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

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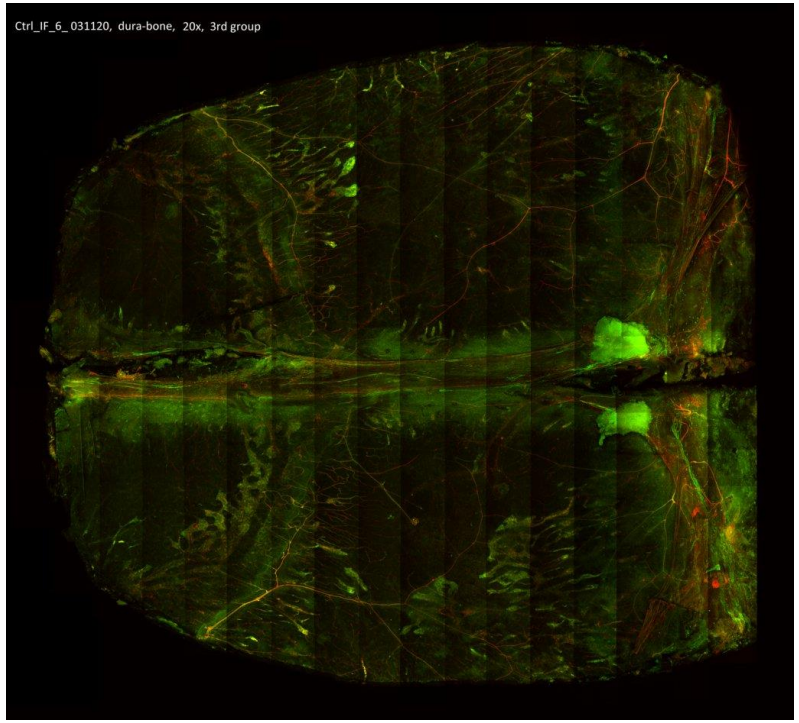
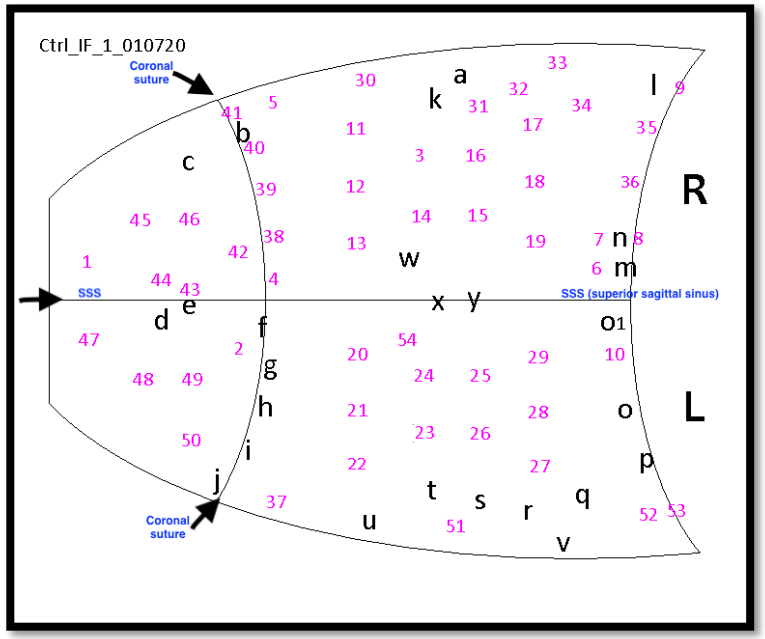
Dura Mater Brain Tissue

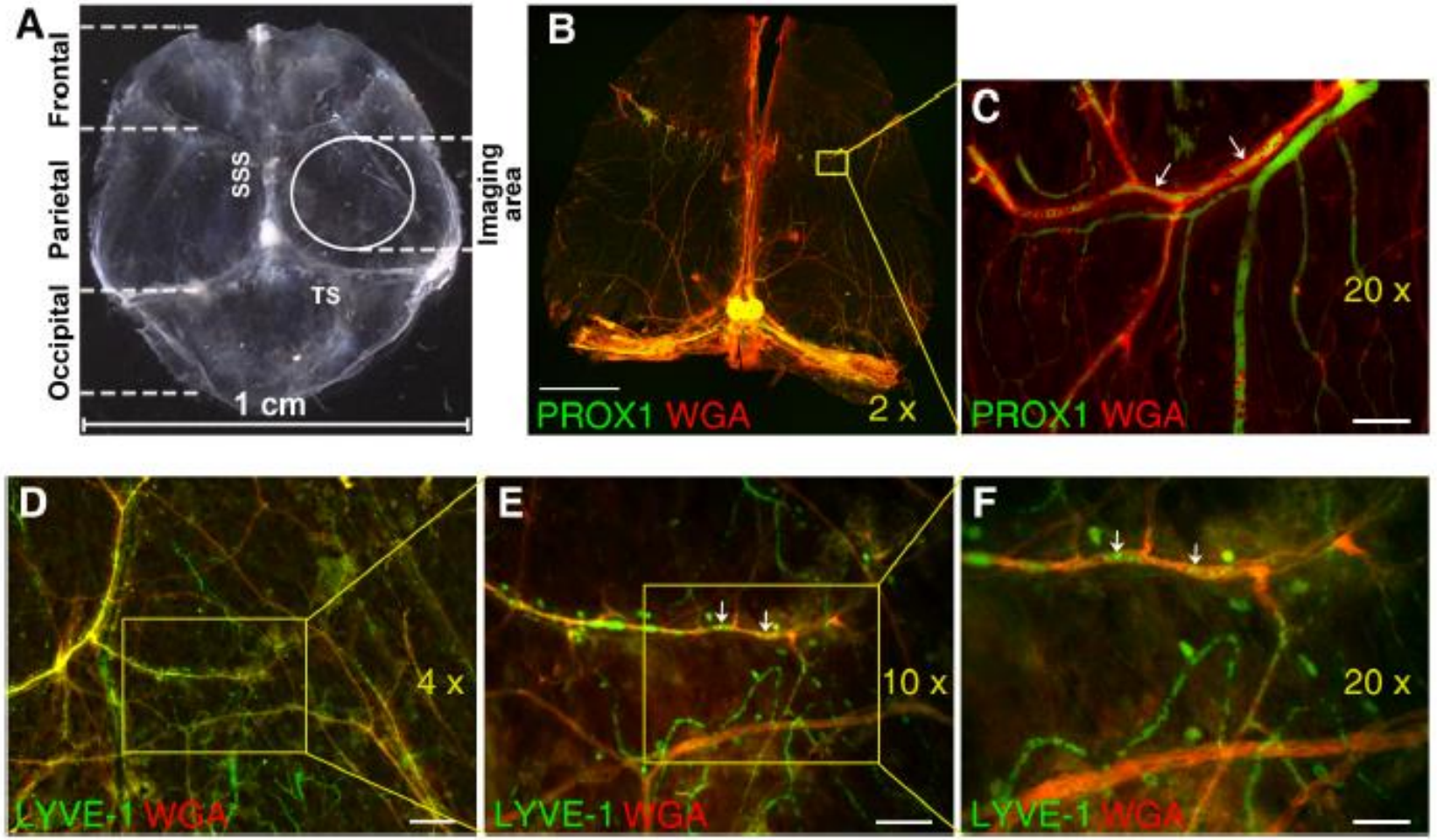


Female 6 week (tissue cut in middle)



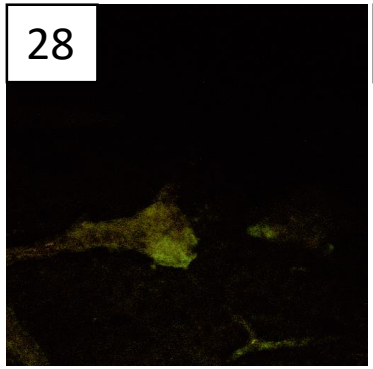
Scattered sampling

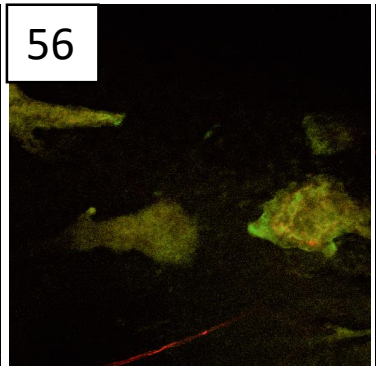
Dura Mater Brain Tissue

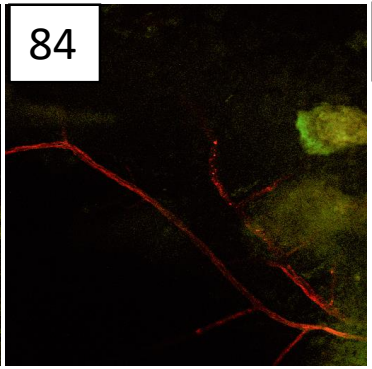


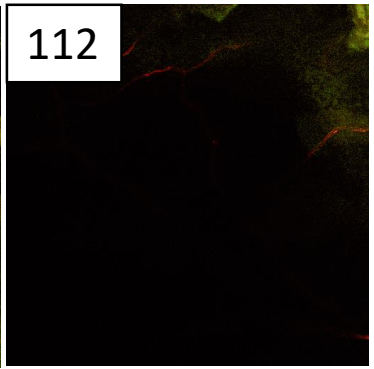
Microscopy Imaging of Dura Mater

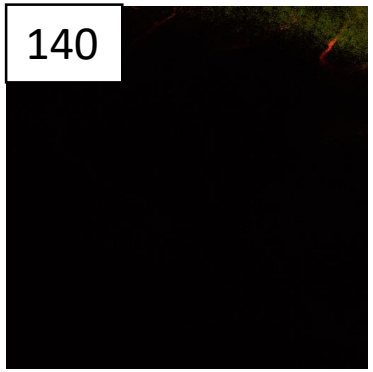
Single Focus Images



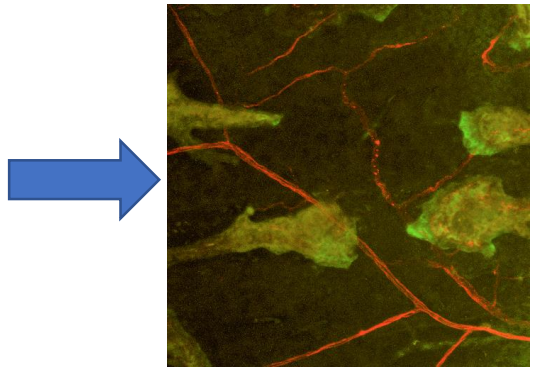




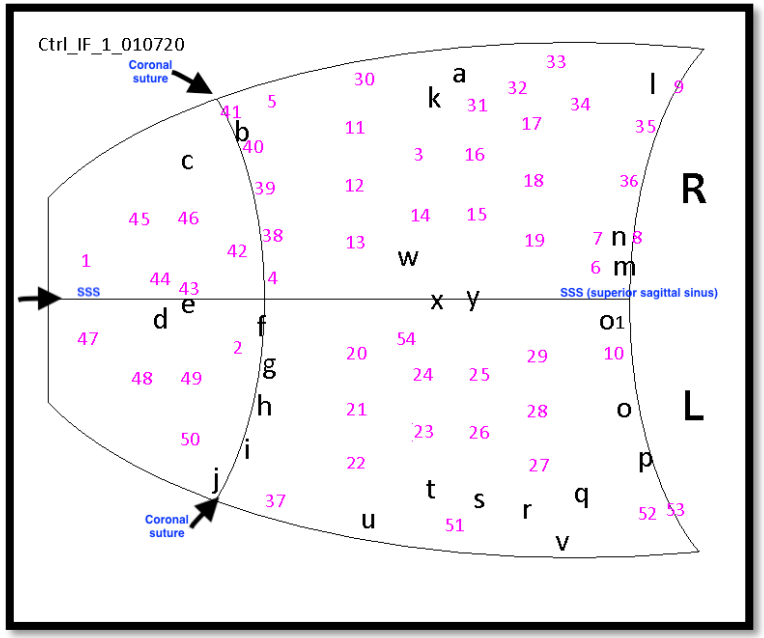




Max Intensity Projection

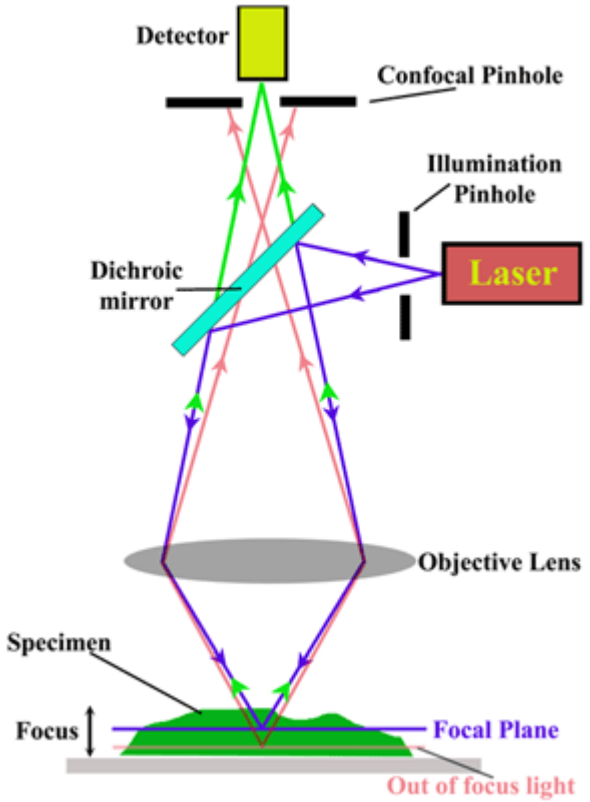


- Confocal microscopy image stack goes from Bone (first slice) towards Dura (last slice)
- 20X, 80 stacks [numbers & letters], 512 x 512, 100 to 500 slices
- 20X, One stack (200 slices) and **two channels** takes 25 minutes
- Staining: vessel red (594nm), lymphatics green (488nm) and combined channels
- 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



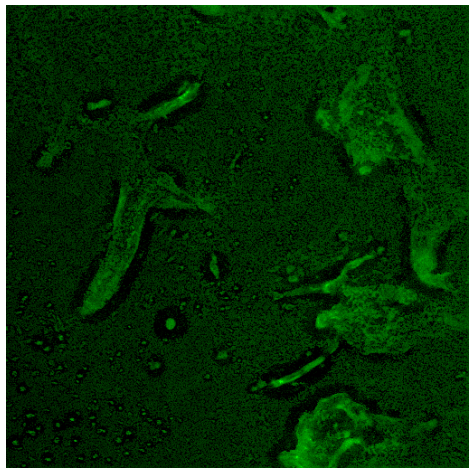
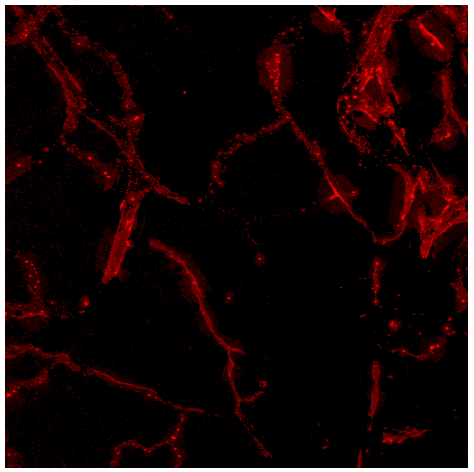
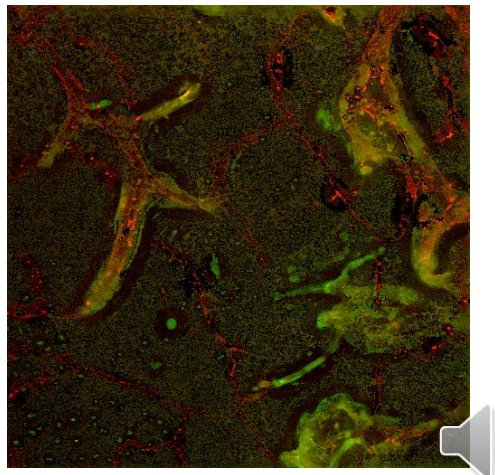
Motivation and Objectives

- 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

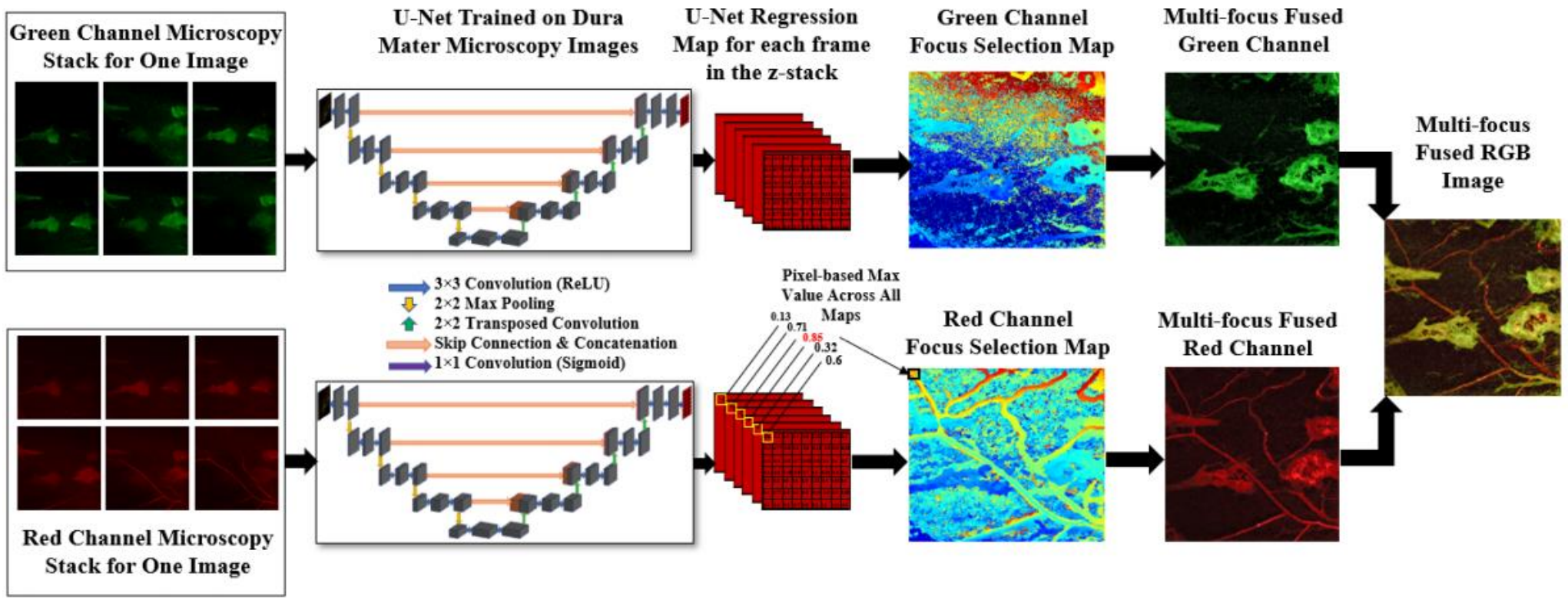
Related Work

- 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.

[3] Y. Li, Y. Sun, X. Huang, G. Qi, M. Zheng, and Z. Zhu, “An image fusion method based on sparse representation and sum modified-laplacian in nsct domain,” Entropy, vol. 20, no. 7, pp. 522, 2018.
[5] J. J. Lewis, R. J. OCallaghan, S. G. Nikolov, D. R. Bull, and N. Canagarajah, “Pixel- and region-based image fusion with complex wavelets,” Information Fusion, vol. 8, no. 2, pp. 119–130, 2007.
[6] Y. Liu, X. Chen, H. Peng, and Z. Wang, “Multi-focus image fusion with a deep convolutional neural network,” Information Fusion, vol. 36, pp. 191–207, 2017.

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

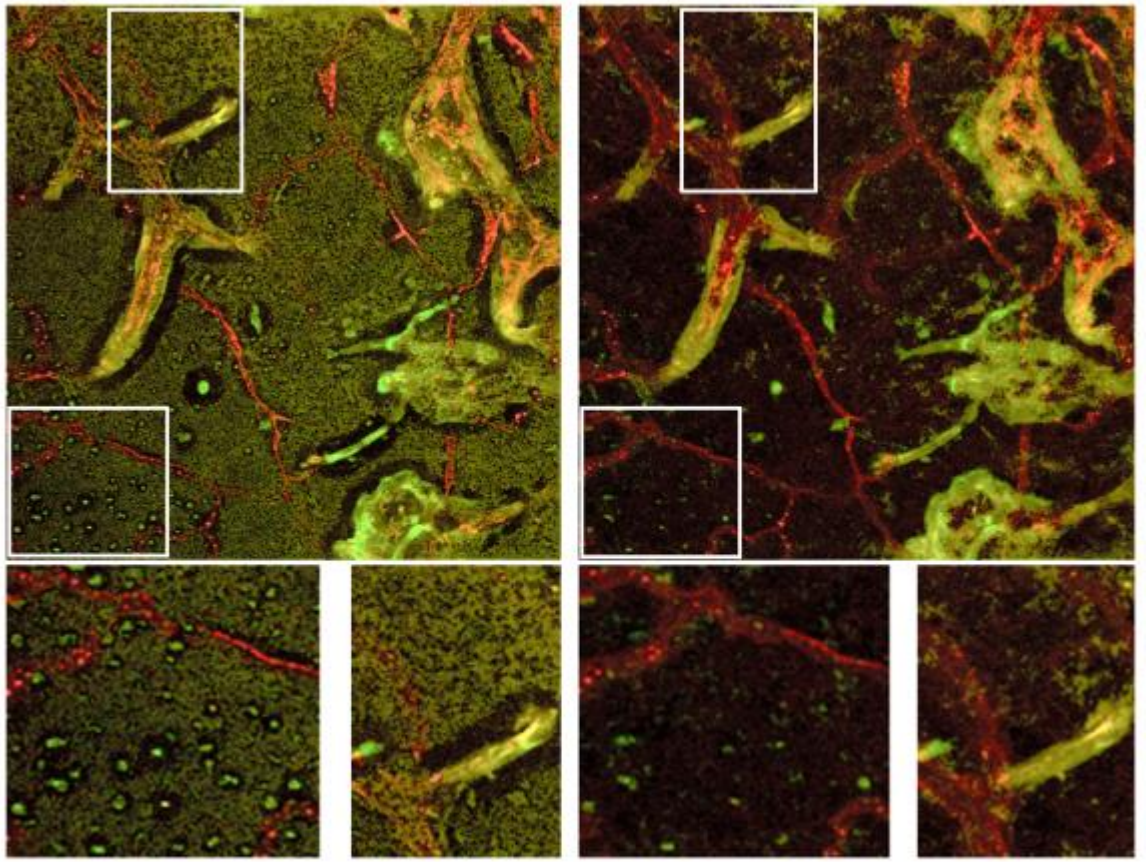
- PIQE: Perception based Image Quality Evaluator [19]
  - A low score value indicates high perceptual quality and high score value indicates low perceptual quality.
  - Calculate Mean Subtracted Contrast Normalized (MSCN) coefficient.
  - Then divide image into nonoverlapping blocks and identify distorted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

[19] N. Venkatanath, D. Praneeth, Bh. M. Chandrasekhar, S. S. Channappayya, and S. S. Medasani. "Blind Image Quality Evaluation Using Perception Based Features", In *Proceedings of the 21st National Conference on Communications (NCC).* Piscataway, NJ: IEEE, 2015.
[20] Mittal, A., R. Soundararajan, and A. C. Bovik. "Making a Completely Blind Image Quality Analyzer." *IEEE Signal Processing Letters*. Vol. 22, Number 3, March 2013, pp. 209–212
[21] Mittal, A., A. K. Moorthy, and A. C. Bovik. "No-Reference Image Quality Assessment in the Spatial Domain." *IEEE Transactions on Image Processing*. Vol. 21, Number 12, December 2012, pp. 4695–4708.
[22] Zhou, W., A. C. Bovik, H. R. Sheikh, and E. P. Simoncelli. "Image Qualifty Assessment: From Error Visibility to Structural Similarity." *IEEE Transactions on Image Processing*. Vol. 13, Issue 4, April 2004, pp. 600–612

Experimental Results

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

Multiscale Hessian Fusion **MCFU-Net Fusion**

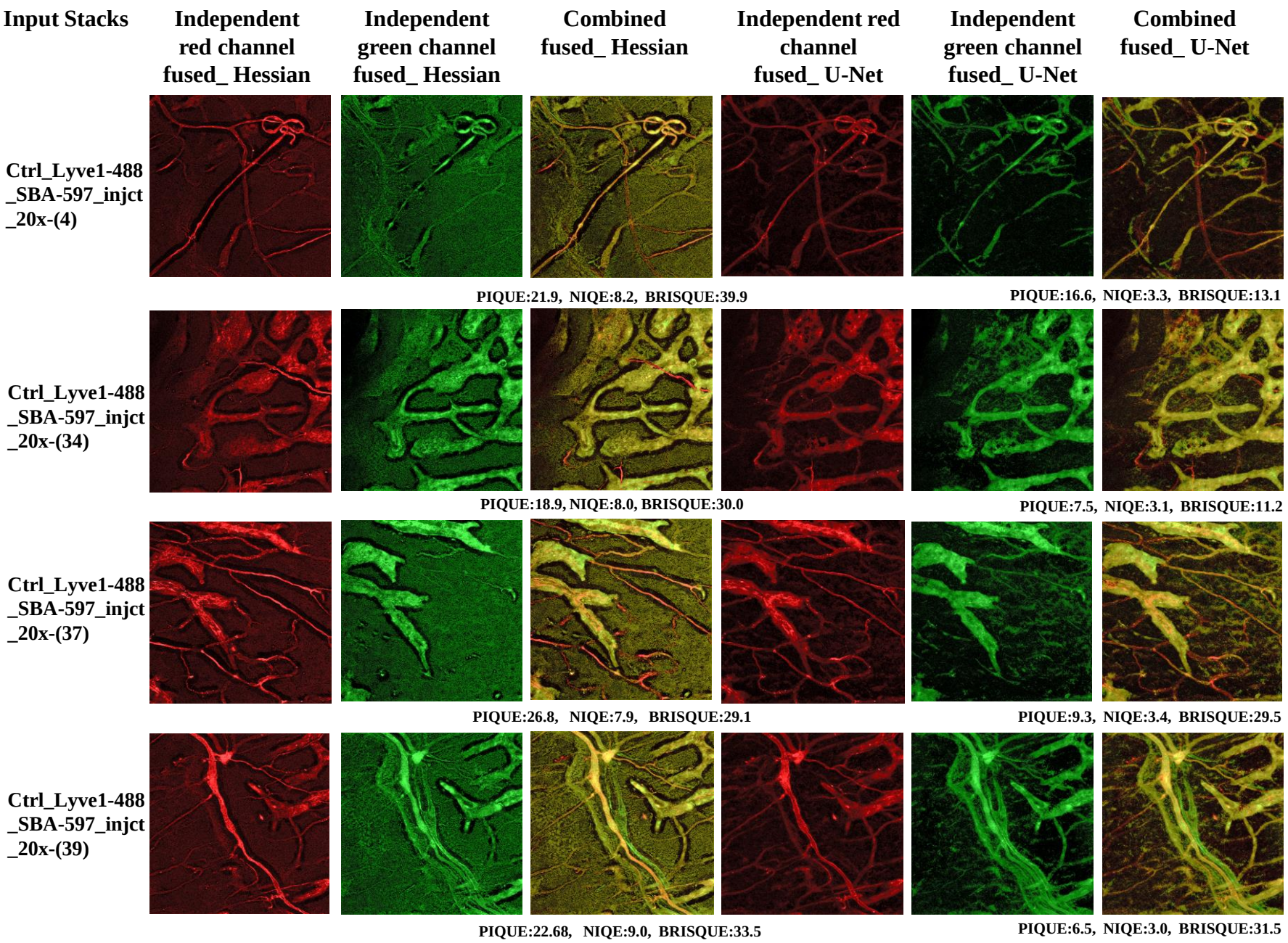


(a) Multi-scale Hessian fusion [8] PIQUE:21.9, NIQE:8.2, BRISQUE:39.9

(b) Our proposed MCFU-Net fusion PIQUE:14.6, NIQE:3.3, BRISQUE:13.2

R. Pelapur et al., "Multi-focus image fusion using epifluorescence microscopy for robust vascular segmentation," Annual International Conference of the IEEE Engineering in Medicine and Biology Society, pp. 4735-4738, 2014

Experimental Results



Quantitative Comparison

- Quality metrices 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

TABLE I: Performance comparison of quality of multi-focus image fusion

Input Stacks	PIQUE		NIQE		BRISQUE		SSIM
	Multi-scale Hessian Fusion	Proposed MCFU-Net Fusion	Multi-scale Hessian Fusion	Proposed MCFU-Net Fusion	Multi-scale Hessian Fusion	Proposed MCFU-Net Fusion	
Ctrl_Lyve1-(2) No. of slices: 157	21.97	**14.64**	8.20	**3.31**	39.96	**13.16**	0.75
Ctrl_Lyve1-(4) No. of slices: 221	26.00	**16.06**	8.64	**4.13**	39.60	**29.99**	0.69
Ctrl_Lyve1-(7) No. of slices: 234	22.04	**14.92**	8.06	**4.55**	34.70	**29.82**	0.74
Ctrl_Lyve1-(16) No. of slices: 140	25.13	**10.45**	8.63	**3.38**	41.06	**20.55**	0.75
Ctrl_Lyve1-(17) No. of slices: 164	22.29	**6.94**	8.49	**3.25**	33.56	**14.89**	0.77
Ctrl_Lyve1-(18) No. of slices: 143	23.07	**10.41**	9.88	**3.49**	31.46	**19.54**	0.77
Ctrl_Lyve1-(27) No. of slices: 130	26.89	**7.61**	9.58	**3.34**	41.39	**24.73**	0.70
Ctrl_Lyve1-(34) No. of slices: 264	18.92	**7.58**	8.01	**3.15**	30.06	**11.21**	0.80
Ctrl_Lyve1-(37) No. of slices: 267	26.83	**9.33**	7.96	**3.4**	**29.19**	29.51	0.73
Ctrl_Lyve1-(39) No. of slices: 185	22.68	**6.58**	9.02	**3.03**	33.55	**31.53**	0.74
Ctrl_Lyve1-(46) No. of slices: 138	26.77	**10.83**	8.55	**3.18**	43.11	**12.87**	0.73
Ctrl_Lyve1-(51) No. of slices: 257	20.04	**5.42**	7.98	**3.59**	**22.27**	25.11	0.80
Ctrl_Lyve1-q No. of slices: 241	22.96	**14.96**	8.40	**3.72**	31.04	**17.1801**	0.75
Ctrl_Lyve1-u No. of slices: 264	25.90	**6.47**	8.48	**3.41**	35.26	**29.22**	0.73
Ctrl_Lyve1-v No. of slices: 229	18.30	**7.35**	8.41	**3.62**	28.51	**18.32**	0.79
Average Scores	23.32	**9.97**	8.55	**3.50**	34.31	**21.84**	0.75

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