PERSPECTIVE

# Directed self-assembly software for single cell deposition

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**Abstract:** Laser direct-write (LDW) bioprinting methods offer a diverse set of tools to design experiments, fabricate tissue constructs and to cellular microenvironments all in a CAD/CAM manner. To date, we have just scratched the surface of the system's potential and for LDW to be utilized to its fullest, there are many distinct hardware and software components that must be integrated and communicate seamlessly. In this perspective article, we detail the development of novel graphical user interface (GUI) software to improve LDW capability and functionality. The main modules in the control software correspond to cell transfer, microbead fabrication, and micromachining. The modules make the control of each of these features, and the management of printing programs that utilize one or more features, to be facile. The software also addresses problems related to construct scale-up, print speed, experimental conditions, and management of sensor data. The control software and possibilities for integrated sensor data are presented.

**Keywords:** CAD/CAM bioprinting, laser direct-write, single-cell printing, GUI software, machine learning

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**Received**: May 2, 2017; **Accepted**: May 19, 2017; **Published Online**: June 5, 2017

**Citation:** Sklare SC, Richey W, Vinson BT, *et al*. 2017, Directed self-assembly software for single cell deposition. *International Journal of Bioprinting*, vol.3(2): 100–108. https://doi.org/10.18063/IJB.2017.02.002.

## 1. Introduction

Laser direct-write (LDW) bioprinting methods offer a diverse set of tools to design experiments and to fabricate tissue constructs and cellular microenvironments all in a CAD/CAM manner. Like extrusion and ink-jet based printing techniques, LDW bioprinting relies on the ability to reproducibly transfer biomaterial, such as cells, to engineer three-dimensional constructs layer-by-layer. To achieve transfer, a quartz disk ("ribbon") is coated on one side with an energy-absorbing (or sacrificial) layer and a biomaterial (or transfer) layer often containing cell-laden materials. The laser beam is focused at the absorbing layer-ribbon interface. The pulsed laser then generates a small pocket of vapor that propels the biomaterial layer as a droplet onto a receiving substrate (Figure 1).

Mounting the ribbon and substrate on computer-controlled three-dimensional stages allows researchers to use LDW to engineer neural networks[1], fabricate microbead cellular microenvironments[2], and study cell tissue interactions[3].

To date, we have just scratched the surface of the system's potential. For the system to be utilized to its maximum, there are many distinct hardware and software components that must be integrated and communicate seamlessly. Programming the computer-

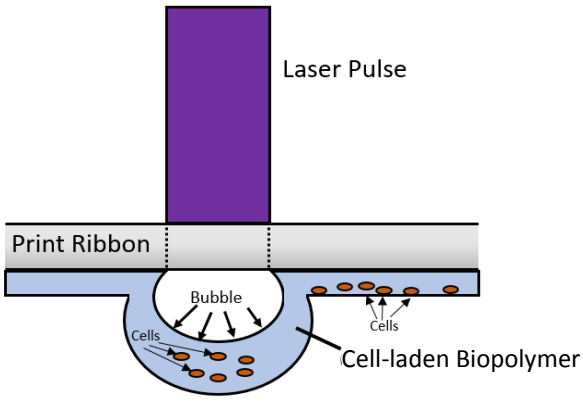


**Figure 1.** Expanding vapor bubble

controlled hardware of a customized bioprinting system to carry out printing or micromachining tasks requires expertise with each advanced programming interface (API) provided by the original equipment manufacturer and knowledge of all the printing and material parameters. Some systems use graphical user interfaces (GUIs) which are only capable of controlling some of the hardware but not in an integrated manner. For example, the laser default fires a pulse at a predefined time rather than waiting for the substrate stage to arrive at a location. Controlling a printing system by loading a prewritten static script or using an awkward GUI without automation or integration is a barrier to LDW's ability to perform high-throughput, parallel biological experiments for applications, such as drug screening, with unambiguous results. The current implementation of the GUI application is built on the Qt framework for C++, which links to C++ programming resources provided by multiple independent manufacturers and incorporates them seamlessly in a Python environment.

While LDW can be used to print individual cells, single-cell resolution printing is a challenge without automation, as each cell must be manually targeted. Print ribbons for single-cell resolution printing are sparsely prepared, making it difficult to find cells to transfer before the ribbon begins to dry out. Manually building intercellular networks or tissue constructs with hundreds or thousands of cells one at a time would be an impossible task without automation, regardless of the availability of a responsive and integrated GUI. However, the importance of this single-cell resolution should not be understated. For example, creating multicellular constructs from single cells allows researchers to study cellular cross-talk in a simplified, well-controlled model rather than as part of a larger tissue with several cell types. Several applications of single-cell resolution printing are discussed in Sklare *et al.*[4]

Laser direct-write is a contact-free printing approach with greater spatial control than contact printing methods such as ink-jet and extrusion bioprinting[5]. In addition to additive manufacturing with micrometer scale transfer accuracy, direct-write systems can process the receiving substrate by subtractively micromachining features into the hydrogels[6] or other substrates, and be used for single-step microbead fabrication[7]. Cell transfer, microbeads, and micromachining are complimentary laser direct-write capabilities that can and should be used together and managed through a convenient interface.

Herein we detail the development of novel GUI software to improve LDW capability and functionality. The main modules in the control software correspond to cell transfer, microbead fabrication, and micromachining (Figure 2). The modules make the control of each of these features and, the management of printing programs that utilize one or more features, to be facile. The software also addresses problems related to construct scale-up, print speed, experimental conditions, and management of sensor data. The control software and the possibilities for integrated sensor data are presented.

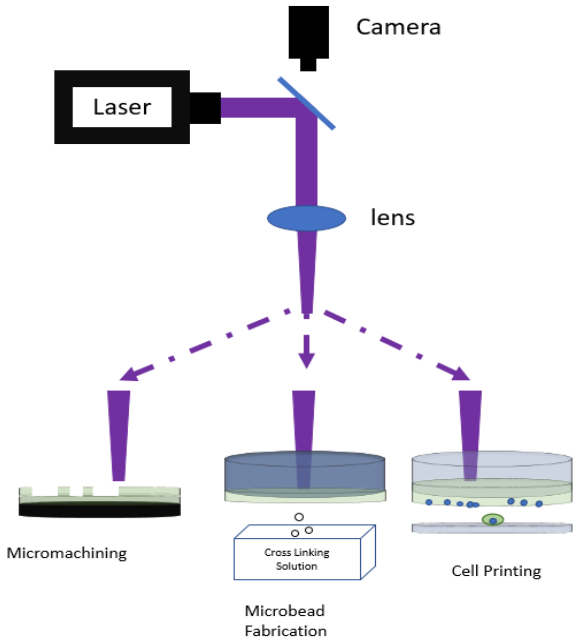


**Figure 2.** Schematic representation of micromachining, microbead fabrication and cell printing with the same system

## 2. Laser Direct-write's Typical System

There are several types of LDW that rely on roughly similar mechanisms for transfer and are incorporated into comparable printing systems. Laser-induced forward transfer (LIFT), absorbing film-assisted LIFT (AFA-LIFT), biological laser processing (BioLP), and matrix-assisted pulsed-laser evaporation direct-write (MAPLE-DW) are examples of similar systems with different transfer mechanisms[8–11]. MAPLE-DW uses an optically transparent "ribbon" as a cell reservoir and a biopolymer-coated receiving substrate. The disks (usually quartz) coated with cells are referred to as "ribbons", in reference to typewriter ribbons that were coated with ink on one side before transfer. The parameters for the pulsed laser and beam delivery optics depend on laser wavelength and ribbon coatings (material composition, viscosity, hydration, and cell suspension layer rheology). Laser-material interaction between the laser and ribbon-coating interface will eject material determined by the resultant vapor bubble's dynamics, again regulated by the laser wavelength, absorbing layer, and cell suspension rheology[12]. The ribbon and

substrate stages are attached to one-, two-, or three-axis computer-controlled actuators for independent movement. Additional ancillary components, such as *in situ* energy meters and environmental (temperature and humidity) control, vary more widely between systems.

MAPLE-DW utilizes several ancillary computer-controlled components: six positioning-motors, two *in situ* imaging devices, two energy meters (one removable and in-line that blocks the beam path and one *in situ*), a motorized iris, a Peltier cooler mounted on substrate stage, a chamber heater, a humidifier, and a temperature/humidity probe (Figure 3). The system is compartmentalized in a fully enclosed area where the ribbon and the substrate area are located for experiments that require aseptic conditions. Two computer monitors, a keyboard, and a mouse are mounted on the exterior of the enclosure.

The current iteration of MAPLE-DW features CAD/CAM controls, environmental controls, and a fully integrated control system to manage each computer-connected component. The main camera, focused on the ribbon, is used with the transverse ribbon stages to select groups of cells or individual cells for printing in real time. Single-cell transfer requires a sparsely populated print ribbon with substantial intracellular spacing to ensure that cellular transfer regions do not overlap. Visually targeting the cells, especially on a low density print ribbon, would remove volumetric probability associated with non-direct-write methods and improve droplet-to-droplet consistency[13].

The receiving substrate is mounted on 3D stages and positioned 500 to 2,000 µm below the ribbon[14]. The receiving side of the substrate is coated in a low viscosity biopolymer or gelatin. Coatings are chosen to mitigate cell impact and promote desired cell behavior by maintaining a moist/humid cellular environment and by mixing culture medium with the coating, preferentially promoting cell adhesion[15]. Alternatively, substrates can be machined with the laser to create a customized cellular environment by moving the ribbon out of the way and refocusing the camera and laser.

With a prepared ribbon and receiving substrate in place, single laser beam pulses are focused at the ribbon-material interface. Methods that use interstitial metallic layers (such as gold) or dynamic release layers (such as triazene and hydrogel) as energy-absorbing layers rely on the localized, rapid evaporation of these layers to produce a vapor bubble. The resulting force from this cavitation bubble ejects a volume of material from the cell suspension layer.

## 3. Laser-assisted Printing Control Software

The general user interface (GUI) control application is composed of three main modules, one for each of the main functions of this laser setup: 1) MAPLE-DW, 2) Micromachining, and 3) Microbead fabrication. These

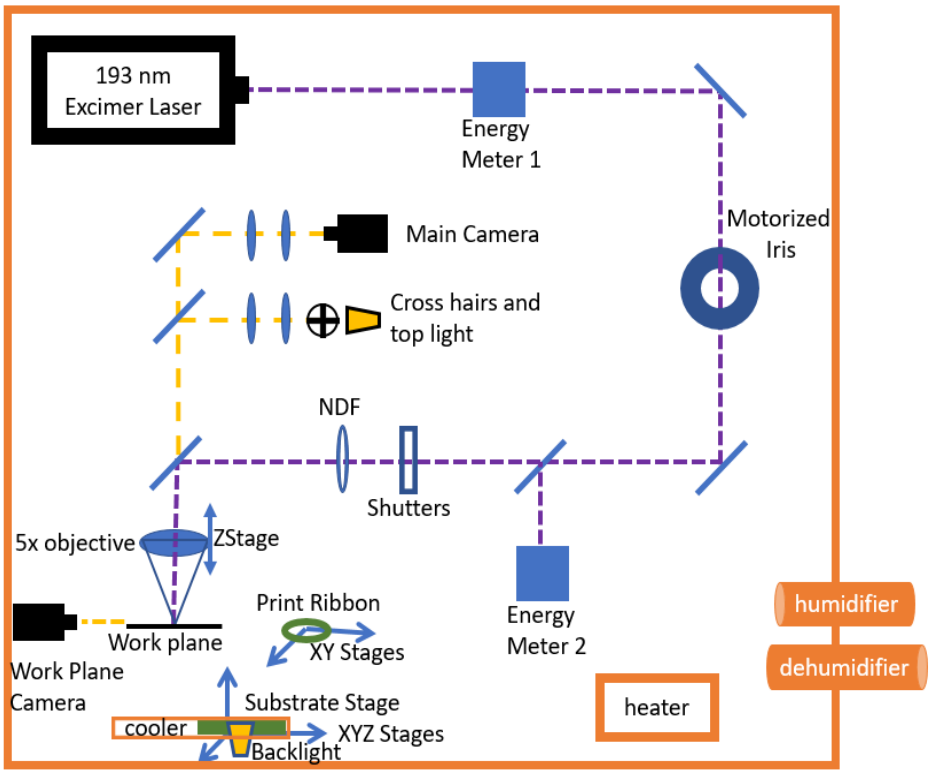


**Figure 3.** MAPLE-DW schematic

modules are written in C++ using the Qt application framework (https://www.qt.io/). With the Qt framework, we were able to create highly customized and detailed user interfaces that controlled our hardware via manufacturer-supplied C++ programming libraries. Matlab (https://www.mathworks.com/) and LabVIEW (http://www.ni.com/labview/) were considered in building the application platforms; however, in contrast to the way C++ development files are handled, hardware manufacturers do not provide Matlab-specific libraries or LabVIEW virtual instruments (VIs) for free. Buying additional Matlab or LabVIEW programming libraries quickly adds up to several thousand dollars for only a few pieces of hardware. Furthermore, when new hardware is added, one would have to check for an available proprietary Matlab or LabVIEW library prior to the equipment purchase.

In addition to cost considerations, Matlab is not well suited for creating complicated GUIs that manage different simultaneous hardware events in different threads. Similarly, while LabVIEW is designed to quickly make control GUIs, there is no standardization across hardware interface VIs. The lack of standard interfacing can lead to problems with timing that requires implementing semaphore time-management loops for each communicating piece of hardware. A hard-coded time management system like this leads to unresponsive GUIs. The Qt framework has several strategies for multi-threading and a signal/slot system that keeps the GUI responsive while handling control requests in different threads, all of which are accomplished without excessive layers of semaphores empirically adjusted for every different piece of hardware.

The current implementation of GUI application links to C++ programming resources provided by Aerotech, Basler, Thor Labs, and Arduino to interface with, and to control, equipment. Python programs are integrated into the control system for analysis and plotting in real time, generating motion-control scripts, and acting as visual guides to help keep track of the printing process, even though the GUI components and equipment controls are written in C++. The Python programming language is great for fast development of analysis tools and works well with both Qt and C++.

This software is written to be useful to as many research groups as possible that use laser direct-write bioprinting and/or micromachining. Different motorized stages, cameras, temperature and humidity probes, humidifiers, heaters, coolers, lasers, and lasers triggers can easily be integrated due to an interstitial layer between the linked libraries and control program. This layer is used to abstract the control program from the hardware libraries. To add new hardware, it is only necessary to copy a hardware's category skeleton file and provide the new hardware API's commands for basic functions listed in the skeleton file. For example, incorporating a new motor would involve identifying the functions in the new API that provide position feedback and motion control, and then creating a new version of the skeleton file with those commands. The source code, not including any hardware-specific libraries, and detailed documentation are being prepared for release on GitHub (https://github.com/) for use in other labs.

## 3.1 GUI Modules

### *3.1.1 Printing Module*

The MAPLE-DW Printing Module is designed to increase print speed, reproducibility and enable single-cell resolution. Print speed is a barrier to scaling up construct size and to performing many sets of parallel experiments, and it is also a critical experimental parameter. Prepared ribbons and receiving substrates tend to dry out, and this increases the inhomogeneity in the absorbing layer material properties and changes the range of acceptable incident fluence for successful transfer. Meanwhile, the receiving substrate's amenability to transferred cells can change with its stiffness and wettability. Reproducibility is improved by the integration and automation of all available sensors. For each initiated transfer, a multitude of data is collected: laser energy, temperature, humidity, images of the ribbon before and after each transfer, distance between the substrate and ribbon, and more. This data is being used as "printing metadata" towards creating smarter printing systems that learn from each failed transfer on their own using machine vision and machine learning, with the goal of optimizing and accelerating LDW.

Other relevant automated features are also facilitated through the Printing Module (Figure 4). In the top left corner of Figure 4, there is a ribbon mini-map, which shows the user the current region on interest (ROI) on the ribbon. The waypoint list feature is in the lower left corner of Figure 4. Users can quickly reposition to any of these points, edit the list, and add their current position.

Printing into grid patterns is automated and controlled via the grid widget. The grid widget guides you using interactive prompts (Figure 5) and generates a graphical guide to help track progress (Figure 6). To print into any arbitrary shape instead of a grid, a motion script converted from a 2D CAD can be loaded for the substrate and incorporated into a printing program.

Ribbon density is determined by application. Sparse print ribbons with low cell density are utilized for applications such as single-cell printing. Conversely, high cell density ribbons allow printing large cell clusters (>200 μm). When using sparse ribbons, it is especially

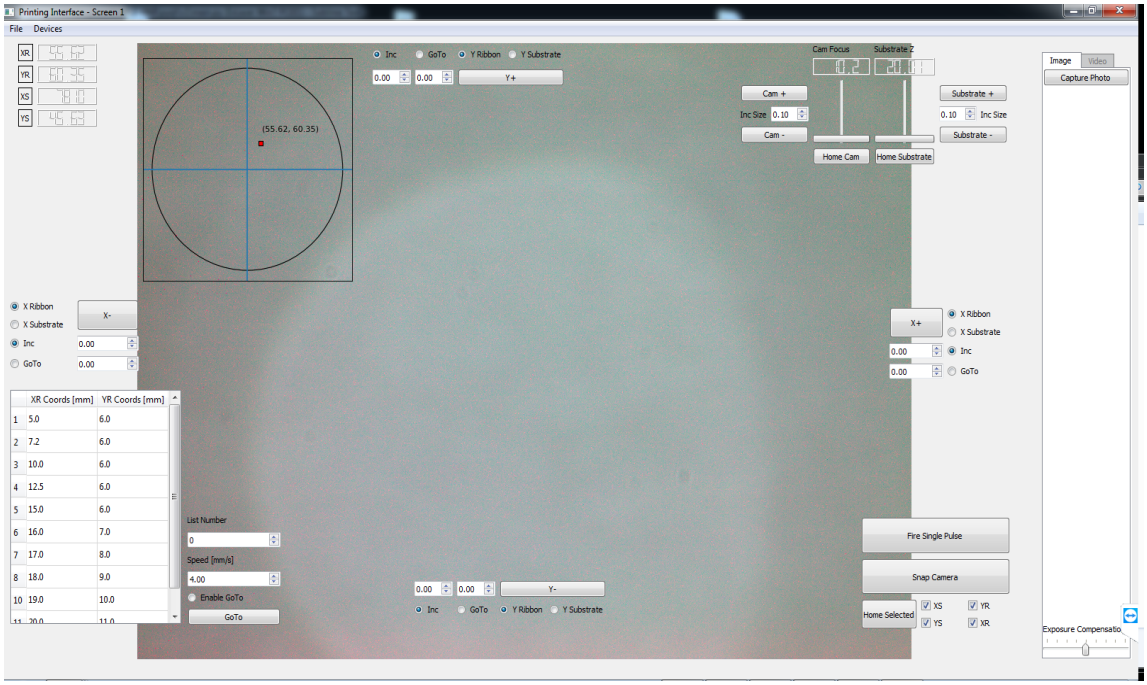


**Figure 4.** Printing module screen

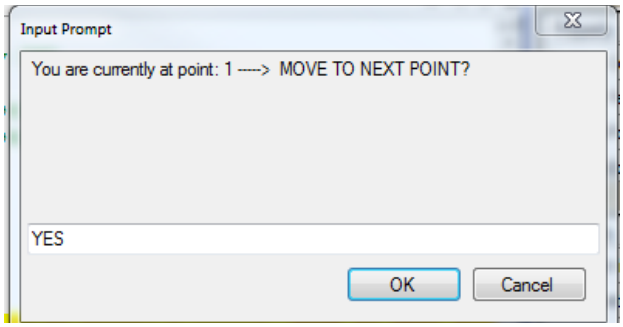


**Figure 5.** Interactive grid-printing prompt

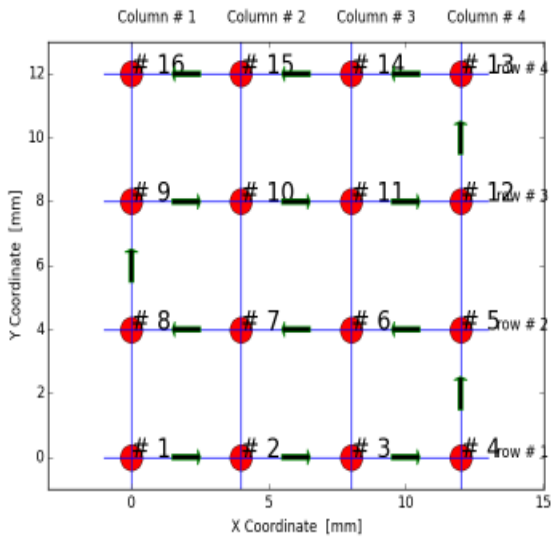


**Figure 6.** Automatically-generated graphical guide to grid program

useful to quickly survey a ribbon to determine cell location prior to printing in order to reduce total print time. The ribbon scan (Figure 7) widget starts a simple raster scan of the ribbon and builds a mosaic composite image. Users can then zoom in/out, navigate, and mark the generated ribbon maps. Waypoints are generated from locations marked on the map by the user. These waypoints can be loaded into the waypoint list widget and used to repeatedly reposition the ribbon to areas of interest, or they can be converted into a printing program that automates the laser firing, ribbon, and substrate motion. In addition, enabling machine vision to identify each cell on a ribbon is currently being implemented using OpenCV. To manually navigate the print ribbon, users can click on the onscreen buttons labeled Y+, Y–, X+, X–, or use the keyboard. The directional keys control the ribbon, while 'w', 'a', 's', and 'd' control the substrate.

### *3.1.2 Microbead-generation Module*

The microbead-generation module interface is similar to the printing module; however, it loads entirely different printing parameters and features a widget to determine parameters for different sizes and material make-up of microbeads (Figure 8).

### *3.1.3 Micromachining Module*

Micromachining requires monitoring and control over the same equipment as printing, but the focus of the GUI is shifted away from navigating the ribbon and managing printing programs (Figure 9). Instead, the focal point is

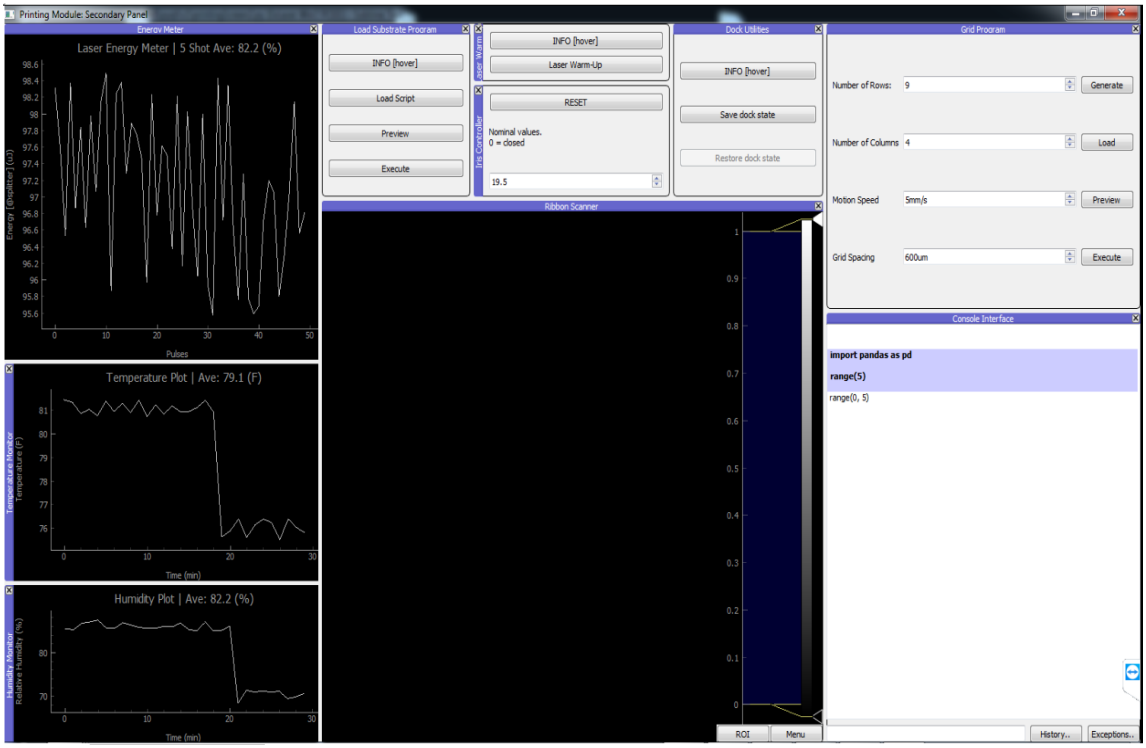


**Figure 7.** Secondary screen

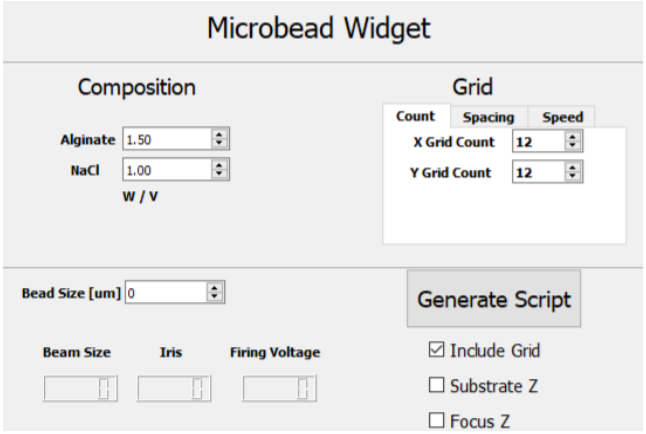


**Figure 8.** Bead parameter calculator

creating material-specific laser tool-paths and closely monitoring the energy throughout the machining process.

Comprehensive and intuitive software is an integral part of higher-order construct fabrication. In particular, such systems can drastically increase experimental throughput by reducing the time from construct inception to fabrication. LDW is a useful platform for fabricating spatially defined biological constructs using low volumes of transferred biomaterials (usually cell-laden biomaterials); essentially, LDW can be used as a rapid prototyping system for tissue constructs and spatially defined biological experiments.

The current iteration requires several different parameter files to be edited and loaded in a specific order. It leans toward monitoring the system (environmental and operational status) and offering powerful semi-automation rather than full-stack automation. Future iterations of the control system should include remote design and operation capabilities. A 3D model of a micromachined feature and grid-printing routine could be created on a remote computer and then transmitted over the internet to the LDW system. Then, a technician could prepare the appropriate print ribbons, load the ribbons into the machine, and start the automated process. Such a decentralized design approach could allow many more researchers to use the same machine and increase the speed of the prototyping process.

## 3.2 Integrated System Data

Continuously collected sensor data is put into a database of "printing metadata" and subsequently analyzed. The goal of this data collection is to build a series of machine-learning and data analytics tools to further automate printing and help us learn from every successful and unsuccessful transfer attempt. A successful transfer occurs when a targeted subsection of cell-laden material is transferred to the receiving substrate, without blowing apart cells, and ejecting rather than delaminating cells.

Using different materials for LDW will alter the optimal printing parameters; thus, every combination

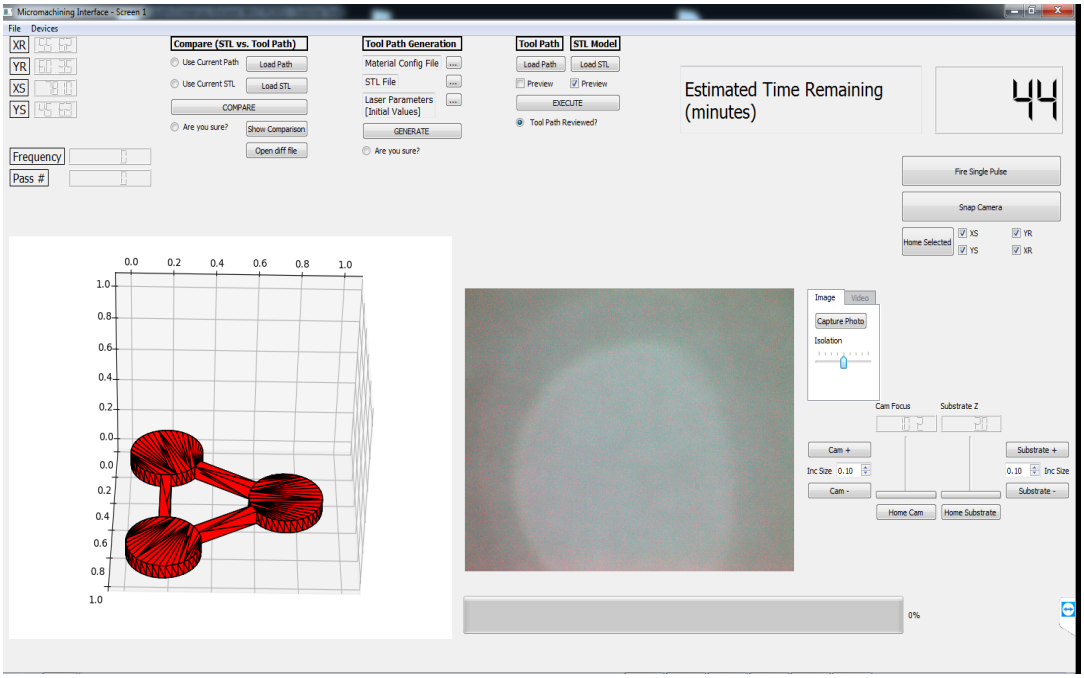


**Figure 9.** Micromachining module screen

requires careful optimization to narrow the operating parameters' space. This information was formerly stored in protocols and entered into printing software for each specific session. Now this information is stored in a database, the Pandas Python module is used to analyze it and appropriate parameters for different cell types and materials are automatically loaded. The stored collected data from a printing session is now tagged with cell types and material composition, in addition to the raw sensor data (laser energy, distance between ribbon and substrate, aperture opening, temperature, humidity, and before/after pulse pictures). When printing sessions include "metadata" collection, it is necessary for the users to judge and indicate if each transfer is successful. Experienced users can tell from the characteristic ablation bubbles on the print ribbon if the transfer is successful for the specific LDW system in use. The most reliable method is to move the ribbon out of the way and focus the camera on the substrate. The examination of the substrate is not yet automated, and this dramatically slows down the printing process. Therefore, printing sessions specifically to collect this data are sometimes desirable. Combining this measured outcome (binary data) with the metadata creates a clear picture of desirable parameters and allows quantitative learning from every printing trial.

Automating the step of classifying prints as successful or unsuccessful is being implemented using machine learning. The database of manually classified prints is being built largely to facilitate this process. Combining, binning, and manually classifying pictures of the ribbon and substrate before and after printing allows the construction of an automated classifier using automated feature extraction and a neural network.

Integrated data collection and machine learning will soon be used to study the entire printing process and downstream experiments. An example implementation involves a simple live/dead experiment:

1. This is a single-cell precision experiment; a sparse ribbon is prepared. After preparing the print ribbon and substrate, the ribbon is scanned and automatically identifies cells using feature recognition. Then, based on an empirical model, the cells are sorted by "printability". Printability is based on the discernable physical appearance of the cell and location, *i.e.*, its proximity to other cells and the condition of hydrogel in that location.

2. A printing program to transfer the cells into a 10×10 grid is loaded and executed; printing metadata is logged throughout.

3. Each grid point now has an associated cell-fate outcome (live/dead data), printing metadata, and printability metric (based on cell appearance, proximity to other cells, and visible local hydrogel conditions). Grid points are discernable due to LDW +/- 5 μm single-cell printing accuracy.

4. Cell fate can now be used to reevaluate printing parameters and incorporated into the entire optimization model.

## 4. Conclusion

The precision and versatility offered by LDW method is exciting. However, when compared to extrusion, pattern-

ing, or even inkjet methods, LDWtransfers a relatively small amount of material[16]. So while the ability to individually target and transfer single-cells or clusters is critical in certain applications, the eventual scale-up capabilities of laser-based methods presents even more challenges to large tissue and organ engineering applications. Because of this, LDW has traditionally been used to create customizable and disposable experiments and micro-environments rather than large-scale 3D printing. The exciting research to create larger additively manufactured constructs, being done by Yan *et al.* and other groups, may advance this paradigm to the next level[17].

Combining the ability to fabricate a cellular microenvironment and precise placement of cells into that microenvironment in one system enables researchers to recapitulate complex cellular constructs automatically and reproducibly. Doing this quickly requires an integrated software designed for bioprinting-specific tasks. The bioprinting control suite presented in this article is an example of integrated software with bioprinting-specific features and will be released as source on GitHub (link will be updated when live). Future iterations of this software will have more tools to rapidly create complex printing programs and utilize robust hardware plugin systems similar to the ones in the open source optics laboratory software packages Itom[18] and Qudi[19]. In addition, the design will be more intuitive: using video game heads-up display aesthetics to redesign the information and control overlays on the video feed. Game controllers with two joy-sticks and triggers will be used to maneuver the ribbon and substrate and to fire laser pulses.

## Conflict of Interest

No conflict of interest was reported by the authors.

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