Optimization of a novel, wide-field, high resolution optical microscopy system

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Introduction

➢Knowledge of the complex fiber structures of soft tissues can lead to greater understanding of basic structure-function relationships and potentially to improvements in tissue engineered constructs and micro-repair techniques.

➢Imaging these structures in fresh, whole-tissue samples is difficult, mainly because current microscopes are designed for small-scale, narrow field imaging of thin, slide-mounted specimens.

➢Several precision stage motaging systems like the CoolScope™ whole slide scanner (Nikon, Inc.), Scanscope XT(Aperio, Inc.) are available but they are only capable of single mode imaging, are often time consuming and can be prohibitively expensive for a small research laboratory**.**

➢The **goal of this project** was to develop a high speed, high resolution imaging system at low cost - capable of imaging thicker, fresh tissue samples as well as prepared slides using both, normal and polarized light.

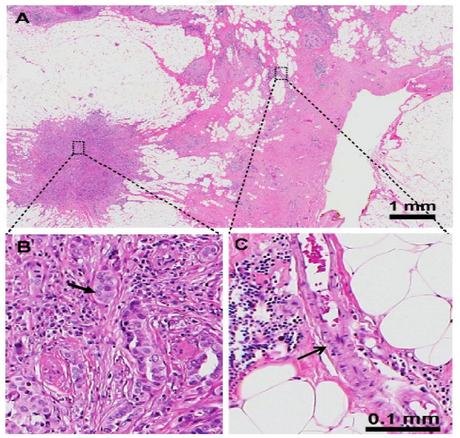
Background

➢A typical Achilles' tendon cross-section may be 15x10 mm in size, while the microscope field of view (using a 10x objective) is only 3x2 mm.

➢These factors motivated us to develop a new mosaic-based imaging system designed to provide rapid, high resolution (0.2 µm), wide field (20x20 mm) images of both standard histology slides and thicker, fresh tissue specimens under normal and transmitted polarized light illumination.

➢ The system is capable of acquiring an image in 10 seconds. The need to speed up the image acquisition system inspired improvisations in the auto-focusing algorithm.

➢Intelligent techniques to select correlation points for stitching the tiles together and eliminate blank spaces are required to make the tiling algorithm more robust.



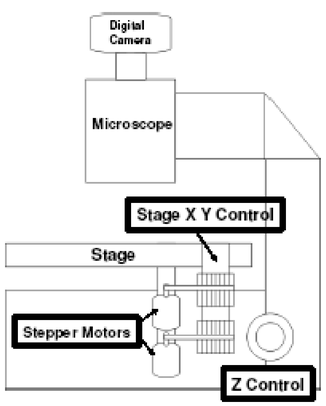


Figure 1. (A) Wide field, high resolution image (22000 x 14000 pixels, appx. 11 x 9 mm) of a breast biopsy histology slide, and full-scale regions showing cellular-scale features such as nucleoli (B, solid arrow), interesting cellular structures (C, open arrow) and (patterning D) system schematic diagram

Hardware

➢ Upright scope - BX50 (Olympus, Inc.).

➢Three stepper motors – X,Y and Z axis control – bipolar chopper drives(Haydon Switch and Instrumentation, Inc.).

➢High resolution camera (PixeLINK-B686CF) attached to the tubus of the microscope via a custom-made adaptor, and interfaced with computer via an IEEE 1394 (firewire) connection.

➢Polarizing filters are added to the light path, including a ½ wave plate to achieve circular polarization.

Stitching Algorithm/Protocol

➢Tiled images are corrected for luminosity and distortion variations.

➢The first and second tiles are digitally overlapped based on the coarse stepper motor position (initial estimate).

➢The overlapping region is then divided into 20 'windows'. These windows are compared by computing digital image correlation (DIC). Then, the X-Y offset of the maximum correlation for each window is computed. This offset represents the positioning error of the stepper motors.

➢Out of these 20 offsets, the three most similar offsets are found by computing the mean and standard deviation.

➢Depending on the accuracy of these offsets (std. dev. <= 0.5 pixels), the location of the seam is calculated, the tiles are cropped and stitched together to form the row.

➢The rows are then joined together to form the montage of image tiles**.**

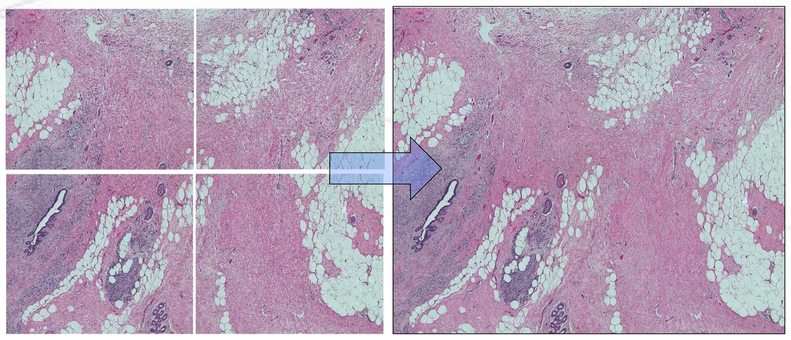


Figure 1. Four image tiles of breast biopsy histology slide and the corresponding wide-field seamless stitched image

Optimizing the Stitching Algorithm

➢A simple edge-based feature selection technique is implemented to facilitate selection of points on the features detected in the overlapping areas.

➢A new algorithm is being developed that changes the tile selection routine to neglect the overlapping regions that contain 'white' spaces (no information) while stitching.

➢The position of the neglected tiles can then be estimated from their neighboring tiles to form the final image, is being developed.

Optimizing the Auto-focus routine

➢The auto-focus algorithm works on the principle that at focus, the image has the maximum number of edges.

➢The routine compensates for small variations of the topography of the tissue slice.

➢The routine works in 2 steps .

- ➢Step 1:- For the first tile , the entire range is scanned for the maximum number of edges for the tile
- ➢Step 2:- For the successive tiles a fine adjustment is done based on the focus setting in the previous step.

➢This reduces the image acquisition time by 50%.

Results

➢ The system produces full montage images of up to 60000 x 30000 pixels (5.2 GigaBytes, 20 x 10 image tiles) in approximately 20 minutes.

➢The effective pixel size is 0.2 µm.

➢The automated image registration algorithm successfully identified and auto-correlated key features (via the DIC algorithm) in the overlapping image tiles, providing sub-pixel accurate image tile registration and a totally seamless montage.

➢The total time to capture an image has reduced by 50 % by optimization of the auto-focusing algorithm.

Discussion

➢Large scale high-resolution imaging has clear advantages:

- ➢Digital Storage of the entire slide.
- ➢Large scale tissue structural analyses
- ➢Analyses can be performed on whole regions, rather than on user selected sub-regions, reducing the potential for human bias.

➢Cost-effective (approximate total cost = $2000) as compared to the available wide-field imaging systems (typically $100k).

➢Unique feature:- Multi-modal Imaging – The system has white light and polarized light capabilities. Future work includes addition of UV capability to the system.

Applications to wide range of fixed and fresh tissue imaging

➢Thicker sections (300-500 µm) and polarized light can be used in this system. This could be highly useful for analysis of the fiber and cellular structure of fresh tissues, for analysis of biopsy and pathology slides, and for improved understanding of **tissue engineered constructs** where large scale structure-function and cell-matrix interactions are of primary importance.

Acknowledgements

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