

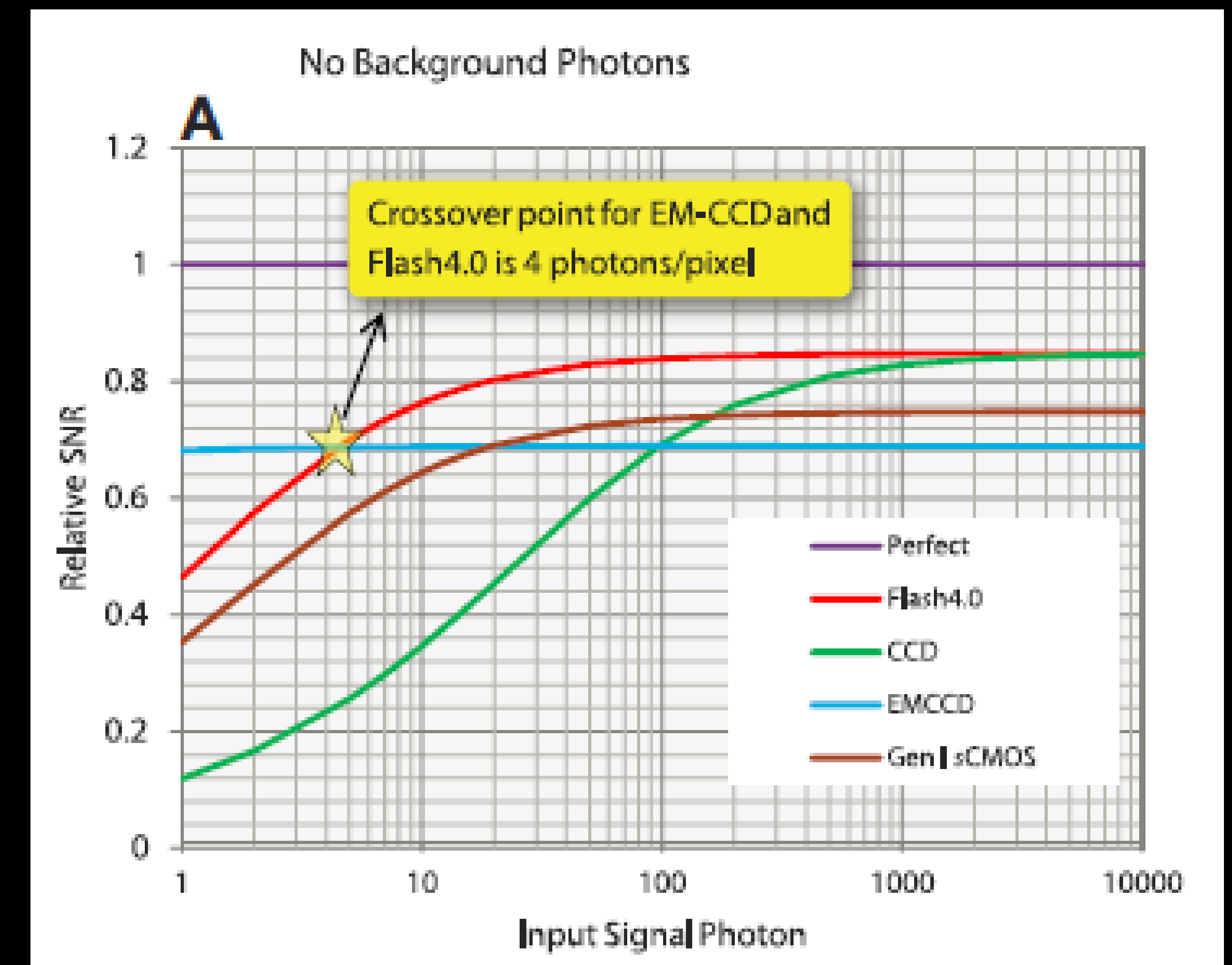
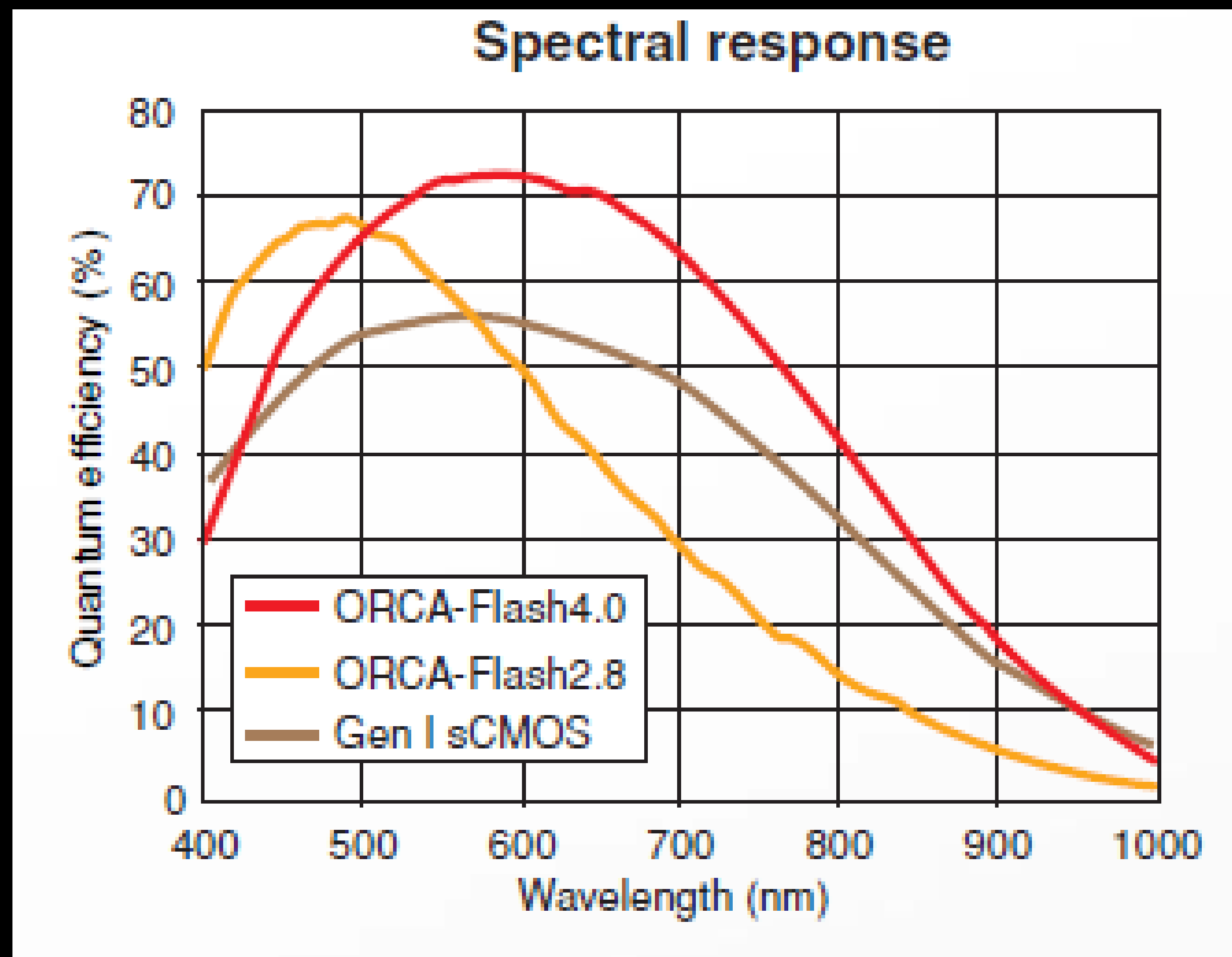
Lightsheet Mode™:
a novel firmware
modification to improve
resolution in lightsheet
microscopy

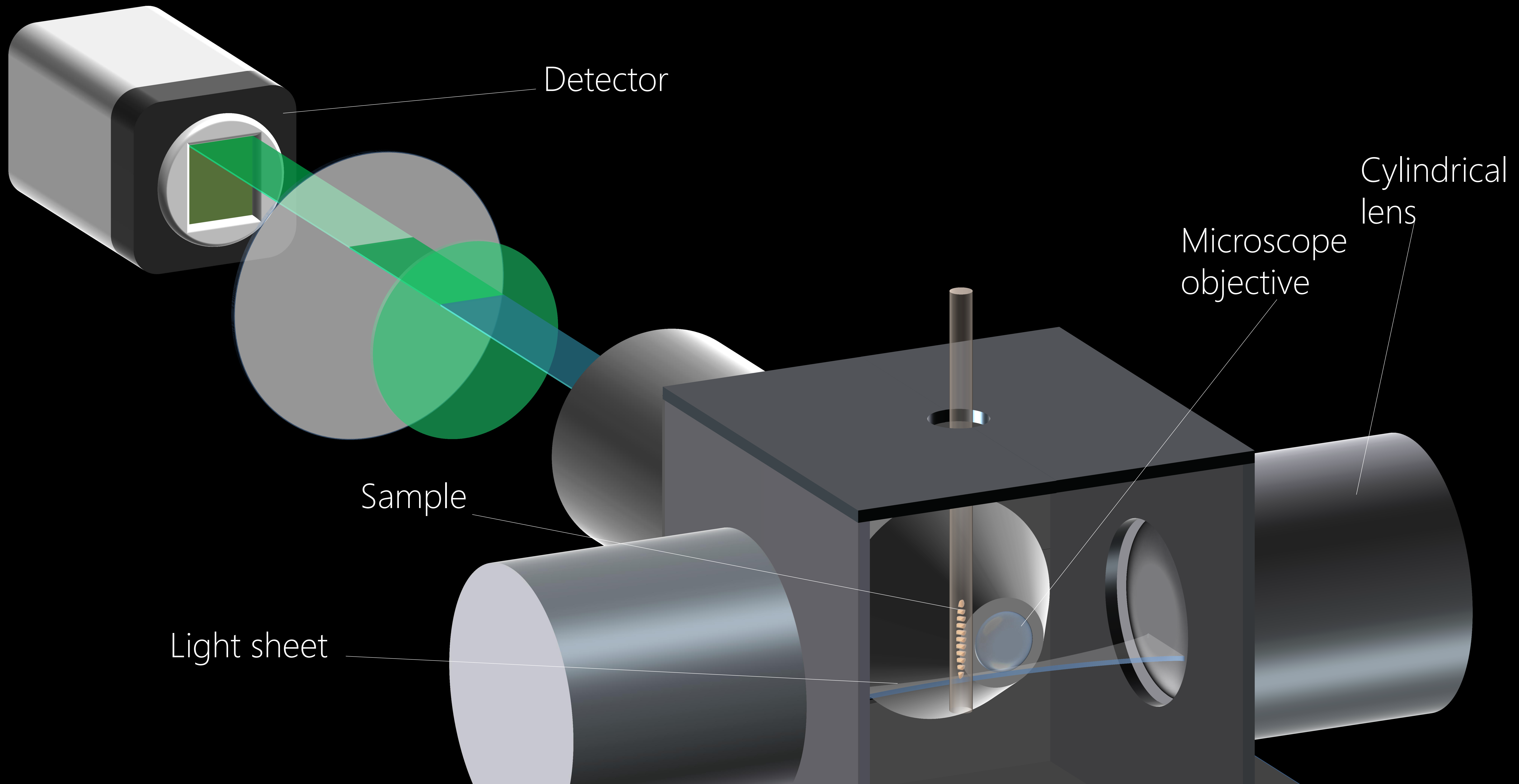


Orca Flash 4.0 - sCMOS

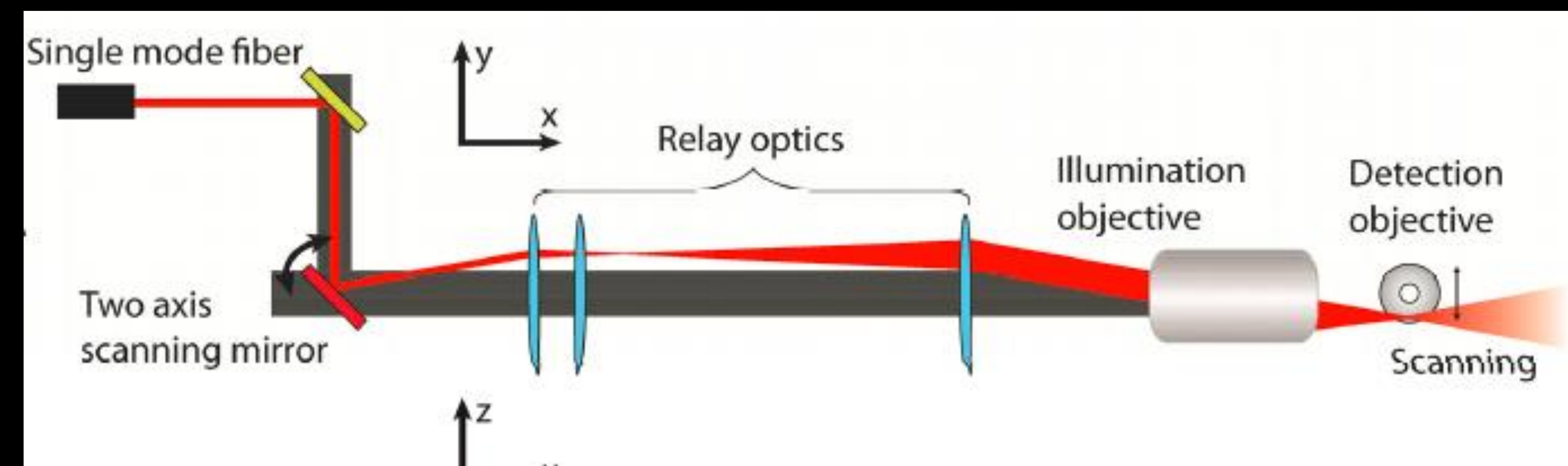
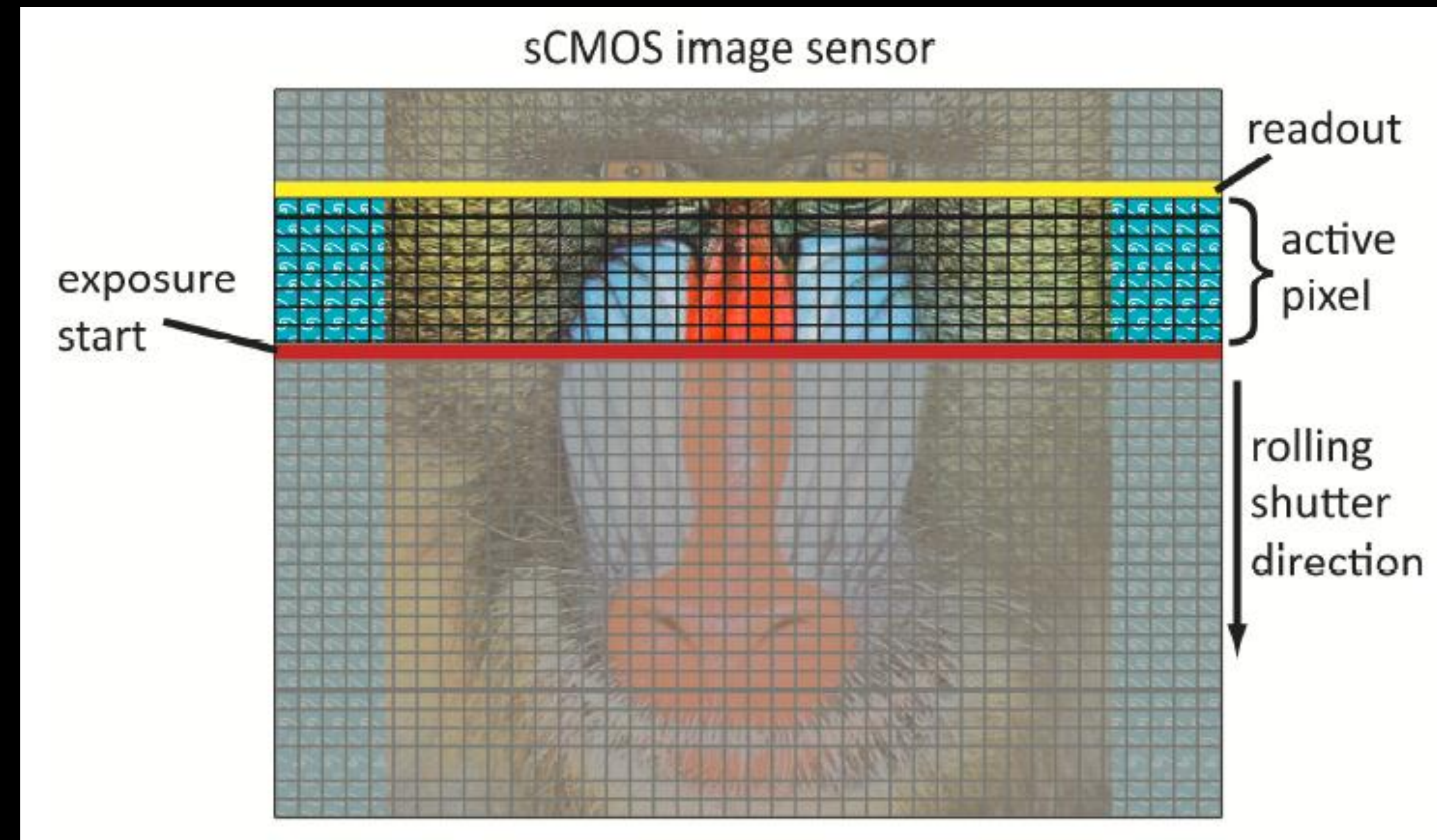
- 100 fps 4mpx readout, 0.9 e- read noise
 - 72% Q.E. – highest available

Most suitable camera for lightsheet microscopy

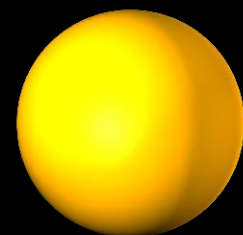




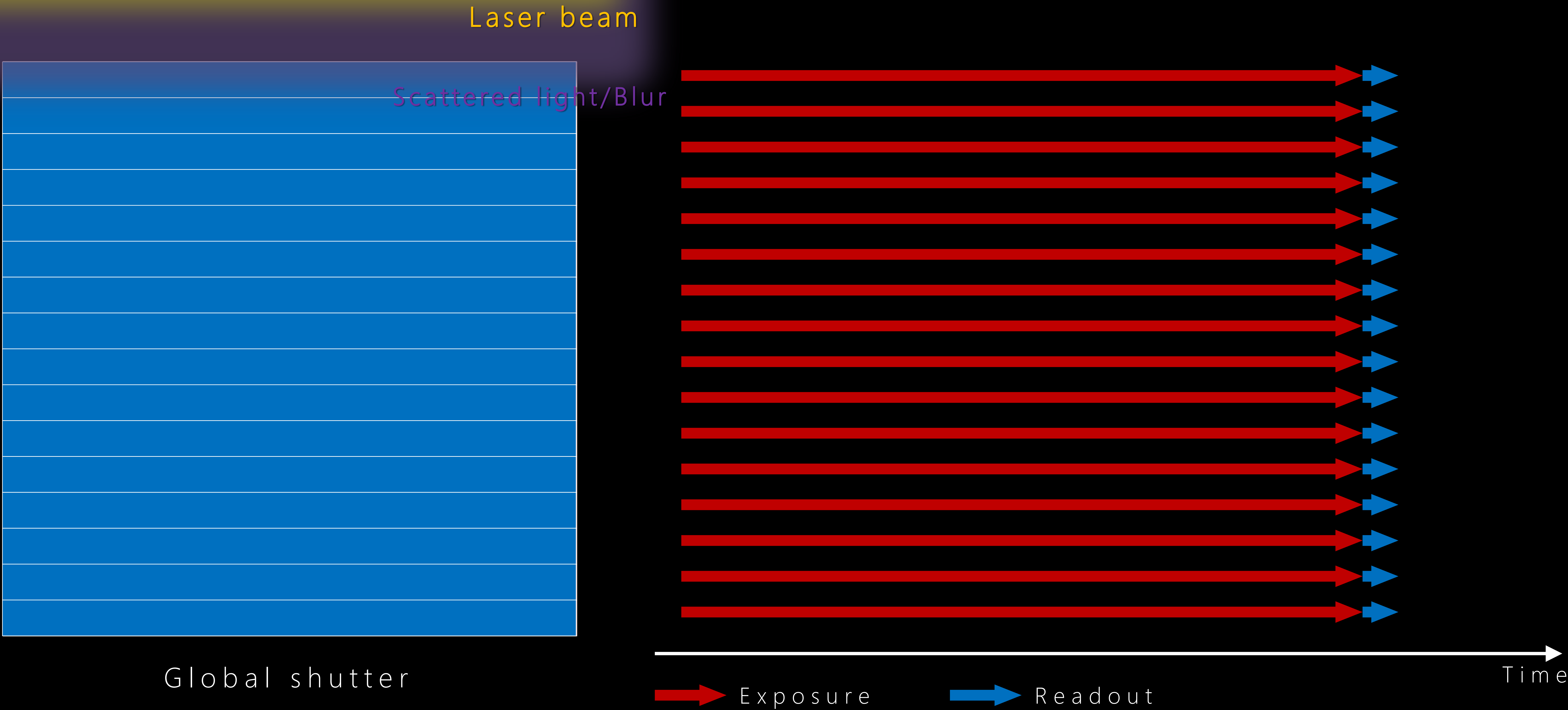
sCMOS rolling shutter

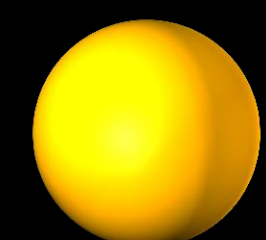


From Baumgart & Kubitscheck. 2012, using Orca Flash 2.8



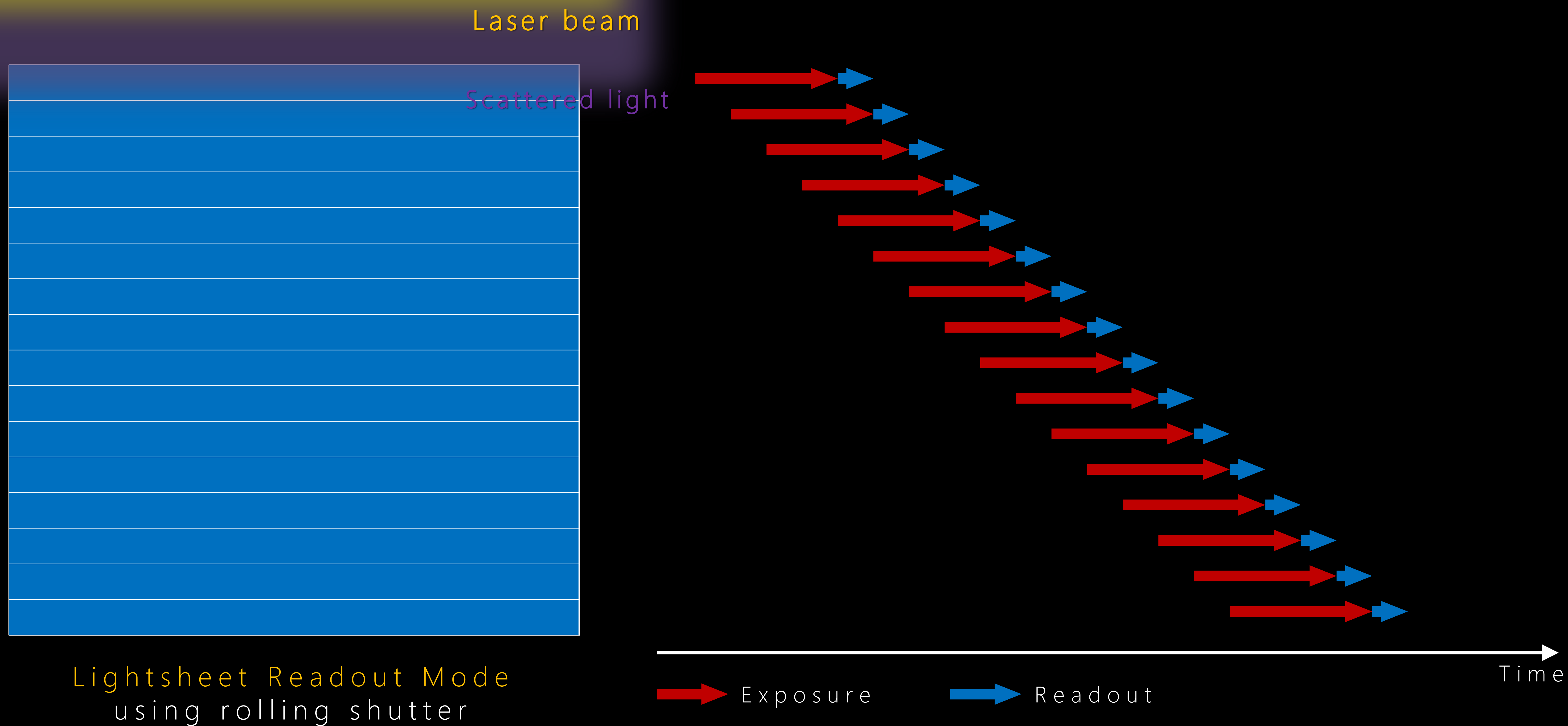
Entire chip exposed = scattered light collected

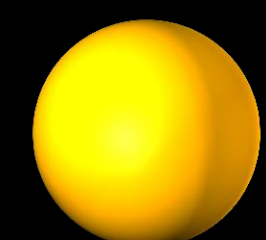




Confocal – like virtual slit

sCMOS chips can be read out line by line. In lightsheet Readout Mode, each line is reset before the next exposure, giving the effect of a 'virtual slit' and removing out of focus light.



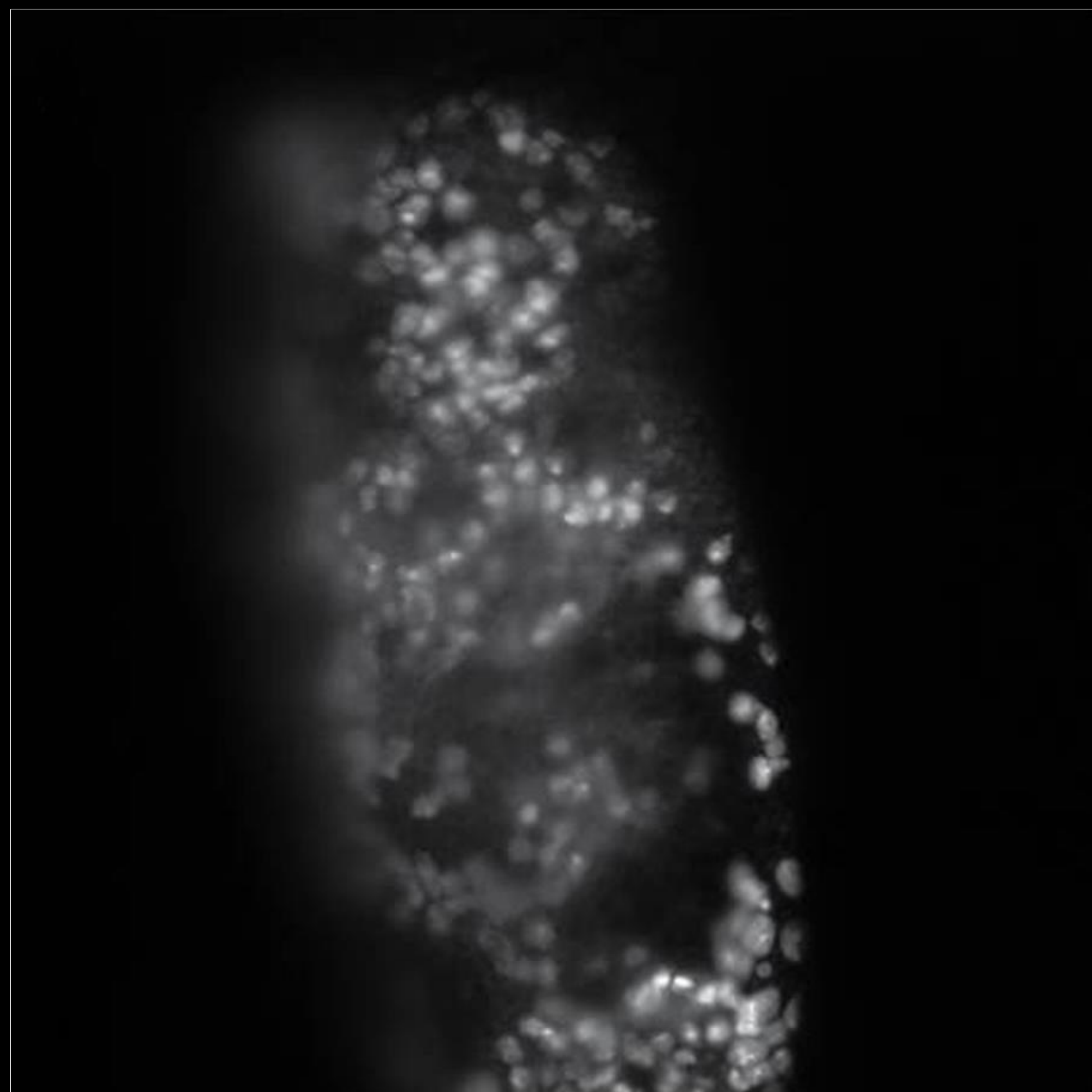


Feature 1: Exposure reset

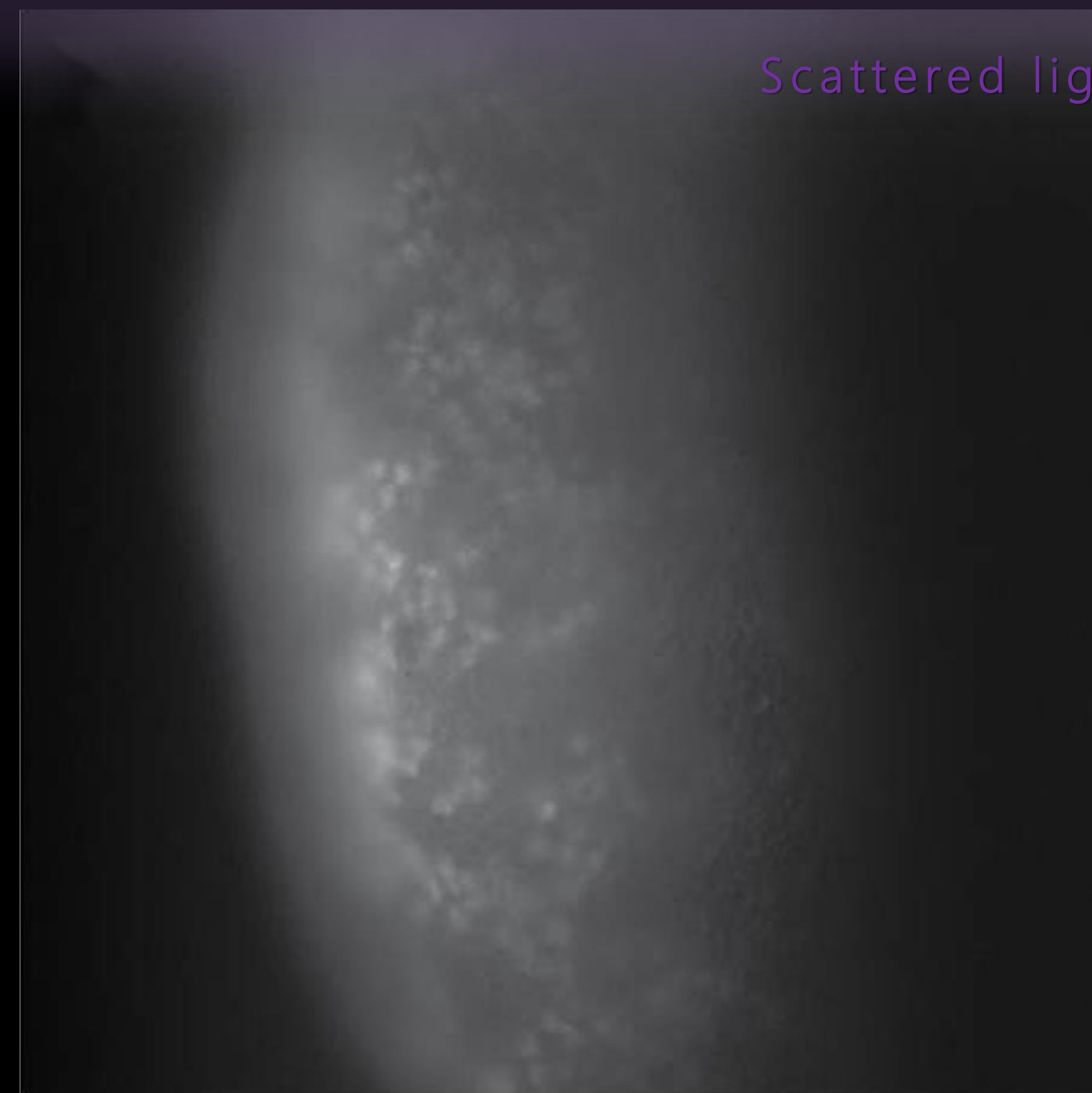
Compared to images acquired with global shutter, much finer detail can be resolved in images acquired with **Lightsheet Readout Mode** since background scattered light is removed.

Laser beam

Scattered light/Blur



Lightsheet Readout Mode
using rolling shutter

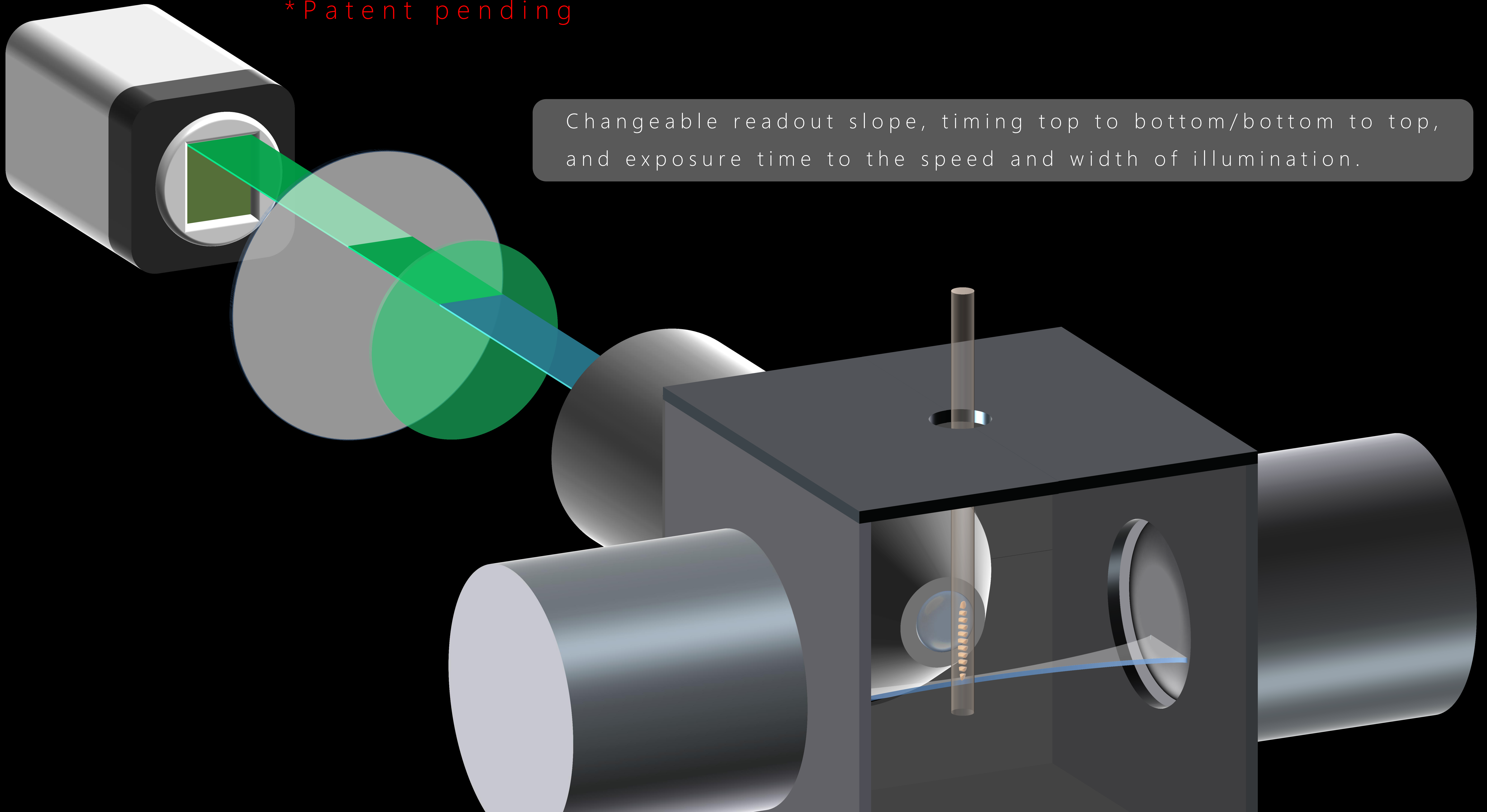


Global shutter

● Feature 2: Adjustment of readout slope and exposure time

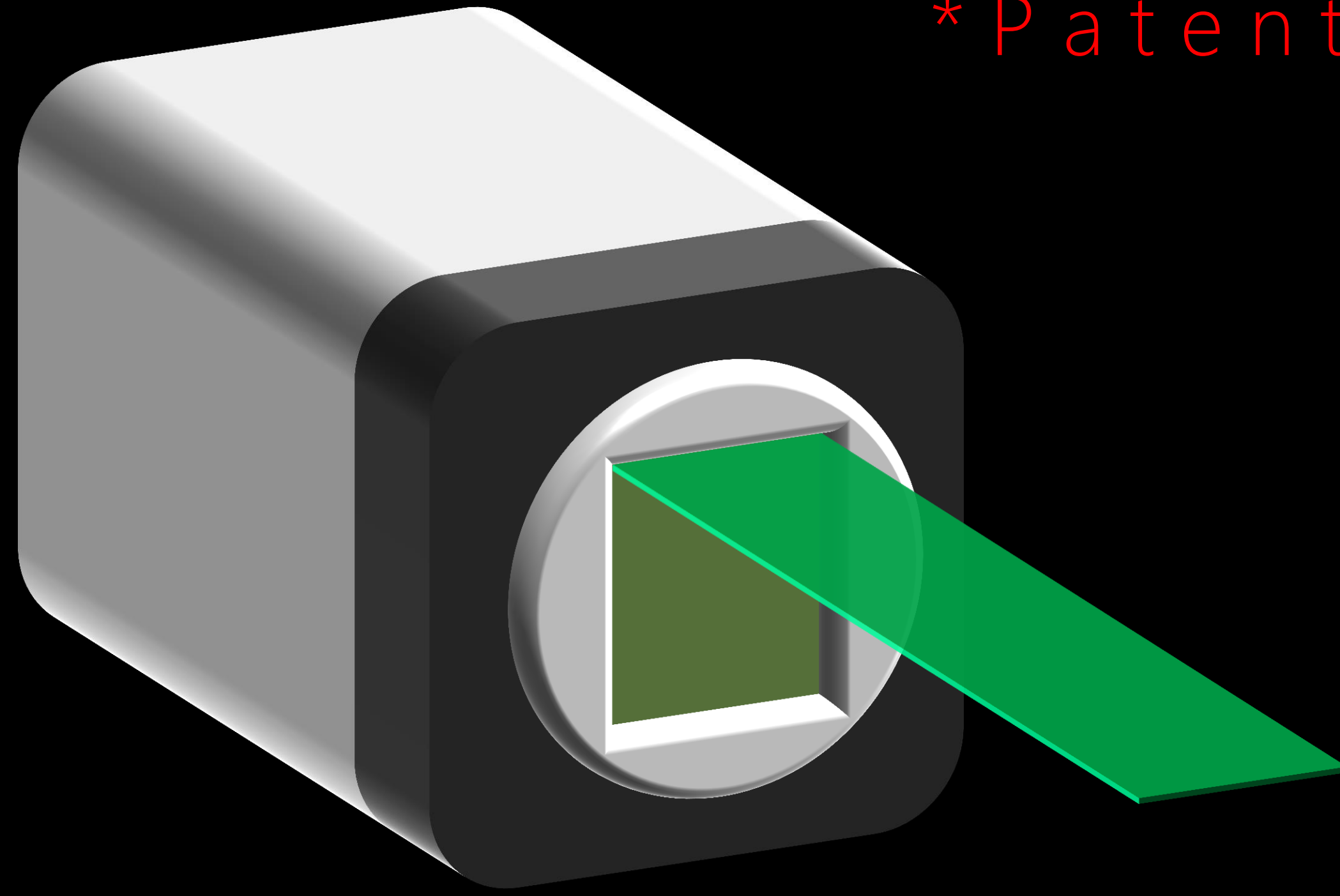
*Patent pending

Changeable readout slope, timing top to bottom/bottom to top,
and exposure time to the speed and width of illumination.

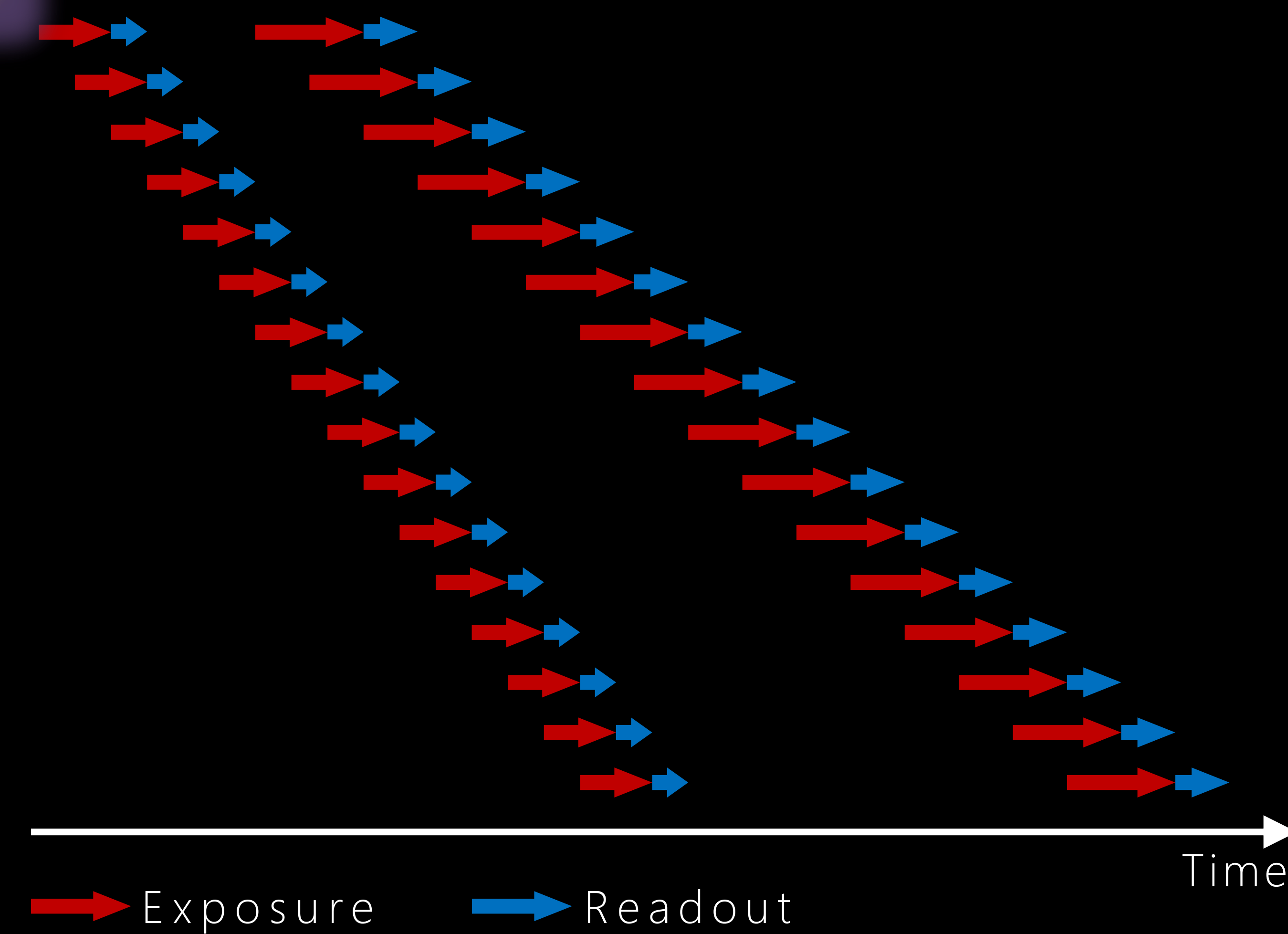
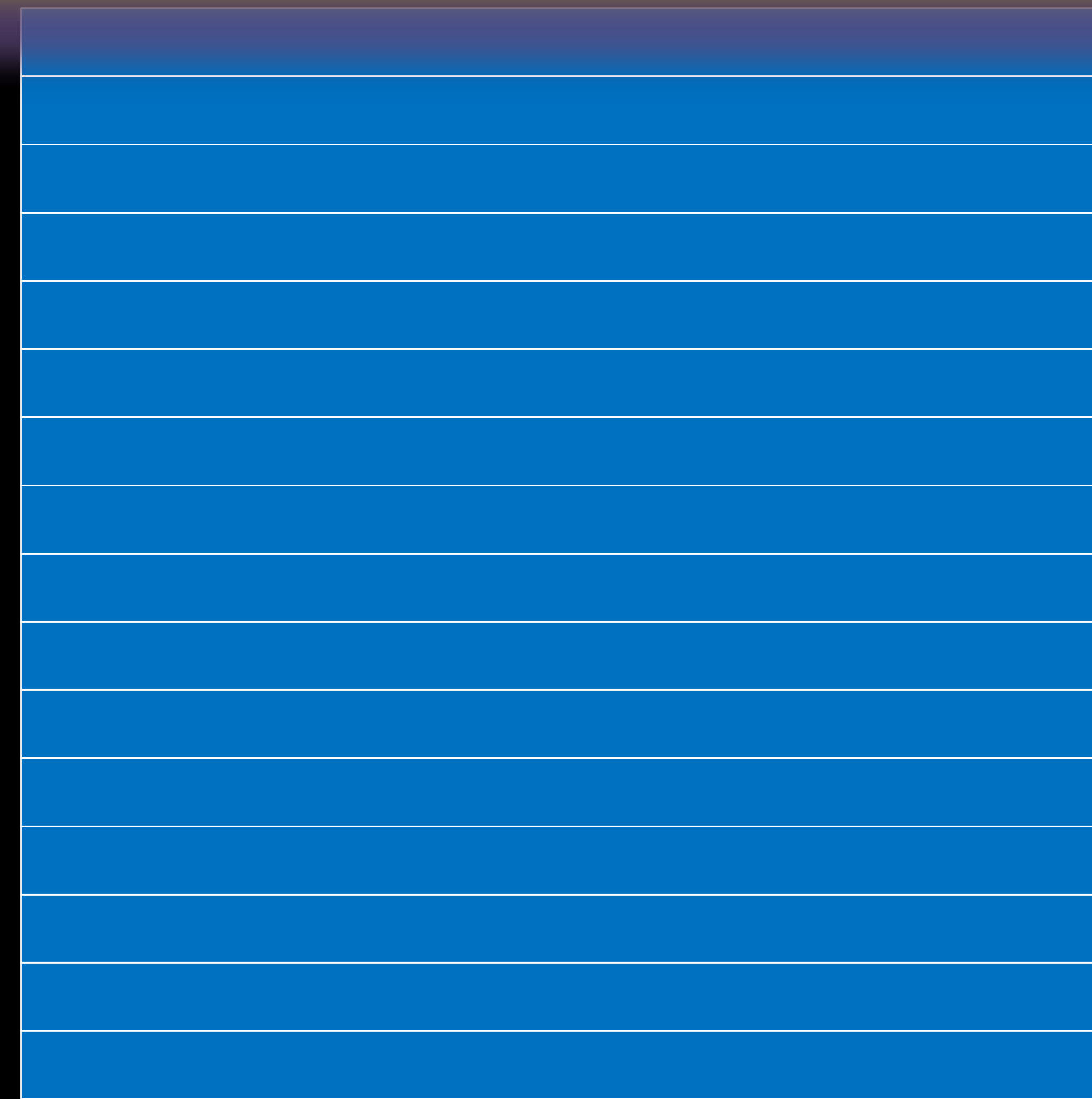


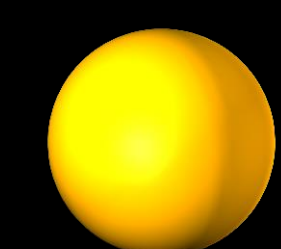
Adjustment of readout slope and exposure time

*Patent pending



Lightsheet Readout Mode is flexible. Setting of readout slope can be adjusted from 10 μ s to 100 ms for each line, so light source can be easily synchronized.

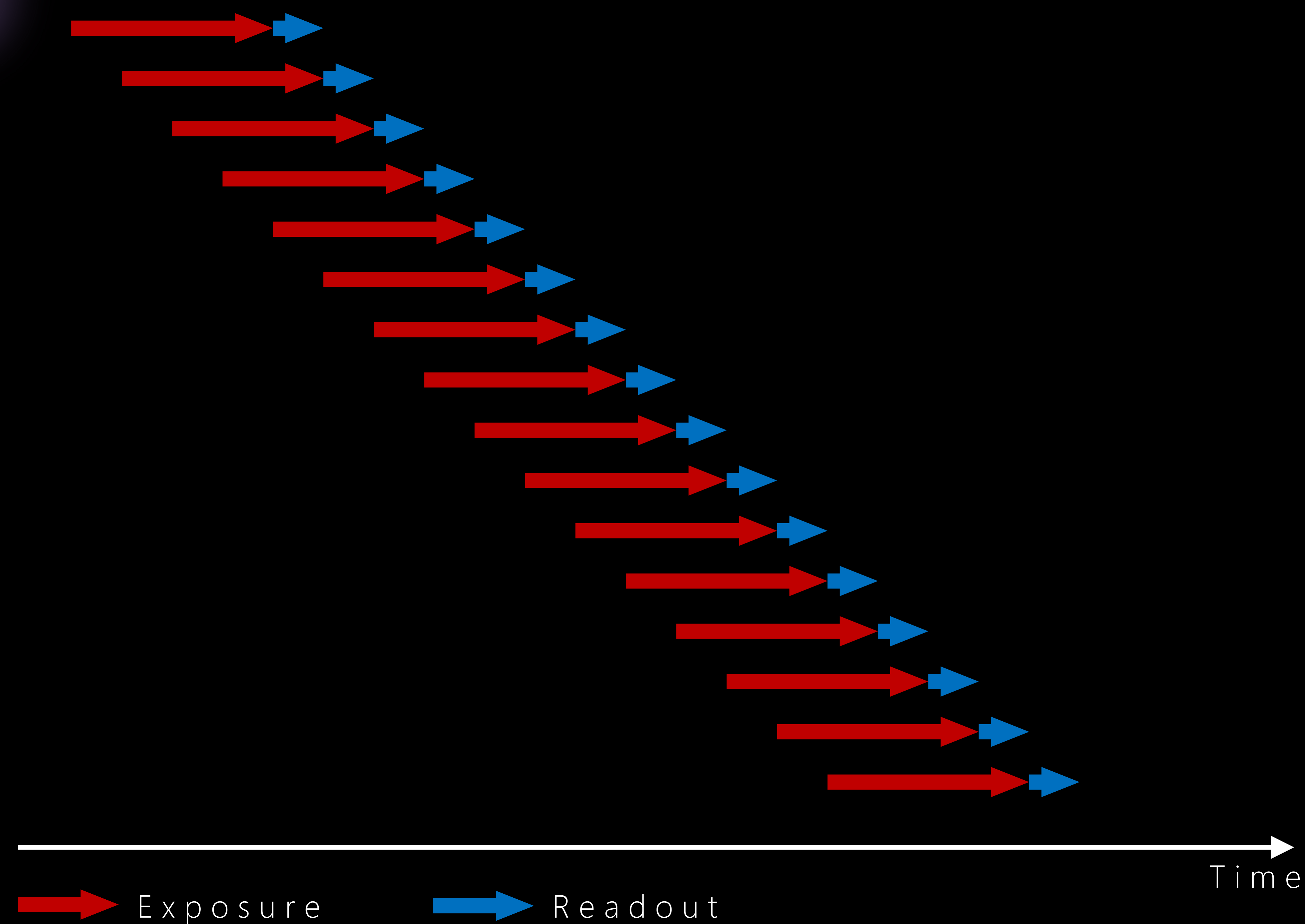
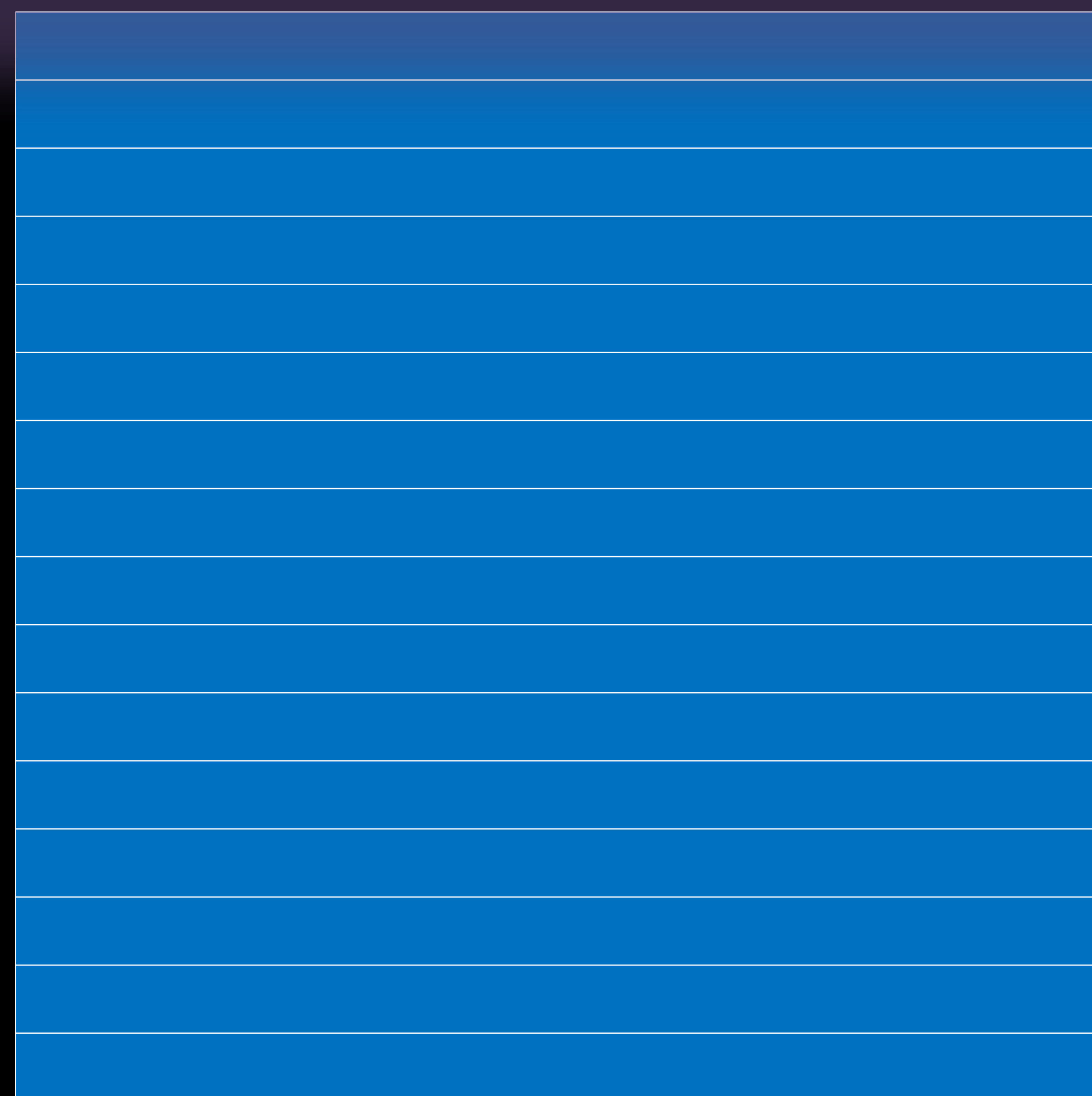




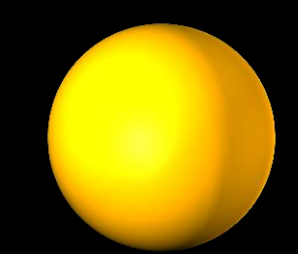
Feature 2: Adjustment of readout slope and exposure time

*Patent pending

Any illumination width is covered by the Light sheet mode with flexible exposure time setting.

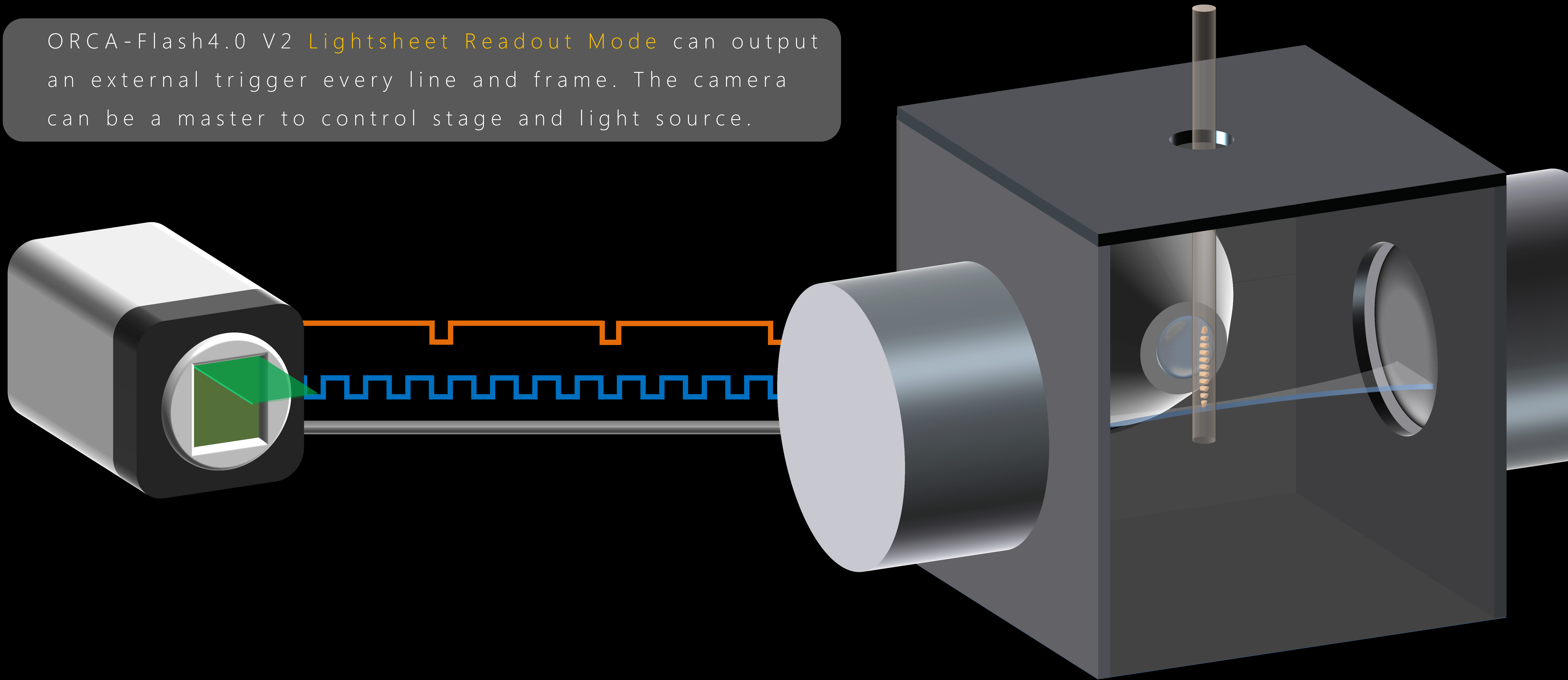


Triggering - Synchronising light
movement to camera chip readout



External trigger output

ORCA-Flash4.0 V2 **Lightsheet Readout Mode** can output an external trigger every line and frame. The camera can be a master to control stage and light source.



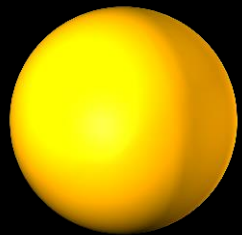
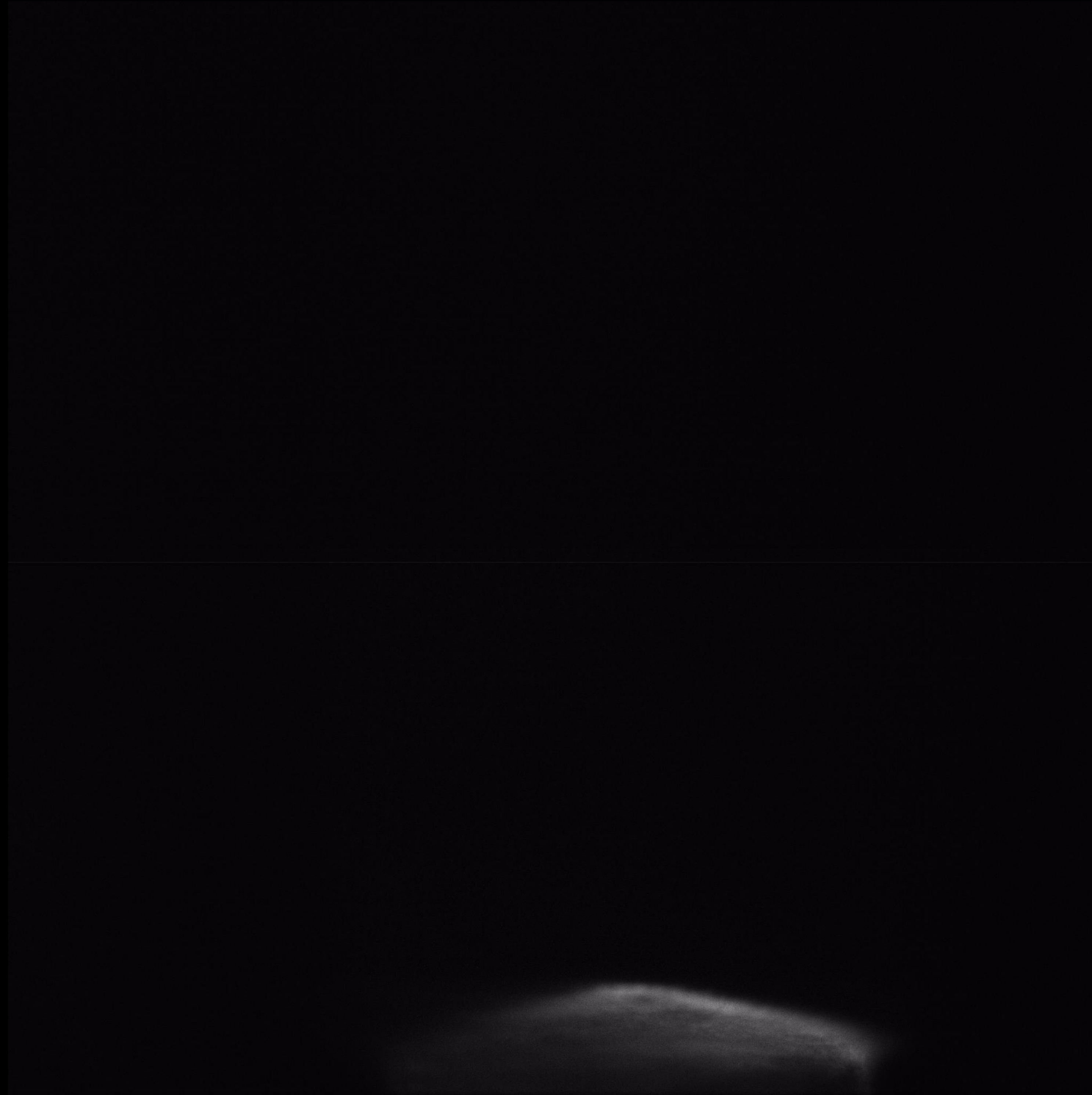
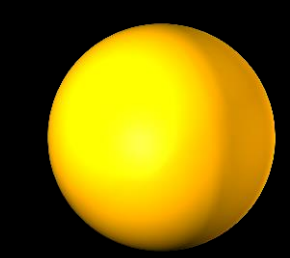


Image samples

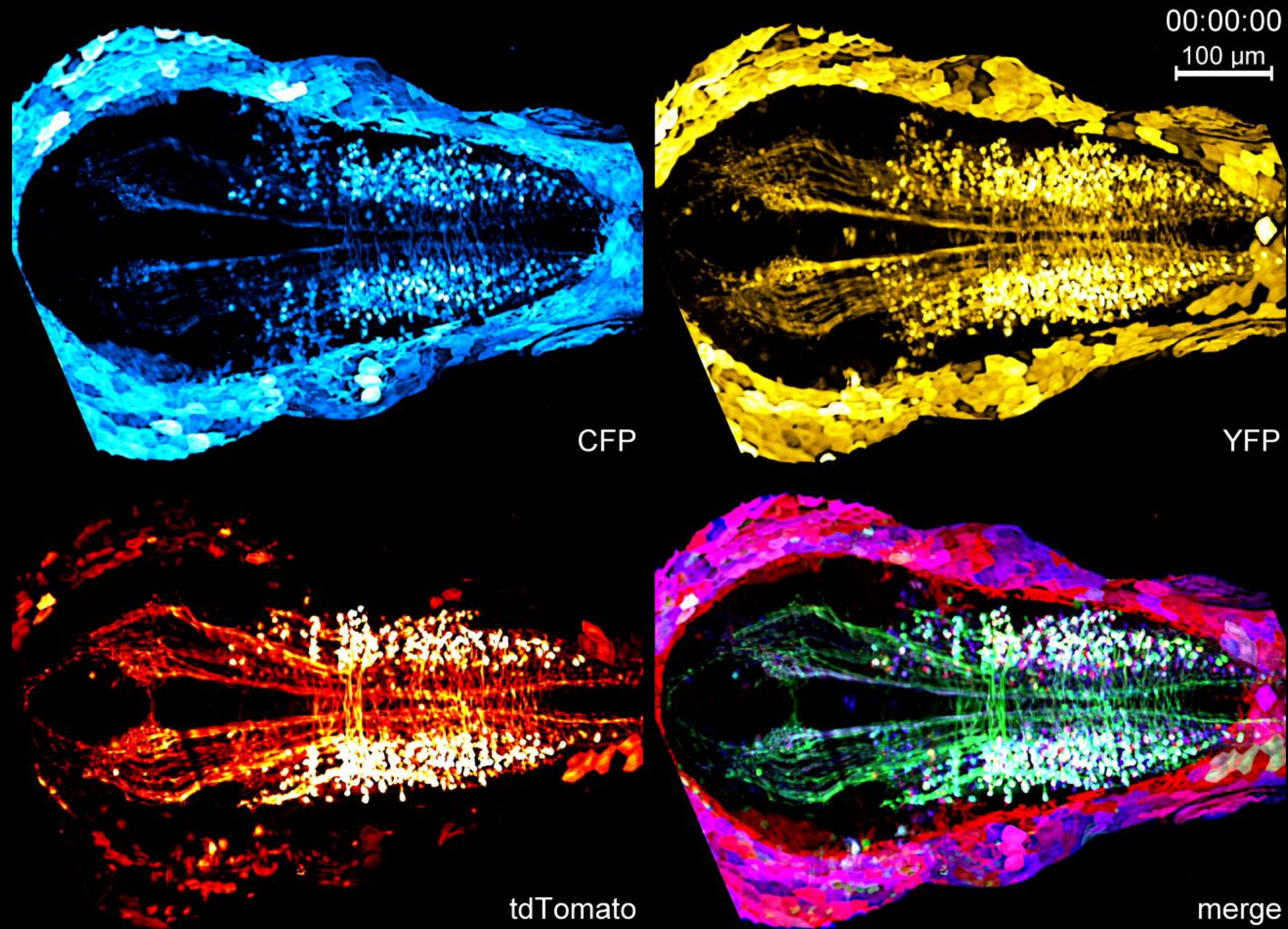
Light sheet readout



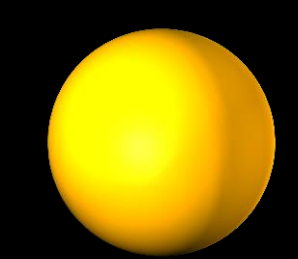
With thanks to Dr. Felix Evers, Aleks Chmliewski, University of Cambridge



Live whole-brain brainbow imaging in zebrafish



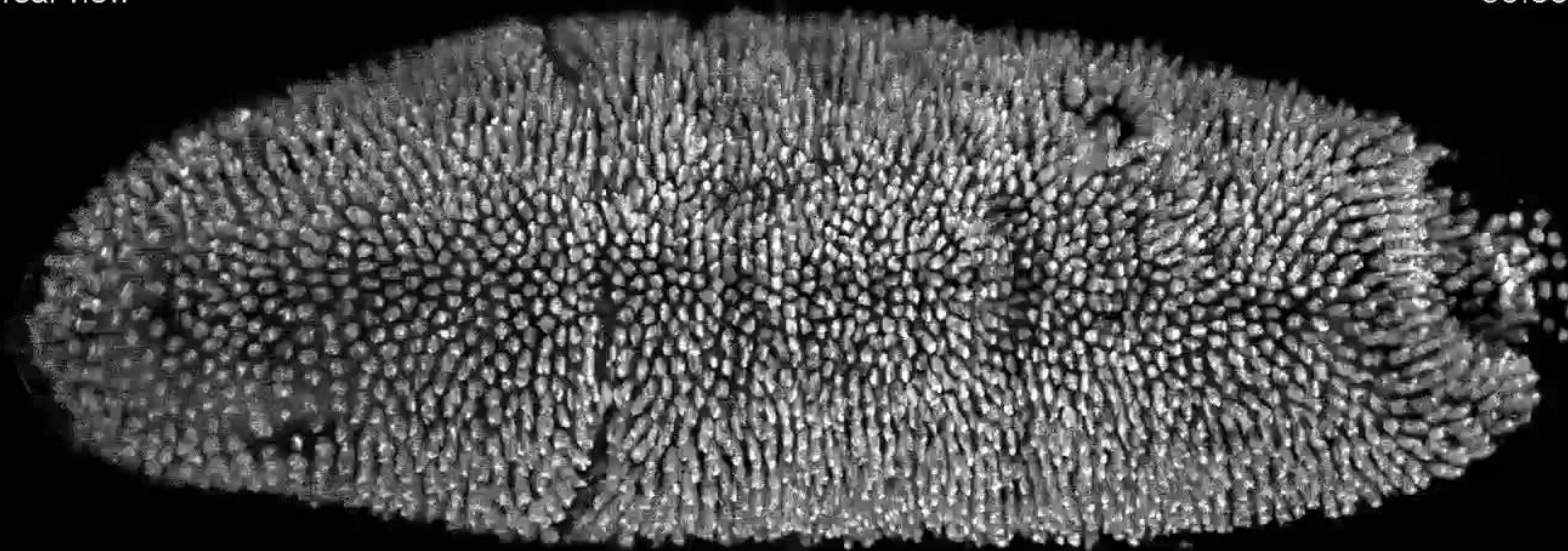
Multi-color SIMView imaging of brainbow-labeled zebrafish embryo at 3dpf; brainbow line developed by Schier lab (Harvard)



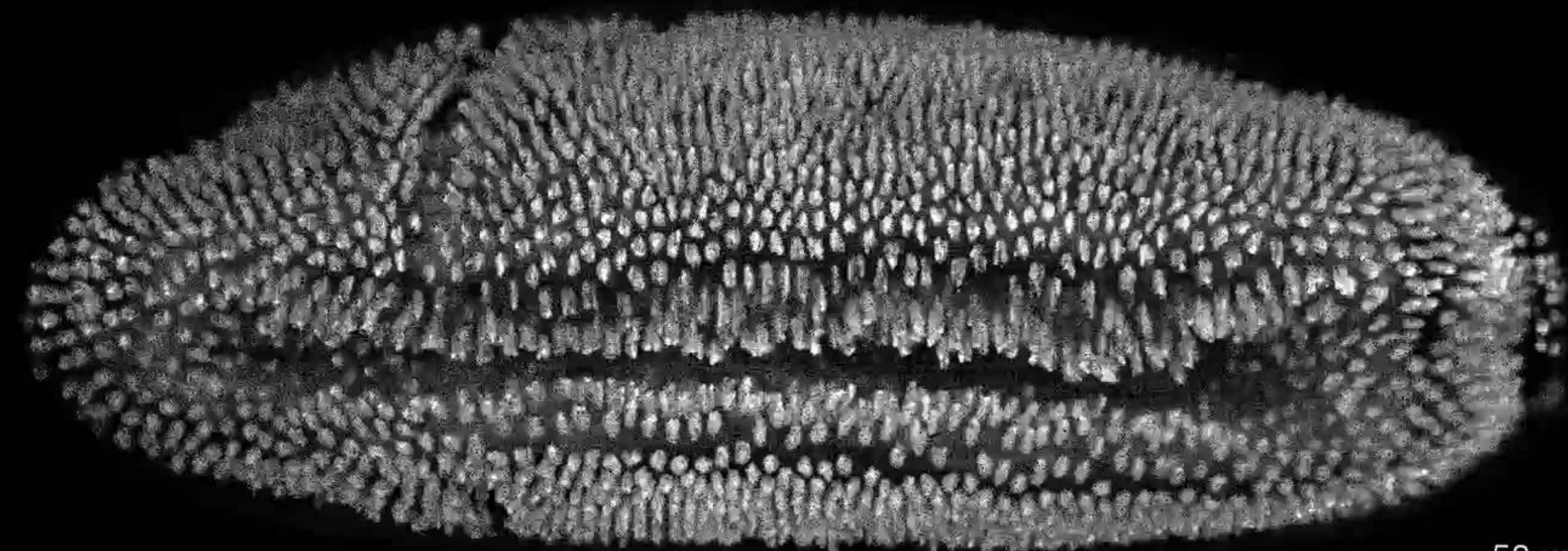
Quantitative high-speed imaging of development

dorsal view

03:00:00

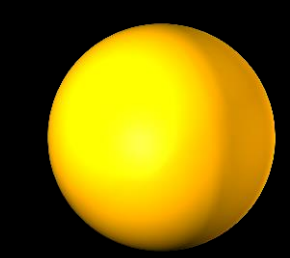


ventral view



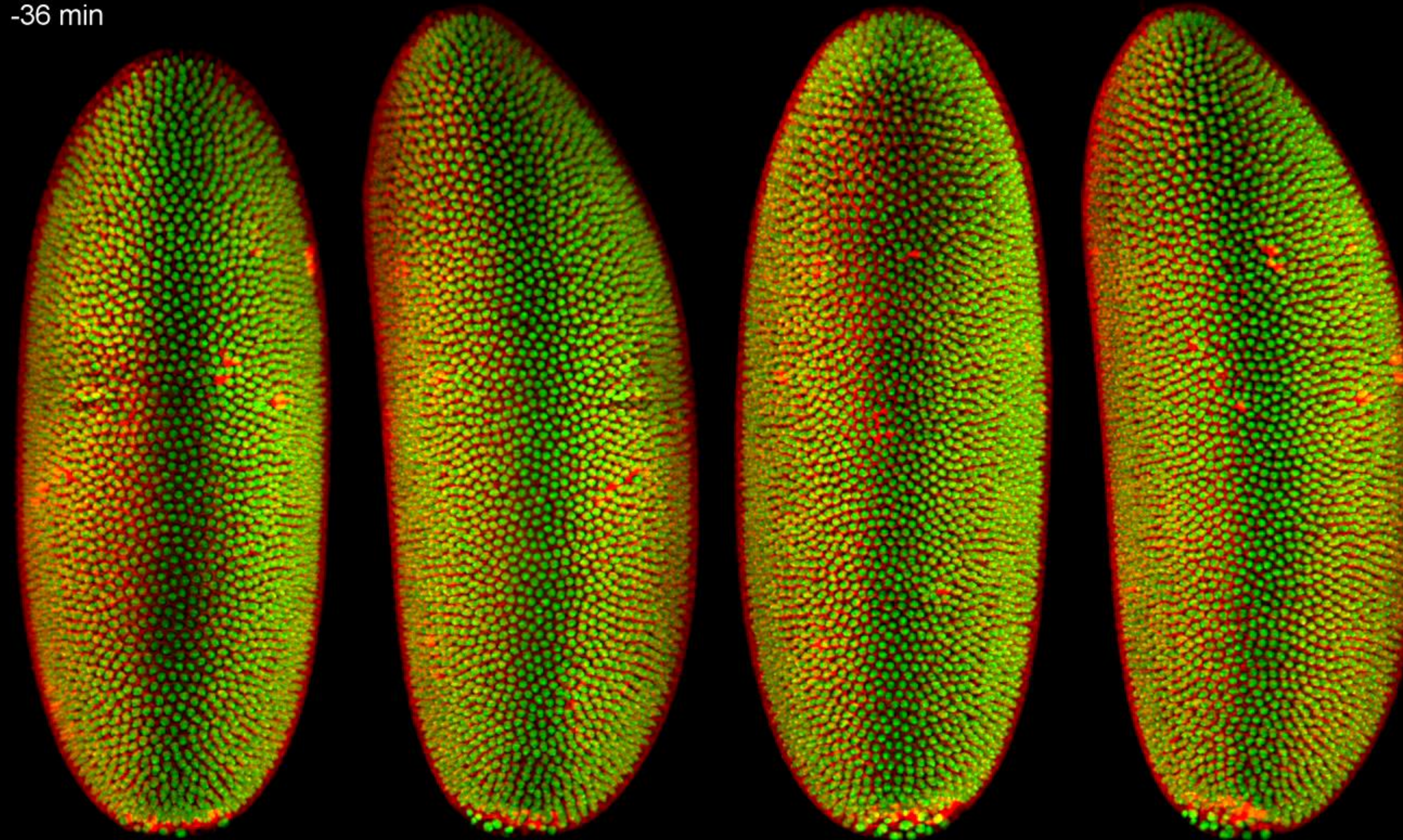
50 μ m

Nuclei-labeled Drosophila embryo, 30 second intervals for 17 hours; 1,100,000 high-resolution images (11 terabytes)



Quantitative high-speed imaging of development

-36 min



0 min = onset of gastrulation

50 μ m



Multi-color multi-angle SiMView imaging of gastrulating *Drosophila* embryo at 3 hpf (H2A-RFP,spider-GFP)

Thank you for your attention!

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