In skin, basal keratinocytes are firmly attached to the basement membrane through adhesion devices called hemidesmosomes (HDs). These structures play a critical role in maintaining tissue integrity and provide resistance to mechanical force.

A model for the spatial organization of the HD components and protein-protein interactions in this ultrastructure has been deduced from functional assays with keratinocytes and in vitro data. Furthermore HDs containing electron dense plaques can be observed by electron microscopy as well as immunofluorescence, using antibodies against constituent proteins that label HD components.

Traditional immunofluorescence however, neither provides sufficient resolution to define the molecular architecture of HD nor characterizes the spatial interrelations between the mentioned HD components. Recently developed Super Resolution (SR) microscopy techniques, in particular Stochastic Optical Reconstruction Microscopy (STORM) and Ground State Depletion Microscopy followed by Individual Molecule return (GSDIM) define approximately 10-fold increased magnification. This technological leap thus makes it possible to study fluorescent preparations at unprecedented detail.

We here not only use two- and three-color GSDIM methods to study the architecture of HD in cultured keratinocytes in great detail, but also apply it to skin tissue imaging, which has remained a challenging step in this field since the very beginning. Moreover in this work we introduce novel methods to quantitatively describe multi-color SR images, including “proximity mapping”, an approach to map average distances between molecular components of HD with precision in the nanometer range.

Our data and novel quantification method show that at the periphery of keratinocytes, where nascent HDs are created, keratin filaments run parallel to the plasma membrane and are decorated with plectin. Strikingly, plectin and β4 integrins localize mainly alongside rather than under the keratin filaments. Similarly, the HD components BP180 and BP230 are associated with keratin filament, but with different characteristic distribution, with several BP180 molecules surrounding tight clusters of BP230 molecules in Type I HDS. The BP180 and β4 integrin-based adhesion structures appear closely interwoven. Thus, based on SR imaging we present an updated model of nascent HD architecture in human keratinocytes in cultured cells and verify the model in developed HDs in tissue.