LIVE AND PRECISE 3D REGISTRATION OF MULTICOLOR 3D STRUCTURED ILLUMINATION MICROSCOPY UNVEILS HIGHER-ORDER STRUCTURAL ORGANIZATION OF FISSION YEAST CHROMATIN

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Recent development of super-resolution fluorescent microscopy set a new practical resolution limit beyond the diffraction limit of light. Among super-resolution microscopy methods, 3D structured illumination microscopy (3DSIM), in particular, is able to capture images of three-dimensions and with multicolor. We have developed live 3DSIM methods allowing direct observation of chromatin within the nucleus of live fission yeast. Chromatin in live cells were organized into <100 nm condensed fibers. To analyze biological functions of this chromatin fibers with immunofluorescence, it is critical to have a highly precise registration method for multicolor 3DSIM in fixed cells. We have developed a chemical fixation buffer to mimic chromatin fibers in live cells. We have then developed an automated 3D registration software to find 6 alignment parameters (translation in X/Y/Z, rotation angles and magnification in X/Y). The accuracy of our software is 4-8 nm in lateral and 30-45 nm in vertical axis over the whole field of view (40 μm²) when examined with multicolor beads test samples. Our observations with nuclei in interphase and meiotic prophase suggested that transcriptionally-inactive regions were organized into a condensed filaments, and that transcriptionally-active regions were relatively decondensed and extended from those filaments. Also, constitutive heterochromatin has similar compaction ratio as inactive euchromatic regions. Our observation in fission yeast is in good agreement with a classical “lamp brush” organization in meiotic prophase as well as interphase.