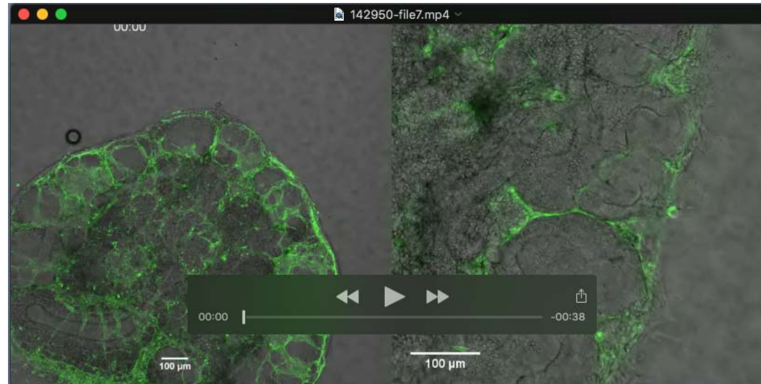
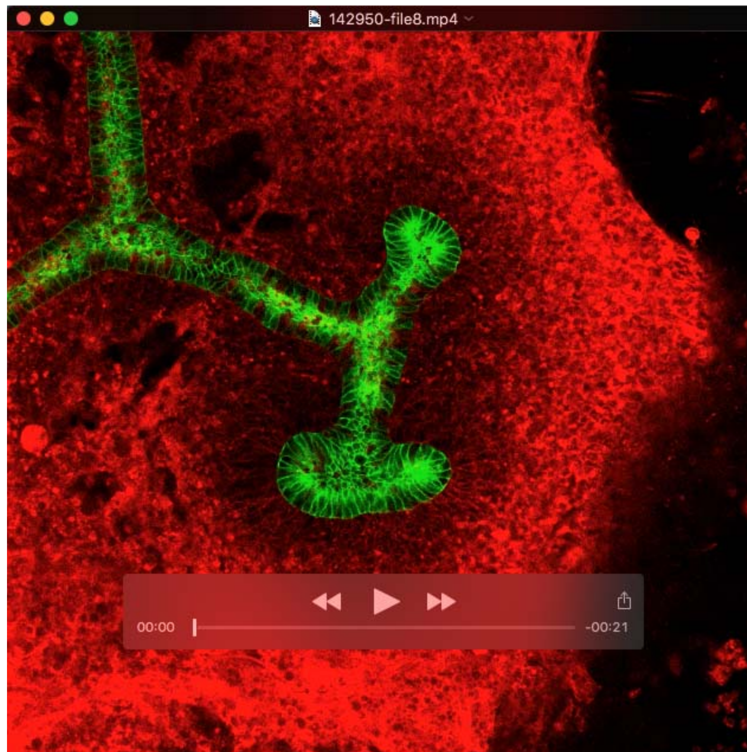
Supplementary data

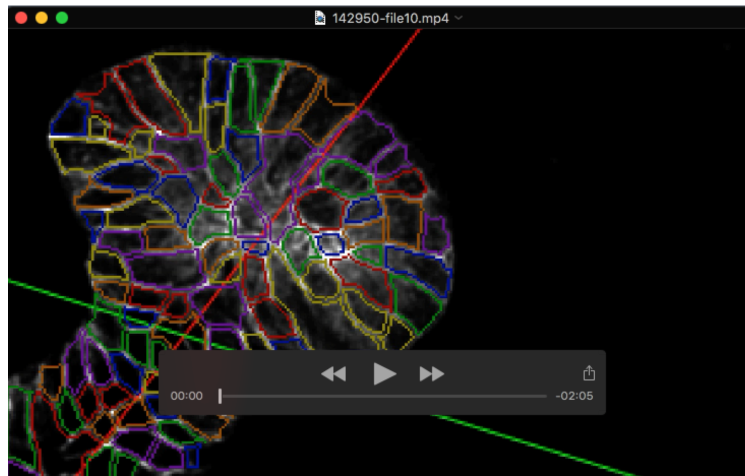


Movie 1. The renal endothelial cells survive and can be observed with high resolution in the FiZD culture. The embryonic kidneys from E11.5 *mTmG*; *Tie1Cre* embryos were dissected and cultured in the FiZD for 6 days. The movie presents the *Tie1Cre* induced GFP expression in the endothelial cells. A stack of 20 z-layers was captured using a 10x/0.45 Zeiss Plan-Apochromat objective, images acquired every 15min. In the movie one bright field focal plane and 5 GFP z-layers are merged. The panel on the right presents a zoomed in to actual resolution close up of a developing kidney where the endothelial cells migrating into vascular cleft of S-shape stage nephrons can be observed. Here one bright field plane and one GFP z-layer are merged. Note that also some blood cells are expressing GFP signal and some of the highly moving cells are likely macrophages (Gustafsson et al., 2001). Voxel size 0.69x0.69x4.13μm.

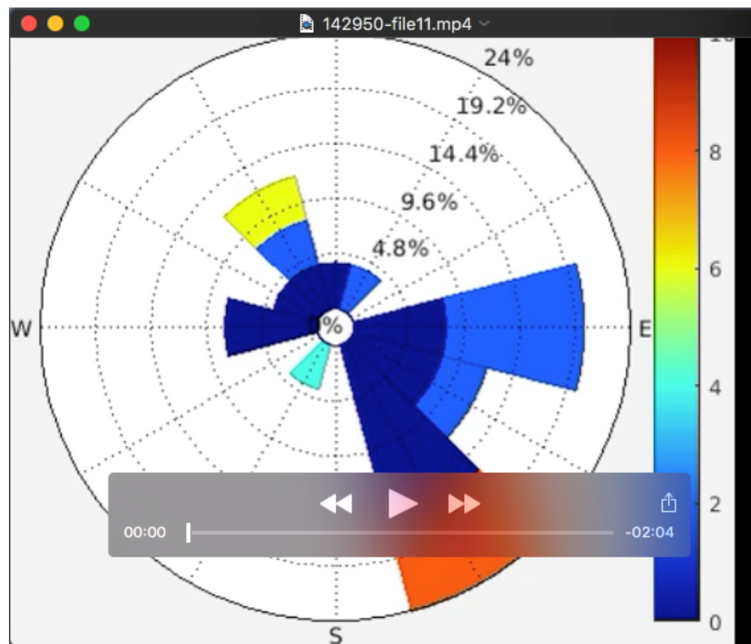


Movie 2. Branching of the UB in the FiZD culture. The embryonic kidneys from E11.5 *mTmG; Hoxb7Cre* embryos were dissected and cultured in the FiZD. Organogenesis was monitored for 5 days. The generated movie illustrates the *Hoxb7Cre* induced GFP expression in the cells of the ureteric bud. A stack of 20 z-layers was captured using a 20x/0.8 M27 Zeiss Plan-Apochromat objective, images were acquired every 5 minutes, Voxel size 0.69x0.69x1.82μm.

Movie 3. The FiZD time-lapse of kidney organoid. The kidneys of the E11.5 *mT/mG;Wn4Cre* embryos were dissected, the metanephric mesenchyme (MM) was separated and subjected to dissociation and reaggregation and BIO mediated induction of nephrogenesis. The nephron precursor cell derived structures are GFP+ due to *Wnt4Cre* mediated activation of the floxed *R26R GFP* reporter. Note that the FiZD culture set up enables tracking of the nephrogenesis process at the single cell resolution in the time-lapse set up. The nephrogenesis was monitored for ten days. The period of time of first 4 days is represented. 10 z-layers were captured; images were acquired every 15 minutes 20x/0.8 M27 Zeiss Plan-Apochromat objective, voxel size 0.69x0.69x1.82µm.

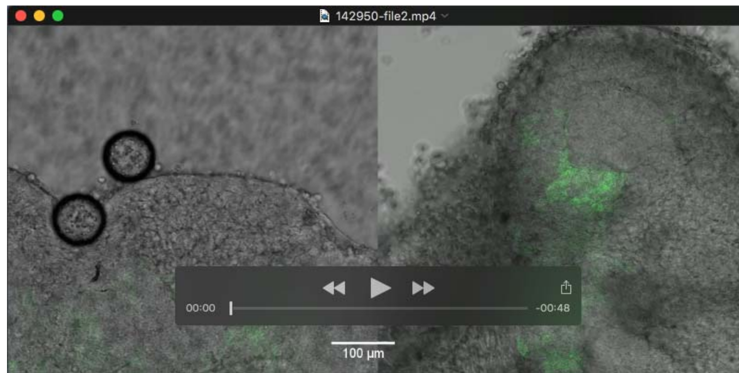


Movie 4. Computer-assisted cell segmentation and tracking for the FiZD derived image data. The quality of the FiZD captured images is good enough for computer assisted analysis of the renal cell behaviour such cell movement during morphogenesis. The movie shows the movement of kidney cells within the ureteric bud branch tip. Green line marks the separation between kidney stem and branch tip. Red line splits the ureteric bud branch tip in the middle to enable the analysis of movement of cells in both halves of the branch tip. Individual cell boundaries and their tracks are also shown.

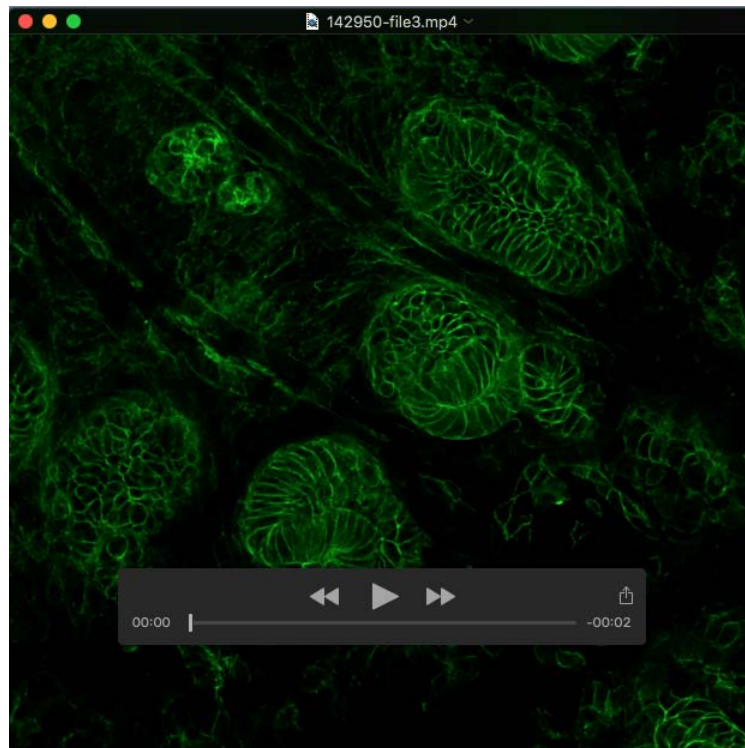


Movie 5. Computer assisted analysis of renal cell migration dynamics can be achieved with the FiZD. Subjection of the time-lapse image stack from the FiZD to computer aided image analysis enabled quantitation of the cell migration dynamics as depicted with the Wind rose plot. The Wind rose plot shows the distribution of magnitude and direction of the cell movements in right half of ureteric bud branch tip for each time point. Length of spokes indicates the proportion of cells moving in a particular direction and thickness of colour bands within a spoke indicates the distribution of speed for these cells.

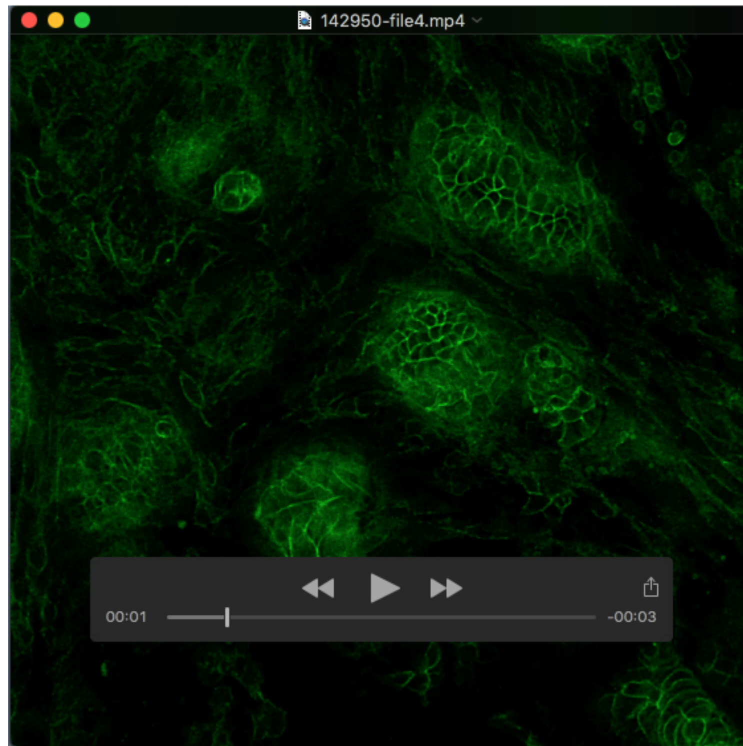
Movie 6. Comparison of low-volume and the FiZD culture systems using intact *ex vivo* embryonic kidney samples. Intact E12.5 kidneys of *Wn4Cre; mT/mG* embryos were placed in both culture systems and grown in time-lapse for 9 days. Left: FiZD, Middle and right: Low-volume method. Note “shrinking” of the kidney cultures in low-volume set up as a result of changing of culture medium (time points: 14:00, 38:00, 59:00, 77:00, 100:00 and 123:00). Note also moving of the sample at right panel out of the field of view. Two beads used as spacers can be seen on left panel. Images were taken every 15 minutes. One out of 20 Z-slices and period of time of 6 days are represented. A 10x/0.45 Zeiss Plan-Apochromat objective was used. Voxel size 0.69x0.69x4.13µm.



Movie 7. Close up from Movie 6 presenting the developing nephrons in both culture systems. Development of few nephrons is shown zoomed in to actual resolution. Left: FiZD and right: low-volume method. All other settings are as in Movie 6.



Movie 8. High resolution short time-lapse of developing nephrons. Intact E12.5 kidneys of *mT/mG; Wn4Cre* embryos were set up in FiZD culture. Images were taken every 5 minutes for 20 time points. A stack of 53 Z-layers was acquired and a single optical plane is represented. A 25x/0.8 Zeiss LCI Plan-Neofluar water immersion objective was used. Voxel size 0.17x0.17x1.13µm.



Movie 9. Scan through all the z-layers during single time point in the same time-lapse experiment as represented in Movie 8. The developing nephron was imaged in time-lapse experiment (see Movie 8). Complete Z-stack of all 53 Z-slices is shown. Other settings are the same as in Movie 8

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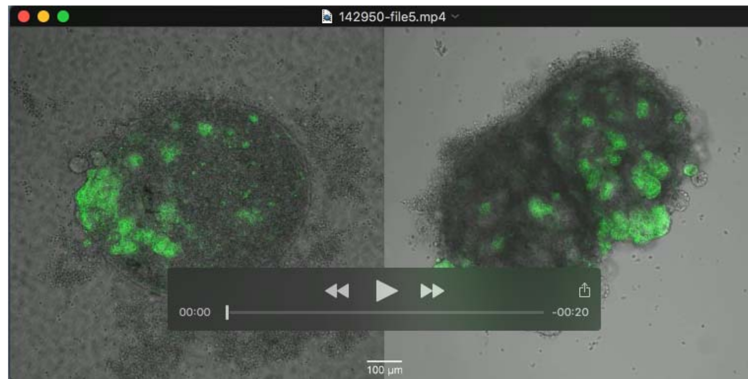
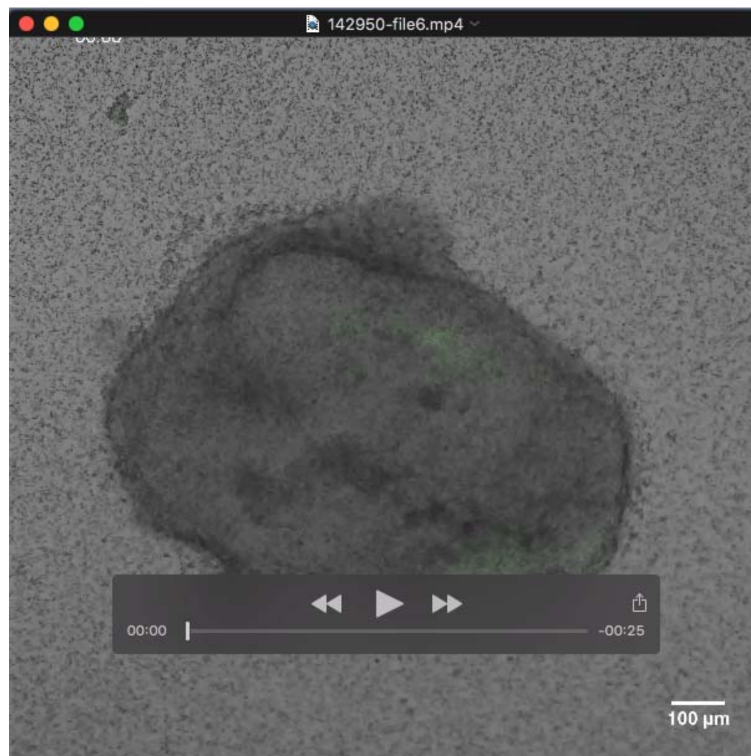


Movie 10. Comparison of silicon chamber and FiZD systems with an embryonic kidney organoid. Kidney organoids prepared from E13.5 frozen embryonic kidneys were placed in both culture systems and grown in time-lapse experiment for 3 days. Images were taken every 15 minutes. A stack of 20 z-layers was captured using a 10x/0.45 Zeiss Plan-Apochromat objective, voxel size 0.69x0.69x4.13µm.



Movie 11. *mTmG;Wnt4Cre* embryonic kidneys cultured on top of Trowell insert. E12.5 kidneys cultured for 3 days on top of the Trowell insert. A stack of 20 z-layers was captured using a 10x/0.45 Zeiss Plan-Apochromat objective, images acquired every 15min. Voxel size 0.69x0.69x4.13µm.

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Supplementary figures

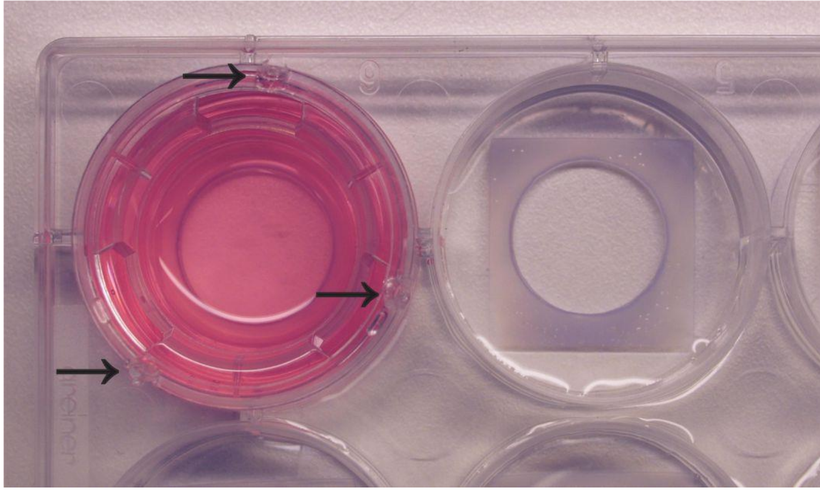

Fig. S1. Preparation of the plate for the FiZD culture.

FiZD is prepared in 6-well CellStar plates (Greiner bio-one) using Transwell inserts (24mm Transwell® with a 0.4μm-pore polyester membrane). A round hole (20 mm diameter) was drilled at the bottom of each well and glass coverslips (24x24 mm) were glued at the upper site of the bottom with using either dental wax or Histoacryl® glue (Braun Ref 1050052). The cover slips need to be cleaned with the protocol presented further in the Supplemental data. This promotes sticking of the organ rudiments to the cover slip glass. Prior to setting up the FiZD culture the processed plates were rinsed with ethanol, distilled water and then dried in a UV hood. Transwell insert is shown placed in the well 1 and the points on the rim where the insert is melted and fixed to the plate are shown with arrows.

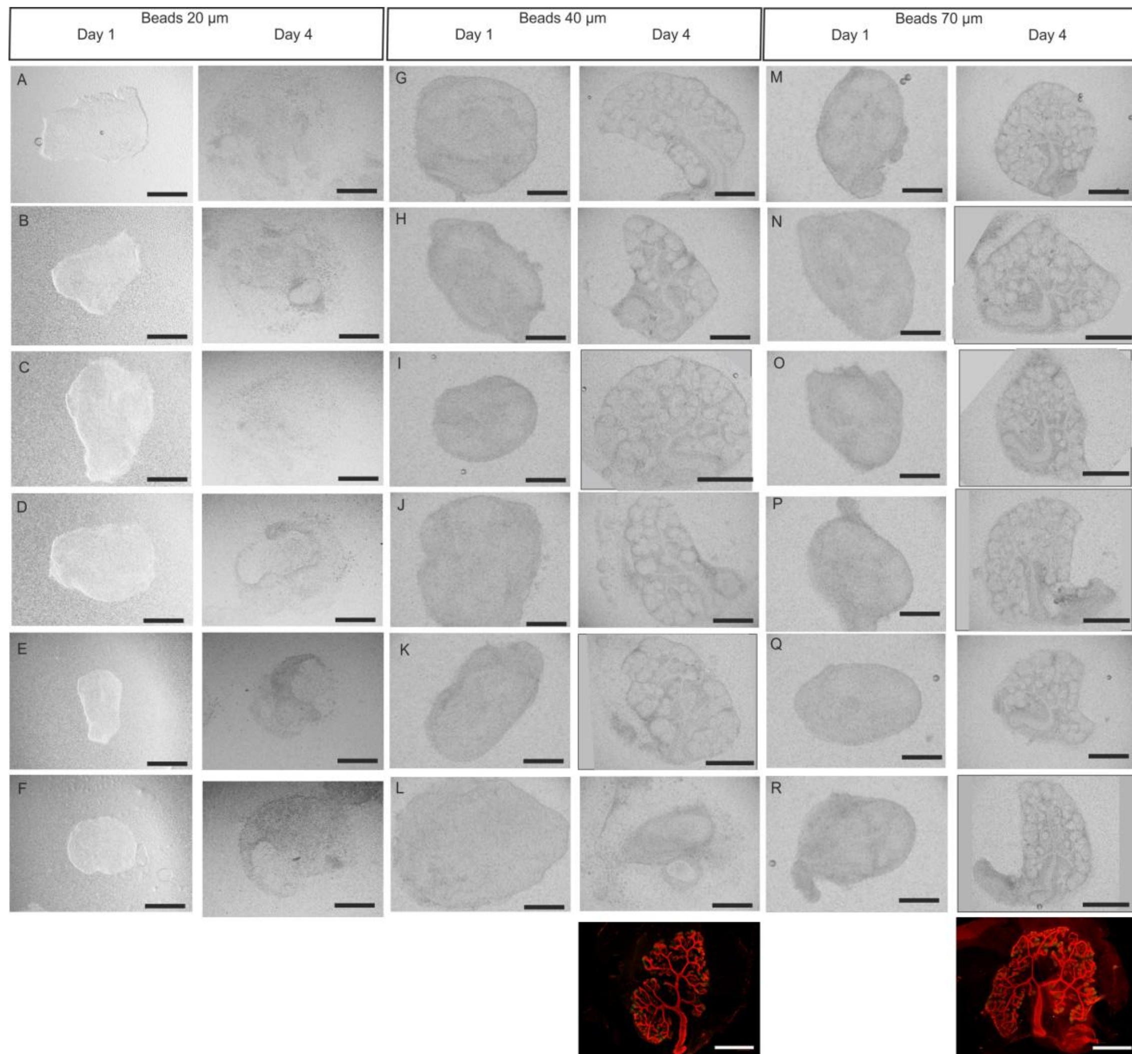

Fig. S2. Comparison of the effect of spacer beads size on the culture. Embryonic kidneys were cultured in planar organ culture with different spacer bead sizes 20, 40, and 70 µm. If the 20 µm beads are used (A-F) the development is ceasing after second day. In the 40 (G-L) and 70 (M-R) µm cases the kidney development is able to proceed, but when using the 40 µm beads there is more variability (L).

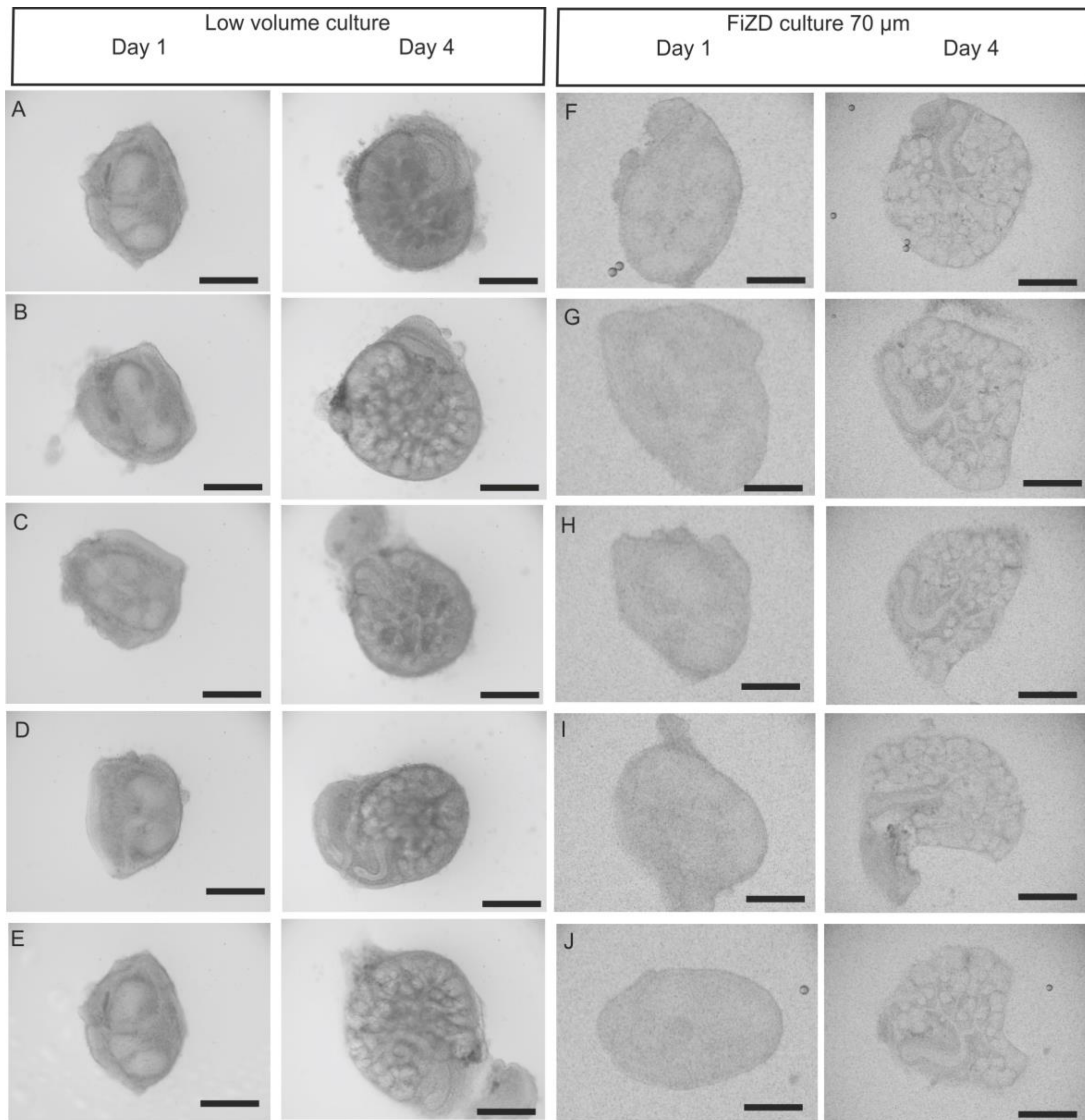

Fig. S3. Comparison of the Low-volume and FiZD methods. Embryonic kidneys were cultured in both Low-volume culture (A-E) and FiZD (F-J) for 4 days. The images show that the development proceeds well in both of the cases. In the FiZD culture the internal morphology of the developing kidneys is more clearly visible.

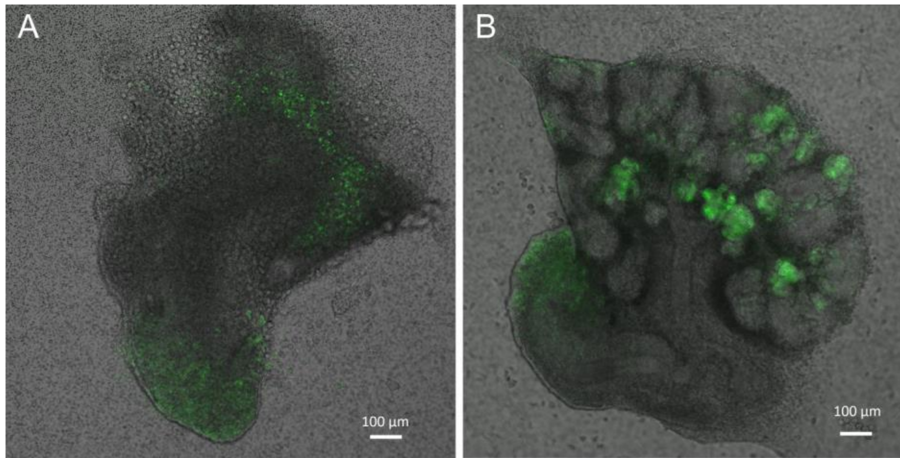

Fig. S4. The effect of increased laser power on the viability of the embryonic kidneys in the Movie 11. Embryonic kidneys were placed on top of a Transwell insert and one of them (A) was imaged for 3 days. The laser power had to be doubled from the FiZD culture settings because the kidney is further away from the objective and located on top of the filter. The development of the kidney was seriously affected when compared to the not imaged (B). A stack of 20 z-layers was captured using a 10x/0.45 Zeiss Plan-Apochromat objective, images acquired every 15min.

Supplementary methods

Segmentation of the image data

The middle slices of the confocal data stacks were used for UB cell tracking. The centroid of the segmented cells in the first frame was used to initialize cell tracks. Hungarian algorithm was used to associate cell tracks in a frame with detections in the next frame. Cell detections which remain nonassociated were used to initialize new tracks. Once all frames had been processed, errors in cell tracking due to missing detections were corrected by associating cell tracks ending at a frame with other cell tracks beginning in the next 3 frames using Hungarian algorithm. The data obtained from the time-lapse cultures is in 3D but the analysis was performed on the 2D slices of the image stacks. The lower resolution in axial direction compared to resolution within a slice, and the blurring

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in axial direction due to chromatic and spherical aberrations made the analysis of data in 3D more challenging.

Wind Rose Plot:

1) The speed and direction of movement for all cells in a frame are computed.

2) Cells are placed into 12 bins (directions) according to their movement direction. 3) For each of the 12 directions, a spoke is drawn. The length of this spoke indicates the proportion of cells moving in the direction covered by the arc of that spoke (in these Wind rose plots, the size of all arcs is 30 degrees, only their direction differs). The circles (circle labels [3.75, 7.5, 11.25, and 15] are shown in the Wind rose plot) can be used to quickly estimate what proportion of cells is moving in a particular direction. 4) All cells within each spoke are placed into 8 bins according to their speed. These bins are shown with different colours (dark blue means cells are moving very slowly with a speed between 0 and 0.2 µm/min and dark brown means cells are moving with speed greater than 1.4 µm/min, the speed for other colours can be checked from the scale on the right side).

If a spoke has only 1 colour e.g. dark blue, it means that all cells moving in that direction have speed less than 0.2 µm/min. Cells with the lowest speed are drawn first, i.e. the dark blue colour band is drawn first and is closest to circle centre than other colour bands and dark brown colour band is drawn last and is furthest from circle centre. Wind rose plots also show that most cells are moving very slowly (thickness of dark blue colour bands is much more than thickness of other colour bands).

Preparation of the cover slips for FiZD culture

1. Heat cover slips in a loosely covered glass beaker in 1M HCl at 50-60°C for 4-16h.
2. Cool to room temperature
3. Rinse out with 1M HCl with ddH_2O

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4. Fill the container with ddH_2O and sonication in water bath for 30 minutes, repeat
5. Fill container with 50% EtOH and 50% ddH_2O and sonicate in water bath for 30 minutes
6. Fill a container with 70% EtOH and 30% ddH_2O and sonicate in water a bath for 30 minutes
7. Fill container with 95% EtOH and 5% ddH_2O and sonicate in water bath for 30 minutes
8. Fill a container with 95% EtOH .
9. Transfer the cover slips into a box with Whatman filter and keep for autoclaving.

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