Figure 1. Sample preparation for imaging IFT in mammalian primary cilia.

(A) The transwell cup is placed upside down in a humid sterile chamber. (B) Put 50 µL of the cell suspension (1×10^4 cells) on the membrane of the inverted transwell cup. (C) Culture cells for 3 hours at 37°C in 5% CO_2. (D) The transwell cup is transferred to 24-well plate and media add into the transwell cup and the 24-well. (E) Culture cells until 90% confluency. (F) Put the transwell cup on a 35-mm glass bottom dish for imaging. We call it transwell imaging chamber.

Figure 2. Image of cilia using transwell imaging chamber.

A representative image of mIMCD-3 cells expressing IFT88-EGFP on the transwell imaging chamber using a TIRF microscope. Most cilia are visible in a single plane. Bar: 5 µm.

Figure 3. Kymograph analysis for measuring IFT velocity

(A) An image of IFT-88EGFP in the primary cilium of mIMCD-3 cells from Movie 1. (B) Kymographs of IFT-88EGFP moving in the cilium of mIMCD-3 cells. The kymographs was assembled from Movie 1. Bars: 5 s (horizontal), 5 µm (vertical). (A' and B') Draw lines along with the cilium or trajectories of IFT trains for making a kymograph and measuring the angle of the trajectory, respectively. (C) The IFT velocity is given from the angle of trajectory of IFT train.

Movie 1. Fluorescent video microscopy of IFT88-EGFP in a cilium of mIMCD-3 cells.

Images were collected at 10 fps for a total duration of 40 s. Playback is set at 10 fps (real-time speed). IFT88-EGFP moves bi-directionally along the cilium.