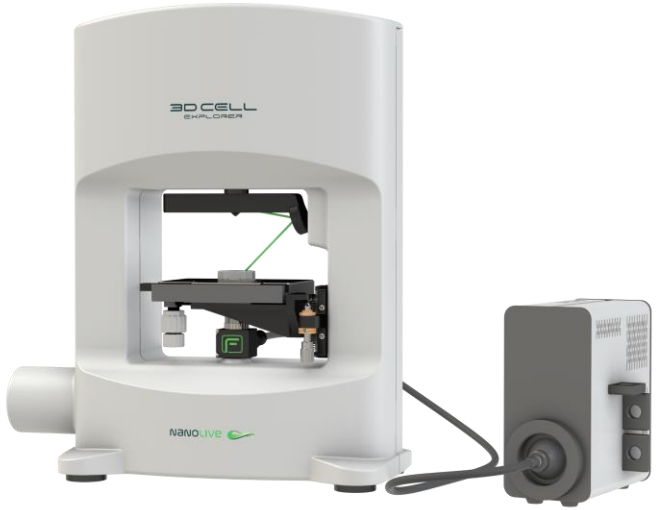
NANOLIVE SA

High precision and quality research devices

NANOLIVE
Looking inside life



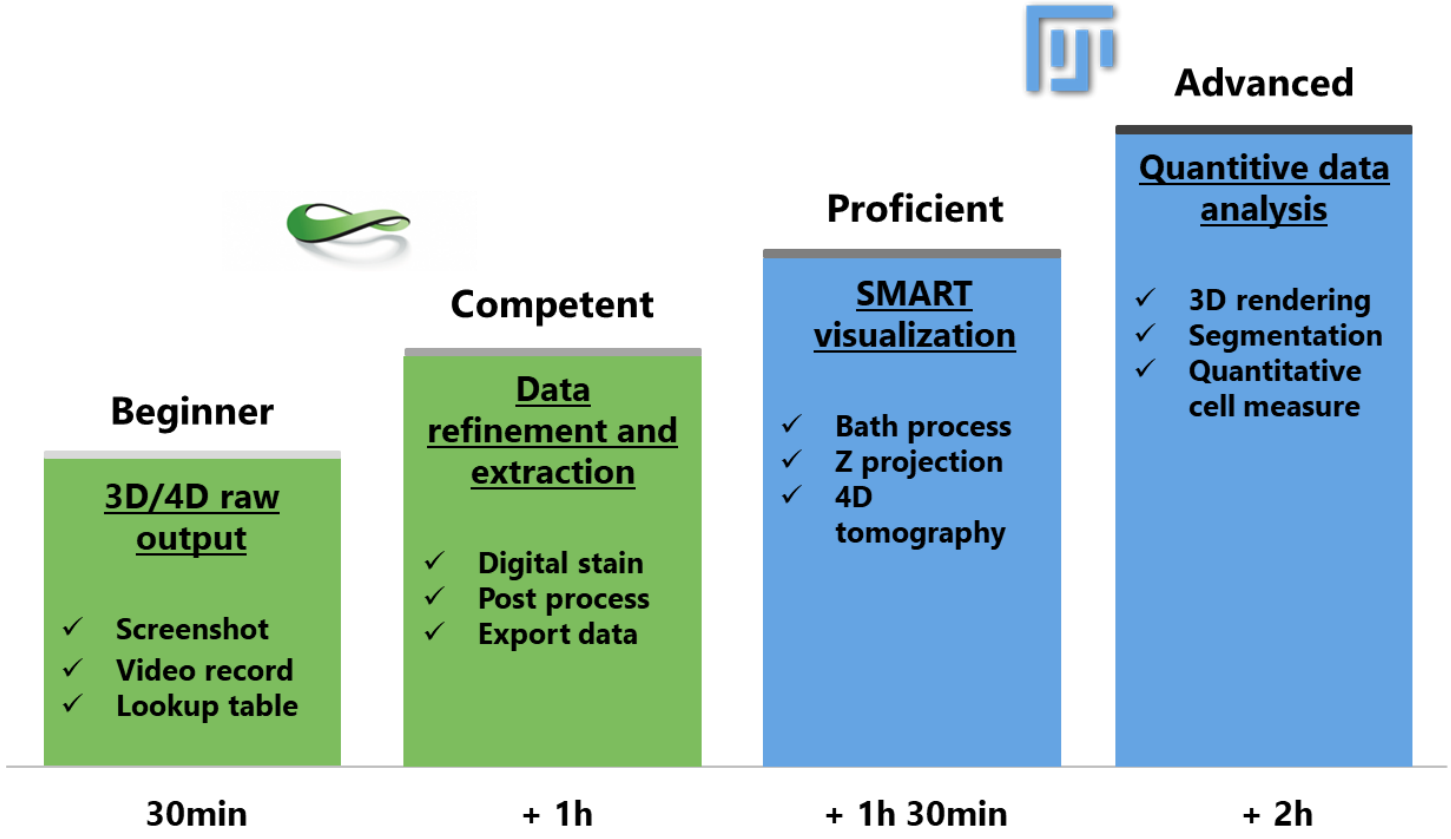
Nanolive is a spin-off company from EPFL with headquarter at EPFL's Innovation Park in Lausanne (Switzerland)

We focus on innovative product development at the highest standards to deliver a high precision and quality research device for our international customers.

Holo-Tomographic Microscopy (HTM) workshop

Practical guidelines to enhance your data from 3D Cell Explorer

Time	Chapter
9:00 – 9:15	• Introduction and set up
9:15 – 9:45	• 3D/4D raw output
9:45 – 10:45	• Data refinement and extraction
11:00 – 12:30	• SMART visualization
12:30 – 14:00	• Lunch break
14:00 – 15.00	• Quantitive data analysis – part I
15:15 – 16:15	• Quantitive data analysis – part II
16:15 – 17.00	• Q&A



Beginner level - 3D/4D Raw Output

Screenshot, video record and lookup table

A. Holotomography introduction

B. 3D Cell Explorer Acquisition

C. **STEVE main features**

1. Open STEVE
2. Drag and drop mESCs_2_150118_single frame
3. Show tips
4. Save 2D/3D Screenshot
5. Grid options
6. Lookup table/Invert color map

D. **Work with dataset to produce 3D/4D raw outputs**

1. Drag and drop mESCs_2_150118_60s_2h
2. Replay movie
3. 3D video record

Competent level – Data refinement and extraction

Digital Stain, post processing and export data

A. Post processing

1. Histogram
2. Background Subtraction
3. Edge Preserving Filter
4. History and Undo
5. Raw/Processed

B. Digital Stain

1. Digital staining with one and multiple channels
2. Load/save/delete panel
3. Info button
4. Color map/Digital Stain
5. Best digital stain practies
6. Xml data format

C. Export data

1. Frame/Content/Format selection
2. Best greyscale 2D and Digital Stain 3D movie contest

D. Fluorescence option

1. mitochondria Fluo_single frame
2. Export 32bit RI
3. 2D RI

TUTORIAL VIDEO:
Post Processing with STEVE software
Exporting video tutorial

Proficient level - SMART VISUALIZATION

Batch process, Z projection and 4D tomography

https://vimeo.com/256424924

BATCH PROCESS AND Z PROJECTION

A. Open ImageJ
B. Update it + plug in
C. Plugin > Macros > Record
D. Open Adipocyte frame 0
E. Retain only useful information
  1. **Make square selection**
  2. **Image > Crop**
  3. **Image > Stacks > Tools > Slide keeper (40/60 by 1)**
  4. **Close previous without saving**

F. Image > Stack > Z projection Max Intensity
G. Image > Adjast > B&C Auto
H. Close without saving
I. Save txt macro
J. Process > Batch > Macro
  1. Reptangular
  2. Crop
  3. Slide Keeper
  4. Max Z project
  5. Window level
  6. Enhance contrast

K. Input and Output Folder
L. Process
M. File > Import Image Sequence, select first [100, sort by name, 8-bit]
N. Image > Adjust > Size x4 [Bilinear]
O. File > Save [AVI, 12fps]
P. VLC opening

4D IMAGE

A. Open ImageJ
B. Open all FRC mitosis frames
C. Image > Stack > Tool > Concatenate [all]
D. Save > Tiff

Advanced level – Quantitative data analysis

Segmentation, 3D rendering and quantitative cell measure

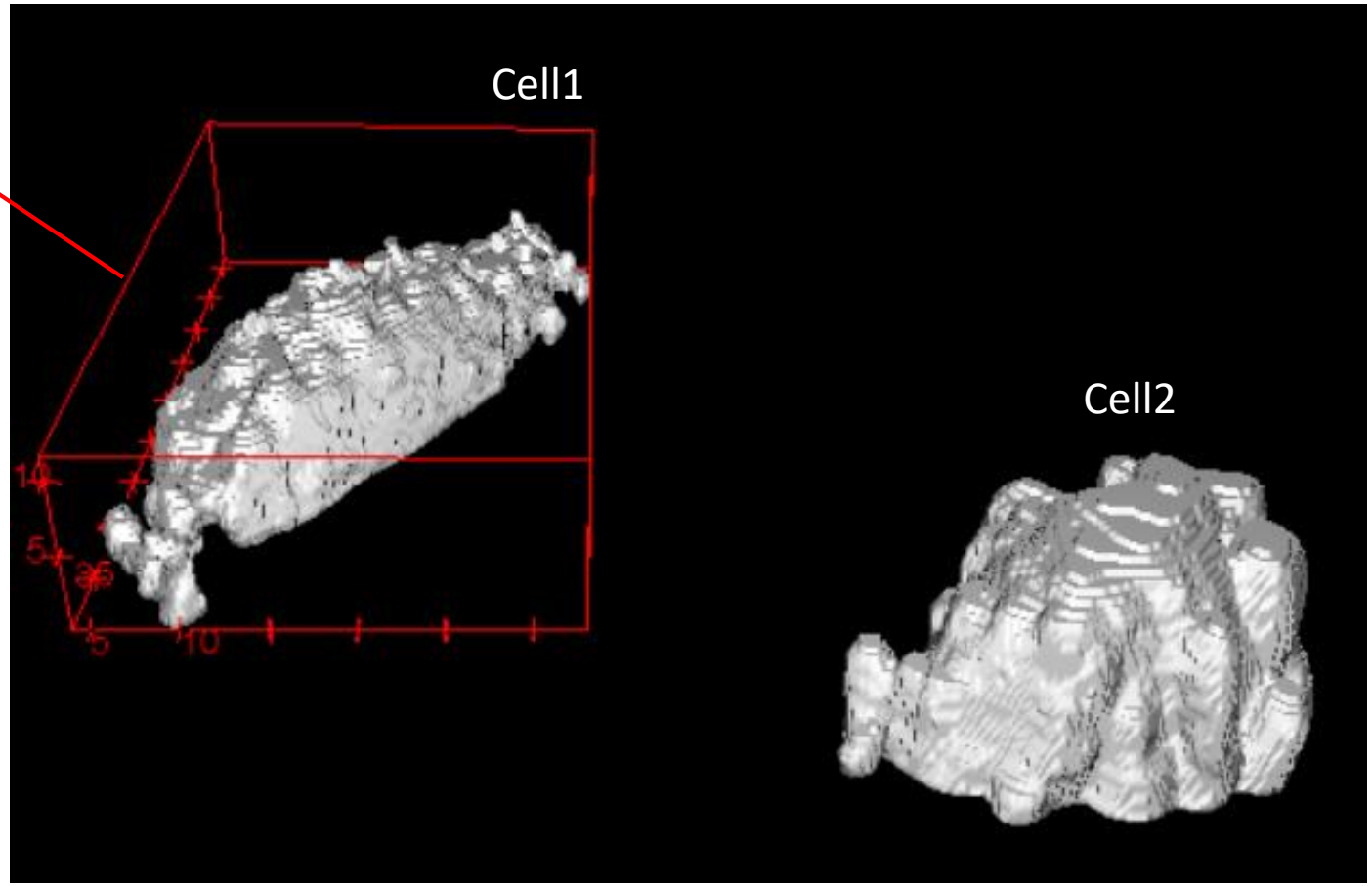
Surface
Volume
Sphericity
Refractive Index
Dry mass

$$[DryMass] = \left\{ \frac{RI}{RI_{H2O}} - 1 \right\} \times \frac{1}{k}$$

where

$k = 0.002$

(Friebel & Meinke, 2006; Phillips, Jacques, & McCarty, 2012)



Advanced level – Quantitative data analysis

Segmentation, 3D rendering and quantitative cell measure

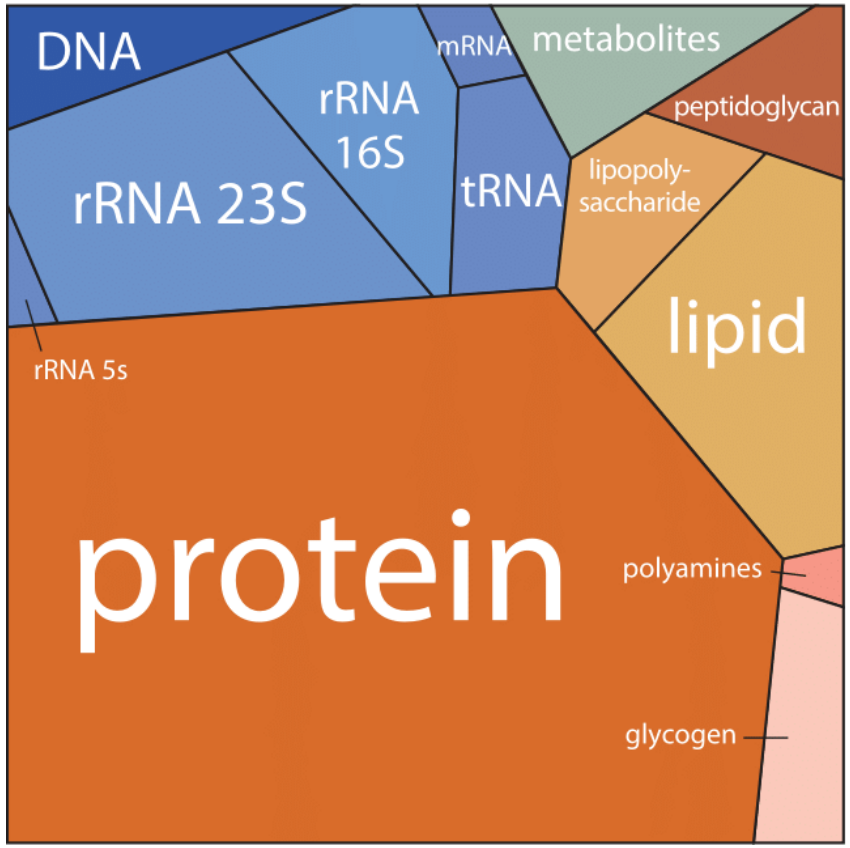


Table 1. Overall macromolecular composition of an average E. coli cell in aerobic balanced growth at 37°C in glucose minimal medium, with doubling time of 40 minutes and 1 pg cell wet weight (≈0.9 µm^3 cell volume).

http://book.bionumbers.org/what-is-the-macromolecular-composition-of-the-cell/

Living cell dry mass measurement using quantitative phase imaging with quadriwave lateral shearing interferometry: an accuracy and sensitivity discussion. Aknoun at all. (2015) J Biomed Opt.

DRY MASS

During the life of a cell, its dry mass, i.e., the mass of all cellular content except water, is deeply modified. These changes are related to all the metabolic and structural cell functions. The study of dry mass consequently reveals important information on cells, either as individuals or as a population.

- Biomass changes of single CD8+ T cells during antigen-specific cytotoxicity
- Stem cell colony studies
- Applied to red blood cells study (hemoglobin concentration)
- Cell death process characterization

Advanced level – Quantitative data analysis

Segmentation, 3D rendering and quantitative cell measure

A. start STEVE
B. upload blebbing .vol file
C. create/upload .xml dstain file (one color/full opacity/full edge softness)
D. export > digital stain [first frame, -4 to 48]
E. export full stack RI in 3D [first frame, -4 to 48]
F. start Fiji
G. update Fiji and 3D ImageJ suite + image science plugin
H. Help > Update > Manage update sites and tick IJPB-plugins
I. Plugin > macro > record
J. import dstain and RI into Fiji
K. Image > properties > 0.182um X 0.182 Y 0.372 Z
L. Image > Type > 8-bit
M. Process > filters > Gaussian Blurr (2-3)
N. Plugins > 3D > 3D Simple Segmentation (min 3000 and thresold 20)
O. Results window > menu > save
P. Plugins > 3D > 3D Binary Close Labels
Q. Plugins > 3D > 3D Manager options (volume, surface, compactness, exclude objects on edges XYZ)
R. Plugins > 3D > 3D Manager > select sample_seg > add image
S. Select the target odject and delete wrong objects
T. 3D manger > select RI 3D tiff > Measure 3D and Quantif 3D
U. Results window > menu > save
V. 3D Manager > 3D Viewer
W. Play animation/ Record 3D
X. Excel>data from [text]
Y. Import data into TEMPLATE and calculate Dry Mass

https://nanolive.ch/application-notes/

Mitochondria segmentation and quantification

A. Open STEVE and load XXX
B. Extract Fluo signal as 2D Tiff and RI as 2D tiff 32bit
C. Open Fiji and load Fluo mito
D. run("8-bit");
E. Image > Adjust > Threshold [light background, 21, 255] Red OK -> mito in black on white background
F. Edit ▸ Selection ▸ Create Mask
G. Edit ▸ Selection ▸ Create Selection
H. Select first the mask, then the original image, and select ⇧ Shift+E to transfer the mask's selections
I. Control which measurements are done using Analyze ▸ Set Measurements.
J. Analyze ▸ Measure
K. Save measures and open with excel

http://imagej.net/Segmentation

Link utili

Thanks!

Application notes: https://nanolive.ch/application-notes/$

Publications: https://nanolive.ch/publications/

STEVE software: https://nanolive.ch/software/

Supporting material and Tutorial videos: https://nanolive.ch/supporting-material/

Thank you for the attention and participation!