**3D visualization for cell biologists: A teaching module**

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3D analysis with existing free computational tools is expected to generate more accurate and conclusive results out of existing microscopy datasets. However, it is discarded in the majority of mainstream studies of cell biology. This situation is to be contrasted with the one in the parallel field of structural biology, in which *quantitative* 3D analysis is, out of obvious necessity, a critical and established method with a history of several decades. The compiled and emerging sets of 3D computational tools (even when exemplified with experimental data) seem to be mostly solutions in search of problems; with little impact on the current paradigm in cell biology *at application level*. Our pedagogical solution is to clarify, simplify, and unify highly multi-disciplinary concepts/tools with significant emphasis on hands-on inductive learning.

Advances in microscopy have made it possible to record full 3D information of cellular structures and processes at different wavelengths and over a time period. Using the full 3D information can be more accurate and conclusive than a 2D (projected) image. Even in a 2D geometry, the shape of an object can change considerably over time, and the full spatio-temporal evolution is better described in a 3D (XYT) coordinate (with distinguishable tracks and clear signatures of velocity regimes). *Observing* (before *thinking*) out of the box is a meaningful way of “[diving deeper, rather than just collecting anecdotes](http://www.nature.com/news/cancer-researchers-revisit-failed-clinical-trials-1.12835)” [1].

Recent advances in computer hardware and software have also facilitated 3D visualization and analysis dramatically and made it a common free tool. Interactive 3D visualization/analysis in a PDF file or with a web browser has been around for a few years. This trend has already affected scientific visualization and data analysis, and is further expected to have a noticeable impact on scientific publications.

The key question for a cell biologist is how to use these tools efficiently; in an easy and informative (not just aesthetically appealing and potentially misleading) way. To this end, we have developed a specific [teaching module](http://www.zmbh.uni-heidelberg.de/Central_Services/Imaging_Facility/ThreeDim.xhtml) [2] to guide cell biologists in a smooth journey from raw microscopy images to the final visualization and interpretation of 3D cell structures.

Unnecessary technical details and terminology have been avoided. For example, “*fast* vs. *high-quality* 3D visualization” is used instead of the common technical terms of “*Open-GL hardware-accelerated rendering* vs. *POV-Ray tracing”.* At the same time, numerous references have been provided for those interested in deeper understanding and technical keywords. The remaining necessary keywords have been clarified with simple pictorial examples and used consistently.

Many new concepts and terms can become familiar after doing a few exercises with computational tools. Nevertheless, the very first encounter (in which new concepts seem abstract) is still a major psycho-pedagogical challenge. We have addressed this issue by using familiar, objective, motivating, and context-creating examples. To exemplify, the notion of “Computed Tomography (CT) scan” and the questions of “Can a 3D printer print a (re-scaled) z-stack?” or “What other scattering patterns of DNA, other than *Photo51*, can be seen?” have been used to motivate “volume rendering”, “surface 3D models”, and “Fourier Transform”, respectively.

Developing the (collaborative) context of *Problem-oriented 3D cell biology* is significantly facilitated by using clear and important take-home messages from *structural* biology. It includes [computer programs](https://www.cgl.ucsf.edu/chimera/) [3], observation/discussion/conclusion examples, reproducing results using online archived data, validation schemes and figures of merit, and [professional multi-disciplinary online communities](http://www.ccp4.ac.uk/ccp4bb.php) [4]. The course materials have been prepared with this consideration.

Some other topics covered in the course (including 10 Appendices for more advanced topics) are

* Creating transitional 4D data out of 3D datasets along a given trajectory (*volume* and *conformation* morphing), and the more practical inverse problem of component analysis of time-lapse microscopy data
* Configuring a workstation with different objectives (fast computation, handling large datasets, or fast visualization) using dedicated online programs
* Different exercises for fast and interactive visualization of microscopy data with a web browser or in a PDF file with the possibility of further inspection and quantification for readers (including peer-reviewers).

Simple, yet helpful guidelines have been provided to translate the subjective perceived 3D information into tangible objective observations. Possible things to look for or to consider in a 3D dataset are

* Existence of special shapes: (Quasi-)Periodic, helical, tubular, fractal, sparse, … (and using image processing schemes enhancing/selecting/quantifying such shapes)
* Clarification of the apparent overlap (or disconnection) between objects in a projected image (tubular example)
* Explicit, clear, and concise observation/conclusion, which has been exemplified using the [recently-reported](http://www.sciencemag.org/content/early/2015/09/09/science.aac5492) [5] ultrafast dynamics in myoglobin, and the deposited data have been used to reproduce the results as an exercise.
* Choice of iso-surface values; disconnected-looking objects (no full coverage with visible fluorophores); and computational/optical/spectroscopic meanings
* [Process of elimination](https://en.wikipedia.org/wiki/Process_of_elimination) [6]: Identification of potential scenarios and the ones that can be proved (or ruled out) using the observations
* Clear and transparent use of hypotheses and prior knowledge, as opposed to taking things for granted or being affected by the negative connotation of “bias”: [Can a bacterium (-looking object) be indeed a virus](https://en.wikipedia.org/wiki/Mimivirus#Discovery) [7]?

Given the uncertainties associated with 3D visualization (parameter-dependence and partial subjectivity), a minimum set of validation tests is presented: Comparing different visualization schemes, viewing along different directions, verification of object visibility, and verification of *stable* visualization (small observed changes after introducing small changes in specific parameters).

This course is even suited for cell biologists *with no knowledge in programming*. Basic knowledge in image analysis and experience with the program ImageJ, however, is a prerequisite. Some specific goals of the course are

* Developing hands-on experience with some visualization programs
* Developing self-confidence and team-spirit in critical evaluation of 3D images
* Developing the prerequisites of the follow-up course on *3D quantification for cell biologists.*

Some examples of (geometrical) quantification have also been included in the course, as for synthesis of spheres with the highest packing density (each with 12 neighbors), the 3D profile of focused light at a given wavelength and for a given numerical aperture, and the distortions in oblique views. 3D *visualization* and 3D *quantification* complete and cross-validate each other. Their combination gives rise to quantitative 3D analysis.

**References**

[1] *Nature* doi:10.1038/nature.2013.12835

[2] http://www.zmbh.uni-heidelberg.de/Central\_Services/Imaging\_Facility/ThreeDim.xhtml

[3] https://www.cgl.ucsf.edu/chimera/

[4] http://www.ccp4.ac.uk/ccp4bb.php

[5] *Science* doi: 10.1126/science.aac5492

[6] https://en.wikipedia.org/wiki/Process\_of\_elimination

[7] https://en.wikipedia.org/wiki/Mimivirus#Discovery