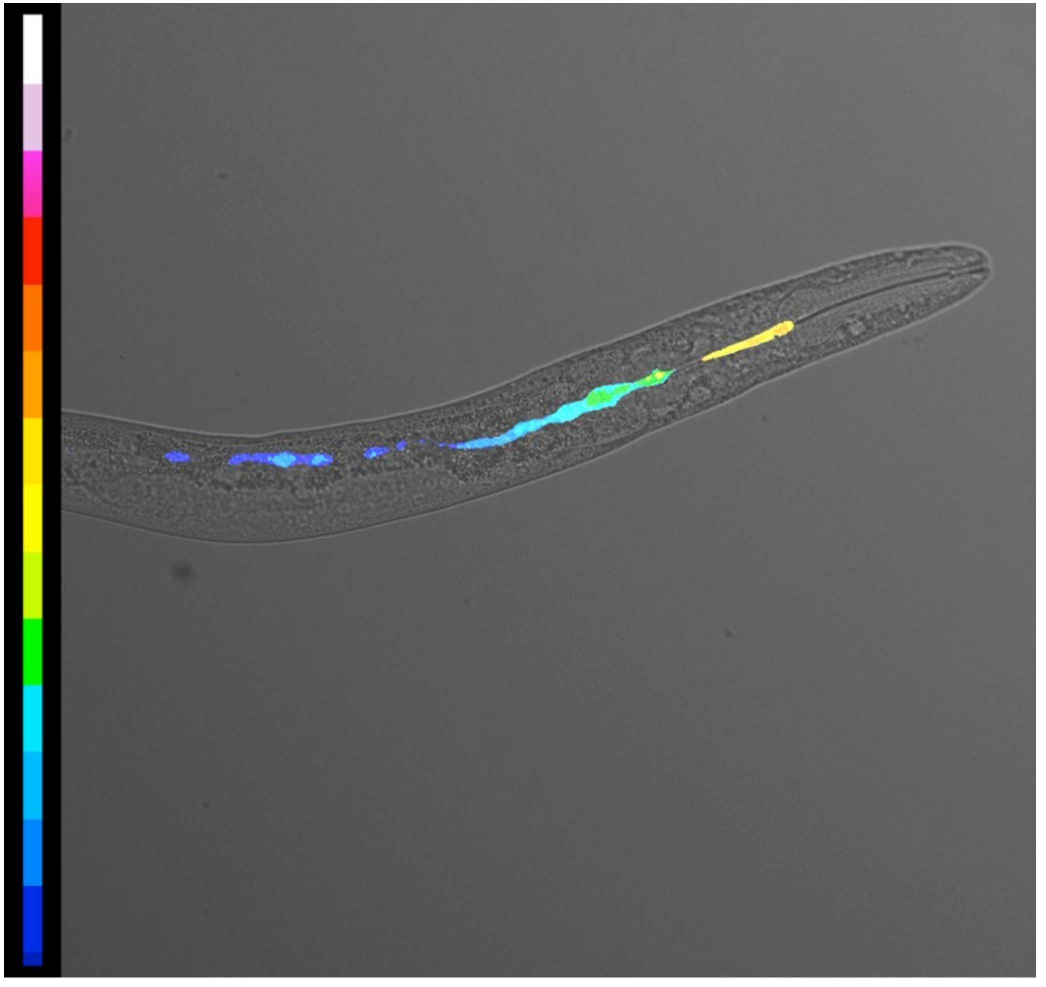
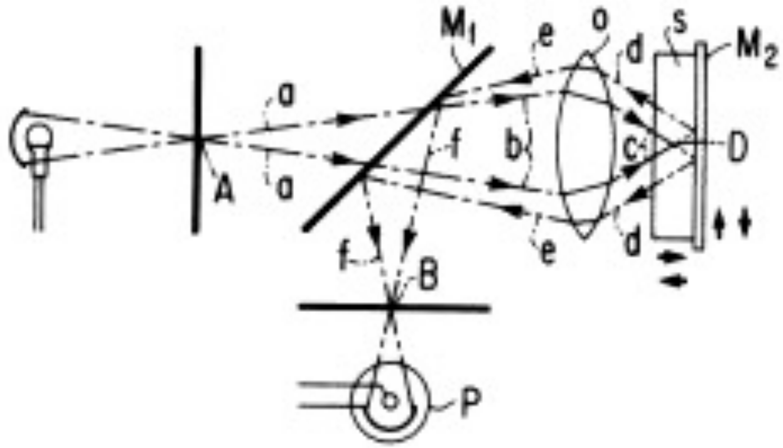
Fluorescence Microscopy

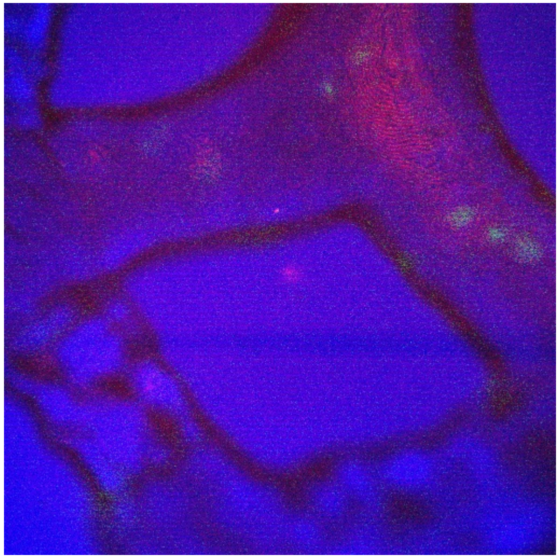
Fluorescence microscopy is used to detect structures, molecules or proteins within the cell. Fluorescent molecules absorb light at one wavelength and emit light at another, longer wavelength. When fluorescent molecules absorb a specific absorption wavelength for an electron in a given orbital, the electron rises to a higher energy level (the excited) state. Electrons in this state are unstable and will return to the ground state, releasing energy in the form of light and heat. This emission of energy is fluorescence. Because some energy is lost as heat, the emitted light contains less energy and therefore is a longer wavelength than the absorbed (or excitation) light. In fluorescence microscopy, a cell is stained with a dye and the dye is illuminated with filtered light at the absorbing wavelength; the light emitted from the dye is viewed through a filter that allows only the emitted wavelength to be seen. The dye glows brightly against a dark background because only the emitted wavelength is allowed to reach the eyepieces or camera port of the microscope.

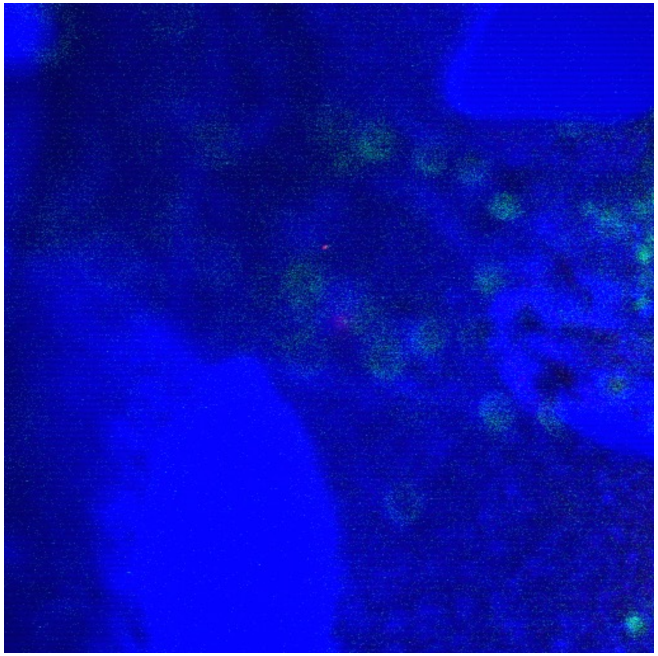
Fluorescence Microscopy



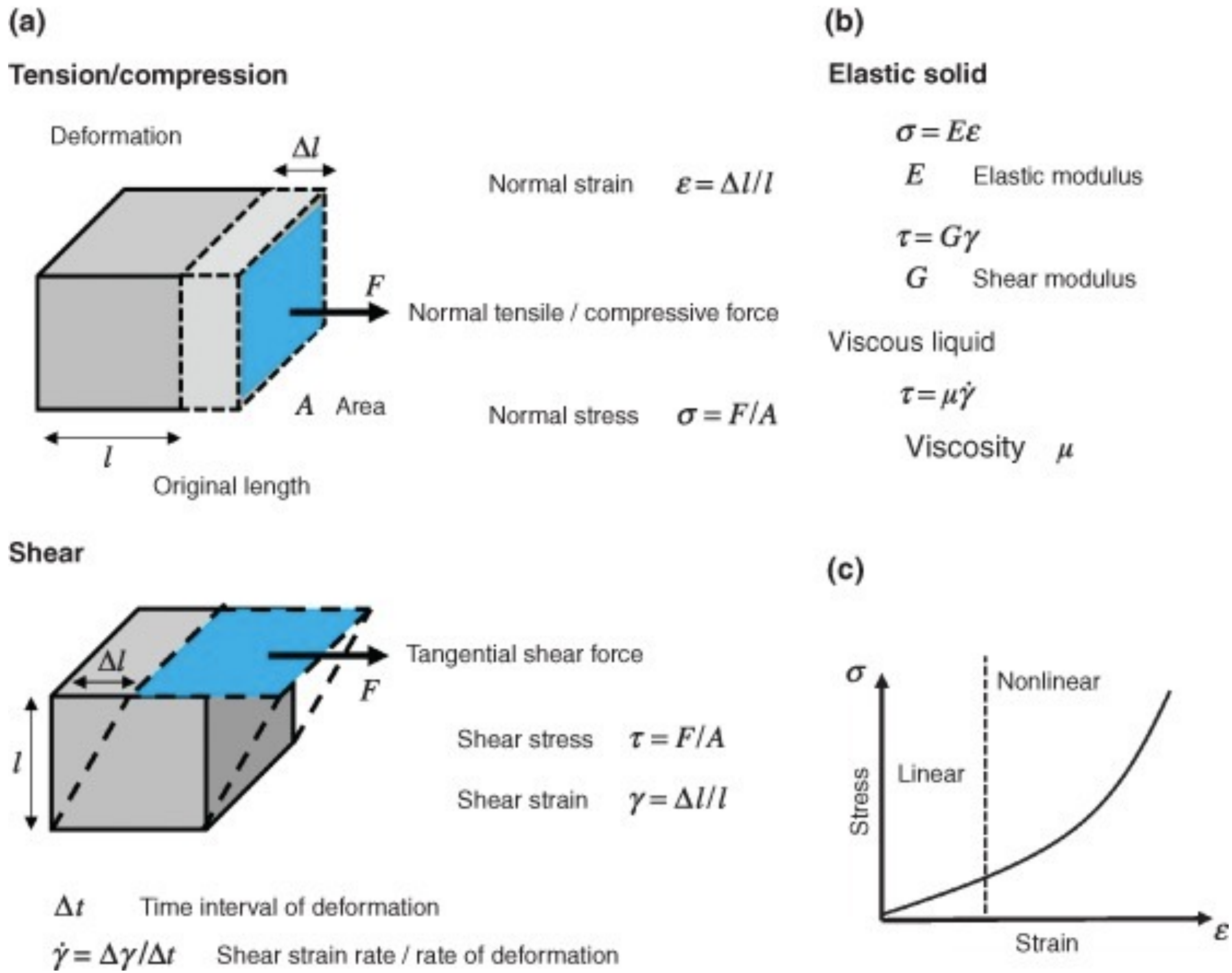
Confocal Microscopy

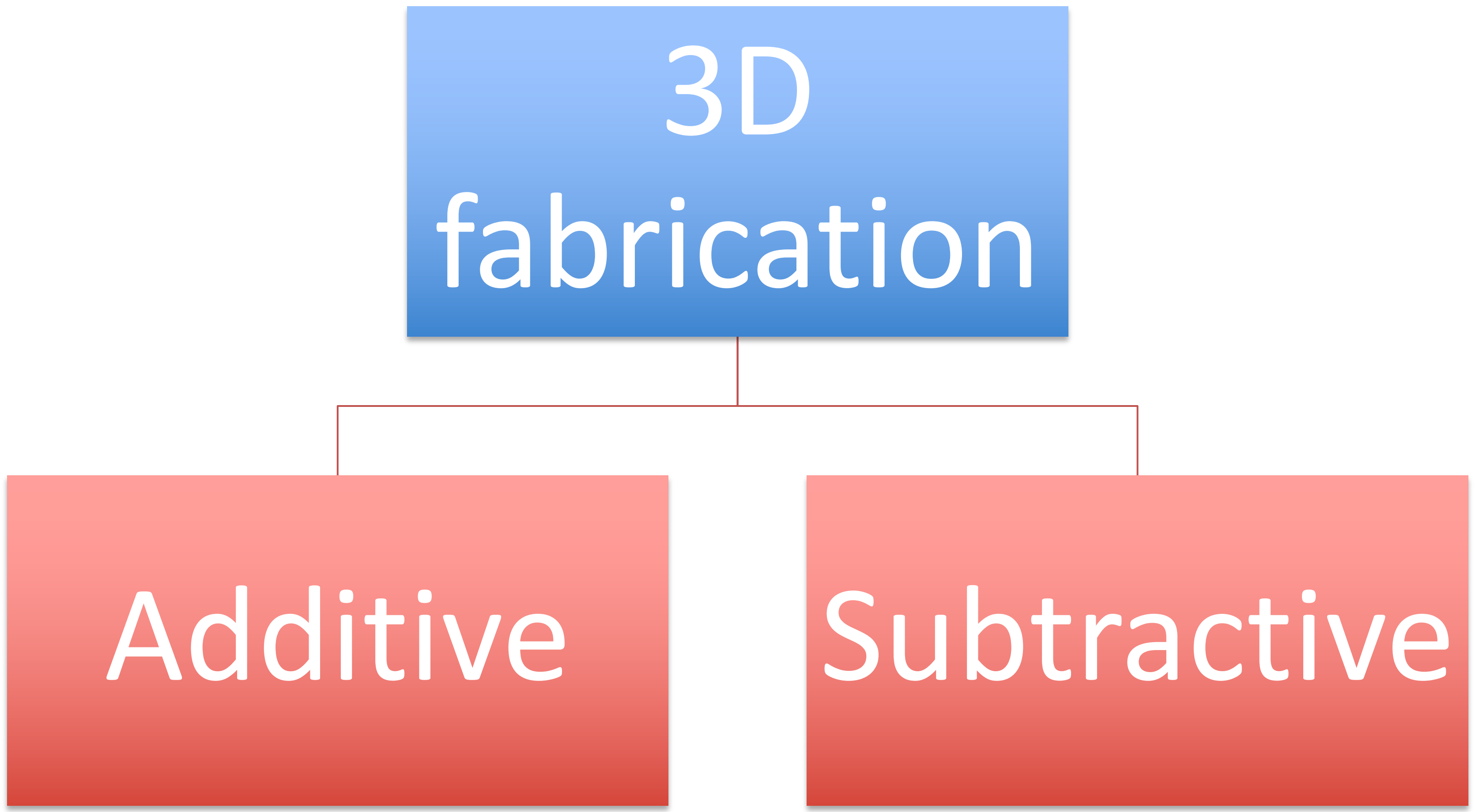




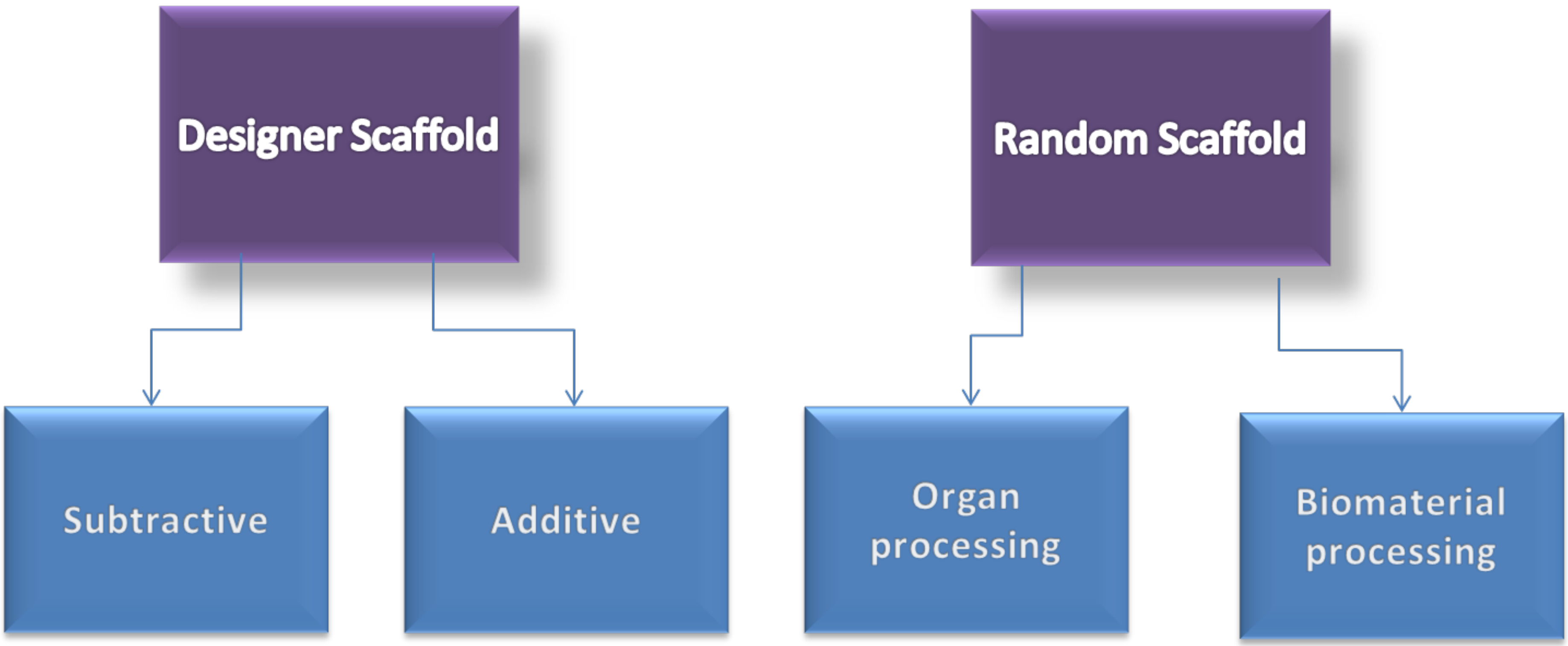


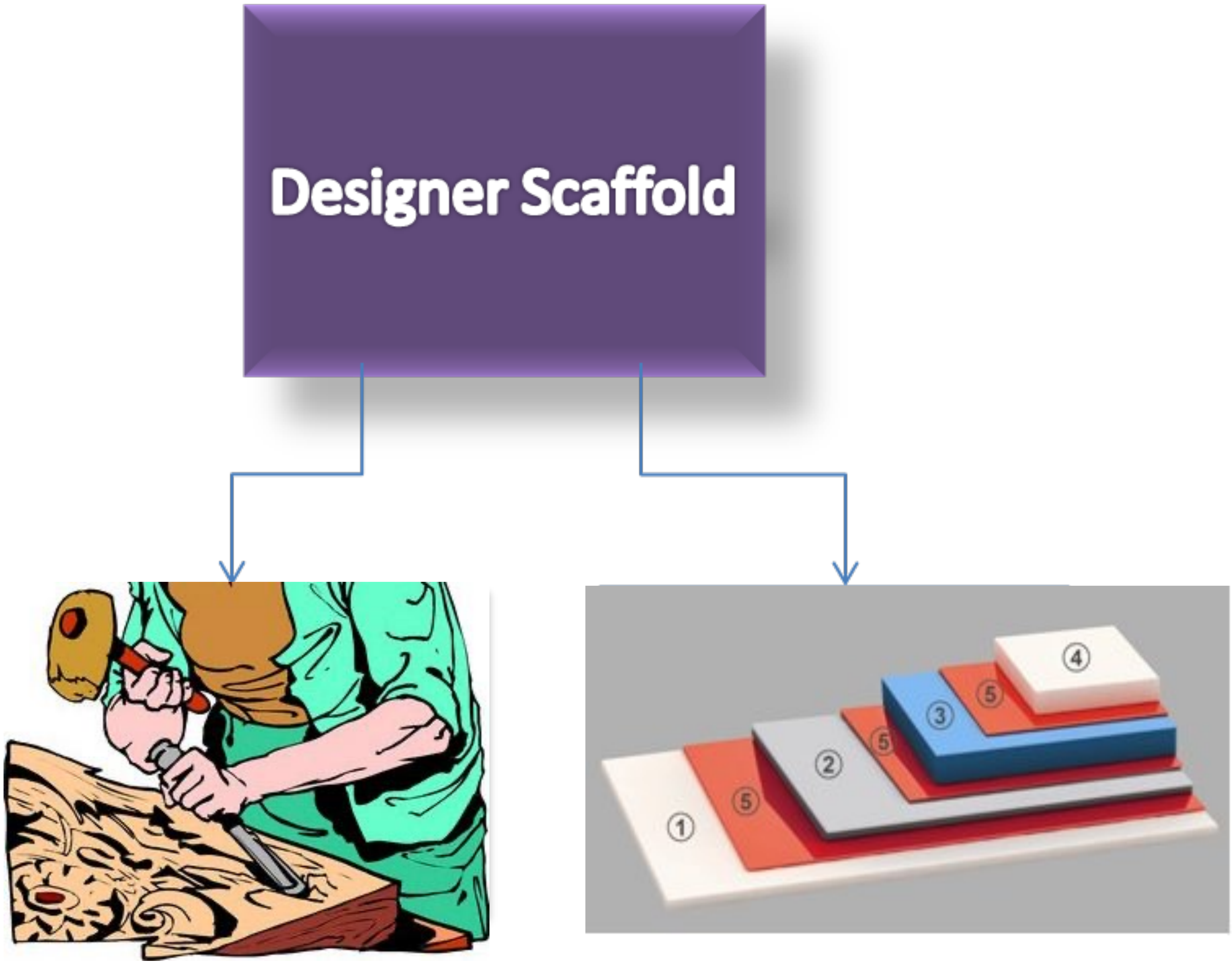
Mechanical properties




3D fabrication
Additive
Subtractive

Methods for generating MS stimuli in scaffolds

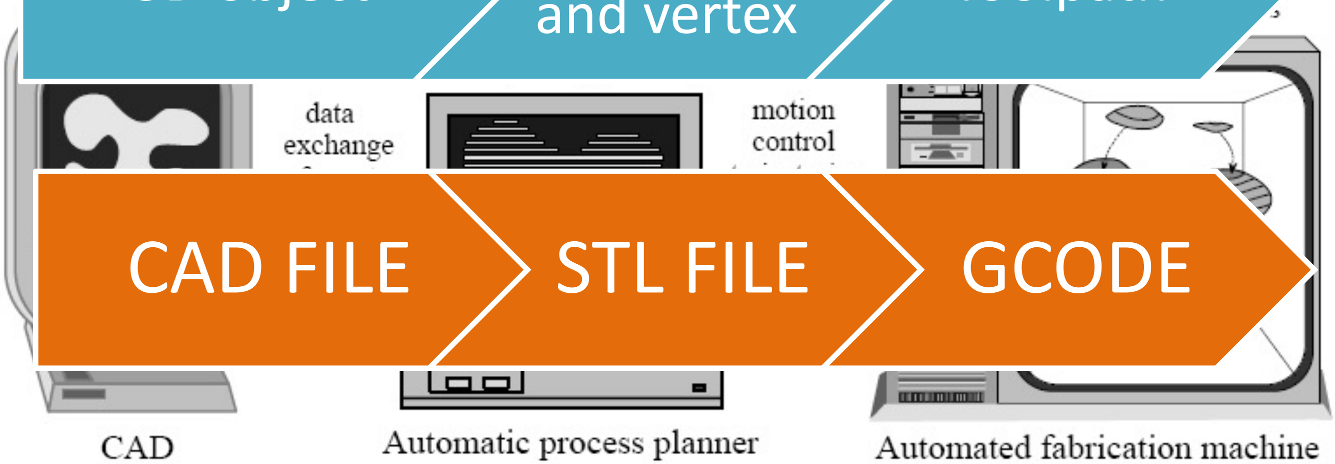



Designer Scaffold

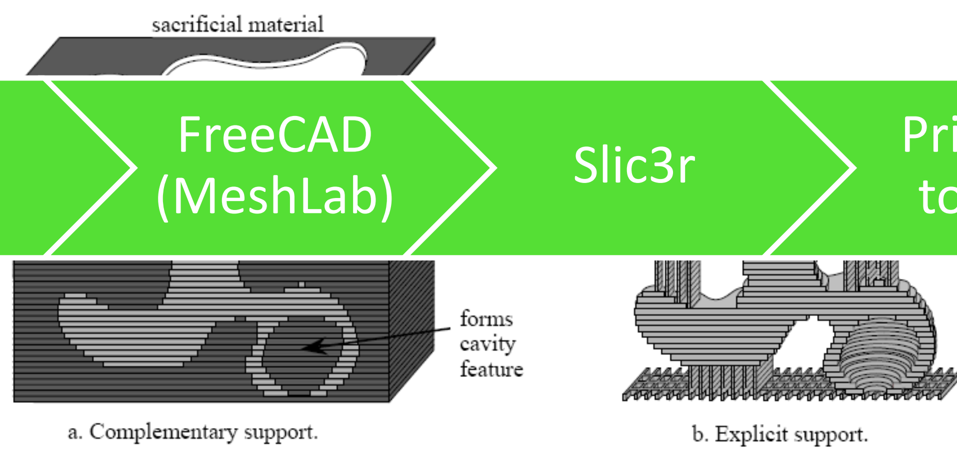
Additive = rapid prototyping

3D object → Triangle and vertex → Toolpath

CAD FILE → STL FILE → GCODE

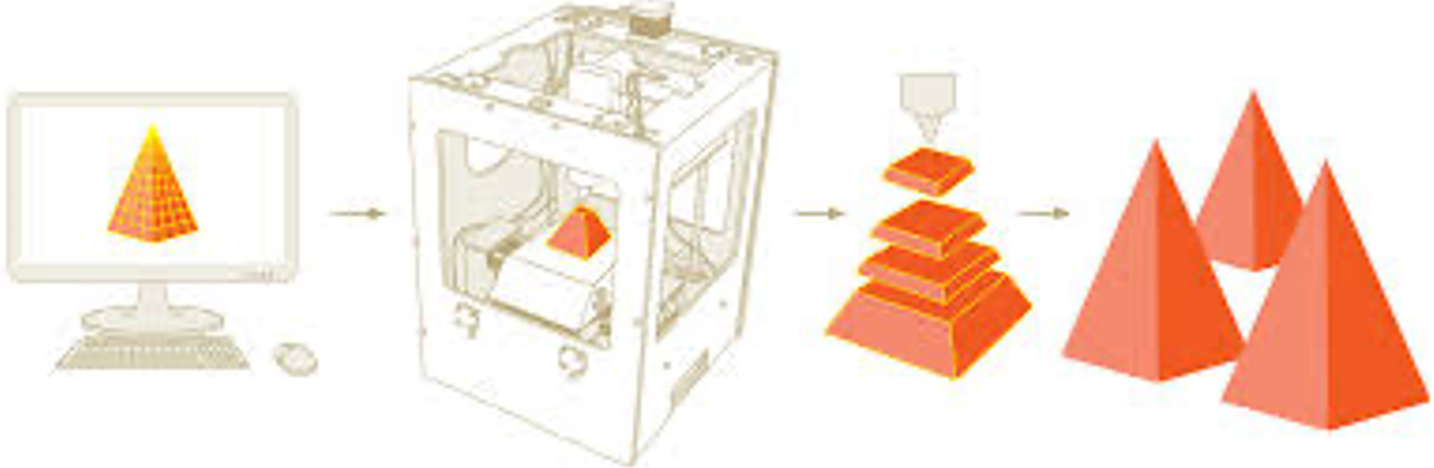


FreeCAD → FreeCAD (MeshLab) → Slic3r → Printrun to 3DP

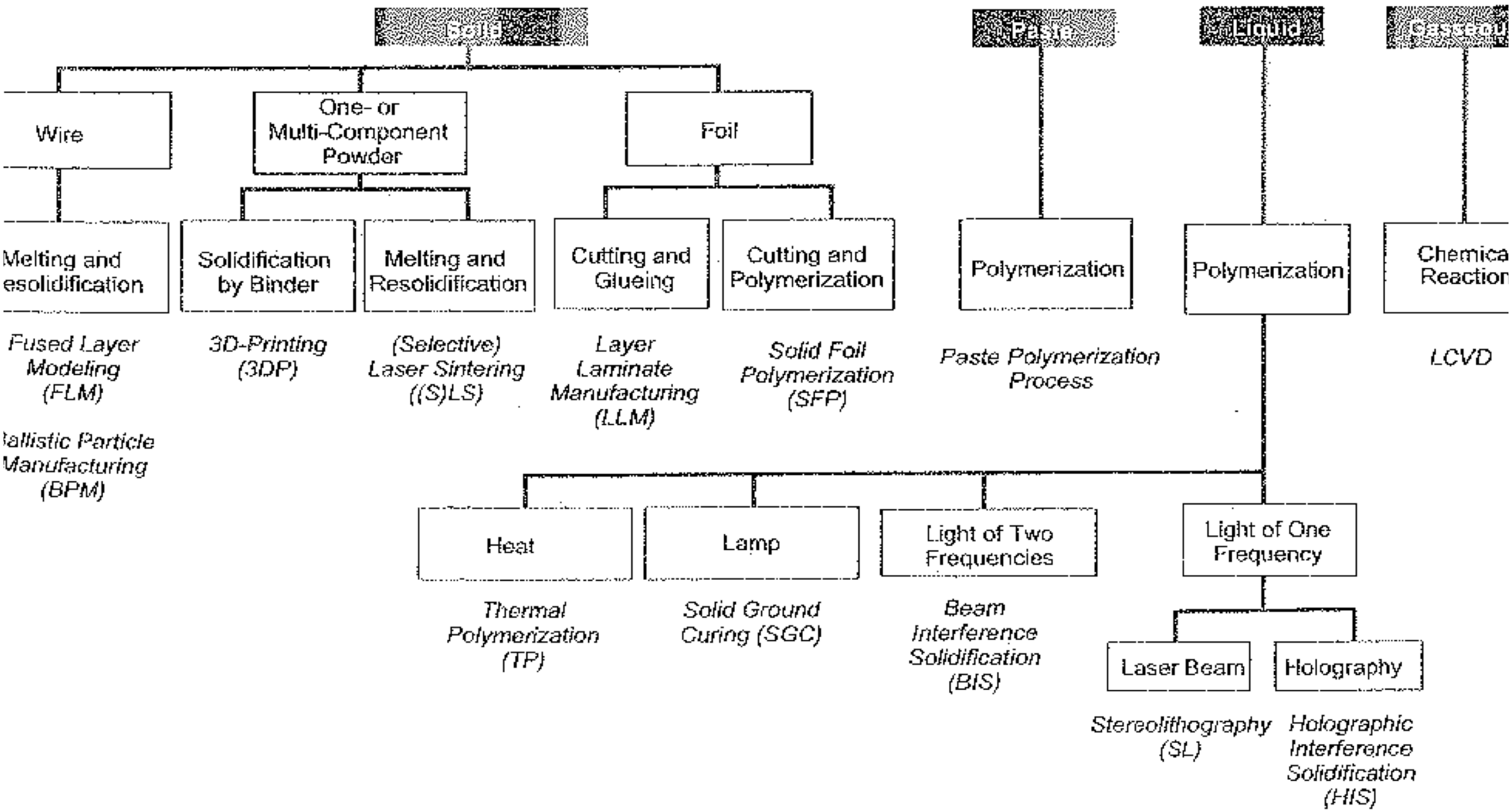


3D printing

- 3D printing (or Additive Manufacturing)is a process of making a three-dimensional solid object of virtually any shape from a digital model.
- 3D printing is achieved using an additive process, where successive layers of material are laid down in different shapes.



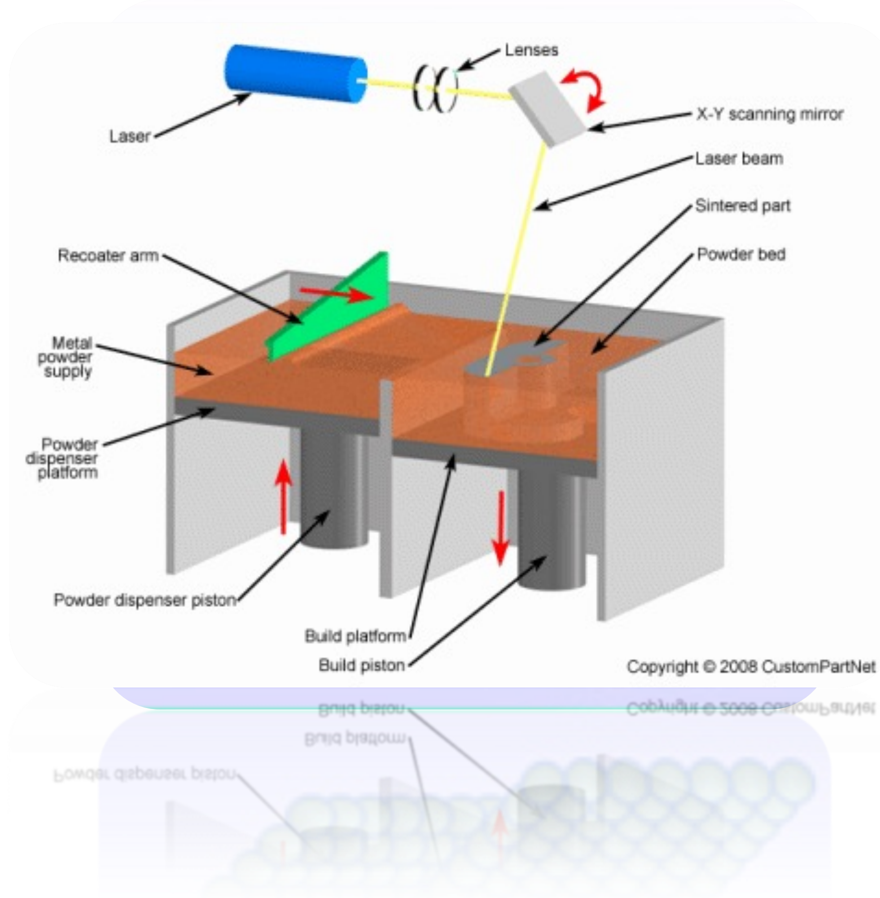
Additive manufacturing world



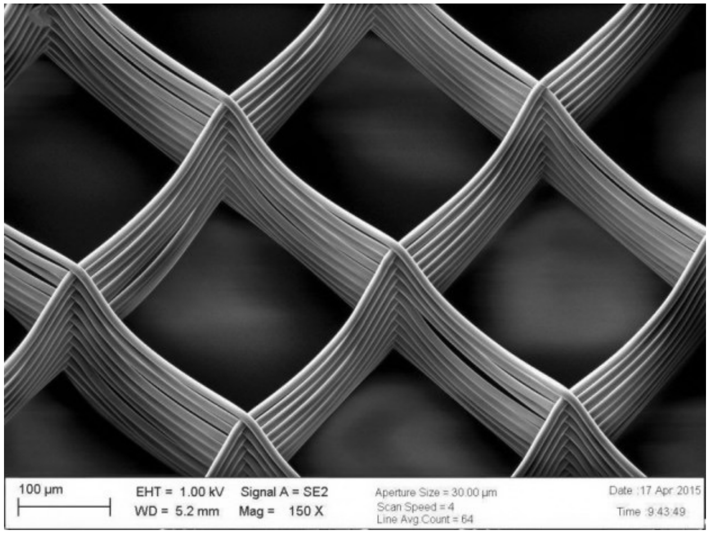
Designer Scaffold

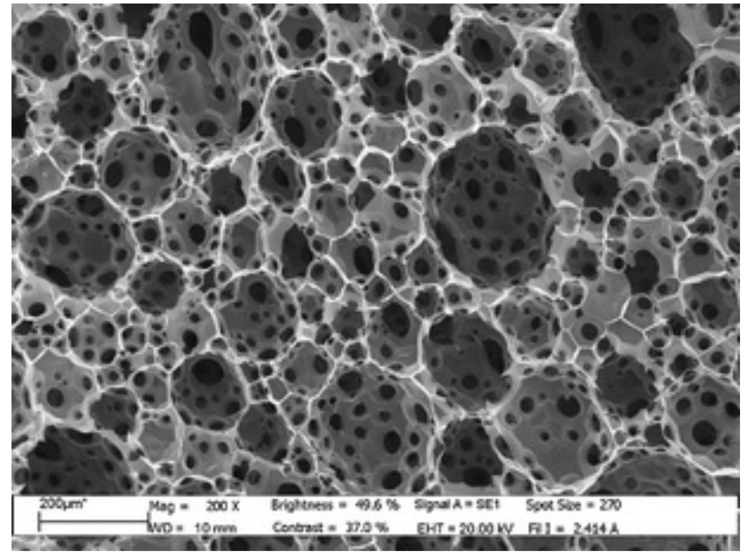
Three main groups:

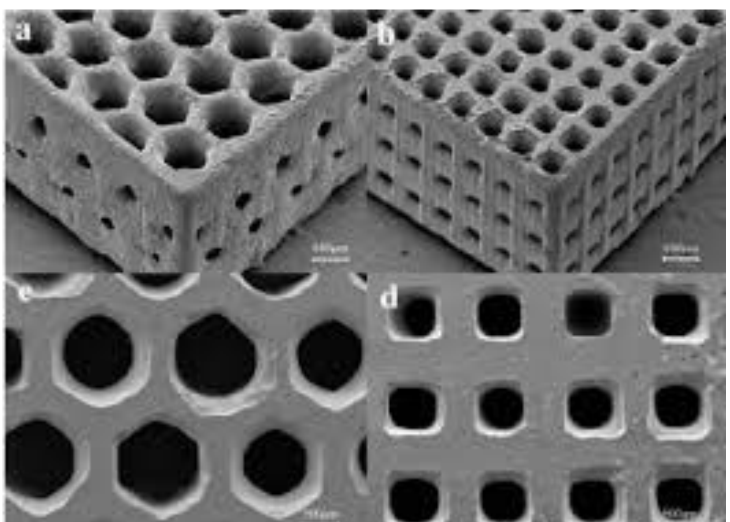
- laser systems
- nozzle based systems
- direct writing systems



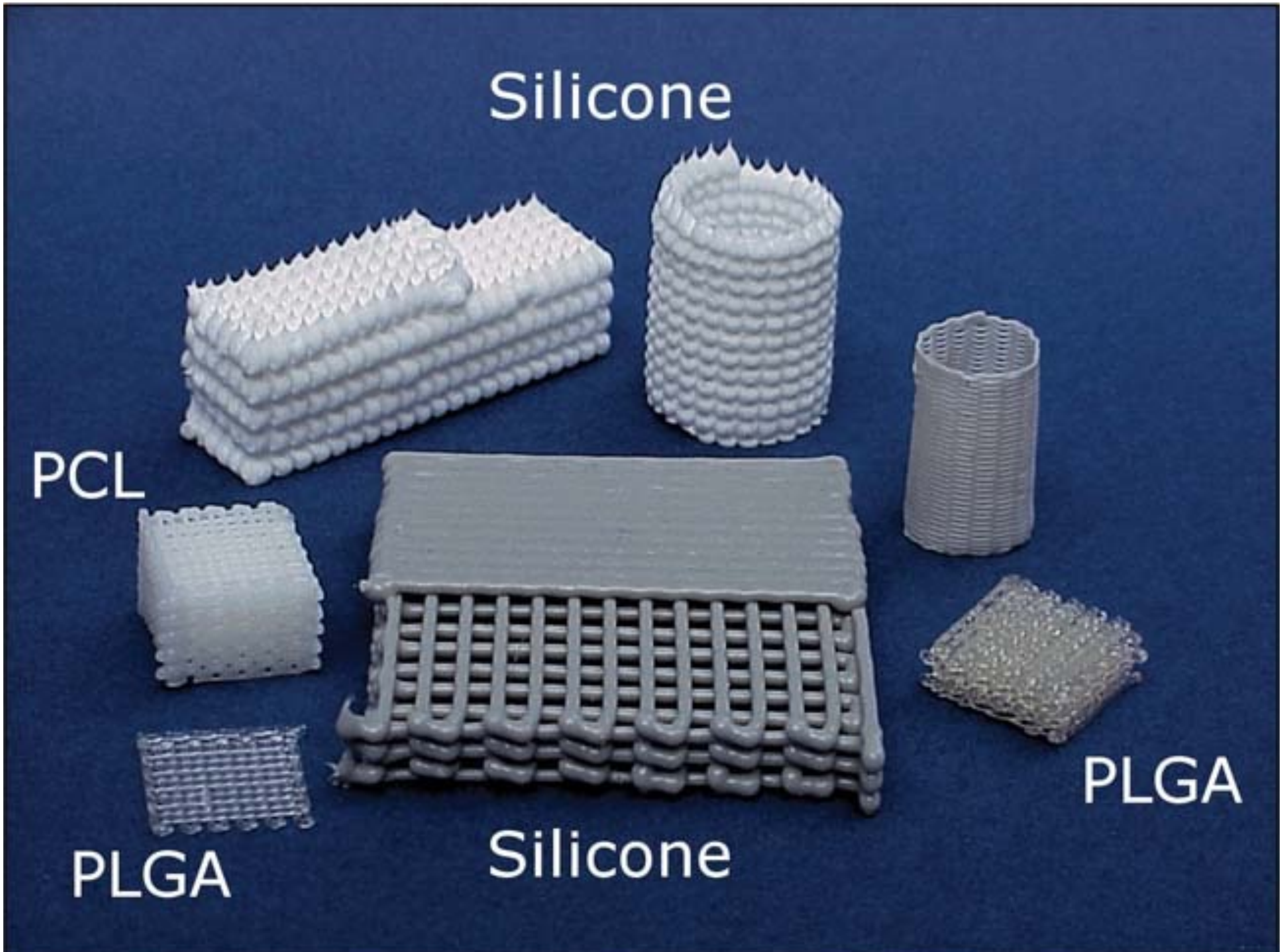
Scaffolds


100 µm
EHT = 1.00 kV Signal A = SE2
WD = 5.2 mm Mag = 150 X
Aperture Size = 30.00 µm
Scan Speed = 4
Line Avg.Count = 64
Date :17 Apr 2015
Time :9:43:49

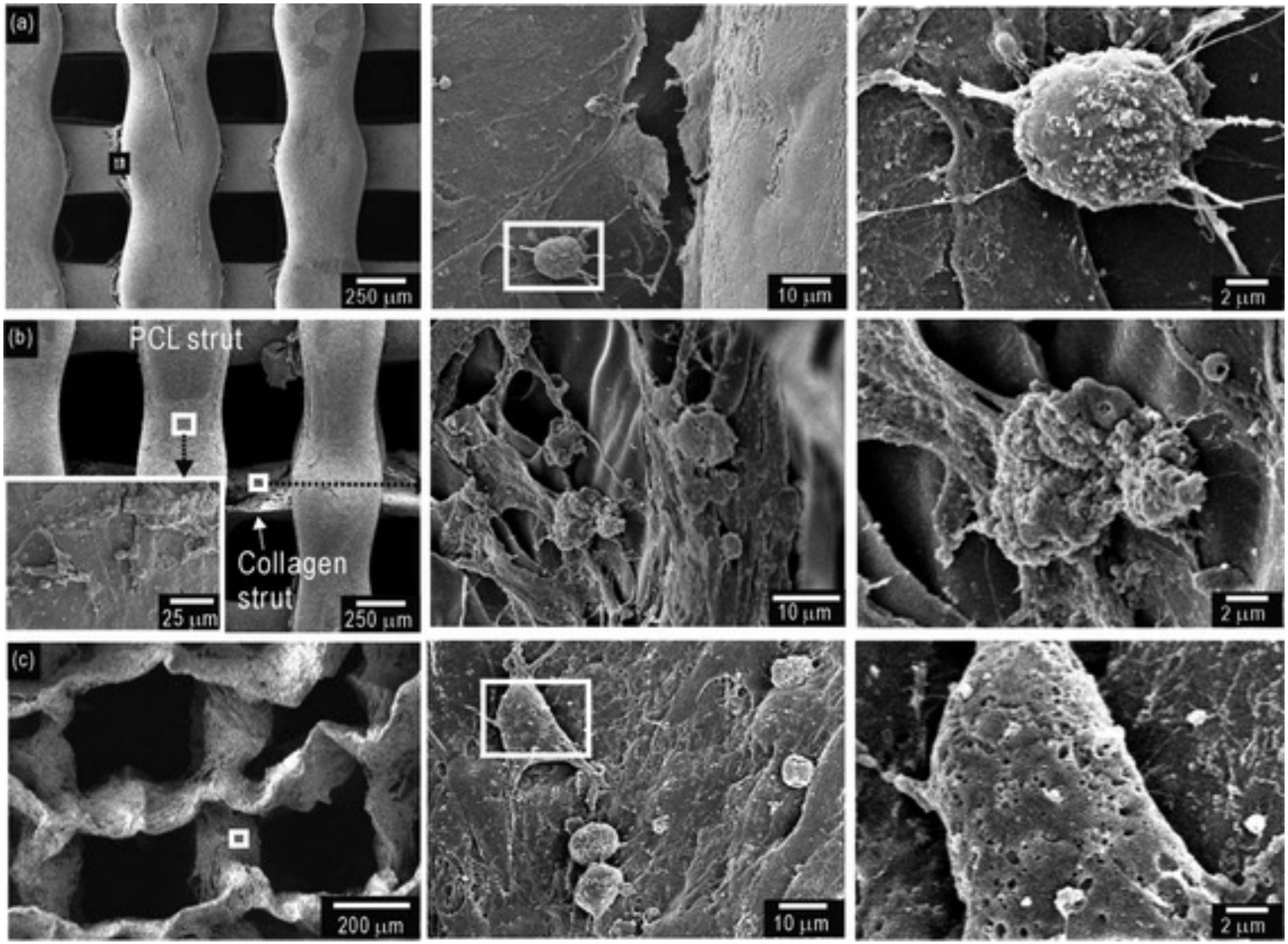

200µm
Mag = 200 X
WD = 10 mm
Brightness = 49.6 %
Contrast = 37.0 %
Signal A = SE1
EHT = 20.00 kV
Spot Size = 270
Fil I = 2.414 A


a
b
c
d

Scaffolds



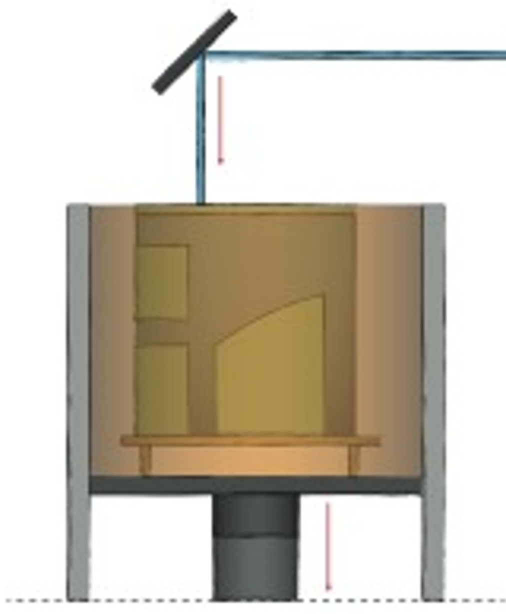
Scaffold

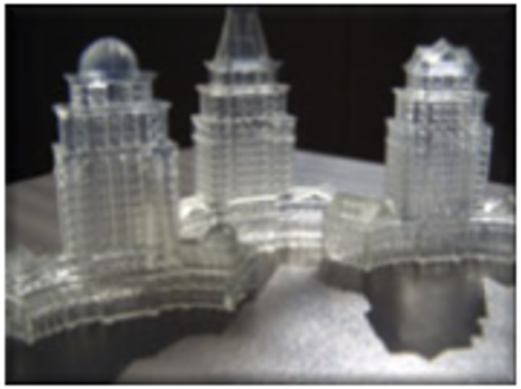


(a)
250 µm
10 µm
2 µm
(b)
PCL strut
Collagen strut
25 µm
250 µm
10 µm
2 µm
(c)
200 µm
10 µm
2 µm

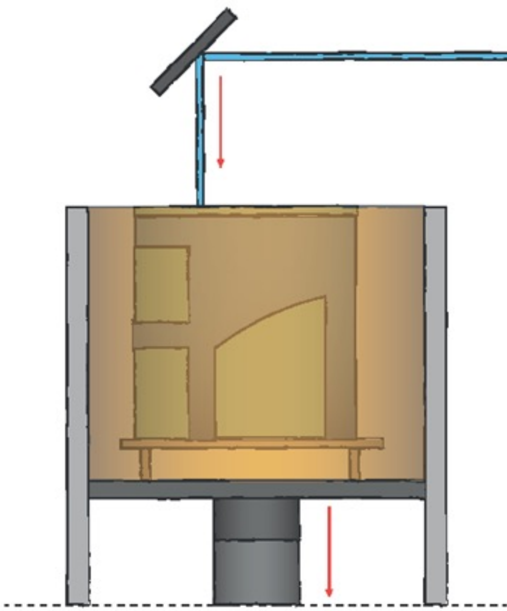
Available technologies

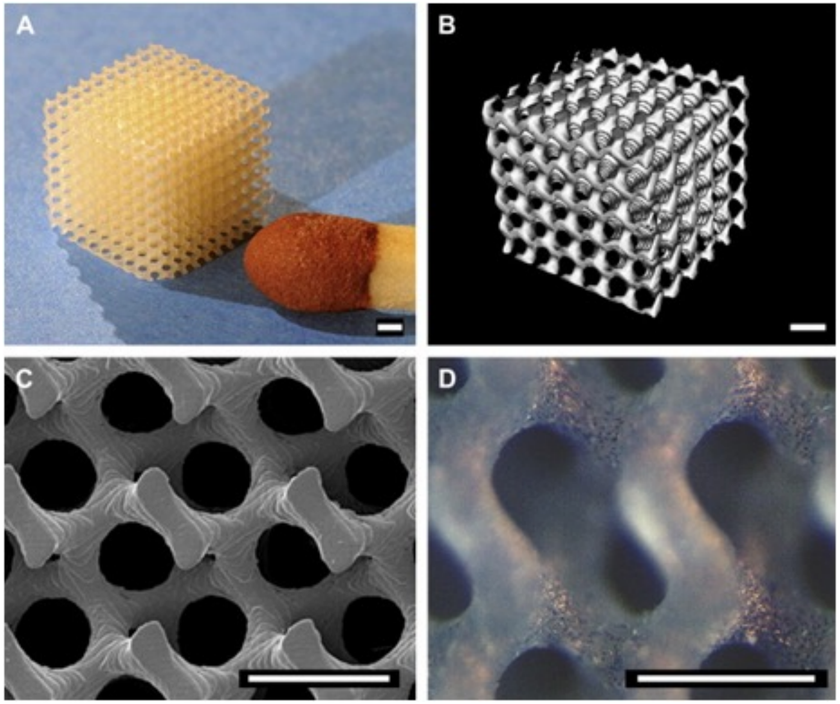
- Solidification of liquid materials
  - Photo-polymerization process





Stereolithography

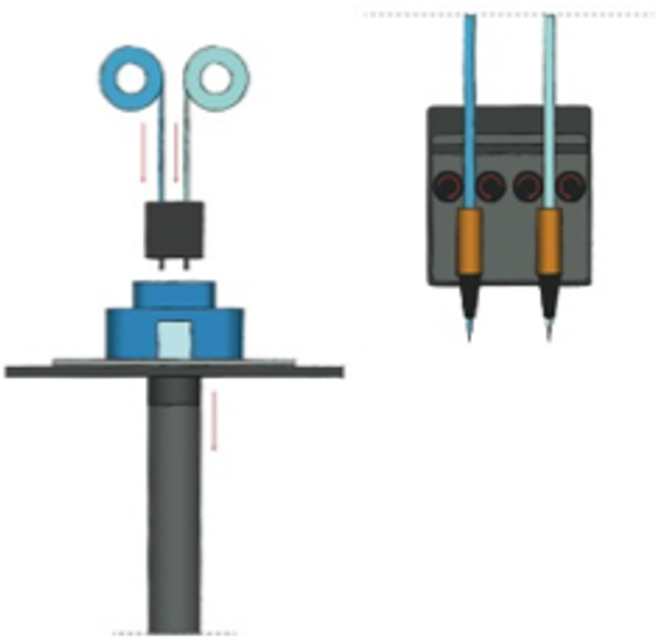




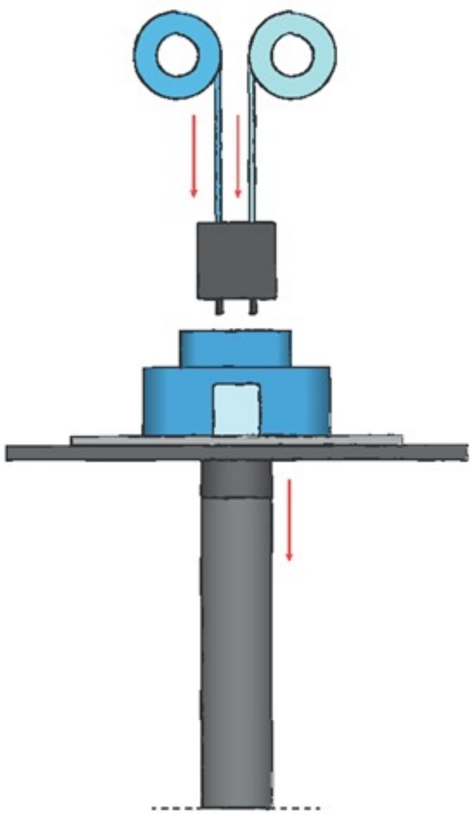
Laser for polymerisation of liquid monomer or resin

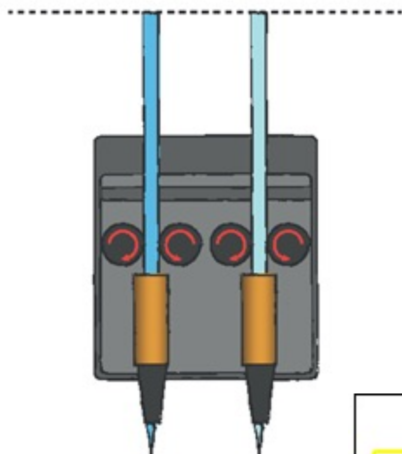
Available technologies

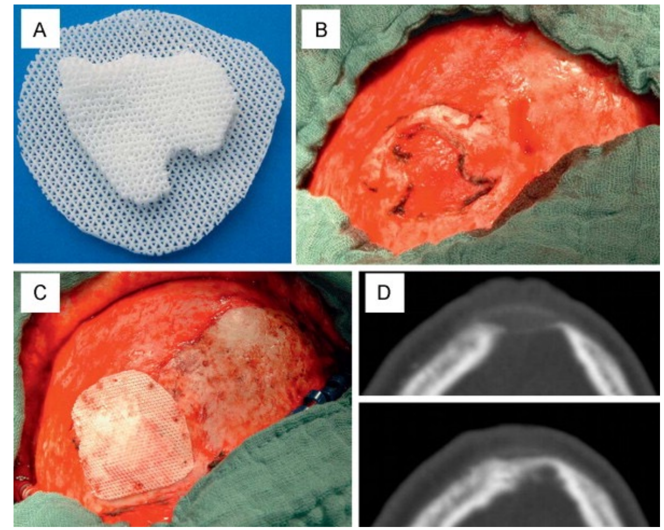
- Generation from the solid phase:
  - incipiently or completely melted solid materials, powder, or powder mixtures:
    - Extrusion (FDM),
    - Ballistic and
    - Sintering processes



Fused Deposition Modeling







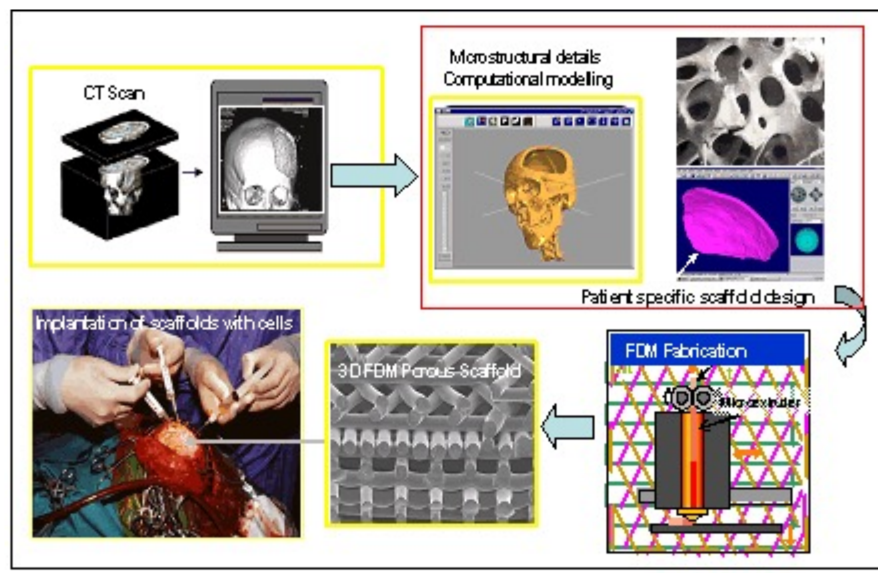
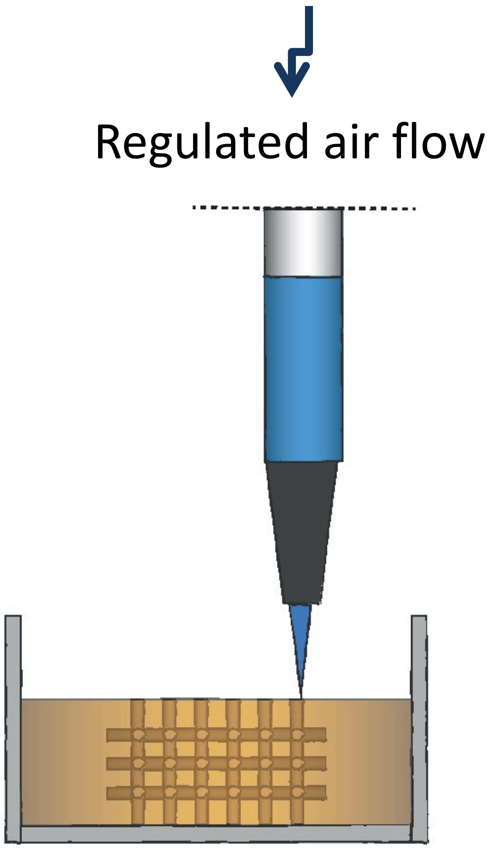


Figure 1: Platform technology for patient specific scaffolds TE.

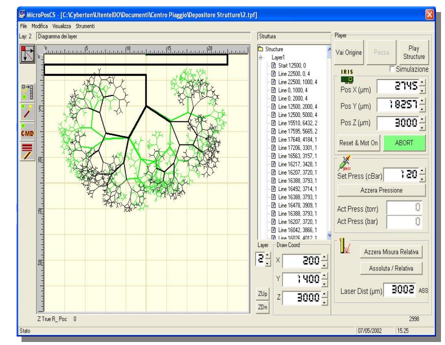
Available technologies

- Generation from the solid phase:
  - incipiently or completely melted solid materials, powder, or powder mixtures:
    - Extrusion (FDM),
    - Ballistic and
    - Sintering processes

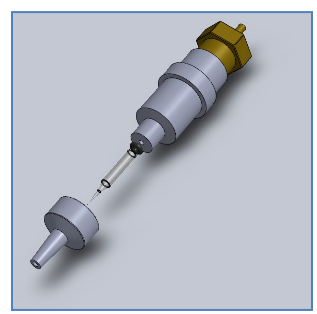
Pressure Assisted Microsyringe (PAM)



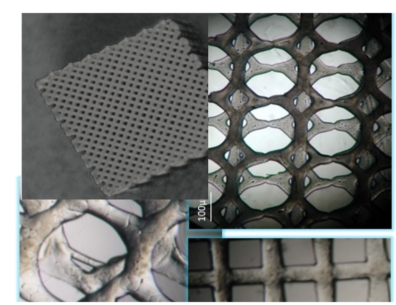
PAM system



Software



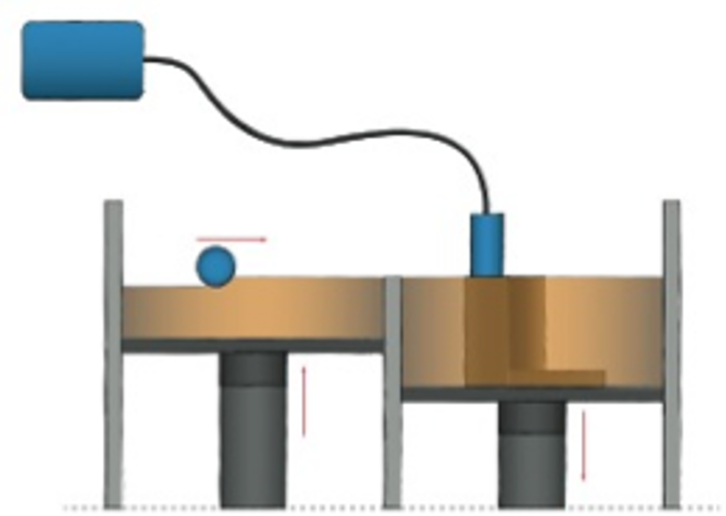
Syringe design



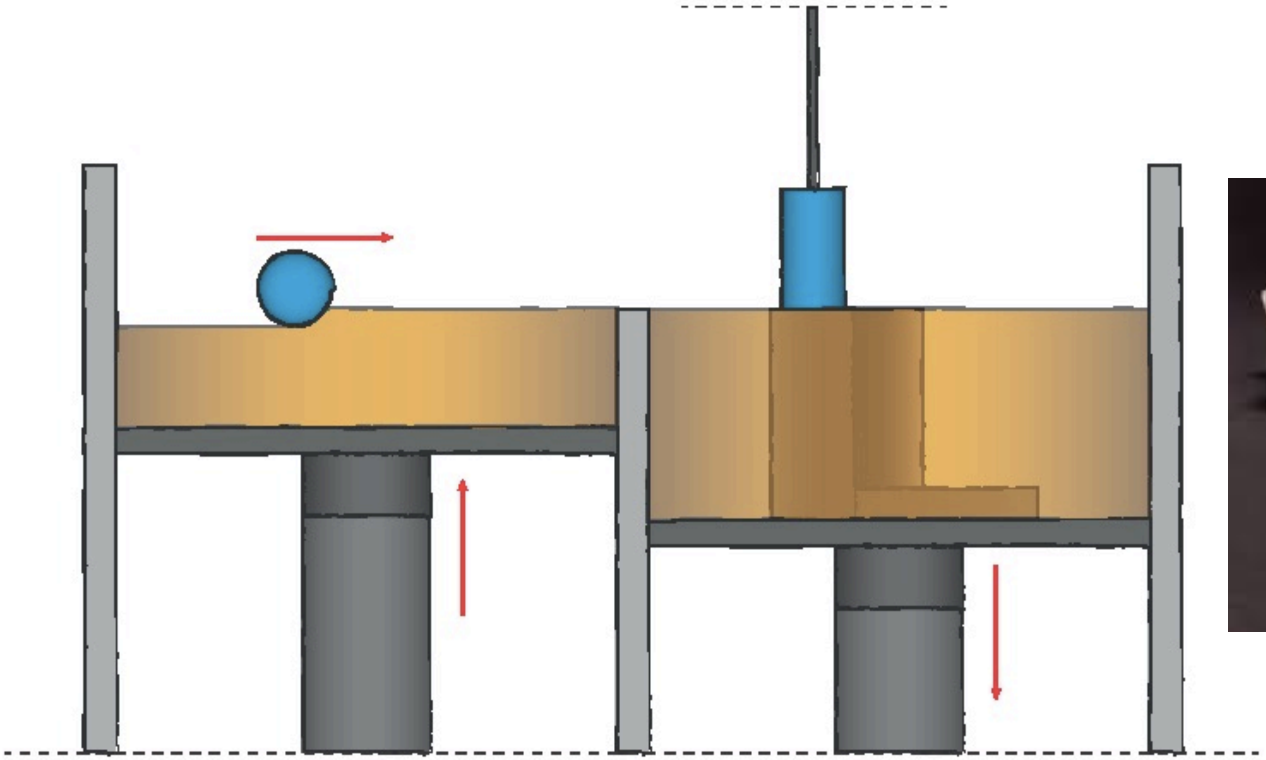
Software

Available technologies

- Generation from the solid phase:
  - Conglutination of granules or powders by additional binders
    - 3D inkjet printer

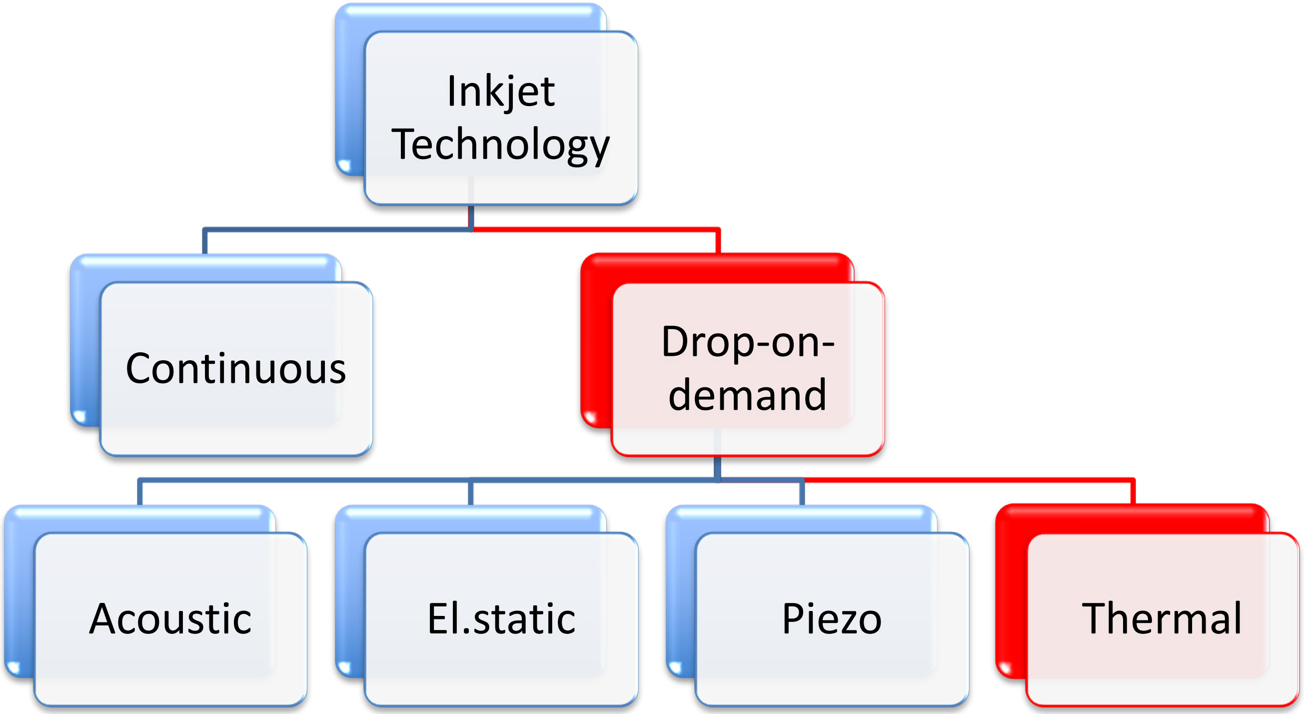


3D Printing™

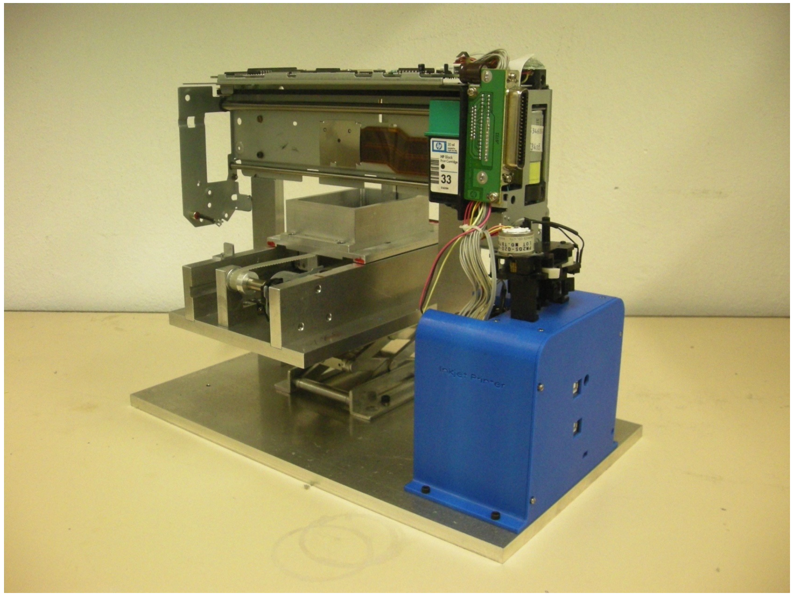


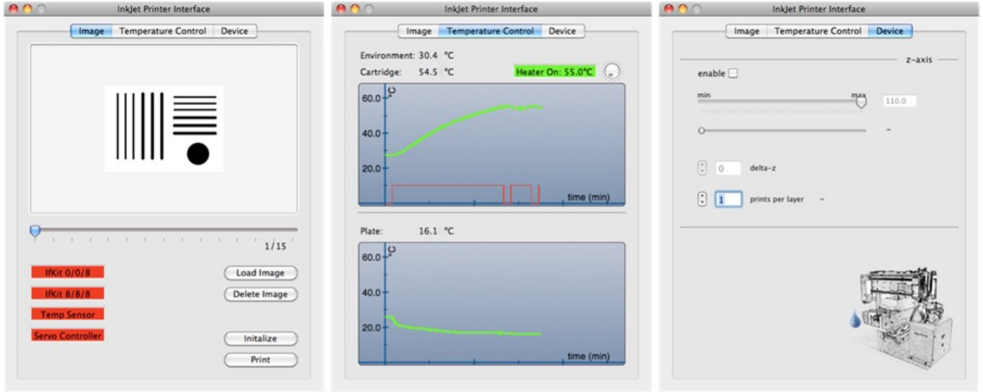
Inkjet Printing

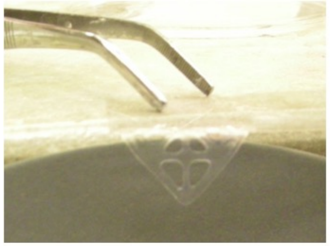
Inkjet technology is a ***contact free dot matrix printing*** procedure. Ink is issued from a small aperture directly onto a specific position on a substrate

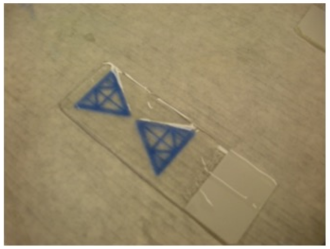


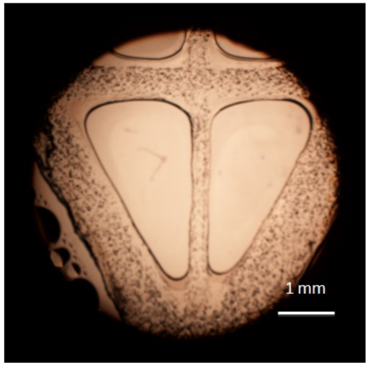
Penelope Ink-Jet printer



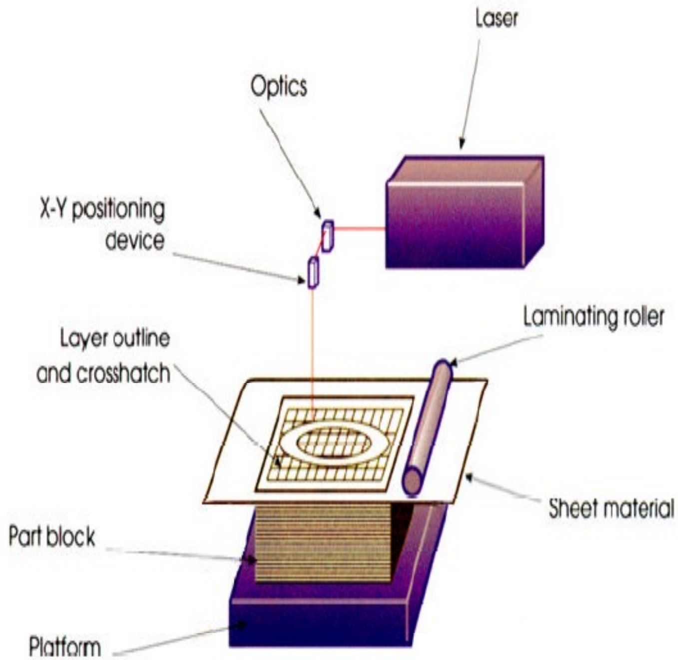


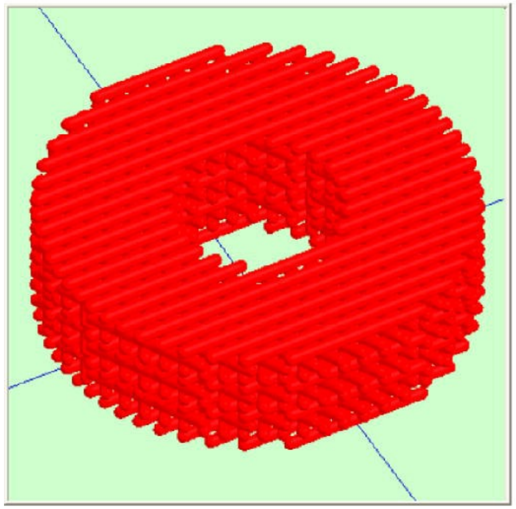


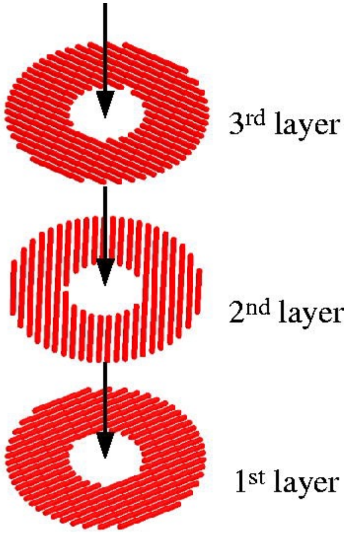




Membrane Lamination



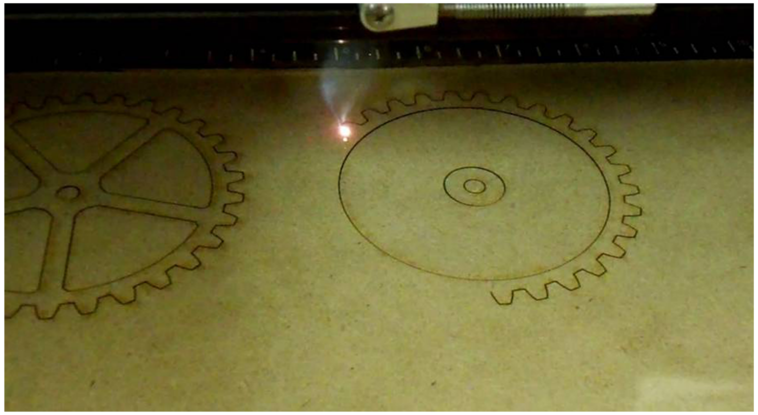


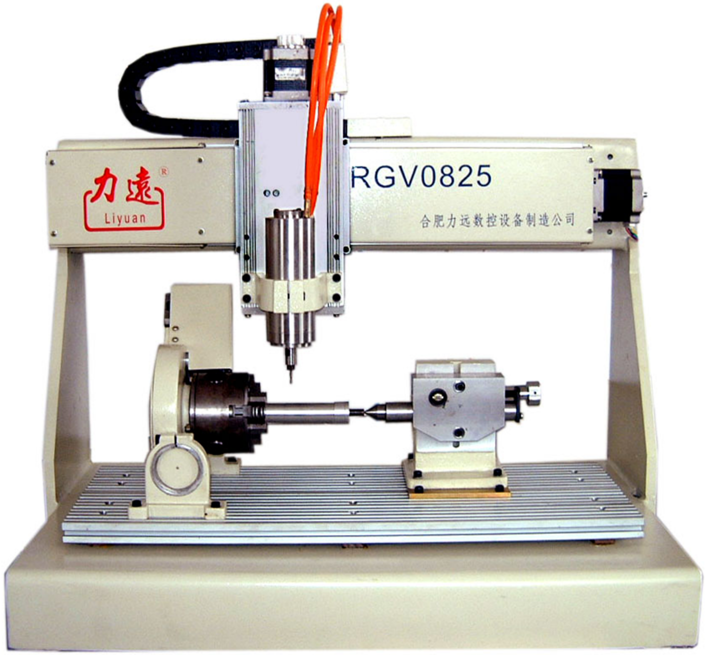


Laser as a cutter

Other fabrication technologies

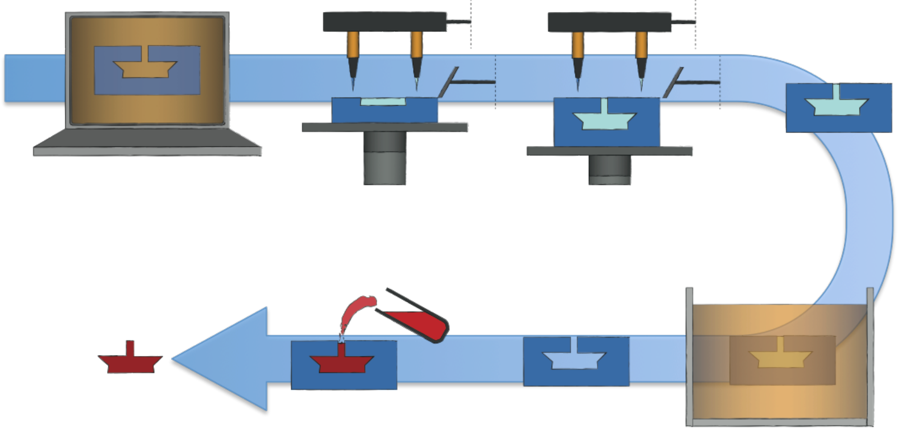
- Subtractive technologies
  - Laser cutter
  - CNC milling machines





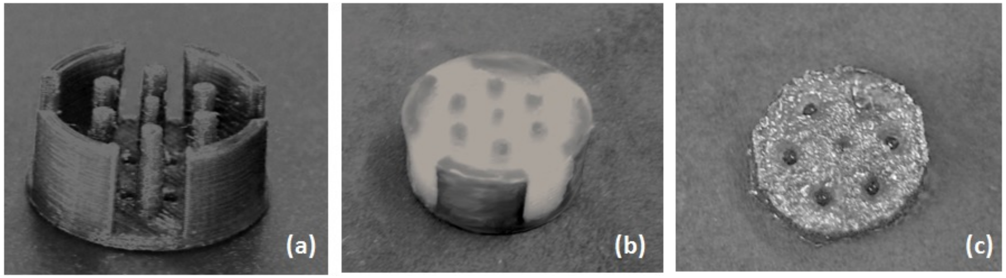
Indirect Rapid Prototyping (iRP)

- Molds realised with RP devices (CAD/CAM)
- Casting of the desired (bio-) material
- Extraction of the final object



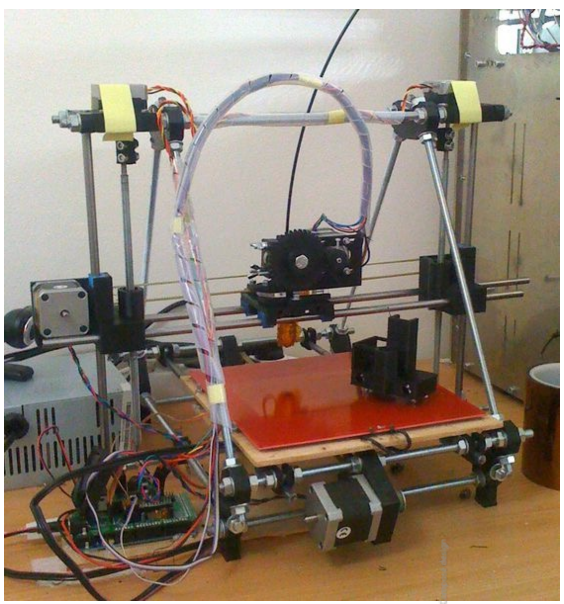
Advantages?
Limitations?

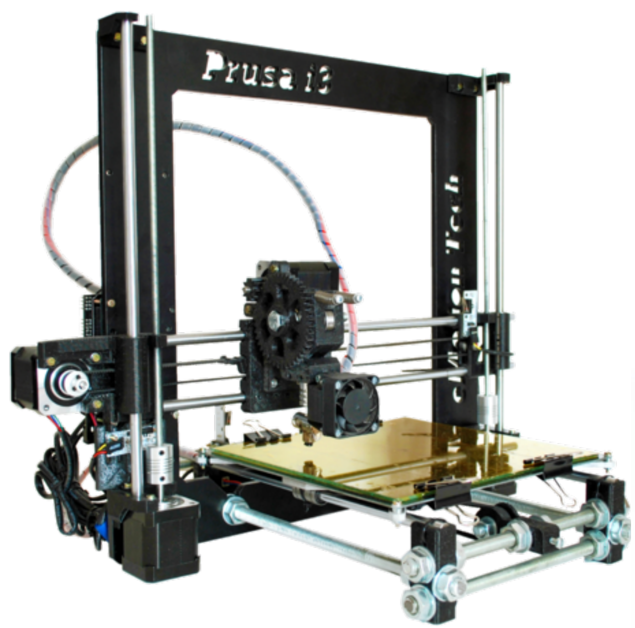
DW Hutmacher et al., Trends in Biotechnology, 22(7):354 – 362, 2004

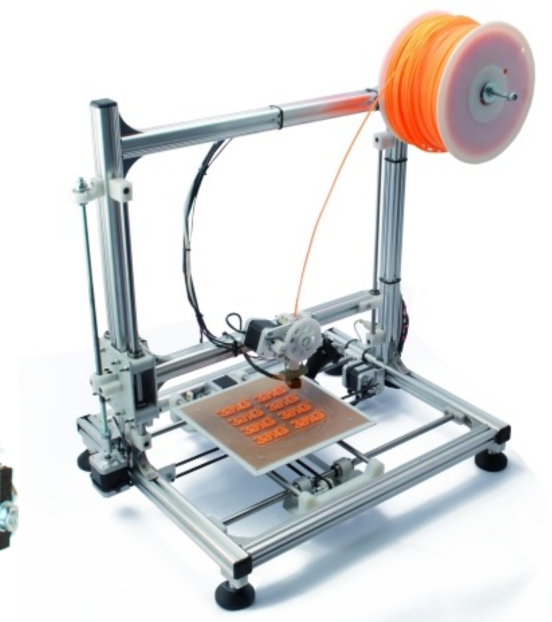


Open source FDM machine: RepRap Project

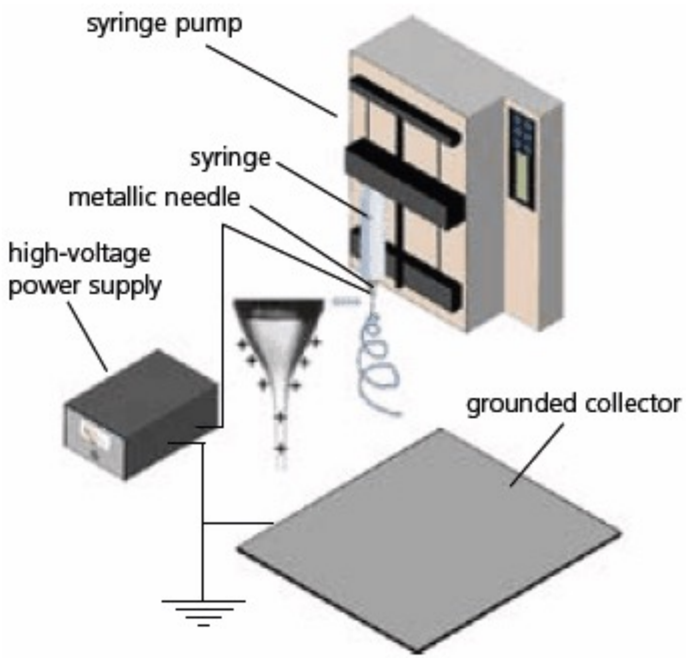
- RepRap is first general-purpose self-replicating manufacturing machine.
- An open source project with several forks

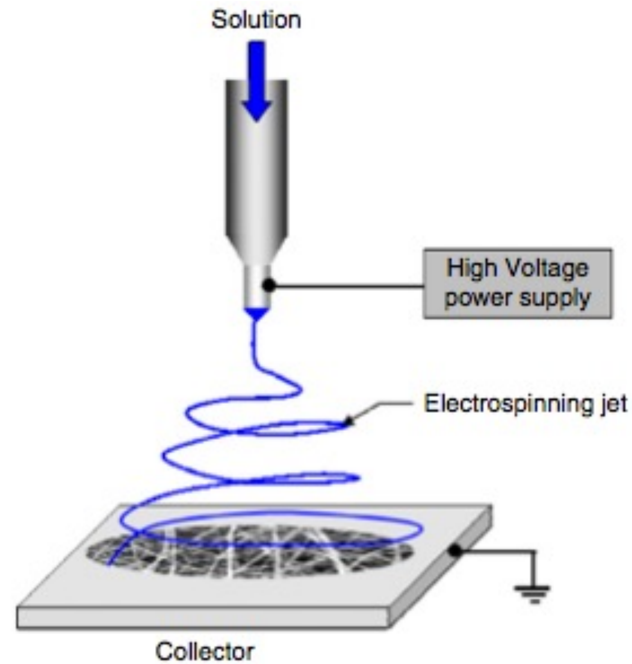


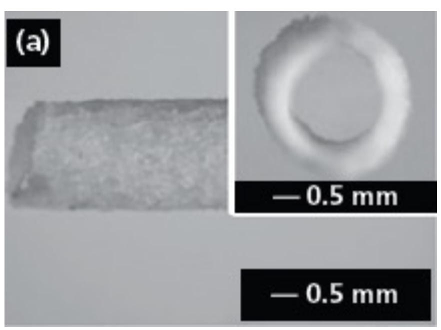


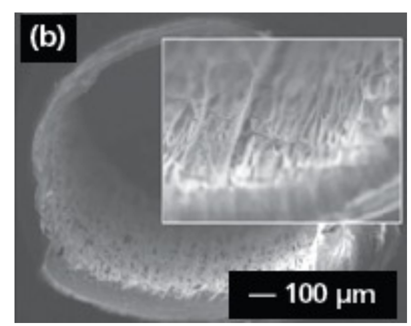


Electrospinning



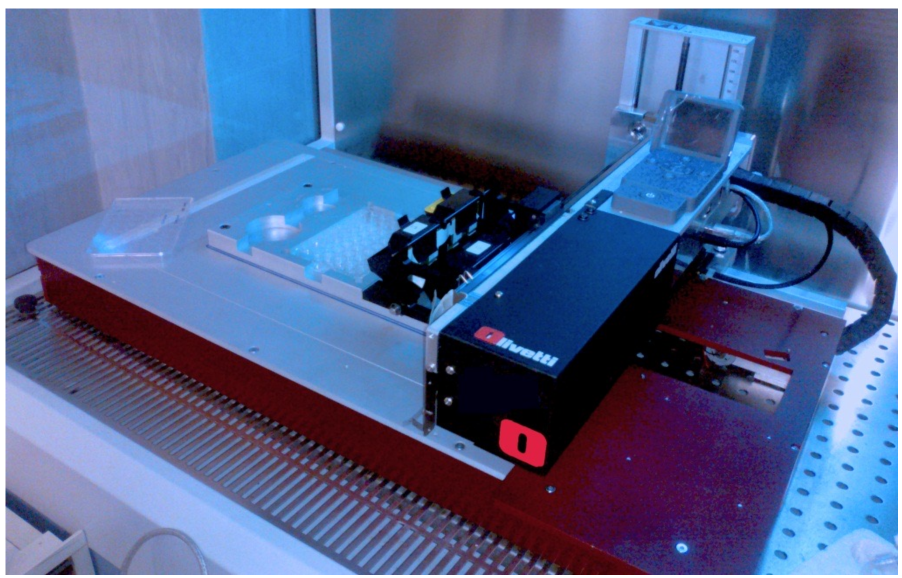






Cell Printing

- Cell Printing (Boland-inkjet)
- Organ Printing (Mironov-Forgacs)
- Living Inks, bioinks, bioprinter, bioplotter



Olivetti NanoBioJet

Cell dispensers and Bioprinters

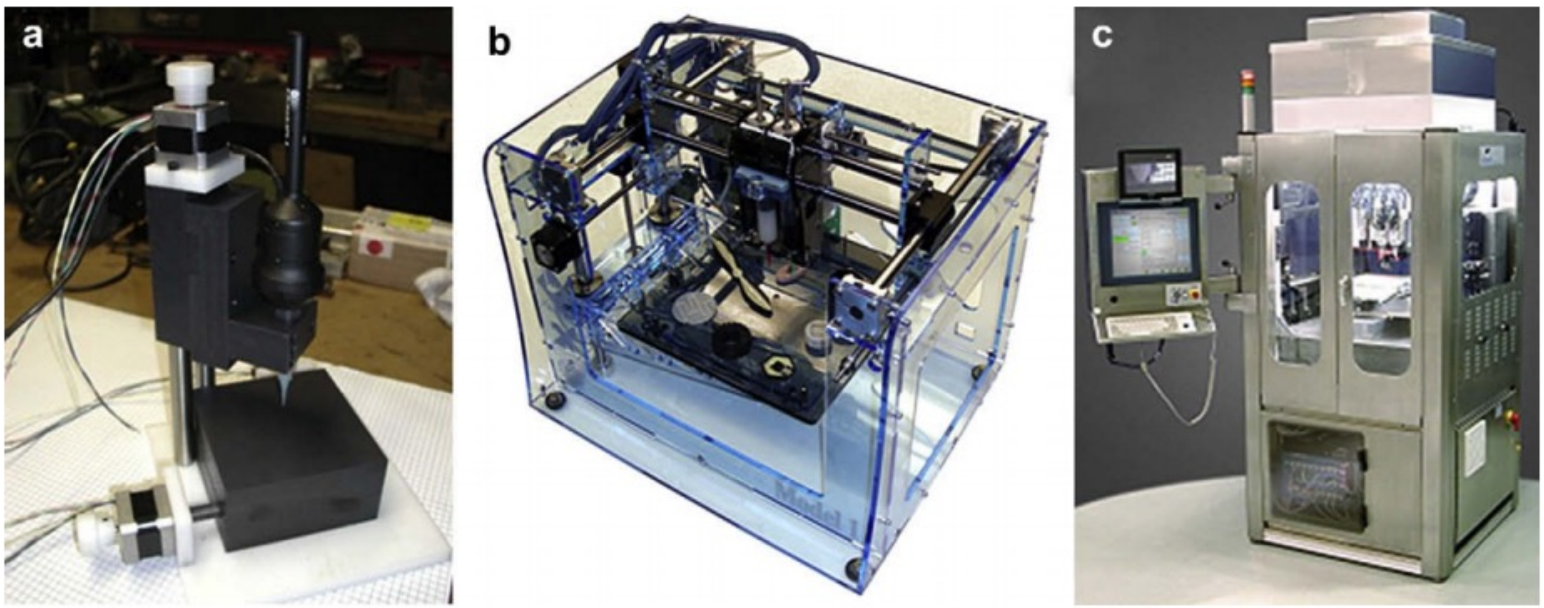
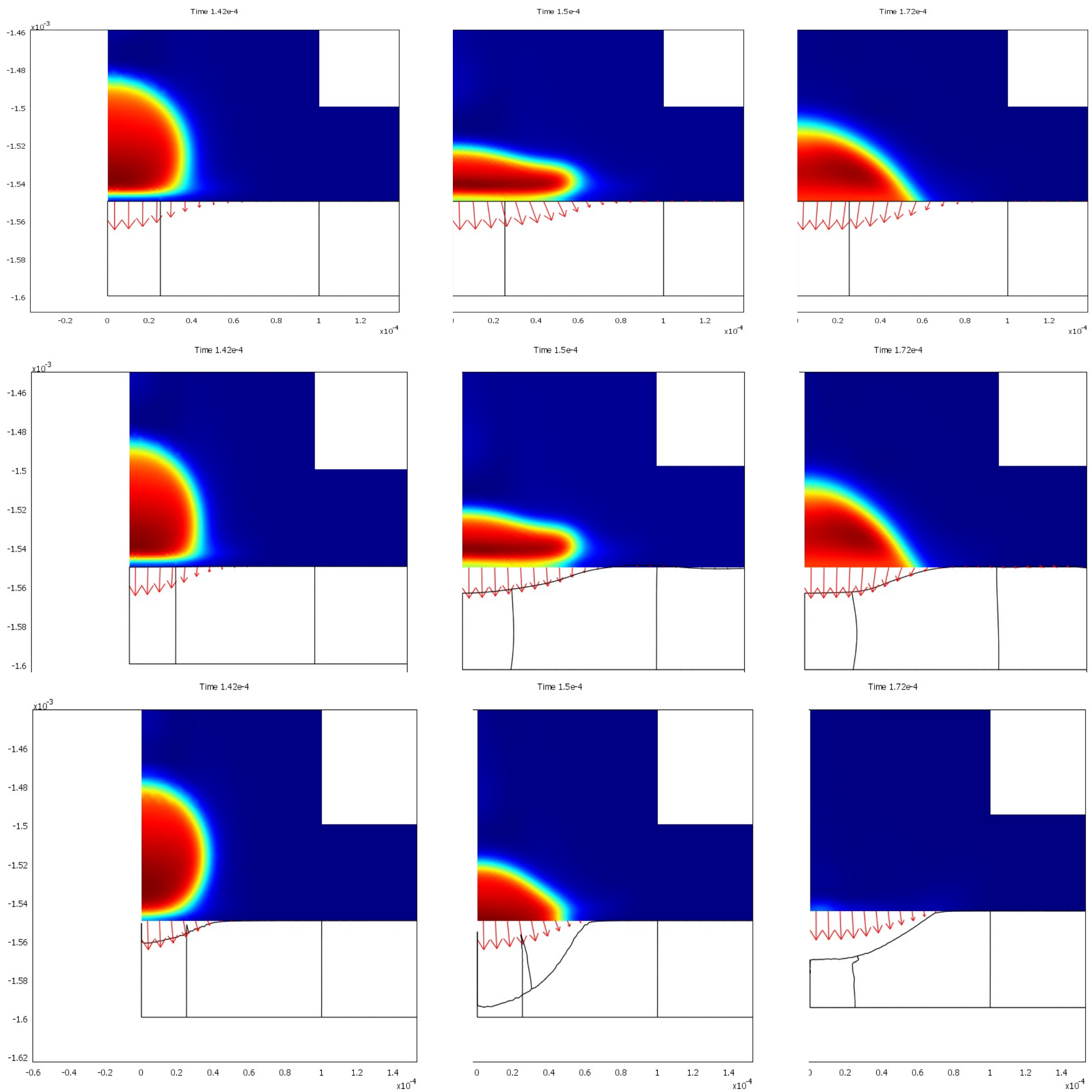


Fig. 3. Bioprinters: a) 3D dispensing Laboratory Bioprinter – 'LBP' (designed by Neatco, Toronto, Canada in cooperation with MUSC Bioprinting Research Center, Charleston, SC); b) 3D robotic printer – 'Fabber' (designed by Cornell University, USA); c) 3D robotic industrial bioprinter — 'BioAssembly Tool' (designed by Sciperio/nScript, Orlando, USA).

Effect of substrate rigidity on drop shape



Hard
(5 GPa)

Medium
(5 MPa)

Soft
(5 kPa)