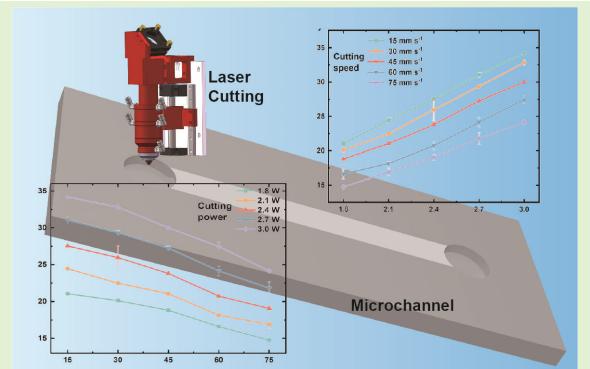


Facile Laser-Cutting Process for Nanocellulose-Paper-Based Microfluidic Microchannel Fabrication

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Abstract—Nanofibrillated cellulose paper (nanopaper) has drawn increasing attention as a potential material for various areas, due to its extremely smooth surface, excellent optical transparency, and sequent nanofiber matrix. To extend nanopaper application as the analytical platform, nanopaper-based microfluidics has quickly advanced recently. However, the current method of patterning microchannels on nanopaper, which is the basic for establishing microfluidic (i.e., 3-D printing and spray coating), still has some limitations, including low precision and long preparation time. So, in this study, we utilized laser cutting to fabricate microchannel patterns on nanopaper by burning the surface of nanopaper. Through systematic parameters (laser-cutting speed and power) optimization, we identified the optimal laser-cutting conditions, enhancing both efficiency and accuracy. The minimum depth and width of the microchannels were reduced to 15 and 58 μm , respectively. The entire fabrication process, including drying, was completed in less than 35 min. Compared to the existing methods, this method has smaller microchannels size, time saving, and no need for additional molds or equipment those advantages, which contribute its novelty and accuracy. By arranging different shapes of lines, microchannels for various sensing were developed. As a proof of concept, we developed two functional nanopaper-based analyzer devices (NanoPADs). With a detection limit of 2.2 mM for glucose and 281 fM for Rhodamine B (RhB), both demonstrate excellent performance and low detection limits. The results indicate that our laser-cutting nanopaper microchannels may serve as a platform for developing high-performance analytical devices, which may spark the development of nanopaper in the future.



Index Terms—Advancements in nanocellulose-paper-based microfluidics, laser cutting, microchannel fabrication.

I. INTRODUCTION

WITH the increasing demand for environmental protection worldwide, materials from plants have been emerging as a popular substrate material for chemical and biochemical analysis [1]. Among them, paper has been extensively studied over the last decade, due to its low

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cost, disposability, and biodegradability [2]. After patterning microchannels on paper, paper-based analytical microfluidic devices (μ PADs) have been quickly advanced in point-of-care detection for various diseases in low-resource settings [3]. Also, many biosensors, including enzyme-linked immunosorbent assay (ELISA) [4], [5], surface-enhanced Raman spectroscopy (SERS) [6], [7], and electrochemical detection [8], [9], have been realized on μ PADs for various biomarkers and biological information. However, μ PADs still suffer from slow flow speeds and solvent evaporation problems, so the novel substrate materials, which contain similar advantages as regular paper and may solve the majority of the above problems, need to be introduced.

In recent times, nanofibrillar cellulose (NFC) paper (nanopaper) has appeared as an ideal matrix material in a variety of evolving fields [10], [11], [12]. As a natural material, nanopaper is not only inexpensive and readily available, but biocompatible, biodegradable, and renewable [13], [14]. Due to the above advantages, nanocellulose has been emerging in wide applications, including supercapacitors [15], energy

devices [16], biomedical and chemical sensors [17], and among others. Microfluidics is a powerful tool that actuates fluids for chemical and biological analysis, such as DNA, cells, and molecules, replicating detection on chip-scale devices. Recently, low-cost and biodegradable substrates have gained interest for microfluidics. Nanopaper also owns its unique advantages for microfluidic substrate material. First, the ultrasmooth surface of nanopaper is ease to generate highly organized and uniform nanostructures on it for fabricating functional nanopaper-based devices [18], [19]. Second, the abundant groups on nanocellulose provide the opportunity for modification for different applications [20]. Third, nanopaper has high optical transparency and a low optical haze and is an excellent substrate material for optical sensors [15]. With the increasing demand for nanopaper microfluidic devices with lower liquid consumption and more sensitive detection, the fabrication of microscale channels has become an important problem. However, how to fabricate high-precision microchannel on nanopaper is the fundamental issue to be solved.

Several methods for fabricating microchannels on nanopaper have been reported. First, a method was reported, in which a skeletonized pattern was first formed on the nanopaper using laser cutting, and then, the skeletonized layer was manually stacked with two other layers of nanopaper to form a microfluidic channel, which solved the problem of the inability of pump-free flow, but it required a lot of manual work [21]. The second, a micropatterned mold is prefabricated using the deep reactive ion etching technique, and then, nanocellulose fibers are sprayed onto the mold to form microchannels, and this process does not require manual operation, but the operation steps are complicated and expensive [22]. The third, a simple microembossing process, using laser cutting to make plastic micromolds, which for the first time improves the microchannel accuracy from millimeter to nanometer level, and can pattern the microchannels up to 0.2 mm [23]. However, none of those methods can reduce the width and depth dimensions to the level of a few or tens of micrometers. Moreover, the aforementioned methods rely on molds or involve multistep preparation procedures, which are time-consuming and pose difficulties for large-scale mass production. To meet the requirements of high precision and low resistance to contamination in microdetection, a simple and efficient method for fabricating microscale channels on nanopaper is of great significance.

This study presents a simple and novel laser-cutting technique for fabricating microchannels on nanopaper. By burning the surface layer of nanopaper, the single channel was developed. Compared to current methods, our technique has three unique advantages. First, this method is characterized by low cost and ease of operation. Second, fabricating microchannels only needs one laser-cutting machine, which is an integrated and one-step method. Third, the minimum width and depth can down to 58 and 15 μm depth, respectively, which performs the high precision of laser cutting. By arranging different numbers of lines and designing different shapes, we can obtain the microchannels of different sizes and patterning for various applications' requirement. Following this, we developed functional nanopaper-based analytical

devices (NanoPADs) focusing on optical colorimetric sensors and SERS. Silver nanoparticles (AgNPs) were synthetically synthesized *in situ* by introducing AgNO₃ and NaBH₄ into the channel for the reaction, and SERS primers were formed from these particles. Rhodamine B (RhB), a common Raman reporter molecule, was used as a model for the detection of the target. Excellent performance and low limit of detection (LOD) (2.2 mM for glucose and 281 fM for RhB) were ensured due to the outstanding optically transparent nature of the nanopaper. This method may inspire the single-molecule or microdroplets detection in the future.

II. RESULTS AND DISCUSSION

A. Laser Cutting of Microchannel Patterns on Nanopaper

In our study, the rapid laser-cutting technique for microchannel on nanopaper consists of three main steps shown in Fig. 1: the (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO)-oxidized NFC gel is diluted to the suspension with 0.1% solid content.

- 1) *Nanopaper Preparation*: We obtained nanopaper gel (diameter 4 cm) by clarifying the above suspension through vacuum filtration.
- 2) *Laser Cutting*: The filtered nanopaper gel without excess moisture was cut using a laser-cutting apparatus at an optimized laser-cutting intensity and speed (optimized laser-cutting intensity and speed will be given later).
- 3) *Drying*: The nanopaper with microchannels was placed in a 75 °C drying oven for 30 min. The whole process did not take more than 35 min (including 30 min of drying time).

B. Effect of Laser Intensity and Speed on Dimensional Accuracy (Microchannel Laser Process Optimization)

There are many parameters affecting the channel size of laser-cut microchannels, such as the intrinsic properties of the nanofilm province (specific gravity of water and thickness of the nanofilm after vacuum filtration). The height of the laser from the nanopaper can also affect the size of microchannels. However, after experimental verification, the effects of those parameters can be ignored or with irregular changes. In our study, we controlled these intrinsic properties parameters to specific values to investigate the effect of two main processing parameters (laser intensity and laser speed) on the channel size. Controlled variable experiments and parameter optimization were performed to improve the channel size of laser-cut microchannels. The channel dimensions are defined as both width and depth parameters of the microchannel we fabricated.

First, we investigated the effect of laser speed on the dimensions of the microchannels. It was found that a faster laser speed (75 mm/s) could result in narrower and shallower channels. As shown in Fig. 2(a) and (b), a faster laser speed applies less energy and heat to the cellulose per unit area, leading to less cellulose being burned and fewer carbonization marks. However, if the laser speed exceeds 75 mm/s, the burn will be too shallow, and the flow channel may be lost due to the nanopaper's self-healing properties. Conversely, a slower laser speed (15 mm/s) causes excessive burning of the nanocellulose outside the desired pattern due to excess

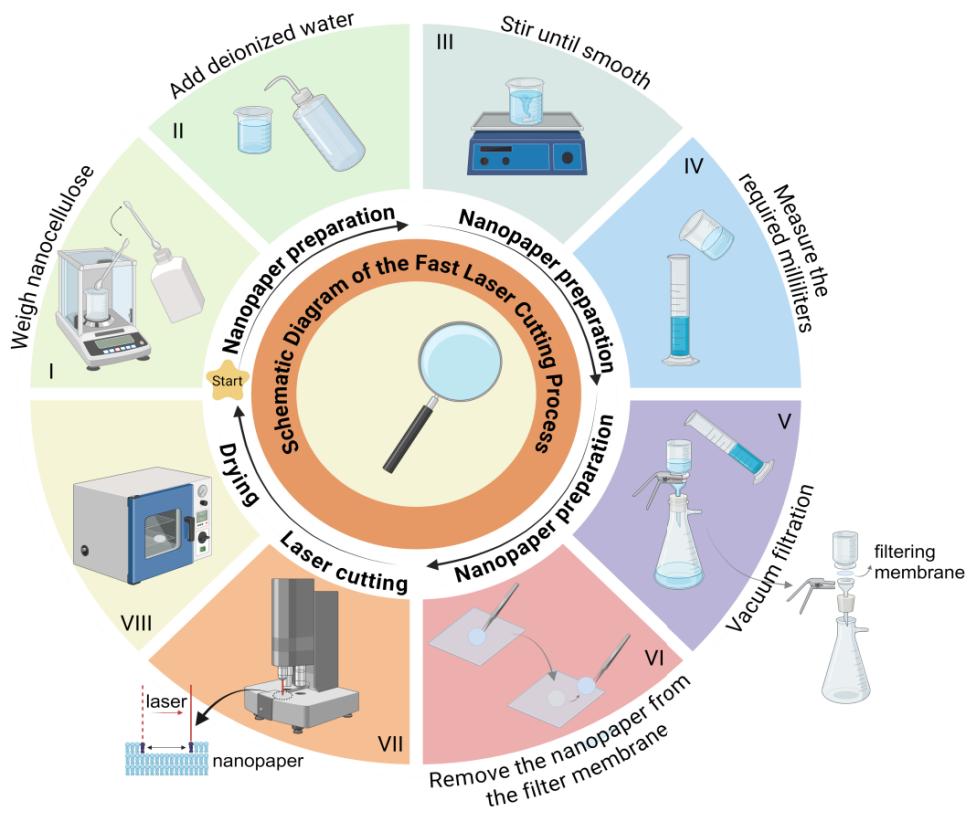


Fig. 1. Schematic of the fast laser-cutting method for forming microchannel patterns on nanopaper: the laser-cutting microchannel process consists of three steps (nanopaper preparation, laser cutting, and drying).

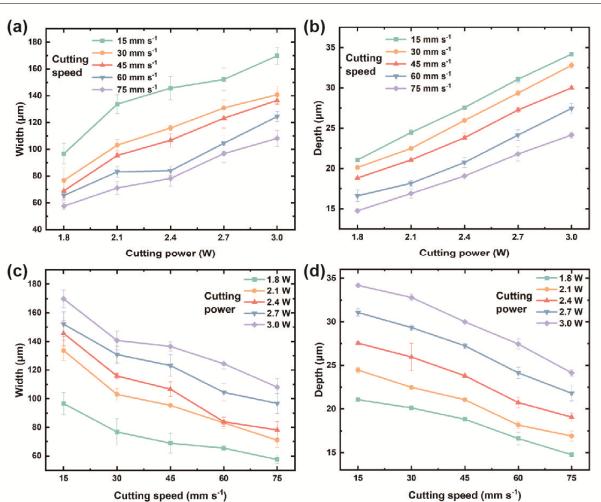


Fig. 2. Optimization of microchannel laser cutting. Effect of (a) and (b) laser-cutting intensity and (c) and (d) laser-cutting speed on width and depth processing accuracy, respectively.

heat, and a longer burn time may penetrate the nanopaper. Also, we can find that at low speed and low power, the width and height of these microchannel change slowly. The tolerance (defining as variance divided by mean) of width and depth of microchannels is shown in Table S1, while the result is within 10%. These tables illustrate the stability and accuracy of our laser-cutting method.

Second, we examined the influence of laser intensity to the accuracy of microchannels in both depth and height level.

The results [Fig. 2(c) and (d)] indicated that a lower laser intensity (1.8 W) produces narrower and shallower channels because less energy and heat are applied to the cellulose per unit area, resulting in less cellulose being burned and fewer carbonization traces. However, if the laser intensity is too low (less than 1.8 W), the channel cannot be successfully cut, or the nanopaper may heal itself after being cut shown in Fig. S1. This is because insufficient energy is delivered to the cellulose, causing incomplete ablation, which, combined with the nanopaper's self-healing properties, leads to the channels either being too shallow or disappearing entirely. Through experimental studies, the optimized laser-cutting parameters were determined to be 75 mm/s and 1.8 W.

From Fig. 3(a), the microchannels with 15 μm depth and 58 μm width are shown. Fig. 3(b) illustrates that we can utilize the arrangement of lines to pattern different designs and sizes microchannels. Fig. 3(c) illustrates the cross section schematic of the microchannel downing to 63 μm width, which shows the high precision of our method.

C. Glucose Colorimetry Experiment

Diabetes mellitus is a chronic disease that requires long-term monitoring and management as there is no permanent cure. Diabetes is a condition, in which the body is unable to digest glucose or blood sugar. As a result, blood glucose levels rise to dangerously high levels. Various NanoPADs glucose colorimetric sensors have been reported as cost effectively, ease-to-utilize, and portable solutions for glucose detection. However, the sensitivity of these sensors is

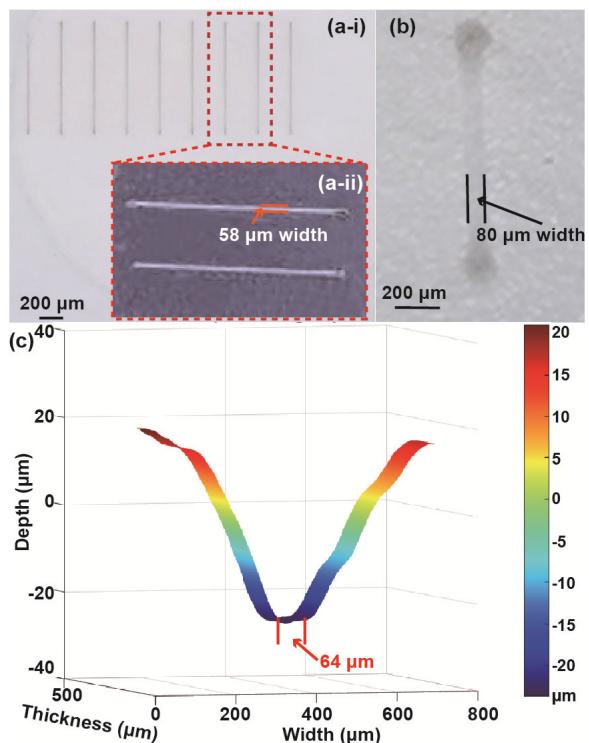


Fig. 3. Photograph of the microchannel fabricated directly by laser cutting. **(a)** 15- μm -depth and 58- μm -width microchannels. **(b)** Microchannel on nanopaper with inlet and outlet zone. **(c)** Fabricated microchannel with a laser microscope generated a 3-D channel view.

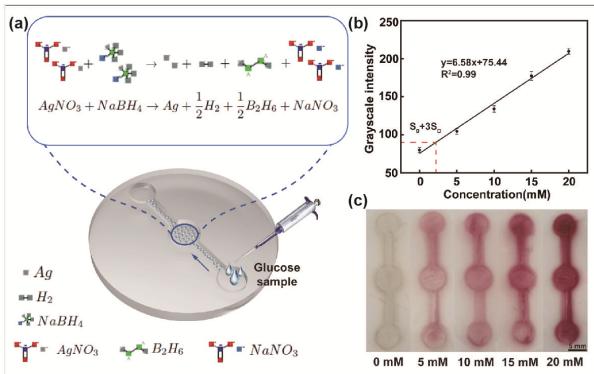


Fig. 4. Colorimetric detection of glucose. **(a)** Schematic of the glucose colorimetric detection mechanism. **(b)** Calibration curve of gray scale intensity of glucose under different concentration ($n = 5$). **(c)** Photograph showing the colorimetric signal in the channel after 10 min of reaction at different concentrations of glucose.

often compromised by the low transparency of conventional paper [24].

Fig. 4(a) illustrates the principle of glucose colorimetric detection using a dual enzyme system, comprising glucose oxidase and type I peroxidase, which amplifies the color signal. These enzymes catalyze the reaction to produce a detectable color change [25]. To illustrate the detection result, 5 μL of enzyme resolution was initially introduced into the lane (25 μm deep) of the reaction zone and allowed to dry for 10 min under room temperature. Then, 5 μL of artificial urine containing various concentrations of glucose was inserted into the lane for the reaction.

Fig. 4(c) displays the photographs of urine samples with different glucose concentrations (0, 5, 10, 15, and 20 mM)

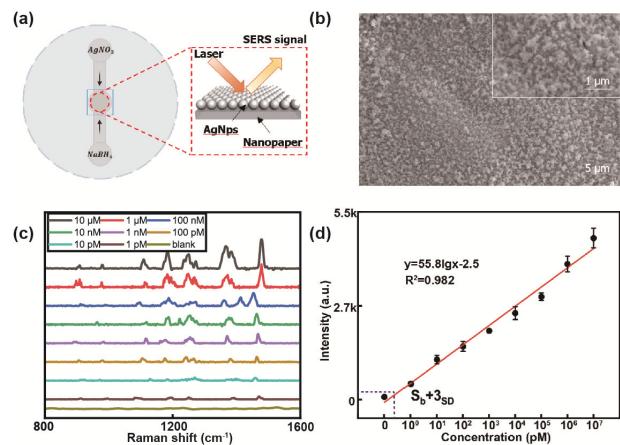


Fig. 5. SERS detection of small molecules (RhB) on NanoPADs by laser cutting method. **(a)** Schematic of AgNPs growth in the reaction zone of NanoPADs. **(b)** SEM photo of AgNPs in reaction zone. **(c)** SERS spectra of RhB detection at concentrations from 1 pM to 100 nM. **(d)** Calibration curve of RhB at 1650 cm^{-1} ($n = 5$).

taken using a desktop scanner (CanoScan LiDE 300, Canon, Japan) after 10 min of reaction. The average gray intensity in the microchannels was analyzed by ImageJ software, and a linear calibration curve of the glucose under different was read by the gray scale intensity as the measurement [Fig. 4(b)]. The calibration curve showed a first-order curve ($y = 6.58x + 75.44$) with an R -squared value of 0.99 for glucose concentrations ranging from 0 to 20 mM, confirming its feasibility in colorimetric sensing applications.

The LOD is comparable to the sensitivity results on PDMS devices and surpasses the most common paper-based detection without chemical functionalization (2.5 mM) due to the exceptional optical transparency and low reflectivity of NanoPADs [26], while retaining the benefits of being economical and ecologically friendly. In the future, NanoPADs are expected to be widely used for trace element analysis, uric acid detection, and other highly sensitive colorimetric assays.

D. Raman Spectroscopy Experiment

SERS is a marker-free, high-sensitivity way to detect as well as analyze biotic and chemic properties [27], [28]. AgNPs with high molar extinction coefficients and excellent optical properties are often regarded as very good SERS substrate materials [29]. In this study, we utilized the in situ growth of AgNPs in the detection region of the NanoPAD to investigate the surface properties of the nanocellulose membrane flow channels after laser cutting. The design of the SERS-NanoPAD is shown in Fig. 5(a), where three circular microchannels are aligned in a straight line and are connected by rectangular flow channels. The in situ method is based on the deposition and growth of silver ions in specific regions to form an effective SERS substrate [30], [31]. As demonstrated in Fig. 5(a), AgNPs are generated through BH₄ reduction on this article surface. The carboxylate group adsorbs silver ions on the nanopaper surface via electrostatic adsorption, with the silver ions replacing the positive ions in the carboxylate reaction region, while BH⁴⁻ effectively reduces the silver ions [32], [33].

In order to validate the usefulness of SERS-NanoPADs for real-world applications, we chose as sensing objects the criteria environmental pollutant and common carcinogen dye RhB. RhB molecules were mixed directly with ethanol. 5 μL of the formation solution was introduced into the channel of the SERS-NanoPADs through the inlet of the sensing solution, a step by which the enhancement of the Raman spectral signal due to these chemicals in the sensing channel was measured. Fig. 5(c) shows the Raman spectra of RhB samples at varying concentrations (1 pM–100 nM) in ethanol revealing peaks at 1200 cm^{-1} (the xanthene ring puckering mode), 1280 cm^{-1} (C–O–C stretching), 1350 cm^{-1} (C–C stretching), 1384 cm^{-1} (C–N stretching), 1520 cm^{-1} (C–H stretching), and 1650 cm^{-1} (aromatic C–C stretching) [34], [35], with pure ethanol being utilized as a blank control. The peak intensity at 1650 cm^{-1} was chosen for recording due to its higher sensitivity to fluctuations in RhB concentrations in comparison with the low background noise. The RhB assay calibration curve shown in Fig. 5(c) shows an LOD of 281 fM, which is the RhB concentration equivalent to the standard deviation of the blank control intensity and three times the blank control Raman intensity. The dashed line represents a linear curve fit to the intensity-concentration data with a regression equation: $y = 55.8(\lg x + 1) - 2.5$ ($R^2 = 0.98$). The result shows that the NanoPADs we fabricated by laser-cutting method have a good performance that can satisfy the requirements in daily life for molecules detection. Regarding the SERS performance, our NanoPADs has picomolar level sensitivity, which is higher than the sensitive regular paper-based SERS substrates ($< 1 \times 10^{-12}\text{ M}$) [36]. Due to the roughness of microchannels by laser-cutting burning process, the LOD is a bit higher than the embossing method (19 fM) [23]. However, the result still proves that the NanoPADs we fabricated occupy significant potential for highly sensitive analytical detection.

III. CONCLUSION

In conclusion, we have proposed a facile and efficient laser-cutting technique for fabricating microchannels on nanopaper, utilizing a fast and convenient process. This work innovatively reduces the width and depth of microchannels to the level of tens of micrometers. Also, the fabrication time of microchannels is greatly reduced compared to other methods and can be achieved in only one step. Notably, we have successfully reduced the microchannel dimensions on nanopaper to $58\text{ }\mu\text{m}$, achieving a threefold improvement over the existing methods. By optimizing parameters during laser-cutting process, including both laser intensity and speed, we were able to create highly accurate and intricate microchannel patterns with minimal variation (2.5% in width and 9% in depth).

We validated two functional analytical sensors on NanoPADs: one for colorimetric glucose detection and the other for SERS biosensing, both demonstrating low limits of detection (2.2 mM for glucose and 281 fM for RhB). The proposed laser-cut nanopaper microchannel fabrication process is not only capable of reducing microchannel sizes to tens of micrometers but also excels in speed, simplicity, and the ability to support the creation of complex patterns. We believe that the laser engraving method for fabricating

microchannels proposed in this article will find applications in the emerging field of nanopaper-based microfluidic chemistry and biosensors, bringing new design inspiration and practical uses to the field. This approach can serve as an effective solution to enhance the precision and integration of nanopaper-based devices.

IV. METHODS

A. Reagents and Materials

Purchased from Tianjin University of Science and Technology: TEMPO-oxidized NFC slurry (1.0 wt% solids). Purchased from Macklin (Shanghai, China): Alginate (>99%). Purchased from Hushi (Shanghai, China): AgNO_3 (99%) and ethanol (>99%). Purchased from Aladdin (Shanghai, China): 3,5-dichloro-2-hydroxybenzenesulphonic acid (>99%) and NaBH_4 (>98%) were obtained from Aladdin (Shanghai, China). Type I peroxidase (200 U/mg) was from horseradish. 4-aminoantipyrine (>98%) and d-(+)-glucose (>99%) were sourced from Meryer (Shanghai, China).

B. Optimization of Laser-Cutting Parameters for Fabricating High Accurate Microchannels

Add 6.0 g of TEMPO-oxidized NFC slurry to distilled water and stir the suspension at 800 r/min for 30 min until no flocculent matter remains. This process results in a final solution with a concentration of 1.0 wt%. Place the PVDF filter membrane (VVLP04700, EMD Millipore Inc., pore size: $0.1\text{ }\mu\text{m}$) on the glass filter holder, then the resulting suspension into the glass filter holder and perform vacuum filtration for 7 h. The filtered nanopaper was then laser cut using a laser cutter (Daqi Yuming Laser CMA0604-BA, China) based on the design. The surface charred cellulose was washed away using Di water. Finally, the nanopaper was placed in an oven and dried at various temperatures for around 1 h.

C. Characterization

To assess the impact of laser cutting on paper structure, the cross-sectional images of the embossed microchannels were examined with laser microscopy (KEYENCE VK-X150, Japan). The channels and AgNPs morphology were further characterized using scanning electron microscopy (SEM) (FEI Scios 2 HiVac, USA) at an operating voltage of 5 kV.

The cross section schematic of microchannel fabricated by laser was taken by laser microscope (KEYENCE VK-X150, Japan).

D. Glucose Colorimetric Detection

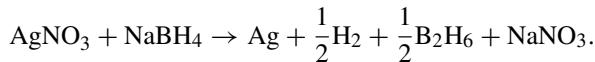
We used d-(+)-glucose as a biomarker in the colorimetric assay. This assay relies on the activity of glucocerebrosidase, which oxidizes glucose to gluconic acid and hydrogen peroxide in the presence of oxygen. The oxidation of the color developer by the resulting H_2O_2 is subsequently measured with the help of peroxidase. For this purpose, we mixed 120 U glucose oxidase (from *Aspergillus Niger*, activity 180 U/mg) and 30 U of type I peroxidase (from Horseradish, activity 200 U/mg) as stabilizers. The oxidation indicator consisted of a mixture of 0.2 M 4-aminoantipyrine and 0.4 M sodium

3,5-dichloro-2-hydroxybenzenesulfonate. Artificial urine consisted of a combination of 2.5-mM calcium chloride, 2-mM citric acid, 90-mM sodium chloride, 1.1-mM lactic acid, 2-mM magnesium sulfate, 10-mM sodium sulfate, 7-mM potassium dihydrogen phosphate, 170-mM urea, 25-mM sodium bicarbonate, a mixture of 7-mM potassium dihydrogen phosphate, and 25-mM ammonium chloride ($\text{pH} = 6$).

We first dissolved the glucose assay reagent in 1× phosphate-buffered saline (PBS). Then, various dilutions of glucose were assayed in artificial urinary fluid. We injected 5 μL of glucose reagent sample into the inlet zone and allowed it to dry at room temperature for around 10 min. Finally, we injected 5 μL of an artificial urinalysis containing different concentrations of the glucose into the lanes for the response. Glucose detection data were taken from the average of five measurements. All spectral data were utilized Origin lab software (OriginLab, USA) to analyze.

E. AgNPs Growth

Silver nanoparticles (AgNPs) were generated and attached to the surface of the flow channel by a modified continuous ionic layer adhesion and reactivity of the process. The chemical reaction for the AgNPs formation is described by the following equation [36]:



We used AgNO_3 as the silver precursor and NaBH_4 as the reducing agent. Briefly, we dropped 5 μL of 20-mM AgNO_3 into the upper side channel port and kept it in the reaction zone for half minutes. Repeating the abovementioned steps five time ensured the uniform distribution of AgNPs, thus explaining the formation of their high intensity bands [18]. Then, we dropped 5 μL of distilled water into the lower side channel opening for washing. After washing, 5 μL of 20-mM NaBH_4 was applied to the lower side channel port.

F. SERS Measurement

We measured all Raman spectra on a Horiba LabRAM Odyssey Nano (Japan) using a 532-nm laser and a 50× optical objective. RhB was dissolved in ethanol and diluted to a concentration range of 10^{-7} – 10^{-12} M; 5 μL of the analyte solution was dropped into the inlet zone of microchannel on NanoPAD and further left to dry in air. We need to note that the Raman spectra were measured in the range of 800–1800 cm^{-1} . Raman spectra were taken from the average of five measurements. All spectral data were analyzed using Origin lab software (OriginLab, USA).

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