ToposNomos Ltd.

Toponome Imaging System (TIS): imaging the proteome with functional resolution

The Toponome Imaging System (TIS) is the latest development in automated multidimensional fluorescence microscopy. It is capable of imaging over 100 different molecular components in a single cell or tissue section at continuous intensity levels for the first time. TIS directly addresses protein-network architecture and function in healthy and diseased states—a completely new dimension in the life sciences.

The organization of complex uni- and multicellular organisms involves the proper spatial distribution of macromolecules, metabolites, ions and cell organelles, as well as the interplay of these components and their individual regulation based on functional requirements. A major goal of biological research is the understanding of these complex cellular mechanisms and spatial arrangements at the molecular level. This is achieved by analyzing the fundamental protein networks encompassing all cellular functionalities. Fluorescence microscopy has revolutionized research in cell biology and histology. The independent observation of the many molecular components characteristic of whole protein networks, however, has been hampered largely by the fact that the spectral isolation of multiple dyes in one biological sample is very limited[1].

A breakthrough in fluorescence imaging has been reported using multi-epitope-ligand cartography (MELC) technology[2]. The basic features of this technology have been described earlier[3–7], and MELC now has been established as an automated imaging technology capable of high-performance, high-content applications. It permits the colocalization of more than 100 proteins or other molecular components in a single cell or tissue section in two or three dimensions. This provides insight into the higher-level combinatorial organization of proteomes *in situ*, which the authors have termed the 'toponome'[1,6]. This technology can work with only one dye to colocalize molecules by using large dye-conjugated tag libraries, and automatically performing sequential rounds of fluorescence protein tagging, imaging and bleaching. Based on the verified robustness of the technology, researchers have been able to study a large variety of eukaryotic cell types, showing that this approach reveals rules of hierarchical protein network organization: state-specific lead proteins appear to control protein network topology and function.

The specific advantage of this technology is not only to provide insight into the contextual organization of proteins (the toponome), but also to pave the way to systematically analyze protein systems directly on a proteome-wide scale in healthy and diseased states. Notably, abnormal lead proteins, which appear to be unique to certain diseases, are new candidate key target proteins that control the topology and whole functionality of molecular networks in pathogenic cells, such as in tumor cells.

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Toponome Imaging System (TIS)

ToposNomos Ltd. (TN) has established TIS (patent pending) as an advanced modular concept for MELC. TIS consists of three essential units: motorized data acquisition, semiautomated image processing and functional toponome annotation. The hardware components comprise a conventional epifluorescence inverted microscope, a multipipette unit, a tag-dye hotel and a high-resolution cooled charge-coupled device (CCD) camera (**Fig. 1**). TIS can be used to detect any molecule

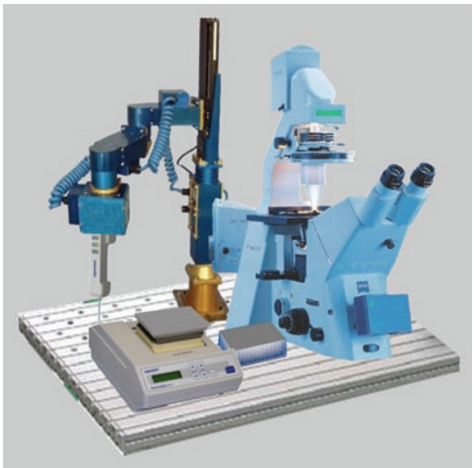

Figure 1 | Modular setup of TIS. TIS resembles a modular construction system, whose hardware and software components are assembled by customers, with the support of TI consultants. The advantages are that the system is automated and highly modular, and offers remote function, component control and annotation software.

APPLICATION NOTES

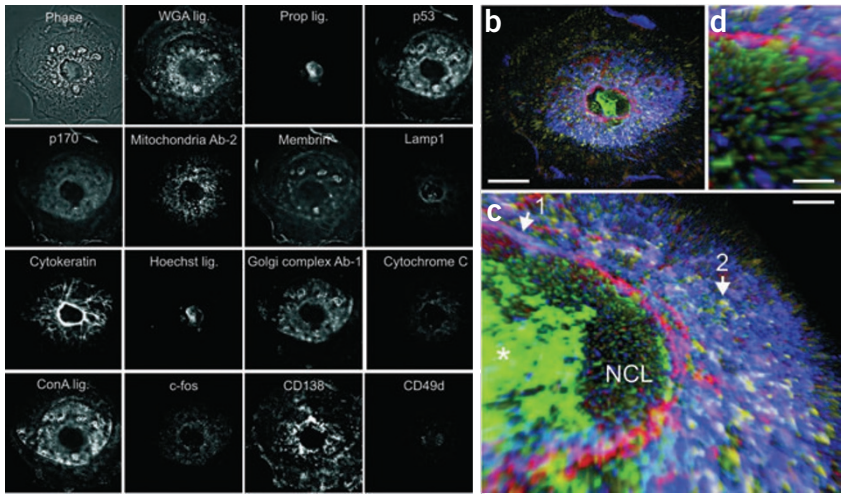

Figure 2 | Three-dimensional toponome map of a human hepatocyte. (**a**) Gallery of the 15 molecular cell components characteristic for different cell compartments of a single cell visualized in three dimensions (perpendicular view). (**b**) Fluorescence intensity image of 15 different molecular components exemplified in one cell giving a toponome reference map of multiple subcellular compartments simultaneously. Whole-cell image constructed by voxel-precise overlays of the 15 molecules. (**c**) Detail showing: the nuclear envelope (arrow 1), parts of the cell nucleus (*) and nucleolus (NCL), and many cytoplasmic vesicles (arrow 2). (**d**) Detail of **c** showing the NCL containing multiple pre-ribosomes. The image has been generated using methods similar to those described in reference 1, and then displayed by TIS three-dimensional software. A navigation demo is available upon request. Scale bars, 20 μm (**a**,**b**); 5 μm (**c**); 2 μm (**d**).

for which a fluorescently labeled ligand is available, such as antibodies, aptamers, lectins or peptides. The entire automated fluorescence procedure is based on bleaching each dye completely after the labeling and imaging steps have terminated, and then applying an additional set of labeled tags to identify the distribution of additional molecules. By repeating these steps, large sets of molecular distribution maps can be accumulated and visualized at light-microscopic resolution (**Fig. 2**). The technology can be used to map hundreds of different proteins in one tissue section or cell sample by performing more than 100 sequential TIS cycles. It is possible to select the most prominent or rare combinatorial patterns by representing the data as vectors, and to depict the distribution of these protein clusters in a 'toponome map' in two or three dimensions. Toponome maps reveal the hierarchical features of protein organization *in situ*. A highly flexible software package controls all functions from automated data acquisition to image processing, semiautomated data analysis of topological information. The technology reported so far[2] is based on binarization of protein signals, thereby 'scratching only the surface of MELC images'[1]. Overcoming this limitation, the new 3D TIS software can be used to visualize each protein in a topological map along its unique signal intensity ranges (**Fig. 2**). TIS resembles a modular construction system that can be assembled by any researcher with the support of TI consultants. The system can easily be customized to tailor to individual scientific needs. One of the new and major features of TIS is a highly modular software package that allows the user to combine different software tools for protein network data mining in individual cells.

Opening new horizons

Understanding how proteins are temporally and spatially arranged, and how this relates to function are major post-genomic challenges[1]. Proteins must be at the right place at the right time and at the right concentration in a cell to form a functional interaction network. This implies that any molecular network exerting a specific cellular function obeys a unique protein colocalization and anti-colocalization code[2,7].

TIS provides direct insight into this functional architecture of molecular networks *in situ*. It makes protein systems *in situ* amenable to combinatorial geometry and statistics[8] and may offer a rapid route to new diagnostic features and targeted therapies. It unravels new lead proteins in healthy and diseased states, and allows the researcher to perform microscopy with functional resolution. Any type of tissue or cell can be visualized as an overlay of all existing kinds of functional protein associations delineating cell types as well as subcellular and tissue compartments in their different functional states.

TN's goal is to make toponome research accessible for the scientific community. It has a worldwide net of scientific collaborations, and has established a team of specialists that supports customers in assembling and developing their own TIS to fulfill individual research requirements.

For additional information, visit our company website (http://www.toposnomos.com).

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