Mosaicking of High-resolution biomedical images acquired from wide-field optical microscope
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Abstract
Large 2D high-resolution color images were acquired from wide-field optical microscope. The specimen was from the field of pathology of tissues. Each large image was obtained by stitching from a grid of smaller images. Separate acquisitions required registration and stitching of adjacent images. The novel use of special order for registration allows for easy processing of images with solely background. The order is determined from graph representation based on the grid. In this way, a reliable registration confidence test could be provided. The final large image was composed by stream stitching process because of imposed memory limitations. The stitches between adjacent images were hidden by meandering technique. The methodology is described and several aspects are discussed in this paper. Our experience, gained from practical application of our system in the department of tissue pathology, supports the claim that the system is robust, fast and accurate.

Introduction
We were acquiring high-resolution 2D color images from optical microscope by composing smaller images (fields) of specimen. This solution enables us to acquire every field at the limits of optical microscope, namely at high magnification and resolution possible. The fields were then acquired with small overlap providing information for correct alignment of fields. Owing to thickness of specimen and almost-perpendicularity of specimen plane, the system had to refocus on every field. Due to this and the imperfection of optics, the overlaps of adjacent fields were not exactly identical and some smoothing had to be performed while stitching fields. Registration was even more complicated because of the structure of specimen. There were fields displaying solely background due to a hole in the specimen or non-convex shape of it.

Method
After the acquisition, all fields were stored into separate files and ready for further three-step processing. The goal was to determine a good order for processing of fields as well as to register adjacent fields resulting in global pixel coordinates attached to every field. Coordinates were then used in the final third step where stream stitching process was creating final large image.

Registration applicability measure
The measure should estimate the quality of parts, high value for parts with tissue in overlap, low value for parts with background in overlap, tissue resembles nonconstant texture, background resembles solid region of almost constant color.

Modified Prim algorithm
We use the vertices processing order of regular Prim algorithm, good for determination of global coordinates of images.

Context of given problem
- acquire grid of color images of given specimen using an optical microscope
- acquire each image at the best resolution and quality possible
- compose images together by stitching them into a single large image
- postprocess the composition for subsequent processing
- for example:
  - annotation by expert in the field of tissue pathology
  - store annotated images into a database

Illustration of stitching problem

Specifications
- 24-bit color images, each of dimension 1282 x 972 pixels
- typically 3-10000 images in one grid (per one specimen)
- 0.35-0.5x0.5mm of image data to be processed
- acquired with 5x-10x overlap of adjacent images
- overlaps do not contain exactly identical data
- exact positions of images (as the pixel level resolution) must be determined to allow resolution of moving stage
- only translation of images is expected
- memory limitations

Processing steps overview
- good order for registration must be determined
- registration of adjacent images
- shift vectors between adjacent images
- determination of global coordinates of each image
- image stitching, according to global coordinates
- large composed image

Illustration of terms

7/overlap:
X = 0.05X - R
Y = 2.00Y

Registration is searching for best-fit alignment
- alignments are sought only within the grey area X < Y
- a part area is used for evaluating alignments
- alignment is given by shift vector (stick-anvou)

Graph representation

Registration method
- voxel-based registration methods
- best results with SAVD and CR (both fast and robust to noise)
- optimized search the X, Y area greatly improves the overall computational demand
- we observed Gaussian distribution of shift vectors
- sounds with expected movement behavior of stage with specimen
- default vectors established for both registration directions
- default vectors used instead when misalignment occurred

Stitching and final composition
- cross-fading over memory stripe used for stitching
- raw is assembled first, then rows together
- two grid lines of images must be stored in memory

Example of image mosaic

1. 10 rows, 9 columns
2. 24-bit image
3. 7% overlap
4. parts rescaled 24MB
5. stitching needed max 240MB
6. up to 4GB of total image size

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