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Rapid interferometric imaging of printed drug laden multilayer structures

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The developments in printing technologies allow fabrication of micron-size nano-layered delivery systems to personal specifications. In this study we fabricated layered polymer structures for drug-delivery into a microfluidic channel and aimed to interferometrically assure their topography and adherence to each other. We present a scanning white light interferometer (SWLI) method for quantitative assurance of the topography of the embedded structure. We determined rapidly in non-destructive manner the thickness and roughness of the structures and whether the printed layers containing polymers or/and active pharmaceutical ingredients (API) adhere to each other. This is crucial in order to have predetermined drug release profiles. We also demonstrate non-invasive measurement of a polymer structure in a microfluidic channel. It shown that traceable interferometric 3D microscopy is a viable technique for detailed structural quality assurance of layered drug-delivery systems. The approach can have impact and find use in a much broader setting within and outside life sciences.
ionizing radiation. None of these methods fulfill the requirements offers sufficient precision but is impractical since it is slow and uses along the z-direction. Nano-scale x-ray tomography potentially tures but does not provide sufficient quantitative spatial precision of layered structures, the adherence between the layers, and the possibility to determine surface roughness, the geometric thickness of layered structures, the adherence between the layers, and the thickness of each individual layer. Here we report the first implementa
tion of scanning white light interferometry to study these features of layered drug-delivery systems.

Results
Our custom-built SWLI optically compensates for the thickness of the roof in the microfluidic channel (Fig. 1) and measures the effective refractive index of the drug-laden structure (Fig. 2). The SWLI method provides 10–20 nm traceable resolution along the z-dimension. This potentially sterile technique requires no sample preparation. Using image stitching it allows, when necessary, high resolution also across a large surface (up to 200×). Moreover, it can determine surface roughness Sq (ISO 25178) for an embedded surface without direct tactile access. It can measure stacked layers that are static or moving. SWLI measures the optical path difference between the reference and sample arm of the interferometer. When the sample is in air and we measure the step height - the single layer thickness – there are two interferences one for the base surface (Point A1, Fig. 1) and one for the top surface of the layer (Point B2, Fig. 1). The distance between these two interferences is equal to the layer thickness directly. When we measure through a well bonded layer there are also two interferences, but the position of the second one is shifted (Point B3, Fig. 1), compared to the interference at the base surface (Point A1, Fig. 1) because the optical thickness is equal to the geometrical thickness multiplied by the refractive index of the layer. The shift of the second interference depends of the refractive index of the layer (case B in the figure). The ratio between geometrical and optical thicknesses is equal to the refractive index of the layer. In the case of an unbonded or missing part of the layer a third interference appears between the first and second one (case C in the figure) and the shift of the last interferogram (Point C6, Fig. 1) depends of the thickness of the nonbonded layer.

To demonstrate the capacity of our proposed method a multi-layer drug-delivery system containing the vitamin B2, riboflavine sodium phosphate (RSP), is shown in Fig. 3a. The trans-
Figure 2 | HPC-Theophylline laden layer on top of a HMPC layer residing on PET. Even without knowing the refractive index of the HPC-Theophylline layer the thickness can be determined (5 μm on the left 10 μm on the right). Sq on the indicated area is 0.157 μm. (Left) Measured interferences. (Inserts top right) Schematic of the samples.

Figure 3 | (a). Multilayer drug delivery system in the form of a crossing strip of printed riboflavine sodium phosphate (RSP) layer between two layers of hydroxypropyl methylcellulose (HPMC) film reconstructed from a SWLI measurement. (b). Structure of hydroxypropyl–cellulose (HPC) film on a PET surface revealing up to 13 μm tall delamination, 0.31 μm (Sq1) and 0.20 μm (Sq2) surface roughness. (Left) Measured interferences, (Inserts top right) Schematic of the samples. We can determine surface topology with high resolution and we can determine presence of disbonds in multilayer structures.
disruption by the top surface makes it appear rougher than it is). Single camera pixel data (Fig. 3a and 3b, leftmost) and a 3D surface plot of the polymer layer are shown (Fig. 3a and 3b, middle). The images were reconstructed pixel by pixel from the interferogram. The depth coordinate of each layer was extracted using Larkin’s algorithm. Fig. 2 shows how the geometric thickness of a structure can be determined even though its refractive index is not known in advance. The SWLI measures optical thickness $d_{opt}$ and the geometric thickness ($d_g$) of the polymer layer is $A_1 - B_3$ since the refractive index of air $= 1$. The effective refractive index of the adhered layer is $(B_3 - B_4)/(A_1 - B_3)$. The same approach can be used to determine both layer thickness and disbend height. Except for the top layer the depth coordinate was corrected using the refractive index. This index was obtained by relying on a discontinuity in the structure, see Fig. 3a. The interferograms from a point on the top layer and from a corresponding point on the bottom surface on that layer are separated on average by $d_{opt} = 6.23 \, \mu m$ whereas the discontinuity on bottom surface is known to be $2.25 \, \mu m$ tall which indicates an estimated layer thickness of $3.98 \, \mu m$. The height of the disbonds was determined by assuming that the refractive index of the disbond was 1.00.

Figure 4 shows a polymer structure measured inside a microfluidic channel during 8 $\mu l$/(1.8 mm/s) water flow. It took a few minutes to obtain the images in Fig. 3 and 4. These images were automatically analyzed by commercial and custom made software to construct and analyze 3D images of the cross-over point of the two crossing strips. The standard uncertainty for the $z$-coordinate in the construct was 0.045 $\mu m$. We assumed the refractive index of water to be 1.33.

Discussion

The proposed method rapidly determined the structure and presence of disbonds in the printed drug-delivery system. There are limitations to the method. A layer separation, of less than $0.5 \, \mu m$ or more than a few millimeters is hard to image. When the number of interfaces exceeds four it is hard to automatically extract the layers. The employed technique to derive the effective refractive index may not always work well. One example is high throughput analysis of systems with different drugs, another example is if the drug concentration exhibits a gradient inside a layer. Finally, the $S_q$ value has not yet been linked in a traceable manner to a common surface roughness index such as $R_q$.

We presented for the first time a practical method for traceable structural quality assurance of printed drug delivery systems. This result opens up a plethora of opportunities to induce quality assured fabrication in the field of tissue engineering, microfluidics, bioMEMS, metamaterials, as well as nano- and micro-sized layered structures in addition to printed pharmaceuticals.

The traceable quantitative method can provide a competitive edge to both research and production carried out in academia and the industry. In practice one can carry out high throughput label-free testing, one can optimize products structurally, and one can assess systems in wet and dry environments. The method has high spatial and temporal precision, and can be applied in a controlled microfluidic environment. It can allow structural and functional quality assurance for a broad range of applications in life sciences.

Methods

Scanning white light interferometry (SWLI). The samples were imaged using a custom-made SWLI instrument, Fig. 2. Briefly, the instrument uses a halogen lamp (Osram G4, driven at 6 Volts, 10 W), a standard 5X Michelson Nikon objective (Nikon CF IC Plan Fl; Japan) or 10X Mirau objective (Nikon CF IC Epi Plan D; Japan), and a piezo translator with 100 $\mu m$ travel (Physik Instrumente - type P-721.CDQ). The system magnification was 3.15 with the Michelson objective and 6.3 with the Mirau objective. The instrument can determine profiles with $\pm 15 \, \mu m$ accuracy along the $z$-direction. A detailed description of the VIS interferometer is given in Ref. 1. In the receiving part we employed a 3.63 $\mu m$ pixel size black and white camera (Hamamatsu C11440 Orca Flash2.8, Hamamatsu City, Japan).

We validated the SWLI for top surface imaging by inspecting a standard sample VLSI 1853 $\pm 2.3 \, \AA$ (model SIS-1800 QC, VLSI Standards, Inc.). Traceability to the national SI standard is ensured using a transfer artifact. The influence of the roof of the channel was removed with a compensating plate identical to the 200 $\mu m$ thick cover glass (CLS2900246, Sigma-Aldrich, Corning) of the microfluidic channel inserted into the reference arm of the interferometer.

Microfluidic channels. The cover glass and 1000 $\mu m$ thick microscope base glass were used (Menzel Microscope slides, ISO 8037/1) to construct the microfluidic channels. Inlet and outlet holes (2 mm in diameter) were drilled in the base glass. Plastic tubing (TubPEEK Blu 1/16 Z226661) was attached by UV cured glue (Thorlabs NOA81). The tubes were sealed to the base glass. A 2 mm wide channel was created using two-sided 50 $\mu m$ thick double-sided tape (Tesa) between the cover and base glass. The sample was placed in the middle of the channel. The cover glass was then attached. Ion-exchanged water was flowed through the channel with a pump (Alladin-1000, World precision Instruments, Astion, Stevenage, UK).

Sample preparation. There were four kinds of samples. The films were created by solvent casting using a manual pipette (BioHit ProLine + Mech, Finland) simulating printing of drug substances and polymers as described in Ref. 1. All samples were dried in ambient conditions (21°C and 40% RH). The aqueous solutions for Figure 3 membranes consisted either of 2 mg/ml riboflavin sodium phosphate (RSP, riboflavin 5’-monophosphate sodium salt, Ph. Eur., Fluka Analytical, Sigma-Aldrich, France) in 1% (w/w) hydroxypropyl methylcellulose (HPMC, Metolose 90SH-4000, Shin-Etsu, Tokyo, Japan) or pure 1% HPMC solution. The sample presented in...
Image reconstruction and processing. We scanned the samples with our custom-made SWLI instrument. Images were acquired with 68.75 nm vertical spacing. At each height we averaged 10 camera frames after which we recorded both the average image and the height coordinate output by the calibrated piezo capacitive sensor.

Surfaces were reconstructed using Larkin’s algorithm on a high-pass-filtered SWLI signal. This modification permitted us to do multi-surface feature extraction. Shortly: the single pixel camera intensity data vector (stacked pixels) was high-pass filtered with a second order derivative filter. The algorithm then identified and stored the maximum of the envelope of the interferences in the filtered vector. The interactive multi-interface extraction process first asked for the maximum number of interfaces to extract, and for the minimum permissible threshold value for envelope maxima and interface separation. For each 3D image the threshold value was found by an iterative procedure. (In Fig. 2 the HPC - HPMC interface was found by pooling 25 pixels prior to extracting envelope maxima. Extracted interfaces were filtered using first a 3 × 3 pixel filter and then a 7 × 7 pixel median filter along the x-y direction. Surface height data was corrected from optical height to geometric height using the method illustrated in Fig. 2. In practice, planes were fitted in least squares sense to the reference surfaces #1 and #3 in the above figure. The distance between these planes was determined analytically. Local surface fitting (second or third order in x and y) was used to patch small areas lacking data. These areas were identified and the size of the patch was decided manually post hoc. The final 3D image was tilt-corrected by requiring that the bottom most interface was horizontal. The Sq parameter was determined from unfiltered surface data as outlined in ISO25178.

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Author contributions

N.S., I.K. and E.H. Conceived the experiments. N.G. prepared the samples, H.E., N.G. and E.H. carried out the experiments. N.S., I.K., H.E. and E.H. analyzed the data, all authors discussed the results and contributed to the manuscript.

Additional information

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