Journal of Visualized Experiments

Electronic measurements of cellular adhesion using Field-Effect Transistor Cell-substrate Impedance Sensing (FETCIS)

--Manuscript Draft--

Manuscript Number:	JoVE52430R5
Full Title:	Electronic measurements of cellular adhesion using Field-Effect Transistor Cell-substrate Impedance Sensing (FETCIS)
Article Type:	Invited Methods Article - JoVE Produced Video
Keywords:	Field-Effect Transistor Cell-substrate Impedance Sensing (FETCIS), label-free, real-time, cell adhesion, cancer cell, confluent culture, single cell, pharmacology
Manuscript Classifications:	5.1.370.225.500.223: Cell Culture Techniques; 5.1.370.225.500.223.500: Primary Cell Culture; 5.5.200.500.223: Cell Culture Techniques; 5.5.200.500.223.500: Primary Cell Culture; 5.5.240.249: Cell Culture Techniques; 5.5.242.223: Cell Culture Techniques; 5.5.242.223.500: Primary Cell Culture; 7.4.299.117: Cell Adhesion; 8.1.181.529.307: Electrochemistry; 93.33.32: field effect transistors (FET)
Corresponding Author:	Sven Ingebrandt, Ph.D. University of Applied Sciences Kaiserslautern Zweibrücken, Rhineland-Palatine GERMANY
Corresponding Author Secondary Information:	
Corresponding Author E-Mail:	sven.ingebrandt@fh-kl.de
Corresponding Author's Institution:	University of Applied Sciences Kaiserslautern
Corresponding Author's Secondary Institution:	
First Author:	Jessica Ka-Yan Law, Ph.D.
First Author Secondary Information:	
Other Authors:	Jessica Ka-Yan Law, Ph.D.
	Anna Susloparova
	Dieter Koppenhöfer
	Xuan Thang Vu, Dr. rer. nat.
	Felix Hempel
Order of Authors Secondary Information:	
Abstract:	In this work, Field-Effect Transistor Cell-substrate Impedance Sensing (FETCIS) is proposed to examine cellular adhesion on protein-coated transistors with improved spatial resolution. Cellular adhesion is often used as an indicator in many in vitro cytotoxicity assays. However, most of those assays involve staining dyes which may interfere with the tested compounds. In our approach, FETCIS is used to detect impedance changes of the transistors due to cellular adhesion and detachment processes. Compared to the commercially available Electrical Cell-substrate Impedance Sensing (ECIS) systems, the size of the detection transistors of FETCIS can reach down to a single cell and even subcellular resolution. In the present study, FET devices with flat topography were designed and fabricated to enhance better cellular attachment and to shift the recorded effects to a lower frequency range. To verify the sensitivity of FETCIS, confluent, low density, and single cell cultures were tested with the system. Chemical and mechanical removal of confluent cultures and single cell from the transistor were performed and clear impedance changes due to the absence and presence of cells were observed. With the help of a simplified, equivalent electrical circuit model, the measured impedance spectra can be explained. Furthermore, the FETCIS system was used for real-time monitoring of the effects of

	anti-cancer drugs and nanoparticles on cancer cells. The results of our study indicate the applicability of FETCIS for cancer research and for analyzing pharmacological effects of the tested compounds opening up new possibilities in future ECIS assays.
Author Comments:	Dear JoVE editor, I would like to submit our manuscript, "Electronic measurements of cellular adhesion using Field-Effect Transistor Cell-substrate Impedance Sensing (FETCIS)" on behalf of all the authors, as discussed with Dr. Eric Veien. In this letter, the sensor technology, the measurement principle, and its biological and pharmacological applications are briefly stated below. Electric Cell-substrate Impedance Sensing (ECIS) is one example for electronic biosensors able to detect the adhesion of a colony of - most of the time confluent - cells on technical substrates, via impedance changes on large metal microelectrodes (250 µm diameter). Here we introduce an alternative, label-free electronic platform named FETCIS to supplement ECIS with the possibility to reach down to a single cell resolution. The used quasi-planar FET devices were fabricated in our group and optimized to measure the attachment and detachment process of a single cell. Confluent and low density cell cultures were used to prove that the sensitivity of the devices and the amplifier system can reach down to single cell resolution. Using impedance spectrometry, significant differences in impedance spectra were observed between the cell-free and cell-covered transistors in both confluent and single cell cultures. The results show that our FET devices and amplifier system are suitable for detecting cell adhesion down to single cells. In order to use the present system as a pharmacological platform for studying cytotoxicity drug effects on cancer cells, the standard anti-cancer drug topotecan hydrochloride and silica nanoparticles were applied to human lung cancer cells cultured on the FET devices. Real-time cell detachment processes due to cytotoxicity were monitored by FETCIS. Our novel measurement technique clearly demonstrates a single cell resolution, which was the aim in the ECIS community for many years. By far, there is no label-free method, which can be used to measure the direct adhesion strength between cell and substrate in a swift and straightforward approach. We hope that by this publication, we reach a lot of researchers in the field and open a whole-new direction within the biosensor community. Sincerely Prof. Dr. Sven Ingebrandt
Additional Information:	
Question	**Response**
If this article needs to be "in-press" by a certain date to satisfy grant requirements, please indicate the date below and explain in your cover letter.	
If this article needs to be filmed by a certain date to due to author/equipment/lab availability, please indicate the date below and explain in your cover letter.	

Fachhochschule Kaiserslautern
University of Applied Sciences

Fachhochschule Kaiserslautern ■ Amerikastraße 1 ■ 66482 Zweibrücken

The Editor, Journal of Visualized Experiments

1 Alewife Center
Cambridge, MA 02140
USA

Contact Person	Contact Tel./Fax	E-Mail	Date
Prof. Dr. Sven Ingebrandt	+49631 37245413	sven.ingebrandt@fh-kl.de	23.06.2014

Dear JoVE editor,

I would like to submit our manuscript, “Electronic measurements of cellular adhesion using Field-Effect Transistor Cell-substrate Impedance Sensing (FETCIS)” on behalf of all the authors, as discussed with Dr. Eric Veien. In this letter, the sensor technology, the measurement principle, and its biological and pharmacological applications are briefly stated below.

Electric Cell-substrate Impedance Sensing (ECIS) is one example for electronic biosensors able to detect the adhesion of a colony of – most of the time confluent – cells on technical substrates, via impedance changes on large metal microelectrodes (250 µm diameter). Here we introduce an alternative, label-free electronic platform named FETCIS to supplement ECIS with the possibility to reach down to a single cell resolution.

The used quasi-planar FET devices were fabricated in our group and optimized to measure the attachment and detachment process of a single cell. Confluent and low density cell cultures were used to prove that the sensitivity of the devices and the amplifier system can reach down to single cell resolution. Using impedance spectrometry, significant differences in impedance spectra were observed between the cell-free and cell-covered transistors in both confluent and single cell cultures. The results show that our FET devices and amplifier system are suitable for detecting cell adhesion down to single cells. In order to use the present system as a pharmacological platform for studying cytotoxicity drug effects on cancer cells, the standard anti-cancer drug topotecan hydrochloride and silica nanoparticles were applied to human lung cancer cells cultured on the FET devices. Real-time cell detachment processes due to cytotoxicity were monitored by FETCIS.

Our novel measurement technique clearly demonstrates a single cell resolution, which was the aim in the ECIS community for many years. By far, there is no label-free method, which can be used to measure the direct adhesion strength between cell and substrate in a swift and straightforward approach. We hope that by this publication, we reach a lot of researchers in the field and open a whole-new direction within the biosensor community.

Sincerely

Prof. Dr. Sven Ingebrandt

Fachhochschule Kaiserslautern
University of Applied Sciences

Fachhochschule Kaiserslautern ■ Amerikastraße 1 ■ 66482 Zweibrücken

List of authors and their contribution to this work is stated below:

Dr. Jessica Ka Yan Law: Organization and writing of manuscript

Anna Susloparova*: Established the data fitting model and performed simulations

Dieter Koppenhöfer*: Performed cell experiments and data analysis

Dr. Xuan Thang Vu: Chip fabrication

Felix Hempel: Manuscript planning

Prof. Dr. Sven Ingebrandt: Corresponding author

*: Equal contribution

Title:
Electronic measurements of cellular adhesion using Field-Effect Transistor Cell-substrate Impedance Sensing (FETCIS)

Authors:
Law, Jessica Ka Yan
Department of Informatics and Microsystem Technology
University of Applied Sciences Kaiserslautern
Zweibrücken, Germany
jessica.law@fh-kl.de

Susloparova, Anna*
Department of Informatics and Microsystem Technology
University of Applied Sciences Kaiserslautern
Zweibrücken, Germany
anna_susloparova@web.de

Koppenhöfer, Dieter*
Department of Informatics and Microsystem Technology
University of Applied Sciences Kaiserslautern
Zweibrücken, Germany
dieter.koppenhoefer@web.de

Vu, Xuan Thang
Department of Informatics and Microsystem Technology
University of Applied Sciences Kaiserslautern
Zweibrücken, Germany

Current address:
Physikalisches Institut IA
RWTH Aachen University
Aachen, Germany
vu@physik.rwth-aachen.de

Hempel, Felix
Department of Informatics and Microsystem Technology
University of Applied Sciences Kaiserslautern
Zweibrücken, Germany
felix.hempel@fh-kl.de

Ingebrandt Sven
Department of Informatics and Microsystem Technology
University of Applied Sciences Kaiserslautern
Zweibrücken, Germany
sven.ingebrandt@fh-kl.de
Tel: +49 631 3724 5413

Corresponding author: Sven Ingebrandt, Prof. Dr.

*Authors contributed equally to this work.

Keywords:
Field-Effect Transistor Cell-substrate Impedance Sensing (FETCIS), label-free, real-time, cell adhesion, cancer cell, confluent culture, single cell, pharmacology

Short Abstract:
Here, we present a protocol to study cellular adhesion using field-effect transistor (FET) devices. This is a label-free and real-time electronic platform complementing the established Electrical Cell-substrate Impedance Sensing (ECIS) method for measurements of adhesion and detachment of confluent cell cultures and is pushing spatial resolution down to single cell.

Long Abstract:
In this work, Field-Effect Transistor Cell-substrate Impedance Sensing (FETCIS) is proposed to examine cellular adhesion on protein-coated transistors with improved spatial resolution. Cellular adhesion is often used as an indicator in many in vitro cytotoxicity assays. However, most of those assays involve staining dyes which may interfere with the tested compounds. In our approach, FETCIS is used to detect impedance changes of the transistors due to cellular adhesion and detachment processes. Compared to the commercially available Electrical Cell-substrate Impedance Sensing (ECIS) systems, the size of the detection transistors of FETCIS can reach down to a single cell and even subcellular resolution. In the present study, FET devices with flat topography were designed and fabricated to enhance better cellular attachment and to shift the recorded effects to a lower frequency range. To verify the sensitivity of FETCIS, confluent, low density, and single cell cultures were tested with the system. Chemical and mechanical removal of confluent cultures and single cell from the transistor were performed and clear impedance changes due to the absence and presence of cells were observed. With the help of a simplified, equivalent electrical circuit model, the measured impedance spectra can be explained. Furthermore, the FETCIS system was used for real-time monitoring of the effects of anti-cancer drug and nanoparticles on cancer cells. The results of our study indicate the applicability of FETCIS for cancer research and for analyzing pharmacological effects of the tested compounds opening up new possibilities in future ECIS assays.

Introduction:
Field-effect devices have been used for several biomedical applications ranging from the detection of cancer marker proteins[1] and DNA[2] to cells[3]. The main advantage of such devices is their high sensitivity to changes in surface potential at the liquid-solid interface. For cell-adhesion studies, a device with comparable size of a single cell is preferable and silicon-based field-effect transistors (FET) fit into these needs. Compared to the commercially available Electrical Cell-substrate Impedance Sensing (ECIS) system which based on gold electrodes[4,5], the FETs are not limited to the size of

the sensors and thus can be applied to study single cells. Typical sizes of the commercial metal electrodes in ECIS range from 25 µm to 250 µm in diameter. Due to the limited size of the detection electrodes, collective instead of individual responses of cells are measured. The data obtained from such averaged cell populations might conceal individual reactions associated to the heterogeneous properties of cells[6]. Therefore it should be of interest for certain specific applications to analyze individual reactions of cells. In the present study, an impedance system using FET, named Field-Effect Transistor Cell-substrate Impedance Sensing (FETCIS), for detecting cell adhesion is presented.

Electronic transistor-transfer function (TTF) was measured in the present study. A combination of bandwidth limiting effect of reference electrode, electrolyte solution, the cell covering the transistor, the transistor itself including contact lines on chip, and the first amplifier stage, are reflected by the measured TTF spectrum. By applying a sinusoidal signal with 10 mV amplitude and varying frequency from 1 Hz to 1 MHz via the reference electrode, changes in bandwidth caused by attaching different biological samples to the gate oxide of the FETs were identified[3,7]. Responses of the biological sample to the AC voltage are frequency-dependent. From the recorded frequency spectrum of the system, characteristics and properties of the attached biological sample can be deduced[8]. Generally an electronic transfer function, denoted as H in the present study, is defined by the ratio of the input and the output voltage of the amplifier. It is the signal transfer of a sinusoidal signal between the reference electrode input U_{in} and the signal output after the transimpedance amplifier stage U_{out} **(Figure 1)**. With our in-house built FETCIS system, only the real part of the TTF is recorded[9]. This system was further optimized and with the help of a fast lock-in amplifier full spectra including amplitude and phase information can be recorded, which were used to interpret the recorded effects[9].

To fully explain the recorded spectra, the FET device in contact with an adherent cell can be described using an equivalent-electrical circuit (EEC) **(Figure 1)**, which is derived from the most simple point-contact model[10]. This model was described before and is typically applied as the easiest approach to explain extracellular recorded action potentials from neurons[11,12] or cardiac myocytes[13]. In brief, the capacitance of the adherent cell membrane C_M, the resistance of the cell membrane R_M, and the resistance of the thin, electrolyte-filled cleft between the cell and transistor R_{seal} represent the passive element of the EEC model. The cellular membrane is further divided into free (C_{FM}, R_{FM}) and attached parts (C_{JM}, R_{JM}). The contact line capacitances and resistances of source and drain are included in the EEC with C_{source}, C_{drain} and R_{source}, R_{drain}, respectively. A combined resistance R_{el} is represented by the reference electrode and the electrolyte. In order to optimize the FETCIS sensor for highest resolution in TTF recordings, different simulations have been performed[14]. Sensitive FETCIS sensors show large TTF differences between the cell-adhered and the cell-free case and in addition these effects are favorable to be observed at lower frequencies due to cheaper instrumentations. Based on the simulation results, a new generation of FET device was designed and fabricated for this project.

In order to understand the TTF spectra, an analytical equation representing the transfer function of the EEC has been derived[15]. In this equation several parameters need to be considered, which result in the typical shape of the measured TTF spectra. These parameters include chip-related parameters, transimpedance circuit parameters, and cell-related parameters. By fitting the measured TTF spectrum to the equation, cell-related parameters (R_{seal} and C_M) can be obtained, which indicate the status of the cell. The R_{seal} value indicates how tight the cell adheres on top of the transistor, while the C_M value indicates the shape of the cell. These two cell parameters change according to the cell attachment and detachment processes. The influences of the R_{seal} and C_M on the TTF spectra have been studied by simulation of the EEC circuit in PSpice, a program for electrical circuit simulation. In **Figure 2** an idealized cell detachment process from the transistor surface is shown where the cell does not change its shape during detachment and is only lifted up, stepwise. The figure shows the simulated TTF spectra varying R_{seal} in a range of 1 MΩ to 400 kΩ. By increasing R_{seal} **(Figure 2, from model 5 to model 1)**, a gradual increase of the signal amplitude between 20 and 200 kHz is observed, while the complete removal of the cell can be clearly distinguished from the cell-attached case **(Figure 2)**. On the other hand, the value of the C_M changes when the morphology of the cells is changing during detachment or as a response to external stimuli. In **Figure 3**, an adherent cell is gradually deformed during the detachment process. Simulation was performed by modelling an adherent cell as a hemisphere and a gradually detaching cell from the transistor surface with different spherical caps but keeping the total surface area of the cell membrane constant. In this model, the C_M value is correlated to the ratio changes of the attached to free membrane area. The simulated TTF spectra show that decreasing C_M values lead to smaller TTF amplitude **(Figure 3, from model 1 to model 5)**. With the above simulation results, the TTF spectra changes due to cell attachment and detachment can now be better understood. For a complete picture, however, recordings on a fixed frequency are not enough. For time-dependent processes, recordings of the full spectra and fitting to the model should be done in order to extract the time evolution of the cell-related parameters from the model[15,16], like it was earlier described for ECIS.

In the following manuscript, new FET devices according to previous simulations were designed and fabricated. In order to show that our FETCIS system is feasible and sensitive enough to measure the cellular attachment and detachment processes, different cell types were cultured on the FET devices and their respective TTF spectra were measured. As mentioned earlier, the detection sensitivity of the FET devices is not limited by the size of the transistors. Therefore, it is possible to study the adhesion of single cell using the present FETCIS system. Furthermore, our FETCIS system has been successfully demonstrated as an electronic platform to monitor real-time drug effects on cell adhesions.

Protocol:

1. Preparation of the FET devices and cell cultures

1.1) Fabrication and encapsulation of the quasi-planar FETs

1.1.1) Clean the 4 inch n-type silicon wafer using a modified protocol based on standard Radio Corporation of America (RCA) protocol[17].

1.1.2) Wet thermally grow silicon oxide (SiO_2) on the silicon wafer in the Centrotherm oven at 1000 ^oC for 5 hours.

1.1.2.1) Load wafers to a quartz wafer holder and load wafer holder to the loader of the oven. Flow N_2 (3 SLM) through the quartz tube of the oven. Heat the tube to 800 ^oC, check temperature homogeneity in the tube (± 5 ^oC)

1.1.2.2) Load wafers to the center of the tube. Increase temperature to 1000 ^oC, check temperature homogeneity in the tube (± 5 ^oC). Heat up the water in the bubbler to 90 ^oC.

1.1.2.3) Turn off N_2 flow, turn on O_2 flow (0.5 SLM) through bubbler. The output of the bubbler was connected to the input of the quartz tube. Start count down time to 0.

1.1.2.4) Turn off O_2 flow and disconnect the output of bubbler to the output of the quartz tube. Connect the N_2 (3 SLM) flow through the tube. Turn off the heating power of the quartz tube and let it evolve.

1.1.2.5) When the temperature of the oven reaches 800 ^oC, unload wafer out of the oven. Wait for 2 hours (wafer temperature is equal to room temperature). Remove wafers from the wafer holder and put to the storage box.

Note: All the valves and connection in the oven were controlled automatically by an oven controller software.

1.1.3) Define the contact lines using optical lithography with a positive photoresist (AR-P3100) (Supplementary note: Mask 1). Expose the wafer with aligned optical mask to UV light. Expose the resist on the contact lines to UV light and develop using a developer (AR 300-26: H_2O_2 =1:1 (v/v)) [17].

1.1.4) Etch SiO_2 of the exposed areas by dipping in buffered hydrofluoric acid (BHF) for 16 min.

1.1.5) Remove the photoresist by acetone and isopropanol and then clean the wafer by RCA cleaning protocol[17].

1.1.6) Implant boron ions on the wafer with a dose of $1 \cdot 10^{16}$ ions/cm^2 and energy of 150 keV using commercial services.

1.1.7) Clean the wafer in piranha solution for 10 min at 60 ^oC and then dip in 1% hydrofluoric acid (HF) for 30 seconds.

1.1.8) Anneal the wafer in in 100% N_2 atmosphere at 1000 °C for 1 h in the Centrotherm

oven.

1.1.9) Define the source and drain electrode areas using a second optical lithography (positive resist AR-P3100) and etch SiO_2 on the exposed areas (same parameters as steps 1.1.3, 1.1.4) (Supplementary note: Mask 2).

1.1.10) Remove the photoresist by acetone and isopropanol and then clean the wafer by RCA cleaning protocol[17].

1.1.11) Implant boron ions on the wafer with a dose of $1 \cdot 10^{15}$ ions/cm^2 and an energy of 80 keV using commercial services.

1.1.12) Etch all SiO_2 layers on the wafer in BHF solution for 15 min as in step 1.1.4. Clean the wafer by the RCA protocol[17].

1.1.13) Anneal the wafer for 5 min in 100% N_2 at 950 °C and then wet oxidation for 60 min at 950 °C. See detail in 1.1.2. Temperature in step 1.1.2.5 was set to 950 ^oC.

1.1.14) Define the gate area and the electrical contacts of source and drain by a third optical lithography using positive photoresist (AR-P3100) (Supplementary note: Mask 3). See detail in 1.1.3.

1.1.15) Wet-etch the wafer by dipping in BHF solution for 3 min and then in 1% HF until the SiO_2 is totally etched. Remove the resist by acetone and isopropanol.

1.1.16) Clean the wafer by the RCA protocol[17]. Afterwards, thermally grow a 6 nm thick SiO_2 as gate dielectrics using a dry oxidation protocol.

1.1.16.1) Heat the oven up to 800 ^oC, flow N_2 (3 SLM) through the tube.

1.1.16.2) Load wafer into the tube. Heat the oven up to 820 ^oC. Turn off N_2 flow, flow O_2 (0.5 SLM) through the oven and wait for 45 min.

1.1.16.3) Turn off O_2 flow, turn on N_2 flow. Turn the heating off.

1.1.16.4) Wait the temperature of the oven reaches 800 ^oC, unload wafer from the tube. Wait for 2 hours (wafer temperature is equal to room temperature). Remove wafers from the wafer holder and put to the storage box.

1.1.17) Spin coat AR U4040 on wafer (2000 rpm, 60 s) and heat the wafer at 90 °C for 2 min. Expose the resist-coated wafer with source and drain contacts mask (Supplementary note: Mask 4) under a broadband UV for 6 s (36 mJ/cm^2) and then post-bake at 115 °C for 5 min.

1.1.17.1) Completely expose the wafer without mask under a broadband UV for 20 s (120 mJ/cm^2). Finally develop the resist in developer (AR-300-46: H_2O_2 = 1:3 (v/v) for

20 s and then rinse in DI water.

1.1.18) Wet-etch the SiO_2 on the source and drain contacts by submerging in 1% HF for 2 min directly before the metal evaporation.

1.1.19) Deposit a metal stack of 200 nm of aluminum (rate 5 A/s, power 48%), then 20 nm of titanium (rate 5 A/s, power 25%) and lastly 100 nm of gold (rate 5 A/s, power 21.5%) using electron beam evaporation.

1.1.20) Lift-off the metal on the resist on the wafer in acetone for 20 min with the help of an ultrasound source. Clean wafer by isopropanol and dry wafer with N_2 gas.

1.1.21) Anneal the wafer at 400 °C in 100% N_2 for 10 min.

1.1.22) Protect the wafer by a resist and cut the 4 inch wafer to obtain the individual 7×7 mm^2 FET chips.

1.1.23) Sonicate the FETs in acetone for 5 min to remove the protection resist. Repeat the sonication step with new acetone and then isopropanol.

1.1.24) Apply a small amount of epoxy 377 to the middle of a 68-pin Leaded Chip Carrier. Glue the FET at the center and bake the FET device for 1 h at 150 °C.

1.1.25) Wire bond the FET to the carrier using aluminum wire. Use wedge-wedge bond techniques for the wire bonding[18].

1.1.26) Mix the silicon adhesive 96-083 in the ratio of 1:10. Apply a thin layer of the silicon adhesive mixture to two glass rings with inner diameters of 3 mm and 17 mm, respectively.

1.1.27) Glue the small glass ring to the center of the FET and the big glass ring on the carrier to form a cell culture chamber using the silicon adhesive 96-083 mixture. Bake the FET device for 30 min at 120 °C.

1.1.28) Fill in the space between the small and big glass rings with silicon adhesive 96-083 mixture. Bake the FET device for 1 h at 130 °C.

1.2) Cleaning and coating of the FET device for cell cultures

1.2.1) Clean the FET surface with 70% ethanol soaked cotton buds. Sonicate the FET devices in distilled water (5 min), 2% Hellmanex III solution (5 min), and then distilled water (5 min).

1.2.2) Dry the FET device and put 20 µl of 20% sulfuric acid to the FET surface. Incubate the FET device for 30 min at 80 °C. Sonicate the FET device in distilled water for 5 min.

1.2.3) Fill the FET chamber with 1 ml of 70% ethanol and wait for 5 min. Replace the 70% ethanol with sterile distilled water. Repeat for 3 times.

1.2.4) Dry the FET devices under the laminar flow.

1.2.5) Add 10 µl of 0.1 mg/ml fibronectin to the FET surface and incubate for 3 h at 37 °C. Afterwards, remove the fibronectin and rinse the FET device with sterile distilled water once.

1.2.6) Add 50 µl of cell suspension (confluent culture: 10,000 to 20,000 cells; low density culture: 1,000 to 3,000 cells) to each FET device and wait for 30 min. Then add 1 ml of respective culture medium to fill up the FET chamber. Incubate for 24 h under standard condition (37 °C, 5% CO_2) before impedance measurement.

1.2.6.1) For human malign melanoma SkMel28 cells, use Dulbecco's Modified Eagle's Medium (supplemented with 10% Fetal Calf Serum, 10,000 U/10 mg Penicillin/Streptomycin and 20 mM L-Glutamine in 100 ml of the medium).

1.2.6.2) For human Embryonic Kidney HEK293 cells, use Minimal Essential Medium (supplemented with 10% Fetal Calf Serum, 10,000 U/10 mg Penicillin/Streptomycin, 20 mM L-Glutamine, 1% Non-Essential Amino Acid, and 50 mg G418 in 100 ml of the medium).

1.2.6.3) For human lung adenocarcinoma epithelial H441 cells, use Roswell Park Memorial Institute medium (supplemented with 10% Fetal Calf Serum, 10,000 U/10 mg Penicillin/Streptomycin and 20 mM L-Glutamine in 100 ml of the medium).

2. Impedimetric analysis of cellular adhesion and detachment

2.1) Connect the portable 16-channel amplifier system to the measurement computer. Connect the FET device with cultured cells to the amplifier. Insert an external Ag/AgCl reference electrode to the cell chamber of the FET and connect to the amplifier.

2.2) Start the BioMol software. Measure the drain-source current of all 16 FET devices by applying gate-source voltage (50 measurement points, from 0 V to -3 V) at constant drain-source voltage (0 V, -1 V, -2 V, -3 V).

2.3) As the program calculates the transconductance value (g_m) automatically by dividing the measured drain-source current with the applied gate-source voltage, set drain-source and gate-source voltages to the values at which transconductance is maximum.

2.4) Apply 10 mV signal ranging from 1 Hz and 1 MHz via the Ag/AgCl reference electrode. Measure TTF from 16 transistors simultaneously within 1 min.

2.5) Switch off the amplifier. Disconnect the reference electrode and the FET device from the amplifier system. Replace the medium in the cell chamber of the FET with 0.5% trypsin. Incubate the FET device for 30 min at 37 °C.

2.6) Switch on the amplifier. Reconnect the FET device and reference electrode to the amplifier. Set the voltages to the same value as in 2.3. Measure the impedance of the transistor again as step 2.4.

3. Verification of the single cell impedance measurement

3.1) Measure the TTF from the FET devices with low density cell culture.

3.2) Pull a patch-clamp pipette with diameter of 10-15 µm (Parameters for the pipette puller: Heat = 400, Filament = 5, Velocity = 20, Delay = 200, Pull = 0).

3.3) Place the patch-clamp pipette next to a single adhered cell on the transistor. Suck the patch-clamp pipette in order to apply under pressure for the single cell to attach on the patch-clamp pipette. Remove the cell by moving away the patch-clamp pipette under the Differential Interface Contrast microscope.

3.4) Measure the TTF in the range of 1 Hz and 1 MHz again as step 2.4.

4. Real-time monitoring of the effect of nanoparticles and topotecan hydrochloride on human lung cells

4.1) Prepare the FET devices as described in step 1.2.

4.2) Dilute NexSil20 nanoparticles to a concentration of 60 µg/ml and 600 µg/ml; and topotecan hydrochloride to a concentration of 10 µg/ml, in H441 culture medium[8,9].

4.3) Characterize the FET device with cultured cells as described in step 2.1 to 2.4.

4.4) Place the portable amplifier system into a regulated incubator (37 °C, 5% CO_2). Replace the culture medium with NexSil20- or topotecan hydrochloride-containing medium. Start the time-dependent TTF measurement at a constant 200 kHz frequency and measure for 2 h and 30 min.

4.5) Measure the TTF in the range of 1 Hz and 1 MHz again as step 2.4.

Representative Results:
Quasi-planar FET devices have been fabricated and used in the present study (**Figure 4**). Each FETCIS has 16 individual transistors (25 µm in width and 5 µm in length) with a distance of 200 µm in between. The FET devices have an almost flat SiO_2 surface **(Figure 5a)** with only a 220 nm step height at the 16 transistors gates **(Figure 5b)**. Flat topography is important for migration and cellular adhesion experiments. Previously, cells preferentially adhered to the edges of the contact lines instead of the sensor area

(Figure 6a). With the optimized flat topography, cells are widely spread on the whole device surface **(Figure 6b)**.

Confluent and low density cell cultures were used in the present study in order to prove that the sensitivity of the FET devices and the amplifier system can reach down to single cell resolution. Human malign melanoma cells with polygonal morphology were used as an example in this case. The FET surface is completely or partially covered by the cells **(Figure 7a and Figure 7b)**. Using FETCIS, significant differences in the spectra were observed between the cell-free and cell-covered transistors in both confluent and single cell cultures **(Figure 7c and Figure 7d)**. The results show that our FET devices and amplifier system are suitable for detecting cell adhesion from confluent down to single cell cultures. To further confirm the single cell measurements, TTF of a transistor with an adhered single cell was measured before and after this cell was mechanically removed by a patch-clamp pipette. A clear difference was observed between the spectra **(Figure 8)**.

In order to use the present system as a pharmacological platform to study cytotoxicity drug effects on cancer cells, the standard anti-cancer drug topotecan hydrochloride and silica nanoparticles NexSil20 were applied to the H441 human lung cancer cells cultured on the FET devices. Real-time cell detachment processes due to drug cytotoxicity were monitored by FETCIS. Compared to the transistors without cells on top (Channel 14), significant changes in the spectra (Channel 9, 10, 13) were observed after the administration of the drug **(Figure 9)**. To extend the application of our electronic platform to study other possible anti-cancer drugs, NexSil20 nanoparticles were tested against the lung cancer cells. The nanoparticles led to an increase of signal amplitude after 30 min **(Figure 10)** indicating cellular apoptosis or necrosis.

Figure 1: An equivalent electronic circuit, which describes an FET device in contact with an adherent cell on top of the transistor gate. The cell membrane is typically divided into a free part (C_{FM}, R_{FM}) and a part in the cellular junction (C_{JM}, R_{JM}). To complete this circuit, the inner resistance of the cell (R_{in}) and the electrolyte-filled cleft of the junction area (R_{seal}) are included. Chip-related elements include gate oxide capacitance (C_{ox}), the transconductance (g_m), and the output resistance (R_{DS}). The parasitic parameters like contact line capacitances and series resistances of source and drain are contributing to the EEC with C_{source}, C_{drain} and R_{source}, R_{drain}, respectively. The reference electrode and the electrolyte solution contribute as a combined series resistance R_{el}. This figure has been modified from[14].

Figure 2: Modelling of the cellular detachment from the transistor surface without changes in cell morphology. The detaching cell from model 1 to model 5 does not change its shape during the detachment process. TTF spectra changes by varying the distance between the cell and the transistor (seal resistance R_{seal}) in a wide range (from 1 MΩ to 400 kΩ).

Figure 3: Modelling of an adherent cell to the transistor surface with simple geometrical shape changes. During detachment, there is a reduction in cell attached

area and an increase in cell height. The simulated TTF spectra for cell-covered transistor gate (model 1), transistor gate with the gradually detached cell (model 2 - 4) and for cell-free transistor gate (model 5) are shown.

Figure 4: Schematic cross-section of the FET device fabrication process. (1) Wet oxidation to grow 1 µm thick of SiO_2 on the n-type silicon wafer. (2) Defining the source and drain contact lines: first optical lithography, wet-etching of SiO_2 in HF, boron ions implantation, and dopen activation. (3) Defining the source and drain of the FET: second optical lithography, shallow boron ions implantation. (4) Complete removal of all SiO_2 layer on the wafer. (5) Growth of 220 nm of SiO_2 as passivation layer. (6) Open the FET's gate and the source and drain contacts against the oxide passivation layer: third optical lithography, wet-etching of SiO_2. (7) Dry oxidation to grow 6 nm SiO_2 as gate insulator. (8) Metallization of the source and drain contacts using lift-off techniques: fourth optical lithography, etching of SiO_2 on the contacts, Al/Ti/Au metal deposition, lift-off in acetone, ohmic contact formation by annealing at 400 °C in N_2 atmosphere. This figure has been modified from[14].

Figure 5: Close-up and AFM images of the planar field-effect transistor surfaces. Zoom in to an individual gate contact opening with the gate dimensions 5×12 μm^2 (Scanning electron microscopy, colored) (a). The modification of the fabrication protocol resulted in a much flatter chip surface, where only the gate areas are embedded in about 220 nm deep holes (b). This figure has been modified from[14].

Figure 6: Microscopic images of the HEK293 cells on the former and the new FET devices. Preferential adhesion of cells to the edges of the contact lines on the surfaces can be observed on the former device (a). Flatter cell morphologies can be observed on the new designed device (b). This figure has been modified from[14].

Figure 7: Microscopic images and transistor-transfer functions (TTF) of the transistor gates covered by confluent and low density of SkMel28 cells. SkMel28 cells have a polygonal morphology and are cultured on fibronectin-coated transistor surfaces. Confluent cell culture (a) and adhered single cells (b) on individual transistors can be achieved by optimizing the number of cells seeded on the FET surfaces. By measuring the TTF, significant differences between the cell-covered and cell-free transistors were measured in a frequency range of 200 to 800 kHz ($p < 0.001$, $n = 20$) (c). Similar result was obtained in low density culture, where significant differences were observed in a frequency range of 500 to 750 kHz ($p < 0.05$, $n = 10$) (d).

Figure 8: Microscopic images and transistor-transfer functions (TTF) of the transistor gates before and after the removal of a single adhered cell with a patch-clamp pipette. Microscopic image of two transistor gates is shown (a). Transistor gate 8 is covered by a single HEK293 cell while transistor gate 7 is cell-free. Comparison of the TTF spectra for the transistor gate 8 before and after removal of the single cell with a patch-clamp pipette (b). Microscopic image after removal of the HEK293 cell from the transistor gate 8 with a patch-clamp pipette is shown (c).

Figure 9: Time-dependent measurement of the anti-cancer drug effects on H441 cells. Real-time measurements at a constant frequency of 200 kHz of the transistors are shown (a). Changes in signal amplitudes are observed within 1 h. Recorded impedance spectra before and after the administration of topotecan hydrochloride (b). Microscopic image of H441 on the FET device is shown (c). Morphological changes of the cells due to the application of topotecan hydrochloride after 2 h and 30 min (d). This figure has been modified from[9].

Figure 10: Real-time transistor-transfer function measurements of H441 covered FET devices with and without nanoparticles NexSil20. Presence of nanoparticles (600 µg/ml) does not affect the TTF of the cell-free gates (a). Negative control shows fluctuations in signal amplitude alone with viable cells on the transistor gates (b); while low dose treatment (60 µg/ml) shows slight signal amplitude increase (c). High dose treatment (600 µg/ml) causes visible amplitude value changes in the first 30 min indicating cell apoptosis or necrosis (d).

Supplementary Figure 1: Mask 1: Define contact lines.

Supplementary Figure 2: Mask 2: Define source and drain.

Supplementary Figure 3: Mask 3: Open source and drain contacts and gate area.

Supplementary Figure 4: Mask 4: Lift-off for the source and drain contacts.

Discussion:

Silicon FETs have been extensively used in the field of biosensors for different biological applications due to the possibility of minimizing the detection electrodes without affecting their sensitivities. By incorporating the miniature FET devices with impedance spectroscopy, cell adhesion strength down to single cell resolution can be measured in the present study. With the help of simulation results, parameters of a new generation of FET devices have been optimized for measuring cell adhesion[14]. With higher R_{seal} values, generally larger differences between the cell-adhered and the cell-free TTF spectrum can be achieved **(Figure 2)**. In this study, new FET devices with almost flat topography were designed and fabricated **(Figure 5)**. With the flat surface, adherent cells can adhere better and form a tighter junction on top of the transistor **(Figure 6b)**. This will in term enhance the differences of the spectra recorded in FETCIS measurements. Besides, smaller resistances and larger capacitances of the contact lines are beneficial for impedance sensing of cellular adhesion. Therefore all these parameters have been considered and optimized during the fabrication of the FET devices. All steps in the fabrication process of the FET devices are important. Otherwise the fabricated devices may not have the same sensitivities and characteristics as described in the present study.

Significant differences between the cell-adhered and cell-free transistors were observed **(Figure 7)**. Adhesion status of the cells to a protein-coated substrate is important to examine the efficacy and the specificity of drug effects on cancer cells. This is usually

studied by microscopic observations. With the presented data it was confirmed that our FETCIS system can be used as an electronic platform for detecting cell adhesion and detachment. In the well-known and established ECIS systems, typically confluent cell cultures are used due to the limitation of recording electrode sizes. However, in many research fields such as immunology and neurosciences, single cell assays would be preferred. Smaller detection electrodes are required for such low density cultures so that individual cell responses can be studied. We presented here, that such low density cultures **(Figure 7)** and even single cell cultures **(Figure 8)** can be applied to our FETCIS system. The sensitivity of FETCIS on single cells is comparable to the sensitivity of ECIS on confluent cultures opening up new possibilities and new assays in Cell-Substrate Impedance Sensing.

Standard methods for studying cell cytotoxicity are normally based on measurements of metabolic activity or membrane integrity of healthy cells using immunostaining techniques and light microscopy. However, results of such methods can be affected by interaction between the tested compounds and the dye. In addition, in most cases end-point live-dead data is obtained. Such methods cannot provide researchers with real-time data. Our FETCIS method is a label-free, fully electronic approach. No additional dye is required for the measurements. This can minimize the possibility of misleading results due to the interaction of the tested drugs and the dye. Furthermore, the FETCIS system provides real-time monitoring function, which allows studying the effectiveness of the drugs easily **(Figure 9 and Figure 10)**. Please bear in mind that, when the long-term pharmacology study is carrying out, the measurement device must be put inside the incubator for proper environment control.

Our FET devices are robust and therefore can be used for several times after proper cleaning. However, the performances of the devices will still decrease gradually due to the use of H_2SO_4 during cleaning. Therefore, it is important to measure the maximal transconductance value of the devices every time before experiment. When the transconductance value is lowered than 0.2 mS, the FET devices are considered defective and should no longer be used.

In the future, FETCIS could be used with many different cell types. Our novel measurement technique clearly demonstrates single cell resolution, which was the aim in the ECIS community for many years. By far, there is no label-free method, which can be used to measure the direct adhesion strength between cell and substrate in a swift and straightforward manner. We hope that by this publication, we reach a lot of researchers in the field and open a whole-new direction within the biosensor community.

Acknowledgements:
We thank the Federal Ministry of Education and Research (BMBF), Germany for financial support via the project "*Entwicklung eins Zell-Chip Hybrid-Testsystems zur Wirksamkeitsanalyse von Krebsmedikamenten*", 17008X10. We thank A. Offenhäusser, N. Wolters, R. Otto, and D. Lomparski (all at the Forschungszentrum Jülich GmbH, Germany) for the support during the initial start of this project and for the support during development of the TTF amplifier box. The authors thank D. Cassel technical support

during chip fabrication and R. Lilischkis (both University of Applied Sciences Kaiserslautern) for SEM imaging.

Disclosures:
The authors have nothing to disclose.

References:
1. Bergveld, P. A critical evaluation of direct electrical protein detection methods. *Biosens. Bioelectron.* **6,** 55–72 (1991).
2. Souteyrand, E. *et al.* Direct Detection of the Hybridization of Synthetic Homo-Oligomer DNA Sequences by Field Effect. *J. Phys. Chem. B* **101,** 2980–2985 (1997).
3. Schäfer, S. *et al.* Time-dependent observation of individual cellular binding events to field-effect transistors. *Biosens. Bioelectron.* **24,** 1201–1208 (2009).
4. Giaever, I. & Keese, C. R. A morphological biosensor for mammalian cells. *Nature* **366,** 591–2 (1993).
5. Wegener, J., Keese, C. R. & Giaever, I. Electric cell-substrate impedance sensing (ECIS) as a noninvasive means to monitor the kinetics of cell spreading to artificial surfaces. *Exp. Cell Res.* **259,** 158–66 (2000).
6. Levsky, J. M. & Singer, R. H. Gene expression and the myth of the average cell. *Trends Cell Biol.* **13,** 4–6 (2003).
7. Ingebrandt, S. *et al.* Label-free detection of single nucleotide polymorphisms utilizing the differential transfer function of field-effect transistors. *Biosens. Bioelectron.* **22,** 2834–40 (2007).
8. Koppenhöfer, D., Susloparova, A., Docter, D., Stauber, R. H. & Ingebrandt, S. Monitoring nanoparticle induced cell death in H441 cells using field-effect transistors. *Biosens. Bioelectron.* **40,** 89–95 (2013).
9. Susloparova, A., Koppenhöfer, D., Vu, X. T., Weil, M. & Ingebrandt, S. Impedance spectroscopy with field-effect transistor arrays for the analysis of anti-cancer drug action on individual cells. *Biosens. Bioelectron.* **40,** 50–56 (2013).
10. Regehr, W. G., Pine, J., Cohan, C. S., Mischke, M. D. & Tank, D. W. Sealing cultured invertebrate neurons to embedded dish electrodes facilitates long-term stimulation and recording. *J. Neurosci. Methods* **30,** 91–106 (1989).
11. Schatzthauer, R. Neuron-silicon junction with voltage-gated ionic currents. *Eur. J. Neurosci.* **10,** 1956–1962 (1998).
12. Ingebrandt, S., Yeung, C. K., Krause, M. & Offenhäusser, A. Neuron-transistor coupling: Interpretation of individual extracellular recorded signals. *Eur. Biophys. J.* **34,** 144–154 (2005).
13. Ingebrandt, S., Yeung, C. K., Krause, M. & Offenhäusser, A. Cardiomyocyte-transistor-hybrids for sensor application. *Biosens. Bioelectron.* **16,** 565–570 (2001).
14. Susloparova, A., Vu, X. T., Koppenhöfer, D., Law, J. K.-Y. & Ingebrandt, S. Investigation of ISFET device parameters to optimize for impedimetric sensing of cellular adhesion. *Phys. status solidi* **211,** 1395–1403 (2014).
15. Susloparova, A., Koppenhöfer, D., Law, J. K., Vu, X. T. & Ingebrandt, S. Electrical cell-substrate impedance sensing with field effect transistors is able to unravel

cellular adhesion and detachment processes on a single cell level.
16. Arndt, S., Seebach, J., Psathaki, K., Galla, H. J. & Wegener, J. Bioelectrical impedance assay to monitor changes in cell shape during apoptosis. *Biosens. Bioelectron.* **19,** 583–594 (2004).
17. Vu, X. T. & Offenhäusser, P. D. A. Silicon nanowire transistor arrays for biomolecular detection. *Faculty of Mathematics, Computer science, and Natural sciences* **Ph.D,** (2011).
18. TIAN, Y. hong, WANG, C. qing & ZHOU, Y. N. Bonding mechanism of ultrasonic wedge bonding of copper wire on Au/Ni/Cu substrate. *Trans. Nonferrous Met. Soc. China (English Ed.* **18,** 132–137 (2008).

Figure 1
Click here to download high resolution image

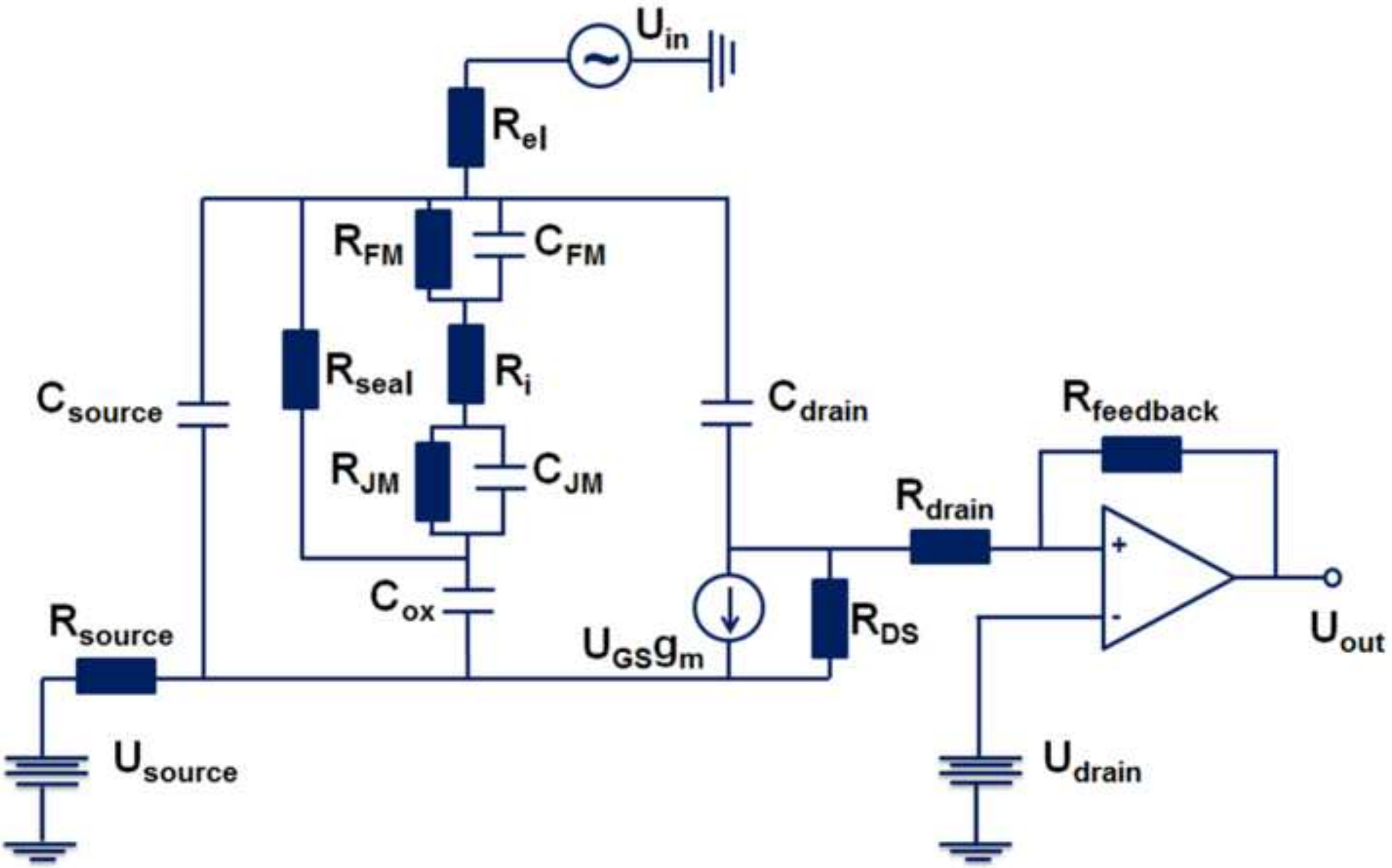


Figure 2
Click here to download high resolution image

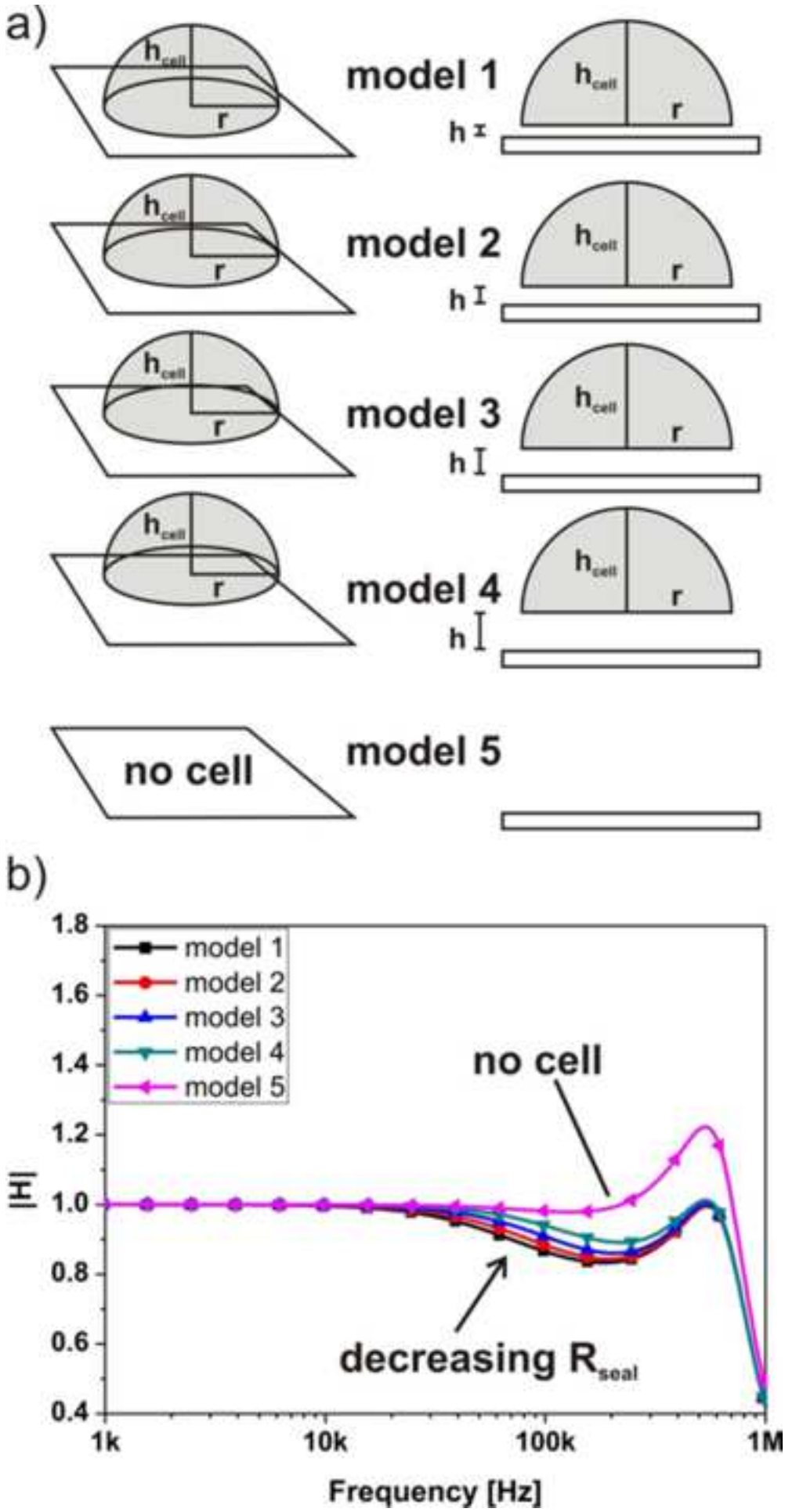


Figure 3
Click here to download high resolution image

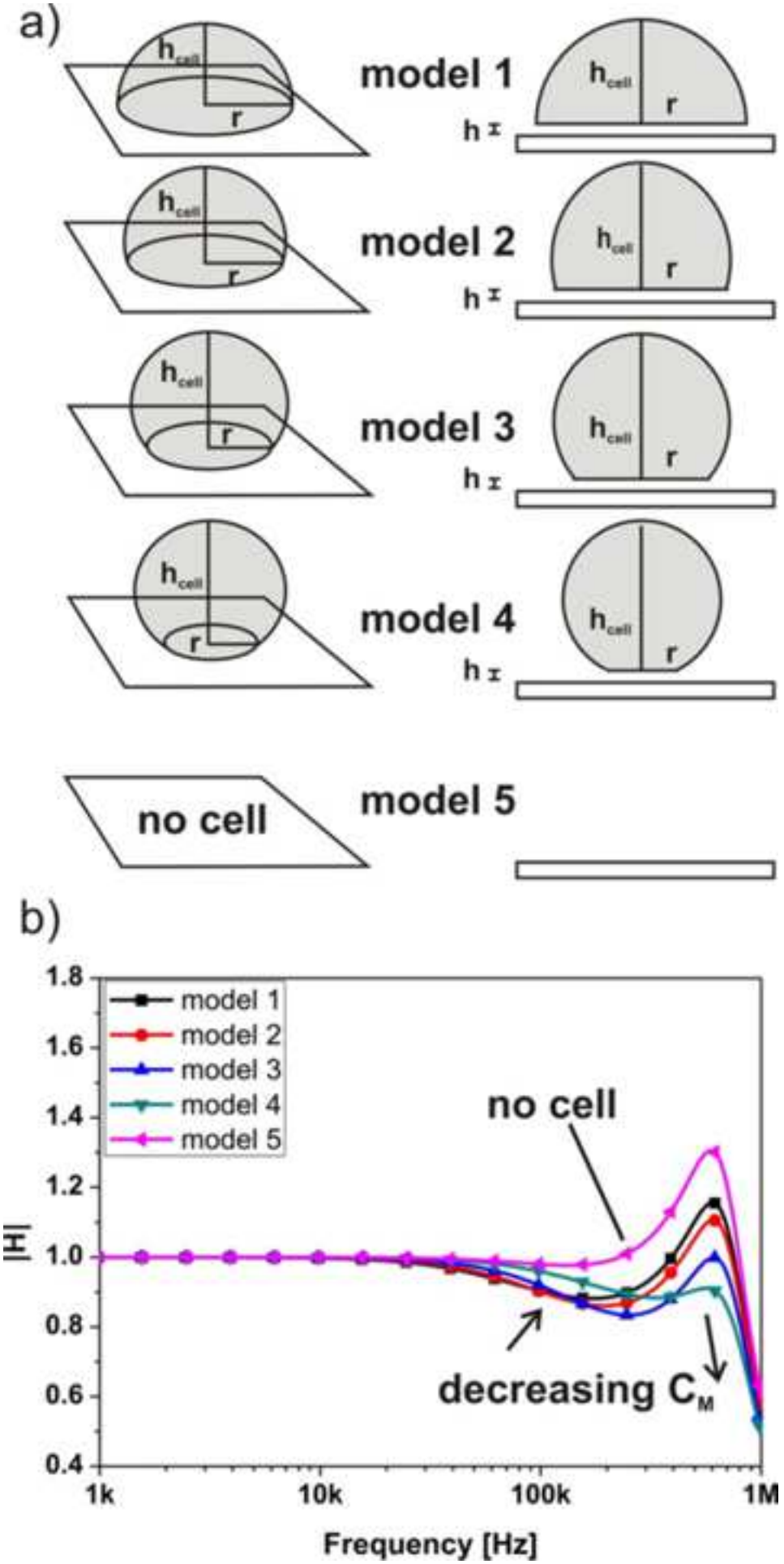


Figure 4
Click here to download high resolution image

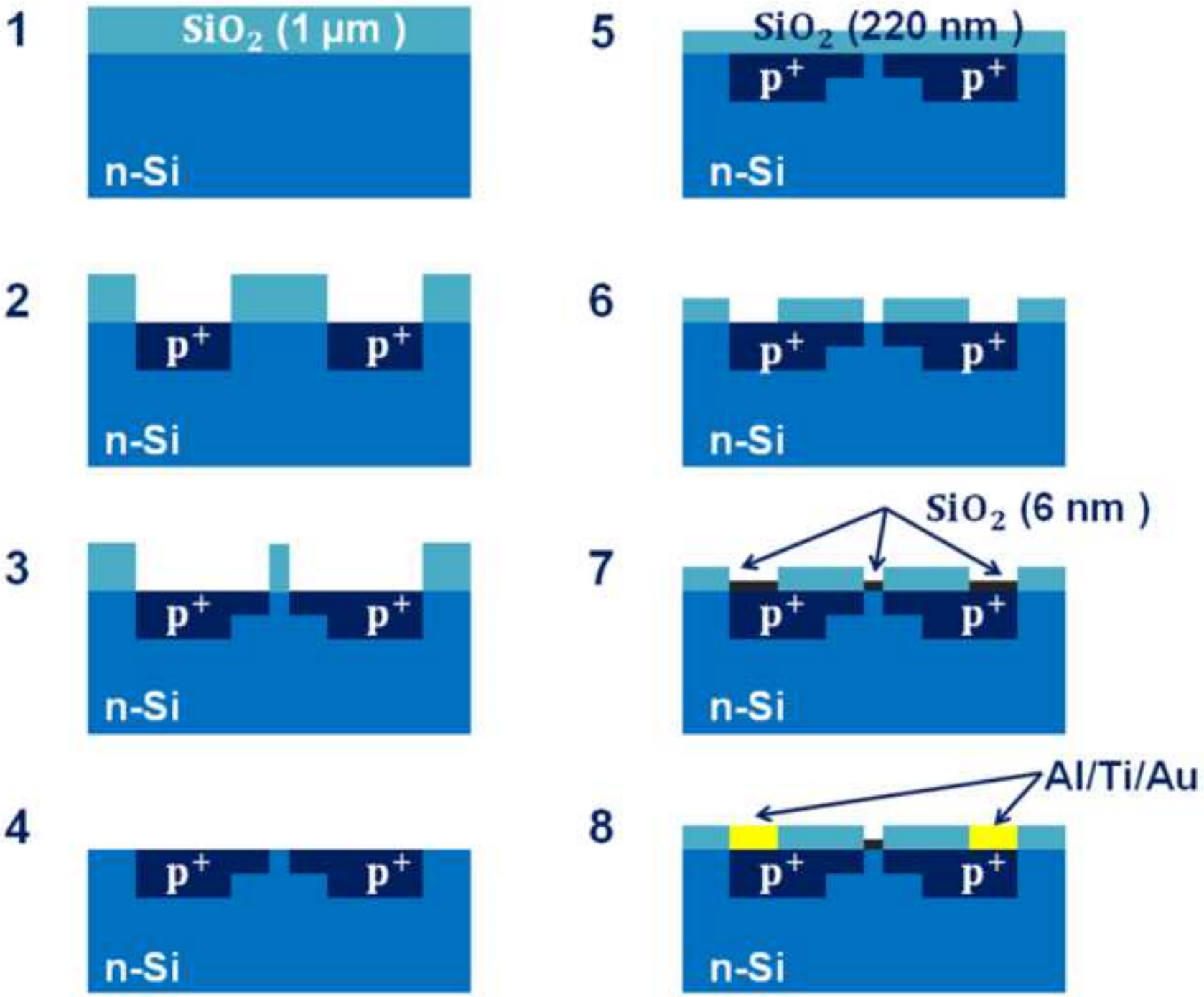


Figure 5
Click here to download high resolution image

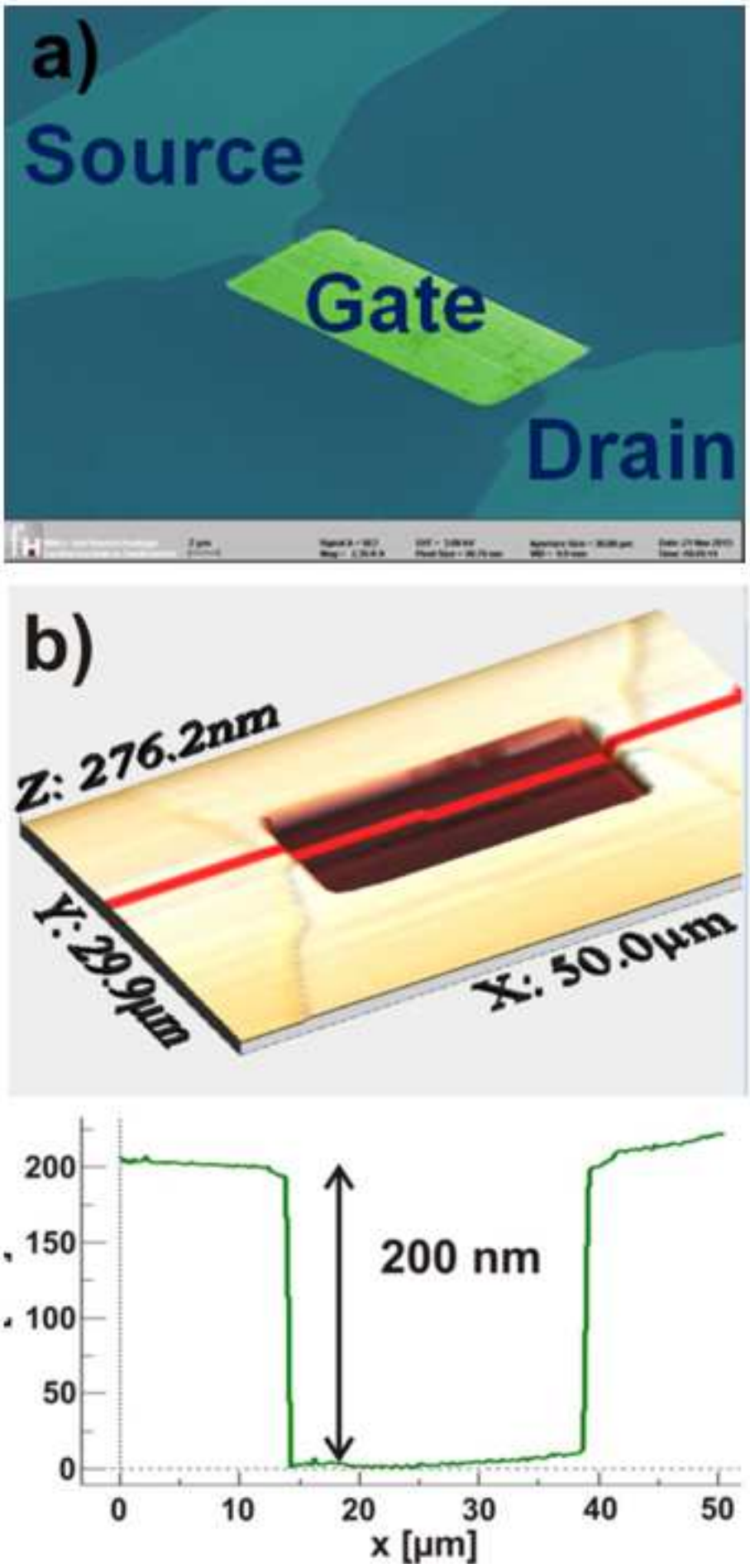


Figure 6
Click here to download high resolution image

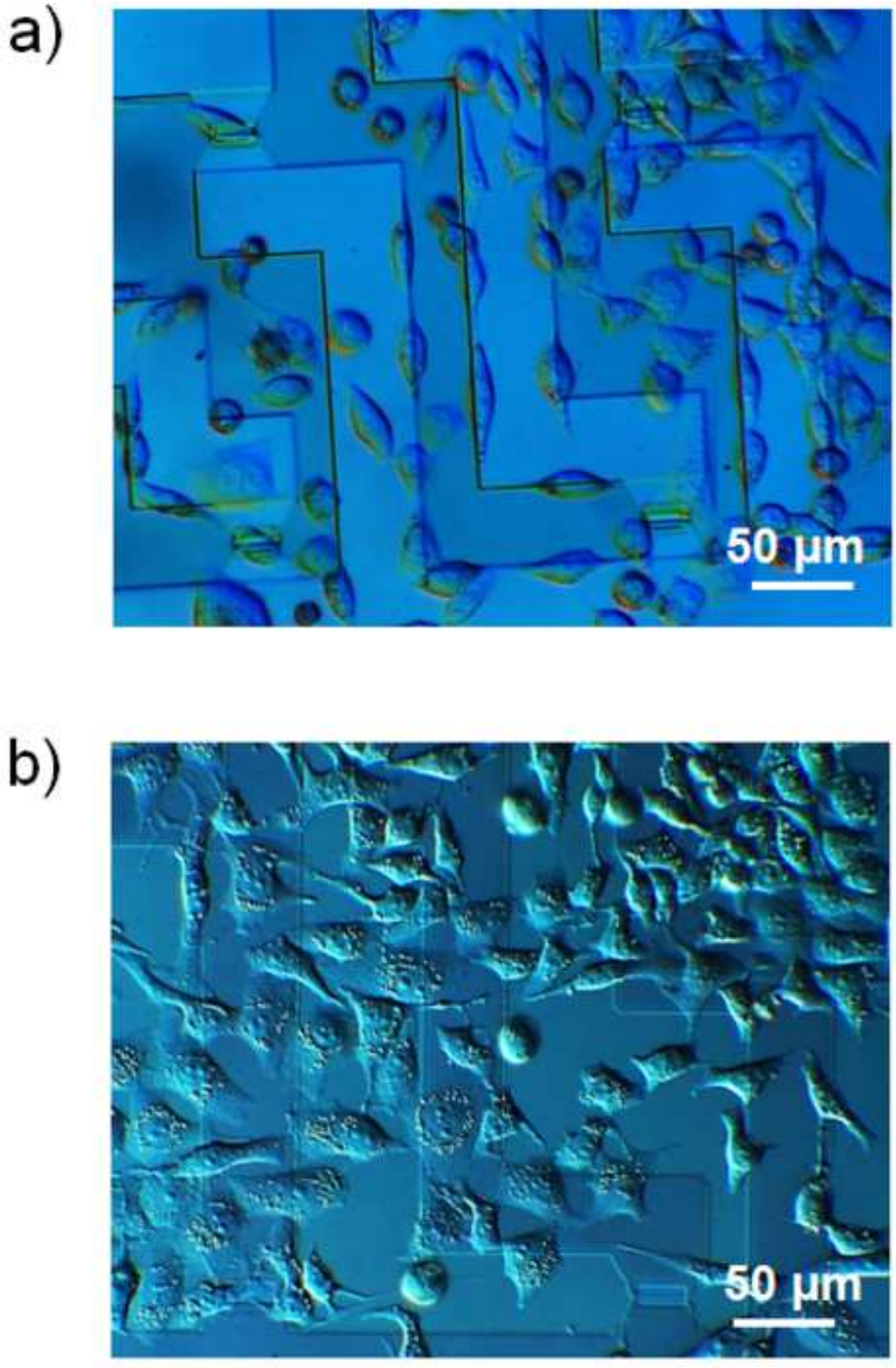


Figure 7
Click here to download high resolution image

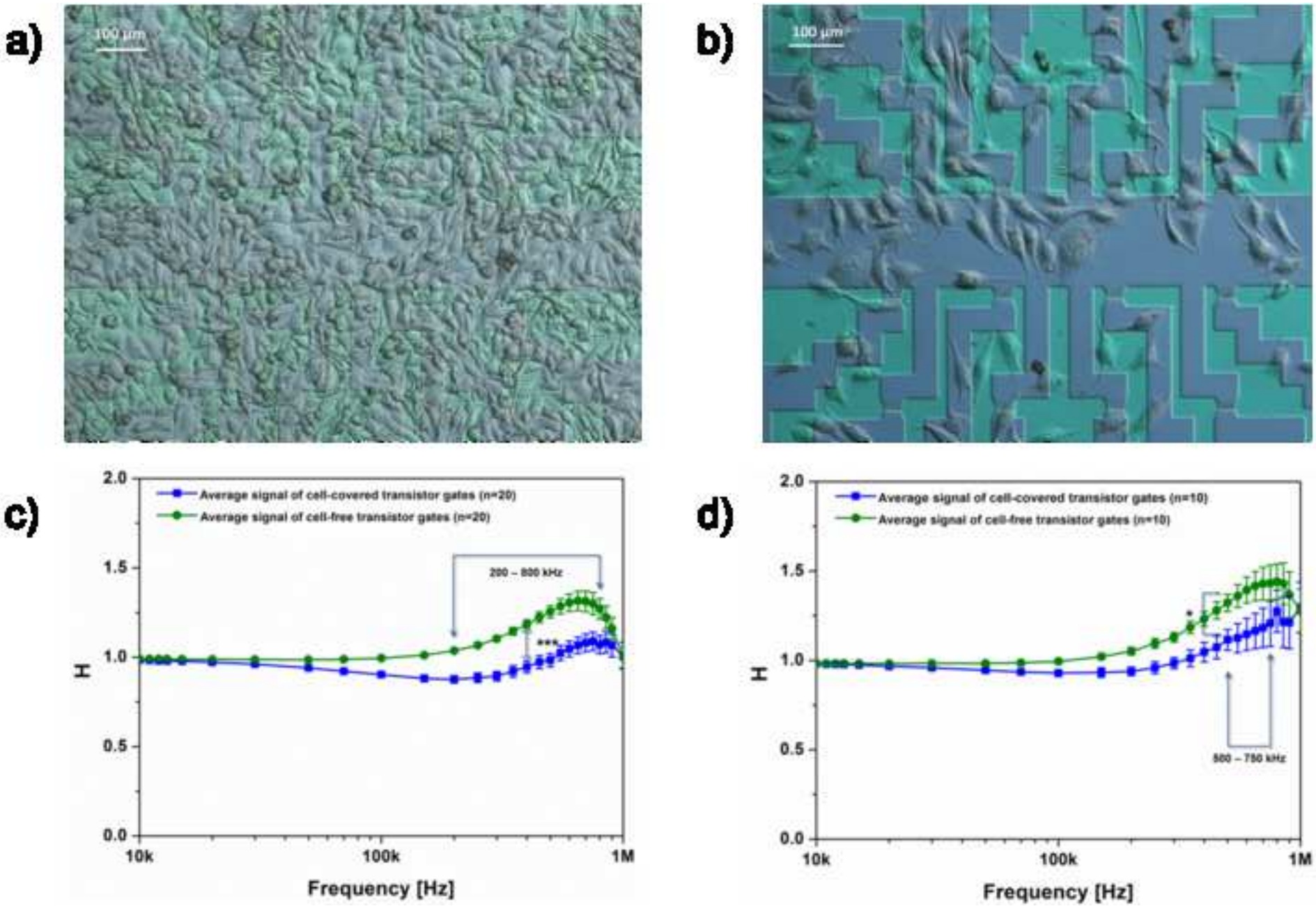


Figure 8
Click here to download high resolution image

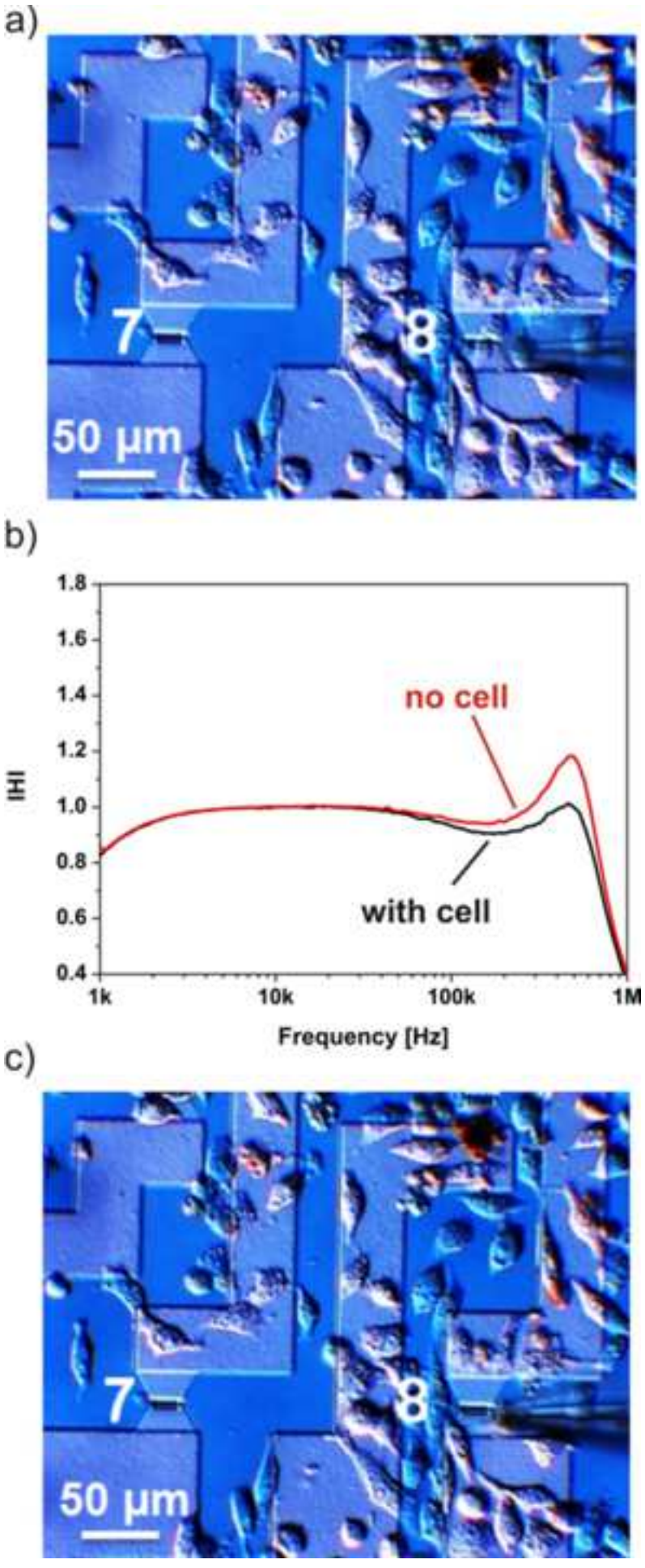


Figure 9
Click here to download high resolution image

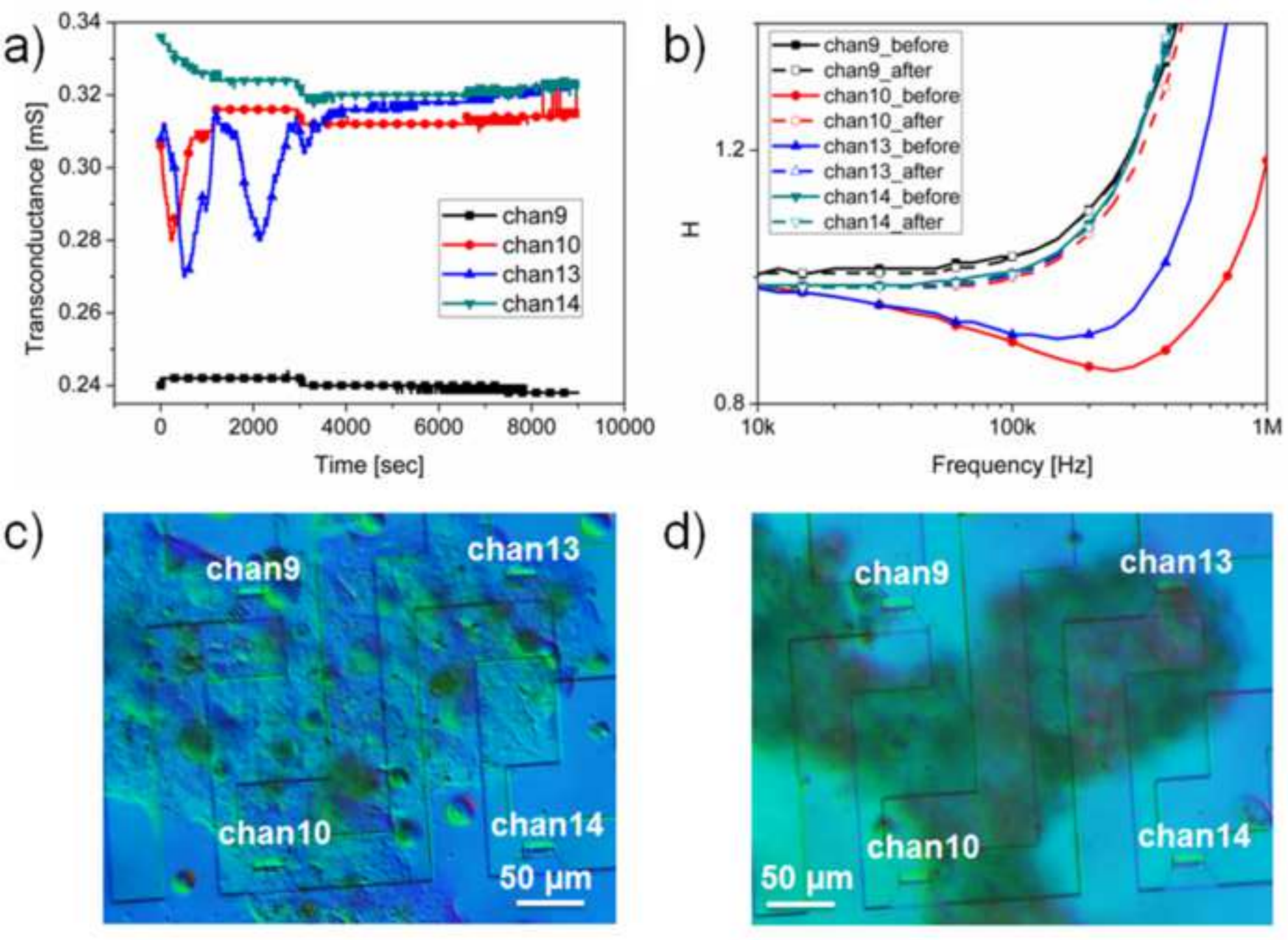
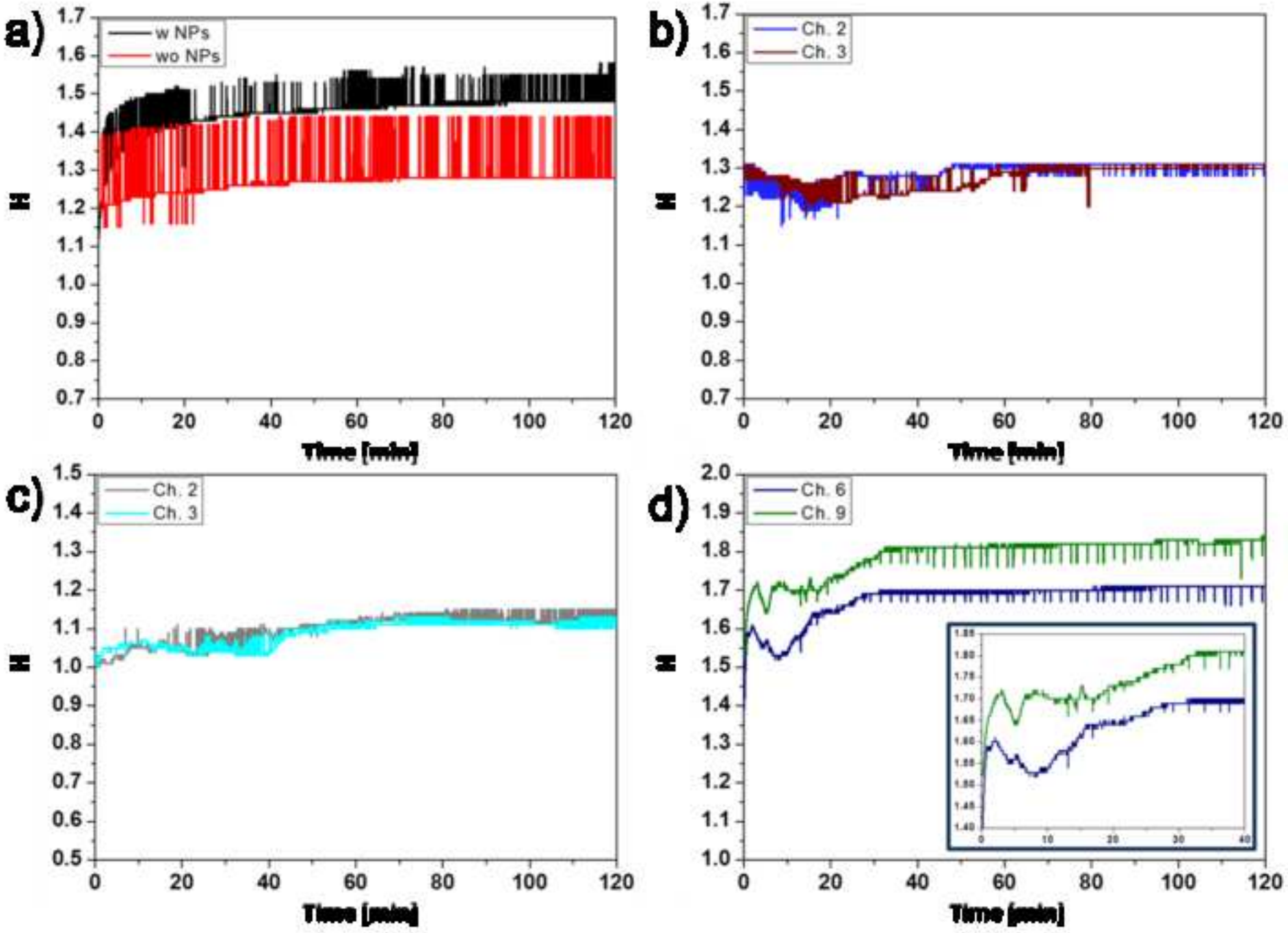
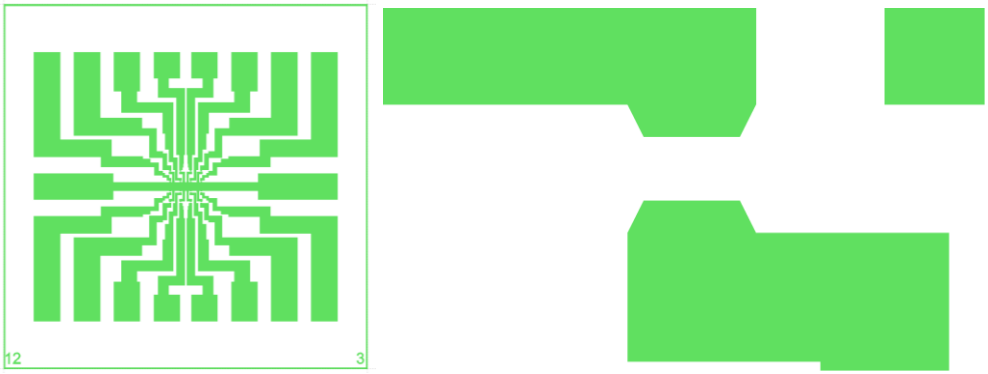


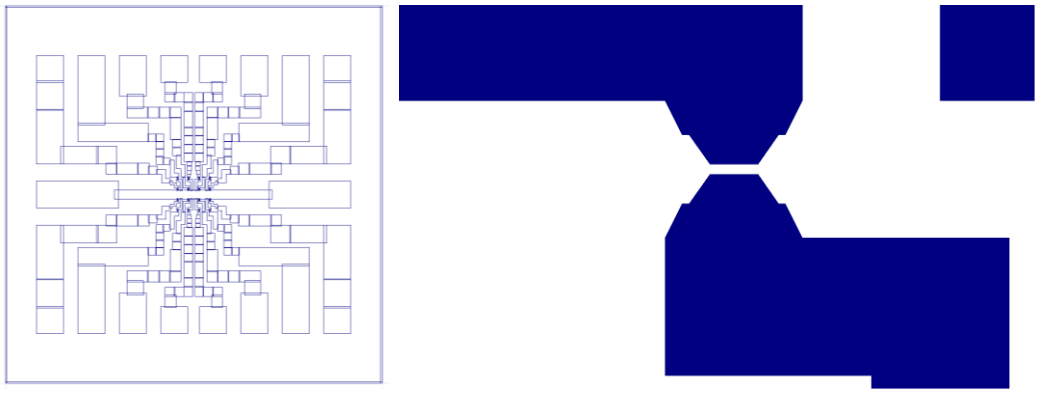
Figure 10
Click here to download high resolution image



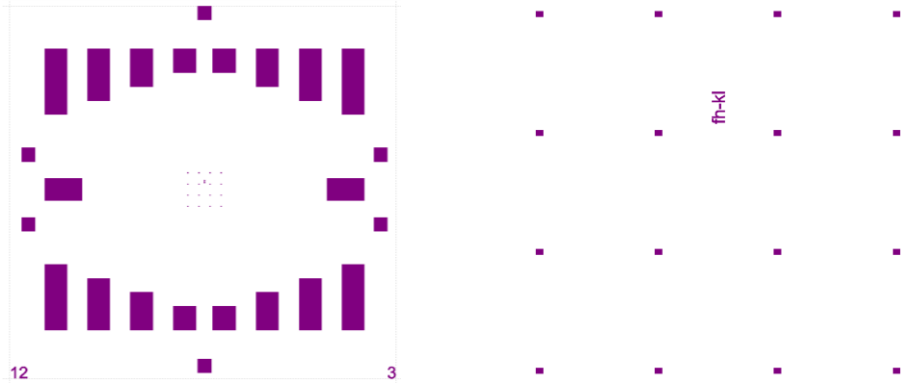
Supplementary Figure 1
Click here to download Figure: S1.pdf



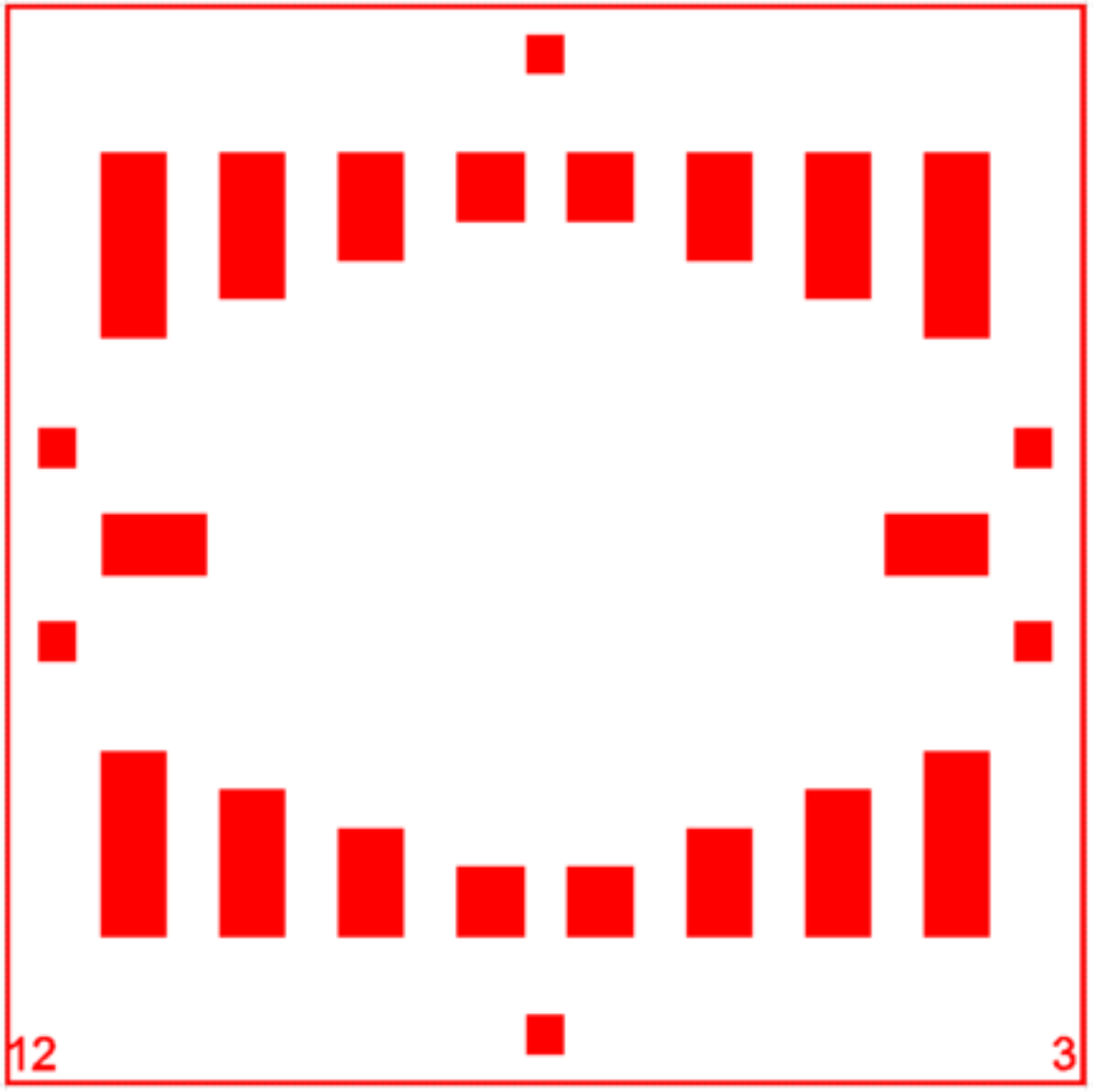
Supplementary Figure 2
Click here to download Figure: S2.pdf



Supplementary Figure 3
Click here to download Figure: S3.pdf



Supplementary Figure 4
Click here to download high resolution image



Excel Spreadsheet- Table of Materials/Equipment

Click here to download Excel Spreadsheet- Table of Materials/Equipment: List of material and equipment.xlsx

Name of the Material/ Equipment	**Company**
Chip fabrication and encapsulation	
n-type silicon wafer	Active business company GmbH
68 Lead Ceramic Chip Carrier	Global Chip Materials
Acetone	ChemSolute
Aluminum wire	Heraeus
Buffered hydrofluoric acid	MicroChemicals
Epoxy 377 1LB Kit	Epoxy Technology
Glass rings	Becker Glas
Hydrogen Peroxide (30 %)	Sigma-Aldrich
Isopropanol	Merck
Photoresist AR-P3100	All resist
Photoresist AR-U4040	All resist
Photoresist developer AR 300-26	All resist
Piranha solution	
Silicon adhesive	Dow Corning
Sulphuric Acid (98 %)	Sigma-Aldrich
Wire bonder	West Bond Inc.
Impedance measurement with cells	
Ethanol	ChemSolute
BioMol software	
Differential Interface Contrast microscope	Carl Zeiss
Dulbecco's Modified Eagle's Medium	PAN Biotech GmbH
Fetal Calf serum	PAN Biotech GmbH
Fibronectin	AppliChem GmbH
G418	Carl Roth GmbH
Hellmanex III	Hellma Analytics
Incubator	NUAIR
Laminar-flow clean bench	Heraeus Instruments
L-Glutamine	PAN Biotech GmbH
Minimal Essential Medium	PAN Biotech GmbH
NexSil20 nanoparticles	NYACOL Nanotechnologies Inc.
Non-Essential Amino Acid	PAN Biotech GmbH
Patch-Clamp Pipette Puller	Sutter Instrument Co.
Patch-Clamp Pipettes	Science Products
Penicillin/Streptomycin	PAN Biotech GmbH
Roswell Park Memorial Institute Medium	PAN Biotech GmbH
Sonicator	Sonovex
Sulfuric acid	AppliChem GmbH
Topotecan hydrochloride	Sigma-Aldrich
Trypsin with EDTA	PAN Biotech GmbH

Catalog Number
WS104-1011002
LCC6830002
2659.5000
BOE 7-1
PB 044542
8.18766.2500
AR-P3100
AR-U4040
AR 300-26
96-083
7602C-79C
22.465.000
180218
P04-02500
P30-3702
A8350
239.3
320003
NU-5500E
HB 2448
P04-80100
P04-08250
P08-32100
P-2000
GB150F-8P
P06-07100
P04-17500
RK 100H
A2762
T2705
P10-024100

Comments/Description
$HF:NH_4F$ =1 : 7 (v/v), VLSI Grade
Degas after mixing; eliminate air bubbles
VLSI Grade
Mask positive photoresist
Image reversal photoresist
Photoresist developer
$H_2SO_4:H_2O_2$= 2:1 (v/v)
Degas after mixing; eliminate air bubbles
VLSI Grade
Read-out software implemented in Delphi 5.0 (Borland Software Corporation)
Prepare 2 % Hellmanex III with distilled water

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article: Electronic measurements of cellular adhesion using Field-Effect ... (FETCIS)

Author(s): Law, Susloparova, Koppenhöfer, Vu, Hempel, Ingebrandt

Item 1 (check one box): The Author elects to have the Materials be made available (as described at http://www.jove.com/publish) via: ☐ Standard Access ☒ Open Access

Item 2 (check one box):

☒ The Author is NOT a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. Defined Terms. As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments;* "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. Background. The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. Grant of Rights in Article. In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

4. Retention of Rights in Article. Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in *JoVE* or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

CORRESPONDING AUTHOR:

Name:	Prof. Dr. Sven Ingebrandt
Department:	Informatics and Microsystem Technology
Institution:	University of Applied Sciences Kaiserslautern
Article Title:	Electronic measurements of cellular adhesion using ... (FETCIS)
Signature:	Sven Ingebrandt
Date:	06/23/2014

Please submit a signed and dated copy of this license by one of the following three methods:

1) Upload a scanned copy of the document as a pfd on the JoVE submission site;
2) Fax the document to +1.866.381.2236;
3) Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02139

For questions, please email submissions@jove.com or call +1.617.945.9051

Rebuttal Comments

Click here to download Rebuttal Comments: Response letter to the reviewers_5.doc

Fachhochschule Kaiserslautern | University of Applied Sciences

Fachhochschule Kaiserslautern ■ Amerikastraße 1 ■ 66482 Zweibrücken

The Editor, Journal of Visualized Experiments

1 Alewife Center
Cambridge, MA 02140
USA

JoVE submission 52430 "Electronic measurements of cellular adhesion using Field-Effect Transistor Cell-substrate Impedance Sensing (FETCIS)"

Contact Person	Contact Tel./Fax	E-Mail	Date
Prof. Dr. Sven Ingebrandt	+49631 37245413	sven.ingebrandt@fh-kl.de	10.11.2014

Dear JoVE editor,

Thank you very much for the effort and suggestion. Hereby, I enclosed the Reviewers' comments and my corresponding responses.

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Answer: Thank you for the suggestions.

2. Please revise the highlighting of the manuscript for the video. Currently, 3.5 pages of protocol text are highlighted and this is beyond the 2.75 page limit of highlighted protocol text for the video. This upper limit exists such that the videography can be performed in one day.

   I would suggest unhighlighting steps that refer to other steps as well as Protocol steps 3 and 4.

Answer: Thank you for the suggestions. The latest provided version is the most simplified one (within 3 pages). Hope that it is acceptable.

Sincerely

Prof. Dr. Sven Ingebrandt

Figures re-use license

Click here to download Supplemental File (as requested by JoVE): Biosensors and Bioelectronics.docx

Figures re-use license

Click here to download Supplemental File (as requested by JoVE): PSS.docx