Two-Photon microscopy with High Depth of Field using an Axicon

P. Dufour¹, Y. De Koninck² and N. McCarthy¹

¹COPL, Université Laval
²Centre de recherche Université Laval Robert-Giffard
Québec, Canada
Contents

- Conventional two-photon microscopy; advantages and limitations
- What is an axicon?
- Two-Photon microscopy with an axicon
- Comparison of an axicon and a microscope objective
- Examples of thick sample images
- Conclusion
Two-photon microscopy

- Scheme

- Laser beam (Ti:sapphire)
- 100 fs pulse
- Dichroic mirror
- Scanning system
- Objective (z-scan)
- Sample
- Focal plane
- PMT
- Filter

June 2005
Two-photon microscopy

Two-photon microscopy (low depth of field)

Projection image

Fluorescence image

June 2005
Two-photon microscopy (limitations)

- Challenge for rapid imaging from several cells at different focal point.
What is an axicon?

- Comparison of thin lens and axicon
What is an axicon? (more details)

- Axicon of diameter D

Mathematical expression:

\[ I(\rho) = I_0 \exp \left[ -2 \left( \frac{(n-1)z \tan \alpha}{w_0} \right)^2 \right] J_0^2 \left[ k_0 \rho (n-1) \tan \alpha \right] \]

\[ I_0 = \frac{4 A_0 (n-1) \lambda z \tan^2 \alpha}{w_0^4} \]
Two-photon microscopy using an axicon

Scheme

- Laser beam (Ti:sapphire)
- Dichroic mirror
- Axicon (transverse scanning)
- Sample
- PMT
- Filter

June 2005
Two-photon microscopy using an axicon

Two-photon microscopy (high depth of field)

Projection image

Fluorescence image

June 2005
Comparison of an axicon and an objective

Axicon: $\rho_0 = 1$ μm
Objective: $\rho_0 = 1$ μm
$I_{\text{max}}/I_{\text{max2}} = 2\%$ (16 % confocal)

Axicon: $L = 3$ mm
Objective: $L = 7$ μm

June 2005
Comparison of an axicon and an objective

Axial resolution

Axicon: 30°

Objective: NA = 0.5
Results with a conventional two-photon

- Twelve scans (300 μm x 300 μm) taken with the conventional two-photon microscope (distance between consecutive plane is 50 μm)
Comparison of both systems

With an objective 
(sum of 12 scans)

With an axicon 
(1 scan)

- Sphere diameter = 15 μm; Sample thickness > 1 mm.

June 2005
Conclusion

- We have demonstrated that a two-photon microscope using an axicon can be used to get fluorescence image of a sample.
- We can increase the depth of field to get a complete image of a thick sample with a single scan.
- The resolution of a 30° axicon is as good as a NA = 0.5 objective.
- It is possible to modify the transverse profile of the incident beam to obtain a constant intensity along the z axis in the sample.
- This system can be use to observe dynamic phenomena over short time scales in thick samples.
- Axicon-based microscopy maintains the advantage of two-photon excitation, namely small excitation volume and low dispersion in biological tissue (allowing deep tissue imaging).
Acknowledgments

CIHR NeuroPhysics Training Program

Le Centre de recherche Université Laval Robert-Giffard

June 2005