LOOKING INSIDE SPHEROIDS:
HISTOLOGY WITHOUT A KNIFE

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We show a mounting technique that enables the optical acquisition of small and cleared objects using the monolithic Digital Scanned Laser fluorescence Microscope (mDSLM) [1].

The low light scatter properties of cleared specimen provide sharp signals deep inside tissues. In contrast to confocal microscopy LSFM provides a fast acquisition of large volume recordings. Low energies prevent the bleaching of fluorescent proteins [2].

Clearing has been mainly used to visualize whole organs with low magnification lenses to observe the distribution of labeled proteins within biological samples in toto [3]. Light scattering limits one photon light microscopy to a range of 100-150 µm inside tissue. Hence, clearing supports the visualization of 3D objects even in the range of a few hundred micrometers.

Spheroids are the specimen of choice in the growing field of the 3D cell cultures [4]. They self-assemble and cluster to a ball shaped structure with 200 to 20,000 cells. Imaging stained and cleared spheroids in toto has enabled a fast and easy observation of morphological events in huge T47D spheroids. Now, we can e.g. determine the absolute cell count and, therefore, the proliferation rate. We observe, that the necrotic core in T47D cells only occurs with a cell count higher than 2,000. Also ducts, which are formed by polarized cells, were characterized in 3D.

References:


