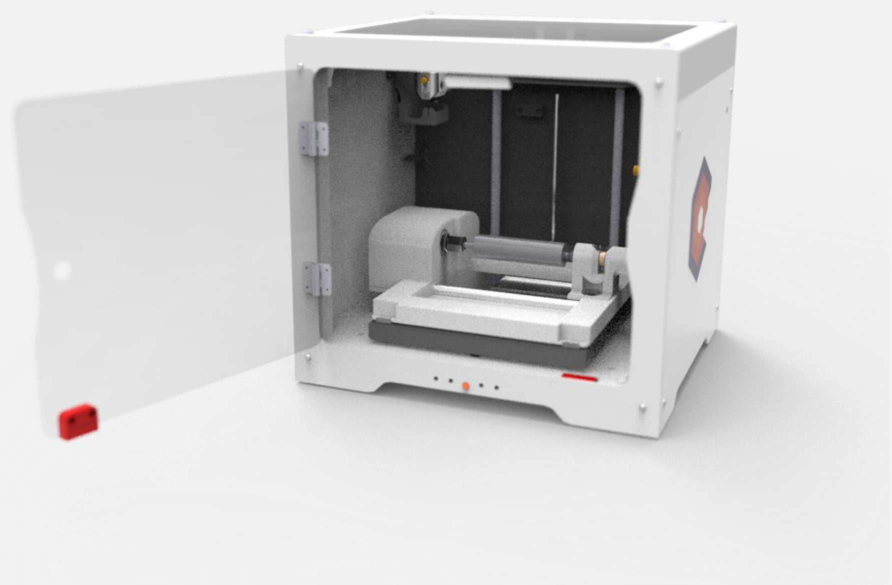
NOVA SPIDER

Advanced Electrospinning Tools



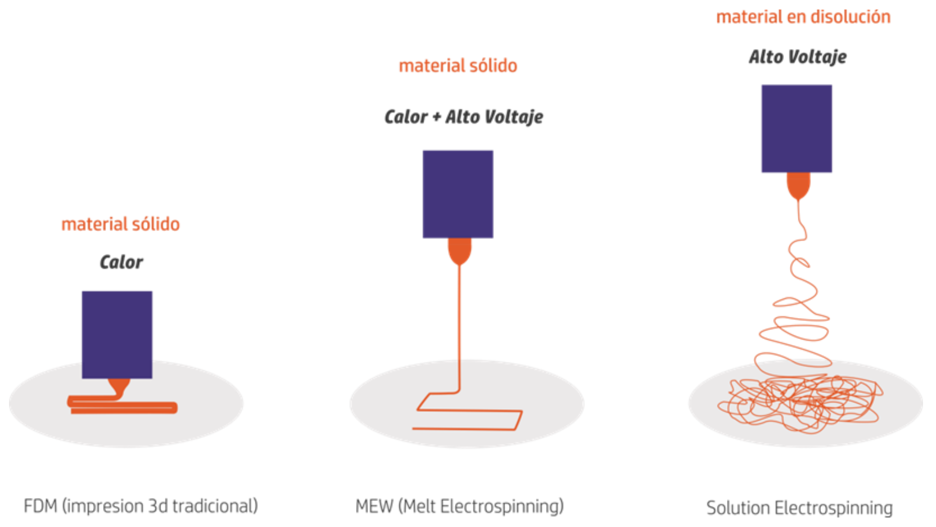
OPERATING MANUAL

NovaSpider Prolab3D

Content

1 Basic Principles of the Applied Techniques

NovaSpider Prolab3D unit uses electrospinning techniques in varieties of electrospinning with solvent and melt-electrospinning to produce fibers of diverse characteristics. Using the combination of both techniques allows to fabricate compound fiber materials in an automatic and repetitive manner.

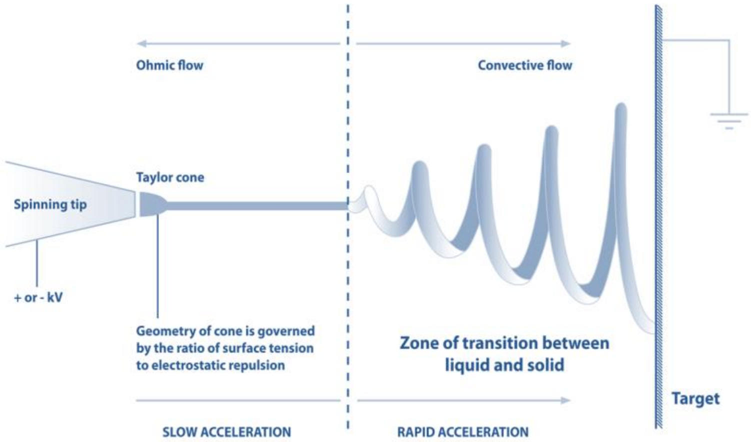


Materials in the form of nano- and micro- fiber acquire special properties such as: ample surface per unit area, porosity, and varied mechanical properties, especially important for several applications.

1.1 Electrospinning

Electrospinning is a method of producing fiber of micro- and nanometric diameter from different materials, mainly polymers. This technique is relatively simple but requires certain parameters of materials and process conditions which must be strictly kept to obtain fiber of given characteristics.

Electrospinning process uses electric force to exctract fibers from a material in a liquid state (in a solvent or from a melted one). When the voltage applied to a droplet is sufficiently high, it causes its stretch as a result of electrostatic repulsion counteracting the surface tension. If the molecular cohesion of the liquid droplet is high enough, once it reaches a critical point, it transforms into a Taylor cone and a liquid jet erupts from its sufrace. The jet stretches in a whipping process caused by electrostatic repulsion before it finally deposits on a collector. In the case of properties of the material not being adequate for electrospinning, micro- or nano-droplets could form instead of fibers. In Pict. 1.0 there is a diagram showing the principles of this method.



Pict. 1.0 Diagram showing fiber formation by electrospinning

The method is non-invasive, it does not require coagulation chemistry nor high temperatures to produce solid threads from liquid materials.

1.1.1 Advantages of Fibers Produced in the Process of Electrospinning

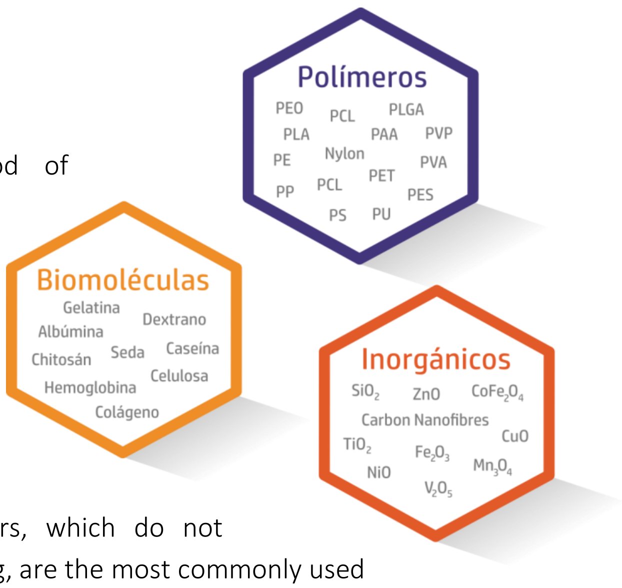
Electrospinning:

- Diameters of nanometer-scale
- High surface to volume ratio (1-100m2/gr)
- Simple and economical process
- High porosity
- Wide range of materials
- Possibility to combine materials
- Easily deposited on other substrates
- Easy to operate

Melt-electrospinning:

- Diameters of micrometer-scale
- High surface to volume ratio
- Economical process
- High porosity
- Mainly polymers
- No need to use solvents
- Controlled deposition
- Respectability

1.1.2 Electrospinning Materials



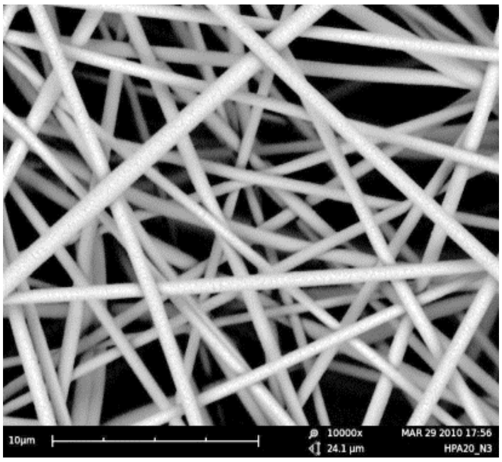
Electrospinning is a very versatile method of producing nanofibers. The materials of large and complexed molecules are the best suited for this technique, which is why polymers are the ones most commonly used. However, other materials, such as metal oxides, carbon, proteins and crystals with a post-treatment, can also be utilized.

In the melt-electrospinning process polymers, which do not degrade in the temperatures necessary for melting, are the most commonly used materials.

1.2 Electrospinning with Solvent: Random Nanofibers

In this technique, the material which we want to use to produce fibers, is dissolved in an appropriate solvent. The properties of the solution, such as: viscosity, surface tension, concentration and conductivity, are crucial for the solution to be proper for electrospinning. The solvent of the solution must evaporate on the trajectory between the emitter and the collector while the thread is lengthening in a whipping process.

The fibers obtained in this process are random, non-aligned, created by threads of nanometer-scale diameter.

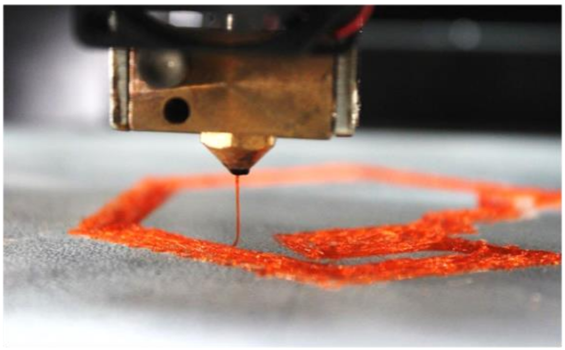


Pict. 1.2 A structure obtained in electrospinning process

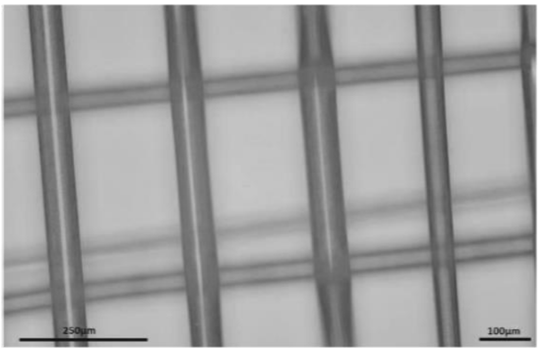
1.3 Melt-electrospinning: Aligned Microfibers

Melt-electrospinning is a technique of electrospinning based on melting. This method allows precise control of deposition of micrometer-scale diameter threads.

In order to obtain a viscous fluid from a solid material without using a solvent, the material is heated to its melting temperature. When an adequate voltage is applied between the emitter and the collector, with a controlled distance between the two, the effect of an electric field results in Taylor's cone appearance and a consequent creation of a fiber from the material to the collector. Due to the high viscosity of the material, the whipping stage used in electrospinning with solvent is not generated, which allows fibers to reach the collector in a direct, straight line. By controlling the movements of the discharge head, with regard to the collector, threads can be arranged in desired drawings, patterns and structures.



Pict. 1.3.1 A discharge head in melt-electrospinning process



Pict. 1.3.2 An image from an optical microscope of a structure generated in melt-electrospinning process

2 Safety Measures

NovaSpider Prolab3D unit is designed to be used in the area of research and development of materials which is why a wide access to the manipulation of its elements and to the modification of the parameters that take part in the processes is allowed. The operator who manipulates the unit must be aware of all the risks that can occur and be able to take necessary measures to avoid them. Risks related to NovaSpider Prolab3D are described in detail in this operating manual. It should also be pointed out that some materials used in the electrospinning process carry their own risks. The operator must also be aware of these risks and be able to take necessary measures in this regard.

Incorrect handling of the equipment can present a serious risk of personal injury. All users of the unit must read and understand the content of this manual before running the machine.

NovaSpider Prolab3D works with a voltage of up to 30kV, the maximum current delivered by the unit is 0.3mA. This power output is too low to harm a human being. Contact with active high voltage parts of the machine would result in an electrical discharge that can be painful.

2.1 Precautions

1 NovaSpider unit should work only with the original power adapter supplied by the manufacturer. Otherwise the equipment can be damaged or even cause fire. Keep the electrical parts of the equipment away from water or sources of high temperature.

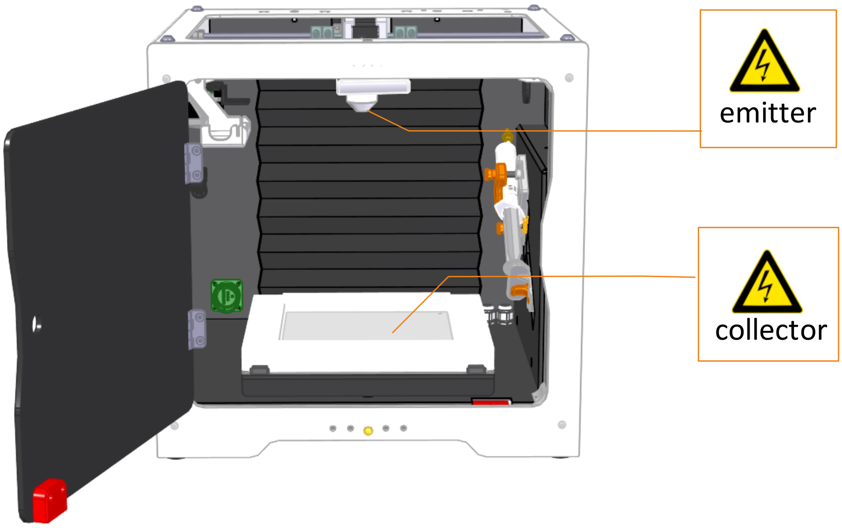
2 Working with high voltage.

Accessible elements that can be electrically active with high voltage are the emitter, the collector and the cables that reach them. These elements are indicated in the image of Pict. 2.1. Avoid contact with them while the high voltage is active or the front door is closed.

After disabling the high voltage sources the circuit will take approximately 2 seconds to discharge, do not touch the active parts during that time.

High voltage

Avoid contact with active parts and discharge them before manipulating.



Pict. 2.1 NovaSpider ProLab 3D unit; the indicated elements can be active with high voltage

3 Static electricity. Static charges that may be present in single components, even when the equipment is switched off, do not pose a risk to the user. They can be discharged by a discharging device before being touched.

Static electricity

Use proper grounding.

Generated fibers get charged during their production process and this charge can be transferred to the storage area. Connect the collector in a correct way so that, at the end of each program, it is properly discharged to the ground.

4 The temperature of the pellet extrusion head can reach 350°C. Do not touch this item with bare hands while it is hot. Beware that the heat can damage protective gloves if they are not suitable for the temperature that is being handled.

Beware

Do not touch, hot surface.

5 During the performance of automatic programs, the head can move at high speed. Avoid touching these items while the unit is working.

Moving parts

Do not touch moving parts while an automatic program is running.

6 Always ensure that there are no flammable gases near the unit while it is working as the spark from a static discharge or an electric arc could cause ignition.

Fire risk

Keep the unit away from flammable gases.

7 Work with hazardous materials. Some of the materials used for electrospinning can be toxic or dangerous. The user is responsible for knowing the risks associated with the materials he is using and must be trained and qualified to work safely with these materials.

Hazardous materials

Use adequate protection regarding the materials in use.

8 The access to the electrical and mechanical elements of the unit placed behind protective parts should only be given to authorised personnel. Any damage or accidents caused by removing or altering these protective parts will be the responsibility of the person who has modified them.

Maintenance and repairs

Must be performed by qualified personnel.

3 Technical Specifications

		NovaSpider Prolab3D
Processes	Electrospinning with solvent	Yes
	Voltage (kV)	0-30
	Melt-electrospinning:	Yes
	Voltage (kV)	0-10
	Temperature range	25-350
	Speed X-Y (mm/min)	24000
	Precision	10 µm
	Programming	CURA / G-code
Collector	Flat plate	190 x 190 mm
	Rotating drum	Optional
	Speed (rpm)	0 - 5000
	Dimensions (length)	110
	Dimensions (diameters)	30, 20, 10, 8 mm
	All parts in range	2- 40 mm
Emitter	Syringe pump (motor)	Double syringe
	Pellet extruder	Yes
Atmosphere	Temperature monitoring (°C)	No
	Humidity monitoring (% RH)	No
	Extraction	Optional
	Extractor	Optional
	Tube connection	Yes
	Methacrylate top cover	Yes
System control	Interface	Web/touch screen
	Secure closing	Yes
	Formulas/programmes	Yes
Data register	Storage	Yes
	Graphics/monitoring	Yes
Video camera	Real-time view	Yes
	Time-lapse videos	Yes
	Taylor’s cone view	No

4 Installation and Running

4.1 Connection

NovaSpider Prolab3D needs a single connection to work, the electric cable supplied by the manufacturer. Attach this cable from the back of the unit to a standard socket which fulfils electrical requirements described below. If necessary, a socket adapter to connect to a different type of a socket can be used. In all cases, beware of meeting the following electrical requirements.

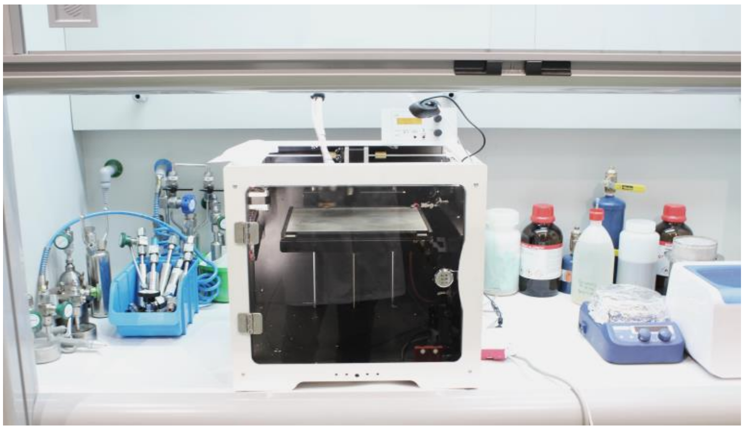
Electrical requirements:

Voltage [V]	100 - 240 V
Intensity [A]	2A
Frequency [Hz]	50 / 60 Hz

Once connected and after activating the unit by a switch located on the back of the equipment, it is powered and ready for use. It takes around a minute for NovaSpider Prolab3D to boot up until all its systems are fully operational.

4.2 Work Environment

NovaSpider Prolab3D should be positioned in an environment adequate to the materials which are going to be used. If considered necessary, it can be placed inside of an extractor hood or in any location that meets the requirements described in this manual. As stated in the safety section, positions where there are flammable gases or where there is a risk of falling water or other liquids on the electrical components of the unit must be avoided.



Pict. 4.2 An image of NovaSpider Prolab3D placed in an extractor hood

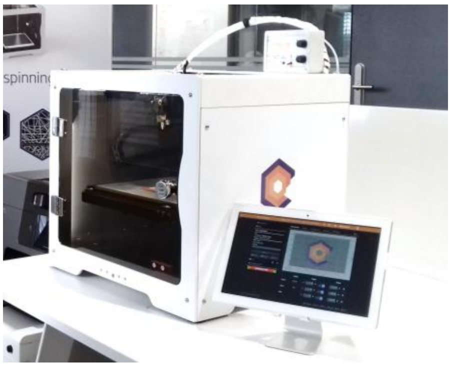
It is recommended to place the unit on a table or a platform of an adequate height to ensure that it can be handled in a comfortable and ergonomic way. One person is enough to run the unit.

Access to the machine is through the transparent front door. The operator can watch the progress of work through them. It can also be done through a NovaScience interface which allows remote management and monitoring of NovaSpider Prolab3D from any device that has a web browser.

5 Operating Instructions

NovaScience user interface

NovaScience user interface allows to control and monitor main parameters of the production process of NovaSpider equipment. This application has been developed from Octoprint, an advanced open source application for controlling 3D printers. Any information about functions of the user interface that are not explained in this manual, can be found on the following website: www.octoprint.org. This tool allows to monitor and control NovaSpider Prolab3D safely from any device or terminal that has a web browser.

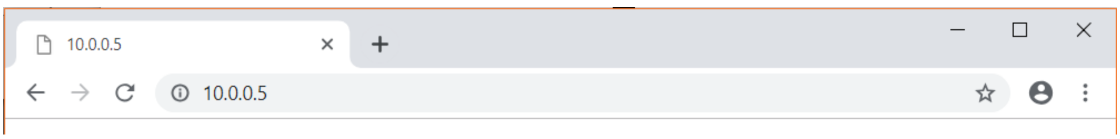


Pict. 6.0 An image of a touch-screen user interface next to a NovaSpider unit

5.1 Step One, Connect to NovaSpider Unit

5.1.1 Option 1: Local Wi-Fi Network Generated by the Unit

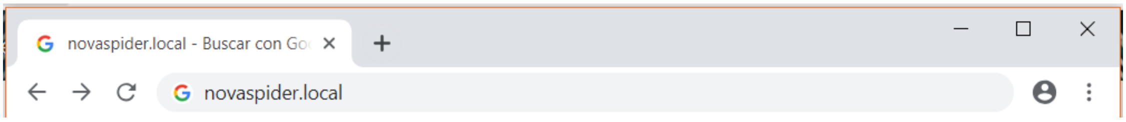
1. Connect to *NovaSpider* Wi-Fi network.
   Password: 1234567890
2. Enter: 10.0.0.5 in the address box of your browser (Pict. 5.1.1).
   After pressing *Enter* key, NovaSpider application will load onto the browser.



Pict. 5.1.1. An image of an address box of a browser connecting to NovaSpider unit

5.1.2 Option 2: Connect through an Existing Wi-Fi Network

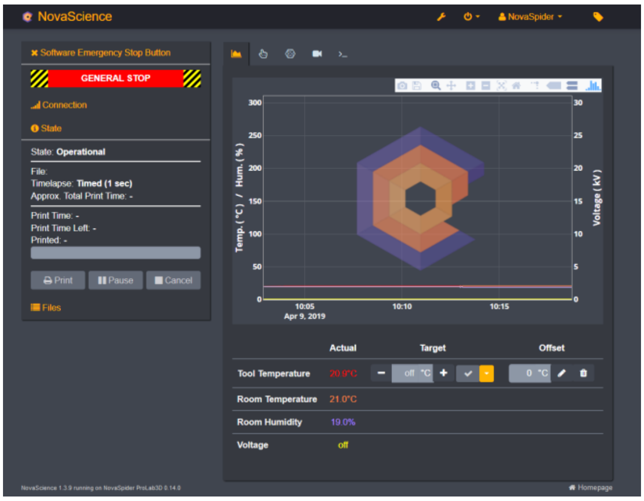
1. Prepare the unit to connect to an existing Wi-Fi network *My Wi-Fi network* (refer to Annex I).
2. Make sure you are connected to your Wi-Fi network *My Wi-Fi network*. If your device supports *bonjour,* enter: *novaspider.local* in the address box of your browser (Pict. 5.1.2). Otherwise, enter IP address of your device, e.g. *192.169.0.10* (to know how to check the IP address of your device refer to Annex II).



Pict. 5.1.2. An image of an address box of a browser connecting to NovaSpider unit

5.1.3 Successful Connection

The home screen of NovaScience application will load onto your browser once you have successfully connected (Pict. 5.1.3). The user has the access to view all the elements and parameters of the process from this screen, but in order to interact with the unit he must log in with a username and a password.

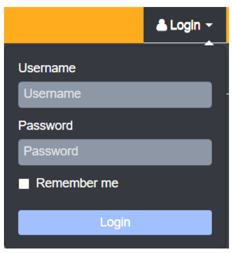


Pict. 5.1.3. An image of the screen after loading the application onto the browser

5.2 Log in

Enter username and password to access to all controles of the application. The access to identify oneself can be found in the right part of the upper tab. By default, enter:

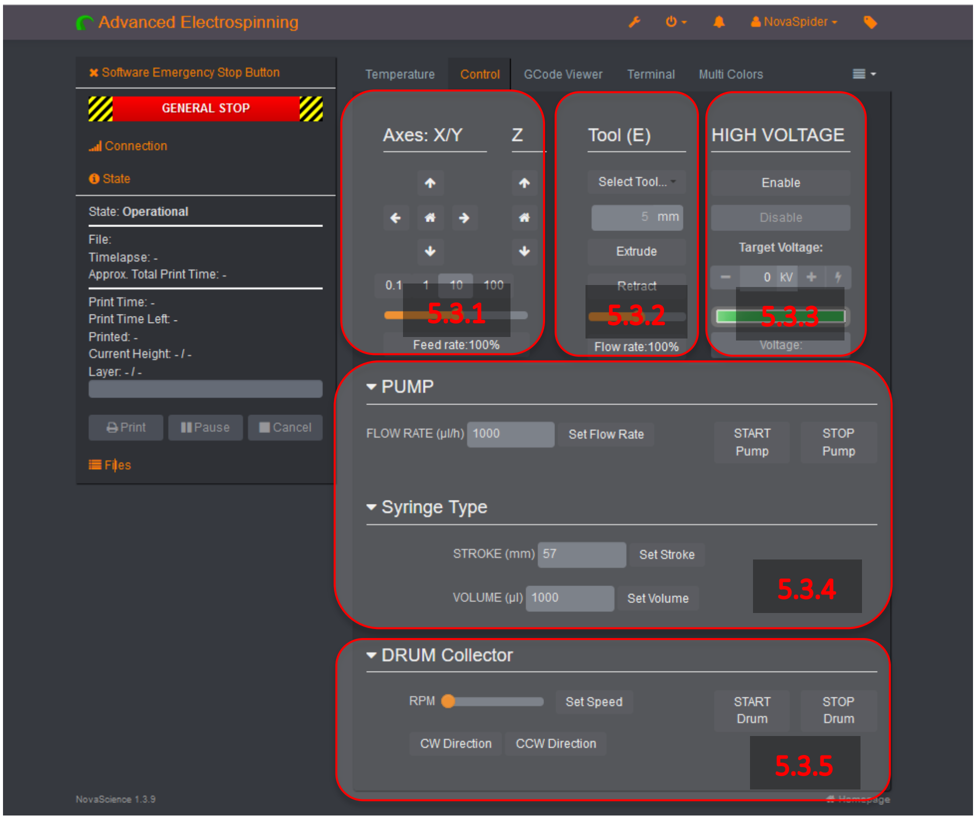
Username: NovaSpider
Password: novaspider



Pict. 5.2 A log in window

5.3 Manual Operating

After starting the session, the user can control different parameters from the *Control* window.



Pict. 5.3.1 An image of the interface screen with *Control* window open

5.3.1 Head and Bed Movements

I. Head movements (axes: x, y)

– Home: send the x and y axes home to position them.

– Direction arrows: click on an arrow to move the head in a chosen direction (by a distance of: 0.1, 1, 10, or 100 mm).

II. Bed movements (axis z)

– Home: send z axis home to position it.

– Direction arrows: click on an arrow to move the bed on z axis (by a distance of: 0.1, 1, 10, or 100 mm).

III. Feed rate flow

– Select the percentage of the speed at which you want the previous movements to be made.

5.3.2 Control of the Pellet Extruder; Tool(E)

I. *Extrude*: extrude the material in an indicated measure (mm).

II. *Retract*: retract the material in an indicated measure (mm).

III. *Flow rate*: select the percentage of the advance speed of the material with respect to last sent value.

5.3.3 HV Power Supply

I. *Enable*: enable the high voltage source.

II. *Disable:* disable the high voltage source.

III. *Target voltage:* enter the desired voltage value and confirm it.

IV. *Indicator bar*: shows real voltage value at the output of the source.

5.3.4 Electrospinning Pump

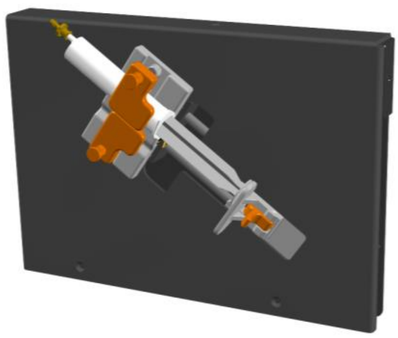
I. *Start pump*: start the pump.

II. *Stop pump*: stop the pump.

III. *Flow rate*: indicate a desired flow and set the value by pressing *Set Flow Rate.*

IV. *Syringe type:*

- *Syringe stroke:* stroke in mm of the syringe in use.
- *Syringe volume*: volume in µl of the stroke indicated for the syringe in use.



Pict. 5.3.4 An image of an electrospinning pump for syringes

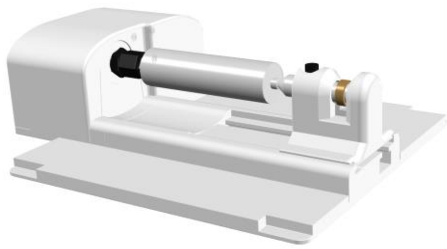
5.3.5 Drum Collector

I. *Set speed:* Set the speed selected from the bar.

II. *Start drum*: start the drum.

III. *Stop drum*: stop the drum.

IV. *CW / CCW direction*: select the direction of rotation.



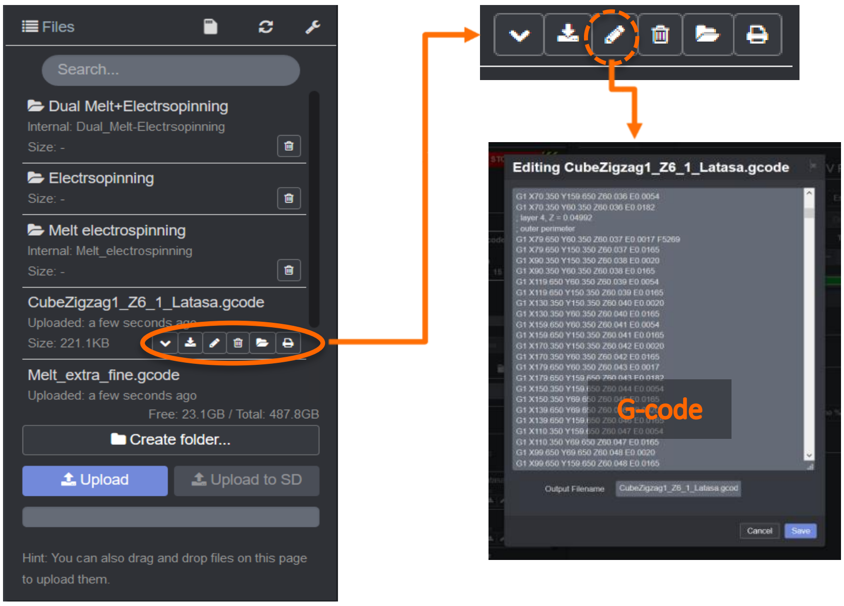
Pict. 5.3.5 An image of a drum collector for nanofibers alignment.

5.4 Automatic Operation

5.4.1 Automatic Programs

This section explains how to handle and execute automatic programs to produce nanofibers. These G-Code programs can be easily generated through a CURA open source application, following the instructions described in Annex V.

To load the program, you should drag it from the folder in which it is located to the browser window where NovaScience application is running. Once loaded, it will appear as tabs selection in *Files* on the left side of the interface screen (refer to Pict. 5.4.1).



Pict. 5.4.1 Steps necessary to edit an automatic program in NovaScience interface.

Tabs selection options in *Files:*

Additional information. Displays all file information.

Download. Downloads programs to your device.

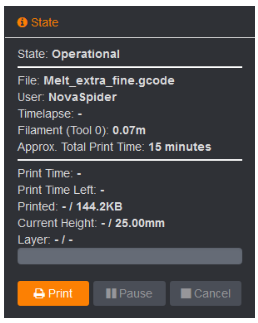
Edit. Allows editing G-code of a program.

Delete. Deletes a program.

Load. Selects a program.

Load and print. Loads and runs a program.

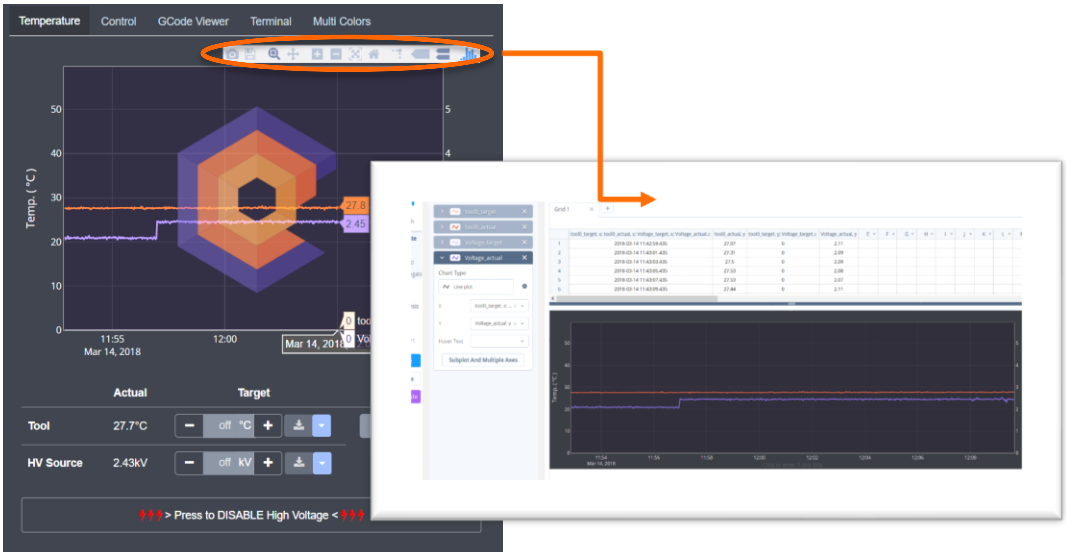
The selected program appears in a section *State* on the left of the screen. You can run, pause or cancel the program from there (Pict. 5.4.2).



Pict. 5.4.2 Information and status of the selected program

5.5 Real-time Monitoring

The integration of the *Plotly* visualization system allows to have interactive graphs based on the system parameters monitoring. The graphs and generated data can be modified and registered in *Web Chart Studio* editor.

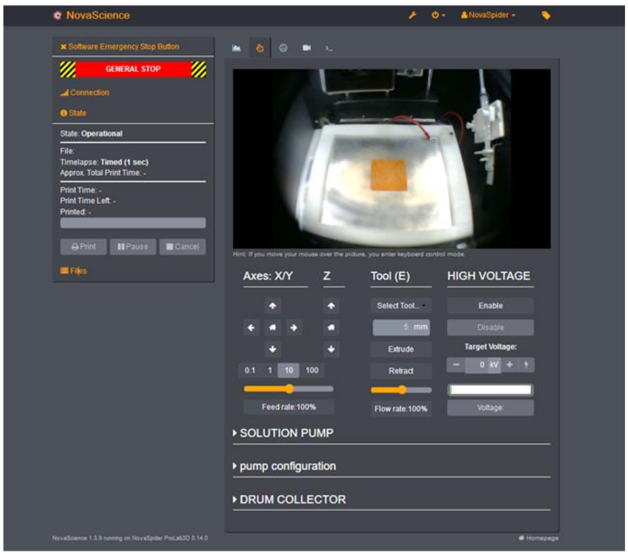


Pict. 5.5 An image of a graph where the variables of the process are registered in real-time

5.6 A built-in Camera. Remote Viewing and Generating of Videos

I. Viewing in real-time: A built-in webcam allows to view the process in real-time from any connected device.

II. Creating a time-lapse video: NovaScience allows for automatic generation of time-lapse videos to view the progress of work. The videos consist of sequences of photographs taken in a consecutive mode. Easy configuration of generating videos is accessible through time-lapse tab.



Pict. 5.6 An image from a NovaSpider built-in camera

6 Cleaning and Maintenance

Maintain the unit in good condition. Clean all of its parts and accessories after each use. Before starting cleaning or maintanence, make sure that the unit is switched off and there aren't any hot or electrically charged parts. The use of solvents or other chemical products may affect the paint or other elements of the unit.

Make sure an adequate personal protective equipment (PPE) is used regarding the materials that had been used and the work environment. Use gloves appropiate for handling high temperatures when manipulating any element that might be hot (extruder and adjacent elements). Do not spill water or other liquids on electrical elements.

Cleaning procedure:

1) Move the piston which drives the syringe to the origin. Extract all the elements used for pumping fluids:

    a. Syringe
    b. Pipes used for supplying the solvent
    c. Needle

2) Remove the collector that you have used (a plate or a rotating drum).

3) Clean and remove any debris embedded in the nozzle of the extruder head.

4) Clean all the elements of the interior of the machine and those that have been removed in the previous steps. Use a damp cloth or an appropriate cleaning product depending on the materials and solvents you have used in the production process. Do not use a scourer on glass and methacrylates.

5) Assemble the system.

7 Annexes

Annex I - Configuration of a Connection to an Existing Wi-Fi Network

The easiest manner to configure NovaScience WiFi is by editing the octopi-wpa-supplicant.txt file. This file can be found in a partition *boot* on the microSD card of the unit. Eject the card from the unit and insert it to the microSD card slot of your device. If it does not appear on the screen, eject and re-insert the micro-SD card. Before proceeding, MAKE A BACKUP COPY of the file you are going to modify. Then, open the file to edit it. It is important NOT to use a default text editor for Windows or Mac, but, for example, Notepad ++. Edit the following lines of the text, replacing the generic names with the name and password of your Wi-Fi.

Before editing:

```
## WPA/WPA2 secured
#network={
#       ssid="put SSID here"
#       psk="put password here"
#}
```

After editing:

```
## WPA/WPA2 secured
#network={
        ssid="your Wi-Fi name"
        psk="your Wi-Fi password"
}
```

Now, in the same file, select the country in which the Wi-Fi connection is made. To do so, edit the following lines:

```
# Uncomment the country your Pi is in to activate
# Wifi in RaspberryPi 3 B+ and above
# For full list see: https://en.wikipedia.org/wiki/ISO_3166-1_alpha-2
country=GB # United Kingdom
#country=CA # Canada
#country=DE # Germany
#country=FR # France
#country=US # United States
```

In the case shown above, if you are not in Great Britain, you should comment on Great Britain (adding the symbol # at the beginning of the line) and uncomment the country you are in (deleting the symbol # from the beginning). If the name of your country is not displayed, comment all and add yours in a new line. Click on this link https://en.wikipedia.org/wiki/ISO_3166-1_alpha-2 to find a list of international codes.

In case the procedure does not work, and after checking that you have followed it correctly, make sure that the characters such as "" and spaces are exactly as they should be.

Annex II - How to Find the IP Address of NovaSpider Unit

After following the steps of Annex I, NovaSpider unit should be connected to the local network. To open NovaSpider interface, you have to know its IP address. To do so, find the dynamic IP that has been assigned to NovaSpider unit in your network. Following options can be used:

- Connect using: novaspider.local as the address.
- Use IP network scanner, such as Nirsoft Wireless Network Watcher (Windows) or Fing (Android).
- Access router configurations and search for the IP of the unit.

In most of the cases, all the above options should apply. However, depending on your network specifications (size, security, etc.) some, or all, may not work properly. If you have access to a computer with Linux or Mac, another option is to mount the SD card and directly configure a static IP.

If none of the above works, connect through the NovaSpider Wi-Fi network, generated by the unit, as described in section 5.1.1 of this operating manual.

Annex III - Specifications for a Ventilation System when Using Hazardous Solvents

Some of the solvents used in electrospinning process are hazardous when coming into contact with or inhaling. To limit any risk caused by the normal evaporation ratio of the solvent to the atmosphere, it is recommended to use an appropriate extraction system for each kind of solvent.

NovaSpider Prolab3D is designed to fit inside a standard laboratory extractor hood. Placing the unit in an extractor hood is recommended to limit user's exposure to solvent vapors. Occasionally, it may be necessary to disconnect the ventilation of the extractor during centrifugation to prevent the fibers from being sucked in by the fan. In those cases, the chimney door must be kept closed during centrifugation.

If there is no access to an extractor hood, evaluate if the ventilation in the laboratory, where the unit is used, is adequate. In order to estimate it, check the limit of a safe exposure to the solvent. This information should be included on the label of each of the materials used to make a solvent. It is also important to know the volume of the room in which electrospinning is going to be conducted.

As an example, we will consider using Hexafluroisopropanol (HFIP) in a room with a volume of 140 m^3. The limit of exposure to this solution is 2.5 mg / m^3 which means the limit will be reached when 350 mg of solvent has evaporated (140 m^3*2,5 mg/m^3). What must be considered next, is time in which this limit will be reached. We can use the rate of evaporation of PVOH in water (60 mg/h) for the electrospinning process. Now the amount of time to reach the limit can be calculated:

$$t = 350 \text{ mg} / 60\text{mg/h} = 5.83 \text{ h} = 5 \text{ h } 49 \text{ min } 48\text{s}$$

This evaporation rate is true only for PVOH, which is why we should apply safety coefficients for this calculation, x5 or x10 are a safe tolerance.

By multiplying an evaporation rate by 5, the following result is obtained:

$$t5 = 350 / 60 \times 5 = 0.58\text{h} = 35 \text{ min}$$

By multiplying an evaporation rate by 10, the following result is obtained:

$$t5 = 350 / 60 \times 10 = 1.17\text{h} = 1\text{h } 10 \text{ min}$$

These results show that the air in the room should be completely renewed in 35 minutes or in 1h and 10 min depending on the level of a required security. Depending on the risks and the amount of the product being used, it is advisable to apply safety factor of 5 or 10. Even if the electrospinning unit is completely isolated, it is highly recommended to always wear a respirator when using any type of hazardous chemicals.

Annex IV - Materials Used with NovaSpider Prolab3D

The following recipes of materials, proportions and parameters give good results to obtain nanofibers from NovaSpider unit.

Material	Solvent	Conc. % (w/w)	Conditions
Gelatin porcine skin	water/acetic acid (2/3)	20%	10cm, 10kV, 0.5mL/h
PVP (Polyvinylpyrrolidone, Mw= 1,300,000)	ethanol	12%	10cm, 7.5kV, 0.5mL/h
PS (Polystyrene, Mw=280,000)	DMF/THF (3/1)	25%	10cm, 15kV, 1mL/h
PCL (Polycaprolactone, Mn=70,000-90,000)	acetic acid	20%	10cm, 12kV, 1mL/h
Nylon 6	HFIP	10%	12cm, 7-8kV, 1mL/h
PVC (Polyvinyl chloride, low molecular weight)	DMF/THF (2/3)	20%	12cm, 12kV, 1mL/h
PMMA (Polymethyl methacrylate, Mw=350,000)	HFIP	5%	15cm, 18kV, 1mL/h

The users of the unit utilise any of these or other recipes under their full responsibility.

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NOVA SPIDER