Long-term, *in toto* live imaging of the developing mouse heart

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Method Article

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Abstract

Mapping holistic cell behaviors sculpting mammalian heart has been a goal, but so far only successes in transparent invertebrates and lower vertebrates. Using a live-imaging system comprising a customized vertical light-sheet microscope equipped with a culture module, a heartbeat-gated imaging strategy, and a digital image processing framework, we realized imaging of developing mouse hearts with uninterrupted cell lineages for up to 1.5 days. Four-dimensional landscapes of cell behaviors revealed a blueprint for ventricle chamber formation in which biased outward migration of outermost cardiomyocytes coupled with cell intercalation and horizontal division. The trabeculae, an inner muscle architecture, was developed through early fate segregation and transmural cell arrangement involving both oriented cell division and directional migration. Thus, live-imaging reconstruction affords a transformative means for deciphering mammalian organogenesis.

Introduction

A central theme in organogenesis is how cell lineages of divergent sources are spatiotemporally deployed to assemble complex organ structures in mammals[1,2]. In particular, the formation of mammalian heart as the first functional organ reflects an exquisite choreography of massive numbers of cells dividing and migrating through the processes of heart tube looping, ballooning, and formation and coalescence of the inner trabecular layer, ultimately creating a four-chambered muscular pump [1-4]. Many congenital malformations result from abnormal cardiac growth[5]. However, cellular mechanisms of heart chamber formation remain controversial because an effective means offering spatiotemporal, holistic information of cell lineages is still lacking.

Ideally, to precisely map the developmental processes of embryogenesis and organogenesis, all single cells should be followed noninvasively through live imaging for a certain period of time. Recent breakthroughs in live imaging coupled with digital reconstructions open a new avenue to decipher morphogenetic rules through precisely tracking cell fate decisions and depicting a holistic lineage landscape[6-9]. Not only it has brought new fundamental understandings of the development of *Caenorhabditis elegans* and *Drosophila* embryos[10,11], great strides have also been made on mouse embryo imaging using light-sheet microscopy, enabling single-cell imaging of yolk sac expansion[12] and gastrulation[9]. Because of technical challenges including rigorous culture conditions to support *ex vivo* development, depth of penetration to image solid organs [9,15,16], and spontaneous heartbeats onset around E8.0 [17], its implementation to mammalian hearts has not been possible, until now.

Here, we 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, we realized a 36-h, all cell-resolved imaging of developing mouse heart at 3-min intervals. Further aided with innovative digital image processing pipelines, Grapebio, we generated,

for the first time, 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). Taking these digital reconstructions, we reveal the cellular basis for the heart chamber ballooning and the cells of origin for the heart trabeculation.

Reagents

NotI (NEB, R3189V)

BamHI (NEB, R3136V)

DMEM/F12 (Sigma, D2906)

Fetal bovine serum (Gibco, 16000-044)

Pen-Strep solution (Hyclone, SV30010)

HEPES (Sigma, H4034-500G)

Sodium bicarbonate (Sigma, S5761)

Glucose (Sigma, G7021)

Low-melt agarose (Sigma, A9414)

OCT (Leica, 14020108926)

Doxycycline (RPI, P6148-500G)

BrdU (Sigma, B5002)

anti-BrdU antibody (Abcam, Ab6326)

EdU (Life Technologies, C10638)

Click-it EdU imaging Kit (Life Technologies, C10638)

TNNI3 antibody (Abcam, Ab56357)

GATA4 antibody (Santa, Sc-25310)

Alexa Fluor 488 donkey anti rabbit (Invitrogen, A11078)

Alexa Fluor 488 donkey anti rat (Invitrogen, A21208)

Alexa Fluor 555 donkey anti goat (Invitrogen, A21432)

Alexa Fluor 647 donkey anti goat (Invitrogen , A21447)

T-A clone vector EZ-T (Vazyme, T168-101)

DIG RNA labeling kit (Roche, 11277073910)

anti-DIG-AP antibody (Roch, 11093274910)

BM Purple AP Substrate (Roche, 11442074001)

Equipment

1.5 ml tube (Axygen, MCT-150-C)

0.2 ml 8-strip PCR tube (Axygen, PCR-0208-C)

Confocal microscope (Zeiss LSM 710)

Nikon SMZ18 microscope

Zeiss Axio Vert.A1 microscope

Zeiss Lumar V12

Centrifuge (Eppendorf)

Thermal cycler (Eppendorf)

ThermoMixer

Procedure

Reagents Setup

• Dissection medium

88% DMEM/F12 (PH 7.2-7.4)

10% fetal bovine serum

2% Pen-Strep solution

25 mM HEPES

• Basic Culture medium-V1

98% DMEM/F12 (PH 7.2-7.4)

2% Pen-Strep solution

15 mM HEPES

• Culture medium-V2

50% basic culture medium

50% rat serum

• Culture medium-V3

83% dissection medium

17% rat serum

Dissection medium and culture medium pre-heated and balanced in an incubator (37°C, 5% CO_2, and 95% air) for 1 h before using.

Detailed Experiment Procedure for embryo preparation

Embryo collection

1. Pregnant mice are euthanized with 4% chloral hydrate.

2. Soak abdomen with 70% ethanol, pinch the skin and make an incision at the midline of the abdomen with surgical scissors, then pull the skin apart.

3. Cut the peritoneum to expose abdominal cavity.

4. Cut the uterus and place it into the dissection medium.

5. Separate each embryo with forceps between implantation sites.

6. Peel back the surrounding muscle layer and expose decidua tissue.

7. Clip off 1/5 portion of the decidua, and expose the embryo;

8. Dissect out the enveloped decidua, Reichert's membrane with fine forceps.

9. Remove ectoplacental cone, yolk sac and amnion with super fine forceps (when embryos are later than E8.25).

Embryo culture

10 Transfer the embryos to a Petri dish/agarose tube with culture medium-V2, put the Petri dish/agarose tube in an incubator (37°C, 5% CO_2, and 95% air).

Embryo mounting

11 Make hollow agarose cylinders: Component B inserted with component A is filled with 2% low melting-temperature agarose. Triangular hollow is formed after removing component A (Figure 5).

12 Suck the mouse embryo with culture medium-V2 to the hollow in the agarose holder.

13 Combine the agarose holder with a base support, and mount the agarose holder and base on the chamber, full the chamber with culture medium-V3.

Live imaging of the mouse embryos

14 Set up the environment on the microscopy: Open and adjust gas mixer to 5% CO_2, 5% O_2 and 90% N_2, pre-heat the chamber to 37°C using water bath which is rolling surrounding the chamber.

15 Image the embryos: Generally, 2.5 μm step size, up to total of ~400 μm, 3 min intervals, lower laser power and exposure time are used to obtain better results.

Troubleshooting

1.

Step: 9.

Problem: The state of mouse embryo is not well when dissecting.

Possible reason: It takes too much time to dissect mouse embryos or destroys the embryo.

Solution: Practice more times and decrease the operating time.

2.

Step: 15.

Problem: The state of mouse embryo is not well when imaging .

Possible reason: The culture condition may be changed during long-term imaging.

Solution: Check the imaging system, containing gas mixture (O_2, N_2, and CO_2) and temperature control.

Time Taken

Day 1

- Dissect mouse embryos

- Mount embryos to V-slot
- Place the V-slot with embryo onto the vLSFM
- Adjust the imaging conditions
- Live imaging

Day 2-3

- Live imaging
- Obtain and analyze the imaging data

Anticipated Results

1 The mouse embryos exhibit good state before imaging.

2 The imaging mouse embryo shows normal heart development.

3 Imaging results show positive signals at single-cell resolution.

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Figures

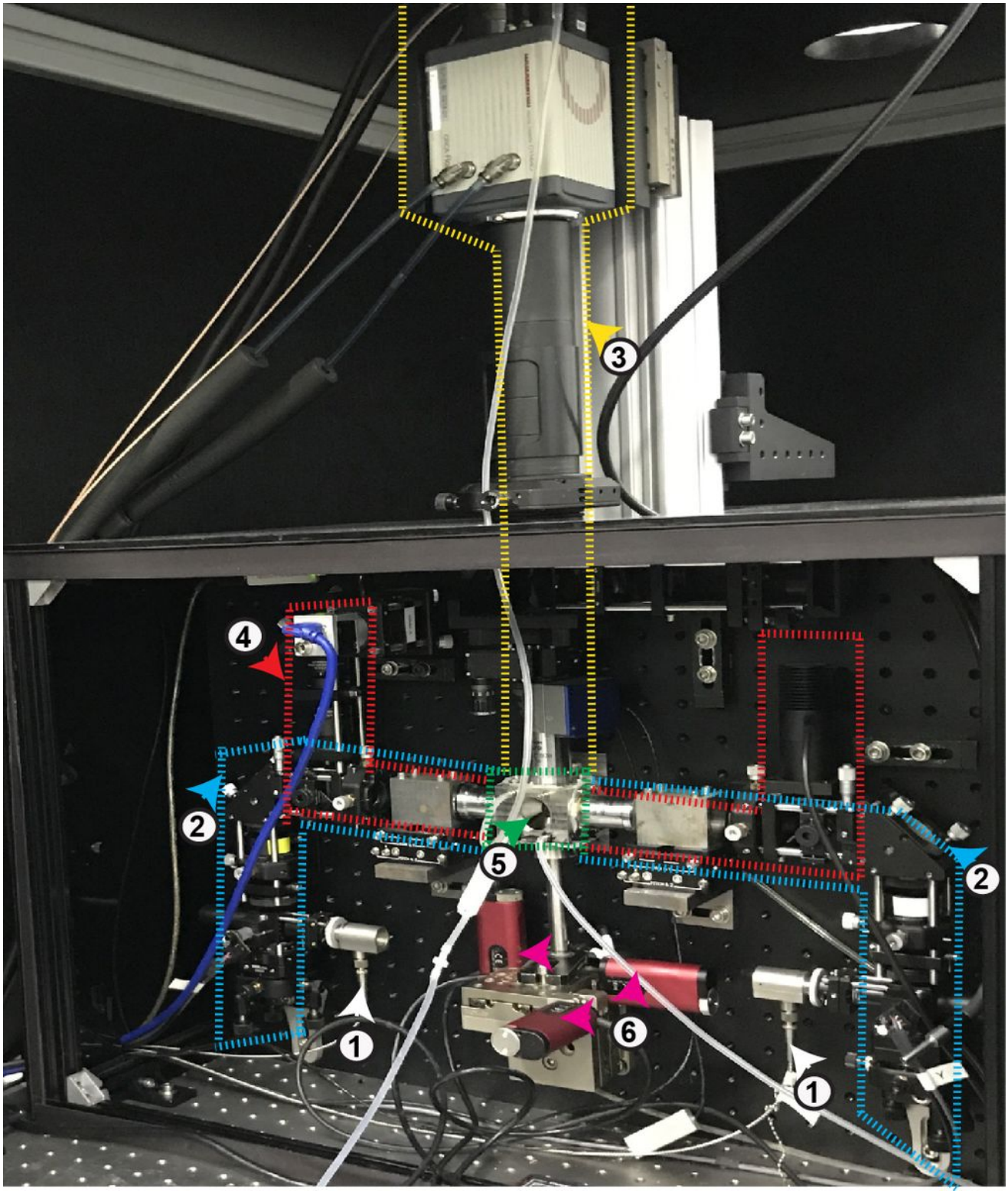

Figure 1

Close-up of the vLSFM imaging system, including the laser light source (1), two illumination arms (2), one detection arm with a sCMOS camera (3), near infrared heart-beating detection path (4), embryo culture and imaging chamber (5), and the 3-axis stage (6). b, 3D model about how to image a mouse embryo by

vLSFM. The anterior, posterior and dorsal view of the mouse sheltered the light path so only the lateral and ventral view of the heart could use for illumination and detection.

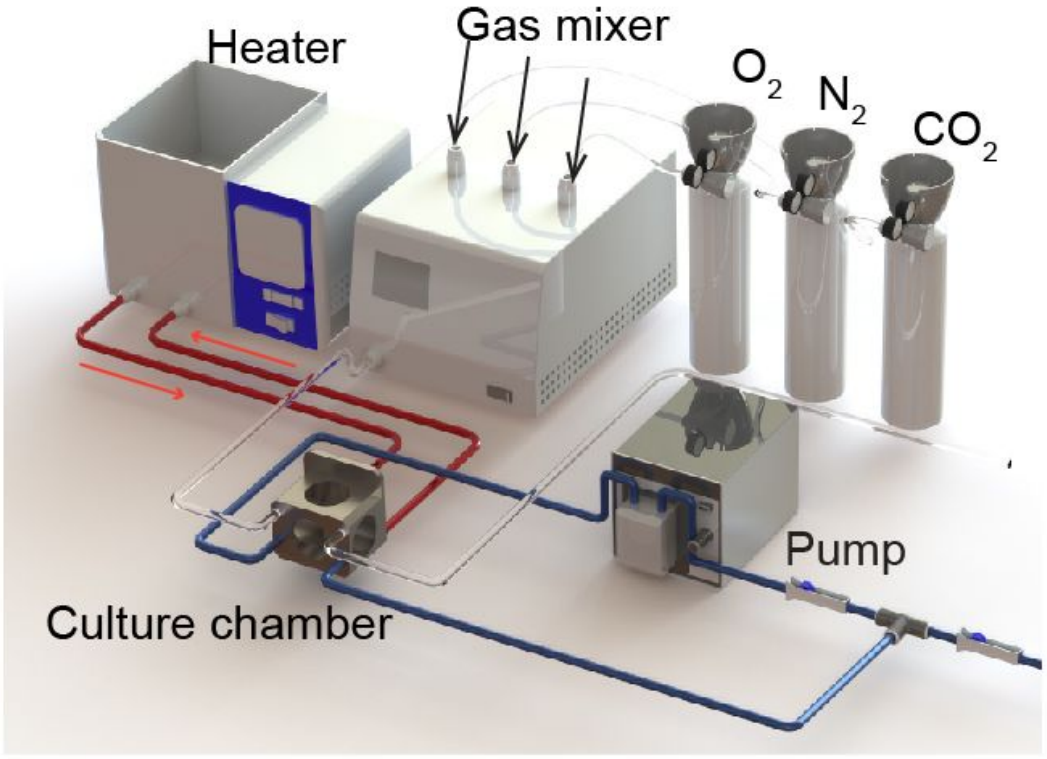

Figure 2

Embryo culture system. The culture system comprises the gas mixer controller, the medium circulation, and temperature controller as well as the embryo culture and imaging chamber.

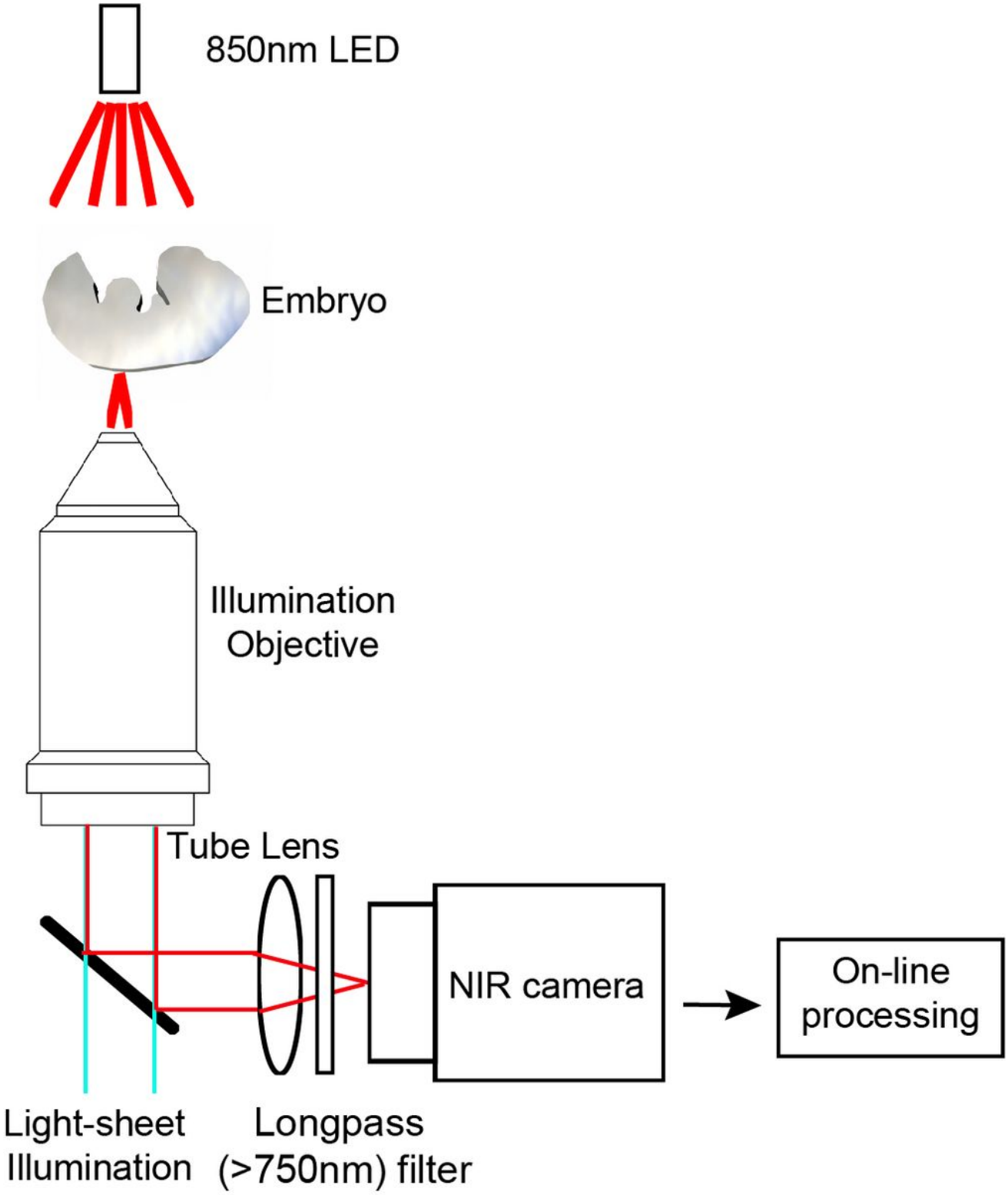

Figure 3

Schematic of the optical tracking of heartbeats using near infrared (850 nm) bright-field imaging at 50 Hz.

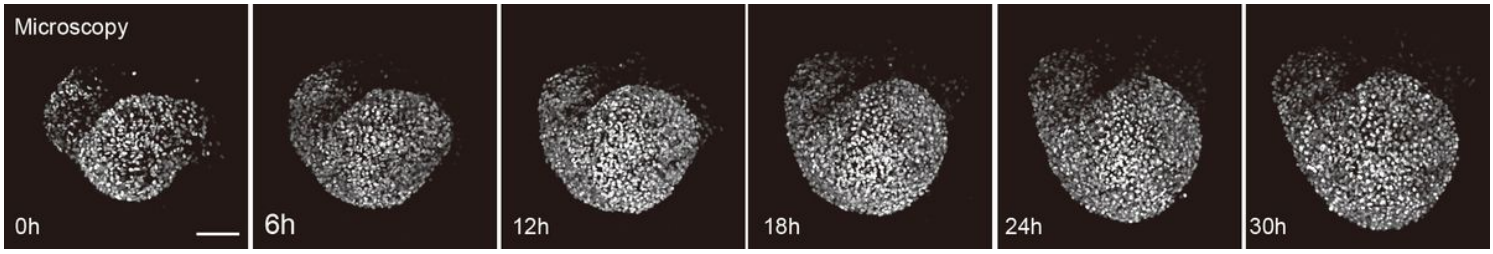

Figure 4

Resolving all nuclei-labeled (H2B-GFP) cardiomyocytes in a developing mouse heart.

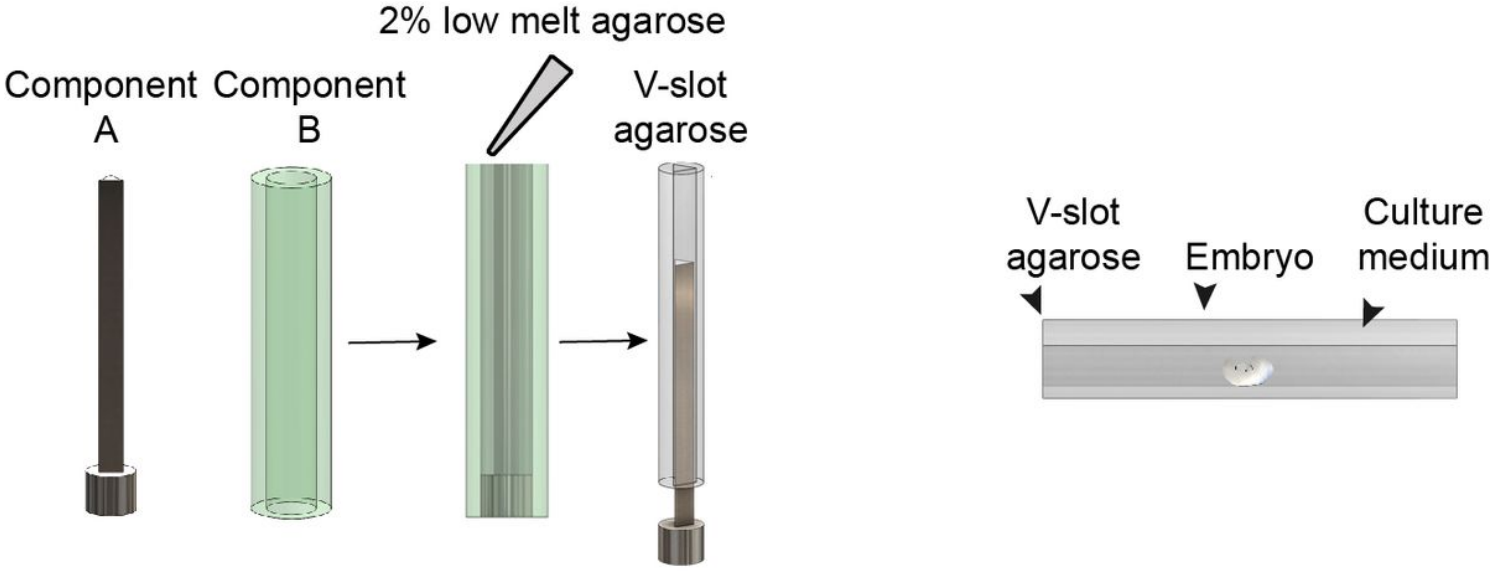

Figure 5

Component B inserted with component A is filled with 2% low melting-temperature agarose. Triangular hollow is formed after removing component A. Sucking the mouse embryo with culture medium to the hollow in the agarose holder.