SOLS optimum* tilt - quadratic estimate

In a single-objective light-sheet (SOLS) style microscope the light-sheet consumes some of the numerical aperture (NA) from the primary objective. i.e. more NA for the light-sheet gives better sectioning but less NA for collection. The trade of light-sheet vs emission NA is governed by the tilt of the 3rd microscope (θ_{tilt}). Historically some builders have used $\theta_{tilt} = 30$ degrees. So the question is... what is the optimal tilt?

Here's a simple analytical estimate for a tilt that gives a light-sheet thickness roughly equal to the axial point spread function.

Primary objective emission:

The numerical aperture of the primary objective (NA_1) and the axial extent of the point spread function (Z_{PSF_1}) can be expressed as,

$$NA_1 = n_1 \sin \theta_1$$

$$Z_{PSF_1} \approx \frac{2\lambda_{em}}{NA_1^2}$$

where n_1 is the index of refraction, θ_1 is the half angle collection of the primary objective and λ_{em} is the emission wavelength.

Light-sheet excitation:

The numerical aperture of a Gaussian light-sheet excitation (NA_{ex}) and beam waist (ω_0) can be written as,

$$NA_{ex} = n_1 \sin \theta_{ex}$$

$$\omega_0 = \frac{\lambda_{ex}}{\pi NA_{ex}}$$

where θ_{ex} is the half angle of the light-sheet and λ_{ex} is the excitation wavelength.

Single-objective light-sheet constraints:

The lower bound on effective numerical aperture (NA_{eff}) is set by:

$$NA_{eff} = n_1 \sin \theta_{eff}$$

for:

$$2\theta_{eff} = 2\theta_1 - \theta_{ex}$$

and

$$\theta_{tilt} = \frac{\pi}{2} - \theta_1 + \theta_{ex}$$

*Optimum tilt selection:

Tilt may be optimized in different ways:

- Minimal tilt gives maximum emission collection, but with a thick light-sheet, poorer sectioning and increased photodose.
- Increasing tilt gives thinner light-sheet options, better sectioning and lower photodose possibilities, but at the expense of emision light.
- Tuning the tilt between minimum and maximum can allow the light-sheet propagation length to be adapted to the sample size. This may be the best way to operate but is practically challenging.

As a starting point we can consider setting the tilt so that the thickness of the light sheet $(2\omega_0)$ is approximately equal to the extent of the effective axial point spread function of the primary objective $(Z_{PSF_{eff}})$:

$$2\omega_0 \approx Z_{PSF_{eff}}$$

or:

$$\frac{\lambda_{ex}}{\pi NA_{ex}} \approx \frac{\lambda_{em}}{NA_{eff}^2}$$

Solve for θ_{tilt}:

Substitute equations for NA and rearrange,

$$\frac{n_1 \lambda_{ex}}{\pi \lambda_{em}} \sin^2 \theta_{eff} \approx \sin \theta_{ex}$$

eliminate θ_{ex} using $\theta_{ex} = 2(\theta_1 - \theta_{eff})$,

$$\approx \sin 2(\theta_1 - \theta_{eff})$$

extract factor of 2 using trig identity ($\sin 2A = 2 \sin A \cos A$),

$$\approx 2 \sin(\theta_1 - \theta_{eff}) \cos(\theta_1 - \theta_{eff})$$

extract single angles using trig identities ($\sin(A - B) = \sin A \cos B - \cos A \sin B$, $\cos(A - B) = \cos A \cos B + \sin A \sin B$),

$$\approx 2(\sin \theta_1 \cos \theta_{eff} - \cos \theta_1 \sin \theta_{eff})(\cos \theta_1 \cos \theta_{eff} + \sin \theta_1 \sin \theta_{eff})$$

multiply out,

$$\approx 2 \sin \theta_1 \cos \theta_1 \cos^2 \theta_{eff} + 2 \sin \theta_{eff} \cos \theta_{eff} \sin^2 \theta_1$$
$$-2 \sin \theta_{eff} \cos \theta_{eff} \cos^2 \theta_1 - 2 \sin \theta_1 \cos \theta_1 \sin^2 \theta_{eff}$$

divide by $\cos^2 \theta_{eff}$ and substitute $\frac{\sin \alpha}{\cos \alpha} = \tan \alpha$ (a valid operation for any real lens where $\theta_{eff} < \frac{\pi}{2}$),

$$\frac{n_1 \lambda_{ex}}{\pi \lambda_{em}} \tan^2 \theta_{eff} \approx 2 \sin \theta_1 \cos \theta_1 \; + 2(\sin^2 \theta_1 - \cos^2 \theta_1) \tan \theta_{eff} - 2 \sin \theta_1 \cos \theta_1 \tan^2 \theta_{eff}$$

re-arrange for the standard quadratic form $ax^2 + bx + c = 0$ and reduce ($\cos^2 A = 1 - \sin^2 A$, $2 \sin A \cos A = \sin 2A$):

$$(\frac{n_1 \lambda_{ex}}{\pi \lambda_{em}} + \sin 2\theta_1) \tan^2 \theta_{eff} + 2(1 - 2 \sin^2 \theta_1) \tan \theta_{eff} - \sin 2\theta_1 \approx 0$$

find the positive root with the quadratic formula:

$$\tan \theta_{eff} = \frac{-b + \sqrt{b^2 - 4ac}}{2a}$$

return the tilt angle with:

$$\theta_{tilt} = \frac{\pi}{2} + \theta_1 - 2\theta_{eff}$$

SOLS reference design, worked example:

The primary objective for the single-objective light-sheet reference design is a 1.35 Silicone oil objective:

In [3]:

```
import numpy as np

NA1 = 1.35 # Nikon 100x1.35 Sil
n1 = 1.4 # Silicone refractive index
lambda_ex = 488 # typical laser
lambda_em = 510 # GFP like

theta1 = np.arcsin(NA1/n1)

a = (lambda_ex * n1)/(np.pi * lambda_em) + np.sin(2 * theta1)
b = 2 * (1 - 2 * (np.sin(theta1) ** 2))
c = - np.sin(2 * theta1)

tan_theta_eff = (- b + (b**2 - 4 * a * c)**0.5)/ (2 * a)
theta_eff = np.arctan(tan_theta_eff)
theta_tilt = np.pi/2 + theta1 - 2 * theta_eff

print("Optimum tilt = ", round(np.rad2deg(theta_tilt), 2), "deg")
```

```
Optimum tilt =  35.68 deg
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In [ ]: