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Current Opinion in Biomedical Engineering

Brain mapping at high resolutions: Challenges and opportunities

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Abstract

Methods for imaging the architecture of the brain at high resolutions, and across large volumes, are rapidly improving. With the convergence of high-resolution datasets and new computational approaches for processing them, fully and semi-automated methods for studying the brain will soon be within reach. However, there are many challenges in developing data-driven strategies for brain mapping with images at cellular and sub-cellular resolutions. This review highlights some key challenges in building models of brain structure from imaging datasets; we describe some existing efforts to tackle these challenges and potential solutions moving forward. Finally, we discuss the need for concerted community efforts to adopt common standards and coordinate systems for brain mapping, which will enable us to achieve robust and scalable solutions that work across different brain models and can accommodate the intrinsic variability both between and within high-resolution neuroimaging datasets.

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Current Opinion in Biomedical Engineering 2019, **12**:126–131

This review comes from a themed issue on **Neural Engineering: High Resolution Cell Imaging**

Edited by **John A White** and **Xue Han**

https://doi.org/10.1016/j.cobme.2019.10.009

Keywords

Brain mapping, Microscopy and sub-cellular imaging, Histology, Machine learning.

Introduction

Ever since Brodmann defined his namesake brain areas in 1909 [56], mapping the function of the brain to its structure has been a primary goal of neuroscience research. Developing brain maps for different populations — healthy, sick, young, old, etc. — may provide us with a way of studying inter-subject neurological variability, as well as processes that affect brain function, including neurodegenerative disease, injury, and aging [41]. Reliable methods for mapping the brain may also play a significant role in personalized medicine [21] and are, therefore, important in both fundamental neuroscience and applied medical research. Magnetic resonance (MR) and other macro-scale imaging methods are popular ways to generate data that can be used to create brain maps [20,19,11,14], but high-resolution histological data are critical for generating maps that contain cellular and sub-cellular information [1,43,11,20]. Brain mapping is now a main focus of the ongoing BRAIN Initiative [10], and suites of tools have been created to facilitate comparisons across datasets and development of brain atlases based on common structural or functional features rather than the unique neuroanatomy of a single individual.

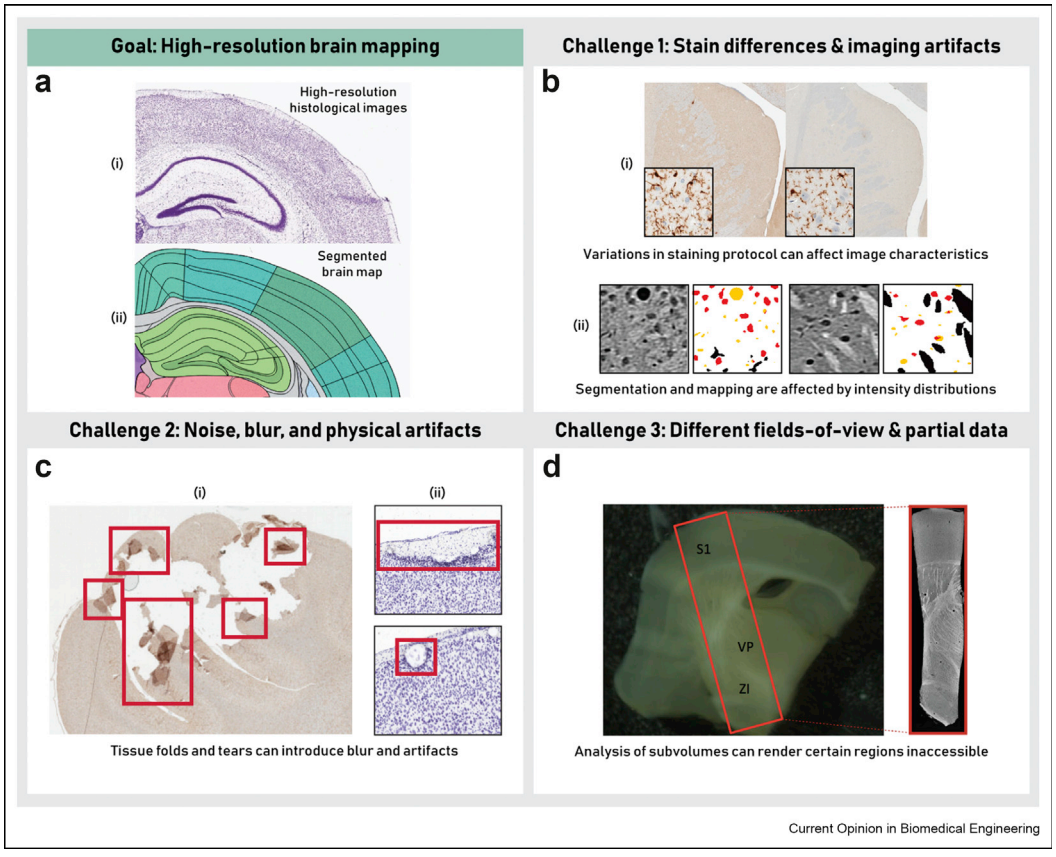
Unfortunately, the inherent variability within and between brains renders the process of defining discrete borders and comparing the brain areas of different individuals extremely difficult. Even in the absence of any pathology, no two brains are alike, and finding adequately descriptive signatures of neural structure that can allow us to faithfully compare brains is difficult. These problems are further exacerbated in high-resolution datasets, as there is an increased ambiguity in boundary definitions at the micro- and nano-scale. Additionally, high-resolution data are more susceptible to artifacts caused by variations in staining and pose the unique problem of having to deal with variable fields of view that arise from imaging different sections or slices of the brain. Consequently, there is an acute need for unbiased, accurate, and standardized methods of representing the microstructure of the brain in a way that allows us to then map and compare it across states and subjects.

In this review article, we highlight several key challenges that arise when building high-resolution maps of the brain (Figure 1) and describe how recent advances, both in machine learning and image analysis, can be leveraged to tackle these problems.

Challenges and potential solutions

When using light microscopy [52] or higher-resolution methods like expansion microscopy [50], nanoCT [54], or electron microscopy [27], slight differences in tissue handling and preparation can produce major changes in the final image. Small variations in the concentration of stains, washes, or contrast agents can result in significant differences in the intensity distribution,

Figure 1



Overview of several challenges encountered in high-resolution brain mapping. **(a)** The overall goal is to generate maps of the brain based on high-resolution histological data. **(b)** (i) Two sections of a porcine brain from the same study, showing differences in stain intensity due to small protocol variations. (ii) Images from a mouse brain demonstrate the sensitivity of segmentation algorithms to changes in image intensity distributions. **(c)** The presence of folds and tears in tissue samples can introduce unwanted artifacts to samples of (i) porcine and (ii) mouse brain. **(d)** Histological data must inherently come from subvolumes/slices of the brain, and not all brain regions can be included in a single sample.

color, contrast, and overall appearance of the sample [15] (Figure 1B). Additionally, during the process of cutting and transferring sections to slides, even minor tears and folds can add undesirable artifacts to the sample or render certain regions of interest (ROIs) inaccessible (Figure 1C). Improper device setup, inconsistencies in section thickness, and lighting variations can also result in blur and other artifacts [25]. These may be troublesome for human pathologists to deal with, but they can dramatically impede automated image analysis and reduce classifier accuracy [8]. In addition to experimental methods that simply bypass the sectioning [45,16,12] or staining [36] steps required in traditional histological preparation, computational techniques have been developed to address problems that arise as a result of process variations in either step.

Stain normalization and imaging artifacts

Two stained tissue samples, even if processed using the same staining protocol and materials, can yield images with dramatically different intensity distributions and visual properties (Figure 1B). Traditional machine learning methods of stain normalization, both supervised [23] and unsupervised [48], have been proposed to address inter-batch differences in staining. In each case, stain-specific transformations are applied to the images based on either prior knowledge or estimations of unique stain matrices that describe the dyes present. These matrices relate stain concentrations to the resulting color change and can be used to separate a red-green-blue (RGB) image into up to three channels. Images are then mapped and aligned with a target distribution. Extensions of this approach include the incorporation of spatial features to account for color variations that result from differences in cell morphology [4]. While these methods may work well, their performance often depends on knowledge of the staining process and a level of domain expertise that can be limiting.

Deep learning approaches have also been applied to the problem of stain variation. These methods include

generative adversarial networks (GANs) [17], in which one deep network attempts to generate synthetic data indistinguishable from the training set, while another network is trained to tell the difference between the two. GANs have been used for stain normalization, including in renal histopathology [9] and breast cancer [42]. Additionally, GANs have been used to perform virtual histological staining, circumventing the need to physically stain sections at all, essentially eliminating the problem of stain variation [40]. Although virtual staining is unlikely to soon replace traditional histopathology, this work holds promise for accelerating pipelines and reducing variation between labs. Style transfer has also been used as a framework to distinguish between sets of images with different stains or staining methods applied [7,42]. In this setup, particular staining conditions and appearances are treated as 'styles.' A GAN is trained to generate images with a selected style and can then be used to normalize input images to that stain's unique style.

In addition to GANs, novel neural network architectures have also been proposed as a way of dealing with stain variations [22]. Domain-adversarial neural networks, for instance, consist of a bifurcating architecture, where one branch assesses the stain and the other evaluates the actual contents of the slide [28]. Other deep learning approaches, such as variational auto encoders (VAEs) [24] and deep convolutional Gaussian mixture models, have been applied to this problem as well [55], in both cases, by generating recolored copies of the input image through nonlinear transformations learned during training.

Noise, blur, and physical artifacts

Noise, blur, and/or physical artifacts can be introduced at many different points in the imaging procedure, and these types of artifacts pose a slightly different problem than staining variations. Sophisticated models exist for denoising images and volumes; for example, [51] is a supervised, deep learning-based denoising algorithm for fluorescence microscopy data that exploits the particular nuances of the problem to train content-aware image restoration networks that perform better than classical content-agnostic approaches. On the other hand, [29] is an example of a network that successfully learns to denoise corrupt images, without ever having to look at any pairs of clean, and noisy images. It is also possible to use deep generative models such as GANs to learn to denoise images, as shown by Ref. [46], which work by finding the closest point on the GAN manifold (essentially, the most similar synthetic image) to the corrupted image.

In addition to noise, blur can also confound automated image analysis and can result from out-of-focus images or nonuniform section thickness. Classification of blurry regions using local image statistics has been successful, permitting these regions to be either left out or evaluated manually, and bypassing the need for a reference image to compare against [53]. Blur can also be a product of tissue tears, bubbles, and folds, which may arise during tissue cutting and mounting (Figure 1C). Preliminary approaches to address these types of artifacts include the segmentation of folds using image features and k-means clustering [26,37], but more recent methods make use of deep learning architectures. For instance, Ref. [3] used convolutional neural networks to extract features from an image before classification was performed by a support vector machine; classification of a single-folded patch within an image flags the image for manual inspection. U-net model architectures have also been used to perform pixel-wise segmentation of regions with blurry, folded, or damaged tissue [44]. These methods represent innovative approaches to a common problem that arises not just in neuroscience research but throughout histology in general.

Different fields-of-view or partial data

Unlike MR and other macro-scale neuroimaging methods that acquire consistent and standardized images of the whole brain, high-resolution or microscopic images can only capture a subset or subvolume of brain tissue at a time. To deal with this, these data are sometimes stitched together into a 3D volume, but in many cases, smaller subvolumes are analyzed based on the design considerations or other constraints on the imaging setup (Figure 1D). This ultimately results in certain ROIs or brain areas becoming inaccessible. Registration of a new test sample to a reference brain is one of the first steps in making a comparison [2,39], but in the absence of an exact match to the reference brain and/or labor-intensive manual annotation of ROIs, it is challenging to register and analyze the sample over large volumes. The fact that samples are collected over limited fields of view is, therefore, a key challenge in automating high-resolution brain mapping.

A seemingly intuitive solution to this problem is to interpolate or fill in the missing data in the imaged volume to allow for registration to the reference brain [47,35]. Methods used in the creation of probabilistic brain atlases, designed to capture inter-subject variations in brain architecture rather than represent a single average brain template that all other brains/ROIs need to be registered to Ref. [38], are a prospective source of ideas. Specifically, combining manual labeling approaches for building atlases [13] with methods for Bayesian inference [32], as seen in Ref. [49], is a potential method of performing automated label interpolation across samples and sections. The field of computer vision also presents a number of ways to

address the partial correspondence problem; methods based on iterative closest point and minimum distortion correspondence [6] have previously been used with some success.

Deep learning has also begun to find use in the problem of partial or missing data. Candidates include deep generative models such as GANs [33], which, in this case, condition their outputs on some aspect of the inputs, which lets them essentially 'fill in the blanks.' VAEs have also been used, for example, in Ref. [18], where the model learns a 'disentangled' representation of the image features (essentially, the model learns an interpretable set of image building blocks rather than acting as a black box), which allows for the explicable generation of synthetic data that match the existing data. Specialized loss functions like those in Refs. [5,31,34] can be used to train these types of networks to model the distribution of the data from partial, noisy, and/or heterogeneous observations and further constrain the outputs of the networks such that they are explicitly conditioned on the inputs and allow for better reconstruction. While deep learning methods for addressing partial or missing data have mostly been applied to a broad variety of nonbiological problems, they form a promising and quickly advancing toolbox for the problem of neuroimaging data interpolation.

Conclusion

Several key strengths of high-resolution histological imaging — its inherent multimodality and ability to capture fine-scale structure — can be problematic when the goal is to develop models of the brain's architecture. Even slight differences in stains or experimental procedures can have pronounced effects on the accuracy of brain mapping methods. Without intervention, blur and other imaging artifacts, as well as tissue folds and similar obstructions, can handicap the ability of automated methods and restrict access to certain ROIs. Variable fields of view pose a similar problem, limiting the space that can be accessed in a single experiment. However, these complications need not impede the use of histological images for brain mapping.

In this review, we focused on the challenges in data integration and mapping that mainly arise due to the imaging or preparation setup. Even when we are able to account for these sources of variation, however, comparing brains at high resolutions is difficult due to the inherent variability in the brain (within an area) and across subjects. A natural consequence of higher-resolution data is the possibility for greater variability between imaging experiments; anatomical structures and boundaries that seem consistent between individuals on the macro-scale level will appear drastically less so at subcellular resolutions. Fortunately, rapid advances in machine learning and image processing are helping to address the challenges in large-scale brain mapping. These methods represent innovative approaches to domain-specific problems, problems that are not typically encountered in macro-scale imaging approaches. However, as we try to answer questions that require larger sample sizes, which is not possible for any one lab to generate, it will become increasingly important to find ways to align and compare multi-modal datasets and to utilize information from other images that have fields of view not present in a single dataset of interest. With such approaches for data integration, it will be possible to tackle the types of problems described in this review.

Leveraging the volume of data being collected across labs and institutes will require a community effort to develop standard frameworks for aggregating and integrating information from different types of imaging datasets at these resolutions. One example of such an effort is the NIH BRAIN Initiative Cell Census Network, whose aim is to build a common repository for the genetic and morphological information about individual cells and cell types. However, consolidation has proven to be difficult due to the wide range of preparation methods and the various types of information represented by these different datasets. This underscores our need for standards and for communities that are willing to work together to build large datasets greater than the sum of their parts, to fully realize the potential of high-resolution brain mapping.

Definitively mapping the brain's multitude of functions to its complex structure is a significant and ambitious objective. The implications for biomedicine are clear, as are the ramifications such an achievement would have for our fundamental understanding of the human brain. Achieving this lofty goal will likely require integration of experimental and computational methods. Rapid advances in both domains provide new opportunities to make progress on this problem, despite the inherent difficulty of working with histological images. In the century since Brodmann's analysis of Nissl-stained sections led him to define the brain areas that bear his name [56], these types of images have continued to represent a critical piece of the brain mapping puzzle.

Conflicts of interest statement

Nothing declared.

Acknowledgements

The authors would like to thank Susan Margulies for the data presented in Figure 1(b)(i) and 1(c)(i) and Judy Prasad for the X-ray microCT images in 1(d). The images presented in Figure 1(a), 1(b)(ii), 1(c)(ii) are selected from the Allen Mouse Brain Atlas [30].

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Atzeni A, Jansen M, Ourselin S, Iglesias JE: **A probabilistic model combining deep learning and multi-atlas segmentation for semi-automated labelling of histology**. *Medical Image Computing and Computer Assisted Intervention – MICCAI 2018* 2018:219–227.

2. Avants BB, Tustison NJ, Song G, Cook PA, Klein A, Gee JC: **A reproducible evaluation of ANTs similarity metric performance in brain image registration**. *Neuroimage* 2011, **54**: 2033–2044.

3. * Babaie M, Tizhoosh HR: *Deep features for tissue-fold detection in histopathology images*. 2019. arXiv preprint, arXiv:1903.07011.
The authors compare five pre-trained CNNs of different depths as feature extractors to characterize tissue folds in histopathological images. They then train an SVM classifier on top of the extracted features to perform classification for diagnosis with much success.

4. Bejnordi BE, Litjens G, Timofeeva N, Otte-Höller I, Homeyer A, Karssemeijer N, van der Laak JA: **Stain specific standardization of whole-slide histopathological images**. *IEEE Trans Med Imaging* 2015, **35**:404–415.

5. Bora A, Price E, Dimakis AG: **AmbientGAN: Generative models from lossy measurements**. In *International conference on learning representations (ICLR)*; 2018.

6. Bronstein AM, Bronstein MM: **Not only size matters: regularized partial matching of nonrigid shapes,**. In *2008 IEEE computer society conference on computer vision and pattern recognition workshops*. IEEE; 2008:1–6.

7. Cho H, Lim S, Choi G, Min H: **Neural stain-style transfer learning using GAN for histopathological images**. *arXiv:1710.08543v2 [cs.CV]* 2017.

8. Ciompi F, Geessink O, Bejnordi BE, de Souza GS, Baidoshvili A, Litjens G, van Ginneken B, Nagtegaal I, van der Laak J: **The importance of stain normalization in colorectal tissue classification with convolutional networks**. In *IEEE 14th international symposium on biomedical imaging*; 2017.

9. de Bel T, Hermsen M, Kers J, van der Laak J, Litjens G: **Stain-transforming cycle-consistent generative adversarial networks for improved segmentation of renal histopathology**. *Proc Mach Learn Res* 2019, **102**:151–163.

10. Devor A, Bandettini PA, Boas DA, Bower JM, Buxton RB, Cohen LB, Dale AM, Einevoll GT, Fox PT, Franceschini MA, *et al.*: **The challenge of connecting the dots in the brain**. *Neuron* 2013, **80**:270–274.

11. * Ding S-L, Royall JJ, Sunkin SM, Ng L, Facer BA, Lesnar P, Guillozet-Bongaarts A, McMurray B, Szafer A, Dolbeare TA, Stevens A, Tirrell L, Benner T, Caldejon S, Dalley RA, Dee N, Lau C, Nyhus J, Reding M, Riley ZL, Sandman D, Shen E, van der Kouwe A, Varjabedian A, Wright M, Zollei L, Dang C, Knowles JA, Koch C, Phillips JW, Sestan N, Wohnoutka P, Zielke HR, Hohmann JG, Jones AR, Bernard A, Hawrylycz MJ, Hof PR, Fischl B, Lein ES: **Comprehensive cellular-resolution atlas of the adult human brain**. *J Comp Neurol* 2016, **524**: 3127–3481. Available: https://onlinelibrary.wiley.com/doi/abs/10.1002/cne.24080.
A high-resolution, manually segmented human brain atlas is constructed through annotation of structural MRI, DWI tractography, and histologically-stained images of 1356 slices from a single brain. At cellular resolution (1um), this atlas is annotated with 862 structures with finely delineated features.

12. Dyer EL, Roncal WG, Prasad JA, Fernandes HL, Gürsoy D, De Andrade V, Fezzaa K, Xiao X, Vogelstein JT, Jacobsen C, *et al.*: **Quantifying mesoscale neuroanatomy using x-ray microtomography**. *Eneuro* 2017, **4**.

13. Evans A: **Three-dimensional correlative imaging: applications in human brain mapping**. In *Functional neuroimaging: technical foundations*; 1994:145–162.

14. Evans AC, Kamber M, Collins D, MacDonald D: **An mri-based probabilistic atlas of neuroanatomy**. In *Magnetic resonance scanning and epilepsy*. Springer; 1994:263–274.

15. * Farhoodi R, Lansdell BJ, Kording KP: **Quantifying how staining methods bias measurements of neuron morphologies**. *Front Neuroinf* 2019, **13**:36.
Studies and quantifies the bias introduced by different staining methods used in imaging by comparing their resultant extracted neuron morphologies.

16. Glaser AK, Reder NP, Chen Y, McCarty EF, Yin C, Wei L, Wang Y, True LD, Liu JT: **Light-sheet microscopy for slide-free non-destructive pathology of large clinical specimens**. *Nat Biomed Eng* 2017, **1**.

17. Goodfellow I, Pouget-Abadie J, Mirza M, Xu B, Warde-Farley D, Ozair S, Courville A, Bengio Y: **Generative adversarial nets**. In *Advances in neural information processing systems*; 2014: 2672–2680.

18. Higgins I, Matthey L, Pal A, Burgess C, Glorot X, Botvinick M, Mohamed S, Lerchner A: **Beta-VAE: learning basic visual concepts with a constrained variational framework**. In *International conference on learning representations*, **3**; 2017.

19. * Iglesias JE, Augustinack JC, Nguyen K, Player CM, Player A, Wright M, Roy N, Frosch MP, McKee AC, Wald LL, Fischl B, Van Leemput K: **A computational atlas of the hippocampal formation using ex vivo, ultra-high resolution mri: application to adaptive segmentation of in vivo MRI**. *Neuroimage* 2015, **115**: 117–137. Available: http://www.sciencedirect.com/science/article/pii/S1053811915003420.
A statistical atlas of hippocampus is developed using manual segmentations of 13 structures, with images taken at 0.13 mm across 15 ex vivo samples. The authors develop a novel atlas building algorithm utilizing Bayesian inference that can analyze multimodal, variable contrast MRI data. To make this multimodal and robust to variable imaging quality, the authors develop a generative image formation process that randomly samples to deform label meshes on the volume.

20. ** Iglesias JE, Insausti R, Lerma-Usabiaga G, Bocchetta M, Van Leemput K, Greve DN, van der Kouwe A, Fischl B, Caballero-Gaudes C, Paz-Alonso PM: **A probabilistic atlas of the human thalamic nuclei combining ex vivo mri and histology**. *Neuroimage* 2018, **183**:314–326. Available: http://www.sciencedirect.com/science/article/pii/S1053811918307109.
This article presents similar techniques to that of Iglesias et al., in 2015, but now applied to thalamus, rather than hippocampus. Atlas construction techniques within are virtually indistinguishable from their work in 2015, using some manual segmentation in addition to Bayesian inference to develop a probabilistic atlas.

21. Irimia A, Chambers MC, Torgerson CM, Filippou M, Hovda DA, Alger JR, Gerig G, Toga AW, Vespa PM, Kikinis R, Van Horn JD: **Patient-tailored connectomics visualization for the assessment of white matter atrophy in traumatic brain injury**. *Front Neurol* 2012, **3**:10.

22. Kamnitsas K, Baumgartner C, Ledig C, Newcombe V, Simpson J, Kane A, Menon D, Nori A, Criminisi A, Rueckert D, *et al.*: **Unsupervised domain adaptation in brain lesion segmentation with adversarial networks**. In *International conference on information processing in medical imaging*. Springer; 2017:597–609.

23. Khan AM, Rajpoot N, Treanor D, Magee D: **A nonlinear mapping approach to stain normalization in digital histopathology images using image-specific color deconvolution**. *IEEE (Inst Electr Electron Eng) Trans Biomed Eng* 2014.

24. Kingma DP, Welling M: *Auto-encoding variational bayes*. 2013. arXiv preprint, arXiv:1312.6114.

25. * Komura D, Ishikawa S: **Machine learning methods for histopathological image analysis**. *Comput Struct Biotechnol* 2018, **16**:34–42.
Reviews machine learning methods that can be applied to digital histopathological image analysis. Discusses problems specific to histopathological image analysis, such as extremely large image sizes, insufficient image labels and colour variations, and suggests potential future directions of research that may provide solutions, such as interpretable deep learning and the use of convolutional neural networks for the discovery of novel objects.

26. Kothari S, Phan JH, Wang MD: **Eliminating tissue-fold artifacts in histopathological whole-slide images for improved image-based prediction of cancer grade**. *J Pathol Inform* 2013, **4**:22.

27. Kuwajima M, Mendenhall JM, Harris KM: **Large-volume reconstruction of brain tissue from high-resolution serial section images acquired by SEM-based scanning transmission electron microscopy**. *Methods Mol Biol* 2013, **950**:253–273.

28. * Lafarge MW, Pluim JP, Eppenhof KA, Veta M: **Domain-adversarial neural networks to address the appearance variability of histopathology images**. *arXiv:1707.06183v1 [cs.CV]* 2019.
The authors propose to deal with staining variation in histopathological images using domain-adversarial neural networks. The hypothesis made and supported through experiments on breast cancer histopathology images is that removing domain information from the model, and ergo the variation that is introduced in the model due to light variations in domain information, would lead to better generalization.

29. Lehtinen J, Munkberg J, Hasselgren J, Laine S, Karras T, Aittala M, Aila T: *Noise2noise: learning image restoration without clean data*. 2018. arXiv preprint, arXiv:1803.04189.

30. Lein ES, *et al.*: **Genome-wide atlas of gene expression in the adult mouse brain**. *Nature* 2007, **445**:168–176. https://doi.org/10.1038/nature05453.

31. Li SC-X, Jiang B, Marlin B: *MisGAN: Learning from incomplete data with generative adversarial networks*. 2019. arXiv preprint, arXiv:1902.09599.

32. MacKay DJ: **Bayesian interpolation**. *Neural Comput* 1992, **4**:415–447.

33. Mirza M, Osindero S: *Conditional generative adversarial nets*. 2014. arXiv preprint, arXiv:1411.1784.

34. Nazabal A, Olmos PM, Ghahramani Z, Valera I: *Handling incomplete heterogeneous data using VAEs*. 2018. arXiv preprint, arXiv:1807.03653.

35. Ólafsdóttir H, Pedersen H, Hansen MS, Larsson H, Larsen R: **Improving image registration by correspondence interpolation**. In *2011 IEEE international Symposium on biomedical imaging: from Nano to macro*. IEEE; 2011:1524–1527.

36. Orringer DA, *et al.*: **Rapid intraoperative histology of unprocessed surgical specimens via fibre-laser-based stimulated Raman scattering microscopy**. *Nat Biomed Eng* 2017.

37. Palokangas S, Selinummi J, Yli-Harja O: **Segmentation of folds in tissue section images**. In *2007 29th annual international Conference of the IEEE Engineering in Medicine and biology society*. IEEE; 2007:5641–5644.

38. * Pauli WM, Nili NA, Tyszka JM: **A high-resolution probabilistic in vivo atlas of human subcortical brain nuclei**. *Scientific Data* 2018, **5**.
Authors build a probabilistic in vivo atlas of subcortical nuclei through diffeomorphic (affine and mirroring) registration of 168 structural images. The resulting atlas has 700 um isotropic resolution.

39. Peng H, Chung P, Long F, Qu L, Jenett A, Seeds AM, Myers EW, Simpson JH: **BrainAligner: 3d registration atlases of drosophila brains**. *Nat Methods* 2011, **8**:493–500.

40. ** Rivenson Y, Wang H, Wei Z, de Haan K, Zhang Y, Wu Y, Günaydõn H, Zuckerman JE, Chong T, Sisk AE, *et al.*: **Virtual histological staining of unlabelled tissue-autofluorescence images via deep learning**. *Nat Biomed Eng* 2019, **3**:466.
The authors propose using a generative adversarial network to transform wide-field autofluorescence images of unlabelled tissue sections into images that are equivalent to the bright-field images of histologically stained versions of the same samples. Results show that the proposed virtual-staining method bypasses the typically labour-intensive and costly histological staining process, and can be used as a blueprint for the virtual staining of tissue images acquired with other label-free imaging modalities.

41. Seghier ML, Price CJ: **Interpreting and utilising intersubject variability in brain function**. *Trends Cogn Sci* 2018.

42. ** Shaban MT, Baur C, Navab N, Albarqouni S: **StainGAN: stain style transfer for digital histological images**. *ISBI* 2019:953–956.
Proposes an end-to-end deep-learning based solution inspired by CycleGANs (generative adversarial networks that use a cyclic loss) for stain style transfer and colour normalization. Subsequent AUC (area under curve) results of performing digitized histological diagnosis even in the presence of colour and stain variations are improved by 12% on a breast cancer classification dataset.

43. ** Spitzer H, Amunts K, Harmeling S, Dickscheid T: **Parcellation of visual cortex on high-resolution histological brain sections using convolutional neural networks,**. In *2017 IEEE 14th international symposium on biomedical imaging (ISBI 2017)*; April 2017:920–923.
A CNN architecture learns texture features from partially annotated 2um histological brain sections of visual areas and then takes advantage of probabilistic atlases to perform nearly automatic cytoarchitectonic mapping with reasonable accuracy.

44. Swiderska-Chadaj Z, Markiewicz T, Gallego J, Bueno G, Grala B, Lorent M: **Deep learning for damaged tissue detection and segmentation in Ki-67 brain tumor specimens based on the U-net model**. *Bull Pol Acad Sci Tech Sci* 2018, **66**.

45. Tanaka N, Kanatani S, Tomer R, Sahlgren C, Kronqvist P, Kaczynska D, Louhivuori L, Kis L, Lindh C, Mitura P, Stepulak A, Corvigno S, Hartman J, Micke P, Mezheyeuski A, Strell C, Carlson JW, Moro CF, Dahlstrand H, Ostman A, Matsumoto K, Wiklund P, Oya M, Miyakawa A, Deisseroth K, Uhlen P: **Whole-tissue biopsy phenotyping of three-dimensional tumours reveals patterns of cancer heterogeneity**. *Nat Biomed Eng* 2017.

46. Tripathi S, Lipton ZC, Nguyen TQ: *Correction by projection: denoising images with generative adversarial networks*. 2018. arXiv preprint, arXiv:1803.04477.

47. ** Tward D, Brown T, Kageyama Y, Patel J, Hou Z, Mori S, Albert M, Troncoso J, Miller M: **Diffeomorphic registration with intensity transformation and missing data: application to 3D digital pathology of Alzheimer's disease**. *BioRxiv* 2019. Available: https://www.biorxiv.org/content/early/2019/01/04/494005.
This paper solves a challenging image registration problem in the presence of multiple tissue artifacts, imaging modalities, and deformations. The work therein performs three key steps: 1) contrast transformation to match modality intensities, 2) filling missing data through expectation maximization, and finally 3) registration through deformation of histological slices to post mortem 3D MRI data.

48. Van Eycke Y-R, Allard J, Salmon I, Debeir O, Decaestecker C: **Image processing in digital pathology: an opportunity to solve inter-batch variability of immunohistochemical staining**. *Sci Rep* 2017, **7**:42964.

49. Van Leemput K: **Encoding probabilistic brain atlases using Bayesian inference**. *IEEE Trans Med Imaging* 2008, **28**:822–837.

50. Wassie AT, Zhao Y, Boyden ES: **Expansion microscopy: principles and uses in biological research**. *Nat Methods* 2018:1.

51. Weigert M, Schmidt U, Boothe T, Müller A, Dibrov A, Jain A, Wilhelm B, Schmidt D, Broaddus C, Culley S, *et al.*: **Content-aware image restoration: pushing the limits of fluorescence microscopy**. *Nat Methods* 2018, **15**:1090.

52. Weisenburger S, Tejera F, Demas J, Chen B, Manley J, Sparks FT, Traub FM, Daigle T, Zeng H, Losonczy A, Vaziri A: **Volumetric ca2+ imaging in the mouse brain using hybrid multiplexed sculpted light microscopy**. *Cell* 2019, **177**:1050–1066.

53. Wu H, Phan JH, Bhatia AK, Cundiff CA, Shehata BM, Wang MD: **Detection of blur artifacts in histopathological whole-slide images of endomyocardial biopsies**. In *2015 37th annual international Conference of the IEEE Engineering in Medicine and biology society (EMBC)*. IEEE; 2015:727–730.

54. Yang X, De Andrade V, Scullin W, Dyer EL, Kasthuri N, De Carlo F, Gürsoy D: **Low-dose x-ray tomography through a deep convolutional neural network**. *Sci Rep* 2018, **8**:2575.

55. Zanjani FG, Zinger S, de With PH, Bejnordi BE, van der Laak JA: **Histopathology stain-color normalization using deep generative models**. In *1st Conference on medical Imaging with deep learning*; 2018.

56. Zilles K: **Brodmann: a pioneer of human brain mapping - his impact on concepts of cortical organization**. *Brain* 2018, **141**:3262–3278.