Inflammation is the body’s adaptive response to noxious and potentially harmful stimuli and conditions such as infection or tissue damage (1–3). What was defined by Celsius as ‘rubor, calor, dolor, and tumor’ has now developed into a challenge for researchers in many fields including systems biology, signal transduction, and physiology. Translation of findings from basic science to clinical applications requires understanding of the underlying processes, development, evaluation, and optimisation of lead compounds as well as diagnosis and staging of patients. All of these stages require a broad spectrum of intravital imaging modalities that cover optical and non-optical, invasive and non-invasive, high resolution and deep tissue penetration techniques. In this theme issue of Thrombosis and Haemostasis we have collected five review articles and two original contributions of experts in the field of intravital imaging of inflammatory diseases such as angiogenesis. In addition, each contribution also describes usage of different imaging modalities covering a broad spectrum from subcellular imaging to detection at tissue levels as well as clinical applications. This variety of herein described imaging techniques may challenge the reader to cross-bridge and interpret the illustrated methodology for application in different fields of research, or ultimately, stimulate the use of complementary imaging modalities and labelling agents for multimodal imaging.

Platelet activation is among the earliest events during the inflammatory processes. Upon vascular injury the haemostatic process must rapidly stanch blood loss while avoiding obstruction of flowing blood within the vessel. In recent years, methods for studying coagulation in vivo such as the ferric chloride injury model and the laser injury model have been standardised. Bellido-Martín et al. highlight imaging modalities that have been employed over the last decade to increase our understanding of fibrin formation and platelet activation (4).

Activation of platelets also induces subsequent activation of neutrophils which are of primary importance in regulation of inflammatory transendothelial permeability changes (5). Increases in vascular permeability contribute to containing and resolving the inflammatory cascade as that way complement factors and immunoglobulins may enter the tissue. On the other hand, overzealous oedema formation in e.g. brain injury or acute lung injury may result in fatal outcomes. Kenne and Lindbom review modalities for intravital imaging of inflammatory permeability changes by use of optical (e.g. fluorescence microscopy) as well as of non-optical imaging (e.g. PET and MRI) techniques (6). While the former are primarily employed in experimental setups, the latter are frequently used in clinical settings. The endothelial glycocalyx is one determinant of the endothelial barrier in the microvasculature. Its degradation is known to facilitate leukocyte adhesion and to increase vascular permeability to fluids and proteins. By use of intravital microscopy of the cremaster muscle, Constantinescu et al. show that hyperlipidaemia, a primary risk factor of atherosclerosis, reduces the thickness of endothelial glycocalyx thereby promoting microvascular permeability (7).

Leukocyte emigration is a crucial process in the inflammatory cascade involving a well-defined multistep cascade involving cell adhesion molecules and chemokines that direct the cell to the site of inflammation (3, 8). Megens and colleagues highlight optical imaging methods that have shed light on many details of the recruitment process allowing for refinement of the cascade that was established over 20 years ago (9). In addition, it is becoming obvious that leukocyte emigration functions differently in various vascular beddings. Such differences are evident when comparing the involvement of chemokine receptors or cell adhesion molecules in neutrophil recruitment studied in model systems such as the cremaster model with or solid organs such as the lungs, liver, or large arteries. Differences in shear forces, capillary sizes, endothelial phenotype, local chemokine environment, and tissue-dependent involvement of resident cells offer explanations of tissue-dependent leukocyte recruitment. In this context, it has recently been shown that neutrophils employ different sets of chemokine receptors when recruited to large arteries as compared with microvascular extravasation in the cremaster muscle (10). Once emigrated, neutrophils may trigger the recruitment of monocytes thereby initiating the second wave of inflammation (11, 12). However, live imaging of such interactions was thus far hampered by lack of suitable models to specifically label and track both phagocyte subsets in parallel. In this issue, Gray et al. generate and characterize a novel transgenic zebrafish model having mCherry-labelled macrophages. Crossing with a transgenic zebrafish carrying GFP-tagged neutrophils revealed a model that allowed for simultaneous intravital imaging of recruit-
The arterial recruitment of leukocyte subsets to atherosclerotic vessels is further complicated by the existence of a rather tight network of microvasculature that originates primarily from the adventitia of large arteries, the vasa vasorum. The classical concept of intimal leukocyte infiltration suggests the emigration of inflammatory cells via the lumen of large vessels. Alternatively, vessel wall inflammation may be initiated in the adventitia and work its way into the media and the intima. Such theory is supported by increases in adventitial neovascularisation and adventitial leukocyte accumulation (14, 15). Zagorchev and Mulligan-Kehoe summarise recent advances in imaging technologies allowing for detection of angiogenesis and inflammation in atherosclerosis (16). Specific emphasis is put on advantages of micro PET, micro CT, and MRI in monitoring inflammation and angiogenesis in atherosclerosis. Arterial leukocyte infiltration via either luminal or adventitial route leads to prominent accumulation of macrophages in the vessel wall. These prominently contribute to atherosprogression and vascular aneurysm formation. By modification of chemokines and cytokines, degradation of extracellular matrix components, and regulation of gene expression, cell differentiation and proliferation, proteolytic enzymes serve as key effectors of many macrophage contributions to cardiovascular diseases. Hence, intravital imaging of protease activity could aid in evaluating the inflammatory state of atherosclerosis. Molecular imaging approaches have focused on the most abundant macrophage protease families, MMPs and cysteiny1 cathepsins. These are commonly imaged by targeting labelled small molecules to the proteases active site or by use of substrates of proteases which become detectable upon proteolytic cleavage. Recording of proteolytic activity is detectable by a broad range of imaging modalities including PET, SPECT, MRI and FMT, all of which are used in clinical and experimental settings. In this issue, Quillard et al. present an update of recent advances in the field of imaging protease activity (17).

Innovations of intravital imaging have contributed considerably to our understanding of the complex and multi-faceted processes of inflammation. Not just has this allowed us to refine and reconsider basic concepts of the inflammatory cascade, but this may also lead to better characterisation of disease states and assess the efficacy of therapeutic interventions.

References