Application note

Deeper insight into cancer metastasis with multimodal correlative microscopy

Metastasis is a critical landmark in cancer evolution, characterized by tumor cells traveling through blood vessels to reach and proliferate at distant secondary sites.[1,2] This is known to occur through a series of steps, but their intricacies have not been fully captured. For instance, extravasation, in which circulating tumor cells leave the vasculature to invade a target tissue, is still poorly understood,[1,3] and there are some steps of extravasation for which there is no direct evidence. Full understanding of these events requires investigation at the cellular scale. Intravital microscopy (IVM) has the potential to capture these dynamic metastatic events, but it does not have sufficient resolution to reveal the interaction of tumor cells with the surrounding tissue, or subcellular events.[1] This can be improved by correlating functional IVM with 3-dimensional electron microscopy (3DEM), creating a powerful technique for the observation of pathophysiological processes *in vivo* at nanometer resolution. However, this approach is hindered by its low throughput, which cannot reasonably produce the quantitative sampling required for translational research. To address this drawback, researchers from the European Molecular Biology Laboratory in Heidelberg, Germany tested a novel approach using micro-computed tomography (microCT) to precisely correlate IVM with 3DEM.[1]

Observing ultrastructural details

Tumor cell extravasation was observed using a mouse model in which GFP-labeled HER2-positive breast cancer cells were intracardially injected.[1] An implated cranial window was used to study these cells within the brain cortex with IVM. Tumor cells were arrested within the vessels prior to extravasation and tracked over several days. The IVM data allowed the position of the tumor cells to be recorded relative to the local vasculature. Biopsies from the region of interest (ROI) were processed for EM analysis and embedded in resin. These samples were then imaged with microCT, and rendered in 3D to display the resin block surface, the embedded tissue, and the included vascular network. Docking the IVM model onto the microCT dataset allowed the position of the biopsied tumor cell to be predicted within the resin block (e.g., the green channel in the IVM data). This information allowed for accurate block trimming within just a few micrometers of the cell – close enough for scanning with focused ion beam scanning electron microscopy (FIB-SEM). This could be used to view the tumor cell's ultrastructure as well as the organization of the capillary and surrounding tissue.

Tumor behavior

This approach was tested further using invasive tumor xenografts in mouse ears, revealing important subcellular features of the arrested tumor cells. The IVM analysis showed actin-rich protrusions at the margins of the tumor mass, and the invasive cells appeared to have distinct morphological features. The microCT volume, meanwhile, revealed critical anatomical features like blood vessels and nerve fibers. The 3D IVM data was combined with the microCT volume, providing the distance of the invasive protrusions from the surface of the

resin block. This allowed the block to be trimmed down to the ROI for EM analysis. The subsequent 3DEM of the subcellular regions allowed ultrastructural features like cytoskeletal fibers and intracellular organelles to be visualized, indicating that the nucleus can deform – an ability that may help its migration through interstitial tissue. Collectively, these findings illustrate the potential of the workflow to unravel metastatic events at the nanoscale.

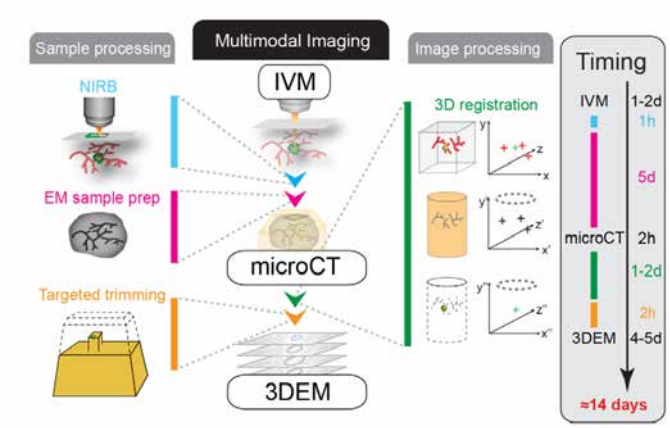

Figure 1. The multimodal correlative microscopy workflow. Once an event of interest is captured with IVM, the ROI is marked at the tissue surface with near-infrared branding (NIRB). A biopsy containing the ROI is then dissected and processed for EM analysis. First, the resin-embedded sample is imaged with microCT and the imaged volume, found with IVM, is registered to the microCT volume by matching pairs of landmarks in Amira Software. This allows the position of the ROI within the resin block to be precisely determined. The resin block is then accurately trimmed to expose the tumor cell for final 3DEM analysis.[1]

Understanding complex processes

Linking functional IVM analysis of tumor cells and their microenvironment to high-resolution EM allows for a greater understanding of the processes that encourage cancer to spread. Although the approach has higher throughput than existing methods that combine IVM with 3DEM (providing results in around two weeks rather than three months) it would still be time-consuming to conduct enough experiments to reveal all of the structural changes a tumor cell undergoes during extravasation. However, the researchers suggest that this is still a worthwhile investment, given the high resolution and success rate of the approach.

"In conclusion, we have established a versatile and precise multimodal imaging approach that allows efficient correlation of in vivo imaging with volume electron microscopy," the team writes in the *Journal of Cell Science*.

They add: "The enhanced throughput of the method will allow routine use in translational research on animal models and holds great potential for understanding multiple biological processes at the ultrastructural level."

Thermo Scientific technology

In this study, the team used Thermo Scientific™ Amira™ Software to register IVM volumes to their corresponding microCT datasets, which allowed for precise determination of tumor cell positions in resin blocks. Amira Software offers a powerful platform for 2D–5D image visualization, analysis, and processing to assist drug discovery pathways across a wide range of imaging modalities including CT, magnetic resonance (MRI), and 3D microscopy.

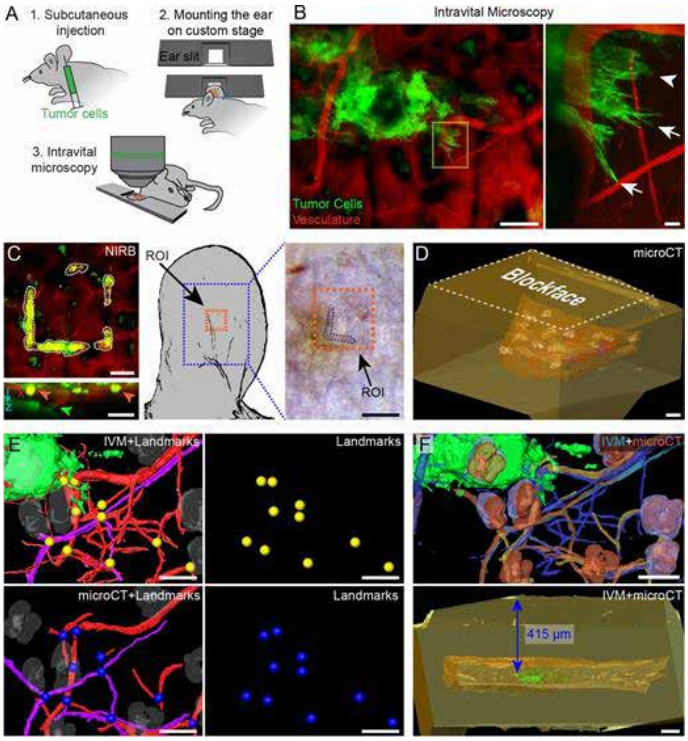

Figure 2. Tumor xenograft correlative microscopy workflow. A) Tumor cells are injected into mouse ears and allowed to grow for 2 weeks, at which point the mouse is sedated and the ear is positioned for imaging. B) IVM reveals both the vasculature and tumor cells, along with tumor cell protrusions. C) The tumor is then marked for further analysis with NIRB. D) After the tumor is embedded in resin, it is further analyzed with microCT and IVM. E) The resulting IVM and microCT volumes are superimposed using registered landmarks (F), providing the precise position of ROIs in the resin block.[1]

References

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3. Miles, FL, *et al. Stepping out of the flow: capillary extravasation in cancer metastasis.* Clin Exp Metastasis 25:305-324 (2008). doi: 10.1007/s10585-007-9098-2

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