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Application note on Functionalization of Graphene

Functionalization of Graphene

Application note

Introduction

In order to design and fabricate graphene sensors, one needs to keep in mind a few key underlying principles. Graphene itself is quite sensitive material due to is atomic thinness. This means that every atom of graphene is a surface atom that can respond to the environment, and also because graphene is a zero band gap semiconductor. The conductivity of graphene responds to changes in carrier concentrations as a result of doping. Additionally, graphene is not intrinsically selective. The majority of research on graphene sensors has moved towards methods of functionalizing graphene with receptor molecules to target selectivity to a desired analyte. The advantage of graphene as a sensor is that it can be fabricated on a wafer-scale and can be miniaturized to submicron dimensions with conventional lithography techniques. Additionaly, the functionalization of graphene can be multiplexed. An array of graphene sensors, each with a different functionality targeted to different analytes, in combination with pattern recognition routines, can result in a very powerful miniaturized electronic sensor system for complex analyte mixtures. However, although sub-micrometer scale physical patterning of graphene can be easily achieved by lithography, sub-micron scale chemical patterning has been challenging.

A critical step in fabrication of graphene-based chemical - and bio-sensors is stable and multiplexed functionalization of graphene. It can however be realized with sub-micron resolution and in well-defined locations by utilizing bottom-up soft lithography techniques. Various tools can be used, but in this application note functionalization of graphene with microchannel cantilevers will be presented. Specifically, the deposition of a biotin azide using click-chemistry (copper-catalysed alkyne-azide cycloaddition (CuAAC) and subsequent binding of fluorescently tagged protein. This approach can be scaled up to multiplex functionalization of graphene devices on a wafer-scale for sensor and biomedical applications.

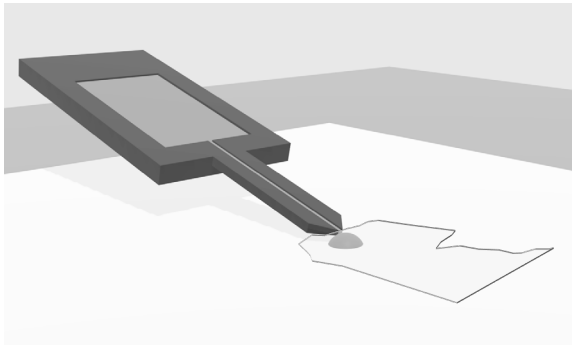
Functionalization of Graphene

Application note

Workflow

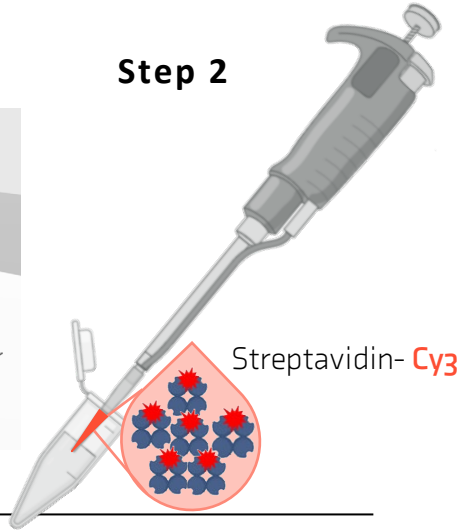
In microchannel cantilever spotting (µCS), the ink is carried down the miocrochannel by capillary liquid flow and is directly transferred to the surface being patterned upon contact of the probe with the surface. The use of microchannel cantilevers in this way allows for the deposition of femtoliter (10^{-15} litres) sized droplets of ink. These minute droplets of ink can act as individual vessels in which a chemical reaction can take place . The workflow for preparing the graphene sensor is shown below and can be divided into three distinct steps:

Step 1



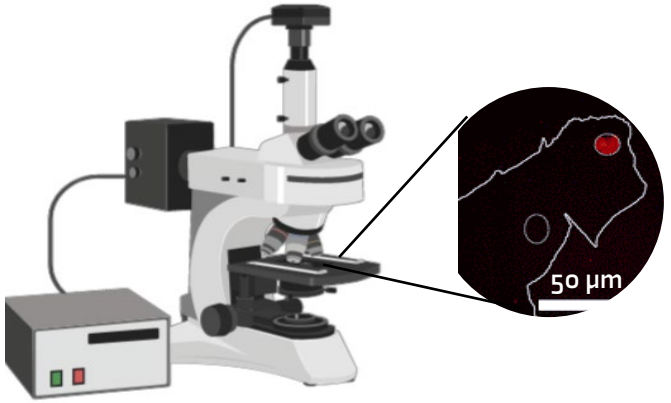
Printing ink on graphene
(e.g. Azdes)

Step 2



Analyte bonding/
Reaction with printed ink
(e.g. Proteins)

Step 3



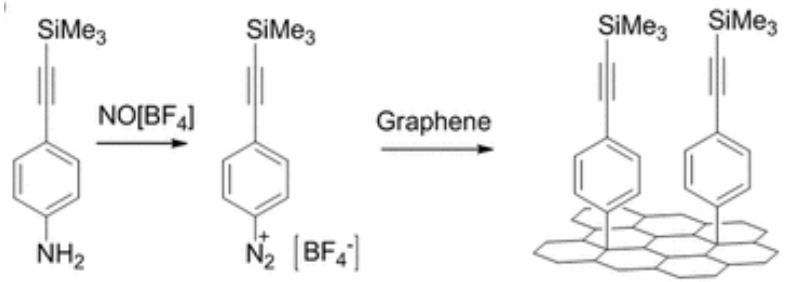
Detection of bound target molecules
(e.g. Fluorescence microscope)

Step 1 - Printing on graphene

Application note

Activation of the graphene surface by diazonium functionalization

To undertake the CuAAC functionalization of the graphene surface, the graphene needs to first be activated to display exposed alkyne groups. This activation is achieved through the reaction with a diazonium tetrafluoroborate salt of 4-(trimethylsilyl)ethynylaniline via an aryl diazonium salt reaction [Paulus at al., 2013]. Directly prior to CuAAC functionalization, the trimethylsilyl (TMS) group is cleaved by immersing the substrate for 2.5 h in tetrahydrofuran (THF) and the addition of tetrabutylammonium fluoride (TBAF) (40 mL THF + 20 µL of 1 M TBAF in THF) [Wang at al., 2011], followed by washing the substrate with THF and blow-drying with nitrogen (reaction scheme outlined in the figure on the right). The extent of functionalization should be evident by viewing the Raman spectrum, that should be done after the activation step.



Ink preparation

The ink composition is water based and contains:

- 100 µg/ml of Azide compound (Jena Bioscience)
- 10 mM $CuSO_4$ and
- 20 mM sodium ascorbate as catalysts.

Additionally to prevent ink from drying out, 30 % v/v glycerol was added (e.g. to 7 µl of ink, 3 µl of glycerol was added).

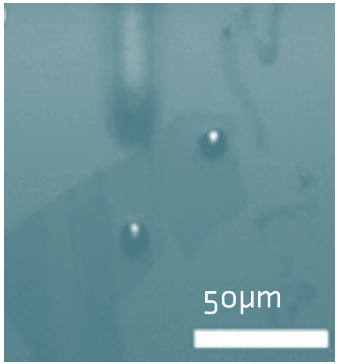
Prior to spotting, the probe (SPT-S-C10S, Bioforce Nanosciences) was activated in oxygen plasma (100 mbar oxygen pressure, 30W power, 2min.). From the ink mixture 0,5 µl was taken out and applied into the reservoir of the activated microchannel cantilever. Such loaded probe was ready for printing.

Step 1 - Printing on graphene

Molecular Printer Set-up

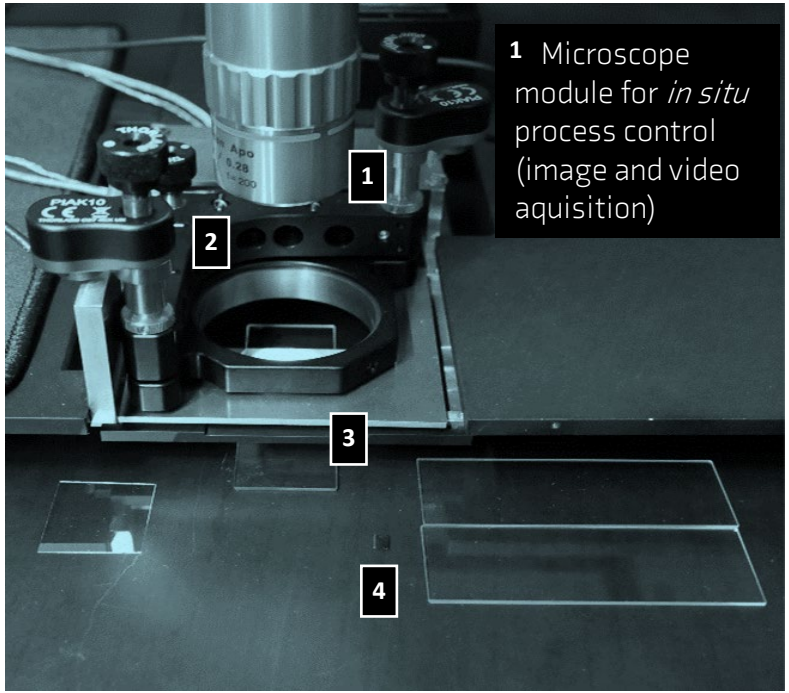
The probe loaded with ink was mounted onto the probe holder.[3] Spotting of the ink onto the functionalized graphene takes place by bringing the tip of the probe in to contact with the surface for a defined time (0,5 s) at the controlled humidity (65 % R.H.).

To ensure the CuAAC takes place as expected, control inks should be spotted in parallel. Here, biotin azide ink with all needed catalysts ('+ Cu') were potted next to a negative control (inks containing no catalysts, "no Cu")(data not shown).



Assuming the ink droplets to be half spheres in profile (as suggested by a contact angle around 90° of water on graphene) and taking a typical droplet diameter of ~8 µm, the typical reaction volumes is estimated to be about 130 femtoliters.

After the spotting step, femtoliter sized droplets of ink are allowed to react with the alkyne groups on the graphene surface for 2h in the dark at a constant humidity of 60% R.H.



1 Microscope module for *in situ* process control (image and video aquisition)

2 High precision module for high resolution control over the printing/spotting process

3 Probe holder for mounting probes

4 Sample table for loading the substrates

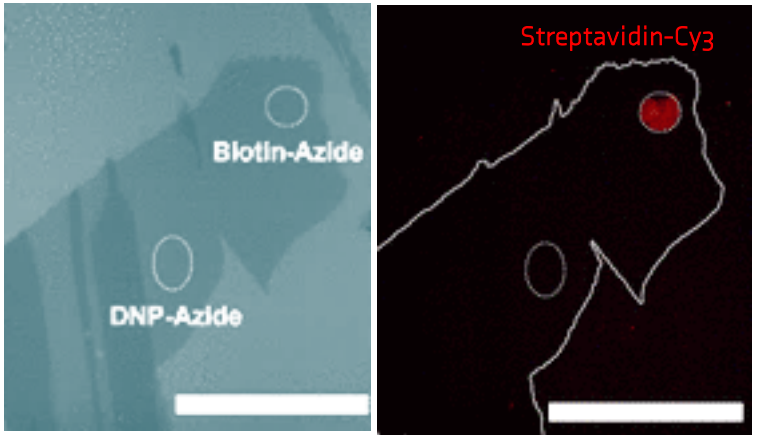
Step 2 - Analyte binding

After the spotting process, the click reaction between the graphene and the ink (biotin-Azide, DNP-Azide) was allowed to take place. After completion of the click reaction, the samples were rinsed with ethanol and phosphate buffered saline (PBS).

For analyte binding experiments, the samples were:

- blocked with BSA (5% in PBS, Sigma Aldrich) for 10 minutes,
- washed with PBS 3 times,
- incubated with fluorescently labelled streptavidin in PBS (10 µg/mL Streptavidin- Cy3, Sigma Aldrich) for 12 minutes,
- washed again in PBS 3 times.

The area functionalized with the biotin-azide is clearly observed by fluorescence of bound Streptavidin -Cy3, whereas just the DNP-azide functionalized area remain dark and serves as a negative control.



Protein binding on a functionalized graphene flake. Imaging: Nikon Upright Microscope (Eclipse 80i), 50x objective. Scale bar equals 50µm.

Step 3 – Detection: Fluorescence microscopy

Fluorescence microscopy images were taken with a Nikon upright fluorescence microscope (Eclipse 80i), equipped with a sensitive camera CoolSNAP HQ2 (Photometrics). The broadband excitation light source (Intensilight illumination) is combined with sets of filters (Texas Red, FITC, DAPI) to separate excitation and emission spectra, depending on the dye molecule used.

Conclusions

Surface immobilization of bioactive molecules by spotting and printing methods has drawn much attention in recent years, as it is a fundamental technique for the preparation of bioactive surfaces. While ink jet printing offers contactless high-throughput spotting at larger length scales (usually >50 µm), techniques like microcontact printing (µCP) and microchannel cantilever spotting (µCS) [Mateos-Gil, et al., 2015] can readily address the scale range down to a few microns [Koehler, et al., 2010; Xu, et al., 2004]. The deposition of micro-reactors onto a surface allows liquid phase coupling chemistry to take place for a prolonged time or the transfer of otherwise unwritable inks [Xu, et al., 2004]. Similarly, µCS was previously demonstrated for the spotting of femtoliter–scale reaction chambers for surface immobilization of bioactive substances into microarrays by "click" chemistry [Hirtz, et al., 2014; Hirtz, et al., 2016]. The click reaction chosen in this application note is the copper-catalyzed alkyne–azide cycloaddition (CuAAC), that has proven enormously useful for biochemical coupling reactions since the introduction of the concept by Sharpless [Taherian, et al., 2013; Kolb , et al., 2004]. In particular, several examples have been reported in which the CuAAC reaction was employed to functionalize the surface of diamond materials, including nanoparticles [Salaita, et al., 2005] or conductive diamond [Yeap, et al., 2014], which provided an avenue to electrochemically stable, functional electrodes.

Here, the CuAAC reaction is shown to be extremely useful in the stable functionalization of graphene for the fabrication of a graphene-based sensor. This approach can be easily modified by using various commercially available azide molecules to target the analyte of choice. In combination with µCS this approach is a promising strategy for the fabrication of high-density arrays that will be useful in the fabrication of a broad spectrum of sensor devices.

Selected literature:

Application note

- Paulus et al., Covalent Electron Transfer Chemistry of Graphene with Diazonium Salts. Acc. Chem. Res. 2013, 46, 160–170.
- Wang et al., Photoactive Graphene Sheets Prepared by "Click" Chemistry. Chem. Commun. (Camb). 2011, 47, 5747–5749.
- Rostovtsev, et al., Stepwise Huisgen Cycloaddition Process: copper(I)-Catalyzed Regioselective "Ligation" of Azides and Terminal Alkynes Angew. Chem., Int. Ed. 2002, 41, 2596– 2599
- Kolb , et al., Click Chemistry: Diverse Chemical Function from a Few Good Reactions Angew. Chem., Int. Ed., 2001, 40 , 2004 —2021
- Koehler, et al., Selective Chemical Modification of Graphene Surfaces: Distinction between Single- and Bilayer Graphene Small 2010,
- Sharma, et al., Anomalously Large Reactivity of Single Graphene Layers and Edges toward Electron Transfer Chemistries Nano Lett. 2010,
- Menanteau, et al., Electrografting via Diazonium Chemistry: The Key Role of the Aryl Substituent in the Layer Growth Mechanism J. Phys. Chem. C 2016, 120, 4423– 4429
- Mateos-Gil, et al., Electroactive Carbon Nanoforms: A Comparative Study via Sequential Arylation and Click Chemistry Reactions Nanoscale 2015, 7, 1193– 1200
- Xu, et al., Microfabricated Quill-Type Surface Patterning Tools for the Creation of Biological Micro/nano Arrays Biomed. Microdevices 2004, 6, 117– 123
- Xu, et al., Microfabricated "Biomolecular Ink Cartridges"—Surface Patterning Tools (SPTs) for the Printing of Multiplexed Biomolecular Arrays Sens. Actuators, B 2006, 113, 1034– 1041
- Hirtz, et al., Click-Chemistry Based Multi-Component Microarrays by Quill-Like Pens Adv. Mater. Interfaces 2014, 1, 1300129
- Hirtz, et al., Click-Chemistry Immobilized 3D-Infused Microarrays in Nanoporous Polymer Substrates Adv. Mater. Interfaces 2016, 3, 1500469
- Salaita, et al., Sub-100 Nm, Centimeter-Scale, Parallel Dip-Pen Nanolithography Small 2005, 1, 940– 945
- Yeap, et al., Boron-Doped Diamond Functionalization by an Electrografting/Alkyne–Azide Click Chemistry Sequence ChemElectroChem, 2014, 1 , 1145 —1154

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Dr. Sylwia Sekula-Neuner

Co-founder, Co-managing Director

Marketing, Customer Relations, Innovation Projects

E: ssn@n-able-innovation.com
T: 0049 (0) 721 608 28897
M: 0049 (0) 176 6400 7660

Dr. Uwe Bog

Co-founder, Co-managing Director

R&D, Process Development

E: ub@n-able-innovation.com
T: 0049 (0) 721 608 28897

More information can be found on our website: **www.n-able-innovation.com**