

**3D dSTORM OF MITOCHONDRIAL NUCLEOIDS AT REDUCED
MITOCHONDRIAL DNA REPLICATION IN PANCREATIC ISLET β -CELLS OF
DIABETIC GOTO KAKIZAKI RATS**

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Hypertrophic pancreatic islets (PI) of Goto Kakizaki (GK) diabetic rats contain lower number of β -cells than non-diabetic Wistar rat PI. Remaining β -cells contain highly reduced mitochondrial (mt) DNA per nucleus (copy number) within the fragmented mt network. Such a profound mtDNA decrease might originate from declining replication machinery or decreased transcription factor TFAM. Moreover, it might be reflected by an altered morphology of nucleoids, which are complexes of mitochondrial DNA with proteins responsible for DNA packaging (TFAM), transcription and replication (DNA polymerase poly, Twinkle helicase, mtSSB, *etc.*). To elucidate these changes, we have immunostained nucleoids of insulin-positive cells by TFAM and Twinkle antibodies and visualized them using a 3D dSTORM imaging. Alternatively, we have employed incorporation of 5-bromo-2-deoxyuridine (BrdU) or 5-ethynyl deoxyuridine (EdU) for staining of newly replicating mitochondrial DNA (mtDNA) itself. The indirect 3D immunocytochemistry was employed with a secondary antibody conjugated to Alexa-647. 3D dSTORM measurement was performed on a Vutara SR-200 nanoscope (currently Bruker). For nucleoids segmentation and their 3D rendering, Delaunay tessellation and subsequent modeling by principal component analysis was used [1].

We have found that despite the profound mtDNA decline, the apparent nucleoid number (spatial density) was constant. In β -cells of GK rats (*vs.* Wistar) Eos-TFAM-visualized nucleoids were by 10% smaller (composed of 72% localized TFAM) while Eos-mtSSB-contoured “nucleoids” were by 25% smaller but 1.25 times denser. TFAM/mtDNA determined by qPCR of TFAM and ND5 subunit remained constant, when both were suppressed equally as the mtDNA copy number in GK samples. The apparent paradox requires further studies.

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[1] 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.* 2016, 45, 443-61.