Long-term, *in toto* live imaging and digital reconstruction of faithful lineage landscapes sculpting the mouse heart chamber

With the support by the National Natural Science Foundation of China, the interdisciplinary team led by Prof. He AiBin (何爱彬) in collaboration with Prof. Cheng HePing (程和平) at the Institute of Molecular Medicine at Peking University and Prof. Xiao WenLei at the School of Mechanical Engineering and Automation at Beihang University, realized long-term, *in toto* live imaging and digital reconstruction of holistic cell behaviors sculpting the embryonic mouse heart, which was published in *Nature Cell Biology* (2020, 22(3): 332—340). This study, for the first time, presented a framework to track uninterrupted cell lineage histories across their past, present, and future to reveal organ-level morphogenetic mechanisms in mammals.

Ideally, to faithfully reveal the developmental processes of organogenesis, all single cells should be followed noninvasively through live imaging for a certain period of time. Formation of a mammalian heart as the first functional organ reflects an exquisite choreography of massive numbers of cells dividing and migrating through the complex processes to ultimately create a four-chambered muscular pump. Many congenital malformations result from any of these anomalies. He's team overcame the technical challenges as previously encountered by optimizing mouse embryo culture and mounting methods, developing a vertical, dual-side illumination light-sheet microscope, equipping it with an integrated embryo culture module, together with a heartbeat-gated imaging module. With this integrative approach, they realized a 36-h, all cell-resolved imaging of the developing mouse heart at 3-min intervals. Innovative digital image processing pipelines, Grapebio, made it possible to uncover all-cell landscapes and uninterrupted cell lineages for the developing mouse heart, through robustly segmenting and accurately locating ~20 000 000 cells of different shapes and intensities in ~7 200 volumetric stacks (obtained over 1.5 days at 3 min intervals).

With digital reconstructions, they unraveled the cellular basis for the heart chamber ballooning and the cells of origin for the heart trabeculation. In contrast to a widely accepted model that hyperproliferation of discrete regions measured by cell cycle labeling leads to local ballooning of the early heart tube, ultimately expanding the chamber, their model supported that the preferentially outward migration of surface CMs coupled with cell intercalation and horizontal cell division, rather than regionalized hyperproliferation, might provide a driving force for this process. Tracing whole uninterrupted cell lineage histories revealed a surprising model in which nearly half trabecular CMs arise from their earlier cell fate segregation from a compact layer, and the second half through oriented cell division and directional cell migration.

Evidently, bolstered by these technical breakthroughs, this study by He's group paves the way for continuous live imaging of multi-scaled grand developmental schemes of organogenesis as well as subtle pathological alterations, e.g., in congenital heart disease models. This offers a new framework across the broad field of developmental biology for deciphering organogenesis in mammals at single-cell resolution, yielding highly accurate cell lineage trees and histories of holistic cell behaviors.

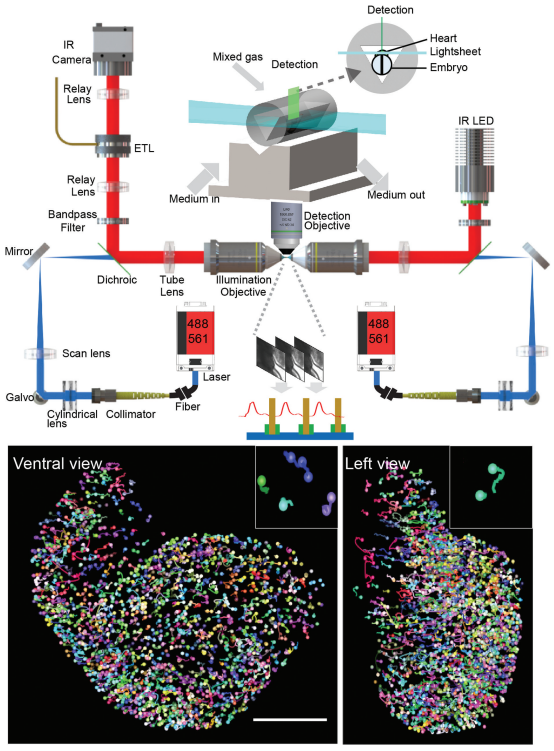

Figure The live imaging system for long-term recording of developing mouse heart at single-cell resolution.