Sectioning-free virtual H&E histology with fiber-based two-photon microscopy

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The routine pathology workflow relies on cutting tissue into single-cell layer thick slices using paraffin or frozen sectioning. We propose a fast method to obtain sections of equivalent quality optically using the strong sectioning capabilities of two-photon microscopy (TPM). Hematoxylin and eosin (H&E) equivalent staining of the tissue is achieved using acridine orange and sulforhodamine 101. We improved our previously presented pulsed fiber laser to deliver adjustable pulse durations of ~30ps at repetition rates of up to 16MHz and kW peak power. We can now image up to one square centimeter of tissue with sub-micrometer resolution within 15 minutes.

Tumors are identified by pathologist using mostly hematoxylin and eosin (H&E) histology. While new immunohistochemical stains are starting to become more important, most parts of pathology still rely on the standard H&E workflow. Biopsies are investigated using two standard techniques: Paraffin or frozen sectioning. While frozen sectioning is fast but comes with imaging artifacts that can hinder a distinct diagnosis, the slow process of paraffin sectioning delivers reliable and high-quality tissue images. When a pathologist wants to investigate a piece of tissue, the first step is to maintain the structures of the cells with a fixative. Then the tissue of interest is selected and trimmed before it has to be embedded in paraffin wax. To do so, one has to dehydrate and clear the tissue and replace the water with paraffin wax, which can take up to 15 hours. The paraffin blocks are cut into single-cell layer thick sections, stained with H&E and imaged with a bright field microscope. The process of paraffin sectioning is labor-intensive and usually takes more than 24 hours in total.

The possibility to replace this process with the much faster digital optical sectioning capabilities of multiphoton microscopy has already motivated others to use a fast-scanning two-photon microscope (TPM) to create H&E like images of tissue [1-3]. We propose a fiber laser-based approach using sub-nanosecond excitation pulses at 1030nm with kW peak power and a repetition rate of up to 16MHz, that is more robust than commonly used fs-laser systems [4]. Our new microscope setup uses two galvanometric mirrors in an 8f relay optic to scan the sample with a 16x microscope objective. We use acridine orange as nuclear stain and sulforhodamine 101 as counterstain [5]. Both fluorescence channels are collected with photomultiplier tubes simultaneously. We have reached a scanning speed of 460000 pixel per second with a square pixel size of 0.5µm. This allows us to create huge mosaics that reach a size of up to 1cm^2 at a resolution of more than 500 megapixel. In theory, we could reach an imaging speed equal to our pulse rate (i.e. ~30s/cm^2) but are currently hindered by a too slow acquisition software and scanners.

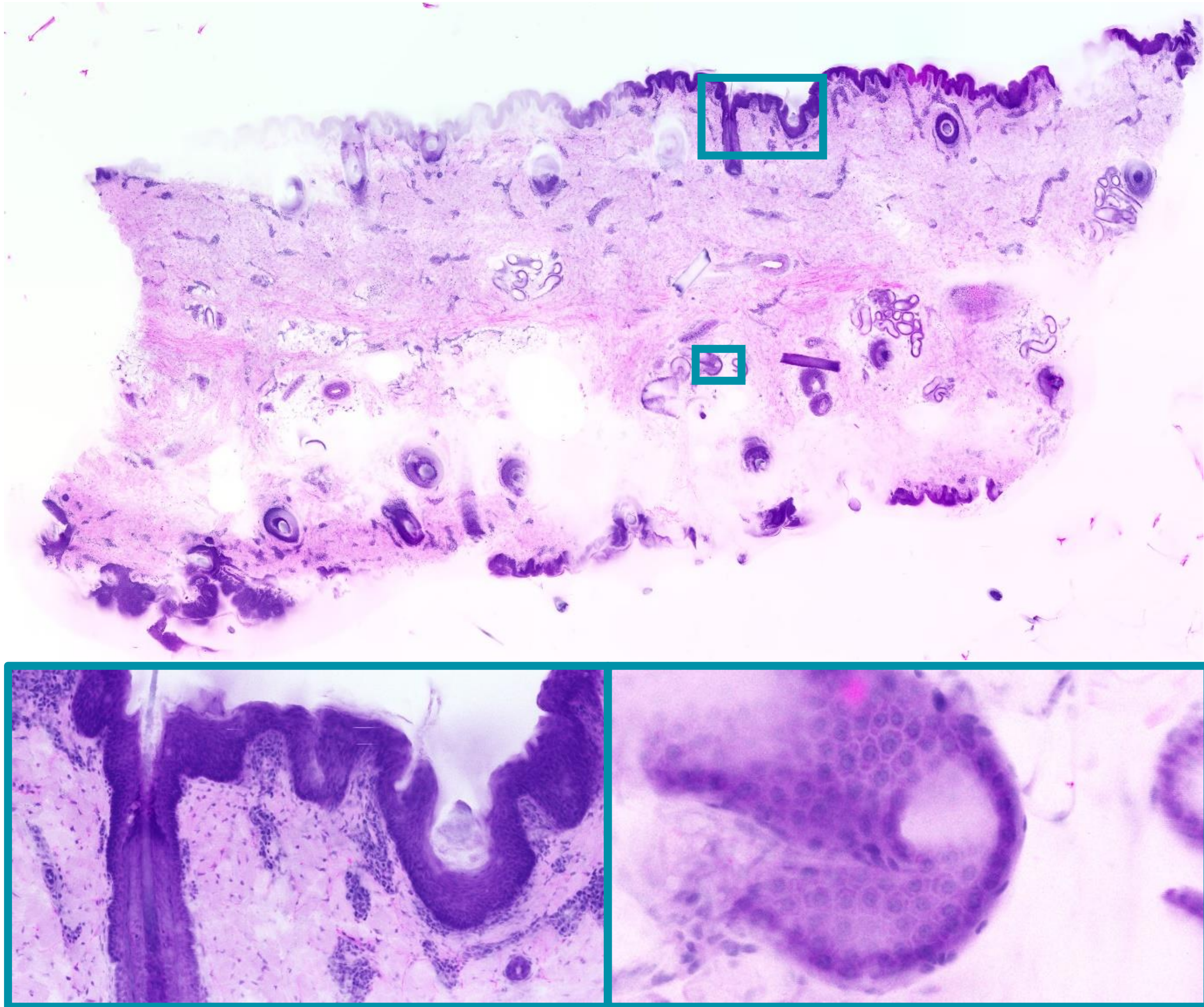

Figure 1: Virtual H&E section of porcine skin (ear): Nuclei are purple/blue and the extracellular matrix and cytoplasm is pink, Top: Image of 11mm x 5mm of porcine skin with an original resolution of 34100px x 18700px. Bottom left: Enlarged image (1mm x 0.5mm) of a hair follicle. Bottom right: Enlarged image (0.4mm x 0.2mm) of a sweat gland.

An example of a porcine ear measured at a pulse repetition rate of 1MHz is displayed in Figure 1.

In the future we intend to further improve the speed of our microscope towards the theoretical limit and to extend our pool of fluorescent dyes and use direct H&E staining.

References

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