Rapid confocal imaging of large areas of excised tissue with strip mosaicing

Sanjee Abeytunge
Yongbiao Li
Bjorg Larson
Ricardo Toledo-Crow
Milind Rajadhyaksha
Rapid confocal imaging of large areas of excised tissue with strip mosaicing

Sanjee Abeytunge,a Yongbiao Li,a Bjorg Larson,b Ricardo Toledo-Crow,a and Milind Rajadhyakshaa,b

aMemorial Sloan-Kettering Cancer Center, Research Engineering Laboratory, 430 East 67th Street, RRL513, New York, New York 10065
bMemorial Sloan-Kettering Cancer Center, Dermatology Service, 160 East 53rd Street, 2nd floor, New York, New York 10022

Abstract. Imaging large areas of tissue rapidly and with high resolution may enable rapid pathology at the bedside. The limited field of view of high-resolution microscopes requires the merging of multiple images that are taken sequentially to cover a large area. This merging or mosaicing of images requires long acquisition and processing times, and produces artifacts. To reduce both time and artifacts, we developed a new mosaicing method on a confocal microscope that images morphology in large areas of excised tissue with sub-cellular detail. By acquiring image strips with aspect ratios of 10:1 and higher, we demonstrated that “stitching” images in software, our method images 10×10 mm² area of tissue in about 3 min. This method, which we call “strip mosaicing,” is currently three times as fast as our previous method.

Keywords: Mohs surgery; confocal fluorescence mosaicing microscopy; basal cell carcinoma; surgical pathology; large area imaging microscopy.

In this report, we eliminate one of the stitching dimensions by acquiring long image strips instead of the standard “square” images. Instead of merging a two-dimensional array of images, a single one-dimensional array of strips is stitched together (see Fig. 1). The benefit of this is threefold: the acquisition time, merging time, and the artifacts due to the illumination variations are all reduced by half. The system described uses a combination of optical and mechanical scanning to generate the images. Preliminary data shows that the system can produce a 10×10 mm² strip mosaic in about 3 min.

Our system is based on a point-scanning confocal microscope with a rotating polygonal mirror and a galvanometrically driven mirror (Vivascope 2000, Lucid Inc.) A laser (Ar + 488 nm) is scanned in the fast (X) direction at 6.8 kHz over 408 μm at the sample through a 30×0.9 NA, water immersion objective lens (Stableview, Lucid Inc.) The captured field of view is 330 μm to limit the intensity falloff at the edges of the scan. The fluorescence signal is captured with a digital acquisition card (DAQ PCI-6110, National Instruments). A custom
Fig. 2 A 10×10 mm² mosaic consisting of 31 fluorescence image strips of excised tissue from Mohs surgery. The tissue was stained with 0.6 mM acridine orange for 20 s (Refs. 9 and 10). Nests of basal cell carcinomas (A) are observed, showing nuclear detail such as increased density, pleomorphism, and palisading. Typical normal features such as hair follicles and sebaceous glands (B) and eccrine ducts (C) can be seen. The mosaic dimensions are 11,415×10,291 (pixels wide x high) with 8 bits/pixel. Note that the magnified areas are digital zooms obtained from the original image showing the detail and resolution of the mosaic. The features in the mosaic compare well to the pathology (Fig. 3), in terms of location, shape, size, nuclear detail and overall morphology of both basal cell carcinomas and normal features.

As reported in our earlier studies the undersampled images are adequate for interpretation by surgeons and pathologists. To obtain square pixels and equal spatial sampling rates in X and Y, the stage speed must be \( \sim 6.8 \text{ mm/s} \) (1 \( \text{\mu m/pixel} \times 6.8 \text{ kHz line rate} \)). Thus, it takes \( \sim 1.5 \text{ s} \) to scan a 10 mm strip plus 0.5 s to move the stage laterally before starting to acquire the adjacent strip. This is about 2 s/strip. A 31 strip (\( \sim 10\text{-mm wide} \)) mosaic takes approximately 1 min to capture.

To acquire a strip image the galvanometric mirror is locked to its center position and the fast (X) scanner is started. The Y stage motor is started and monitored by a hardware counter. On reaching a constant speed after \( N \) steps, the DAQ starts acquiring image lines on the next valid horizontal line trigger from the scanner. This ensures that the strips do not have more than

As reported in our earlier studies the undersampled images are adequate for interpretation by surgeons and pathologists. To obtain square pixels and equal spatial sampling rates in X and Y, the stage speed must be \( \sim 6.8 \text{ mm/s} \) (1 \( \text{\mu m/pixel} \times 6.8 \text{ kHz line rate} \)). Thus, it takes \( \sim 1.5 \text{ s} \) to scan a 10 mm strip plus 0.5 s to move the stage laterally before starting to acquire the adjacent strip. This is about 2 s/strip. A 31 strip (\( \sim 10\text{-mm wide} \)) mosaic takes approximately 1 min to capture.

To acquire a strip image the galvanometric mirror is locked to its center position and the fast (X) scanner is started. The Y stage motor is started and monitored by a hardware counter. On reaching a constant speed after \( N \) steps, the DAQ starts acquiring image lines on the next valid horizontal line trigger from the scanner. This ensures that the strips do not have more than

As reported in our earlier studies the undersampled images are adequate for interpretation by surgeons and pathologists. To obtain square pixels and equal spatial sampling rates in X and Y, the stage speed must be \( \sim 6.8 \text{ mm/s} \) (1 \( \text{\mu m/pixel} \times 6.8 \text{ kHz line rate} \)). Thus, it takes \( \sim 1.5 \text{ s} \) to scan a 10 mm strip plus 0.5 s to move the stage laterally before starting to acquire the adjacent strip. This is about 2 s/strip. A 31 strip (\( \sim 10\text{-mm wide} \)) mosaic takes approximately 1 min to capture.

To acquire a strip image the galvanometric mirror is locked to its center position and the fast (X) scanner is started. The Y stage motor is started and monitored by a hardware counter. On reaching a constant speed after \( N \) steps, the DAQ starts acquiring image lines on the next valid horizontal line trigger from the scanner. This ensures that the strips do not have more than

As reported in our earlier studies the undersampled images are adequate for interpretation by surgeons and pathologists. To obtain square pixels and equal spatial sampling rates in X and Y, the stage speed must be \( \sim 6.8 \text{ mm/s} \) (1 \( \text{\mu m/pixel} \times 6.8 \text{ kHz line rate} \)). Thus, it takes \( \sim 1.5 \text{ s} \) to scan a 10 mm strip plus 0.5 s to move the stage laterally before starting to acquire the adjacent strip. This is about 2 s/strip. A 31 strip (\( \sim 10\text{-mm wide} \)) mosaic takes approximately 1 min to capture.

To acquire a strip image the galvanometric mirror is locked to its center position and the fast (X) scanner is started. The Y stage motor is started and monitored by a hardware counter. On reaching a constant speed after \( N \) steps, the DAQ starts acquiring image lines on the next valid horizontal line trigger from the scanner. This ensures that the strips do not have more than
a single line of misalignment. M lines are acquired and then the stage is stopped. The X stage motor moves the sample laterally by 330 µm (80% of the imaged field) and the process starts anew. Thirty-one strips were acquired in the example shown in this paper (Fig. A). After completing the acquisition, the images are loaded into the MosaicJ open-source mosaicing software together with information on their relative positions. The program automatically places the images in their acquired positions and merges the images with corrections for any remnant misalignments and variations in signal level. The merging time depends on the power of the processing computer. On an Mac Pro (2 x 2.93 GHz Quad-Core Xeon, 16 GB RAM) it took 35 s to load 31 images and 92 s of processing by MosaicJ to form Fig. B. Although our system is designed to accurately synchronize the scanner and stage to acquire strip images that are adjacent to each other with a vertical misalignment of no more than one line, there remain some misalignments that are entirely due to inadequate precision in our stages. This, of course, can be easily corrected with newer and better quality hardware.

Figure shows a strip mosaic of a skin excision from Mohs surgery. The total time for acquisition and merging of the 31 image strips was about 3.1 min. The mosaic shown is in fluorescence contrast. Acridine orange was used to stain because its excitation matches the 488-nm wavelength in our system. Any contrast agent can be used, such as methylene blue with a suitable excitation source. The mosaic shows the morphology of a basal cell carcinoma and typical normal features (Fig. A). The nuclear morphology is not readily visible in small figures but is clearly seen on a large monitor. To show the detail as seen on a monitor, we present the magnified inserts A, B, and C. The morphologic features in the mosaics compare well to the corresponding pathology of Fig. A. The features corresponding to areas A, B, and C can be identified across the two figures.

Our results demonstrate that strip mosaicing is three times as fast as the previously reported two-dimensional mosaicing method with square images. Starting the merging process as the strips are acquired through the integration of the acquisition and merging programs will further reduce the turnaround time to produce mosaics. This, together with more precise stages, will allow us to generate 10 x 10 mm² mosaics in less than the 3.1 min reported here. The projected acquisition time for a 20 x 20 mm² mosaic would be 3.5 min and we anticipate a linear increase of acquisition time with sample area.

Acknowledgments
The authors gratefully acknowledge support from NIH Grant No. R01EB012466 from NIBIB’s Image Guided Interventions Program (Program Director Dr. John Haller). We thank Dr. Kishwer Nehal and Dr. Erica Lee for supplying discarded tissue from Mohs surgery (under an IRB-approved protocol), and William Fox, Scott Grodevant, and Zachary Eastman at Lucid Inc. for technical support.

References