LETTER TO THE EDITORS

# Imaging cell biology in transplantation

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Dear Editors,

We here present a miniature suction-assisted endoscope suitable for imaging cellular events in the beating heart graft of the mouse and we demonstrated the applicability of our imaging method for a wide range of cardiovascular research [1–3].

Over the past decade, intravital optical microscopy (IVM) has emerged as a powerful tool in animal research. By monitoring the complex behavior of cells, it will be possible to answer many outstanding questions in the field. Despite their earlier success with open-heart vasculature imaging [4], scientists in cardiovascular research have been largely unable to benefit from this technology owing to the difficulty of obtaining minimally invasive access to the heart and owing to the motion artifacts induced by the heartbeat.

We developed the applicability of new imaging method for transplant immunology using our IVM system. To date, there are no published studies to evaluate the real-time dynamic imaging of a vascularized allograft. This system has an advantage compared with others in the use of beating heart imaging because of suction attachment (Fig. 1a left). We used the same incision and inserted the microscopy into abdominal cavity (Fig. 1a right).

We have employed the system in our heterotopic cardiac transplant model [5] using several unique mouse strains available in our laboratory. B6 background (H-$2^b$) CD11c-YFP [6] and CX3CR1$^{GFP/+}$ [7] fluorescently labeled reporter mice were used as donor to track dendritic cell (CD11c-YFP) or monocyte (CX3CR1$^{GFP/+}$). This allows us to offer a unique opportunity to examine dendritic cell (DC)–T cell interactions over time and space in their physiological microenvironment when five million fluorescently labeled syngeneic T cells are injected intravenously into fully allogenic BALB/c (H-$2^d$) recipients (Fig. 1b, panels C and D). The application of intravital imaging will allow us to directly assess and quantitate DC–T cell interactions that cannot be assessed using any other approach (Ueno *et al.*, *American Transplant Congress* 2012, 2013) (Fig. 1b, panel D). Imaging was performed at several different time points (i.e., right after reperfusion 3, 24, and 72 h; later 5, 7, 14, and 28 days of transplant) with inhalational isoflurane anesthesia. It also provides us a long duration of each imaging session (up to 60 min), which allows us to observe the changes in cells morphology and numbers at the same location. We can control and change the dose of suction, and this suction surrounded the tip of camera so that cellular imaging of the beating heart in vivo is possible with minimal motion-induced artifacts. At each imaging site, z-stack images were acquired at the depths from 0 to 100 μm by changing the imaging plane.

Importantly, mice survive the procedure in our model, thereby permitting imaging of the same recipients between the beating cardiac allograft and secondary lymphoid tissues via the systemic circulation over time, which demonstrates the feasibility and safety of long-term serial imaging for more than 100 days post-transplantation (Video S1). In addition, intravenous injection of TAMRA dextran conjugate further allows us to investigate migration of monocyte (panel A) and DC (panel B) within the local circulatory compartment and across the vascular endothelium into cardiac myocytes (Fig. 1b).

In summary, live imaging of the transplanted allograft and secondary lymphoid tissues by IVM provides high-resolution information on the dynamic single-cell interactions that take place during an alloimmune response *in situ*.

These studies promise to enhance our understanding of immune cell migration and communication and provide the basis for new therapeutic strategies for inducing immune tolerance.


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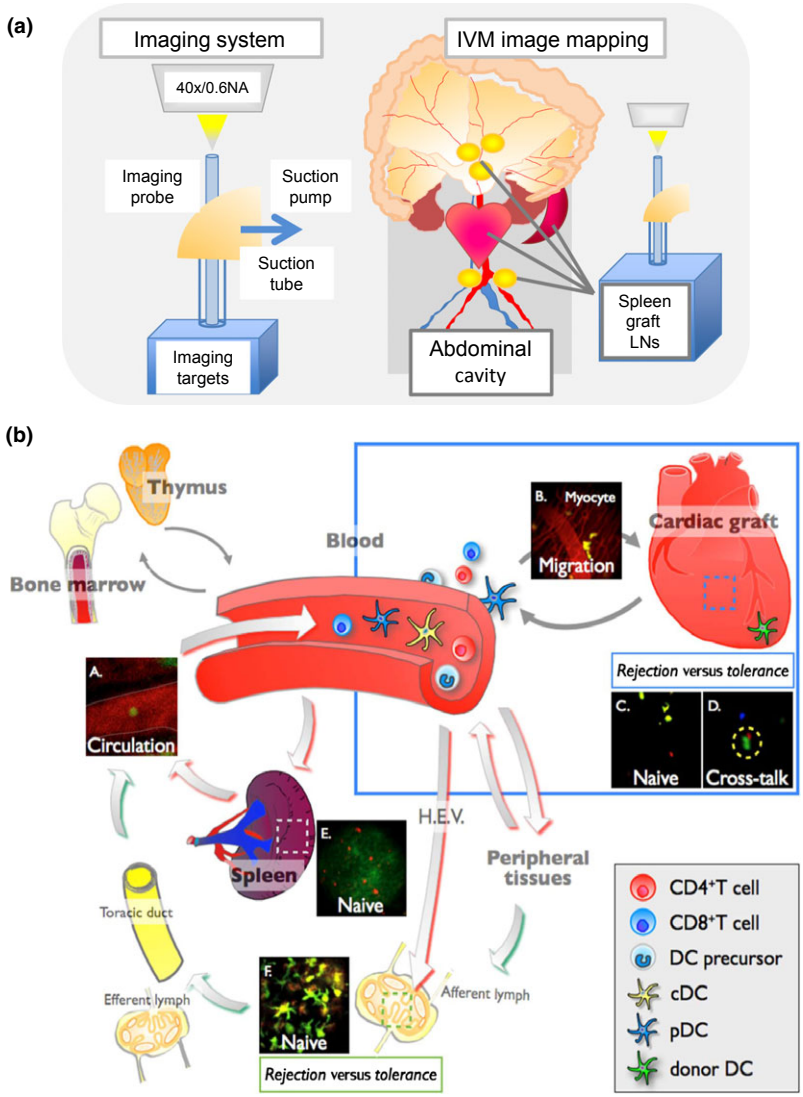


**Figure 1** (a) **Schematic of IVM system and experimental design** Left: Schematic of motion-stabilizing endoscopy with suction assist. The optical endoscope is made of graded index (GRIN) lense with a diameter of 1 mm. A motion stabilization scheme was implemented so that cellular imaging in vivo is possible with minimal motion-induced artifacts. Right: Image mapping of abdominal cavity in cardiac transplant model: We can explore wherever we want to investigate with the IVM system with safety. (b) Programmatic Outline of DC and DC Precursor Trafficking **Routes in cardiac transplantation**. In cardiac transplant model, those DCs and other cells including T cells start migration into transplanted graft after reperfusion via anastomosed vessels, which causes alloimmune responses between donor vs recipient. The event has been visualized at cardiac graft as shown as "Cross talk." A: Circulation of monocyte (GFP) in peripheral blood in CX3CR1$^{GFP/+}$ donor transplant model. B: Migration of DC (EYFP) into cardiac graft in CD11c-YFP donor transplant model. C, F: Images of CD11c-YFP heart and abdominal LN following harvesting. D: Cellular interaction between DC and injected T cell at graft in CD11c-YFP donor transplant model. E: Live image of spleen following T-cell injection.

## Authors' contributions

T.U. and K.J. participated in the performance of the research, performed the data collection, performed the statistical analysis, and contributed to the writing of the manuscript; M.Y.Y. helped in design of the study and performed the data collection; M.M.M. participated in the writing of the manuscript and performed review; P.K. and T.S. participated in the statistical analysis; M.H.S. helped in the design of the study; and A.C. and S.H.Y. participated in review.

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## Conflicts of interest

The authors have declared no conflicts of interest.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

**Data S1.** Materials and Methods.

**Video S1.** Live image of cardiac graft (>100 days survival).

## REFERENCES

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