UNIVERSITY OF AMSTERDAM

Unraveling Intermediate Filaments. The Super Resolution Solution.

L. Nahidiazar

Thesis Abstract

Intermediate Filaments (IFs) carry out major functions in cells, and these functions are closely linked to the cell types. In general, they are important in maintaining cell integrity, provide mechanical support for the plasma membrane, interact with microtubules, regulate gene transcription, protect cell from damage and indirectly contribute to cell migration and adhesion. Several diseases have been associated with malfunctioning IFs in the cells and among them are certain sub types of cancer. Light microscopy has been widely used to study IFs. Yet due to the limited optical resolution of the microscope, IFs and their role in diseases has not been fully understood.

To determine the structure and organization of IFs, we have used Single Molecule Localization Microscopy (SMLM) . In the first study, we have shown that keratin IF plays a key role in Hemidesmosomes organization (HDs) in cultured keratinocytes. Unlike accepted models in text books, SMLM has revealed that non-looping keratin single filaments in the cell periphery interact with *plectin* and β*4 integrin* simultaneously and asymmetrically in a tent-and-peg model. We have found a unique distribution pattern of the *plakin protein BP230* (which directly binds to keratin IF) and the *transmembrane protein BP180* which is distinct from the one between *plectin* and β*4 integrin*. By investigating human skin sections using SMLM for the first time, we have presented the similarity between HD structure in cultured cells and human tissue. Due to our newly developed analysis methods (proximity mapping and distance distribution analysis), we were also able to map other HD components in unprecedented detail.

In addition, we have studied the orientational alignment between vimentin, another abundant IF protein, and MT. With SR, we observed spatial proximity between single vimentin and MT filaments. Furthermore, we have introduced an analysis approach in which the alignment of the structures in super resolved images is considered as an extra information dimension. Adopting this method, so called *co-orientation* analysis, we have quantitatively shown that interaction between vimentin and MT are cell-type dependent. Co-orientation between two cytoskeleton networks is predominant in the cell periphery and not in the perinuclear region in certain cell types.

Before arriving at these results, we first addressed several critical issues in SMLM imaging. First, we have developed a new buffer which supports multi-color imaging. OxEA, an Oxyrase based imaging buffer, elevates localization precision for commonly used dyes and enhances blinking of other dyes, including some that have not been successfully used for SR imaging before (such as FITC). OxEA does

not produce harmful by-products and it does not acidify the environment, unlike commonly-used buffers like Gloxy. This enabled much more prolonged imaging. Second we have improved a dedicated chamber for SR imaging. The Oxygen Tight Chamber (OTC) benefits from a better aluminum tape-based sealing method and blocks Oxygen influx entirely. Especially the *Cyanine-based dyes* have better performance in the OTC, since they blink effectively in the total absence of Oxygen. In the OTC, we also minimized drift by introducing an alternative curing procedure that reduces stress in the bond between glass and plastic. Long-term storage of the OTC preparation is practical and possible. The OTC helps microscopists to record millions of frames and several continues hours non-stop. Due to considerably higher number of photons, it is possible to reconstruct 3D stacks and bigger fields of view (image stitching) with considerable less axial and lateral drift. The OTC has been launched to the market by WillCo Wells in 2016 (WillCo-dish NANO, glass bottom, GWSB-3512N).

In brief, in this thesis we have optimized imaging condition and post processing analysis for SMLM microscopy. With the resulting high quality SR images and precise quantification methods, we have contributed to the biological knowledge in the IF field.