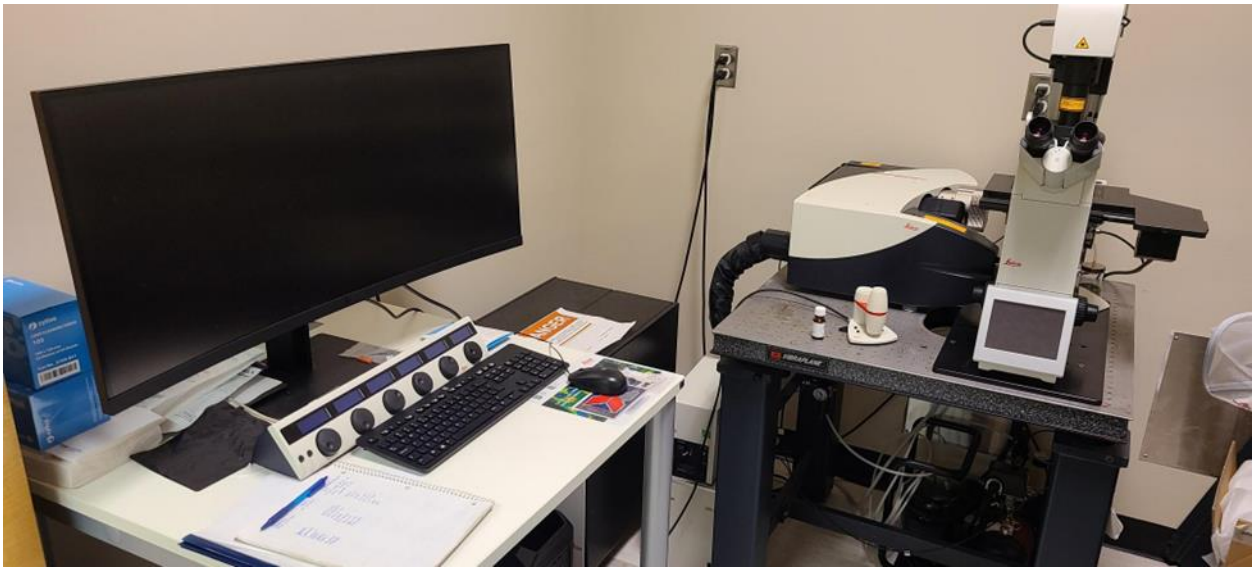
In 2019, the ALS Society of Windsor-Essex generously donated funds that contributed to the purchase of a Leica SP8 confocal microscope to Dr. Michael Strong's lab at Western University in London. This specific type of imaging is an extremely important tool by which we can examine individual cells for development of pathology. Throughout the last 30 years, we have used imaging extensively to document pathology in human tissues, track proteins, DNA and microRNA in culture cells, document pathology arising in cells expressing ALS related RNA and protein, and development of pathology in animal models to determine the effects of expressing ALS-related molecules on normal cellular function.

Confocal imaging is so powerful that almost every research group employs it in their research, which results in very competitive booking schedules for existing machines, and sometimes quite lengthy delays in getting time on the relatively few microscopes available. Since confocal imaging is central to at least 5 individual projects within Dr. Strong's lab itself, the purchase of a confocal system was vital to maintaining the speed with which our research projects are progressing. The SP8 confocal microscope (Figure 1) allows us to look directly at a broad range of targets, from individual cells to specific molecules or proteins within the cell. In fact, we can detect structures as small as 1 nanometer (one millionth of a millimeter) in diameter. The power of the confocal also allows us to build a 3-dimensional picture of structures within the cell/tissue which in turn gives us information about whether our targets are simply "close" or co-localized, and how they are associated in a 3-dimensional space (see for example Movie 1. In this short movie there are several 5nm optical sections that have been stacked on top of each other, and then 3D rendered to create this movie that allows for us to look at the structures in 3D from different vantage points).

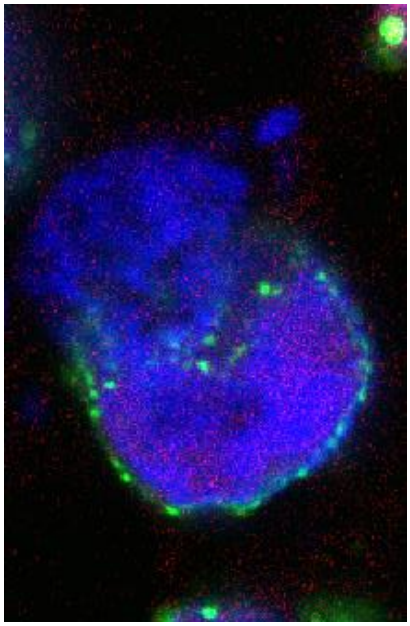
Figure 1: The Leica SP8 scanning laser confocal microscope.



An example of how this is used in our research is highlighted by one of our recent projects aimed to determining the effect within an ALS family in which one member developed

ALS and other members had other neurodegenerative diseases. The family possessed 3 different genetic variants with 2 genes directly linked to ALS, and another mutation in a gene not apparently linked to ALS (called *LMNA*). Curiously, only the individual bearing all 3 genetic variants developed ALS, while other members of the family had different combinations of the mutations but did not develop ALS (Volkening et al., Mol Cell Biochem 476(7):2633-2650). It was the first real evidence that ALS can be induced by multiple genetic variants occurring within the same individual, whereas any one occurring in isolation was insufficient to do so. To prove this, we used confocal imaging to demonstrate the changes in the nuclei of cells that we had genetically modified to express the genetic variants observed in this family, either as single variants or any combination (for an example of this, please see Figure 2).

Figure 2: An abnormal nucleus seen by confocal imaging.



In this confocal image, we were able to observe multiple structures and molecular markers simultaneously (the nucleus itself is blue; a protein at the margins of the nucleus is green (the new gene associated with ALS – called LMNA); and the known genes associated with familial ALS are seen as red (mutant FUS) or lilac (mutant SETX). Both FUS and SETX have moved from their normal location of inside the nucleus due to loss of the nuclear envelope (to the upper left of the DNA, lacking green signal) and the DNA is now also becoming less dense and spilling into the cytosol of the cell. The smaller blue structure is DNA that has fragmented from the other DNA.

This is but one example of the many projects being conducted in the Strong lab for which the ability to undertake confocal imaging has been crucial. By combining the biochemical and molecular aspects of our work with the imaging and cellular biology information from the confocal microscope, we have been able to conduct thorough investigations on all our projects without delays to the work.

The Strong lab thanks the ALS Society of Windsor-Essex for their generous donation, it is greatly appreciated, and this microscope will be heavily used for the years to come in our ongoing research.