

Normalized Covariance Image Stitching Technique for Rigid Registration of Microscope Tiles

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I. INTRODUCTION

Modern microscopes can achieve very high magnification to distinguish fine biological structures. However, high magnification also captures only a small field of view since the imaging sensor sizes are limited. To obtain a fine detailed image that spans a large area, a technique called tiling or stitching is applied. A series of images are taken in a grid pattern where adjacent image sections have some overlap. Microscope stage positions stored along with each image can be used to stitch these images together into a large mosaic. However, the stage position alone is insufficient to make a final reconstruction because of drift of the position encoders and deformation of the specimen. Each overlap section's position needs to be fine-tuned to reconstruct a seamless mosaic from each individual image.

II. METHODS

Normalized covariance is a measure of the pixel-wise similarity of two images. This can be used to determine the best overlap position of two adjacent images taken from a single microscope stage. The parameter space for inferring general transformations between two images is too large to be searched using a normalized covariance measure. However, the microscope acquisition process is constrained by mechanics and thus only a subset of transformations are possible. The microscope stage only moves at right angles (it does not tip or rotate), there is minimal time between images (taken with no delay between sections), the specimen will not shear between sections (objects deform in a lateral motion), and the physical size represented by each pixel remains constant. So for the image tiling problem only translational motion needs to be considered.

Adjacent images are first evaluated by calculating the normalized covariance of the two overlapping regions at position given by the microscope. In the case of the image stitching challenge, this means grid position (row,column) and percent overlap. The normalized covariance is obtained by finding the mean of each overlapping section,

$$\mu = \frac{\sum_{x,y,z} I}{|I|}, \quad (1)$$

where $|I|$ indicates the number of pixels in the overlap section. The standard deviation of the same sections are calculated by,

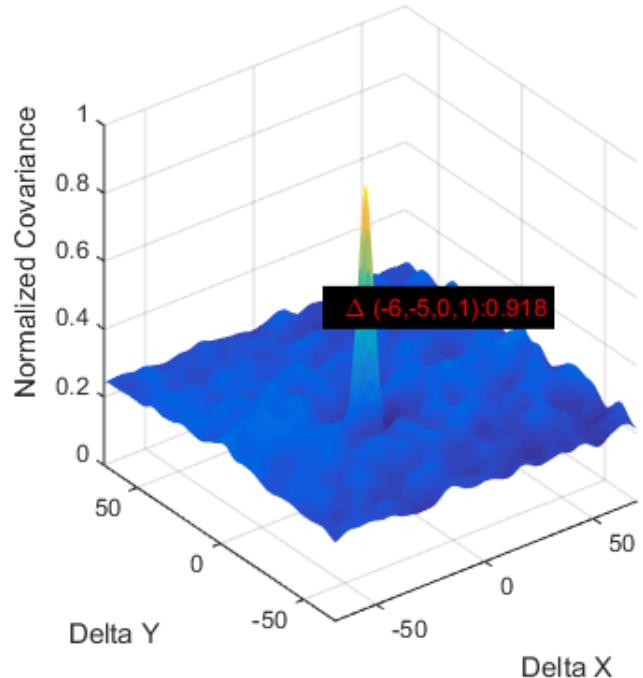


Fig. 1. A surface plot showing the normalized covariance at each change in overlap position. The $(0, 0)$ position is the initial overlap given by the microscope stage position. The maximal point corresponds to a $(-6, -5, 0, 1)$ (x, y, z, λ) , where λ represents the channel, change from this initial position. It is clear that the images match better at this point given a normalized covariance of 0.918 compared to the ≈ 0.23 everywhere else.

$$\sigma = \sqrt{\frac{1}{|I|} \sum_{i=1}^{|I|} (I_i - \mu)^2}. \quad (2)$$

Each overlapping region has its respective mean subtracted. The new mean subtracted images are multiplied together and the result is summed (numerator in eq. 3). The standard deviations, calculated in eq. 2, are multiplied together and used to normalize the sum (denominator in eq. 3).

$$\text{normcov}(A, B) = \frac{\sum (A - \mu_A) \times (B - \mu_B)}{\sigma_A \times \sigma_B} \quad (3)$$

The algorithm searches within ± 100 pixels in x and y beginning from $\Delta(0, 0)$, the starting offset indicated by the microscope stage encoders. A normalized covariance score is calculated for each possible offset between the two images and stored in a matrix. These scores for images *img_Phase_r001_c001.tif* and *img_Phase_r001_c002.tif* are plotted as a 3-D surface in fig. 1.

It is clear from the plot that there is a single point that is optimal under this criterion. We have found this to be a global optimum, meaning that if stage position is given and both images are allowed to pass over one another in both x and y , the same offset is found. The peak in this instance is at $(-6, -5)$ with a normalized covariance of 0.918, which means that the best overlap occurs at a position that is six pixels less in x and five pixels less in y than the initial position. Fig. 3 shows the two entire images with their intensities normalized between $[0, 1]$ for better reproduction here. The red lines indicate the initial overlap position in each and the green lines show the best overlap position. The two strips between the images are the regions of interest (ROIs) at the best position.

All adjacent images are evaluated and stored as a graph where each image is a node and there are edges between images that have some overlap. Each edge in this graph has a weight equal to the normalized covariance score (eq. 3). From this graph, we create a max spanning tree. When an edge is dropped that would disconnect a node from the graph, the second lowest is then dropped. This is done until there are no more edges that can be dropped and keep a connected graph, see fig. 4. When this is complete, a root image is selected (this can be any image) and each edge is traversed creating a new image position. This graph traversal accumulates the offset necessary for each image to be registered to its neighbor relative to the root image. These offsets are then applied to the original stage positions and a mosaic created, fig. 5.

III. CONCLUSION

We have found that this technique works for 2-D and 3-D images. Multiple channels can also be beneficial where the maximum covariance across all channels can be used as an edge weight. A final mosaic has been created from the challenge dataset in fig. 5. The phase contrast channel has been represented in black and white where the CY5 fluorescence channel was overlaid in green. The variance in brightness is most likely due to the light falloff of the given objective and could be accounted for during reconstruction. This reconstructed image has no blending applied other than to choose the brighter section where there is an overlap. Even without blending techniques, the seams are difficult to find when zoomed in, fig 2. Fig. 2 shows a colony of cells that are situated on the border of four images. Red lines have been included to add emphasis to where the seams exist. We have run this technique on 3-D images with the same results [1].

The program to register these images was written in MATLAB and runs on a Windows 8.1 computer.

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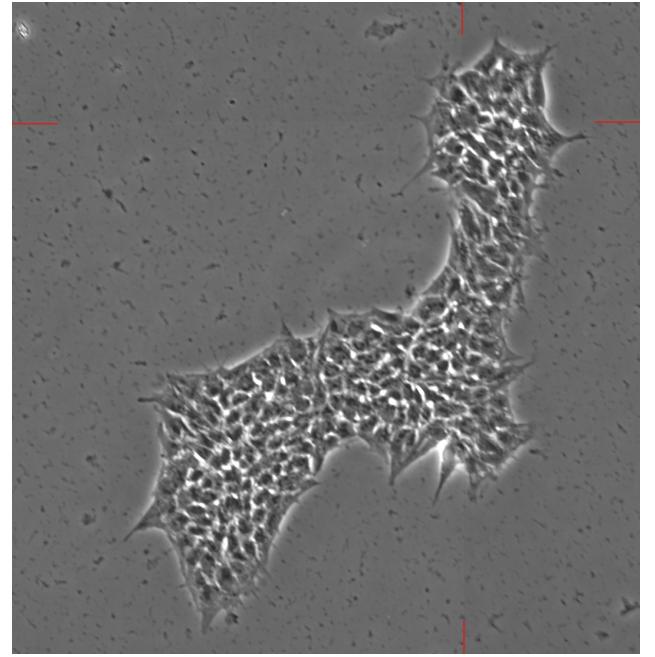


Fig. 2. A single colony of cells that spans four images: *img_Phase_r007_c009.tif*, *img_Phase_r007_c010.tif*, *img_Phase_r008_c009.tif*, and *img_Phase_r008_c010.tif*. The red lines represent the seams. There was no blending applied, the brightest of the two overlapping sections was chosen and placed in the resulting mosaic. Note that artifacts are within one pixel and could only be improved by a sub-pixel blending.

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REFERENCES

- [1] E. Wait, M. Winter, C. Bjornsson, E. Kokovay, Y. Wang, S. Goderie, S. Temple, and A. Cohen, "Visualization and correction of automated segmentation, tracking and lineage from 5-D stem cell image sequences," *BMC Bioinformatics*, vol. 15, no. 1, p. 328, 2014.

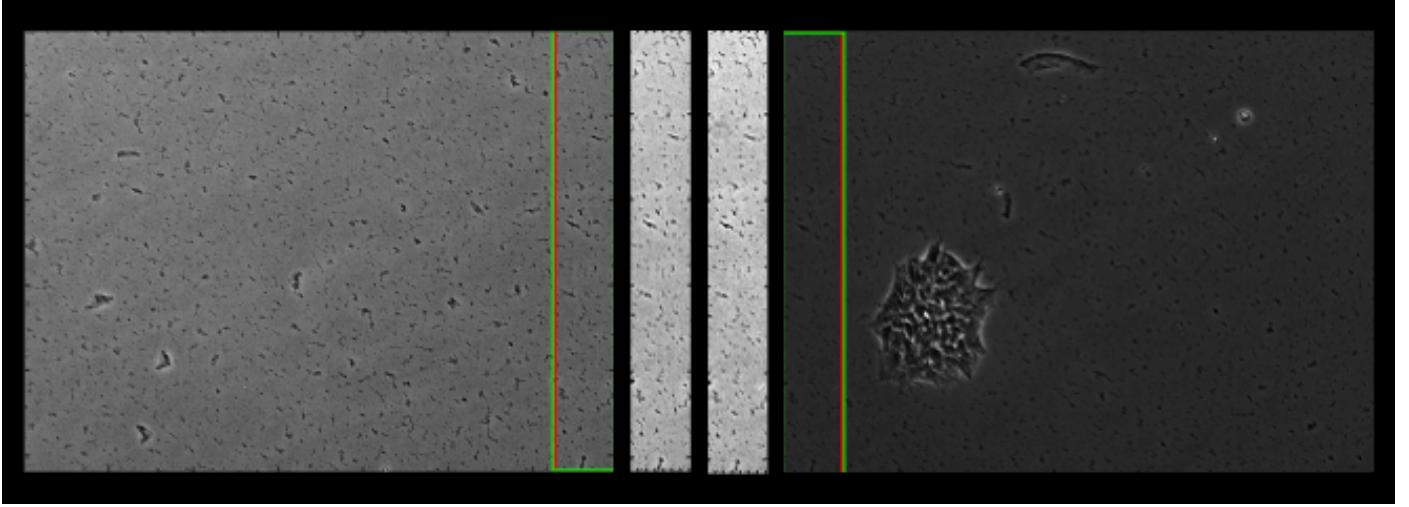


Fig. 3. The overlap of images *img_Phase_r001_c001.tif* and *img_Phase_r001_c002.tif* are shown on the left and right, respectively. The red lines represent the initial overlap given the stage position. The green line represents the maximal normalized covariance position. The two strips in the middle are the regions of interest outlined by the green lines in their respected images. It is clear that these two overlap regions are close to identical.

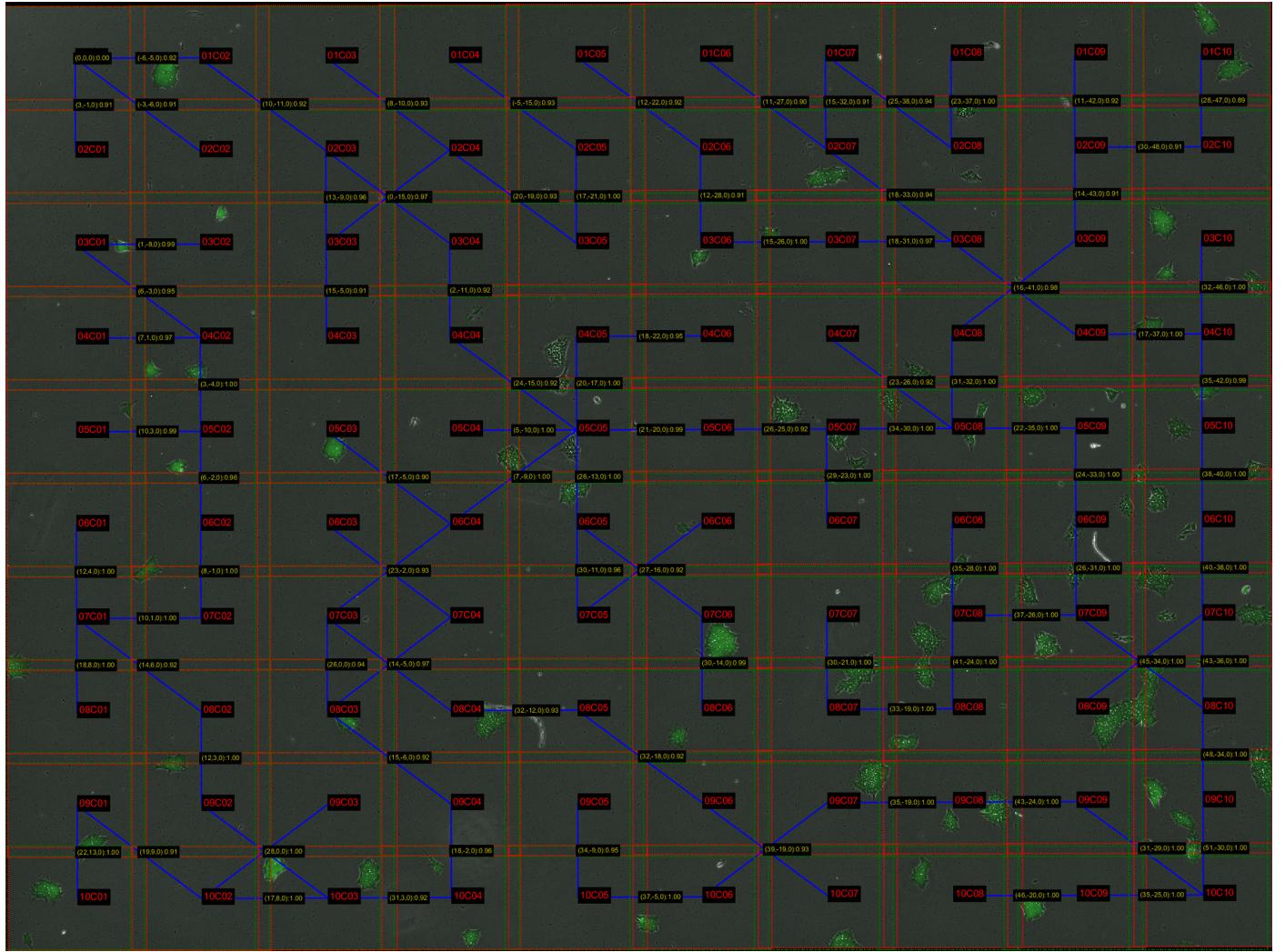


Fig. 4. The max spanning tree that preserved the highest normalized covariance scores across edges is represented in blue. The global change in position and normalized covariance score is in gold. The red numbers correspond to the image location where the first two digits are the row and the last two digits are the column, which also correspond to the file name, e.g. *img_Phase_r001_c001.tif*. The green dotted lines are the initial location of the images given by position and percent overlap. The red dotted lines are where the image was ultimately positioned to make the final montage.

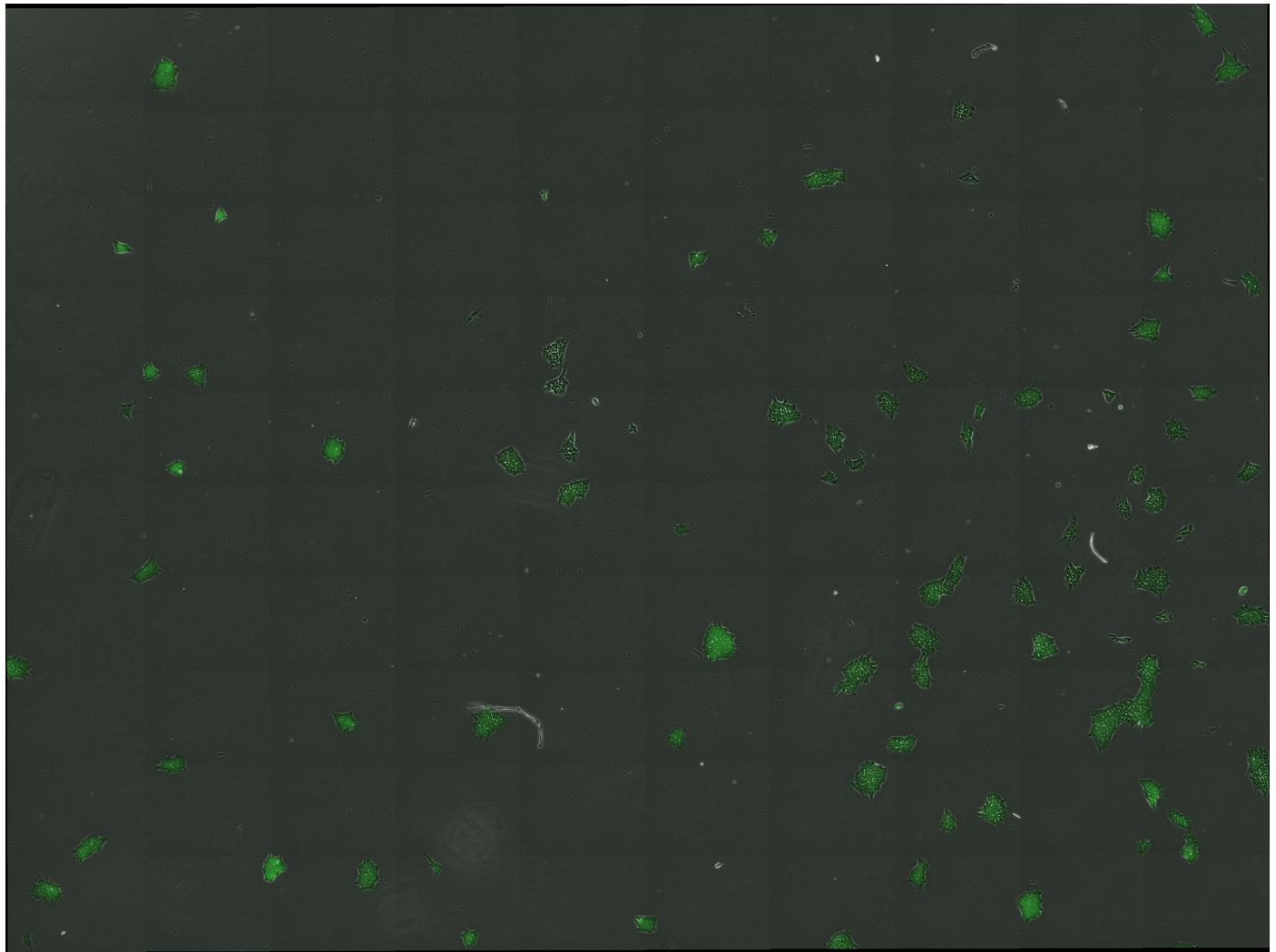


Fig. 5. The resulting image is created using the max spanning tree in Fig 4. Phase contrast is in black and white and the fluorescent channel, CY5, has been overlaid in green. Artifacts of edge brightness is typically due to the light falloff inherent in the objective and can typically be mitigated by sampling over a smaller section of the projected image.