

“Intravital imaging and photomanipulation of tumor invasion and intravasation microenvironments”

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We are interested in comparing tumor behavior inside different tumor microenvironments *in vivo*. In the past, we discovered invasion and intravasation microenvironments by intravital imaging of tumor cells at high resolution at time scale of hours [1, 2]. However, quantitative measurements in specific populations of tumor cells are only possible via monitoring these microenvironments on the time scale extended to days. To acquire images at day scale and throughout multiple imaging sessions, we have recently developed a Mammary Imaging Window (MIW, [3]) that is inserted on top of the palpable tumor (Figure 1a, b). This implant allows us to use both orthotopic tumor xenografts and transgenic animals growing breast tumors. The MIW consists of a plastic base and a glass coverslip attached on the top. To properly position the MIW on top of the microscope objective and immobilize the animal, we place the animal inside the stereotactic imaging box (Figure 1c). A flow of anesthesia enters the box through the inlet, and exits towards the vacuum due to the negative pressure, passing through a carbon filter which removes isoflurane from the mix. Tumors with MIW implant do not show significant difference in rate of tumor growth, macrophage density, angiogenesis or necrosis compared to tumors without the implant [3].

Over days, tissue topology of the tumor changes due to angiogenesis and cell proliferation and migration, which complicates microenvironment recognition over several imaging sessions. To solve this we use a photoswitchable fluorescent protein Dendra2 [4], stably expressed in breast cancer lines MTLn3 and MDA-MB-231, to enable direct photomarking of the cells of interest. Dendra2 resembles GFP in its spectrum prior to photoswitching, but exposure to blue light can induce an irreversible red shift, creating an RFP-like protein and increasing red fluorescence *in vivo* up to 250 fold.

Regions of interest of desired size (one to thousands of cells) and position (relative to blood vessels or immune cells) can be selectively photoswitched and visualized through the MIW (Figure 2). As cells in the tumor migrate and invade, the distribution of these (red) cells relative to blood vessels and other cells changes, which can be quantified as change in cell position or in number of red cells over time. Our experiments so far demonstrate that cell behavior is determined by the surrounding microenvironment, and that the vascular microenvironment promotes invasion and intravasation of tumor cells [2, 3]. We are currently working on determining spatio-temporal dimensions of intravasation microenvironments by using an artificial blood vessel [5].

The combination of photoswitchable proteins with the MIW allows for a quantitative long-term analysis of a distinct group of cells photomarked in the primary tumor, and tracked over time without long term anesthesia [6].

References:

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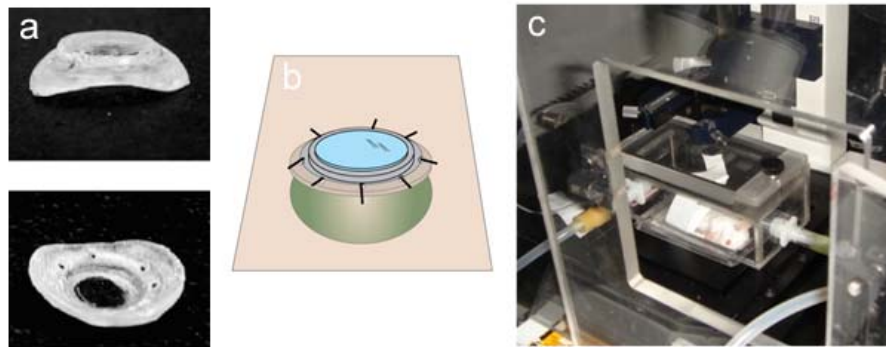


FIG.1 MIW implantation and use. (a) The side and bottom views of MIW. MIW consists of a plastic mount with eight evenly distributed holes to facilitate suturing into the skin, and a glass coverslip attached on the top which assures the

optimal working distance for high resolution imaging; (b) The view of the MIW as it would appear sutured into the skin (beige) on top of a growing tumor; (c) The view of the mouse with MIW implant inside the imaging box. Prior to imaging, the animal is placed inside the box, MIW is immobilized between two sliding doors on the bottom of the box, the box is connected to the anesthesia machine (right) and to the vacuum (front left) and placed inside the environmental chamber, built around the microscope set up.

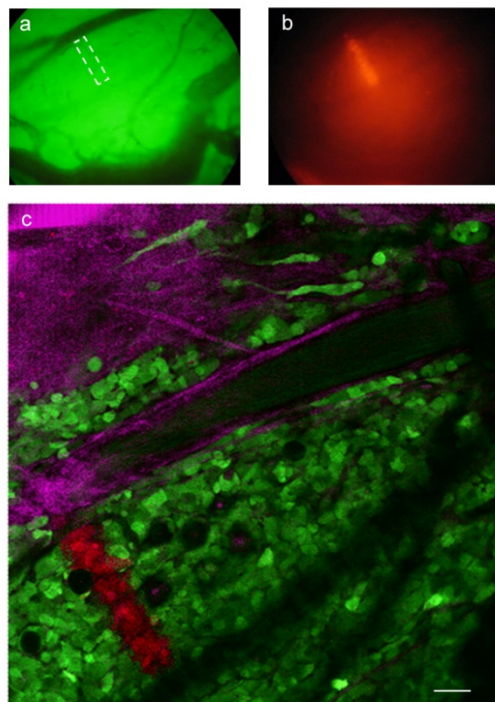


FIG. 2. Intravital imaging and photomanipulation of tumor intravasation microenvironment (a) ROI to be photomarked is chosen using 10x ocular view through the MIW in the green channel; ROI is perpendicular to a flowing blood vessel (white outline); (b) Same field viewed in red channel after the photomarking; (c) High-resolution image of the tumor intravasation microenvironment: Dendra2-MDA-MB-231 cells (green), ECM (purple), blood vessels (black) and photomarked region (red).