

3D dSTORM SEQUENCE-SPECIFIC VISUALIZATION OF HIGHLY REDUCED MITOCHONDRIAL DNA IN NUCLEOIDS OF PANCREATIC ISLET β -CELLS ISOLATED FROM DIABETIC GOTO KAKIZAKI RATS

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Goto Kakizaki (GK) rats represent a useful model of type 2 diabetes, having hypertrophic pancreatic islets (PI), lower number of β -cells in which highly reduced mitochondrial (mt) DNA exists within the fragmented mt network. We applied the dSTORM nanoscopy for sequence-specific super-resolution visualization of mtDNA in combination with short DNA probes capable of self-hybridization, termed molecular beacons. To visualize mtDNA by 3D dSTORM, we immunostained nucleoids of insulin-positive cells by TFAM, Twinkle and antiDNA antibodies. To visualize the specific sequences of mtDNA, we have constructed specific probes (ND5, DLOOP, 7S and ND6) by standard PCR reaction with incorporation of labeled nucleotides either with Alexa Fluor 647, Cy3B, CF568 or biotin. The typical probe had length between 100 and 1500 bp. The employed fluorophores were able to photoblink in reducing media, hence were suitable for dSTORM nanoscopy with ~25 nm x,y-resolution after their hybridization to specific mtDNA sequences. We have combined *in situ* hybridization [1] technique in the first channel with the dSTORM immunocytochemistry in a second channel, visualizing nucleoids by either *anti*-DNA, Twinkle or TFAM. 3D dSTORM measurement was performed on a Vutara SR-200 nanoscope. For nucleoids segmentation and their 3D rendering, Delaunay tessellation and subsequent modeling by principal component analysis was used [2]. Using such double color 3D imaging, we characterized positioning on/inside the nucleoid spheroids for so-called D-loop sequences of mtDNA, and we compared it with the positioning of ND5 gene encoding sequences. We were also able to resolve a distinct size distribution of nucleoids of the diabetic Goto Kakizaki β -cells.

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[1] Alán L, Zelenka J, Ježek J, Dlasková A, Ježek P., *Fluorescent in situ hybridization of mitochondrial DNA and RNA. Acta Biochim Pol* 57(4):403-8; 2010.

[2] Alán L, Špaček T, Ježek P., *Delaunay algorithm and principal component analysis for 3D visualization of mitochondrial DNA nucleoids by Biplane FPALM/dSTORM. Eur Biophys J* 45(5):443-61; 2016.

Figure shows nucleoids of mtDNA with diameter of each particle approx. 45nm. Anti-DNA dSTORM (white) together with probe against D-loop of mtDNA (7S-DNA; dark gray).

