AI in Microscopy: A BioImaging Guide

Table of contents

Welcome

This is an initial outline for the welcome page:

- Include a very brief introduction of the book (this is not the introduction chapter).
- Explain how to interact with the book. For example, explain how code snippets work for the reader, links to figures, glossary terms, etc.
- State the licensing and use restrictions.
- How to cite the book.

1 Introduction

AI at Every Stage of the Microscopy Workflow

Under your first header, include a brief introduction to your chapter.

Starting prompt for this chapter: Chapter 1 outlines how AI can span experimental design, image acquisition, image processing, and analysis (without discussing what AI is from a technical perspective). This chapter will also outline the roadmap of the book which will largely focus on acquisition and processing.

Topics suggested during the authors' meetings: Discuss that AI is not always solution and talk about when it is actually useful. Discuss that there are many types of microscopy images and each will have their own AI considerations (e.g., imaging modality, 2D vs 3D, static vs time lapse).

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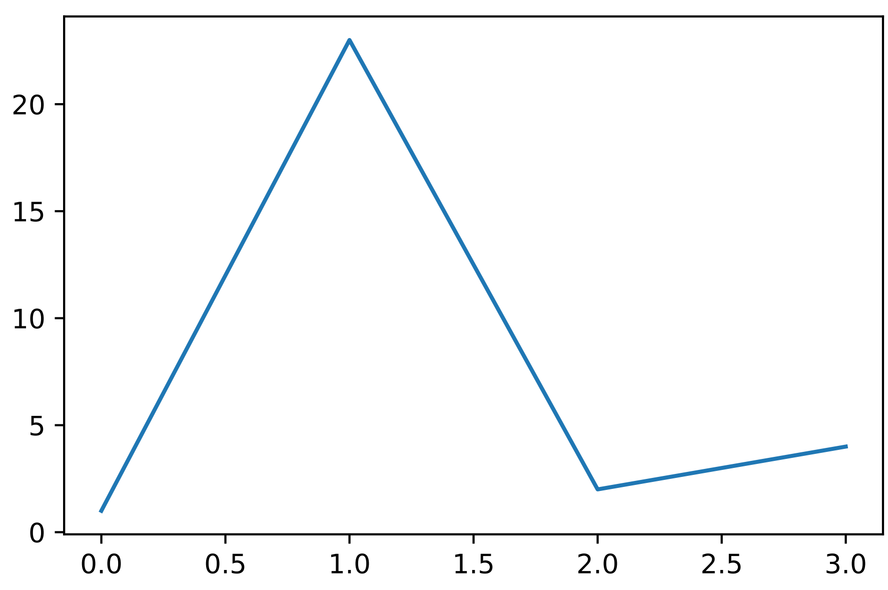

Figure 1.1: Simple Plot

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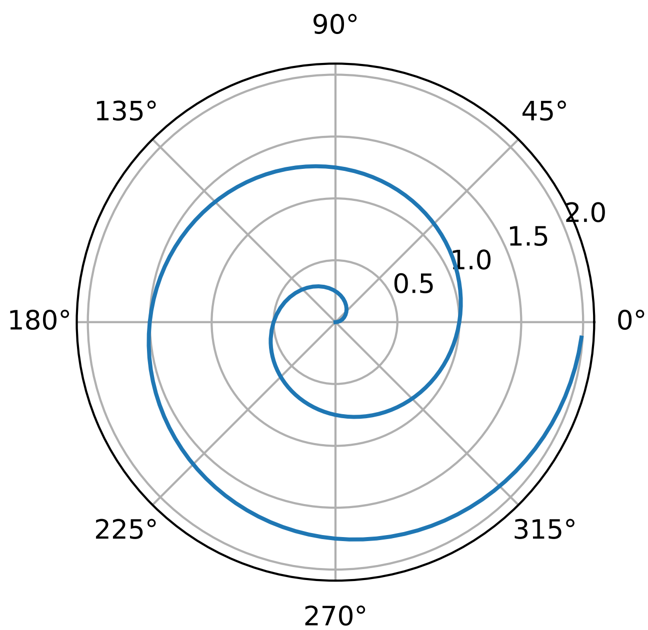

Figure 1.2: A spiral on a polar axis

Here is an example equation.

$$s = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \overline{x})^2} \tag{1.1}$$

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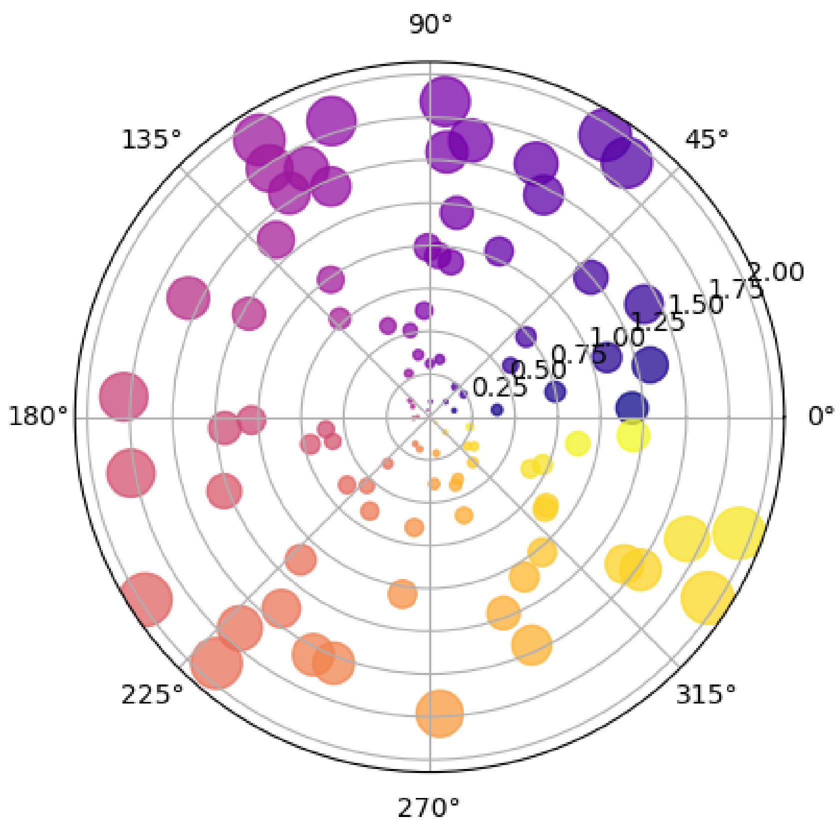

Figure 1.3: Polar plot of circles of random areas at random coords

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> **Important**
>
> This is key information.

Part I

Getting Started with AI

2 AI Primer

An Introduction to Artificial Intelligence

Under your first header, include a brief introduction to your chapter.

Starting prompt for this chapter: Chapter 2 demystifies Artificial Intelligence for microscopy users. It should define terms (e.g., machine/deep learning, supervised/unsupervised learning) without programming details such that an educated scientist without AI experience can understand how these concepts apply to microscopy in life sciences. The use-cases and strengths of different approaches for different applications should be discussed (e.g., contrasting unsupervised clustering vs supervised segmentation). This chapter should broadly introduce image restoration and segmentation, as they will be themes throughout.

Suggestion from authors' meetings: This chapter can draw on the outlines from other chapters to introduce key topics for the following chapters.

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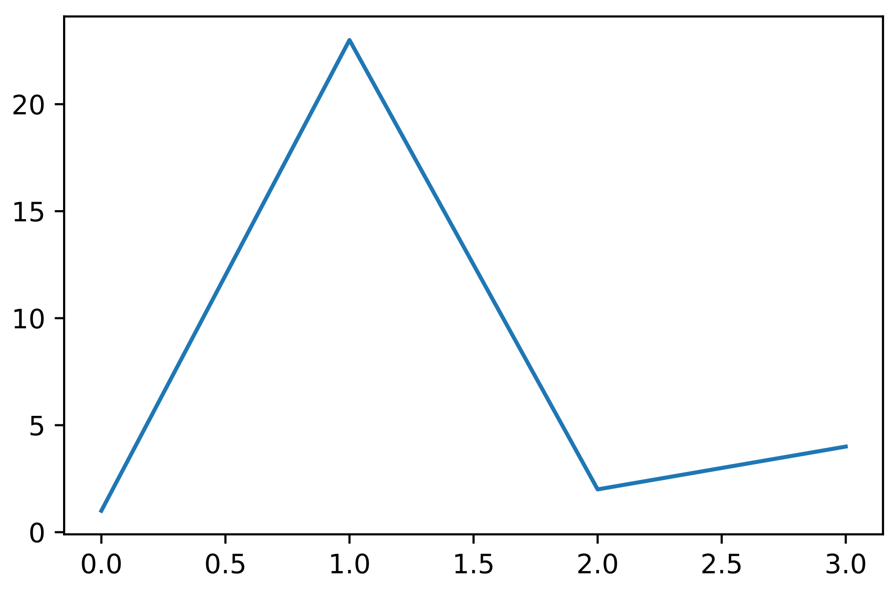

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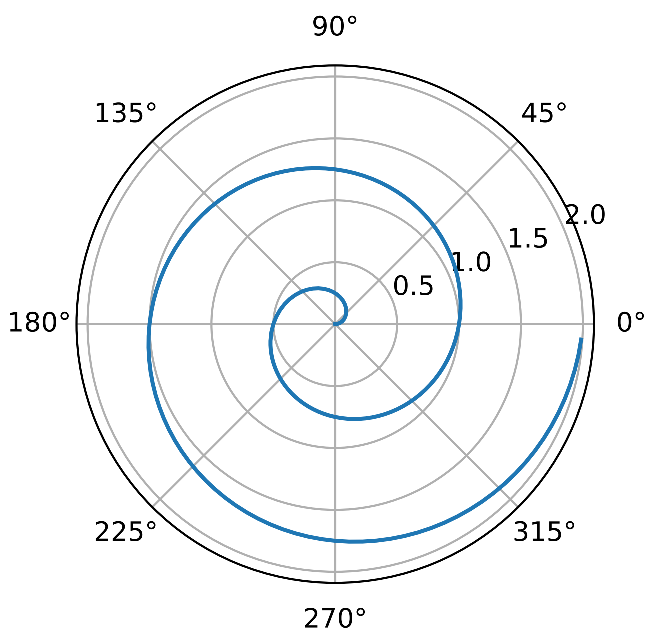

Figure 2.2: A spiral on a polar axis

Here is an example equation.

$$s = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \overline{x})^2} \tag{2.1}$$

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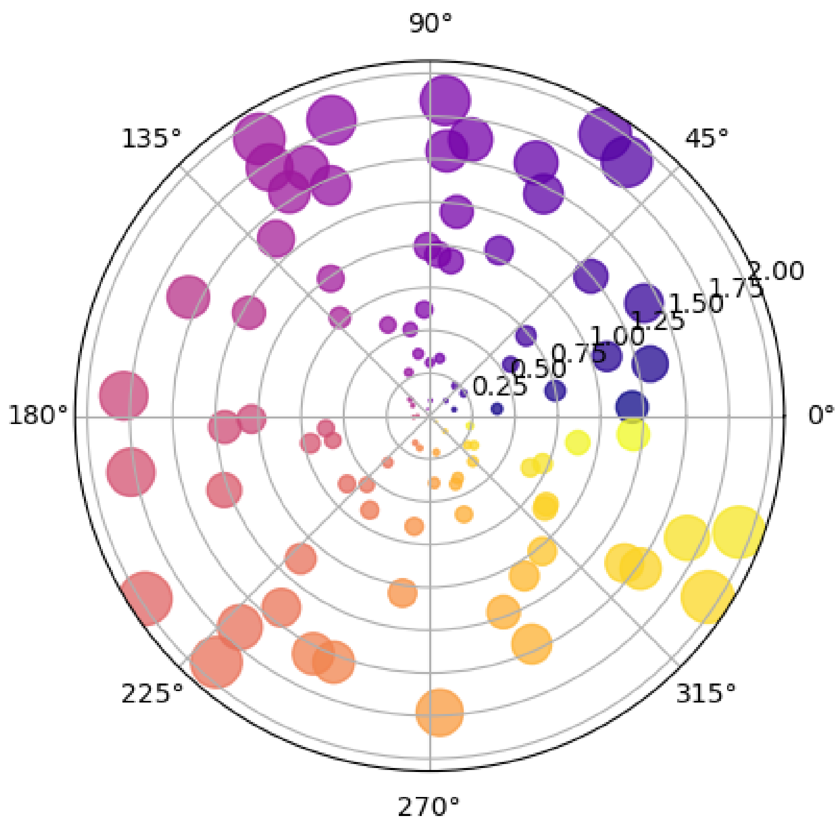

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3 Foundations of Large Language Models

Large Language Models and AI Agents for Microscopy Imaging

In recent years, large language models (LLMs) have revolutionized how we interact with technology, bringing unprecedented capabilities to scientific research including microscopy. This chapter explores how microscopists can leverage these powerful AI tools to enhance their workflow, from learning concepts to automating analysis tasks. We'll discuss both general-purpose and microscopy-specific tools while highlighting practical applications and potential pitfalls.

This section introduces the fundamental concepts behind modern language models, focusing on transformer architectures that power tools like ChatGPT. We'll explain how these models function, their capabilities for understanding scientific text, and their emerging role in generating code for image analysis tasks. We'll demonstrate how microscopists can effectively use LLMs to learn new concepts, troubleshoot methods, and generate starting points for analysis scripts.

3.1 Multi-modal AI: Vision-Language Models and Generative AI

Moving beyond text-only interfaces, multi-modal models combine language understanding with visual processing capabilities. This section explores how Vision-Language Models (VLMs) like GPT-4o can "see" and interpret microscopy images, assist with image annotation, and even aid in experimental design. We'll also cover generative AI technologies including diffusion models that can create synthetic training data, perform style transfer, or convert microscopy images into vector graphics for publications.

3.2 AI Agents for Microscopy Workflows

AI agents represent the next evolution - autonomous systems that combine language understanding with specialized scientific knowledge and the ability to execute actions. We'll examine microscopy-specific tools like Omega and the BioImage.io chatbot that can perform complex bioimage analysis workflows through natural language instructions. This section will explore chain-of-thought reasoning, code generation and execution capabilities, and how these agents use visual feedback to iteratively improve results.

3.3 Challenges and Limitations

While powerful, AI assistants come with significant limitations that microscopists must understand. This section addresses critical challenges including: - and factual errors in generated content - The "black box" nature of models and concerns about reproducibility - Alignment problems when tools lack domain-specific knowledge - The need for human validation and the dangers of overreliance - Practical strategies for steering models toward scientifically valid outputs

3.4 Future Directions

The intersection of LLMs and microscopy is rapidly evolving. This final section examines emerging capabilities and future possibilities, including: - Generalist vision-language models capable of performing diverse analysis tasks - Models that can directly transform input images into processed outputs - The integration of AI agents with microscope hardware for fully autonomous imaging - Smart microscopy systems that adapt acquisition parameters based on real-time image understanding - Ethical considerations and best practices for responsible AI adoption in biological research

3.5 Practical Guide: Getting Started with LLMs for Microscopy

This hands-on section provides step-by-step guidance for microscopists to begin leveraging LLMs effectively, including: - Crafting effective prompts that produce reliable, scientific outputs - Using ChatGPT and similar tools to learn imaging concepts and generate analysis code - Getting started with BioImage.io tools and microscopy-specific AI agents - Strategies for validating and verifying AI-generated solutions - Example workflows demonstrating LLM integration into real microscopy analysis tasks

4 Architectures and Loss Models

optionally add a subtitle

Under your first header, include a brief introduction to your chapter.

Starting prompt for this chapter: Chapter 4 introduces architectures and loss models, defining them and providing examples through two practical case studies: image restoration and segmentation. Although this chapter will include code snippets/exercises, the presentation of essential concepts should communicate the philosophy behind the choice of a model for non-programmers.

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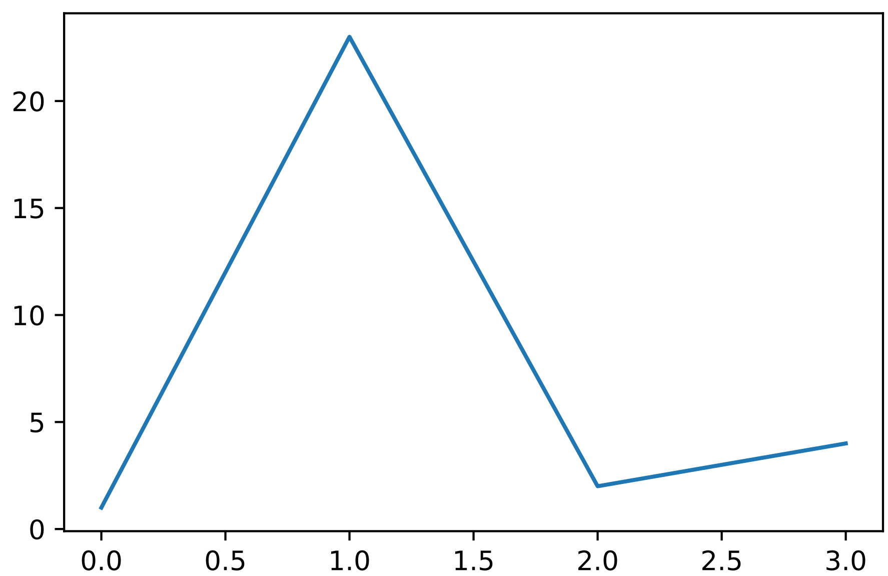

Figure 4.1: Simple Plot

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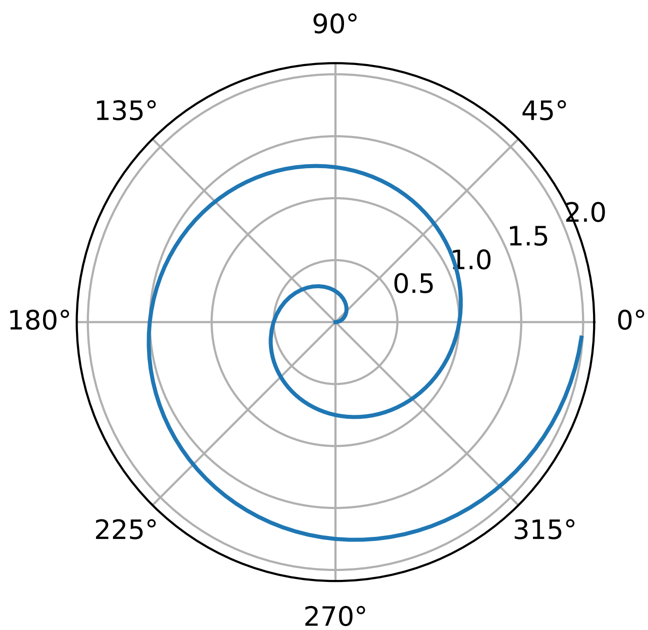

Figure 4.2: A spiral on a polar axis

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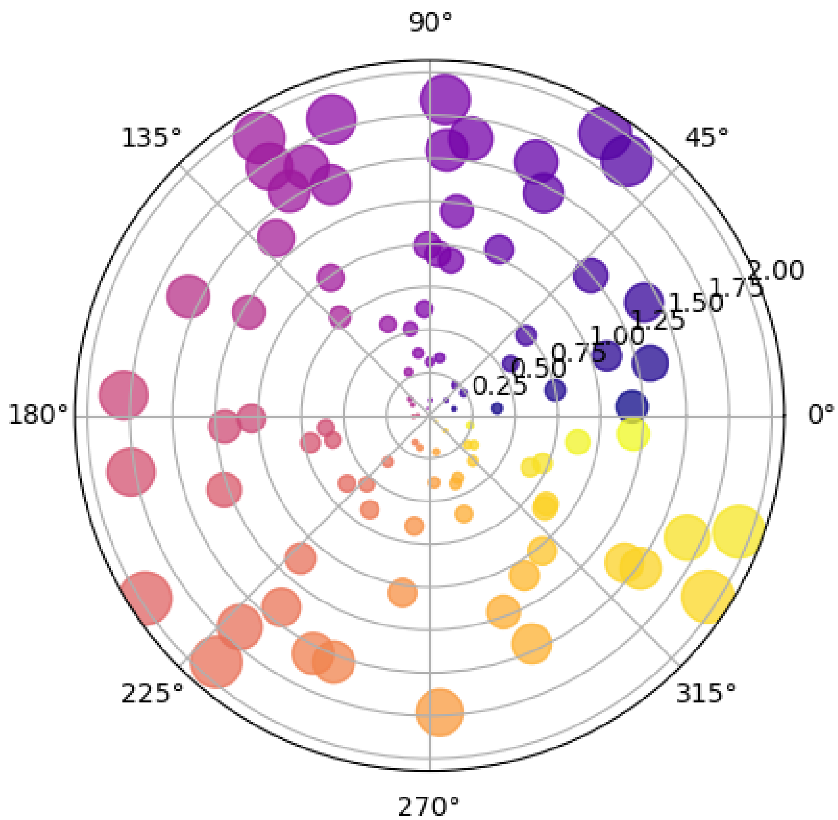

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Part II

Image Acquisition

5 Training Data

Image Collection and Considerations

Under your first header, include a brief introduction to your chapter.

Starting prompt for this chapter: Chapter 5 discusses collecting, annotating and validating training data. It should highlight potential pitfalls such as balanced data sets, out-of-distribution problems, etc. It should also address the question: how do you collect training data on your microscope? For example, this chapter should discuss collecting low/high-laser power pairs for the purpose of training an image restoration model.

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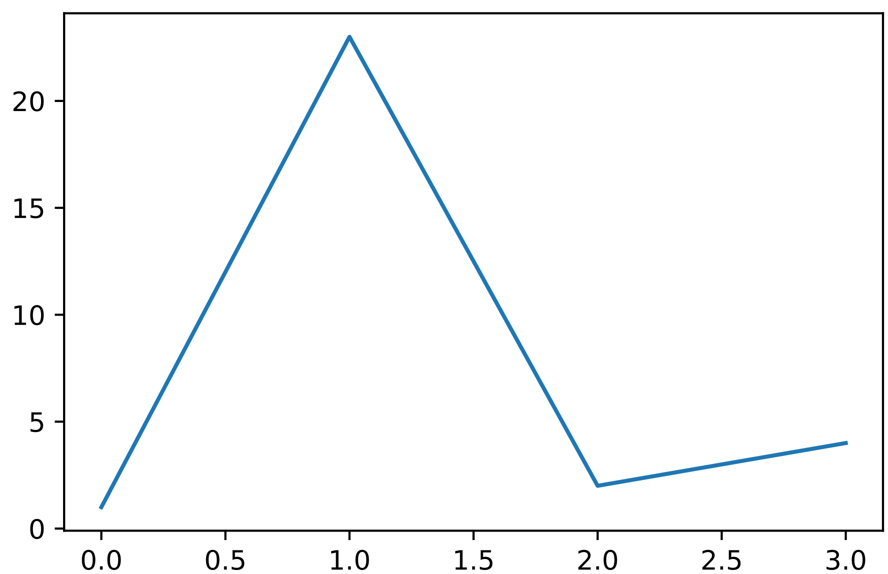

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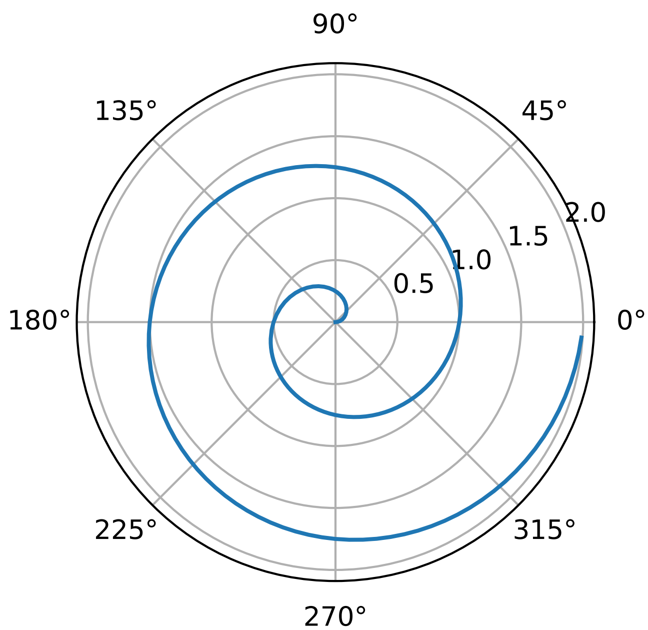

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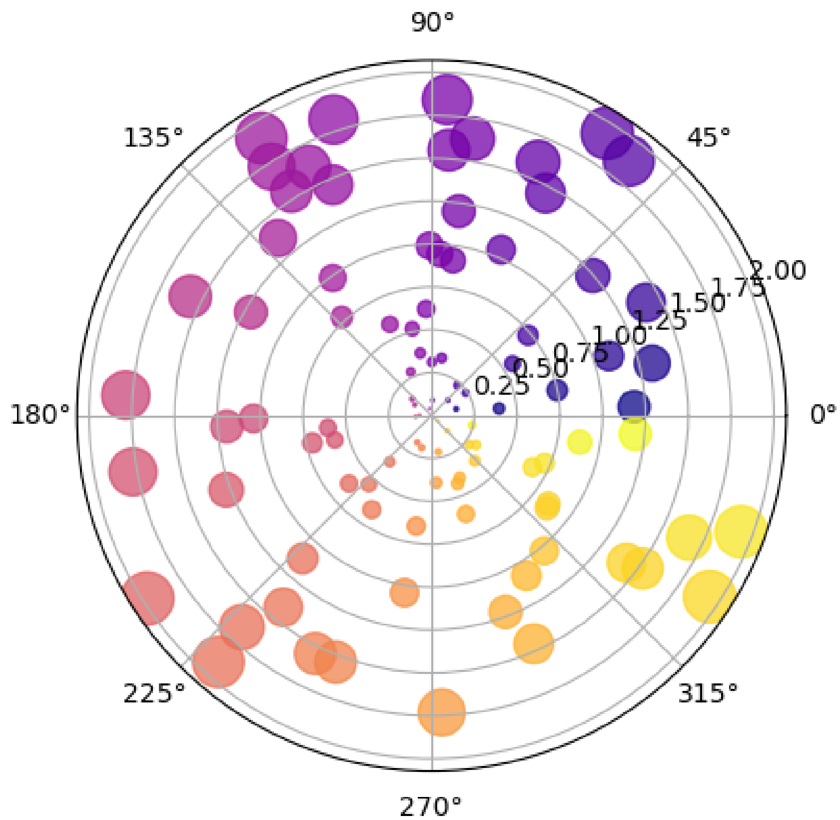

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theta = 2 * np.pi * r
```

Warning

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Important

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6 Extending Your Hardware With AI

Image Restoration and Related Tools

Under your first header, include a brief introduction to your chapter.

Starting prompt for this chapter: This image restoration focused chapter motivates how AI can improve the image quality beyond hardware limitations. This chapter should include image restoration (denoising) and sensor-less AO (deep-learning-based AO); other topics can be included at the author's discretion.

Notes from the authors' meeting: This chapter can start with a broader overview of the topic, which will reference existing reviews on the topic. Then the chapter will focus on a denoising tutorial, which will include discussions of potential pitfalls and best practices.

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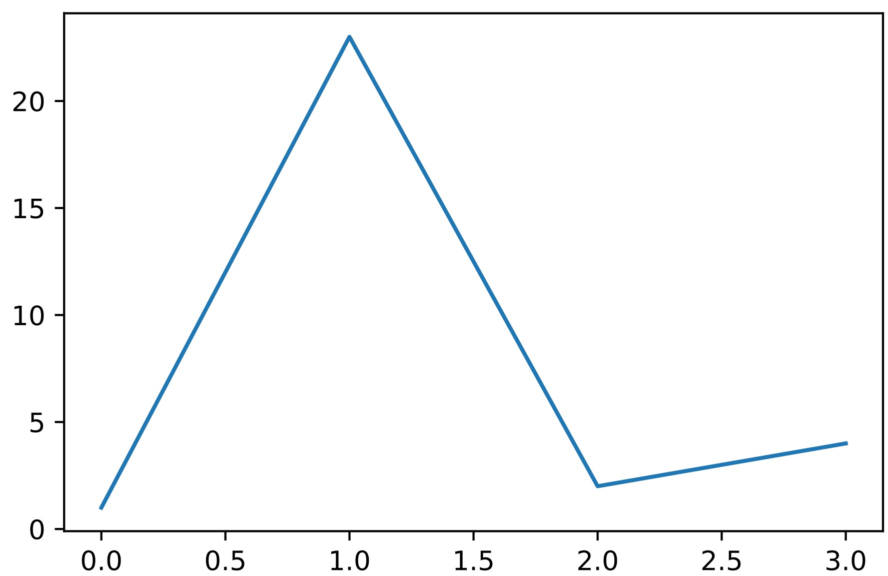

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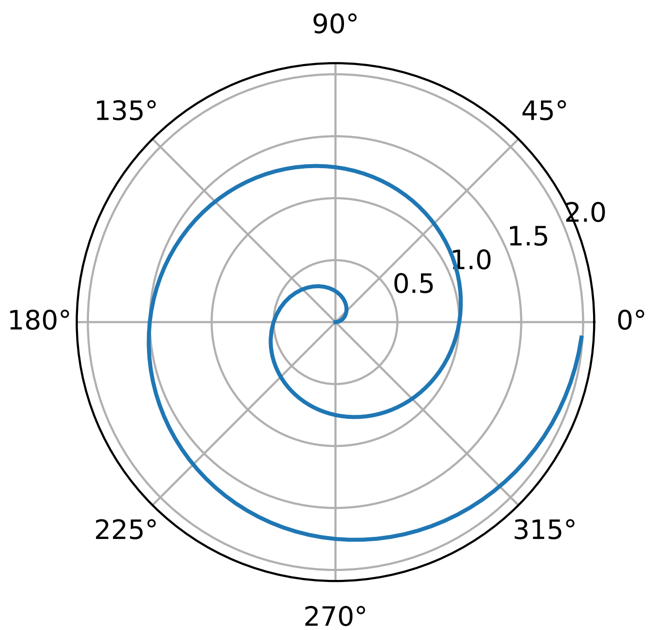

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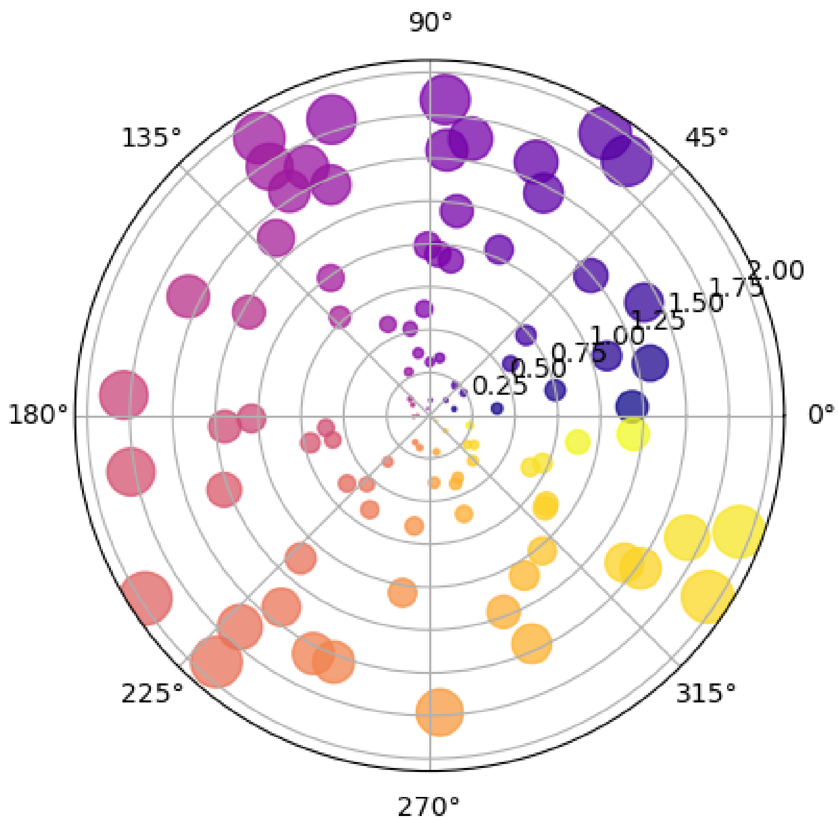

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> **Warning**
>
> Be careful to avoid hallucinations.

> **Important**
>
> This is key information.

7 Adding AI to Your Hardware

An Introduction to Smart Microscopy

- Introduction
  - Define and motivate the "problem"
    - What is a Biological "Event"?
      - Why is it important to study these?
      - How is it distinct from an "Object"?
- Setting the bounds
  - What kinds of signals are we looking to extract events from?
  - What spatiotemporal scales are relevant?
  - What types of microscopes/imaging assays are in the scope of this chapter?
    - Limiting the scope
- Brief history/evolution of automated event/object detection in general
  - Offer some background to current methods
    - Including classical up to SVM
      - e.g micropilot and limitations
      - Case Study #1 : CellProfiler
      - Case Study #2 : Micropilot
- The need for new methods
  - Deep learning based and ML approaches
    - Where the algorithm learns what is important
    - What are the advantages of these methods?
      - What has been done recently in the literature?
    - What is required to label, train and implement these methods?
      - Special considerations for event detection modesl
      - Specifics of event detection in contast the other ML tasks
- Real-time vs a posteriori inference

  - Challenges, opportunities
  - Event detection as the first step in the microscopy workflow

- Limitations and notes of caution related to inference

  - "Trusting the algorithms"

- Conclusion

Part III

Image Analysis

8 Finding and Using Existing Tools

Under your first header, include a brief introduction to your chapter.

Starting prompt for this chapter: Chapter 8 should discuss pre-existing tools that do not require programming knowledge. The chapter should start with a brief discussion of what to consider when searching for pre-trained models and software packages on sites (e.g., BioImage Model Zoo, Bioimage Informatics Index (BIII), image.sc). This should be followed by a discussion of a few specific tools (e.g., CellProfiler, Cellpose, ImageJ's Weka Segmentation), highlighting what to look for in a tool, potential pitfalls, etc.

8.1 Include section headers as appropriate

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plt.show()
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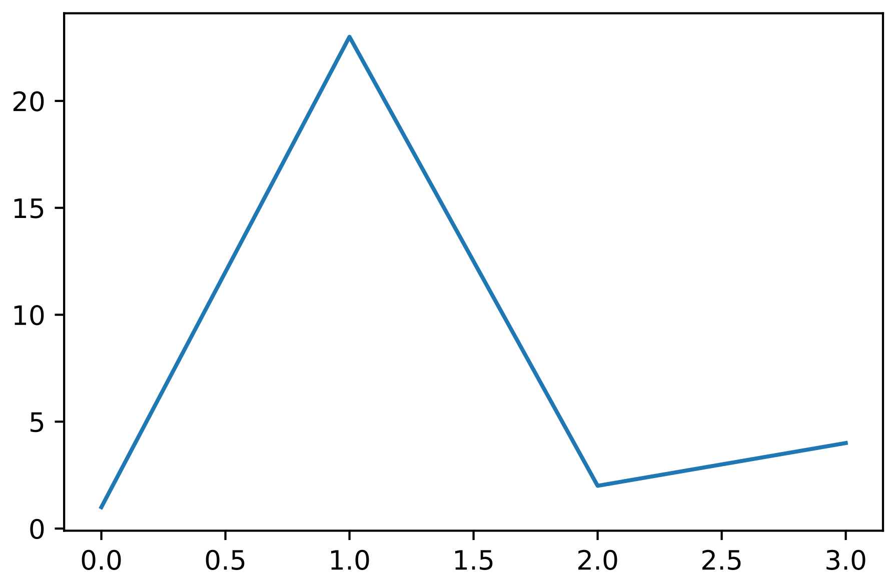

Figure 8.1: Simple Plot

In some cases, you may want to include a code-block that is not executed when the book is compiled. Use the `eval: false` option for this.

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plt.show()
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import matplotlib.pyplot as plt

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  subplot_kw = {'projection': 'polar'}
)
ax.plot(theta, r)
ax.set_rticks([0.5, 1, 1.5, 2])
ax.grid(True)
plt.show()
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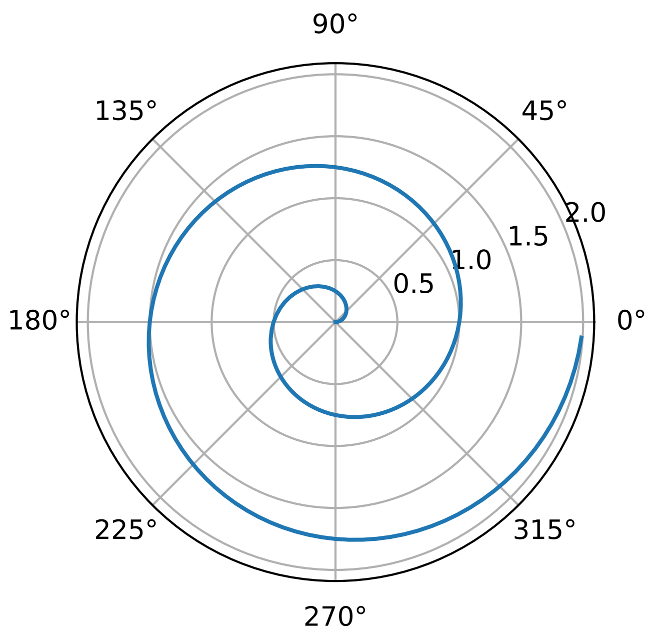

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Here is an example equation.

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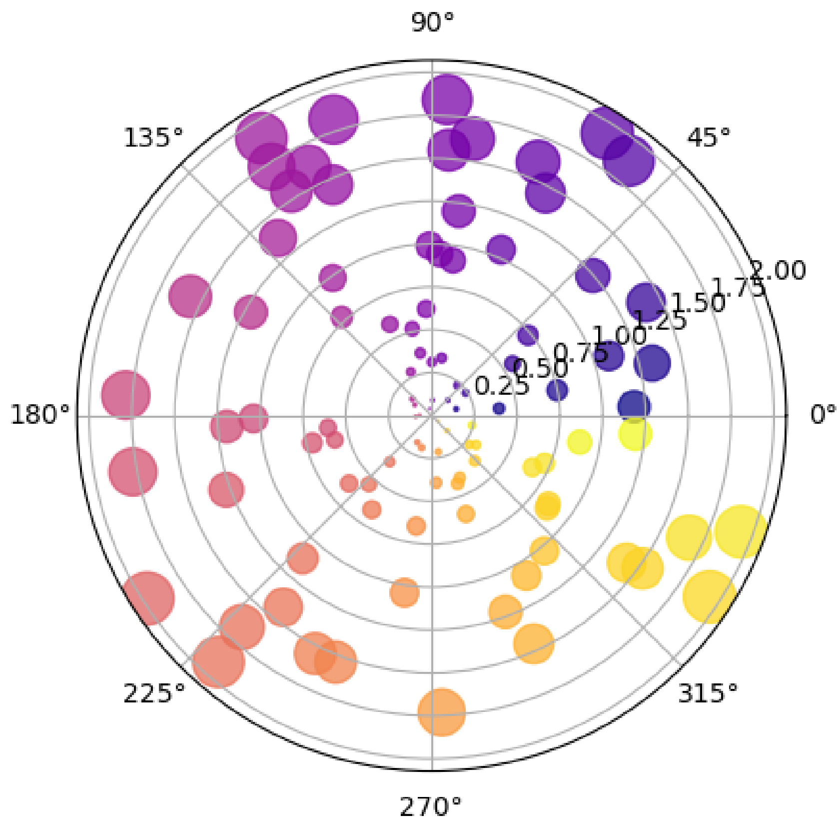

Figure 8.3: Polar plot of circles of random areas at random coords

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Caution

There are caveats when applying these tools. Expand the code below to learn more.

```
r = np.arange(0, 2, 0.01)
theta = 2 * np.pi * r
```

Warning

Be careful to avoid hallucinations.

Important

This is key information.

9 Training and Using Your Own Models

optionally add a subtitle

Under your first header, include a brief introduction to your chapter.

Starting prompt for this chapter: Chapter 9 discusses the considerations (e.g., over/under-fitting, parameter choices) for training a new model and tools to help getting started (e.g. DL4MicEverywhere). It should include a primer on cloud-based computing tools such as Docker, Google Colab, etc. to highlight the potential for training models without bespoke hardware in house. This chapter should demonstrate a walk-through of training a model for segmentation, following the throughline of the book.

9.1 Include section headers as appropriate

Use markdown heading level two for section headers. You can use standard markdown formatting, for example *emphasize the end of this sentence*.

This is a new paragraph with more text. Your paragraphs can cross reference other items, such as Figure 11.1. Use `fig` to reference figures, and `eq` to reference equations, such as Equation 11.1.

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9.2 Bibliography and Citations

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9.4 Code and Equations

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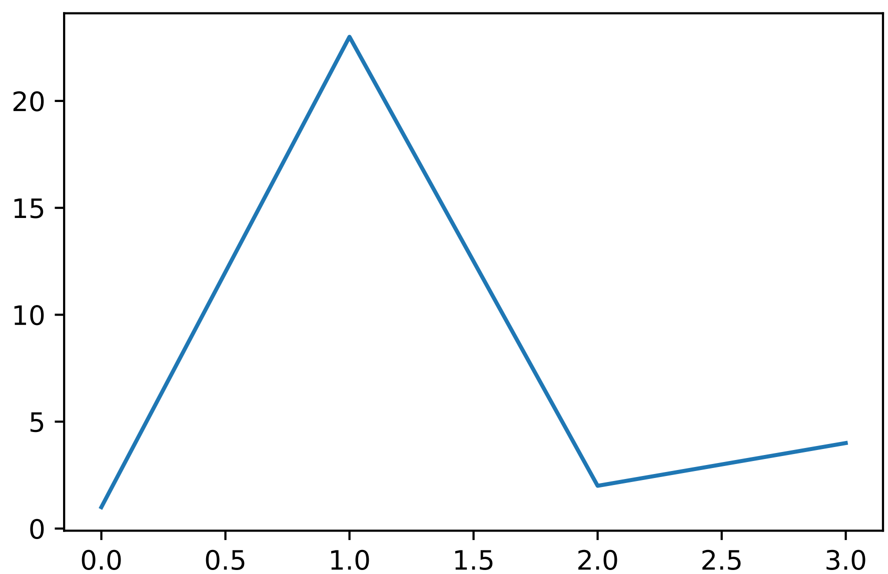

Figure 9.1: Simple Plot

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)
ax.plot(theta, r)
ax.set_rticks([0.5, 1, 1.5, 2])
ax.grid(True)
plt.show()
```

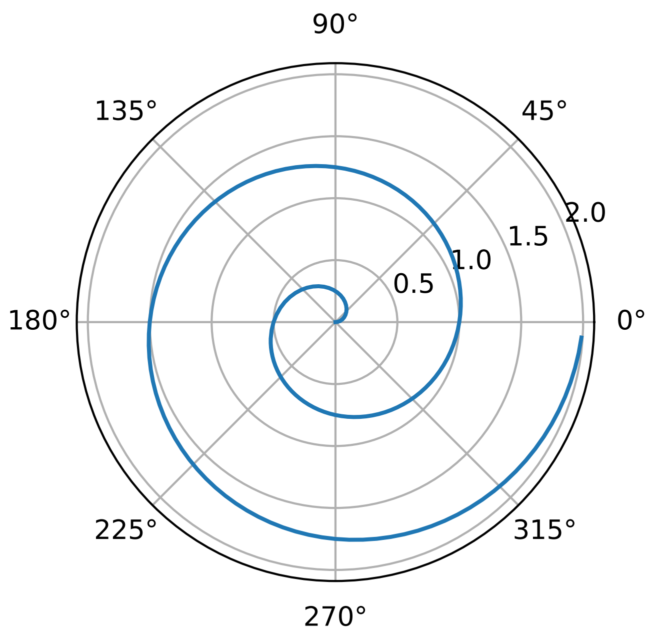

Figure 9.2: A spiral on a polar axis

Here is an example equation.

$$s = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \overline{x})^2} \tag{9.1}$$

9.4.1 Embedding Figures

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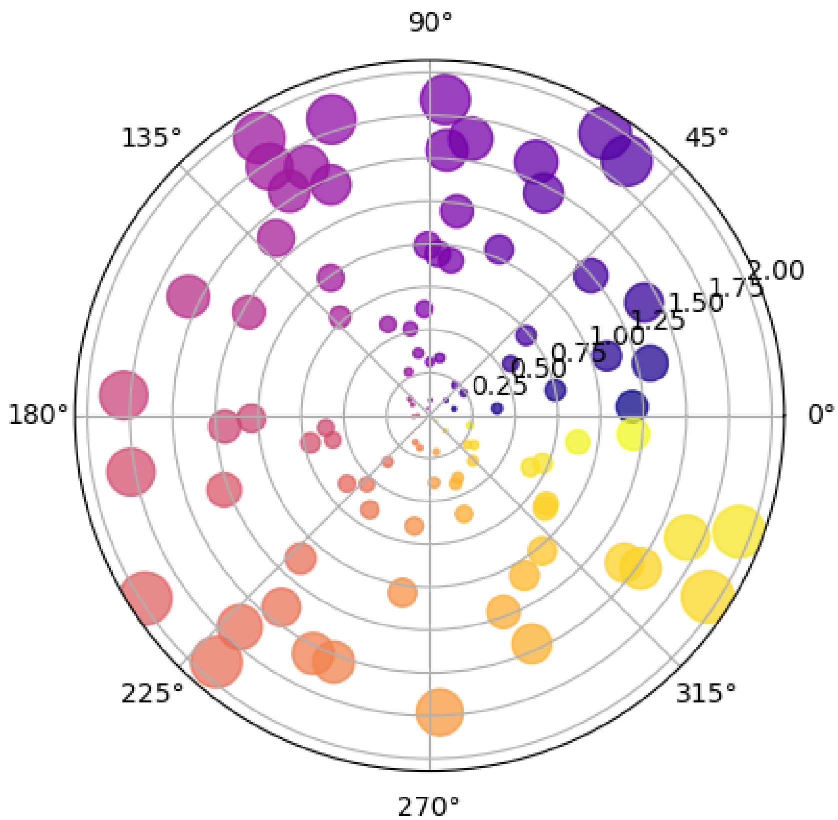

Figure 9.3: Polar plot of circles of random areas at random coords

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>
> Be careful to avoid hallucinations.

> **Important**
>
> This is key information.

10 Output Quality

Through the Lens of Segmentation

Under your first header, include a brief introduction to your chapter.

Starting prompt for this chapter: Chapter 10 addresses how to assess the quality of a model's output, mentioning Metrics Reloaded. This chapter should address the question: how do I know my model is good enough? It should frame this discussion using the example of a segmentation model and discuss how tools can identify uncertain decisions from a model.

10.1 Include section headers as appropriate

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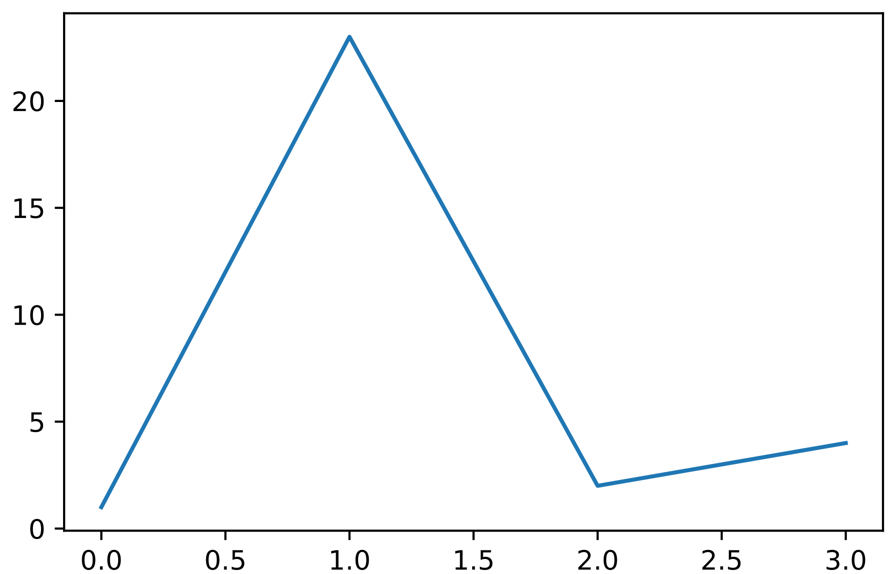

Figure 10.1: Simple Plot

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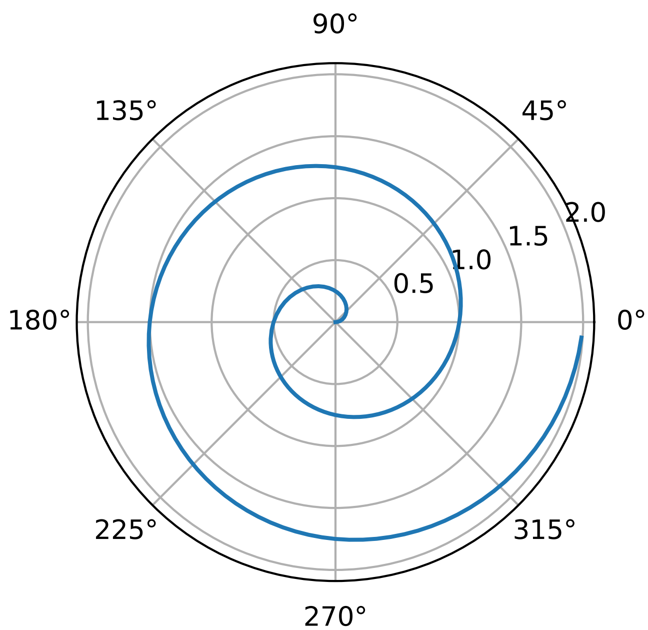

Figure 10.2: A spiral on a polar axis

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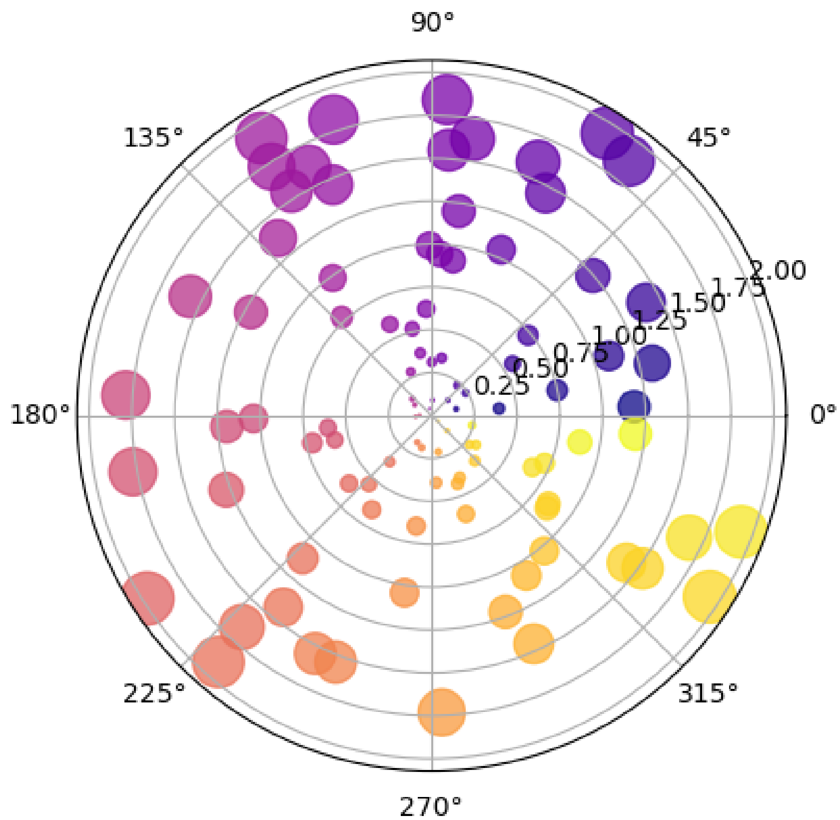

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11 Outlook

What can AI enable for biology?

Under your first header, include a brief introduction to your chapter.

Starting prompt for this chapter: Chapter 11 concludes the book with a forward-looking assessment of AI in microscopy, focusing on how it can/will enable biological discovery. It should highlight a few motivational examples of discoveries that AI has already enabled and discuss opportunities to which the reader is primed to contribute after reading this book.

11.1 Include section headers as appropriate

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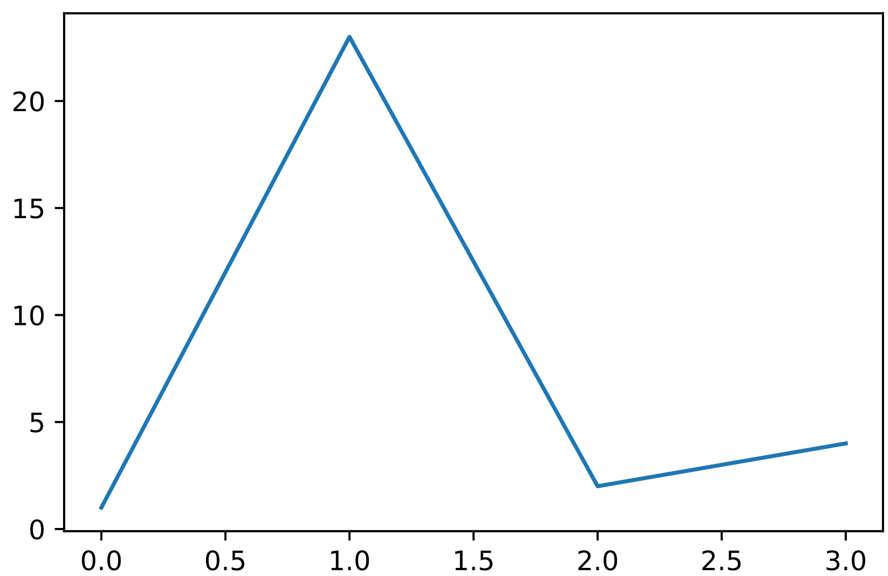

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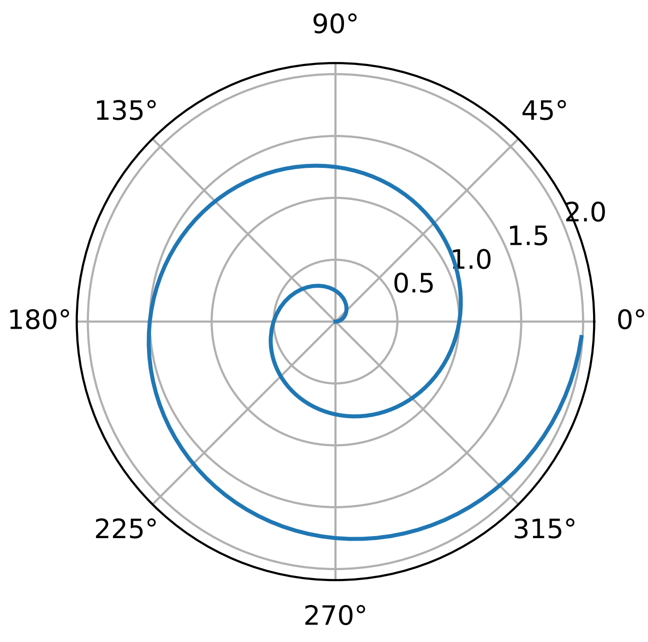

Figure 11.2: A spiral on a polar axis

Here is an example equation.

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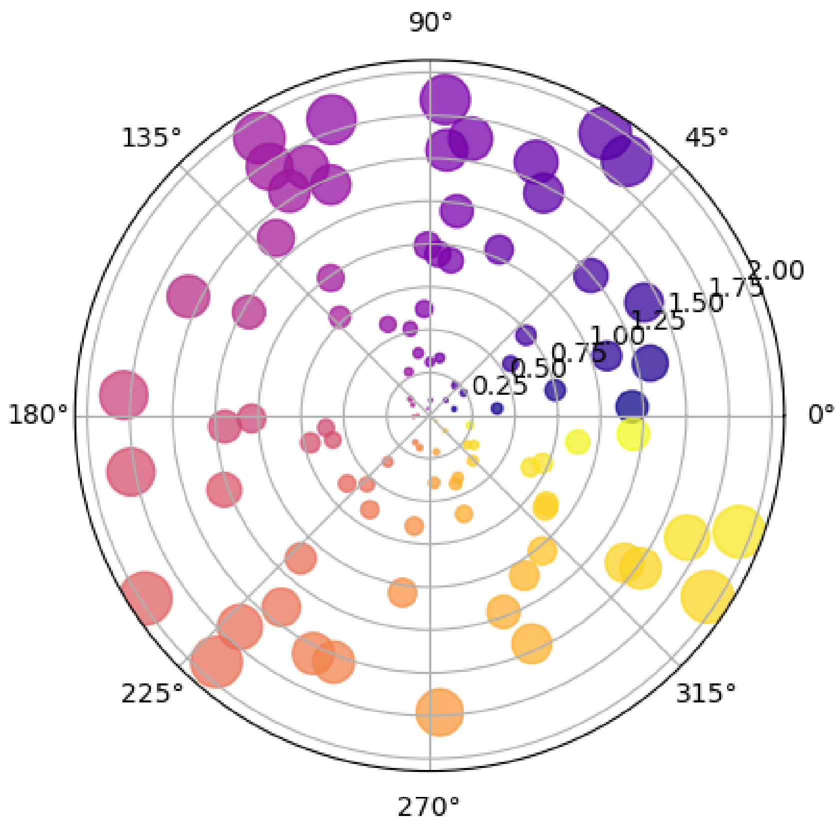

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Glossary

The glossary table will be automatically generated here after we resolve formatting issues.

Temporary Troubleshooting Table

See https://github.com/debruine/quarto-glossary/issues/10

```
Hallucinations

Outputs from a model that do not have a basis in the input data and may contain false or misl


Training Data

Data used to train an algorithm to make predictions.
```

References

1. Stringer, C., Wang, T., Michaelos, M. & Pachitariu, M. Cellpose: A generalist algorithm for cellular segmentation. *Nature Methods* **18**, 100–106 (2021).
2. Chamier, L. von *et al.* Democratising deep learning for microscopy with ZeroCostDL4Mic. *Nature Communications* **12**, 2276 (2021).