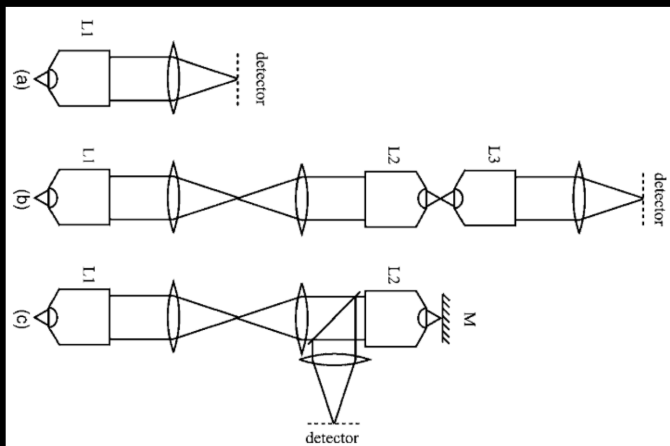
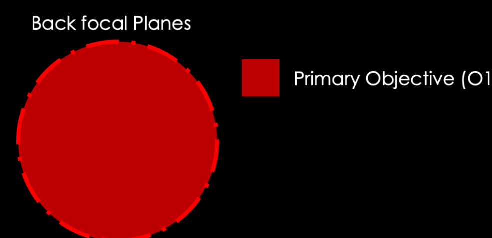
Remote focusing

- These two papers instrumental in seeding the idea for what follows (OPM, SCAPE):

  Botcherby EJ, Juskaitis R, Booth MJ, Wilson T (2007) Aberration-free optical refocusing in high numerical aperture microscopy. Opt Lett 32:2007

  Botcherby EJ, Juškaitis R, Booth MJ, Wilson T (2008) An optical technique for remote focusing in microscopy. Opt Commun 281:880–887





- Remote scanning of the imaging plane of a microscope with no movement of the objective lens relative to the sample

Remote focusing

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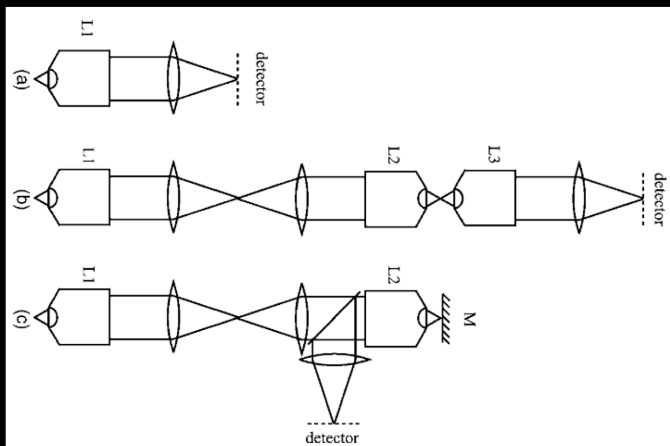

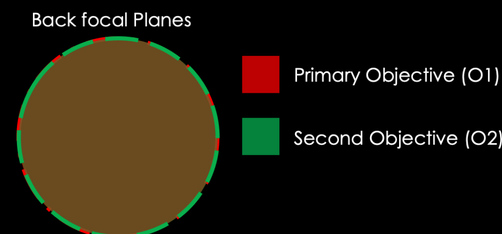

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Remote focusing

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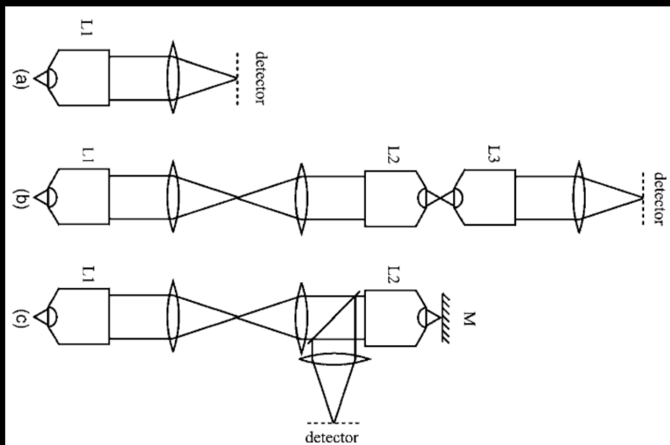

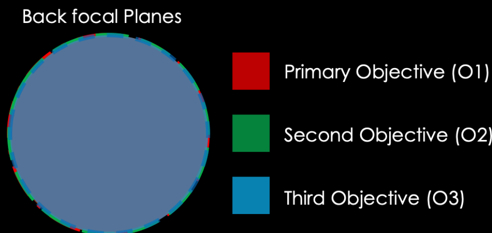

- Remote scanning of the imaging plane of a microscope with no movement of the objective lens relative to the sample

Remote focusing

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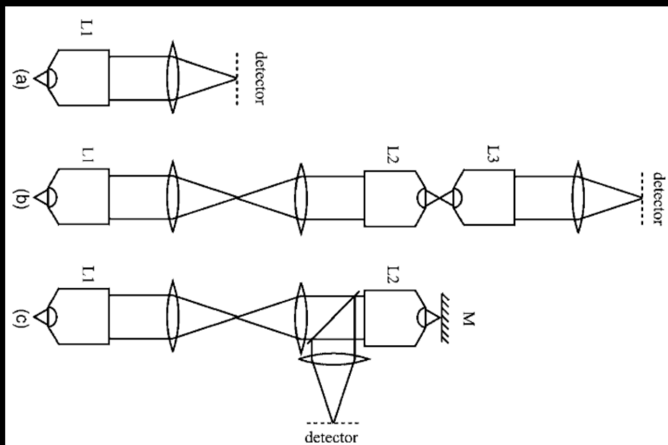
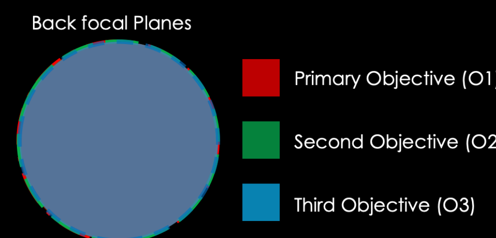
  Botcherby EJ, Juškaitis R, Booth MJ, Wilson T (2008) An optical technique for remote focusing in microscopy. Opt Commun 281:880–887

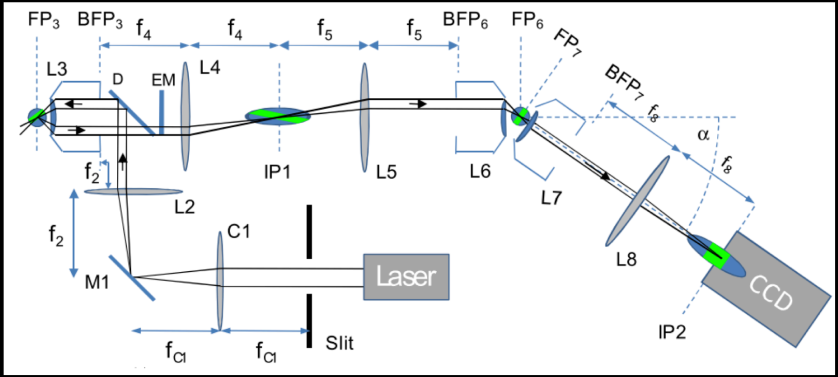


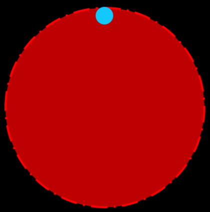
Re-image condition:
Magnification from object to intermediate image = n_1/n_2



- Remote scanning of the imaging plane of a microscope with no movement of the objective lens relative to the sample

Oblique plane microscopy: OPM (2008)





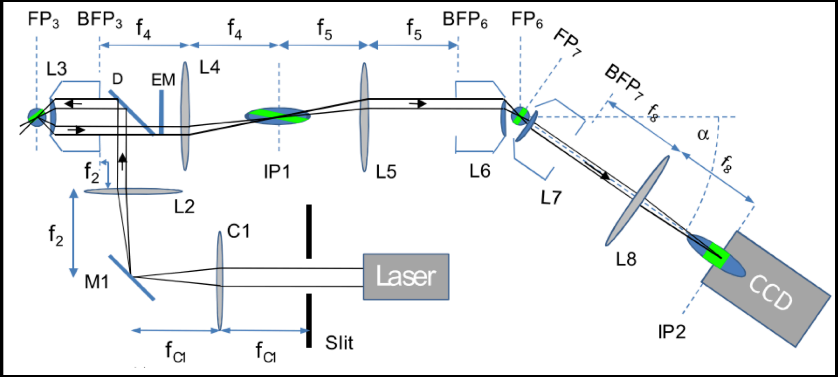
- Light-sheet (NA of 0.0785) at the edge of the primary objective
  light-sheet makes an angle of 60° with the optic axis

Primary Objective (O1)
Olympus 60x/1.35NA oil

Excitation beam

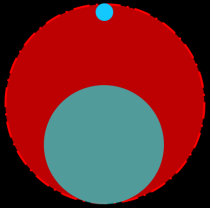
Dunsby C (2008) Optically sectioned imaging by oblique plane microscopy. Opt Express 16:20306

Oblique plane microscopy: OPM (2008)



- Light-sheet (NA of 0.0785) at the edge of the primary objective
  light-sheet makes an angle of 60° with the optic axis

- Orthogonal detection cone, half-angle of 33°
  Corresponds to NA(potential) of 0.82



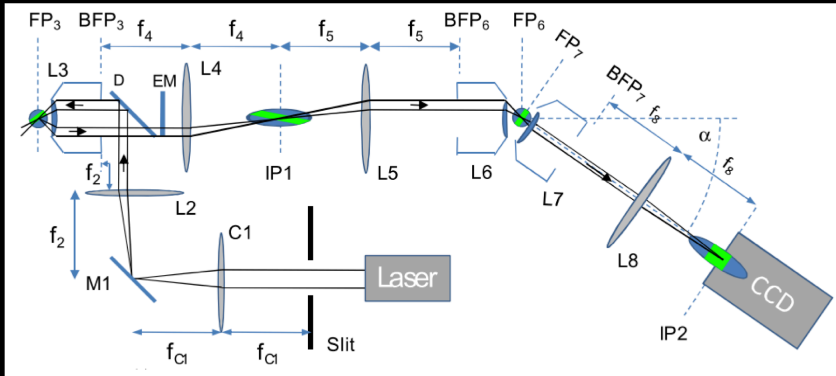
Primary Objective (O1)
Olympus 60x/1.35NA oil

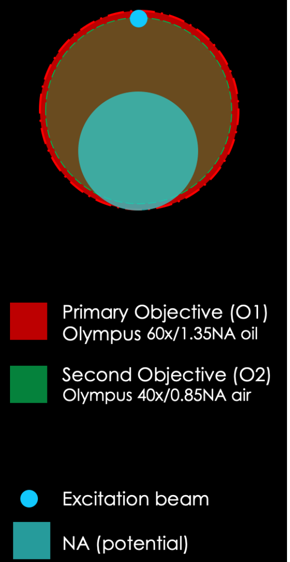
Excitation beam

NA (potential)

Dunsby C (2008) Optically sectioned imaging by oblique plane microscopy. Opt Express 16:20306

Oblique plane microscopy: OPM (2008)



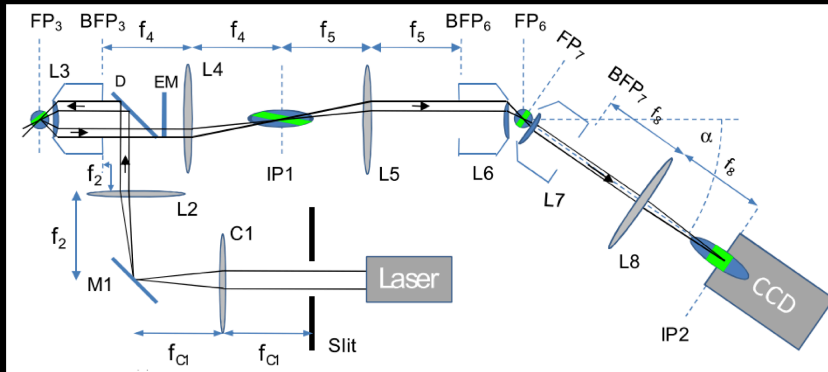


- Light-sheet (NA of 0.0785) at the edge of the primary objective
  light-sheet makes an angle of 60° with the optic axis

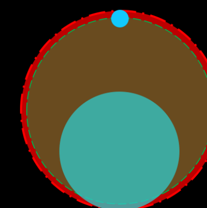
- Orthogonal detection cone, half-angle of 33°
  Corresponds to NA(potential) of 0.82

Dunsby C (2008) Optically sectioned imaging by oblique plane microscopy. Opt Express 16:20306

Oblique plane microscopy: OPM (2008)



- Light-sheet (NA of 0.0785) at the edge of the primary objective
  light-sheet makes an angle of 60° with the optic axis
- Orthogonal detection cone, half-angle of 33°
  Corresponds to NA(potential) of 0.82
- n_1/n_2 = magnification from object to intermediate image = 1.5 ✓



Primary Objective (O1)
Olympus 60x/1.35NA oil

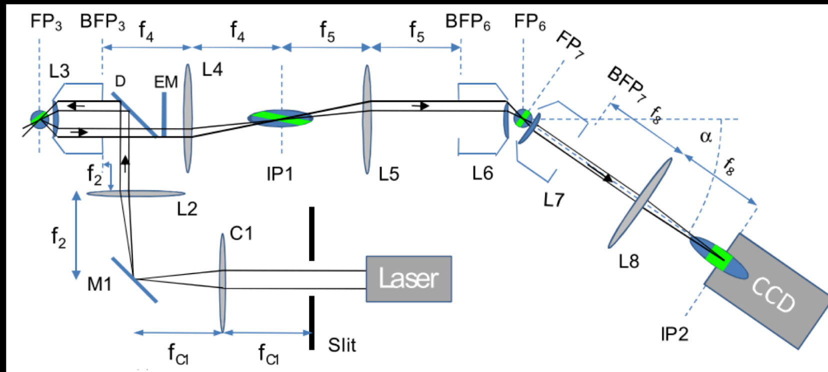
Second Objective (O2)
Olympus 40x/0.85NA air

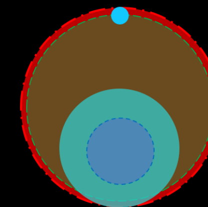
Excitation beam

NA (potential)

Dunsby C (2008) Optically sectioned imaging by oblique plane microscopy. Opt Express 16:20306

Oblique plane microscopy: OPM (2008)





- Light-sheet (NA of 0.0785) at the edge of the primary objective
  light-sheet makes an angle of 60° with the optic axis
- Orthogonal detection cone, half-angle of 33°
  Corresponds to NA(potential) of 0.82
- n_1/n_2 = magnification from object to intermediate image = 1.5 ✓
- 3rd objective tilted by 30° to capture the oblique emitted photons

Primary Objective (O1)
Olympus 60x/1.35NA oil

Second Objective (O2)
Olympus 40x/0.85NA air

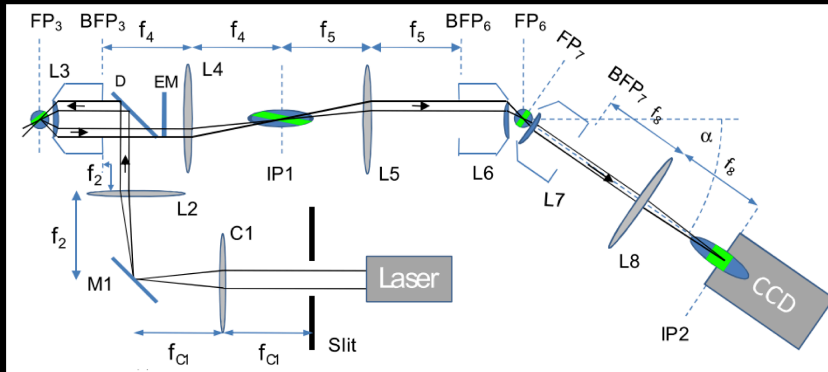
Tilted Objective (O3)
Olympus 10x/0.3NA air

Excitation beam

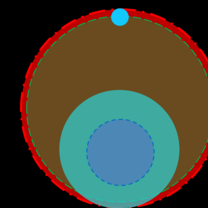
NA (potential)

Dunsby C (2008) Optically sectioned imaging by oblique plane microscopy. Opt Express 16:20306

Oblique plane microscopy: OPM (2008)



- Light-sheet (NA of 0.0785) at the edge of the primary objective
  light-sheet makes an angle of 60° with the optic axis
- Orthogonal detection cone, half-angle of 33°
  Corresponds to NA(potential) of 0.82
- n_1/n_2 = magnification from object to intermediate image = 1.5 ✓
- 3rd objective tilted by 30° to capture the oblique emitted photons
- Effective NA limited by the 3rd objective



Primary Objective (O1)
Olympus 60x/1.35NA oil

Second Objective (O2)
Olympus 40x/0.85NA air

Tilted Objective (O3)
Olympus 10x/0.3NA air

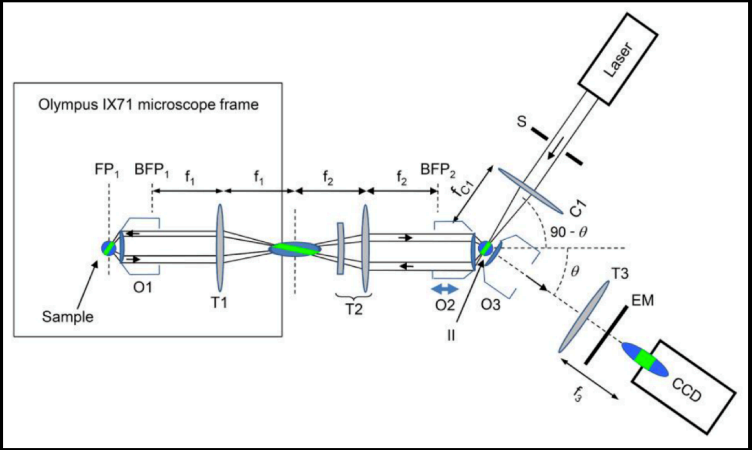
Excitation beam

NA (potential)

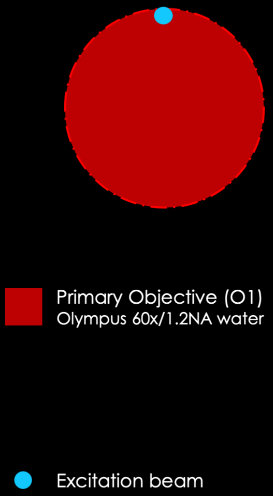
Effective NA 0.45
Collection efficiency of 31%

Dunsby C (2008) Optically sectioned imaging by oblique plane microscopy. Opt Express 16:20306

OPM, v2 (2011)

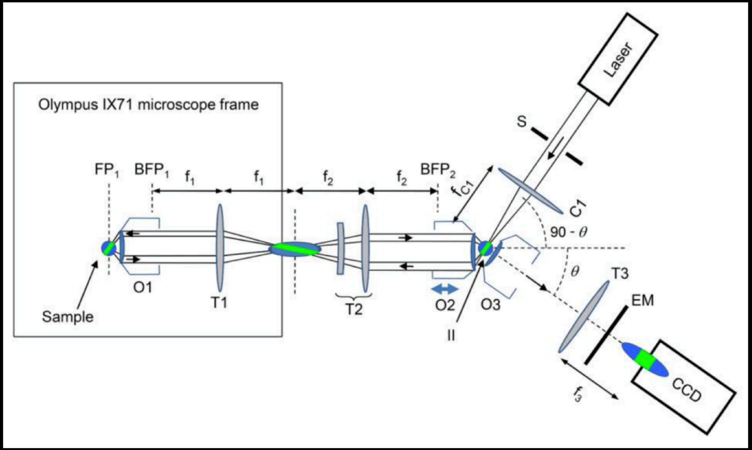


- Light-sheet (NA of 0.12) at the edge of the primary objective
  light-sheet makes an angle of 59° with the optic axis



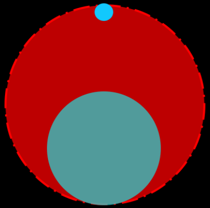
Kumar, S., Wilding, D., Sikkel, M. B., Lyon, A. R., MacLeod, K. T., & Dunsby, C. (2011). High-speed 2D and 3D fluorescence microscopy of cardiac myocytes. *Optics Express*, *19*(15), 13839

OPM, v2 (2011)



- Light-sheet (NA of 0.12) at the edge of the primary objective
  light-sheet makes an angle of 59° with the optic axis

- Orthogonal detection cone, half-angle of 33.4°
  Corresponds to $NA_{potential}$ of 0.7



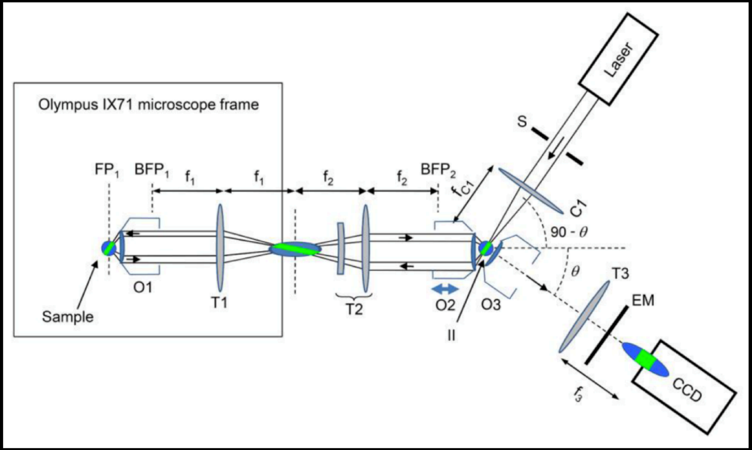
Primary Objective (O1)
Olympus 60x/1.2NA water

Excitation beam

$NA_{potential}$

Kumar, S., Wilding, D., Sikkel, M. B., Lyon, A. R., MacLeod, K. T., & Dunsby, C. (2011). High-speed 2D and 3D fluorescence microscopy of cardiac myocytes. *Optics Express*, *19*(15), 13839

OPM, v2 (2011)



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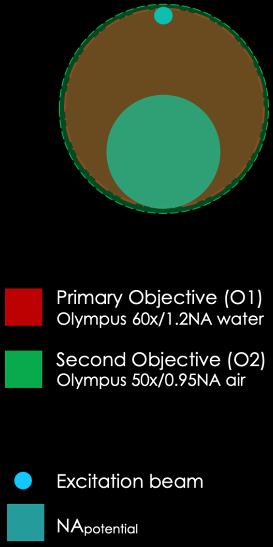
- Orthogonal detection cone, half-angle of 33.4°
  Corresponds to $NA_{potential}$ of 0.7



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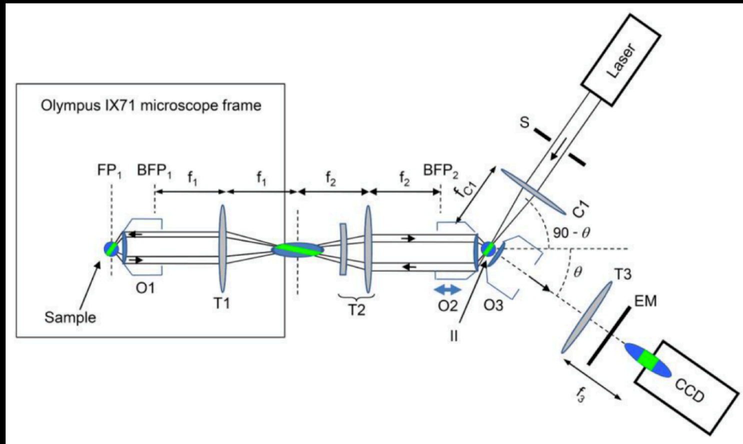
OPM, v2 (2011)



- Light-sheet (NA of 0.12) at the edge of the primary objective
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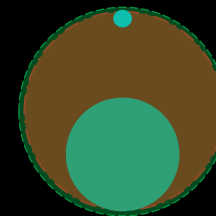
- Orthogonal detection cone, half-angle of 33.4°
  Corresponds to $NA_{potential}$ of 0.7

- n_1/n_2 = magnification from object to intermediate image = 1.333 ✓



Primary Objective (O1)
Olympus 60x/1.2NA water

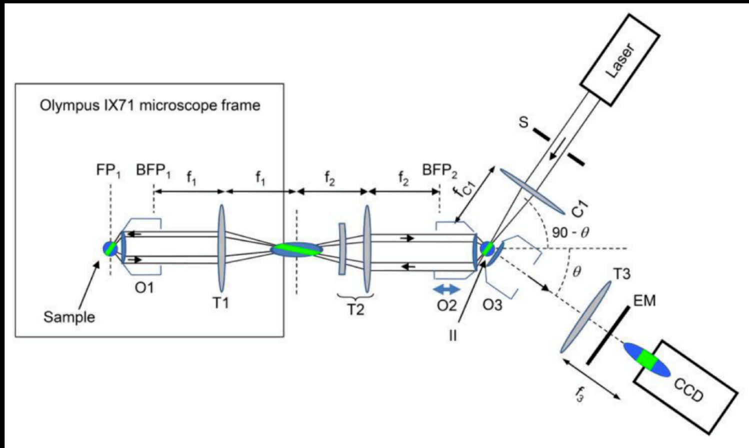
Second Objective (O2)
Olympus 50x/0.95NA air

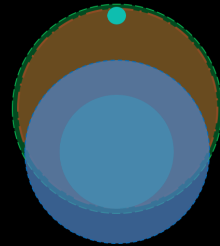
Excitation beam

$NA_{potential}$

Kumar, S., Wilding, D., Sikkel, M. B., Lyon, A. R., MacLeod, K. T., & Dunsby, C. (2011). High-speed 2D and 3D fluorescence microscopy of cardiac myocytes. *Optics Express*, *19*(15), 13839

OPM, v2 (2011)





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- Orthogonal detection cone, half-angle of 33.4°
  Corresponds to $NA_{potential}$ of 0.7

- n_1/n_2 = magnification from object to intermediate image = 1.333 ✓

- 3rd objective tilted by 32° to capture the oblique emitted photons

Primary Objective (O1)
Olympus 60x/1.2NA water

Second Objective (O2)
Olympus 50x/0.95NA air

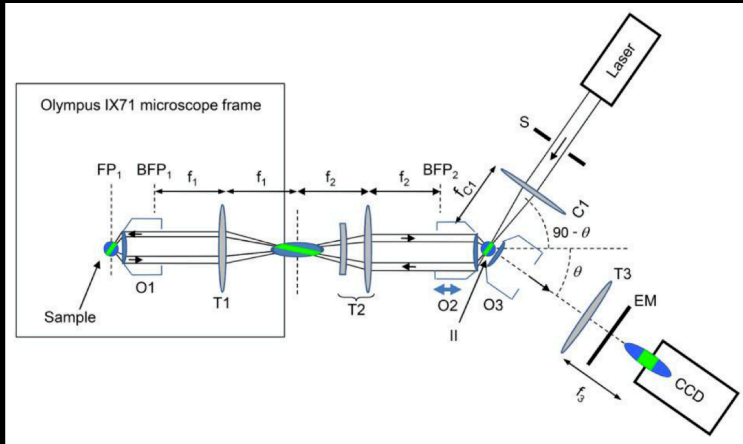
Tilted Objective (O3)
Nikon 40x/0.6NA air

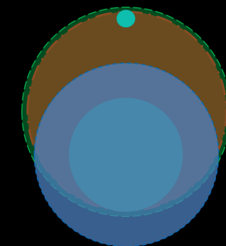
Excitation beam

$NA_{potential}$

Kumar, S., Wilding, D., Sikkel, M. B., Lyon, A. R., MacLeod, K. T., & Dunsby, C. (2011). High-speed 2D and 3D fluorescence microscopy of cardiac myocytes. *Optics Express*, *19*(15), 13839

OPM, v2 (2011)





- Light-sheet (NA of 0.12) at the edge of the primary objective
  light-sheet makes an angle of 59° with the optic axis
- Orthogonal detection cone, half-angle of 33.4°
  Corresponds to $NA_{potential}$ of 0.7
- n_1/n_2 = magnification from object to intermediate image = 1.333 ✓
- 3rd objective tilted by 32° to capture the oblique emitted photons
- Effective NA not limited by the 3rd objective in this case- captures all of $NA_{potential}$

Primary Objective (O1)
Olympus 60x/1.2NA water

Second Objective (O2)
Olympus 50x/0.95NA air

Tilted Objective (O3)
Nikon 40x/0.6NA air

Excitation beam

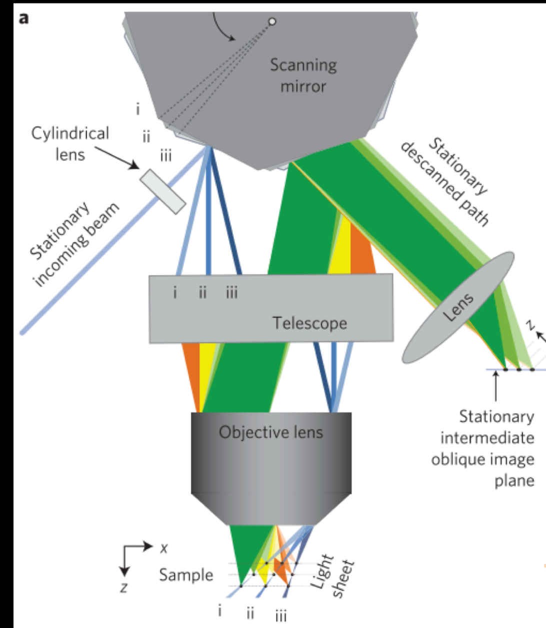
$NA_{potential}$

Effective NA 0.7
Collection efficiency of 35%

Kumar, S., Wilding, D., Sikkel, M. B., Lyon, A. R., MacLeod, K. T., & Dunsby, C. (2011). High-speed 2D and 3D fluorescence microscopy of cardiac myocytes. *Optics Express*, *19*(15), 13839

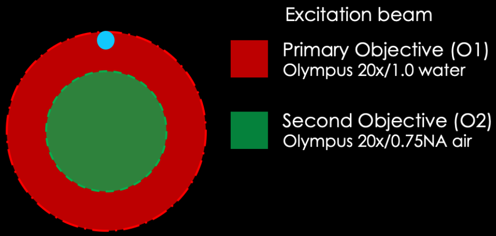
SCAPE (2015)

- A single-objective, oblique light-sheet approach
- Light-sheet plane and detected beam cones aren't orthogonal
- Light-sheet angle and position changes as the mirror is scanned: i.e., scanner not conjugate to the objective's back focal plane
- Scanning element is a 12-sided polygon mirror; 2 sides are involved
- Excitation sheet and co-aligned detection plane swept with a back and forth motion of the polygon mirror (10-40 Hz)
- No sample or objective lens motion
- The polygon mirror splits the objective's aperture in half i.e., detected NA (max) of 0.5



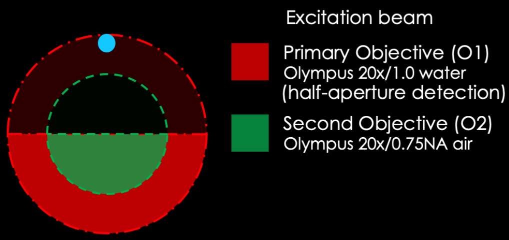
Bouchard MB, Voleti V, Mendes CS, Lacefield C, Grueber WB, Mann RS, Bruno RM, Hillman EMC (2015) Swept confocally-aligned planar excitation (SCAPE) microscopy for high-speed volumetric imaging of behaving organisms. Nat Photonics 9:113–119

SCAPE resolution, effective NA



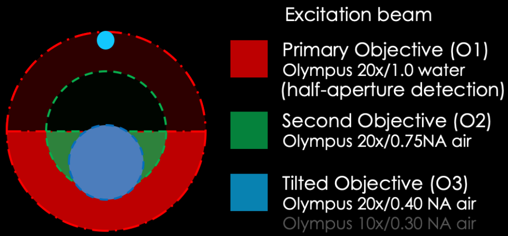
- Only half-aperture of Obj1 utilized (NA_det = 0.5)
- BFP_Obj2 is 1.333x smaller than BFP_Obj1, yet the two are relayed 1:1. Thus, NA_det=0.375
- Obj3 tilted to capture the oblique beams
- All of NA_det should make it through Obj3 since:
    - BFP_Obj3 > Half of BFP_Obj2
    - f_Obj2 = f_Obj3

SCAPE resolution, effective NA



- Only half-aperture of Obj1 utilized (NA_det = 0.5)
- BFP_Obj2 is 1.333x smaller than BFP_Obj1, yet the two are relayed 1:1. Thus, NA_det=0.375
- Obj3 tilted to capture the oblique beams
- All of NA_det should make it through Obj3 since:
  - BFP_Obj3 > Half of BFP_Obj2
  - f_Obj2 = f_Obj3

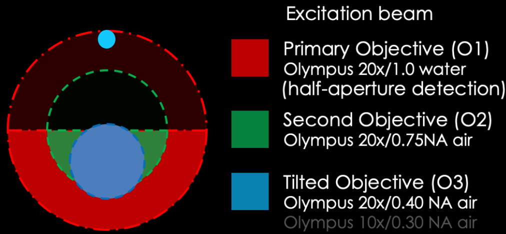
SCAPE resolution, effective NA



Effective NA = 0.375
Collection efficiency = 14%

- Only half-aperture of Obj1 utilized (NA_det = 0.5)
- BFP_Obj2 is 1.333x smaller than BFP_Obj1, yet the two are relayed 1:1. Thus, NA_det=0.375
- Obj3 tilted to capture the oblique beams
- All of NA_det should make it through Obj3 since:
    - BFP_Obj3 > Half of BFP_Obj2
    - f_Obj2 = f_Obj3

SCAPE resolution, effective NA



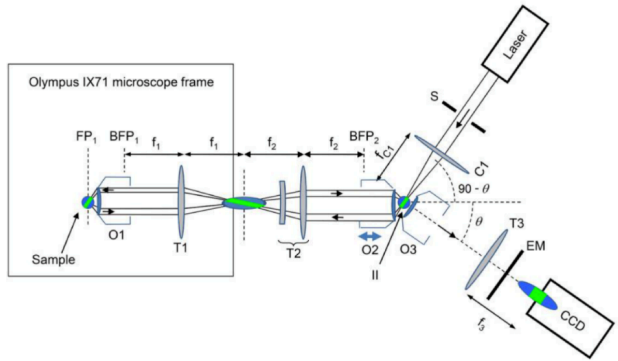
Effective NA = 0.375
Collection efficiency = 14%

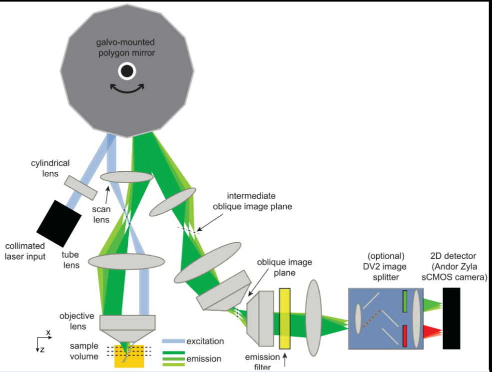
- Only half-aperture of Obj1 utilized (NA_det = 0.5)
- BFP_Obj2 is 1.333x smaller than BFP_Obj1, yet the two are relayed 1:1. Thus, NA_det=0.375
- Obj3 tilted to capture the oblique beams
- All of NA_det should make it through Obj3 since:
    - BFP_Obj3 > Half of BFP_Obj2
    - f_Obj2 = f_Obj3

Between object space and intermediate image space:
- Ratio of refractive index = 1.333
- Magnification = 1x

Violates the condition necessary to re-image from sample to intermediate image location

OPM v2 (2011) and SCAPE (2015) differences





OPM v2 (2011)	SCAPE (2015)
Remote objective scans the light-sheet and the co-aligned detection plane	Oscillating polygon mirror sweeps (scan & tilt) the light-sheet and the co-aligned detection plane
Excitation beam in tight space between two objectives; Light-sheet and detection cones orthogonal	Excitation beam arrangement simplified; However not orthogonal to the light-sheet plane
Light-sheet angle stays the same in sample space	Scan-position dependent light-sheet tilt in sample plane
Limited by the speed of the camera, not the piezo!	Limited by the speed of the camera

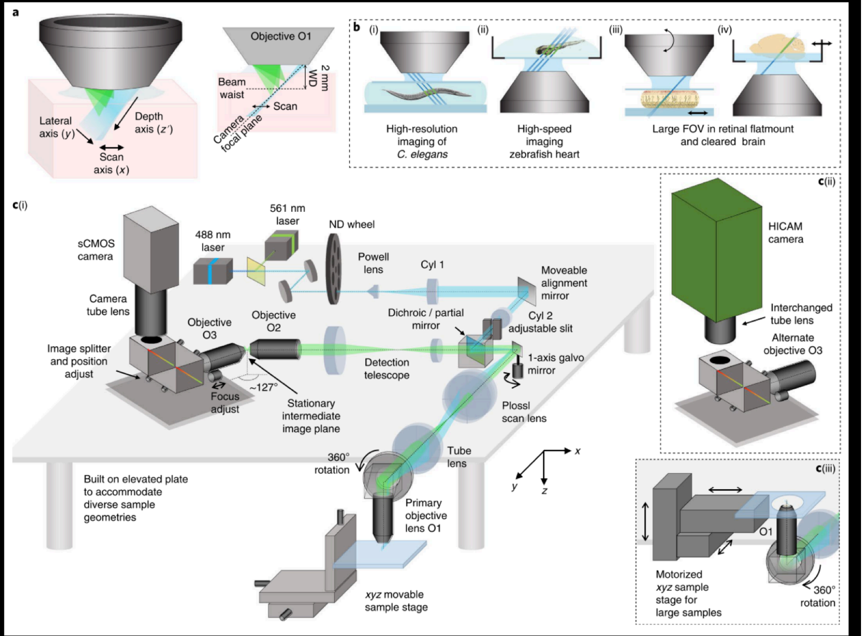
SCAPE 2.0: Improvements over SCAPE

1. Polygon mirror replaced by a 10 mm galvo mirror that is now conjugate to the objective's back focal plane. So, fixed the position-dependent light-sheet tilt in sample space

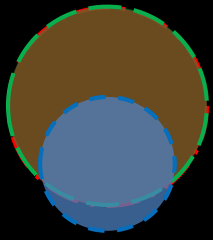
2. Fixed the re-image condition (Magnification = n1/n2)

3. Fixed the mismatch between Obj1 and Obj2 back focal plane that can otherwise reduce the effective NA further

4. Effective NA of 0.35 (high-res) or 0.23 (low-res)



SCAPE 2.0 resolution, effective NA



Primary Objective (O1)
Olympus 20x/1.0 water

150mm→75mm→60 mm→100mm

Second Objective (O2)
Nikon 20x/0.75NA air

Tilted Objective (O3)
Nikon 10x/0.45NA(eff NA 0.23)
Edmund 20x/0.60NA (eff NA 0.35)
Mitutoyo Plan Apo HR 50x/0.75NA (eff NA 0.48)

- Obj1 and Obj2 BFPs match exactly

Between primary and intermediate image location:
- Ratio of refractive indices = 1.333
- Magnification = 1.333x

Satisfies the condition necessary to re-image from sample to intermediate image location

Effective NA of 0.23
Collection efficiency max = $(0.23/1.0)^2$ = 5.3%

Effective NA of 0.35
Collection efficiency max = $(0.35/1.0)^2$ = 12.25%

Effective NA of 0.48 (max)
Collection efficiency max = $(0.48/1.0)^2$ = 23%

Other OPMs between 2015 and 2019

OSLM, Oblique Scanning Laser Microscopy (2017)

Zhang L, Capilla A, Song W, Mostoslavsky G, Yi J (2017) Oblique scanning laser microscopy for simultaneously volumetric structural and molecular imaging using only one raster scan. Sci Rep 7:8591

SOPi, Scanned Oblique Plane illumination (2018)

Kumar M, Kishore S, Nasenbeny J, McLean DL, Kozorovitskiy Y (2018) Integrated one- and two-photon scanned oblique plane illumination (SOPi) microscopy for rapid volumetric imaging. Opt Express 26:13027

e-SPIM, Epi-illumination SPIM (2019)

Yang B, Chen X, Wang Y, Feng S, Pessino V, Stuurman N, Cho NH, Cheng KW, Lord SJ, Xu L, Xie D, Mullins RD, Leonetti MD, Huang B (2019) Epi-illumination SPIM for volumetric imaging with high spatial-temporal resolution. Nat Methods 16:501–504

Future directions

AMS-AGY objective aka“Snouty”

https://andrewgyork.github.io/high_na_single_objective_lightsheet/