Round-robin testing of commercial two-photon polymerization 3D printers

Federico Cantoni\textsuperscript{a}, Daniel Maher\textsuperscript{b}, Eugenia Bosler\textsuperscript{c}, Stefan Kühne\textsuperscript{c}, Laurent Barbe\textsuperscript{a}, Dirk Oberschmidt\textsuperscript{c}, Christophe Marquette\textsuperscript{d}, Rafael Taboryski\textsuperscript{b}, Maria Tenje\textsuperscript{a}, Ada-Ioana Bunea\textsuperscript{a,b,*}

\textsuperscript{a} Department of Materials Science and Engineering, Science for Life Laboratory, Uppsala University, Box 35, 751 03 Uppsala, Sweden
\textsuperscript{b} National Centre for Nano Fabrication and Characterization (DTU Nanolab), Technical University of Denmark, Ørsteds Plads 347, 2800 Kgs. Lyngby, Denmark
\textsuperscript{c} Institute of Micro and Precision Devices, Technical University of Berlin, Pascualstraße 8-9, 10587 Berlin, Germany
\textsuperscript{d} 3d.FAB, Univ Lyon, Université Lyon1, CNRS, INSA, CPE-Lyon, ICBMS, UMR 5246, 43, Bd. du 11 Novembre 1918, 69622 Villeurbanne Cedex, France

**ARTICLE INFO**

Keywords:
Two-photon polymerization
Direct laser writing
3D printing
Microfabrication

**ABSTRACT**

Since its introduction in the 1980s, 3D printing has advanced as a versatile and reliable tool with applications in different fields. Among the available 3D printing techniques, two-photon polymerization is regarded as one of the most promising technologies for microscale printing due to its ability to combine a high printing fidelity down to submicron scale with free-form structure design. Recently, the technology has been enhanced through the implementation of faster laser scanning strategies, as well as the development of new photoresists. This paves the way for a wide range of applications, which has resulted in an increasing number of available commercial systems. This work aims to provide an overview of the technology capability by comparing three commercial systems in a round-robin test. To cover a wide range of applications, six test structures with distinct features were designed, covering various aspects of interest, from single material objects with sub-micron feature sizes up to multi-material millimeter-sized objects. Application-specific structures were printed to evaluate surface roughness and the stitching capability of the printers. Moreover, the ability to generate free-hanging structures and complex surfaces required for cell scaffolds and microfluidic platform fabrication was quantitatively investigated. Finally, the influence of the numerical aperture of the fabrication objective on the printing quality was assessed. All three printers successfully fabricated samples comprising various three-dimensional features and achieved submicron resolution and feature sizes, demonstrating the versatility and precision of two-photon polymerization direct laser writing. Our study will facilitate the understanding of the technology maturity level, while highlighting specific aspects that characterize each of the investigated systems.

1. Introduction

3D printing, also referred to as additive manufacturing, was originally employed primarily for small-scale manufacturing of plastic components. The more recent development of new 3D printing technologies has led to a significant upscaling of the fabrication capabilities. The more recent development of new 3D printing technologies has led to a significant upscaling of the fabrication capabilities. The more recent development of new 3D printing technologies has led to a significant upscaling of the fabrication capabilities. The more recent development of new 3D printing technologies has led to a significant upscaling of the fabrication capabilities. The more recent development of new 3D printing technologies has led to a significant upscaling of the fabrication capabilities. The more recent development of new 3D printing technologies has led to a significant upscaling of the fabrication capabilities.

Typically, the resolution of 3D printing technologies is in the range of tens to hundreds of micrometers. The only 3D printing technology that can provide sub-micrometer resolution and lithographic fabrication of feature sizes beyond the diffraction limit [5,6] is two-photon polymerization (2PP) direct laser writing (DLW). In 2PP DLW, the focal spot of a visible or near-infrared pulsed laser beam is scanned in 3D through a liquid resin, hence the name DLW. This triggers the photo-polymerization of the resin in the areas where a rapid subsequent absorption of two photons occurs, hence the term 2PP. The localized absorption of two photons allows for accurate fabrication of micro-sized objects with complex geometries without the need for supporting structures. A noteworthy aspect is that the lengthy fabrication, which
has been one of the greatest obstacles to scalable microfabrication by 2PP, has been addressed with the implementation of digital mirror devices. This strategy enables multiple laser foci for parallel fabrication instead of a sequential laser scanning process, accelerating the process rate [7].

Previous work describes, in detail, the theoretical considerations for maximum resolution achievable [5], as well as the main factors influencing processing accuracy [8]. Typically, when using high-resolution oil immersion objectives, the 2PP minimum feature size is ∼200 nm in the XY plane and ∼500 nm in the Z direction, whereas the XY resolution is ∼500 nm. However, higher resolution can be achieved by post-processing, and feature sizes below 100 nm have been demonstrated using secondary processing steps based on pyrolysis and/or isotropic plasma etching [9]. The focal spot of the laser beam typically has an ellipsoidal shape with an aspect ratio between 1.5 and 3.5, depending on the numerical aperture (NA) of the laser focusing objective [10]. This means that the 2PP DLW resolution is 1.5–3.5 times lower on the Z-axis, along the laser beam’s propagation direction, compared to the XY plane. This aspect is also quite different in 2PP DLW as opposed to other 3D printing techniques, where the Z-resolution is typically superior to the XY-resolution.

2PP DLW has previously been employed for e.g. biomedical applications [11,12], microbotics [10,13], biosensing [14,15], bioinspired structural colors [16,17], and metamaterials [18,19]. Epoxy-based and acrylic polymers used to be the typical 2PP DLW materials, but the choice of photoresists has expanded tremendously over the recent years [20,21]. With the current broad applications in different fields, commercial 2PP photoresists include hybrid organic-inorganic materials, biocompatible hydrogels, and fused silica precursors, whereas home-made formulations often focus on functional materials [22] or on responsive materials for 4D printing [23], such as certain liquid crystalline elastomers [24], hydrogels [25], or shape memory polymers [26].

The first experimental demonstration of 2PP DLW came in the late ’90 s from Maruo et al. [27]. Nanoscribe, a spin-off from the Karlsruhe Institute of Technology in Germany, launched the first commercial equipment for 2PP DLW in 2007, and today provides several 2PP printer models, as well as a selection of resins for 2PP. In the past decade, other companies followed suit and launched alternative systems for 2PP DLW and as of today there are seven main players on the market. Multiphoton Optics, a spin-off from Laser Zentrum Hannover, Germany, and Femtika, a Lithuanian company and a spin-off from the Vilnius University Laser Research Centre, were both established in 2013. Femtika provides a combination of additive and subtractive manufacturing using femtosecond pulsed lasers. More recent 2PP DLW commercial system companies are Microlight3D, a French spin-off from the University of Grenoble Alpes established in 2016, and UpNano, an Austrian spin-off from the Technological University of Vienna established in 2018. LightFab, a company from Aachen, Germany established in 2013, and WOP Altechna, a laser micromachining Lithuanian company established in 2003, both offer multifunctional 3D printers primarily meant for the selective laser etching of glass, while also capable of 2PP DLW.

With the rapid growth of the field and the fast appearance of new 2PP printer models, it is not obvious from the perspective of new users what the technology can enable or how different systems actually differ. In this paper, we therefore aim to provide an overview of 2PP DLW commercial equipment, together with a round-robin style experimental comparison of three such systems, namely the NanoOne200 from UpNano, the Photonic Professional GT+ (PPGT+) from Nanoscribe, and the FemtoLAB from WOP Altechna (Table 1). It must be noted that there are several other types of 2PP DLW equipment commercially available both from these companies and from other manufacturers. The three printers described herein were selected in the study to cover a varied range of solutions, from a rather commonly-used printer produced by the first commercial 2PP printer manufacturer Nanoscribe to models from newer manufacturers which offer unique technological solutions such as high printing speed and embedded HEPA filters (UpNano) or hybrid additive and subtractive 2PP technology (WOP Altechna). While a complete evaluation of 2PP systems would not be realistic to obtain, given the pace the technology is evolving at, this work aims to address the most relevant types of features currently encountered in commercial 2PP printers and should thus prove highly valuable to anyone interested in the latest developments in the field. Furthermore, this work addresses suggestions for possible future improvements.

### 2. Materials and methods

#### 2.1. Design

The round-robin test structures were designed using SolidWorks 2021 (Dassault Systems) and exported as STL files. The six different designs investigated are described below.

#### 2.1.1. Resolution test

An array of cylindrical pillars and rectangular walls (Fig. 1), simply called Pillars and walls from here on, was designed to evaluate the capability of the printer to print basic structures at the sub-micrometer scale. Both geometries were printed with different spacing to identify the printing resolution, in addition to the minimum feature size. For a more detailed schematic, see S.I. Fig. S1.

### Table 1

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>UpNano</th>
<th>Nanoscribe</th>
<th>WOP Altechna</th>
</tr>
</thead>
<tbody>
<tr>
<td>System</td>
<td>NanoOne200</td>
<td>Photonic Professional GT+</td>
<td>FemtoLAB</td>
</tr>
<tr>
<td>Laser wavelength (nm)</td>
<td>780</td>
<td>780</td>
<td>514</td>
</tr>
<tr>
<td>Pulse duration (fs)</td>
<td>95</td>
<td>100–200</td>
<td>&lt;250</td>
</tr>
<tr>
<td>Repetition rate (MHz)</td>
<td>80</td>
<td>80</td>
<td>0.6</td>
</tr>
<tr>
<td>Average laser output power (mW)</td>
<td>125</td>
<td>50–150</td>
<td>10–700</td>
</tr>
<tr>
<td>Minimum XY feature size (nm)*</td>
<td>220</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>XY resolution (nm)*</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Z resolution (μm)*</td>
<td>0.45</td>
<td>1.5</td>
<td>3</td>
</tr>
<tr>
<td>Maximum object height (mm)</td>
<td>42</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>Maximum scan speed (mm/s)</td>
<td>250</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Surface roughness (nm)</td>
<td>10</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Special features</td>
<td>HEPA filter, plug in for biological applications. Tilt compensation stage</td>
<td>Wide online repository of documentation, including literature from system users (NANOguide)</td>
<td>Infinite field of view (FOV) mode for stitchless printing, Hybrid additive and subtractive manufacturing</td>
</tr>
<tr>
<td>System size (cm)</td>
<td>58.5 × 70 × 66</td>
<td>110 × 110 × 130</td>
<td>150 × 135 × 140</td>
</tr>
<tr>
<td>Other available models</td>
<td>NanoOne200</td>
<td>Photonic Professional GT, GT2, QuantumX, QuantumX Shape</td>
<td>FemtoMPP</td>
</tr>
</tbody>
</table>
2.1.2. True 3D test

The Multiplane structure was designed to evaluate the minimal achievable feature sizes on a true 3D structure (Fig. 2). For this purpose (1) pillars and (2) holes with both circular and square footprints were included on planes printed at different angles (0°, 30°, 60°, and 90°). In addition, (3) a spiral geometry, (4) high-aspect ratio columns with a base of 5 × 5 µm², aspect ratio 1:12, and pitches of 0.3, 0.5, 1, 2, and 4 µm, (5) concentric trenches (size 4, 2, 1, 0.5 µm), and (6) a horizontal ladder with step widths of 1, 0.6, 0.3, and 0.15 µm were included on a vertical wall to evaluate the printer’s ability to produce such features in the Z-direction. The dimensions shown and listed here are for the high NA objective. The structure dimensions for the low NA objective was obtained by scaling up the design 7 times in all directions (S.I. Fig. S2).

2.1.3. Free-hanging test

The Free-hanging lines structure design (Fig. 3) evaluated the capability of the systems to print 0.1 µm wide free-hanging lines. The structure was oriented during fabrication so that the free-hanging lines were printed at different angles within the XY plane: 60°, 90°, and 120°. For simplicity, in the Results section, we refer to the lines which were printed as straight lines as “0°”. Because of this coordinate change, we refer to the lines printed at 60° as “−60°” and to those printed at 120° as “+60°”.

2.1.4. Surface roughness test

The AFM lens (Fig. 4) was printed to determine the achieved roughness on curved surfaces using atomic force microscopy (AFM). For the high NA objective, the structure had a diameter of 100 µm and a maximum height of 10 µm. For the low NA objective, the structure was 7 times larger (S.I. Fig. S3), with a diameter of 700 µm and a maximum height of 70 µm.

2.1.5. Stitching test

A Honeycomb pattern (Fig. 5) was designed to evaluate the stitching capability of the systems for different types of geometries, i.e., straight walls and truncated pyramids with a hexagonal base. Individual fields-of-view employed during the printing are squares with a size of 82.5 × 82.5 µm².

2.1.6. Multiscale and multimaterial test

To evaluate the capability of the printers to fabricate structures comprising feature sizes at different scales, as well as the possibility to obtain multimaterial objects, the Multiscale structure (Fig. 6) was designed. The large trapezoidal prism base structure was printed with a low NA objective, and the array of mushroom-like objects on its top was printed using a high NA objective. The trapezoidal prism had a side length of 600 µm. The top circular feature, i.e. platter, was also printed with the low NA objective and had a diameter of 100 µm and a height of 100 µm.

Fig. 1. Pillars and walls array. a) Isometric view. b) Top view. The pillars have a diameter of: (1) 1 µm, (2) 0.5 µm, (3) 0.3 µm. (4) Walls with 0.3, 0.5 and 1 µm widths and with 0.5 µm height. (5). Walls with 0.3 µm width, aspect ratio 5, and 0.3, 0.5, and 1 µm pitch. (6) Walls with 0.5 µm width, aspect ratio 5 and with 0.5, 1, and 2 µm pitch. (7) Walls with 1 µm width, aspect ratio 5, and 1 and 2 µm pitch.

Fig. 2. Multiplane structure. a, b) Isometric view of the a) front side and b) back side. c) Top view. d) 30° view The features included in this design are: (1) Array of cube/circle based pillars with feature sizes of 0.3, 0.5, 1, 2 and 4 µm. (2) Array of cube/circle based holes with feature sizes of 0.3, 0.5, 1, 2 and 4 µm. (3) Spiral. (4) High aspect ratio columns with 0.3, 0.5, 1, 2 and 4 µm pitch. (5) Concentric trenches of 0.3, 0.5, 1, 2 and 4 µm width. (6) Horizontal step structure (step width 0.15, 0.3, 0.5, 1 µm). The dimensions shown are for the high NA prints.
The mushroom-like pillars on top of the platter consist of a pillar with a 6 \( \mu \text{m} \) base diameter and 15 \( \mu \text{m} \) height, and a top with a 10 \( \mu \text{m} \) base diameter and 1.5 \( \mu \text{m} \) height.

2.2. Fabrication

2.2.1. NanoOne250 system

The STL files were imported into the Think 3D software (UpNano), where the print parameters were set and the thinkage job file for 3D printing was generated. Fabrication was done by 2PP DLW on a Nano-One250 system (UpNano GmbH, Vienna, Austria), which uses 95 fs pulses emitted at 80 MHz by a 780 nm Ti-Sapphire laser. All prints were performed on a cube-shaped borosilicate glass substrate (10 \( \times \) 10 \( \times \) 10 mm\(^3\)) (Fig. 7a), b), g)). Before printing, the glass substrates were surface functionalized with methacrylated silane to improve structure adhesion, as recommended by the manufacturer.
using the following protocol. After an air-plasma cleaning (Power: 200 W, time: 1 min) using a Model 3 Atto, (Diener electronic GmbH, Ebhausen, Germany) the glass cubes were immersed in a solution of 47.7 % w/w deionized water, 50 % w/w ethanol, 2 % w/w (3-mercapto-propyl) trimethoxysilane, and 0.3 % w/w acetic acid for 20 min at room temperature. Afterwards, the samples were washed three times with deionized water, dried under nitrogen gas and finally placed on a hot plate at 60°C for 3 h. The functionalized substrates were stored protected from light and used within 1 month from the functionalization. For printing, the glass substrates were mounted on the printer holder and all the prints were performed in vat mode with UpPhoto and UpBrix resin for the lowest NA objective (Olympus, 10 ×/0.40 NA Apochromat) and highest NA objective (Olympus, 60 ×/1.42 N.A, Apochromat), respectively. For the multiscale structure, after the removal of the non-crosslinked resin from the base structure no further surface salinization was done before printing with the high NA objective. For all the
prints the substrate-resin interface was identified by automated focusing. In the case of the multiscale structures, the autofocusing system was calibrated by exploiting the fluorescence intensity difference between the UpPhoto structure and the UpBrix resin.

After the 2PP DLW process, the substrates containing the 3D printed structures were developed by immersing the samples in two subsequent batches of propylene glycol methyl ether acetate (PGMEA) of 10 min each to accelerate the development, followed by a 5 min isopropanol bath and drying at room temperature.

2.2.2. Photonic Professional GT+ system

The STL files were imported into the DeScribe software (version 2.7), where the print parameters were set and the .GWL job file for 3D printing was generated. Fabrication was done by 2PP DLW on a Nanoscope Photonic Profession GT+ (PPTG+) system (Nanoscope GmbH, Karlsruhe, Germany), which uses 150 fs pulses emitted at 100 MHz by a 780 nm Ti:Sapphire laser. For the Plan-APOCHROMAT 63 x/1.40 Oil DIC objective (Carl Zeiss, Oberkochen, Germany) objective, 30 x 30 x 0.7 mm3 fused silica glasses were used as 3D printing substrates in dip-in configuration (Fig. 7c, d), h), and the IP-Dip2 photoresist (Nanoscope GmbH, Karlsruhe, Germany) was employed as polymer precursor. For the 10 x/0.30 NA objective, 30 x 30 x 0.7 mm3 silicon pieces were used as 3D printing substrates in dip-in configuration, and the IP-Q photoresist (Nanoscope GmbH, Karlsruhe, Germany) was employed as polymer precursor. For all the prints, except for the Multiscale mushroom-like pillars, the substrate-resin interface was identified by automated focusing. For the mushroom-like pillars of the Multiscale structure, the substrate-resin interface was identified by automated focusing and a Z-axis offset equal to the approximate height of the base was added to the print such that the pillars were printed at the top of the base.

After the 2PP DLW process, the substrates containing the 3D printed structures were developed by 20 min immersion in PGMEA, followed by 5 min in isopropanol, then placed horizontally and allowed to dry at room temperature.

2.2.3. FemtoLAB system

The STL files were imported into the SCA software provided by WOP (Altechna, Vilnius, Lithuania), where the print parameters were set and the structure was sliced for the fabrication process. The structures were fabricated via 2PP DWL on a FemtoLAB system (WOP Altechna, Vilnius, Lithuania), which uses 244 fs pulses emitted at 601.8 kHz by a laser using a 514 nm wavelength. All prints were performed on a 170 x170 mm2 glass substrate (18 x18 mm2 thickness of ~ 15 nm using an evaporator (DV-5028 High Vacuum Evaporator from Denton Vacuum, Moorestown, NJ, USA). The samples for the Multiscale and Honeycomb pattern were instead coated with a 25 nm thick gold layer. The imaging was done using a Hitachi S-2700 (Hitachi High-Tech, Tokyo, Japan) SEM and a Gemini Ultra 55 (Carl Zeiss AG, Oberkochen, Germany). The images were acquired either using the secondary electron detector or the InLens detector. An acceleration voltage of 3 kV in high vacuum mode was used for both detectors.

The FemtoLAB samples were coated with a thin layer of aluminum (70 nm) using an evaporator (DV-5028 High Vacuum Evaporator from Denton Vacuum, Moorestown, NJ, USA). The samples for the Multiscale and Honeycomb pattern were instead coated with a 25 nm thick gold layer. The imaging was done using a Hitachi S-2700 (Hitachi High-Tech, Tokyo, Japan) SEM and a Gemini Ultra 55 (Carl Zeiss AG, Oberkochen, Germany). The images were acquired either using the secondary electron detector or the InLens detector. An acceleration voltage of 4 kV in high vacuum mode was used for both detectors.

2.3. Characterization

2.3.1. Scanning electron microscopy

Prior to scanning electron microscopy (SEM) imaging, the NanoOne250 structures were coated with a layer of gold with a thickness of ~ 3 nm using a sputter coater (Polaron SC7640, Quorum Technologies, Newhaven, UK). Imaging was done using a Zeiss LEO 1550 (Carl Zeiss AG, Oberkochen, Germany) SEM and the images were acquired either using the secondary electron detector or the InLens detector. An acceleration voltage of 3 kV in high vacuum mode was used for both detectors.

The PPGT+ samples were coated with a layer of gold with a thickness of ~ 15 nm using a sputter coater (Cressington Scientific Instruments, Watford, UK). Imaging was done using a Zeiss Supra 40 VP (Carl Zeiss AG, Oberkochen, Germany) SEM and the images were acquired either using the secondary electron detector or the InLens detector. An acceleration voltage of 3 kV in high vacuum mode was used for both detectors.

2.3.2. Atomic force microscopy

An atomic force microscopy (AFM) system was used to measure the surface roughness of the AFM lens structures. The AFM lens structures were characterized by AFM (Dimension Icon-PT from Bruker, Billerica, MA, USA) prior to metallization for SEM imaging, to ensure that the structure roughness achieved by the printers is not affected. Two 10 x 10 µm2 areas on two separate structures were chosen approximately halfway between the center and the edge of the lens-like structure and scanned to calculate the average roughness, presented as average ± standard deviation. Tapping mode imaging with Tap150-Al probe (BudgetSensors®, Innovative Solutions Bulgaria Ltd, Sofia, Bulgaria) with a normal spring constant of 5 N/m and a resonance frequency of 150 kHz was performed to extract the topology of the specific area on the surface. The software NanoScope Analysis, an AFM data processing software, was used to first flatten the measured data to a planar surface by removing the bow and tilt, and subsequently for computing the root-mean-square roughness (Rq). Rq is defined as the root-mean-square average of height deviations taken from the mean image data plan [29]:

\[ R_q = \sqrt{\frac{\sum_{i=1}^{n} (Z_i - \overline{Z})^2}{n}} \]

where \( Z_i \) is the current Z value, \( \overline{Z} \) is the mean Z value of all points within the area and \( n \) is the number of points.

2.3.3. Optical profilometry

A Profilm3D optical profiler from Filmetrics (KLA Corporation, Milpitas, CA, USA) equipped with a 10 x/0.30 NA objective was used for 3D topographic imaging of the large AFM lens structures in vertical scanning interferometry (VSI) mode. The data acquisition was done using the Profilm software, which was subsequently used to compute the maximum height of each structure from a line profile passing through the center of each AFM lens.

2.3.4. Image analysis

The computer-aided manufacturing vs. computer-aided design (CAM/CAD) mimicry was investigated using CellProfiler [30] and FIJI
analysis of various SEM images of the different structures [31]. The eccentricity of all circular features was analysed using CellProfiler analysis. The quantitative analysis of the different objects features was performed as follows. For the pillars and walls structure, all features were analysed with CellProfiler. For the Multiplane structure, the pillars and holes (0° and 30° planes) were analysed with CellProfiler, whereas the distance between the columns and the step width of the horizontal ladder were measured using FIJI. For the Free hanging lines structure, the width of the segments and the distance between segments were measured using FIJI. The mushroom-like pillars on the top of the Multiscale structure were analysed with CellProfiler. All CellProfiler pipelines are available as SI.

3. Results and discussion

The aim of this work was to evaluate a large range of fabrication needs for diverse applications of 2PP DLW in the micro-, meso- and macroscale, and thus to provide information on the printing possibilities to interested users of 2PP as an additive manufacturing technique. In order to address different size scales, two distinct microscope objectives were chosen for the fabrication: the highest NA objective of each printer (60×- or 63×-), and a lower NA objective (10×-), which is the standard solution for macroscale fabrication. In order to address different printing needs, which would reflect in different features of the 3D printed objects, 6 different designs were made, as described in Section 2.1. Most designs were fabricated using the highest NA objective, and two selected designs (the Multiplane and AFM lens structures) were also fabricated using the macroscale fabrication objective. Finally, one design (the Multiscale structure) was fabricated using the macroscale objective and the highest NA objective serially.

The fabrication parameters for all structures were optimized by users of each instrument. The aim of structure optimization was to improve the processing accuracy, i.e., dimensional accuracy, shape accuracy, and surface roughness [8]. Several fabrication parameters were considered in the optimization: laser power, scan speed, infill line direction, hatching and slicing distances, and number of contour lines. For both the NanoOne250 and the PP GT+ systems, the starting point for the optimization was the standard recipe provided by the company (S.I. Table S1).

The overall goal for the optimization was to identify fabrication parameters that allowed for a good trade-off between printing accuracy and printing time. However, several important aspects should be noted here. First, the investigated printers present different printing modes, laser sources and objectives making a comparison with the same printing parameters not suitable for a valid evaluation. Consequently, each user had to obtain the optimal structure with a trade-off of the different printing parameters specific to the printer. Second, different printers from the same manufacturer can be slightly different, so it is possible that simply using a different piece of equipment could generate slightly different results. Third, it is possible that the structures described herein could be further optimized, possibly by considering other fabrication parameters, such as the laser beam path, hatching angle, surface pre-treatment, using an improved development procedure with additional UV exposure during the isopropanol development step [32], or other post-processing methods such as UV curing and thermal treatments [9]. Furthermore, the companies are continuously providing software updates, which contribute to improving the sample slicing, the laser path and tuning, and fabrication times. Finally, this study compares preset configurations wherever possible, i.e. the use of a certain microscope objective together with a certain resin, as recommended by the manufacturer. It is possible that installing a different microscope objective or employing a different resin could further improve the fabrication performance. However, in the context of this paper, preset configurations were deemed to be the most relevant, as this is what most new users of such equipment would normally have access to.

The make and model of every printer, as well as details about the microscope objectives, are listed in Section 2.2. For simplicity, this section will simply refer to the different printers using the printer model name and, where relevant, the objective employed for the fabrication. The printing parameters are reported in S.I. Table S2.

Besides the printing parameters discussed in Section 3.1, the hardware, software and resin employed for the fabrication process also play a key role in determining the quality of a print. Consequently, these aspects are discussed in Section 3.2–3.4, respectively.

3.1. Optimized structures

To assess the highest resolution and the minimal feature sizes achievable by each system, the highest NA objectives were employed to fabricate several designs, as described below. All three systems have a high NA objective, but there are differences in the magnification and NA of these objectives (see Section 2.2). One of the main parameters for evaluating the printing accuracy of the systems is the CAM/CAD ratio, which is defined as the measured size of a feature of the fabricated structure divided by the size of the same feature in the CAD 3D drawing. A CAM/CAD ratio of 1 signifies a perfect match between the designed and fabricated size of a feature. Another noteworthy parameter for circular features is the eccentricity. A perfect circle has an eccentricity of 0. An eccentricity higher than 0 means that the shape deviates from that of a perfect circle, with higher values meaning that the object is more elliptical.

3.1.1. Pillars and walls

The pillars and walls design provides information about minimal feature sizes, resolution, and the influence of the aspect ratio (A.R.) over these two structure characteristics. Fig. 8 shows SEM top views of the pillars and walls design printed using all systems discussed in this paper, as well as results from the data analysis. Structures that were not resolved, i.e. high A.R. 0.3 µm pillars printed on all systems and high A. R. 0.3 µm walls printed on the PP GT+ and FemtoLab systems were excluded from the data analysis. For all high A.R. structures, the analysis was performed using the set with a higher separation between the structures.

All the investigated structures were printed with a low and high A.R. to investigate the A.R. influence over the minimal achievable feature size and printing fidelity. In addition, the structure stability and printer resolution were investigated by printing the A.R. structures at different distances (0.3, 0.5 and 1 µm).

As a general observation, both the eccentricity and CAM/CAD ratio improved as the feature size increased. The low A.R. structures generally showed a CAM/CAD mimicry closer to 1 than the high A.R. samples, Fig. 8(d), e) and f). The A.R. influence over the resolution and printing fidelity was associated with three main mechanisms. The first is related to the galvanometric scanning resolution and precision [8]. The second is that the position-laser mismatch can cause submicron differences over multi-layer structures [33]. The third is that undesired resin polymerization can occur due to voxel overlapping in multi-layer structures [34]. These mechanisms can occur simultaneously, influenced by the printing technique and fabrication parameters. Finally, vibrations in the environment can also influence the process, but all the systems described herein are equipped with a vibration isolation system.

For the pillar structures, no relevant differences in CAM/CAD mimicry were observed among the investigated systems and all printers displayed average diameter values bigger than the intended ones, Fig. 8(e). All the systems presented a clustered array for the shallow 0.3 µm pillars and only the FemtoLab system managed to resolve the high A.R. pillars with a 0.5 µm inter-pillar distance. The higher fidelity was attributed to the lower wavelength of the FemtoLab system, which ensures a smaller voxel size and thus enables higher resolution and minimal feature size [35]. Such samples represented a challenge since they are at the systems’ printing limit in terms of resolution and minimal feature size. The pillars were printed in 5 × 5 arrays to investigate the repeatability for small batches. The NanoOne250 presented 2 detached
pillars in the 0.5 µm feature size pillars, which is most likely due to the development process, Fig. 8a).

The NanoOne250 system presented the closest wall CAM/CAD mimicry for both the low and the high A.R. structures, with values around 1 and 1.3, respectively. The FemtoLAB system displayed CAM/CAD values closer to 1 than the PPGT+ printer, Fig. 8d). Interestingly, both the NanoOne250 and FemtoLAB systems achieved 0.3 µm resolution for the low A.R. walls, while the PPGT+ presented a lower resolution, 1 µm, for the wall structures, S.I. Fig. S4. For the low A.R. pillars with 0.5 µm spacing, the FemtoLAB print resolved all structures, whereas the NanoOne250 prints presented a few merged pillars, and the PPGT+ had the majority of pillars connected by a thin resin line, S.I. Fig. S5. The different outcome is probably related to the simpler wall geometry when compared to the pillar design, underlining the feature geometry’s influence on printing fidelity [36].

The FemtoLAB system displayed straight walls over all the tested conditions, while the PPTG+ and NanoOne250 presented for some conditions no resolved and collapsed walls, respectively, S.I. Fig. S5). The collapsing/adhesion of dense and high aspect ratio structures is also a well-known challenge in single photon lithography caused by the surface tension of the developer solution [37].

The FemtoLAB system generally showed a uniform and smooth surface with well-defined edges for all the printed geometries. On the other hand, the structures obtained with NanoOne250 and PP GT+ generally presented a more irregular surface with a lower accuracy over the pillar eccentricity, Fig. 8a,b, c) and f). The different results can be associated with different printing strategies. For the NanoOne250, a slower writing speed (10 mm/s) was chosen for writing the contours, while a faster printing speed (40 mm/s) was implemented for the structure core. On the PP GT+ , both the contours and the structure core were written using the same parameters and a speed of 7.5 mm/s. The FemtoLAB system printed the structures as a monolith, i.e. with no contour lines, but with fine hatching and slicing dimension. Despite resulting in high-quality printing, this approach resulted in a lengthier fabrication time. The NanoOne250 and PP GT+ systems were around 23 and 17 times faster than the FemtoLAB printer, respectively. It should be noted that after a software update, the fabrication time for the Pillars and walls structure on the FemtoLAB printer was reduced to 1 min 58 s, which is still 11 and 7 times slower than the NanoOne250 and PP GT+ , respectively. However, the use of contour lines for writing small features resulted in artefacts, which can be seen as an uneven surface at the edges of some structure geometries, e.g. the 1 µm diameter pillars (S.I. Fig. S6).

For a more detailed list of methods to improve the 2PP processing accuracy in terms of surface roughness, achievable feature sizes and resolution, the reader may refer to the following review [8].

The shown printer capabilities can find applications in the functionalization of surfaces with micron or submicron features, including wetting properties [38], antifouling and bacterial resistance [39], diagnostic in microfluidic devices [40] and diffraction/focusing of light [41]. In addition, the systems have the potential to create templates with structures down to submicron structures for replica molding to achieve high throughput [42].

3.1.2. Multiplane structures

The Multiplane structure comprises numerous different features with both straight and curved lines and thus represents a more comprehensive assessment of the systems’ capability to print true 3D objects with different features on the microscale, as the features are positioned at various angles, perpendicular to a plane ranging from the XY plane (0°) to the Z-plane (90°). For all printers, the structures were fabricated layer by layer, starting from the base of the structure.

The ability to produce pillars such as those on the Multiplane structure is relevant for e.g. metasurfaces [43], cell interaction with surfaces [44], or mold fabrication [45], while producing small holes can be of interest for e.g. human physiology mimicry [46], filtration systems [47], and microfluidics [48]. The pillar and hole features were repeated on planes at different angles for a more comprehensive assessment of the processing accuracy, given the fact that the laser focal spot is known to have an ovoid shape [10]. Due to stage tilting limitation in the SEM imaging, the quantitative analysis was performed just on the 0° and 30°
planes. Compared to the pillars and walls design, the multiplane structure includes convex and concave objects with submicro-, micro- and mesoscale feature sizes. Consequently, selecting a set of fabrication parameters that accommodate all such features on a relatively large structure represented a challenge.

The printing quality was indeed best on the 0° plane, as expected. The average CAM/CAD ratio was slightly higher on the 30° plane compared to the 0° plane, while the eccentricity was significantly higher for the 30° plane, Fig. 9(g)–j). On the tilted planes, the surface also appeared rougher and the features were less uniform. An influence of the printing plane over the printing quality was expected as the voxel geometry presents an elongated shape along the Z-axis, with a voxel height roughly 3.5 times larger than the voxel diameter [10]. The fact that the voxel height is larger than its diameter leads to the fact that feature sizes and resolution are better in XY than in the Z direction. In particular, the layers on the NanoOne250 30° plane were more visible than for the 30° plane fabricated using the other two systems, Fig. 9(a)–f). The typical 3D printing layer-by-layer structure observed on the NanoOne250 system resulted from a different slicing distance, 1.7 and 2.5 times bigger than the PPGT+ and the FemtoLAB, respectively. Such parameters probably also caused the absence of the 0.3 and 0.5 µm pillars on the NanoOne250 for 60° and 90° planes, while contributing to the reduced printing time (NanoOne250: 00:10:33, PPGT+: 1:01:06 and FemtoLAB: 0:38:20). Despite the less stringent slicing interval, the NanoOne250 was the only system to display the submicron holes on the tilted planes, probably due to using the lowest area hatching (2.25 and 9 time smaller than the PPGT+ and FemtoLAB, respectively). Occluded cavities in the PPGT+ and FemtoLAB prints could also occur as a consequence of resin over-exposure, due to the reduced layer thickness / increased number of layers employed in the fabrication. The PPGT+ prints displayed results comparable to the Pillars and walls structure, while the FemtoLAB prints showed clogged cavities for diameters below 1 µm. The different result was mainly attributed to the higher scattering of the laser beam focal point during printing, which occurs due to the bottom-up printing technique employed on the FemtoLab system [49]. In this case, unlike for the NanoOne250 and PPGT+ prints, the laser beam needs to travel through the solid printed structure, which has a different refractive index than the unexposed resin. This leads to improper focusing of the beam and defects in the printed structure [12]. Additionally, the FemtoLab print resulted in an overall lower structure height compared to the design. The structures fabricated with the FemtoLab also show a shape deviation of the 0° plane, Fig. 9(e). This curvature is a result of the printing configuration, as well as of the SZ2080 resist preparation. The SZ2080 tempering test indicated that the influence of time and temperature is significant for producing a flat surface. Further testing must be performed to improve the preparation of this resist for fabricating tall structures. The Multiplane structure from FemtoLAB shown in this paper had the lowest overall curvature achieved among structures tested with various tempering parameters. However, it is likely that further optimizing the tempering process could result in a Multiplane structure of better overall quality, as the issues encountered seem to come from resist tempering rather than from the printing capabilities of the FemtoLAB system itself.

All three systems successfully printed all pillar sizes on the 0° plane, while the 0.3 µm pillars were broken for the NanoOne250 system on the 30° plane, Fig. 9(a)–f). The FemtoLAB system printed pillars with a smaller height than the CAD file, associated with either an unknown software artefact or the oil immersion printing mode, Fig. 9(e)–f). The holes with sizes below 1 µm were clogged on both planes of the PPGT+ system and the FemtoLAB system prints, Fig. 9(d)–j). The NanoOne250 system printed all the holes down to 0.3 µm and 0.5 µm on the 30° and 0° planes, respectively, Fig. 9(b).

On the 0° and 30° planes, both the CAM/CAD ratio and the eccentricity generally improved as the feature size increased, Fig. 9(g)–j), as also observed for the pillars and walls samples (Section 3.1.1). Specifically, the fabricated pillars had a higher diameter than the CAD drawing, while the holes had a lower diameter. This behavior can be attributed to two factors. First, in the hardening of a structure, a “point” corresponds to the center of the laser beam focal spot, so it is expected that polymerization will occur in the surrounding area. Second, including contour lines during fabrication stabilizes and improves the features, but presents the drawback of increasing the pillar thickness and decreasing the hole aperture [50]. Naturally, this effect is more noticeable for smaller features. The NanoOne250 and PPGT+ systems displayed similar results for the standing pillars, whereas the FemtoLab system showed slightly worse CAM/CAD ratios. For the holes, the NanoOne250 and FemtoLab systems presented a better CAM/CAD mimicry for holes with a size > 1 µm on both the 0° and 30° planes, whereas the PPGT+ showed slightly worse CAM/CAD ratios. The NanoOne250 system performed well with both pillars and holes structures, and it was the only one able to produce holes with sizes < 1 µm using the selected printed parameters. Interestingly, for both the NanoOne250 and the PPGT+ systems, square pillars displayed a higher CAM/CAD mimicry compared to round pillars, while the opposite can be seen for the FemtoLab system. This could be explained by differences in the laser scanning path defined in the different software. For the holes, all printers showed a better CAM/CAD mimicry for square features. Such behavior can be related to the simpler geometry as also observed when comparing the resolution between pillars and walls in Section 3.1.1.

The tested features demonstrated the capability of the systems to create pillars at different angles that may find application as a platform to investigate the interaction and adhesion of cells and small organisms with materials, as shown in previous studies [44]. In addition, the obtained holes at different plane angles pave the way for the fabrication of complex microfluidic platforms with channels not confined to the XY plane [51,52].

In the back of the Multiplane structure, the spiral feature was fabricated to investigate the capability of the systems to fabricate intersecting curved surfaces at the microscale. All the systems successfully printed the spiral and the trenches running along the structure. However, the PPGT+ and FemtoLAB spirals resulted smoother than that printed using the NanoOne250 system, similar to what was observed on the 30° plane, Fig. 10(a)–c).

The column structure combines high-aspect ratio and distance intervals down to the submicron scale, which represents a challenge for the printers, in terms of resolution and structure stability. The smallest resolved distance between the columns was 0.3 µm with the NanoOne250, 0.5 µm with the PPGT+ and 1 µm with the FemtoLab system, Fig. 10(d). Both the NanoOne250 and PPGT+ systems presented a bending column, Fig. 10(a) and b). The bending of the column caused CAM/CAD ratio bigger than 1 for one of the distance intervals in the NanoOne250 and PPGT+ systems. The columns displayed a solid base-substrate adhesion excluding a detachment issue for both systems. Deformations caused by shrinking/swelling of the material were also discarded as defects such as bending, cracks or structure detachment over the printed samples were not observed. Considering the fact that the bending occurs for a tight spacing between the columns, the deformation was hypothesized to occur due to the capillary forces exerted during the drying of the development solution.

The recent development of droplet microfluidics has benefited from the development of concentric channels for the fabrication of droplets [53]. Concentric trenches can represent a simplified design of such a system. For this structure, the NanoOne250 system obtained trenches down to 0.3 µm while the PPGT+ and FemtoLAB systems displayed clogged sections in the 0.3 and 0.5 µm features localized along the Z-axis of the trenches.

The horizontal staircase step scope was to investigate the XY minimal feature size of the printers. The NanoOne250 and FemtoLAB systems displayed a better CAD/CAM ratio than the PPGT+ for features sizes below 0.5 µm. The systems displayed comparable results starting from 0.5 µm steps sizes, Fig. 10(e).
Fig. 9. Multiplane structure printed with the high magnification objective. a-f) Scanning electron micrographs. a, c, e) Overview of the structure. b, d, f) Pillar and hole arrays (scale bar = 5 µm). The structures were fabricated using the a, b) NanoOne250, c, d) PPGT+, and e, f) FemtoLAB systems. g-l) Results from data analysis: g - j) CAM/CAD ratio for the g, i) pillars and h, j) holes with g, h) square and i, j) round base. k, l) Eccentricity of the round i) pillars and l) holes.
As observed for the features printed on the front of the structures, the structure tolerance drastically reduces when the feature size moves from submicron to micron scale.

The same multiplane structure design was printed with the low NA objective with a 7 × larger size compared to the high NA print. This was meant to demonstrate the capability of the systems to fabricate a millimeter-scale sample and to investigate the influence of the NA on the printing fidelity.

Similarly, as in the case of the high NA Multiplane structure, the surface quality was superior for the 0° plane than the 30° plane. No relevant differences were observed between the pillar and hole eccentricity of the two systems, Fig. 11 h). The NanoOne250 system displayed an apparently rougher surface on the 0° plane, which can be attributed to the 1.6 times bigger hatching dimension compared to the PPGT+ printer. On the other hand, a 4 times smaller slicing interval than the PPGT+ printer resulted in a smoother surface on the tilted printing surface, Fig. 11 c) and d).

Interestingly, based on visual inspection, the pillars on the NanoOne250 0° plane showed a reduced surface quality when compared to the top plane surface of the bulk structure fabricated using the same printer. Since the printing parameters employed were the same for the entire structure, this quality difference can only be attributed to the printing software or to the properties of the resin employed for the fabrication. The holes resulted in CAM/CAD ratios smaller than 1 for both systems, with the exception of the 3.5 µm diameter square holes printed using the PPGT+ system. Overall, the PPGT+ printer achieved hole diameters closer to the intended size than the NanoOne250 system that did not resolve the smallest circular holes on the 0° plane, Fig. 11f). The pillar bottom and tops resulted in bigger and smaller diameters, respectively, than the intended value in both systems, with the NanoOne250 displaying a slightly better pillar CAM/CAD mimicry compared to the PPGT+ printer, Fig. 11e) and g). As observed for the high NA, both printers fabrication quality improved as the feature size increased.

Both systems presented pillars with a conical pyramid shape, with more tilted side walls for the PPGT+ printer than the NanoOne250 system, Fig. 11c) and d). This observed shape is related to the bigger and more elongated voxel size of low NA objectives. It is also expected that the effect is more noticeable on the PPGT+ system, which has a NA of 0.3 as opposed to 0.4 on the NanoOne250 system. Interestingly, the lower NA of the PPGT+ did not result in a faster print when compared to the NanoOne250, despite the fact that the PPGT+ used the highest available printing power, Table S2. The low NA of the objectives caused the bottom part of the pillars to print bigger in diameter, because of undesired material crosslinking during the laser fabrication of the plane surface, while the top of the pillars were smaller than intended, Fig. 11e) and g). The smaller NA effect on the printing quality was even more visible on the 30° tilted plane of both printers. The pillar side facing the substrate displayed a tilted wall instead of being perpendicular to the plane, Fig. 11c) and d). A similar artefact is expected to happen inside the holes as well, even if it was not possible to visualize with the SEM microscope.

Despite the spiral being printed by both printing systems, the trenches were poorly visible (top) or absent (side), Fig. 12a) and c). Similar to the observation made for the 30° plane, the smaller slicing dimension resulted in a better spiral surface quality for the NanoOne250 system.
Fig. 11. Multiplane structure printed with the low magnification (10 ×) objective. a-f) Scanning electron micrographs. a, b) Overview of the structure. c, d) Side view, scale bar 50 µm. The structures were fabricated using the a, c) NanoOne250 system, and b, d) PPGT+ systems. e-g) CAM/CAD ratio for the features fabricated on the e) square pillars, showing measurements from both the top and the bottom of the pillars, f) holes, and g) round pillars showing measurements from both the top and the bottom of the pillars. h) Eccentricity of the round pillars and holes.
system. Regarding the columns, both systems presented upright columns without any bending. The PPGT+ presented CAM/CAD ratio closer to 1 for distances ranging from 3.5 µm up to 7 µm than the NanoOne250, Fig. 12 c), g) and i). The trenches of both printers presented clogged sections in the Z direction due to the elongated shape of the voxel. The PPGT+ system also presented a more layer-by-layer structure visible at the edge of the trenches, Fig. 12 d) and h). For the staircase steps the PPGT+ system presented a better CAD mimicry for the smaller features while the NanoOne250 printer performed better for the bigger staircase steps, Fig. 12 l). The structure was printed in 29 min and 44 min by the NanoOne250 and the PPGT+, respectively.

As observed for the high NA, the two printers’ tolerance reduced as the feature size increased. The low NA objective allowed faster printing for the fabrication of objects to achieve an mm-scale object with micron resolution features. The bigger voxel drastically increases the printing speed but also limits the printing fidelity for features below 10 µm. In particular, the obtained pillars on the 30° plane demonstrated how structures on tilted planes can be affected. The small NA objective still maintains the capability to realize free-standing objects and hollow structures but the generation of artefacts of a few microns due to the voxel size has to be taken into account [54].

3.1.3. Free-hanging structure

Free-hanging structures, which are necessary designs in some fields, are challenging to produce by lithography, extrusion or single photon-based printing techniques. The possibility to generate such structures at different angles by 2PP DLW, without the need for supports or sacrificial layers, has paved the way for studies in fields including sensor [55], photonics [56], two-component metamaterial [57] and biology with scaffolds [44,58–60] for cell force measurements [61]. Thus, a Free-hanging lines design was included in this study.

All the systems successfully fabricated free-hanging structures, Fig. 13a–c). However, the 0.1 µm thickness of the overhanging segments represented a challenge for all printers, which is natural, given that the voxel size in the XY plane is bigger than 0.1 µm. The PPGT+ printer displayed the closest CAD mimicry, with a CAM/CAD ratio of ~3 and a decreasing printing quality from 60° to +60°. The NanoOne250 and FemtoLab systems showed a CAM/CAD ratio of ~4 and ~5, respectively. No significant difference was measured between the overhanging lines printed at different angles for the PPGT+ and the NanoOne250, whereas the FemtoLAB system displayed better results for the lines printed at 0°, Fig. 13d).

No significant bending of the overhanging lines was observed, as demonstrated by the CAM/CAD pitch values of ~1 measured for all printers. The PPGT+ system properly resolved the lines as placed entirely on the top of the pillars. At the opposite end, the overhanging lines were fully embedded into the cylindrical support pillars for the FemtoLAB print. The NanoOne250 print lines appear to be also on top of the pillars, albeit less defined than on the PPGT+. This could be due to the reduced exposure dose used on the PPGT+ to fabricate the thinnest overhanging lines, which likely resulted in an incomplete crosslinking of the pillar structure, Fig. 13b) and S.I. Table S2. The printing time was
Fig. 13. a–c) Scanning electron micrographs of the Free hanging structure taken at an angle of 30°. The structures were printed using the a) NanoOne\textsubscript{250}, b) PPGT\textsuperscript{+}, and c) FemtoLAB systems. d) CAM/CAD ratio for the hanging lines and the pitch between them.

3.1.4. AFM

The roughness of a structure plays a key role in e.g. cell biology \cite{62}, the wetting behavior \cite{63} of a surface, or the quality of optical components \cite{64}. Optical components typically require a very low roughness tolerance and defects to reduce artefacts. The AFM lens structure is a simple design that allows us to investigate the surface roughness of a curved object fabricated by the 2PP DLW systems investigated here. The AFM measurement was conducted at the half height of the lens.

From a visual inspection, the FemtoLAB prints displayed a very smooth surface with no noticeable Z-layer lines, Fig. 14c. Z-layer lines are instead visible on the PPGT\textsuperscript{+} prints, Fig. 14b) and the NanoOne\textsubscript{250} prints, Fig. 14a). The root-mean-square roughness (Rq) obtained using the high NA objective was of $8.6 \pm 2.5, 11.3 \pm 0.1$ and $7.6 \pm 0.8$ for the NanoOne\textsubscript{250}, PPGT\textsuperscript{+} and FemtoLAB prints, respectively, Fig. 14f). The measured roughness is likely a consequence of several factors, including fabrication parameters, as well as properties of the resin and factors related to the software which controls the printing process. The presence of surface roughness could be further reduced by post-processing treatment using thermal reflow of polymer-based resins \cite{65} and pyrolysis \cite{66}.

As expected, the $10 \times$ objectives fabricated structures with higher roughness and a more visible step-like profile, with an Rq of $154 \pm 31$ and $29.3 \pm 15$ for the NanoOne\textsubscript{250} and PPGT\textsuperscript{+} prints, respectively, Fig. 14f). The resulting roughness might not be suitable for fabricating optical components or other structures where surface roughness represents a critical factor. The NanoOne\textsubscript{250} print obtained with the low magnification objective, Fig. 14d) showed a Rq around 5 times higher than the equivalent print from the PPGT\textsuperscript{+} system, Fig. 14e).

The lens displayed the longest printing time when normalized to the structure volume due to the very stringent printing parameters to achieve the smoothest surface (High NA: FemtoLAB: 27:40, PPGT\textsuperscript{+}: 07:38, NanoOne\textsubscript{250}: 08:53, Low NA: PPGT\textsuperscript{+}: 09:53 NanoOne\textsubscript{250}: 02:17).
3.1.5. Stitching

The first 2PP systems were extremely slow, and the single microscale object fabrication took several hours. With the implementation of faster printing strategies enabled by galvanometric mirror systems, 2PP started allowing for mesoscale structure fabrication with micron feature sizes in more reasonable time intervals. Every microscope objective has a certain field of view, as well as a certain effective writing field for 2PP DLW when using galvanometric mirror scanning. Outside of this effective writing field, aberrations occur, so large objects need to be fabricated by stitching several independent print areas [67]. This can lead to imperfections, i.e. stitching errors, in the final product. The Honeycomb pattern was made to investigate this effect. As an alternative, the FemtoLAB system uses the infinite field of view (IFOV) technology to print structures larger than the field of view by simultaneously using stage motion together with the galvanometric scanner to create a continuous processing flow [68].

As can be seen in Fig. 15, all systems successfully printed the structure, even if stitching lines can be seen in the higher magnification images for the NanoOne250 and PPGT+ systems. From a visual inspection, the NanoOne250 prints displayed more surface artefacts and poorer surface quality in the overlapping zones. On the other hand, The PPGT+ system prints presented a smoother surface, but a higher degree of misalignment, Fig. 15a-b). While for most applications, the quality should be suitable, a more thorough study should be performed for structures where small imperfections might be critical, such as optical or microfluidic components, or structures intended for mechanical properties testing, where such imperfections are likely to be the source of mechanical failure. In contrast, the FemtoLAB system displayed a remarkable structure without showing any defects or alignment marks thanks to the IFOV system, Fig. 15c). The IFOV setting extends the field of view of by combining the motion of both linear axes with the galvanometric scanner. While the slow stage is moving in the direction of the hatching path, the fast galvanometric scanner controls the laser path and writes the lines that are in the field of view while the stage is in motion. Thus, simple lines that go beyond the field of view of a single stage position can be printed following the hatching path.

3.1.6. Multiscale structure

The need to combine large structures with micro-sized features still represents a challenge in 3D printing. Fabrication of submicron features requires a high NA objective that in turn results in a remarkable increase in printing time and risk of obtaining stitching errors. As an alternative, a two-step printing strategy combines the printing speed of a low NA objective for large structures with the fabrication of the smallest features by using a high NA objective. This approach, which involves the delicate step of aligning the small feature print with the already printed object, was investigated with the Multiscale design. The alignment was performed by the operator as neither system is equipped with an automated aligning system. However, because of the sequential nature, this strategy can cover applications where multi-material structures are required, such as Metamaterials [57]. This structure was not printed using the FemtoLAB system because this was not equipped with a low NA objective.

The fabrication of the Multiscale structure was successful with both printers, and no significant printing fidelity differences were identified. In both cases, all of the mushroom-like pillars remained upright with a CAM/CAD ratio of ~1 (1.02 ± 0.00 and 0.99 ± 0.02 for the NanoOne250 and PPGT+ systems, respectively), and an eccentricity of ~0.2 (0.18
\[ \frac{\pm 0.03}{0.06} \] and \[ \frac{0.21}{0.06} \] for the NanoOne\textsubscript{250} and PPGT\textsuperscript{+} systems, respectively, Fig. 16 a) - f. The base surface of the NanoOne\textsubscript{250} sample displayed radial lines as STL file facet artefacts, Fig. 16 a). Interestingly, the PPGT\textsuperscript{+} presented mushroom stems with a larger base than the top, and some of the top features were tilted towards the centre of the array, Fig. 16 f). The base structure, obtained with the low NA objective, displayed bent corners indicating deformation and a local detachment of the structure, Fig. 16 d). The total printing time was of 2 min 22 s and 9 min on the NanoOne\textsubscript{250} and PPGT\textsuperscript{+}, respectively. However, this strategy resulted in a time-consuming multistep protocol involving the removal of the sample from the printer for development and drying steps, followed by reintroducing the structure into the system with the new resin and writing the fine features with the high NA objective. The overall fabrication process took several hours on both systems, with the sample drying being the most time-consuming step. Besides being a lengthy process, this entailed that the sample was exposed to contaminants and possible damage. As an alternative, the use of a single resin removes the laborious resin exchange while still requiring the delicate objective exchange and structure alignment. On the other side, fabricating everything with the high NA objective would also be time-consuming - for example, the total print time of the base using the PPGT\textsuperscript{+} high NA objective would be approximately 4.5 h, which is \( \sim 45 \) times higher than the print time when using the low NA objective. Furthermore, using the high NA objective requires stitching, since the base structure is significantly larger than the effective printing field of the high NA objectives [67]. This can introduce further fabrication errors and requires additional optimization.

Beyond the alignment, the interaction of the different materials and the resin exchange technique can play a key role. While the first was resolved here by using materials (polyacrylates) with similar chemistry and shrinking/swelling to prevent deformations, the second aspect still represents a challenge despite the multiple applications for multi-

Fig. 15. Scanning electron micrographs of the Honeycomb pattern printed using the a) NanoOne\textsubscript{250}, b) PPGT\textsuperscript{+} and FemtoLAB systems. The higher magnification images show the stitching lines in more detail.
material printing, including 4D printing [57,69,70] and biology [58]. More examples can be found in a recent review [21].

Another approach includes the use of microfluidics, as previously done for stereolithography printers [71], to quickly exchange the resins in situ. This strategy uses small volumes of resin to allow a fast exchange of the resin while maintaining the sample in a protected environment. However, the print quality can be negatively affected due to the sub-optimal printing distance between the substrate and the objective. Moreover, the structure height would be confined to the geometry of the microfluidic device. Recently, a commercial solution for efficient and automated resin replacement directly at the objective in dip-in mode, currently available only for the PPGT̂ system, was developed and is commercialized by HETEROMERGE [72]. The system simplifies the resin exchange significantly when using a single objective but does not solve the problem of switching objectives when various feature sizes are required.

3.2. Hardware

In addition to the design of the structures printed, laser power and wavelength have a significant influence on a system’s capability. Besides increasing the printing speed, a powerful laser enables the use of low NA objectives for the generation of millimeter and centimeter scale objects. In addition, a powerful laser increases the range of resin availability, enabling the printing of poorly sensitive materials, such as hydrogels and single-photon resins with low two-photon absorption [73]. The laser wavelength, which is 780 nm for the NanoOne250 and the PPGT̂, and 514 nm for the FemtoLAB system, contributes to defining the light penetration depth and the voxel size (V ~ λ^2) and, therefore, the minimal achievable feature dimension [35]. It should be noted that the reduction of undesired light penetration results in printing depth limitations for the bottom-up and top-down modes, commonly used for direct 3D printing inside a carrier such as a microfluidic chip [74].

The laser scanning to generate the object layer-by-layer is performed by galvanometric scanners (NanoOne250, PPGT̂, FemtoLAB) or by using a piezoelectric XY stage (PPGT̂, FemtoLAB). The galvanometric scanners provide printing rates around 2 orders of magnitude higher than the stage scanning technology [33]. However, the galvanometric scanners have the major drawback of vignetting and stitching lines for samples bigger than the objective effective writing field [34]. If submicron features are not critical, objectives with a small NA offer a larger field of view, which reduces the number of stitching lines needed, as well as the print time.

The laser scanning process to generate the sample represents the technology’s throughput bottleneck. New developments have shown promising results in integrating digital micromirror devices (DMD) to increase the technology’s productivity, as previously shown for single photon applications [75,76]. Alternatively, the fabrication time can be decreased by using the “core-shell” printing mode, i.e., writing only the outer shell of the structure, developing and then crosslinking the remaining resin trapped in the core by flood UV light exposure [77].

The printer optics’ configuration has an influence in defining the suitable application of the printer. An inverted system (NanoOne250, PPGT̂), with the objective located under the sample, is more suitable than an upright printer (FemtoLAB) for printing 2PP resin collected in an open container-like structure, i.e. a well-plate and open microfluidics. However, in such a system the components are more exposed to spillage or leakage.

The printing mode is influenced by the system, the application and the resin. Upright systems, such as the FemtoLAB, are compatible with dip-in and oil printing modes, where the objective is immersed in the resin or in oil, respectively. In addition to such methods, inverted
F. Cantoni et al.

3.3. Software

All the investigated printers have proprietary software to visualize the structure and set the printing parameters. It is important to underline that among the different factors influencing the printed structure fidelity, the STL file plays a key role. The finer the mesh size, the better the surface mimicry for curved structures. In the presented study, the mesh was set to “fine” while exporting the STL file, in order to ensure sufficient detail for the features of interest.

The software from UpNano for the NanoOne250+ and PPGT+ can also operate in vat mode. The investigated printers used different modes for the fabrication of the structures discussed herein: the NanoOne250 prints were done in vat mode, the PPGT+ prints were done in dip-in (DILL) mode, and the FemtoLAB prints were done in oil immersion mode. In the oil immersion printing mode (FemtoLAB), the objects are built in a bottom-up approach. This approach requires transparent substrates that match the ZPP resin refractive index to achieve submicron resolution. The structure height achievable with the oil approach is limited by the objective working distance and artefacts may appear for structures above a few microns since the laser light passes through the crosslinked material [49]. In contrast to the oil immersion printing mode, the dip-in (PPGT+) and vat mode (NanoOne250) are top-down modes. In both approaches, the objective is always at optimal printing distance to ensure the highest printing performance. In the dip-in mode, the objective is in direct contact with the resin during the printing process, resulting in the need to clean the objective after every print session, which in turn might compromise the quality of the objective over time.

The vat mode avoids the direct objective-resin contact by collecting the resin in a tank where the print is performed. The vat approach gives more freedom regarding the resin choice while removing the dip-in mode’s need of cleaning the delicate objective at the end of each print. However, the storage of the resin in the vat system generally requires a higher initial resin amount and suffers from dust contamination over several uses if not properly handled in a dust-free environment. A HEPA filter (NanoOne250) is implemented in the printing chamber to reduce the possible contamination while opening to biological applications as well. The dip-in mode allows printing on large XY areas, i.e. several centimeters, while limiting the height to a few millimeters according to the resin surface tension. On the contrary, the vat mode enables the fabrication of structures up to a few centimeters tall, with a limitation in the XY plane to a few centimeters, according to the vat diameter. While in principle it is possible to increase the vat diameter, this comes with the drawback of increasing the initial volume of resin.

A levelled substrate is essential to avoid printing issues related to structure partial printing or detachment. The PPGT+ system performs automatically autofocus at each tile to detect the substrate-resin interface. Despite preventing structure detachment, such a strategy can lead to the generation of objects with “steps” at each Z-adjustment performed on a tilted substrate. The NanoOne250 system offers an autofocus system combined with a stage tilting system to level the substrate before starting the printing ensuring optimal and faster printing of the object. Despite a motorized tilting stage being available, the used system presented automated focusing with manual levelling before printing. The FemtoLAB system has an integrated autofocus device to detect sample tilts. The SCA software performs an algorithm to find the focus at three points, which can be used to correct the machining algorithm or adjust the stage for levelling. The PPGT+ system offers a similar option for stage levelling in piezo mode.

2PP has been explored as an innovative and flexible technology in different fields for a wide range of applications that requires different substrates. The investigated printers present holders for glass slides, silicon/glass wafers and direct printing on optical fibers for photonic applications. In addition, printer functionalities can be added for bio-printing (NanoOne250 – biomodule) and multi-material printing (PPGT+ – HETEROMERGE). It is expected that further modules will be developed to satisfy the increasing application-driven demand from the scientific community and industry.

3.3. Software

The software from Nanoscribe for the PPGT+, DeScribe, allows users to load and process STL files. Users can orient and scale the model within the software itself, as well as adjust print parameters such as the slicing, hatching, contour count etc. Core-shell printing is also possible, and the parameters for the core structure can be adjusted manually if the default parameters are not satisfactory. The laser power and scan speed can be adjusted in a text editor. The text editor can also allow users to program special instructions that the printer can execute, such as printing different structures in a single print job without the need to issue commands manually. Likewise, the print job can be previewed and the software provides an estimated print time which is usually quite accurate, within 10% of the fabrication time. Users can also select a predefined recipe for each proprietary resin from a list given by the company. This allows inexperienced users to print structures with acceptable quality without having to modify the parameters themselves. Furthermore, the software also provides a feature to facilitate the optimization of certain print parameters, called a parameter sweep. For example, a single structure can be printed several times on a substrate, but with different laser powers and scan speeds. The structures can then be inspected after development to determine the optimal exposure dose for the best print quality. Nanoscribe also provides access to an online repository for customers that contains guides regarding advanced printing techniques. For printing structures, another software by Nanoscribe called NanoWrite is used. NanoWrite communicates with the PPGT+ and sends commands to the printer’s components based on the print job that was loaded in the software.

The process parameters of the FemtoLAB system can be controlled with the SCA software. A fabrication code has to be written to produce structure, where the different system settings can be defined using variables. The user has the option to create structures out of predefined shapes (lines, circle) or to upload different types of 3D models into the software. Based on programming languages, loops can be created and structures can be produced in an array with different parameter settings. The software allows to see a preview of the structures and hatching (slice by slice), while displaying the approximate fabrication time. The software has two predefined writing strategies, the density and the frequency mode, in which one of these parameters is constant and the other is adjusted according to the writing speed. Once the system is prepared for fabrication, the writing process can be started using conventional remote access software. The manufacturer WOP Altechna provides an online support for problems or questions regarding the system or the software, including timely solutions or the option to test fabrication files on their own system.

Both think3D and DeScribe have set of standard recipes for fabrication, unlike the SCA software. A visual comparison between the structures produced using the standard recipes and the optimized
parameters discussed in this paper are shown in the S.I., Figs. S7-S15.

3.4. Resins

The selection of resin to use for the printing also plays an important role in defining the success of a print. Since the commercialization of the first 2PP DLW system, new resins have been developed to meet the requirements of ever-expanding applications. Commercially available 2PP inks are commonly in a liquid state and contain a photoinitiator, a monomer, a crosslinker and a solvent. Most of the resins can be directly used to fabricate the structure without requiring any preparation. The most commonly available resins on the market are acrylate-based (UpNano, Nanoscribe), organic-inorganic hybrids (Microresist technology and OrmoComp materials) [78]. For a more in-depth analysis of the mentioned resins, the reader may refer to the following review [79]. After polymerization, the non-crosslinked resin is removed by development with organic solvents such as propylene glycol methyl ether acetate (PGMEA), isopropanol or ethanol. The solvent is chosen so as to remove the residual resin while preventing deformations due to capillary forces and structure swelling/shrinking [36].

Among the analysed systems, Nanoscribe and UpNano commercialize resins developed specifically for these systems, in collaboration with various resin producers, S.I. Table S3. The resins cover feature sizes ranging from submicron up to the macro scale. The UpNano resins require the substrate to be pre-treated with a methacrylated silanization coating after a surface activation by plasma to enhance the structure adhesion and prevent detachment.

Despite the high performances with submicron feature size structures and resolution provided by traditional resins, polymer-based photoresists often present poor transparency, reduced thermal and electrical conductivity, and low radiation stability, which limits the practical applications of 2PP. The recent use of 2PP DLW systems for electrical conductivity and low radiation stability, which limits the possible voxel shape. The bigger voxel size of low NA objectives enabled feature sizes below 1 µm. In particular, printing on a tilted surface generally - from Bio-INX®, Ghent, Belgium. Despite such resins allowing cell encapsulation, a highly-efficient and biocompatible photoinitiator suitable for a water-based printing environment still represents an unaddressed challenge.

4. Conclusions

In this study, the printing capabilities of three commercial two-photon printers have been investigated. Even if all the tested systems achieved submicron feature sizes and resolution when using the highest NA objective available, the printing fidelity drastically decreased for feature sizes below 1 µm. In particular, printing on a tilted surface negatively affected the structure fidelity, as a consequence of the ellipsoidal voxel shape. The bigger voxel size of low NA objectives enabled faster printing, with the downside of a reduced printing quality. Both the NanoOne250 and the PPGT+ systems successfully allowed for multi-material printing with the alignment capability of consecutive prints in the micrometer range. The FemtoLAB prints presented the lowest surface roughness, below 8 nm, and benefitted from the IFOV technology to not display stitching artefacts. The NanoOne250 system generally displayed the fastest printing time without compromising the printing fidelity when compared to the other two systems.

The successful printing of all the designed test structures shows the versatility of the systems for a wide range of potential applications. New technical developments and photoresists are expected in the coming years to further increase the capability and freedom of the two-photon polymerization technology.

Funding

A.-I.B. acknowledges funding from Villum Fonden (grant number 54424). A.-I.B. and D.M. acknowledge funding from Danmarks Frie Forskningsfond (grant number 1134-0001B). M.T., L.B. and F.C. acknowledge funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation program (grant number 757444) and the Knut and Alice Wallenberg Foundation (grant number WAF 2016.0112). D.O., S.K. and E.B. acknowledge internal funding from TU Berlin. Myfab is funded by the Swedish Research Council (2019-00207) as a national research infrastructure.

CRediT authorship contribution statement


Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgements

The authors would like to acknowledge several people who have contributed to the success of this work: Berit Herstrøm from DTU Nanolab – for her help with AFM and optical profilometry, Daniel Alexandre Rolón from TU Berlin – for help with SEM imaging of the FemtOlab structures, Sönke Steenhusen and Gunnar Böttger – for help establishing a collaboration with the FemtOlab users, and Milena De Albuquerque Moreira from Uppsala University – for support with the NanoOne250 system. We acknowledge Myfab Uppsala for providing facilities and experimental support.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.addma.2023.103761.

References
