METHOD FOR REMOVING A HIGH DEFINITION NANOSTRUCTURE, A PARTLY FREESTANDING LAYER, A SENSOR COMPRISING SAID LAYER AND A METHOD USING SAID SENSOR

The present invention is in the field of a method for removing a high definition nanostructure in a partly free-standing layer, the layer, a sensor comprising said layer, a use of said sensor, and a method of detecting a species, and optional further characteristics thereof, using said sensor. The sensor and method are suited for detecting single ions, molecules, low concentrations thereof, and identifying sequences of base pairs, e.g. in a DNA-strand.
DESCRIPTION

FIELD OF THE INVENTION

The present invention is in the field of a method for removing a high definition nanostructure in a partly free-standing layer, the layer, a sensor comprising said layer, a use of said sensor, and a method of detecting a species, and optional further characteristics thereof, using said sensor.

The sensor and method are suited for detecting single ions, molecules, low concentrations thereof, and identifying sequences of base pairs, e.g. in a DNA-strand.

BACKGROUND OF THE INVENTION

Graphene has attracted a lot of research interest because of its promising electronic applications related to its superior electron mobility, mechanical strength and thermal conductivity. It may have wide range of applications, for instance, field-effect transistors, photonic or optoelectronic device, sequencing DNA through nano-holes in graphene etc. Most of these applications demand modification of a graphene sheet into specific nano-patterns.

In a published paper, inventors used a transmission microscope operating in high resolution mode to sculpt graphene, showing a possibility of sculpting for multi-layer graphene. It was also found that for monolayer graphene, nanometer precision sculpting could not be obtained using the same method due to a width of an electron beam and unpredicted e-beam damage during an accompanying imaging process.

Various documents recite production of nanostructures. For instance, B. Song et al, in 'Atomic-Scale Electron-Beam Sculpting of Near-Defect-Free Graphene Nanostructures', 2011, 11 (6), pp. 2247-2250, recites fabrication of a range of graphene nanostructures into multilayer graphene, also known as graphite. In the method no contamination occurs upon electron beam exposure, and the graphitic material remains crystalline up to edges of a nanostructure formed (i.e., the graphite does not get amorphous). However, the nanostructure formed can not be programmed in advance (for example using a computer script). A further disadvantage is that upon electron beam exposure that nanostructure will change in shape, fold, etc. It is noted
that, although the publication recites graphene, it recites a multilayer graphene which is physically and chemically very different from the unique mono- or bilayer graphene. In this respect it is e.g. noted that if the method in the paper is applied to monolayer graphene (i.e., that lithography is carried out in a bright field mode of an electron microscope), it has been found that the material is extremely sensitive upon electron beam exposure, making it virtually impossible to fabricate a predetermined structure in the material; e.g., even a simple hole (e.g. 5 nm in diameter) could not be made (see figure 1, showing that if one hole is intended in the bright field mode, always several holes appears, making the above technique for graphene nanostructure design useless.

Further, e.g. WO2011/046706 recites use of a nanopore in graphene for DNA analysis. A transmission electron microscope in the bright field mode is used.

The graphene undergoes the problems raised in the above publication, namely contamination, amorphization and lack of control. Also, use is made of synthetic graphene which is generally multilayer graphene. The method does not provide perfect control and reproducibility. It is not possible to produce controlled and perfectly crystalline nanopores in single layer graphene.

In CN101872120 a method for preparing patterned graphene is recited. In the method, a photoresist is patterned on a device substrate by a microelectronic process such as UV lithography, and windows are formed at positions needing graphene; by a graphene transfer method, large-area graphene is transferred onto the patterned photoresist; and the photoresist and the graphene thereon are stripped to obtain a patterned graphene. Compared with the prior art, the method has the advantages of accurate positioning, and does not require etching or manufacturing an imprint template so as to have low cost.

However, UV lithography does not allow the fabrication of features smaller than approximately a wavelength of light used (UV, e.g., 200 nm). No single atom resolution is provided. Further, edge structures will be amorphous and contaminated. Third, graphene needs a support, typically a wafer or a resist, constituting another source of contamination.

In Liu et al, 'Nanosphere Lithography for the Fabri-
cation of Ultranarrow Graphene Nanoribbons and On-Chip Bandgap Tuning of Graphene’, Advanced materials, 2011, 23, 1246, an approach for high throughput, rapid, and low-cost fabrication of ultranarrow graphene nanoribbons (GNRs) using nanosphere lithography (NSL) nanopatterning in combination with low-power O₂ plasma etching is presented.

Further Ning Lu et al. in “In situ studies on the shrinkage and expansion of graphene nanopores under electron beam irradiation at temperatures in the range of 400-1200 °C”, CARBON, vol. 50, nr 8, 3 March 2012, pp. 2961-2965 process graphene. For this article similar disadvantages as above apply.

It is noted that nanospheres therein are deposited on graphene acting as a mask. An oxygen plasma is used to etch graphene that is not protected by the nanosphere. By principle a plasma (especially O₂ plasma) is reacting (in this case oxidizing) the graphene surface and importantly edges thereof as well, which convert into graphene oxide (an insulator while graphene is conducting). Therefore the chemical nature of graphene (sp2, honeycomb bonded graphene) is changed.

In general it is noted that current techniques to fabricate any given shape in graphene lack required sub-nanometer precision for obtaining atomically sharp and controlled regular edges, with an appropriate crystal orientation. They also lack the control over the shape to be made. Even further, the graphene, especially a thin layer thereof, such as a mono-layer, can not be sculpted using prior art techniques, without destroying at least part of the graphene.

The present invention therefore relates to a method which overcomes one or more of the above disadvantages, without jeopardizing functionality and advantages, as well as products obtainable thereby, and use of said products.

SUMMARY OF THE INVENTION

The present invention relates in a first aspect to a method for removing a high definition nanostructure in a partly free-standing layer, a layer obtainable accordingly, a sensor comprising said layer, use of the sensor and a method of detecting.

Therewith a solution has been found to fabricate a wide-range of nanostructures (e.g. graphene) with structural control at atomic level, without inducing amorphization and
without contamination. It was so far impossible to control-
lably pattern e.g. graphene with atomic resolution.

The present invention relates e.g. to formation of nano-ribbons or nano-pores with desired sizes and precision, or desired crystallographic orientation, for instance, a nano-ribbon with zigzag edges, requiring to cut along a crystallographic (e.g. [100]) direction. Typically the structure comprises one or more edges, such as an edge along a crystallographic direction of a monolayer. The structure may comprise a geometry, such as circ-

ular, hexagonal, triangular, etc. The size of the structure is typically from less than 1 nm, e.g. 1 atom, to a few hundred nm. It is noted that in principle also larger structures, comprising nano- and/or microstructures, can be made using the present method, such as a MEMS.

It is noted that the present method is also applicable using more than one radiation sources, such as 2 or more. Using e.g. software multiple beams can be used to sculpt structures parallel in time. Such is e.g. extremely useful when sculpting repetitive structures, such as a sequence of nano-

holes, e.g. in one or two dimensions.

The precision is in the order of 1 atom (e.g. 0.1 nm) or better (0.05 nm), whereas the relative determination of a location is also in the order of 1 atom. Such accuracy and control thereof is not obtainable by any prior art method, according to the knowledge of the inventors. That is a radiation source can be focused on such a small area (0.1 nm) relative to a known or predetermined location (x,y). Such requires precise control of heating of the sample, and damping of external fac-
tors, such as vibration. Such may also require also forming of an image during sculpting or in between sculpting. It is noted that theoretical calculations show that properties of a gra-

phene device depend very strongly on the exact geometry at nano scale. At present an highly accurate and highly reproducible technique is provided to make a range of such geometries, e.g.

for testing theoretical predications. A way to achieve this is to use electrons or other radiation damage to sculpt graphene into desired nano patterns; it is noted that a state of art process does not precisely control radiation damage, contrary to the present invention.

Inventors demonstrate that by using e.g. scanning
transmission electron microscopy, surprisingly one can fully control the e-beam induced damage and combine such with a self-repairing effect of graphene at elevated temperature higher than e.g. 500 °C. Thereby inventors achieved site and orientation specific nano-scale pattern sculpting of mono-layer graphene with reproducibly for the first time. Such a self-repair effect is typically not obtained using an e-beam, unless the effect is activated. Also providing an elevated temperature in a controllable and reliable manner is typically not considered in the prior art. If control and activation are not properly provided self-repair will typically absent or only be partially and insufficiently present.

By using e.g. a scanning electron probe, graphene could be sculpt into all kinds of e.g. pre-defined patterns with sub-nanometer resolution (precision) and simultaneously form an image of the sculpted result in same resolution. The present invention therewith provides a full control of (electron) radiation damage of graphene such that the destructive nature of sculpting and in principle non-destructive imaging can be achieved in a same mode (electron beam current, beam energy, etc.), without further a need for e.g. adjustments and alignment. Thus an imaging feed-back controlled sculpting system is provided, that allows automatically fast pattern writing on graphene, and is suitable for large scale graphene device fabrication.

At present it is to the knowledge of the inventors impossible to predefine and then fabricate graphene nanostructures with sub-nanometer accuracy. More importantly it is not possible to program such a structure using a computer script, as is provided by the present invention. The computer script provides e.g. for at least one predefined structure, communication with the radiation source, processing of an optional image formed, feedback to the radiation source, optimization of e.g. sculpting steps to be performed, control, e.g. of heating, focusing, localization of radiation, and quality determination, e.g. in terms of shape, size and location of a structure. Nanometer size graphene nanostructure with sub-nanometer resolutions are for example important in nanoscience and bionanoscience. One particular example is in the field of biomolecule analysis.
with nanopores and nanogaps. Other examples include narrow/sub-nanometer electronics.

Although the present method may appear to be very simple to use, the present invention is not obvious, even for a skilled artisan in the field. For instance, sculpting into monolayer graphene has been made possible only by combining heating of a sample and self-repair thereof and a scanning (electron) probe.

The present invention also relates to a design of a software platform e.g. to allow more parameters to be tuned during sculpting (shapes, different beam sizes, different exposition times per exposed spots). It also relates to sculpting nanostructures on a substrate (using electron or ion beams). It can be scaled-up to (12") wafer scales. Further a combination of electrical measurement and sculpting is provided. Also atomic resolution sculpting is provided, i.e. one can design defects on graphene atom by atom. Such is regarded totally unique.

A nanostructure typically relates to a structure of intermediate size from molecular to microscopic (micrometer-sized) structures. In describing nanostructures one may differentiate between the numbers of dimensions on the nanoscale, e.g. a nanotextured surfaces, nanotube, nanoparticle, etc. Typical dimensions are between 0.1 and 100 nm; its length could be much greater. The present layer, however, typically has dimensions of a few mm width and length up to a few cm, and a thickness in the nanoscale.

It is noted that terms as “upper”, “lower”, etc. are relative terms.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates in a first aspect to a method for removing a high definition nanostructure according to claim 1. The present invention provides a heating means having a shift of less than 0.1 nm/10 sec. Such provides for sculpting with the present accuracy. The support typically is an electrical insulator, such as SiN. Therewith an electrical current, if a voltage is being applied, will mainly run through the conducting layer.

The present method is extremely clean, e.g. hardly any or no
impurities are introduced. Such is essential for the characteristics of the layer. Further, almost no carbon contamination is produced as well.

In an exemplary embodiment the microscope is operated at 20-2500 kV, preferably at 50-1000 kV, more preferably at 100-500 kV, such as at 200-400 kV. If a relatively low voltage is applied, such as with a SEM, a gas may be present to assist sculpting, for instance water vapor may be added. It has been found that a somewhat lower voltage causes less damage.

In an exemplary embodiment the current of the microscope is 0.05-10 nA, e.g. 0.25-2 nA.

In an exemplary embodiment the sample is modified defect free on nanometer scale, in a time frame of less than 500 ms, preferably from 5-250 ms, more preferably from 10-100 ms, such as 20-50 ms.

In an exemplary embodiment the radiation dose is less than $10^9$ "items"/atom, wherein items relates to e.g. number of electrons, ions, and the like.

In an exemplary embodiment the microscope for forming a nanostructure in a monolayer, comprises a vacuum chamber, and a means for holding the sample to be provided, such as a stage. Such a holder is especially designed by the inventors, in order to obtain desired characteristics of the nanostructures.

In an exemplary embodiment the means for heating is one or more coils, such as a Pt-coil. It has been found that a Pt-coil provides superior reproducibility and reliability.

In an exemplary embodiment the microscope comprises a further source, such as a combination of ions and electrons. There with multiple sources may be provided, each being capable of sculpting. Also a first source may be used for sculpting, and a second for imaging.

In an exemplary embodiment of the present method the radiation source is an electron gun of an electron microscope, preferably a SEM, a HREM, a TEM, a HRTEM, a HRSTEM, and combinations thereof, such as a STEM, HREM and SEM, and STEM and HRSTEM. Although other radiation sources may be applicable, such as a focused ion-beam (FIB), such as using He, or Ga, an electron microscope is preferred in view of higher accuracy.

The abbreviations above relate to Scanning Electron Microscope, High Resolution Electron Microscope, Transmission Electron MI-
croscope, High Resolution Transmission Electron Microscope, and High Resolution Scanning Transmission Electron Microscope, respectively. These terms are considered well known in the field, whereas typical features of the invention are not known in the field, at least not in the combinations as claimed.

In an exemplary embodiment of the present method radiation is focused to an area of less than 2 nm, such as less than 1 nm, such as less than 0.1 nm. Effectively atoms can be removed one by one. Some care has to be taken not to damage the layer; therefore the dwell time is preferably limited. It is preferred that markers are provided on the graphene and/or on the support, in order to improve positioning of the sample. The markers may for instance be a multiple of horizontal and vertical lines, spaced apart, or likewise diagonal lines.

In an exemplary embodiment of the present method an energy used for removing one atom in the layer from $1 \times 10^{-18}$ J to $1 \times 10^{-16}$ J, preferably from $2 \times 10^{-18}$ J to $5 \times 10^{-16}$ J, more preferably from $3 \times 10^{-18}$ J to $1 \times 10^{-17}$ J. It has been found that surprisingly a relatively low energy level is sufficient to remove e.g. atoms, i.e. sculpt a nanostructure, the energy being much lower than expected or typically considered. By controlling the energy at such a relative low level an improved control and accuracy is obtained. It has also been found experimentally that the present method involves a chance process, in that radiation, e.g. electrons, have a certain chance of "hitting" e.g. an atom, and thereby sculpting said atom. It has been established by inventors that said chance is relatively small, e.g. $10^{-9}$ to $10^{-4}$, in other words in an example only one out of very many electrons hit an atom. It has also been found experimentally that only a fraction of the energy of a radiation species is transferred to the layer, e.g. to an atom. Said energy fraction is in the order of $10^{-6}$ to $10^{-3}$, depending on species used more or less energy of said species is transferred to e.g. an atom. Further, it has been found that the amount of energy transferred does not depend linearly on the energy of a species, e.g. higher energies may provide a lower transfer. Also due to e.g. temperature fluctuation e.g. a position of an atom may vary, at least on a nanometer scale, and therefore a focused bundle may be focused (slightly) on a wrong position, e.g. not exactly on an atom, or
nucleus thereof. By carrying experiments and/or calculations suitable values for energy, current, dwell-time, etc. have been obtained, as e.g. detailed throughout the description.

In an exemplary embodiment of the present method sculpting per single point is performed during a period of 0.01-1000 mseconds, preferably from 2-500 mseconds, such as from 5-300 mseconds. Examples of times used are 10, 25, 35, 50, 82, 100, 120, and 250 mseconds. The process of sculpting (at a certain point or location) can be interrupted by a time for forming an image, e.g. of a surrounding area. Typically a size of said location is in the order of a few atoms, or 1 atom, such as 1 nm, or less. The image forming time is typically in the order of 1-1000 μseconds, such as 2-500 μseconds, e.g. 5-100 μseconds. It is preferred that image forming takes place in a time small enough to allow the layer to relax. Thereafter sculpting may be continued, e.g. until a desired structure is sculpted. Throughout the present application a time between sculpting period is also referred to as a “dwell time”.

Some examples of settings for an EM are 500 ms/nm at a beam current of 0.15 nA (3Å resolution), 2ms/nm at beam current of 5nA (1Å resolution), about 2nm/s at beam current of 0.15 nA current, with 3Å resolution, and 500nm/s at a beam-current of 5 nA, with 1Å resolution.

In an exemplary embodiment of the present method after focusing

d) the radiation bundle is moved to a next position on the layer, and wherein optionally steps c) and d) are repeated.

In an exemplary embodiment of the present method the bundle is moved from a first to a further position, which movement is repeated from 1-10^5 times. Also the shape of the nanostructure may be adapted accordingly. As such a single atom may be removed. In an example a complete structure may be removed, such as a structure wherein a relative large number of atoms is removed, e.g. 10^{10} atoms. Typical structures sculpted may have dimensions in the order of nm by nm to 500 μm by 500 μm.

In an exemplary embodiment of the present method further an image is formed of the layer, such as by detecting forward or backward scattered radiation, such as by an annual detector, and/or
providing feedback control to the means for directing radiation.

Such is a well appreciated feature, as almost in the same time frame a user is capable of checking results of the sculpting, and/or optionally adjusting said sculpting, if considered necessary. The sculpting can be followed "real time", effectively after removal of e.g. each atom. It is noted that only a small delay is involved, e.g. a time needed to form an image, to process data, and the like. The delay is therefore in an order of μsec-msec. The feedback control loop may comprise software for analyzing an image obtained, e.g. in view of quality of the sculpture, in view of a crystallographic direction to be followed during sculpting, etc. The feedback loop and/or computer associated therewith may further comprise a predetermined shape to be sculpted, which shape is than sculpted according to the present method. The feedback may also provide valuable information, e.g. on quality, of an intermediate product being formed. Such is not available in the prior art.

The image formed accordingly may also be used as a means for quality control. In an example a formed imaged may be characterized e.g. in terms of position, orientation, shape, size, width, length, etc. Using an electron microscope this can be done with high precision, e.g. with an accuracy of about 0.01 nm. Also one or more images may be formed during sculpting, and/or in between sculpting, and/or in a final stage.

The present invention relates in a second aspect to a free-standing layer comprising one or more nanostructures formed therein obtainable by the present method, wherein the one or more nanostructures are defined with a precision of less than 1 nm, preferably less than 0.5 nm, more preferably less than 0.25 nm, such as of about 0.1 nm, wherein the one or more nanostructures are selected from the group comprising a hole, a bridge, two or more parallel bridges, a ribbon, a bridge in a crystallographic direction [hkl], and combinations thereof, and wherein the layer is from one monolayer - 10 monolayers thick, preferably from 1-5 monolayers, such as from 1-2 monolayers.

In an exemplary embodiment of the present layer the layer is a monolayer of graphene, a bilayer of graphene, or a
layer of graphene on a layer of a further material, such as BN. As such combinations of one or more layers having the same, similar, or different materials.

The layer may for instance comprise one or more of nanoholes, nanoslits, e.g. along a crystallographic direction [hkl], nanobridges, e.g. between a first and a second part of the layer, and nano rasters, such as a hexagonal or trigonal raster comprising one or more holes therein.

The layer is preferably one atom or molecule thick, optional two atoms or molecules. A somewhat thicker layer provides e.g. better mechanical strength. A monolayer has somewhat better electro-magnetically properties.

The layer may also relate to a so-called 2-dimensional crystal or the like. Such crystals are considered to have an atomic flat structure. In other words, a crystallographic layer may be formed according to the invention, wherein an option of self-repair is available. Examples relate to boron nitride, graphite, graphene, dichalcogenides, such as sulfides, selenides and tellurides, having a metal such as Cd, In, Zn, Na, Nb and Mo, such as NbSe₂, MoS₂, and complex oxides, such as MeCu₂Oₓ, such as Bi₂Sr₂CaCu₂Oₓ.

The present invention relates in a third aspect to a sensor for detecting charged species in a fluid, comprising a free-standing layer according to the invention.

In an exemplary embodiment the sensor comprises an electro-magnetically conducting layer. In an exemplary embodiment the present sensor further comprises an electrical power supply, and a means for detecting direct or indirect fluctuations in one or more of electrical field and magnetic field, such as in current, resistance, potential, charge, inductance, capacitance, magnetic field, frequency, power and flux. As fluctuations are typically very small, the sensitivity and selectivity of the means for detecting electro-magnetic variations is preferably very high. In principle such means and attributes for measuring e.g. nano ampere or lower are at present available.

The layer of the sensor may comprise one or more nanostructures. Typically the layer is somewhat less wide in a middle thereof. The support beneath said middle typically will comprise a hole for letting e.g. a fluid pass through. The hole in
the support is typically at least one order of magnitude larger than the nanostructures. In an example the middle part of the layer may comprise one or more bridges, the one or more bridges preferably being aligned. A fluid may pass alongside said bridges, optionally causing a variation in electro-magnetic behavior thereof. The variation can be measured and is indicative for the nature of the fluid, and/or species therein, passing by. The layer may be provided with a thin conductor attached there- to, such as a metal wire, such as a (nm) Pt wire. Such provides improved reliability and reproducibility.

In an exemplary embodiment the present sensor is for detecting one or more of a single ion, a DNA-base pair, a RNA-base pair, an enzyme, a protein, a nucleotide, a gene, a molecule, such as ethene, CO₂, CO, poisonous gas, O₂, and volatiles, a plasmid, and a virus. With the present sensor and/or method, and typically a calibration curve or the like, the above ions and molecules can be analyzed.

The present invention relates in a fourth aspect to a use of a sensor according to the invention for detecting one or more of a single ion, a DNA-base pair, a RNA-base pair, an enzyme, a protein, a nucleotide, a gene, a molecule, a plasmid, and a virus.

The present invention relates in a fifth aspect to a method of detecting a species such as one or more of single ion, a DNA-base pair, a RNA-base pair, an enzyme, a protein, a nucleotide, a gene, a molecule, a plasmid, and a virus, comprising the steps of:

providing a sensor according to the invention,
providing a sample comprising the species,
detecting presence of the species, and optionally one or more further characteristics of the species, such as concentration, base-pair sequence, or absence of the species.

Thereby the present invention provides a solution to one or more of the above mentioned problems.

Advantages of the present description are detailed throughout the description.

EXAMPLES

The present inventors operated a transmission elec-
tron microscope in Scanning Transmission Electron Microscopy mode at 300 keV and 200 keV, in which electrons are focused into a fine spot of 0.1 nm. In this mode, the electron dose exposed onto graphene (4) could be simply controlled over a time the electron probe residual in a given position, the dwell time. Thus, setting different scanning dwell times, the present inventors achieved a slow scan for a destructive sculpting and a fast scan for a non-destructive imaging of the sculpted structure without a need for changing electron beam condition (300 keV beam energy and 0.15 nA beam current in most of the experiment). Optionally sculpting is chemically assisted. A schematic diagram is given in Figure 2a, showing a present configuration of feed-control sculpting in STEM mode, comprising scanning coils (1), incident electrons (2), back scattered electrons (3), heating coils (5), preferably made of Pt, a SiN support (6), an annual detector (8), an image forming step (9) typically using a computer, and a feedback control (10). The image is formed (9) by fast scanning a subÅngstrom electron probe over an interested region and collecting all the forward scattered electrons (7) using an annular detector (8). The dwell time of imaging is usually set as 5~30 µs, giving a radiation dose as ~10^4 electrons/atom. It has been found that the possibility of inducing one carbon atom displacement by a 300 keV electron is less than 10^{-7}, and therefore the total sputtering possibility by the radiation dose during imaging process is rather rare (less than 10^{-3}). It has been found experimentally that easy achieving of non-destructive imaging is preferred for fast and controllable sculpting, because one can immediately image the geometries of a sculpted pattern without inducing extra unwanted electron beam damage. Therefore, based on imaging a feedback (10) control is built to correctly and precisely adjust the sculpting process. The typical sculpted nano-structures of graphene (4) are respectively shown in Figure 2b-f, including three nano-ribbons with defined ribbon directions along crystallographic [100], [110] and [210] directions giving edges of a zigzag, armchair and a mixed type pattern, respectively, and an ordered nano-hole pattern, with each nano-hole of the same diameter (2 nm). The width of the nanoribbon and the diameter of the nanoholes can be controlled within sub-nano-meter accuracy and they can be easily repro-
Another important component of controllable sculpting is that the sample is heated above e.g. 500°C, because this allows a self-repairing effect. During a fast scan for imaging, the chance of inducing e.g. carbon knock out damage is found to be rare, but it may still initialize few point defects of carbon vacancies on graphene (the density of carbon vacancies is typically around $10^{-3}$). It has been found that without heating the sample, around these point defects e-beam damage can be easily developed in a next scan. However, at a high temperature protecting full integrity of a graphene lattice in an imaging process is protected. This effect of heating can be visualized from Figure 3, in which an atomic resolution STEM imaging at 300 kV of defect free graphene lattice using a rather long dwell time of 240 μs.

Whereas a fast scan of STEM provides an easy imaging of a shape of sculpted structures, atomic resolution imaging of what was made, for instance, crystallinity and sharpness of an edge is more difficult to obtain, because of a long exposure time needed; for instance, a 300 keV electron beam certainly changes edges of a sculpted structure (SI). For this reason, it has been established to image details using an acceleration voltage of less than ~100 keV, which is found to be about the graphene damage threshold.

Figure 4 shows HREM images of sculpted ribbons at 80kV and a graphene sample is also heated to 600°C for further protecting the edge of a ribbon. It is clearly observed that crystallinity of the graphene lattice stops close to the nano-ribbon edge. Inventors have often observed that an atomic sharp edge was obtainable for a [100] direction (zigzag) and [110] direction (armchair). However, despite of sculpting with the same setting, the edges of the ribbons along other orientations are not atomically sharp. An instability of the edge along these directions is observed. It is therefore believed that a stable edge in a random direction can only be constructed by combining two stable zigzag and armchair edges, resulted in the roughness of the edge.

In summary, inventors have demonstrated a full control of the scanning electron beam technique to sculpt monolayer graphene into size, site (position) and orientation
specific nano-patterns, an advance in view of the prior art that allows automatic pattern writing on graphene sheet and
the like e.g. for large scale application. This capability
opens new applications of graphene in nano-electronics and
nanophysics.

Sample preparation and transferring:
Graphene flakes were prepared by exfoliation of
natural graphite (NGS graphite) on a 285 nm thermally grown
SiO$_2$/Si wafer.

Graphene flakes of interest were selected using op-
tical interference microscopy. A selected graphene flake was
then transferred on top of a hole in a supporting SiN mem-
brane using a wedging transfer technique. The crystallinity
and the single-layer graphene were further checked using
electron diffraction.

A heating holder with a MEMS heater was used for
in-situ experiments.

For in-situ heating, a SiN membrane was used with
an embedded, coiled Pt wire. In the SiN membrane, a 2 μm di-

20
25

Parameters for STEM sculpting and imaging:
STEM imaging of nano-patterns (in Figure 5) was
performed in a cubed FEI Titan microscope with a post-
specimen was corrector operated at 300 keV. Spherical aber-
ration is always set below 1 micron (μm). A convergent angle
of focused electron is set at 10 mrad for achieving a very
fine electron beam. The camera length is set to 470 mm in
order to allow the annual detector to record a maximum num-
ber of diffracted electron beams of graphene, in order to
obtain a good signal. The electron beam current was set at
~0.15 nA for both STEM imaging and sculpting. The time was
set at 5~30 μs for imaging and 10 ms for sculpting.

A HRSTEM image of monolayer graphene was obtained
in a Titan3 G2 60-300 TEM, equipped with both image and
probe correctors and a monochromator. The microscope was operated at 300 kV with a beam current at ~0.2 nA. The convergent angle is set at 20 mrad. For collecting a maximum number of diffracted electron beams of graphene best signal, the camera length is set at 185 mm. The imaging time was set at 240 μs, resulting in a total of 52 seconds for recording a 512x512 pixels image.

The high resolution transmission electron microscopy (HRTEM) was performed in a Titan 60-300 PICO TEM equipped with a high brightness electron gun, Cs probe correctors and a monochromator unit together with a Cs-Cc achro-aplanat image corrector. The microscope was operated at 80 kV. No apparent beam damage was observed during image recording, however, a longer expose time of nano-ribbons under a high energy/high current electron beam may cause breakage of ribbons. Inventors took 10 images for each nano-ribbon with 2 seconds exposure time using a 4k by 4k Gatan CCD camera with a binning set to 2. Then these image sequences are aligned and summed up to give an image with a high signal-noise ratio, such as by using software (e.g. ImageJ).

Control of knock out damage:

While operating in STEM mode, an easy control over a time an electron probe resides in a given position, the dwell time, was achieved. By tuning the dwell time, a control of a total number of electrons exposed on a carbon atom (dose) by giving a fixed electron beam current (typically 0.1-0.2 nA) was obtained. It is noted that only a very tiny portion of incident high-energy electrons exactly hit a core of an (C) atom and are then back-scattered. The electrons can induce a so-called knock out damage. Most of incident electrons are only slightly scattered and can be used to form an STEM image. The huge ratio between forward forward-scattered electrons for imaging and the back-scattered electrons for sculpting allows inventors to set a dwell time to less than a critical value. Under a critical dwell time the fraction of the weakly scattered electrons is found to be sufficient to provide a good contrast of an STEM image, whereas it has been found that the fraction of the back scattered electrons hardly create carbon vacancies. Such vacancies if required could be self-repaired when the graphene
is at elevated temperatures.

Control Parameters for STEM sculpting and imaging:

When an electron beam is fixed at one position (no scanning but static), it is believed that electron beam damage relies on how long the electron beam stays on said position, which is may be the dwell time only. When the dwell time is longer than a critical time, it has been found that an electron beam will create a hole around the electron beam position. The size of hole grows with increasing dwell time, up to a final size, determined by a whole by electrons exposed region, usually being about several nanometers (in diameter) in an STEM mode. It is noted that a real electron beam exposed region is typically much larger than a “spot”, where only 80% of a number of electrons are focused at. It is observed that once vacancies initially are created at a center spot, carbon atoms near the vacancies are not fully bonded and can therefore be removed easier. Thus an an e-beam created hole can grow out to the whole by electrons exposed area, even though an outer region thereof is only weakly exposed by electrons. For this reason, an electron beam is preferably blocked or kept in fast scanning mode for protecting integrity of e.g. graphene, except when sculpting is required.

It has been found that when an electron beam is scanning on e.g. graphene, another parameter, namely scanning resolution, will also play an important role in e-beam damage. During scanning, an electron beam does not continuously move over a sample. Instead, the electron beam stays at a given position for a certain time, jumps to a next position, being a certain distance away from the previous position. This step size between neighboring scanned positions is typically termed as the scanning resolution. In order to have a continuous cutting through of graphene lattice, it has been found that the scanning resolution cannot be set too large, e.g. not larger than a size of a hole etched by an e-beam. Otherwise only discrete holes will be created. On the other hand, it has been found that a small scanning step size results in overlapping of a by electrons exposed region for neighboring scanning points. The common area of these points suffers in the example double electron exposure or
even more. This is similar to an effect of increasing dwell time. Thus, in the sculpting process, a smaller scanning resolution will result in a wider cutting line, which is not desired. More seriously in the imaging process, if a very small scanning step size is used, heavy overlapping is thereby induced, which may dramatically increase an effective dwell time, resulting unexpected e-beam damage in imaging process. This often happens for recording an atomic resolution STEM. In order to reach a high magnification for imaging carbon atom, the scanning resolution is normally set as 0.15 Å/pixel. Since the size of one carbon atom is ~1.4 Å (approximated by using C-C bonding length), an effective electron exposed dwell time for one carbon atom will in an example be 10 times higher than a static dwell time setting.

In the present HRSTEM experiment, a relatively long dwell time of 240 µs was once used for achieving single C atom contrast. This resulted in actually exposing the single one carbon atom by an electron beam up to ~ 2.4 ms, which is comparable to the time used for sculpting (10 ms). Thus, although inventors had observed that graphene sheet keeps its integrity after taking 3-4 HRSTEM images from one area of the graphene, it has been observed that further scanning of same area always produces collapse of graphene lattice, being undesirable.

The invention is further detailed by the accompanying figures, which are exemplary and explanatory of nature and are not limiting the scope of the invention. To the person skilled in the art it may be clear that many variants, being obvious or not, may be conceivable falling within the scope of protection, defined by the present claims.

FIGURES

Figures 1, 2b-g, 3-5 show microscope images, figure 2a a schematical layout of a microscope, and figure 6 represents an example of the present method.

DETAILED DESCRIPTION OF THE DRAWINGS / FIGURES

Figure 1 shows a STEM of monolayer graphene.

Figure 2 Schematic diagram sketches the configuration of sculpting graphene using scanning transmission electron microscopy: a high energy electron beam is focused and scanned on a graphene sheet. Back scattered electrons induce
a knockout of carbon atoms, used for sculpting the nano-pattern and forward scattered electrons are collected to form a STEM image, which may be used for control of the sculpting process. The graphene sheet is laid on a SiN MEMS, which is heated by embedded Pt coils. In this configuration, nano-ribbons are created along three specific orientations, [100] (B) [210] (C) and [110] (D), referenced to the diffraction of the graphene (E); An ordered nano-hole pattern with controlled diameter size of about 2 nm is also obtained (F); further a bridge like structure of about 20 nm are shown, which structure can be produced with high reproducibility and accuracy (G).

Figure 3 shows a high resolution scanning transmission electron microscopy of a mono-layer graphene heated at 650 °C being recorded by operating the microscope at 300 kV (A). The denoised image (B), being an image processed from image (A), clearly indicates a nice arrangement of carbon hexagon rings without visible atoms vacancies. Such is highly desired.

Figure 4 shows a high resolution electron microscopy of Nano-ribbons obtained at 80 kV.

Reproducibility of controllable Sculpting:

Inventors have performed controllable sculpting on different graphene samples. A good repeatability and accuracy is achieved. Another example is given in Figure 5. A further way of making a nano-ribbon is demonstrated.

Figure 5 (A) shows a STEM image for an electron etched nano-ribbon with defined ribbon orientation along [100]. Figure (B) shows a HREM image of a part of the ribbon of figure (A) for indicating crystallinity of the ribbon edge and an inset of FFT (Fast Fourier Transform) of the image at right upper corner is provided showing the crystal orientation of the graphene. The illumination region of (B) is also outlined by a white enclosed frame in (A). Figure (C) shows a STEM image of another ribbon along [-120]. Figure (D) shows an ordered pattern of nano-holes with 6 nm diameter.

Figure 6 shows the influence of scanning resolution. ds denotes the scanning resolution that a distance between two neighboring electron beams exposed positions in
the scanning. $d_h$ denotes a size of an e-beam etched hole on a graphene sheet. In order to get a continuous cutting through graphene, the scanning resolution is set to be not less than a certain value. In fig. 6a $d_h < d_s$, implying that the scanning resolution is larger than the size of a hole. A first hole is sculpted, followed by subsequent holes 2-4. As such, during sculpting, some material may remain in between holes. On the other hand, in fig. 6b, $d_h \geq d_s$, implying that the scanning resolution is smaller than the size of a hole. Therewith a "continuous" removal of material is obtained (holes 1-5).

The invention although described in detailed explanatory context may be best understood in conjunction with the accompanying figures.
CLAIMS

1. Method for removing a high definition nanostructure in a partly free-standing layer with a thickness of less than 5 nm, comprising the steps of:

   a) providing a radiation source, a means for high precision directing radiation, a sample, the sample comprising the free-standing layer, a support for largely supporting the layer, and one or more means for self-repairing of the layer, such as heating means for heating the layer,

   b) activating said means for self-repairing, and

   c) focusing said radiation in a bundle on the sample during a period sufficient for removing the high definition nanostructure,

   wherein an energy used for removing one atom in the layer is preferably from $1 \times 10^{-18}$ J - $1 \times 10^{-16}$ J.

2. Method according to claim 1, wherein the partly free-standing layer is a monolayer, such as of graphene, BN, a dichalcogenide and a complex oxide,

   wherein the radiation source is a source of electrons, such as an electron microscope,

   preferably wherein the radiation source is an electron gun of an electron microscope, preferably a SEM, a HREM, a TEM, a HRTEM, a HRSTEM, and combinations thereof, such as a STEM, HREM and SEM, and STEM and HRSTEM,

   wherein the one or more means for self-repairing of the layer is a heating means for heating the layer,

   wherein the means for self-repairing is by heating the sample, such as to above 400 °C.

3. Method according to any of the preceding claims, wherein radiation is focused to an area of less than 2 nm, such as less than 1 nm, such as less than 0.1 nm.

4. Method according to any of the preceding claims, wherein an energy used for removing one atom in the layer is from $2 \times 10^{-18}$ J - $5 \times 10^{-16}$ J, more preferably from $3 \times 10^{-18}$ J - $1 \times 10^{-17}$ J.

5. Method according to any of the preceding claims, wherein sculpting per single point is performed during a period of 0.01-1000 mseconds, preferably from 2-500 mseconds, such as
from 5-300 milliseconds.

6. Method according to any of the preceding claims, wherein after focusing
d) the radiation bundle is moved to a next position
on the layer, and wherein
optionally steps c) and d) are repeated.

7. Method according to claim 6, wherein the bundle is moved from a first to a further position, which movement is repeated from 1-10x10⁸ times.

8. Method according to any of the preceding claims, wherein further an image is formed of the layer, such as by detecting forward or backward scattered radiation, such as by an annual detector, and/or
providing feedback control to the means for directing radiation.

9. Free-standing layer comprising one or more nanostructures formed therein obtainable by a method according to any of the preceding claims, wherein
the one or more nanostructures are defined with a precision of less than 1 nm, preferably less than 0.5 nm, more preferably less than 0.25 nm, such as of about 0.1 nm, wherein the one or more nanostructures are selected from the group comprising a hole, a bridge, two or more parallel bridges, a ribbon, a bridge in a crystallographic direction [hkl], and combinations thereof, and
wherein the layer is from one monolayer - 10 monolayers thick, preferably from 1-5 monolayers, such as from 1-2 monolayers.

10. Free-standing layer according to claim 9, wherein the layer is a monolayer of graphene, a bilayer of graphene, or a layer of graphene on a layer of a further material, such as BN.

11. Sensor for detecting species in a fluid, comprising a free-standing layer according to any of claims 9-10.

12. Sensor according to claim 11, further comprising an electrical power supply, and a means for detecting direct or indirect fluctuations in one or more of electrical field and magnetic field, such as in current, resistance, potential, charge, inductance, capacitance, magnetic field, frequency,
power and flux.

13. Sensor according to any of claim 11-12 for detecting one or more of a single ion, a DNA-base pair, a RNA-base pair, an enzyme, a protein, a nucleotide, a gene, a molecule, a plasmid, and a virus.

14. Use of a sensor according to any of claims 11-13 for detecting one or more of a single ion, a DNA-base pair, a RNA-base pair, an enzyme, a protein, a nucleotide, a gene, a molecule, a plasmid, and a virus.

15. Method of detecting a species such as one or more of a single ion, a DNA-base pair, a RNA-base pair, an enzyme, a protein, a nucleotide, a gene, a molecule, a plasmid, and a virus, comprising the steps of: providing a sensor according to any of claims 11-13, providing a sample comprising the species, detecting presence of the species, and optionally one or more further characteristics of the species, such as concentration, base-pair sequence, or absence of the species.
Fig. 2b-g

Fig. 4a-c
Fig. 5a-d

\[ d_s \leq d_h \]

Fig. 6a

\[ d_h < d_s \]

Fig. 6b

\[ d_h \geq d_s \]
### A. CLASSIFICATION OF SUBJECT MATTER
INV. G03F7/00 G03F7/20 G01N33/487 H01L29/16

**ADD.**

According to International Patent Classification (IPC) or to both national classification and IPC

### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G03F H01L G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>NING LU ET AL: &quot;In situ studies on the shrinkage and expansion of graphene nanopores under electron beam irradiation at temperatures in the range of 400-1200°C&quot;. CARBON, [Online]. vol. 50, no. 8, 3 March 2012 (2012-03-03), pages 2961-2965, XP055043166, ISSN: 0008-6223, DOI: 10.1016/j.carbon.2012.02.078 abstract; figures 1,2 page 2962, left-hand column, paragraph 2 - page 2963, right-hand column page 2964, left-hand column - right-hand column; figure 4 the whole document</td>
<td>1-3,6-10</td>
</tr>
</tbody>
</table>

- Further documents are listed in the continuation of Box C.

- * Special categories of cited documents:
  - "A" document defining the general state of the art which is not considered to be of particular relevance
  - "E" earlier application or patent but published on or after the international filing date
  - "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  - "O" document referring to an oral disclosure, use, exhibition or other means
  - "P" document published prior to the international filing date but later than the priority date claimed

- *"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

- *"X" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is taken alone

- *"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

- "*8" document member of the same patent family

Date of the actual completion of the international search
7 May 2013

Date of mailing of the international search report
29/05/2013

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk, Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

Authorized officer
Paisdor, Bernd
<table>
<thead>
<tr>
<th>Category**</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>BO SONG ET AL: &quot;Atomic-Scale Electron-Beam Sculpting of Near-Defect-Free Graphene Nanostructures&quot;, NANO LETTERS, vol. 11, no. 6, 8 June 2011 (2011-06-08), pages 2247-2250, XP055042632, ISSN: 1530-6984, DOI: 10.1021/nl200369r cited in the application abstract page 2247, left-hand column, paragraph 2 - page 2248, right-hand column, paragraph 2 page 2248, right-hand column, paragraph 2 - page 2249, right-hand column, paragraph 4 -----</td>
<td>1-3,6-10</td>
</tr>
<tr>
<td>A</td>
<td>JI FENG ET AL: &quot;Patternning of graphene&quot;, NANO SCALE, vol. 4, no. 16, 20 July 2012 (2012-07-20), page 4883, XP055043157, ISSN: 2040-3364, DOI: 10.1039/c2nr30790a the whole document page 4885, right-hand column figures 1,2 -----</td>
<td>1-10</td>
</tr>
<tr>
<td>L</td>
<td>TAO XU ET AL: &quot;Size-Dependent Evolution of Graphene Nanopores Under Thermal Excitation&quot;, SMALL, 20 August 2012 (2012-08-20), pages n/a-n/a, XP055043158, ISSN: 1613-6810, DOI: 10.1002/smll.201200979 figures 1-4 the whole document page 5, column 1 -----</td>
<td>1-15</td>
</tr>
<tr>
<td>Patent document cited in search report</td>
<td>Publication date</td>
<td>Patent family member(s)</td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>-----------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2772789 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 102630304 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 2470899 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 2013505448 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KR 20120069720 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2012234679 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 2011046706 A1</td>
</tr>
</tbody>
</table>