Novel carbon materials and microstructures for electrochemical sensors

Joonas J. Heikkinen
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Abstract

Carbon thin films have gained a lot of attention since the discovery of carbon nanotubes in 1991, which ignited widespread research on the many forms of carbon. The most well-known form of carbon is diamond, and its synthetic version has been adopted in applications that require high wear resistance. However, carbonaceous materials have a lot more potential than just that. There is a broad spectrum of carbonaceous materials available which properties can be tailored according to need.

In this thesis, three different carbon thin films were studied from the microfabrication and patterning perspective. These selected thin films were especially electrically conductive as their performance was studied as electrode material in multielectrode arrays (MEA). These three materials were nanocarbon (nC), tetrahedral amorphous carbon (ta-C), and pyrolytic carbon (PyC). In microfabrication and thin film technologies, the methods and equipment for carbon thin film patterning are limited. This thesis presents patterning methods for all three studied carbon materials. The nC film was plasma etched, the ta-C film was patterned with lift-off, and PyC was patterned while it was still photoresist before pyrolysis.

Most carbon materials are naturally biocompatible without any additional surface coatings. These three materials were used in electrochemical measurements to detect the minor presence of neurotransmitters like dopamine or other biological analytes. From an electrochemistry point of view, carbon is an appealing material as it exhibits a wide potential window that allows the measurement of multiple analytes simultaneously. PyC and ta-C show promising results in dopamine detection from bulk concentrations, but there is still a need for improvement if interfering molecules and impurities are present.

These three carbon materials were utilized in multielectrode arrays, which are formed of many microelectrodes close to each other. MEA devices can be used to follow the signaling between neuronal cultures, follow the activity of brain slices, or measure even the minuscule concentration of biological analytes. This thesis presents biological measurements only for nC-MEA (with brain slice). PyC and ta-C materials were successfully implemented in MEAs, but their performance was only tested in preliminary experiments.

Keywords Microfabrication, carbon, pyrolysis, multielectrode arrays

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After 12 years of studying at Aalto University, it is finally the time to finish this chapter of my life in the form of a doctoral thesis. The journey has been long to get to this point, but also very rewarding. I would not have reached this point without the help and support from many individuals along the way.

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To me, science and research are more than just publishing articles, it is also collaboration and teamwork. Therefore many thanks go to the teammates I got to work with during all the years, but with whom I do not (yet) share any published articles. The shared findings in the cleanroom and endless debates about insignificant process steps kept the motivation
They are not only co-workers but very good friends: Sasha, Giovanni, Mario, Ashkan, Farzin, Anas. A very special one goes to Ville who has, since 2013, been my office roommate, lunch buddy, cleanroom pal, personal trainer, and a very close friend.

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Espoo, October 4, 2021,

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List of Publications

This thesis consists of an overview and of the following publications which are referred to in the text by their Roman numerals.


Author’s Contribution

Publication I: “Plasma etched carbon microelectrode arrays for bioelectrical measurements”

JH designed and executed etching experiments, analysed nanocarbon material, and fabricated carbon MEAs under supervision of SF and VJ. TK performed electrophysiological experiments and analysed the data under the supervision of TT. AL, TS, and SK performed biocompatibility studies and staining experiments under the supervision of CR. SIK fabricated the nC films under the supervision of JGH. SF, VJ, HH, CR, and SL planned the research. JH wrote the original manuscript, all authors reviewed and commented.

Publication II: “SU-8 Based Pyrolytic Carbon for the Electrochemical Detection of Dopamine”

JH fabricated all Pyrolysed carbon films and did Raman and contact angle measurements under supervision of SF and VJ. EP designed the experiments, did electrochemical measurements and cell cultures, and wrote the original manuscript under TL's supervision. KS did parts of the electrochemical experiments under the supervision of EP. SS did surface characterization. AA did simulations. All authors reviewed and commented on the original manuscript. JH, EP, VJ, SF, and TL planned the research.

Publication III: “Fabrication of micro- and nanopillars from pyrolytic carbon and tetrahedral amorphous carbon”

JH fabricated and analysed carbon samples, designed sample layouts (together with EP and TL), optimized fabrication procedures, and wrote the
original manuscript under the supervision of VJ and SF. EP performed and analysed cell viability studies under the supervision of TL. NW did ta-C deposition under the supervision of JK. EP, TL, and VJ planned the research. All authors reviewed and commented on the original manuscript.

**Publication IV: “Fabrication and design rules of three dimensional pyrolytic carbon suspended microstructures”**

JH designed the structures and fabrication procedure, executed initial tests, analysed the data, and wrote the original manuscript under the supervision of SF and VJ. JK executed sample fabrication and did all data acquisition under the supervision of JH. SF and VJ planned the research. All authors reviewed and commented on the original manuscript.
Abbreviations

2D Two dimensional
3D Three dimensional
AA Ascorbic Acid
AFM Atomic Force Microscopy
bSi Black Silicon
C-MEA Carbon Multielectrode Array
C-MEMS Carbon Microelectromechanical Systems
CE Counter Electrode
CFUBM Close-Field Unbalanced Magnetron
CNT Carbon Nanotube
CTE Coefficient of Thermal Expansion
CV Cyclic Voltammetry
CVD Chemical Vapor Deposition
DA Dopamine
DBS Deep Brain Stimulation
DIV Days In Vitro
DIW Deionized Water
DLC Diamond Like Carbon
DNQ Diazonaphthoquinine
EAT Electrode Area Test
Abbreviations

**ECIS**  Electrochemical Cell-Impedance Spectroscopy

**EIS**  Electrical Impedance Spectroscopy

**FCVA**  Filtered Cathodic Vacuum Arc

**FWHM**  Full Width Half Maximum

**GBL**  Gamma-Butyrolactone

**HF**  Hydrofluoric acid

**HMDS**  Hexamethyldisilazane

**hPDMS**  hard Polydimethylsiloxane

**IC**  Integrated Circuit

**ICP-RIE**  Inductively Coupled Plasma Reactive Ion Etching

**IPA**  Isopropanol

**IR**  Image Reversal

**ISR**  Inner Sphere Probe

**LPCVD**  Low Pressure Chemical Vapor Deposition

**MEA**  Multielectrode Array

**MEMS**  Microelectromechanical Systems

**mNSC**  Mouse Neural Stem Cell

**nC**  Nanocarbon

**OSR**  Outer Sphere Probe

**PAC**  Photoactive Compound

**PEB**  Post-Exposure Baking

**PECVD**  Plasma Enhanced Chemical Vapor Deposition

**PEI**  Polyethylenimine

**PGMEA**  Propylene Glycol Methyl Ether Acetate

**PI**  Propidium Iodide

**PLL**  Poly-L-Lysine

**PDMS**  Polydimethylsiloxane

**PyC**  Pyrolytic Carbon
Abbreviations

**RE**  Reference Electrode  
**RF**  Radio Frequency  
**RIE**  Reactive Ion Etching  
**RMS**  Root Mean Square  
**rpm**  Revolutions Per Minute  
**S/N**  Signal-to-Noise  
**SEM**  Scanning Electron Microscopy  
**ta-C**  Tetrahedral Amorphous Carbon  
**UA**  Ultraviolet light  
**UV**  Ultraviolet  
**WE**  Working Electrode  
**WHO**  World Health Organization
1. Introduction

The miniaturization of diagnostic tools and analytical devices has enabled the deployment of portable laboratories and accelerated the gaining of results. These miniaturized devices are often called microchips, or in specific cases, lab-on-a-chip because the coin-sized chips can contain all the necessary analytical methods that a full-sized laboratory would perform. Miniaturization has been possible through decades of development and breakthrough research in multiple disciplines like microfabrication technologies, microfluidics, materials science, electrical engineering, chemistry, information technologies, and many more. The most recent need for portable point-of-care diagnosis tools emerged with the SARS-CoV-2 "Coronavirus" outbreak in 2019-2020, and many interdisciplinary testing and analysis methods have been proposed to help identify the virus spreading [108, 153, 158]. The pandemic just made the need for point-of-care clinical diagnostic tools urgent, but microchips have been developed for years for many purposes like immunodiagnostics [36, 37], drug discovery and development [22, 163], portable blood glucose monitoring [94], and studying and treating neuropathologies [41, 120, 125].

The data from the World Health Organization (WHO) indicate that brain disorders account for one-third of the burden caused by all of the diseases in the wealthy part of the world. [112, 113] These disorders include mental and neurological disorders, where the latter are related to neurotransmitter communication malfunctions between the neurons in the brain, meaning disorders like Alzheimer’s, Huntington’s and Parkinson’s disease, schizophrenia, epilepsy, and migraine. The costs of these disorders were around 800 billion euros for 179 million people afflicted in Europe (2010) and 800 billion US dollars for 100 million people afflicted in the US (2011). [21, 39, 42] The risk of getting afflicted by brain disorders is higher with increased age, and with declined fertility rate, the overall burden of the brain disorders for the societies becomes significantly higher in the following decades.

Accurate measurements of neurotransmitters, the small molecules that transduce the signal from one neuron to another, inside the brain are
crucial for implementing treatments for brain disorders. Most neurological
diseases are beyond rehabilitation, and their treatments mostly focus on
alleviating the symptoms and preventing the disease progression. Early
detection and identification of a disease is a key subject to guarantee a
patient’s autonomy as long as possible. [114] Current treatments are
mainly temporary, and the effect of some treatments, like deep brain
stimulation (DBS) used to alleviate the motor symptoms in Parkinson’s
disease, are examined only by following the presence in symptoms. [84, 86,
88]

Many neurological diseases have been connected to the dysfunction of
neurotransmitter release or uptake: glutamate contributes to cellular
death, leading to epilepsy and Huntington’s, Parkinson’s, and Alzheimer’s
disease [98], and loss of dopaminergic neurons is connected to Parkin-
son’s disease. [6, 8, 35, 88]. The current ways of measuring the levels of
neurotransmitters in vivo are microdialysis, imaging techniques, and elec-
trochemical methods. [134, 140] The benefits of electrochemical methods
lie in the simple instrumentation with low tissue damage, lower costs, and
high temporal resolution, whereas their sensitivity and selectivity over a
certain molecule become a challenge as they are dependent on the electrode
material. [134] Thus, by material selections, it is possible to tailor the elec-
trochemical sensors to meet the high criteria of in vivo neurotransmitter
measurements.

The sensor criteria for successful neurotransmitter monitoring are de-
manding and include i) sensitivity and temporal resolution in micro- and
nanomolar concentrations in milliseconds timescales [40, 135], ii) selectiv-
ity over interfering molecules [134], iii) biocompatibility, and iv) material
integration and durability. Common choices for electrode materials are
usually metals like platinum (Pt)[15, 90] and metallic ceramics [152] like
titanium nitride (TiN). These example materials have their own distinct
benefits: Pt can be used to detect multiple neurotransmitters and other bio-
logical molecules [13, 142, 149], but it wears out easily over time, whereas
TiN is more robust and can take multiple cleaning cycles, but it is not
electrochemically very active material. Therefore they are used in their
own respective applications.

Carbonaceous materials combine the best properties of both Pt and TiN.
Carbon provides natural biocompatibility, and it has been utilized in many
electrochemical sensors [40, 69, 95]. The reason carbon has not reached
the same popularity as Pt and TiN is the complexity of patterning it. Pt
and TiN have been used in IC-industry for decades, and their properties
and fabrications techniques are well known. In contrast, carbon-based
materials are considered non-IC-compatible, their widespread research
emerged only upon the discovery of carbon nanotubes (CNT) in 1991, and
due to their strong and stable bonding nature, they are extremely difficult
to pattern as electrodes.
Patterning, tailorability, and modification are material properties that are needed in electrochemical sensor materials which aim for neurotransmitter detection; for example, glutamate cannot be directly measured with the electrode material, but a specific enzyme has to be immobilized on the electrode surface to produce hydrogen peroxide, which can be electrochemically detected [58]. This requires the electrode material to be compatible with enzyme immobilization.

Carbon materials used in electrochemistry include graphite [12], graphene [144], graphene oxide [167], pyrolytic carbon [4], tetrahedral amorphous carbon [116, 122], single- and multi-walled carbon nanotubes [162], and carbon nanofibers [133]. They are all different forms of carbon and differ mostly on their physical appearances; the chemical structures are usually a mix of sp\(^2\) or sp\(^3\) bonds. Most of these materials are characterized and fabricated as thin films, which can also be used as electrochemical sensors. But for in vivo use and to study the miniaturization of the electrodes, parts of the thin films must be removed and selectively leave a certain area remaining on the substrate (patterning and etching). In IC-industry and microfabrication technologies, there are several ways how this can be achieved, but they are uniquely targeted for a single type of carbon thin film, and therefore they can not be widely exploited. Removal of carbon thin films by chemical wet etching is not possible due to carbons’ chemical resistance, and more energetic reactions like plasma etching must be used.

The physical shape and topography of a material affect its performance in electrochemistry, which brings new dimensions into the discussion about the ideal electrode. Most of the electrochemical sensors available now rely on chemical bond structure and activity on the electrode surface but do not take into account the shape of the electrode in all dimensions. Physical modifications on the electrode (like surface roughness to increase the surface area or pillars to modify the geometry from 2D to 3D) can change the electrochemical performance drastically. Most of the IC-techniques focus on depositing and patterning of thin films (thicknesses usually in nanometer scale), and therefore there lies a great challenge using the same methods to create structures several micrometers high, and the process becomes even more complicated when the structures are made of carbon (which is extremely difficult to pattern).

Electrodes for electrochemical measurements can be realized in several ways. One method is to create a multielectrode array (MEA) which consists of multiple microelectrodes in a small space. MEAs are generally divided into two main classes, implantable in vivo [14, 106] and non-implantable in vitro [56, 110, 147]. For material characterization and basic studies about electrochemical performance, the in vitro type provides a more controllable environment. This type of MEAs is used for electrophysiological signal measurements from acute [56, 110] and organotypic [25, 127, 150] brain slices, from cultivated neuron populations, electrochemical cell-impedance
spectroscopy (ECIS) from any mobile cell type, or cell migration. MEA layouts, number of electrodes and materials varies depending on applications, from custom layout with 8 electrodes [2] to square-grid layout with over 59 000 electrodes [23], and from transparent with indium-tin oxide electrodes to opaque 3D nanorough platinum black electrodes [1]. Recent advancements in MEA technologies include perforated membrane to introduce negative pressures for tissue attachment or to introduce local chemical treatments, 3D electrodes to penetrate acute slices dead cell layer for better signal acquisition, variable material selection to provide targeted electrochemical species detection, and multiwell format for multiplexing variable samples. [3, 102] Different carbon materials have been utilized in MEA format by multiple research groups. Generally the carbon is in the form of nanotubes [7, 18, 34, 43, 143, 161], but also carbon thin films have been used [46, 55, 70, 71, 74], which is also the main scope of this thesis.

1.1 Objectives and overview of publications

In this study, we used three carbon-based materials and developed patterning methods for them to be used in microelectrode format. This study focuses on the patterning possibilities and changes in materials properties and performance in microelectrode format. We present three different patterning methods for three different carbon materials, namely nanocrystalline carbon (nC), tetrahedral amorphous carbon (ta-C), and pyrolytic carbon (PyC). The different patterning methods were used due to differences in carbon thin films and their deposition procedures. We have used two of the carbon materials, ta-C and PyC, in electrochemical detection of neurotransmitters to fabricate a 3D carbon MEA platform with superior selectivity and sensitivity compared to the current state-of-the-art MEAs.

The research questions in this study were:

1. What are the optimal patterning methods for the selected carbon materials?
2. How well do the carbon materials hold their specific properties after patterning?
3. How do the selected carbon materials differ from each other from the fabrication perspective?
4. How can carbonaceous materials be fabricated as 3D microelectrodes and multielectrode arrays?

In Publication I, we studied plasma etching possibilities for nanocrystalline carbon (nC) thin film. We used several plasma etching recipes with two different etching equipment. The optimal conditions were determined by the Taguchi method. We used the most optimal etching recipe and
fabricated several carbon multielectrode arrays for electrophysiological measurements. The results indicate that the material properties are suitable for cellular experiments after etching and microelectrode fabrication procedures.

Publication II presents an electrochemical characterization of pyrolytic carbon (PyC) thin films. We established a fabrication procedure of PyC in our facilities based on literature and characterized the material properties with multiple methods. We also studied the electrochemical detection of dopamine (DA) with two different PyC surfaces: native and oxidized. Results show that oxidized PyC surfaces provide slightly better signals, but the surface treatment is not permanent and diminishes over time.

In Publication III, we studied how two different carbon materials, ta-C and PyC, can be fabricated into identical structures. The chosen target structures were nanorough surface, micropillars in two scales (2 μm and 20 μm diameters), and hybrid (pillars with nanorough surface). For ta-C, the structures were first made on a silicon wafer, and then they were coated with a thin ta-C layer. For PyC, the structures were first patterned in a photoresist, which was then pyrolysed into PyC. The differences between these two materials and their patterns were studied with cell viability experiments. Results show that the different patterns and structures exhibit only minor differences in cellular responses. The structures achieved with these two materials were almost identical even though the fabrication methods were greatly different.

In Publication IV, we present how complex 3D structures can be fabricated from PyC. We used sacrificial process flow to create suspended microstructures in varying shapes and sizes from the precursor resist before pyrolysis. PyC electrical and structural/geometrical properties were characterized, and parameter spaces for successful fabrication were provided. During pyrolysis, material shrinkage plays a crucial role. Therefore it was studied extensively, and we propose a three-stage model for the impact of temperature on the material properties and level of pyrolysis. In this research, we fabricated significantly smaller suspended structures closer to the substrate than in previous studies and demonstrated the limits for successful fabrication with our process.
2. Background

2.1 Microfabrication techniques

Microfabrication is a set of techniques used to fabricate structures, patterns, and devices in the micrometer range. These techniques include (but are not limited to) deposition of thin films, etching of material, modifying substrates, and thermal modification of materials. The most common substrate in integrated circuit (IC) and microelectromechanical system (MEMS) industry is silicon, which is usually in the form of a single crystalline wafer, and it serves as a foundation stone to which all subsequent steps are performed on. Silicon is a semiconducting material, and its conductivity can be tailored by doping, and it easily forms an insulating oxide layer in elevated temperatures. It also allows the deposition of multiple different materials on top of it due to good material compatibility and adhesion. [63]

The following sections will explain briefly the microfabrication techniques used in this research. For a more thorough introduction to the techniques, please see the books by Franssila [33] and Madou [87].

2.1.1 Thin film deposition

The thickness of thin films is on micrometer or nanometer scale. Thin films can be made from various materials: pure metals like aluminium, chromium, copper, alloys like titanium-tungsten, ceramics like silicon nitride and silicon dioxide, aluminium oxide, and titanium nitride, or special films like diamond-like and teflon-like, or other polymers like photoresists. Thin film properties are different from the material’s bulk properties and give rise to many possibilities that cannot be achieved in larger structures. There are a plethora of different deposition techniques available for many different materials, but here we highlight only the ones that were crucial for this research.
Spin coating
Spin coating is a very widely adopted method for photoresist and polymer deposition due to the simplicity of the process [136]. Viscous material is dispensed on top of the substrate, and it is spun 2000-9000 rpm, usually up to one minute. The resulting film thickness is controlled by resist viscosity, spinning speed and time, and solvent evaporation rate. The film thicknesses can vary between 0.1 - 500 μm. During spinning, the liquid resist spreads from the center towards the edges and finally is expelled over the edge. The film thickness can be controlled with a few percent accuracy, except for the edge bead, which forms at the edge of the wafer.

Sputtering
Sputtering is a deposition method to create thin films from solid materials, usually from metals like chromium, copper, aluminium, gold, and similar [145]. Also, metal alloys can be used. The basic principle is to collide argon ions with the target material and eject atoms into the vacuum. When the substrate is placed close to the target, the ejected atoms will hit the surface and condense, which starts producing a film. Typical deposition rates are 1-10 nm/s, depending on Ar collision power (bias voltage), target material, and vacuum level (higher pressure inside the chamber leads to more collisions of ejected atoms). The films are created with high energy flux, which can have beneficial or detrimental effects: the resulting film will be denser, which leads to compressive stress, but too high energies can damage the substrate. Due to process simplicity and speed, sputtering can be considered one of the most important physical vapor deposition techniques.

Magnetron sputtering
Close-field unbalanced magnetron (CFUBM) sputtering is a special type of sputtering system [11, 68, 105, 121]. In such a system, the targets (usually two or more) are located at the sides of the substrate, and several magnetrons are placed behind the targets. When the sputtering gas is ionized, the magnetrons confine the sputtering plasma near the surface of the targets, which leads to a higher density plasma and faster deposition rates. This setup allows the deposition of hard and dense coatings with good adhesion, for example, diamond-like films with extreme hardness.

Cathodic arc deposition
Cathodic arc deposition uses an electric arc to vaporize the material from the cathode target [117, 128, 129]. The vapor is then condensed on the substrate to create a thin film. A special type of equipment is a filtered cathodic vacuum arc (FCVA) deposition system where the target vapor is directed through a 90°quarter-torus duct surrounded by filtering electromagnetic coils. Impurities (macroparticles and neutral particles) are filtered through this coil, and ionized target material is focused on the
substrate. This setup is actively used to synthesize extremely hard films to protect a high-wear surface (like cutting tools), but also for carbon atom deposition to create diamond-like films. Due to the high-energy electric arc, the target surface can eject a larger cluster of atoms. These clusters are filtered with the duct, and only single carbon atoms can reach the substrate surface, and high sp$^3$ content films can be created (also known as tetrahedral amorphous carbon, ta-C). Filtering the material lowers the deposition rate, which is not desired when as thick as 1 μm layer is required for high-wear tools, but as a coating for electrochemistry, several nanometers are sufficient.

**Chemical vapor deposition**

Chemical vapor deposition (CVD) is a process where the material is synthesized on the substrate with chemical reactions between two or more molecules (compared to physical vapor deposition where the material is deposited from the target as atoms) [126]. The precursors in CVD are in gaseous form, and they are pumped into the reactions chamber, where they are activated to react on the substrate surface. CVD deposition rates usually vary between 0.1 - 10 nm/s, and it is limited either by reaction rate on the substrate surface or by precursor gas flow (mass transport limited). The chemical reaction is temperature dependant, as the reaction requires external energy to take place.

**LPCVD** Low pressure CVD (LPCVD) furnaces usually work in the temperature ranges of 600 - 1200 °C, and due to high temperature, they provide dense and good quality films. The film growth is surface reaction dependent, and the temperature plays a crucial role in precursor decomposition and reaction. Many LPCVD furnaces can support batch processing, but the equipment throughput is usually slow due to thermal stabilization times. The high temperature sets limitations to materials that can be inserted in the equipment, like borosilicate glass wafers with a melting temperature around 500 °C. Amorphous silicon films can be deposited with the LPCVD technique.

**PECVD** Plasma enhanced CVD (PECVD) decomposes the precursor gases in lower temperatures, usually around 300 °C, resulting in worse film quality than in LPCVD. New precursor gas chemistries can be used, which react in lower temperatures ignited by plasma. It also provides one more parameter (RF power) to control the film deposition rate and quality. Due to the complexity of the RF generator and required electrodes, the PECVD equipment usually processes a single wafer. Silicon nitride and silicon dioxide films can be deposited with the PECVD technique.
2.1.2 Lithography

Optical lithography is a technique where light is used to pattern a photosensitive film on top of a substrate [151]. Three elements are usually present in the lithography process: a light source (usually in the ultraviolet range), a photomask (an object which blocks parts of the light), and the photosensitive material (photoresist, usually thin polymer film on top of a substrate). The pattern of the photomask is transferred to the photoresist through UV-light exposure. Depending on the resists’ chemical formula, the UV-exposure can either make the resist more soluble to developers or initiate polymer cross-linking (and reduce solubility). Lithography process is a basic method to create patterns and structures in IC- and MEMS-industries and complex devices can go through lithography process 40 times.

Photoresists are commonly divided into two groups: positive tone resist and negative tone resists. Resists are formed from three main elements: the base resin (which defines the mechanical and thermal properties), photoactive compound (PAC, which defines the sensitivity to UV-light), and solvent (which defines the viscosity and handling of the resist). Photolithography process with resist includes the deposition of resist (usually by spin coating), soft baking (removal of solvents), exposure (to activate the PAC), development (to remove soluble parts of the resist), and hard baking (to harden the remaining resist).

![Diagram of lithography process](image)

**Figure 2.1.** Basic steps in lithography with positive and negative tone resists. The resist type determines if the exposed (positive, d-f) or unexposed (negative, g-i) parts dissolve into a developer. The two different resist types generate different transferred patterns.
Positive resists
In positive tone resist, the photoactive compound is usually diazonaphthoquinone (DNQ), which acts as an inhibitor and prevents the base resin (usually phenolic novolak) solubility to alkaline developers. When exposed to UV-light, DNQ reacts with water (moisture in the air) to form an acidic compound which makes the resist soluble. The name of "positive resist" therefore comes from the pattern reproduction from the mask: the resist remains under the areas of the mask which block the UV-light. The standard processing of positive resist is spin coating, soft baking, exposure, development, and hard baking. The positive resist generates either vertical or positive sidewall slopes because of the trajectory and diffraction of light through and from the mask.

Negative resists
Negative tone resists work in vice versa compared to positive ones: the UV-exposure makes the resist less soluble. This is usually due to increased molecular weight, namely cross-linking of resist (either via free-radical or acid-catalyzed polymerization). Thermal treatment after exposure increases polymer cross-linking, makes the resist more stable, and complicates resist removal. Therefore developers usually do not affect the exposed resist, and soluble parts can be dissolved into solvents. The standard processing of negative resist is spin coating, soft baking, exposure, post-exposure baking, development, and hard baking. Negative resists generally produce negative sidewall slopes.

A common negative tone resist SU-8 comes in various viscosities, and it allows film thicknesses from 300 nm up to 500 μm depending on the amount of solvent in the resist. During UV-exposure, the epoxide-groups in the resist cross-link and create a polymer matrix with very high chemical resistance. SU-8 molecule comprises of carbon, oxygen, and hydrogen (monomer: C₈₇O₁₆H₉₈), and the reactive groups are eight epoxide-groups (more precisely Bisphenol A Novolac epoxy) which connects to other SU-8 molecules under UV-exposure forming a highly cross-linked matrix. In liquid form, the SU-8 is usually dissolved in γ-butyrolactone (GBL), a simple molecule with carbon, oxygen, and hydrogen. When used as a photoresist, a photoinitiator triaryl sulfonium/hexafluoroantimonate salt, which consists of carbon, sulphur, iron, and antimony, is added to the mixture (up to 10 wt-%). The fully cross-linked resist has thermal stability with $T_g > 200 \, ^\circ\text{C}$, it is optically semi-transparent, and it has found great use in MEMS (microelectromechanical systems) structures due to high structural durability. [77, 83, 91]

Image reversal resists
Image reversal resists are a special type of resists. When processed with the positive tone resist steps, they behave as positive tone resist. However,
the resist image can be reversed when a baking step and a second exposure step are added (similar to negative tone). The key to this lies in the chemical formula of the resist: when the resist is baked after exposure, the exposed parts become insoluble to the developer, whereas the unexposed parts remain photoactive. During the second exposure step, which is done without a mask (flood exposure), the unexposed parts become activated and turn soluble for developers, whereas the already exposed and baked areas have already lost their photoactivity. Therefore when the resist is developed after second exposure, only the once-exposed areas are dissolved. Resist AZ5214 is an image reversal resist [38] which can generate both polarity patterns.

**Soft lithography**

Soft lithography is a set of techniques that transfer the patterns to target substrate with elastomeric stamps, molds, and conformable photomasks. To create the patterns to the elastomeric material (usually polydimethylsiloxane, PDMS), the standard lithography technique is usually used first, and the elastomeric material is employed on the 3D structures of the photoresist. [165]

**Replica molding**  
Replica molding is a pattern copying from solid structures (Schematic illustration in Figure 2.2). First, the structures are created on the substrate either with standard lithography techniques or through etching. Then the patterns are usually coated with an anti-adhesive layer like fluoropolymer to ease the stamp release. The liquid form elastomer material is poured on the 3D resist structures and hardened (for example with UV-light or thermal treatment). After cool down, the solid elastomer is peeled off from the master mold, and the (opposite) structures are copied on the elastomer. This elastomer replica can be used as a stamp for processes like imprint lithography and embossing.

![Figure 2.2. Schematic illustration of the replica molding process. a) Patterned Cr layer on Si wafer. b) Si etching through Cr hard mask. c) Cr removal. d) Liquid PDMS is poured on the Si master and then e) baked. f) The solidified PDMS is then peeled off from the master.](image)

**Embossing**  
In embossing, a stamp is used to create structures on polymer film. When the stamp is made of PDMS, the process is known as soft
embossing (Schematic illustration in Figure 2.3). In this process, the stamp is first made with the replica molding technique, and then it is pressed on a resist which is still in liquid form. Then the resist is baked and exposed through the PDMS stamp and finally peeled off. The structures from the stamp can be copied with very high detail, and no development step is needed in the embossing technique. The whole process is also known as double casting, and the final structure is a replica of the original silicon master mold. Figure 2.4 shows SEM images of SU-8 structures fabricated with PDMS stamp.

**Figure 2.3.** Schematic illustration of the embossing process. a) A replica stamp is made with the replica molding technique. b) SU-8 is spin-coated on a Si wafer (or any other substrate). c) While the SU-8 is still in the glass transition state, the replica stamp is pressed firmly on top of the wafer and then cooled down. SU-8 is exposed through the stamp and post-exposure baked before the stamp is peeled off.

**Figure 2.4.** SU-8 structures made with embossing. a) 2 μm pillars with nanoroughness on top. b) 2 μm pillars without nanoroughness. Scale bars 2μm.

### 2.1.3 Thin film patterning

The standard pattern transfer from the designed mask to the target material consists of two steps: photoresist patterning with lithography followed by etching. During etching, the photoresist protects some areas, and only areas open from the photoresist are etched. The etchant can attack the target material chemically and/or physically, and the used method affects the final profile of the pattern. Usually, it is desired to find an etching
method with a low energetic etchant, as this causes less damage to other structures and films, but sometimes a high energy method like ion beam bombardment is necessary to pattern a specific film. Also, the etchant selectivity between target and mask material is crucial: the used mask material (usually photoresist) must withstand the whole etching process. When choosing a proper protocol, the selectivity and damage caused by etching are the main factors, but special techniques can go around these questions and produce almost identical patterns and profiles, like lift-off.

**Wet etching**

Wet etching is a process in which liquid etchant reacts with the target material with chemical reactions. Almost all materials can be etched by wet etching, and in lithography, the development step can also be considered wet etching of soluble part of the resist with the developer. The etch rate of wet chemical reactions is defined either by surface reactions or etchant availability, much like the deposition rate in CVD. Wet chemical etching usually occurs spontaneously when the etchant becomes into contact with the etched material, and external energy (like heat or ultrasonic agitation) is not needed, but it can increase the etch rate and change the etch profile.

Example wet etching processes are silicon dioxide (SiO₂ etching with hydrofluoric acid (HF) (Equation 2.1), chromium (Cr) etching with ceric ammonium nitrate (NH₄)₂Ce(NO₃)₆ (Equation 2.2) with perchloric acid (HClO₄) as an optional additive and aluminum (Al) etching with phosphoric acid/nitric acid mixture H₃PO₄/HNO₃ (Equation 2.3) [59].

\[
SiO₂ + 6HF \rightarrow SiF₆ + H₂ + 2H₂O \tag{2.1}
\]

\[
Cr + 3(NH₄)₂Ce(NO₃)₆ \rightarrow Cr(NO₃)₃ + 3(NH₄)₂Ce(NO₃)₅ \tag{2.2}
\]

\[
Al₂O₃ + H₃PO₄ \rightarrow 2AlPO₄ + 3H₂O
\]

\[
2Al + 2HNO₃ \rightarrow Al₂O₃ + H₂O + 2NO \tag{2.3}
\]

**Plasma etching**

Plasma etching (or reactive ion etching, RIE) is carried out with excited neutrals and reactive ions, which are bombarded towards the etched surface. Chemical (reactive) and physical (bombardment) processes are both important for the etching to take place, but there are also etching combinations in which the generated plasma etch the target material spontaneously, like silicon nitride (Si₃N₄) etching with CF₄. For plasma etching to work properly, the reaction product volatility is an important factor. The reaction products must be able to pump away from the substrate surface and out of the chamber. Different etching gas combinations can be used to alter the
etch rate and etch profile. [16]

Examples of plasma etching processes are silicon dioxide (SiO₂) etching with CHF₃ and O₂ (Equation 2.4) with sidewall CFₓ passivation, silicon nitride etching with CF₄ and O₂ (Equation 2.5) and silicon etching with SF₆ and O₂ (Equation 2.6) [159].

\[
2SiO₂ + 4CHF₃ + O₂ \rightarrow 2SiF₄ + 2CO₂ + 2CFₓ + 2H₂O \quad (2.4)
\]

\[
Si₃N₄ + 4CF₄ + 4O₂ \rightarrow 3SiF₄ + 2N₂F₂ + 4CO₂ \quad (2.5)
\]

\[
3Si + 2SF₆ + 2O₂ \rightarrow 3SiF₄ + 2SO₂ \quad (2.6)
\]

**Lift-off**

Lift-off is a special patterning method used when the substrate (or other existing materials and structures on the substrate) cannot withstand highly reactive etchants, or the patterned material is very challenging to etch chemically (like diamond). In lift-off, the lithography step is performed first, and film deposition takes place on top of the resist. When the resist is removed, the film on top of the resist is removed, and the film attached to the substrate remains. This process brings many restrictions and requirements to work correctly: the resists can usually withstand only low temperatures (below 200 °C), the resist can contaminate the deposition process due to monomer outgassing in high vacuum, the resist sidewall profile must be negative, so the overlaying film does not cover the resist completely, and the resist must be thicker than the deposited film.

An example process of lift-off patterning is to create a pattern with image reversal resist (like AZ5214) to acquire negative sidewall slopes of 75°–85° (normal positive resist slope) while preserving easy removal of the resist (negative resists are characteristically hard to remove due to cross-linking). A thin film of 30 nm of chromium is sputtered on top of the wafer with patterned resist (resist thickness around 1.4 μm). The sputtering of Cr must be short enough to prevent substrate heating due to high energy collisions of Cr atoms. Sputtering has decent step coverage, and usually, the negative slope of the resist remains uncoated. The resist can be easily removed with wet etching methods, like immersion in resist strippers (like AZ100 remover) or solvents like acetone (10 minutes with ultrasonic). Resist removal will also remove the deposited Cr on top of the resist as flakes, and areas without resist will be coated with Cr.
2.2 Carbon thin films

Carbon thin films are carbonaceous thin layers that consist primarily of carbon atoms, they are usually less than 1 μm thick, and they can be deposited on top of a target substrate. On the scope of this research, only thin films and nanoscale materials are considered, for example, materials like graphite, graphene, carbon nanotubes (CNT), diamond, glassy carbon, etc. A carbon atom can bond to another carbon atom through three different bonding types, called sp₁, sp² and sp³ bonds, which is a unique property and enables carbon compounds to have very different formulas even though the carbon content is the same. A carbon-carbon bond is also known as an organic bond. Carbonaceous materials can have unique combinations of properties like tailorable electrical conductivity, biocompatibility, high friction coefficient, chemical inertness, and high hardness, among many. [24]

Carbon thin films are characterized by the amount of sp² and sp³ bonds between atoms and hydrogen content in the ternary phase diagram (Figure 2.5). With 100 % sp² content, the material is considered graphite/graphene, and with 100 % sp³ content, the material is diamond-like, and if the hydrogen content becomes greater than 60 %, the film is hydrocarbon polymer, or there is no film growth at all. [65] In this research, three groups of carbon materials were used: pyrolytic carbon (PyC) with high sp² content, tetrahedral amorphous carbon (ta-C) with high sp³ (belongs to the group of diamond-like carbons, DLC) and nanocrystalline carbon (nC) with a slightly higher percentage of sp² bonds than sp³ (with properties similar to amorphous carbon). The deposition and patterning of these carbon materials are different, and due to their different chemical structure, also their material properties and application performance varies.

![Figure 2.5. Ternary phase diagram of sp², sp³, and hydrogen content of various carbon films. Figure from [65].](image-url)
2.2.1 Pyrolytic carbon

Pyrolytic carbon (PyC) is a material made with high-temperature degradation of organic source material, which is usually in solid form. In the scope of this research, we consider PyC material made from solid polymer, namely epoxy-based resist SU-8. The pyrolysis process requires an inert atmosphere in order to degas volatile elements out from the source without chemical reactions with the atmosphere (internal chemical reactions will take place). Typical pyrolysis atmospheres are nitrogen, argon and forming gas (5 % H₂ in N₂). The presence of oxygen would lead to oxidation of the material, or "burning," and would also remove the carbon content as CO and CO₂. An extreme case of pyrolysis, which leaves mostly carbon remaining from the source material, is called carbonization. [66, 73]

During pyrolysis (in an inert atmosphere), the ordered polymer structure starts to decompose and rearrange. Below 300 °C, the moisture and remaining GBL are evaporated from the film. Fully cross-linked SU-8 has a thermal degradation temperature around 380 °C [77]. Above this temperature, the decomposition starts, and volatile products are released. The oxygen in the film reacts with carbon causing combustion and forms CO and CO₂. Also nitrogen atmosphere and sulphur can react with the oxygen forming nitrogen oxides like NO₂ and N₂O₃ or sulphur oxides like SO₂. Metals present in the source material usually remain in the final product as oxides or carbonates; thus, PyC made from SU-8 has minor traces of iron and antimony. The carbon backbone is rearranged and becomes mostly sp²-bonded matrix with properties similar to glassy carbon (Schematic illustration in Figure 2.6), while a fraction of the material can remain sp³-bonded. [168, 169, 170]

![Figure 2.6. Schematic illustration of glassy carbon. The entangled graphitic ribbons give rise to extraordinary properties like structural stability and high surface area. La = intraplanar microcrystalline size, Lc = interplanar microcrystalline size. Figure copyright IJ Cambria Scientific Ltd [62, 85].](image)

The most important properties of PyC are high temperature resilience
Background

(due to a high temperature synthesis), low electrical resistance (due to high content of sp²-bonded carbon atoms), biocompatibility, high tolerance to chemicals, and low density. The material is widely used as an electrode material, especially in electrochemistry. PyC is an excellent material for cellular studies due to biocompatibility together with low electrical resistance.

### 2.2.2 Tetrahedral amorphous carbon

Tetrahedral amorphous carbon (ta-C) is a material with high sp³-content. The high amount of carbon-carbon single bonds makes the material diamond-like (DLC), and it has many similar properties to diamond, like high chemical stability, wear resistance, and thermal conductivity. From DLC materials, ta-C is the hardest, strongest, and slickest of them all since it consists purely of sp³-bonds in two crystalline polytypes: cubic-lattice and hexagonal-lattice. Also, less-pure forms of ta-C exist, which have a 10-50% proportion of sp²-bonds. [72, 96, 97, 111]

The applications for ta-C are mainly wear-resistant coatings (around 2–5 μm thicknesses) in tooling components like endmills and drill bits, but it is also used for tribological purposes like artificial joints. It is also used as an electrode material (especially in electrochemistry) even though it has high electrical resistivity (ρ ≅ 10⁸ Ωm [97]). But when it is used as a thin coating (down to 7 nm), the electric current does not need to pass a long distance through the thin film [117]. This has enabled the use of the material in electrochemistry, where electric signals from the surface of the electrode need to be transmitted through the electrode to amplifying circuitry [79]. The thin films of ta-C are deposited with filtered cathodic vacuum arc (FCVA) process with thickness control of few nanometers [128, 129].

### 2.2.3 Nanocrystalline carbon

Nanocrystalline carbon (nC) is an amorphous mixture of sp² and sp³-bonds. Nanocrystalline carbon is easier and cheaper to manufacture than ta-C because the presence of sp²-bonds are allowed. Generally, the film consists mostly of sp²-bonds with 20-30% of sp³-bonds, and therefore the material has properties from both diamond and graphite, like high hardness, wear resistance, biocompatibility, tailorable electrical conductivity, and chemical inertness. [61, 67, 121]

The combination of these properties makes nC desirable as a coating for applications with high wear (endmills, drill bits), corrosion protection (optical materials, electronic devices), and solid lubricants. The deposition method of the material greatly affects which kind of properties it possesses. The natural biocompatibility of carbonaceous materials has also increased the use of nC in many bio-applications like biological signal sensing, tissue
engineering, and cell proliferation. In medical use, the nC coating can be applied on re-usable instruments and devices which need sterilization after use since nC film is non-toxic, does not launch immunogenic response, and is chemically inert (it can be sterilized multiple times).

The film thicknesses with nC are usually tens of nanometers. Usually, less energy is needed for the film formation, and multiple methods can be utilized, like chemical vapor deposition (electron cyclotron resonance and plasma-enhanced), laser ablation, and magnetron sputtering.

### 2.2.4 Carbon patterning techniques

Carbon thin films are very challenging to pattern due to their high resistance to chemicals and corrosion. This is because the carbon atoms are connected with stable covalent bonds, and no chemical reaction will break it without additional energy. This makes the wet chemical etching of carbon films practically impossible, and methods that provide more energy to the carbon bonds are needed (reactive plasma or ion bombardment), or methods that do not need to break the atomic bonds can be used.

**Pyrolytic carbon**

Patterning of pyrolytic carbon is done before transforming the source material into carbon. Any carbon-containing resist can be used as a source material for pyrolytic carbon, but the negative tone resist SU-8 with epoxy backbone is by far the most used. SU-8 can be patterned with basic lithography techniques, and by choosing different solvent content, the film thicknesses can be varied. SU-8 crosslinks upon UV-exposure and subsequent post-exposure baking (PEB) finalizes the polymer matrix. The unexposed resist can be developed with propylene glycol methyl ether acetate (PGMEA, 1-methoxy-2-propanol acetate).

The high thickness of SU-8 requires ramped baking steps as the resist is prone to substrate delamination and film cracking and buckling with rapid temperature shifts. After PEB, the resist has a very high glass transition temperature, and the film/resist shape is fixed (but is still prone to delamination).

The pyrolysis of SU-8 is usually done in a nitrogen atmosphere, but other inert gases can also be used. The level of SU-8 carbonization is highly dependent on pyrolysis temperature, which can vary from 700 °C to 1200 °C. Below 700 °C, the outgassing of volatile elements is imperfect and resist-like properties will remain in the film. Also, long enough time must be allowed for the pyrolysis, usually one hour, but it can also vary up to five hours. Figure 2.7 shows SEM images of SU-8 micropillars before and after pyrolysis.

The pyrolysis conditions pump out the volatile elements from the film like oxygen, nitrogen, and even hydrogen, leaving mostly carbon remaining.
The loss of these elements is also visible as a mass loss of the structures: depending on pyrolysis temperature and time, the film thickness and structures can shrink from 60% to 80%. The shrinkage is not only because of loss of mass but also the rearrangement of carbon atoms into denser structure shrinks the film. The thermal energy during pyrolysis creates mostly sp²-bonds into the material, but higher temperatures like 1000 °C can also produce a fraction of sp³-bonds. The pyrolytic carbon material is electrically very conductive, opaque, nanorough, and biocompatible.

**Overhanging structures** With pyrolytic carbon, the patterning possibilities are extensive because of SU-8. This resist is well suited for high aspect-ratio structures, with minimized UV-photon scattering during exposure. Therefore SU-8 provides exceptional patterning possibilities. High compatibility with other resists and chemical inertness after cross-linking grants SU-8 the possibility to pattern it into three-dimensional structures with overhangs and suspended parts. 3D-structures can be made, for example, with sacrificial processing, greyscale lithography, bonding, and overexposure.

The sacrificial processing requires a sacrificial support material which is removed after it is no longer needed. The material can be anything whose removal is compatible with overhanging SU-8, like another photoresist, silicon dioxide or nitride, or metal. A common sacrificial process starts with patterning the sacrificial layer, for example, positive resist on top of a silicon wafer (Figure 2.8a-c). After patterning the sacrificial layer, SU-8 can be spin-coated on the substrate, followed by exposure and baking (Figure 2.8d-e). After developing SU-8 (Figure 2.8f), there should be openings made for the etchant to remove the sacrificial layer; in case of many positive resist an acetone bath could be used (Figure 2.8g). Removal of sacrificial layer can create overhangs and suspended parts in SU-8, and depending on created dimensions, removing liquids from underneath the suspended parts can create stiction problems that should be taken into account in process planning. Also, the suspended parts are left without
support and can collapse. The sacrificial process is one of the most common techniques for the fabrication of suspended structures due to its simplicity, but material compatibility can be an issue for some applications.

**Figure 2.8.** Sacrificial lithography process flow. a) Spin coating of sacrificial photoresist. b) Exposure of sacrificial resist. c) Development and pattern generation for sacrificial resist. d) Spin coating of SU-8. e) Exposure of SU-8. The exposure of sacrificial resist patterns takes place at the same time, but it is not critical as long as the resist’s physical shape does not alter. f) Development and pattern generation for SU-8. g) Sacrificial resist removal.

Greyscale lithography is a technique that relies on partial exposure of the resist. The process requires a single spin-coated film that is exposed through two photomasks or a greyscale mask. With two masks, the first exposure is done through the whole film to create support points for the suspended structure, and with second exposure, the exposure dose is kept lower than what is needed to expose the whole film (meaning, leaving the bottom of the film which is on contact with the substrate unexposed). A greyscale mask has partial openings which block part of the UV-light, thus allowing a smaller dose to go through it. A greyscale mask can create overhanging structures with a single exposure. During development, also the bottom of the unexposed film is removed. The process is simple because only one material is needed to create the overhangs, but the exposure dose must be strictly controlled because even the smallest variations can significantly influence the exposure depth. [132]

Suspended SU-8 structures fabricated through bonding require two substrates with patterned resists: one with anchor points and one with suspended parts. The wafers are aligned on top of each other, and SU-8 structures bonded in high temperature (100-120 °C) and high pressure (around 3 bars) because the resist is already fully cross-linked prior to bonding. After successful bonding, the substrate with suspended structures is removed. The method is not widely used because it requires two
substrates, and aligning them is challenging even with dedicated wafer bonder equipment. The benefit comes from easy lithography steps and no need for buried layer removal, which allows the creation of enclosed structures. [155, 156]

Overexposure of a film is not usually desired because it can distort the pattern designed on the photomask, but this distortion can also be exploited. During exposure, a phenomenon occurs at the top of the exposed resist known as T-topping. T-topping makes the exposed and crosslinked areas wider than in the mask because either the photomask diffracts the UV-photons to wider areas, or the crosslinking ignites a chain reaction that propagates under the mask. If two adjacent structures are designed with small spacing, the T-topping from both structures can encounter and create a crosslinked pathway between the structures. After development, this pathway remains there. This technique’s benefit is the possibility of creating extremely narrow bridges between structures, but only between closely packed ones. The disadvantages are poor control over the exposed area and strict limitation to the shape and size of the structures that can be used. [91, 92]

*Embossing* Generally, the embossing molds could be made from any material, but with SU-8, the mold needs to be transparent for UV-light to activate the crosslinking. In microfabrication, it is quite common to create the mold in two steps: first, create the desired patterns on a silicon wafer, and then make a negative copy of these structures into a soft polymer (Figure 2.9). This solid polymer is then used to emboss the structures on SU-8 film. With SU-8, commonly, PDMS is used as a mold because it does not adhere to SU-8, is permeable to air, and allows easy replication from the silicon master. [130]

As an elastomer, plain PDMS can be too soft for feature replication as the smallest structures can collapse in the mold, or they are not replicated in high enough resolution (sub-micrometer scale). Therefore a hybrid mold can be used, which has a thin layer of high-resolution material in contact with the silicon mold, and PDMS on top of this material to provide structural rigidity. A widely accepted method is to use hard PDMS (hPDMS) as the high-resolution material. The hPDMS is mixed from several components and spin-coated on top of the silicon master mold, and basic 10:1 PDMS is cast on top of hPDMS to create a hybrid mold. The hPDMS has the ability to copy structures on a 100 nm scale, and high aspect ratio features do not collapse. [130]

*Tetrahedral amorphous carbon* Tetrahedral amorphous carbon is deposited as a thin film, and very different patterning methods should be used than with PyC. Etching with wet chemistry is not possible due to very stable sp³-bonds in the material, and therefore plasma etching should be considered. For ta-C plasma etching,
different forms of reactive ions can be used. However, due to a weak adhesion of the ta-C film to a silicon substrate, commonly, a thin adhesion layer is used (usually a metal like titanium or compound like titanium-tungsten). When an adhesion layer is used under ta-C film, plasma etching becomes more complicated because the etching procedure has to take into account all etched materials. If the adhesion layer cannot be etched with plasma etching, it should be removed in other ways (like wet etching). This kind of double etching for a single pattern is not desirable but can be used if there are no other patterning methods.

In this work, we used lift-off to pattern ta-C film with an adhesion layer of Ti. The lift-off method can be used because the deposition is done at room temperature. The used thin films are usually tens of nanometers in thickness, and most lift-off resists are over hundreds of nanometers thick (we used 1.4 μm thick resist for 20 nm Ti + 7 nm ta-C). This method can be considered easier for patterning multi-layer thin films like ta-C with an adhesion layer than etching.
**Nanocrystalline carbon**

Nanocrystalline carbon is deposited with sputtering, and in many cases, especially magnetron sputtering like CFUBM (see Section 2.1.1). The nC film has similar restrictions like ta-C, and wet chemical etching can not be used. Also, lift-off is challenging because of the deposition method: sputtering is done in a process vacuum of $10^{-3}$ mbar, and ejected atoms from the target must have higher energy in order to reach the substrate compared to high vacuum processes. This high energy is absorbed at the substrate and transformed into heat. Even though the rise of the temperature cannot be measured from the substrate, there will be local heating, especially at the corners like photoresist patterns. This makes the photoresist removal with wet processes almost impossible due to implanted and absorbed atoms and changed chemistry.

On the other hand, nC film has better adhesion to silicon or silicon dioxide surface than ta-C, and it does not require adhesion promoter films. When sputtered on a silicon wafer, the patterning can be done with plasma etching. Oxygen is a key element in carbon patterning as CO and CO$_2$ are the main volatile compounds made in chemical reactions on the surface. High energy is also needed to break the organic bonds between carbon atoms in nC film.

### 2.2.5 Carbon materials in device applications

Carbon materials are present in many different forms, and some of them are more easily applicable to different applications than others. For example, carbon nanotubes (CNT) are very stable “particles” that can be attached or grown to many surfaces to unlock the marvelous properties of carbon, and glassy carbon is a long known electrode material. However, the applications are more limited with carbon thin films due to more demanding substrate requirements and processing. Due to the unique properties of carbon, it is widely used in many electrical applications like supercapacitors [118] and electrochemical sensors [17, 45, 57, 107], and also in increasing amount in biosensor applications [45, 115, 123].

Carbon electrodes have extraordinary electrochemical features, which makes them an appealing choice of material for many devices. Electrochemical sensing of neurotransmitter dopamine (DA) in physiological conditions has been studied intensively during the last half of a century, and it has been connected to many central nervous system diseases. Different carbon electrodes have shown great results with DA detection, mainly because of the antifouling properties, tailorable electrical conductivity, wide potential window, and comprehensive compatibility with other materials and molecules. [28, 44, 52, 53, 103, 122, 124] Other carbon electrode applications are electrical impedance spectroscopy (EIS) [47, 48, 100], deep brain stimulation (DBS) [109], and multielectrode array (MEA) [74].
2.3 Electrochemistry

Electrochemistry is physical chemistry that studies the relationship between electricity and chemical changes. If the chemical reaction spontaneously produces an electric current or an externally supplied current drives the reaction, the phenomenon is called an electrochemical reaction. When electrons are transferred directly between molecules and materials, it is called an oxidation-reduction (redox) reaction. The field of electrochemistry is vast, and here we focus only on a fraction of it by describing the principles for electrochemical detection of molecules and a cyclic voltammetry (CV) method briefly. More thorough electrochemistry descriptions can be found in a book by Bard [5] and a book by Bockris [10].

The signal acquired through electrochemical detection of molecules is dependant on i) electron transfer speed between the electrode and the molecule, ii) the number of molecules on the surface, iii) diffusion of new molecules from the bulk on the surface, and iv) noise from the solution. These processes are illustrated in Figure 2.10. The electron transfer between the electrode and the molecule is responsible for the resulting chemical changes, and the flow of electrons in the electrode can be measured. The electron transfer takes place at the working electrode (WE), and the WE potential is measured between a reference electrode (RE) while the flow of current is measured between a counter electrode (CE).

Figure 2.10. Electrochemical measurement setup usually includes three electrodes: work (WE), reference (RE), and counter (CE) electrode. The material selection and shape of the electrodes can vary a lot depending on the application. On the working electrode surface, there are reactions that affect the detection of molecules (yellow stars) in the solution: i) electron transfer speed, ii) the number of molecules on the surface, iii) diffusion of new molecules, and iv) noise from the solution.

The electron transfer speed is dependent on the electrode material and detected molecule and can be improved by choosing the most prominent ma-
terial for each molecule. Metals like platinum and gold are prevalent, but also titanium is used, and carbon is becoming more prominent, especially in neurotransmitter detection. The number of molecules on the surface can be increased by increasing the surface area of the electrode. Nanoporous materials are very common (like platinum black), but micro/nanostructured surfaces (like pillars and spikes) are also used. The diffusion of new molecules on the surface is generally not an electrode-related issue but more about the solvent and mobility of the measured molecule in it. The reaction layer can also be breached with the electrode geometry by adding tens of micrometers long and narrow structures like pillars on the electrode surface. This would create a more complex reaction and diffusion layers, enhancing the flow of new molecules on the electrode surface. The noise from the solution is related to the used solution and electrode material, and the surface area. Especially the electrode’s footprint area (area without surface textures) is important with the signal-to-noise ratio. Therefore, if the electrode surface area can be increased with nanoroughness and microstructures without increasing the footprint area, the noise from the solution would decrease while more molecules could be detected on the electrode surface, and thus the signal would be strengthened. [69, 95, 166]

2.3.1 Cyclic voltammetry

Cyclic voltammetry is a method where the electrode potential is linearly ramped over time, and when a setpoint is reached, the potential is ramped in the opposite direction to reach to initial potential. These ramps can be performed as many times as needed. The working electrode’s changed potential can lead to an electron transfer with the molecule, and when the potential is ramped back, the electron transfer can occur in the opposite direction (from molecule to the electrode). This requires the redox couple to be reversible, and the electron transfer occurrences can be measured as a flow of electrical current. The measured current and used voltage are then plotted to get a cyclic voltammogram which can be used to study analytes in a solution or molecules immobilized on the electrode surface.

CV relies on rather fast scans, as it is not desired that the reacted molecule diffuses away from the electrode surface because then the redox-cycle can not be finished. If the electron transfer on the surface is fast and the process is limited by the flow of new molecules on the surface, the CV can only work with the small amount of molecules already on the electrode surface, and this yields a specific shape voltammogram which can be used to further analyse the molecule.
3. Experimental

3.1 Microfabrication processes

3.1.1 Tools and chemicals

The patterning of three different carbon materials required a combination of many different techniques. The tools used in this thesis are in Table 3.1 and chemicals in Table 3.2.

Table 3.1. Tools used in this research.

<table>
<thead>
<tr>
<th>Tool</th>
<th>Manufacturer</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Priming oven</td>
<td>YES</td>
<td>HMDS priming</td>
</tr>
<tr>
<td>Spinner</td>
<td>BLE</td>
<td>Photresist spinning</td>
</tr>
<tr>
<td>Mask aligner MA-6</td>
<td>Suss</td>
<td>Resist exposure</td>
</tr>
<tr>
<td>Furnace RS 170/1000/13</td>
<td>Nabertherm</td>
<td>Pyrolysis</td>
</tr>
<tr>
<td>Sputter Plasmalab</td>
<td>Oxford Instruments</td>
<td>Deposition of chromium</td>
</tr>
<tr>
<td>RIE Plasmalab 80+</td>
<td>Oxford Instruments</td>
<td>Plasma etching</td>
</tr>
<tr>
<td>ICP-RIE Plasmalab 100</td>
<td>Oxford Instruments</td>
<td>Plasma etching</td>
</tr>
<tr>
<td>PECVD Plasmalab</td>
<td>Oxford Instruments</td>
<td>Deposition of SiO₂ and Si₃N₄</td>
</tr>
<tr>
<td>CFUBM sputter</td>
<td>home-built</td>
<td>Deposition of nC</td>
</tr>
<tr>
<td>FCVA</td>
<td>Kurt J. Lesker</td>
<td>Deposition of ta-C</td>
</tr>
<tr>
<td>SEM Supra 40</td>
<td>Zeiss</td>
<td>Sample imaging</td>
</tr>
<tr>
<td>Raman Alpha300 RA</td>
<td>WITec</td>
<td>Raman analysis</td>
</tr>
<tr>
<td>Profilometer Dektak XT</td>
<td>Bruker</td>
<td>Step height measurement</td>
</tr>
<tr>
<td>4-point probe MCP-T400</td>
<td>Loresta AP</td>
<td>Resistivity measurement</td>
</tr>
<tr>
<td>AFM Dimension 3100</td>
<td>Digital Instruments</td>
<td>Roughness measurement</td>
</tr>
<tr>
<td>Dicing saw DAD3220</td>
<td>Disco</td>
<td>Wafer dicing</td>
</tr>
</tbody>
</table>
Table 3.2. Chemicals used in this research.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Use</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>SU-8</td>
<td>Insulator or PyC precursor</td>
<td>Kayaku</td>
</tr>
<tr>
<td>AZ5214</td>
<td>Resist mask or lift-off resist</td>
<td>MicroChemicals</td>
</tr>
<tr>
<td>AZ4562</td>
<td>Sacrificial layer</td>
<td>MicroChemicals</td>
</tr>
<tr>
<td>SF9</td>
<td>Plasma durable resist</td>
<td>Kayaku</td>
</tr>
<tr>
<td>PDMS</td>
<td>Elastomer stamp</td>
<td>Dow</td>
</tr>
<tr>
<td>AZ351B</td>
<td>AZ resist developer</td>
<td>MicroChemicals</td>
</tr>
<tr>
<td>PGMEA</td>
<td>SU-8 developer</td>
<td>Dow Chemical</td>
</tr>
<tr>
<td>HF</td>
<td>Native oxide etchant</td>
<td>Honeywell</td>
</tr>
<tr>
<td>Ceric ammonium nitrate</td>
<td>Chromium etchant</td>
<td>Honeywell</td>
</tr>
<tr>
<td>Acetone</td>
<td>Resist removal</td>
<td>Honeywell</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>Wafer cleaning</td>
<td>Honeywell</td>
</tr>
<tr>
<td>Nitric acid</td>
<td>Al etch bath</td>
<td>Honeywell</td>
</tr>
<tr>
<td>Phosphoric acid</td>
<td>Al etch bath</td>
<td>Honeywell</td>
</tr>
</tbody>
</table>

3.1.2 Basic protocols

The following protocols were used throughout this research: lithography for AZ5214 (positive tone Protocol 3.1, image reversal Protocol 3.2) and SU-8 (Protocol 3.4), pyrolysis of SU-8 (Protocol 3.5), chromium patterning (Protocol 3.3), and dicing of wafers (Protocol 3.6).

Procedure 3.1. AZ5214 basic patterning protocol

<table>
<thead>
<tr>
<th>BASIC AZ5214</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Priming with HMDS, 25 minutes 170 °C</td>
</tr>
<tr>
<td>2. Spin coating of AZ5214, 4000 rpm for 30 s (approx. 1.4 μm)</td>
</tr>
<tr>
<td>3. Soft baking on a hotplate, 90 °C for 2 min</td>
</tr>
<tr>
<td>4. UV-exposure (365 nm) with 90 mJ/cm² dose (contact exposure mode)</td>
</tr>
<tr>
<td>5. Development in 1:5 AZ351B:DIW for 1 min</td>
</tr>
<tr>
<td>6. Rinsing with DIW and drying in spindryer</td>
</tr>
<tr>
<td>7. Hard baking on a hotplate, 120 °C for 3 min</td>
</tr>
</tbody>
</table>
**Procedure 3.2.** AZ5214 image reversal basic patterning protocol

### AZ5214 IMAGE REVERSAL

1. Priming with HMDS, 25 minutes 170 °C
2. Spin coating of AZ5214, 4000 rpm for 30 s (approx. 1.4 μm)
3. Soft baking on a hotplate, 90 °C for 2 min
4. UV-exposure (365 nm) with 30 mJ/cm² dose through a photomask (contact exposure mode)
   **[pattern generation]**
5. Reversal bake on a hotplate, 120 °C for 2 min
   **[most critical step]**
6. UV-exposure (365 nm) with 900 mJ/cm² dose (flood exposure mode)
   **[non-critical]**
7. Development in 1:5 AZ351B:DIW for 1 min
8. Rinsing with DIW and drying in spindryer, moisture removal in 90 °C oven for 10 min
9. (Optional hard baking on a hotplate, 120 °C for 3 min. This will cause resist reflow and negative sidewall slope is lost)

**Procedure 3.3.** Basic procedure for chromium patterning with resist mask and wet etching

### BASIC CR PATTERNING

1. Silicon wafer (100 mm) moisture removal in 120 °C for 5 min
2. Chromium sputtering, 50 nm
3. Basic lithography for AZ5214 (Protocol 3.1)
4. Cr etching in ceric ammonium nitrate 90 s, rinsing with DIW
5. Resist removal in an ultrasound acetone bath for 10 min, cleaning with IPA
**Procedure 3.4.** Processing of SU-8 with different thicknesses

### BASIC SU-8 PROCESSING

1. Silicon wafer dipping in 10:1 DIW:HF for 1 minute, rinse with DIW, dry with spindryer
2. Moisture removal in an oven of 120 °C for 5 min
3. Spin coating of SU-8

<table>
<thead>
<tr>
<th>Resist SU-8</th>
<th>5</th>
<th>5</th>
<th>50</th>
<th>50</th>
<th>2000.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>thickness (μm)</td>
<td>2</td>
<td>3</td>
<td>13</td>
<td>20</td>
<td>0.8</td>
</tr>
<tr>
<td>rpm</td>
<td>4000</td>
<td>5000</td>
<td>9000</td>
<td>9000</td>
<td>3000</td>
</tr>
<tr>
<td>time (s)</td>
<td>40</td>
<td>30</td>
<td>45</td>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>

4. Soft baking with ramped temperature: from 20 °C to 65 °C for 15 °C / min, hold, ramp up to 95 °C for 10 °C / min, hold, cool down to 20 °C naturally

<table>
<thead>
<tr>
<th>Resist SU-8</th>
<th>5</th>
<th>5</th>
<th>50</th>
<th>50</th>
<th>2000.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hold 65 °C (min)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Hold 95 °C (min)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>

5. UV-exposure (365 nm) (flood or contact mode)

<table>
<thead>
<tr>
<th>Resist SU-8</th>
<th>5</th>
<th>5</th>
<th>50</th>
<th>50</th>
<th>2000.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mJ/cm²)</td>
<td>150</td>
<td>150</td>
<td>240</td>
<td>240</td>
<td>240</td>
</tr>
</tbody>
</table>

6. PEB with ramped temperature: from 20 °C to 95 °C for 15 °C / min, hold, cool down to 20 °C naturally

<table>
<thead>
<tr>
<th>Resist SU-8</th>
<th>5</th>
<th>5</th>
<th>50</th>
<th>50</th>
<th>2000.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hold (min)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>5</td>
</tr>
</tbody>
</table>

7. Development in PGMEA, rinsing with IPA and DIW

<table>
<thead>
<tr>
<th>Resist SU-8</th>
<th>5</th>
<th>5</th>
<th>50</th>
<th>50</th>
<th>2000.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>time (min)</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Procedure 3.5.** Basic process flow for pyrolysis

### BASIC PYROLYSIS

1. Sample loading into the processing chamber
2. Chamber purging with N₂ (either with the assistance of vacuum pumps or high volume gas flow)
3. Constant gas flow through the chamber must be kept on throughout the process
4. Pre-heating to stabilization temperature: 200 °C / h to 300 °C
5. Holding for 40 min to stabilize the sample and the chamber
6. Heating to pyrolysis temperature: 200 °C / h to 900 °C
7. Holding for 60 min to let the pyrolysis take place
8. Cooling down to room temperature naturally
Procedure 3.6. Silicon wafer dicing with dicing saw

<table>
<thead>
<tr>
<th>BASIC SILICON DICING</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Silicon wafer attachment into dicing tape from backside</td>
</tr>
<tr>
<td>2. Wafer dicing with 150 μm resinoid blade (spindle speed 30 000 rpm, feed speed 5 mm/s)</td>
</tr>
<tr>
<td>3. Rinsing with DIW</td>
</tr>
<tr>
<td>4. Wafer cleaning by wiping with DIW soaked cleanroom wipe (if possible)</td>
</tr>
<tr>
<td>5. Tape detachment with backside UV-exposure</td>
</tr>
<tr>
<td>6. Sample cleaning in ultrasonic acetone/isopropanol bath for 10 min</td>
</tr>
<tr>
<td>7. Rinsing with DIW, drying with a nitrogen gun, moisture removal in an oven of 50 °C</td>
</tr>
</tbody>
</table>

3.2 Carbon material processing and characterization

3.2.1 Pyrolytic carbon

Planar PyC films
In Publication II, we used the planar PyC films for electrochemical detection of neurotransmitter dopamine (DA). We used epoxy-based photoresist SU-8 as a source material for PyC. The films were created on top of a 100 mm silicon wafer by spin coating a 13 μm thick SU-8 50 layer according to Protocol 3.4 (Spin 9000 rpm 45 s, exposure in flood mode, no development). The first step (DIW:HF dip) is to remove the native silicon oxide layer (cleaning) and make the surface more hydrophobic, ensuring good SU-8 adhesion to the surface. Then the wafer was cut with a dicing saw according to Protocol 3.6 into 10 x 10 mm² chips and cleaned by wiping gently with DIW soaked cleanroom wipe. Finally, the chips were pyrolysed according to Protocol 3.5.

Patterned PyC films
In Publication III, we created PyC structures with variable surface roughness with two methods: standard lithography and embossing. We used these structures to study the effect of surface topography on cell viability. We created PyC pillars in two scales (2 μm and 20 μm diameter) with standard lithography and nanoscale roughness with embossing technique (which required an elastomer master replicated from silicon master).
**Experimental**

**Standard lithography**  The SU-8 micropillars were created with negative tone photoresist lithography. A two-layer structure required two lithography steps. A 3 μm thin SU-8 base layer was used to increase the pillar adhesion to the silicon wafer, and it was fabricated according to Protocol 3.4 from SU-8 5. Second SU-8 layer was patterned on top of the base layer according to Protocol 3.4 (2 μm from SU-8 5, 20 μm from SU-8 50). The pillars were protected with a thick layer of AZ5214 by spinning it at 500 rpm for 30 s and soft baking at 90 °C for 5 min. The wafer was diced with Protocol 3.6, the protective resist was removed in an ultrasonic acetone bath for 10 min, and samples were pyrolysed with Protocol 3.5.

**Embossing**  In Publication III, three types of silicon masters were created to achieve three different surface topographies: nanorough, 2 μm pillars with nanorough top surface, and 20 μm pillars with nanorough top surface.

The nanorough black silicon (bSi) masters were fabricated with maskless anisotropic silicon etching in ICP-RIE (Inductively Coupled Plasma Reactive Ion Etching) equipment with a process described by Sainiemi et al [137]. Standard 100 mm <100> silicon wafers were used without pretreatments. The etching recipe was: SF₆ flow 40 sccm, O₂ flow 18 sccm, pressure 10 mTorr, temperature -110 °C, RF-power 6 W, ICP-power 1000 W, etch time five minutes. After etching, the silicon wafers were coated with fluoropolymer to decrease the adhesion forces on the stamp. Fluoropolymer coating was deposited in Oxford Plasmalab 80+ etch reactor using CHF₃ as a source gas.

The 2 μm and 20 μm pillars with nanorough top surface were fabricated with two-step silicon etching. The first etching step creates the pillar structures, and after etch mask removal, the second etching step takes place and creates the bSi surface. A chromium hard mask was patterned on a 100 mm silicon wafer with Procedure 3.3 and Si substrate was etched through the hard mask with fluorine-based reactive ion etching (target depths 2 μm and 20 μm with corresponding pillar diameters). After etching, Cr hard mask was removed in ceric ammonium nitrate, and the wafer was nanoroughened with maskless anisotropic etching and finally coated with a fluoropolymer.

The PDMS stamps were fabricated with a dual-layer process: first, a thin layer of hard-PDMS (hPDMS) was spin-coated on the surface of a fluoropolymer-coated silicon wafer, and then normal 10:1 PDMS was cast to provide a bulk structure for the stamp.

The components for hPDMS stamp are (amounts for single 100 mm silicon wafer): 3.4 g vinyl PDMS prepolymer ((vinylmethylsiloxane)-dimethylsiloxane copolymer), 17.5 μl platinum catalyst (platinum divinyltetramethyldisiloxane), 8 μl modulator (1,3,5,7-tetravinyl-1,3,5,7-tetramethylcyclotetrasiloxane), and 1 g hydrosilane prepolymer ((methylhydrosiloxane)-dimethylsiloxane copolymer). The addition of the hydrosilane prepolymer starts the polymerization process, and it should be added last. The mixture
Experimental

has to be spin-coated on a 100 mm substrate within 10 minutes from the addition of the last component. Normal PDMS must be cast on top of the hPMDS layer while both are still in liquid form. A container with high walls must be used in order to get the stamp thick enough. The hPDMS-PDMS mixture is baked in an oven of 50 °C for 2 hours to solidify the stamp and cut and peeled off.

A 13 μm SU-8 50 layer thickness was used as a substrate for embossing. The resist was handled according to Protocol 3.4 until the soft baking step, at which the cooldown was stopped above SU-8 glass transition temperature, and the hPDMS-PDMS stamp was embossed. The embossing was done at two different temperatures, 65 and 75 °C, to study the process yield. After embossing, the process was continued. The exposure dose (flood mode) was 480 mJ/cm² to make sure all photoactive components are activated. The stamp was peeled off after PEB. For dicing, the wafer was protected with an AZ5214 layer to prevent residual debris attachment on the surface and protect the delicate structures, and the protective resist was removed with acetone after dicing. The samples were diced and pyrolysed according to Procedures 3.6 and 3.5.

Suspended PyC Structures

In Publication IV, we created 3D PyC structures with suspended overhangs by sacrificial processing. We used the suspended structures to study the SU-8 behavior during pyrolysis, and we created parameter spaces for successful overhang dimensions. The overhanging structures were created on top of a 100 mm silicon wafer by first creating chromium alignment marks with Procedure 3.3, and then the sacrificial layer from AZ4562 positive tone resist. The wafer was first primed with HMDS, then AZ4562 was spin-coated with 2000 rpm for 30 s, which created approximately 10 μm layer. Resist was soft baked on a hotplate of 90 °C for 4 min, exposed with 900 mJ/cm² dose in contact mode, developed in 1:5 AZ351B:DIW for 15 min, rinsed with DIW, and dried in spin-dryer. The hard baking of AZ4562 is optional as the temperature of 120 °C leads to resist reflow and changes the pattern profile drastically.

The SU-8 50 was deposited according to Procedure 3.4 for 13 μm layer with a six hours long development step. The long PGMEA immersion combines the development of SU-8 and the removal of sacrificial resist AZ4562 since the developer removes the AZ4562 even after hard baking. The development and the following rinse have to be performed carefully as not to bend, break or remove the fragile suspended SU-8 microstructures. After the last rinse, the wafer was left for 24 hours at room temperature to completely dry off the sample before pyrolysis (performed according to Procedure 3.5 with final pyrolysis temperature ranging from 800 °C to 1100 °C).
Experimental

Characterization methods

**Profilometry** The vertical and lateral shrinkage of SU-8 during pyrolysis is analysed with profilometry. A stylus profilometer Dektak XT from Bruker is used. The same microstructures are measured before and after pyrolysis. With suspended microstructures and smaller than 5 μm structures, scanning electron microscopy (SEM) assists in shrinkage analysis.

**Imaging** An optical microscope with a camera is used to analyze the lateral shrinkage and structure reproducibility. Also, possible adhesion problems and multilayer structures can be identified with a top-illuminated microscope. A scanning electron microscope SUPRA 40 from Zeiss is used to measure and image the smallest features down to 50 nm (black silicon surface roughness). Photoresists before pyrolysis had to be coated with 20 nm sputtered Cr to enhance the SEM imaging and prevent charging.

**Raman spectroscopy** The bond structure of carbon films and structures were analyzed with Raman spectroscopy. We used an Alpha300 RA micro-Raman spectrometer (WITec) with a green laser (wavelength 532 nm). The scans are performed as line scan with multiple measurement points (by default, 50 points per line, 10 accumulations per point, 0.5 s integration time for accumulation). The laser power had to be kept low 1 mW not to damage the surface and alter the results (high power levels like 10 mW proved to damage the surface). The Raman spectra are analysed and fitted with Origin Pro by following the research done by Ferrari and Robertson [30, 31, 32].

**Electrical resistivity** The electrical resistivity of the resulting PyC films is measured with a four-point probe. We used a four-point probe MCP-T400 from Loresta AP. The measurements were done on unpatterned films, which were deposited on top of insulated (300 nm Si₄N₄ or SiO₂ film) silicon wafer in order to eliminate the effect of semiconducting silicon. Measurements were performed at least 2 cm away from the sample edge, with a minimum of five measurements per sample.

**Atomic force microscopy** The surface roughness of SU-8 before pyrolysis and resulting PyC materials surface roughness is measured with atomic force microscopy. We used an AFM Dimension 3100 from Digital Instruments. Three measurements were performed with tapping mode at random locations at least 2 cm away from the sample edge. A surface area of 5 x 5 μm² is scanned, from which the surface roughness is analyzed with Nanoscope Analysis.

3.2.2 Tetrahedral amorphous carbon

In Publication III, we created micropillars in two scales (2 μm and 20 μm diameter) and nanorough surfaces from silicon and coated these sam-
samples with ta-C film. We used structured surfaces to study the effect of topography on cell viability. Niklas Wester carried out the ta-C deposition with FCVA technique and characterization of film quality, and they are described in greater detail in [117].

**Patterning of ta-C**

We used ta-C thickness of 7 nm with and without 20 nm Ti adhesion layer. The diamond-like film ta-C is extremely hard to pattern due to high sp³ content, which makes the material inert to any chemical reactions, and a high acceleration voltage is needed when bombarding with ions (to sputter-etch). Generally, the ta-C film also needs an adhesion layer made from titanium, which can be magnetron sputtered in the same chamber as ta-C. The adhesion layer brings great challenges for the patterning of the film, as both layers, 20 nm Ti and 7 nm ta-C, should be patterned with the same mask without damaging the surrounding structures. Therefore an easy way around the patterning difficulties is provided by the lift-off procedure. We used photoresist AZ5214 in image reversal process flow as a lift-off resist. First, the patterns are created with Procedure 3.2, followed by Ti magnetron sputtering for 20 nm and ta-C FCVA deposition for 7 nm. AZ5214 is then dissolved in an ultrasonic bath either with acetone (10 min) or AZ100 remover (30 min), followed by clean acetone, IPA, DIW rinsing, and moisture removal in an oven of 120 °C for 30 min.

**3D ta-C structures**

Because of the small layer thickness of ta-C films, it is not possible to create high 3D structures made entirely of it. Therefore, we used silicon structures as a backbone for high features and coated those with ta-C. In Publication III, we used three different structure scales: nanoscale rough surface (black silicon), 2 μm pillars, and 20 μm pillars. The samples were fabricated on highly boron-doped 100 mm silicon wafers with a similar process as described for embossing silicon master mold fabrication in 3.2.1. 7 nm of ta-C film was deposited without adhesion layer directly on structured silicon. Samples were diced after ta-C coating with Procedure 3.6. The structures were protected with AZ5214 before dicing, and the resist was removed after it.

**3.2.3 Nanocrystalline carbon**

In Publication I, we studied the patterning of nanocrystalline carbon (called nanocarbon, nC). In nanocarbon, the sp² content is present, and it can be etched with reactive ions due to a more reactive nature of the material. We created multielectrode arrays (MEA) from nanocarbon thin film.
Experimental

**Nanocarbon deposition**
The nanocarbon film was deposited with the CFUBM method by the group of Jeon Geon Han in SKKU, Korea. In short, two high purity graphite targets are bombarded with magnetically targeted Argon plasma which ejects the carbon atoms towards the rotating substrate. The process is explained in greater detail in [11, 105, 121].

**Nanocarbon patterning**
Nanocarbon patterning was done with reactive ions (RIE and ICP-RIE). In reactive ion etching, a correct gas composition must be chosen to have proper reactive species accelerated towards the carbon film. After deposition of nC, a hard etch mask was created from Cr with Procedure 3.3. The etch rate was studied between two tools, RIE and ICP-RIE, and the Cr mask was removed after etching with ceric ammonium nitrate.

We analyzed the optimal etching recipe for nC patterning while taking into account the Cr-mask selectivity. We created a Taguchi-method orthogonal array with two parameter levels. In RIE, we varied five parameters, and in ICP-RIE six. We calculated the response values and signal-to-noise ratios for all parameters and compared them to determine the optimal etching recipe.

**Reactive ion etching** RIE was carried out with Oxford Instruments Plasmalab 80Plus. Etching parameters with two levels were plasma power: 40 and 80 W, gas flows: O₂ 20 and 40 sccm, SF₆ 40 and 80 sccm, CHF₃ 40 and 80 sccm and pressure: 50 and 100 mTorr. The temperature was kept at room temperature and argon flow in 80 sccm at all experiments. The etch time in every experiment was two minutes.

**Inductively coupled plasma reactive ion etching** The ICP-RIE was carried out with Oxford Instruments Plasmalab 100 - ICP 180 and parameters with two levels were temperature: -110 and 20 °C, ICP power: 200 and 500 W, RF power: 100 and 150 W, gas flows: O₂ 10 and 40 sccm, SF₆ 10 and 40 sccm and pressure: 10 and 15 mTorr. ICP-power refers to the inductive coil power, and RF-power to the power applied to the wafer electrode (responsible for ion bombardment). The etch time in every experiment was two minutes.

**3.3 Multielectrode array design and fabrication**
All three presented carbon materials were implemented into a multielectrode array format. The implementation required controlled patterning of each material but also required full process flow compatibility with other materials. The used MEA layouts and fabrication procedures are presented below, and fabrication notes and analysis are presented in chapter 4.
3.3.1 MEA layouts

Two different types of MEA chips were fabricated in this research. The first design mimicked the commercial Alpha MED Scientific MED64 probe, and the second design was our own creation to study the interference of adjacent electrodes (named Electrode Area Test, EAT). MED64 probe is a 5 x 5 cm² chip designed to fit a probe station, whereas EAT is designed to save space on a 100 mm silicon wafer and to maximize the electrode density per 100 mm wafer.

Multielectrode array: MED64

The MED64 probe has 64 measurement electrodes in a square grid array and four reference electrodes outside the array. In our design, we had the measurement electrodes’ XY-distance from each other 250 μm and 450 μm, and they were wired to the outer edge of the chip where the 1 x 6 mm² output pads were located. Figure 3.1 shows the layout.

Multielectrode array: EAT

The EAT array has four individual chips in a single 100 mm wafer (Figure 3.2a). Each chip has 44 measurement electrodes radially distributed around a center reference electrode (Figure 3.2b, c, d), surrounded by a counter electrode. The measurement electrodes are laid in pairs, and the distance between the electrodes in a pair is varied between the chips (100, 200, 400, and 800 μm) (Figure 3.2e). The radial distribution allows all electrode pairs to be at an equal distance from the counter and reference electrodes, making every electrode pair almost identical inside the EAT chip. The electrodes are finally coated with top insulation material with openings at the electrode (Figure 3.2f).
3.3.2 Fabrication protocols

The fabrication process for both types of MEAs was dependent on the materials to be patterned. In MED64 design, the carbon film was patterned on top of bottom insulation, and supporting metal wiring is fabricated on top of that, whereas in EAT, the supporting metal is buried under the carbon film.

Multielectrode array: MED64

Nanocarbon  The MEA fabrication from nanocarbon utilizes similar procedures as explained earlier. The conducting nC thin film was deposited on a thermally oxidized silicon wafer. Nanocarbon patterns were defined by optical lithography and dry etching (with Cr as a hard mask, Procedure 3.3).
Figure 3.3. Schematic cross-section picture of EAT. All arrays are made on top of electrically insulated silicon wafers. The bottom insulator was thermal SiO$_2$ with or without PECVD Si$_3$N$_4$ coating. The chromium wiring was always sputtered, but the patterning was done with either wet etching or lift-off. The carbon coating was deposited only on top of the measurement sites, and the output pads were left with chromium. In the case of ta-C, the thickness was 27 nm (including 20 nm of Ti adhesion layer sputtered in the same chamber), whereas in the case of PyC, the final layer was 1 - 3 μm. The PyC version also has a possibility to use carbon pillars at the measurement electrode. The top insulator was either SU-8 2000.5 (patterned directly with photolithography) or a combination stack of SiO$_2$ and Si$_3$N$_4$ (patterned with plasma etching). All numbers in the schematic are μm.

After dry etching, an insulating layer was deposited: either silicon oxide or SU-8 2000.5. A silicon oxide layer (100-200 nm) was deposited with PECVD (Plasmalab 80Plus, Oxford Instruments), and insulation openings were done on both ends of the electrodes with optical lithography and RIE. Photoresist AZ5214 (Procedure 3.1) served as an etching mask in RIE of silicon oxide with parameters: plasma power 200 W, gas flows CHF$_3$ 25 sccm, Ar 25 sccm, pressure 30 mTorr and temperature in room temperature with etch rate of 35 nm/min. If SU-8 2000.5 (800 nm) was used as an insulation layer, it was fabricated according to Protocol 3.4. Cr was removed from the recording electrodes with 3:1:21 Ce(NH$_4$)$_2$(NO$_3$)$_6$:HClO$_4$:H$_2$O wet etching in room temperature for approximately 90 seconds. If Al was used as a conducting wire, it was removed from the recording electrodes with 80:4:16 H$_3$PO$_4$:HNO$_3$:H$_2$O in 50 °C for one minute. MEA device was diced from the silicon wafer into 5x5 cm$^2$ piece with a Loadpoint Microace 3 dicing saw, and a quartz ring was glued with polydimethylsiloxane (PDMS) to serve as cultivation well.

Tetrahedral amorphous carbon Fabrication of MED64 on top of a thermally oxidized silicon wafer from ta-C required two consecutive lift-off processes: first for Ti+ta-C layer and second for supporting Cr wiring (both with Procedure 3.2 + deposition + lift-off in acetone). The top insulation layer was made from PECVD SiO$_2$ which required a third lithography step (Procedure 3.1) with plasma etching. The silicon substrate can be replaced by a glass wafer which removes the requirement for silicon dioxide bottom insulation. Due to this change and the fact that the layer thicknesses are
20 nm Ti and 7 nm ta-C at the measurement electrode, the measurement site (opening in top insulation) is optically transparent. This type of MEA is discussed more in Chapter 4, Section 4.5.2.

**Multielectrode array: EAT**

*Pyrolytic carbon*  The pyrolysis of SU-8 is a high-temperature process, and therefore it requires substrate and thin-film compatibility for this processing. In EAT design, the supporting metal wire is buried under the carbon layer, which in the case of PyC EAT means that the supporting metal must withstand high-temperature processing. The chosen metal was chromium (Procedure 3.3) due to good electrical properties and high temperature resistance. The carbon surface area is reduced to only near the measurement sites (Procedures 3.4 and 3.5). If a 3D PyC electrode was created, dual-layer processing for SU-8 was used with Procedure 3.4 to create 3 μm thick base layer from SU-8 5 with 13 μm high pillars from SU-8 50. The insulation was done with SU-8 2000.5, where the development time was increased tenfold to remove all unexposed resist between the pillars.

*Tetrahedral amorphous carbon*  With ta-C, the same supportive metal placement was used as with PyC EAT. The carbon surface area was increased compared to PyC EAT to cover all supportive Cr from the area of a 2 cm diameter quartz experiment chamber. Chromium was patterned on a thermally oxidised silicon wafer with Procedure 3.3, and Ti+ta-C was patterned with lift-off (Procedure 3.2 + deposition + lift-off in acetone). Multilayer PECVD Si₃N₄ and SiO₂ was used as a top insulator, which required highly controlled plasma etching with two steps: first to etch through three topmost insulator layers with high power, and then to etch the remaining Si₃N₄ with low power. The high power etching recipe was power: 200 W, gas flows: CHF₃ 45 sccm, O₂ 3 sccm, pressure: 50 mTorr with etch time of 7.5 min. The low power etching through the last Si₃N₄ layer was power: 30 W, gas flows: CF₄ 19 sccm, O₂ 1 sccm, pressure: 150 mTorr with etch time of 1 min. Two resists were used as etching masks: plasma-resistant SF9 and AZ5214. SF9 was spin-coated 3000 rpm for 45 s and soft baked 170 °C for 5 min, and AZ5214 was patterned with Procedure 3.2, except the development was done with MIF726, which develops both resists. The reasoning for this complicated processing is explained in Chapter 4, Section 4.5.3.
Procedure 3.7. Insulator stack etching in two steps

**PECVD INSULATOR STACK ETCHING**

1. Deposition of insulator stack with PECVD
   100 nm $\text{Si}_3\text{N}_4$ + 100 nm $\text{SiO}_2$ + 100 nm $\text{Si}_3\text{N}_4$ + 100 nm $\text{SiO}_2$
2. Spin coating SF9 with 3000 rpm for 45 s
3. Soft baking SF9 at 170 °C for 5 min on a hotplate
4. Lithography with AZ5214 (Procedure 3.1 without development)
5. Development in MIF726 developer for 1 min (both resists), rinse with DIW and dry
6. Insulator stack reactive ion etching in two steps

<table>
<thead>
<tr>
<th>Recipe</th>
<th>High power</th>
<th>Low power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Power (W)</td>
<td>200</td>
<td>30</td>
</tr>
<tr>
<td>$\text{CHF}_3$ (sccm)</td>
<td>45</td>
<td>0</td>
</tr>
<tr>
<td>$\text{CF}_4$ (sccm)</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>$\text{O}_2$ (sccm)</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Pressure (mTorr)</td>
<td>50</td>
<td>150</td>
</tr>
<tr>
<td>time (s)</td>
<td>450</td>
<td>60</td>
</tr>
</tbody>
</table>

7. AZ5214 removal in acetone, SF9 removal in MIF726, rinse and dry
4. Results and Discussion

4.1 PyC processing

4.1.1 Patterning

The patterning of PyC is done by patterning the source material SU-8 before pyrolysis. In Publication III we patterned SU-8 with direct photolithography and embossing techniques, and in Publication IV we created suspended and overhanging structures and 3D structures with sacrificial processing.

**SU-8 patterning with direct photolithography**

Two-layer SU-8 lithography and pyrolysis are shown in Procedure 4.1.

**Procedure 4.1.** Processing of two layer SU-8

<table>
<thead>
<tr>
<th>TWO-LAYER SU-8 LITHOGRAPHY AND PYROLYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Dipping of 100 mm p-type silicon wafer in BHF mixture for 1 min</td>
</tr>
<tr>
<td>2. Drying with spindryer, moisture removal in an oven of 120 °C for 5 min</td>
</tr>
<tr>
<td><strong>Base layer</strong></td>
</tr>
<tr>
<td>3. Spin coating SU-8 5 5000 rpm for 30 s (approx. 3 μm)</td>
</tr>
<tr>
<td>4. Ramped soft baking, hold times 65 °C for 3 min, 95 °C for 5 min</td>
</tr>
<tr>
<td>5. UV-exposure with 150 mJ/cm² dose (Flood exposure mode)</td>
</tr>
<tr>
<td>6. Ramped PEB, hold time 95 °C for 6 min</td>
</tr>
<tr>
<td>7. Development in PGMEA for 3 min, rinse with IPA and DIW, drying in 90 °C</td>
</tr>
</tbody>
</table>
Results and Discussion

**Pillar layer**

\[ 2 \mu m \]

8. Spin coating SU-8 5 4000 rpm for 40 s (approx 2 \( \mu m \))
9. Soft baking same as step 4
10. Exposure through photomask with 150 mJ/cm\(^2\) dose
11. Ramped PEB: hold time 95 °C for 4 min, cool down 3.75 °C/min
12. Development in PGMEA for 5 min

\[ 20 \mu m \]

8. Spin coating SU-8 50 9000 rpm for 30 s (approx. 20 \( \mu m \))
9. Soft baking, hold times 65 °C for 5 min, 95 °C for 8 min
10. Exposure through photomask with 240 mJ/cm\(^2\) dose
11. Ramped PEB: hold time 95 °C for 8 min, cool down 3.75 °C/min
12. Development in PGMEA for 10 min
13. Rinsing with IPA and DIW, drying in 90 °C oven for 10 min
14. Pyrolysis in 900 °C N\(_2\) atmosphere for one hour (Procedure 3.5)

The direct photolithography of SU-8 is a very controlled process because the resist allows high aspect ratio structure fabrication with almost straight sidewalls. Despite that, problems during structure fabrications can emerge if the following aspects are not taken into account.

**Surface termination** The adhesion of SU-8 onto the substrate can be compromised if the substrate lacks the pre-treatment before spin coating. The first aspect to consider is wafer cleanliness and surface termination. A dirty wafer can have variable, undesirable, and uncontrollable surface chemistry, which leads to compromised SU-8 adhesion. Even a clean silicon surface can have poor adhesion due to unfavorable surface termination. We found out that if a silicon wafer is spin-coated with SU-8 right after unboxing it from the factory packaging, the resist does not adhere properly but starts to coalesce right after spinning. The effect is enhanced by soft baking.

Multiple different processes can be utilized in order to prevent this. We used native oxide removal by HF dipping prior to spin coating. The process creates a fresh native oxide after washing and drying, but the surface becomes hydrogen-terminated and hydrophobic, supporting SU-8 adhesion on the surface. Another proposed method is wafer exposure to oxygen plasma which cleans the surface, making it oxygen terminated. This surface has to be baked (for example 120 °C for 2 hours) to hydrogenate the surface because the SU-8 will not adhere properly to the hydrophilic surface. The HDMS treatment is also proposed [19], but with our experiments,
we did not observe drastic changes in adhesion with and without HMDS treatment.

**Material compatibility**  The surface termination of silicon with HF is a straightforward and reliable method to increase the adhesion, but it cannot be done on all materials. For example, silicon oxide and nitride are etched in HF solutions. Therefore if SiO$_2$ or Si$_3$N$_4$ are used as a bottom layer beneath SU-8 (typically for electrical insulation), the adhesion can be increased either by spin coating SU-8 right after SiO$_2$ or Si$_3$N$_4$ deposition, or by treating the surface with oxygen plasma and baking in an oven of 120 °C for 2 hours. We did not observe any adverse effects of SU-8 adhesion on metals with or without any treatments. When we used small footprint structures (like 2 μm diameter pillars), we observed that most of the structures lost adhesion with the mentioned treatments, and therefore we overcame the issue by depositing and patterning a thin SU-8 base layer beneath the small footprint structures. The SU-8 on SU-8 adhesion proved to work extremely well, and even after rigorous washing and rinsing steps, the small features were still present. We also used AZ4562 under SU-8 in Publication IV and observed that the two resists somewhat react with each other, but no adhesion issues were encountered.

**Thermal relaxation**  The thermal treatments to remove the solvent and cross-link the monomers can create intrinsic stresses in SU-8. If these stresses are not relaxed, they can affect the shape of the final structures, crack the resist or even cause delamination. The residual stresses in SU-8 are usually minimized by ramping the baking steps during heating and cooling. Thin layers like 800 nm with SU-8 2000.5 can be baked directly with a pre-heated hotplate, but thick layers from 3 μm upwards require ramped baking steps (Procedure 3.4). Thicker layers require longer ramping steps to let the intrinsic stresses relax, even 12 hours with 500 μm thick layers.

**SU-8 embossing**  In publication III, we used the embossing method to create two kinds of structures from SU-8: nanorough surfaces with and without micrometer-scale pillars. The master mold fabrication from silicon did not produce any notable problems or issues, and successful processes are shown in Procedure 4.2. We studied the evolution of black silicon formation (silicon nanoscale pyramids) by varying the etching time. We observed two major changes in the formation: the first micrometer-sized pyramids emerge after 5 minutes of etching, and these pyramids are cleaved into smaller pyramids after 6 minutes of etching, and when finally our initial target etching time of 7 minutes was reached, all the created nanoscale pyramids were submicron diameter with nanoscale rough sidewalls. The sidewall roughness played a crucial role in hPDMS-PDMS stamp creation (Procedure 4.3).
Results and Discussion

If the PDMS mixtures were cast on top of the 7 minutes etched and fluoro-polymer coated silicon master, the sidewall roughness of the nanoscale pyramids created very high adhesion forces on solidified PDMS, which can overcome the cohesion forces inside the elastomer, thus rupturing and breaking it apart. We observed a compromise between surface roughness and resulting stamp adhesion forces around 5 minutes of nanoscale pyramids etching, which is just before the cleavage of micrometer-sized pyramids takes place.

**Procedure 4.2.** Fabrication of silicon master with structures in two scales

### DUAL SCALE SILICON MASTER FABRICATION

#### Hard mask
1. Moisture removal from 100 mm silicon wafer in 120 °C oven for 5 min
2. Chromium sputtering, 50 nm
3. HMDS priming, 25 minutes 170 °C
4. Spin coating AZ5214 4000 rpm for 30 s (approx. 1.4 μm)
5. Soft baking on a hotplate, 90 °C for 2 min
6. UV-exposure (365 nm) with 90 mJ/cm² dose (contact mode)
7. Development in 1:5 AZ351B:DIW for 1 min
8. Rinsing with DIW and drying in spindryer
9. Hard baking on a hotplate, 120 °C for 3 min

#### Cr wet etching
10. Etching of Cr in ceric ammonium nitrate 90 s, rinsing with DIW
11. Removal of AZ5214 mask in ultrasound acetone bath for 10 min, cleaning with IPA

#### Silicon etching
12. Etching of silicon with ICP-RIE, target depths 2 μm and 20 μm
13. Removal of Cr in ceric ammonium nitrate 5 min, rinsing with DIW
14. Maskless anisotropic silicon etching in ICP-RIE
15. Fluoropolymer coating in RIE chamber by providing gas CHF₃ and ionizing it
Procedure 4.3. Elastomer hPDMS-PDMS stamp fabrication

<table>
<thead>
<tr>
<th>PDMS mixing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Mixing PDMS prepolymer and curing agent in 10:1 weight ratio</td>
</tr>
<tr>
<td>2. Degassing 10 min in vacuum desiccator (or longer if necessary)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>hPDMS mixing</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. Mixing 3.4 g of vinyl PDMS prepolymer, 17.5 μl platinum catalyst and 8 μl of modulator</td>
</tr>
<tr>
<td>4. Degassing in vacuum desiccator for 5 min</td>
</tr>
<tr>
<td>5. Addition of 1 g hydrosilane prepolymer, mixing, and degassing for 2 min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Casting</th>
</tr>
</thead>
<tbody>
<tr>
<td>6. Spin coating hPDMS on silicon master with 1000 rpm for 20 s</td>
</tr>
<tr>
<td>7. Casting liquid PDMS on top of hPDMS (target for 1 mm layer thickness)</td>
</tr>
<tr>
<td>8. Degassing 10 min in vacuum desiccator if necessary</td>
</tr>
<tr>
<td>9. Curing the hPDMS-PDMS stamp in 50 °C for 2 hours</td>
</tr>
<tr>
<td>10. Removal of the solid stamp from the silicon master mold</td>
</tr>
</tbody>
</table>

Figure 4.1 shows SEM images of silicon master mold and PDMS stamp from Publication III.

![Figure 4.1](image)  
**Figure 4.1.** Replica molding from a silicon master. a) Silicon master mold with etched 2 μm pillars and submicron nanoroughness. b) PDMS stamp made from silicon master has opposite structure than the master. Scale bars 2μm. Reproduced from [51] with permission from MDPI.

The second transfer of features takes place at soft baking of SU-8 (Procedure 4.4). The hPDMS-PDMS stamps had to be pressed on the SU-8 while it was still above the glass transition temperature of 50 °C (before exposure [77]). We studied the embossing in two temperatures: 65 °C and 75 °C. There are two aspects to consider when choosing the proper temperature: yield in copied surface area and stamp adhesion on SU-8.
First, we found out that 65 °C embossing copied approximately 50 % of the nanorough surface, whereas the success area was increased to 90 % when we increased the temperature to 75 °C. However, while the temperature increase leads to a better stamp fill rate (softer resist "fills" the stamp features better than stiff one), it also increased the stamp adhesion on SU-8, which resulted in stamp ruptures during detaching. We did not observe major differences between the copied structures (size and shape) on either temperature; only the copied area differed. All these issues and notes are valid for both master mold features we used.

**Procedure 4.4.** Processing steps for embossing

<table>
<thead>
<tr>
<th><strong>EMBOSSING</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Precleaning of silicon wafer with 1 min HF dip</td>
</tr>
<tr>
<td>2. Spin coating of SU-8 on the substrate (13 μm, Procedure 3.4)</td>
</tr>
<tr>
<td>3. Ramped soft bake to 95 °C on a hotplate</td>
</tr>
<tr>
<td>4. During cool down, the hotplate is stopped at 65 °C, and the hPDMS-PDMS stamp is embossed on SU-8</td>
</tr>
<tr>
<td>5. When most of the air bubbles are squeezed out, the cooldown is continued</td>
</tr>
<tr>
<td>6. Overexposure at least 3x the normal dose without photomasks (flood exposure mode)</td>
</tr>
<tr>
<td>7. PEB is done normally, and the hPDMS-PDMS stamp is removed at room temperature. No development is needed.</td>
</tr>
</tbody>
</table>

**Overhanging and suspended structures**

In Publication IV, we created suspended SU-8 structures with the AZ4562 sacrificial layer (Procedure 4.5). We first patterned the positive tone AZ4562 and then spin-coated and patterned the SU-8 on top of that. When a resist is used as a sacrificial layer, the processing is straightforward, but as a downside, the material compatibility can be compromised. We encountered four types of problems when creating the SU-8 overhangs: thermal reflow of the sacrificial layer, air pocket formation, dissolution of the sacrificial layer, and SU-8 residual stress (Figure 4.2).
**Procedure 4.5.** Fabrication of suspended SU-8 structures with sacrificial process flow

<table>
<thead>
<tr>
<th><strong>SUSPENDED SU-8 STRUCTURES</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cr alignment marks</strong> (described in Procedure 3.3)</td>
</tr>
<tr>
<td>1. Moisture removal bake in 120 °C for 5 min</td>
</tr>
<tr>
<td>2. Chromium sputtering, 50 nm</td>
</tr>
<tr>
<td>3. Basic lithography for AZ5214 (Procedure 3.1)</td>
</tr>
<tr>
<td>4. Wet etching of Cr and AZ5214 mask removal</td>
</tr>
<tr>
<td><strong>AZ4562 sacrificial layer</strong></td>
</tr>
<tr>
<td>5. HMDS priming, 25 minutes 170 °C</td>
</tr>
<tr>
<td>6. Spin coating AZ4562 2000 rpm for 30 s (approx. 10 μm)</td>
</tr>
<tr>
<td>7. Soft baking on a hotplate, 90 °C for 4 min</td>
</tr>
<tr>
<td>8. UV-exposure (365 nm) with 900 mJ/cm² dose (contact mode)</td>
</tr>
<tr>
<td>9. Development in 1:5 AZ351B:DIW for 15 min</td>
</tr>
<tr>
<td>10. Rinsing with DIW and drying in spindryer</td>
</tr>
<tr>
<td>11. Optional: Hard baking on a hotplate, 120 °C for 10 min</td>
</tr>
<tr>
<td><strong>Suspended SU-8 layer</strong></td>
</tr>
<tr>
<td>12. Wafer dip into 10:1 DIW:HF for 1 min</td>
</tr>
<tr>
<td>13. Moisture removal in 120 °C oven for 5 min</td>
</tr>
<tr>
<td>14. Spin coating SU-8 50 9000 rpm for 45 s (approx. 13 μm)</td>
</tr>
<tr>
<td>15. Ramped soft bake, hold times 65 °C for 3 min, 95 °C for 5 min</td>
</tr>
<tr>
<td>16. UV-exposure (365 nm) with 240 mJ/cm² dose (contact mode)</td>
</tr>
<tr>
<td>17. Ramped PEB: hold times 95 °C for 4 min, cool down 3.75 °C/min</td>
</tr>
<tr>
<td>18. Development in PGMEA for 6 hours</td>
</tr>
<tr>
<td>19. Rinsing with DIW, drying with N₂ gun and 24 hour room temperature moisture removal</td>
</tr>
<tr>
<td>20. Pyrolysis in 900 °C N₂ atmosphere for one hour (Procedure 3.5)</td>
</tr>
</tbody>
</table>

*Thermal reflow of AZ4562*  
After development, AZ4562 resist has a rectangular cross-section with a slightly positive sidewall slope, as is typical with this resist. However, during hard baking in 120 °C, the resist undergoes a thermal reflow, transforming the rectangular shape into a rounded one. After SU-8 patterning and AZ4562 removal, the suspended structures obtain the arch-shaped profile caused by refloved resist profile (Figure 4.2a-b). Therefore we produced most of our suspended structures without AZ4562 hard baking, but this exposed more compatibility issues between the resists.
Results and Discussion

**Figure 4.2.** Fabrication problems with sacrificial processing. a) 50 μm holes in AZ4562 before hard bake and b) same holes after hard bake. The resist has gone through thermal reflow. c) 40×50 μm SU-8 bridge before pyrolysis shows an arch-shaped profile because of a rounded sacrificial layer and a small gap between SU-8 and the substrate. d) 40×50 μm pyrolysed bridge. e) Spinning SU-8 on top of sacrificial layer AZ4562 leaves a small pocket of air trapped between the resists. f) 300×250 μm PyC cantilever stuck to the substrate. g) Bottom surface of pyrolysed SU-8 shows undulations. Pyrolysis was done in 900 °C. Reproduced from [50] with permission from IOP Publishing.

**Air pocket formation between SU-8 and the substrate** After removing the sacrificial layer and during SEM inspection, we observed an unintended increase in the width of the structures. This was concluded to be due to the spin-coating process and physical flow of the resist. During spin-coating of SU-8 on top of AZ4562, the liquid and viscous resist flows over the solid AZ4562 structures and "lands" further away from the sacrificial resist on the substrate, leaving air trapped at the base of the sacrificial layer (Figure 4.2c-e). This air pocket was always found from the side facing away from the center of the wafer. This air pocket increases the size of the suspended part as it restricts the SU-8 to form an anchor closer to the AZ4562. The air pocket size ranged from 3 to 30 μm depending on the angle where the SU-8 was spin-coated on top of AZ4562, 90 °angle producing the biggest pocket.

**Dissolution of AZ4562** We inspected the underside of suspended SU-8 structures with SEM imaging which showed clear undulations on the struc-
Results and Discussion

Features which were in direct contact with the sacrificial layer (Figure 4.2f-g). This leads us to believe that the solvent in SU-8 attacks the solid AZ4562 upon spin coating, and the effect is enhanced if the AZ4562 is not hard-baked. The fine features under the SU-8 structures remain even after the removal of the sacrificial layer. The extreme case of this phenomenon was the fabrication of 10 μm long bridges; in most cases, the sacrificial layer was completely dissolved into the spin-coated SU-8, thus preventing us from fabricating these small bridges successfully.

We exploited the material compatibility issues during SU-8 development. The developer used was PGMEA, which also dissolves AZ4562. Therefore we could do the development of SU-8 and removal of the sacrificial layer in a single step, but it required more time (SU-8 was removed in a matter of minutes, whereas AZ4562 removal took several hours). The long immersion time in PGMEA did not have any effect on polymerized SU-8 structures.

**SU-8 residual stress** As explained earlier, the SU-8 processing can lead to residual stresses in the resist. We observed this effect from long and narrow bridges and cantilevers. The bridges tend to bend and move one way or another due to the stresses. Cantilevers usually exhibit an upward bend when released from the sacrificial layer (if they do not collapse to the substrate due to stiction forces).

### 4.1.2 Processing parameters vs. material properties

We fabricated pyrolytic carbon in Publications II, III, and IV, and we have gathered data about the correlation between PyC pyrolysis temperature and resulting material properties. The results show that the final temperature affects many of the properties and should be chosen accordingly.

**Shrinkage of PyC**

The source material for our pyrolytic carbon is epoxy-based negative tone photoresist SU-8, which holds a great carbon content, but also other elements like oxygen, nitrogen, and hydrogen. During the high-temperature pyrolysis in inert N2 atmosphere, the volatile elements O, N, and H are removed from the carbon backbone. Since SU-8 contains a lot of -OH groups, the mass loss is significant, resulting in material shrinkage in all dimensions. The relative shrinkage of the material compared to the original structure size is stronger with larger structures and strongest in the z-direction (thickness) with all structures. We studied the pyrolysis of SU-8 in multiple pyrolysis temperatures: 600 °C, 700 °C, 800 °C, 900 °C, 1000 °C, 1100 °C (Figure 4.3). We did not observe any correlation with the level of shrinkage with the used pyrolysis temperature or heating rate.

**Shrinkage of PyC thin film** The plain sheets of PyC shrunk during pyrolysis approximately 85 %. This applied to all our tested film thicknesses...
Results and Discussion

from 3 μm to 20 μm. The pyrolysis time showed a correlation with the shrinkage. Our standard pyrolysis time was one hour, but we did few point tests with longer pyrolysis time and different atmospheres (like argon, forming gas, and vacuum). Longer pyrolysis time leads to thinner film, and in an extreme case of 17 hours pyrolysis in 1000 °C, the film was almost completely lost (only small residues remaining). We have not examined the crystallinity nor roughness of these samples, only physical appearance and film survival.

Shrinkage of patterned films The level of vertical shrinkage with patterned structures was also 85 % with structure width over 20 μm, but structures with smaller width shrunk approximately 50 % in height. The restricted vertical shrinkage with these smaller structures is due to lateral shrinkage affecting and partly inhibiting vertical shrinkage. The lithography of SU-8 creates almost vertical sidewalls. During pyrolysis, the shrinkage of the material is greater at the top of the structures (always 10 μm per side with a film thickness of 13 μm) because the bottom is bound to the substrate. This leads to a change in the sidewall slope into a positive angle. The lateral shrinkage at the structure’s sidewall piles up the carbon material, which leads to a noticeable burr to the top of the structure (Figure 4.3). This burr is approximately 1 μm higher than the rest of the PyC layer, and it is 5 – 8 μm wide. Due to the constant width of the burr, the structures with a width smaller than 20 μm shrunk vertically less than bigger structures. The 2 μm pillars did not show any signs of burr, and they shrunk 40-50 %. Figure 4.4 shows different PyC pillar samples made in Publication III.

The nanorough SU-8 surfaces created with embossing preserved their nanorough nature after pyrolysis and resulting shrinkage, but the nanopillar density decreased (Figure 4.4b). The individual nanopyramids in the final PyC material were <1 μm in width, but the height was decreased compared to embossed SU-8. Both are due to the shrinkage of SU-8 during pyrolysis; the density decreased because the smallest pillars are lost into the bulk film beneath the rough surface.

The dual-scale structures (micropillars with nanorough surface) experienced a more drastic effect by losing most of the nanoroughness. Figure 4.5 shows SEM images from all of the steps in the embossing process. Figure 4.5a shows the formation of nanopillars on a silicon wafer in random order on top and between micropillars. During the first copying process from silicon to hPDMS-PDMS stamp, the nanopillars on top of micropillars are lost, most probably due to air being trapped in the hollow cup on top of the micropillar (Figure 4.5b). The second copying process from the hPDMS-PDMS stamp to the SU-8 layer was successful, and all features from the stamp are copied in great detail (Figure 4.5c). During pyrolysis, most of the nanoroughness between the micropillars is lost because of similar reasons as the density decreases with plain nanopillar PyC surface: the bottom
bulk swallows the surface roughness due to shrinkage (Figure 4.5d).

**Shrinkage of suspended structures** The shrinkage of suspended 3D features had parts unbound to the substrate, which allowed more freedom for shrinkage. This freedom leads to drastic geometrical effects due to residual stresses and higher deformations due to shrinkage. We tested four types of suspended structures, which were bridges, mushrooms, membranes, and cantilevers.

The Table 4.1 presents the dimensions of the tested bridges with width-to-length ratios and shows the failure mechanisms. The bridges failed either by collapsing to the substrate, bending sideways until they stuck to an adjacent bridge, or cracking. The bridge’s collapse is attributed to the relaxation of residual stresses in SU-8 (partly glass transition) and thermal expansion, which would deflect the bridge downwards, and upon contact with the substrate, it would stick. Quang et al. [131] explain that a CTE mismatch between SU-8 (102 ppm/K [27]) and silicon (2.6 ppm/K) would cause the compressive stress and resulting buckling. The fact that a bridge can deflect without cracking indicates that this occurs at initial stages of pyrolysis (below 600 °C) because SU-8 possesses a significant amount of flexibility [54, 82]. The final pyrolysis temperature did not affect the collapse probability, supporting the claim that the collapse happens at lower temperatures.

The long and narrow bridges with a width:length ratio of 1:10 and larger experienced mostly sideways bending due to a long and fragile suspended part. The movement is attributed to aggressive processing and residues of
Results and Discussion

Figure 4.4. SEM images of PyC structures made with direct lithography a), c)–h) and UV-embossing b), i)–k). a) Planar reference sample with silicon particle on the surface to show the absence of structures (scalebar: 1 μm). b) PyC nanopillars made with embossing (scalebar: 2 μm). All pillar structures with three layouts: c)–e) 2 μm pillars (scalebar: 2 μm), f)–h) 20 μm pillars (scalebar: 10 μm) and i)–k) Dual-scale pillars (scalebar: 2 μm). Reproduced from [51] with permission from MDPI.

PGMEA, which can act through capillary forces [160]. When the bridges come into contact with each other, they would stay attached due to stiction forces. The sideways movement was observed with less than 25 % for bridges with dimensions of 10 μm × 100 μm and more than 70 % with 10 μm × 500 μm. In our experiments, the spacing between the adjacent bridges was 50 μm except with 10 μm wide bridges it was 40 μm. The cracking of a bridge was a stochastic effect, and less than 1 % of bridges failed because of this. The pyrolysis temperature had only a minor effect on cracking: higher temperature slightly increased the number of cracked bridges.

The shrinkage of anchor points is similar to the standard patterned film, but the suspended part experiences drastic deformations. Before pyrolysis,
all the bridges show straight profiles in all dimensions (except the area defined by sacrificial layer due to rounding of AZ4562 or solubility to SU-8). After pyrolysis, the bridges are usually narrower from the center than the edges, resulting in the bridge acquiring a shape resembling an hourglass: the ends of the bridge are attached to the anchors and cannot shrink more than that, but the middle has more freedom, and it shrinks more than the ends. This phenomenon was noted mostly with bridges wider than 30 μm. This is because of lateral shrinkage taking place perpendicular to the bridge length and lateral elongation towards the anchor points (the shrinkage of the anchor point stretches the bridge). The narrowing of a bridge was more significant with wider bridges. The evolution of bridge shapes is shown in Figure 4.6.

The Table 4.2 presents the dimensions of the fabricated mushrooms with stem and cap diameter. The cap is always 1.5 times wider than the stem. The SU-8 mushrooms possess a relatively flat topside without any signs of bending, but after pyrolysis, especially larger mushrooms exhibit clear bending of overhanging parts. The mushrooms with overhanging part shorter than 20 μm retain flat topside after pyrolysis, but longer caps

Figure 4.5. Carbon micro- and nanopillar fabrication by embossing and pyrolysis. a) Dual-scale pillar-bSi structures had two kinds of topography: either the interpillar space had only a few nanopillars (left), or it was full of them (right). b) The hPDMS-PDMS stamp. c) The copy process into the SU-8 layer was also successful with even the smallest of features. d) Dual-scale pillar-bSi structures after pyrolysis. All scale bars: 2 μm. Reproduced from [51] with permission from MDPI.
Results and Discussion

Table 4.1. Bridge width-to-length ratios with success and failure mechanisms. The number in the cell shows the bridge’s designed width-to-length ratio. The background color indicates the time and type of possible problems. Reproduced from [50] with permission from IOP Publishing.

<table>
<thead>
<tr>
<th>Width [µm]</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>300</th>
<th>400</th>
<th>500</th>
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<tbody>
<tr>
<td>500</td>
<td>*50:1</td>
<td>*25:1</td>
<td>*50:3</td>
<td>*25:2</td>
<td>10:1</td>
<td>5:1</td>
<td>5:2</td>
<td>5:3</td>
<td>5:4</td>
<td>1:1</td>
</tr>
<tr>
<td>400</td>
<td>*40:1</td>
<td>20:1</td>
<td>*40:3</td>
<td>10:1</td>
<td>*8:1</td>
<td>4:1</td>
<td>2:1</td>
<td>4:3</td>
<td>1:1</td>
<td>4:5</td>
</tr>
<tr>
<td>200</td>
<td>20:1</td>
<td>10:1</td>
<td>*20:3</td>
<td>5:1</td>
<td>4:1</td>
<td>2:1</td>
<td>1:1</td>
<td>2:3</td>
<td>1:2</td>
<td>2:5</td>
</tr>
<tr>
<td>100</td>
<td>10:1</td>
<td>5:1</td>
<td>*10:3</td>
<td>5:2</td>
<td>2:1</td>
<td>1:1</td>
<td>1:2</td>
<td>1:3</td>
<td>1:4</td>
<td>1:5</td>
</tr>
<tr>
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<td>5:2</td>
<td>5:3</td>
<td>5:4</td>
<td>1:1</td>
<td>1:2</td>
<td>1:4</td>
<td>*1:6</td>
<td>*1:8</td>
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<td>4:5</td>
<td>2:5</td>
<td>1:5</td>
<td>*2:15</td>
<td>1:10</td>
<td>*2:25</td>
</tr>
<tr>
<td>20</td>
<td>2:1</td>
<td>1:1</td>
<td>1:2</td>
<td>1:4</td>
<td>2:5</td>
<td>1:5</td>
<td>1:10</td>
<td>*1:15</td>
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<td>1:5</td>
<td>1:10</td>
<td>1:20</td>
<td>*1:30</td>
<td>*1:40</td>
<td>*1:50</td>
</tr>
</tbody>
</table>

AZ4562 dissolution before pyrolysis
Successful
Collapse during pyrolysis
Sideways bending before and during pyrolysis

*Unique ratios in this list, others have two to ten instances.

exhibit clear upward bending. The cap shrinks laterally approximately 50 % from the original diameter. The round mushrooms bend isometrically (similar from all directions), but the square caps show more upward deflection at the corners.

Table 4.2. Dimensions for mushrooms. The diameter of the cap is 1.5 times larger than the stem. A small area of the stem results in loss of adhesion to the substrate. The stem of square mushrooms has a 27 % bigger footprint area than round mushrooms. The number in the cell indicates the measured cap diameter after pyrolysis and percentage from the original diameter. The results presented are from 900 °C pyrolysis. Reproduced from [50] with permission from IOP Publishing.

<table>
<thead>
<tr>
<th>Shape</th>
<th>Cap %</th>
<th>Diameter [µm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Round</td>
<td>9:6</td>
<td>18:5 56:3</td>
</tr>
<tr>
<td>Square</td>
<td>9:3</td>
<td>18:0 26:7</td>
</tr>
</tbody>
</table>

Mushroom structures are an ultimate version of a cantilever: they have a single anchor point, but the overhang is 360°around the anchor in mush-
Results and Discussion

The shrinkage with mushroom structures bent the cap when the overhang was longer than 12.5 μm. In round mushrooms, the overhang bent upwards radially and formed edges around the stem. In square-shaped mushrooms, the shrinkage and bending were similar. The corners of the cap were further away from the stem, and thus after bending, they created higher peaks. The evolution of mushroom shapes is shown in Figure 4.6.

**Figure 4.6.** The evolution of overhang deformation with bridges and mushrooms. The value with dark background shows the design value for dimension (dashed lines in the second row), whereas the value with white background represents the actual measured value after pyrolysis. All samples pyrolysed in 900 °C. Reproduced from [50] with permission from IOP Publishing.
The dimensions of fabricated membranes are presented in Table 4.3. Before pyrolysis, the SU-8 membranes possess a relatively flat profile, except for the larger ones that have a slight deflection towards the substrate. The shrinkage in the whole structure is similar to bridges, except that there are four anchor points preventing shrinkage of the whole structure. Because the membrane has holes in the suspended part, it resembles a suspended grid. During pyrolysis, the holes in the membrane increase 50% in size because the SU-8 between the holes shrinks 70%. This yields tensile stresses and movement of the grid, which can eventually lead to cracking. 5% of the bridges cracked due to similar reasons as with bridges. The Table 4.3 shows a clear line that divides the dataset between successful and collapsed membranes: when the overall membrane size increases beyond 1 mm in length. The collapse was observed with less than 25% of the membranes with a hole dimension of 62.5 μm and was increased to approximately 75% for the membranes with hole dimension of 112.5 μm. The collapse is similar to bridges, where the biggest roles are internal stress relaxation and capillary forces. The evolution of membrane shapes is shown in Figure 4.7.

Table 4.3. Dimensions for suspended membranes. Total size indicates the width/length of the whole membrane structure, and the symbol defines the shape of the holes. Membranes with critical dimension X higher than 50 μm experienced significantly more fabrication problems. The grid between the holes shrunk by an average of 70% from the original width, and the hole size increased by an average of 50% from the original width. The numbers in the cells indicate the actual measure widths of the holes and grids after pyrolysis and their respective percentage from the design value. The results presented are from 900 °C pyrolysis. Reproduced from [50] with permission from IOP Publishing.

<table>
<thead>
<tr>
<th>Total size (μm)</th>
<th>Critical dimensions [μm]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12.5</td>
</tr>
<tr>
<td>238</td>
<td>69.0</td>
</tr>
<tr>
<td>213</td>
<td>41.7</td>
</tr>
<tr>
<td>Round</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>42%</td>
</tr>
<tr>
<td></td>
<td>20.7</td>
</tr>
<tr>
<td></td>
<td>166%</td>
</tr>
<tr>
<td>Square</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>34%</td>
</tr>
</tbody>
</table>

The SU-8 cantilevers show clearly the residual tensile stresses remaining in the cross-linked matrix: cantilevers with length below 100 μm possess a relatively straight profile, whereas over 100 μm long cantilevers exhibit a positive deflection at an angle between 5° and 10°. The elevated temperature releases the stresses and pyrolysis shrinks the material, which

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Results and Discussion

Figure 4.7. The evolution of overhang deformation with membranes. The value with a dark background shows the design value for dimension, whereas the white background represents the actual measured value after pyrolysis. All samples pyrolysed in 900 °C. Reproduced from [50] with permission from IOP Publishing.

together results that not a single cantilever longer than 50 μm survived the process. The cantilevers most often collapsed to the substrate, or in few cases, bent 180° over themselves. The few survived short cantilevers expressed upward deflection of 45°, which is due to anchor point shrinkage and resulting transverse stress gradient pulling the cantilever upwards as discussed in [76, 81]. The dimensions of fabricated cantilevers are shown in Table 4.4. Most of the fabricated cantilevers collapsed either before or
Results and Discussion

during pyrolysis. The few surviving cantilevers always had less than 100 μm overhang, and they always bent upwards.

**Table 4.4.** Cantilever's width to length ratios. The number in the cell shows the cantilever width:length ratio. Cantilevers with lengths over 50 μm experienced significant fabrication problems. Reproduced from [50] with permission from IOP Publishing.

<table>
<thead>
<tr>
<th>Width [μm]</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>100</td>
<td>500</td>
<td>10</td>
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<tr>
<td>400</td>
<td>250</td>
<td>200</td>
<td>20</td>
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<td>300</td>
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<td>20</td>
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<td>10</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>500</td>
<td>10</td>
</tr>
</tbody>
</table>

- **Collapse during pyrolysis**
- **Successful**
- **Collapse and extensive bending before pyrolysis**

*Unique ratios in this list, others have two to ten instances.

**Atomic force microscopy**

In Publication IV we observed a correlation between the surface roughness and used pyrolysis temperature. Original SU-8 film is smooth with a mean surface roughness of 0.34 nm, and during pyrolysis the roughness increases to 0.46 nm @ 800 °C and 1.75 nm @ 1100 °C (Figure 4.8). These values are within the expected range and are comparable to other pyrolysed photoresists. According to the literature, the surface roughness of PyC material should not be dependent on the final pyrolysis temperature but rather the heating rate [48, 73, 80]. In our experiments regarding the roughness, the heating rate was always kept constant 200 °C/h. The reason for changed roughness is mostly due to increased outgassing of volatile atoms in higher temperatures, leading to a more ruptured surface.
Results and Discussion

Figure 4.8. Surface roughness increases with increased pyrolysis temperature (Inserts from AFM measurements). Reproduced from [50] with permission from IOP Publishing.

Raman spectroscopy
We used Raman spectroscopy to analyze the carbon bond structure of PyC samples pyrolysed in different temperatures (Figure 4.9a). The results show that increased temperature yields larger crystallite sizes and fewer defects in graphitic planes, which are responsible for the decreased resistivity. We made peak fitting analysis and $I_D/I_G$ intensity ratio comparison with peak maximum intensities and peak integrated areas, which both require a bit different fitting curves and calculation methods, which can show different results. The $I_D/I_G$ ratio decreased in both calculation methods with increased temperature, which corresponds to increased crystallinity and crystallite size $L_a$ (Figure 4.9b). The FWHM-analysis shows a slight change in FWHM$_D$ from 240 to 191 cm$^{-1}$ with increased temperature (Figure 4.9c), which means fewer disorders [99, 157]. The D and G peaks center positions move with increased pyrolysis temperature (Figure 4.9d). However, the analysis of this movement is contradictory: according to Ferrari and Robertson, the G peak should move towards lower frequencies with increased pyrolysis temperature, but according to DelTorro et al. [20], Ferralis et al. [29], and Vallerot et al. [157], the local stress and strain in the material can move the peaks in one way or another.

Electrical resistivity
The electrical resistivity of PyC films was decreased when the pyrolysis temperature was increased. The resistivity for PyC was $12.9 \times 10^{-5}$ Ωm @ 800 °C, $4.8 \times 10^{-5}$ Ωm @ 900 °C, $3.6 \times 10^{-5}$ Ωm @ 1000 °C and $2.9 \times 10^{-5}$ Ωm @ 1100 °C. The biggest change in resistivity was observed between 800 °C and 900 °C, which is also the reported temperature range where the outgassing of hydrogen takes place [66, 119]. In higher temperatures
the resistivity drop is more gradual which is due to increased ordering of the crystal structure, growth of graphitic crystallite sites and elimination of the sp$^3$ bonds [64, 89]. The graphitization and ordered structure is confirmed with Raman spectra (Figure 4.9). The electrical properties are particularly important in electrochemistry, where lower material resistivity allows more heterogeneous electron transfer amplifying redox-current thus improving sensor quality [95]. The resistivity on PyC in our studies is $2.9 \times 10^{-5} – 1.3 \times 10^{-4}$ Ωm which is in line with other pyrolytic carbons $3.2 \times 10^{-5} – 6.0 \times 10^{-3}$ Ωm [48, 89, 119, 146]. Compared to other carbon materials, PyC falls between amorphous carbon (a-C, $5.0 \times 10^{-4} – 8.0 \times 10^{-4}$ Ωm [141]) and graphite ($4.0 \times 10^{-7} – 1.7 \times 10^{-3}$ Ωm [95]).

4.2 Processing of ta-C

Direct etching of ta-C films is extremely difficult. We found compatible methods to pattern the film in 2D and 3D forms, which did not require
Results and Discussion

The patterning of ta-C was always carried out with the lift-off process (Procedure 4.6) because the material required the titanium adhesion layer, and there were no practical limitations to perform lift-off. The following parts focus on full MEA fabrication with ta-C as the active electrode material rather than the material properties (which are characterized in other studies).

**Procedure 4.6.** Lift-off procedure for Ti+ta-C layer

<table>
<thead>
<tr>
<th>LIFT-OFF WITH IMAGE REVERSAL AZ5214</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AZ5214 patterning with IR process</strong></td>
</tr>
<tr>
<td>1. Procedure 3.2</td>
</tr>
<tr>
<td><strong>Ti/ta-C deposition</strong></td>
</tr>
<tr>
<td>2. Deposition of 20 nm Ti with magnetron sputtering</td>
</tr>
<tr>
<td>Deposition of 7 nm ta-C with FCVA</td>
</tr>
<tr>
<td><strong>Lift-off</strong></td>
</tr>
<tr>
<td>3. Lift-off either with:</td>
</tr>
<tr>
<td>- Acetone in ultrasonic bath for 10 min</td>
</tr>
<tr>
<td>- AZ100 resist remover in ultrasonic bath for 30 min</td>
</tr>
<tr>
<td>4. Rinse with clean acetone, IPA and DIW, dry in spindryer. Remove moisture in 120 °C oven for 30 min</td>
</tr>
</tbody>
</table>

4.2.1 **2D patterning of ta-C**

We used the lift-off method with image reversal resist AZ5214 to pattern ta-C film with titanium adhesion layer for a MED64 MEA probe (Figure 3.1, Procedure 4.7). The resist must not be hard-baked in temperatures over 100 °C as this will prevent the resist from undergoing thermal reflow, which would destroy the desired negative sidewall slope. The benefit of using this resist is the easy removal/lift-off procedure, which can be done either in acetone or specific AZ100 remover. Acetone works faster than the remover, sometimes too fast, leaving large chunks of lift-off flakes floating in the liquid. Using an ultrasonic bath during lift-off breaks these flakes into smaller particles and also enhances the process. The wafer must be rinsed with clean acetone immediately after taking it out from the bath as the solvent dries out very quickly, and upon drying, the residual flakes can attach permanently to the surface of the wafer.
**Procedure 4.7.** MED64 probe fabrication with ta-C as electrode material

<table>
<thead>
<tr>
<th>SILICON MED64 PROBE WITH TA-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Thermal oxidation of 100 mm Si wafer (500 nm)</td>
</tr>
<tr>
<td>2. Patterning of Ti/ta-C layer for carbon electrodes Procedure 4.6</td>
</tr>
<tr>
<td>3. Patterning of Cr layer to create supportive metal conductor Procedure 4.6 except with 50 nm Cr sputtering</td>
</tr>
<tr>
<td>4. Deposition of 200 nm PECVD SiO2</td>
</tr>
<tr>
<td>5. Lithography with AZ5214 (Procedure 3.1)</td>
</tr>
<tr>
<td>6. Plasma etching of SiO2 with AZ5214 mask, mask removal</td>
</tr>
<tr>
<td>7. Wafer dicing into 5 x 5 cm² chip (Procedure 3.6)</td>
</tr>
<tr>
<td>8. Quartz ring (diameter 2 cm) attachment with 10:1 PDMS</td>
</tr>
</tbody>
</table>

4.2.2 3D Patterning of ta-C

The 3D structuring of ta-C was done as a coating on top of pre-existing structures. Typically FCVA deposited films are ultrasmooth [101], and in our research RMS surface roughness of ta-C was 0.1 - 0.2 nm [26]. The ta-C deposited with FCVA can also copy the roughness of the underlying substrate in a few nm roughness range [128]. The underlying silicon substrate had eight different structures fabricated prior to deposition: planar, nanorough pillars, 2 μm pillars in three layouts (actual height 2.2 μm), and 20 μm pillars in three layouts (actual height 21.7 μm). The silicon etched with ICP-RIE produces a slight positive sidewall slope, allowing the ta-C deposition to occur to some extent on the sidewalls, producing fully coated structures.

4.3 Processing of nC

In Publication I we studied the best way to etch the nanocarbon film. We used two processing equipment, RIE and ICP-RIE, and applied the acquired recipes for multielectrode array fabrication.

4.3.1 Patterning of nC

For etching recipe optimization, we used a test mask with variable-sized patterns. For RIE, we used 1×1.5 cm² samples with Cr coverage of 0.54, 0.65, 0.83 and 0.98 cm², and for ICP-RIE 1×3 cm² samples with Cr coverage of 1.19 and 1.80 cm². The Cr coverage for all samples is important to take
the loading effect into account. The used recipes and their corresponding etch rates and selectivities were calculated, and the results are presented below. The etching sample fabrication is presented in Procedure 4.8.

**Procedure 4.8.** Plasma etching of nanocarbon thin film

<table>
<thead>
<tr>
<th>NANOCARBON PATTERNING WITH REACTIVE IONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Nanocarbon deposition on 100 mm silicon wafer with CFUBM Hard mask</td>
</tr>
<tr>
<td>2. Patterning of 80 nm Cr with Procedure 3.3 nC etch</td>
</tr>
<tr>
<td>3. Etching of nC film with Cr mask (with RIE or ICP-RIE)</td>
</tr>
<tr>
<td>4. Removal of Cr mask in ceric ammonium nitrate 120 s, rinse with DIW</td>
</tr>
</tbody>
</table>

Both etching methods (RIE and ICP-RIE) successfully patterned nanocarbon thin film, but there were clear differences in etch rates between the processes. We also got clear results about parameter importance within the equipment. The loading between the samples had minor variations. Still, these were considered insignificant, as the size of the reaction chamber and the used gas flows and pressures were considered high enough to cover these small variations in nC surface area. Therefore, the loading has not been taken into account in the result analysis.

**RIE of nC**

The Taguchi orthogonal array for RIE etch rates are shown in Table 4.5. Each parameter has a calculated signal-to-noise (S/N) ratio to determine which parameters affects most to the etching process. Also, material selectivities were calculated because it is important that the mask is not sputtered and redeposited on the samples.
Table 4.5. Taguchi L8(2^5) orthogonal array with two levels for RIE results. All experiments were done at room temperature. 80 sccm argon flow was used in all runs except in “Max” 50 sccm Ar flow was used. Reproduced from [49] with permission from Elsevier.

<table>
<thead>
<tr>
<th>#</th>
<th>P W</th>
<th>Gas Flow</th>
<th>Pres.</th>
<th>Rate</th>
<th>Sel.</th>
<th>S/N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sccm</td>
<td>mTorr</td>
<td></td>
<td>n/min</td>
<td></td>
<td>dB</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>20</td>
<td>80</td>
<td>50</td>
<td>4.7</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>20</td>
<td>80</td>
<td>100</td>
<td>1.7</td>
<td>2.7</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>40</td>
<td>80</td>
<td>50</td>
<td>3.9</td>
<td>2.4</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>40</td>
<td>80</td>
<td>100</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td>20</td>
<td>80</td>
<td>50</td>
<td>4.6</td>
<td>0.1</td>
</tr>
<tr>
<td>6</td>
<td>80</td>
<td>20</td>
<td>80</td>
<td>100</td>
<td>8.5</td>
<td>1.7</td>
</tr>
<tr>
<td>7</td>
<td>80</td>
<td>40</td>
<td>40</td>
<td>50</td>
<td>7.5</td>
<td>3.9</td>
</tr>
<tr>
<td>8</td>
<td>80</td>
<td>40</td>
<td>80</td>
<td>100</td>
<td>13.4</td>
<td>7.7</td>
</tr>
</tbody>
</table>

\[ \Delta nC \]

5.4 1.9 2.0 1.3 3.6

\[ \Delta Cr \]

1.3 2.7 2.1 1.7 0.9

OnC 80 40 40 80 50 14.0 0.7 20:1 32 11
O Sel 80 40 80 40 50 12.1 0.3 40:1 31 -38
Max 200 50 0 0 30 92.7 2.1 44:1 45 -15

The experiment numbers 1–8 are part of the Taguchi orthogonal array to determine the parameter effect. The recipes "OnC" and O Sel" are recipes derived from the Taguchi array to optimize the etching for high nC etch rate and high selectivity, respectively. In these recipes, the same parameter ranges are used as in the Taguchi array. The recipe “Max” is derived from all previous recipes to maximize the nC etch rate by altering the parameter ranges to ultimate values within equipment parameter boundaries.

The reactive ion etching of nC was analyzed in terms of nC etch rate and selectivity with a Cr mask. The results in Table 4.5 show that experiment 8 had the best etch rate for nC (13.4 nm/min), and experiment 5 had the best selectivity (36:1). The \( \Delta \)-values are calculated for all parameters by subtracting the average etch rate of "low level" experiments from all "high value" experiments. The \( \Delta \)-value is a measure of impact: higher \( \Delta \)-value signifies that the changes in this parameter affect more to the process. The response diagram is presented in Figure 4.11, which shows each parameters’ impact on nC and Cr etch rate. The steepness of the slope is the \( \Delta \)-value, and the direction of the slope indicates which parameter level (high or low) produces a higher etch rate. All recipes had a constant 80 sccm Ar gas flow.

The best response for high nC etch rate are produced with high power level, low pressure level, and low SF6 flow rate, whereas for low Cr etching, the most effective parameters were the gas flow rates: low O2 and CHF3, and high SF6. Based on this information we created two optimal recipes (within our chosen parameter levels "high" and "low"): optimal for nC etching (OnC) and optimal for high selectivity (O Sel). These recipes indeed worked as planned, and OnC had etch rate of 14.0 nm/min, and O Sel had selectivity of 40:1. It is worth to note that the O nC recipe is the same
Results and Discussion

Figure 4.11. Response diagram of Taguchi experiments for both nC and Cr with reactive ion etching. nC and Cr trends are similar, but power and pressure effects are substantially bigger for nC. Reproduced from [49] with permission from Elsevier.

as in experiment 8, but for some unknown reason, the Cr etch rate was hugely different. We produced a final "Max" recipe from all this data, which is meant to balance between high nC etch rate and Cr selectivity. The parameter values were maximized (or minimized) within equipment parameter boundaries, and Ar gas flow was reduced to 50 sccm (1:1 ratio with O₂ flow). The acquired etch rate was 92.7 nm/min, which is almost 7 times faster than with O₉. By removing the other gases from the plasma, the selectivity was also very good 44:1. These results are in accordance with Sandhu et al., [139] who report that Ar:O₂ gas mixture has a good etch rate (∼ 50 nm/min) for diamond.

ICP-RIE of nC

The Taguchi orthogonal array for ICP-RIE results are shown in Table 4.6 with S/N-ratios for all parameters.

The ICP-RIE experiments with nC show greater values for nC etch rates compared to RIE. Experiments 4 and 8 clearly have the best nC etch rates (160 nm/min and ∼ 180 nm/min), but the best selectivities were obtained with experiments 1 and 3. We calculated the Δ-values for all parameters, presented in Table 4.6 and plotted in Figure 4.12.

The best responses for high nC etch rate are produced by high ICP-power level, low pressure level, and high oxygen flow. The most effective
Results and Discussion

Table 4.6. Taguchi L₈(2⁶) orthogonal array for ICP-RIE results. Reproduced from [49] with permission from Elsevier.

<table>
<thead>
<tr>
<th>#</th>
<th>T °C</th>
<th>Power W</th>
<th>Gas Flow sccm</th>
<th>Pres. mTorr</th>
<th>Rate nm/min nC</th>
<th>Sel.</th>
<th>S/N dB</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-110</td>
<td>200 100</td>
<td>10 10 10 10</td>
<td>34 0 135:1</td>
<td>31 12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-110</td>
<td>200 100</td>
<td>40 40 15 15</td>
<td>25 1 34:1</td>
<td>28 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-110</td>
<td>500 150</td>
<td>10 10 15 15</td>
<td>97 1 99:1</td>
<td>40 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-110</td>
<td>500 150</td>
<td>40 40 10 10</td>
<td>160 31 5:1</td>
<td>44 -30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>20 200 150</td>
<td>10 40 10 10</td>
<td>47 6 8:1</td>
<td>33 -16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>20 200 150</td>
<td>40 10 15 15</td>
<td>49 2 34:1</td>
<td>34 -3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>20 500 100</td>
<td>10 40 15 15</td>
<td>95 4 25:1</td>
<td>40 -12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>20 500 100</td>
<td>40 10 10 10</td>
<td>&gt;180 19 10:1</td>
<td>45 -25</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ΔnC 2.4 10.6 2.0 1.9 1.0 3.0
ΔCr 10.3 15.5 6.5 10.2 9.6 11.7

OnC 20 500 150 40 10 10 ~160 16 10:1 50 -29
OSel 20 500 100 10 10 10 80 1 109:1 42 -6
Max 50 800 200 50 0 10 ≫390 12 >32:1 52 -19

Figure 4.12. Response diagram of Taguchi experiments for both nC and Cr with inductively coupled plasma reactive ion etching. ICP power is the dominant parameter for nC etch rate. Reproduced from [49] with permission from Elsevier.

parameters for low Cr etch rate are high pressure level, low ICP-power level, and low oxygen flow. The optimized recipes for nC etch rate (OnC) and Cr selectivity (OSel) were created, and they produced nC etch rate of 160 nm/min and selectivity of 109:1, respectively. These values are not the best from all the experiments (experiment 8 has a better etch rate and similar selectivity as OnC, and experiment 1 has better selectivity, but worse etch
rate than \( O_{\text{Sel}} \)). To maximize the nC etch rate with decent selectivity, we created a "Max" recipe by altering the parameter values within equipment limitations. The etch rate was incredible \( \gg 390 \) nm/min with good Cr selectivity of 32:1. The exact etch rate could not be determined because the nC film was completely etched during the 2 min etching run.

### 4.4 Carbon thin film processing comparison

We have patterned the three different carbon materials with different methods, which all have their benefits and disadvantages. The most distinct difference between the materials is between PyC vs. nC and ta-C because the latter can only be used as thin film coating (with thicknesses of tens of nanometers). In contrast, PyC can be used as a 3D structure where the bulk is also made from carbon. The thicknesses with PyC can be varied from less than a micron to tens of micrometers.

All our patterning methods require photolithography to define the structures, but only direct etching (like we did with nC in Publication I) requires expensive plasma processing tools. PyC patterning requires a high-temperature furnace with an inert gas option in addition to lithography. Lift-off goes around the need for plasma etching tools. Still, lift-off is considered a dirty process as the resists can leak monomers and other chemicals into vacuum chambers (where deposition is done) and thus contaminate the resulting film. Also, wet processing in lift-off is not always a feasible method. On the other hand, pyrolysis produces good quality carbon films, but it requires high temperature, which must be taken into account in material selection. The most complicated method to pattern a film is etching, which requires an etchant compatible mask on top of the etched material (like Cr in plasma etching or AZ5214 in wet etching). Especially plasma etching with a hard mask is a multistep process, which requires 1) photolithography, 2) hard mask patterning (generally wet etching), 3) resist removal, 4) plasma etching of target film, and 5) hard mask removal.

In Publication III we demonstrated how to fabricate similar structures from two different carbon materials (PyC and ta-C). PyC was patterned with direct photolithography but also with the embossing method. The embossing utilized a silicon master mold, which was also used as a substrate for ta-C deposition. Also nC could be deposited similarly on top of patterned Si substrate to obtain 3D nC structures. The key difference between the ta-C/nC and PyC structures is the embossing resolution and shrinkage during pyrolysis, which will alter the PyC structures copied from the original master mold.

The following sections describe the use of these patterning methods to create multielectrode arrays. The differences in patterning methods played a crucial role in the functionality of the final device. All the MEAs had
a conducting metal layer to enhance the electrical conductivity. In nC MEA, the carbon layer was etched through a metal mask. This masking material was later used as the conducting metal layer by removing the metal from the measurement site. In the best version of ta-C MEA, the material is deposited as the last step through insulation openings on top of the underlying conducting metal layer. In PyC MEA, the metal layer is deposited and patterned first, and the precursor SU-8 is patterned on top of the metal and pyrolysed. From these three different carbon MEAs, the fabrication of ta-C MEA proved to be the most feasible. In nC MEA, the measurement site had metal contamination from the mask and thus could have interfered with electrochemical measurements. The high temperature required in PyC MEA changed the conductivity of underlying metal film (through dissolution into underlying insulator) and hindered the electrical conductivity in some chips. The lift-off of ta-C kept the underlying metal layer intact and provided as-deposited carbon film for measurements.

4.5 Fabrication of multielectrode arrays

4.5.1 Fabrication of PyC EAT

The fabrication of MEA-chip with PyC electrodes requires the other materials to be compatible with high temperatures. Especially the supporting metal wire needs to have high temperature tolerance because it provides electrical conductivity for a long wire. We chose chromium, which provides good electrical conductivity as a thin layer and high temperature tolerance. However, we found two issues arising with the chromium. The first issue was bottom insulation compatibility with the Cr film. We used either silicon dioxide (thermal and PECVD) or nitride (PECVD) as a bottom insulator. We found out that the Cr layer dissolves into the insulator during pyrolysis, especially when PECVD was used as the insulator deposition tool. Nitride is known to prevent ionic movement into the material, but we believe our PECVD does not produce dense enough film to prevent it in this case. We grew PECVD nitride and oxide at 300 °C. Oxides are known to allow more ionic movement into them than nitrides, and we clearly observed the Cr dissolving into the film with the change of color, decreased height, and decreased electrical conductivity. Thermal oxide provided better insulation due to denser film.

The second issue with high-temperature pyrolysis with Cr wires was CrN formation. We mostly used nitrogen as a pyrolysis atmosphere (as is recommended by many studies), and we found out that chromium nitride is formed around 400 to 800 °C. The CrN film is usually used as an extremely hard coating due to fantastic corrosion and wear resistance
but also because of non-adhesive properties. CrN has electrical resistivity around $6.4 \times 10^{-6}$ Ωm [9, 93], which is a tenfold increase over plain Cr. These two issues combined, we often found out that the chromium wires leading to the PyC electrodes were not conductive enough for microelectrode applications. However, we did manage to fabricate few successful PyC MEA chips and did preliminary and promising electrochemical measurements with those (unpublished results, work in progress).

**Flat PyC microelectrodes** In the microelectrodes, the signal conducting metal wire was buried underneath the patterned SU-8 prior to pyrolysis (Figure 4.13a and b). In flat electrodes, we used 13 μm thick SU-8 layer. We could not use the HF dip nor oxygen plasma to increase the SU-8 adhesion on the surface because HF would have etched the bottom insulator, and oxygen plasma would have oxidized the chromium. Due to these factors, we found out that the SU-8 electrodes started to delaminate from the edges even before pyrolysis. Pyrolysis enhanced the delamination due to changes in stresses inside SU-8/PyC (Figure 4.13c), and many of the electrodes were lost during processing. We increased the SU-8 adhesion on the bottom Cr/insulator by keeping the sample in a 120 °C oven overnight prior to spin-coating, removing the moisture from the surface. The top insulator SU-8 2000.5 was patterned on top of PyC electrodes (Figure 4.13d). A successful fabrication process for PyC EAT is shown in Procedure 4.9.

**Procedure 4.9.** Multielectrode array made with pyrolytic carbon working electrodes

<table>
<thead>
<tr>
<th>SILICON EAT PROBE WITH PYC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Thermal oxidation of 100 mm Si wafer (500 nm)</td>
</tr>
<tr>
<td>2. Patterning of 50 nm Cr with Procedure 3.3</td>
</tr>
<tr>
<td>3. Lithography with SU-8 50 for 13 μm thickness (Procedure 3.4)</td>
</tr>
<tr>
<td>To create microelectrode pads</td>
</tr>
<tr>
<td>4. Pyrolysis in 900 °C N₂ atmosphere for one hour (Procedure 3.5)</td>
</tr>
<tr>
<td>5. Lithography with SU-8 2000.5 (Procedure 3.4)</td>
</tr>
<tr>
<td>6. Quartz ring (diameter 2 cm) attachment with 10:1 PDMS</td>
</tr>
</tbody>
</table>

**3D PyC microelectrodes** The 3D PyC microelectrodes were in the form of pillars on top of a base layer to increase pillar adhesion. The base layer was 3 μm and covered the whole underlying Cr wire. The pillars were created on the second lithography step, and they had the height of 20 μm and diameter of 20 μm (Figure 4.14a). The thinner base layer had clearly fewer adhesion problems than the thicker layer used with flat electrodes (the area was the same). With PyC electrodes, we used thin SU-8 2000.5 as a
Results and Discussion

Figure 4.13. Flat PyC microelectrodes. a) In a single MEA layout, there are duplicates of similar electrodes radially distributed around the center. b) The SU-8 was patterned on top of underlying Cr wiring. c) The adhesion of SU-8/PyC was compromised without proper surface treatments prior to deposition. d) Successful electrodes were insulated with SU-8 2000.5 to have only the PyC electrode visible.

top insulator material (Figure 4.14b and c). The development time or SU-8 2000.5 was around 15 minutes to remove all unexposed resist between the pillars (followed by microscope inspection after every 3 minutes of development). A successful fabrication process for 3D PyC EAT is shown in Procedure 4.10.
**Results and Discussion**

**Figure 4.14.** PyC microelectrodes with pillars. a) The electrodes were fabricated from SU-8 with a two-step lithography process: first, the 3 μm base layer was patterned and then 20 μm pillars. The base layer experienced minor adhesion problems. b) The SU-8 pillars shrunk during pyrolysis, but most of the pillars were preserved during processing. The electrodes were insulated with SU-8 2000.5 layer with extended development time. c) The small electrodes had only a single pillar.

**Procedure 4.10.** Multielectrode array made with pyrolytic carbon working electrodes with pillars

<table>
<thead>
<tr>
<th>SILICON EAT PROBE WITH 3D PYC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Thermal oxidation of 100 mm Si wafer (500 nm)</td>
</tr>
<tr>
<td>2. Patterning of 50 nm Cr with Procedure 3.3</td>
</tr>
<tr>
<td>3. Lithography with SU-8 50 for 13 μm thickness (Procedure 3.4) To create microelectrode pads</td>
</tr>
<tr>
<td>4. Lithography with SU-8 50 for 20 μm thickness (Procedure 3.4) To create pillars on pads</td>
</tr>
<tr>
<td>5. Pyrolysis in 900 °C N₂ atmosphere for one hour (Procedure 3.5)</td>
</tr>
<tr>
<td>6. Lithography with SU-8 2000.5 (Procedure 3.4)</td>
</tr>
<tr>
<td>7. Quartz ring (diameter 2 cm) attachment with 10:1 PDMS</td>
</tr>
</tbody>
</table>
4.5.2 Fabrication of ta-C MED64

The motivation to include ta-C in the MED64 probe was the pre-existing probe station and fully operational measurement software (all from Alpha MED Scientific). We fabricated the ta-C MED64 probes on two different substrates, glass and silicon, which both required a bit different fabrication approaches.

Silicon substrate MEA on a silicon substrate is shown in Figure 4.15a. The silicon substrates were used mostly to testing the process flow and design feasibility. The biggest drawback with silicon is the non-transparency, which is required to confirm the slice orientation or presence of cells on a single electrode. We used silicon dioxide as a bottom insulator, and we varied the location of the signal conducting metal wire (below or on top of the ta-C layer). The top insulator was SU-8 2000.5, which covered all areas except the measurement pads (Figure 4.15b). The fabrication procedure is shown in Procedure 4.7.

![Figure 4.15. MEAs made on opaque silicon and transparent glass. a) MED64 probe made on top of a silicon substrate. The patterned top insulation shows as reddish. b) Close-up from the measurement electrodes. c) MED64 probe made on top of glass substrate allows easier optical sample positioning and measurements. d) Part of the probe on top of blue ink demonstrates the transparency of the Ti/ta-C layer. The ink can be seen through glass and Ti/ta-C, but not through Cr.](image)
Glass substrate  The glass substrate (100 mm wafer, 0.7 mm thick, double side polished Borofloat 33) offered the highly valued transparency for an MEA chip (Figure 4.15c). It was also noted that 20 nm Ti + 7 nm ta-C is partly transparent (Figure 4.15d), which made the measurement electrode transparent. A commonly used transparent electrode material is ITO, and our carbon material (even with Ti adhesion layer) could be a novel alternative for that. Naturally, the signal conducting metal wire is not transparent, but the metal is not under the measurement pad in our design. The downside of the Ti/ta-C layer is the electrical conductivity, which is poor in thin films. Thus, the signal conducting metal wire needs to be patterned as close to the measurement pad as possible to minimize the electrode resistance [117]. With silicon substrate, the signal conducting metal wire can be placed under the measurement electrode, in which the electrical current needs only to go through the Ti/ta-C layer (and not to conduct along it). The fabrication procedure with a glass substrate is similar to Procedure 4.7, except the bottom insulation was not created separately.

4.5.3 Fabrication of ta-C EAT

The EAT design was made by the author to have several different-sized electrodes in variable distances from each other to distinguish how the adjacent electrodes interfere with each other. The used elements were similar to in all MEAs: a bottom insulator, signal conducting metal, carbon electrode, top insulator. We developed two different versions of ta-C EAT: one with carbon underneath the insulator layer to ensure no underlying Cr is visible from the opening, and one where ta-C was not buried under the insulator.

Version 1: ta-C under insulator

The process for ta-C EAT fabrication with Si₃N₄/SiO₂ top insulator is shown in Procedure 4.11. The Cr metal support was buried under Ti/ta-C layer to provide electrical conductivity for a long wire but hide it from the measurement sites. Cr was left visible to the wafer periphery where the output pads are located. We decided to pattern the Cr layer with lift-off for yield reasons even though we had the possibility for wet etching of Cr. During lithography, we used plastic masks. If the AZ5214 is processed like positive resists, the small defects in the mask’s transparent areas (bubbles and scratches) are also copied on the resist because these defects refract UV-light and prevent the resist exposure from the bottom. Therefore, if the resist is used as a wet etch mask, Cr spots remain where plastic masks had spot defects. In our case, this leads to short-circuit between several electrodes. When the AZ5214 is used in image reversal mode, the small defects are not copied in such detail because of resist exposure mechanisms.
Results and Discussion

and light diffraction, and the Cr can be patterned through lift-off.

**Procedure 4.11.** Multielectrode array made with ta-C working electrodes

<table>
<thead>
<tr>
<th>SILICON EAT PROBE WITH TA-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Thermal oxidation of 100 mm Si wafer (500 nm)</td>
</tr>
<tr>
<td>2. Patterning of 50 nm Cr with Procedure 3.3</td>
</tr>
<tr>
<td>To create bottom supportive metal conductor</td>
</tr>
<tr>
<td>3. Patterning of Ti/ta-C layer with Procedure 4.6</td>
</tr>
<tr>
<td>To create carbon electrodes</td>
</tr>
<tr>
<td>4. Deposition of insulator stack with PECVD</td>
</tr>
<tr>
<td>100 nm Si₃N₄ + 100 nm SiO₂ + 100 nm Si₃N₄ + 100 nm SiO₂</td>
</tr>
<tr>
<td>5. Spin coating SF9 with 2000 rpm for 30 s</td>
</tr>
<tr>
<td>6. Soft baking SF9 at 170 °C for 5 min on a hotplate</td>
</tr>
<tr>
<td>7. Image reversal lithography with AZ5214 (Procedure 3.2)</td>
</tr>
<tr>
<td>8. Development in MIF726 developer for 1 min, rinse with DIW and dry</td>
</tr>
<tr>
<td>9. Etching of Si₃N₄/SiO₂ with RIE (High power)</td>
</tr>
<tr>
<td>Etching to halfway of undermost Si₃N₄ layer</td>
</tr>
<tr>
<td>10. Etching remaining Si₃N₄ with RIE (Low power)</td>
</tr>
<tr>
<td>11. Removal of AZ5214 in acetone for 10 min, rinse with DIW</td>
</tr>
<tr>
<td>12. Removal of SF9 in MIF726 for 10 min, rinse with DIW and dry</td>
</tr>
<tr>
<td>13. Quartz ring (diameter 2 cm) attachment with 10:1 PDMS</td>
</tr>
</tbody>
</table>

The ta-C layer was also patterned with lift-off (Procedure 4.6), and the process worked very well every time. The top insulation is composed of multiple insulating thin films where SiO₂ provides an excellent electron barrier whereas Si₃N₄ prevents the movement of ions and diffusion of material into the insulator. We used four 100 nm layers by alternating these two materials, where nitride was in contact with the supporting metal (to prevent metal diffusion), and oxide was the topmost (to provide the most biocompatible surface).

We patterned the insulator stack with reactive ion etching (Procedure 3.7). To minimize the damage on the underlying ta-C layer, we had to do the etching in two steps: first, etch through the three topmost layers (oxide-nitride-oxide) with high power, and then change the etching recipe to low power on the last layer (nitride). This method takes advantage of the reactivity of silicon nitride: Si₃N₄ etches spontaneously in the presence of CF₄. Therefore we timed the high power etching with CHF₃ and CF₄ to go halfway through the last layer and then changed the recipe to low power. The high power recipe had etch rates of 52 nm/min for SiO₂ and 67 nm/min for Si₃N₄. Due to uneven etch rates throughout the chamber and
slight variation in layer thicknesses, over-etching was done for the first three layers to make sure no SiO₂ remains (which would mask the next etching step). The second recipe used only low bombardment power, which should not cause serious damage on ta-C (which has a surface of weak sp² hybridized carbon) once the last nitride layer is breached.

**Version 2: Double resist for ta-C lift-off**

One of the applications required the final MEA structure to be heated to 750 °C at very fast ramp rates in the presence of plasma (final processing steps). This process grows carbon nanofibers on the ta-C surface [138]. Upon first tests, we found out that the top insulator stack delaminates from areas on top of the underlying ta-C film (Figure 4.16a). Therefore we created a design where this deficiency is removed (Figure 4.16b). From an electrochemistry point of view, it is crucial that the supporting metal wire is not visible from the top insulator opening, or it would dominate the electrochemical response.

![Figure 4.16. Layer designs of ta-C MEA. a) In high-temperature processes, if ta-C is buried under the top insulation SiO₂-Si₃N₄ stack, the thermal expansion and adhesion problems cause the insulation to crack severely. Image credit Elli Leppänen. b) In improved design, the ta-C is patterned with lift-off directly on top of the electrode openings with the same mask which was used to etch the openings (to minimize the misalignment and improve the coverage). Upon initial tests, no underlying Cr is visible through the insulation opening.](image)

In designs where the ta-C layer covers the surroundings of metal wire, and the top insulator is opened only from areas where ta-C is visible, there is no risk for the metal to dominate the electrochemical response. However, if the ta-C is deposited after top insulator patterning, there are several issues to consider (mask alignment accuracy, step coverage, measurement
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electrode area). To go around these fabrication problems, we used the top insulator stack etching mask (SF9 + IR AZ5214) for ta-C lift-off because it provided no need to align a new mask, and it defined the electrode area as well (Figure 4.16b). Also, the FCVA deposition of ta-C had a good step coverage, and no underlying metal was detected being visible upon first experiments. The procedure is presented in Procedure 4.12.

Procedure 4.12. Multielectrode array made with ta-C working electrodes

SILICON EAT PROBE WITH TA-C

1. Thermal oxidation of 100 mm Si wafer (500 nm)
2. Patterning of 50 nm Cr with Procedure 3.3
   To create bottom supportive metal conductor
3. Deposition of 100 nm PECVD Si$_3$N$_4$
4. Deposition of 100 nm PECVD SiO$_2$
5. Deposition of 100 nm PECVD Si$_3$N$_4$
6. Deposition of 100 nm PECVD SiO$_2$
7. Spin coating SF9 with 2000 rpm for 30 s
8. Soft baking SF9 at 170 °C for 5 min on a hotplate
9. Lithography with AZ5214 (Procedure 3.1)
10. Development in MIF726 developer for 1 min, rinse with DIW and dry
11. Etching of Si$_3$N$_4$/SiO$_2$ with RIE (High power)
    Etching to halfway of undermost Si$_3$N$_4$ layer
12. Etching remaining Si$_3$N$_4$ with RIE (Low power)
13. Deposition of Ti/ta-C layer with FCVA
14. Lift-off of ta-C by removal of AZ5214 in acetone for 10 min, rinse with DIW
15. Removal of SF9 in MIF726 for 10 min, rinse with DIW and dry
16. Subsequent high temperature processes –>

Plastic masks and image reversal resist

In standard processing of AZ5214 (Figure 4.17 S1 – S2), the resist structures have a positive slope. This can be because of multiple reasons: the light diffracts from the mask pattern, the exposed resist can bend the light, or the light can reflect from the bottom of the wafer. Generally, this is not crucial because the bottom of the unexposed resist pattern has the same linewidth as the mask, and therefore the resist can be used as an etching mask. In a more realistic process, the photomask (especially plastic photomask) can have defects like bubbles and thin scratches, which can
block the UV-light. Because these defects are generally so small, the resist underneath is only partially exposed (Figure 4.17 R1), and the bottom of the resist stays unexposed. When this resist mask is used for etching, the defects are copied to the underlying films (Figure 4.17 R2). To overcome this issue, AZ5214 can be processed in IR mode with the same photomask. During the first exposure, the exposed pattern of the resist is similar to normal positive tone processing (Figure 4.17 IR1). During image reversal bake, the exposed parts are made invulnerable to consequent exposure and development. During the second exposure (flood), all remaining resist is exposed to UV-light and made soluble to the developer (Figure 4.17 IR2). Upon development, the big features are developed normally (with resulting negative sidewall slope to the remaining features), but since the patterns created by the small defects are trapped inside the already hard-baked AZ5214, the developer can not reach those areas, and therefore the defects are not copied with the lift-off material (Figure 4.17 IR3).

Figure 4.17. AZ5214 resist handling with plastic photomasks. Plastic photomasks can have defects (bubbles and scratches) that change light’s course through the photomask. There are no defects in the ideal process (S1-S3), and therefore, the subsequent etching procedures yield planned structures. In a realistic process (R1-R3), the defective photomask creates undesired patterns which can mask the subsequent etching procedures and create dots (R2) and pinholes (R3). When a defective photomask is used in image reversal mode (IR1-IR4), the effect of bubbles and scratches can be avoided. Resist patterned with image reversal mode can be used similarly in wet and plasma (IR4) etching, but it also enables the use of lift-off process (IR3).

In a different scenario, the resist can be used as a plasma etching mask (Figure 4.17 S3, R3, IR4, plasma etching of SiO₂). In the standard process, the resist mask is consumed during plasma etching. Still, if the selectiv-
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ities are calculated correctly, the resist thickness is enough to etch the whole underlying film (Figure 4.17 S3). In a realistic process with plastic masks, the small defects produce problems. The defects at transparent areas are copied similar to the wet etching procedure explained above, but during plasma etching, the resist thickness is too thin to act as a mask (Figure 4.17 R3). Plastic masks are usually printed with ink, and the ink can contain defects and openings as well. These small openings produce small pits on the surface of the unexposed resist (Figure 4.17 R2). When the plasma etching consumes the resist, these pits will become fully open and expose the underlying film for etchants producing undesired etch patterns (Figure 4.17 R3). This can also be avoided with image reversal processing. The small pits will turn into a hard-baked resist, which are lifted off during development (Figure 4.17 IR2 – IR3), and buried exposed resist will prevent plasma etchant from reaching the underlying film (Figure 4.17 IR4). An important factor to note is that using IR processed resist as an etch mask requires opposite polarity photomask (for clarity, Figure 4.17 IR4 shows a pattern for a similar mask with all other processes showed).

During plasma etching of ta-C EAT SiO2-Si3N4 stack top insulator, we used photoresist as an etching mask. Plain AZ5214 did not work due to similar reasons as explained above. The bubbles in the plastic mask created thinner areas in the underlying resist (Figure 4.18a), which are breached during plasma etching, and several pinholes are created in the top insulator (Figure 4.18b). Therefore, we made a procedure for a double resist mask: A plasma resistant resist SF9 is used under IR-processed AZ5214 (Figure 4.18c). SF9 is not photopatternable by itself, and it needed another resist for patterning (Procedure 4.11). It is highly dissoluble to common resist developer MIF-726, which can also be used to develop AZ5214. The IR processing for AZ5214 was used to minimize the mask defect interference. This combination produced a negligible amount of pinholes on the top insulator (Figure 4.18d), and both resists could be removed with wet chemicals (AZ5214 in acetone and SF9 in MIF-726).
4.5.4 Fabrication of nC MED64

We fabricated the nC MED64 probe with Procedure 4.13. The nanocarbon layer was etched with ICP-RIE optimized recipe for selectivity because Cr was needed in the final device. The recipe provided a fast enough process for 100 nm and 500 nm nC film etching.
Procedure 4.13. MED64 probe fabrication with nanocarbon as electrode material

**SILICON MED64 PROBE WITH NANOCARBON**

1. Thermal oxidation of 100 mm Si wafer (500 nm)
2. Nanocarbon deposition and patterning without Cr mask removal (Procedure 4.8)
3. Deposition of SiO₂ with PECVD (200 nm)
4. Lithography with AZ5214 (Procedure 3.1)
5. Etching of SiO₂ with RIE
6. Removal of AZ5214 in acetone for 10 min, rinse with DIW
7. Etching of Cr from insulation openings in ceric ammonium nitrate 120s, rinsing with DIW
8. Wafer dicing into 5 x 5 cm² chip (Procedure 3.6)
9. Quartz ring (diameter 2 cm) attachment with 10:1 PDMS

4.5.5 Carbon as microelectrode material

Microelectrode arrays can be used in many applications, and their effectiveness is highly dependent on used electrode materials. For example, in electrical impedance spectroscopy, the electrode material does not need to support/promote cellular life as long as it is not toxic [78], in electrophysiological measurements the material needs to support cellular life more in order to allow cells to adhere on it (also required in organotypic slice measurements, but not with acute brain slices) [148] and in electrochemical measurements the material needs to possess correct electrochemical potential window to allow the detection of specific analytes and metabolites [10]. Our studies have targeted the carbon materials to fulfill the needs of many different applications, and the Table 4.7 summarizes the different C-MEA designs. The purpose of each design is discussed below.

**Table 4.7. MEA designs**

<table>
<thead>
<tr>
<th>Material</th>
<th>Layout</th>
<th>Structure</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>PyC</td>
<td>EAT</td>
<td>Flat</td>
<td>Electrochemistry</td>
</tr>
<tr>
<td>PyC</td>
<td>EAT</td>
<td>3D pillars</td>
<td>Electrochemistry</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acute slices</td>
</tr>
<tr>
<td>ta-C</td>
<td>EAT</td>
<td>Flat</td>
<td>Electrochemistry</td>
</tr>
<tr>
<td>ta-C</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acute slices</td>
</tr>
<tr>
<td>ta-C</td>
<td>MED64</td>
<td>Flat</td>
<td>Opaque, Organo. slices</td>
</tr>
<tr>
<td>ta-C</td>
<td>MED64</td>
<td>Flat</td>
<td>Transparent, Organo. slices</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cultured cells</td>
</tr>
<tr>
<td>nC</td>
<td>MED64</td>
<td>Flat</td>
<td>Organo. slices</td>
</tr>
</tbody>
</table>

The PyC microelectrodes were only fabricated in EAT format, which was
meant to study the electrochemical performance of materials and electrode interference to each other (simultaneous measurements).

In electrochemistry, the electrode size affects the reaction kinetics, and microelectrodes can exhibit desirable properties regarding the selectivity of molecules and redox kinetics. We used PyC microelectrodes in electrochemical detection of DA in the presence of interfering molecules AA and UA, which provides novel information for designing in vivo carbon microelectrodes.

3D pillar electrodes fabricated from PyC and ta-C are meant for acute slice experiments. It would be beneficial for the electrode to penetrate the dead cell layer and perform electrochemical or electrophysiological measurements in close proximity to alive cells in a slice. The MED64 format is designed to fulfill the needs of brain slice measurements, and we tailored our electrode layout to fit mouse hippocampal brain slices specifically.

We fabricated flat carbon microelectrodes in MED64 layout from ta-C and nC. Both are meant for organotypic brain slice measurements where the slice is cultivated on top of the MEA for several days (DIV8 in our Publication I). The transparency is a really desired property for an MEA because it allows better slice visual alignment on top of the electrodes, and in the case of cultivated cells, the cell count per electrode can be easily identified with an optical microscope.

### 4.6 Application demonstrations

#### 4.6.1 Biocompatibility

All tested carbon materials exhibit good biocompatibility without any surface coatings. In Publication I, we used PEI-coating (polyethylenimine) to increase the cell adhesion on nC electrode pads, and in Publication II we grew cells on flat PyC surfaces. In Publication III we grew the cells on top of patterned surfaces to observe the cellular responses.

We tested the cell viability on flat PyC and ta-C samples in Publication II and III with mouse neural stem cells (mNSC), neuroblastic PC-12 cells, and C6 glial cells. All these cells show a higher viability rate on untreated ta-C compared to untreated PyC. Both materials have an extremely smooth surface (average roughness $1.15 \pm 0.14$ nm for ta-C [154] and $0.9 \pm 0.6$ nm for PyC), but the chemical functionalities differ: ta-C has significantly more oxygen functionalities (8.9 %) than PyC (1.5 %). In Publication II, we observed that oxidizing the surface of PyC will increase the amount of all cell types, supporting that oxygen has a critical influence on cell biocompatibility.
In Publication III, we tested the cellular response of mNSC on micro- and nanopatterned PyC and ta-C surfaces, and we observed clear differences between the two materials. On PyC, the cell number and morphology are not affected by the different surface topographies, and cell distribution is quite homogeneous in all surfaces, even though we have structures from nanometer-sized pyramids to 20 μm sized pillars. But on the contrary, the cells grown on 2 μm sized ta-C coated pillars show higher MTT reading than the planar reference ta-C surface. There is also clear cell aggregation and increased viability rate, especially on two specific pillar layouts (hex-grid and random-grid).

In Publication I, we assessed the neurocompatibility of nC surface with dissociated neuronal cultures and compared the results to the same tests on reference glass coverslips. The MAP2-GFAP immunostainings (Figure 4.19a) demonstrates that the culture density and cell morphology were similar on both substrates and KCC2-NeuN immunostainings (which represent the cellular maturation tests) show no difference either (Figure 4.19b). KCC2 is sensitive to pathological conditions, and our experiments show that the nC supports neuronal growth and maturation at least equally viable as glass.

4.6.2 Electrical measurements

In Publication II, we carried out electrochemical experiments with oxidized and native PyC surfaces. PyC showed linear electrochemical response to dopamine in the tested range of 50 nM to 1 μM, which is in physiologically relevant concentrations (0.01 - 1 μM in extracellular fluid). The oxidized surface expressed improved electrochemical performance compared to the native PyC surface, which was visible as increased current in the surface redox process. We compared the two surfaces with two common molecules found in the brain, ascorbic acid (AA) and dopamine (DA), from which AA is the major interferent in the electrochemical detection of neurotransmitter DA. The flat surfaces did not show significant selectivity between these two molecules with cyclic voltammetry (CV), and interfering AA dominated the electrochemical response. In other studies, we performed similar tests with AA, DA, and UA (uric acid) by using PyC microelectrodes (electrode area as small as 314 μm²). The microelectrode format showed more clear selectivity between the molecules and the possibility to separate DA peaks from the CV data. This is due to different diffusion and redox dynamics.

Specifically in this research we did not study the electrochemical response of ta-C, but other studies show the ta-C thin film as an electrochemical electrode for dopamine detection [79, 116, 123], H₂O₂ detection [154] and drug discovery [104, 164].

The performance of nC MEA was studied with organotypic hippocampal slices in Publication I. We compared the results with commercial MED64
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Figure 4.19. Hippocampal neuron culturing on nC and glass substrate with a) anti-MAP2 (red) and anti-GFAP (green) immunostaining, and b) anti-KCC2 (green) and anti-NeuN (red) antibodies. Strong color intensity represents viable cells, and there are no clear differences between the two substrates. Reproduced from [49] with permission from Elsevier.

MED-P515A MEA-probe. The slices on both MEAs were imaged with a bright field microscope, which showed the slices had sharp edges, even surface, and well preserved hippocampal morphology. After recordings, the slice cultures were stained with propidium iodide (PI) to observe the slice viability and detect the damaged cells, which showed similar survival of the cells in both cultures, indicating that the nC MEA provides good biocompatibility. The network activity was recorded in DIV8, which showed spontaneous neuronal bursting activity with silent periods. Our nC MEA acquired these events with $0.07 \pm 0.004$ mV peak amplitude and P515A with $0.25 \pm 0.020$ mV, ($p < 0.05$). The RMS noise was similar in both MEAs, nC MEA: $9.5 \pm 0.9$ $\mu$V and P515A: $10.5 \pm 0.5$ $\mu$V, $p > 0.05$. Thus, the signal-to-noise ratio for our nC MEA was $13 \pm 1.4$ and for P515A $59 \pm 7.0$. Our nC MEA had less sensitive event detection, and the resulting signal-
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to-noise ratio was approximately 1/4 from the commercial P515A. The high difference between the SNR values is mostly because of a different active surface area between the electrodes: nC MEA is mostly flat, whereas P515A is made from rough platinum black. The power spectrum also revealed that the nC MEA can detect the slow components, but the detection of higher frequencies is weak. Finally, we confirmed that the recorded activity was of a neuronal origin and added 2 nM tetrodotoxin to block voltage-gated sodium channels, which resulted in a complete loss of electrical activity.
5. Conclusions

In this study, we have successfully demonstrated the patterning methods and possibilities of carbon thin films nanocarbon (nC), tetrahedral amorphous carbon (ta-C), and pyrolytic carbon (PyC). The patterning methods were common microfabrication techniques and included lithography, wet chemical etching, and reactive ion etching. Different patterning methods were required to shape a specific carbon material, but similar structures could be achieved despite different processing protocols.

In Publication I, we studied plasma etching of nanocarbon thin film. We etched nC with two etching processes and fabricated multiple multielectrode arrays (MEA) with nC measurement electrodes which were then utilized in mouse brain slice measurements. Nanocarbon was etched with reactive ions, and we could achieve an etch rate of 160 nm/min with high selectivity of 109:1 to chromium when using inductively coupled plasma. Nanocarbon supports cell culturing, and MEAs made from nC show promising characteristics in organotypic hippocampal slice measurements. This type of etching and patterning could be targeted for carbon electronics and circuitry.

Publication II examined how PyC and ta-C possess distinct chemical functionalities on the surface despite having a similar elemental backbone. PyC surfaces had only a small amount of oxygen (1.5 %), ketone, hydroxyl, and epoxide groups, whereas ta-C surfaces had almost 9 % oxygen functionalities, 41 % ketone, and 7 % carboxyl groups. The oxygen content on the ta-C surface was further increased with increased surface roughness. Both materials were electrochemically tested with outer-sphere (OSR) and inner-sphere reactions (ISR) to reveal the electron transfer kinetics and electrode fouling. The different surface functionalities change the hydrophobicity, thus affecting protein adhesion. PyC was more hydrophobic and made stronger interactions with proteins with a larger footprint but still maintained unchanged OSR electron tunneling kinetics. In contrast, ta-C was significantly less hydrophobic and made denser protein film on the surface, which hindered the OSR electron tunneling through the film. Dopamine was used as an ISR, and it showed faster electron transfer
kinetics on PyC than ta-C, and it was strongly affected by biofouling on all surfaces by slowing down the electron transfer.

In Publication III, we studied the micro- and nanoscale patterning of two carbon materials, PyC and ta-C, and how the surface topography affects cell adhesion and viability. The patterns used were 20 $\mu$m, 2 $\mu$m, and <1 $\mu$m pillars, which are in the correct scale to produce a cell response. Different types of cells adhered on all surfaces, and there was a clear difference between cell behavior between the materials: The viability of cells was similar on all PyC surfaces, whereas with ta-C, the 2 $\mu$m features showed increased cell count and aggregation. Application of PLL coating on the ta-C surface did not affect cell response but slightly increased cell count on the PyC surface. These results highlight the different roles of surface geometry and chemistry.

In Publication IV, we created 3D suspended and overhanging carbon microstructures from PyC with sacrificial lithography and pyrolysis. The transformation from negative photoresist SU-8 to PyC during pyrolysis appears to take place in three stages: (i) at around 300 °C, the remaining solvent is evaporated from the resist, and SU-8 is under thermal expansion, whereas (ii) at higher temperatures up to 600 °C there is voluminous outgassing of volatile elements which result into shrinkage of the material. (iii) The last step takes place above 600 °C, where the internal bonds rearrange (crystallization and graphititization) and electrical resistance decreases significantly. The studies on structure dimensions show that increasing the anchor points from one to four also increases the possible length of overhangs from 50 $\mu$m to 1 mm. Due to uneven lateral shrinkage of PyC, the structures lose their original shape and become deformed.

A systematic study of different pyrolysis temperatures with varying suspended structures helps us better understand the correlation between the pyrolysis conditions and the mechanical behavior of 3D carbon structures. There is still room for optimization of suspended structures to improve structural stability and increase the fabrication success rate, but our study will help develop more complex suspended C-MEMS structures.

All Publications I-IV form a solid entity that provides methods for carbon thin film patterning and integration for electrical applications. This thesis focuses on carbon patterning and its usage in electrical devices, but all the developed fabrication procedures can be implemented in various other applications. Especially pyrolytic carbon was studied extensively during this research, and very detailed fabrication and characterization parameters and protocols are provided. Also, more general fabrication guidelines and practical approaches are offered to help the worldwide microfabrication community.

Carbon materials, in general, are already implemented in various applications: carbon fibers as reinforcement in plastics, graphite in nuclear power plants, or lead pencils and diamond in jewelry or grinding and
cutting machinery. Graphene and carbon nanotubes have shown great potential in the fields of electronics and electrical engineering, but they are not yet widespread on an industrial scale. The results from this thesis could be a small step towards carbon breakthrough in the electronic industry, where the use of rare metals could be replaced with carbonaceous alternatives. When more patterning and deposition possibilities are made available, this could be a reality.
References


References


References


