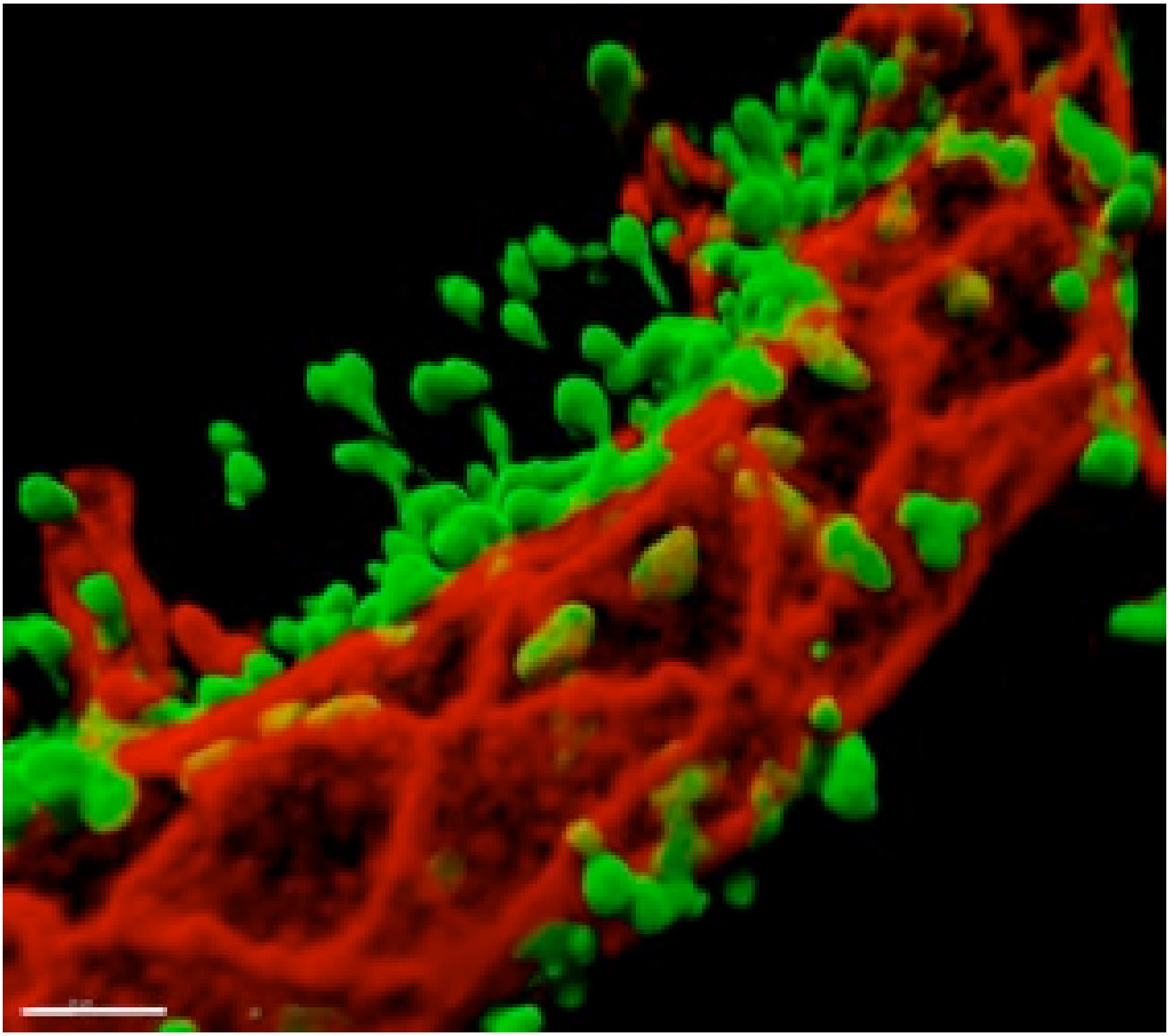
Tracking Leukocyte Migration in 3D

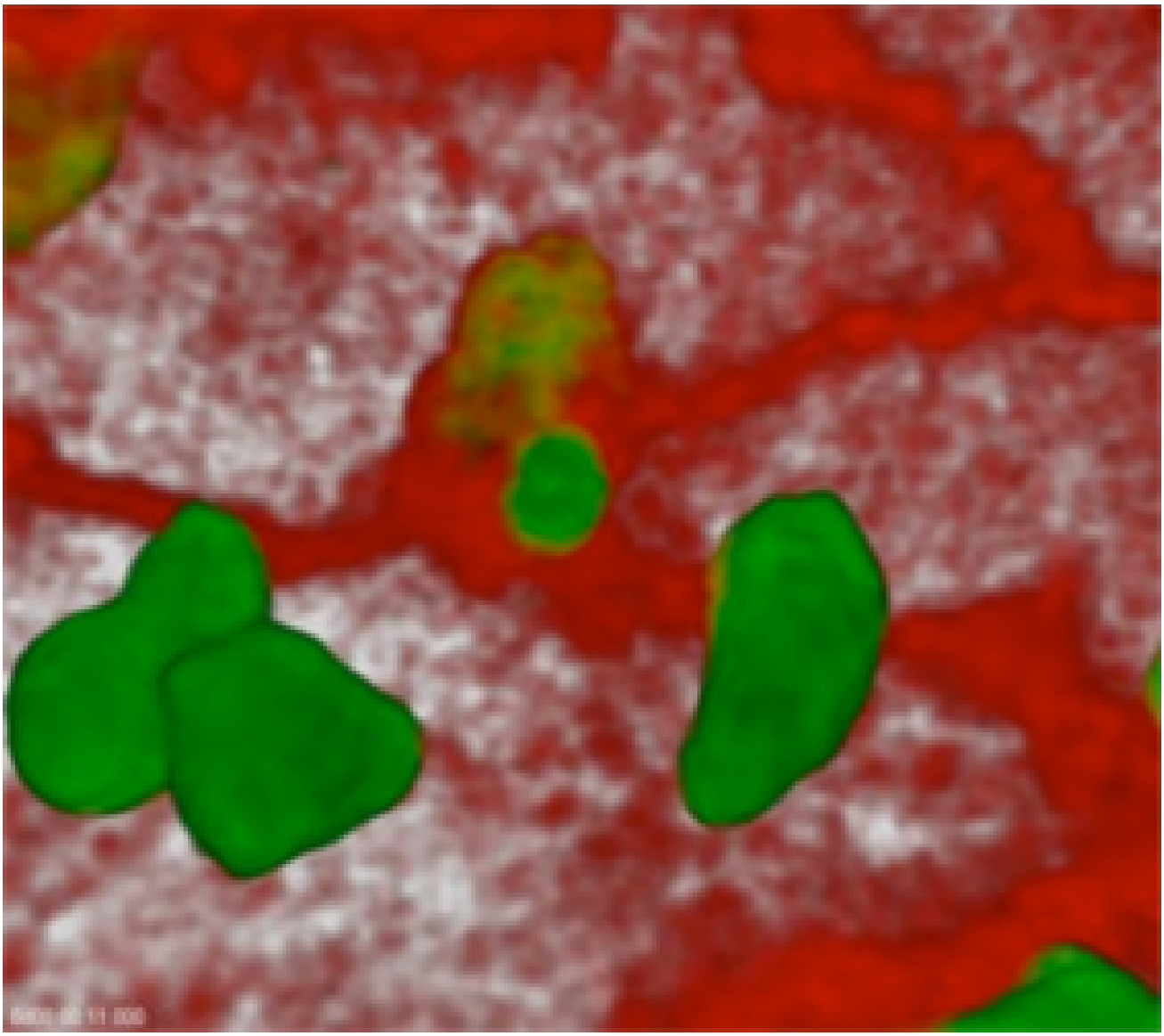
Immunology

Dr. Abigail Woodfin, Queen Mary University of London, and colleagues.

As part of the body's reaction to injury and infection, leukocytes leave the blood and migrate to the affected tissue. This migration also plays a significant role in the development of pathologies where inappropriate inflammation contributes to tissue damage and disease progression. "In vitro studies of the mechanisms governing this process have provided many insights, but the complex structure of the vessel wall, and the contribution of environmental factors such as blood flow and local chemokine production limits the in vivo relevance of these studies," says Dr. Abigail Woodfin from Queen Mary University of London.

Dr. Woodfin was part of a research team that investigated the in vivo mechanisms and dynamics of leukocyte migration through vessel walls by using immunofluorescent labeling and real-time confocal microscopy of inflamed post capillary vessels. The team, which also included Dr. Mathieu-Benoit Voisin and Prof. Sussan Nourshargh from Queen Mary University of London, captured sequential 3-D images of the inflamed vessels and used Imaris software to create dynamic 3-D models so that they could observe and analyze the inflammatory response as it progressed.

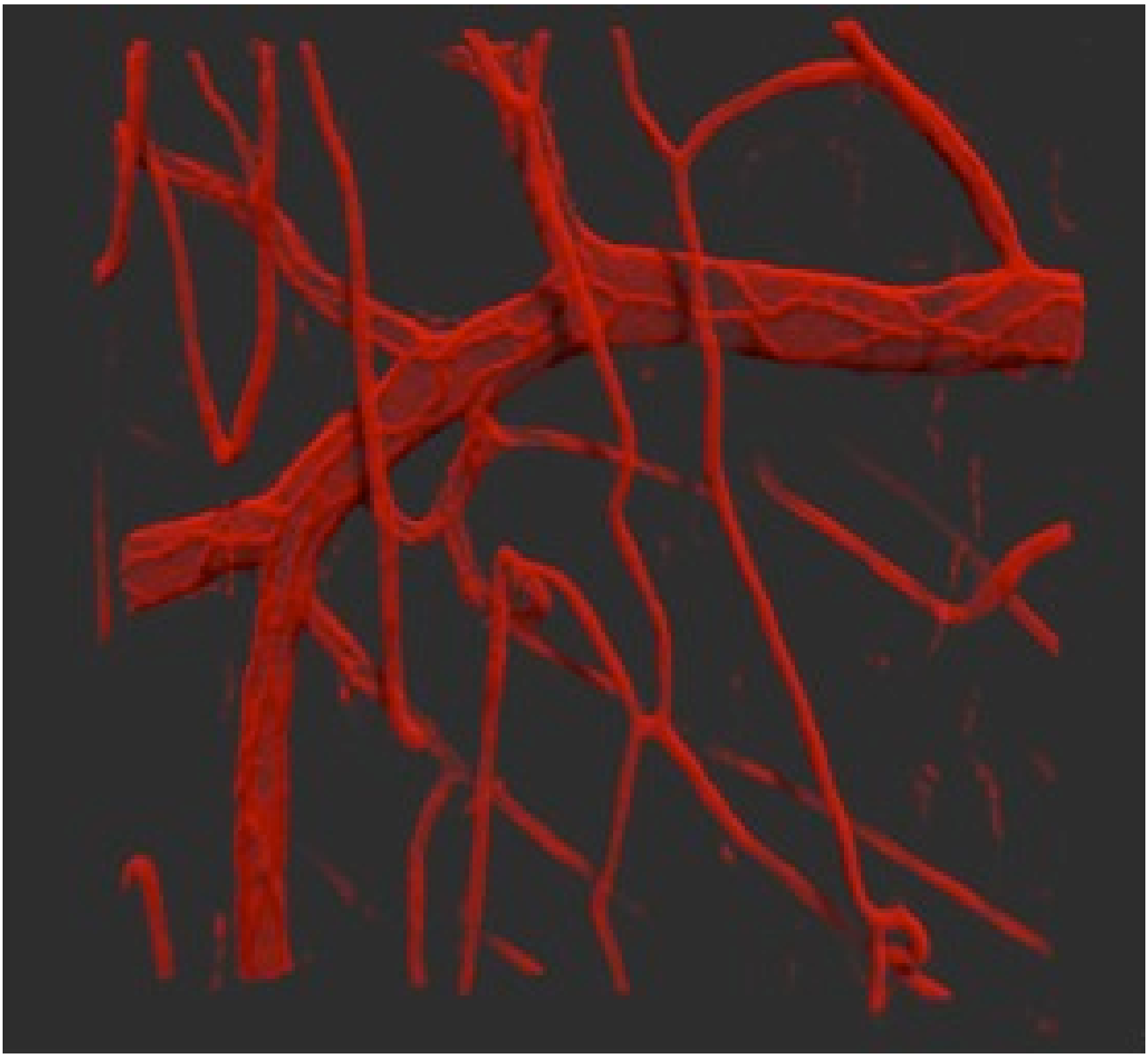


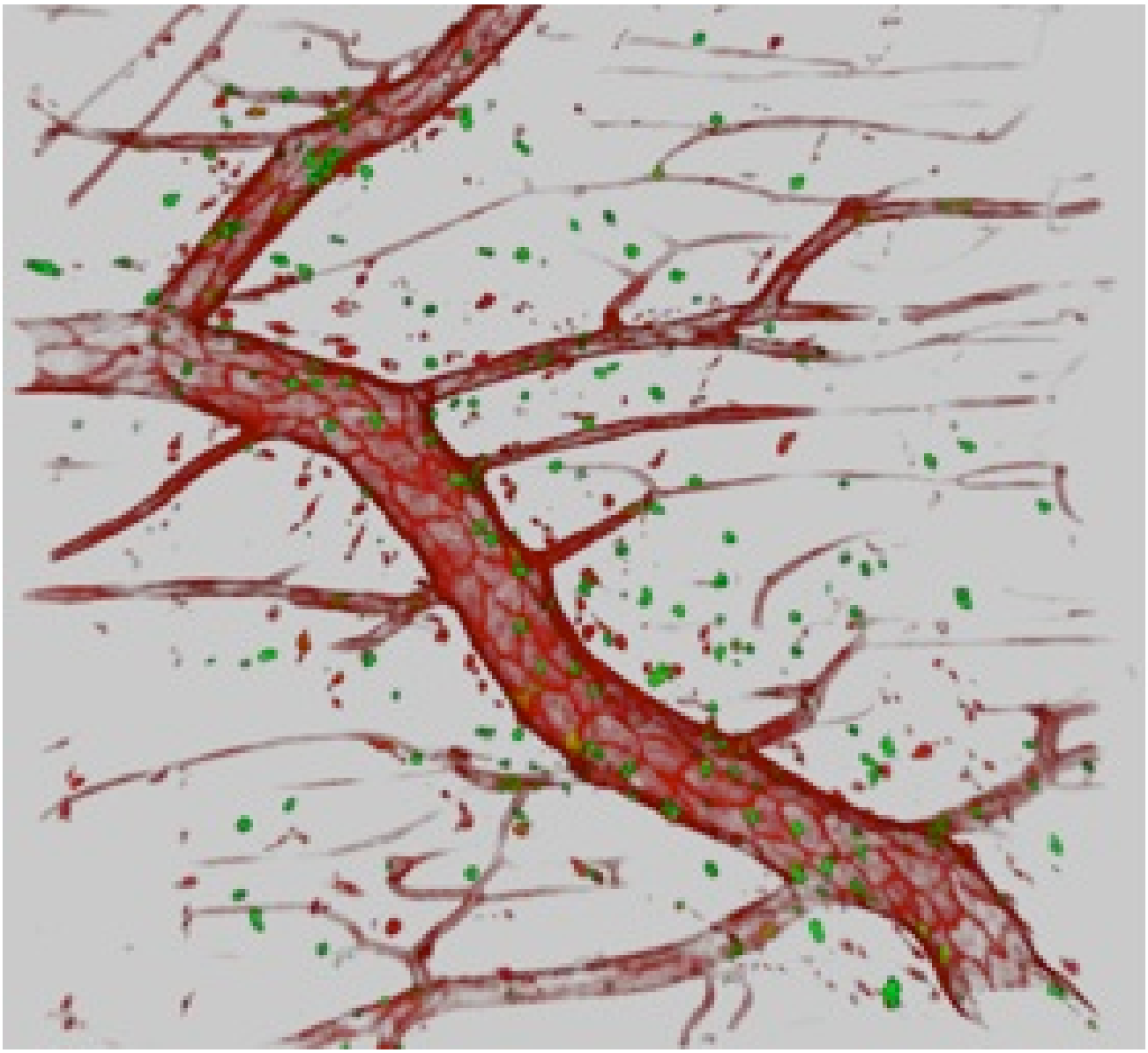


The researchers used the mouse cremaster model, and in all the images the endothelial junctional protein PECAM-1 is immunofluorescently labeled (red). Leukocytes, predominantly neutrophils, express green fluorescent protein (green). Each green cell is approximately 7 µm in diameter. In the left image, an inflamed post-capillary venule is seen from the abluminal side. Leukocytes migrate through the vessel wall and into the surrounding tissues. The right image shows a luminal view of a leukocyte migrating through the junction between three endothelial cells to the sub-endothelial space. Other leukocytes can be seen crawling on the luminal surface of the venule.

“By converting the data into 3-D models Imaris enabled us to track the movement of leukocytes relative to the endothelial cells lining the vessel wall and to analyze the dynamics of leukocyte migration though the vessel wall with a high degree of spatial and temporal resolution,” Dr. Woodfin says. The virtual 3-D object created by Imaris, the most powerful and versatile 3D and 4D image visualization and analysis software solution on the market for researchers in life sciences, could be fully manipulated in terms of rotation position, zoom, and the intensity at which each channel is displayed. This was crucial to the analysis because it allowed the researchers to determine the interaction of objects for many sequential time points. “Traditional maximum intensity projections of a Z-stack do not allow the way in which objects interact with each other to be determined/observed in this way,” Dr. Woodfin says.

In addition, they used the Imaris drift correction function to correct for movement within the living tissues during imaging and were able to produce video files illustrating the 3-D models (see videos here). Their analysis revealed that junctional adhesion molecule C (JAM-C) was a key regulator of the migration of leukocytes through the vessel wall.





These low magnification views show a post capillary venule and capillaries in the mouse cremaster. In the left image PECAM-1 is enriched at the junctions between endothelial cells and expressed at lower levels on the body of the endothelium. In the right image leukocytes can be seen crawling on the luminal surface of the vessel, and migrating through the extravascular tissues.

“Our work has combined improved spatial and temporal resolution at the level of image capture with the presentation of this data as 3-D models, enabling more in-depth analysis of the nature and dynamics of cellular interactions in vivo than has previously been possible,” Dr. Woodfin says.

Research Paper: The junctional adhesion molecule JAM-C regulates polarized transendothelial migration of neutrophils in vivo, Nature Immunology 12, 761–769 (2011) doi:10.1038/ni.2062.