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4 D imaging of insulin secretory granule dynamics and secretion in primary beta cells with lattice light sheet microscopy

Total internal reflection microscopy (TIRFM) has been the method of choice for many years to image insulin secretory granule (SG) dynamics and secretion in primary beta cells and insulinoma cell lines. However, it only allows for imaging of SGs located <200 nm from the surface of the cell attached to the glass, thereby restricting the view only to events happening on one side of the cell. Since beta cells have a polyhedral shape with a diameter of several µm, by TIRFM imaging events happening in the major part of the cell remain invisible. Furthermore, prior to TIRFM imaging pancreatic islets are usually dissociated into single cells – a procedure that affects cell-to-cell interaction and signaling.

These limitations can be overcome with novel microscopy techniques that allow for imaging insulin SGs within primary beta cells of isolated islets at sub-cellular resolution and high speed. Specifically, we have used lattice light sheet microscopy (LLSM) to resolve insulin SGs, which have a mean diameter of 250 nm. LLSM allows for fast TIRFM-like sectioning of cells in 3 dimensions with low photo-toxicity. In this way we could image SNAP-labelled insulin SGs in isolated SOFIA (Study of Insulin Ageing) mouse islets within the whole cell volume. Use of a novel pH-sensitive SNAP-substrate further enabled us to image insulin SGs undergoing exocytosis. Hence, this is the first report for the use of LLSM in a primary mouse tissue at sub-cellular resolution in order to address insulin SG turnover within whole beta cells. Ultimately, this approach might be exploited to study peptide hormone turnover in other model systems, thus providing novel insights into the physiology of regulated secretion in health and disease.

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Terms and Conditions

Yes

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