Aligned silver nanowires for plasmonically-enhanced fluorescence detection of photoactive proteins in wet and dry environment

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Abstract

We developed a method of aligning silver nanowires in a microchannel and fixing them to glass substrates via appropriate functionalization. The attachment of nanowires to the substrate is robust with no variation of their angles over minutes. Specific conjugation with photoactive proteins is observed using wide-field fluorescence imaging in real-time for highly concentrated protein solution, both in a microchannel and in a chip geometry. In the latter case we can detect the presence of the proteins in the dropcasted solution down to single proteins. The results point towards possible implementation of aligned silver nanowires as geometrically defined plasmonic fluorescence sensing platforms.

1. Introduction

In recent years application of metallic nanoparticles as building blocks of functional biosensing devices has gained considerable interest across various research disciplines. Metallic nanoparticles have been used for detecting proteins, molecules, and viruses [1-4] due to their unique physical and chemical properties, such as high thermal and chemical stability, as well as the presence of a plasmon resonance [5,6]. In particular, the plasmon resonance can enhance the optical response of analytes improving the signal-to-noise ratio, lowering thus detection limits and increasing sensitivity. Among metallic nanoparticles, silver nanowires (AgNWs) are particularly interesting in the context of sensing. First of all, their lengths reaching hundreds of micrometers facilitate straightforward localization of nanowires on a substrate. At the same time, the plasmon resonance of silver nanowires covers the whole visible range, reaching even

to near-IR. Last but not least, the nanowires have a comparatively large surface available for functionalization towards desired biorecognition [7,8].

Recently, the ability to correlate position of silver nanowires on a substrate with the fluorescence image, provided a way to follow biorecognition of bacteriophages down to single virus level [9]. On the other hand, for nanowires deposited randomly on a substrate, detection of fluorescence of single proteins was demonstrated [10]. In this case, the effect of plasmonic enhancement of fluorescence was utilized due to the short distance between the protein and the surface of silver nanowire [11]. However, also in this case it was necessary to correlate the fluorescence image with the transmission image. One of the methods devised to overcome this limitation concerns alignment of silver nanowires.

Assembly of silver nanowires on glass or plastic surfaces can be achieved with simple methods such as spray-coating, as the AgNW attaches to clean glass surfaces without any necessary surface treatment [12]. However, with this method the AgNWs are randomly oriented. For alignment of the nanowires on the surface, an early method was the use of the Langmuir-Blodgett technique to assemble monolayers of AgNWs on a Si-wafer [13]. More control of the positioning and density of the NWs is offered by the use of microfluidic deposition, where competition between shear forces on the nanowires from the flowing liquid and the electrostatic interaction between the wires and the substrate determines the level of alignment [14]. By aligning a film of nanowires on a substrate Xu and co-workers could create a coating with both optical and electrical anisotropy. By changing the flowrate, thereby controlling the ratio between shear force and surface attraction, they show that the order of the wires can change from basically random to well-aligned as the flowrate increases [14]. Using a system of hydrodynamic focussing, with a three-nozzle setup where the central sample flow is squeezed between two outer guiding flows Liu et al. could control both the position and the width of the deposited nanowire assembly [15]. With this method they could deposit nanowires onto the set of pre-patterned electrodes on the surface and measure the electrical properties of single nanowires [16,17].

In this work we apply microchannel technology to align silver nanowires and also to bind them specifically to the substrate without losing their biorecognition function. Such an approach yields perfectly aligned silver nanowires. Wide-field fluorescence imaging is used for studying attachment of photoactive proteins to such aligned silver nanowire, with the experiments being carried out both in a microchannel (in wet conditions) and in a chip geometry (in dry conditions). Both approaches yield clear biodetection activity of the substrates. In addition, in the second experimental architecture also detection of single proteins is demonstrated. This result indicates that we can design a universal and efficient real - time sensing platform with plasmonically active aligned silver nanowires.

2. Materials and Methods

Photoactive protein

For all experiments we used peridinin-chlorophyll-protein with streptavidin tag (PCP) photosynthetic complexes (BD-Biosciences). This photosynthetic complex was described in detail previously [8,18]. Relatively simple structure, excellent optical properties, and solubility in water render this protein a model system for studying interactions/conjugation in hybrid nanostructures.

Synthesis of silver nanowires

Synthesis of silver nanowires (AgNWs) was carried out using the polyol method, which yields AgNWs with diameters of approximately 100 nm and lengths up to 50 µm [19]. In the beginning, 5 ml of ethylene glycol (EG) was heated in a glass vial to 155 ˚C in an oil bath with continuous stirring. After 1 h, 40 µl of 4 mM copper (II) chloride dihydrate ($CuCl_2$ x H_2O) was added and the stirring speed was increased. Next, after 10 min, 3 ml of 94 mM silver nitrate ($AgNO_3$) and 3 ml of 114 mM polyvinylpyrrolidone (PVP) were added dropwise simultaneously by syringe pumps (speed 27 ml/h). To grow nanowires the mixture was left for 1 h at the same temperature and with constant stirring. The process of growing was stopped by cooling the glass vials in cold water. The mixture was washed by centrifugation, first in acetone and next, twice, in distilled water at 2000 rpm for 20 min. Finally, the sediment of AgNWs was dispersed in 4 ml of distilled water. The nanowires studied in this work are characterized with diameters of around 100 nm and lengths of tens of microns, both of which are typical values.

As long as their lengths are sufficient to visualize the nanowires on a surface and their diameters are small enough to facilitate the plasmon resonance, the two key requirements are achieved.

Surface modification of silver nanowires

In the next stage, the surface of AgNWs was modified with specific chemical groups. To chemically bind biotin the following procedure was used. 100 µl of AgNWs suspension was centrifuged at 2000 rpm for 20 min. The deposit was dispersed in 100 µl of ethanol (EtOH) and 100 µl of cysteamine (0.5 mM/EtOH) and kept at 4 ˚C for 2 h and then was centrifuged at 2000 rpm for 20 min. Next, the deposit was re-dispersed in 100 µl dimethyl sulfoxide (DMSO) and 100 µl biotin N-hydroxysuccinimide Ester (NHS-Biotin) (1 ul/ml DMSO). The mixture was left overnight at room temperature, and then was centrifuged at 6000 rpm for 20 min. The deposit was dispersed in 200 µl of distilled water.

Surface modification of glass coverslips by APTES

Glass coverslips were placed in a rack and cleaned by immersion in 2% Hellmanex aqueous solution in an ultrasonic bath at 35˚C for 30 min. This was followed by 15 min long rinsing in distilled water at the same temperature. Clean glass coverslips were attached to the polystyrene Petri dish and placed in a desiccator over two containers with (3-Aminopropyl)triethoxysilane (APTES) end triethylamine in 3 : 1 v/v ratio. The reaction took place under nitrogen atmosphere at room temperature for 2 h. Next, according to the procedure described in [20], the reagents were removed from the desiccator and the samples were left for 48 h under argon atmosphere for curing of the silane functional groups.

Surface modification of glass coverslips by OTMS

Glass coverslips were purified in an ultrasonic bath with acetone, then with dichloroethane for 10 min each, and in the end 3 times in distilled water (3 x 2 min). After that, glass coverslips were rinsed in a hot water bath (70 ˚C) for 1 h in mixture: water : Hydrogen peroxide (30%) : NH_2 (25%) in ratio 40:8:8. Next, glass coverslips were dried under a stream of argon and were placed in a laboratory oven at 100 ˚C for 4 h. Subsequently, glass coverslips were immersed in n-Octadecyltrimethoxysilane (OTMS, 98 %) diluted in toluene (2,4 : 57,6 v/v) in a beaker,

which was placed in a desiccator filled with silica gel for one week. Finally, the glass coverslips were washed with dichloroethane in an ultrasonic bath (20 min.) and dried with nitrogen.

Preparation of PDMS channels

The microchannels were created by standard soft lithography. A mold was fabricated by spin coating SU-8 2100 photoresist on a silicon wafer. The mold was irradiated with ultraviolet light through an overlaying mask to crosslink the SU-8 and subsequently the pattern was developed in an AZ 400 K bath. The dimensions of the microchannel mold were 1 mm wide, 100 um high, and 1.3 cm long.

The Elastomer Kit (184 Silicone) used in the experiment was bought from SYLGARD. In the first step, Silicone Elastomer Base (PDMS) was mixed with Elastomer Curing Agent in proportion 10 : 1 and put in a vacuum desiccator to remove air bubbles. The silicon wafer with the mold was placed in a Petri dish or a 3D-printed PLA platform and then were poured by PDMS mixture, and they were placed in a laboratory oven at 65 ˚C for 90 min. After that, PDMS on Petri dishes was cut to the size of glass coverslips (around 24 mm). Next, the surface of PDMS and glass coverslips was activated in plasma ozone cleaner for 1 min and stuck together (A picture is included in Supplementary Materials).

Sample preparation

The microfluidic channel was put on the amine-modified glass [19,21]. Next, streptavidin (10 µl/980 µl phosphate buffered saline (PBS)) activated by N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) for 10 min (40 mg/ml PBS) was injected into the channel and left for 90 min. After that, channel was washed with 1 ml of PBS. Immediately afterwards, the microfluidic device was placed on wide field fluorescence microscope sample holder and filled with biotin-modified AgNWs via a syringe pump (200 µl / min.) (Harvard Apparatus, Pump 11 Elite). Non-bounded AgNWs were washed with PBS. In the end, peridinin-chlorophyll-protein complexes tagged with streptavidin (0,002 mg/ml) were injected by a syringe pump and the interaction between biotin-modified AgNWs and PCP-streptavidin was monitored in real-time. PCP, which was not conjugated to the AgNWs was washed with PBS.

For experiments on a chip, OTMS-modified glass was used. PDMS channel was used for AgNWs alignment by injection of 100 µl of biotin-modified AgNWs via a syringe pump (250 µl/min.). Next, non-bounded AgNWs were washed out with distilled water. Then, PDMS channel was removed and this device was ready for real-time fluorescence investigations of conjugation between biotin-modified AgNWs and PCP-streptavidin using wide field fluorescence microscopy.

Optical measurements

Wide Field Fluorescence Microscopy used in this experiment enables measurement of transmission and fluorescence intensity kinetics, i.e: recording movies in real-time. The microscope is based on the Nikon Ti-U inverted body. Excitation wavelength was 480 nm provided by LED illuminator (Mic LED, Prizmatix Ltd., Givat-Shmuel, Israel). The power of 100 µW (chip experiment) and 180 µW (channel experiment) was used. The excitation beam was reflected from a dichroic mirror (T650 LPXR, Chroma) to the microscope objective. In the chip experiment we used an oil-immersion objective (Nikon Plan Apo 100x, NA 1.4), while in the experiments carried out in a microchannel, we used an air objective (Nikon L Plan 100x, NA 0.7) in order to reduce any influence of glass movements while injecting solutions. Emission filters 670/10 (channel experiment) or 675/20 (Chroma) (chip experiment) and FELH650 (Thorlabs, Newton, USA), were placed in front of the detector. Fluorescence was detected using a sensitive EMCCD camera (iXon3, Andor, Abingdon, GB), which allows for improving the signal-to-noise ratio with Electron Multiplication gain (EM gain). In the channel experiment, the parameters were EM gain equal to 500 with the acquisition time of 0,1 s. In the chip experiment for highly concentrated PCP solution, the EM gain and acquisition time of 100 and 0,1 s. were used, respectively, while for highly diluted PCP solution, the EM gain was 800.

3. Results

3.1. Aligning silver nanowires

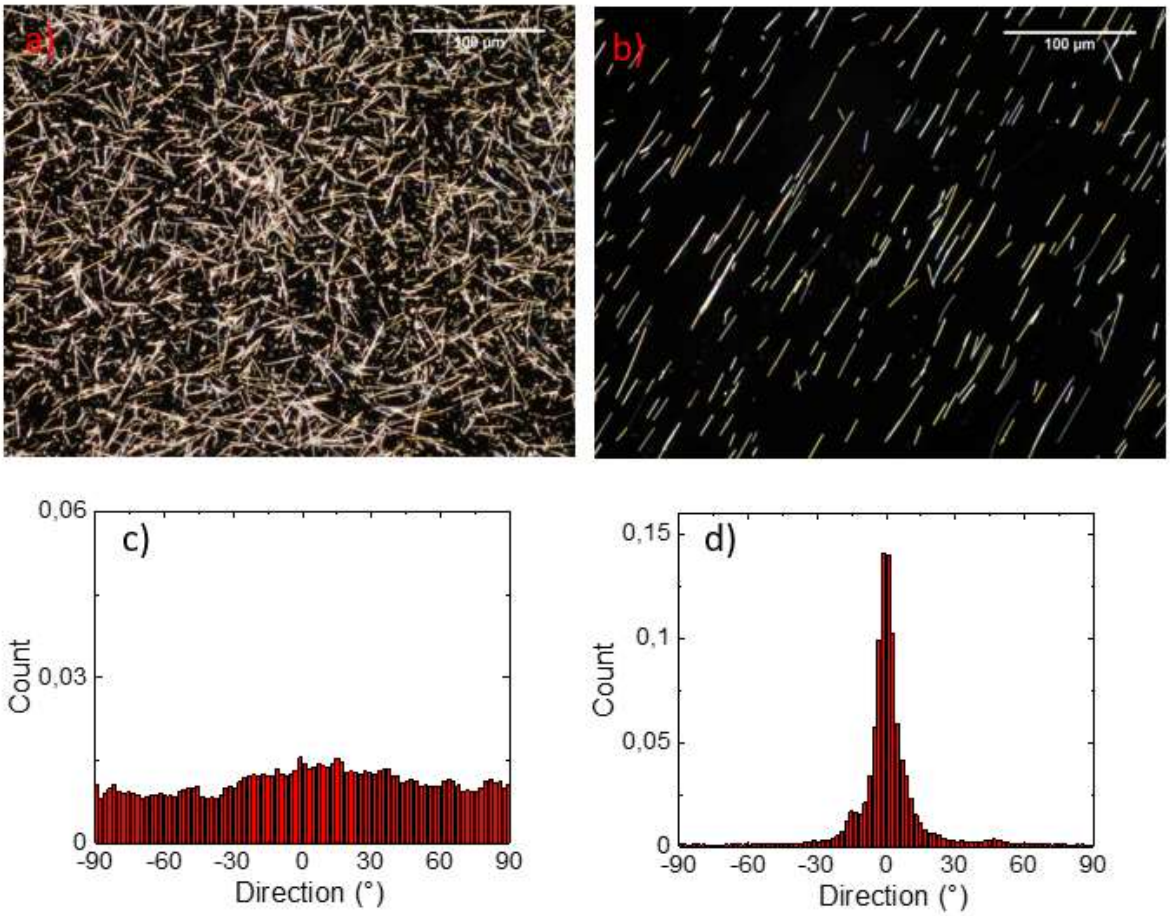

Figure 1. Dark-field optical microscopy image of randomly oriented AgNWs (a) compared with the sample, where AgNWs were oriented in a microchannel (b). Histograms displayed in (c) and (d) correspond to analysis of angle distribution of AgNWs alignment, as estimated from the images obtained for the respective substrates.

Suitability of introducing a microchannel for aligning silver nanowires on a glass substrate is displayed in Figure 1, where we compare dark-field microscopy images of substrates, where a microchannel was used to inject silver nanowires and a substrate where silver nanowires were deposited in a random way, i.e.: by drop-casting the solution. The images are accompanied with statistical analysis of the angle distribution of the nanowire position. The movie of depositing nanowires in a microchannel is included in Supplementary Material. It can be seen in this movie that over a microscopic region the injected nanowires are attaching to the surface, making the overall coverage denser and denser. While in the case of drop-casted sample the direction of nanowires is purely random, with no dominant alignment axis, application of a microchannel yields almost perfect alignment of silver nanowires on a substrate. The maximum of the angle

distribution is clearly visible and any other angles are just a minute contribution. Successful alignment of silver nanowires on a substrate is the first step in fabrication of a sensor chip, which utilizes the fluorescence enhancement associated with plasmon excitations in the nanowires.

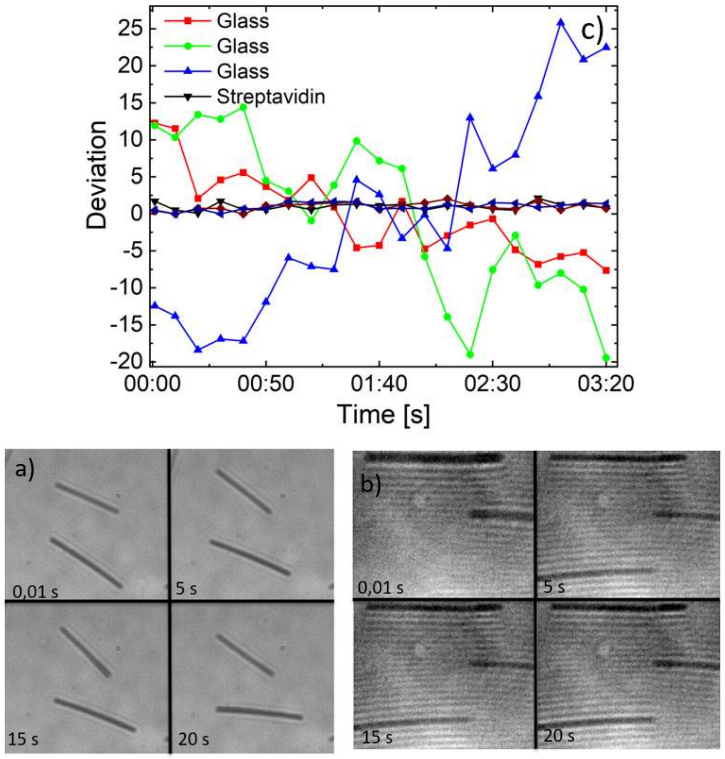

Figure 2. Snapshots of images obtained in a transmission mode acquired at given time intervals for a substrate, where silver nanowires were injected into a microchannel without glass substrate functionalization (a) and with the glass substrate being functionalized with streptavidin (b). Temporal evolution over 300 s of the angle of single AgNW alignment measured for three typical nanowires for each substrate is shown in (c).

A critical aspect in implementing such an approach for sensing concerns the influence of surface functionalization of the glass substrate in the microchannel on the properties of the nanowires, including their attachment and stability. In particular for longer detection times the nanowires should be robustly fixed to the surface preventing any movement, such as rotation or translation. In order to test the quality of nanowire attachment to a glass substrate, we monitored temporal variation of the angle of single nanowires in two samples. In the first case, the glass substrate

was functionalized with streptavidin before injecting biotin-functionalized silver nanowires into the microchannel. This result was compared against a sample where the glass substrate was not functionalized. In Fig. 2 we display snapshots of images taken at given time intervals of single nanowires in these two microchannel devices. The corresponding movies are included in the Supplementary Material. For the sample, where the substrate was functionalized with streptavidin, both the position and the orientation of the silver nanowires is constant, there is no variation of any parameter over time of several minutes. It is confirmed by the results shown in Fig. 2c, where the orientation of three typical silver nanowires are plotted (black points). Indeed, we find no deviation of the angle over several minutes. The situation is radically different for the sample, where no substrate functionalization was applied (Fig. 2a). As can be seen, while the positions of the nanowires in the microchannel are more or less constant over time, their orientation shows considerable variation, which means that the physisorption is less robust. In fact, the variation amounts to 40 degrees over the time of several minutes, which effectively diminishes applicability of such a geometry as a reliable sensing platform. We note that in both cases, there was no flow of nanowire solution applied during the measurement.

3.2. Fluorescence imaging in microchannel

The first round of experiments was focused on fluorescence imaging of proteins specifically conjugated to silver nanowires in a microchannel. Upon attaching the nanowires to the glass substrate, the microchannel was filled with the buffer solution before injecting the solution of photoactive proteins. The PCP complexes were tagged with streptavidin, facilitating specific attachment to biotin-coated silver nanowires. Upon injection of the protein solution a preselected area of the sample was imaged continuously by acquiring a movie (Supplementary Material). In Figure 3a we show a snapshot of the microchannel with silver nanowires upon injecting the solution of PCP proteins. The image was measured after 35 seconds from the injection. Importantly, the positions of the silver nanowires are clearly visible as bright vertical lines placed on the background of the fluorescence of proteins in solution. Stronger fluorescence emission of PCP complexes upon conjugation with silver nanowires can be indicative of metal-enhanced fluorescence due to plasmon excitations in the metallic nanostructures [22,23]. However, regardless of the metal-enhanced fluorescence effect, the contrast with the background is rather weak due to the presence of the protein solution in the microchannel. This can be quantified by extracting a cross-section along the line marked by black in Fig. 3a, shown in Fig. 3c. The actual signal-to-noise ratio is approximately 0.5 for the four nanowires studied.

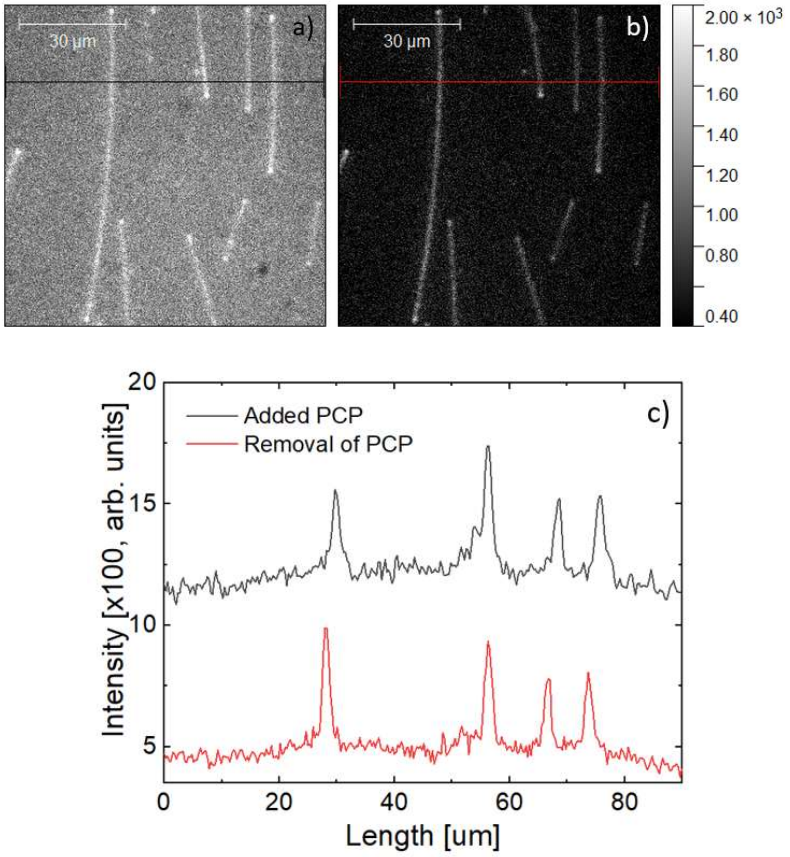

Figure 3. Fluorescence intensity maps of PCP conjugated with oriented modified AgNWs in a PDMS channel: snapshots of the same sample area after injection of PCP solution (a) and upon removal of PCP solution (b) are compared. (c) Cross-sections of fluorescence intensity across selected four AgNWs after injecting (black) and removing (red) the PCP solution.

The signal-to-noise ratio can be improved by purging the microchannel with the buffer solution. In this way, non-bonded proteins in the solution are removed and the background emission is substantially reduced. The resulting image of the same area is displayed in Fig. 3b with the corresponding cross-section. After removing the protein solution the signal-to-noise ratio is improved by a factor of 2 and it is equal to 1. Future work will focus on obtaining single protein sensitivity in such a sensor geometry. Robust attachment of silver nanowires, which inhibits any reconfiguration of the nanowires upon injecting protein or buffer solution, together with real-time fluorescence imaging provide a solid framework for devising and performing such experiments in the future.

3.3. Fluorescence imaging in a chip geometry

An important extension of the results obtained for AgNWs aligned in a microchannel in conditions, where the protein solution was injected to the microchannel, concerns applicability of such substrates as dry detection chips. This is critical as upon removing the PDMS channel the nanowires dry out rather quickly, which may result in diminishing their capability to capture proteins. In Fig. 4 we present a sequence of images obtained for aligned nanowires after removing the PDMS channel. As a result, the access to the surface is easier, however the risk is – as pointed out already – that upon drying of the nanowire the sensing functionality is lost. The images were taken before depositing of the droplet of the protein solution as well as at given time intervals after protein solution deposition. First of all, the nanowires are nicely aligned, as expected from the fabrication method. At the moment the protein solution is deposited on the chip the nanowires can be distinguished as dark lines in the background of protein fluorescence in the droplet. As time progresses, the situation channels qualitatively. Namely, the nanowires – upon conjugation with proteins – start to glow, and the fluorescence intensity is considerably stronger than that of the background. After 90 sec, the image is very clear with bright elongated shapes containing proteins conjugated to silver nanowires.

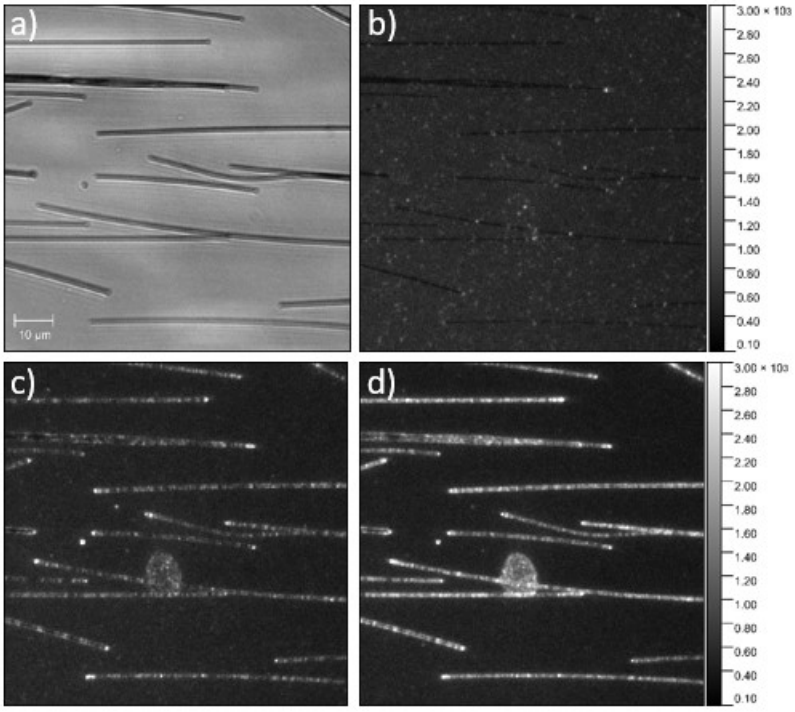

Figure 4. (a) Transmission image of aligned silver nanowires accompanied with fluorescence intensity maps of PCP (0.2 µg ml^{-1}) on a chip at several time intervals of 0 s (b), 20 s (c), and 90 s (d) from drop-casting the protein solution.

The difference in temporal dependence of fluorescence intensity between areas containing silver nanowires and those of bare glass substrate is quantified in Fig. 5, where a cross-section along the selected AgNW is shown as a function of time. The bright vertical stripes correspond to fluorescence of proteins that conjugate to the nanowire, and, as can be seen, the intensity attributed to the end of the nanowire is considerably stronger than along the nanowire. The cross-sections of fluorescence intensity are plotted in Fig. 5b. They feature saturation behavior of the intensity, perhaps indicating that almost the whole area of the nanowire probed in the experiment is fully coated with specifically attached protein. At the same time, the intensity profile extracted from the region free of silver nanowires features first an increase of the intensity (ascribed to deposition of the droplet of protein solution) and then the intensity is reduced as the amount of the protein in the background decreases at the expense of proteins that are conjugated to silver nanowires. Analogous conclusion can be drawn from the cross-sections displayed in Fig. 5c, which were obtained along the distance. Indeed, the buildup of the intensity on the silver nanowire is clearly visible and distinguishable from the region of the bare glass substrate. We note that for this highly concentrated protein solution the contrast is almost an order of magnitude. Such a dramatic difference in fluorescence intensity allows to test the feasibility of this approach for detection of single proteins [10].

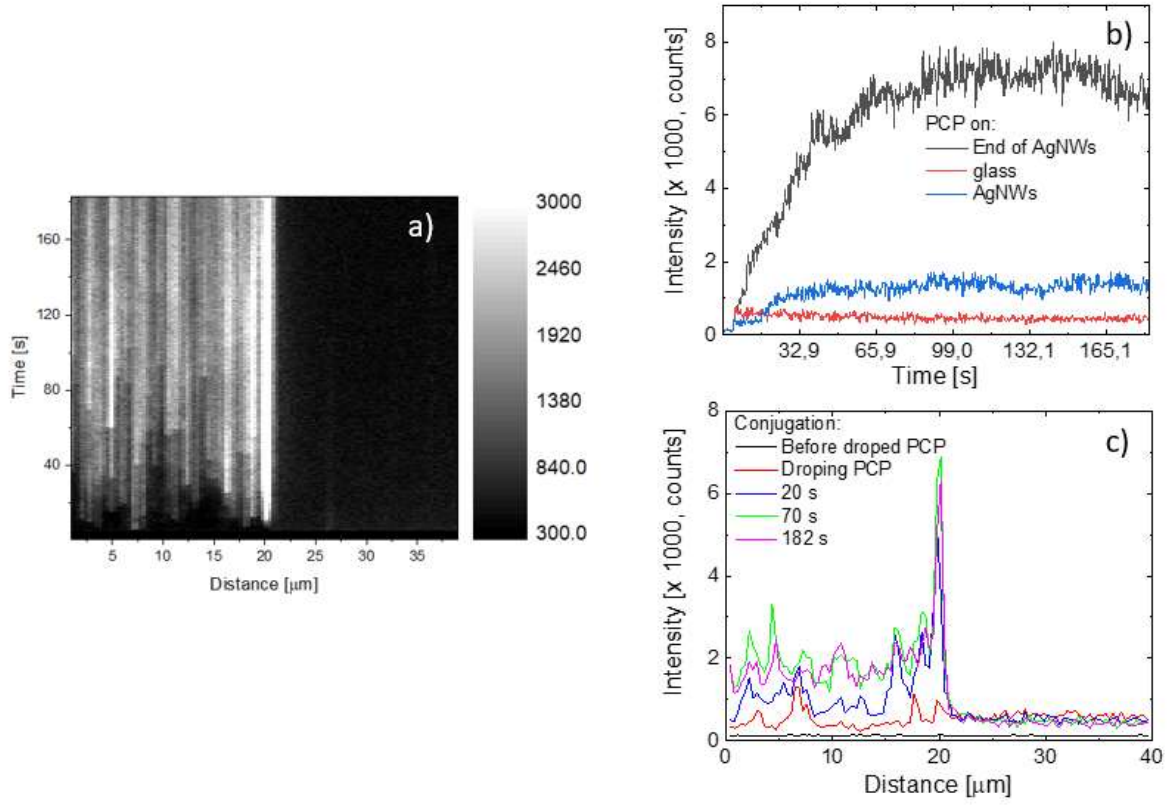

Figure 5. (a) Transient behavior of fluorescence intensity of PCP (0.2 μg ml^{-1}) measured for a single nanowire on a chip, (b) Time-traces of PCP fluorescence intensity extracted for selected positions on the silver nanowire (end and middle) and on the glass substrate, (c) Fluorescence intensity cross-sections along the silver nanowire obtained for selected times.

The result of this experiment is displayed in Fig. 6. The concentration of proteins in the solution, which was dropcasted on the previously aligned silver nanowires, was over two orders of magnitude less compared to the previous measurement. In this case we observe individual emission spots located on silver nanowires, however the alignment of the nanowires allows for assignment of the emission spots to proteins indeed conjugated to the nanowires. Based on the previous experiments [10], we attribute these spots to fluorescence of single proteins attached specifically to silver nanowires. While this is the proof-of-concept attempt, it shows that alignment of the nanowires combined with better spatial control of their position can be considered as an attractive method for enhancing the efficiency of fluorescence detection. Indeed, even without any prior knowledge about the actual positions of the silver nanowires on the substrate, the information that they are aligned in the horizontal direction is sufficient for unambiguous interpretation of the observed emission spots as related to proteins conjugated to silver nanowires.

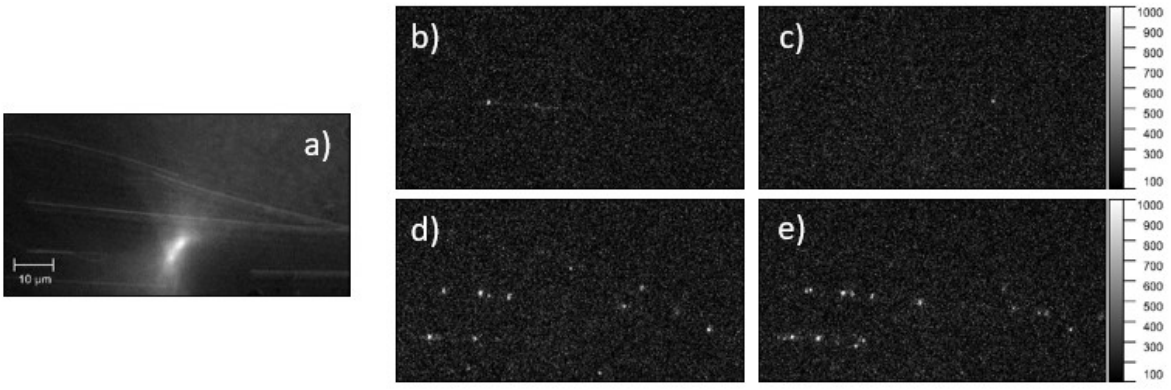

Figure 6. Fluorescence intensity map of highly diluted PCP solution upon drop-casting on a chip with aligned silver nanowires: (a) Transmission image and fluorescence images before drop-casting PCP solution (b), at the time of drop-casting (c), and after 20 (d) and 70 seconds (e) from the drop-casting.

Conclusions

We demonstrate and describe a method of aligning silver nanowires in a microchannel and fixing them to glass substrates via appropriate functionalization. The attachment of nanowires to the substrate is robust with no variation of their angles over minutes. Specific conjugation with photoactive proteins is observed using wide-field fluorescence imaging in real-time for highly concentrated protein solution, both in a microchannel and in a chip geometry. In the latter case we can detect the presence of the proteins in the dropcasted solution down to single proteins. The results point towards possible implementation of aligned silver nanowires as geometrically defined plasmonic fluorescence sensing platforms.

Acknowledgements

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References

1. M. Bauch et al, Plasmon-enhanced fluorescence biosensors: a review, Plasmonics (2014), 9, 781-799

2. M. Amiri et al, Electrochemical methodologies for the detection of pathogens, ASC Sens. (2018), 3, 1069-1086

3. J. N. Anker, et al, Biosensing with plasmonic nanosensors, Nature Materials (2008), 7, 442-453

4. Y. Nasu, Y. Shen, L. Kramer, R. E. Campbell, Structure and mechanism guided design of single fluorescent protein-based biosensors, Nature chemical biology (2021), 17, 509-518

5. P. Tan, L. HeSheng, J. Wang, S. C.B. Gopinath, Sliver nanoparticle in biosensor and bioimaging: Clinical perspectives, Biotechnology and applied biochemistry (2020),1-7

6. K. McNamara, S. A. M. Tofail, Nanoparticles in biomedical applications, Advances in Physics: X, (2017), 2(1), 54-88

7. Y. Joo et al, Silver nanowire-embedded PDMS with a multiscale structure for highly sensitive and robust flexible pressure sensor, Nanoscale (2015), 7, 6208

8. Y. Sun et al, Uniform silver nanowires synthesis by reducing AgNO3 with ethylene glycol in the presence of seeds and Poly (Vinyl Pyrrolidone), Chem. Mater. (2002), 14, 4736-4745

9. J. Grzelak et al, Capturing fluorescing viruses with silver nanowires, Sensors and Actuators B: Chemical (2018), 273, 689-695

10. K. Sulowska et al, Real-time fluorescence sensing of single photoactive proteins using silver nanowires, Methods Appl. Fluoresc. (2020), 8, 045004

11. E. A. Coronado, E. R. Encina, F. D. Stefani, Optical properties of metallic nanoparticles: manipulating light, heat and forces at the nanoscale, Nanoscale (2011), 3, 4042

12. A.R. Madaria, A. Kumar, C. Zhou, Large scale, highly conductive and patterned transparent films of silver nanowires on arbitrary substrates and their application in touch screens, Nanotechnology. 22 (2011) 245201. https://doi.org/10.1088/0957-4484/22/24/245201.

13. A. Tao, F. Kim, C. Hess, J. Goldberger, R. He, Y. Sun, Y. Xia, P. Yang, Langmuir−Blodgett Silver Nanowire Monolayers for Molecular Sensing Using Surface-Enhanced Raman Spectroscopy, Nano Letters. 3 (2003) 1229–1233. https://doi.org/10.1021/nl0344209.

14. Y. Xu, D. Ge, G.A. Calderon-Ortiz, A.L. Exarhos, C. Bretz, A. Alsayed, D. Kurz, J.M. Kikkawa, R. Dreyfus, S. Yang, A.G. Yodh, Highly conductive and transparent coatings from flow-aligned silver nanowires with large electrical and optical anisotropy, Nanoscale. 12 (2020) 6438–6448. https://doi.org/10.1039/C9NR09598E.

15. M. Liu, Y. Chen, Q. Guo, R. Li, X. Sun, J. Yang, Controllable positioning and alignment of silver nanowires by tunable hydrodynamic focusing, Nanotechnology. 22 (2011) 125302. https://doi.org/10.1088/0957-4484/22/12/125302.

16. M. Liu, Y. Chen, Q. Guo, R. Li, X. Sun, J. Yang, An Economic Method for Large-Scale Patterning and Electric Measurement of Nanowires, Journal of Nanoelectronics and Optoelectronics. 6 (2011) 144–151. https://doi.org/10.1166/jno.2011.1153.

17. M. Liu, Y. Peng, Q.Q. Guo, J. Luo, J. Yang, Large-Scale Patterning and Electric Test of Silver Nanowires by Microfluidic Hydrodynamic Focusing, Advanced Materials Research. 148–149 (2010) 1310–1314. https://doi.org/10.4028/www.scientific.net/AMR.148-149.1310.

18. M. Olejnik et al. Imaging of fluorescence enhancement in photosynthetic complex coupled to silver nanowires, Appl. Phys. Lett (2013), 102(8), 08703

19. M. Szalkowski et al, Wide-Field fluorescence microscopy of real-time bioconjugation sensing, Sensors (2018), 18(1), 290

20. A. Ebner, P. Hinterdorfer, H. J. Gruber, Comparison of different aminofunctionalization strategies for attachment of single antibodies to AFM cantilevers, Ultramicroscopy 2007, 107, 922-927

21. M. Focsan et al, A simple and efficient design to improve the detection of biotin-streptavidin interaction with plasmonic nanobiosensors, Biosensors and Bioelectronics, (2016), 86, 728-735

22. Y. Fu, J. Zhang, J. R. Lakowicz, Plasmonic enhancement of single-molecule fluorescence near a silver nanoparticle, J. Fluorescence (2007), 17(6), 811-816P.

23. P. Anger, P. Bahardwaj, L. Novotny, Enhancement and quenching of single-molecule fluorescence, PRL (2006), 96, 113002 J. Li et al, Spectral variation of fluorescence lifetime near single metal nanoparticles, Scientific Reports (2016), 6, 21349