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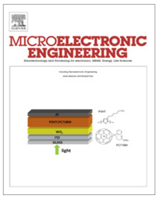

Review Article

Direct laser writing: Principles and materials for scaffold 3D printing

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ABSTRACT

For a great variety of research fields extending from photonics to tissue engineering applications, the requests for the construction of three-dimensional structures with high resolution grow more and more imperative. Towards this aim, the direct laser writing technique by multi-photon polymerization, due to its unique properties and characteristics, has proven to be an indispensable tool to high accuracy structuring and has been put on the map as an emerging technology for scaffold 3D printing. In the present review, the basic principles of multi-photon polymerization are presented, the experimental set-up requirements are described and the employed materials demands are thoroughly mentioned as well as the most representative examples of the recent developments in the field.

1. Introduction

Direct laser writing (DLW) by multi-photon polymerization (MPP) is a three-dimensional (3D) printing technology which allows the construction of readily assembled structures with sub-100 nm resolution [1,2]. It is based on the nonlinear photon absorption by photopolymers; the beam of an ultra-fast laser is tightly focused inside the volume of a transparent material, causing it to absorb two or more photons and polymerize locally. Moving the beam according to a path representing a Computer Aided Design/Computer Aided Manufacturing (CAD/CAM) model, one can fabricate a realistic micromodel of this design.

DLW, along with classic stereolithography [3–5] and selective laser sintering [6,7], make up a versatile class of laser-based 3D printing techniques [8] which enable the fabrication of tailored structures directly from a computer data via a CAD/CAM design. Compared to those two, DLW offers superior resolution and does not require the need of recoating or layer-by layer fabrication; on the negative side, it is much slower and requires expensive and specialized equipment.

Pioneering work on single-photon DLW was performed in the early ‘90s [9,10]. DLW by multi-photon polymerization was first demonstrated in 1997 [11], and it was immediately embraced by the photonics community, as its capability for the fabrication of fully 3D, nanostructures was obvious [12,13]. More recently it has found application in more diverse fields such as micro-optics [14,15], microfluidics [16], biomedical implants [17,18], and in 3D scaffolds for cell cultures and tissue engineering [19–21].

Tissue engineering is defined as the discipline which applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ [22]. Creating an engineered tissue requires imitating the extracellular matrix (ECM); this entails finding a material suitable for the fabrication of a scaffold for a specific tissue engineering application, fabricating the scaffold, and seeding it with cells for cell culturing *in vitro* or *in vivo* [8,23,24]. Micro- and nano-topography have been found to influence cell survival and proliferation [25]; this is the main reason for which the use of DLW in scaffold fabrication has found explosive increase over the last few years.

In this review, we describe direct laser writing and its applicability in cell culture and tissue engineering. We present the theory of multiphoton polymerization, and describe a typical experimental set-up. We then describe the most widely used biodegradable and/or biocompatible materials for DLW. We finish by detailing recent developments in the field and the future prospects of the technology.

2. Multiphoton absorption

The physical phenomenon behind the operation of DLW is multiphoton absorption. In this review, for simplicity reasons we shall concentrate on the theory of two-photon absorption; this can be easily extrapolated to three or more photons.

Two-photon absorption (TPA) is defined as the simultaneous absorption of two photons of identical or different frequencies in

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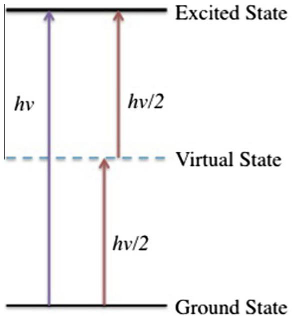

Fig. 1. Diagram of energy transitions in two-photon absorption.

order to excite a molecule from one state (usually the ground state) to a higher state [26]. The energy difference between these states is equal to the sum of the energies of the two photons (Fig. 1). Two-photon absorption is a second-order process and as such, its strength depends on the square of the light intensity. It is several orders of magnitude weaker than linear absorption.

TPA was theoretically predicted in 1931 by Maria Göppert-Mayer in her doctoral dissertation [27], however, it was not verified experimentally until 30 years later by Werner Kaiser, when the invention of the laser permitted the generation of two-photon excited fluorescence in a europium-doped crystal [28].

The TPA cross section is measured in Göppert-Mayer (GM) units: $1\ \mathrm{GM} = 1 \times 10^{-50}\ \mathrm{cm}^4\ \mathrm{s}\ \mathrm{molecules}^{-1}\ \mathrm{photon}^{-1}$. TPA can be measured by several techniques; the most common being two-photon excited fluorescence and nonlinear transmission (z-scan) [29]. As TPA is a second-order nonlinear optical process, and therefore is most efficient at very high intensities, pulsed lasers are usually employed in its measurement [26].

3. Equipment and experimental set-up

A typical DLW experimental set-up is shown in Fig. 2. The main and necessary components of a DLW system are (i) an ultrafast laser, (ii) a beam/sample motion system, (iii) beam focusing optics, (iv) beam intensity control and beam shutter, and (v) control software. These components are described below:

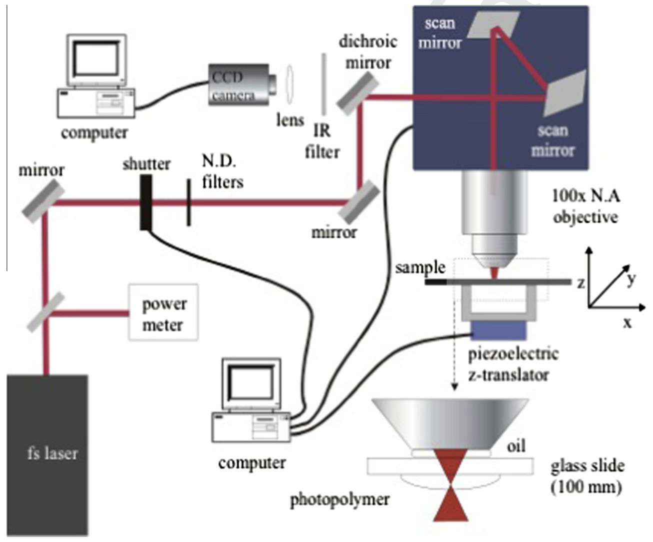

Fig. 2. A typical experimental set-up consisting of a light source, beam and sample movement components, beam control and focusing optics, and a vision system.

3.1. Laser source

Until recently, the laser source typically would be a Ti:Sapphire femtosecond oscillator operating at around 800 nm. However, as second-harmonic fibre lasers have become more reliable and affordable, they have taken over; they would typically operate at 780 nm. DLW laser sources usually have a pulse length of less than 200 fs and a repetition rate of 50–80 MHz. The energy required for the polymerization process depends on the material, the photoinitiator and the focusing, but is usually in the order of a few nanojoules per pulse.

There are also examples of an amplified ultrafast YAG (yttrium aluminium garnet) laser operating at a green wavelength (second harmonic) being used. In this case, the pulse length is sub-picosecond, and the laser repetition rate lies in the kHz range. The use of this type of light source is not very common in DLW, most likely due to its high cost and the availability of alternative, low cost sources.

3.2. Motion system

In order to 'write' the 3D structure into the photopolymer using the focused laser beam as the pit of a pen, one needs to move either the beam focus, or the sample. The former can be done with galvanometric scanners, while the latter with high-resolution *xyz* stages.

When using galvo scanners, the photopolymerized structure is generated in a layer-by-layer format. The CAD/CAM file is 'sliced' and each slice is exported as an .stl file (STereoLithography). After the 'writing' of each slice is completed, the sample moves on the *z*-axis using a high-resolution translation. In addition to the *z*-axis movement stage, *xy*-axes movement stages are also required, to allow large-scale movement.

When using *xyz* stages for structure writing, there is no need to slice the design, as the stages can move in all directions. It is possible to use piezoelectric stages, which have nm-scale accuracy. These stages can travel small distances-a few hundred micrometers at best, at tens of microns per second speed. In this case, it is also useful for these stages to be positioned on large-scale movement stages (such as linear or step-motor stages).

Using galvo scanners has the advantage of high scanning speeds (up to 5 m/s) and ease to fabricated curves. However, as the beam moves, it can be distorted as the objective, even if it a-planar, does not provide a completely flat field-of-view; for this reason, galvo scanners are not used for applications where high accuracy is needed (such as photonics applications). On the other hand, they are very commonly used in bio-applications, where nanometer accuracy is not essential, and where scales are large, so high building speed is desired.

In practice, most systems have both galvanometric scanners and high-accuracy *xyz* stages, and switch between them depending on the application requirements. Both systems have high reproducibility.

3.3. Focusing optics

The high intensities required for multi-photon absorption and high structuring resolution necessitate a tightly focused laser beam and, thus, a microscope objective has to used; when the numerical aperture (N.A.) of the objective is higher than 1, immersion oil is used for index matching. Galvo scanners have to be adapted to accommodate microscope objectives, as usually they are designed to take lenses with long focal lengths.

One disadvantage of using microscope objectives is the short working distance (typical values: 170 μm–1 mm), which limits the height of the fabricated structures (typical values: 30 μm–1 mm). To address this issue, "Dip-in" Lithography [30]

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