Biopolymer-Templated Deposition of Ordered and Polymorph Titanium Dioxide Thin Films for Improved Surface-Enhanced Raman Scattering Sensitivity

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Titanium dioxide (TiO\textsubscript{2}) is an excellent candidate material for semiconductor metal oxide-based substrates for surface-enhanced Raman scattering (SERS). Biotemplated fabrication of TiO\textsubscript{2} thin films with a 3D network is a promising route for effectively transferring the morphology and ordering of the template into the TiO\textsubscript{2} layer. The control over the crystallinity of TiO\textsubscript{2} remains a challenge due to the low thermal stability of biopolymers. Here is reported a novel strategy of the cellulose nanofibril (CNF)-directed assembly of TiO\textsubscript{2}/CNF thin films with tailored morphology and crystallinity as SERS substrates. Polymorphous TiO\textsubscript{2}/CNF thin films with well-defined morphology are obtained by combining atomic layer deposition and thermal annealing. A high enhancement factor of $1.79 \times 10^6$ in terms of semiconductor metal oxide nanomaterial (SMON)-based SERS substrates is obtained from the annealed TiO\textsubscript{2}/CNF thin films with a TiO\textsubscript{2} layer thickness of 10 nm fabricated on indium tin oxide (ITO), when probed by 4-mercaptobenzoic acid molecules. Common SERS probes down to 10 nm can be detected on these TiO\textsubscript{2}/CNF substrates, indicating superior sensitivity of TiO\textsubscript{2}/CNF thin films among SMON SERS substrates. This improvement in SERS sensitivity is realized through a cooperative modulation of the template morphology of the CNF network and the crystalline state of TiO\textsubscript{2}.

1. Introduction

Surface-enhanced Raman scattering (SERS) substrates based on semiconductor metal oxide nanomaterials (SMONs) have attracted substantial attention for their potential applications in sensing of biomolecules,[1] trace detection of pollutants,[2] and monitoring catalytic reactions[3] due to their low-cost, high stability, and biocompatibility.[4] In addition, SMONs show a greater variety of crystalline states,[5] as well as a wider range of optical and electrical properties in comparison to noble metals,[6] which can be tailored to meet the specific demands in SERS applications. Titanium dioxide (TiO\textsubscript{2}) nanoparticles (NPs) are among the most appropriate materials for fabricating high-performance SERS substrates in the area of SMONs.[7] However, the bottleneck problem of TiO\textsubscript{2} NP-based SERS
substrates is the low sensitivity compared to noble metal nanomaterials. The theoretical maximum enhancement factor (EF) for SMON SERS substrates based on the charge-transfer (CT) mechanism has been estimated to be 10^6,[1] while the experimental EF values for practical TiO_2 SERS substrates are mostly only in the range of 10^3-10^4.[9]

Two strategies have been carried out to improve the EF values of TiO_2-based SERS substrates. The first option is to tailor the crystalline state of TiO_2 for improving the interactions between TiO_2-based substrate and the analytes. The most investigated crystalline parameters include the crystallinity, crystallite size, as well as the polymorph ratio, for example, the rutile to anatase crystalline phase ratio of TiO_2.[10] Nonetheless, both the control over the crystallinity and crystallite size could be a “double-edged sword” for improving the SERS performance of TiO_2 substrates. The high degree of crystallinity as well as a high concentration of surface defects are two desired but contradictory properties for a superior SERS performance of SMONs.[13] When the annealing temperature raises, the crystallinity of TiO_2 increases but the concentration of surface defects decreases. At high annealing temperature, also the anatase-to-rutile phase transformation of TiO_2 has to be taken into account. Although the rutile phase displays a much lower SERS activity compared to the anatase phase, the synergistic effect between the rutile and anatase TiO_2 leads to a higher SERS activity than pure anatase TiO_2 due to the additional CT from rutile to anatase TiO_2 despite the intrinsic CT from anatase TiO_2 to the analytes. Whereas polymorphous TiO_2 with a low rutile-to-anatase ratio could not bring about the synergistic effect, an overly high rutile-to-anatase ratio will lead to a decreased SERS activity as well. Moreover, the SERS intensity of TiO_2 increases within a certain range of particle size, reaches a maximum and declines for even larger sizes.[12] Therefore, the preparation of TiO_2 thin films with desired crystallinity state is a crucial requirement for fabrication. Thermal annealing is one of the most commonly used methods for controlling the crystalline state of TiO_2.[13] However, the morphology and optical properties of the TiO_2 thin films could be significantly affected by thermal annealing.[14]

Thus, the preservation of the tailored morphology is another important requirement for the fabrication of TiO_2 despite its crystalline state. This leads to the second option, which is to regulate the surface morphology of the TiO_2 layer to enhance the interactions between the laser and the substrate. The specific surface area of the TiO_2 layer influences the density of available active sites for SERS activity,[15] and the interconnected network within the TiO_2 layer influences the rate of charge-carrier transfer. The most investigated morphological parameters include the shape, size, and ordering of TiO_2.[16] The control over the morphology of TiO_2 nanomaterials can be achieved by precise surface engineering methodologies. An effective method for controlling the dimensions, periodicity, and structure at both nano- and micro-scale is to use templates with desired 3D architectures.[17] Commonly used templating material range from hard/inorganic materials to soft/organic materials. Representative inorganic templates for TiO_2 fabrication include carbon materials, as well as metal oxide particles such as ZnO and Al_2O_3.[18] In contrast to hard templates, soft templates such as synthetic and natural polymers are particularly attractive due to the relative ease of chemical modification. A few biotemplates including the proteins in tobacco mosaic virus, ferritin,[19] and butterfly wings[20] have been successfully exploited to prepare porous TiO_2 thin films. Being engineered from the most abundant biopolymers,[21] nanocellulose represents another type of biotemplates,[22] which typically includes cellulose nanocrystals (CNCs), cellulose nanofibrils (CNFs), and bacterial cellulose.[23] Although CNCs[24] and bacterial cellulose[25] have demonstrated promising possibilities for fabricating 3D templates of TiO_2 thin films, the progress over CNF-templated TiO_2 thin films is still very limited.

Nonetheless, CNFs hold great potential for templating the morphology of TiO_2 thin films in three aspects. 1) CNFs feature high flexibility and mechanical strength,[26] which creates an interconnected network to support the complex morphologies of TiO_2.[27] 2) CNFs can be fabricated into hierarchically ordered materials, which provides a wide range of templating substrate architectures[28] including 2D arrays,[29] and 3D scaffolds.[30] 3) CNFs could be used as a standard deposition matrix for NPs with a homogeneous distribution at a low agglomeration rate,[31] which confers the resulting composite films with distinct optical properties. However, the use of CNF templates presents a challenge for traditional coating processes such as sol–gel,[32] layer-by-layer (LBL) deposition,[33] chemical vapor deposition,[34] sputtering,[35] lithography,[36] and their combinations,[37] as they are limited in their ability to preserve the topology of the template. On the contrary, atomic layer deposition (ALD) is a powerful coating technique based on self-limiting surface reactions, which enables deposition of highly conformal thin-films onto 3D templates with high precision.[38] ALD coating enables a well-defined thickness and the fulfillment of deposited material into the nanometer-sized pores within the template. With the improvement in ALD technology, which made the deposition at low temperature possible, TiO_2 could be deposited without destruction of the biotemplate.[19] However, the CNF-templated assembly of TiO_2 as SERS substrates has not been reported yet.

In the present study, we report a versatile strategy for the fabrication of TiO_2/CNF thin films that feature an improved SERS sensitivity with a precisely controlled morphology and crystalline state. The 3D network of CNFs with well-defined morphology was templated to fabricate a conformal TiO_2 layer via ALD coating, which is thermally annealed to achieve the desired crystalline state (Figure 1). The influence of three key factors on the SERS performance of TiO_2/CNF thin films is studied: 1) The morphology of the CNF template, which regulates the ordering of the TiO_2 layer, is achieved via oblique incidence spraying (OIS) of CNFs onto Si and indium tin oxide (ITO) substrates. ITO substrates are commonly used as electrodes, and the nanostructures on ITO provide a patterned template for the OIS of cellulose in comparison to the smooth Si substrates. 2) Thermal annealing of TiO_2/CNF thin films to control the crystalline state of TiO_2. Since the crystal size and rutile-to-anatase ratio of TiO_2 increase simultaneously with annealing temperature, an annealing temperature of 450 °C was applied to achieve a relatively higher crystal size of anatase TiO_2 together with a relatively lower rutile-to-anatase ratio.[41] 3) The deposition thickness of the TiO_2 layer is expected to influence the specific surface area of the layer due to limited preci-
sion of the ALD coating, and therefore the crystallinity of TiO$_2$. In this respect, a thickness of 10 nm represents a 2D TiO$_2$ thin layer with high specific area whereas a thickness of 60 nm represents a bulky 3D layer with a lower specific area considering the 6.5 nm radii of the TiO$_2$ nanograins (NGs), as deduced from grazing-incidence small-angle X-ray scattering (GISAXS). The as-prepared TiO$_2$/CNF thin films are probed by atomic force microscopy (AFM) and scanning electron microscopy (SEM) for the local examination of the surface, cross-sectional morphology as well as estimation of the specific surface area. As a complementary method, GISAXS and grazing-incidence wide-angle X-ray scattering (GIWAXS) techniques are used to investigate the in-plane 3D ordering and crystalline state of the thin films. Finally, the performance of TiO$_2$/CNF thin films as SERS substrates is investigated, and the impact of the template morphology, crystallinity state, and specific surface area of the TiO$_2$/CNF thin films on the EFs of the analytes are characterized by Raman spectroscopy. Collectively, this strategy offers a high tunability in the tailoring of the SERS performance of SMONs by modulating the template morphology and the crystalline properties of SMONs.

2. Results and Discussion

2.1. Morphology

We adopted the following name conventions for samples as indicated in Table S1, Supporting Information: 1) The Si and ITO substrates are denoted as “Si” and “ITO.” 2) The CNF thin films OIS-coated on Si and ITO are referred as “Si/CNF” and “ITO/CNF” as SERS substrates is investigated, and the impact of the template morphology, crystallinity state, and specific surface area of the TiO$_2$/CNF thin films on the EFs of the analytes are characterized by Raman spectroscopy. Collectively, this strategy offers a high tunability in the tailoring of the SERS performance of SMONs by modulating the template morphology and the crystalline properties of SMONs.

Figure 1. Fabrication of the titanium dioxide/cellulose nanofibril (TiO$_2$/CNF) composite films. a) Preparation of CNF thin film through oblique incidence spraying (OIS) at an angle of 10°. b) The as-prepared CNF thin film is composed of CNF matrix with a well-ordered network structure (left). TiO$_2$ nanograins are coated by atomic layer deposition (ALD) onto CNF thin film with a thickness of $\delta_\text{T}=10$ nm and $\delta_\text{T}=60$ nm (middle). Samples with $\delta_\text{T}=10$ nm thickness are shown as an example. The ALD-coated TiO$_2$/CNF thin films are annealed at $T = 450 \, ^\circ\text{C}$ for 0.5 h to obtain a desired crystalline state (right). The upper and lower rows indicate the fabrication of TiO$_2$/CNF thin films on Si and ITO, respectively.
sizes. While the small TiO$_2$ nanostructures bind to cellulose bundles to form a necklace architecture, the large and uniformly distributed TiO$_2$ nanostructures are more densely distributed in the Si/CNF/TiO$_2$10a ($d_{\text{TiNP1}} = 174 \pm 18$ nm) than in the Si/CNF/TiO$_2$60a sample ($d_{\text{TiNP1}} = 201 \pm 9$ nm), as shown in Figure S4, Supporting Information. On the other hand, the ITO-based ITO/CNF/TiO$_2$10a and ITO/CNF/TiO$_2$60a samples display a terrace-like morphology (Figure S5, Supporting Information), with discs ($\Omega_{\text{TiNP1}} = 193 \pm 22$ nm) which are composed of closely packed TiO$_2$ NPs with a diameter of $\Omega_{\text{TiNP2}} = 34 \pm 5$ nm (Figure S1, Supporting Information). The distinct morphology of the TiO$_2$ layer in Si-based (Si/CNF/TiO$_2$10a, Si/CNF/TiO$_2$60a) and ITO-based (ITO/CNF/TiO$_2$10a, ITO/CNF/TiO$_2$60a) samples is related to the crystallization degree of TiO$_2$ NPs, which will be discussed in detail in the Section 2.2.

In agreement with the line-cut results, analysis of the fits to the PSD data reveals similar sizes and distributions of TiO$_2$ NGs among the Si/CNF/TiO$_2$10, Si/CNF/TiO$_2$60, ITO/CNF/TiO$_2$10, and ITO/CNF/TiO$_2$60 samples (Figures S6 and S7, Supporting Information). The nanostructure with radii (R) of $R_{\text{TiNG2}} = 59 \pm 9$ nm and center-to-center distance ($D$) of $D_{\text{TiNG2}} = 295 \pm 55$ nm are observed, with “R = $\Omega$/2” and “D = 2 \times R + d” corresponding to the parameters in the line-cut analysis (Table S2, Supporting Information). After thermal annealing, the PSD distributions of TiO$_2$/CNF thin films display similar patterns as their corresponding templates. Especially, the extracted parameters in the ITO/CNF/TiO$_2$10a sample ($R_{\text{TiNP1}} = 77 \pm 11$ nm and $D_{\text{TiNP1}} = 247 \pm 55$ nm) are almost consistent with the ITO/CNF sample ($R_{\text{CNF1}} = 79 \pm 9$ nm $D_{\text{CNF1}} = 245 \pm 39$ nm), indicating the replication of the knot-like morphology of the CNF template. The values from PSD fits are smaller than that from the line-cut analysis due to averaging of the nanostructures of different sizes.

The second difference between Si- and ITO-based samples lies in the distribution of the TiO$_2$ nanostructures, which directly reflects the template morphology and the template replication degree. Two levels of structural replication exist in our systems: 1) The CNF layer is expected to template the morphology of TiO$_2$ layer by ALD coating, and 2) the substrate morphology is expected to transfer to the CNF template by OIS coating. At the first level, the necklace architecture signifies the morphology of TiO$_2$ templated by the CNF layer. According

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**Figure 2.** Morphology analysis of the TiO$_2$/CNF thin films. Atomic force microscopy (AFM) images of the TiO$_2$/CNF thin films on a) Si and b) ITO substrates, respectively. Abbreviations of the sample names are shown in Table S1, Supporting Information. Taking the Si-based samples as an example, “Si” and “Si/CNF” represent the bare Si substrate and CNF thin film OIS-coated on Si substrate. “Si/CNF/TiO$_2$10” and “Si/CNF/TiO$_2$60” are samples with TiO$_2$ ALD-coated on the Si/CNF sample with $\delta_T = 10$ nm and $\delta_T = 60$ nm, respectively. “Si/CNF/TiO$_2$10a” and “Si/CNF/TiO$_2$60a” are the thermally annealed “Si/CNF/TiO$_2$10” and “Si/CNF/TiO$_2$60” samples. SEM cross-sectional images of the c) ITO/CNF/TiO$_2$10 and d) ITO/CNF/TiO$_2$10a samples showing the layer replication at the TiO$_2$/CNF and the CNF/ITO interfaces. e) Root-mean-square (RMS) roughness ($\sigma_{\text{rms}}$) analysis of the TiO$_2$/CNF thin films.
to Figure 2a, the necklace architecture is more obvious in Si- than ITO-based samples, and is better seen in samples with $\delta_T = 10 \text{ nm}$ than that with $\delta_T = 60 \text{ nm}$ (Figure S3, Supporting Information). In Si-based samples, the morphology of the TiO$_2$ layer does not reveal the fibrous architecture of CNFs, but reflects the knot-like morphology of the ITO and the CNF thin film coated on ITO, which can be observed from the strong morphological similarities among the ITO/CNF/TiO$_2$10a, ITO/CNF/TiO$_2$60a, ITO/CNF, and ITO samples (Figure 2b). This indicates a weaker interaction between TiO$_2$ nanostructures and individual cellulose bundles due to the large diameter of TiO$_2$ nanostructures in the ITO/CNF/TiO$_2$10a and ITO/CNF/TiO$_2$60a samples. However, due to the larger diameter of the TiO$_2$ nanotube ($d_{\text{TiO}_2} = 193 \pm 22 \text{ nm}$) than the CNF knots ($d_{\text{CNF}} = 190 \pm 9 \text{ nm}$), and the smaller edge-to-edge distance of TiO$_2$ nanotube ($d_{\text{TiO}_2} = 35 \pm 10 \text{ nm}$) than the knots ($d_{\text{CNF}} = 65 \pm 10 \text{ nm}$), TiO$_2$ terraces are more likely to grow on the CNF knots, leading to the replication of the CNF template (Figure S5, Supporting Information). Furthermore, the cross-sectional SEM images clearly show the knot-like morphology (Figure 2c) and the terrace morphology of the TiO$_2$ layer in the ITO/CNF/TiO$_2$10a samples (Figure 2d). The similarity in the distribution patterns of the ITO/CNF/TiO$_2$10 and ITO/CNF/TiO$_2$10a samples indicates the successful structural templating at the TiO$_2$/CNF and the CNF/ITO interfaces (Figure S8, Supporting Information). Collectively, both the Si- and ITO-based samples successfully replicated the layer morphology of CNFs, but the layer replication is more obvious in ITO-based samples due to the knot-like morphology. In Si-based samples, the small TiO$_2$ nanostructures dominate, and their smaller diameter compared to the distance between cellulose bundles made it possible to replicate the individual cellulose bundles. On the other hand, in ITO-based samples, the sizeable TiO$_2$ nanostructure dominates, and the larger diameter of this nanostructure compared to the distance between cellulose bundles blocked the replication of the individual cellulose bundles.

Apart from the morphological evolution parallel to the film surface, the surface roughness of the TiO$_2$/CNF thin films also varies with $\delta_T$ and the template morphology (Figure 2e). Obtained from the WsXm software v5.0,[89] we found that the root-mean-square (RMS) roughness of the ITO/CNF sample ($\sigma_{\text{rms}} = 5.2 \pm 0.3 \text{ nm}$) is almost two times of the ITO ($\sigma_{\text{rms}} = 2.6 \pm 0.2 \text{ nm}$). The increase in roughness of the OIS-coated CNF template compared to the substrate indicates that the surface morphology is transferred and enhanced from ITO to the CNF layer. Furthermore, the ITO-based TiO$_2$/CNF thin films display a higher roughness than their Si-based counterparts (e.g., Si/CNF/TiO$_2$10 and ITO/CNF/TiO$_2$10, and Si/CNF/TiO$_2$10a and ITO/CNF/TiO$_2$10a), which is also related to a lower roughness of the CNF template in the Si/CNF sample ($\sigma_{\text{rms}} = 3.7 \pm 0.2 \text{ nm}$). This can be rationalized by the fact that cellulose bundles are more likely to distribute onto the convex surface of ITO particles due to the mutual effect of shear strength and spatial effect, while they distribute uniformly on Si substrate under the same fabrication process. Before annealing, the roughness of the Si- or ITO-based samples is slightly smaller than their corresponding CNF template (Si/CNF and ITO/CNF). Also the roughness in samples with $\delta_T = 10 \text{ nm}$ are marginally higher than that with $\delta_T = 60 \text{ nm}$, which decreased from $\sigma_{\text{rms}} = 3.5 \pm 0.3 \text{ nm}$ (Si/CNF/TiO$_2$10) to $\sigma_{\text{rms}} = 3.4 \pm 0.2 \text{ nm}$ (Si/CNF/TiO$_2$60) on Si, and from $\sigma_{\text{rms}} = 4.6 \pm 0.4 \text{ nm}$ (ITO/CNF/TiO$_2$10) to $\sigma_{\text{rms}} = 4.3 \pm 0.3 \text{ nm}$ (ITO/CNF/TiO$_2$60) on ITO with the increase of $\delta_T$, respectively. This is due to the limitation in size of TiO$_2$ NGs determined by the ALD conditions, leading to a decreased ability for TiO$_2$ NGs to fulfill the underlying layer, and thus a decreased template replication efficiency with an increased ALD thickness.

After thermal annealing, the roughness in all TiO$_2$/CNF thin films increased, which are $\sigma_{\text{rms}} = 5.0 \pm 0.2 \text{ nm}$ in the Si/CNF/TiO$_2$10a, $\sigma_{\text{rms}} = 8.4 \pm 0.4 \text{ nm}$ and $\sigma_{\text{rms}} = 8.4 \pm 0.5 \text{ nm}$ in the ITO/CNF/TiO$_2$10a and ITO/CNF/TiO$_2$60a samples, respectively. This might originate from the partial collapse of the voids within the CNF network formed during OIS coating, which leads to a higher roughness contrast between the collapsed and remaining part among the CNF network. The increase is more significant in ITO- than in Si-based samples, which might be related to the larger TiO$_2$ nanostructures’ size in ITO-based samples.

2.2. Crystallinity

GIWAXS is performed to investigate the effect of thermal annealing on the crystalline state of ALD-coated TiO$_2$ and CNFs (Figure S9, Supporting Information). Figure 3a and Figure S10, Supporting Information, illustrate the GIWAXS patterns of TiO$_2$/CNF thin films fabricated on Si and ITO, respectively. The GIWAXS patterns of the CNF templates and their corresponding substrates are shown in Figure S11, Supporting Information. To investigate the influence of ALD-coated TiO$_2$ on the crystallinity of cellulose, the GIWAXS patterns of pristine CNF thin film without TiO$_2$ coating are also investigated (Figure S12, Supporting Information). In the GIWAXS patterns of Si-based samples, the broad peak in the range of 1.5 Å$^{-1}$ < $q$ < 2.5 Å$^{-1}$ is observed in the Si/CNF/TiO$_2$10 and Si/CNF/TiO$_2$60 samples, which is attributed to the (100) scattering plane of CNFs and the amorphous state of ALD-coated TiO$_2$ NGs. However, two sharp peaks become prominent after thermal annealing, which indicate the evolution of the (100) and (200) scattering planes of CNFs. Two broad and pronounced peaks in the range of 2.5 Å$^{-1}$ < $q$ < 2.8 Å$^{-1}$ are identified as the (101) and (110) scattering planes of anatase and rutile phase of TiO$_2$ crystallites, respectively. Despite the weakening of the anatase and rutile scattering planes of TiO$_2$ due to the strong scattering of ITO in the ITO/CNF/TiO$_2$10a and ITO/CNF/TiO$_2$60a samples, the peak in the range of 1.7 Å$^{-1}$ < $q$ < 2.2 Å$^{-1}$ clearly indicates the crystallization of
Figure 3. a) 2D GIWAXS data ($q_r$, $q_z$ maps) of the TiO$_2$/CNF thin films fabricated on Si. $q_r$, $q_z$ maps of the ITO-based samples are shown in Figure S9, Supporting Information. The scattering pattern of CNF and TiO$_2$ are designated with "C" and "T," respectively. Radial integration of the reshaped $q_r$, $q_z$ map of the b) Si- and c) ITO-based samples, respectively. Light-green and green arrows represent the crystalline patterns on type I and type II cellulose, respectively. Light-orange and orange arrows show the crystalline patterns of amorphous/anatase TiO$_2$ and anatase/rutile TiO$_2$, respectively. d) Crystalline domain size (DnP) of the anatase and rutile crystalline phase of TiO$_2$ calculated from the FHWM of the (101) and (110) peaks. e) Crystalline phase ratio ($\chi_{R/A}$) of rutile to anatase TiO$_2$. 
CNFs. The agreement between Si- and ITO-based samples demonstrates the important role of annealing conditions in the phase transition of TiO₂ and crystallization of CNF.

To further analyze the evolution of the crystallinity of TiO₂ and CNFs, the 2D GIWAXS profiles are radially integrated using the GIXSGUI software v1.71 (Figure S1, Supporting Information). Before thermal annealing, the Si/CNF/TiO₂, 10 and Si/CNF/TiO₂, 60 samples show a broad peak over the range of 0.5 Å⁻¹ < q < 2.5 Å⁻¹ in the 1D GIWAXS patterns (Figure 3b), which is attributed to the amorphous state of TiO₂ and the Si substrate. Additionally, the marked small peak at q_TA = 2.5 Å⁻¹ (110) indicates the existence of a small amount of anatase TiO₂. After annealing, the rutile TiO₂ peak appears at q_TR = 2.8 Å⁻¹ (110), and the anatase peak at q_TA = 2.5 Å⁻¹ becomes more pronounced. This suggests an increase in the crystallite size, and a partial thermally induced anatase-to-rutile phase transition of TiO₂. The situation for ITO-based samples is quite similar, indicating polymorphic TiO₂ after annealing (Figure 3c). Moreover, the crystallinity of CNFs undergoes tremendous changes after thermal annealing. In TiO₂/CNF thin films, the CNF crystalline domains show three broad peaks at q_CNFA = 1.6 Å⁻¹ (110), q_CNFA = 2.1 Å⁻¹ (200), and q_CNFR = 3.4 Å⁻¹ (004), which belong to type I cellulose structure. After annealing, two prominent characteristic peaks at q_CNFR = 2.1 Å⁻¹ and q_CNFA = 2.0 Å⁻¹ (110) are detected, demonstrating the formation of type II cellulose. The decrease in the peak intensity of (004) and (110) is due to the transformation of cellulose from type I to II and a partial carbonization of CNFs, suggesting that the remaining CNFs became more thermodynamically stable (Figure S13, Supporting Information). On the other hand, the annealed CNF thin films without ALD-coated TiO₂ display no characteristic peaks of cellulose, indicating the total carbonization of CNFs (Figure S14, Supporting Information). Collectively, the ALD-coated TiO₂ offers a protecting layer for CNFs, which leads to a limited carbonization degree, meaning an increased thermal stability providing a strong support for the crystallized TiO₂ nanostructures.

To investigate the formation of TiO₂ polymorph, the Bragg peaks in the range 0.5 Å⁻¹ < q < 3.0 Å⁻¹ are deconvoluted (Figure S15, Supporting Information). The crystalline domain size (DnP) of TiO₂ is estimated from the full width at half maximum (FWHM) of the fitted (110) and (110) scattering peaks deducing the instrumental resolution measured from LaB₆₆ via the adapted Debye–Scherrer formula for grazing-incidence scattering. Before annealing, the DnP of amorphous/anatase TiO₂ NGs varies in the range of DnP = 14.2 ± 0.4 nm among the Si/CNF/TiO₂, 10, Si/CNF/TiO₂, 60, ITO/CNF/TiO₂, 10, and ITO/CNF/TiO₂, 60 samples (Figure 3d). After annealing, a slight increase in DnP of anatase TiO₂ NP is observed, reaching DnP = 17.5 ± 0.3 nm. This is consistent with the Ostwald ripening effect, in which atomic diffusion leads to the growth and coalescence of the TiO₂ crystalline phase. The DnP values of rutile TiO₂ NP range from DnP = 34 ± 3 nm in spite of the differences in δT and template morphology. The DnP of rutile TiO₂ is smaller in Si- than in the ITO-based samples, corroborating the AFM results. Besides, the rutile-to-anatase ratio (χ_R/A) of TiO₂ polymorph are χ_R/A = 0.21, χ_R/A = 0.45, χ_R/A = 0.26, and χ_R/A = 0.59 among the Si/CNF/TiO₂, 10a, Si/CNF/TiO₂, 60a, ITO/CNF/TiO₂, 10a, and ITO/CNF/TiO₂, 60a samples (Figure 3e), which are within the suitable range (17.6% < χ_R/A < 33.3%) of rutile-to-anatase ratio for optimal SERS activity of TiO₂.[49] The relatively higher χ_R/A of the ITO-based samples coincides with the higher δ_T from AFM analysis, contributing to a higher specific surface area to act as the active sites for nucleation of rutile TiO₂ crystallites (Table S3, Supporting Information). The values of surface area are obtained by the Gwyddion software v2.55 from AFM images under the scan size of 3 × 3 μm². By assuming that the mass of TiO₂ increases proportionally with δ_T, we are able to compare the relative specific area among the samples.

2.3. Topography

Complementary to the AFM and SEM measurements, which study the local morphology of the film surface or cross section, GISAXS is performed to probe into the topography of the entire film (Figure S9, Supporting Information). 2D GISAXS data of the TiO₂/CNF thin films and their corresponding templates are shown in Figure 4a,b and Figure S16, Supporting Information, respectively. To obtain a quantitative analysis of the nanostructures of TiO₂ and the CNF template, horizontal line-cuts were taken along q_y direction at q_x = 0.65 nm⁻¹ in Si-based samples and q_x = 0.75 nm⁻¹ in ITO-based samples (Figure S7, Supporting Information). The horizontal line-cuts are subsequently analyzed by a model described in our previous studies to obtain statistically averaged values of the morphological features of TiO₂ and CNFs in real-space.[48] The model is based on cylindrical objects describing the cellulose-based nanostructures and spherical objects describing the TiO₂-based nanostructures and ITO (Table S4, Supporting Information). The lower layer is fitted prior to the upper layer to reduce the complexity of the fits and reduce the number of free parameters in the fits. For example, the ITO/CNF/TiO₂, 10 sample is fitted by the successive addition of the fit functions of ITO, CNFs and TiO₂. Based on this model, the best fits are shown as the green dashed lines in Figure 4c,f, and the center-to-center distances “D” (Figure 4d,e) as well as the radii “R” (Figure 4f,g) are extracted.

In agreement with our previous results (Figure S18, Supporting Information),[52] three cylindrical structures are identified for the CNF template on Si, with R_CNFI = 27 ± 3 nm, R_CNF2 = 11.0 ± 1.9 nm, R_CNF3 = 2.1 ± 0.3 nm, and D_CNFI = 0.9 ± 2 nm, D_CNF2 = 27 ± 3 nm, and D_CNF3 = 11.0 ± 0.9 nm in the Si/CNF sample, which correspond to the empty voids within the CNF network (3D), individual cellulose bundle (2D), and cellulose fibrils (1D), respectively. Similarly, three cylindrical structures are observed for the CNF template on ITO besides the prominent nanostructure of ITO with R_ITO = 50 ± 5 nm and D_ITO = 150 ± 9 nm in the ITO/CNF sample. Apart from the nearly identical 1D and 2D nanostructures of CNFs compared to the Si/CNF sample, the cylindrical and knot–like nanostructure (R_CNFI = 90 ± 13 nm, D_CNFI = 255 ± 20 nm) is identified, which agrees with our AFM analysis. Before annealing, TiO₂ shows a nanostructure with 5.1 nm < R_TNG2 < 7.9 nm and 12.9 nm < D_TNG2 < 17.6 nm. The “R” and “D” values of this nanostructure correspond to individual TiO₂ NGs that build up the conformal TiO₂ layer. In the range of 0.01 nm⁻¹ < q < 0.06 nm⁻¹, a superstructure with 49.5 nm < R_TNG1 < 870 nm is observed.
which corresponds to orderly assembled TiO$_2$ agglomerates, and the size TiO$_2$ agglomerates ($R_{\text{TiNG}1}$) varies significantly among samples with $\delta_T = 60$ nm and $\delta_T = 10$ nm. In the Si/CNF/TiO$_2$10 sample, the TiO$_2$ agglomerates with $R_{\text{TiNG}1} = 55 \pm 6$ nm in a distance of $D_{\text{TiNG}1} = 340 \pm 10$ nm and individual TiO$_2$ NPs with $R_{\text{TiNG}2} = 6.5 \pm 1.1$ nm in a distance of $D_{\text{TiNG}2} = 16 \pm 2$ nm are identified. In the Si/CNF/TiO$_2$60 sample, the agglomerates show $R_{\text{TiNG}1} = 66 \pm 6$ nm in a distance of $D_{\text{TiNG}1} = 340 \pm 15$ nm, and individual TiO$_2$ NPs show $R_{\text{TiNG}2} = 6.8 \pm 0.9$ nm in a distance of $D_{\text{TiNG}2} = 15.5 \pm 1.5$ nm (Figure 4d,e). The TiO$_2$ agglomerates in the Si/CNF/TiO$_2$60 sample display larger radii ($R_{\text{TiNG}1} = 66 \pm 6$ nm) compared to the Si/CNF/TiO$_2$10 sample ($R_{\text{TiNG}1} = 55 \pm 6$ nm), indicating that the ALD-coated TiO$_2$ NPs gradually assemble into orderly arranged nanostructures horizontally as the layer.

Figure 4. Topographical study of the TiO$_2$/CNF thin films at the nanoscale. 2D GISAXS data of the TiO$_2$/CNF thin films on a) Si and b) ITO substrates, respectively. Horizontal line-cuts of the 2D GISAXS data of the c) Si- and f) ITO-based samples, respectively. Details about the line-cut region are shown in Figure S16, Supporting Information. The green dashed lines present the best fit to the data. d,e,g,h) Extracted structure parameters for CNFs, TiO$_2$, and ITO (in [g] and [h] only). The fit method for the representative Si/CNF sample is shown in Figure S17, Supporting Information. d,g) Center-to-center distances ($D$) and e,h) radii ($R$) of the ITO, CNF, and TiO$_2$ nanostructures. The following symbols are used to represent the ITO, CNF, and TiO$_2$ nanostructures in TiO$_2$/CNF thin films for simplicity. “wo/a” and “wa” represent the CNF nanostructures before and after annealing. “10” and “10a” represent TiO$_2$ nanostructures in samples ($\delta_T = 10$ nm) before and after annealing, which correspond to the “Si or ITO/CNF/TiO$_2$10” and “Si or ITO/CNF/TiO$_2$10a” samples. “60” and “60a” represent TiO$_2$ nanostructures in samples ($\delta_T = 60$ nm) before and after annealing, corresponding to the “Si or ITO/CNF/TiO$_2$60” and “Si or ITO/CNF/TiO$_2$60a” samples.
thickness increases on Si. The ITO-based ITO/CNF/TiO210 and ITO/CNF/TiO260 samples show similar parameters compared to the Si/CNF/TiO210 and Si/CNF/TiO260 samples (Table S5 and S6, Supporting Information), with the radii of TiO2 agglomerates being R_{TiNG1} = 60 ± 7 nm and R_{TiNG1} = 75 ± 12 nm, as well as the radii of TiO2 NPs being R_{TiNP1} = 6.2 ± 1.1 nm and R_{TiNP1} = 70 ± 0.5 nm, respectively. Besides, the same center-to-center distance of D_{TiNG1} = 350 ± 32 nm and D_{TiNG1} = 150 ± 21 nm between the ITO/CNF/TiO210 and ITO/CNF/TiO260 samples are detected (Figure 4g,h).

As observed from the AFM and GIWAXS analysis, the TiO2/CNF thin films undergo substantial structural changes, including the partial carbonization of the CNFs, and the crystallization of TiO2 after annealing. For CNFs, the individual cellulose fibrils (R_{CNF1} and D_{CNF1}) in both Si- and ITO-based samples are preserved. The cellulose bundles with R_{CNF2} = 78 ± 2.5 nm and D_{CNF2} = 75 ± 3 nm which are hardly visible in the GISAXS data of the ALD-coated samples (Si/CNF/TiO210, Si/CNF/TiO210, and ITO/CNF/TiO260), are clearly identified in the Si/CNF/TiO210a, ITO/CNF/TiO210a, and ITO/CNF/TiO260a (Figure 3c,f and Figure S19, Supporting Information). Besides, despite the identical D_{CNF2} of cellulose between the annealed samples (Si/CNF/TiO210a, Si/CNF/TiO210a, ITO/CNF/TiO210a, and ITO/CNF/TiO260a) and their corresponding CNF templates (Si/CNF and ITO/CNF), the radii of these nanostructures drop to R_{CNF2} = 78 ± 2.5 nm compared to the CNF template (R_{CNF2} = 11.0 ± 1.9 nm). This reflects the increase of the electron density contrast due to the tubular structure of the partially carbonized cellulose bundles or the decrease in the radii of cellulose bundles (Figure S20, Supporting Information). At a higher order, 3D CNF nanostructures’ preservation varies between Si- and ITO-based samples. The empty voids of the 3D CNF network in Si-based samples (Si/CNF/TiO210a and Si/CNF/TiO260a) disappear after annealing (seen as R_{CNF1} = 27 ± 3 nm, D_{CNF1} = 73 ± 2 nm in Figure 4e), which might be the result of the collapse of the 3D CNF architecture during the intensive crystallization of TiO2. This agrees with the increased RMS roughness after thermal annealing in the Si-based samples from the AFM analysis. However, in ITO-based samples, the 3D nanostructure of CNF knots is preserved, with R_{CNF1} = 85 ± 10 nm and D_{CNF1} = 123 ± 5 nm. For TiO2-related nanostructures, the radii of individual TiO2 NPs on Si-based samples (Si/CNF/TiO210a and Si/CNF/TiO260a) slightly increase to R_{TiNP1} = 8.1 ± 1.3 nm and R_{TiNP1} = 8.3 ± 2.0 nm with the center-to-center distance being constant of D_{TiNP1} = 18.5 ± 3.3 nm and D_{TiNP1} = 18.0 ± 2.5 nm, signifying the formation of anatase TiO2 NPs with larger crystallite sizes after annealing. The fitted “R” and “D” values of rutile TiO2 crystallites are nearly consistent between the Si/CNF/TiO210a and Si/CNF/TiO260a samples, which are R_{TiNP2} = 16.5 ± 2.9 nm with D_{TiNP2} = 39 ± 5 nm, and R_{TiNP2} = 16.9 ± 2.0 nm and D_{TiNP2} = 36 ± 5 nm. This can be rationalized with the DnP analysis from GIWAXS measurement, which suggests the diameter of rutile TiO2 crystallites in the range of 30.7 nm < DnP < 36.8 nm. Besides, the agglomerates of rutile TiO2 are identified, with R_{TiNP1} = 95 ± 5 nm, D_{TiNP1} = 180 ± 8 nm in the Si/CNF/TiO210a, and R_{TiNP1} = 105 ± 4 nm, D_{TiNP1} = 200 ± 10 nm in the Si/CNF/TiO260a samples, which corroborates the line-cut results from the AFM analysis. Similar to the Si-based samples, two rutile TiO2 nanostructures and one anatase TiO2 nanostructure are observed in ITO-based samples, with the “terrace” of rutile TiO2 composed of densely clustered rutile TiO2 crystallites. In the ITO/CNF/TiO210a sample, the anatase TiO2 NPs show R_{TiNP3} = 8.3 ± 2.1 nm and D_{TiNP3} = 19.6 ± 4.2 nm, while the rutile TiO2 NPs display a radius of R_{TiNP2} = 170 ± 2.5 nm with a distance of D_{TiNP2} = 39 ± 3 nm, and R_{TiNP1} = 113 ± 5 nm with a distance of D_{TiNP1} = 275 ± 25 nm (Figure 4g,h). Similarly, the ITO/CNF/TiO260a sample contains anatase TiO2 NPs with R_{TiNP3} = 8.4 ± 1.7 nm and D_{TiNP3} = 19.0 ± 3.2 nm, as well as two rutile TiO2 nanostructures with R_{TiNP2} = 176 ± 2.0 nm and D_{TiNP2} = 37 ± 3 nm, as well as R_{TiNP1} = 125 ± 15 nm and D_{TiNP1} = 255 ± 25 nm. The sizes and distributions of rutile TiO2 crystallites confirm the DnP results from GIWAXS measurements.

2.4. Model of Evolution in Morphology and Crystallinity

At the same annealing conditions, the morphological and crystalline parameters of TiO2 are dominated by the ALD coating conditions and the template morphology of the CNFs (Figure 5). The structural evolution of TiO2 is affected by the structural replication of the CNFs at three different scales. First, as indicated by the AFM and SEM analysis, the TiO2 layer replicates the morphology of the CNF layer in both the Si- and ITO-based samples due to the conformity of ALD coating.

Second, the replication of individual cellulose bundles depends on the interaction between TiO2 and CNF nanostructures, which could be influenced by the morphology of CNF and the crystallization of TiO2. The morphology of the CNF template is significantly dominated by the 3D architectures of CNFs, which includes the empty voids of the CNF network in Si-based samples, and the knots in ITO-based samples. The empty voids in the CNF template on Si disappeared after annealing, while the knots on ITO are preserved. The ratio of the removed 3D architectures in the CNF template is estimated to be higher on Si than on ITO substrates. This is due to the weaker support of Si compared to the knot structure supported by ITO particles.

For the evolution of TiO2 crystallites, the replication of the individual cellulose bundles depends on the size of TiO2 NPs, with the small anatase TiO2 NPs in Si-based samples displaying a better replication of these bundles compared to the large rutile TiO2 NPs in ITO-based samples, in which the size of rutile TiO2 is almost two times of anatase TiO2. The rutile TiO2 (R_{TiNP2} and D_{TiNP2}) on ITO grows into large and coalescent nanostructures, which limit their horizontal growth. This results in a terrace morphology due to its higher specific area to create more nucleation sites at the anatase/rutile interface in ITO-based samples. In Si-based samples, the lower rutile-to-anatase ratio of TiO2 crystalline phases, and the smaller diameter of anatase TiO2 compared to the distance between cellulose bundles lead to a better replication of these bundles. However, in ITO-based samples, the higher specific surface area of the ITO/CNF sample leads to a larger rutile-to-anatase ratio, and the larger diameter of rutile TiO2 crystallites compared to the distance between cellulose bundles weakens the interaction.
between TiO\textsubscript{2} and CNFs. This results in a poor replication of the individual cellulose bundles. While AFM techniques focus locally on the surface morphology, GISAXS provides statistical information of the complete film. The variation in the size and distance of the nanostructures extracted from the PSD analysis of AFM images and GISAXS fit results are interpreted as a gradient distribution of the TiO\textsubscript{2} nanostructures along the thickness direction of the film, which shows larger radii on the film surface than the ones underneath (Table S7, Supporting Information).

Third, the replication of the topography of cellulose bundles is preserved in spite of the TiO\textsubscript{2} crystallization and the template morphology. The GISAXS and GIWAXS results reveal the partially carbonized cellulose bundles with a smaller radius underlying the TiO\textsubscript{2} layer compared to the pristine bundles, which is undetectable from AFM analysis. The results from the above measurements are in good agreement and are sufficient to study the size and distribution of the TiO\textsubscript{2} and CNF nanostructures. Therefore, TiO\textsubscript{2} polymorphs with desired crystallite size and rutile-to-anatase ratio are obtained by controlling the template morphology of the CNF layer and the deposition thickness of TiO\textsubscript{2}. Besides, although the morphology of cellulose bundles is replicated at different degrees due to the crystallization of TiO\textsubscript{2}, the topography of cellulose bundles at the TiO\textsubscript{2}/CNF interface is preserved during annealing. The layer replication of TiO\textsubscript{2} layer on both Si and ITO substrates demonstrates the ability of CNFs as a robust and precise structure-directing template.

### 2.5. Surface-Enhanced Raman Scattering Performance

Different from the noble metal nanomaterials, the SERS performance of SMONs depends significantly on the combination of morphological and crystalline properties including the shape, size, ordering, and crystallinity of the SMON nanostructures.\(^{[16]}\) Therefore, the influence of these parameters on the SERS performance of the TiO\textsubscript{2}/CNF thin films are investigated by using 4-mercaptobenzoic acid (4-MBA) and crystal violet (CV) as the standard probes. The SERS spectra

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**Figure 5.** Schematic representation of the morphology and crystallinity evolution of TiO\textsubscript{2} and CNF nanostructures on Si (top) and ITO (bottom) substrates, respectively.
with continuously varying concentrations of 4-MBA (from $1 \times 10^{-4}$ to $1 \times 10^{-8}$ m) on the TiO$_2$/CNF thin films are provided in Figure 6a and Figure S21, Supporting Information. The marked peak positions are consistent with the characteristic SERS signals of 4-MBA molecules.\cite{10,53} The strong bands at 1594 and 1078 cm$^{-1}$ are assigned to aromatic ring vibrations, and the weak bands at 1148 and 1182 cm$^{-1}$ are attributed to the C$=\text{H}$ deformation modes (Table S9, Supporting Information). The SERS spectra of 4-MBA adsorbed on TiO$_2$/CNF thin films are enhanced\cite{12} and is different from the typical Raman spectra of 4-MBA ethanol solution in both the intensity and position of the peaks.\cite{54} Furthermore, the selective enhancement of the 1148 cm$^{-1}$ peak suggests the CT from TiO$_2$ to 4-MBA molecules.\cite{55} Additionally, the narrow 1078 cm$^{-1}$ peak of 4-MBA overlaps with the broad peak characteristic of CNF (1097 cm$^{-1}$). Thus, the Raman spectra of CNFs are deducted in the following analysis of the EF.

It can be observed from Figure 6b that 4-MBA molecules exhibit a higher enhancement at the 1594 and 1078 cm$^{-1}$ bands when adsorbed on the annealed ITO/CNF/TiO$_2$10a and ITO/CNF/TiO$_2$60a samples than on their pristine ITO/CNF/TiO$_2$10 and ITO/CNF/TiO$_2$60 samples. The highest SERS enhancement is received on the ITO/CNF/TiO$_2$10a sample at the Raman shift of 1078 cm$^{-1}$, in which the concentration of 4-MBA as low as (1 $\times$ $10^{-8}$ m) can still be clearly distinguished (Figure 6a). Figure S22a, Supporting Information, suggests a linear relationship between the peak intensity and the concentration of 4-MBA, demonstrating its potential application for quick analysis. To quantitatively analyze the SERS performance of TiO$_2$/CNF thin films, the EFs at the 1078 and 1594 cm$^{-1}$ bands of 4-MBA at the concentration of $10^{-5}$ m were calculated. According to the standard equation,\cite{56} the ITO/CNF/TiO$_2$10a sample shows an EF value of 1.79 $\times$ 10$^6$ at 1078 cm$^{-1}$ (Figure 6e), which indicates an outstanding sensitivity among plasmon-free TiO$_2$-based SERS substrates (Table S9, Supporting Information). The high EF achieved by TiO$_2$/CNF is even comparable to some noble metal-based SERS substrates that typically show much higher EF than TiO$_2$-based SERS substrates.\cite{57} In contrast, the EF value of the Si/CNF/TiO$_2$10 sample is estimated to be 5.25 $\times$ 10$^5$ under the same conditions. Additionally, after storing in a sealed sample box at ambient condition for 45 days, the SERS spectra of the ITO/CNF/TiO$_2$10a sample shows a substantial similarity to that of the freshly fabricated samples (Figure 6f). These results demonstrate excellent stability of TiO$_2$/CNF thin films as a practical SERS substrate, which could originate from the support of CNF matrix to the polymorphous TiO$_2$ NPs.

Similar results of TiO$_2$/CNF thin films are obtained by using CV as the analyte (Figure 6c and Figure S23, Supporting Information). In Figure 6d, the SERS intensities of the C$=$C stretching band at 915 cm$^{-1}$, in-plane C$=$H stretching band at 1180 cm$^{-1}$, and C$=$C stretching band at 1623 cm$^{-1}$ in the ITO/CNF/TiO$_2$10a sample at $10^{-5}$ m of 4-MBA shows a substantial similarity to that of the freshly fabricated samples (Figure 6f). These results demonstrate excellent stability of TiO$_2$/CNF thin films as a practical SERS substrate, which could originate from the support of CNF matrix to the polymorphous TiO$_2$ NPs.
The lowest detection limit of the ITO/CNF/TiO$_2$10a sample is at $10^{-7}$ m level, which is slightly lower than the sensitivity with 4-MBA as the analyte. This could be related to a stronger interaction between TiO$_2$ and 4-MBA than that between TiO$_2$ and CV molecules. The superior SERS performance of the ITO/CNF/TiO$_2$10a sample can also be observed by comparing the EF values calculated from the three characteristic bands of CV. The EF values measured at 10$^{-5}$ m CV in ITO-based samples follow the identical trend as the one of 4-MBA: ITO/CNF/TiO$_2$10a $>$ ITO/CNF/TiO$_2$60a $>$ ITO/CNF/TiO$_2$10 $>$ ITO/CNF/TiO$_2$60 (Figure 6d). For comparison, the EF values of plasmon-free TiO$_2$ SERS substrates previously reported are shown in Table S9, Supporting Information.

The SERS enhancement of TiO$_2$/CNF thin films is proposed to stem from the CT of the TiO$_2$ NPs assisted by multiple light scattering at the film surface. To further understand the variation of the CT efficiency between the ALD-coated and thermally annealed samples, we measured the sheet resistance of the TiO$_2$/CNF thin films. The sheet resistance of the ALD-coated Si/CNF/TiO$_2$10, Si/CNF/TiO$_2$60, ITO/CNF/TiO$_2$10, and ITO/CNF/TiO$_2$60 samples are $R_s = 21.6 \pm 0.04$ $\Omega$ $\text{sqr}^{-1}$, $R_s = 16.6 \pm 0.05$ $\Omega$ $\text{sqr}^{-1}$, $R_s = 19.5 \pm 0.04$ $\Omega$ $\text{sqr}^{-1}$, and $R_s = 15.1 \pm 0.05$ $\Omega$ $\text{sqr}^{-1}$. After thermal annealing, the sheet resistance decreased to $R_s = 7.1 \pm 0.06$ $\Omega$ $\text{sqr}^{-1}$, $R_s = 6.9 \pm 0.05$ $\Omega$ $\text{sqr}^{-1}$, $R_s = 6.7 \pm 0.04$ $\Omega$ $\text{sqr}^{-1}$, and $R_s = 6.6 \pm 0.04$ $\Omega$ $\text{sqr}^{-1}$ in the Si/CNF/TiO$_2$10a, Si/CNF/TiO$_2$60a, ITO/CNF/TiO$_2$10a, and ITO/CNF/TiO$_2$60a samples, respectively (Figure S24, Supporting Information). The decrease in sheet resistance is attributed to a more favorable crystallinity state which enables higher electron mobility,[56] and the generation of free electrons to improve the carrier concentrations for SERS enhancement. The relatively lower sheet resistance of the ITO/CNF/TiO$_2$10a and ITO/CNF/TiO$_2$60a compared to the Si/CNF/TiO$_2$10a and Si/CNF/TiO$_2$60a samples could be related to the higher crystallinity (larger crystallite size) and the higher rutile-to-anatase ratio of TiO$_2$.

To summarize, the SERS activity of the TiO$_2$/CNF thin films depends on the layer morphology, topography, and the crystalline state of TiO$_2$. The superior SERS performance of the ITO/CNF/TiO$_2$10a sample originates from the CT resonance based on the combination of appropriate crystallite size of anatase TiO$_2$ and an appropriate rutile-to-anatase ratio, which is further amplified by a relatively high degree of ordering at the film surface. The crystalline property of TiO$_2$ is the most crucial factor in the SERS performance of TiO$_2$/CNF thin films. The small crystallite size of anatase TiO$_2$ in the ALD-coated ITO/CNF/TiO$_2$10 sample is less favorable for SERS enhancement of the adsorbed molecules than the thermally annealed ITO/CNF/TiO$_2$10a sample. Besides, one reason for the higher SERS performance of the ITO/CNF/TiO$_2$10a than the Si/CNF/TiO$_2$10a sample is related to its higher rutile-to-anatase ratio (Figure 3e). The synergistic effect between rutile and anatase phases of TiO$_2$ provides an additional CT from rutile to anatase TiO$_2$ besides the intrinsic CT from anatase TiO$_2$ to the probe molecules, which is responsible for the higher SERS enhancement in the polymorphous than monophased TiO$_2$-based substrates. However, an overly rutile-to-anatase ratio is unfavorable for improving the CT resonance in polymorph TiO$_2$ due to the lower SERS activity of rutile compared to anatase phase. Therefore, the ITO/CNF/TiO$_2$60a sample is less sensitive in the analyte’s SERS enhancement than the ITO/CNF/TiO$_2$10a sample.

Template morphology is another essential factor in the SERS performance of TiO$_2$/CNF thin films in amplifying the surface resonance of Raman signals,[68] and in providing access to high specific surface area. On the one hand, the regularity of the TiO$_2$ nanostructures with the support of CNF knots leads to a higher SERS activity in ITO- than Si-based samples by multiple light scattering. This is another reason for the higher SERS enhancement of the ITO/CNF/TiO$_2$10a than the Si/CNF/TiO$_2$10a sample. On the other hand, the ITO-based samples generally show a larger specific area than the Si-based samples. The difference in specific surface area is enlarged by the crystallization of TiO$_2$, in which the samples with larger specific surface area create more anatase-to-rutile nucleation sites, and thus a higher crystallite size of TiO$_2$ is obtained (Figure 3d). Nonetheless, the effect of template morphology appeared to be less critical than the crystalline state of TiO$_2$. Besides, the ITO/CNF/TiO$_2$10 sample shows a higher SERS activity than the ITO/CNF/TiO$_2$60 sample, demonstrating the vital role of the specific surface area regardless of the crystalline state and template morphology.

3. Conclusion
This study demonstrates a new strategy to fabricate biotemplated, well-ordered, and polymorphous TiO$_2$/CNF thin films with significantly enhanced SERS sensitivity through the combined action of 1) OIS to prepare a highly ordered CNF template, 2) ALD to transfer the template morphology into the TiO$_2$ layer, and 3) thermal annealing to obtain a desired crystalline state. The template morphology shows a significant influence on the order and morphology of the resulting TiO$_2$ layer. Thermal annealing exhibits a profound effect on the topography and crystalline state of TiO$_2$ and CNFs. An improved templating effect of CNFs is observed after annealing, with a higher RMS roughness due to the collapse of the empty voids in the 3D CNF network. And the partially carbonized cellulose bundles of the remaining CNF network contribute to the higher electron density of the polymorphous TiO$_2$ NPs. Collectively, the TiO$_2$/CNF thin film with a TiO$_2$ layer thickness of 10 nm on ITO substrate shows an excellent SERS performance with an EF value of $1.79 \times 10^6$, which is higher than the average EF for TiO$_2$-based, plasmon-free SERS substrates via CT mechanism. This is achieved by the appropriate rutile-to-anatase ratio and crystallite size of anatase TiO$_2$, accompanied by the high ordering degree of the TiO$_2$ layer. These results demonstrate the important roles of the layer morphology, topography, and crystallinity of TiO$_2$ in the SERS performance of CNF-templated TiO$_2$ thin films. A high SERS sensitivity is achieved at the orderly assembled and polymorphous TiO$_2$ layer, which opens the possibility of fabricating SMONs with tailored SERS sensitivity at an industrial scale. For future studies, we believe that the method of CNF-directed assembly of TiO$_2$ layer could be applied to fabricate a wider range of semiconductor metal oxide films, and the templating role of CNFs could be extended from the bottom to the top layer in a multiple-layered thin film via LBL deposition.
4. Experimental Section

Chemicals and Materials: 0.07 wt% aqueous solution of 2,2,6,6-tetramethylpiperidine-1-oxyl radical-CNF with a surface charge of 800 μmol g⁻¹ and a radius of around 2.5 nm was dispersed by sonication and centrifugation to prepare CNF thin films. Si wafers (Silicon Materials, USA) and ITO glass slides (SOLEMS, France) with the same surface area of (25 × 25 mm²) were used as substrates. Si wafers were sonicated with acetone for 15 min and then sequentially cleaned with isopropanol and deionized water, followed by an acid bath (87.5 mL hydrogen peroxide 30%, 190 mL 96% sulfuric acid, and 37.5 mL deionized water) at 80 °C for 15 min. ITO glass slides were cleaned with deionized water and then sequentially treated with alcohol, acetone, and isopropanol for 10 min. All solvents were purchased from Carl Roth GmbH, Germany. Deionized water was used throughout the experiment.

Oblique Incidence Spraying: LBL spray-coating was performed by a spray-coating device (Compact JAU D555000, Spray Systems, Germany). In the authors’ previous work, a standard deposition procedure for spray-coating of uniform CNF thin films with a thickness of 200 nm was developed. Herein, in order to fabricate a CNF thin film with well-defined orientation, the standard spray-coating procedure was slightly modified. The substrate was placed at an oblique angle of 10° with respect to the spray-coating direction. The distance between the nozzle and the substrate was kept at 100 mm. The preparation was conducted at room temperature (T = 23 °C, RH = 50%). For each spray pulse, the deposition condition was 0.1 s spraying and the waiting time was elongated to 45 s for complete evaporation of the solvent. To minimize the film inhomogeneity resulting from the spray-cone, the substrate was rotated at 90° after each spray pulse. Therefore, after four spray pulses, the substrate was rotated back to the starting direction, which the authors’ noted as a spray cycle. The spray cycles were repeated 40 times to prepare a CNF thin film with a thickness of 200 nm.

Atomic Layer Deposition and Thermal Annealing: The ALD of TiO₂ layer was conducted at the GSI Helmholtzzentrum für Schwerionenforschung, Darmstadt, Germany with a Picosun R200 Basic ALD device. The ALD of the TiO₂ layer was performed at 110 °C, and the tetraisopropoxide precursor was preheated to 70 °C in the reservoir. The procedures were set as follows: a) A 5.2 s dose of titanium tetraisopropoxide (TTIP) with 60 standard cubic centimeter per minute (sccm) N₂ carrier gas flow; b) a 0.5 s purge of oversupplied TTIP with 120 sccm N₂; c) a 0.4 s dose of H₂O with 200 sccm N₂ as carrier gas flow; d) a 50 s purge of oversupplied H₂O and by-products. The four-step sequence constitutes one ALD cycle and the number of ALD cycles were adjusted for obtaining TiO₂ layer with a desired thickness. For the TiO₂ layer, with Δh = 10 nm, the number of cycles was 909, and the uncertainty of the layer thickness was ±0.19 nm; and for the TiO₂ layer with Δh = 60 nm, the number of cycles was 3454, and the uncertainty of the layer thickness was ±1.09 nm. The as-prepared TiO₂/CNF thin films were thermally annealed in a furnace (FP1100, Borel Swiss) at 450 °C for 30 min. The annealing condition was chosen based on literature to produce a mixed-phase TiO₂ layer with the desired rutile-to-anatase ratio.

Morphology Characterization (Atomic Force Microscopy and Scanning Electron Microscopy): The AFM images were acquired with an NTegra probe Nano-Laboratory (NT-MDT, Russia) in the semi-contact mode. ETALON cantilevers with a tip radius of 10 nm and a resonant frequency of 90 kHz (NT-MDT, Russia) were used for all samples. For each sample, five pictures of 2D topography maps were taken with a scan size of 3 x 3 and 1 x 1 μm². AFM data analysis, including line-cuts, and average roughness was done by WSXM v5.0 software. For the cross-sectional analysis, the samples were frozen in liquid nitrogen to avoid delamination of the film upon fracture. SEM images were taken with a Zeiss Gemini NVision 40 field emission microscope operated at 5 kV and a working distance of around 5.6 mm.

Grazing-Incidence X-Ray Scattering: Since the as-prepared TiO₂/CNF samples were thin films, grazing-incidence X-ray scattering techniques were used to study the structural information at the TiO₂/CNF interface. The in-depth morphology and crystallinity of the samples were investigated with GISAXS and GIWAXS, respectively. The X-ray scattering experiments were performed at the P03 beamline of the PETRA III storage ring at DESY, Hamburg.[59] The energy of the X-ray was 11.8 keV (λ = 1.05 Å) with a beam size of 80 × 56 μm² (horizontal × vertical). One beam stop was used to shield the specular reflected beam. A sketch of the GISAXS and GIWAXS setup is shown in Figure S9, Supporting Information, with αi and αf representing the incident and exit angles, respectively. The sample-to-detector distance (SDD) was kept at SDDGISAXS = 3930 ± 2 mm in GISAXS and SDDGIWAXS = 312 ± 2 mm in GIWAXS measurements. 2D GISAXS data were collected by PILATUS 300k detector (Dectris Ltd., Switzerland) with a pixel size of 172 × 172 μm² and GIWAXS data were collected by LAMDBA 4.5M detector (X-spectrum GmbH, Germany) with a pixel size of 55 × 55 μm². The incident angle αi was set at 0.4° for GISAXS, and 0.11° for GIWAXS measurements. A lateral scanning with a step size of 100 μm by moving the sample through the beam in the lateral direction was performed to avoid X-ray beam induced alteration of the samples. Each GISAXS pattern was acquired for 1 s, and each GIWAXS pattern was acquired for 0.1 s. 1D intensity distributions were extracted from the 2D GISAXS data using the Directly Programmable Data Analysis Kit software (DPDAK) v1.4[49] and fitted by minimum chi-square estimation with cumulative cylindrical and spherical form factors including 1D structure factors as previously reported by Schaffer et al.[51] For GIWAXS, the q₁, q₂, plots were transformed by Grazing-incidence X-ray Scattering Graphical User Interface software (GIXSGUI) v1.7.135. Peak positions and intensity of the peak in the q₁, q₂ plots were extracted to provide information about crystal structure, degree of crystallinity, and the rutile-to-anatase ratio in the TiO₂/CNF thin films. The rutile-to-anatase ratio (δR/AR) was calculated based on a formula reported by Khatun et al.[46]

\[ \delta_R = \left( I_{101} - I_{110} \right) / \left( I_{101} + I_{110} \right) \] (1)

where \( I_{101} \) and \( I_{110} \) are the intensities of the (110) and (101) diffraction peaks of rutile and anatase TiO₂ in the 1D GISAXS data, respectively.

Crystallite domain size (DnP) of TiO₂ was calculated with an adapted form of the Debye–Scherrer formula,[60]

\[ D_{nP} = \frac{2πK}{β_{3/2}} \] (2)

where \( β_{3/2} \) was the FWHM of the (101) or (110) peaks, and K the particle shape factor, which was taken as 0.94 valid for spherical crystallites with cubic symmetry.

Conductivity Measurement: The measurements were performed using a four-point setup T2001A (Ossila Ltd., UK) and the raw data were analyzed using Ossila Sheet Resistance (v2.0.3.3, Ossila Ltd, UK) software. The probes were spring-loaded and separated by 1.27 mm, the set target current was 10 mA, and the voltage increment was 0.1 V. Sheet resistance measurements were performed 25 times and 2048 samples per point were measured. The sample size for conductivity measurements was 20 × 80 mm².

Surface-Enhanced Raman Scattering and Raman Spectroscopy: The samples were cut into 4 × 4 mm² for SERS measurement. Aqueous solution of CO₂, and ethanol solution of 4-MBA were diluted to desired concentrations as analytes for SERS (10⁻₅–10⁻₄ m) and Raman (10⁻¹⁰–5⁻¹⁰ m) analysis. To make the surface of the substrates covered with a monolayer of CO₂ and 4-MBA sufficiently, 3 μL of the analyte solutions were directly dropped on the surface of the TiO₂/CNF thin films and their corresponding templates and substrates. For Raman analysis, 3 μL of the solution of analyte was dropped onto the Si and ITO substrates, respectively. After the evaporation of water or ethanol, Raman signals were collected on the surface of these samples. SERS spectra were collected on a Raman spectrometer (SENTERRA, Bruker GmbH) with an excitation wavelength of 532 nm at a laser power of 4 mW and an objective of 50× magnification. The accumulation time was 20 s and the time for data acquiring was 4 s. Raman spectra were collected and averaged collected from five different points. Raman data was analyzed by OPUS
where $I_0$, $c_0$, and $N_0$ were the intensities of the characteristic vibration bands, the concentration, and the corresponding number of molecules in Raman spectra. $N_{\text{SERS}}$, $c_{\text{SERS}}$, and $N_{\text{SERS}}$ were the intensities of the characteristic vibration bands, the concentration, and the corresponding number of molecules in SERS spectra. $h$, $S$, $S'$, and $N_0$ were the focused depth, the area of the laser spot, the area of the TiO$_2$-containing substrate, the volume of analyte, and Avogadro constant.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

**Acknowledgements**

Q.C., S.V.R., and M.M. acknowledge the financial support from the Helmholtz-OCPC program (No. 2019T004). Q.C. and S.V.R. thank Ilya Sergeev, the responsible scientist at P01 of PETRA III, for giving them access to Raman spectroscopy. S.V.R. acknowledges the kind financial support from the DESY strategic fund (DSF) “Investigation of processes for spraying and spray-coating of hybrid cellulose-based nanostructures”. M.M.B. acknowledges the financial support from the DSF “Investigation of processes for fabricating functional multicomponent stacks based on nanopaper and spray-coating”. V.K. and P.M.-B. acknowledge funding from TUM.solar in the context of the Bavarian Collaborative Research Project Solar Technologies Go Hybrid (SolTech), the Deutsche Forschungsgemeinschaft (DFG) under Germany’s Excellence Strategy-EXC 2089/1-390776260 (e-conversion), and the International Graduate School for Environmentally Responsible Research Training Group 2022 Alberta/Technical University of Munich (ATUMS). N.M.U. acknowledges the support from the HGS-HIRe Graduate School. All GISAXS and GIWAXS measurements were carried out at the light source PETRA III at DESY; a member of the Helmholtz Association (HGf).

**Conflict of Interest**

The authors declare no conflict of interest.

**Data Availability Statement**

Research data are not shared.

**Keywords**

atomic layer deposition, biotemplates, semiconductor metal oxide nanomaterials, surface-enhanced Raman scattering, X-ray scattering.
