Crossing Borders: Imaging Single Tumor Cells at the Blood-Brain-Barrier Using Multimodal Correlative Microscopy

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Metastasis, the spreading of tumor cells (TCs), is the main cause of mortality in cancer patients. Following release from the primary tumor, the tumor cells enter the bloodstream and exit the bloodvessel (extravasation) at a distant site. A small subset of these cells proliferates and grows out into metastases [1]. Extravasation is a rare and transient event, making it difficult to study this process *in vivo*. Combining intravital microscopy (IVM) to 3D Electron Microscopy (3DEM) enables to correlate functional and dynamic in vivo imaging to high-resolution of the tumor cells and their microenvironment. However, keeping track of single tumor cells when moving from IVM to EM imaging is highly challenging in complex tissue samples [3].

Here, I will demonstrate Multimodal Correlative Microscopy, an imaging workflow that combines x-ray microscopic computer tomography (microCT) [4] to correlate IVM to EM [5,6]. Using this approach, we studied extravasation of TCs in mice brain *in vivo*. Here, IVM was performed of intracardially-injected fluorescent tumor cells, arrested in the brain vasculature of a living mouse. After perfusion fixation, brain biopsies containing the tumor cells were processed for EM. Next, microCT scans were obtained from the resin-embedded sample. The microCT 3D scans were correlated to the FM volumes, based on structural features of the sample visible in both datasets. 3D registration of both datasets allowed to determine the position of the tumor cell inside the resin block, allowing to accurately approach this area and studying it at high resolution with EM [5,6]. Significantly speeding up the correlative workflow, this method allows performing high-resolution imaging of a statistically relevant number of samples.

References:
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