Type-2 diabetes is characterized by insulin resistance in peripheral tissues frequently combined with impaired insulin secretion of pancreatic β-cells. These cells control glucose metabolism by insulin secretion using mitochondrial oxidative phosphorylation as glucose sensor. Hence, pathophysiology of diabetes must involve β-cell mitochondrial dysfunction. The functional impairment of mitochondrial metabolism might be reflected in their structure and network organization. Therefore, we attempted to evaluate possible morphological changes in diabetic β-cells mitochondrial network, here represented by isolated Langerhans islets of Goto Kakizaki diabetic rats. 3D 4Pi microscopy possesses sufficient 3D resolution for visualization of mitochondrial networks. We have analyzed 4Pi images of mitochondria-targeted GFP (achieved by lentiviral expression) of primary β-cells within the Langerhans islets isolated from Wistar (control) rats and Goto Kakizaki (diabetic) rats. The diabetic β-cells showed highly disintegrated pattern of the mitochondrial reticulum (counting numerous small spherical and short segment objects), reflecting pathogenic changes, namely the impaired glucose-stimulated insulin secretion and respiration and mitochondrial membrane potential responses on glucose stimulus.