Microscopy Journal club

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RAPID: Real-time image-based autofocus for all wide-field optical microscopy systems

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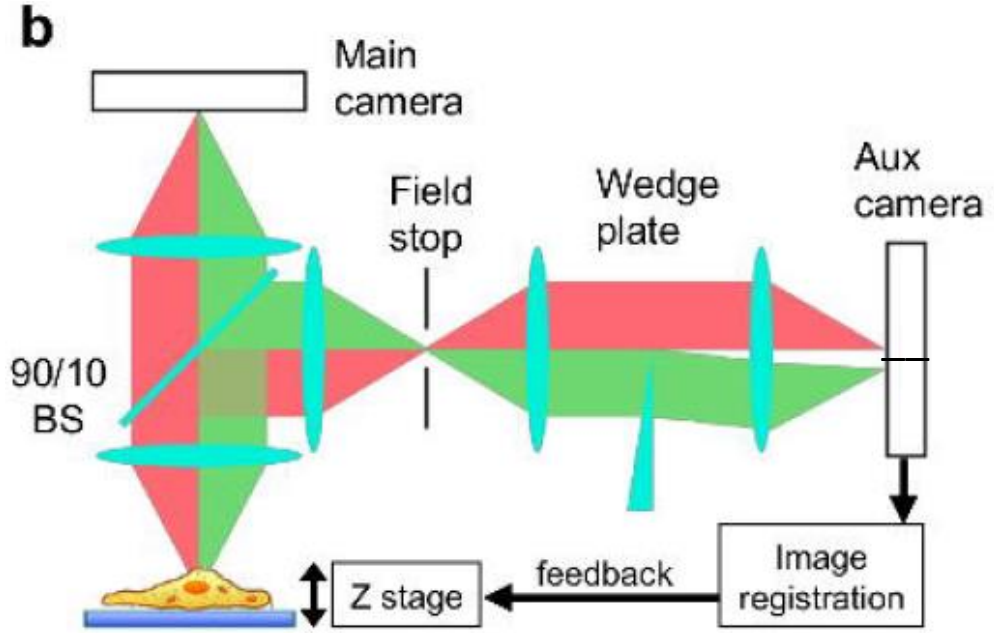
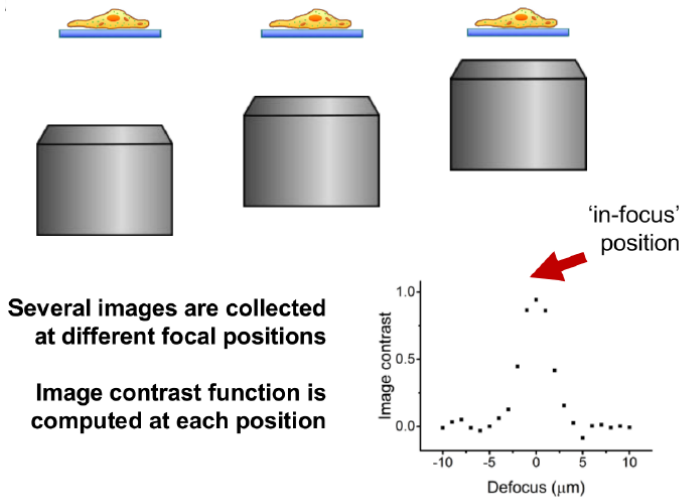


Autofocusing in microscopy (and photography)

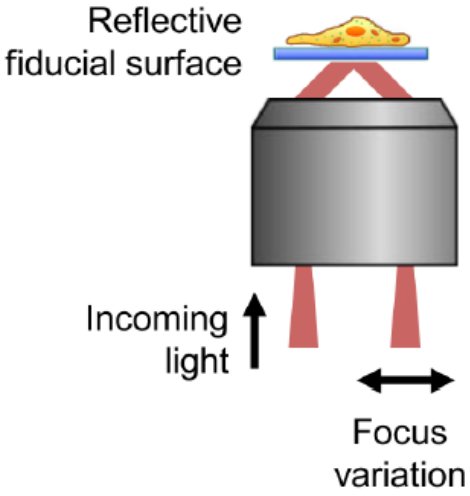
Common methods

Contrast-based



- ✓ Image-based
- X Slow
- X User must define contrast function

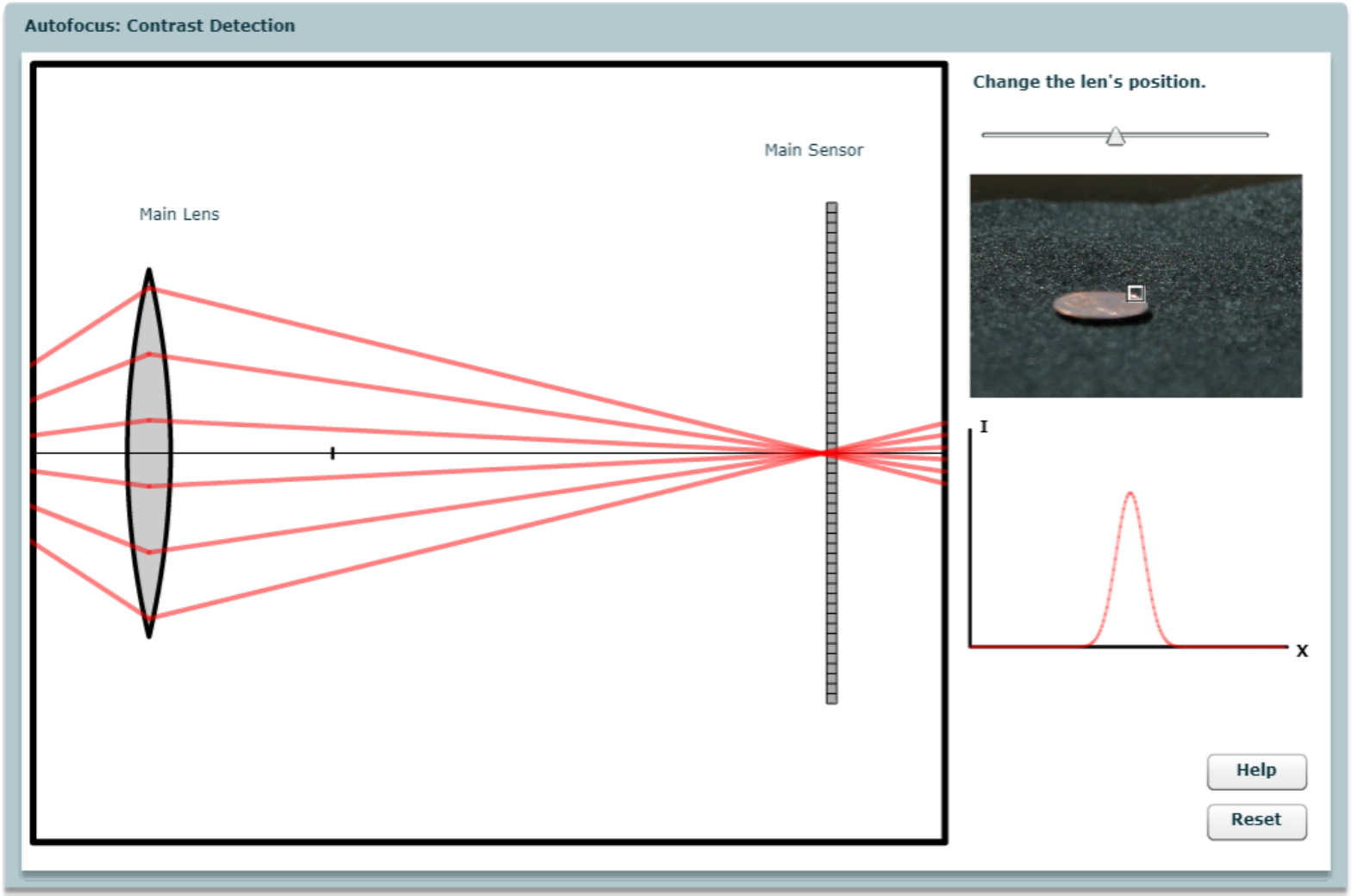
Triangulation-based



- X Based on reflective surface (~~SPIM~~)
- X Media R.I. should **not** match coverslip (for reflection)
- ✓ Fast
- ✓ Independent of the imaging

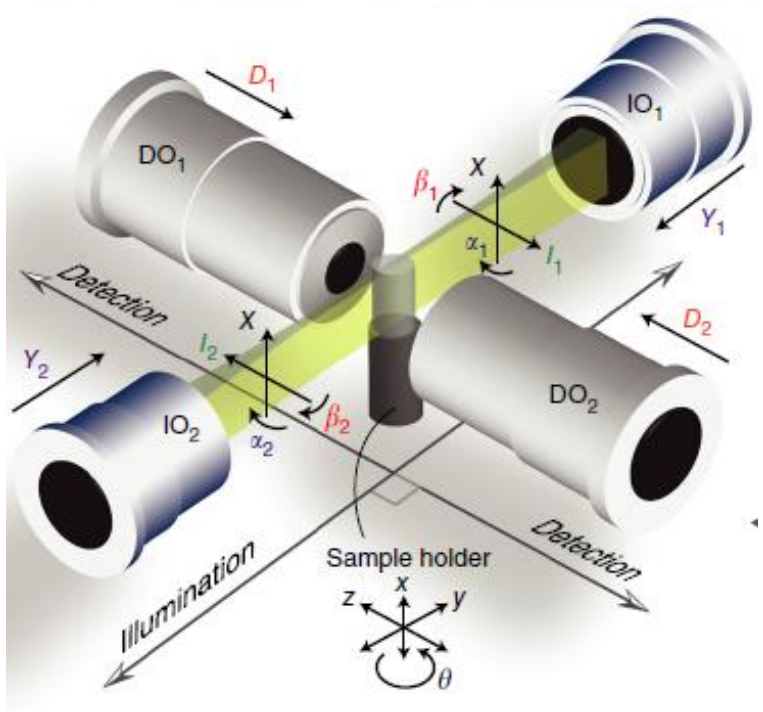
Phase detecton…

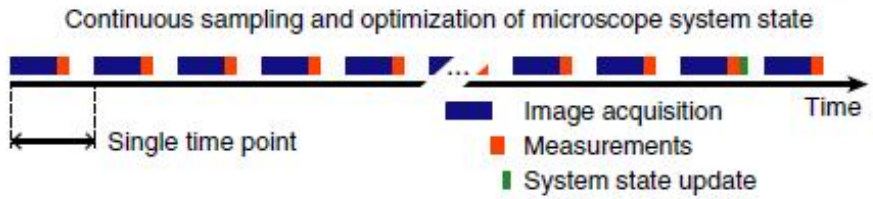
Contrast-based autofocus



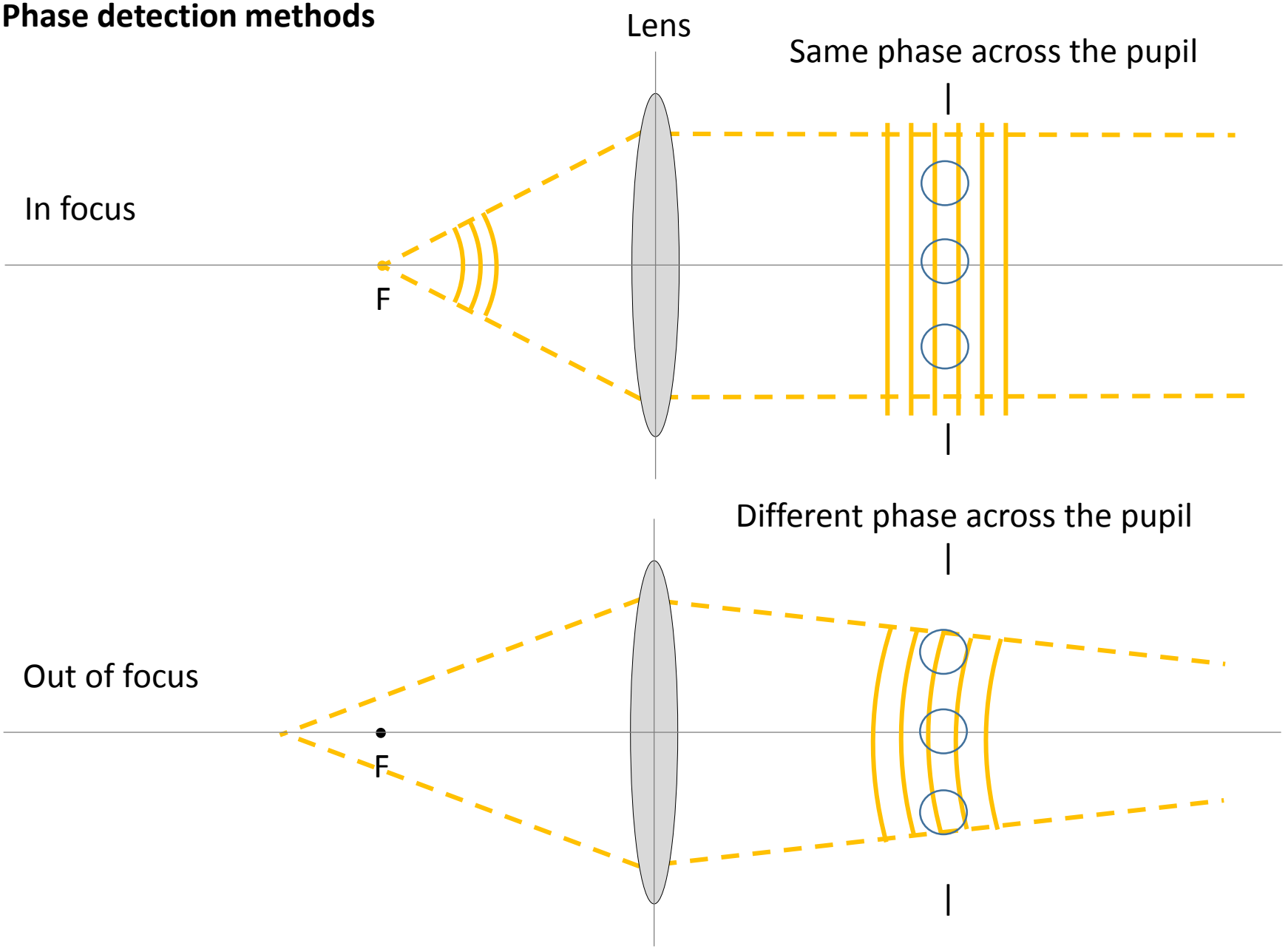
http://graphics.stanford.edu/courses/cs178-10/applets/autofocusCD.html

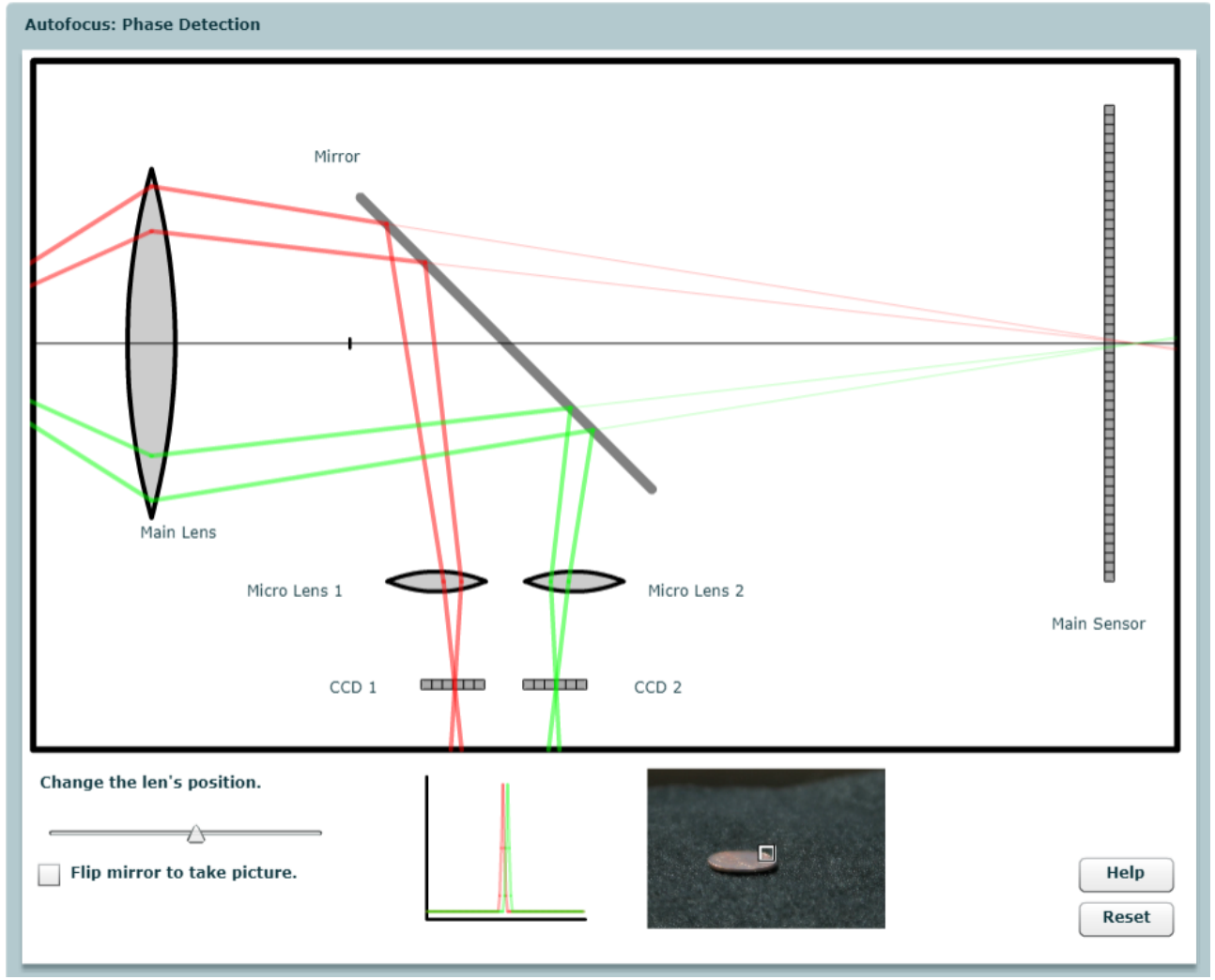
Contrast-based AF in SPIM system (Royer et al, *Nat. Biotech.,* 2016)





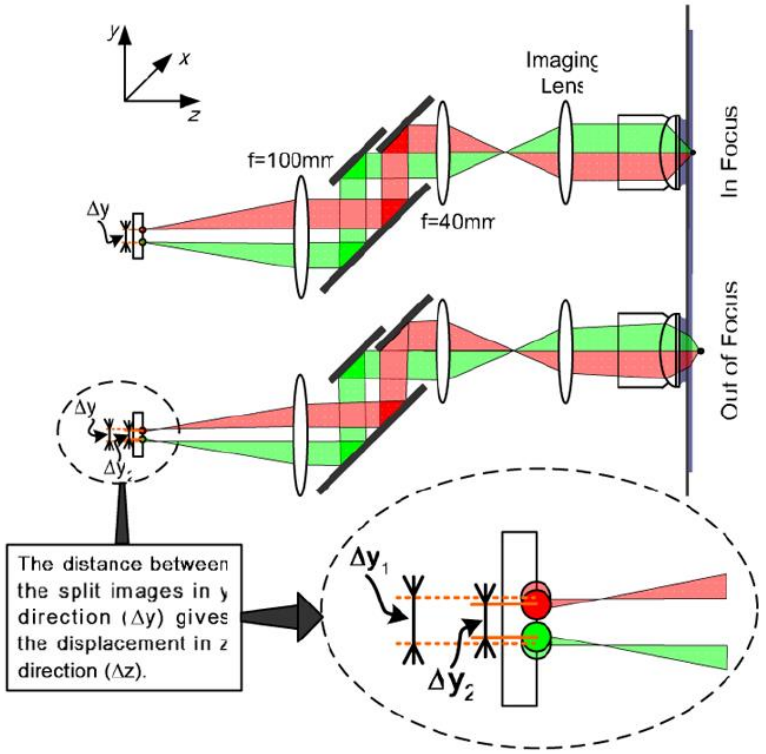
Phase detection methods





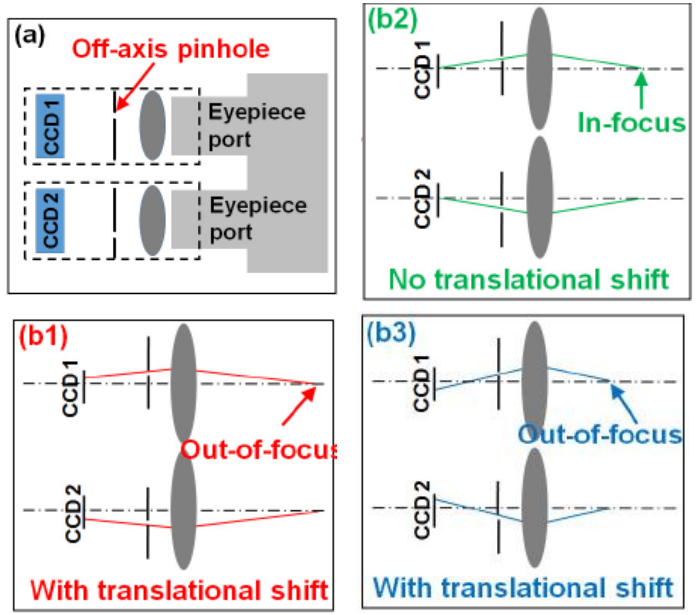
http://graphics.stanford.edu/courses/cs178-10/applets/autofocusPD.html

Phase-detection autofocus in microscopy (some other methods)



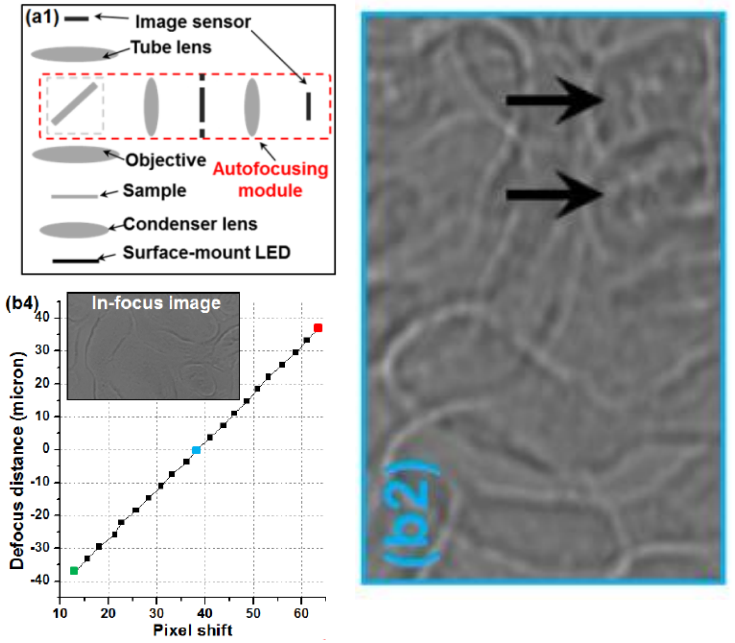
Parallax method: Sun et al, *Nano Lett.*, 2009

X Usable only in sparse samples (single-molecule conditions)
✓ Very accurate
✓ Allows sub-pixel localization
X Slow



Dual-cam **Instantscope**:
Guo et al, *Biomed Optics Express*, 2015

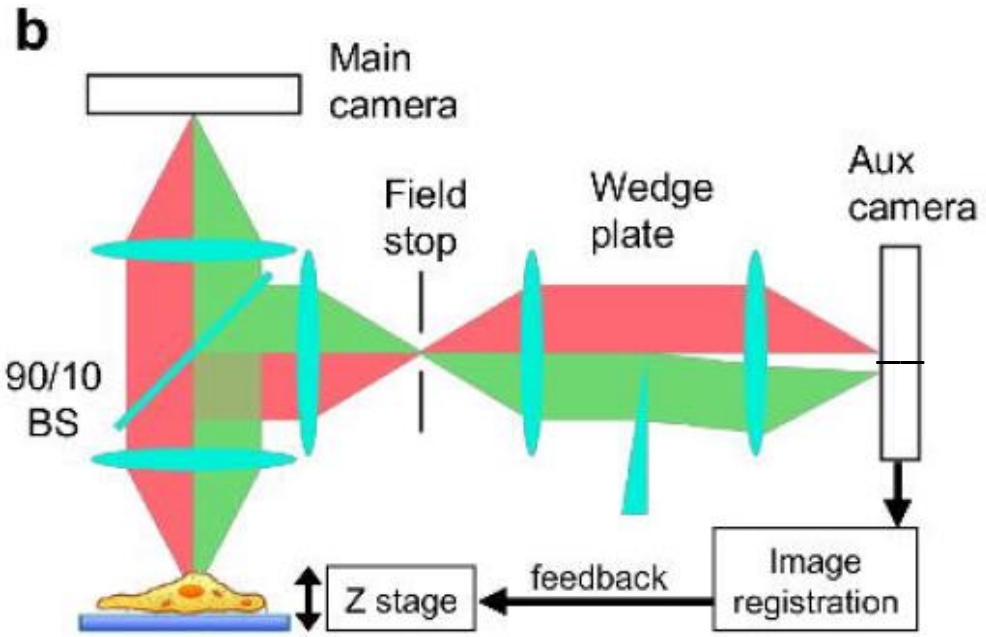
X Usable only in high light conditions
✓ Very accurate
✓ Very fast
X Requires 2 extra CCDs

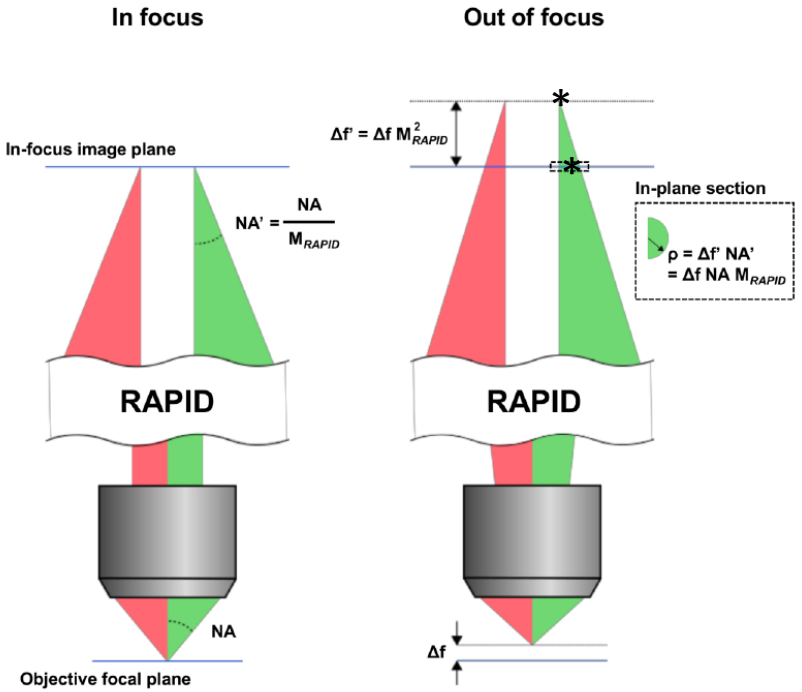


Single-cam **Instanstscope**
Liao et al, *Biomed Optics Express*, 2016

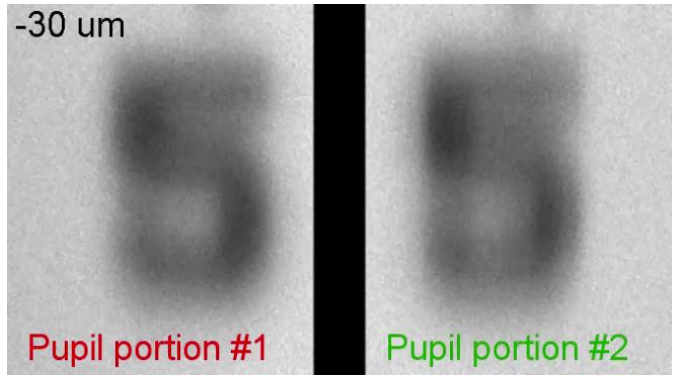
X Uses pinholes: only in high light conditions
✓ Very accurate
✓ Very fast
✓ Requires 1 extra CCD

Silvestri et al, 2017 setup:

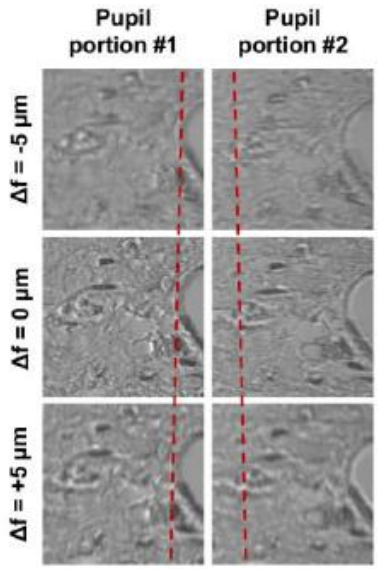




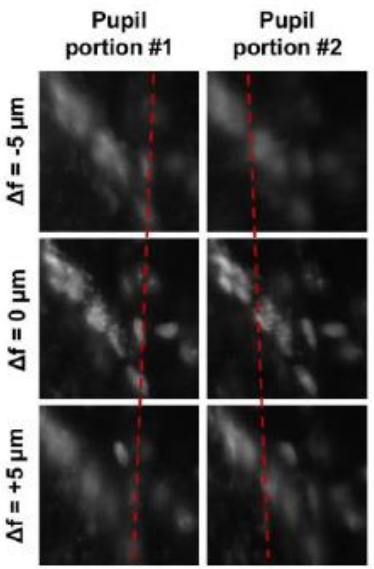
- ✓ Accurate
- ✓ Fast
- ✓ Works in low-light conditions



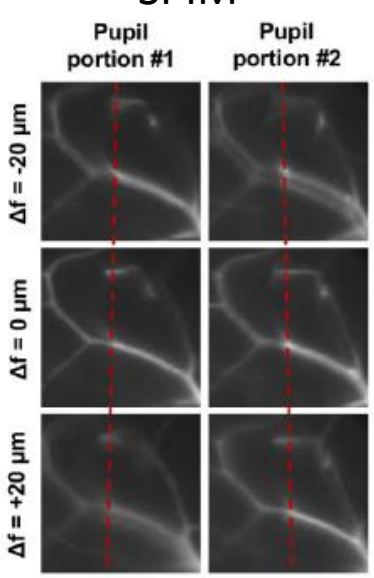
Bright-field



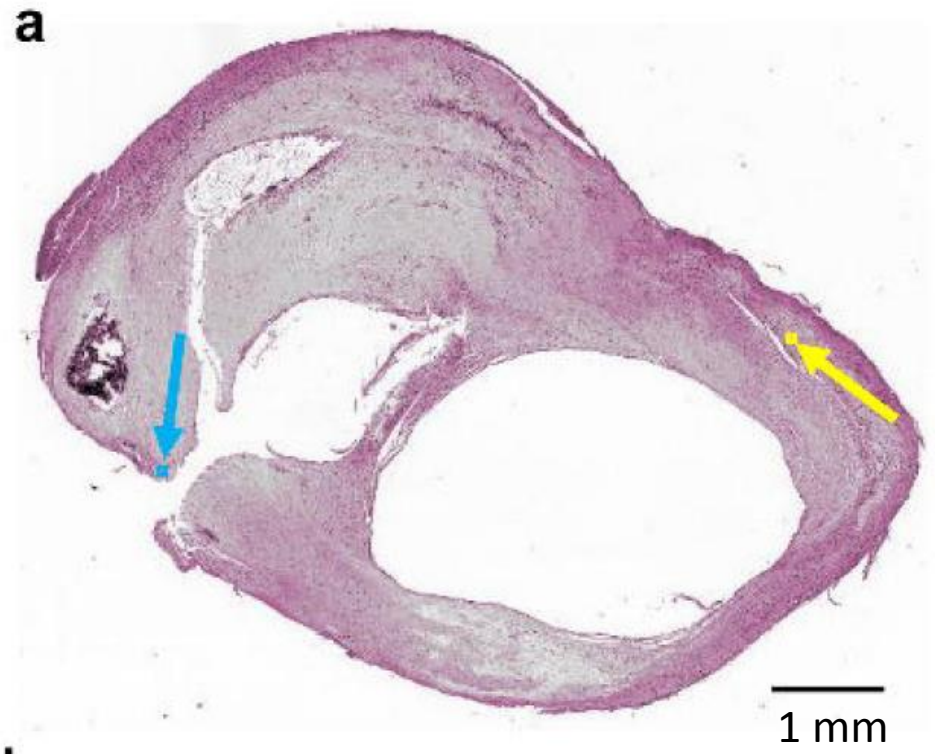
Epi-fluorescence



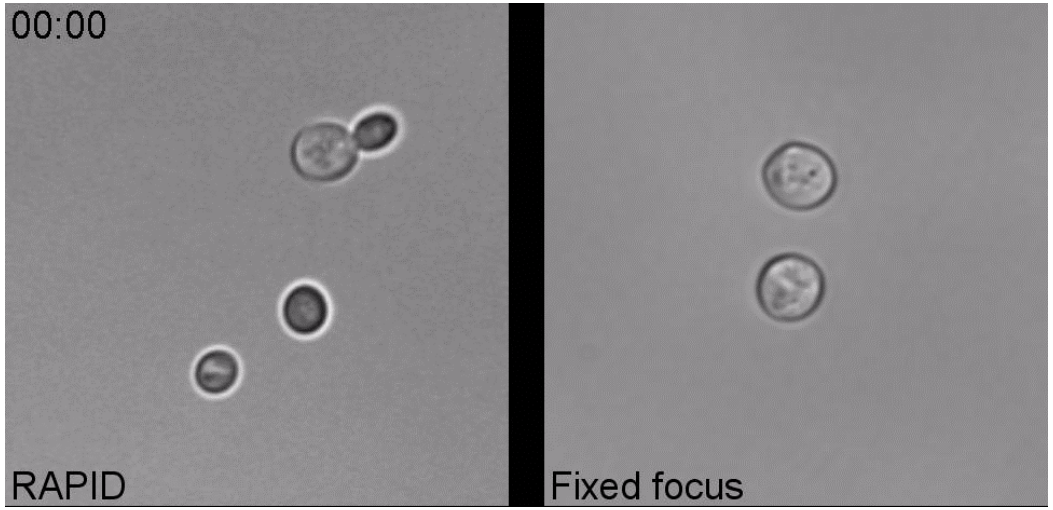
SPIM

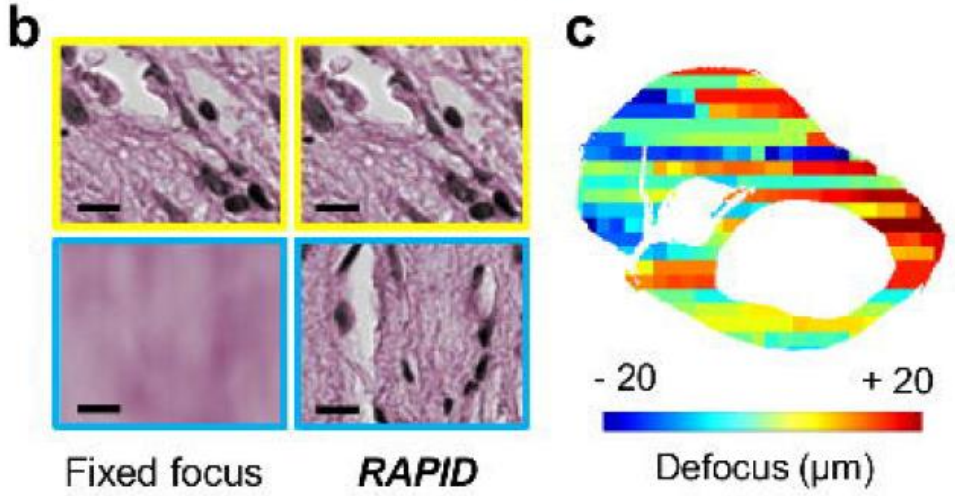


Histology slice

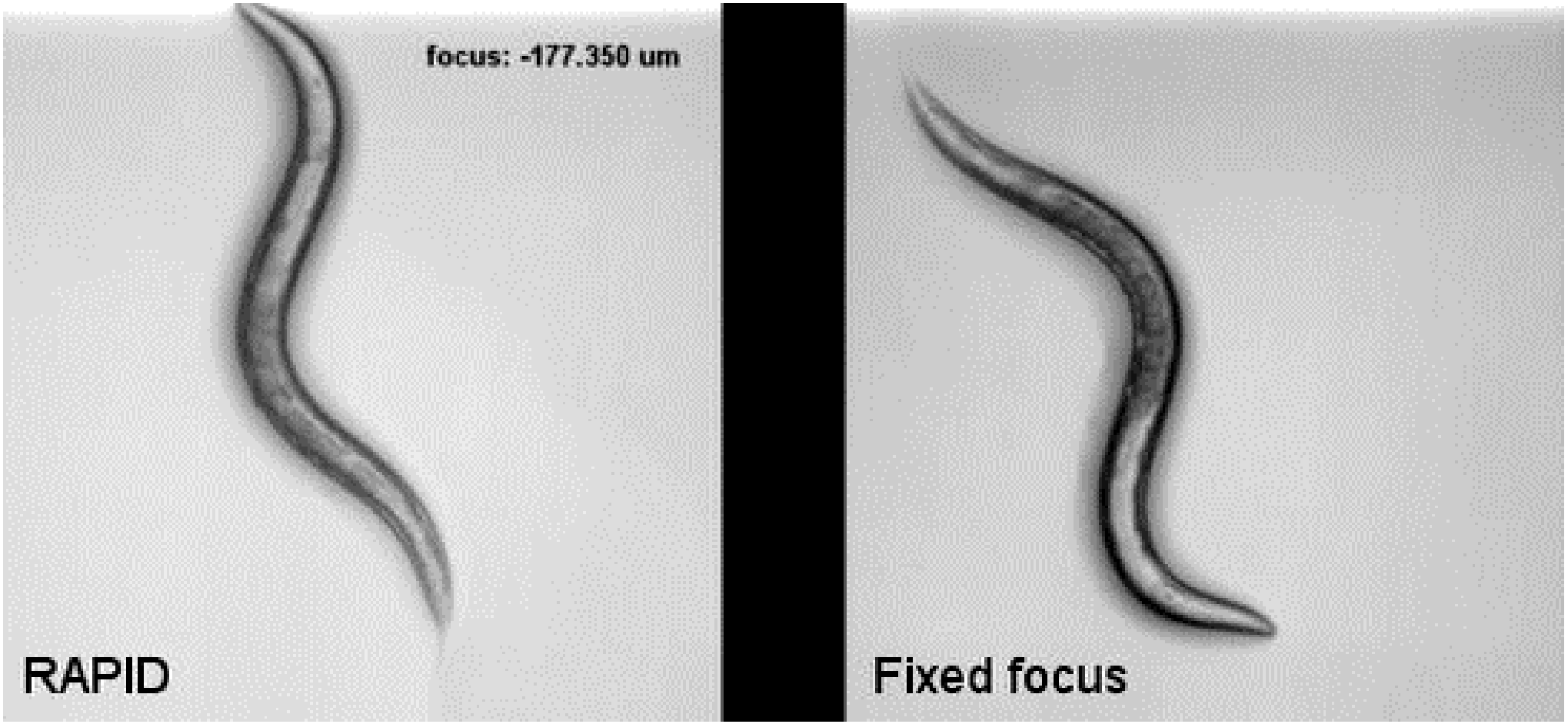


Cell culture

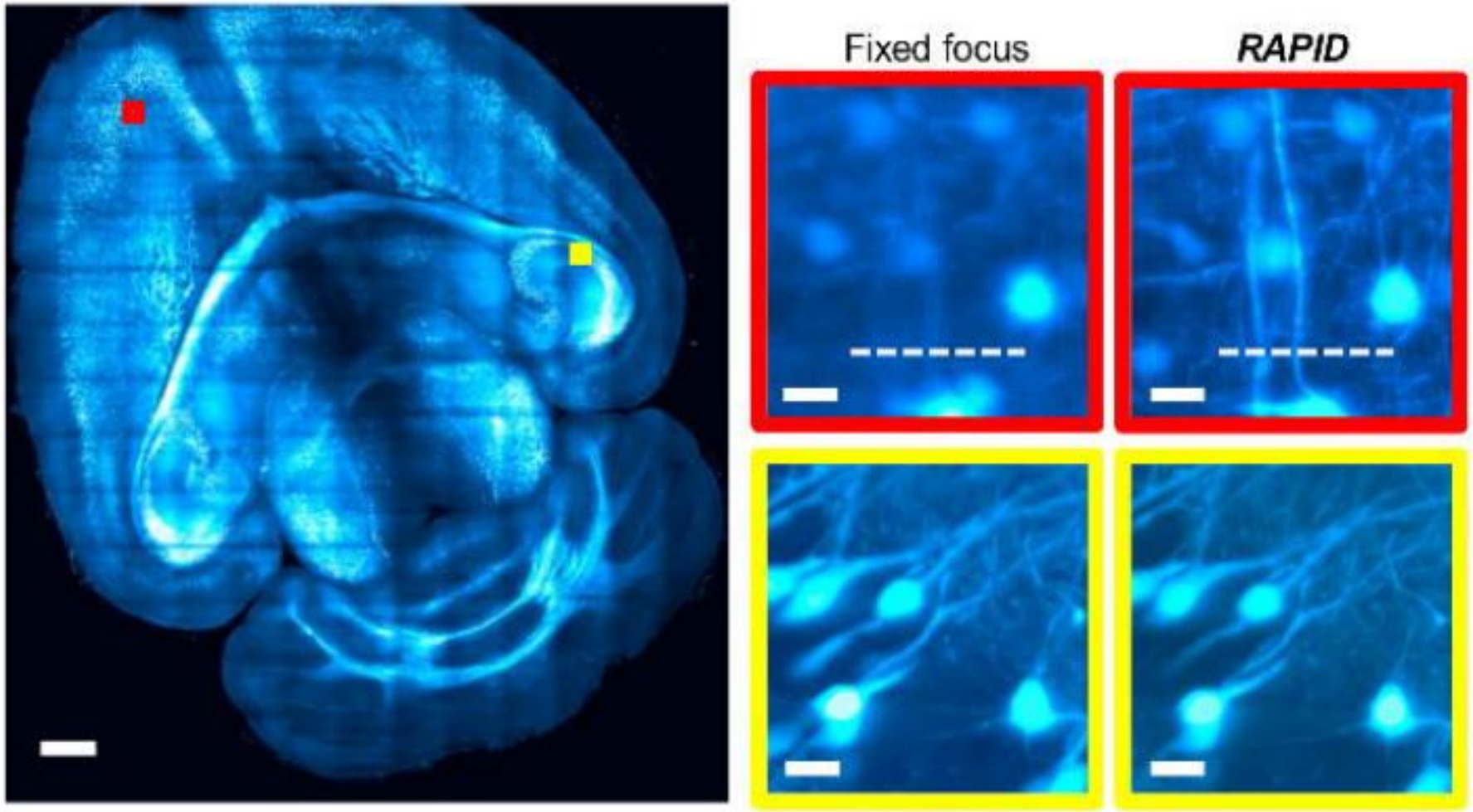




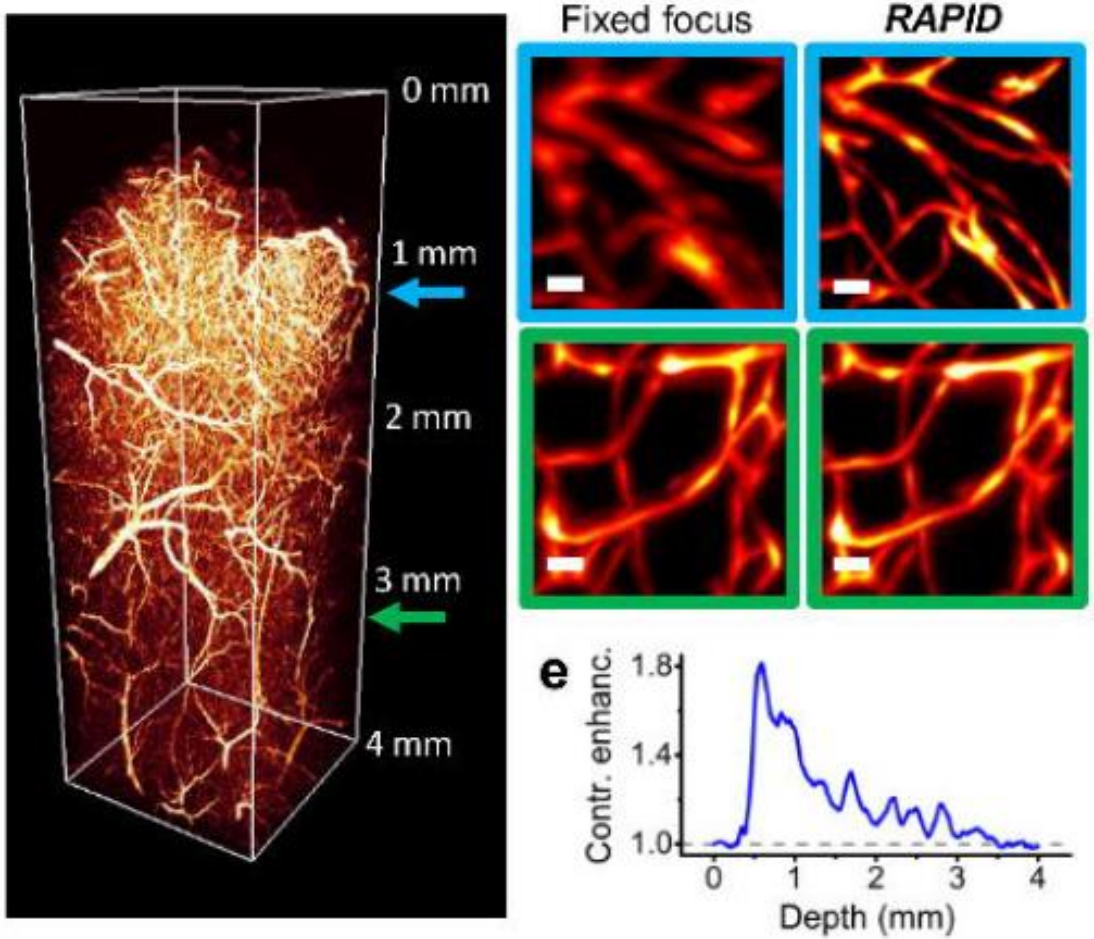
C.elegans tracking



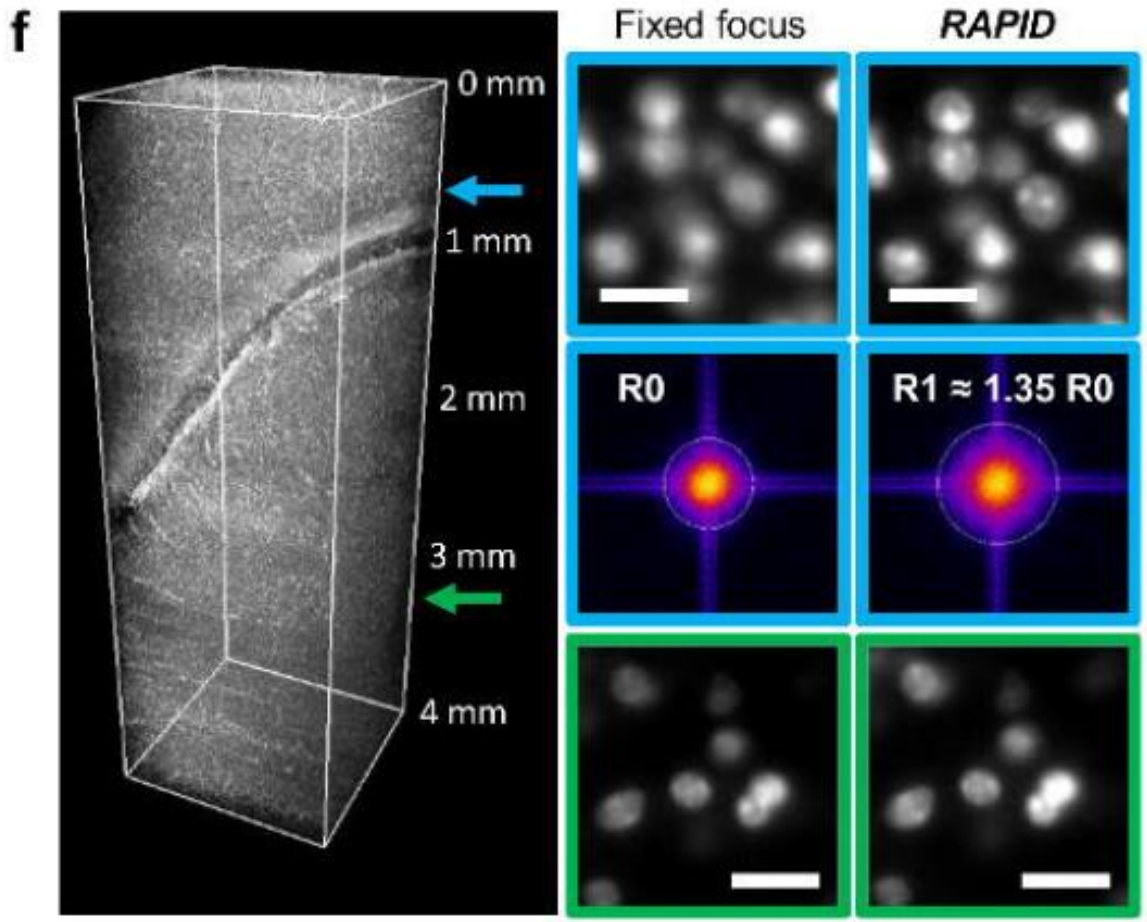
SPIM, cleared mouse brain



SPIM, vasculature in mouse brain



SPIM, nuclear staining in mouse brain



Disadvantages?

- Unlikely to work in low-light conditions (single-molecule) – using 10% of the collected light
- Not super-accurate, 0.7 of DOF

Method	*Accuracy (DOF)*	*Range (DOF)*	*Demonstrated time stability*	*Min S/N*	*Min S/B*	*Fluorescence*	*Dense samples*
Parallax[17]	~ 0.01	~ 8	minutes	~ 120	~1300	Yes	No
Instantscope[18]	~ 0.3	~ 300	N/A	N/A	N/A	No	Yes
Single-camera *Instantscope*[19]	~ 0.11	~ 25	N/A	N/A	N/A	No	Yes
RAPID	~ 0.7	~ 70	> 12 hours	~ 4.5	~ 1.7	Yes	Yes

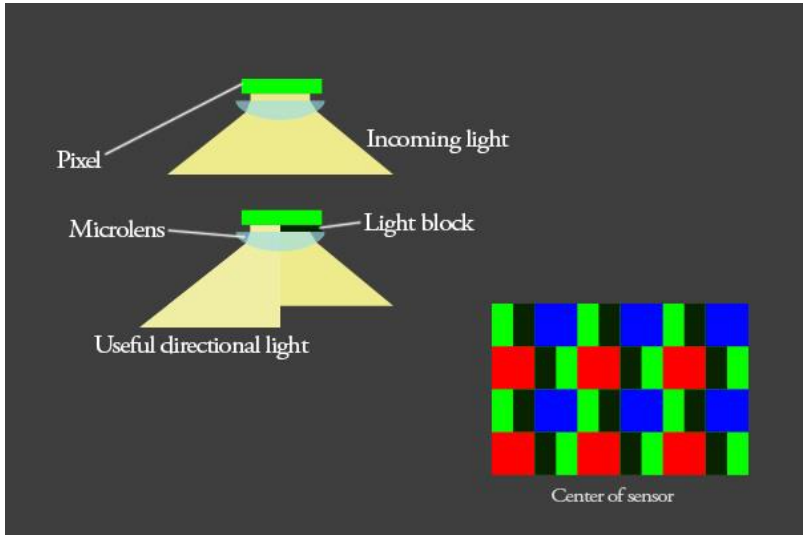
Other options?

AF requires *information* from the sample

AF photons can be separated from data photons in various ways:

- in **time**: spend some imaging time (contrast-based methods, Parallax)
- in **photon budget**: sacrifice some data photons (Parallax, RAPID)
- in **spectrum**: separate the AF wavelength
    - IR beam reflected from glass (triangulation, e.g. ASI Crisp)
    - IR image of the sample (McGorthy et al, 2013)
    - time of flight for a laser pulse (LG smartphones)

Hybrid systems: split time and photon budget (modern Canon, Sony high-end cameras)



Summary

The ‘best’ AF method depends on your application:

- Photon budget: how much light you can sacrifice?
- Speed: how fast you need to re-focus?
- Accuracy: what’s your goal AF precision?
- Sample density: do you have points sources?
- Any reliable fiducials surfaces or beads?
- Can AF use a spare band of spectrum?