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EDITORIAL

In focus in HCB

Douglas J. Taatjes[1] · Jürgen Roth[2]

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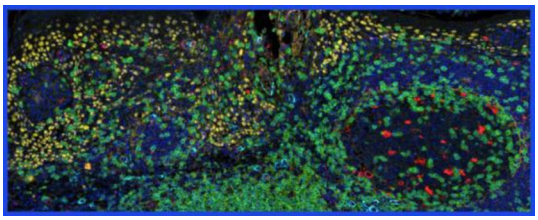

In this October 2022 Editorial, we offer highlights from a review describing state-of-the-art methods for imaging fluorescently labeled live samples (Robert Feulgen Prize 2020 awardee); and two Short Communications describing (1) a novel use for the fluorescence signal of eosin dye to identify and quantitate red blood cells in sections of formalin-fixed and paraffin-embedded tissues; and (2) the specific expression of S100A1 mRNA and protein in terminally differentiated superficial cells in the urothelium of the murine bladder and ureter during embryonic and postnatal development. We hope that you find these brief highlights interesting!

Life in technicolor

The Robert Feulgen Prize is an internationally announced prize of the Society for Histochemistry, awarded for work of outstanding scientific merit in the field of microscopic histochemistry (see Taatjes and Roth 2021 for more historical information). The 2020 Robert Feulgen Prize was awarded *ex aequo* to H. Shroff, National Institute of Biomedical Imaging and Bioengineering, NIH, Bethesda, MD, USA, and to Ch. Mühlfeld, Hannover Medical School, Hannover, Germany. Professor Mühlfeld's award manuscript was published in 2021 (Mühlfeld 2021), and we now have the honor to present Professor Shrof's prize-winning award manuscript. In this beautifully crafted and illustrated review, together with Yicong Wu, Professor Shrof presents a state-of-the-art synopsis of multiscale (representing temporal and spatial scales) fluorescence imaging of living samples, ranging from cells to organisms to animals (Wu and Shrof 2022). The review is divided into sections describing (1) overall challenges and basic considerations in imaging fluorescent tags in living samples; (2) an "instrument" section in which the myriad of available technologies are concisely explained through examples from the literature illustrating their successful application in multiscale fluorescence imaging; and (3) suggestions and tips for those planning their own multiscale fluorescence imaging of living samples. Optical techniques including widefield microscopy, total internal reflection fluorescence (TIRF) microscopy, point-scanning, spinning disk, and multiphoton confocal microscopies, various super-resolution microscopy iterations, light-sheet microscopy, and adaptive optics (AO) are described in detail, noting advantages and disadvantages of each fluorescence imaging technique. The review concludes with sections on powerful computing methods and algorithms for improving multiscale fluorescence imaging, the creation of multiscale image atlases for organisms such as *C. elegans* and embryonic mouse development, and a final summary of useful tips when considering embarking on a fluorescence multiscale imaging project. The frequent inclusion of published examples provided in the review very nicely illustrate the power and utility of the various imaging methods described. It is probably not too soon to speculate that this review, which flows like a textbook chapter, will become a "must read" for anyone involved in fluorescence imaging of live (and of course) fixed biological samples.

✉ Douglas J. Taatjes
douglas.taatjes@med.uvm.edu

[1] Department of Pathology and Laboratory Medicine, Larner College of Medicine, University of Vermont, Burlington, VT 05405, USA

[2] University of Zurich, 8091 Zurich, Switzerland

"Seeing Red" in a non-pejorative sense: "Red" shift of a "Red" dye to identify "Red" blood cells in tissue sections

Eosin is the "E" component of the classic H&E histochemical stain ubiquitous in histology and diagnostic pathology labs (the "H" component of course refers to hematoxylin). While hematoxylin stains the nucleus purple, eosin stains cytoplasm and extracellular matrix pink, providing color contrast and thus rendering cells and tissues visible by transmitted light microscopy. Interestingly, eosin stain also possesses inherent fluorescence properties when exposed to specific wavelengths of visible light. This is well known to those of us using hospital laboratory facilities for embedding research samples in paraffin, where an eosin staining step is typically incorporated into the automated tissue processing. Using sections from these samples for immunofluorescence staining procedures typically results in very high background staining due to the eosin fluorescence signal. Francis et al. (2022) have now taken advantage of the fluorescence properties of eosin when bound to erythrocytes to introduce a method to quantify blood and red blood cells in sections from tissues fixed with formalin and embedded in paraffin. Using control hamster lung tissue, as well as SARS-CoV-2 infected hamsters as a model due to the extreme hemorrhage present in these lungs, they first characterized the spectral emission properties of eosin upon excitation with 470-nm laser light in the control tissue sections. They found that eosin displayed an emission maximum of 550 nm over most of the lung tissue, whereas over areas where blood was present a red-shift to an emission maximum of 570 nm was detected. They then used this information when imaging sections by both conventional wide-field fluorescence microscopy and two-photon confocal microscopy. Employing standard FITC (520–540 nm) and TRITC (570–610 nm) filter sets on both microscopes, they found that the eosin signal associated with blood deposits was visualized specifically with the TRITC filter. Finally, they developed an image-processing algorithm providing a quantitative evaluation of the total pixels in the sample associated with areas of blood deposition. The authors propose that the method can be used to measure areas of microthrombi and hemorrhage in routinely formalin fixed/paraffin embedded tissues, ultimately providing important information in both clinical diagnostics and research applications.

Is that why they are called urothelial "S"-cells?

The urothelium is a transitional stratified epithelial layer lining the bladder, renal pelvis, ureters, and proximal urethra. As recently outlined very nicely by Dalghi et al. (2020), the urothelium is characterized by three distinct cellular layers (stratified-multiple layers thick): a superficial layer ("S"-cells or "umbrella" cells); an intermediate cell layer of variable thickness layers; and a single basal layer sitting atop a basement membrane. Interestingly, since these cell layers can undergo morphological changes as fluid is transported through the urinary tract, originally the urothelium was classified as transitional epithelium, since morphologically it seemed to fall between a stratified columnar and stratified squamous epithelium (Dalghi et al. 2020). This term has been replaced by the more generally acceptable urothelium to reflect these transitory cellular morphological changes occurring, principally in the surface S-cell layer. In addition to the morphological distinction of the three cell layers, the cells themselves display characteristic mRNA and protein phenotypes. For instance, basal cells are KRT5-positive (intermediate filament protein), ΔN isoform of TRP63 (ΔNP63; runt-type transcription factor)-positive, UPKs (uroplakins)-negative; intermediate cells are KRT5-negative, ΔNP63- and UPKs- slightly positive; and S cells are KRT5- and ΔNP63-negative, and highly UPKs-positive (Gandhi et al. 2013; Bohnenpoll et al. 2017). In this light, Qasrawi et al. (2022) have now performed a detailed in situ hybridization and immunohistochemical analysis of mouse urothelium during embryonic and postnatal development. They focused particularly on S100A1, a protein of the S100 family, which as a group are small calcium-binding proteins. Using multiple mRNA probes and colocalization of antibodies as described above to identify specific cell types, they characterized their expression during development of the urothelium components bladder and ureter. Their results showed (1) specific expression of *S100a1* mRNA in luminal S-cells of the ureter, starting with embryonic day 17.5 onwards, and from embryonic day 15.5 onwards in the bladder; (2) by multiple label immunofluorescence, S100A1 protein was found to be expressed in the cytoplasm and nucleus, exclusively in terminally differentiated S-cells in both ureter and bladder. Importantly, the in situ hybridization and immunohistochemical results were confirmatory of each other. Since S100A1 as been proposed as a useful marker for various cancers, the authors suggest that given its specific localization to S-cells in the urothelium, it may also serve as a specific marker for urothelial carcinomas.

Finally, as an aside, we would like to point out the very detailed descriptions of the materials and methods in this manuscript. Particularly, the antibody validation and image documentation sections are "textbook" descriptions in the realm of rigor and reproducibility, and we recommend their careful scrutiny as a model for emulation when preparing your next manuscript!

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