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Properties of coatings on RFID p-Chips that support plasmonic fluorescence enhancement in bioassays

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Abstract

Microtransponders (RFID p-Chips) derivatized with silver island film (SIF) have previously seen success as a platform for the quantification of low-abundance biomolecules in nucleic acid-based assays and immunoassays. In this study, we further characterized the morphology of the SIF as well as the polymer matrix enveloping it by scanning electron microscopy (SEM). The polymer was a two-layer silane-based matrix engulfing the p-Chip and SIF. Through a series of SEM and confocal fluorescence microscopy experiments we found the depth of the polymer matrix to be 1–2 μm . The radiative effects of the SIF/polymer layer were assessed by fluorescence lifetime imaging (FLIM) of p-Chips coated with the polymer to which a fluorophore (Alexa Fluor 555) was conjugated. FLIM images showed an 8.7-fold increase in fluorescence intensity and an increased rate of radiative decay, the latter of which is associated with improved photostability and both of which are linked to plasmonic enhancement by the SIF. Plasmonic enhancement was found to extend uniformly across the p-Chip and, interestingly, to a depth of about 1.2 μm . The substantial depth of enhancement suggests that the SIF/polymer layer constitutes a three-dimensional matrix that is accessible to solvent and small molecules such as fluorescent dyes. Finally, we confirmed that no surface-enhanced Raman scattering (SERS) is seen from the SIF/polymer combination. The analysis provides a possible mechanism by which the SIF/polymer-coated p-Chips allow a highly sensitive immunoassay and, as a result, leads to an improved bioassay platform.

Keywords

ELISA; multiplex; SIF; surface plasmon resonance; FLIM; RFID

1. Introduction

Fluorescence-based techniques have become an indispensable tool for the modern biologist. They are the ideal methodologies for medical diagnostics, DNA sequencing, genetic analysis, forensics, and cellular/molecular imaging. Many approaches involve the use of a solid phase to facilitate manipulations and data acquisition. The solid phase that has been recently successfully implemented in multiplex bioassays [1,2] is an ultra-small RFID

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silicon p-Chip. These light-activated microtransponders are $500\text{ }\mu\text{m} \times 500\text{ }\mu\text{m} \times 100\text{ }\mu\text{m}$ integrated circuits that have electronic elements on one side of the chip. The electronic circuits on the p-Chip include photocells, read-only memory (ROM), a loop antenna, and control elements. Their role is to transmit the ID of the chip when the chip is illuminated by laser light during the assay to reveal the type of biological material carried on the chip. In the assays, biological material (oligonucleotides, antibodies, antigens) is conjugated to the p-Chip, the chip is exposed to the sample and fluorescently stained, and the chip's fluorescence intensity is quantified in a flow-based custom analyzer [3] revealing concentrations of analytes in the sample. The benefits of p-Chips in bioassays are clear, as they allow a high-level multiplexing to obtain concentration of a large number of analytes in the sample [1–3]. The chips have been also used to tag small laboratory animals [4–6].

Aside from their obvious advantages in multiplex bioassays, the p-Chips also provide a means of fluorescence enhancement, improving the sensitivity of assays for low-abundance biomolecules. In traditional fluorescence assays, fluorophores are examined in the free-space condition, i.e., the fluorophore may be modeled as an oscillating dipole radiating energy into a transparent, homogeneous medium. The free-space spectral properties are not modified by local polarity, quenching, or energy transfer. In the case of p-Chip-based assays, it is possible to place the fluorophore in close proximity to a conducting metal surface, thereby altering the free-space properties through surface plasmon resonance [7–10]. This refers to the interaction between excited state fluorophores and mobile electrons on the surface of the metal, and it has been shown to increase quantum yields, increase photostability, and decrease lifetimes [11–24].

Metal-enhanced fluorescence (MEF) has been extensively studied over the last two decades, and these studies often revolved around the morphology of the metal particles providing the enhancement. Great success has been seen from not only silver island film (SIF) substrates [25–31] but also silver colloids [32–33], nanorods [34], nanotriangles [35–36], and silver fractal surfaces [10]. However, these systems usually involve nanoparticles resting on a substrate, while the flow-based p-Chip assays require that silver particles be attached to the surface in a robust manner and on a mass scale. One such method, in which p-Chips are coated with a SIF and the film is held in place by a coating of polymer above and below, has been previously demonstrated [2]. The polymer prevents the SIF from being removed during an assay as a result of mechanical forces acting on the polymer. It also provides a bed for the conjugation of biological molecules, e.g., DNA or protein. The polymer provides another added benefit in that the fluorophore is separated from the silver and located at optimal distances for fluorescence enhancement. The optimal distance for enhancement between a fluorophore and a metal surface is $\sim 100\text{ }\text{\AA}$, while a fluorophore is severely quenched within $50\text{ }\text{\AA}$ [11].

It is known that the size and shape of silver particles have a direct effect on plasmon resonance and the subsequent fluorescent spectral properties [37]. It is also known that the separation between the fluorophore and the metal particles will affect the enhancement [23]. The structure and geometrical properties of the SIF and the polymer are thus the focus of the present study. We also investigated fluctuations of both fluorescent intensity and fluorescent lifetimes across the chip's surface to better understand the significance of silver particle clusters within the polymer on fluorescence enhancement.

2. Materials and methods

2.1. p-Chips

p-Chips were provided by PharmaSeq, Inc. (Monmouth Junction, NJ, www.pharmaseq.com). The properties of the p-Chips and two types of p-Chip readers (flow-

based bench-top analyzer and hand-held ID reader) have been described [1–4]. The 10-bit p-Chips used in the work described allowed a maximum 1,024 different IDs.

2.2. Coating p-Chips with polymer

The p-Chips were coated by aminopropyltriethoxysilane (APTS) and 3-glycidoxypentyltrimethoxysilane (GPTS) as previously reported [2] with several modifications. Briefly, p-Chips were washed with 99.5% methyl alcohol at room temperature (RT) for 5 min three times. The p-Chips were then rinsed with toluene/dimethylformamide (DMF) mixture with 0.01% distilled water and 0.9% APTS at RT four times. After rinsing, p-Chips were immediately treated with a coating solution (mixture of toluene and DMF with 0.01% distilled water, 0.9% APTS, and 0.3% GPTS) at 80°C for 20 min. After the coating reaction, p-Chips were washed once with toluene, three times with DMF, and three times with acetonitrile at RT, followed by air drying. The procedure places both amino and hydroxyl groups within the polymer and on the surface of p-Chips.

To introduce the carboxyl groups, the derivatized p-Chips were treated with 10% succinic anhydride in dry pyridine:DMF (1:9) and placed on a tissue culture rotator at RT for 30 min. This step was repeated once using fresh reagents. After the reaction, the carboxylated p-Chips were washed with DMF four times and acetonitrile twice, followed by air drying.

All chemicals used for coating and carboxyl conversion were purchased from Sigma-Aldrich, St. Louis, MO.

2.3. Deposition of SIF on p-Chips

In order to make SIF-coated p-Chips, the chips were coated with polymer first as described above. Then SIF was deposited on the surface of polymer-coated p-Chips as reported previously [2] with several modifications. Briefly, 60 μ L of 5% NaOH was slowly added to 18 mL of 0.83% AgNO₃ solution with intensive stirring at RT in a 50 mL reaction tube. Then 400 μ L of 30% NH₄OH was added with intensive stirring at RT. The clear solution was incubated in an ice bath for 10 min, followed by the addition of 4.5 mL of a fresh 4.8% glucose solution with intensive stirring. Polymer-coated p-Chips were incubated in this solution in a 1.5 mL Eppendorf tube on a tissue culture rotator at RT for 50 min. After the silver deposition, the p-Chips were immediately washed with distilled water three times followed by air drying. The dried SIF-deposited p-Chips were coated with another polymer layer and carboxylated as described above to seal the SIF layer.

2.4. IL-6 immunoassay

We performed a standard IL-6 assay on polymer coated p-Chips and SIF/polymer coated p-Chips in order to test the two platforms under identical conditions. To conjugate an antibody, the carboxylated p-Chips were incubated with 100 μ L 261 mM N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and 230 mM N-hydroxysulfosuccinimide (NHSS) in 0.1M HEPES buffer (pH 7.5) on a rotator for 30 min at RT. The p-Chips were then washed with 200 μ L PBS three times and incubated with 30 μ L 200 μ g/mL monoclonal anti-human IL-6 antibody (R&D Systems, Minneapolis, MN) for 2 hrs at RT on a tissue culture rotator. The p-Chips were washed with PBS three times and blocked with SuperBlock solution (Thermo Fisher Scientific, Waltham, MA) for 5 min at RT on a rotator three times. The p-Chips were then washed with PBS three times and stored in TBS with 3% BSA at 4 °C.

Anti-IL-6-conjugated p-Chips were incubated with 50 μ L 100ng/mL recombinant human IL-6 protein standard (R&D Systems) in TBS with 3% BSA for 1 hour at RT on a rotator.

After incubation, the p-Chips were washed with 200 μ L of Tris-buffered saline with 0.05% Tween-20 (TBST) three times.

The detection antibody solution was prepared by diluting biotinylated anti-human IL-6 antibody (R&D System) to 5.0 μ g/mL with PBS. The p-Chips were then incubated with 50 μ L of this solution for 1 hour, followed by washing with TBST three times. The p-Chips were pooled and incubated with 50 μ L of 8 μ g/mL streptavidin-Alexa Fluor 555 conjugate in TBST for 30 min at RT in the dark. After incubation, the p-Chips were washed with TBST three times and with distilled water twice and were stored in PBS at 4°C between measurements. All measurements except SEM were conducted with the p-Chips immersed in PBS.

2.5. SEM

Samples were imaged with an FEI model XL30 (Hillsboro, OR) scanning electron microscope (SEM). The SEM is equipped with a tungsten filament operating at accelerating voltages up to 30 kV. The samples were fixed to aluminum sample stubs via carbon conductive tape, which provided a continuous, conductive path from the sample through the stage. When p-Chip cross-sections were imaged, the p-Chip was first cut in half with a razor blade before being attached to the carbon tape, cut side up towards the primary electron beam. The chips were then placed into the sample chamber, where they were measured under vacuum with pressures always below 1 microtorr. The working distance between the bottom of the column and the sample was 10 mm. The spot size and accelerating voltage were varied as needed, and these values are indicated on each particular image. As is typical for SEM imaging, all images reported here were collected from the detection of secondary electrons, emitted as the primary electron beam rasters across the sample surface.

2.6. Elemental analysis in SEM

The SEM was equipped for energy dispersive spectroscopy (EDS) with a detector manufactured by EDAX (Mahwah, NJ). Because semi-conductive and nonconductive materials exhibit very low signals in EDS, the accelerating voltage used for EDS was always 30 kV so as to provide the highest possible count rate.

2.7. Fluorescence microscopy: FLIM and intensity data

Fluorescence lifetime imaging (FLIM) data were collected by the MT200 system (PicoQuant GmbH, Berlin, Germany). This system was coupled to an Olympus (Shinjuku, Tokyo, Japan) IX71 confocal microscope with a 100 $\times$ oil objective, unless it was noted that a 60 $\times$ water objective was used. A single photon avalanche detector (SPAD) manufactured by Micro Photon Devices (Bolzano, Italy), model PD1CTC, was chosen for its sensitivity. A 470 nm pulsed laser diode with a 20 MHz repetition rate was utilized for excitation. The emission was filtered by a 470 long pass liquid filter as well as a 560/40 band pass filter. For reflection images, the laser power was reduced and a 470/10 band pass filter was used. The system is capable of picosecond time resolution, and the piezo stage is accurate to within 1 nm.

Symphotime (version 5.2.4) software by Picoquant was used to process all FLIM data and to extract intensity information from the FLIM data. FLIM images are produced with grayscale intensity data and false color overlaid to indicate the lifetime calculated for every pixel. The enhancement in fluorescence intensity was measured with the MT200 simultaneously with FLIM data.

2.8. Fluorescence lifetime measurements

Fluorescence lifetime was measured with a Fluorotime 200 fluorometer, also from Picoquant. The FT200 was equipped with a 470 nm pulsed laser diode, a microchannel plate photomultiplier ultrafast detector, and a monochromator. The p-Chips were placed between two coverslips, a drop of buffer was added, and the coverslips were taped together. The sample was then arranged in a front face attachment with a 495 nm long pass filter on emission. The monochromator was set to 565 nm. The response was measured with the monochromator set to 470 nm along with a 470/10 band pass filter.

2.9. Raman scattering measurements

Raman scattering measurements were collected on a custom-built system consisting of a dispersive spectrometer from Kaiser Optical Systems, Inc. (Ann Arbor, MI) with 4.0 cm^{-1} resolution and a front-illuminated CCD detector from Roper Scientific (Sarasota, FL). The p-Chips were illuminated with an argon-ion laser (514 nm) emitting 220 mW of power. The light was focused by an Olympus BH-2 microscope with a 40 $\times$ objective, resulting in a 1.5 μm collection area. The system was capable of measuring Raman shifts up to 2000 cm^{-1} , corresponding to the 572 nm wavelength. The experiments were conducted on p-Chips coated with SIF and polymer in the manner described above but using a different assay. Briefly, the polymer coating the SIF and non-SIF p-Chips was carboxylated and further derivatized by conjugating streptavidin to the carboxyl groups (see sections 2.3 and 2.4 for the conjugation methods). The streptavidin-conjugated p-Chips were then incubated in 50 μL of 10 $\mu\text{g}/\text{mL}$ Cy3-labeled biotinylated oligonucleotide at RT in a dark enclosure for 30 min. The p-Chips were washed with TBST three times and air dried. The sequence of the oligo was 5'-Cy3-TTT TTT TTT T-biotin-3'.

3. Results and discussion

3.1. SEM

To avoid the suspected complex effects of electronic circuitry on the front of the p-Chips, the back of the p-Chips were viewed under SEM at high magnification in order to evaluate the morphology of the SIF and polymer coatings. Further, p-Chip cross-sections were viewed to measure the thickness of the coatings. When investigated via SEM (Figure 1), the surfaces of the p-Chips derived with SIF and polymer coatings were not smooth, as we expected them to be. Instead, they consisted of irregularly shaped nanoparticles as well as dispersed aggregates on top of nanoparticle layers. The sizes of the nanoparticles were measured according to their shortest axis, and their average was found to be around 270 nm when a log-normal distribution was fitted to 216 measurements (Figure 2). To determine the thickness of the SIF and polymer layer, we cut the SIF/polymer coated p-Chip in half and imaged the cross-section by SEM. Figure 3 shows that the particles were deposited in a 1–2 μm thick layer. This layer was confirmed by EDS mapping to be silver, with traces of carbon and oxygen likely originating from the polymer coating (Figure 4). Surprisingly, we were unable to detect an expected smooth, polymer coating on top of the silver particles. We expected to see this layer because the SEM images were formed from low-energy secondary electrons produced from the bombardment by the primary electron beam. These electrons are capable of traveling around 100 nm in insulators [30]. Secondary electrons originating at the silver would be lost as they traveled through the polymer. We hypothesize that when this polymer coating is placed into the high vacuum environment of the SEM (10^{-5} bar), the moisture was removed, leaving only a very thin and undetectable coating surrounding the silver islands.

In another independent measurement, we determined that the polymer coating is around 1.0–1.5 μm thick. This was done by stacking several polymer coated p-Chips, measuring the

thickness, and then comparing that thickness to a stack of uncoated p-Chips (data not shown). These measurements were conducted immediately after removing the p-Chips from PBS buffer solution before the polymer had dried. The results are consistent with the SEM-based measurements for SIF/polymer p-Chips.

3.2. Fluorescence enhancement and lifetime change

To evaluate the fluorescence enhancement by the silver nanoparticles in bioassays, we performed IL-6 ELISA assays on both SIF and non-SIF p-Chips. IL-6 is a pleiotropic cytokine involved in inflammation, hematopoiesis, and immune response [38]. Aberrant IL-6 levels have been observed in many autoimmune disorders and cancers [39]. The IL-6 ELISA assays are widely used in cancer studies and basic biological research and have been demonstrated to work well on the p-Chip platform [2]. In the assays, Alexa Fluor 555 was used as the fluorescent dye. We used an IL-6 standard of known concentration to ensure the conditions of the assay were identical for both platforms. After the complete assay was performed (please see section 2.4), fluorescence intensity was determined by averaging the count rate into the SPAD during collection of the corresponding FLIM image in Figure 5. To measure the enhancement, the laser intensity had to be reduced 15.6-fold while imaging the SIF p-Chip to avoid detector damage, and so the average count rate was scaled accordingly for comparison with the non-SIF p-Chip. We observed an 8.7-fold enhancement from the SIF-derived p-Chips over chips without SIF, which is consistent with previous findings [2].

FLIM images were recorded from both the SIF-polymer coated p-Chips from the IL-6 assay and from p-Chips covered with polymer only. The z-distance was adjusted for the brightest focal plane before the image was collected, and the reflection image was recorded at the same plane. The color seen in Figure 5 reflects the fluorescence lifetime. The results indicate that the lifetime was reduced from 0.62 ns in the polymer-only p-Chip to 0.36 ns in the polymer/SIF p-Chip. The sensitivity required for FLIM imaging necessitated the use of detectors that, when coupled with the electronics of our FLIM system, unfortunately had responses around 100 ps in FWHM, thereby making it impossible to resolve the full extent of the plasmonic effect on radiative decay. However, the system's temporal resolution was adequate for microscopic mapping of the enhancement, and we direct the reader to a previous study for more information regarding the radiative decay rates on SIF-enhanced p-Chips [2]. The 10-fold reduction was confirmed in the present study as well with a Fluotime 200 system from PicoQuant.

While the increase is well understood and has been quantified to a high degree of precision previously [2], the FLIM images show that the fluorescence enhancement is, surprisingly, relatively uniform across the p-Chip surface, both with and without the SIF layer. We had expected to see a pattern of "hot spots" following the morphology of the SIF where intensity and lifetime were dramatically different. The deposition of the SIF produces a very rough surface (Figures. 1, 3, and 5b), and enhancement hot spots were seen previously [40] and found to be correlated with morphological features. Instead, we found that the intensity and lifetime do not follow the morphology of the SIF and that there are no obvious "hot spots" present on the SIF surface. This uniform enhancement is presumably due to the dense packing of silver particles on the surface. The gaps between silver particles (Figure 1) are much smaller than the lateral resolution of the microscope system, which is around 300 nm. Therefore the diffraction-limited optics lead to a spreading out of the fluorescence across the p-Chip.

Fluctuations in the intensity of the reflection image (Figure 5) result from the varying height of the silver particles, as evidenced by Figure 3, so the depth of the fluorescence emission was investigated as well. While adjusting the focus, it was obvious that the depth of the

fluorescence signal extended over 1 μm . To quantify this thickness, the p-Chip was scanned in the X and Z directions (see Figure 6). The photon counts were averaged along the X axis and plotted as a function of the axial position. Assuming a Gaussian distribution, the full width at half maximum was $\sim 1.9 \mu\text{m}$. As a calibration of this approach, the same procedure performed on 20 nm fluorescent nanobeads showed that the axial resolution of our system in this configuration was $\sim 700 \text{ nm}$. Thus we may conclude that the thickness of the polymer was at least 1.2 μm . Even more interestingly, the enhancement from the SIF extended throughout this 1.2 μm layer. Given that the metal-enhanced fluorescence is limited in range to hundreds of angstroms, this evidence suggests that the polymer and dye molecules are wrapped around the SIF particles in a three-dimensional matrix above the surface of the p-Chip. This structure is very beneficial to the fluorescence enhancement because the three-dimensional morphology increases surface area, thereby maximizing the number of dye molecules that can bind to the chip and experience enhancement.

3.3. Raman measurements

While the polymer coating itself did not contribute appreciably to the fluorescent signal observed on the coated p-Chips, a Raman scattering effect introduced by the polymer would add complications to the type of simple bioassay for which the p-Chips are intended. Raman scattering occurs when the excitation light is inelastically scattered, and thus a weak, red-shifted signal is produced that may be confused with fluorescence in the absence of spectral decomposition. Raman scattering has been previously observed and studied in silane-based polymers [41]. The concern is that, when in contact with the SIF, the well-known surface-enhanced Raman scattering effect [42] would greatly increase the signal. Therefore Raman scattering measurements were conducted at several places on the back side of a SIF/polymer coated p-Chip to determine if such an effect would interfere in bioassay experiments; the data from an example spot is shown in Figure 7. The intensity data was measured in terms of the shift with respect to the 514 nm excitation source. The signal observed was not typical of Raman scattering—the signal was broad and corresponded to the emission from the fluorophore used in the assay. After several minutes, the fluorophore was bleached (bleaching is not seen in Raman scattering), and no signal remained. It may be noted that the pileup of intensity on the low end of the x-axis is due to the bandwidth of the intense 514 nm laser. Raman scattering would be independent of the assay performed on the p-Chip, thus we can conclude that it will not present any problems for broad use of the derivatized p-Chips.

4. Conclusions

In this study, we explored the morphology and radiative properties of our SIF and polymer coatings enveloping p-Chips. In SEM experiments we found that the SIF layer itself consisted of silver particles averaging 270 nm in diameter. Direct measurement of the polymer coating on top of the SIF via SEM was not feasible, as the high vacuum likely collapsed the polymer by the removing moisture. However, FLIM images showed that the enhancement was extended to a depth of 1.2 μm , thus the actual depth of the polymer layer must have been greater than or equal to this. This polymer depth was also supported by direct measurements of the thickness of several p-Chips in a stack.

We observed a significant, 8.7-fold plasmonic fluorescence enhancement as evidenced by the increased intensity and the decrease in lifetime. We expected that silver particles would create “hotspots” of plasmonic enhancement when an ELISA assay (IL-6 assay with Alexa Fluor 555) was performed. Therefore, the fluorescence intensity and lifetime were expected to vary greatly with the relief of the SIF layer. FLIM images showed that fluorescence enhancement was relatively uniform over the surface. All this lends evidence to the idea that the SIF/polymer layer exists as a three-dimensional matrix over the p-Chip surface. This matrix is accessible to solvent and allows fluorophores to surround the silver particles,

resulting in an increased number of dye molecules that are exposed to the plasmonic enhancement than would be the case if the polymer surface were flat and impenetrable to solvent and dyes.

This work has shown that a robust platform may be created for immunoassays. The polymer layer protects the silver island film from being scratched off, while together, the SIF and polymer layer create a volume accessible to a greater number of dye molecules for plasmonic enhancement. Future improvements of the SIF/polymer system on p-Chips may involve increasing the relief of the SIF layer or alterations to the layering process.

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Fig. 1.

SEM image of the backside of a p-Chip. The p-Chip was imaged with the electron beam perpendicular to the back side of the p-Chip. The accelerating voltage was 10.0 kV and the magnification was around 2,500 $\times$. Inset: A higher magnification image in which the accelerating voltage was increased to 30 kV. The particles shown are representative of the silver nanoparticles on the surface, i.e., not the large aggregates

Fig. 2.

Log-normal distribution of silver particle sizes in SIF. The data was collected by measuring the shortest axis of 216 of the irregularly shaped particles

Fig. 3. SEM of p-Chip cross-section. The SIF-coated p-Chip was cut in half with a razor blade, and the cross-section was imaged with SEM under 10.0 kV accelerating voltage and at 5000 $\times$ magnification

Fig. 4.

EDS mapping p-Chip cross-section. The area displayed corresponds to the area shown in Fig 3. The elements present in the sample emit x-rays corresponding to discrete energy transitions after the bombardment of electrons in SEM. The spectral line widths of these transitions are very narrow, and so the contribution from each element is easily separated. False-colors are added to distinguish between the wavelengths corresponding to particular elements: blue corresponds to Silicon, yellow corresponds to silver, red corresponds to carbon, and green corresponds to oxygen. The images show that the top layer of silver also contained oxygen and a small amount of carbon as well as silver. The carbon seen above the p-Chip results from the carbon tape used to fix the sample

Fig. 5.

FLIM and reflection images of a p-Chip (10 $\mu\text{m} \times 10 \mu\text{m}$). a) FLIM image (in false color) of a p-Chip coated with the SIF/polymer layer. The amplitude-averaged lifetime from this image was 0.36 ns and the average intensity was 268 cts/ms. This intensity average was scaled by a factor of 15.6 to account for the change in laser power. b) Reflection image of the p-Chip in Panel (a). Silver nanostructures can be seen. c) FLIM image (in false color) of a p-Chip coated with the polymer only. The amplitude-averaged lifetime from this image was 0.62 ns and the average intensity was 30.8 cts/ms. d) Reflection image of the p-Chip in Panel (c). The stripe pattern is due to backgrounding performed to reduce the thickness of the chip. A comparison of the results shown in Panels (a) and (c) indicates that the SIF coating reduces the lifetime. The lifetimes and intensities of both chips are relatively uniform across the chip, and the SIF/polymer coated chip does not exhibit intensity and lifetime modulation with the morphology of the silver particles seen in Panel (b). NOTE: Laser power differs between the panels due to a necessity to maintain the signal level in a proper range

Fig. 6. Left: Fluorescence image of the cross-section of a p-Chip. The p-Chip was coated with SIF and polymer; the area shown is $10\mu\text{m} \times 10\mu\text{m}$. The p-Chip was not physically cut but scanned along the x and z axes. This measurement was conducted using a 60 $\times$ water objective. Right: The graph depicts the average intensity along the x-axis as a function of axial position (open circles). The solid line represents the Gaussian fit to these data, whose FWHM is $1.9\mu\text{m}$

Fig. 7.

Raman spectra of SIF/polymer coated p-Chip. The signal seen at 0 min exposure is due to the emission of the fluorophore used in the assay. The fluorophore is bleached after 6 min exposure to the laser. The intensity data is measured and presented in terms of the shift with respect to the 514 nm excitation source. The pileup of intensity at low end of the x-axis is due to the bandwidth of the excitation