Metrology and Sensing

Lecture 15: Confocal sensors
2019-02-05
Herbert Gross
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<td>Metrology of aspheres and freeforms</td>
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<td>Principle, resolution and PSF, microscopy, chromatical confocal method</td>
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Content

- Principle of confocal imaging
- Resolution and PSF
- Pinhole size
- Impact of aberrations
- Scanning
- Examples / applications
- Chromatical confocal method
- Laser scan microscope
- Depth resolution (sectioning) with confocal pinhole
- Transverse scan on field of view
- Digital image
- Only light coming out of the conjugate plane is detected
- Perfect system: scan mirrors conjugate to pupil location
- System needs a good correction of the objective lens, symmetric 3D distribution of intensity
Confocal 3D Image Collection

- Contact free optical sectioning
- 3D information collection & reconstruction
- 3D measurement and analysis
- The laser focus is moved over the sample (flying spot method)
- The measured intensity at each spot forms an xy image frame

Ref: M. Kempe
Confocal Images

Depth resolved images

Ref.: M. Kempe
Confocal Microscopy

3-D volume imaging with reconstruction in confocal Laser scan microscope

a) Classical microscopy
depth of object : 300 μm

b) Confocal microscopy
with 3-D reconstruction

Ref: M. Kempe
Examples

- Microelectronic circuit
- Abrasive paper
- Smooth paper

Ref.: R. Leach
Examples

- Silicon surface with stitching
- Microlens array
Laser scan microscope produces only images in combination with software for the image processing.
Realtime image gathering is possible today.

Usually the illumination is a scanning laser beam.

Usually the detection/observation uses the same lens.

The confocal pinhole detection guarantees:
- a z-sectioning capability
- a good suppression of straylight out of other planes in the sample

In scanning systems:
- the field is generated by transverse scanning with a mirror in a pupil-conjugated plane
- in case of volume imaging, the z-scan is performed by moving the stage
- the signal beam is descanned after a beam splitter
- primary image gathering is monochromatic in a plane-by-plane z-scan

Due to the very small pinhole, the sensitivity of the microscope is high:
- strong impact on residual aberrations
- large environmental sensitivity
Confocal Laser Scan Microscope

- Complete setup: objective / tube lens / scan lens / pinhole lens
- Scanning of illumination / descanning of signal
- Scan mirror conjugate to system pupil plane
- Digital image processing necessary
Fourier optical model:
- illumination with point spread function $h_{ill}$
- object function plane, $t_{obj}$, scanned
- detection with point spread function $h_{det}$
- detector function by pinhole size $D_{ph}$

General transform of amplitudes

$$U_2 = U_1 * h_{ill}$$

$$U'_2 = U_2 \cdot t_{obj}$$

$$U_3 = U'_2 * h_{det}$$

$$U'_3 = U_3 \cdot D_{ph}$$
- Amplitude of PSF: \( h \)
  - Illumination and observation with same lens: identical PSF
- Confocal intensity in image
- Real conditions:
  - thick sample, straylight from other z-planes
  - apodization of source due to laser illumination
  - residual aberrations of lenses
  - finite size of the pinhole
  - special shapes of detectors (circle, square, slit,..)
  - Partial coherence of illumination
  - high-NA, vectorial PSF
  - wavelength shift for fluorescence
- Other/different imaging modes:
  - 2-photon
  - 4\(\pi\)
  - interference
  - structured illumination
  - ...

\[
H_{\text{psf}} = H_{\text{ill}} = H_{\text{obs}}
\]

\[
I_{\text{conf}} = |H_{\text{psf}}|^4
\]

\[\lambda_1 < \lambda_2\]
Image Formation Confocal LSM

Special cases:

- Brightfield, perfectly small pinhole
  \[ D = \delta(x) \delta(y), \text{ imaging coherent} \]

- Fluorescence, coherence destroyed
  perfectly small pinhole

- Point like object
  \[ t_{\text{obj}} = \delta(x) \delta(y) \]

- Point object and perfectly small pinhole

- Plane mirror object
  \[ t_{\text{obj}} = \text{const.} \text{ perfectly small pinhole} \]

\[ I_{\text{ima}} = \left| \left( h_{\text{ill}} \cdot h_{\text{det}} \right) \otimes t_{\text{obj}} \right|^2 \]

\[ I_{\text{ima}} = \left| \left( h_{\text{ill}} \cdot h_{\text{det}} \right) \right|^2 \otimes t_{\text{obj}} \quad \lambda_{\text{ill}} < \lambda_{\text{det}} \]

\[ I_{\text{ima}} = \left| h_{\text{ill}} \right|^2 \cdot \left| h_{\text{det}} \right|^2 \otimes D_{\text{ph}} \]

\[ I_{\text{ima}} = \left| h_{\text{ill}} \right|^2 \cdot \left| h_{\text{det}} \right|^2 \]

\[ I_{\text{ima}} = \iint \left| h_{\text{det}}(x, y, 2z) \right|^2 \, dx \, dy \]

\[ \lambda_{\text{ill}} = \lambda_{\text{det}} \quad h_{\text{ill}} = h_{\text{det}} \]

Ref: M. Wald
- Simple model of confocal imaging:
  - illumination with coherent PSF $H_{\text{ill}}$
  - object function $T_{\text{obj}}$
  - observation with coherent PSF $H_{\text{obs}}$

- Rearrangement
  spatial domain
  transfer in frequency domain

\[
I_{\text{conf}} = \left| \left( H_{\text{obs}} \cdot H_{\text{ill}} \right) \otimes T_{\text{object}} \right|^2
\]

\[
I_{\text{conf}}(x, y) = \left| \int \int T_{\text{obj}}(v_x, v_y) \cdot H_{\text{conf}}(v_x, v_y) \cdot e^{2\pi i(xv_x + yv_y)} \, dv_x \, dv_y \right|^2
\]

\[
H_{\text{conf}}(v_x, v_y) = H_{\text{ill}}(v_x, v_y) \otimes H_{\text{obs}}(v_x, v_y)
\]
Lateral and Axial Resolution

- Intensity distributions

Ref: U. Kubitschek
Confocal PSF

- Change of intensity distributions by confocal mode
  1. lateral
  2. axial

Ref: A. Szameit
Lateral and Axial Resolution

- Tradeoff between:
  1. lateral resolution
  2. axial resolution
  3. signal to noise ratio
     (detection yield)

Ref: U. Kubitschek
Confocal microscopes:
- Lateral resolution is a complicated function.
- Not only optical influence functions.
- Normalized transverse coordinate $v$
- Usual PSF: Airy
- Confocal imaging:
  Identical PSF for illumination and observation assumed

$$v = \frac{2\pi}{\lambda} \cdot x' \cdot \sin \alpha$$

$$I(v) = \left( \frac{2J_1(v)}{v} \right)^2$$

Resolution improvement be factor 1.4 for FWHM
Confocal Microscopy: Axial Sectioning

- Normalized axial coordinate

- Conventional wide field imaging:
  Intensity on axis
  
  \[ I(u) = \left( \frac{\sin(u/2)}{u/2} \right)^2 \]

  Axial resolution
  
  \[ \Delta z_{\text{wide}}^{(\text{approx})} = \frac{0.45 \cdot \lambda}{n' \cdot (1 - \cos \theta)} \]

- Confocal imaging:
  Intensity on axis
  
  \[ I(u) = \left( \frac{\sin(u/2)}{u/2} \right)^4 \]

  Axial resolution improved by factor 1.41 for FWhM
  
  \[ \Delta z_{\text{confo}} = \frac{0.319 \cdot \lambda}{n' \cdot (1 - \cos \theta)} \]

\[ u = \frac{8\pi}{\lambda} \cdot z \cdot \sin^2(\alpha / 2) \]
Microscopic Resolution

- Signal, lateral and axial resolution depends on imaging mode

<table>
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<tr>
<th>Imaging mode</th>
<th>signal</th>
<th>lateral resolution</th>
<th>axial resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>classical wide field</td>
<td>$S = I_{ill}$</td>
<td>$\Delta x = \frac{0.61 \cdot \lambda}{n \cdot \sin \theta} = 0.5 \cdot D_{airy}$</td>
<td>$\Delta z = \frac{2 \cdot \lambda}{n \cdot \sin^2 \theta} = 2 \cdot R_E$</td>
</tr>
<tr>
<td>confocal</td>
<td>$S = I_{ill} \cdot I_{obs}$</td>
<td>$\Delta x = \frac{0.40 \cdot \lambda}{n \cdot \sin \theta} = 0.33 \cdot D_{airy}$</td>
<td>$\Delta z = \frac{1.4 \cdot \lambda}{n \cdot \sin^2 \theta}$</td>
</tr>
<tr>
<td>2 photon</td>
<td>$S = I_{ill}^2$</td>
<td>$\Delta x = \frac{0.70 \cdot \lambda}{n \cdot \sin \theta} = 0.43 \cdot D_{airy}$</td>
<td>$\Delta z = \frac{2.3 \cdot \lambda}{n \cdot \sin^2 \theta}$</td>
</tr>
<tr>
<td>2 photon confocal</td>
<td>$S = I_{ill}^2 \cdot I_{obs}$</td>
<td></td>
<td></td>
</tr>
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</table>

- Approximation in these formulas: wavelength shift by fluorescence

- Lateral resolution and coherence general formula:
  $$\Delta x = k \cdot \frac{\lambda}{n \cdot \sin u}$$

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<tr>
<th>factors</th>
<th>coherent</th>
<th>incoherent</th>
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<tr>
<td></td>
<td>Rayleigh</td>
<td>Sparrow</td>
</tr>
<tr>
<td>Classical</td>
<td>0.82</td>
<td>0.74</td>
</tr>
<tr>
<td>confocal</td>
<td>0.56</td>
<td>0.48</td>
</tr>
</tbody>
</table>
- Ideal coherent transfer function: complex pupil function

- Confocal transfer function: product in spatial domain, convolution in frequency domain identical to incoherent OTF

- Confocal system has higher spatial resolution

\[
H_{coh}(v) = P\left( \frac{x_p}{\lambda \cdot f} \right)
\]

\[
H_{conf}(v) = \frac{2}{\pi} \cdot \left[ \arccos \left( \frac{v}{2} \right) - \frac{v}{2} \cdot \sqrt{1 - \left( \frac{v}{2} \right)^2} \right]
\]
- Increased resolution:
  - axial by factor 2
  - lateral by factor 2
  - no longer missing cone
- In general also improvement of contrast:
  suppression of straylight by pinhole

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<tr>
<th></th>
<th>lateral</th>
<th>axial</th>
</tr>
</thead>
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<tr>
<td>Conventional</td>
<td>2 NA/(\lambda)</td>
<td>(NA^2/\lambda)</td>
</tr>
<tr>
<td>Confocal</td>
<td>4 NA/(\lambda)</td>
<td>2NA^2/(\lambda)</td>
</tr>
</tbody>
</table>

Ref: M. Kempe
CTF in Microscopy

Brightfield

\[ P \times P^* \]

Incoherent laser scan microscope

\[ (P \times P^*) \times (Q \times Q^*) \]

Coherent laser scan microscope

\[ P \times Q \]

\[ P \times Q^* \]
Lateral Resolution in Confocal Imaging

- Comparison of PSF in wide field and confocal imaging
- Improved 2-point resolution in confocal mode
Generalized Depth Criterion

- $H_{CTF}$: coherent transfer function/PSF
- Integration over spatial frequencies function of the defocussing $z$
- Depth discrimination: FWHM of function $J(z)$ decrease with $z$

$$J(z) = \int_0^1 |H_{CTF}(v_x, z)|^2 dv_x$$

$$\Delta z = \frac{\lambda}{2\pi \cdot n \cdot \sin^2 \theta_o} \bigg|_{J(z)=1/2}$$
- Large NA:
  confocal, depth discrimination by NA

- Small NA:
  OCT, depth discrimination by axial coherence
Confocal Depth Signal

- Measurement of the axial confocal signal by using a lateral shifted tilted mirror
- Detection of spherical aberration degradation
Confocal Pinhole Size

- Change of pinhole size: Observation PSF changed
- Changing relative sizes of illumination and observation PSFs

\[ \Delta z_{\text{ill}} \quad \Delta z_{\text{obs}} \quad \Delta x_{\text{ill}} \quad \Delta x_{\text{obs}} \]

- Decreasing pinhole, detection PSF shrinking

Geometrical optical confocality

Transition range

Wave optical confocality

Illumination PSF quite smaller

PSF of observation and illumination of nearly same size
- Large pinhole: geometrical optic
- Small pinhole:
  - Diffraction dominates
  - Scaling by Airy diameter $a = D/D_{\text{Airy}}$
  - Diffraction relevant for pinholes
  \[ D < D_{\text{Airy}} \]
- Confocal signal:
  Integral over pinhole size
  \[ S(u) = \int_{0}^{a} |U(u,v)|^2 2\pi v \, dv \]

\[
\begin{array}{c|c|c|c|c|c}
\Delta x / D_{\text{Airy}} & NA = 0.30 & NA = 0.60 & NA = 0.75 & NA = 0.90 \\
\hline
0 & 0.5 & 1.0 & 1.5 & 2.0 \\
0.5 & 1.0 & 1.5 & 2.0 & 2.5 \\
1.0 & 1.5 & 2.0 & 2.5 & 3.0 \\
1.5 & 2.0 & 2.5 & 3.0 & 3.5 \\
2.0 & 2.5 & 3.0 & 3.5 & 4.0 \\
2.5 & 3.0 & 3.5 & 4.0 & 4.5 \\
3.0 & 3.5 & 4.0 & 4.5 & 5.0 \\
3.5 & 4.0 & 4.5 & 5.0 & \end{array}
\]

\[
\begin{array}{c|c|c|c|c|c}
D_{PH} / D_{\text{Airy}} & a = 0.5 & a = 1 & a = 2 & a = 3 \\
\hline
0 & 0 & 0 & 0 & 0 \\
0.5 & 1.25 & 2.5 & 5 & \end{array}
\]
- Numerical result for different sizes $a$ of the fiber radius
- The width increases with the fiber diameter
- The diffraction fine structure disappears with growing $a$

\[
S(\lambda) = \begin{cases} 
0.58 & a = 0 \\
0.585 & a = 5 \, \mu m \\
0.59 & a = 10 \, \mu m \\
0.595 & a = 20 \, \mu m \\
0.6 & \text{otherwise}
\end{cases}
\]
Confocal Signal and Pinhole Size

- Confocal signal $S(z)$ without aberrations as a function of the pinhole size $a$
- Smaller pinhole:
  - low signal (bad SNR)
  - better z-resolution (sectioning)
  - centroid remains constant in case of perfect imaging
Wilson's Formula

T. Wilson, Jour. of Microsc. 244 (2011) p113, Resolution and optical sectioning in the confocal microscope

Empirical formula for the width of the confocal signal in the case of a finite size pinhole and a fluorescence object
( self luminous, phase information lost )
First factor: diffraction
Second factor: finite size object

\[ D_{FWMH} = \frac{0.67 \cdot \lambda}{n - \sqrt{n^2 - NA^2}} \cdot 3^{\frac{1+1.47 \cdot \left( \frac{D_{ph}}{D_{airy}} \right)^3}{}} \]

\[ \Delta z_{FWMH} \]

\[ \lambda = 675 \text{ nm} \]

geometrical regime

diffraction regime

\[ \lambda = 450 \text{ nm} \]
Wilson's Formula: Critical Review

- Formula is valid for:
  1. one wavelength
  2. self luminous object (fluorescence molecule)
  3. perfect corrected spherical aberration

- A different object interaction changes the pre-factor:
  mirror:
  \[
  D_{FWHM} = \frac{0.45 \cdot \lambda}{n - \sqrt{n^2 - NA^2}} \cdot \sqrt{1 + 1.47 \left( \frac{D_{ph}}{D_{airy}} \right)^3}
  \]
  point reflector:
  \[
  D_{FWHM} = \frac{0.62 \cdot \lambda}{n - \sqrt{n^2 - NA^2}} \cdot \sqrt{1 + 1.47 \left( \frac{D_{ph}}{D_{airy}} \right)^3}
  \]

This causes errors of the first factor in the range of 30%

- Incorporation of spherical aberration:
  t.b.d., PCA analysis as approach seems to be promising
Variable Pinhole Diaphragm

- Real shape of pinhole: quadratic or circular signal depends on shape
- Variable pinhole easy to really quadratic
- Typical size: \(D_{\text{pinhole}} = 0.5...2 \ D_{\text{airy}}\)
- Easy to fabricate: approx. 30 mm very small numerical aperture in pinhole objective lens helps
Confocal Signal with Spherical Aberration

- Spherical aberration:
  - PSF broadened
  - PSF no longer symmetrical around image plane during defocus

- Confocal signal:
  - loss in contrast
  - decreased resolution

![Graph showing the effect of spherical aberration on the signal with different pinhole sizes.](image)
Confocal Signal with Spherical Aberration

- Spherical aberration with Zernike coefficient $W_{40}$
- Integration over finite size pinhole with radius $a$
- Asymmetry and width depends on $a$ and $W_{40}$
- Large pinhole:
  - depth discrimination decreased
  - fine structure disappears

- Sphärische Aberration mit Koeffizient $W_{40}$
Confocal Signal with Spherical Aberration

1. $c_9 = 0.3 \lambda$
   re-normalized

2. $c_9 = 1.0 \lambda$
   re-normalized

3. $c_9 = 1.0 \lambda$
   not normalized
Signal Errors due to Spherical Aberration

- In the case of spherical aberration, the confocal signal curve $S(z)$ is degraded:

  1. in position
     measurement error
     possible criteria:
     a) centroid
     b) midpoint of 50% threshold

  2. in width
     loss of accuracy
     possible criteria:
     a) $2^{nd}$ moment
     b) 50% threshold (FWHM)
Numerical Results

- Width of the confocal signal in the spectral domain

- Location of the sample z position
Confocal Distance Sensor

- Principle of the confocal distance sensor
Confocal Depth Measuring System

- The system is described by
  - Zernike $c_4$, gives the defocus
  - Zernikes $c_9$, $c_{16}$,... describe the correction of the system

- The point spread function is calculated with the help of the Zernike coefficients as

$$h_{psf}(a, \Delta z) = \int \int_{r < a} A_o \cdot e^{2\pi i [c_4 Z_4(x,y) + c_9 Z_9(x,y) + c_{16} Z_{16}(x,y) + ...]} \, dx \, dy$$

- Approximations of the model:
  1. psf considered as shift invariant
  2. perfectly incoherent fiber source
  3. perfectly homogeneous fiber source
  4. in reality, the sample is not a perfect mirror but introduces scattering contributions

![Diagram showing finite object size, pinhole diameter, total profile, and finite size PSF]
- Polychromatic illumination
- Airy diameter changes of measuring range
- Measuring accuracy varies over range
- Larger relative influence for small pinholes
Surface Smoothness

- Smooth / polished surface:
  - only reflected light is measured
  - maximum acceptable slope of the sample surface

\[ \alpha_{\text{max}} = \arcsin(\text{NA}) \]

<table>
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<tr>
<th>NA</th>
<th>maximum angle ( \alpha )</th>
</tr>
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<tr>
<td>0.3</td>
<td>18°</td>
</tr>
<tr>
<td>0.4</td>
<td>24°</td>
</tr>
<tr>
<td>0.5</td>
<td>30°</td>
</tr>
<tr>
<td>0.6</td>
<td>37°</td>
</tr>
<tr>
<td>0.7</td>
<td>44°</td>
</tr>
</tbody>
</table>

- Diffuse surface:
  - larger slopes can be measured
  - quantitatively the BRDF determines the limit
Ghost Foci

- If parts of a polished sample are spherical in shape:
  - ghost foci with high intensity
  - wrong interpretation of the depth out of the signal
- Setup with fiber and plane mirror for autocollimation
- Change of distance between test lens and fiber
- Analysis of the recoupled power into the fiber (confocal) gives the focal point
Chromatical Confocal Sensor

- Spectral sensitive sensor
- Objective lens with large axial chromatical aberration
Confocal Imaging with Hyper Chromate

- Wide field 20x0.5
- Confocal with chromate at low aperture 20x0.5
- Confocal with chromate at high aperture 50x0.9

Ref: R. Semmler
Goal:
1. large chromatical spreading (large CHL) $\Delta z$
2. large numerical aperture
3. corrected spherochromatism

In the case of a large ratio $\Delta z / f$, the numerical aperture shows a considerable change in the measuring interval

Design approach:
1. Achromate with positive flint and negative crown
2. Achromates cascaded
3. Improved spherochromatism by asphere
4. monochromatic lens with buried surface adapter

Principle

$$\Delta z = \frac{644 \text{ nm}}{\lambda} = \frac{546 \text{ nm}}{\lambda} = \frac{480 \text{ nm}}{\lambda}$$
Comparison

- Confocal signal as a function of distance and wavelength

- Cases:
  1. single lens / gauss-aberration corrected
  2. pinhole size 1 Airy
  3. no quadrature of confocal psf
Case 1-1

$NA_{image} = 0.3$, $NA_{object} = 0.22$

$\Delta z = 3$ mm, $f = 13$ mm

$z_{free} = 16.3$ mm
## Specifications

<table>
<thead>
<tr>
<th>NA</th>
<th>Δz</th>
<th>z\text{_free}</th>
<th>Δz = 3.9mm</th>
<th>Δz = 2.5mm</th>
<th>Δz = 0.5mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>3</td>
<td>16 mm</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0.4</td>
<td>0.4</td>
<td>10 mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>1</td>
<td>10 mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>0.12</td>
<td>3 mm</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Fourier optical model:
- object/sample to be assumed as a plane mirror
- fiber source incoherent, diameter $D_{fib}$, uniformly radiating
- optical system with point spread function $h_{psf}$
- confocal detection by fiber (pinhole) size $D_{fib}$

Incoherent imaging model to get the intensity of at the fiber

Calculation of the confocal signal by integration over the pinhole

$$I_{ima}(a, \Delta z) = I_{fib}(a) \otimes |h_{psf}(\Delta z)|^2$$

$$S_{conf}(a, \Delta z) = \iint_{r<a} I_{ima}(a, \Delta z) \, dx \, dy$$
Time is Over
Feedback

nothing clear?

too complicated?

too much stuff?

Ref: D. Shafer