Point spread function engineering

Linear systems

• A system is mapping of a set of input functions into a set of output functions
  \[ g_2(x_2, y_2) = S[g_1(x_1, y_1)] \]

• Linearity: For all input functions \( p, q \) and complex constants \( a, b \)
  \[ S[ap(x_1, y_1) + bq(x_1, y_1)] = aS[p(x_1, y_1)] + bS[q(x_1, y_1)] \]

• Sifting property of \( \delta \) function
  \[ g_1(x_1, y_1) = \int \int g_1(\xi, \eta)\delta(x_1 - \xi, y_1 - \eta)d\xi d\eta \]

• Linear combination of weighted and displaced \( \delta \) functions

• Response of the linear system:
  \[ g_2(x_2, y_2) = S\left[ \int \int g_1(\xi, \eta)\delta(x_1 - \xi, y_1 - \eta)d\xi d\eta \right] \]
  \[ g_2(x_2, y_2) = \int \int g_1(\xi, \eta)S[\delta(x_1 - \xi, y_1 - \eta)]d\xi d\eta \]

• Impulse response or Point Spread Function is the system’s response to a point source:
  \[ h(x_1, y_1; \xi, \eta) = S[\delta(x_1 - \xi, y_1 - \eta)] \]

• Linear system completely characterized by \( h \)
  \[ g_3(x_2, y_2) = \int \int g_1(\xi, \eta)h(x_1, y_1; \xi, \eta)d\xi d\eta \]
Space (or shift) invariant linear systems

• A linear (imaging) system is space invariant (or isoplanatic) if the response depends only on the distances between the excitation point and the response point

\[ h(x_1, y_1; \xi, \eta) = h(x_2 - \xi, y_2 - \eta) \]

– Response to arbitrary located point source excitation changes in location but not in functional form

• System response is now:

\[
g(z, y) = \int \int g(\xi, \eta) h(x_1 - \xi, y_1 - \eta) d\xi d\eta
\]

\[
g_z = g \ast h
\]

\[
G_z(f_x, f_y) = H(f_x, f_y) G(f_x, f_y)
\]

\[
H(f_x, f_y) = \mathcal{F}[h(x, y)]
\]

is the transfer function

Decomposition in complex exponentials (eigenfunctions of linear space invariant systems) and multiplication by \( H \) - transfer function represents the eigenvalues of the system.
(Spatial) frequency analysis

Impulse response of a (defocused) optical imaging system

Object is a point source on axis. At aperture the complex amplitude is

\[ U(x, y) \approx h_1 \exp \left( -jk \frac{x^2 + y^2}{2d_1} \right) \]

After crossing transmittance function of a lens with a pupil function \( p(x, y) \):

\[ U_1(x, y) = U(x, y) \exp \left( jk \frac{x^2 + y^2}{2f} \right) p(x, y) \]

Propagation (Fresnel) to distance \( d_2 \) behind the lens

\[ h(x, y) = h_2 \int_{-\infty}^{\infty} U_1(x', y') \exp \left[ -j \frac{\pi(x-x')^2 + (y-y')^2}{\lambda d_2} \right] dx' \, dy' \]

After substitution and using the FT:

\[ h(x, y) = h_1 h_2 \exp \left( -j \frac{\pi x^2 + y^2}{\lambda d_2} \right) \mathcal{F} \left( \frac{x}{\lambda d_2}, \frac{y}{\lambda d_2} \right) \]

\( \mathcal{F} \) is the FT of \( p_1 \), the generalized pupil function

\[ p_1(x, y) = p(x, y) \exp \left( -j \frac{\pi x^2 + y^2}{\lambda} \right) \]

\( \varepsilon \) is the focusing error

\[ \varepsilon = \frac{1}{\sigma_1} + \frac{1}{\sigma_2} - \frac{1}{f} \]
Impulse response of a focused coherent optical imaging system with circular aperture

\[ h(x, y) = h(0, 0) \frac{2J_1(\pi Dp/\lambda d_2)}{\pi Dp/\lambda d_2} \]

Transfer function of a coherent imaging system with circular aperture

\[ H(\nu_x, \nu_y) \approx p_1(\lambda d_2 \nu_x, \lambda d_2 \nu_y) \]

Imaging with a finite aperture

Point Spread Function (PSF)

**Numerical Aperture**

\((NA) = n(\sin \mu)\)

NA is a measure of an optical system’s ability to gather light and of its resolution.

**Resolution – Rayleigh criterion**

\[ \delta_x \approx \frac{\lambda}{2NA} , \quad \delta_z \approx \frac{2\lambda n}{NA^2} \]

The resolution of an imaging system is classically defined as the smallest distance between two points that can still be distinguished as two separate entities.
**F number**

\[ F\# = \frac{f}{D} \]

The f-number is a measure of the light gathering capability for far-away objects and of its resolution. The greater the f-number, the less light per unit area reaches the image plane of the system.

The working f\# (object not at infinity) is

\[ F_w\# = \frac{1}{2 \text{ NA}} \]

Comparison of \#32 (top-left corner) and \#5 (bottom-right corner). From http://en.wikipedia.org/wiki/F-number

**Relation between PSF and MTF**

The modulation transfer function is the magnitude response of the optical system to sinusoids of different spatial frequencies.

For incoherent illumination:

\[ \text{OTF} = \mathcal{F}(\text{PSF}) \text{ (normalized)} \]

\[ \text{MTF} = |\text{OTF}| \]

Adapted from http://micro.magnet.fsu.edu/
OTF of a diffraction limited system with a square aperture

OTF of a diffraction limited system with a circular aperture

Adapted from Goodman, *Introduction to Fourier Optics*

Aberrations

Diffraction limited system – a point source object generates a perfect spherical wave at the exit pupil

Aberrations - departure from perfect spherical wave

Generalized pupil function

\[ \tilde{P}(x, y) = P(x, y) \exp[jkW(x, y)] \]

Adapted from Goodman, *Introduction to Fourier Optics*
Wiener deconvolution

The goal is to minimize the effect of deconvolution noise at frequencies which have a poor signal to noise ratio.

 Imaging model

$$im(u, v, z) = psf(u, v, z) \otimes ob(u, v) + n(u, v)$$

 Restore $ob(u,v)$ via knowledge of $psf(u,v,z)$:

$$\hat{ob}(u, v) = g(u, v, z) \otimes im(u, v, z)$$

In the frequency domain, the Wiener filter is

$$\hat{g}(f_u, f_v, z) = \frac{psf(f_u, f_v, z)^{\dagger} im(f_u, f_v, z)}{|psf(f_u, f_v, z)|^2 im(f_u, f_v, z) + \tilde{n}(f_u, f_v)}$$

Then the reconstruction is:

$$\hat{ob}(f_u, f_v) = \hat{g}(f_u, f_v, z) \hat{im}(f_u, f_v, z)$$
Some applications of 3-D PSFs

Imaging with extended depth of focus

Point Spread Function engineering


Implementation

Diffractive micro-optics

Continuous surface relief optics

Phase-only

Binary amplitude

Spatial light modulators

Volume optics
Synthesis of 3D light fields

- **Analysis**: given the incident field and the system, determine the 3-D field
- **Synthesis**: Given the desired 3-D field and tolerances, determine the system subject to technological constraints.

---

Coherent vs. Incoherent

- Coherent light produces specified 3-D field

\[ h(x,y,z_0) \]
Coherent vs. Incoherent

- Slight modification produces optical system with Coherent impulse response: \( h(x,y,z_o) \) …

Input point source

3-D impulse response (coherent)

\( f \)

… and Incoherent impulse response: \( |h(x,y,z_o)|^2 \)
- in general shift variant

Incoherent illumination

Input plane

Phase mask

3-D impulse response (Incoherent)

Similarly for partially coherent impulse response \( h_{PC}(x,y;x',y';z_o) \)
Wavefront coding for extended depth of field

\[ P(x) = \begin{cases} \frac{1}{2} e^{j\pi s^3} & \text{for } |s| \leq 1 \\ 0 & \text{otherwise} \end{cases}, \quad |s| >> 20 \]

Cubic phase mask

The problem of depth
Obtain 3D information from 2D data - “images”

System
Optical path length
z (x,y)
brightness

Context
Parallax
Focus/Defocus

Depth cues
Engineering the Point Spread Function

Every optical image has 3D information encoded in the defocus of the image

- Design the optical response (PSF) to ease the estimation of defocus
- PSF that changes quickly through focus
- PSF constrained to satisfy the wave equation
- Implementation using phase diffractive/refractive mask

Typical PSF changes slowly
Designed PSF changes fast

Double-helix PSF

Positive defocus  In-focus  Negative defocus

(a) DH-PSF
(b) Standard PSF

Qualitatively, the rotating PSF changes faster in depth (for the same numerical aperture)

**Depth from diffracted rotation**

- **Double-helix PSF**
  - Generated by a mask in Fourier plane
  - Exhibits two main lobes that rotate with defocus

- **Depth is estimated from rotation angle**

- **“Axial super-resolution”**
  - Rate of rotation maximum in focal plane!
  - Standard PSF: constant within the depth of field
  - Information theoretical analysis shows improved 3D position estimation accuracy

---

**3D Imaging - Algorithm**

- In general two image frames are necessary for the reconstruction of continuous 3D objects
- Needed to separate the contribution of the object from the PSF
  - Under-constrained problem, infinite solutions
  - Blurry image of a sharp object vs. sharp image of a blurry object

\[ \tilde{h}_{rot}(y') = \mathcal{F}^{-1} \left( \frac{I_{ref}}{|I_{ref}|^2 + \sigma^2} I_{rot} H_{ref} \right) \]
Experimental Images and PSF estimation

- Need a practical (fast) algorithm to estimate defocus
  - Measure angle of rotation

Experiment - Microscopy

A. Greengard, R. Piestun, to be published
Effect of spectral bandwidth

- Real scenes have in general broad spectral bandwidth – produce smeared images with DOE
- Mask designed for 550nm exhibits rotating lobes for 100nm bandwidth!

- Spatial light modulator (SLM) phase mask
- On-axis mask encoding to avoid dispersion
- Allows 2ms update of optical function

* SLM from Boulder Nonlinear Systems

Gauss Laguerre Modes

The Modal Plane:
Rotating PSFs

- Gauss Laguerre (GL) basis
  - All paraxial fields are superposition of GL modes
- Rotating beams
  - A GL mode superposition along a straight line rotates upon propagation

Intensity Phase

Intensity Phase


High efficiency DH-PSF

Exact rotating PSF Initial phase-only design Optimal DH-PSFs

\[ \eta_{TF} \] 1.84% \[ \eta_{TF} \] 42.07% \[ \eta_{TF} \] 57.01%

- Design Method - Iterative optimization in 3 domains
  - Gauss Laguerre modal plane
  - Fourier domain
  - 3D spatial domain
- Desired features
  - Phase only transfer function
  - Lobes rotate with defocus
  - Minimal side lobes
- High-efficiency phase mask exhibits 7 phase singularities

**High efficiency DH-PSF**

Exact GL superposition  Initial estimate  Optimized transfer function.

GL modal plane decompositions of the transfer functions in (a), (b), and (c)

**Three-dimensional particle localization**

- Object: Discrete points in a volume
- Phase mask in Fourier plane
- Axial position estimated from PSF’s rotation angle
- Transverse position is the midpoint of PSF lobes

3D position localization of multiple object points with a single image

**Brightfield experimental results**

- Standard PSF image blurs with defocus
- Double-helix PSF image encodes depth in PSF’s rotation angle
- 1° rotation every 35nm
- Standard deviations calculated from 100 successive estimations with 239ms exposure

![Standard and Double-helix PSF images](image)

**Standard deviations**

\[ \sigma_x : 8\text{nm} \]
\[ \sigma_y : 4\text{nm} \]
\[ \sigma_z : 8\text{nm} \]

**Nanometer scale accuracy in 3D!**

![3D positions](image)


---

**Fluorescent particle localization**

*Estimate the 3D position of fluorescent micro-particles*

- Biology
  - Intracellular structure
  - Flow cytometry
- Chemistry
  - Molecular dynamics
- Physics
  - Forces at the nanoscale
- Engineering
  - Nanofabrication

![Intracellular Imaging](image)

Intracellular Imaging

*Science 313, 1642 (2006)*

![Single molecule Imaging](image)

Single molecule Imaging

*Moerner Lab, Stanford Univ.*

![Particle trapping](image)

Particle trapping

*Perkins Lab, CU Boulder*
Fluorescent particle localization and tracking

- Fluorescent sample
  - 1 μm diameter, yellow-green microspheres

- Fluorescent particle tracking
  - Limit on emitted photons
  - Limit on exposure time

- Blazed encoding of DH-PSF mask
  - 1st order: DH-PSF image
  - 0th order: Standard PSF image


Information theoretical analysis

Double-helix PSF carries more information about 3D positions than standard PSF

Effect of bandwidth

- Fluorescence has broad bandwidth (~100nm)
- Mask designed for 550nm exhibits rotating lobes for both 500nm and 600nm (100nm bandwidth!)
- Spatial light modulator (SLM) mask implementation
  - Blazed mask encoding to avoid non-ideal SLM response
  - 10nm bandwidth filter corrects blazed mask chromaticity
  - Blazed mask and 10nm filter not required with on-axis implementation*

Fluorescent particle localization

- Fluorescent microspheres embedded in cured optical cement
- Standard deviations computed from 100 estimations

<table>
<thead>
<tr>
<th>Particle</th>
<th>X±σX (nm)</th>
<th>Y±σY (nm)</th>
<th>Z±σZ (nm)</th>
<th>σX (nm)</th>
<th>σY (nm)</th>
<th>σZ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.141</td>
<td>0</td>
<td>2.055</td>
<td>14</td>
<td>11</td>
<td>41</td>
</tr>
<tr>
<td>2</td>
<td>2.246</td>
<td>7.716</td>
<td>0</td>
<td>11</td>
<td>11</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>9.670</td>
<td>715</td>
<td>19</td>
<td>19</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>1.902</td>
<td>12.961</td>
<td>962</td>
<td>13</td>
<td>11</td>
<td>31</td>
</tr>
</tbody>
</table>

**Fluorescent particle tracking**

- Microspheres moving in water, which is trapped inside cured optical cement voids

![Images of fluorescent particle tracking](image)

---

**Velocity measurements**

- Three-dimensional time-varying velocities
  - Microsphere 1 exhibits low velocities → bound to optical cement
  - Microspheres 2 and 3 have relatively higher velocities

---