

## Supporting information

### Plasma polymerization of 1,1,1-Trichloroethane yields coating with robust antibacterial surface properties

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#### Experimental details

##### Materials

1,1,1-Trichloroethane  $\geq 99\%$  (TCE) was purchased from Sigma-Aldrich (St. Louis, MO) and used as received. The coverslips were stamped out with a 13 mm punch from a 0.05 mm thick PET foil supplied from Goodfellow Cambridge Ltd. (Huntingdon, England) and washed with Ethanol twice prior to their use. Oxoid™ Nutrient Agar (CM0003) and cold filterable Oxoid™ Tryptic Soy Broth (TSB, CM1065) were purchased from Thermo fisher (Scoresby, Australia). Phosphate buffered saline (PBS, SLBB6584) tablets and Safranin stain were purchased from Sigma Aldrich. The BacLight™ staining kit was purchased from Invitrogen (Mulgrave, Australia) and used according to specifications. All chemicals were used as received and Milli-Q™ filtered water was used to prepare solutions, with the aforementioned chemicals, according to their recommended concentration. In the case of antibacterial testing, all solutions were autoclaved prior to their usage in order to ensure sterility. The bacteria strain used was *Staphylococcus epidermidis* ATCC® 35984™. 24 well plates were NUNC™ brand, purchased from Thermo fisher.

##### Plasma polymerization and post-plasma processing

The reactor design used and the deposition method of a thin plasma polymer coating was performed as previously reported.<sup>1,2</sup> The standard plasma polymerization was carried out as follows: PET coverslips and one silicon wafer were placed into the plasma chamber. The silicon wafer was used for determination of polymer thickness by ellipsometry. First, the plasma chamber was pumped down to its base pressure of 30 mTorr. Afterwards the inlet valve was carefully opened to allow for air flowing into the chamber and stabilize the pressure at 200 mTorr. Subsequently,

an air plasma with a RF frequency of 13.56 MHz, at a vapour pressure of 200 mTorr, input power of 50 W and treatment time of 1 minute was used to oxidize the surface of the PET coverslips in order to ensure proper bonding with the subsequent plasma polymer; followed by the evacuation of the plasma chamber down to its base pressure of 30 mTorr. Lastly, TCE vapours were introduced into the plasma chamber and the pressure was observed to be stable at 200 mTorr for at least one minute. This was followed by plasma polymerization with a RF frequency of 13.56 MHz, at a vapour pressure of 200 mTorr, input power of 25 W and deposition time of 2 minutes. Samples which were soaked or washed were done so with 1 ml of distilled water per well. In the case of Wet samples, the coverslips were immersed once; in case of the washed samples, this procedure was repeated 20 times with a time interval between the washes of at least 15 minutes.

## **Antibacterial testing**

### **24h**

Small amounts from a frozen aliquot (-20 °C) of bacteria were thawed then plated out on an agar plate and incubated overnight at 37 °C. The frozen aliquots were replaced fresh ones from the -78 °C freezer every month in order to ensure the genetic pedigree of the bacteria. The next day a single colony was picked and transferred into 10 mL of TSB and incubated at 37 °C for 18 h. 1 mL of the bacteria solution was transferred into 5 mL of fresh TSB and incubated at 37 °C for 2 h to reach exponential bacteria growth. The optical density was measured at 600 nm and the bacteria solution was diluted with fresh TSB so its optical density corresponded to  $OD_{600}=0.2$  which translated into a bacterial concentration of approximately  $10^8$  CFU/mL. This mother solution was then further diluted by 10 times to the desired concentrations; 600 µl per well of those solutions were then used to inoculate the samples. The samples were then incubated at 37 °C for 4 hours, followed by twice washing the samples with 600 µl of PBS to rinse off non-adhering bacteria. The wells were refilled with 600 µl of fresh TSB and the samples were incubated further for 20 hours. Afterwards, the coverslips were washed twice with 600 µl of PBS each; followed by 600 µl of Safranin stain for 15 minutes. Upon drawing off the excess stain, the samples were washed three times with an excess of distilled water and analyzed in a wellplate reader (ELx800, BioTek) using a 5\*5 area scan protocol at a absorbance wavelength of 490 nm.

### **Time lapse**

Small amounts from a frozen aliquot (-20 °C) of bacteria were thawed then plated out on an agar plate and incubated overnight at 37 °C. The frozen aliquots were replaced fresh ones from the -78 °C freezer every month in order to

ensure the genetic pedigree of the bacteria. The next day a single colony was picked and transferred into 10 mL of TSB and incubated at 37 °C for 18 h. This was followed by centrifugation at 1000rpm for 10 minutes, replacing the supernatant with PBS and two subsequent centrifugation/wash cycles with PBS. The optical density of the bacteria in PBS solution was measured at 600 nm and the bacteria solution was diluted with fresh PBS so its optical density corresponded to OD<sub>600</sub>=0.02 which translates into a bacterial concentration of approximately 10<sup>7</sup> CFU/mL. The samples to be studied were placed in quadruplicates in a 24-well plate and inoculated with 600 µl of the bacteria solution. Air plasma treated PET coverslips were used as the reference. Afterwards the well plate was shaken at a frequency of 2 Hz while incubated for 1h at a temperature of 37 °C to ensure the homogenous attachment of bacteria to the surfaces. After this time period the bacteria solution was drawn off and the samples were washed twice with 600 µl of PBS followed by the addition of 600 µl of TSB into each well. This time point was defined as “0 h”. Samples for analysis were placed into a separate 24 well plate and the remaining samples were placed back into the shaking-incubator for further incubation. BacLight™ stain was prepared according to specification and 300 µl were pipetted into each well. After 20 minutes incubation at room temperature each sample was washed three times with excess deionized water, then placed into 600 µl of deionized water to prevent the stain drying out, causing an alteration of the results. Afterwards the samples were imaged one at a time with a Nikon Eclipse Ni™ microscope, equipped with a green/red filter and with the Nikon digital sight DS-L3™ at a 490 nm excitation wavelength. This procedure was repeated for all samples after an incubation time of 2 and 6 hours in TSB.

## **Cytotoxic testing**

To determine if TCEpp surfaces were cytotoxic to mammalian cells we quantified the impact the surfaces had on both cell expansion and viability. KG1a cells, a human myeloid leukemia cell line<sup>3</sup>, were cultured in 24-well plates that did or did not contain a TCEpp coated coverslip. KG1a are a suspension cell line, so they do not require attachment to a surface in order to remain viable or to proliferate. KG1a cells were seeded in 1 ml of Xvivo medium (Lonza, Basel Switzerland ) either at a density of 50,000 cells/ml. Replicate cultures were maintained with or without 10% foetal bovine serum (FBS, ThermoScientific, Waltham, MA). Cells were then cultured in a humidified incubator at 37°C and 5% CO<sub>2</sub>. Following four days of cultures the cell number and viability was quantified via flow cytometry. 180 µl from eight replica cultures was transferred to individual wells in a 96-well plate. 20 µl of Flow-Count Fluorospheres (Beckman Coulter, Pasadena, California) plus 1 µg/ml Propidium iodide (PI, Invitrogen Carlsbad, CA) was added to each well. Cells and beads were then analyzed using a FC 500 Series flow cytometer (Beckman Coulter). Cell numbers and viability (PI negative) were quantified using CXP software (Beckman Coulter) and Flow-Count beads used as a reference.

Results:

TCEpp surface mammalian cell cytotoxicity was quantified following four days of culture. KG1a cells underwent approximately four-fold expansion on control surfaces and zero cell expansion on TCEpp surfaces. Additionally, >1% of the TCEpp cultured cells were viable (PI negative) after four days culture. Cell expansion and viability on TCEpp surfaces could not be rescued through the addition of FBS supplementation. These results suggest that TCEpp surfaces are cytotoxic to mammalian cells.

Figure S1. The chart contrast KG1a fold expansion following four days culture on either control tissue culture plastic surfaces or on TCEpp coated cover slips (n = 8,  $\pm$  standard deviation,  $P < 0.05$ ).

## **Analytical methods**

### **XPS**

Surface analysis was conducted using a Kratos Axis Ultra DLD spectrometer utilizing a monochromatic Al K $\alpha$  X-ray source running at 225W which corresponds to an energy of 1486.6 eV. The size of the analysed area was 0.3\*0.7 mm and an internal flood gun was used to minimize the charging of the samples. All survey spectra were collected at 160 eV pass energy at steps of 0.5 eV and dwell time of 55 ms. The data was processed and analysed with CasaXPS (ver.2.3.16 Casa Software Ltd.) using Shirley baseline correction. All spectra were compensated for charging effects by offsetting the C-C peak to 284.8 eV. All atomic percentages were rounded to one decimal after the comma.

### **Tof-Sims**

Surface analysis was conducted using a Physical Electronics Inc. PHI TRIFT V nanoTOF instrument, utilizing a pulsed liquid  $79^+$  Au primary ion gun which was operated at 30kV. A dual charge neutralization was in place in form of an electron flood gun and  $Ar^+$  ions (both 10 eV). Two spots at random were chosen for each sample for the collection of negative secondary ions and six different random spots were chosen for the collection of positive secondary ions. Collection time per spot was 1 minute and the raster size was  $10^{-4}$  cm<sup>2</sup>. The spectra were processed using WincadenceN software (ver. 1.8.1.3 Physical Electronics Inc.)

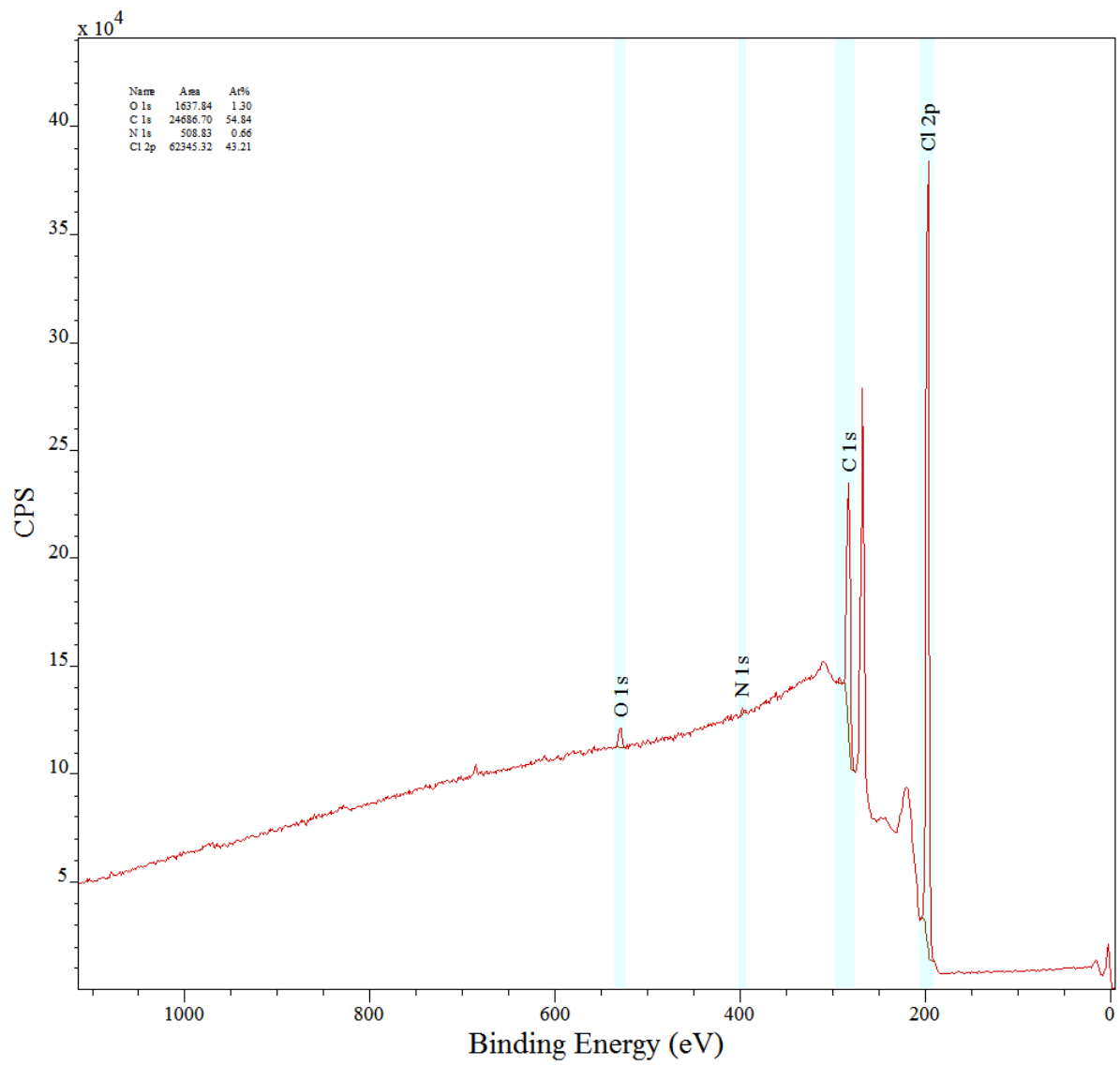
## **Ellipsometry**

The thickness of the deposited plasma polymer was determined using a J.A. Woollam (Model MC-200) V-Vase ellipsometer. For this purpose TCEpp was deposited onto a silicon waver under standard conditions as described above; followed by ellipsometry measurement of the silicon waver over a wavelength range of 400-1100 nm in 10 nm steps at alignment angles of 65°, 70° and 75°. The experimental data was then fitted using the supplied modelling software WVASE32 (Ver. 3.770) using a two layer Cauchy model. From refining the optical parameters, the mean squared error of the fit was minimized from which the plasma polymer thickness was obtained.

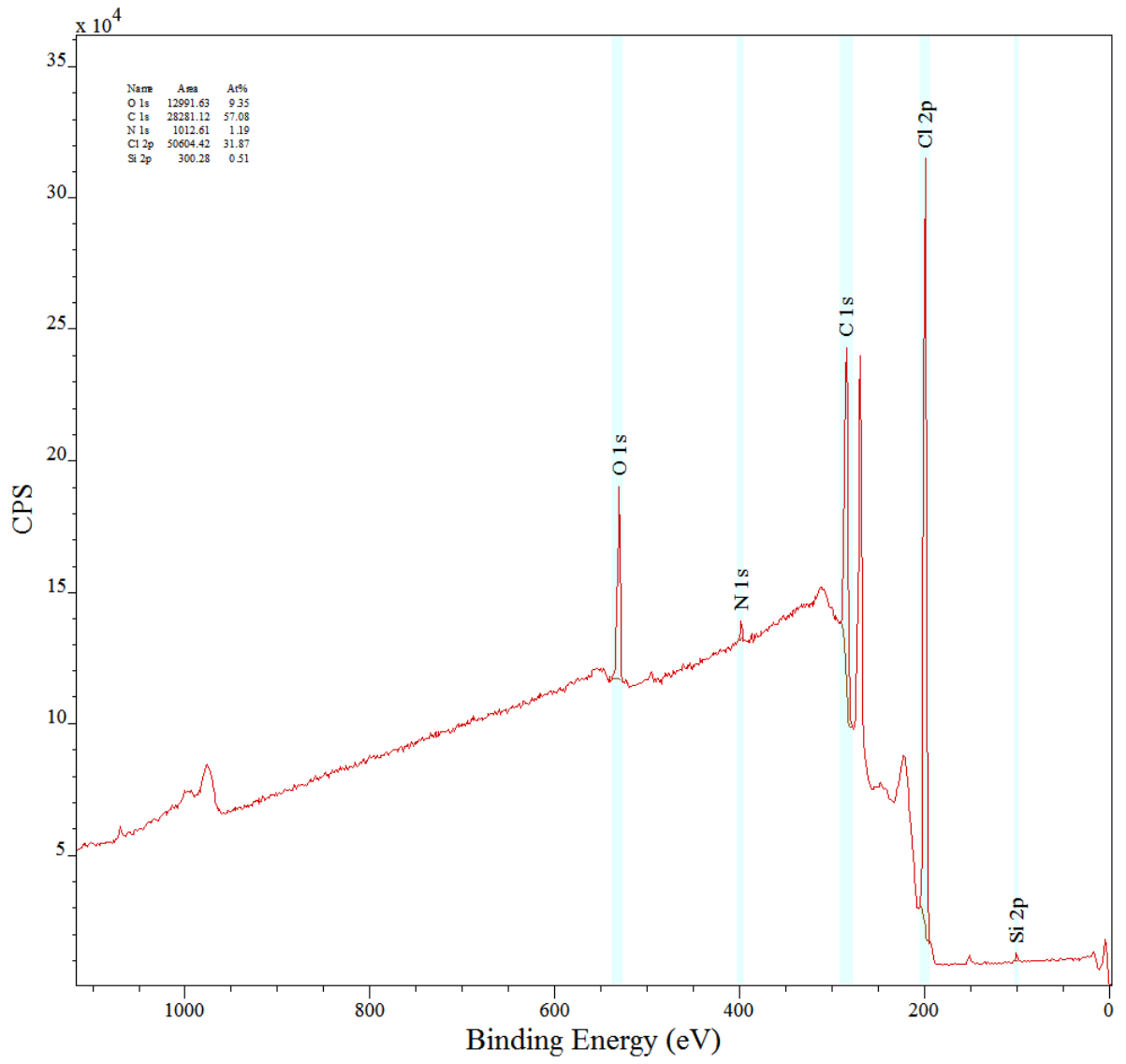
# Spectra

## XPS

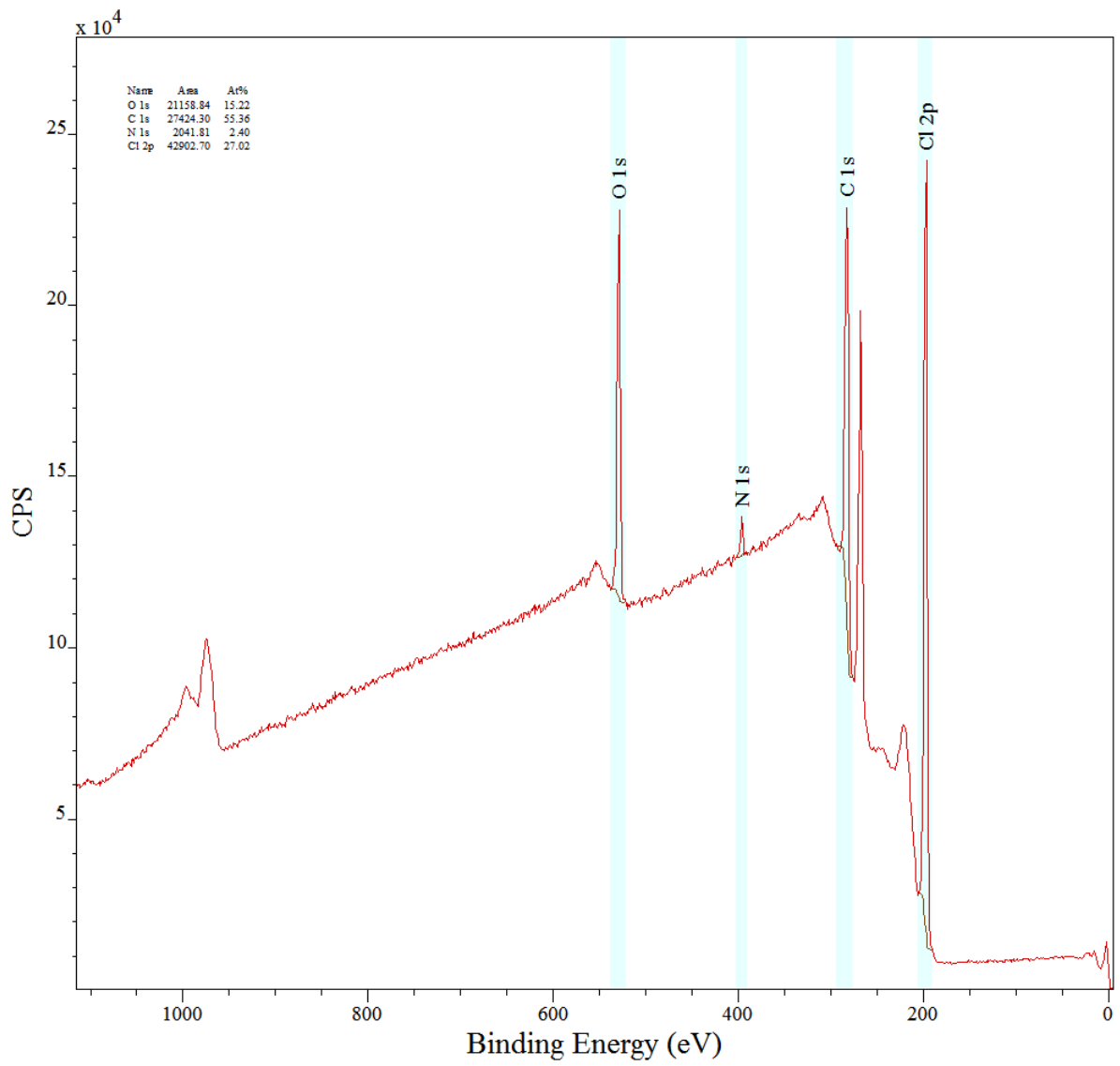
TCE Dry



# TCE Wet



# TCE Washed

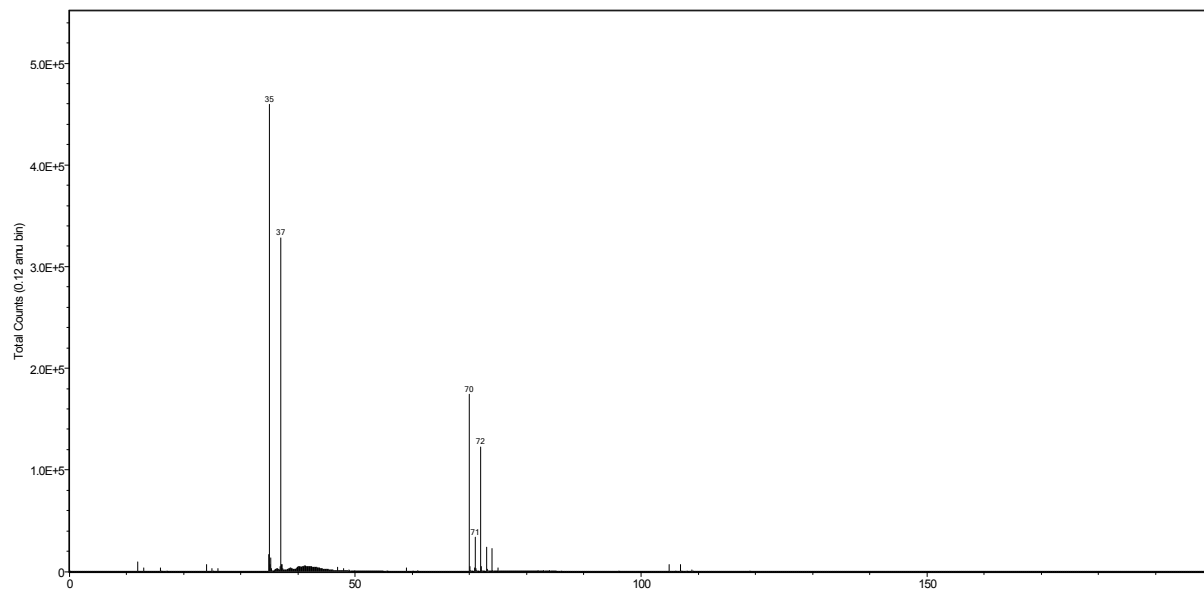




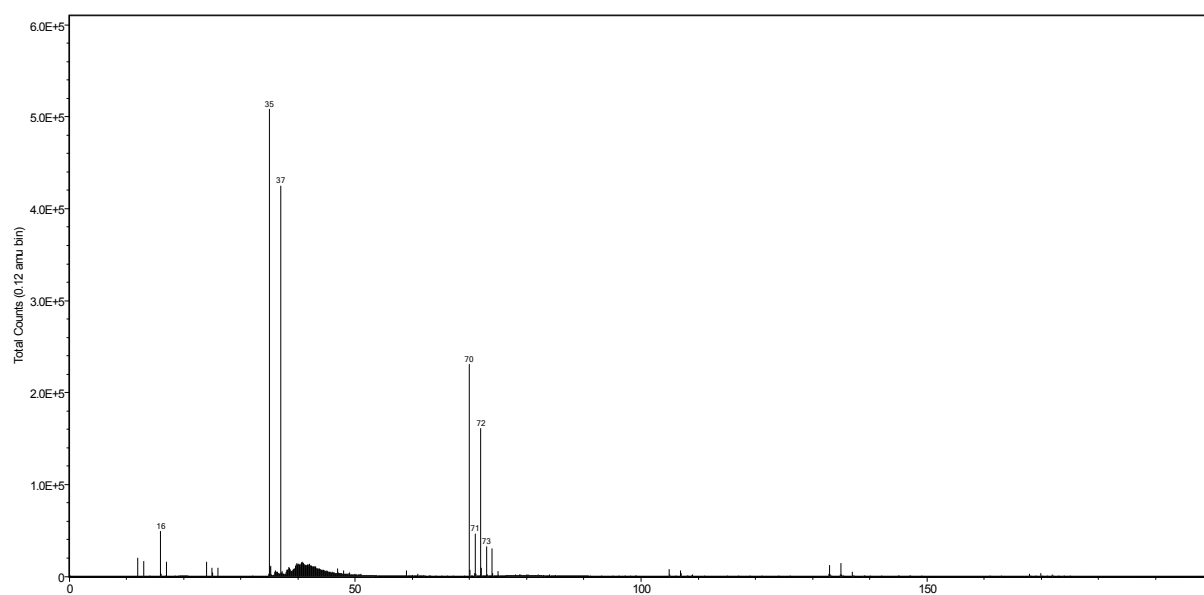
Tof-Sims

Negative mode

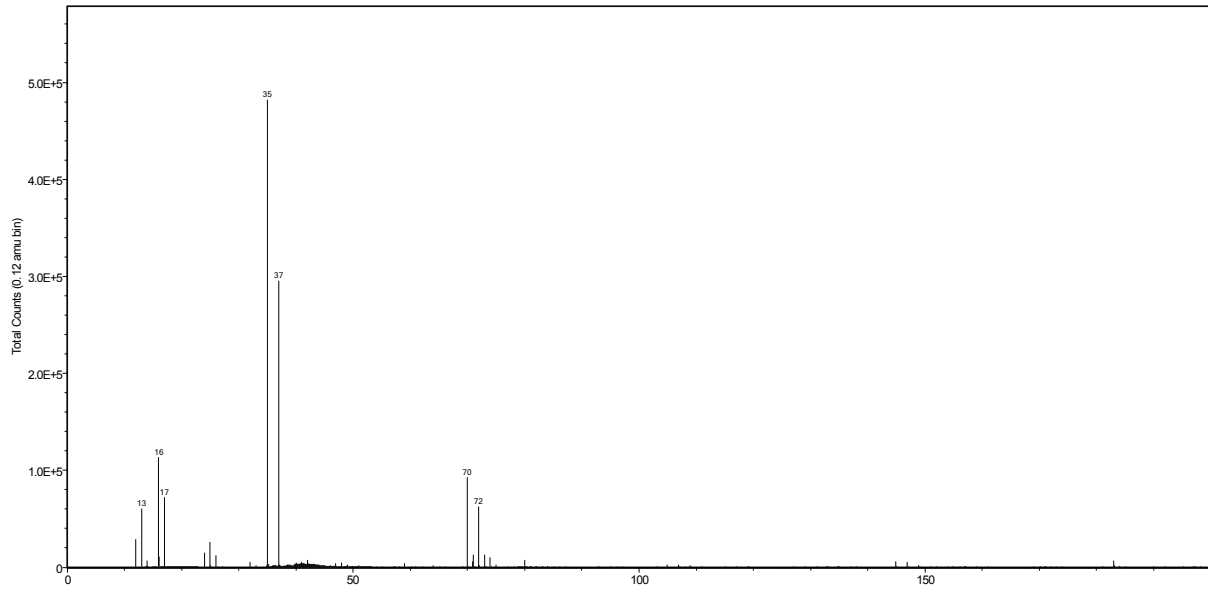
*TCE dry*



*TCE wet*

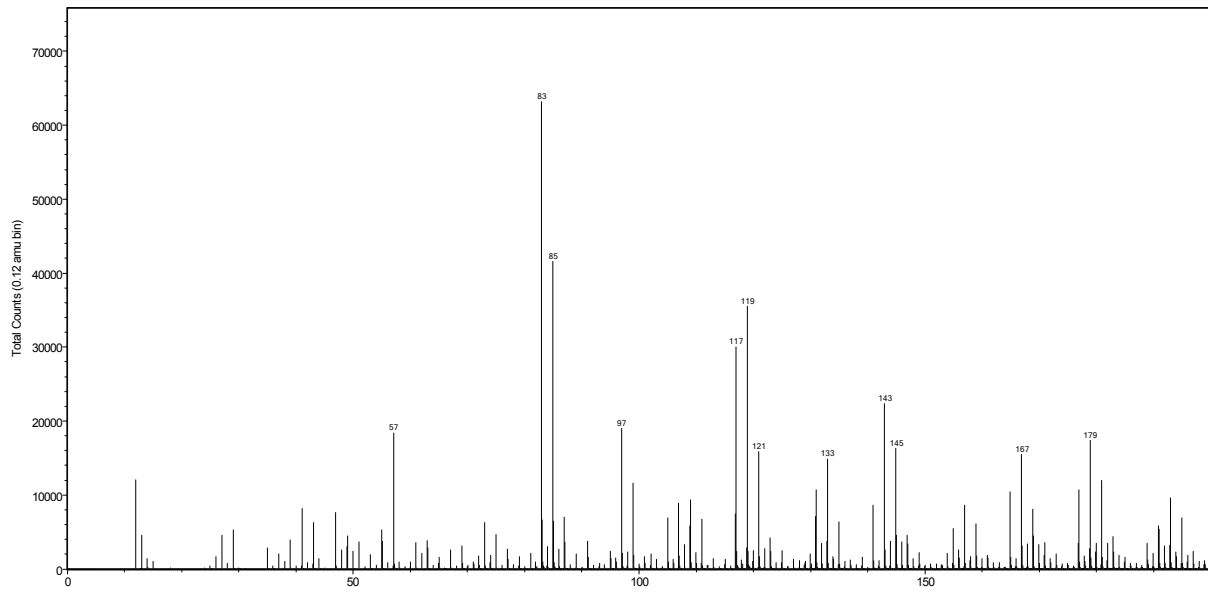


***TCE washed***

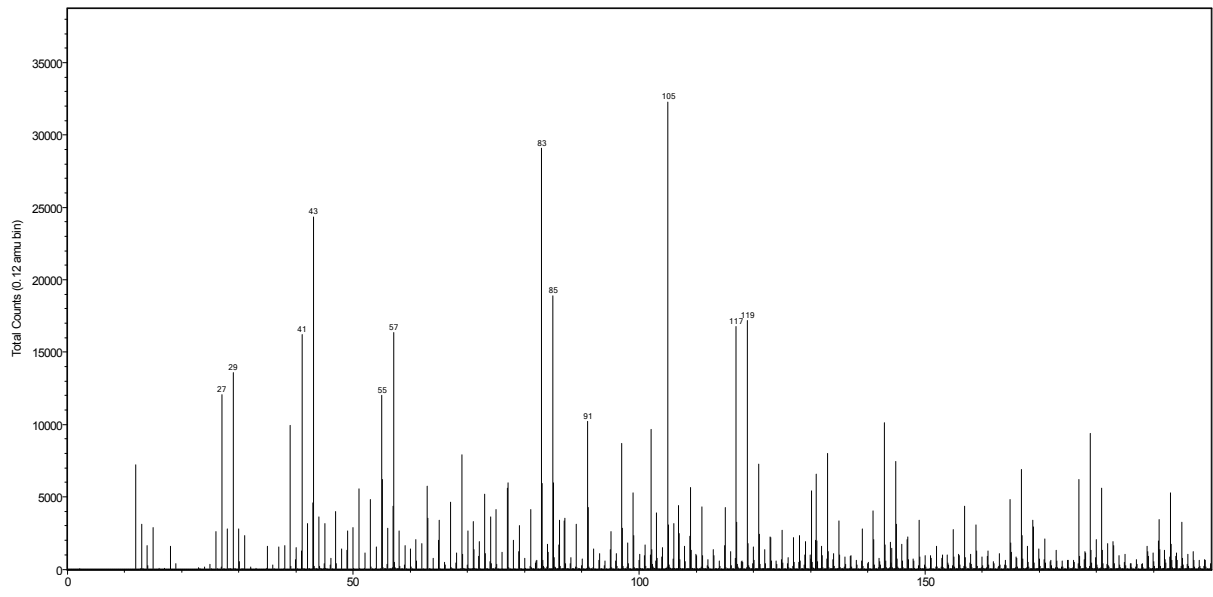


**Positive mode**

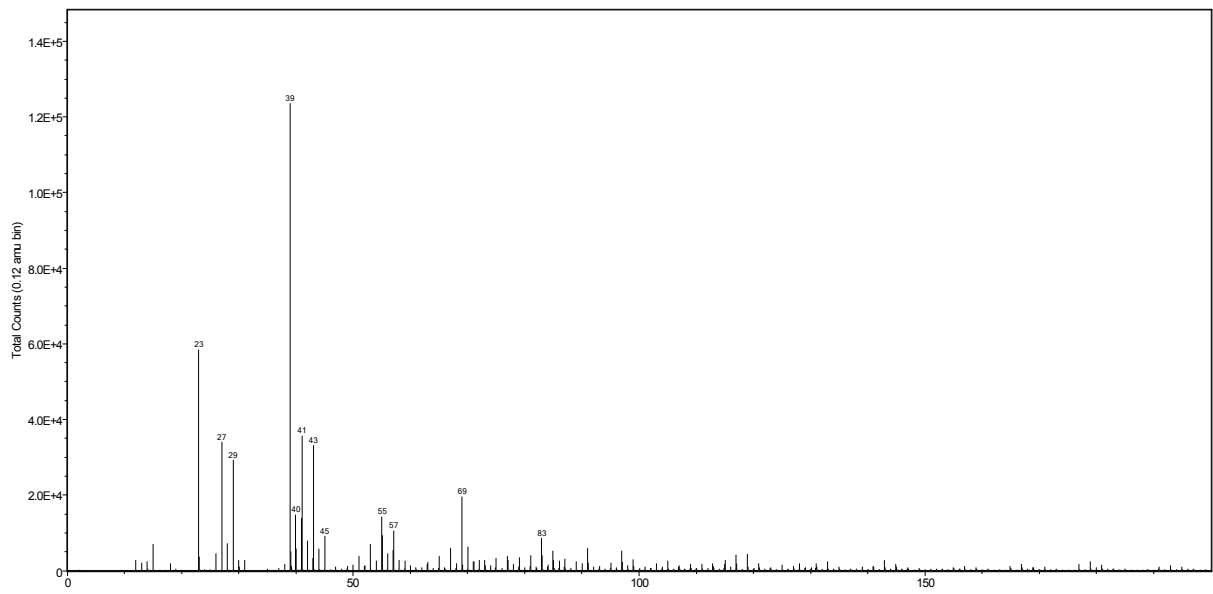
***TCE dry***



*TCE wet*

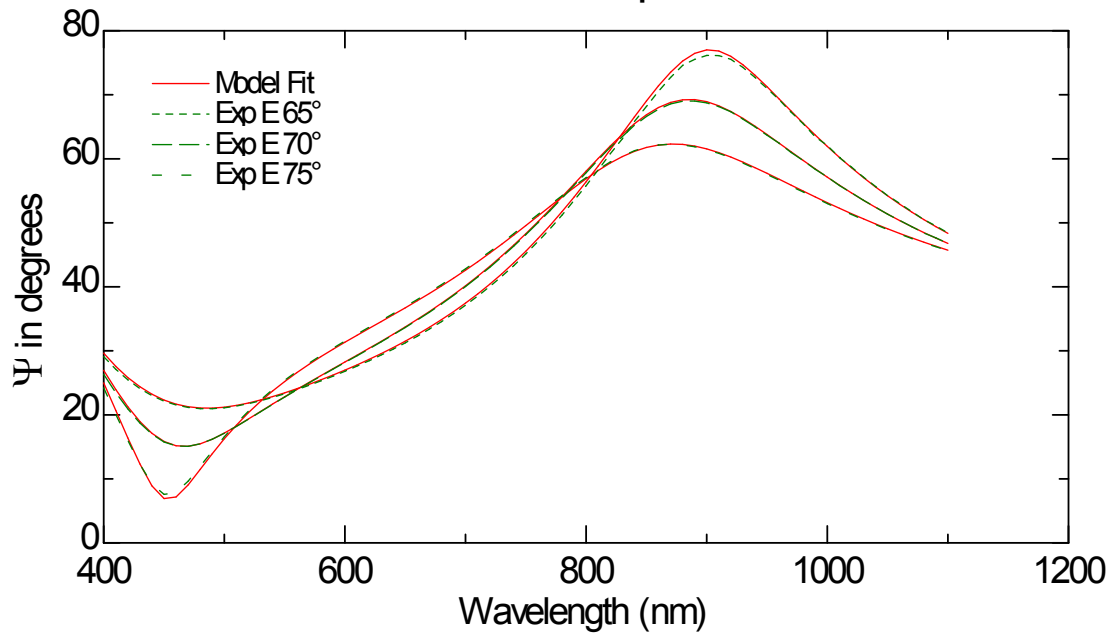


*TCE washed*

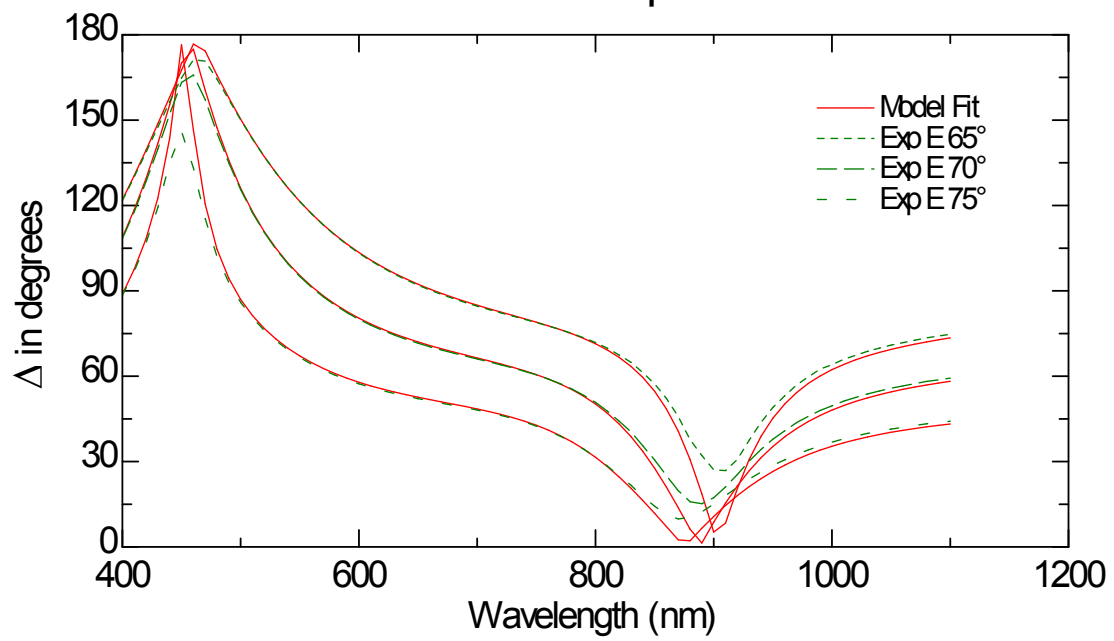


# Ellipsometer

## Generated and Experimental



## Generated and Experimental



The Cauchy equation:

$$n(\lambda) = A + \frac{B}{\lambda^2} + \frac{C}{\lambda^4}$$

Fitting Parameter	Constant	Software
Wavelength independent index of refraction	A	An
Wavelength dependent index of refraction	B	Bn
Wavelength dependent index of refraction	C	Cn

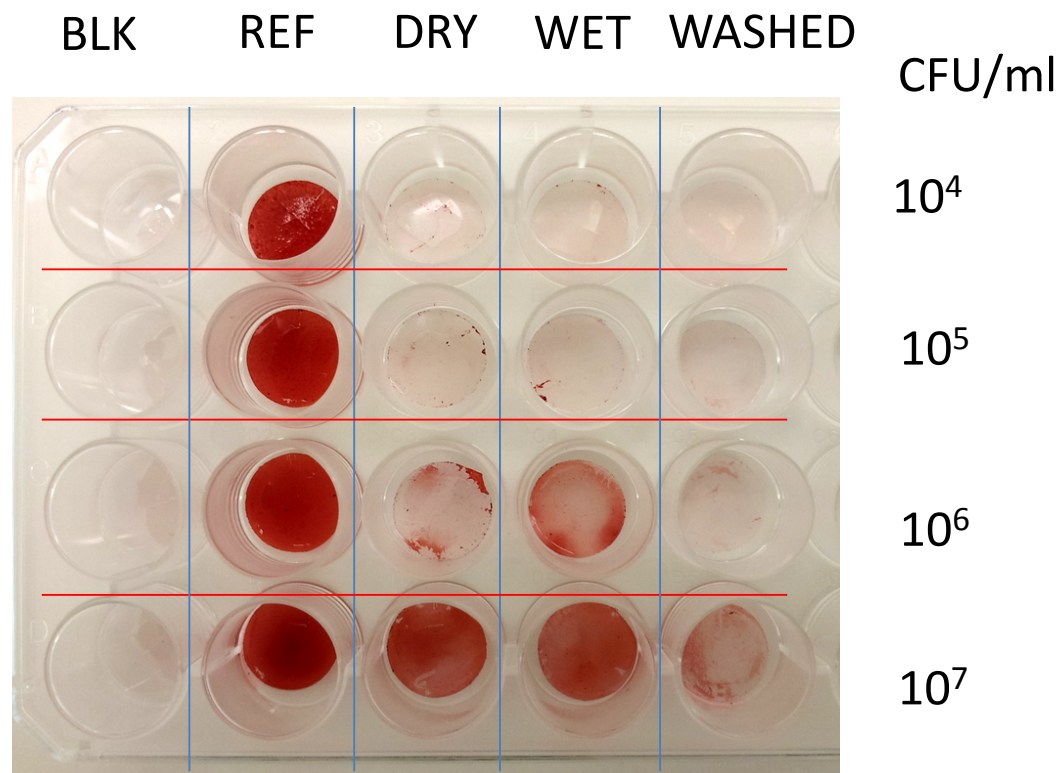
MSE=42.54

Thick.1      169.403±0.203  
An.1          1.6084±0.00239  
Bn.1          0.0020923±0.00196  
Cn.1          0.00095622±0.000305

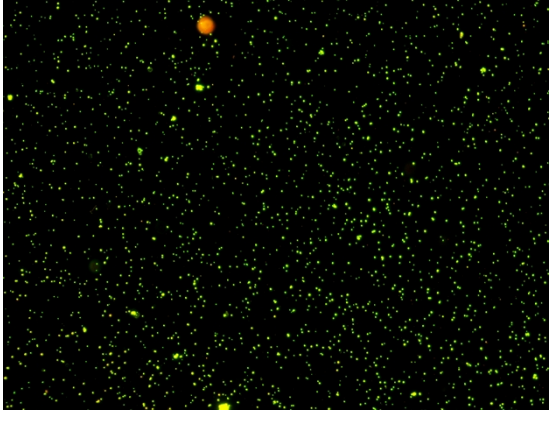
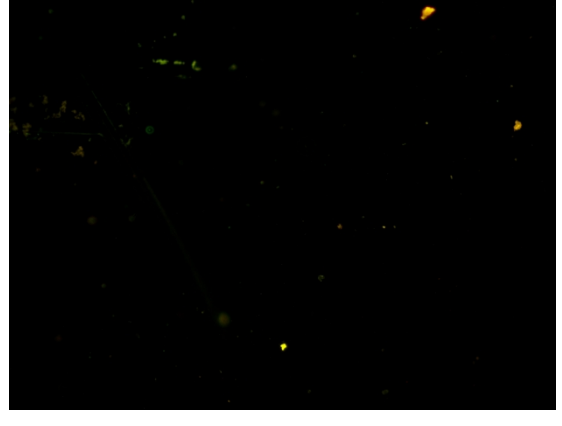
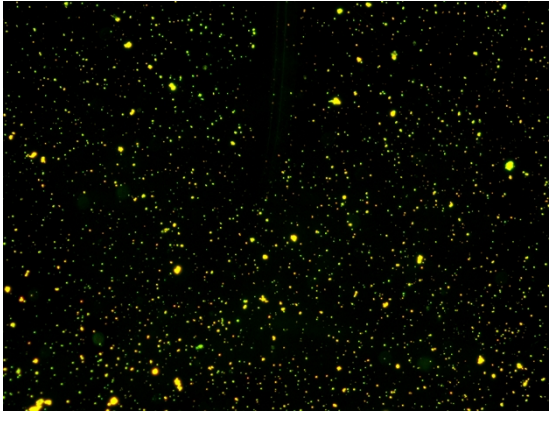
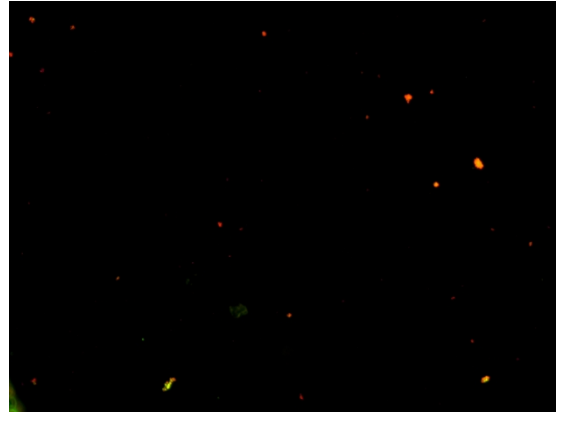
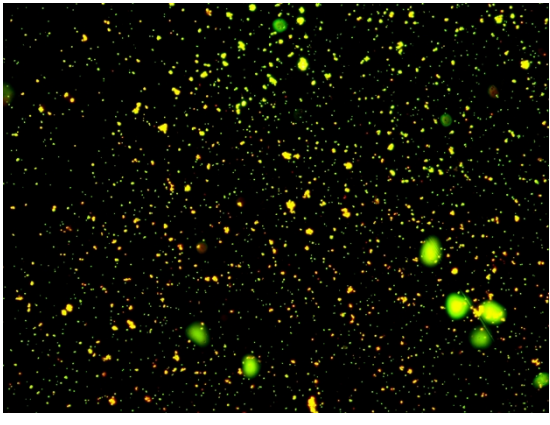
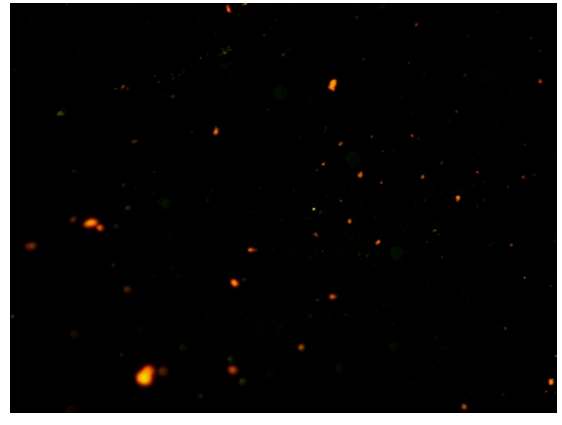
# Images

## Bacterial testing

24h



# Time lapse

Ref		TCEpp dry
	0h	
	2h	
	6h	

(1) Griesser, H. J. *Vacuum* 1989, 39, 485.

(2) Coad, B. R.; Scholz, T.; Vasilev, K.; Hayball, J. D.; Short, R. D.; Griesser, H. J. *ACS Applied Materials & Interfaces* 2012, 4, 2455.

(3) Koeffler, H. P.; Billing, R.; Lusic, A. J.; Sparkes, R.; Golde, D. W. *Blood* 1980, 56, 265.