Suction-assisted Imaging Window for Stabilized Intravital Imaging of Lung and Heart in a Live Animal

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Intravital microscopy is a unique imaging technique to visualize various *in vivo* cellular-level dynamics such as cell trafficking, cell-to-cell or cell-to-microenvironment interactions in a live animal. Intravital imaging of cellular dynamics in a natural physiological microenvironment can provide unprecedented insights in the dynamic pathophysiology of human diseases those were impossible to obtain through conventional histological observation of *ex vivo* sample or *in vitro* culture system. However, unlike the organs those can be mechanically fixed without disturbing their natural physiology, the lung and heart are challenging for a microscopic intravital imaging due to unfixable motion of breathing and beating, respectively.

In this talk, the All-in-One real-time intravital two-photon and confocal microscopy system with a suction-assisted imaging window for a stabilized imaging of beating heart and a breathing lung in a live anesthetized mouse. First, the anesthetized mice were ventilated with an inspiratory pressure of 24~30 mmHg, a respiratory rate of 120~130 breaths per minute, and a positive-end expiratory pressure of 2 cmH_2O. 2% isoflurane was delivered to maintain anesthesia status for several hours. To expose the lung or heart, mice were positioned in right lateral or dorsal decubitus followed by thoracotomy. Skin and muscle were dissected until the exposure of rib and then incision was made between 3rd and 4th rib. Inside the suction-assisted imaging window, a negative pressurization of 920~960 mbar was achieved by suction of air through the tube connected to a vacuum pump, which immobilize the lung surface of 5 mm diameter with the transparent cover glass as shown in following figure. For a beating heart, another window of a similar design with smaller open imaging diameter of 2 mm was used with negative pressurization of 880~900 mbar. After achieving the suction-assisted stabilization of lung and heart surface, the residual motion of several tens of micrometer could be further removed by frame-by-frame image registration. Finally, cardiovascular and pulmonary vasculature and microcirculation, circulating immune cells in normal and pathological condition were successfully visualized.