Modelling, Simulation, and Prototyping of Hollow Microfluidic Channel for Investigation of Blood Cells

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Abstract: *The current publication presents an approach for the elaboration of a disposable microfluidic hollow micro-channel using 2 photon polymerization technology and Photonic Professional GT2 (Nanoscribe, Germany) equipment. The design of a 3D model of a microchannel is realized by the CAD analysis software – SOLIDWORKS. A suitable laminar flow is generated by using computational fluid dynamics (CFD) software. As a result, the critical points of the pressure, velocity, and wall shear stress into the microfluidic channel are obtained. A real prototype of the hollow microfluidic device is created, using a highly innovative technology of 3D nanoprinting by two-photon polymerization. Experimental studies with dilute erythrocyte suspensions are conducted to test the functionality of the developed prototype of nano 3D printed microchannel.*

***Keywords**: MICROFLUIDICS, HOLLOW MICROCHANNELS, 3D MODELING, 3D NANOPRINTING, TWO PHOTON POLYMERIZATION, RED BLOOD CELLS*

1. Introduction

Microfluidic devices can be used for a variety of studies because they operate with a small volume of fluid and with channels typically between 1 µm to 500 µm wide [1]. Traditionally, microfluidic devices are fabricated from PDMS through a soft lithography fabrication process. By this fabrication method, it is extremely difficult to create 3D microfluidic devices due to the challenges in aligning the different PDMS layers [2].

3D printing of microfluidic devices provides design freedom which is difficult or impossible to achieve using traditional manufacturing techniques. Fabrication of microfluidic devices is usually expensive and labor intensive, while 3D printing technology has the potential to reduce costs while offering the possibility of more complex microfluidic devices [3]. This allows the development of truly three-dimensional microfluidic designs and the fabrication of intricate and customizable vascular networks that can mimic the complexity of natural blood vessels [4].

3D-printed hollow microchannels are a promising alternative for complex tissue vasculature engineering and other complex regenerative medicine applications [5, 6]. Microfluidic hollow devices and 3D printing technologies enable the perfusion and regulation of fluids through these blood vessel models, creating a more realistic in vitro environment for studying various biological processes and drug development [7, 8].

The Two Photon Polymerization (2PP) technique is considered an advanced 3D printing technique and is based on the Two Photon Absorption (TPA) process [9]. This technique is applied for micro- and nano-scale 3D printing. This involves the usage of a laser to selectively polymerize a liquid or photosensitive material, enabling the precise production of complex three-dimensional structures with high resolution.

It is possible to manufacture 3D microchannels with diameters size less than 1 µm - nano-3D printing, by the application of 2PP. This is a hybrid process that combines digital light projection 3D printing with nanoscale-relief patterning. Nano-3DP enables a variety of novel applications with higher-resolution surface details than currently available [10].

This publication aims to model, simulate, and prototype a hollow microchannel mimicking a microvessel that can be used to conduct various blood cell studies.

2. Materials and Methods

The 3D modeling of hollow microchannel is realized based on computer-aided design (CAD) software such as SOLIDWORKS. It is generated .stl file format, by this software and after that, it is imported into the computations fluid flow dynamics (CFD) software environment - FLOW 3D. The selected 3D microchannel design is tested for suitable inlet fluid flow parameters (such as velocity, pressure, and shear stress) which will create the required laminar flow for the study of dilute suspensions of blood cells.

The 3D model of the microchannel, designed in SOLIDWORKS (.stl file) is imported into a specialized software (DeScribe) of the 3D nanoprinter - Photonic Professional GT2 (Nanoscribe, Germany).

A highly innovative two-photon polymerization (2PP) technology is used for 3D printing of the designed microfluidic hollow micro-channel. By two-photon, polymerization, a filigree structure from a 3D shape via high-precision 3D printing is created. The 2PP 3D printing is a direct laser writing (DLW) technique in which a solid structure is written into a liquid resin voxel by voxel by scanning a femtosecond pulsed tightly focused laser beam.

By applying, the 3D nanoprinter, the prototype of the hollow microchannel is printed on a glass substrate. The selected recipe IP-S 25x ITO Solid (3D MF) is suitable for manufacturing of 3D medium features (3D MF) using solid mode. The glass substrate is coated with an Indium Tin Oxide (ITO) conductive thin layer. The main advantage of ITO coatings is their chemical stability and durability. The photosensitive resin used for the development of the micro-channel is IP-S which has to be combined with the optical magnification x25.

To increase the printing quality, adjustments to laser power and scanning speed can be made. Additionally, the 3D printing process can be simulated for thorough investigation and necessary corrections.

The holder is placed in the 3D printing machine. PPGT2 3D printer is controlled by an easy-to-use graphical user interface of the specialized software (NanoWrite) from Nanoscribe. NanoWrite is launched, which calibrates the overall system, loads the work file from DeScribe, and performs the nanoprinting operation.

The dimensions and structure of the prototype of the hollow micro-channel are analyzed and measured using a Primostar 3HDcam optical microscope.

To assess the micro-channel's patency, it is infused with a diluted suspension of erythrocytes, and the subsequent flow of cells is observed and recorded using an optical microscope.

3. Results

The SOLIDWORKS software environment is applied to generate the 3D model of the hollow microfluidic channel, as shown in Fig. 1. The outer dimensions of the micro-channel are length/width/height - 5 / 0.35 / 0.1 mm. The inner dimensions are height/width - 0.08 / 0.25 mm. The thickness of the walls of the microchannel is 0.05 mm.

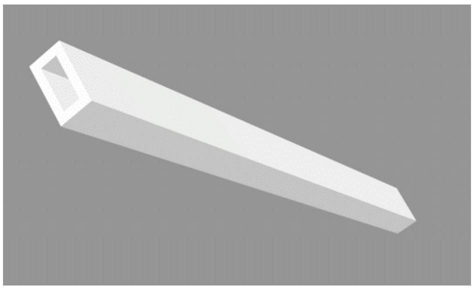


Fig. 1 *3D design of a straight hollow micro-channel by SOLIDWORKS.*

The .stl file created in the SOLIDWORKS is imported into FLOW 3D (CFD software). A suitable mesh for the simulation of the fluid flow in the microchannel is generated (mesh cell size of 0.025 x 0.025 mm).

The flow through the microchannel is simulated using water as the testing fluid at a temperature of 20°C. The boundary conditions of the model are defined by setting a fluid flow rate of 0.026 mm^3/s, which is obtained at a Reynolds number <1 (a necessary condition for laminar flow). As a result, the pressure contours (Fig. 2), x-velocity contours (Fig. 3), and wall shear stress contours (Fig. 4) are obtained.

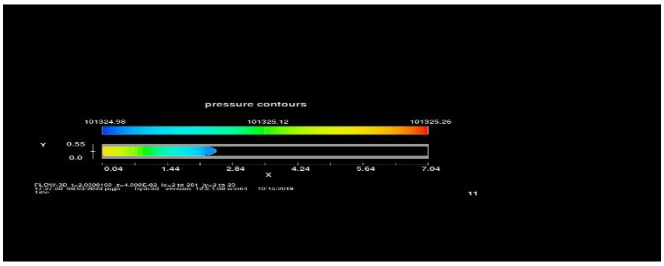


Fig. 2 *Simulation results of the pressure (g/mm/s2) in the microfluidic channel.*

Based on the simulation results, the pressure along the microchannel is approximately 101325 g/mm/s² (Fig. 2). Within the central region of the flow, the fluid velocity increases to 1.41 mm/s, while at the channel walls, it decreases to 0.02mm/s (Fig. 3).

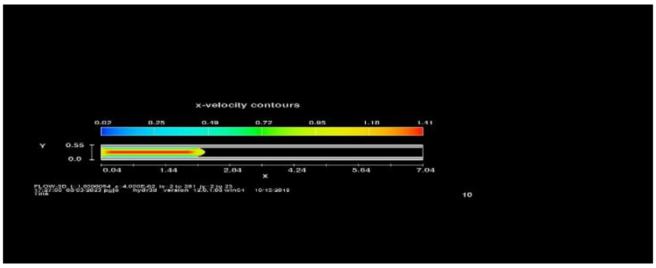


Fig. 3 *Simulation results of the velocity (mm/s) in the microfluidic channel.*

The wall shear stress on the bottom of the microchannel has a maximum value of 0.04 $g/mm/s^2$ (Fig. 4).

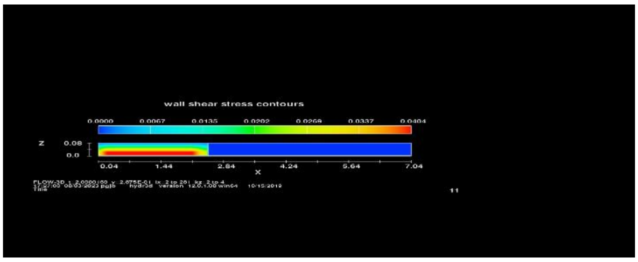


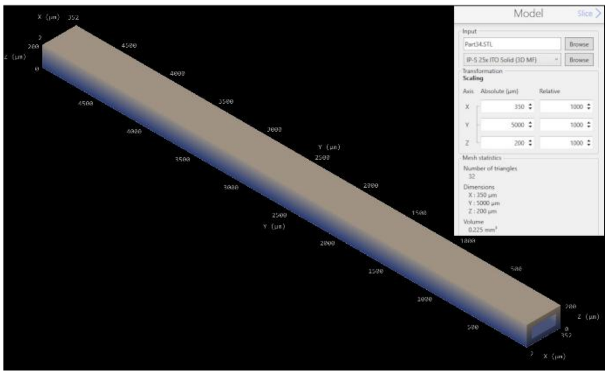
Fig. 4 *Simulation results of the wall shear stress ($g/mm/s^2$) in the microfluidic channel.*

After the assessment of the hollow microchannel model using FLOW 3D software to determine optimal parameters for laminar flow, the designed .stl file in SOLIDWORKS is imported into the 3D nanoprinter's software – (DeScribe).

DeScribe reads the .stl file and slices the object by intersecting the 3D solid surfaces with a series of parallel planes. For each plane, DeScribe computes the intersection contours of the surfaces with the plane and subsequently fills each obtained contour with hatching lines. DeScribe creates a compiled (machine-readable) version of all required files and a specially created GWL file can be loaded in NanoWrite or uploaded to the print project list, respectively.

The import wizard is divided into five steps: Model, Slice, Fill, Scaffold, and Output.

The first step "Model" allows for resizing of the dimensions, as well as changing the orientation of the STL model file (Fig. 5).



Input
Part34.STL
IP-S 25x ITO Solid (3D MF)

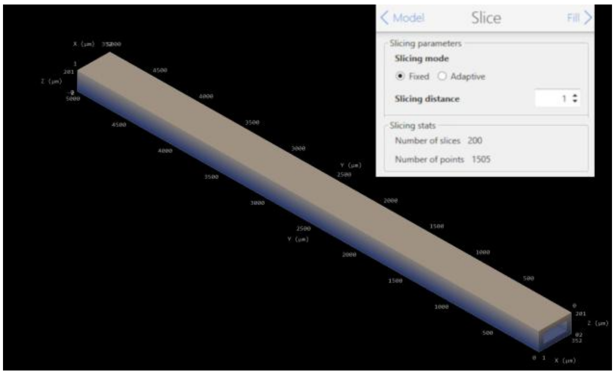
Transformation
Scaling

Axis	Absolute (µm)	Relative
X	350	1000
Y	5000	1000
Z	200	1000

Mesh statistics
Number of triangles
32
Dimensions
X : 350 µm
Y : 5000 µm
Z : 200 µm
Volume
0.225 mm^3

Fig. 5 *DeScribe wizard - Model view*

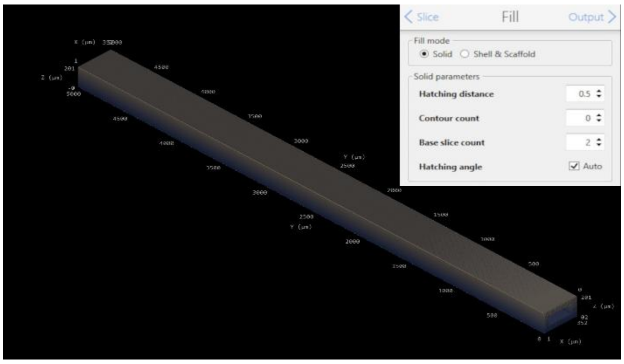
In "Slice" mode, the micro-channel is sliced in a stack of 200 layers (slices) along the *z* direction. The slicing distance is fixed to 1µm (Fig. 6).



Slicing parameters
Slicing mode
● Fixed ○ Adaptive
Slicing distance 1
Slicing stats
Number of slices 200
Number of points 1505

Fig. 6 *DeScribe wizard - Slice view*

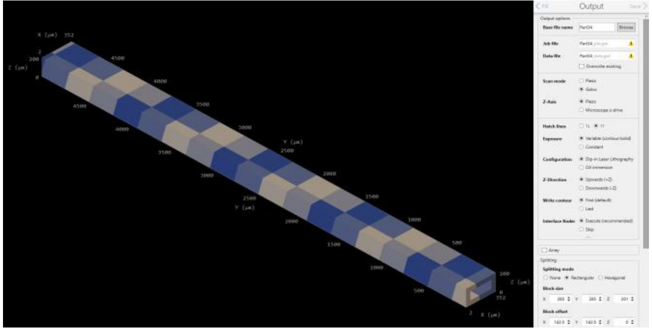
The "Fill" step has two options ("Solid" and "Scaffold") and the "Solid" option is used. For "Solid", the core of the structures is filled with adjacent lines, and spacing is defined by the Hatching distance (0.5 µm). To increase the mechanical stability of the structure a Hatching angle and a Hatching angle offset can be defined (Fig. 7).



Fill mode
● Solid ○ Shell & Scaffold
Solid parameters
Hatching distance 0.5
Contour count 0
Base slice count 2
Hatching angle ☑ Auto

Fig. 7 *DeScribe wizard - Fill View*

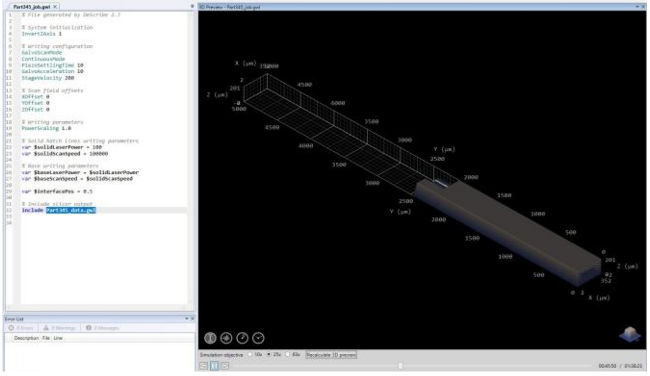
The “Output” menu is the last step of the import wizard. It defines the units of the system that will be used for printing. The standard combination consists of Galvo for the Scan mode and Piezo for the Z-Axis option. The hatching lines are set in a one-way direction. The duration of the laser exposure is set to be constant. Dip-in Laser Lithography (DiLL) is set where the 2PP resin is used as the immersion medium. The nonpolymerized photosensitive resin is placed on top of the ITO glass substrate. The substrate is fixed to a specially developed holder. The main purpose of the holder is to position the glass substrate containing the resin over the optical objective x25. The objective is immersed into the nonpolymerized resin and after setting the Z direction parameter to be Upwords (+Z), the printing of the micro-channel starts from the substrate surface down to the Z axis. The microchannel is separated into blocks that are connected. The printing is realized in blocks by polymerizing the hatching lines of each one of the layers sequentially. The length of each hatching line is equal to the length of the predefined block (Fig. 8)



Base file name: Part34
Job file: Part34_job.gwl
Data file: Part34_data.gwl
☐ Overwrite existing
Scan mode: ○ Piezo ● Galvo
Z-Axis: ● Piezo ○ Microscope z-drive
Hatch lines: ○ ↑↓ ● ↑↑
Exposure: ● Variable (contour/solid) ○ Constant
Configuration: ● Dip-in Laser Lithography ○ Oil immersion
Z-Direction: ● Upwards (+Z) ○ Downwards (-Z)
Write contour: ● First (default) ○ Last
Interface finder: ● Execute (recommended) ○ Skip
Splitting mode: ○ None ● Rectangular ○ Hexagonal
Block size: X 285 Y 285 Z 201
Block offset: X 142.5 Y 142.5 Z 0

Fig. 8 *DeScribe wizard - Output View*

In Describe a special job file is generated that is used as an input to the nanoprinting process (Fig. 9).



```
% File generated by DeScribe 2.7

% System initialization
InvertZAxis 1

% Writing configuration
GalvoScanMode
ContinuousMode
PiezoSettlingTime 10
GalvoAcceleration 10
StageVelocity 200

% Scan field offsets
XOffset 0
YOffset 0
ZOffset 0

% Writing parameters
PowerScaling 1.0

% Solid hatch lines writing parameters
var $solidLaserPower = 100
var $solidScanSpeed = 100000

% Base writing parameters
var $baseLaserPower = $solidLaserPower
var $baseScanSpeed = $solidScanSpeed

var $interfacePos = 0.5

% Include slicer output
include Part345_data.gwl
```

Fig. 9 *DeScribe wizard - job file (machine readable) and virtual simulation of the 3D printing procedure*

The nanoprinting procedure is monitored in real-time using a specialized digital camera integrated into the PPGT2 3D printer. The microchannel is perpendicular to the surface of the glass substrate. In this way, the contact points are smaller and this potentially will reduce the defects on the printed microstructures (Fig. 10).

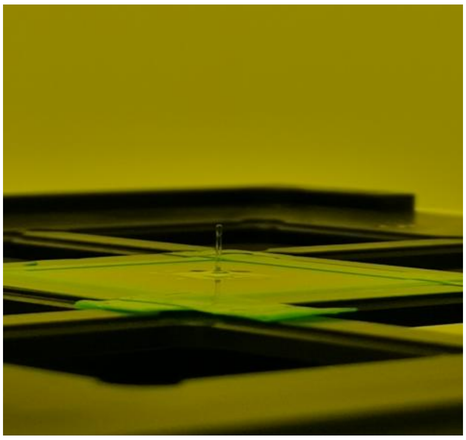


Fig. 10 *Hollow micro-channel, vertically printed on a glass substrate*

After finishing the 3D printing process, the holder with the polymer microstructure is removed from the 3D printer. The glass substrate with the microchannel is washed exclusively with an isopropanol solution, and the geometry and structure of the printed microchannel are analyzed under an optical microscope (Fig. 11).

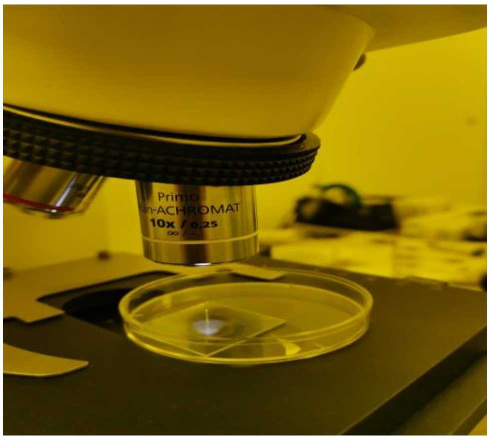


Fig. 11 *The hollow micro-channel investigated under an optical microscope Primostar 3HDcam*

The optical examination shows that the microstructure of the hollow channel printed in the “Solid” has no defects. The same process is repeated in “Scaffold”, and it is found out the existence of many defects. Hence, the nanoprinting of the hollow microchannel is better to be realized in the “Solid” operation and vertically printed regarding the glass substrate (Fig. 12).

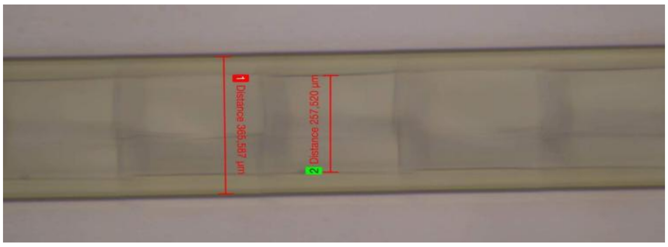


Fig. 12 *Image of the geometry and structure of the developed hollow micro-channel taken by optical microscope Primostar 3HDcam*

The functionality of the constructed hollow microchannel is assessed by filling it with a diluted erythrocyte suspension. The flow of erythrocytes is created with a syringe pump and observed using an optical microscope Primostar 3 Zeiss with an integrated digital camera (Fig. 13).

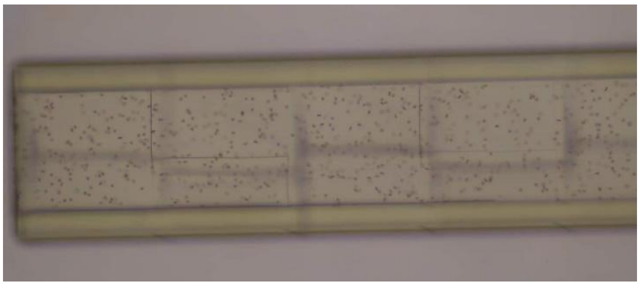


Fig. 13 *Image of the developed hollow micro-channel filled with a diluted erythrocyte suspension*

Experimental results with diluted erythrocyte suspensions show that the channel is passable and the flow through it is laminar. The hollow channel could be used as an experimental model of microvessel for studying the rheological properties of blood cells, such as erythrocyte aggregation and deformability.

4. Discussion

The study of the rheological properties of blood and mimicking the erythrocyte flow at *in vitro* conditions is essential for the design, performance, and optimization of microfluidic devices. Valuable progress in personalized medicine for new treatments and diagnosis approaches can be achieved by combining the unique features of hemorheology and microfluidic technology for cell analysis [11].

The creation of affordable 3D printers revolutionizes the field of microfluidics, allowing for the creation of complex and customized microfluidic devices that were previously difficult or impossible to fabricate using traditional methods [11]. The key advantages of 3D printing are freedom of design, mass customization, and the ability to print complex structures with minimal waste [4].

Two-photon polymerization-based direct laser writing is an excellent technological solution for the fabrication of micro-, meso-, and even macroscale structures with extremely fine features. However, the quality of the PPGT2 printed structures depends on many different fabrication parameters, starting with the employed microscope objective, printing configuration, substrate, and resin. Additionally, other fabrication parameters, including the laser power and scan speed, as well as post-processing solutions, are likely to affect the final printed product [12].

The unique capabilities associated with 2PP come from the ability to process materials with sub-micrometer resolution, as complex features can be made in regions of structures that are impossible to access using other fabrication techniques [13].

One set of applications of 2PP is fabrication within a microfluidic channel. 2PP-based structuring is performed within a photopolymer-filled channel, followed by exposure to the developing solution [14]. 2PP is also used to generate porous microchannels for the study of chemotaxis in dendritic cells [15]. A 2PP-fabricated hypodermic microneedle is integrated with a microfluidic device; this device can uptake solutions containing physiologically relevant K^+ solutions for detection with an ion-selective electrode [16].

The present study suggests and constructs a 3D nanoprinting hollow microfluidic channel by innovative two-photon polymerization 3D printing technology. This is an important technological approach for the rapid production of low-cost microfluidic channels with the help of which the rheological properties of blood cells can be studied in various diseases.

5. Conclusion

A hollow micro-channel is developed using 2 photon polymerization 3D printing technology and PPGT2 Nanoscibe equipment. Every stage of 3D modeling and simulation of the prototype is described. The simulation results of the pressure, velocity, and wall shear stress measured in the microchannel are of great importance for laminar flow generation. The whole process of development of a machine-readable job file for 3D printing is described in detail. Experimental studies using dilute erythrocyte suspensions are applied to assess the functionality of the developed prototype of a hollow micro-channel. This microfluidic device has potential applications in rheological investigations of blood cells.

Acknowledgment

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