Abstract of the Project of Prof. Dr. med. Michael Sixt

Funded by the Peter Hans Hofschneider Foundation the laboratory developed a number of novel experimental setups to investigate cell motility; we mainly employed leukocytes as a model system as these cells are prototypic examples of rapidly moving cells, displaying extraordinary plasticity in locomotion strategies. The approaches we developed range from reductionist *in vitro* assays that allow us to image membrane and cortical dynamics with total internal reflection microscopy, to *ex vivo* (tissue explants) and *in vivo* preparations where cells migrate in response to endogenous signals within the physiological environment.

We used artificial 3D environments like gels, channels and micropatterned substrates to mimic isolated aspects of homeostatic as well as inflammatory tissues. Most of these assays allow pharmacological intervention and all of them are accessible to live cell imaging. The model cell type we mainly focused on were dendritic cells. These are primary cells that can be expanded from bone marrow stem cells or embryonic stem cells and we established genetic manipulation protocols for gene knockdown and transgene expression. Guided by only one chemokine receptor (CCR7, that binds the chemokines CCL19 and CCL21), dendritic cells naturally go through a stereotypic migratory program that leads them through various types of tissues: they i) pass through the epidermal basement membrane ii) traverse the dermal interstitium iii) enter the lymphatic vessel iv) enter the lymph node sinus and v) migrate through the lymph node parenchyma into the T cell area. With our established experimental tools we are now able to efficiently analyze all these steps.

Our published key findings: We studied basic mechanisms of force generation and force transduction (Lämmermann et al., *Nature*, 2008 and Renkawitz et al., *Nat Cell Biol.*, 2009), actin flow polarization in complex 3D environments (Lämmermann et al., *Blood*, 2009), entry strategies of leukocytes into afferent lymphatic vessels (Pflicke and Sixt, *J Exp Med*, 2010) and the role of soluble vs. immobilized chemokine gradients in lymph node cellular traffic (Schumann et al., *Immunity*, 2010). We also developed novel tools and approaches to visualize cortical actin dynamics in chemotactic leukocytes (Riedl et al., *Nat Methods*, 2008 and 2010) and started to combine this with biophysical and pharmacological manipulations of the cytoskeleton.