The main cause of cancer mortality is not the primary tumor, but metastasis: the spreading and colonization of cancer cells at a distant site. The major events in cancer metastasis involve detachment of cells from the tumor (invasion), intrusion into vessels (intravasation), circulation, exit from the vessel, and secondary site colonization. Metastatic processes involve structural and functional transformations to the invading cell, and also the tumor microenvironment is found to be an active member of the complex cellular ecosystem shaping cancer progression.

There is a strong need of the cancer research community to gain more insight into metastatic processes in vivo. Whereas fluorescence microscopy offers intravital imaging of cancer cells in tissue, it suffers from a limited resolution and is restricted to imaging of fluorescent probes. Electron microscopy, on the other hand, reveals the complete architecture of the region of interest (ROI) at the ultrastructural level. Combining these two imaging modalities results in a tool that correlates the dynamic and functional recordings of tumorigenic events in vivo to the sample’s most-detailed ultrastructure.

We have recently developed a correlative microscopy workflow that complements the advantages of intravital two-photon excitation microscopy (2PEM) of murine tumor xenografts, with volume electron microscopy (EM). Fluorescently labeled cancer cells are injected subcutaneously into a mouse ear and imaged using 2PEM. Using near-infrared branding, the position of areas of interest within the sample is labeled at the skin level, allowing for their full preservation. Concerted usage of these artificial brandings and anatomical landmarks enables targeting and approaching the cells of interest while serial sectioning through the specimen. Full volume correlation is then performed between the 2PEM and EM datasets. Upon retrieval of the cancer cells, their structure and microenvironment could be revealed in 3D at high resolution through electron tomography. Our approach correlates intravital microscopy to 3D electron microscopy, uniquely demonstrating in vivo formation of invasive protrusions in cancer cells and enabling visualization of cell-matrix contacts. This study therefore provides unique and unprecedented insights into tumorigenic processes, which could benefit to the cancer research community.

Acknowledgement: We thank M. Koch and P. Kessler, IGBMC imaging platform, D. Hentsch, S. Taubert and F. Egilmez and the animal facility at INSERM U1109. We are greatful to W. Hagen, R. Mellwig and the Electron Microscopy Core Facility of the EMBL Heidelberg.
Fig. 1: (A,B) Fluorescent cancer cells were xenotransplanted into mouse ear skin and imaged with intravital 2PEM. (C) An invasive cancer cells was retrieved with electron microscopy and the structural organization of invasive protrusions were revealed with electron tomography (D). Scale bars are 100 µm in B, 5 µm in C and 500 nm in D.