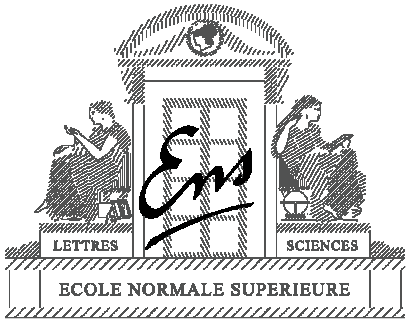
Journées Recherche Industrie de l'Optique Adaptative, Nantes – 19th/20th Nov 2008

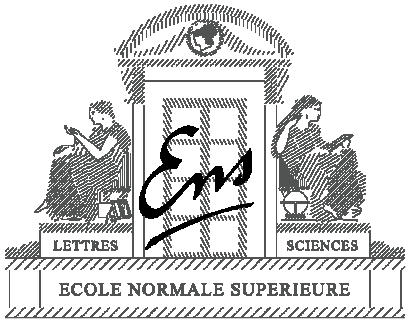
Sample-introduced spherical aberration in high-NA objectives

Jonas BINDING

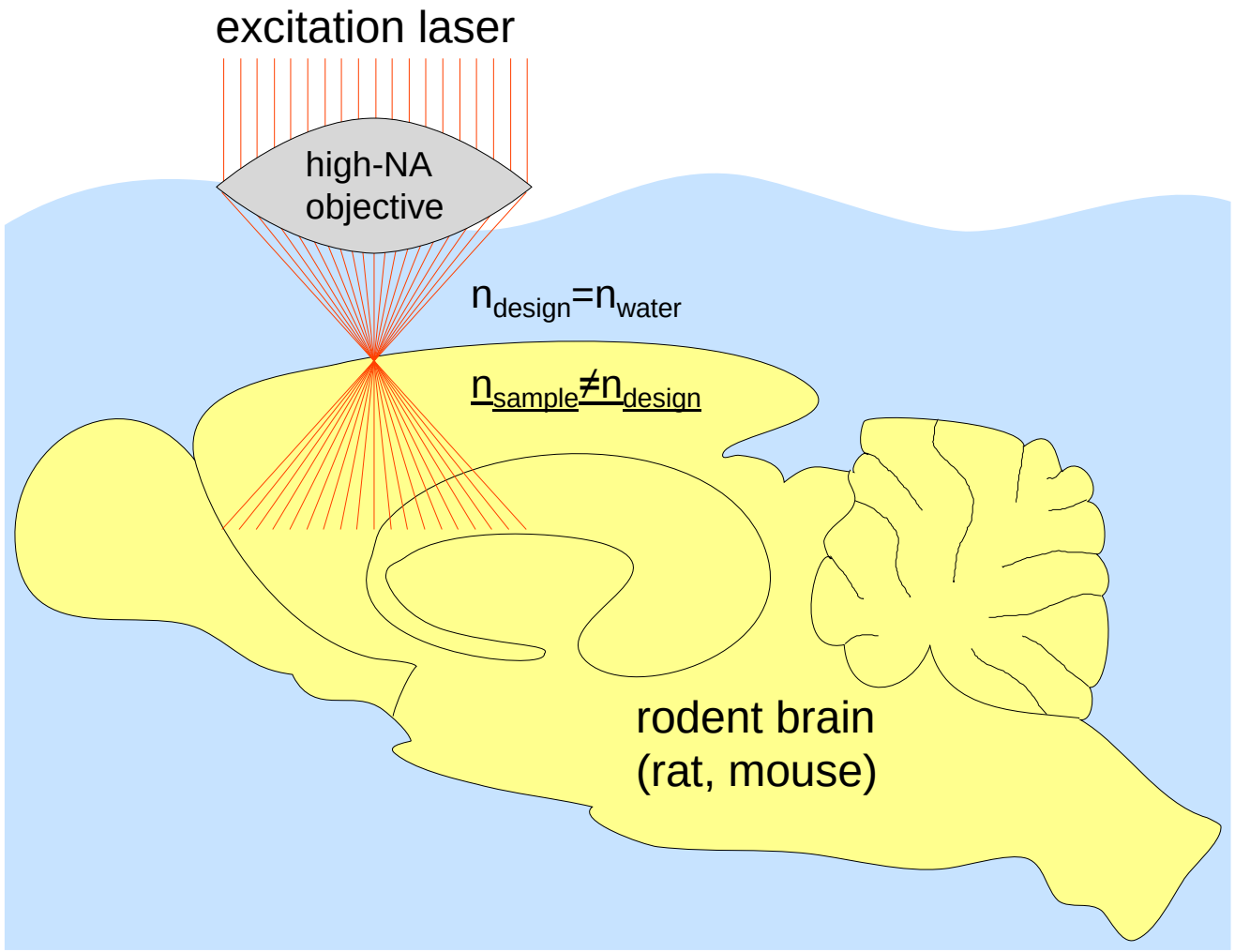


Compensation d'aberrations en microscopie à deux photons

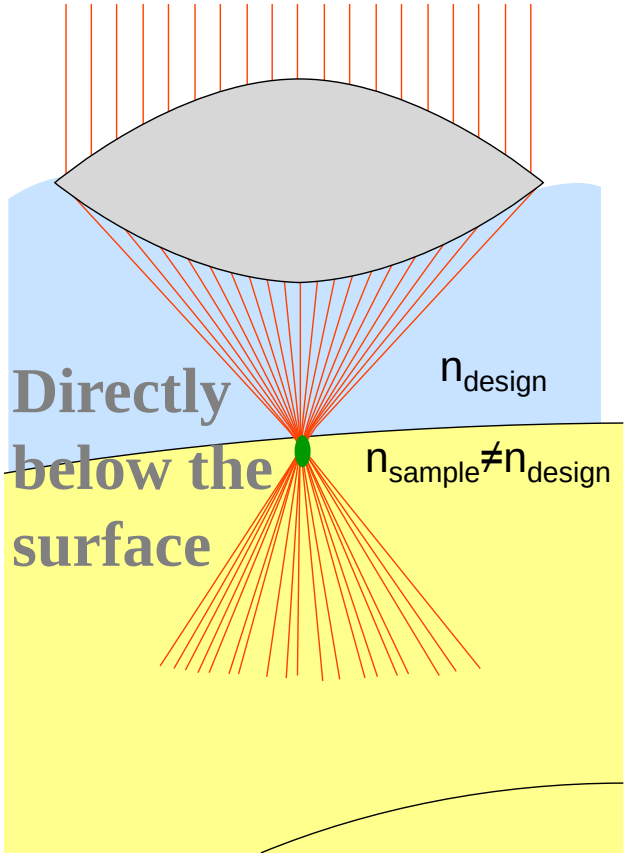
Jonas BINDING



The goal: two-photon imaging deep inside the living brain



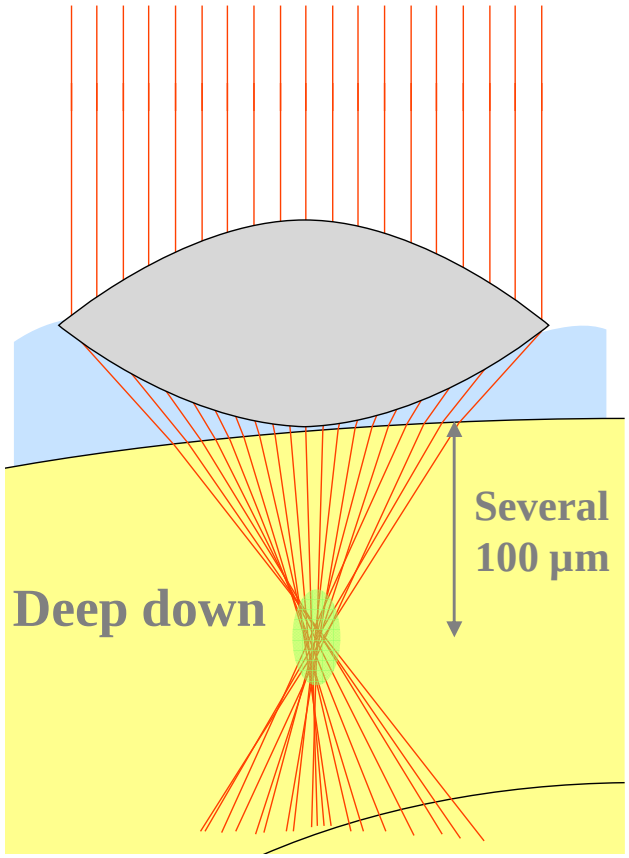
The biological sample causes aberrations



Good focus

→ high fluorescence

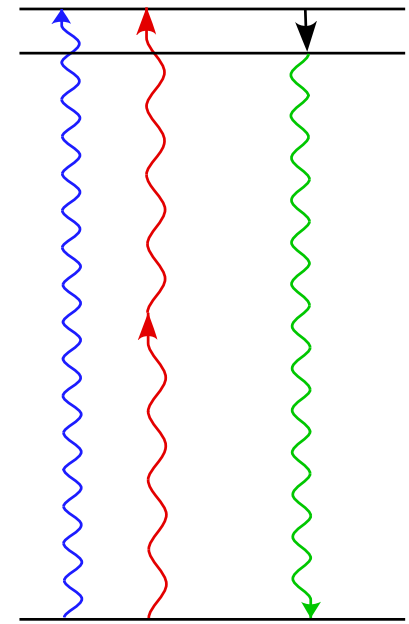
Aberrations decrease two-photon signal



Bad focus

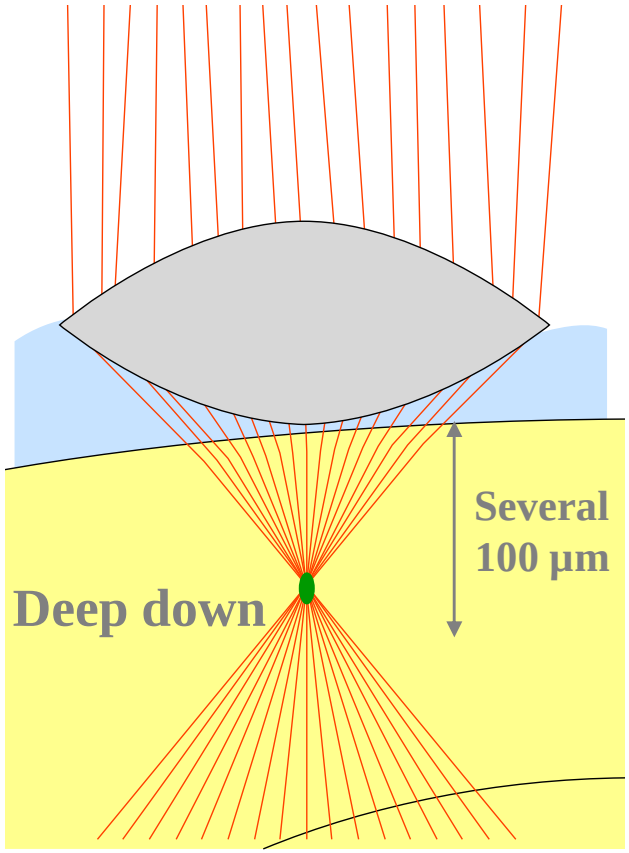
→ **low fluorescence**

- Aberrations decrease focus quality
- Two-photon (2P) fluorescence signal depends on squared excitation intensity
- Decreased focus quality means decreased 2P fluorescence



1P absorption 2P fluores-cence

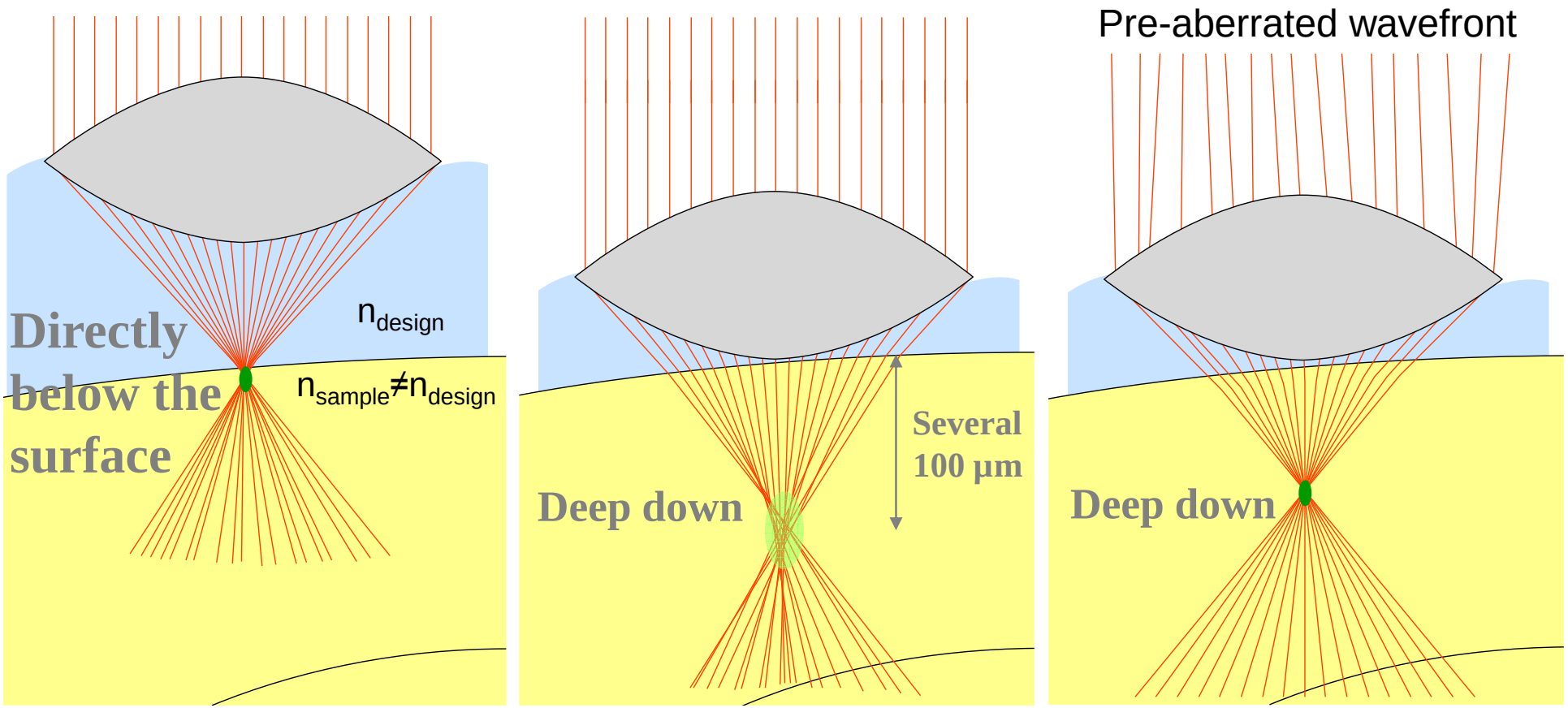
Wavefront correction can restore two-photon signal



Restored focus

→ high fluorescence

Wavefront correction can restore two-photon signal



Good focus

→ high fluorescence

Bad focus

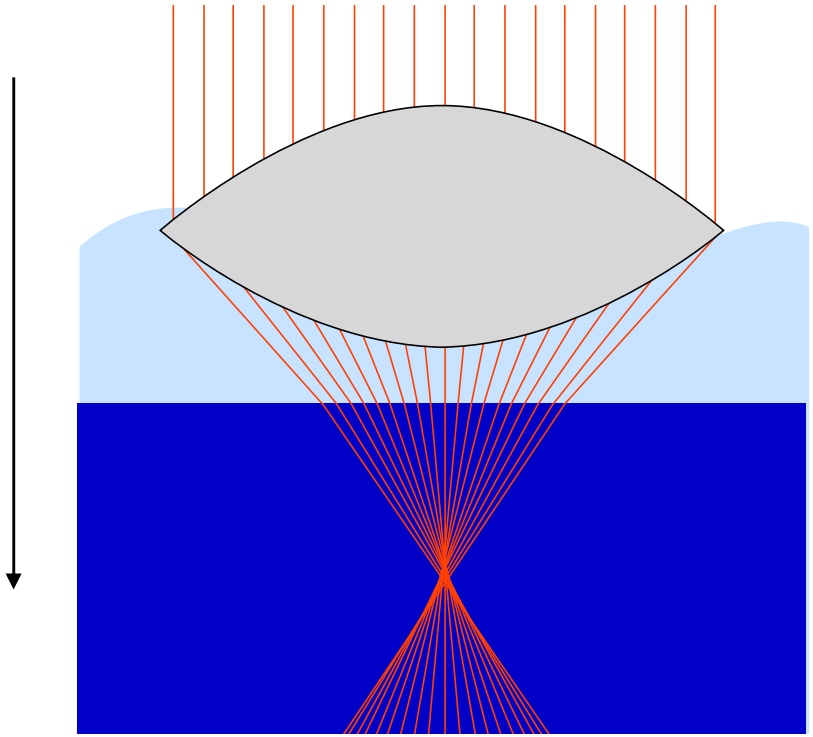
→ low fluorescence

Restored focus

→ high fluorescence

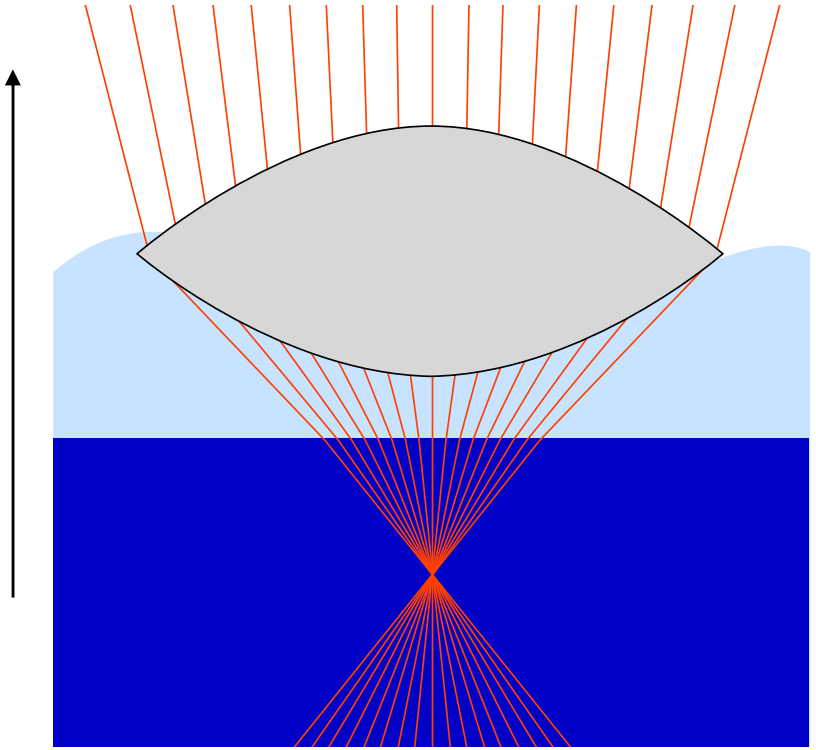
Two approaches to aberrations in microscopy

What does the focus look like for a flat wavefront?



Ling, H and SW Lee (1984). JOSA A **1**(9): 965-973.
Hell, SW, G Reiner, et al. (1993). JoMicrosc **169**: 391-405.

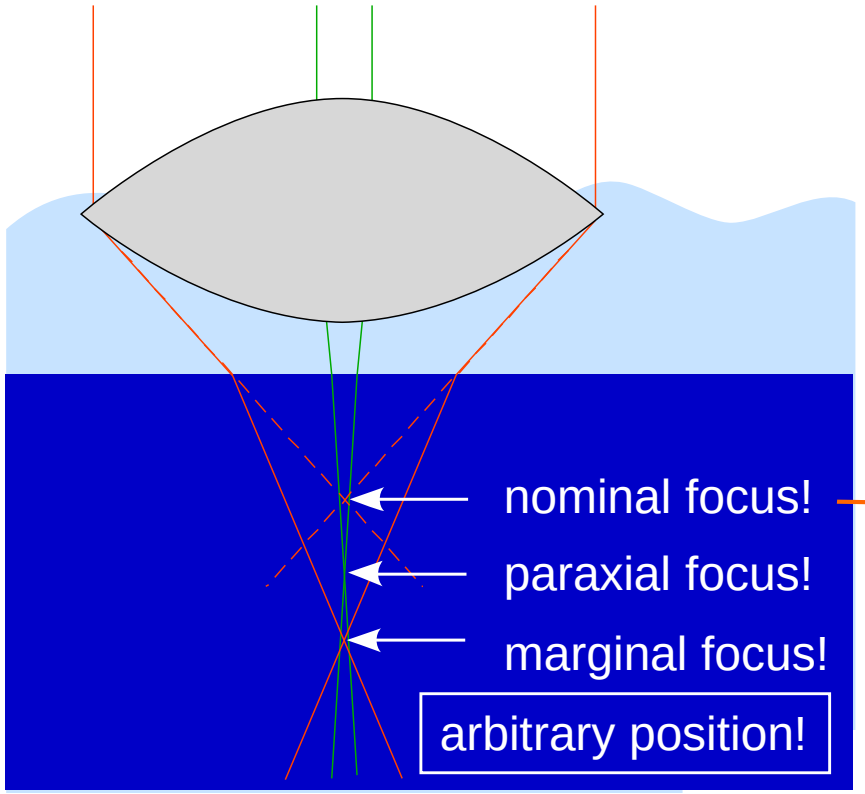
Which wavefront corresponds to a diffraction-limited focus?



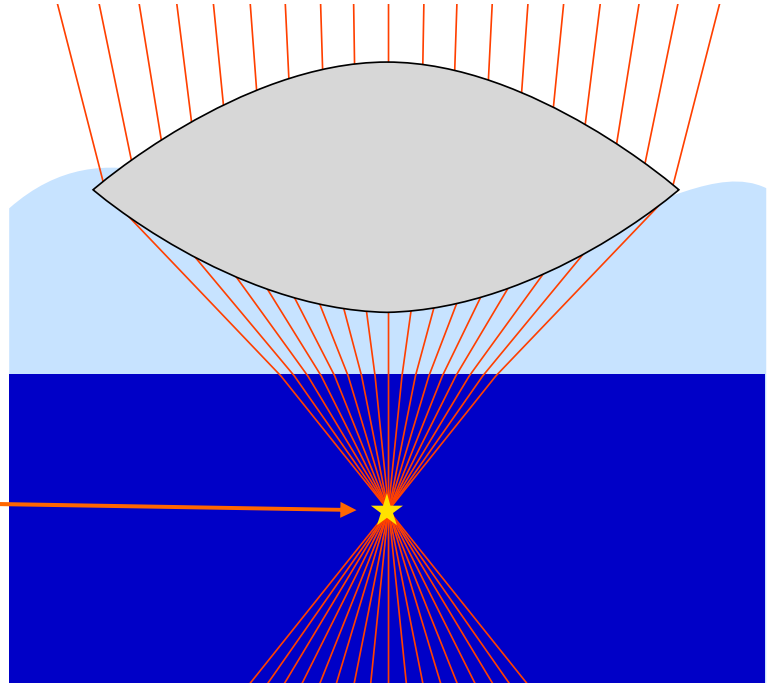
Török, P, P Varga, et al. (1995). JOSA A **12**(12): 2660-2671.
Booth, MJ, MAA Neil, et al. (1998). JoMicrosc **192**: 90-98.

Where should we focus?

Are there no other options for the actual focus position?



Which wavefront corresponds to a diffraction-limited focus **at the nominal focus position**?



Török, P, P Varga, et al. (1995). JOSA A **12**(12): 2660-2671.
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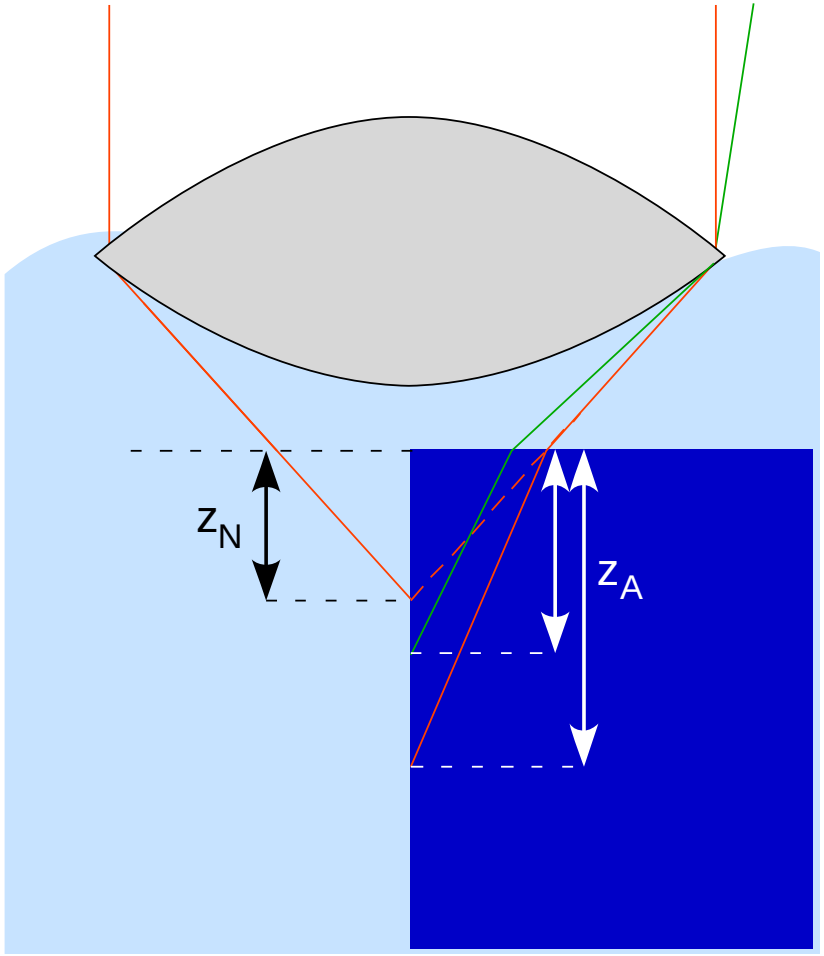
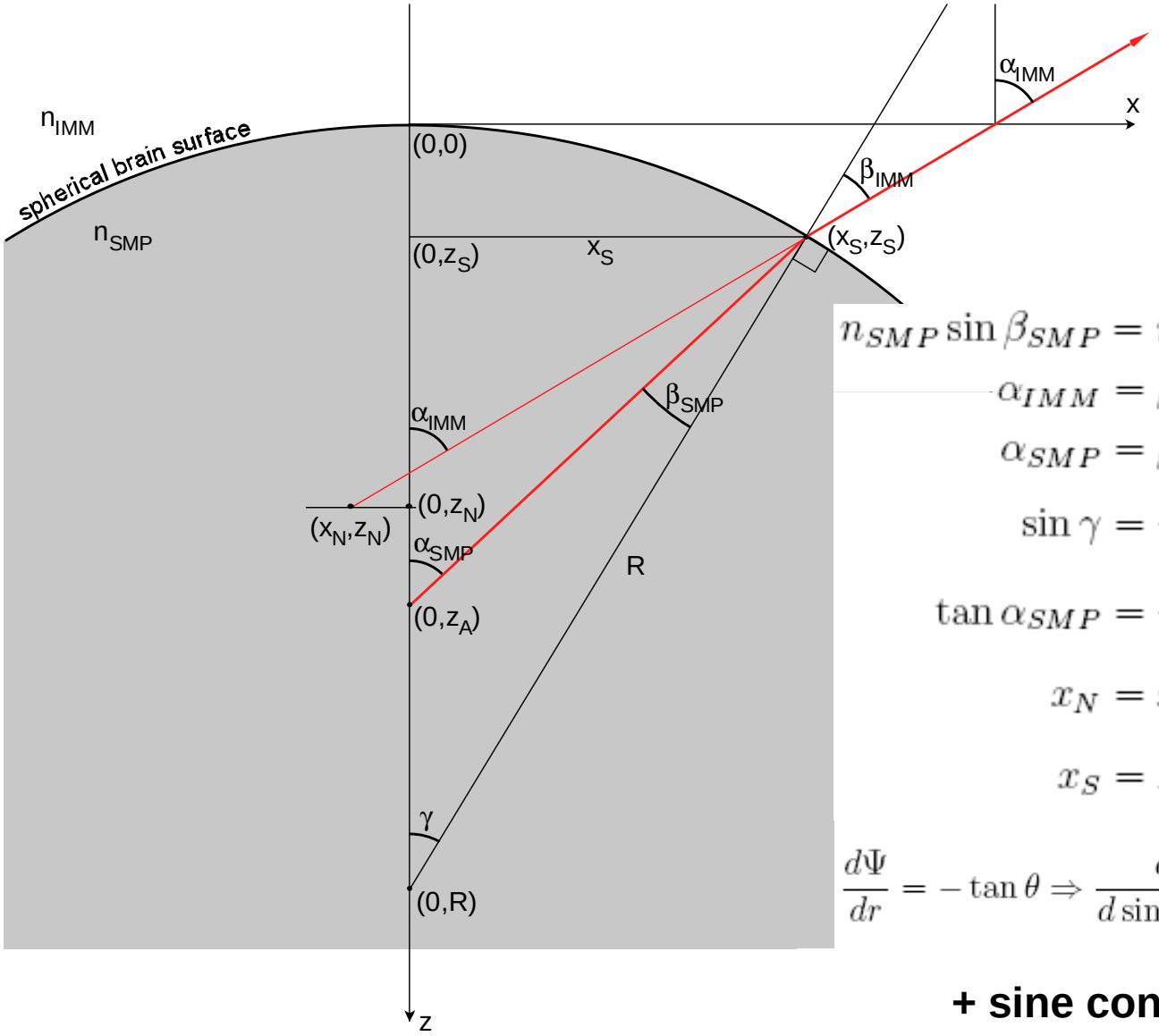
Definitions: nominal vs. actual focus position



- actual focus position z_A: distance of the focus from the refractive index mismatch
- nominal focus position z_N: corresponding distance for a flat wavefront with matched refractive index

Ray tracing for a spherical sample



$$n_{SMP}\sin\beta_{SMP} = n_{IMM}\sin\beta_{IMM}$$

$$\alpha_{IMM} = \beta_{IMM} + \gamma$$

$$\alpha_{SMP} = \beta_{SMP} + \gamma$$

$$\sin\gamma = \frac{x_S}{R}$$

$$\tan\alpha_{SMP} = \frac{x_S}{z_A - z_S}$$

$$x_N = x_S - (z_N - z_S)\tan\alpha_{IMM}$$

$$x_S = R - \sqrt{R^2 - x_S^2}$$

$$\frac{d\Psi}{dr} = -\tan\theta \Rightarrow \frac{d\Psi}{d\sin\alpha_{IMM}} = -n_{IMM}x_N$$

+ sine condition objective

Ray tracing for a spherical sample

$$\Psi(\rho) = \int_0^{\frac{\mathrm{NA}\,\rho}{n_{IMM}}} \frac{d\Psi}{d\sin\alpha_{IMM}} d\sin\alpha_{IMM}$$
$$= -n_{IMM} \int_0^{\frac{\mathrm{NA}\,\rho}{n_{IMM}}} \left[x_S - \left(z_N - R + \sqrt{R^2 - x_S^2} \right) \frac{\sin\alpha}{\sqrt{1-\sin^2\alpha}} \right] d\sin\alpha$$

where

$$\frac{x_S}{z_A - R + \sqrt{R^2 - x_S^2}} = \tan\left\{ \arcsin\frac{x_S}{R} + \arcsin\left[\frac{n_{IMM}}{n_{SMP}} \sin\left(\alpha_{IMM} - \arcsin\frac{x_S}{R} \right) \right] \right\}$$

→ Only numerically solvable!

Special case: flat sample surface

- Wavefront for diffraction-limited focus in nominal focus depth (index-mismatched):

$$\Psi(\rho) = z_N \left(\sqrt{n_2^{\,2} - NA^2\rho^2} - \sqrt{n_1^{\,2} - NA^2\rho^2} \right)$$

Török, P, P Varga, et al. (1995). JOSA A **12**(12): 2660-2671.
Booth, MJ, MAA Neil, et al. (1998). JoMicrosc **192**: 90-98.

- Wavefront for diffraction-limited focus in arbitrary focus depth (index-matched):

$$\Psi(\rho) = \left(z_A - z_N \right) \sqrt{n^2 - NA^2\rho^2}$$

Feierabend, M (2004). PhD thesis.
Botcherby, EJ, R Juskaitis et al. (2008). OptComm **281**: 880–887.

$$\Psi(\rho) = z_A \sqrt{n_2^{\,2} - NA^2\rho^2} - z_N \sqrt{n_1^{\,2} - NA^2\rho^2}$$

High-NA defocus with planar refractive index mismatch

Ψ: wavefront, ρ: normalized radial coordinate, NA: numerical aperture, z_N: nominal focus depth, z_A: actual focus depth, n_1, n_2: refractive indices

Decomposition into Zernike modes

$$\Psi(\rho) = A_{00} + \sum_{\substack{n=2 \\ n \text{ even}}}^{\infty} A_{n0} Z_n^0(\rho)$$

with

$$A_{n0} = NA\left[z_N B_n(\arcsin\tfrac{NA}{n_1}) - z_A B_n(\arcsin\tfrac{NA}{n_2}) \right]$$

where

$$B_n(\gamma) = \left[1 - \left(\frac{n-1}{n+3} \right) \tan^4(\tfrac{\gamma}{2}) \right] \frac{\tan^{n-1}(\tfrac{\gamma}{2})}{2(n-1)\sqrt{n+1}}$$

All orders of spherical aberration are affected by changing the focus position!!

Adapted from Booth, MJ, MAA Neil, et al. (1998). JoMicrosc **192**: 90-98.

Example: deep brain imaging

- n_1=1.33 (water), n_2=1.37 (brain tissue), NA=1.0 (20x water objective), z_A=1mm, $\Delta z = z_N - z_A$

  → leading Zernike coeffcients:

  A_{02}=5.428µm z_A[mm] – 0.130µm Δz[µm]

  A_{04}=0.827µm z_A[mm] – 0.007µm Δz[µm]

  A_{06}=0.137µm z_A[mm] – 0.001µm Δz[µm]

All orders of spherical aberration are affected by changing the focus position!!

Consequences for aberration correction

- One free parameter: Difference between nominal and actual focus position
- Possible uses:
  - Remove Zernike defocus
  - Remove one order of spherical aberration
  - Minimize wavefront RMS
  - Optimize for abilities of wavefront correction element and objective

Example: deep brain imaging

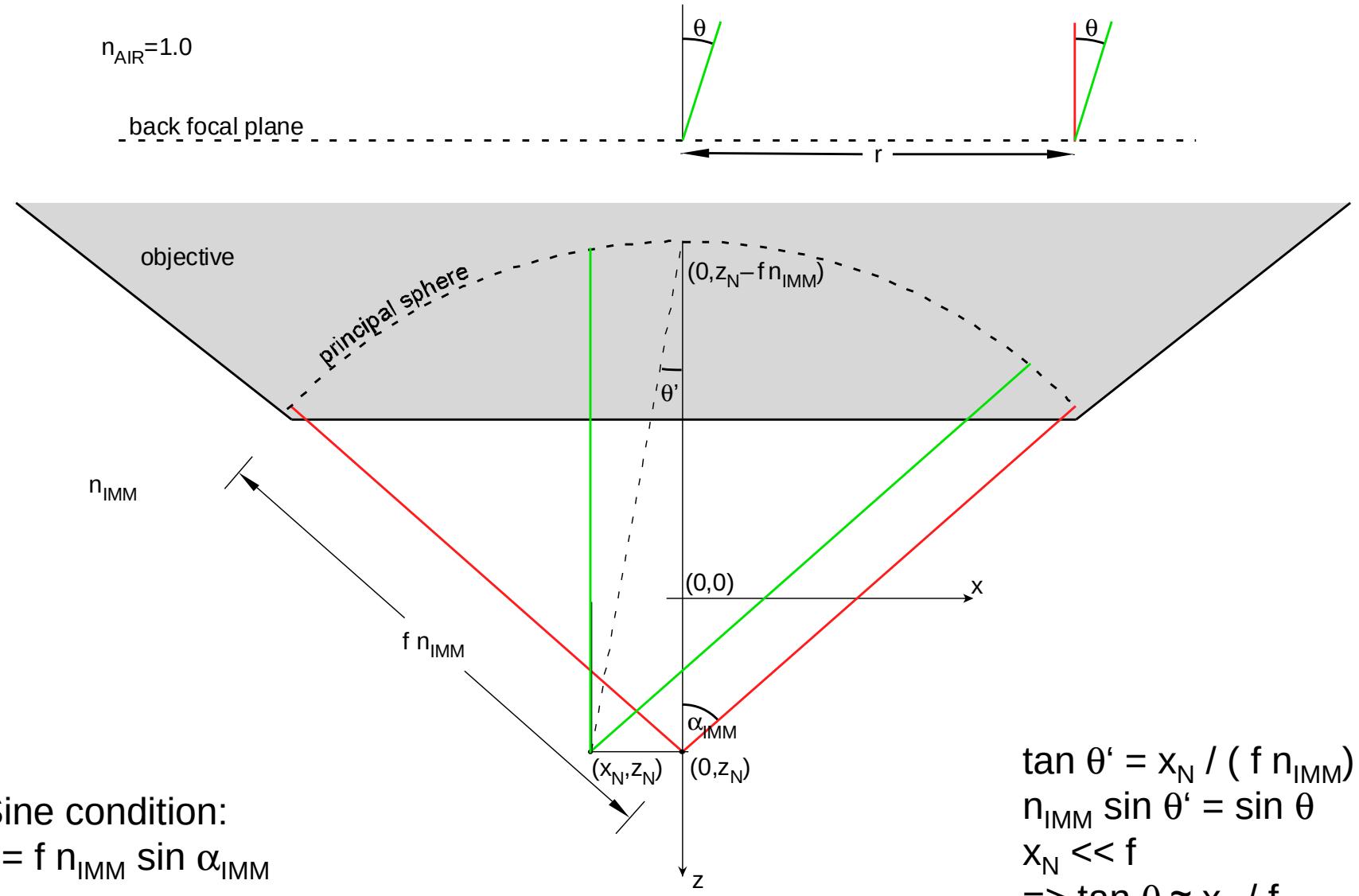
- A_{02}=5.428µm z_A[mm] – 0.130µm Δz[µm]
  A_{04}=0.827µm z_A[mm] – 0.007µm Δz[µm]
  A_{06}=0.137µm z_A[mm] – 0.001µm Δz[µm]
- High-NA refocusing:
  A_{02}=0 µm
  A_{04}=0.541µm z_A[mm]
  A_{06}=0.108µm z_A[mm]
- Zernike refocusing:
  A_{02}=0 µm
  A_{04}=0.827µm z_A[mm] → 50% too large!
  A_{06}=0.137µm z_A[mm] → 27% too large!

Summary

- High-NA defocus is what shifts the focus of a high-NA objective
- High-NA defocus is not equal to Zernike defocus
- Correct treatment decreases the predicted wavefront amplitudes necessary to correct for index-mismatch-caused aberrations

Thank you!

Assumptions about the objective

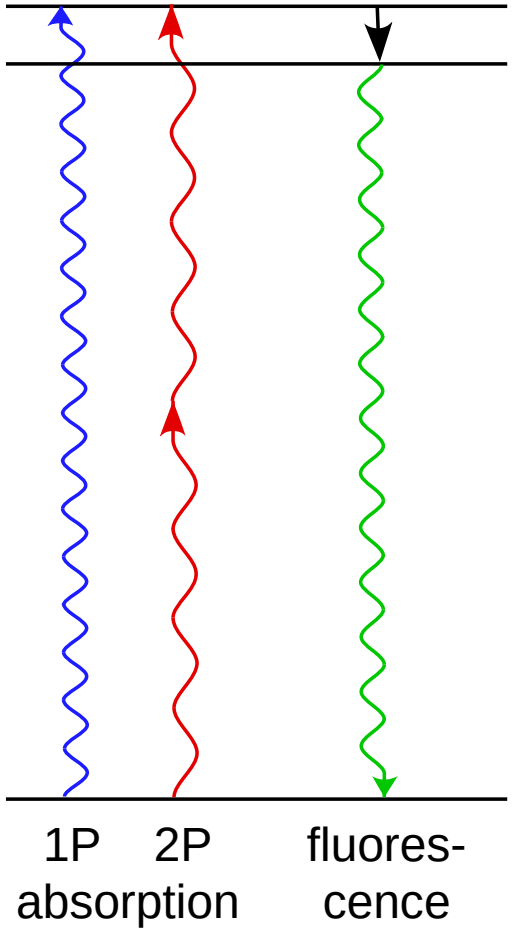


Sine condition:
$r = f\,n_{IMM} \sin \alpha_{IMM}$

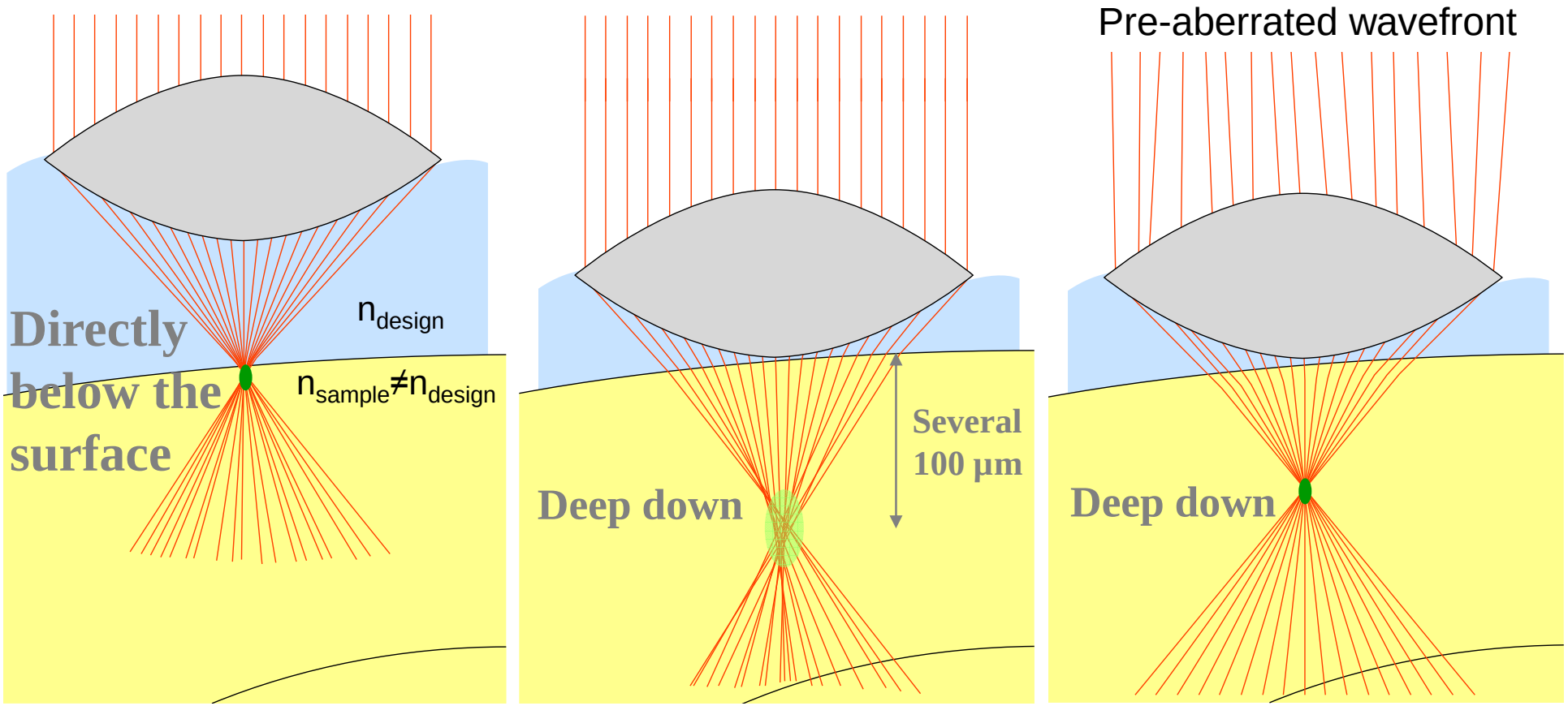
$\tan \theta' = x_N / (f\,n_{IMM})$
$n_{IMM} \sin \theta' = \sin \theta$
$x_N << f$
$\Rightarrow \tan \theta \approx x_N / f$

Aberrations decrease 2P signal

- Aberrations decrease focus quality
- Two-photon (2P) fluorescence signal depends on squared excitation intensity
- Decreased focus quality means decreased 2P fluorescence



The goal: two-photon imaging deep inside the living brain



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Restored focus
→ high fluorescence