Multi-focus Image Fusion for Confocal Microscopy using U-Net Regression Map

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Dura Mater Brain Tissue

Female 6 week (tissue cut in middle)

Scattered sampling
Dura Mater Brain Tissue
Microscopy Imaging of Dura Mater

- Confocal microscopy image stack goes from Bone (first slice) towards Dura (last slice)
- 20X, 80 stacks [numbers & letters], 512 x 512, 100 to 500 slices
- 20X, One stack (200 slices) and **two channels** takes 25 minutes
- Staining: vessel red (594nm), lymphatics green (488nm) and combined channels
- Sampling resolution, 20X/0.45: 1 or 7 micron in z, 0.672 x 0.672 resolution in x and y; for oil lens 60X (0.212 x 0.212 x 0.896 micron)
- 56GB of storage for one tissue sample, scattered stacks takes 2 weeks
- Grid sampling takes 1 month (Ctrl_6, 20X, 224 stacks, 180GB = 60GB raw+ 120GB TIFF)
- Green fluorescence channel images lymphatics structures: sinusoids either pure (no blood vessels) or has one blood vessel or multi blood vessels
- To reveal details of these complex anatomy, multi-focus fusion is important
Motivation and Objectives

• Confocal microscope is often used to image the dura mater tissues.
• Each single focus image captures details of specimen regions that lie close to its focal plane, remaining regions imaged with poor contrast.
• Multi-focus image fusion aims to create a single sharp and detailed composite image that captures the essential 3D structure information in a set of single focus images
• Fusion is necessary to characterize spatial relationship between dura mater blood vessel and lymphatic vascular structures
• To overcome the limitations of derivative based fusion methods
Challenges of Multi-focus Image Fusion

- Multi-focus image fusion to capture the highly complex structures of the blood and lymphatic microvasculature is challenging due to
  - Contrast level variation caused by lectin stain diffusion
  - High variance in vessel intensity
  - Fine microvascular structures
  - Leakage of stain from vessels
  - Variable depth of focus, and
  - Complex 3D structure
Related Work

- Existing approaches can be broadly categorized as:
  (i) Transform domain-based,
  (ii) Spatial domain-based, and
  (iii) Neural networks-based

- Transform domain approaches first transform source images into a desired domain, then fuse transform coefficients, and finally apply inverse transform to obtain fused image [3]
- Spatial domain approaches rely on focus measures based on first and second order image derivatives such as energy of gradients, sum of modified Laplacian, energy of Laplacian[5]
- These focus measures highly depend on block sizes and perform poorly at object boundaries
- The neural network-based fusion approaches exploit learned, data-driven features to produce fusion results, that often lead to reduced fusion artifacts[6]
- Our approach relies on a novel vesselness likelihood index computed using a U-Net convolutional neural network trained to segment vascular networks in microscopy images.

Proposed Architecture: DUAL U-Net Streams

- U-Net applied independently to each channel of multi-focus images in confocal microscopy Z-stack to produce set of vesselness likelihood maps.
- Composite fused image constructed by selecting focus layer for each pixel with maximum vesselness likelihood value.
Quality Metrics

- PIQE: Perception based Image Quality Evaluator [19]
  - A low score value indicates high perceptual quality and high score value indicates low perceptual quality.
  - Calculate Mean Subtracted Contrast Normalized (MSCN) coefficient.
  - Then divide image into nonoverlapping blocks and identify distorted and non distorted blocks using MSCN.
  - PIQE score is mean of scores in distorted blocks.

- NIQE: Naturalness Image Quality Evaluator [20]
  - Quality is expressed as distance between a multivariate Gaussian (MVG) fit of Natural Scene Statistics (NSS) features extracted from test image, and MVG model of quality aware features extracted from set of natural images.
  - Lower values of score reflect better perceptual quality.

- BRISQE: Blind/Referenceless Image Spatial Quality Evaluator [21]
  - Predicts BRISQUE score by using a support vector regression (SVR) model trained on an image database containing compression artifacts, blurring, and noise.

- SSIM: Structural Similarity Index (SSIM) [22]
  - Quality assessment index based on computation of three terms: luminance, contrast, and structure.


Experimental Results

- Visibility and shape of microvascular structures significantly improved and revealed
- Noisy background problem and scattered disconnected component problem of multiscale Hessian fusion have been addressed

**Experimental Results**

<table>
<thead>
<tr>
<th>Input Stacks</th>
<th>Independent red channel fused_ Hessian</th>
<th>Independent green channel fused_ Hessian</th>
<th>Combined fused_ Hessian</th>
<th>Independent red channel fused_ U-Net</th>
<th>Independent green channel fused_ U-Net</th>
<th>Combined fused_ U-Net</th>
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<tbody>
<tr>
<td>Ctrl_Lyve1-488 _SBA-597_injct _20x-(4)</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
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<tr>
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<tr>
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<td><img src="image23.png" alt="Image" /></td>
<td><img src="image24.png" alt="Image" /></td>
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Quantitative Comparison

- Quality metrics for fifteen Z-stacks from different parts of whole dura mater
- Average SSIM score of 0.75, indicating structural differences between two fusion.
- For each quality metrics our MCFU-Net fusion resulted in lower scores, indicating better image quality
- PIQUE score within range of 0 to 20, image perceptual quality marked as ‘Excellent’
- Proposed MCFU-Net fusion for all experiments fell in the ‘Excellent’ category
Conclusions

- Presented a new approach for multi-focus image fusion of confocal microscopy images that includes extremely complex structures of blood vessels, and lymphatics like structure.
- Classical derivative-based methods, such as multi-scale Hessian based image fusion, fail to capture the structural complexities of these images, particularly for the green channel corresponding to lymphatics like structures.
- Our proposed solution, MCFU-Net, relies on a novel vesselness likelihood index computed using a U-Net convolutional neural network trained to segment vascular structures in microscopy images.
- The learned and data-driven nature of the MCFU-Net fusion approach allows us to better capture the complexities of microvascular structures, and other staining and imaging characteristics.
- Future work is to do multi-class segmentation using the fused images to identify blood vessels and lymphatic like structures.