# Bioprint Design & Use of Imaging in 2022

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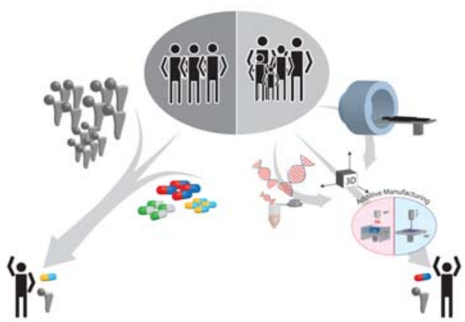
http://saliterman.umn.edu/

*Prof. Angela Panoskaltsis-Mortari's BMEn 5361, 3D Bioprinting*

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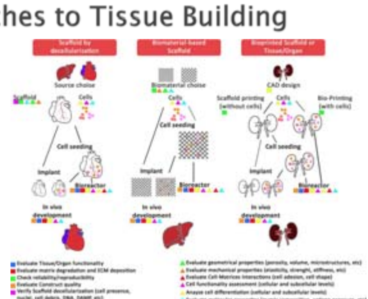
Guzzi EA, Tibbitt MW. Additive Manufacturing of Precision Biomaterials. *Advanced Materials.* 2020;32(13).

## Approaches to Tissue Building

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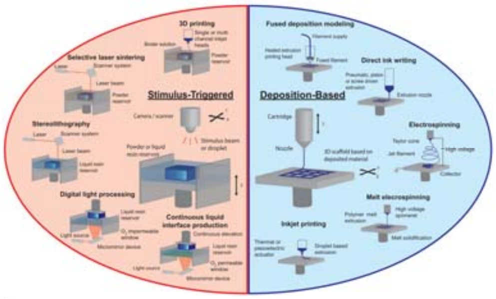
Teodori, L., A. et al. "3D Imaging Technologies: A Priority for the Advancement of Tissue Engineering and a Challenge for the Imaging Community." *J of Biophotonics* 10, no. 1 (Jan 2017): 24-45.

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Guzzi EA, Tibbitt MW. Additive Manufacturing of Precision Biomaterials. *Advanced Materials.* 2020;32(13).

## Workflow

*Pre*

1) Design – What are your trying to achieve? What bioinks and printing method?
2) Images – Useful from the subcellular to organ level. Apply CAD tools for segmentation, freeform and other space-filling methods.

*Processing*

3) Slicer Software – G-code generation for controlling toolpath, speed, valves, droplet patterns, laser pulse, photoinitiator lights *(e.g. Ingracure and other gels)*, temperature etc.
4) Special setups – Extruder, tips, light source (specific nm), pressure & calibration, cooling *(Pluronic Gel)* etc.
5) Bioprinting

*Post*

6) Bioreactor – Incubation, nutrients, growth factors, oxygen supply, environment, etc. *(Your 4th dimension –time!)*
7) Observation – Fluorescent and transmitted light *(confocal microscopy, bright-field, dark-field, confocal laser microscopy etc.).*
   - Automatic imaging with control of ambient air, humidity and temperature.
8) Characterization – Histology, growth, mechanical properties etc.

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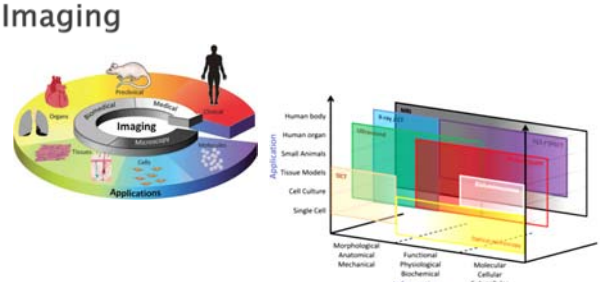
## Imaging

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Nam, S. Y., et. al. "Imaging Strategies for Tissue Engineering Applications." *Tissue Engineering Part B-Reviews* 21, no. 1 (Feb 2015): 88-102.

## Some Common Imaging Methods…

- Magnetic Resonance Imaging (MRI) or NMR
  - Human max. is 3T (Tesla) – resolution of 250μm x 250μm 0.5mm.
  - High spatial resolution μMRI, 7-10T, 5-200μm.
  - Magnetic nanoparticles.
- Computed tomography (CT)– *Computer Axial Tomography*
  - Typical resolution of 0.24 – 0.3mm.
  - μCT, resolution of 1-200μm.
- Ultrasound (less useful in bioprinting)
  - Resolution of 1mm x 1.mm x 0.2mm.
- PET – Positron emission tomography
- SPECT – Single photon emission computed tomography
- Optical Coherence Tomography (OCT)
- Traditional optical techniques.

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## Components of Medical Imaging…

- Image *formation* & *reconstruction* – using machines to create 2D and 3D images.
- Image *processing* and *analysis* – algorithms to enhance image properties (like noise removal); extracting quantitative information or a set of features from the image for object identification and classification.
- Input into *Machine Leaning* and *Deep Learning* systems for more advanced analysis.

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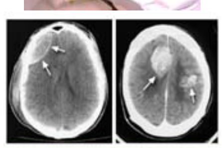
## Magnetic Resonance Imaging (MRI) & Computed tomography (CT)…



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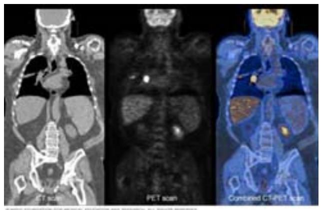
## Positron Emission Tomography (PET)…



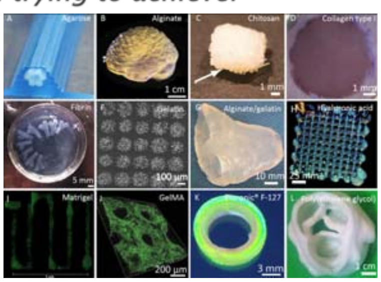

CT scan/PET Scan/ Combined



Mayo Foundation for Medical Education and Research

## What are you trying to achieve?

Bioprinted Hydrogel Examples






Hospodiuk, M. et al. "The Bioink: A Comprehensive Review on Bioprintable Materials." *Biotechnology Advances* 35, no. 2 (Mar-Apr 2017): 217-39.

## Pathway for Bioprinting 3D Tissue




Murphy, S. V., and A. Atala. "3d Bioprinting of Tissues and Organs." *Nature Biotechnology* 32, no. 8 (Aug 2014): 773-85.

Shape Fidelity

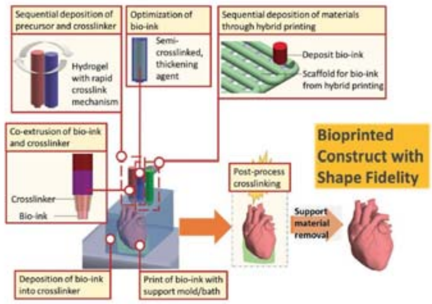



**Figure 3.** A bioprinting process that uses combination of different printing strategies to improve print fidelity and resolution.

Lee, J. M., and W. Y. Yeong. "Design and Printing Strategies in 3d Bioprinting of Cell-Hydrogels: A Review." *Advanced Healthcare Materials* 5, no. 22 (Nov 2016): 2856-65.

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## *Design Techniques…*

- Underlying methods in CAD systems:
  - Constructive solid geometry (solid primitives and boolean operators)
  - Boundary representation (vertices, edges and faces)
  - Spacial enumeration (cubic elements)
- Image-based design
- Implicit surfaces
- Space-filling curves
- Irregular porous structures

Giannitelli, S. M., et. al.. "Current Trends in the Design of Scaffolds for Computer-Aided Tissue Engineering." [In English]. *Acta Biomaterialia* 10, no. 2 (Feb 2014): 580-94.

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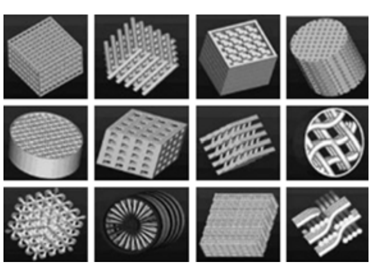
## *Library of CAD-based Primitives…*



Nam Jet.al. Computer aided tissue engineering for modeling and design of novel tissue scaffolds. Computer-Aided Design & Applications 2004;1:633-40.

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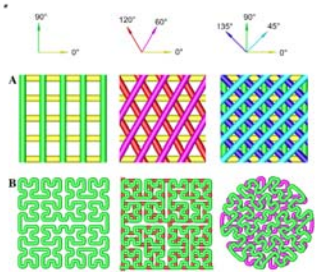
## *Lay-down Patterns…*

Honeycomb Pores

Hilbert Recursive Curves



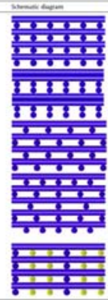

Giannitelli, S. M., et. al.. "Current Trends in the Design of Scaffolds for Computer-Aided Tissue Engineering." [In English]. *Acta Biomaterialia* 10, no. 2 (Feb 2014): 580-94.



Different Fiber Arrangements for Extrusion

Table 2
Different fiber arrangements implemented using extrusion-based manufacturing techniques.

| Typology | Arrangement | Schematic diagram | Refs. |
|---|---|---|---|
| Single material scaffolds | Homogeneous fiber spacing | | [48,51,52,85] |
| | Double layer configuration | | [52] |
| | Staggered fiber spacing | | [51,55,64] |
| | Pore size gradients | | [65] |
| Hybrid scaffolds | Bi/multimaterial | | [58,117] |



Giannitelli, S. M., et. al.. "Current Trends in the Design of Scaffolds for Computer-Aided Tissue Engineering." [In English]. *Acta Biomaterialia* 10, no. 2 (Feb 2014): 580-94.



## Segmentation

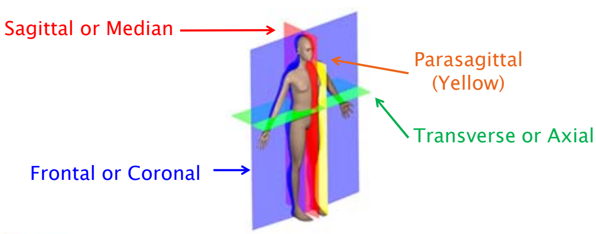
- Purpose
  - To delineate and isolate anatomical features within an imaging database- e.g. bone, cartilage, soft tissue, edema; muscle, lung, brain & other organs, and tumors.
- Categories
  - Manual, Semi-automatic and fully automatic.
- Techniques
  - Thresholding
  - Clustering based approach
  - Edge-based

## Segmentation with Available Software…

- Extract images from DICOM files (ITK-Snap, Onis) and possible deindentifying them for HIPPA regulations (DICOMCleaner).
- Segmentation Software (ITK-Snap, Seg3D2, Materialise Mimics, Materialise 3-matic).
  - Pre-segmentation Phase – identify parts of image as foreground and background.
  - Active Contour Phase – manual and semiautomatic methods.
- Editing and fixing mesh files (.STL) – Autodesk Meshmixer.
- Slicer software – Simplify3D and Repetier.
  - G-coding for the specific bioprinter – e.g. Slic3R (printer customized interface to control what happens in a sequence of control steps.)



## Know the Main Anatomical Planes…



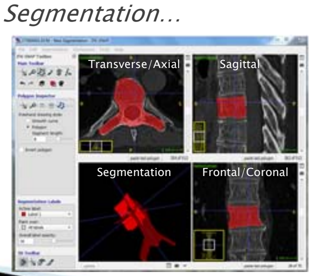

*ITK Snap Demo Next…*

Image, Wikipedia

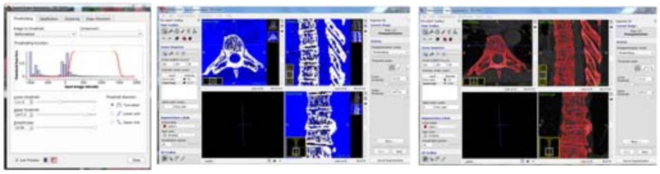


## Segmentation with ITK-Snap (freeware).

*Manual Segmentation…*



## *Semiautomatic – Contrast Adjustment…*



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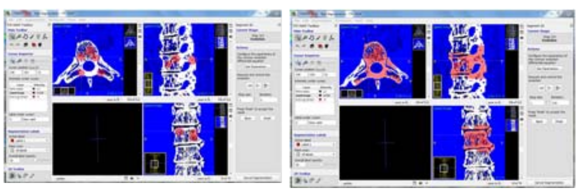
## *"Balloon" Placement & Inflation…*



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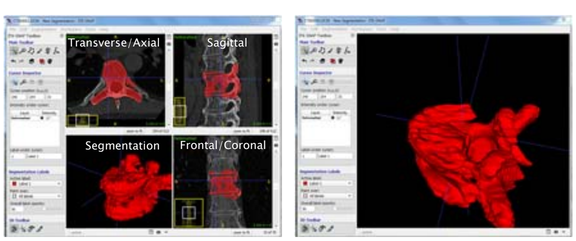
## *3D Rendering…*

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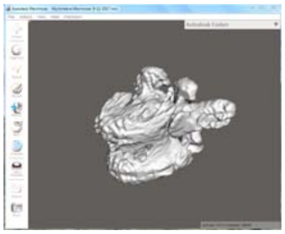
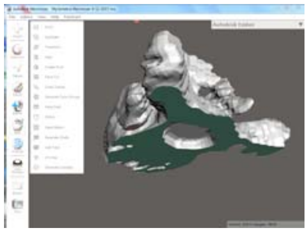
## *Editing with Meshmixer (freeware)…*



Import the STL Mesh file generated by ITK-Snap.



Edit feature – here slicing in a plane, bottom view.

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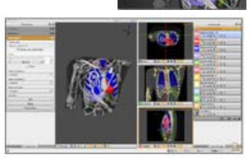
Segmentation of the Cerebellum




Seg3D is a free volume segmentation and processing tool developed by the NIH Center for Integrative Biomedical Computing at the University of Utah Scientific Computing and Imaging (SCI) Institute. Seg3D combines a flexible manual segmentation interface with powerful higher-dimensional image processing and segmentation algorithms from the Insight Toolkit. Users can explore and label image volumes using volume rendering and orthogonal slice view windows.

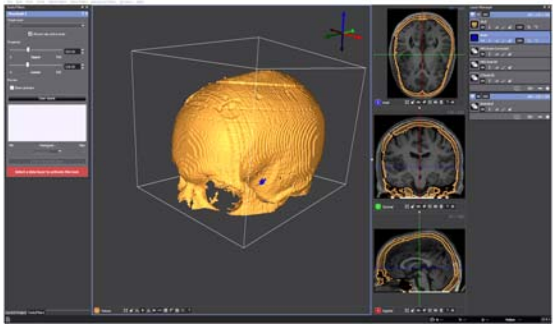


Seg3D at a glance:

- Fully 3D interface with multiple volumes managed as layers
- Automatic segmentation integrated with manual contouring
- Volume rendering with 2D transfer function manipulation in real-time
- Image processing and segmentation from the Insight Toolkit (ITK)
- Real time display of ITK filtering output allows for computational steering
- 64-bit enabled for handling large volumes on large memory machines
- Supports many common biomedical image formats
- Open source with BSD-style license
- Cross platform: Windows, OSX, and Linux

# Seg3D

https://www.sci.utah.edu/cibc-software/seg3d.html



## Toolpaths

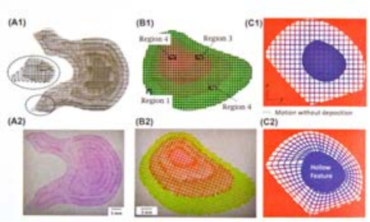
- Bioprinting rasters may be in Cartesian vs parametric* form.
  - Consider extrusion-based (EBB) rather than droplet-base (DBB) or laser-based bioprinting (LBB) which are less common and based on manufacturer specific tool paths.
- Why use one method vs the other?
  - Issues arise with resulting printed *gradients* as excess accumulation of bioink can occur at directional changes.
  - Parametric* modeling/toolpath may be helpful for *lumen* and other *hollow shape* object printing.
  - Control of porosity (e.g. bones)

*Parametric implies a variable is dependent on other variables – commonly used to express the coordinates of the points that make up a geometric object such as a curve or surface.



## *Toolpath in Cartesian Coordinates…*

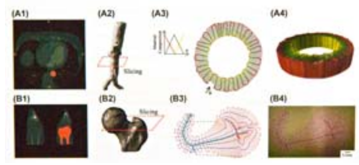
A. Design of a continuous toolpath and bilayer bioprinted vertebra.
B. Toolpath for graded wound device and bilayer printed device.
C. Comparison of toolpath using cartesian vs parametric coordinates.




Ozbolat, I.T. 3D *Bioprinting Fundamentals, Principles and Application*. Elsevier, Amsterdam 2017.

## *Toolpath in Parametric Form…*

A. CAD model of aorta with controlled material composition along the parametric distance *u*.
B. Femur model with toolpath for controlled porosity along the distance *u*; sample double layer structure bioprinted using sodium alginate hydrogels.



Ozbolat, I.T. 3D *Bioprinting Fundamentals, Principles and Application.* Elsevier, Amsterdam 2017.



## Image J1 & J2 Software

- Image processing software developed by the NIH.
- Display, edit, analyze, process, save and print color and grayscale images.
- Able to read TIFF, PNG, GIF, JPEG, BMP, DICOM and FITS files.
- Calculate area and pixel value statistics of user-defined selections and intensity-threshold of objects.
- Measure distances and angles.



- Create density histograms and line profile plots.
- Supports standard image processing functions such as logical and arithmetical operations between images, contrast manipulation, convolution, Fourier analysis, sharpening, smoothing, edge detection, and median filtering.
- Geometric transformations such as scaling, rotation, and flips.
- Useful in evaluating 3D printability of gels though image analysis of lattice structures.

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2020, VOL. 15, NO. 3, 340–358
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REVIEW

## Deep learning for fabrication and maturation of 3D bioprinted tissues and organs

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**ABSTRACT**
Bioprinting is a relatively new and promising tissue engineering approach to solve the problem of donor shortage for organ transplantation. It is a highly-advanced biofabrication system that enables the printing of materials in the form of biomaterials, living cells and growth factors in a layer-by-layer manner to manufacture 3D tissue-engineered constructs. The current workflow involves a myriad of manufacturing complexities, from medical image processing to optimisation of printing parameters and refinements during post-printing tissue maturation. Deep learning is a powerful machine learning technique that has fuelled remarkable progress in image and language applications over the past decade. In this perspective paper, we highlight the integration of deep learning into 3D bioprinting technology and the implementation of practical guidelines. We address potential adoptions of deep learning into various 3D bioprinting processes such as image-processing and segmentation, optimisation and *in-situ* correction of printing parameters and lastly refinement of the tissue maturation process. Finally, we discuss implications that deep learning has on the adoption and regulation of 3D bioprinting. The synergistic interactions among the field of biology, material and deep learning-enabled computational design will eventually facilitate the fabrication of biomimetic patient-specific tissues/organs, making 3D bioprinting of tissues/organs an impending reality.

**ARTICLE HISTORY**
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**KEYWORDS**
3D bioprinting; 3D printing; biofabrication; deep learning; machine learning



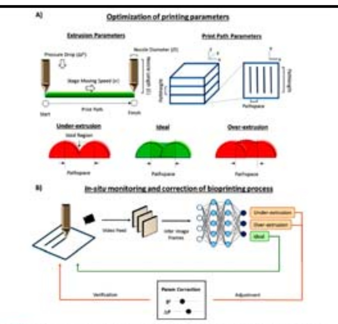


Figure 3. (A) Systematic drawing of quantitative optimization of 3D bioprinting process. (B) In-situ monitoring and correction of 3D bioprinting process using computer vision and deep learning.



Ng WL, Chan A, Ong YS, Chua CK. Deep learning for fabrication and maturation of 3D bioprinted tissues and organs. *Virtual Phys Prototype.* 2020;15(3):340-358.

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## Deep learning approaches to biomedical image segmentation

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## Machine & Deep Learning

- Identification and quantification of patterns in medical images.
- Implications for clinical diagnosis with automated and enhanced throughput, and applications of segmentation – including 3D model building and bioprinting.
- Simple machine learning includes automated analysis, beginning with *feature extraction* based on visually distinct regions – color, gray scale, texture, contrast and size. These regions have semantic meaning for the given problem. This becomes input into an *ML classifier*, which can determine optimal boundaries.
- With a *deep learning-based classifier (DLC),* raw image data is processed without pre-processing, segmentation and feature extraction.

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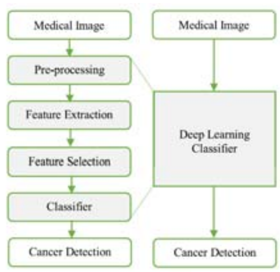


**Fig. 1.** Change in classifier approach using typical machine learning algorithm and deep learning. Figure adapted from Ref. [14].

Haque, IR, J. Neubert, Deep learning approaches to biomedical image segmentation. Informatics in Medicine, 18 (2020).

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frontiers in Bioengineering and Biotechnology

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# Engineering Tissue Fabrication With Machine Intelligence: Generating a Blueprint for Regeneration

Joohyun Kim[1*], Jane A. McKee[2], Jake J. Fontenot[2] and Jangwook P. Jung[2*]

[1] Center for Computation and Technology, Louisiana State University, Baton Rouge, LA, United States, [2] Department of Biological Engineering, Louisiana State University, Baton Rouge, LA, United States

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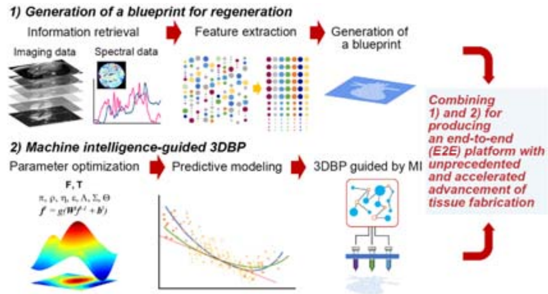


Kim J, McKee JA, Fontenot JJ, Jung JP. Engineering Tissue Fabrication With Machine Intelligence: Generating a Blueprint for Regeneration. *Frontiers in Bioengineering and Biotechnology.* 2020;7.

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This paper was submitted as an invited paper to IEEE Reviews in Biomedical Engineering on April 6, 2020. 1

Review of Artificial Intelligence Techniques in Imaging Data Acquisition, Segmentation and Diagnosis for COVID-19

Feng Shi *, Jun Wang *, Jun Shi *, Ziyan Wu, Qian Wang, Zhenyu Tang, Kelei He, Yinghuan Shi, Dinggang Shen*

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## Summary

- Approaches to Tissue Engineering.
- Workflow
- Imaging
- Design
- Segmentation
- Tool Paths
- Image J1 & J2 Software
- Application of Machine Learning and Deep Learning

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