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Lattice Light-Sheet Microscopy as a Key Method for the Analysis of the Mislocalization of TDP-43 in Amyotrophic Lateral Sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease) is a neurodegenerative disorder that is characterized by the progressive loss of motor neurons. Consequently, patients afflicted with this disease eventually lose the motor function associated with moving, speaking, eating, and even breathing. One neuroscience team identified a common mechanism in nearly all sporadic ALS patients in which transactive response DNA binding protein 43 kDa (TDP-43) was mislocalized from the nucleus to the cytoplasm. This suggests that the mislocalization of TDP-43 into the cytoplasm is central for the onset of ALS in patients, but the underlying factors that result in this process are not fully understood as there are no current laboratory methods to study the progression of TDP-43 mislocalization. However, the development and refinement of lattice light-sheet microscopy (LLSM) as a laboratory technique has significant potential in helping scientists elucidate the unknown processes behind ALS. This review discusses the implications of lattice light-sheet microscopy and its optimization methods as a solution to the issue and identifies LLSM as a key method that could be significant in the field of ALS research.

Introduction

According to the CDC, there are five-thousand new cases of amyotrophic lateral sclerosis (ALS) every year in the United States. While this is a minor statistic when considering the size of the population, ALS is still a fatal disease at large as it currently has no cure. ALS is characterized by the rapid cell death of motor neurons which leads to the loss of muscle control and eventual paralysis. In 2010, Barmada *et al*. discovered that the disease is commonly followed by the mislocalization of a protein known as transactive response DNA binding protein 43 kDa (TDP-43) into the cytoplasm of neurons which contribute to cytotoxicity or neuronal cell death (1).

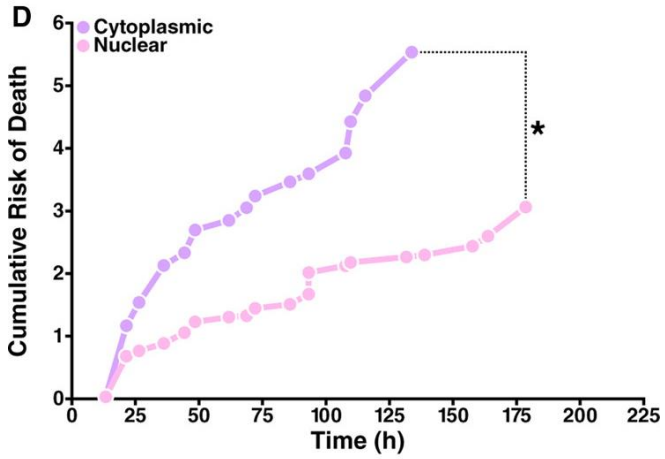

Figure 1. Cumulative risk of increased neuronal cell death is associated with the location of TDP-43 in cytoplasm. Barmada et al. 2010.

However, we do not fully understand this process or the mechanisms behind it. This is mostly due to the fact it is impossible to observe this sensitive cellular process in real time with traditional imaging techniques. Recently, a new microscopy method known as lattice light-sheet was developed that could address the issue. Lattice light-sheet microscopy is a recently developed microscopy method that allows for the four-dimensional observation of biological molecules as operators can image cells or molecules in three dimensions over a time scale. After processing individual images and knitting them together, scientists can produce a realistic ‘live’ view of biomolecules functioning in their environment and specific biological

context (2). However, this method does require the optimization of several steps to function ideally. From sample care and preparation to imaging and processing, each step is critical in the overall process. Despite this, lattice light-sheet microscopy has incredible potential for the biosciences field, ideal for observing cells that display both sentient and transient properties as they are rapidly evolving specimen, whereas traditional structural imaging methods cannot provide the whole picture. For this reason, scientists should consider using this strategy to overcome their obstacles in studying the process of TDP-43 mislocalization and other important ALS mechanisms. LLSM *in vivo* (referring to procedures performed using living or whole organisms) imaging will allow scientists to make new investigations on the spatially sophisticated physiology of afflicted motor neurons.

The Mechanisms and Value of LLSM

Studying cells and especially motor neurons at the nanoscale is an arduous process, and electron microscopes can reach this minute scale at the cost that one cannot view them while they are alive. To address this issue, Chen *et al*. has developed a new method that will allow scientists to view incredibly small molecules and live specimens at the nanoscale (2). This newly developed method is known as lattice light-sheet microscopy and its functioning mechanism is newly developed as its ‘light’ source is derived from a beam of electrons that will illuminate individual 2D sheets of the organism or cell one by one until a 3D dimensional image is formed. Using image processing software, these images can then be connected over time to permit 4D observations over a timescale. The key value of this method is the minimization of phototoxicity or the phenomenon that imaging could kill the cell and photobleaching as extended exposure to a light source during imaging could damage the specimen. These two processes could negatively

affect the cell's physiology or prevent an operator from observing important aspects of the cell as fluorescence (light being emitted from the cell) drops off after some time. One limitation of this method highlighted performance deficiency due to sample aberrations from defects or heterogeneity which prevent the rays from converging at a single point of focus. If samples are not prepared properly, any artifacts or damages introduced to the specimen will not allow an operator to have a clear image of what is being looked at. However, this implies that this limitation is not restricted to this method alone, and other microscopy methods are subject to careful processing of samples. In addition to other benefits, this strategy is orders of magnitude faster than comparable methods, making it a key method in the fluorescent imaging of live cells. Faster processing and low phototoxicity propels lattice light-sheet microscopy to the forefront of live cell imaging and opens the door for scientists to investigate the biological mechanisms behind ALS. However, there still exists a few limitations in the sample preparation process that hinder the potential of LLSM that scientists are working to address.

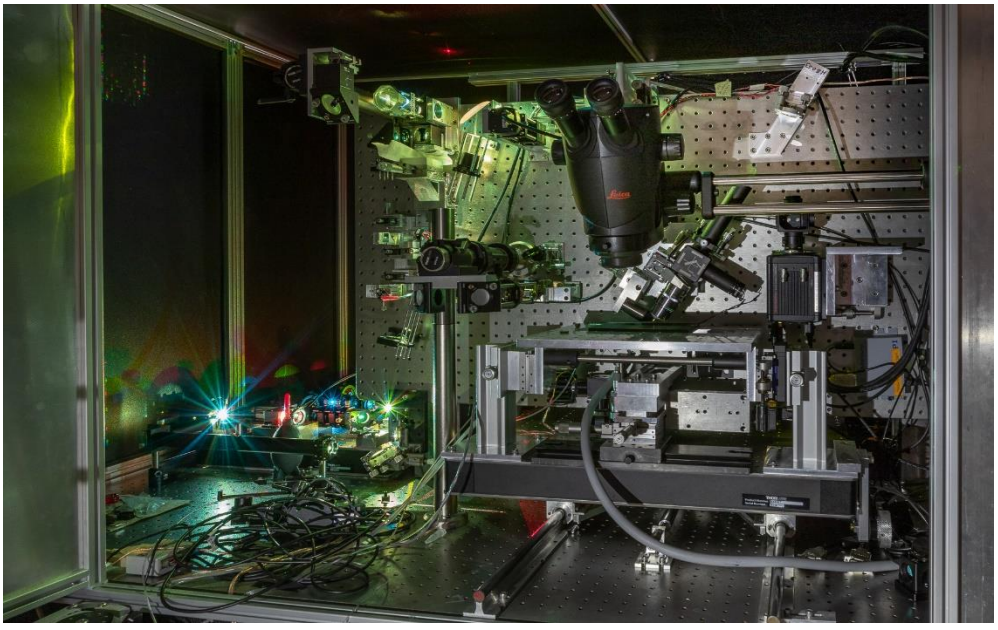

Figure 2. Lattice light-sheet microscope designed by Dr. Eric Betzig. ©Randal C. Jaffe 2018.

Optimizing Sample Preparation

Although lattice light-sheet microscopy (LLSM) is an incredible method to be used for the observation of cells in their standard physiological state, there is a significant limitation of this method that becomes more apparent as the depth of the study increases. Optical heterogeneity or the fact that the sample or specimen is different in certain parts will introduce aberrations to the system. Aberrations in the field of optics refers to the inability of light rays to converge at a single point because of limitations or defects. The resulting images then lose its optimal resolution and contrast. To address this issue, Liu *et al*. utilizes adaptive optics (AO), which detects distortions caused by the sample with reference to a single fluorescent point in the volume known as a 'guide star' (4). These distortions are then compensated for through alterations in a mirror's shape to create an equal and opposite distortion in the microscope's image negating the aberration. AO-LLSM is a faster and less intrusive technique to image cellular mechanisms in the context of optically intricate biological specimens. While this method addresses sample-induced aberrations, it can also correct for those introduced during the mounting process, submersion within the media, and even distortions caused by an imperfect optical path inside the microscope.

To expand the ability of adaptive optics LLSM, Tsai *et al*. perfected the sample preparation process of mounting lattice light sheet microscopy specimens to improve imaging performance for expanded specimens (6). Their strategies correct for two main limitations of LLSM as the procedure is not optimized for imaging larger specimens even as fast imaging speeds and resolutions are achievable on smaller samples that are in the range of dozens of microns. The first limitation is that LLSM's imaging ability is reduced considerably when the length of the

lattice light sheet increases in light propagation direction when illuminating the complete range of the specimen. As a result, larger samples require translation to be imaged at higher spatial resolutions with shorter LLS. Sample translation in imaging larger specimens reduces the speed of imaging while also increasing the complexity of the process and potentially introducing image registration problems after acquiring these images. The second limitation has to do with the design choice to maximize photon collection efficiency with the placement of two high numerical aperture objectives at right angles to the specimen. This design hinders a researcher's ability to move the specimen freely for imaging. The combination of these limitations leaves LLSM a less than ideal strategy for imaging expanded specimens. To address the first limitation, this group employs the use of a tiling LLS method so that larger specimen can be imaged with minimal sample translation, eliminating any technical limitations associated with this issue (6). As twin LLSs are generated in this tiling technique, imaging speed of this method is doubled which reduces the time needed for image acquisition. The second limitation is amended through their optimized sample preparation technique so that the expanded specimens can be loaded onto the microscope easier, and any optical aberrations introduced from the gel is reduced considerably. The strategies used by this team significantly improve the performance capabilities of LLSM in speed and post-image processing.

Despite this, Liu *et al*. assert that the greatest limitation of this method is the processing aspect of the immense size of the data set (4). Reducing the effort required in this process could make AO-LLSM less expensive and costly, as the authors emphasize that production of a few images required 0.62 terabytes of raw storage, and that data had to be deconvoluted to create a second copy, and finally imported into 3D visualization software which had generated a third copy. Any reasonable advances in this field would stem from reducing the cost of processing and

continued developments in commercial hardware so that imaging analysis and quantification would be more viable.

Refining the Post-Imaging Process

Originally, molecular graphics was a procedure commissioned by structural biologists, but as the field of bioscience has developed, this became a skill that every life scientist needed to master to become a competitive researcher in the field. This method was developed to be used for the visualization and interpretation of atomic-scale data, but now it is at the heart of presentation and education for distinct types of biological studies involving microscopy and imaging. As the scientific discipline of biology continues to evolve, more limitations are introduced. In the past, imaging and visualization techniques were used on single molecules to yield density maps. Now, optical microscopy techniques produce higher quality images which are associated with larger data sets requiring more computational and processing potential. However, current molecular modeling programs are unable to successfully integrate newer and significantly larger data sets as the data managed by these packages did not exist at the time when these programs were originally developed. In addition, the amount and access of online resources such as protein databases and other molecular repositories has increased extensively.

To deal with the limitations associated with large data sets and newer microscopy methods, Goddard *et al*. is developing the next-generation system for interactive molecular modeling known as UCSF ChimeraX (3). The goal of this project is to develop a program that effortlessly integrates investigation techniques for visualization and analysis of biomolecules, while resolving different resolutions on distinct scales starting at atomic and ending with the cellular scale. Alongside these advantages, UCSF ChimeraX provides much adaptability as it can be

customized for the processing of microscopy data as this type of data confers various performance hinderances. LLSM data is often processed in terabytes and requires a significant effort to analyze, but UCSF ChimeraX allows for the development of custom code to fit this larger data set. In addition, this new program employs a wide variety of performance optimizations to be better able to manage LLSM data. With the emphasis on the program's extensibility, independent developers can formulate and disperse their own distinct code packages based on UCSF ChimeraX's foundation.

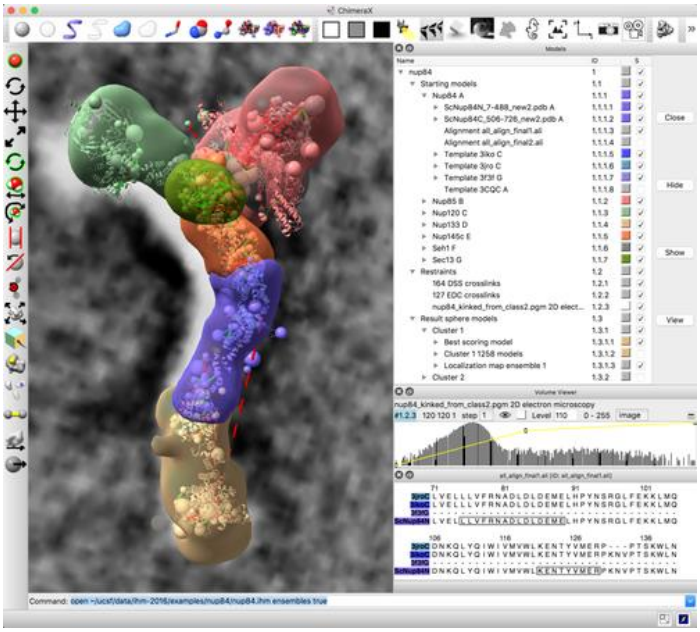

Figure 3. Integrated modeling with UCSF ChimeraX program. Goddard et al. 2017.

Schöneberg *et al*. expand on the work from Goddard *et al*. by addressing the limitation of using semimanual analysis such as ImageJ/Fiji for 2D datasets in the size range of hundreds of megabytes (5). This size range is prohibitive for the analysis of gigabytes or terabytes of 4D datasets that were imaged using LLSM. A combination of sophisticated analysis algorithms, 4D visualization tools, and new data storage is adapted and paired to new code, resulting in the pyLattice package which also interfaces with UCSF ChimeraX. The resulting code package features a user-friendly Python interface and support for large AO-LLSM data sets and support for membrane detection, and even membrane classification into basal, apical, and lateral assignments. PyLattice is a library of custom tools for image and data analytics, and it is specialized for handling large data sets as part of addressing the big data image analysis issue.

This program was designed with four principles in consideration: accessibility, interactivity, reusability, and generalizability.

As LLSM gains popularity in modern imaging techniques, a larger group of scientists will require access to sufficient image analysis and processing programs. To address the concern of expensive software and less recognized programming languages, pyLattice was built using Python which is one of the most popular coding languages today and it can interface seamlessly with ChimeraX for 4D visualization. The creators of this coding package also wanted users to be able to sample a variety of parameters, while also reusing existing code to minimize any redundancies to free up more processing and storage space for the data sets that will be analyzed (5). As this code package is also generalizable, it was built to be used beyond the task of adaptive optics lattice light-sheet microscopy on clathrin-mediated endocytosis, and it is possible to extend pyLattice to include other modules of LLSM imaging strategies being developed such as membrane trafficking. With this added component to the toolkit of any researcher using 4D imaging, AO-LLSM continues to be optimized for a wider breadth of research applications in the biological field being the ideal method for the study of molecular mechanisms of ALS.

Conclusion

With the development and optimization of lattice light-sheet microscopy, scientists can make vast leaps into understanding the molecular mechanisms of amyotrophic lateral sclerosis. The key advantage of using LLSM is that researchers can now observe cells and subcellular processes in real-time as the process is minimally invasive and less phototoxic to the cells. This is ideal as neurons are extremely sensitive cells, and any harsh environmental alterations or effects that introduce direct changes to the neuron will kill it. Despite this, LLSM had two

limitations associated with the sample preparation process as heterogenous samples induced aberrations in the imaging process, and the orientation of the objectives also make it difficult for the sample to be moved during imaging. By applying a tiling LLS method and adaptive optics, sample-induced aberrations were eliminated completely and orientation issue that prevented movability was also addressed. In addition, the development and expansion of UCSF ChimeraX's program capabilities prove to be a significant contribution to the LLSM field as it addresses post-imaging data processing issues connected with the use of large data sets in the range of terabytes. By combining the research of these teams, the door is open for scientists to now perform in-depth research on the mislocalization of TDP-43 and discover the molecular causes of this negative process. With the uncovering of the reasons behind TDP-43 mislocalization and the study of other subcellular processes associated with ALS, the progress of developing a therapeutic for the disease is accelerated significantly.

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