**Title:** Mechanisms of immune cell migration: how actomyosin contractility controls podosome-mediated protrusion and mechanosensation in dendritic cells.

**Department:** Cell Biology

**Supervision:** Prof. Dr. A. Cambi (alessandra.cambi@radboudumc.nl)
Dr. K. van den Dries (koen.vandendries@radboudumc.nl)

**Duration:** 6 months

**Main aim:** To study the localization and dynamics of myosin within podosome clusters at high spatiotemporal resolution using state-of-the-art microscopy techniques.

**Background:**

Podosomes are dynamic multi-molecular complexes formed by cells that need to cross and degrade tissue boundaries to perform their biological function. They are used by dendritic cells (DCs) to extravasate, by osteoclasts to degrade bone and by endothelial cells to initiate new vessels. Loss or gain of podosome-mediated degradation has been associated with a diverse range of pathologies including osteoporosis, Wiskott-Aldrich syndrome, Crohn's disease and Frank-Ter Haar syndrome. Moreover, podosome-like structures called invadopodia are used by cancer cells to initiate cell invasion and metastasis.

Podosomes have a circular architecture consisting of a protrusive actin-rich core surrounded by an adhesive integrin ring. We and others have shown before that podosome protrusive activity is controlled by an interplay between actin polymerization in the core and myosin contraction in the ring. Interestingly, while it has been shown that myosin localizes to podosome clusters, it is still very much unclear where myosin is exactly located and how it exerts its function within podosomes. This internship aims at identifying the role of myosin within podosome clusters and study its function in relation to sensing substrate stiffness and topography.

**Research plan:**

Human monocyte-derived DCs will be labelled for myosin and actin and their organization will be studied at high resolution with super-resolution techniques such as Airyscan (**Fig. 1**), STORM and electron microscopy. Furthermore, fluorochrome-tagged proteins will be transfected into DCs and live-imaged at high resolution to acquire detailed insight into the spatiotemporal organization of myosin within podosome clusters (see http://tinyurl.com/ActinAiryscan for actin Airyscan live-imaging in DCs). To study the mechanosensory role of myosin, cells will be seeded on substrates with different stiffness or with topographical features. Western blot analysis will be exploited to further study myosin activation and expression in DCs.

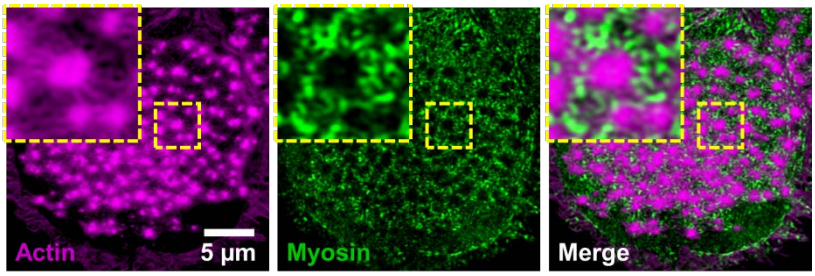


**Fig. 1. Actin and myosin localization in dendritic cells.** Super-resolution imaging (AiryScan) of actin and myosin in human monocyte-derived DCs. Note the stress fibers radiating from the podosome core and the detailed structures of myosin that can not be resolved with conventional microscopy.

**Techniques:**

- State-of-the-art fluorescence microscopy:
  - Super-resolution microscopy
  - Live cell imaging
  - Electron microscopy
- Molecular cloning
- SDS-Page and western blot
- Substrate design

**References:**


1. Murphy et al. Nat Rev Mol Cell Biol. 2011
2. van den Dries et al. Nat Commun. 2013
3. Meddens et al. Nat Comms. 2016
4. Beach et al. Nat Cell Biol. 2017