Resolution and Magnification

Rayleigh criterion

Minimum resolvable distance

Empty magnification

Magnifying beyond resolution limit

Nyquist sampling

2× sampling above highest frequency

Digital resolution

Pixel size vs optical resolution

Super-resolution preview

Breaking diffraction barrier

1. Rayleigh Criterion

The Rayleigh criterion defines the minimum distance at which two point sources can be distinguished as separate entities in an optical system. This fundamental principle is essential for understanding the resolution limits of microscopes and other imaging devices.

Mathematical Formula

$$d = 0.61\lambda / NA$$

Where:
- d = minimum resolvable distance
- λ = wavelength of light
- NA = numerical aperture

Physical Interpretation

Two point sources are considered "just resolved" when the central maximum of one Airy disk coincides with the first minimum of the other. This occurs when the intensity dip between the two peaks is approximately 26.5% of the peak intensity.

Key Points:

- Shorter wavelengths provide better resolution
- Higher NA objectives improve resolution
- Diffraction limits all optical systems

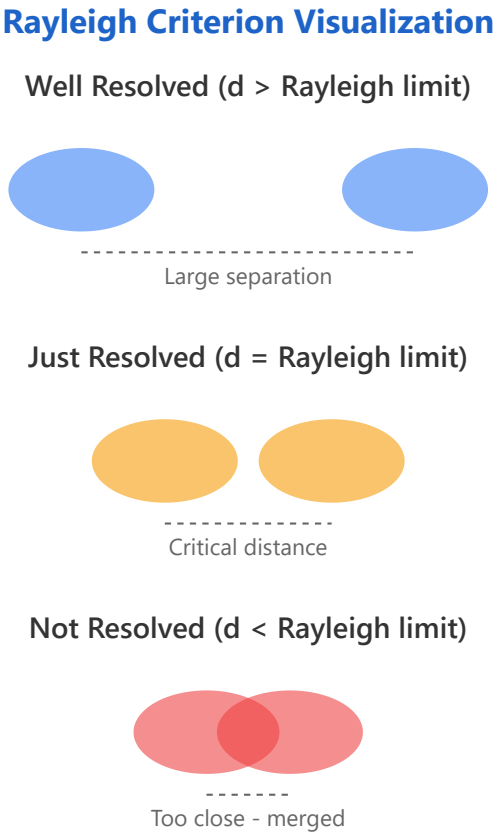

Illustration of the Rayleigh criterion showing three scenarios: well resolved, just resolved, and unresolved point sources

- Immersion media can enhance NA up to ~1.5

Example:

For green light (λ = 550 nm) with NA = 1.4:
d = 0.61 × 550 / 1.4 ≈ 240 nm
This represents the best lateral resolution achievable with conventional light microscopy.

2. Empty Magnification

Empty magnification occurs when an optical system magnifies an image beyond its resolution limit. While the image becomes larger, no additional detail is revealed—similar to digitally zooming into a low-resolution photograph.

Useful vs. Empty Magnification

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Useful magnification range:
500 × NA to 1000 × NA

Example for NA = 1.4:
• Minimum useful: 700×
• Maximum useful: 1400×
• Beyond 1400×: Empty magnification
```

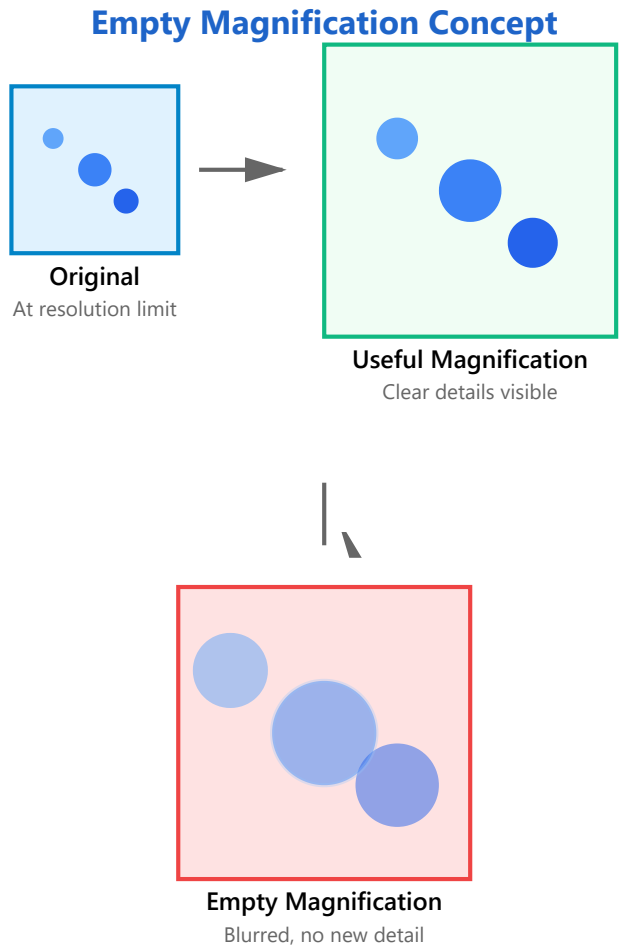
Empty magnification wastes optical performance and can actually degrade image quality by magnifying aberrations, noise, and artifacts without providing any additional structural information.

Consequences of Empty Magnification:

- No increase in resolvable detail
- Magnified diffraction patterns (Airy disks)
- Amplified noise and aberrations
- Reduced image brightness per unit area
- Potential eye strain for observers

Practical Example:

Using a 100×/1.4 NA objective with a 10× eyepiece gives 1000× total magnification (ideal). Adding a 2×



Comparison showing how useful magnification reveals detail while empty magnification only enlarges blur

intermediate magnifier creates 2000× total—this is empty magnification that adds no detail.

3. Nyquist Sampling

The Nyquist sampling theorem states that to accurately capture a signal, you must sample at least twice the highest frequency present. In microscopy, this means your pixel size must be at least 2× smaller than the smallest resolvable feature.

Sampling Requirements

```
Nyquist sampling criterion:
Pixel size ≤ (Resolution / 2)

For Rayleigh resolution d:
Pixel size ≤ d / 2 = 0.61λ / (2 × NA)

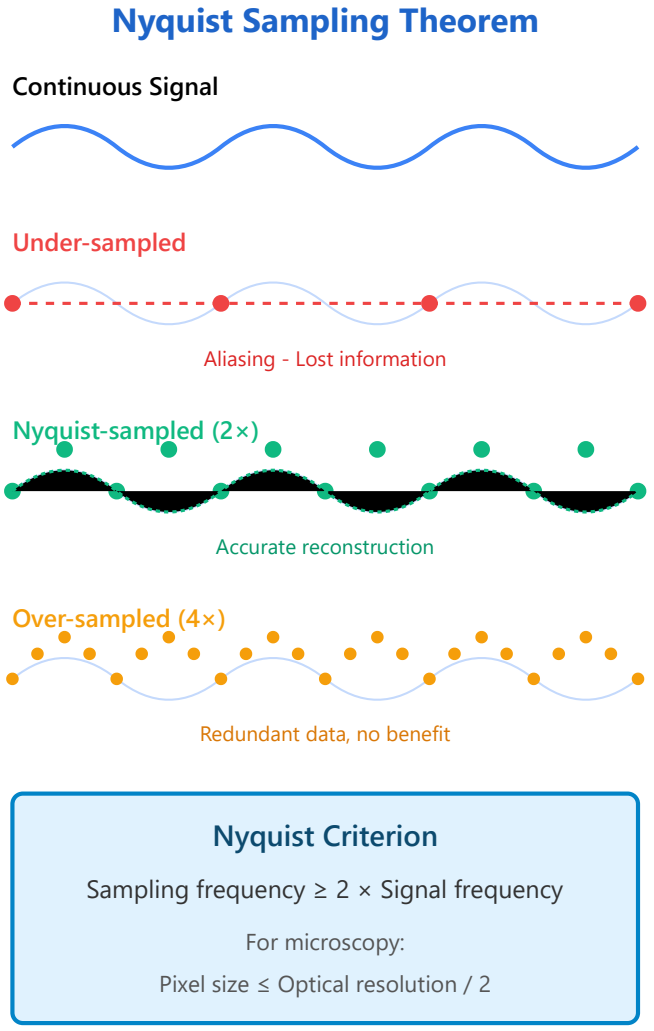
Practical recommendation:
Pixel size = d / 2.3 (Nyquist-Shannon)
```

Under-sampling vs. Over-sampling

Under-sampling (pixel size too large) leads to aliasing artifacts and loss of fine detail. **Over-sampling** (pixel size too small) wastes storage space, increases acquisition time, and provides no additional information beyond noise.

Sampling Guidelines:

- Under-sampled: Pixel size > d/2 → Aliasing
- Nyquist-sampled: Pixel size = d/2.3 → Optimal
- Over-sampled: Pixel size < d/3 → Diminishing returns
- 3× over-sampling rarely justified except for deconvolution



Demonstration of under-sampling (aliasing), Nyquist sampling (optimal), and over-sampling

Calculation Example:

For 100×/1.4 NA objective with green light (550 nm):
• Optical resolution: d = 240 nm
• Nyquist pixel size: 240/2.3 ≈ 104 nm
• Camera: Use 6.5 µm pixels → requires ~63× magnification

4. Digital Resolution

Digital resolution refers to the relationship between the physical pixel size of a camera and the optical resolution of the microscope. The key is to match your camera's sampling to the microscope's optical capabilities.

Calculating Effective Pixel Size

```
Effective pixel size = Camera pixel
size / Total magnification

Example:
• Camera: 6.5 µm pixels
• Objective: 63× / 1.4 NA
• Camera adapter: 1×
• Effective pixel: 6.5 / 63 = 103 nm
• Optical resolution: 240 nm
• Ratio: 240 / 103 = 2.3× ✓ (Optimal!)
```

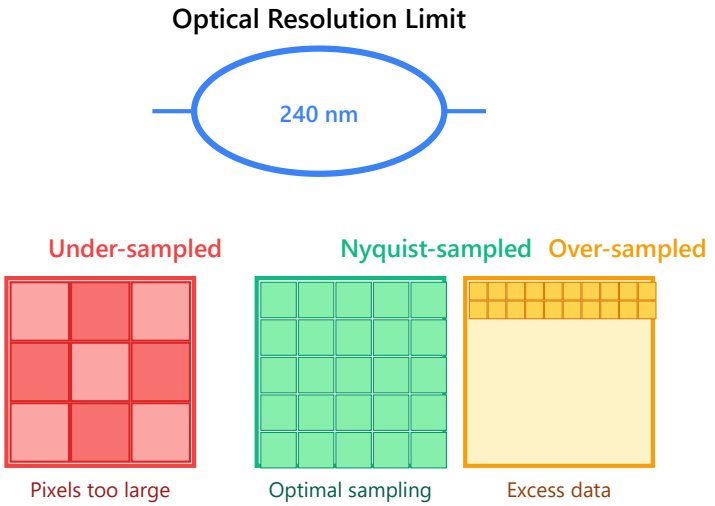
Matching Digital and Optical Resolution

The goal is to ensure your camera's effective pixel size is approximately 2-3× smaller than the optical resolution. This can be achieved by selecting appropriate magnification or using camera adapters.

Optimization Strategies:

- Use higher magnification objectives for small pixels
- Add camera adapters (0.5×, 0.63×, 1×, 1.6×)
- Consider camera binning for low-light conditions
- Match sensor size to field of view requirements

Digital vs Optical Resolution



Pixel Size Comparison

Condition	Pixel Size	Result
Under-sampled	> 120 nm	Aliasing
Nyquist	~100 nm	Optimal
Over-sampled	< 80 nm	Redundant

Effective pixel size = Camera pixel / Magnification

Target: 2-3× smaller than optical resolution

Visualization of how pixel size relates to optical resolution in digital imaging

- Balance between resolution, field of view, and speed

Practical Scenario:

Camera with 3.45 µm pixels, 100×/1.4 NA objective:
• Effective pixel: 3.45/100 = 34.5 nm
• Optical resolution: 240 nm
• Sampling: 240/34.5 = 7× over-sampled
Solution: Use 0.5× adapter → 69 nm effective (3.5× sampling) ✓

5. Super-resolution Microscopy Preview

Super-resolution microscopy techniques overcome the diffraction limit defined by the Rayleigh criterion, achieving resolution down to 10-20 nm. These methods revolutionized biological imaging and earned the 2014 Nobel Prize in Chemistry.

Main Super-resolution Techniques

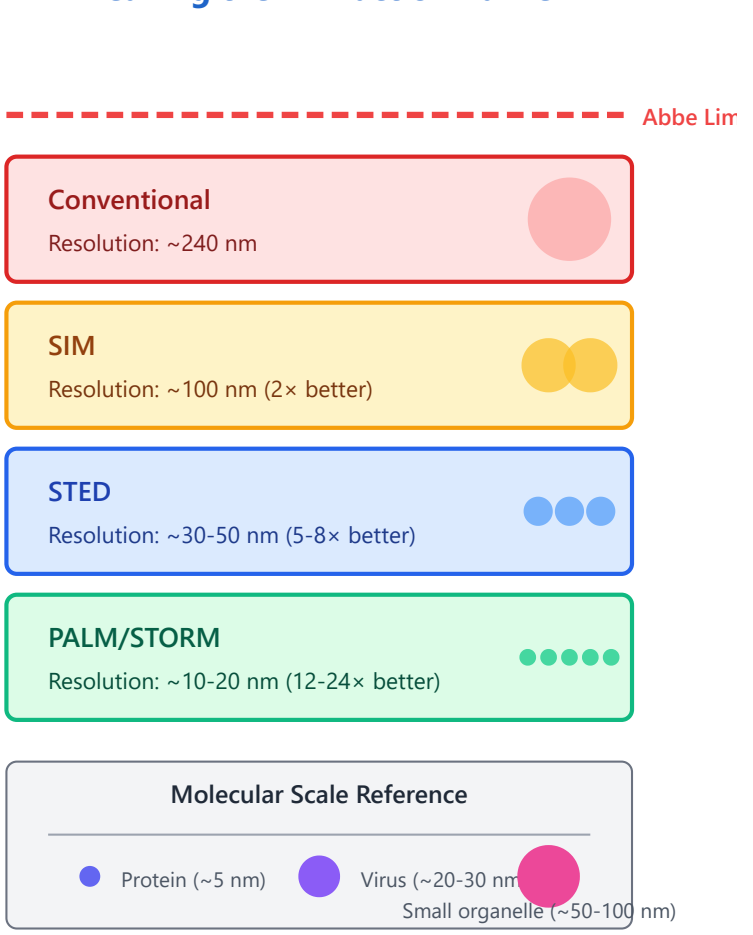
1. STED (Stimulated Emission Depletion)
Uses a depletion beam to narrow the effective point spread function, achieving ~30-50 nm resolution. This deterministic method can image in real-time.

2. PALM/STORM (Photoactivated/Stochastic Localization)
Activates and localizes individual fluorophores stochastically, then reconstructs a super-resolved image from thousands of frames. Achieves ~10-20 nm resolution.

3. SIM (Structured Illumination Microscopy)
Projects patterned light to extract higher frequency information, doubling resolution to ~100 nm. Fast and gentle on samples.

Key Advantages:

- Resolution 10-20× better than conventional microscopy
- Can visualize molecular-scale structures
- Compatible with live-cell imaging (some methods)



Comparison of resolution capabilities across different microscopy techniques, showing how super-resolution methods surpass the diffraction limit

- Reveals previously invisible cellular details

Current Limitations:

- Requires specialized equipment and expertise
- Often slower than conventional microscopy
- May need special fluorophores
- Higher photobleaching in some techniques
- Complex data processing requirements

Resolution Comparison:

• Conventional (Rayleigh): ~240 nm
• SIM: ~100 nm (2× improvement)
• STED: ~30-50 nm (5-8× improvement)
• PALM/STORM: ~10-20 nm (12-24× improvement)
• Expansion microscopy: ~70 nm (physical expansion)