Photometrics Customer Profile

In vivo Fluorescence Imaging of Microvasculature

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>
> **– Waleed Gaber,** associate professor at Baylor's College of Medicine and co-director of the small animal imaging facility at Texas Children's Hospital

BACKGROUND

Radiation therapy is one of the most successful treatments for malignant tumors. In addition to causing tumor death it also weakens and collapses the rapidly forming vasculature around the tumor. For tumors in the central nervous system (CNS), radiation-induced vascular weakening and activation of astrocytes can cause acute and long-term damage to normal brain tissue.

M. Waleed Gaber, an associate professor at Baylor's College of Medicine and co-director of the small animal imaging facility at Texas Children's Hospital, is investigating factors that influence the health of vasculature surrounding CNS tumors to optimize efficacy and safety of anti-cancer therapies. His team recently identified that tumor necrosis factor-alpha is linked to acute microvascular damage and astrocyte activation following radiotherapy.

The discovery stemmed from intravital fluorescence microscopy techniques that Gaber's team developed. Their goal was to visualize long-term changes in the health of the same section of cerebral microvasculature in live laboratory mice.

"By testing the same vessel or network for the duration of therapy, we can reduce variability and enable correlative studies of key markers of vascular health. This is not possible using ex-vivo sections," explained Gaber. "One key marker is leukocyte adhesion to vascular epithelium. We visualize this by fluorescently labeling leukocytes with Rhodamine 6B, which stains their mitochondria."

CHALLENGE

The illumination intensity for Gaber to visualize the labeled leukocytes flowing through blood vessels transferred toxic levels of heat. Over the long intervals that he needed to monitor these and other interactions, the energy from the illumination could photobleach fluorophores in the labeled cells and could ultimately interfere with the functionality of those cells.

OVERVIEW

Using Photometrics cameras, M. Waleed Gaber of Baylor's College of Medicine, studies the brain vasculature of live mice to optimize anti-cancer therapies.

FACILITIES

Baylor College of Medicine, Texas Children's Hospital

CAMERAS

Photometrics CoolSNAP™ and Cascade® II cameras for low-light fluorescence microscopy

KEY FEATURES

- *Image dimly fluorescing samples under low-light conditions (high quantum efficiency)*
- *Capture clear images at low and high frame rates (low dark current and read noise)*

SOLUTION

Gaber integrated Photometrics' CoolSNAP™ and Cascade® II cameras for low-light fluorescence microscopy with his custom-engineered stereomicroscope for intravital imaging. For maximum sensitivity and lowest noise, the cameras are thermoelectrically cooled – without the need for bulky circulators or cryogenics – and provided quantum efficiencies upwards of 60% and 90%, respectively.

"By integrating a Photometrics high-quantum efficiency camera, I could visualize the weak fluorescence signals within the vascular bed without phototoxicity risk and perform the experiments I needed," explained Gaber.

Gaber integrated the cameras with his novel hybrid imaging system with guidance from the Photometrics imaging team. "What impressed me the most was their commitment to us," said Gaber. "It was an evolving process to determine our requirements, but knowing that a company is going to invest time in supporting our needs was essential to the success of our work."

RESULTS

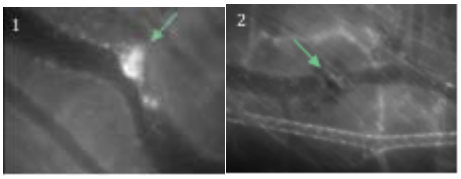
The high quantum efficiencies and low noise levels of the Photometrics cameras enabled high-resolution, low-light, non-cytotoxic in vivo imaging of leukocyte trafficking and interactions over longer time periods at up to 50 to 60 frames per second.

"I attribute the success of several recently published papers to integrating the high quantum efficiency Photometrics CoolSNAP and Cascade II cameras into my fluorescence intravital microscopy system," Gaber said. "By paying careful attention to the technology behind my experiments, I'm able to investigate questions that were difficult to tackle before."

"These cameras deliver the final image that proves our concept, which seals the deal – beyond what ex-vivo sections or our analog-era microscope systems can deliver. If that leukocyte is at all touching that blood vessel wall, I can catch it," Gaber explained.

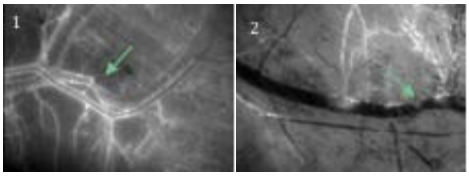
Leukocyte recruitment in blood vessels post-radiation injury

A.



Vessels were injected with Rhodamin-6-G to preferentially stain Leukocytes. Vessels were imaged (at 100X magnification) immediately after (Panel 1) and 24 hrs after (Panel 2) radiation injury. Note the lack of clot in the irradiated animal's micro-vessel (Panel 2).

B.



Images were taken (40X Magnification) 24 hours post injury following injection of FITC-dextran 150-KDa. The control (Panel 1) reached full perfusion within seconds; while irradiated (Panel 2) was badly perfused (both are acquired at three minutes post-dye injection).

C.

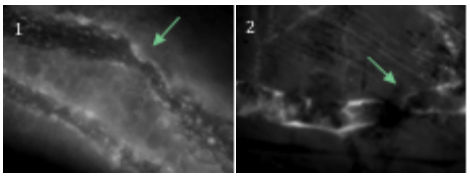


Figure 1C shows vessels immediately after (Panel 1) and 24 hours after (Panel 2) irradiation injury (at 100X magnification) after injection of rhodamine-6G which stains leukocytes. Similar to figure 1A, note the lack of clot in the irradiated animal's micro-vessel (Panel 2). Leukocyte activity was lacking in irradiated (Panel 2) as compared to control (Panel 1).

LOOKING FORWARD

Gaber's team recently introduced fluorescent beads coated with specific leukocyte cell surface receptors to murine models. They imaged the interactions between those receptors and the vascular epithelium. Gaber has been recently funded to study the combined effect of irradiation and wound injury on vascular integrity. His team will soon publish the time scale at which the vasculature expresses these adhesion molecules after radiation – in addition to other insights.

Gaber plans more advanced uses of these cameras, "I see potential for even more complex and sensitive fluorescence experiments, such as FRET, to evaluate specific molecular interactions in my murine CNS cancer models and in whole body radiation exposure."

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