# THREE-DIMENSIONAL CHROMATIC CORRECTION FOR FLUORESCENCE MICROSCOPY ENABLING PRECISE DISTANCE MEASUREMENTS IN THE NUCLEAR PORE COMPLEX

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Chromatic aberration had been underestimated in conventional light microscopy in biology. Emerging new technologies with increasing spatial resolution, penetration depth, and simultaneous acquisition by multiple cameras require more accurate yet robust methods for chromatic correction than required ever before. Here, we present a marker-free, simple, yet accurate method for measurement of chromatic shifts at the focus of interest in biological samples for fluorescence microscopy. We obtained reference images containing the same object in all color channels using the hallmark of fluorescence in which the emission spectrum always contains longer wavelengths. To obtain registration parameters from the reference images, we developed a robust method named "quadrisection phase correlation" to identify translation, rotation, and magnification in x, y, and z-dimension in a single operation. In addition, this method made it possible to measure local chromatic shifts by splitting images into smaller pieces and measuring phase correlation between two channels. This user-friendly, open-source software package is available for download (https://github.com/macronucleus/Chromagnon). Although the size of a chromatic shift between biological samples was found to be as much as 440 nm, the software corrected it at the three-dimensional accuracy of 10.0–19.0 nm depending on the method. Thus, after chromatic shift removal, distances between differentially labeled objects could be directly measured with precision far beyond the classical resolution of light microscopes. Using our chromatic correction software, we demonstrated the mean distances among proteins in the nuclear pore complex in live cells using conventional microscopy with precision of 0.5–3.5 nm.