Electronic Supplementary Information (ESI):

Cost-effective Smart Microfluidic Device With Immobilized Silver Nano-particles and Embedded UV Light Sources for Synergistic Water Disinfection Effect

1. Synthesis of Silver Nanoparticles:

Colloidal silver is made by adding an excess of the reducing agent sodium borohydride, $NaBH_4$ to silver nitrate, $AgNO_3$.

The procedure followed for the experiment:

- Glassware was cleaned by soaking in an alcoholic KOH bath.
- Two solutions were prepared -
  - 0.0010 M $AgNO_3$ (aq.) and 0.0020 M $NaBH_4$ (aq.)
- Using a graduated cylinder, 30 mL 0.0020 M sodium borohydride was poured into a 250 mL Erlenmeyer flask. The Erlenmeyer was placed into an ice bath and allowed to cool for about 20 minutes.
- A stir bar was placed in the Erlenmeyer, and the assembly was centred on a stir plate and stirring was begun.
- 10 ml 0.0010 M $AgNO_3$ solution was added drop wise, about 1 drop/second to $NaBH_4$.
- The product was clear yellow once the reaction got completed.

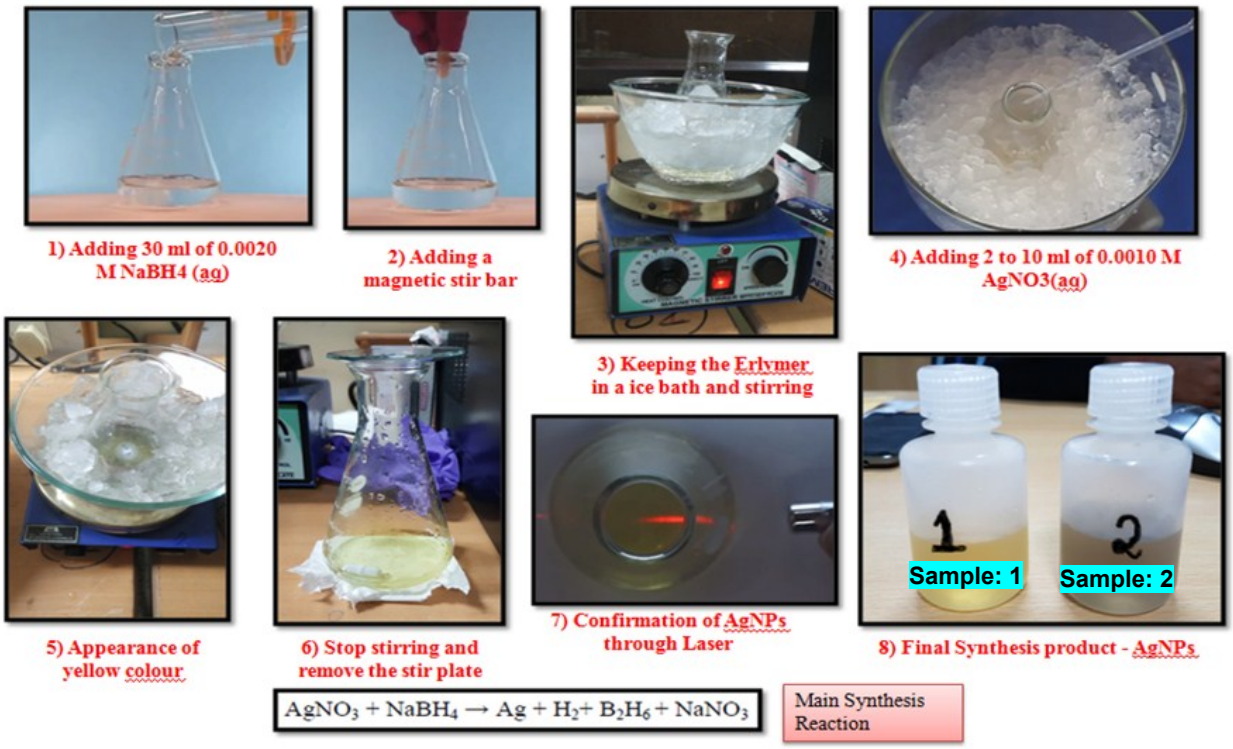


Figure S-1. Synthesis Process of silver nanoparticles.

The brown colour *sample no.:-2* in *Figure S-1*, actually shows the aggregated AgNPs, which appeared during one of our experiments for testing the stability of the freshly prepared AgNPs colloidal suspension. In this experimental process, a small amount of freshly prepared yellow AgNPs suspension (*sample no.:-1 in figure S-1*) taken into a clean test tube, and was further mixed with few

drops of 1.5M NaCl solution. This process caused AgNPs nanoparticles (*sample no.:-1 in figure S-1*) to aggregate and turned their darker yellow suspension to orangish grey (*sample no.:-2 in figure S-1*). Similarly, a small amount of yellow colloidal silver nanoparticle solution taken in another clean test tube was added with 0.3% polyvinyl pyrrolidone solution (PVP). Subsequently, on adding few drops of 1.5M NaCl solution into yellow colloidal silver did not turn them into orangish grey.

Theoretically, the Ag nanoparticles which are formed after the standard synthesis process are stabilized by a protective layer of borohydride ions; however, the salts such as NaCl or KI shield the negative charges allowing the particles to clump together to form aggregates. Further, to prevent the aforesaid aggregation the nanoparticles are coated with a polymer such as polyvinyl pyrrolidone (PVP), which inhibits aggregation and stabilizes the colloidal silver even when salt is added.

1.1. Characterization of colloidal silver nanoparticles:

In the aforesaid synthesis process, 10 ml of 10^{-3} M AgNO3 was reduced with 30 ml of 2 x 10^{-3} M $NaBH_4$. Total silver (At. wt. 107.8) content of the reaction volume would be 1.078 x 10^{-3} g in 40 ml which is equivalent to 26.95 µg/ml.

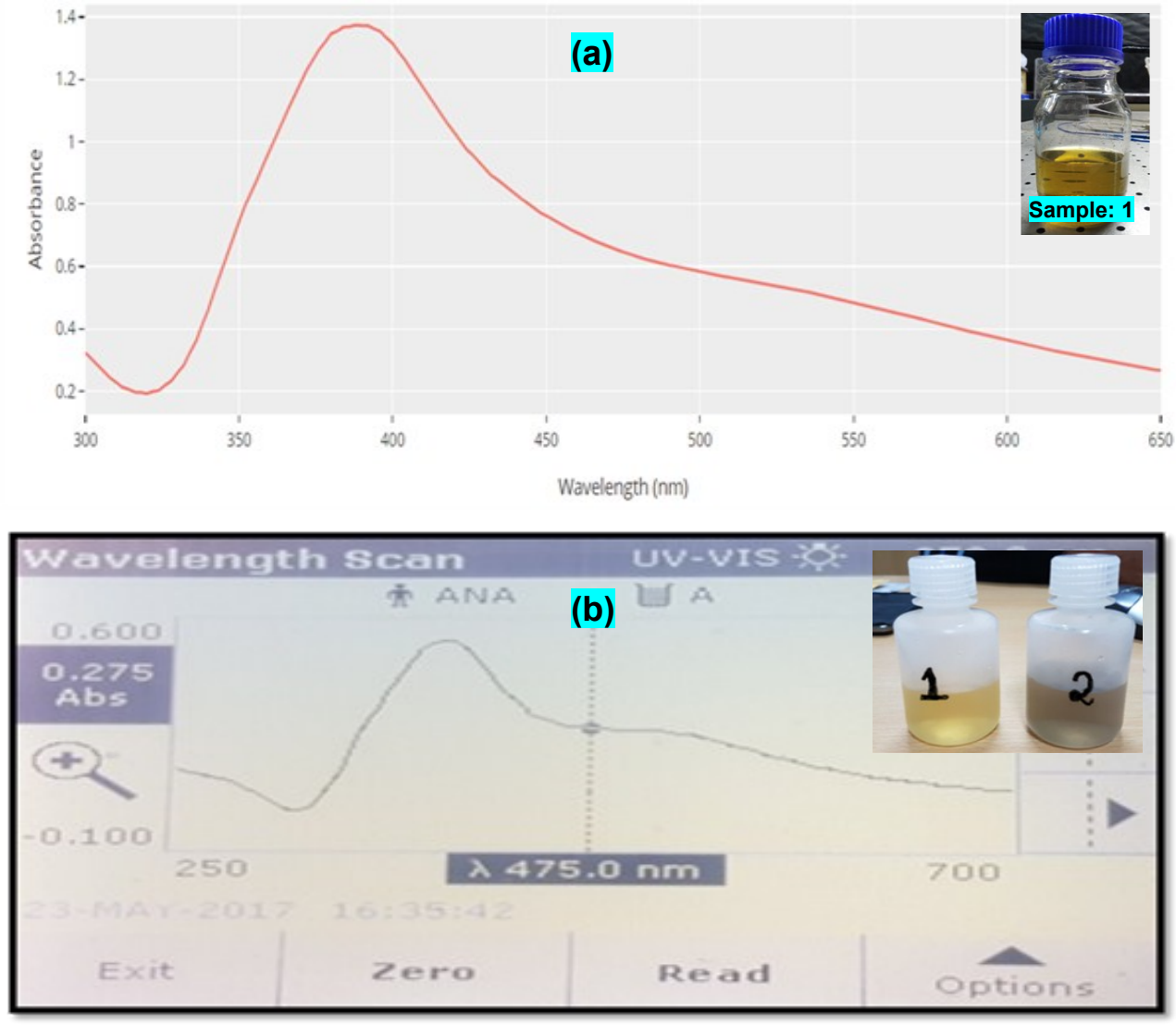


Figure S-2. UV-Vis Spectrum of AgNPs synthesized in lab

1.1.1. Optical properties/ UV-Vis Spectroscopy:

Studies report the appearance in the electronic absorption spectrum of a band located at 396 nm, associated with the presence of small spherical silver nanoparticles. The optical properties of colloidal silver nanoparticle solution was determined in a UV-Vis spectrophotometer. The spectra recorded are presented in figure S-2.

1.1.2. Particle Size Analysis (PSA) Results:

The NANO-flex® 180° DLS System (Figure S-3), which measures size distributions in the range of 0.3 nm to 10 µm, was used for the particle size analysis of silver nano-particles synthesized in abovementioned process. The particle size as observed in the system came out to be between 20.83 nm to 42.10 nm (Figure S-4), which is in accordance with the size of the AgNPs given in literature. The particle size distribution and summary table auto-generated by the aforementioned DLS-system is shown in the figure S-3(b).

Several batches of AgNPs suspension were recovered after the synthesis of AgNPs, using the standard protocol [14]. Sometimes, due to unfavourable synthesis/storage condition, high polydisperisity of the AgNPs sample appeared, which is evident from the UV-VIS spectroscopy and

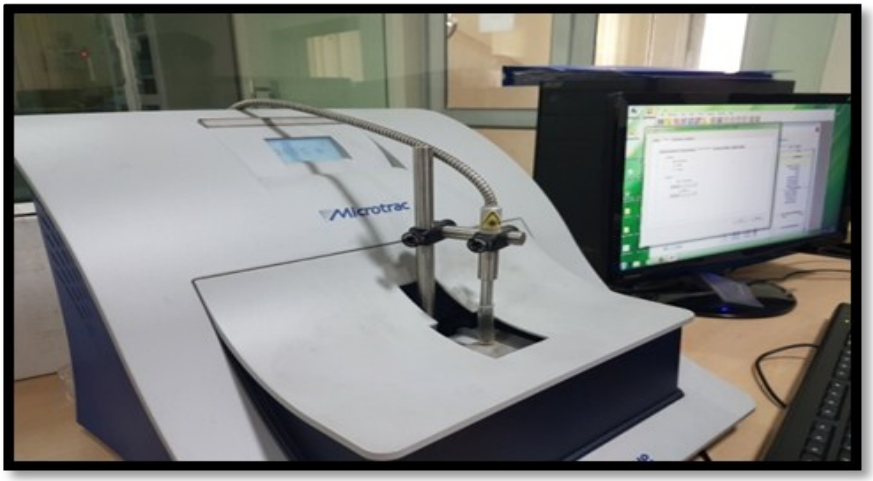


Figure S-3 (a). Nanotrac Flex DLS System

Summary	
Data	Value
MI(nm):	224.8
MN(nm):	20.83
MA(nm):	42.10
CS:	142.4
SD:	30.90
PDI:	84.76
Mz:	57.22
si:	28.42
Ski:	-193.08042
Kg:	729.9

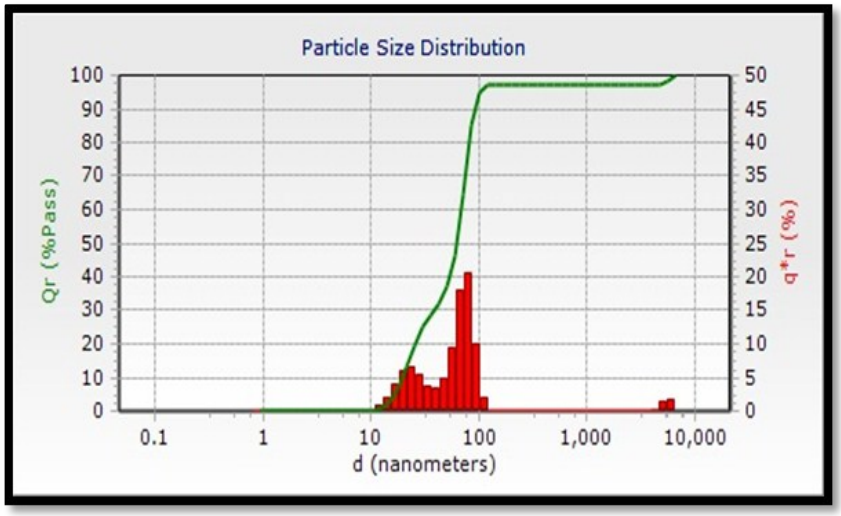


Figure S-3 (b). PSA Result of the aforesaid synthesized silver nanoparticles, obtained from Nanotrac Flex DLS System

[i.e. multiple peak at around 396 nm and 500 nm in figure: S-2(b)] DLS results (Figure S-3). However, under the favourable synthesis/storage condition of AgNPs preparation, the graph revealed only one peak at around 396 nm and no other elongated peaks, (figure: S-2(a)).

Polydispersity index (PDI) or heterogeneity index is used to describe the degree of non-uniformity of a size distribution of particles. In Figure S-3(b), the PDI value is about 85% (i.e. PDI = 0.85) which indicate the high dispersion of the sample. In general, this number expected to be *less than one* to specify a mono dispersion of NPs population, owing to the followed standard AgNPs synthesis methods [14]. The automatic data generated from DLS-analysis via Nanotrac Flex DLS System (figure: S-3 (a&b)), indicates MN = 20.83 nm, i.e. the Mean Diameter of the Number distribution (which is calculated using the volume distribution data and is weighted to the small particles). This type of average particle size is related to the population. Additionally, the automatic data generated from DLS-analysis (Figure: S-3 (b)), also indicates: MA = 42.10 nm, i.e. the Mean Diameter, of the Area distribution (which is calculated from the volume distribution). This Mean diameter represents a particle surface measurement. Hence, the automated generated DLS data suggest that AgNPs size-range is expected to be in between 20.83 and 42.10 nm. The higher value of PDI (i.e. 85 percent) is possibly due to aggregation under the favourable synthesis/storage condition of AgNPs preparation. The TEM images of the synthesized Silver-nanoparticles *(Figure S-4)* corroborates with the aforesaid results.

Under the appropriate synthesis/storage condition of AgNPs preparation (figure: S-4), the graph revealed only one standard peak at around 396 nm, without any other elongated peaks, (figure: S-2(a) & Figure 2(e)). Moreover, as disinfection process discussed in our present reported study were mostly dependent on compactly immobilised AgNPs (inside the microcannel surface); hence, in condition of any possible variation from the uniformity of nano-particle size (i.e. either in situation-

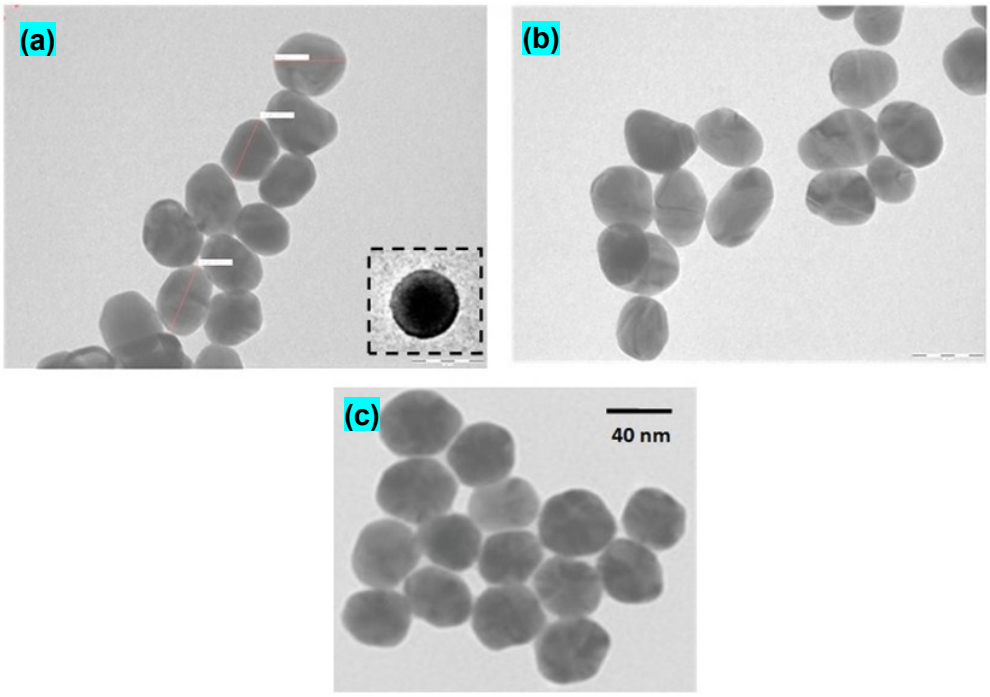


Figure S-4. Showing the TEM images of the (a) Synthesised Silver Nanoparticles, and (b)&(c) standard Sigma-Aldrich AgNPs; used for the fabrication of microdevices; *scale bar for all images: 40 nm.*

of AgNPs poly-disperisity, or its uniform size), any probable variation in the disinfection process has not been observed, experimentally. Moreover, we were greatly intended with the issue of compact immobilisation of AgNPs over microchannel surfaces; which was achieved, via keeping the AgNPs nanoparticle suspension in contact with the silanized microchannel surface for a sufficiently higher time-period.

Further, considering the abovementioned concerns over high value of PDI, we also used a standard Silver nanoparticles (AgNPs) dispersion (figure: S-4) of 40 nm particle size (TEM), purchased from Sigma Aldrich (Product Number: 730807), for the fabrication of our few standard microdevice. Additionally, following the aforesaid AgNPs immobilisation process, a very similar disinfection capability of the microdevice was realised. It also effectively approved our theoretical assumptions, that either polydispersity or uniform nano-particle size does not highly affect the device efficiency if the given AgNPs are compactly immobilised inside the device.

2. AgNPs Immobilization Protocol:

Although surface modification by oxygen plasma treatment is a popular technique, wet chemical treatment of PDMS surface by piranha solution is an inexpensive alternative approach. However, the former gives a better result than the latter in terms of hydrophilicity of the PDMS surface. Thus, to improve the hydrophilicity, stability and hydrophobic saturation level of the PDMS surface, a new approach of two-step wet chemical treatment involving piranha solution and KOH solution was performed. This PDMS surface activation processes mostly involves cleavage of the non-polar hydrophobic methyl (–CH3) group of the siloxane polymer chain and oxidation of the cleaved sites to polar hydrophilic silanol (Si–OH) groups, resulting in considerable increase of the surface energy of the polymer, thereby rendering it hydrophilic.

In general, after making PDMS microchannels hydrophilic, via the piranha solution and KOH solution treatments,–OH groups appears on the PDMS surface; which were later functionalised with the help of silanization process. Self Assembled Monolayers created by using organosilane, form intermediate silanol groups during the reaction which then react with the hydroxyl groups present on the surface of PDMS microchannel. It thus results in covalently immobilizng the organosilane (Figure S-5). During the silanization process the reactive end of the organosilane bonds to the –OH group of PDMS microchannel surface, while the tail end becomes the dominant surface chemical species. The organosilane used for silanization purpose in the current device is APTES ((3-Aminopropyl) triethoxysilane), which introduces amine functional groups onto the surface. Once the microchannels become functionalised, they can be easily immobilized with silver nanoparticles for disinfection studies (Figure S-6), as the negatively charged AgNPs could interact electrostatically with the silanized PDMS surface.

Particularly, for the aforesaid silanization process, 2% v/v APTES solution was prepared in 5:2 ethanol to acetic acid solution. The ethanol-acetic acid mixture acts as an aqueous catalyst to catalyze hydrolysis. The microchannels were treated with this 2% APTES for more than 30 minutes. After surface modification, modified PDMS is preferably left overnight under ambient conditions before further steps, to allow for complete drying and condensation of the silica precursor. Before first use, channels are preferably flushed with a continuous flow of DI water for 5 min to remove any residue adhering to the channel walls. After silanization, the solution of silver nanoparticles is passed through the microchannels and left overnight for proper immobilization of the nanoparticles. A

similar immobilisation technique has been elucidated/ characterised in our earlier reported studies [18], which highlight appropriate facts.

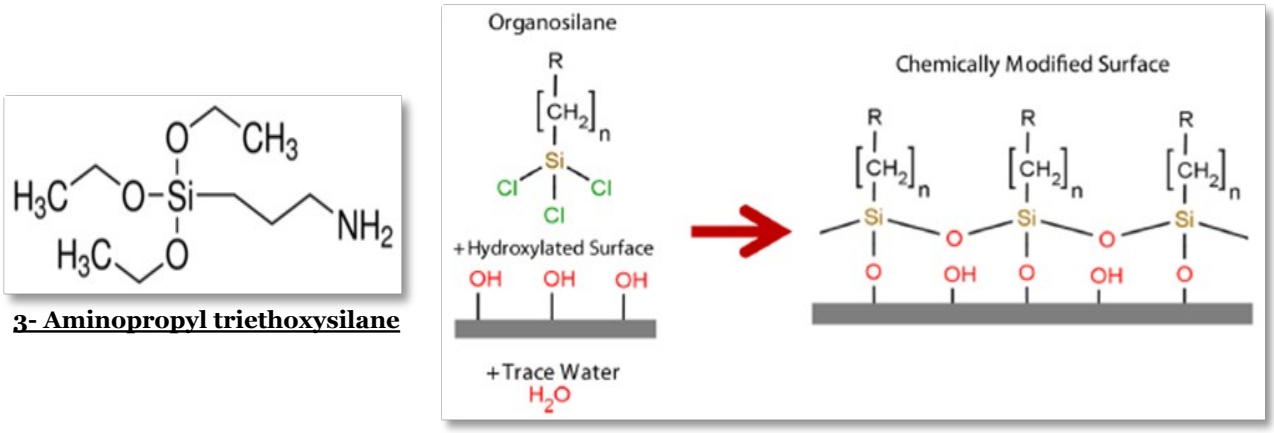


Figure S-5. APTES organosilane and its reaction of with hydroxylated surface [19]

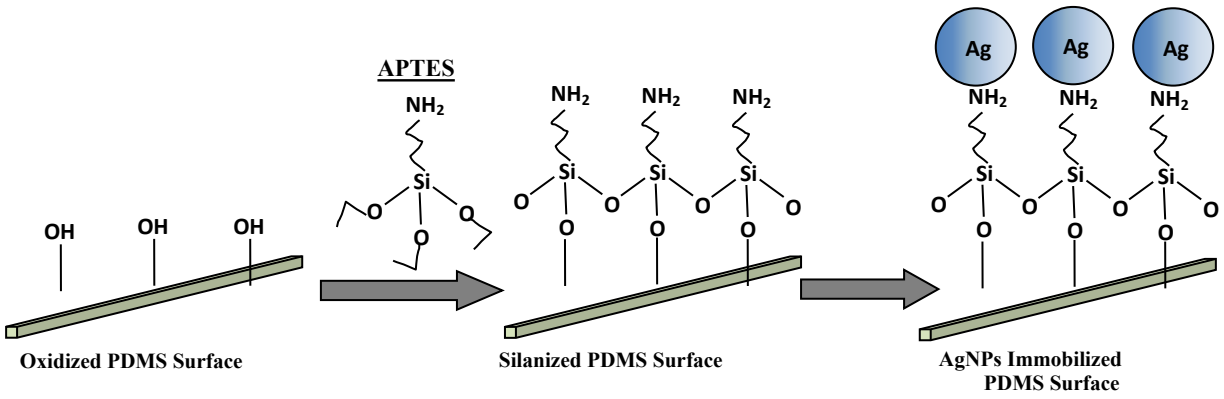
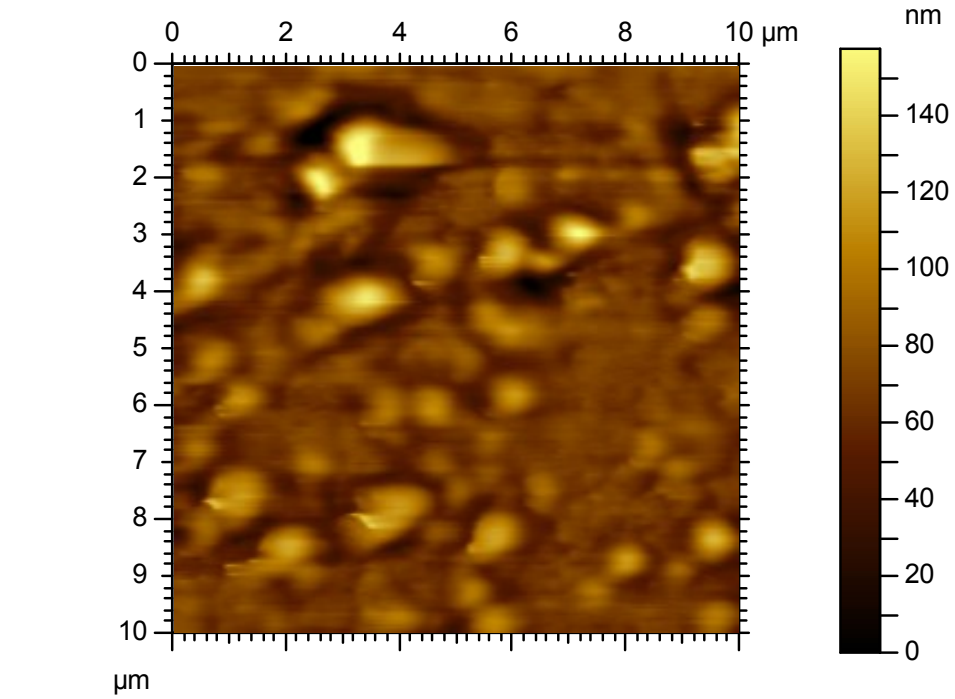


Figure S-6. Schematic of AgNPs immobilisation Reaction on oxidised PDMS surface via APTES.

2.1. Characterization of AgNPs Immobilization:

To confirm the immobilization of AgNPs, the same immobilization protocol was followed on the same but unsealed replica of PDMS microchannels to get AFM images of untreated PDMS surface and immobilized PDMS surface. *Figures: S-7.1, S-7.2, S-8.1, & S-8.2* show the AFM images of the AgNPs immobilised PDMS surface and *Figures: S-9.1, S-9.2, S-10.1, & S-10.2* show the AFM images of plain PDMS surfaces, which reveal a clear difference between them. The AgNPs-immobilized PDMS surface has more pits and falls (Figures: S-7.1, S-7.2, S-8.1, & S-8.2) as compared to plain PDMS surface (Figures: S-9.1, S-9.2, S-10.1, & S-10.2). The final immobilization protocol followed for the immobilization of silver nanoparticles is shown in *figure 3* of main manuscript.



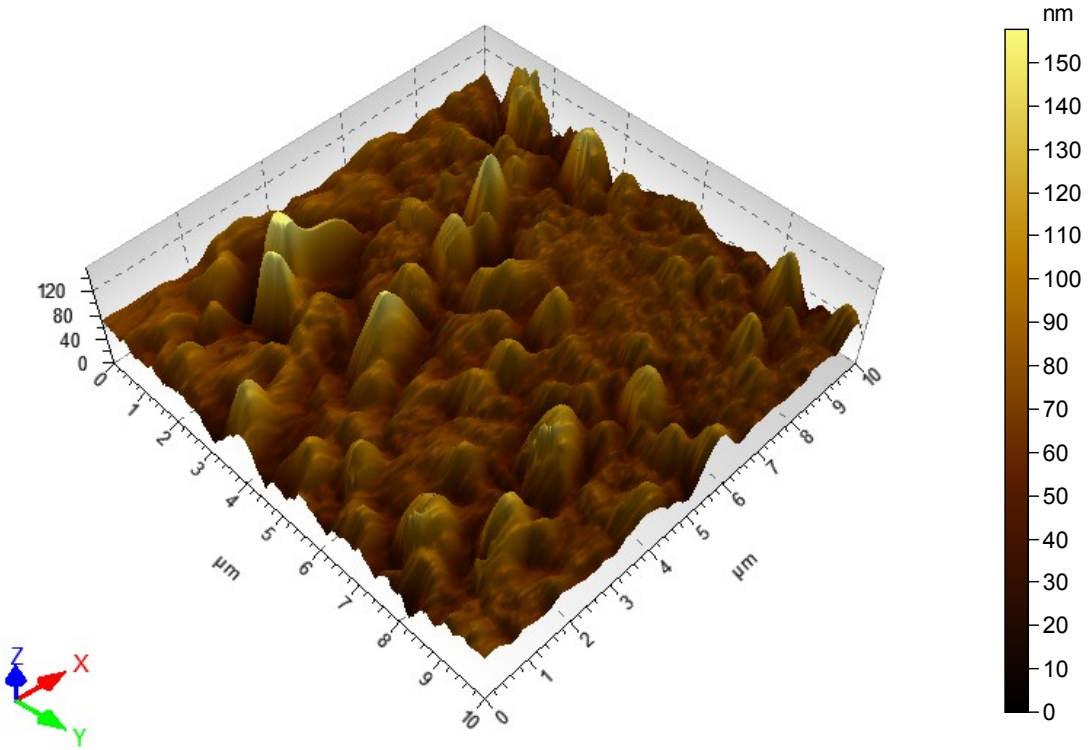


Figure S-7.1: 2D and 3D AFM images of AgNPs immobilized PDMS surface (*Scan range 10×10 µm)*

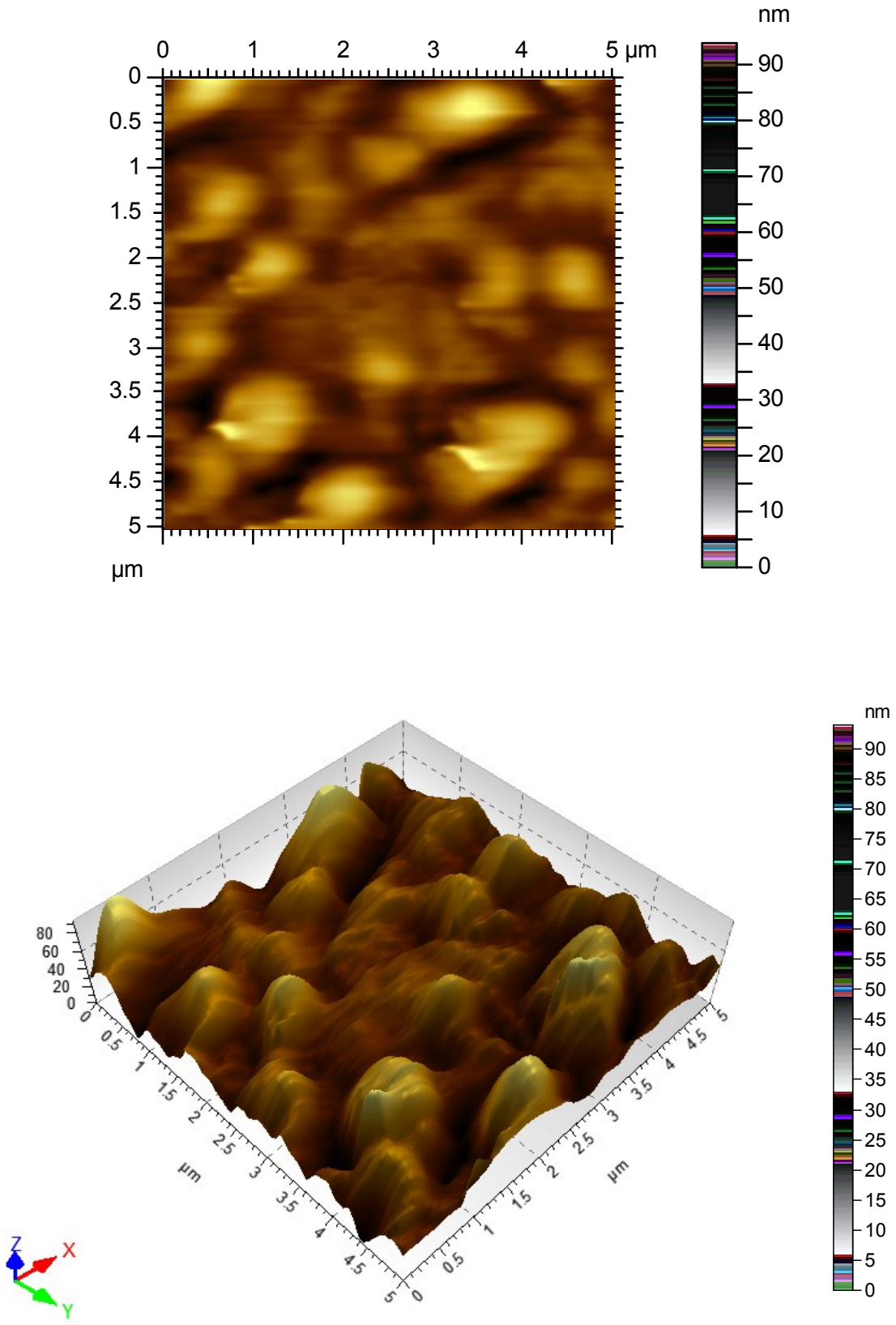
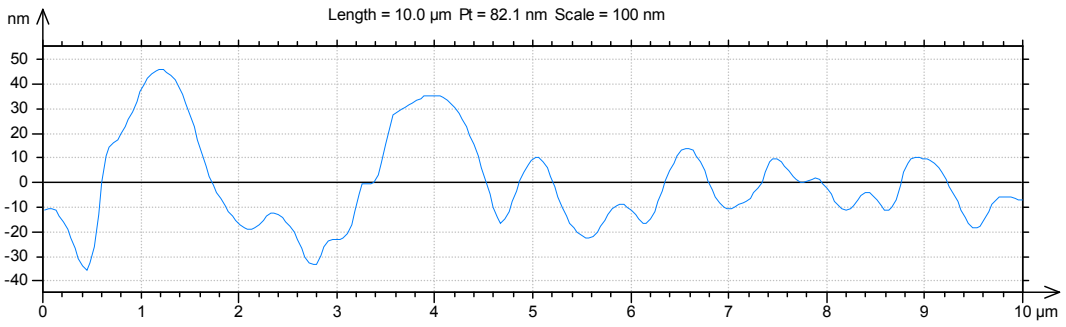
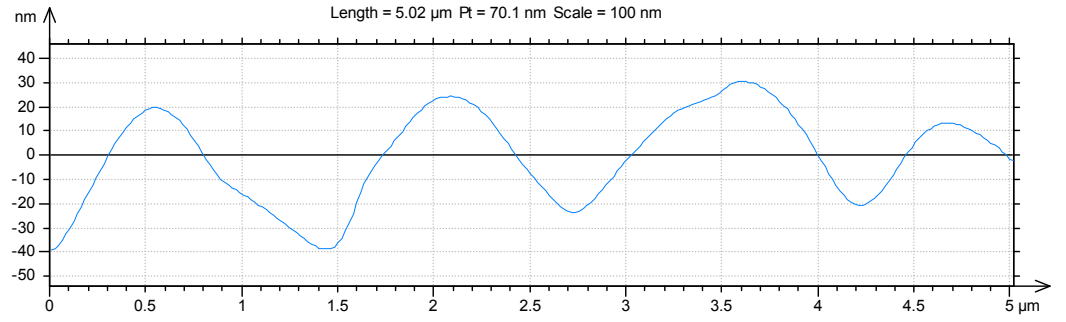


Figure S-7.2: 2D and 3D AFM images of AgNPs immobilized PDMS surface *(Scan range 5×5 µm)*



ISO 4287			
Amplitude parameters - Roughness profile			
Rp	25.3	nm	*Gaussian filter, 0.0025 mm*
Rv	21.9	nm	*Gaussian filter, 0.0025 mm*
Rz	47.1	nm	*Gaussian filter, 0.0025 mm*
Rc	27.3	nm	*Gaussian filter, 0.0025 mm*
Rt	47.1	nm	*Gaussian filter, 0.0025 mm*
Ra	8.47	nm	*Gaussian filter, 0.0025 mm*
Rq	10.2	nm	*Gaussian filter, 0.0025 mm*
Rsk	0.342		*Gaussian filter, 0.0025 mm*
Rku	2.28		*Gaussian filter, 0.0025 mm*
Material Ratio parameters - Roughness profile			
Rmr	100	%	*c = 1000 nm under the highest peak, Gaussian filter, 0.0025 mm*
Rdc	20.4	nm	*p = 20%, q = 80%, Gaussian filter, 0.0025 mm*

Figure S-8.1: AFM Profile of AgNPs immobilized PDMS surface for *Scan range 10×10 µm.*



ISO 4287			
Amplitude parameters - Roughness profile			
Rp	22.6	nm	*Gaussian filter, 0.0025 mm*
Rv	28.3	nm	*Gaussian filter, 0.0025 mm*
Rz	50.8	nm	*Gaussian filter, 0.0025 mm*
Rc	50.8	nm	*Gaussian filter, 0.0025 mm*
Rt	50.8	nm	*Gaussian filter, 0.0025 mm*
Ra	14.6	nm	*Gaussian filter, 0.0025 mm*
Rq	16.5	nm	*Gaussian filter, 0.0025 mm*
Rsk	-0.309		*Gaussian filter, 0.0025 mm*
Rku	1.66		*Gaussian filter, 0.0025 mm*
Material Ratio parameters - Roughness profile			
Rmr	100	%	*c = 1000 nm under the highest peak, Gaussian filter, 0.0025 mm*
Rdc	36.5	nm	*p = 20%, q = 80%, Gaussian filter, 0.0025 mm*

Figure S-8.2: AFM Profile of AgNPs immobilized PDMS surface for *Scan range 5×5 µm.*

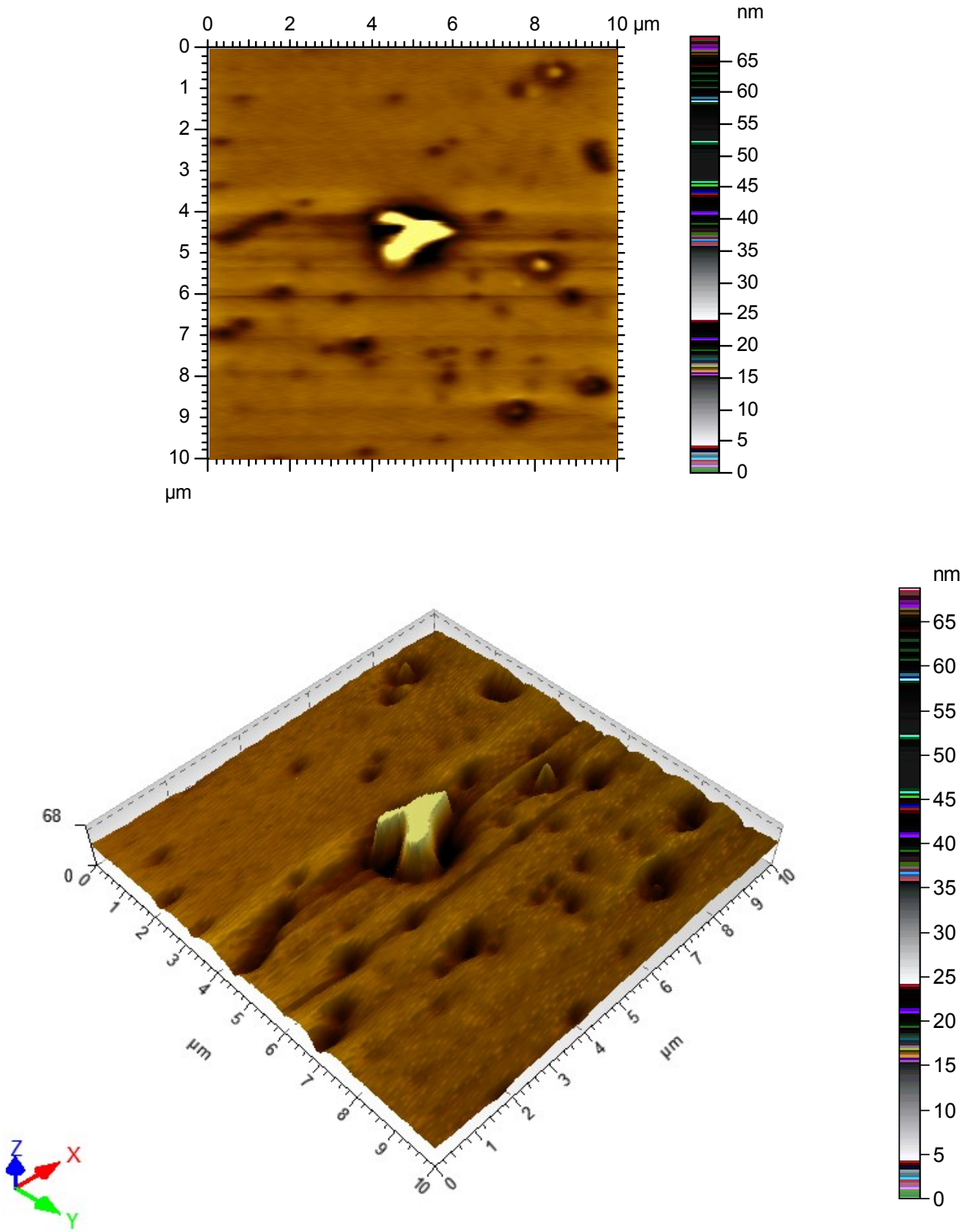


Figure S-9.1: 2D and 3D AFM images of plane PDMS surface for *Scan range 10×10 µm.*

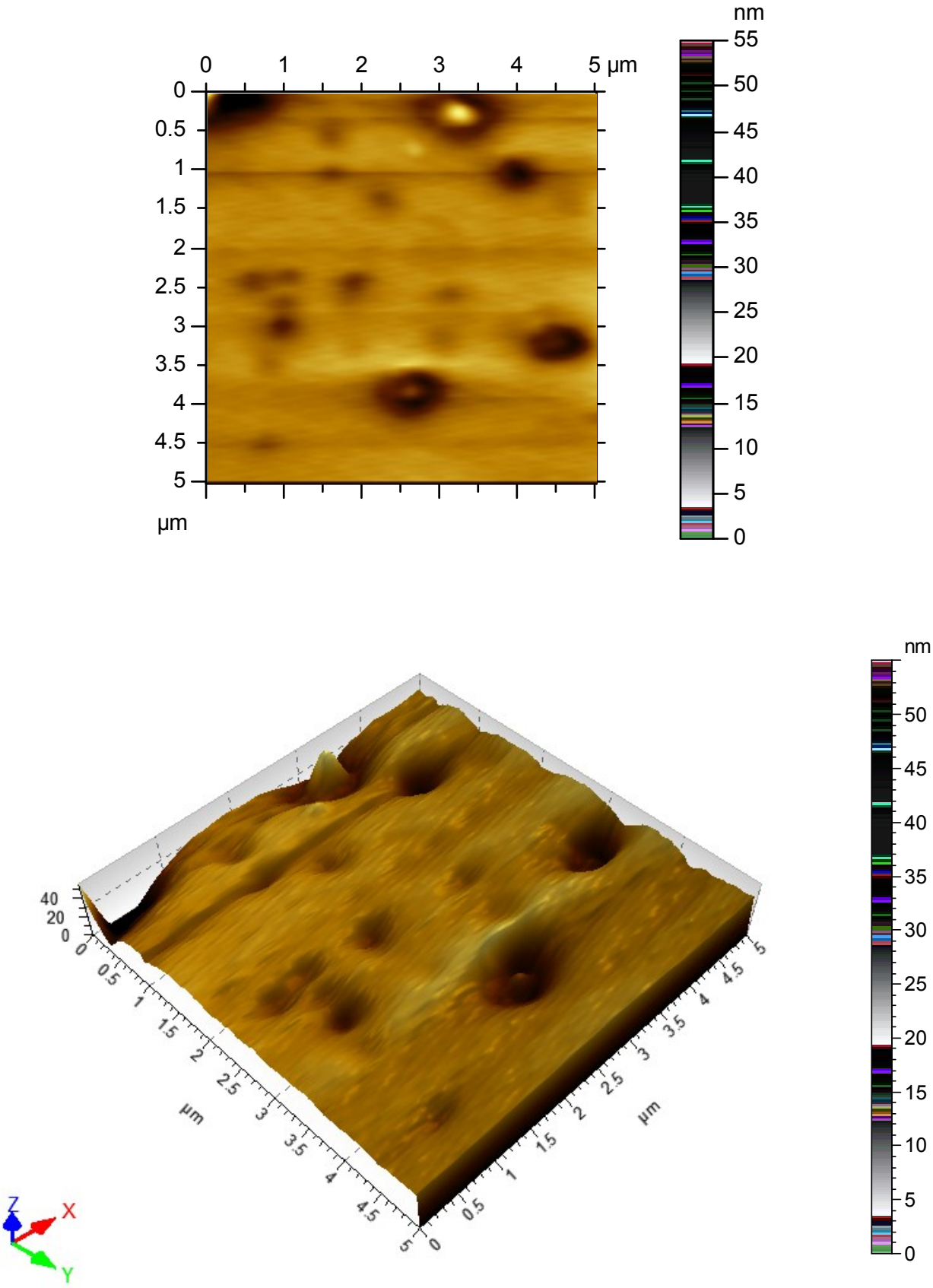
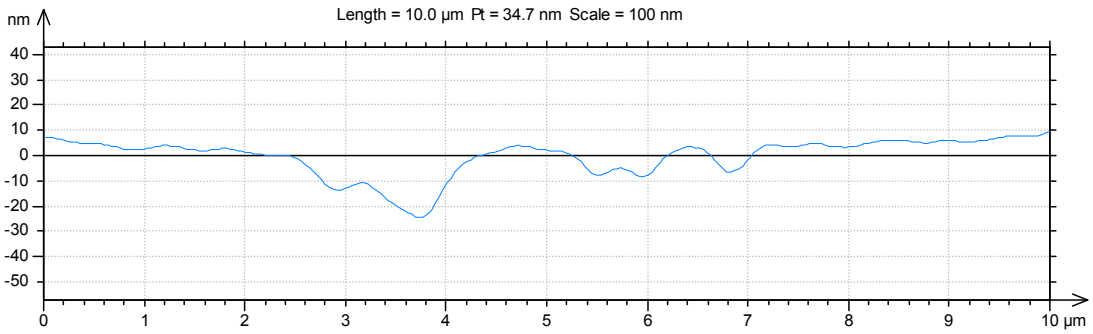
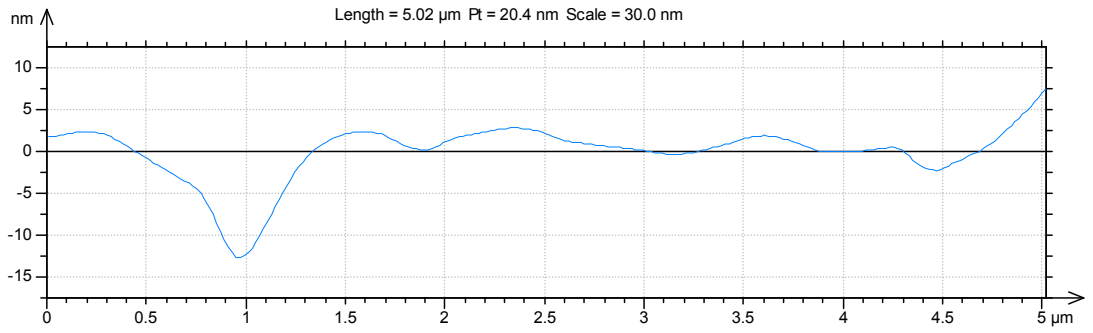


Figure S-9.2: 2D and 3D AFM images of plane PDMS surface for *Scan range 5×5 µm.*



ISO 4287			
Amplitude parameters - Roughness profile			
Rp	4.96	nm	*Gaussian filter, 0.0025 mm*
Rv	11.3	nm	*Gaussian filter, 0.0025 mm*
Rz	16.3	nm	*Gaussian filter, 0.0025 mm*
Rc	9.97	nm	*Gaussian filter, 0.0025 mm*
Rt	16.3	nm	*Gaussian filter, 0.0025 mm*
Ra	2.63	nm	*Gaussian filter, 0.0025 mm*
Rq	3.43	nm	*Gaussian filter, 0.0025 mm*
Rsk	-1.03		*Gaussian filter, 0.0025 mm*
Rku	3.99		*Gaussian filter, 0.0025 mm*
Material Ratio parameters - Roughness profile			
Rmr	100	%	*c = 1000 nm under the highest peak, Gaussian filter, 0.0025 mm*
Rdc	5.53	nm	*p = 20%, q = 80%, Gaussian filter, 0.0025 mm*

Figure S-10.1: AFM Profile of plain PDMS surface for *Scan range 10×10 µm.*



ISO 4287			
Amplitude parameters - Roughness profile			
Rp	2.63	nm	*Gaussian filter, 0.0025 mm*
Rv	1.69	nm	*Gaussian filter, 0.0025 mm*
Rz	4.32	nm	*Gaussian filter, 0.0025 mm*
Rc	3.14	nm	*Gaussian filter, 0.0025 mm*
Rt	4.32	nm	*Gaussian filter, 0.0025 mm*
Ra	0.961	nm	*Gaussian filter, 0.0025 mm*
Rq	1.18	nm	*Gaussian filter, 0.0025 mm*
Rsk	0.630		*Gaussian filter, 0.0025 mm*
Rku	2.62		*Gaussian filter, 0.0025 mm*
Material Ratio parameters - Roughness profile			
Rmr	100	%	*c = 1000 nm under the highest peak, Gaussian filter, 0.0025 mm*
Rdc	1.81	nm	*p = 20%, q = 80%, Gaussian filter, 0.0025 mm*

Figure S-10.2: AFM Profile of plain PDMS surface for *Scan range 5×5 µm.*

3. Initial bacterial count and Counting Living and Dead Bacteria:

The disinfection experiments were carried out on Gram negative bacterial strains, E. coli MTCC 443 (ATCC 25922), and a Gram-positive bacterial strain B. subtilis MTCC 441 (ATCC 6633), which were procured from the Institute of Microbial Technology (Chandigarh, India).

3.1. Bacterial Culture:

The protocol (figure S-11 & figure S-12) followed for performing bacterial culture is as follows:

- Prepared 40 ml Luria Broth solution (for E.coli) and Nutrient Broth solution (for B.Subtillis) in two test tubes.
- Prepared 50 ml Nutrient Agar solution in three test tubes.
- Autoclaved at 120ºC for 15 minutes at 15 psi with proper cotton plugging.
- After autoclaving, kept the agar test tubes in slanted manner to make slants. Also kept the broth solutions for cooling.
- Breaking the seal of the bacterial culture vial inside a laminar flow and using an inoculation loop, mixed small amount of lyophilized bacterial powder into the broth.
- After the slants have solidified, streaked them with an inoculation loop with respective bacterial cultures.
- Incubated the streaked slants and broth solutions for 24 hours at 37ºC.

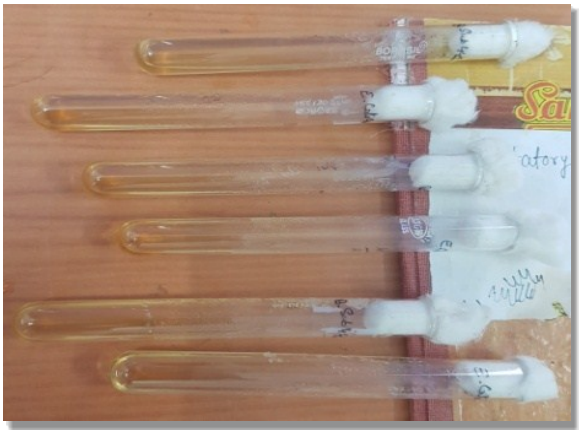


Figure S-11. Preparation of Agar slants.

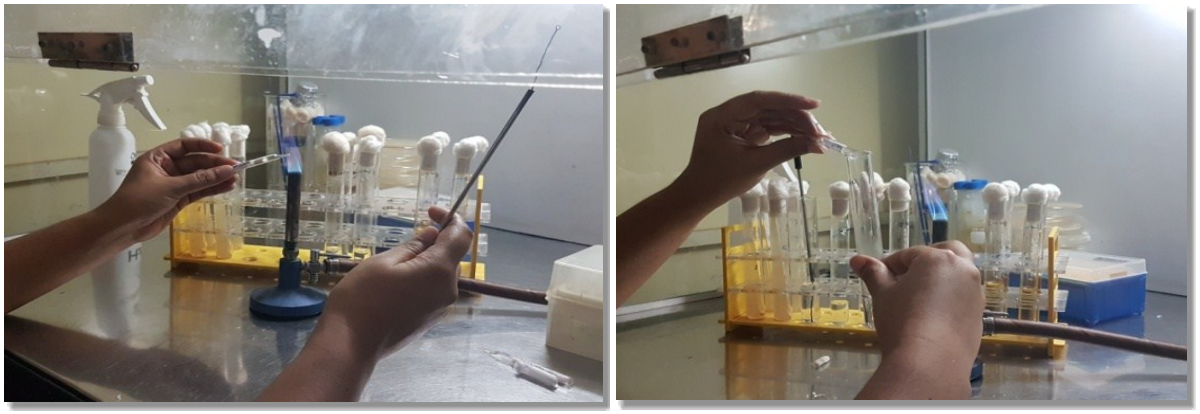


Figure S-12. Breaking the seal of a vial and inoculating lyophilized bacteria into the broth

3.2. Performing initial bacterial count:

Initial bacterial count was performed using Serial Dilution Method (figure S-13) and bacterial colonies were counted as CFU/ml at the end. The protocol for serial dilution method used is as follows:

- Taken 9 ml DI water in ten test tubes.
- Prepared 150 ml Nutrient Agar solution with 1% Agar, agar type 1 in a beaker.
- Autoclaved for 120ºC for 15 minutes at 15 psi.
- Poured 25 ml. of autoclaved agar solution in six petri plates inside a laminar flow.
- Kept the petri plates to solidify overnight.
- Inoculated 1 ml. of bacteria from the above cultures broth solution and pour it into the first DI water test tube. Marked the test tube as 10^{-1}.
- Taken 1 ml from the first test tube and pour in second test tube. Marked the second test tube as 10^{-2}.
- Repeated the above step serially and marked the test tubes as 10^{-3}, 10^{-4} and so on till 10^{-6}.
- Plating - Spread 30 µl from each of the above labelled test tubes into the six petri plates and mark the corresponding petri plates the same i.e. 10^{-1}, 10^{-2} and so on.
- Incubate the inoculated petri plates for 24 hours at 37ºC.

Note: Dilution Factor is reciprocal of dilution i.e. each of the above petri plates have the dilution factor of 10^1, 10^2,10^3,10^4,10^5,10^6 respectively.

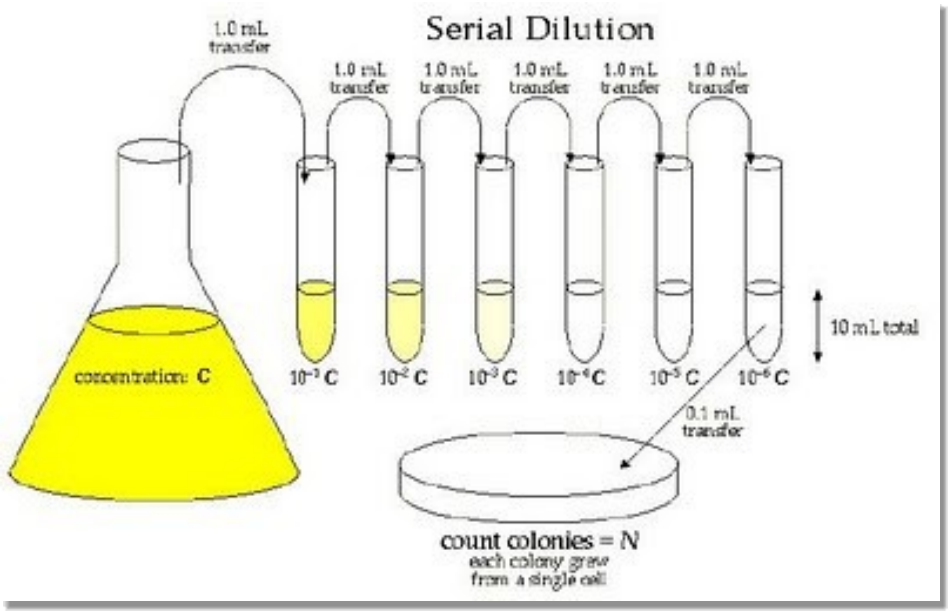


Figure S-13. serial dilution method [11]

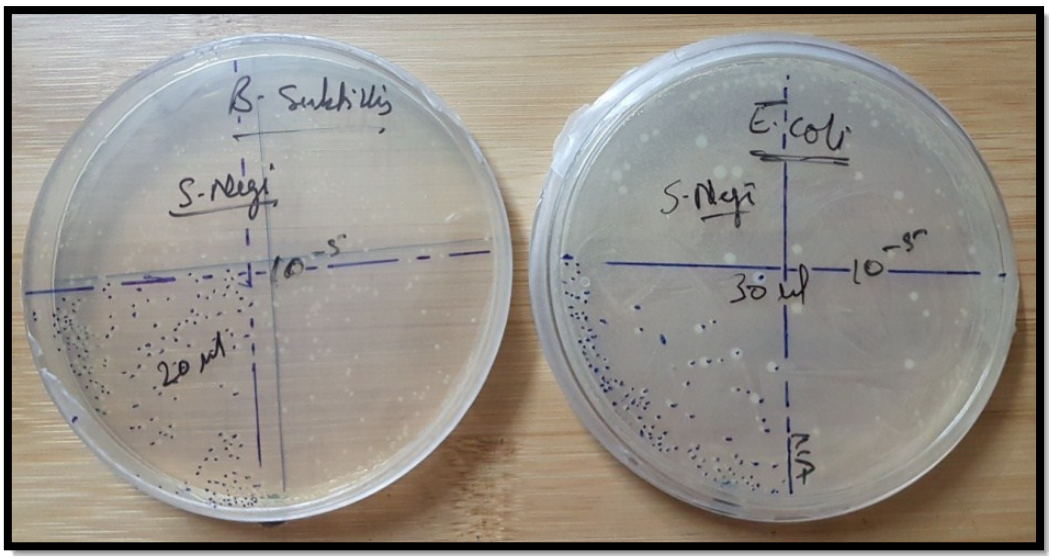


Figure S-14. Colonies formed after incubation at a dilution factor 10^5.

- Divided the Petri-plate with countable colonies into 4 quadrants (figure S-14) and counted the number of colonies in any one quadrant and multiply by 4. These are the number of colonies.
- Applied the formula –

No. Of cells/ml or CFU/ml = No. Of colonies × Dilution Factor

3.3. Results Obtained –

- **E.coli –**
  No. Of colonies in one quadrant = 180
  Total colonies = 180 * 4 = 720
  CFU/ml = 720 * 10^5
  = 7.20 * 10^7
- **B.subtilis –**
  No. Of colonies in one quadrant = 182
  Total colonies = 182 * 4 = 728
  CFU/ml = 728 * 10^5
  = 7.28 * 10^7

3.3. UV – Visible Spectroscopy of the cultured bacteria:

The culture strains of E.coli and B.subtilis were characterized using UV – Visible spectroscopy (Figure S-15). Spectrally resolved emission from dilute suspensions of washed Bacillus subtilis and Escherichia coli were measured by use of UV – Visible Spectrophotometer between 200 and 500 nm. Generally the spectrum obtained for E.Coli and B.Subtilis is indistinguishable.

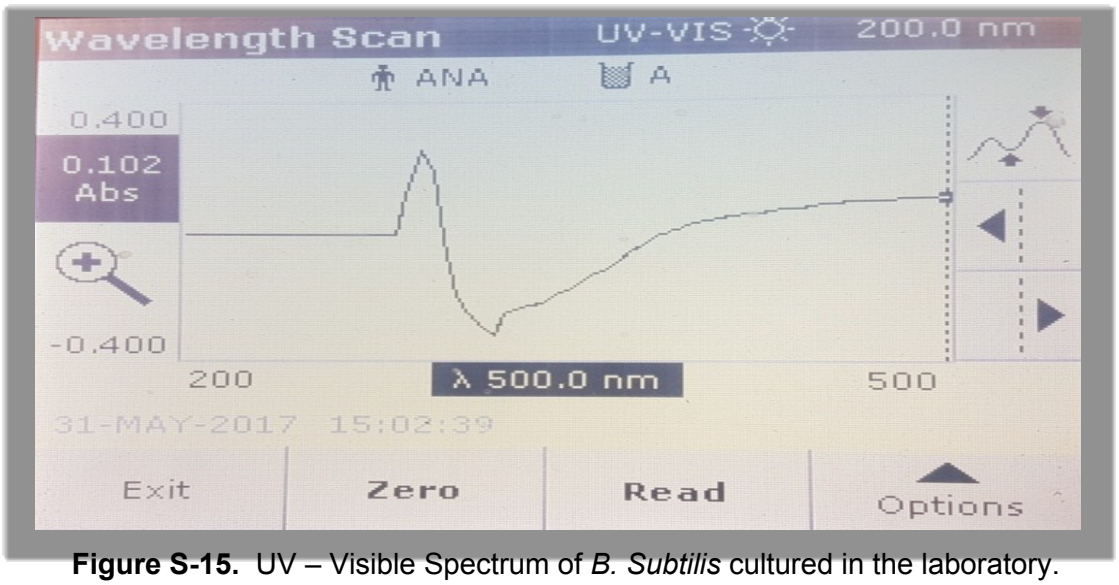


Figure S-15. UV – Visible Spectrum of *B. Subtilis* cultured in the laboratory.

3.4. Counting Living/ dead bacteria:

In the samples collected at the output, several methods could be used to differentiate living/dead bacteria such as:

- Performing serial dilution method of the samples before and after passing through the micro-channels and calculating the difference.
- Flow cytometry
- Fluorescence Microscopy
- Hemocytometry
- PI Staining

The method used in this project is Propidium Iodide (PI)- Staining. Propidium iodide (PI) is a popular red-fluorescent nuclear and chromosome counterstain, which is not permeable in live cells, it is also commonly used to detect dead cells in a population.

PI binds to DNA by intercalating between the bases with little or no sequence preference. In aqueous solution, the dye has excitation/emission maxima of 493/636 nm. Once the dye is bound, its fluorescence is enhanced 20- to 30-fold, the fluorescence excitation maximum is shifted ~30–40 nm to the red and the fluorescence emission maximum is shifted ~15 nm to the blue, resulting in an excitation maximum at 535 nm and fluorescence emission maximum at 617 nm. PI is widely used in fluorescence microscopy, confocal laser scanning microscopy, flow cytometry, and fluorometry.

The steps used for PI Staining are as follows:

- The AgNPs and UV treated samples collected at the outlet were centrifuged at 7000 RPM for 10 minutes at a temperature of 4ºC. All the bacterial cells (living or dead) get collected in the pellet.
- The supernatant is discarded and the pellet is stained with 30 µl PI dye.
- The stained samples are kept aside for about 15 minutes to allow the dye to enter into the dead bacterial cells.

- After incubation, slides are prepared with 30 to 50 µl of the stained samples and allowed to dry. The excess PI is washed away to obtain a clear image.
- The slides are then viewed under a fluorescent microscope with green fluorescent light. Green light is used since the absorption maxima of PI (around 496 nm) lies in the wavelength range of green light (495 – 570 nm) in the visible spectrum.
- Since PI stains the dead bacterial cells only, they will appear red under the microscope.