Image Analysis (Physics 410/510)
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Final Project

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Goals

- Learn more about an Image Analysis topic, exploring algorithms and applications.
- Practice implementing image analysis methods.

Format, Timeline, and Guidelines

- **Topic choice:** Your topic should **not** be closely related to your research. Please **talk to me** if you'd like to discuss possibilities, if you'd like suggestions, or if you'd like to chat about what "closely" means!
- **Topic distribution:** Each of the suggested topics can be claimed by *at most* one group. The signup sheet is on Canvas, see the Final Project page.
- By Tuesday of Week 7 (**Nov. 12**): Select a topic, and be ready to give a 1-2 minute summary of why you picked it and / or the general idea in class. Note that this is just to share topics; you don't need to have finished your readings.
- **Groups:** Work in groups of 2 (optional, strongly recommended).
- **Presentation:** In class, 15 minutes.
    - Tuesday, December 10, 12:30-2:30 p.m., and Wed. Dec. 11, 11:00-12:30pm.
    - The signup sheet will be on Canvas, see the Final Project page.
- **Written part:** Due Thursday, Dec. 12, 2024 11:59 pm. (Through Canvas; PDF.) You only need to submit one PDF per group; clearly indicate everyone's names!

Presentation Components

- **[10/100] Background.** Give a general background to the topic and explain key concepts.
- **[30/100] Methods.** How does the technique work?
- **[40/100] Code / Pseudocode / Analysis.**
- **[20/100] Overall clarity**

Note: Don't be too concerned about this rubric. There's a lot of flexibility in what you decide to do.

Paper Components

Same as the presentation, but with two additional sections (highlighted):

- **[10/100] Background.** Give a general background to the topic and explain key concepts.
- **[25/100] Methods.** How does the technique work?
- **[35/100] Code / Pseudocode / Analysis.**
- **"Learning Goals" for the presentation [10/100]** – what was your intent for what your classmates should get from the presentation? What would you hope they retain a year from now? Be specific – not "students should learn about deconvolution," but for example "students should understand the goal of deconvolution as being ..., and issues that arise with its implementation such as noise artefacts and computational speed." You should of course write the learning goals before working on your presentation!
- **[15/100] Overall clarity**
- **Group tasks [5/100] –** Write how each of your group members contributed to the project.

Note: Don't be too concerned about this rubric. There's a lot of flexibility in what you decide to do.

Topics

Note: You can choose a topic that's not on this list, but I must approve it first. Feel free to contact me, and be ready with a description of the topic, references to articles, and ideas for how to tackle it. – Prof. Parthasarathy

De-noising

Images have noise. The goal of de-noising is to remove noise and get something more similar to the "true" image. We've already seen two simple approaches to de-noising: low-pass filtering and median filtering. There are many more sophisticated approaches – this is an active area of research.

(i) Illustrate the limitations low-pass filtering and median filtering (loss of resolution and non-linearity, respectively).

(ii) Research various de-noising methods (see References below for suggestions; look up other papers as well). Describe at least three – how they work, and what the results are like.

(iii) Pick one of the de-noising methods of part (ii) and write code that implements its algorithm, or a simplified version of this. If this is too difficult, contact Prof. Parthasarathy – it may be sufficient to implement just part of it, or to sketch pseudocode.

SUGGESTED REFERENCES

Review papers

- L. Fan, F. Zhang, H. Fan, C. Zhang, Brief review of image denoising techniques. *Visual Computing for Industry, Biomedicine, and Art*. **2**, 7 (2019).

- B. Goyal, A. Dogra, S. Agrawal, B. S. Sohi, A. Sharma, Image denoising review: From classical to state-of-the-art approaches. *Information Fusion*. **55**, 220–244 (2020).

Primary papers

- E. Cohen, R. Heiman, M. Carmi, O. Hadar, A. Cohen, When physics meets signal processing: Image and video denoising based on Ising theory. *Signal Processing: Image Communication*. **34**, 14–21 (2015).
- J. Chen, H. Sasaki, H. Lai, Y. Su, J. Liu, Y. Wu, A. Zhovmer, C. A. Combs, I. Rey-Suarez, H.-Y. Chang, C. C. Huang, X. Li, M. Guo, S. Nizambad, A. Upadhyaya, S.-J. J. Lee, L. A. G. Lucas, H. Shroff, Three-dimensional residual channel attention networks denoise and sharpen fluorescence microscopy image volumes. *Nat Methods*. **18**, 678–687 (2021).

Other references

- "Total Variation Denoising" – https://en.wikipedia.org/wiki/Total_variation_denoising
- Markov-Chain Monte Carlo denoising – https://uwaterloo.ca/vision-image-processing-lab/research-demos/image-denoising

Localization-based Super-Resolution Microscopy: 3D!

We've seen in class how localization microscopy works in 2D. How can we get three-dimensional information? There are several interesting ways!

(i) Describe briefly two or three methods for achieving 3D localization-based super-resolution microscopy.

(ii) Illustrate one of these methods (at least roughly) using simulated images and an analysis algorithm. I especially suggest techniques based on astigmatism (Huang 2008, for example) or PSF-shaping (e.g. Pavani 2009).

SUGGESTED REFERENCES

- K. Temprine, A. G. York, H. Shroff, "Three-Dimensional Photoactivated Localization Microscopy with Genetically Expressed Probes," in *Advanced Fluorescence Microscopy: Methods and Protocols*, P. J. Verveer, Ed. (Springer, New York, NY, 2015; https://doi.org/10.1007/978-1-4939-2080-8_13), *Methods in Molecular Biology*, pp. 231–261 – available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5234325/
- B. Huang, W. Wang, M. Bates, X. Zhuang, Three-Dimensional Super-Resolution Imaging by Stochastic Optical Reconstruction Microscopy. *Science*. **319**, 810–813 (2008).
- S. R. Pavani, M. A. Thompson, J. S. Biteen, S. J. Lord, N. Liu, R. J. Twieg, R. Piestun, W. E. Moerner, Three-dimensional, single-molecule fluorescence imaging beyond the diffraction limit by using a double-helix point spread function. *Proc Natl Acad Sci U S A*. **106**, 2995–9 (2009).

Localization-based Super-Resolution Microscopy: Further Topics

We've seen in class how localization microscopy works, and how it's basically an image analysis method. Beyond localizing objects, there are still more analysis challenges, for example determining whether objects are part of the same cluster or not. Look into these issues.

(i) Describe briefly one or two cluster analysis methods.

(ii) Illustrate one of these methods (at least roughly) using simulated images and an analysis algorithm. (This may be feasible only with pseudocode.)

SUGGESTED REFERENCES

- M. Khater, I. R. Nabi, G. Hamarneh, A Review of Super-Resolution Single-Molecule Localization Microscopy Cluster Analysis and Quantification Methods. *Patterns*. **1**, 100038 (2020).
- P. Rubin-Delanchy, G. L. Burn, J. Griffié, D. J. Williamson, N. A. Heard, A. P. Cope, D. M. Owen, Bayesian cluster identification in single-molecule localization microscopy data. *Nat Meth*. **12**, 1072–1076 (2015).

Image Analysis in Cryo-Electron Microscopy

Cryo-electron microscopy uses electron microscopy and image analysis algorithms to reconstruct nearly atomic-level structures from images of randomly oriented molecules. It has been extremely successful (see e.g. the 2017 Nobel Prizes).

(i) Describe (briefly) the experimental technique.

(ii) Describe image analysis algorithms and their output.

(iii) Implement one of the analysis algorithms, or provide pseudocode.

SUGGESTED REFERENCES

About the experimental technique:

- E. Callaway, The revolution will not be crystallized: a new method sweeps through structural biology. *Nature* **525**, 172 (2015). [http://www.nature.com/news/the-revolution-will-not-be-crystallized-a-new-method-sweeps-through-structural-biology-1.18335]
- Eva Nogales' web site: http://cryoem.berkeley.edu/cryoem
- *Nature Methods'* collection of articles on cryo-EM ("Method of the Year" in 2015): http://www.nature.com/nmeth/focus/moy2015/index.html
- Electron microscopy in biology (video): https://www.ibiology.org/ibioseminars/techniques/eva-nogales-part-1.html

Image analysis

- Philip Nelson has an excellent description of this in his textbook, Physical Models of Living Systems (2nd edition, Chapter 8). See also: https://www.youtube.com/watch?v=Q6zs6U_cDeA , and https://www.physics.upenn.edu/~pcn/24-01-12SyracuseInferenceLong.pdf .

- F. J. Sigworth, Principles of cryo-EM single-particle image processing. *Microscopy*. **65**, 57–67 (2016).
- M. Serna, Hands on Methods for High Resolution Cryo-Electron Microscopy Structures of Heterogeneous Macromolecular Complexes. *Frontiers in Molecular Biosciences*. **6**, 33 (2019)

Deconvolution

We'll introduce this in class. The basic idea is *inverting* the convolution with the point spread function and thereby reconstructing the "true" image (if possible!). This is a very active area of research.

(i) Describe two or three deconvolution algorithms that we don't discuss in class.
(ii) Illustrate the performance of at least two methods using simulated images. You can use toolkits written by others.

Suggested References

- P. Sarder, A. Nehorai, Deconvolution methods for 3-D fluorescence microscopy images. *IEEE Signal Processing Magazine*. **23**, 32–45 (2006)
- D. Sage, L. Donati, F. Soulez, D. Fortun, G. Schmit, A. Seitz, R. Guiet, C. Vonesch, M. Unser, DeconvolutionLab2: An open-source software for deconvolution microscopy. *Methods*. **115**, 28–41 (2017)
- M. Guo, Y. Li, Y. Su, T. Lambert, D. D. Nogare, M. W. Moyle, L. H. Duncan, R. Ikegami, A. Santella, I. Rey-Suarez, D. Green, A. Beiriger, J. Chen, H. Vishwasrao, S. Ganesan, V. Prince, J. C. Waters, C. M. Annunziata, M. Hafner, W. A. Mohler, A. B. Chitnis, A. Upadhyaya, T. B. Usdin, Z. Bao, D. Colón-Ramos, P. La Riviere, H. Liu, Y. Wu, H. Shroff, Rapid image deconvolution and multiview fusion for optical microscopy. *Nature Biotechnology*, 1–10 (2020)

Image Reconstruction in Tomography

Tomography involves reconstructing three-dimensional images from two-dimensional projections and is important for a variety of medical imaging methods (such as CT scans.)

(i) Explain how at least one tomographic imaging method, such as CT scanning, works.
(ii) Describe the central slice theorem, also known as the projection-slice theorem, and its proof.
(iii) Write a program to illustrate the central slice theorem. It's fine to do the 2D version of this (with 1D projections).

Suggested References

- R. K. Hobbie, B. J. Roth, *Intermediate Physics for Medicine and Biology* (Springer-Verlag, New York, ed. 4, 2007). (I think UO has access to the electronic edition.)
- There's a lot of good stuff out there! (Medical imaging is important and popular.)

Segmentation – beyond Watersheds

We'll introduce segmentation in class. There are a lot of segmentation methods out there; explore.

(i) Describe two or three segmentation algorithms that we don't discuss in class.

(ii) Illustrate the performance of at least one method using either simulated images or images from some image library with known properties. You can use toolkits written by others. Suggested methods: snakes, graph cuts.

SUGGESTED REFERENCES

- Y. Boykov, G. Funka-Lea, Graph Cuts and Efficient N-D Image Segmentation. *Int. J. Comput. Vision*. **70**, 109–131 (2006).
- V. Ulman, M. Maška, K. E. G. Magnusson, O. Ronneberger, C. Haubold, N. Harder, P. Matula, P. Matula, D. Svoboda, M. Radojevic, I. Smal, K. Rohr, J. Jaldén, H. M. Blau, O. Dzyubachyk, B. Lelieveldt, P. Xiao, Y. Li, S.-Y. Cho, A. C. Dufour, J.-C. Olivo-Marin, C. C. Reyes-Aldasoro, J. A. Solis-Lemus, R. Bensch, T. Brox, J. Stegmaier, R. Mikut, S. Wolf, F. A. Hamprecht, T. Esteves, P. Quelhas, Ö. Demirel, L. Malmström, F. Jug, P. Tomancak, E. Meijering, A. Muñoz-Barrutia, M. Kozubek, C. Ortiz-de-Solorzano, An objective comparison of cell-tracking algorithms. *Nature Methods*. **14**, 1141 (2017)
- There is a lot out there on-line about segmentation!

Light Field Imaging

Light field imaging is a new technique for recording three-dimensional data and reconstructing a variety of two-dimensional images – different focal planes, for example. It involves interesting optics, for example arrays of sensors, and interesting image analysis methods. Explore this and describe some of the image analysis challenges and methods. These may be challenging to implement, so it is probably sufficient to explain them and show others' results. (We can discuss this.)

SUGGESTED REFERENCES

- I. Ihrke, J. Restrepo, L. Mignard-Debise, Principles of Light Field Imaging: Briefly revisiting 25 years of research. *IEEE Signal Processing Magazine*. **33**, 59–69 (2016).
- G. Wu, B. Masiá, A. Jarabo, Y. Zhang, L. Wang, Q. Dai, T. Chai, Y. Liu, Light Field Image Processing: An Overview. *IEEE Journal of Selected Topics in Signal Processing* (2017), doi:10.1109/JSTSP.2017.2747126
- R. Prevedel, Y.-G. Yoon, M. Hoffmann, N. Pak, G. Wetzstein, S. Kato, T. Schrödel, R. Raskar, M. Zimmer, E. S. Boyden, A. Vaziri, Simultaneous whole-animal 3D imaging of neuronal activity using light-field microscopy. *Nat Methods*. **11**, 727–730 (2014).
- "10.1 Light Fields | Image Analysis Class 2013" (Univ. of Heidelberg) https://www.youtube.com/watch?v=q_zVV89nU3g
- Zhou, K. C. *et al.* High-speed 4D fluorescence light field tomography of whole freely moving organisms. Preprint at https://doi.org/10.1101/2024.09.16.609432 (2024). A recent preprint involving light fields (and more).

Multi-spectral / Hyper-spectral Imaging

Often one wants to image colors whose spectra overlap – this comes up a lot in satellite imaging, where signatures of different landscapes overlap, and biological imaging, where one might use many fluorescent probes whose emission wavelengths overlap. Many methods have been developed to analyze these "multi-spectral" or "hyper-spectral" datasets – i.e assessing what combination of signals gave the observed images in different spectral channels. Some methods require knowing the component spectra; some don't. Explore the state of the field!

SUGGESTED REFERENCES

- Y. Garini, I. T. Young, G. McNamara, Spectral imaging: Principles and applications. *Cytometry Part A*. **69A**, 735–747 (2006).
- Neher RA, Mitkovski M, Kirchhoff F, Neher E, Theis FJ, Zeug A. Blind Source Separation Techniques for the Decomposition of Multiply Labeled Fluorescence Images. *Biophys J*. **96**, 3791–3800 (2009). This is a good example of an unmixing method. It's not hard to implement.
- Code for the "PoissonNMF" method: http://neherlab.github.io/PoissonNMF/Documentation/NMF/PoissonNMF_documentation.html
- Another method: Spectral Phasor, with a nice tutorial for an ImageJ plugin: https://research.stowers.org/imagejplugins/spectral_imaging.html

Structured Illumination Microscopy

Changing the spatial pattern of light used to illuminate a sample can, surprisingly, allow us to exceed the diffraction limit of resolution by a factor of 2. This technique, developed in the early 2000s, is called structured illumination microscopy, and it involves patterned illumination plus image reconstruction algorithms. It's also a great example of the power of understanding Fourier Transforms, which help make sense of how the method works. There are several commercial microscopes that incorporate structured illumination microscopy, such as the Zeiss Elyra 7 and the GE DeltaVision OMX (which we have in the Imaging Core Facility at UO).

(i) Describe the experimental technique. (It's very simple.)

(ii) Briefly describe why the method breaks the diffraction limit.

(iii) Implement one of image reconstruction algorithms, or provide pseudocode.

(iv) [Optional; if you do this (iii) can be shorter] Describe how at least two of the commercial systems implement structured illumination microscopy. What's the patterning? Is processing done in real time?

SUGGESTED REFERENCES

- "Superresolution Structured Illumination Microscopy" in https://zeiss-campus.magnet.fsu.edu/articles/superresolution/supersim.html
- Gustafsson, M. G. Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. *J. Microsc.* **198**, 82–87 (2000).
- Manton, J. D. Answering some questions about structured illumination microscopy. *Philos. Trans. R. Soc. Math. Phys. Eng. Sci.* **380**, 20210109 (2022). [Just the first parts.]

Digital Holographic Microscopy

Holography involves using light interference to preserve phase as well as amplitude information from a sample. It can be used for microscopy, in certain contexts.

(i) Describe the experimental technique and a few applications.

(ii) Describe image reconstruction or analysis methods.

POSSIBLE REFERENCES

- https://manoharan.seas.harvard.edu/holographic-microscopy, a brief description
- Martin, C. *et al.* In-line holographic microscopy with model-based analysis. *Nat. Rev. Methods Primer* **2**, 1–17 (2022) [You'll have to request a PDF from interlibrary loan / UO article request.]
- Kim, M. K. Principles and techniques of digital holographic microscopy. *SPIE Rev.* **1**, 018005 (2010)