Emerging Techniques in Modern Microscopies

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Direct, spatial imaging of randomly large supermolecules by using parameter unlimited TIS imaging cycler microscopy

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The recent development of parameter-unlimited functional super-resolution microscopy TIS™ (Toponome Imaging System) is based on the Imaging Cycler™ principle [1,2]. It provides direct access to protein networks of randomly large supermolecules at high 2D and 3D resolution in a single tissue section or inside cells, with many thousand distinct protein-complexes in one cell [1,2], as featured in Fig. 1 (cover stories). TIS™ is a device that can overcome both the spectral and the resolving power of conventional light microscopy without having to change hardware. It is the first ready-to-use Imaging Cycler™ technology for dimension- and parameter-unlimited histological diagnostics and systematic decoding of the toponome at functional super-resolution (toponome: defined as the spatial protein network code in morphologically intact cells and tissues providing direct access to the supramolecular order of biological systems). TIS™ is a highly flexible machine that can adapt to the needs of the researcher: a 4-in-one microscope including (1) routine transmitted light functions, (2) conventional epifluorescence functionalities, (3) paramater-unlimited protein network visualization in realtime and at relational stoichiometric resolution for at least 100 distinct molecules (approx. 40 nm resolution) (Fig. 2), and (4) functional super-resolution of subcellular structures and protein complexes in tissue sections, cultured or isolated cells at 3D (Fig. 3). It is a novel platform providing the robustness needed for the human toponome project, combining industry partners and research institutions. The technology has shown to solve key problems in cell-, tissue-, and clinical toponomics by directly decoding cellular (disease) mechanisms *in situ/in vivo*, in particular at the target sites of cancer in human tissue ([3-9] reviewed in [10]). Several next-generation toponome biomarkers and toponome drugs are on the way to clinic. The human toponome project has at its goal to unravel the complete toponome in all cell types and tissues in health and disease. The technology is scalable as large cooperative parallel screening devices extracting the most relevant disease targets from protein network hierarchies in situ: a novel efficient way to find selective drugs, by escaping the low content trap in current drug target and diagnostic marker discovery strategies, which, as yet, have disregarded the spatial topology of the protein network code.

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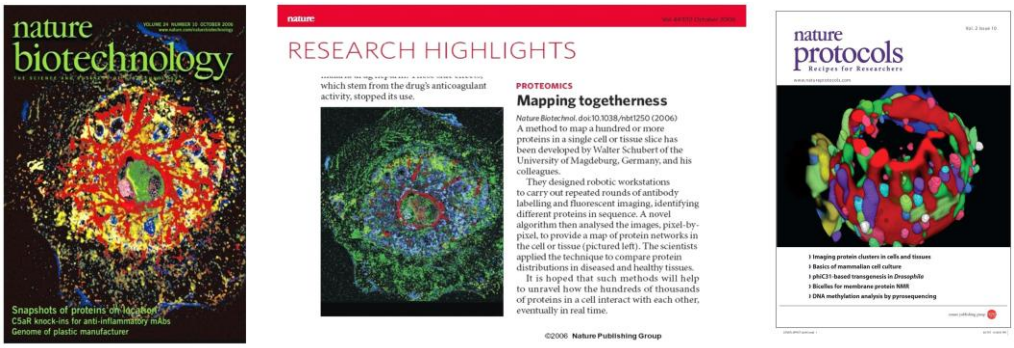

Figure 1. Featured Toponome maps: (a) Cover image from [1, above]: over 7,000 Protein clusters in a single human liver cell; (b) Corresponding Research Highlight referring to (a) (text of this highlight is found in Abbot A. Nature, 443, 609, 2006); (c) Cover image from [2, above] showing a cell surface protein cluster network of a single human peripheral blood T lymphocyte. Applications in real time at 20 – 40 nm resolution are shown on the web page of the human toponome (HUTO) project www.huto.toposnomos.com): the highest ever shown resolution of giant supermolecules at stoichiometric measures in real time in a tissue section using traditional fluorescence microscopy.

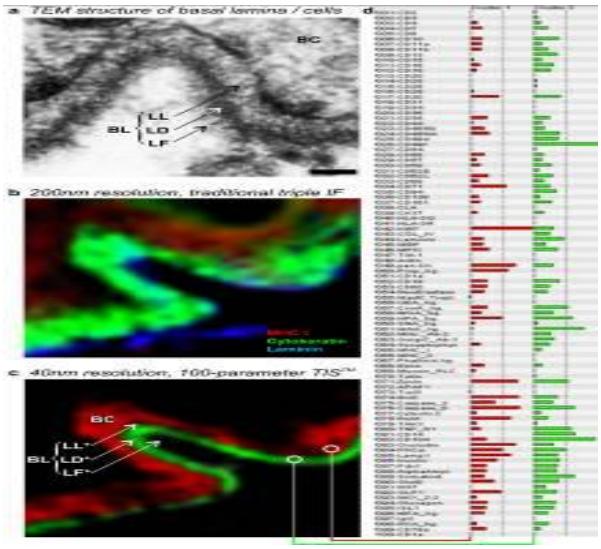

Figure 2. Detection of skin lamina densa as a giant supermolecule
(a) TEM micrograph of the dermoepithelial junction (b) traditional epifluorescence image of this area (c) TIS functional super-resolution image of this area in a human skin tissue section showing the relational stoichiometric molecular profiles of 100 distinct proteins and glyostructures captured by cyclical imaging at approx. 40 nm resolution (from Schubert et al, N Biotechnol. [9]) lamina lucida (LL), Lamina densa (LD, green profile) and lamina fibroreticularis (LF). Bar: 50nm (a)

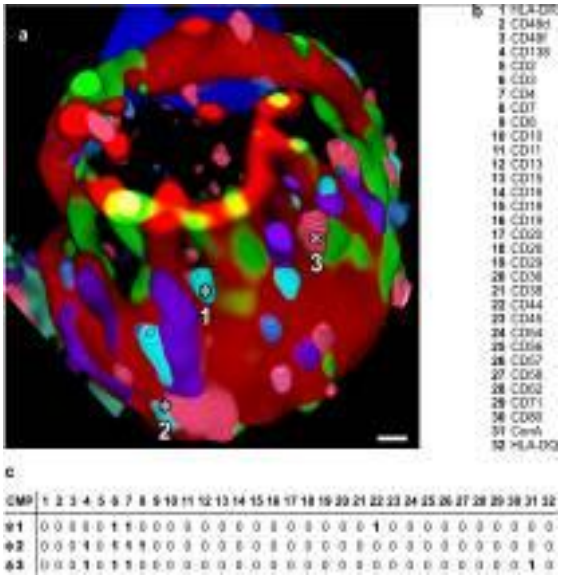

Figure 3. Cell Surface protein complexes (a) different spherical bodies formed by differential clustering of 27 distinct simultaneously mapped proteins (b) in a human blood T lymphocyte, from [2, 9]. (c) Note: CMP = protein com-plexes with colocation and anticolocation code (1/0). Bar: 1µm