REAL-TIME, MULTIDIMENSIONAL, *IN-VIVO* AND *IN-SITU* IMAGING OF PANCREATIC ISLET USING THREE-PHOTON MICROSCOPY AND THIRD HARMONIC GENERATION

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The pancreatic islet of Langerhans is a multicellular system that plays a key role in the maintenance of blood glucose homeostasis. Malfunctions of it lead to serious diseases such as diabetes. Hence, the study of pancreatic islet function is a fundamentally important task in medical research.

Even though *in vitro* imaging of isolated islets has made great progress in investigating its calcium dynamics and sensitivity to glucose, they are still limited owing to their isolation from the surrounding pancreatic tissue. The vacuum window has been used in intravital imaging, which can on one hand stabilize the pancreas for imaging, and keep the loss of fluid during the imaging can to the least on the other hand, since only a small incision in the peritoneal wall is required [1,2].

Due to its scattered distribution throughout the pancreas, the pancreatic islet of Langerhans brings forward high request of the deep tissue imaging at single-cell resolution. Here we used three-photon microscopy and Third Harmonic Generation (THG) to extend imaging depth in scattering tissues. We used *Ins-cre*$^{+/-}$*; Gcamp6s*$^{f/f}$ mouse to record the calcium oscillation of beta cell under the three-photon excitation. Adopting glucose clamp technique to control the blood glucose and perfuse insulin, we found islets displayed regular and adjustable Ca^{2+} oscillation *in vivo*. In details, fast blood glucose induced high frequencies Ca^{2+} oscillation with a period of 1-3 min. While，elevated glucose increased the plateau time of high Ca^{2+} level and decreased the time of low Ca^{2+} level with a period of 6-10 min, which is different from that high glucose induced long-time stable high Ca^{2+} plateau *in vitro*. We collected the THG signal, which was generated from optical heterogeneities along with the three-photon excitation, to monitor the single red blood cell *in vivo.* We realized blood flow imaging without labeling and observed a "stop-and-go" blood flow in pancreatic islet. In summary, we established a novel approach with single cell resolution allowing for real-time imaging of islet function within the living mouse, which has not to our knowledge been attainable by other methods.

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