These surfaces are very flat, stable and are chemically similar to the undecyl-capped SiNCs. This facilitates height measurements ($\simeq$ 5nm) of the particles; although the particles do have a tendency to form clusters on the surface, some isolated particles or islands one particle high are detectable (line section (B)).



(A)



(B)

Figure 8: (A) Tapping mode AFM image of alkyl-SiNCs deposited from the vapor onto undecyl-capped Si(111) surfaces. The grayscale corresponds to 20 nm and the image area is 3×3 μm. (B) zoom and line section of an island one particle high.

2.7 Uptake of alkyl-SiNCs by HeLa cells - additional data

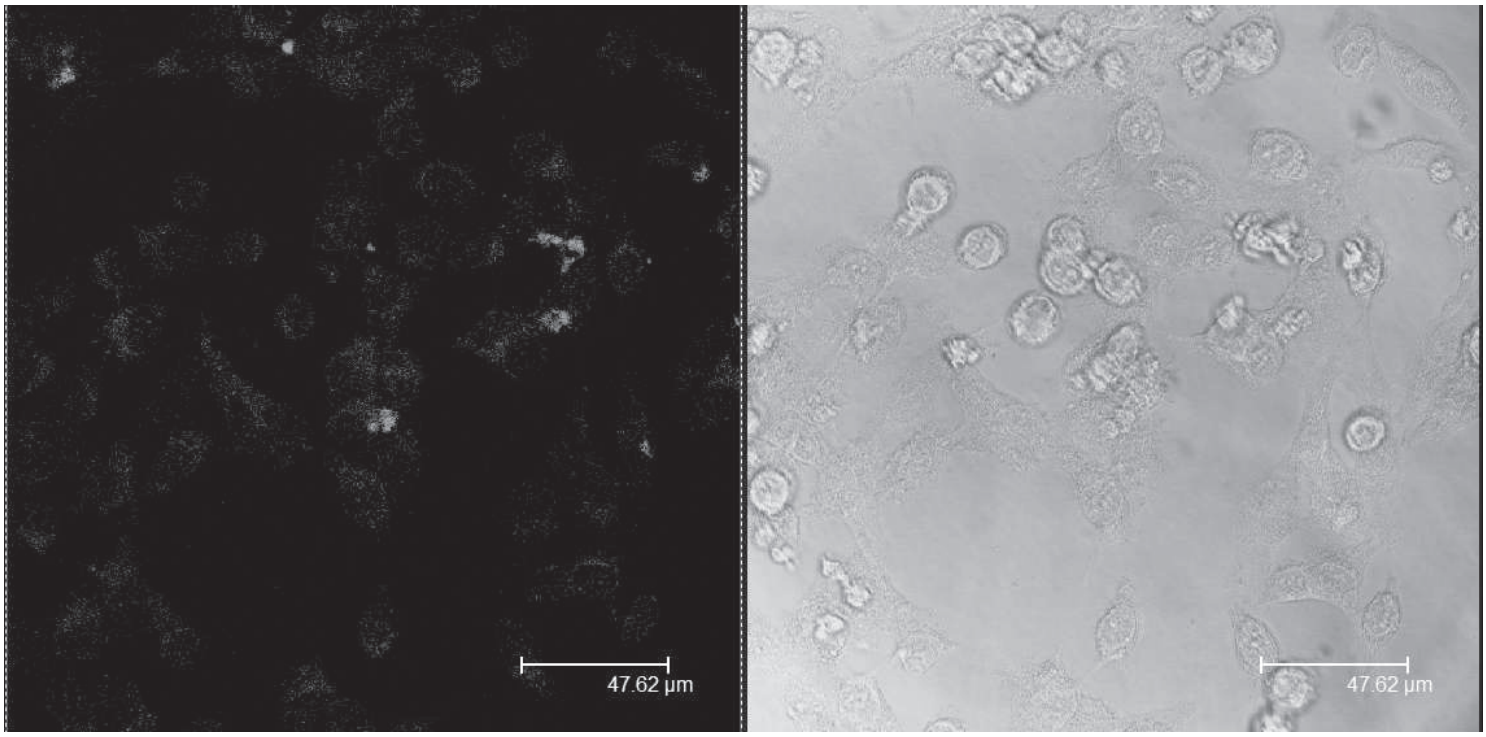


Figure 9: Confocal luminescence images and normal optical images of a field of HeLa cells after incubation in the presence of 0.2% THF v/v for 2h.

Figure 9 shows a confocal fluorescence image (λ_{ex} =488 nm; emission bandpass = 550-650 nm) of HeLa cells after 2h exposure to alkyl-SiNCs ($\simeq$ 5pmol) in 0.2%THF / medium. The cells all show strong luminescence because of internalization of the alkyl-SiNCs, but about 30% of those in the field also show very evident necrosis (rounded appearance). When ether is used as the vehicle, no necrosis or other acute toxicity is observed; a detailed account of this data will be published elsewhere.

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