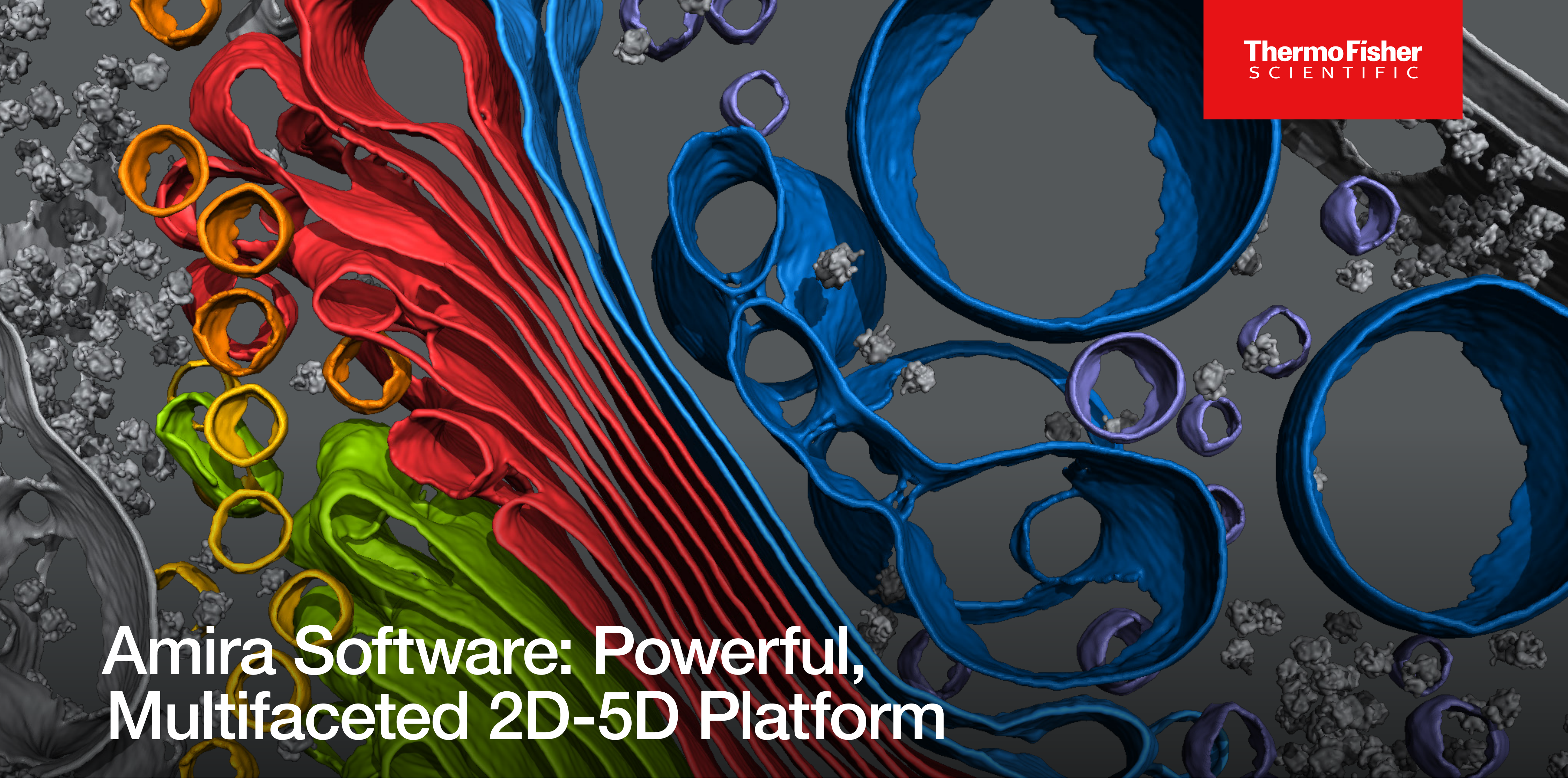


Amira Software: Powerful, Multifaceted 2D-5D Platform

Visualizing, manipulating, and understanding life science research data

Today’s life science researchers need to gather massive amounts of high-quality data to properly assess and identify an ever-growing array of complex biological samples. Manual analysis of high-resolution imaging data, like those provided by cryo-electron microscopy or high-content screening, could take weeks or even months. Thermo Scientific™ Amira™ Software is a powerful, multifaceted 2D-5D platform that can shorten these processing times to a manageable level. An imaging analysis toolbox such as Amira Software allows pharma users to define precise search parameters in their imaging data, so data segmentation time can be reduced to a matter of days, even on large data sets. With its state-of-the-art automated software, Amira Software supports research across disciplines as diverse as neuroscience, immuno-oncology, or membrane protein study. The advanced image analysis enabled by Amira Software helps biological researchers make faster, more accurate, structure-based discoveries.

A powerful, multifaceted 2D-5D platform for visualizing, manipulating, and understanding life science research data

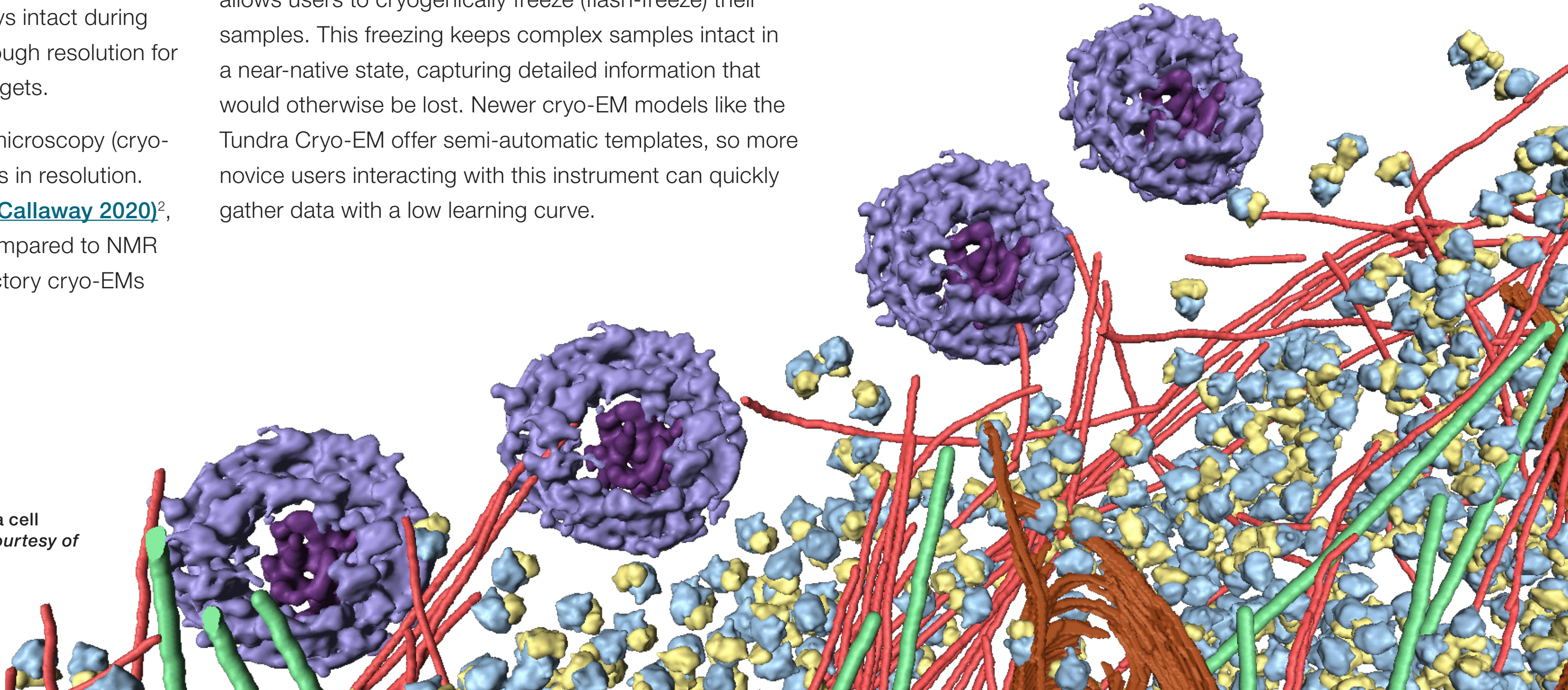
Studying membrane proteins with cryo-EM, cryo-ET, and Amira Software

Obtaining accurate sample images of membrane proteins is key to implementing accurate structure-based drug designs. Traditional methods such as nuclear magnetic resonance (NMR) and X-ray crystallography can provide incredible resolution but those methods require specific sample preparation to acquire data from membrane proteins. Additionally, membrane proteins account for over 60% of drug targets but make up only 2% of existing crystal structures (Aguayo-Ortiz 2021).[1] This gap in imaging membrane protein data illustrates how pharmaceutical research needs imaging techniques that will ensure a membrane protein stays intact during sample preparation and have a high enough resolution for researchers to identify potential drug targets.

Recent developments in cryo-electron microscopy (cryo-EM) have led to significant improvements in resolution. Thanks to this "resolution revolution" (Callaway 2020)[2], cryo-EM is now a competitive option compared to NMR and X-ray crystallography. Even introductory cryo-EMs such as the Thermo Scientific™ Tundra™ Cryo-TEM offer competitive resolutions as fine as five angstroms. The Tundra Cryo-TEM's resolution is high enough to acquire membrane protein processes such as revealing binding sites and conformations, assisting with hit finding, and other day-to-day protein functions that unlock a meaningful observation of cell and protein function.

Another advantage of cryo-EM is that pharma researchers do not need to rely on the "perfect crystal" when preparing their samples. The cryo-EM analytical process allows users to cryogenically freeze (flash-freeze) their samples. This freezing keeps complex samples intact in a near-native state, capturing detailed information that would otherwise be lost. Newer cryo-EM models like the Tundra Cryo-EM offer semi-automatic templates, so more novice users interacting with this instrument can quickly gather data with a low learning curve.

For a broader view of cell functioning, cryo-electron tomography (cryo-ET) enables 3D visualizations and analysis of cellular structures. This clear overview gives researchers a better understanding of cellular processes and thus a better understanding of cell function. Cryo-ET samples are preserved in a native state through label-free cryogenic imaging, without the need for sample staining or chemical fixation. The structural and exact spatial arrangement of organelles and proteins remains intact.

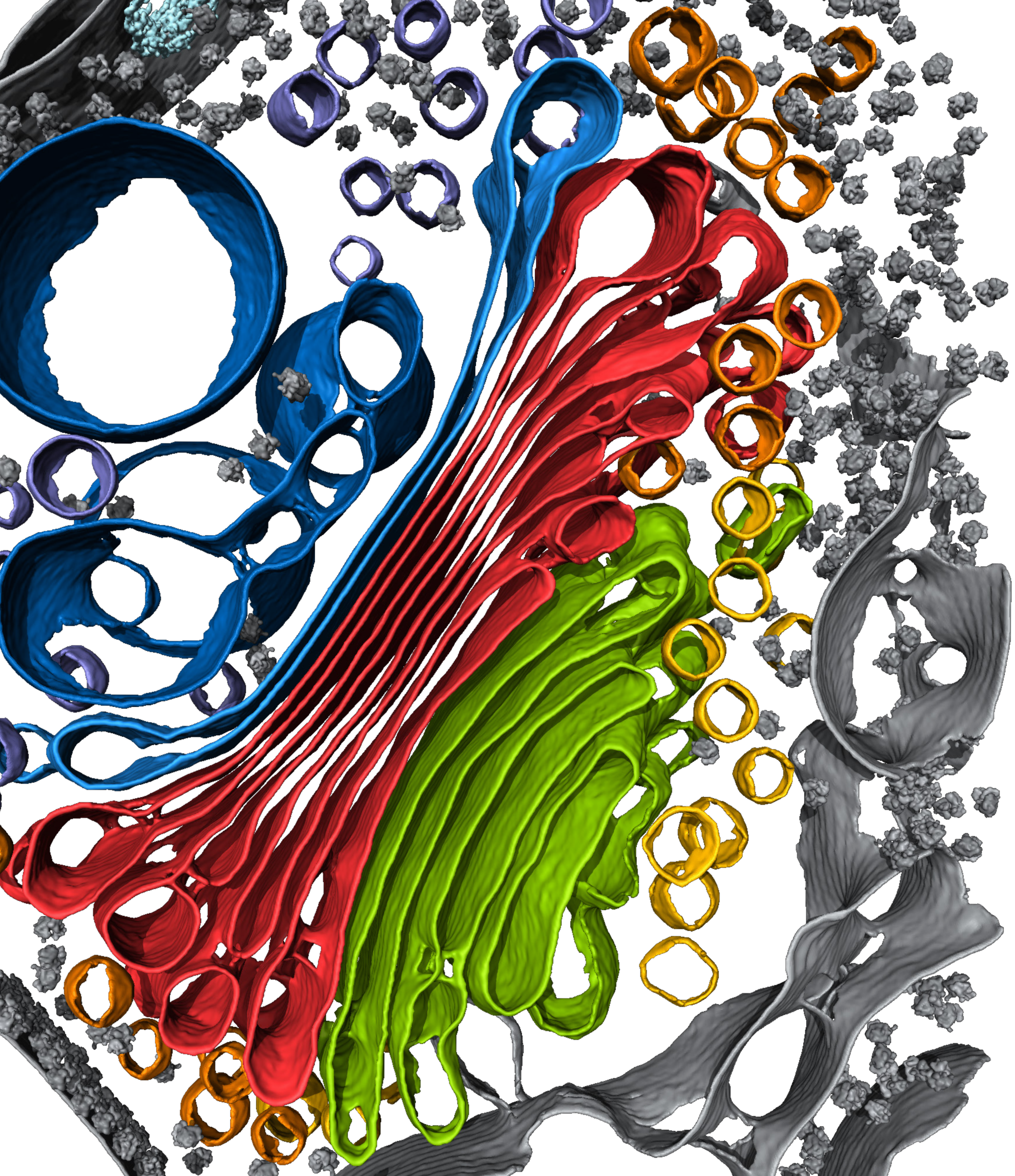


Visualization of the nuclear periphery of a HeLa cell revealed by cryo-electron tomography. ***Data courtesy of Dr. J. Mahamid, EMBL.***

This generation of previously unseen, high-resolution imaging data with an abundance of information for pharma researchers means that manually analyzing imaging data gathered from cryo-ETs could take months. An imaging analysis toolbox such as Amira Software allows pharma users to define the search parameters in their imaging data, so automatic data segmentation can be reduced to mere days on large data sets.

Amira Software also allows for a multifaceted approach by enabling pharma research teams to process the same sample with multiple modalities. For example, a team could employ cryo-EM, confocal microscopy, and X-ray crystallography imaging on the same sample. No matter how many modalities a user may implement, Amira Software can automatically sort through the relevant information that the user has specified.

Especially when used with Amira Software, cryo-EM and cryo-ET offer pharma researchers the ability to analyze membrane proteins, cell processes, and cell functions. Using cryo-EM or cryo-ET with Amira Software also streamlines analysis so pharma researchers can make faster, more accurate, structure-based drug discoveries. These unique, cutting-edge imaging techniques hold enormous potential for imaging cell biology.



In situ* cryo-electron tomography reveals the molecular architecture of the Chlamydomonas Golgi apparatus with native morphology. *Data courtesy of Dr. Benjamin Engel, Helmholtz Zentrum München.
https://doi.org/10.1126/science.aaq1392

High-content screening and Amira Software

High-content screening (HCS) also known as High-content imaging, is a widespread technique employed in drug discovery and other biological research to identify inter- or intracellular morphology, key characteristics of cell migration, druggability, and drug safety especially within the fields of oncology, neuroscience, and toxicity studies (BCC Research Sep 2021)[3]. Because this technique must generate, test automatically, and segment thousands to millions of high-resolution samples, employing a variety of imaging modalities, such as fluorescence and confocal microscopy, helps illuminate specific aspects of cell functionality.

Additionally, having imaging analysis software with sufficient throughput and processing power to effectively sort through and automatically interact with these very large data sets is also imperative for streamlined High-Content screening campaigns. The Thermo Scientific™ CellInsight™ CX7 Pro HCS Platform offers imaging modalities of LED fluorescence illumination, widefield and brightfield modes, and confocal capabilities to enable high-resolution fluorescent 3D imaging.

Below are two application examples of how Amira Software offers seamless functionality with the CX7 Pro HCS Platform to deliver high-quality data sets to immuno-oncology and neuroscience studies.

HCS and Amira Software for immuno-oncology

Immuno-oncology, or the study of stimulating the body's natural immune response to preventing, halting, and eliminating cancers, is currently one of the most promising cancer therapy programs. This field of study requires HCS to target abnormal cell characteristics effectively. Immuno-oncology researchers use 3D tumor spheroid models to train the immune system to identify, target, and kill tumor-embedded metastatic cells.

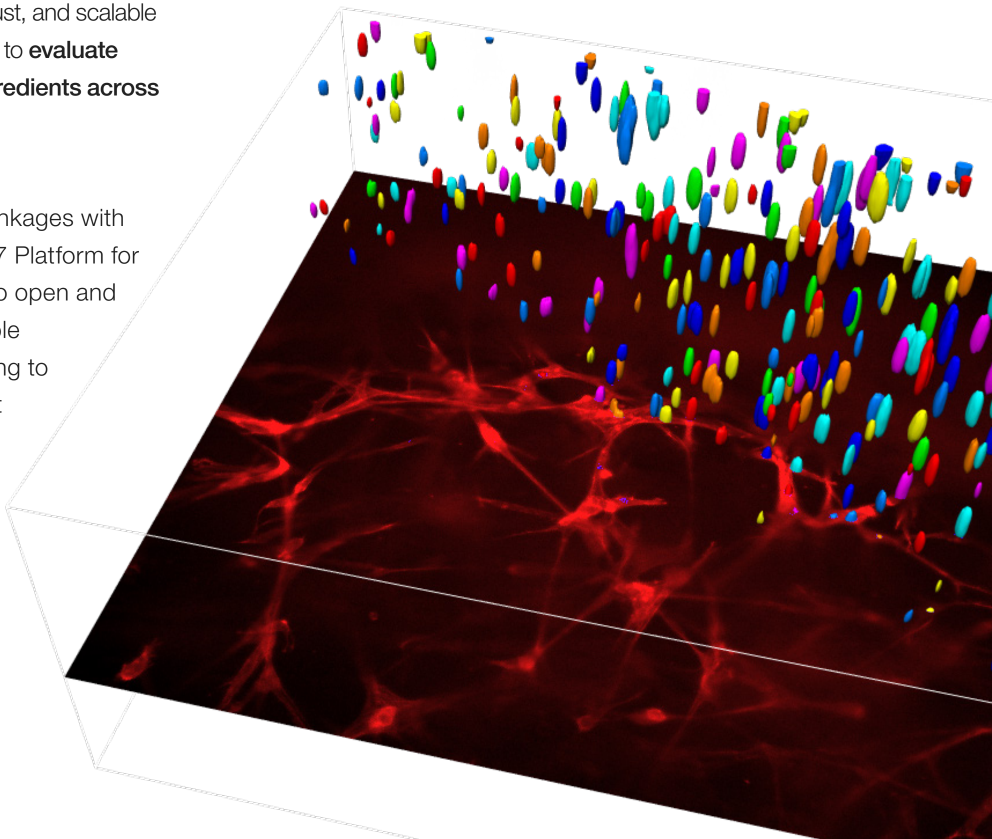
To create these accurate 3D tumor spheroid models, immuno-oncology researchers need an accurate, robust, and scalable 3D software solution such as Amira Software to **evaluate hundreds of potential pharmaceutical ingredients across dose-response curves.**

Key benefits:

Amira Software provides permanent file linkages with HCS Studio Analysis Software for the CX7 Platform for a secure imaging data channel. It can also open and process HCS Studio native files and enable multichannel 3D visualization and rendering to provide Amira Software users with almost immediately available imaging data.

In addition, users can implement time-course analysis capabilities to permit kinetic studies for "4D" measurements necessary for chemotaxis studies. Amira Software also offers 3D morphological evaluations necessary for more accurate immune cell activation studies. To ease the analysis of data from complex, cell-rich samples, Amira Software can evaluate co-localization statistical analysis in 3D samples including the co-localization of immune cells infiltrating a cancerous tumor model.

As noted in the examples above, Amira Software provides users with an extensive quantitative toolbox to tackle tasks such as cell count, morphology, and segmentation for more targeted cancer therapy studies.



HCS and Amira Software for neuroscience

Neuroscience research continues to evolve as new technologies are developed. Traditional in vivo animal testing, while very useful, has become both time- and resource-prohibitive. *In vitro* human-model neuroscience studies yield faster, more accurate models of neuronal networks than animal-based testing. Accordingly, the amount of *in vitro* neuroscience research in neurodegenerative, neurogenesis, and neurotoxicology applications has increased considerably.

Studies performed using human-derived 3D neuronal models generate even better outcomes than either monolayer cell culture models or animal-based models. Research using these 3D models is aimed at generating meaningful, realistic outcomes. Specifically, the studies aim to develop, within the models, healthy neurite growth and synaptogenesis that closely mimic *in vivo* results.

However, identifying—let alone targeting—neuronal outgrowth morphology is difficult, even *in vitro*. Neuroscience researchers have attempted to trace neurites and synaptogenic areas, but manual tracing could not keep up with the increased volume of compounds needing to be characterized. To address this issue, three-dimensional software toolboxes such as Amira Software were specifically developed.

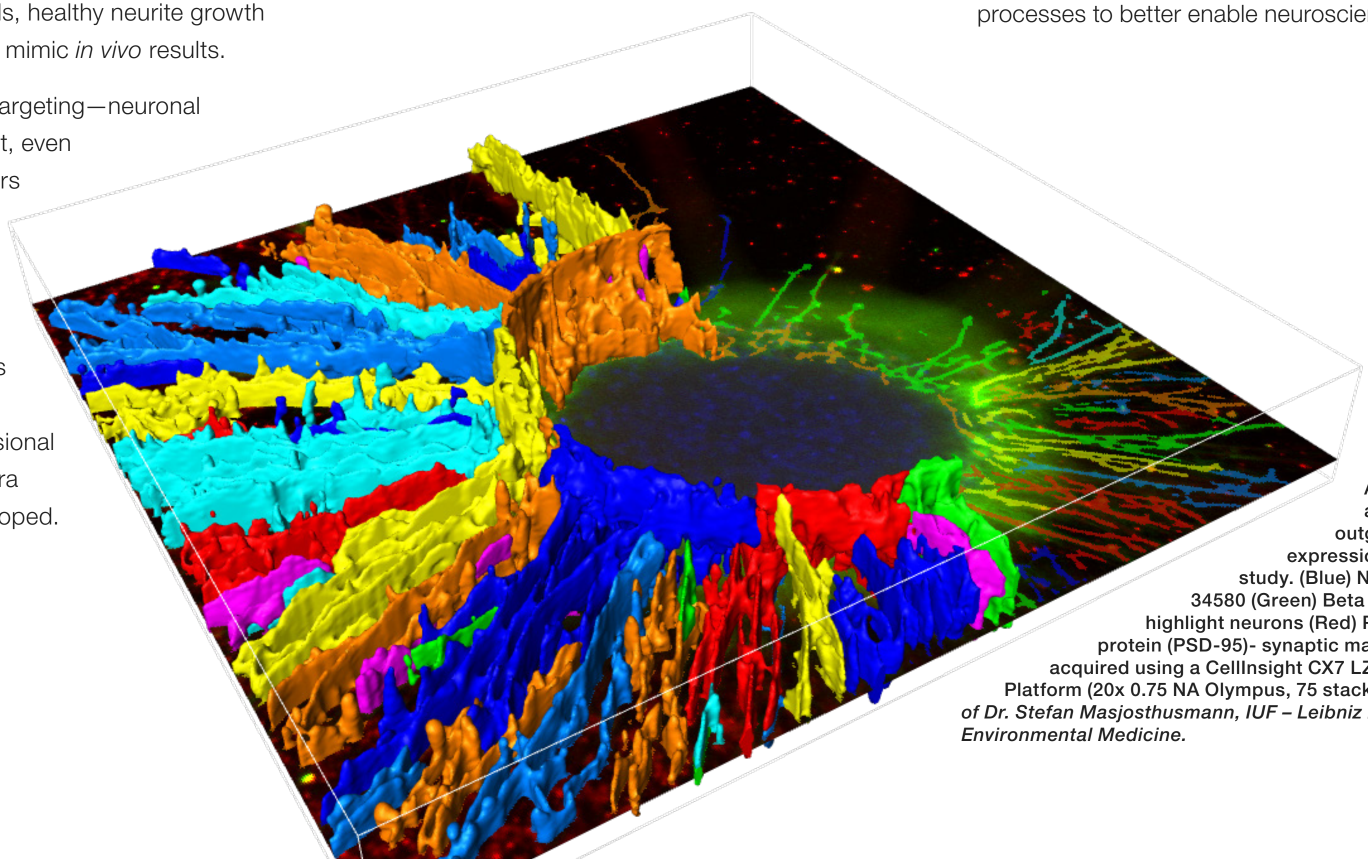
The software's 3D neuroscience abilities have been designed to quantify neuronal morphology accurately and efficiently, to facilitate the high throughput demands of the research. Amira Software offers overarching support in a neuroscience workflow by automating standardized imaging analysis, reducing the end-user learning curve, and improving model accuracy.

Key benefits:

Amira Software has built-in tools to detect primary and secondary neurite morphology and alignment in both 2D and 3D cell culture models. Amira Software also performs automatic detection and segmentation, allowing users to save time and automate their workflows in a way that supports accuracy and reproducibility of analysis.

The Amira Software toolbox can also be adapted to evaluate multi-channel fluorescent markers, allowing for the selective identification of both pre- and post-synaptic (puncta) fluorescent markers. This colocalization capability is imperative to understanding neurodevelopmental disorders such as Autism Spectrum Disorder or neurodegenerative disorders like Alzheimer's Disease. For a faster analysis of large data sets, users can scale Amira Software to enable high-throughput screening of multiple well plate formats, including 96-, 384-, and 1536-well plates.

These features can alleviate some of the pressure points in acquiring and accurately segmenting notoriously complex data sets from neurites while streamlining the associated processes to better enable neuroscience research.

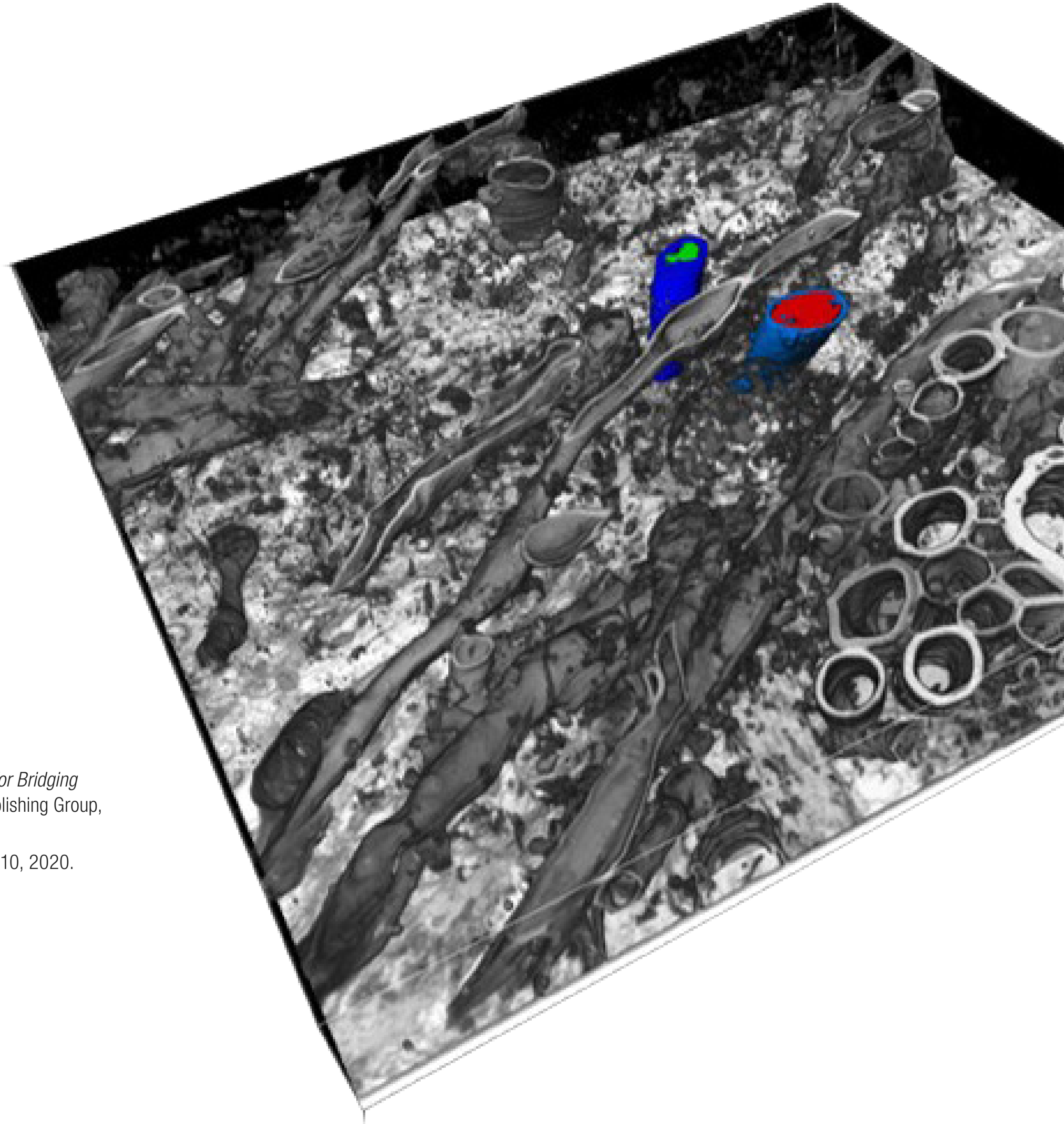


Amira Software 3D analysis of neuronal outgrowth and synapse expression in a toxicological study. (Blue) Nuclear staining Hoechst 34580 (Green) Beta tubulin staining to highlight neurons (Red) Postsynaptic density protein (PSD-95)- synaptic marker. These images were acquired using a CellInsight CX7 LZR High Content Analysis Platform (20x 0.75 NA Olympus, 75 stacks at 3 µM). *Data courtesy of Dr. Stefan Masjosthusmann, IUF – Leibniz Research Institute for Environmental Medicine.*

In summary

Thermo Scientific Amira Software is a comprehensive imaging analysis toolbox for pharmaceutical and life science researchers that enables a multifaceted approach to discovery by providing them the ability to process images of one sample that were acquired with multiple imaging modalities. Even if a user employs multiple modalities on the same sample—including cryo-EM, confocal microscopy, X-ray crystallography, HCS, or more—Amira Software can automatically sort through user-specified parameters to generate relevant information quickly and accurately.

Thermo Fisher Scientific continues to partner with the scientific community to effectively develop technologies that drive pharmaceutical research.



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