

Live-cell imaging of astrocytic reactivity adaptations under space conditions using FLUMIAS-ISS

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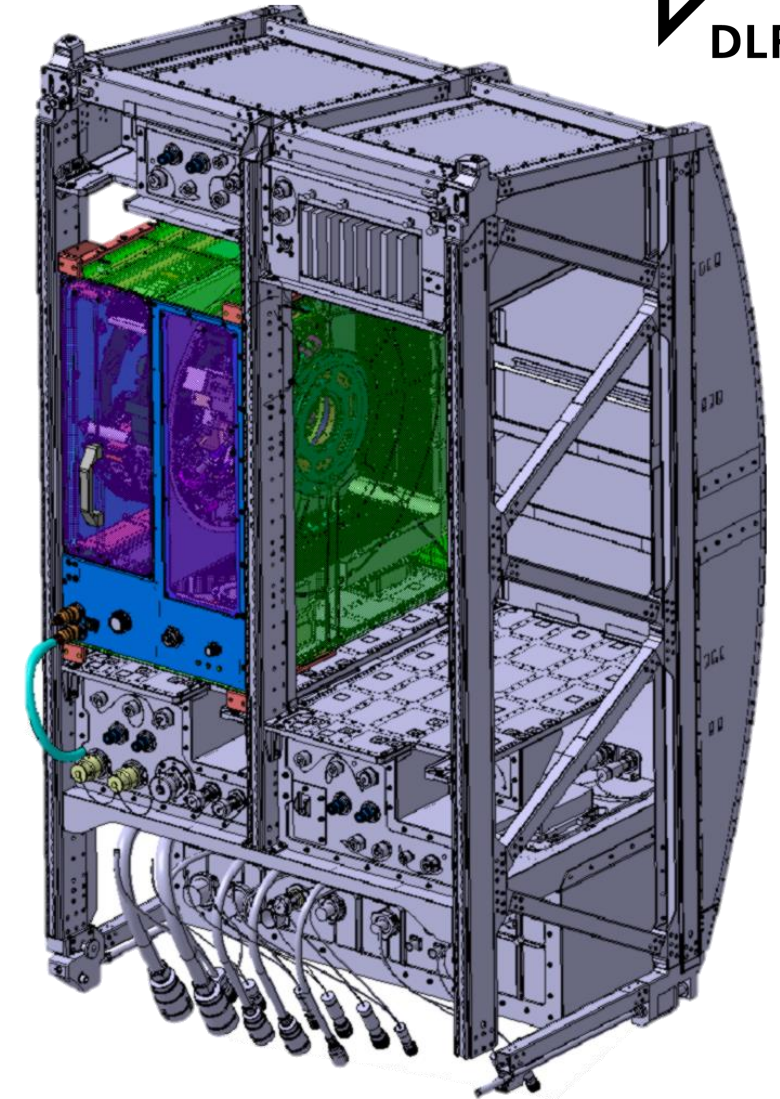
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DLR Aerospace Medicine



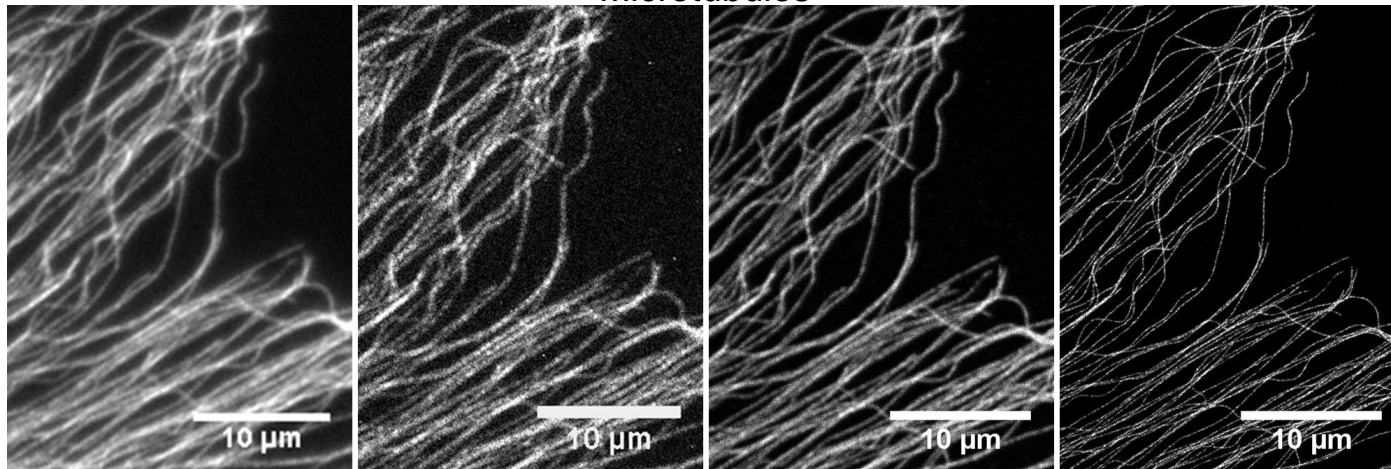
FLUMIAS aka. Live Cell Imaging (LCI)



- Live-cell fluorescence microscope on ISS
- Built by Airbus for DLR Space Agency
- Centrifugation from μg to 1g
- Included life support (medium, pumps, heating)
- Confocal-like SIM microscopy



Microtubules



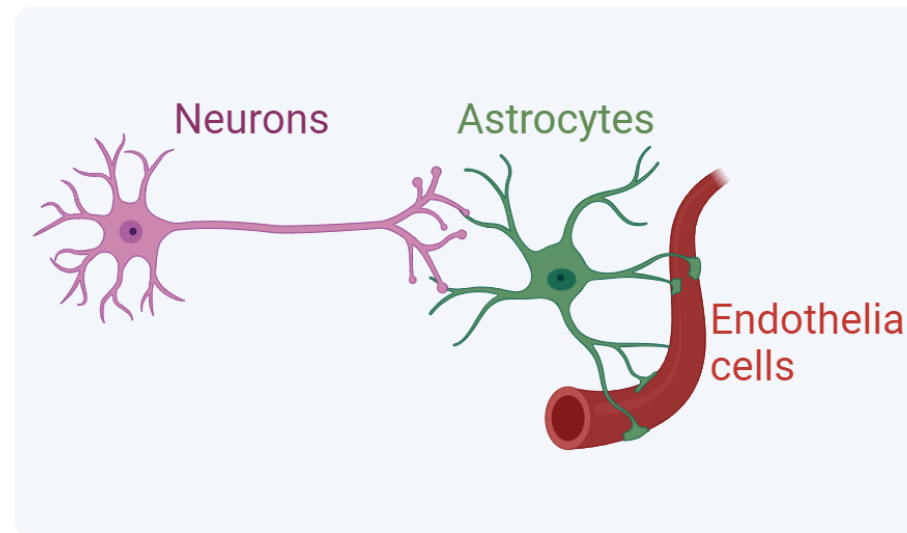
Epi 40x Oil

LCI 40x Air

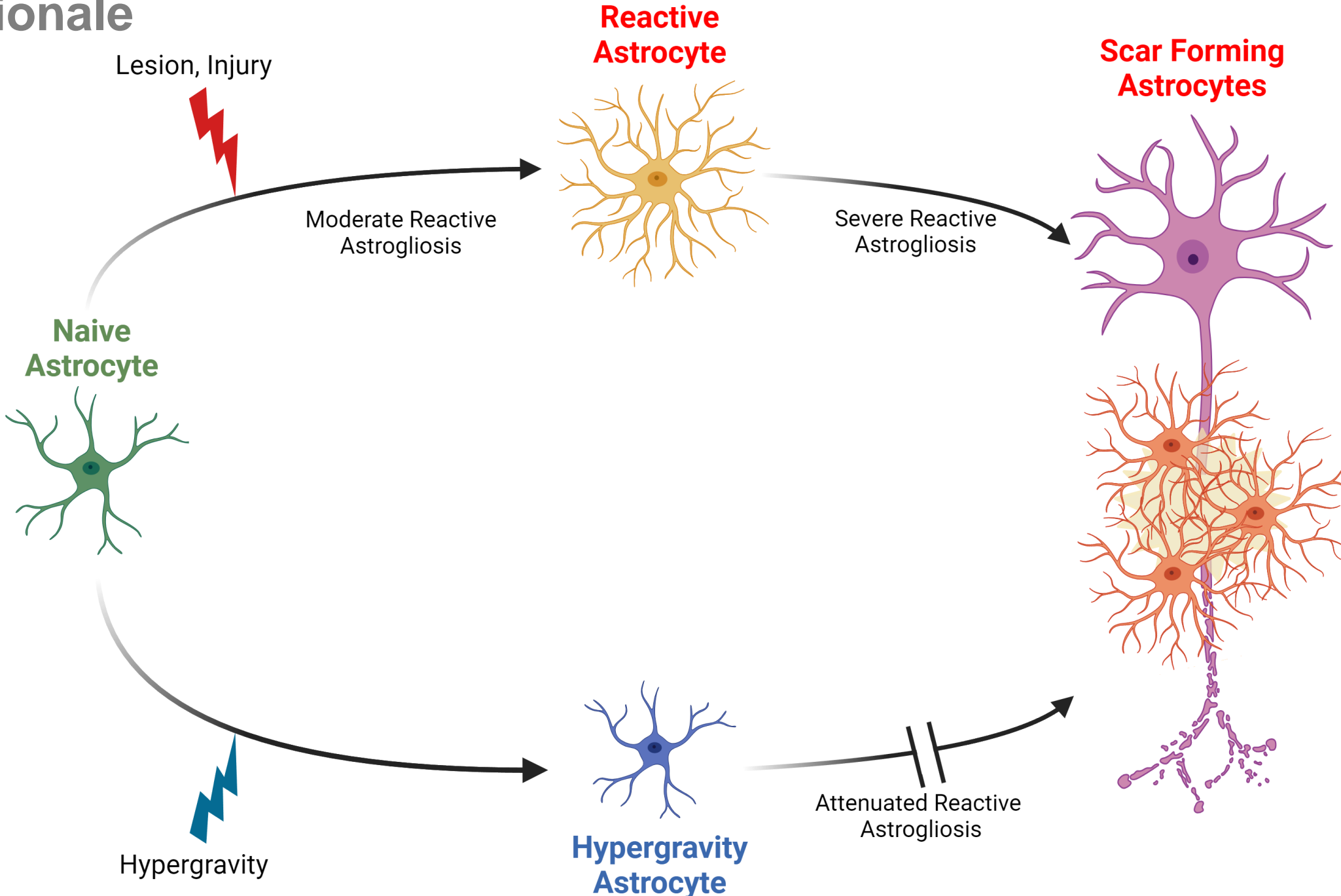
LSM 60x Oil

STED 60x Oil

Astrocyte reactivity and astrogliosis

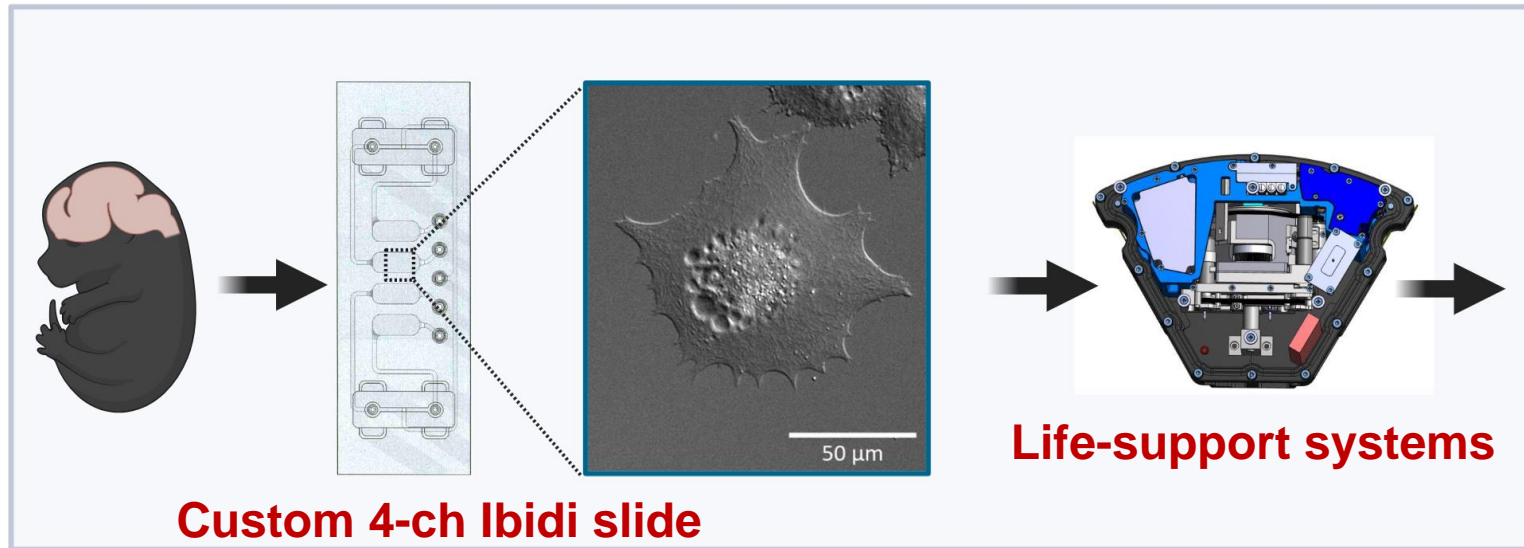


Rationale



Procedures and Caveats

Launch Site Activities

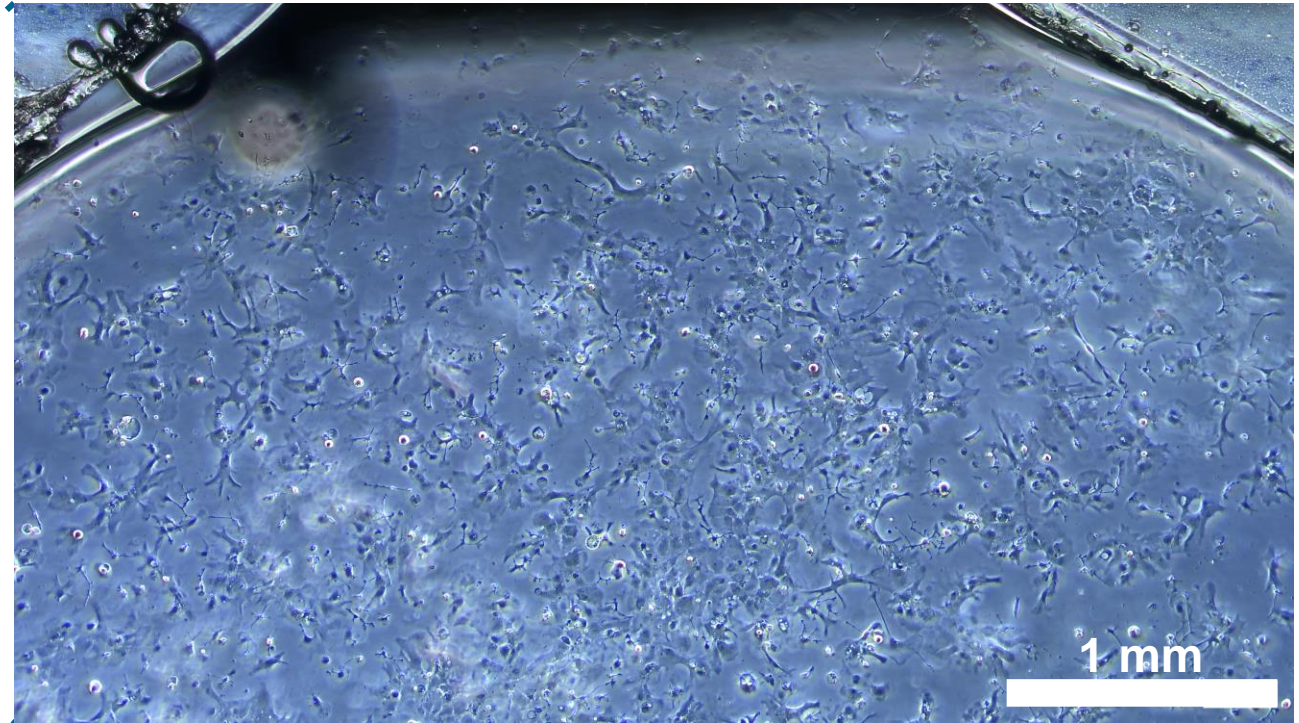
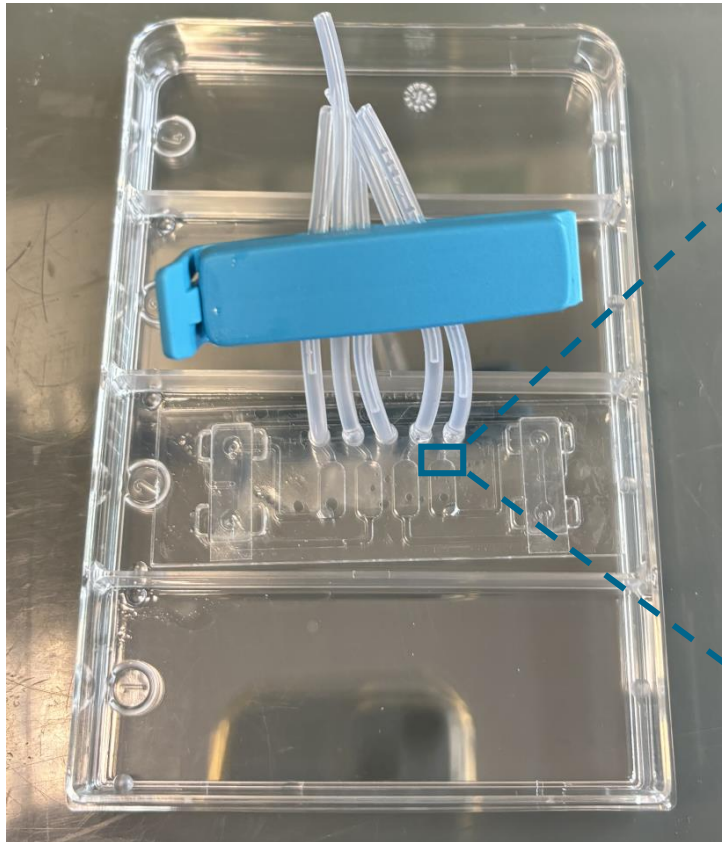


General and Experiment Specific Tests



Number	Name	Parameter	Adjustments [range]
1	Specimen adherence	% of adherent specimens	incubation time, pump speed [$<70\mu\text{L}/\text{min}$ - TBD], slide material [ibitreat/glass]
2	LSS biocompatibility	Specimen viability	slide material [ibitreat/glass] tubing [silicone, TBD]
3	Slide coating	Specimen adherence, viability	slide material [ibitreat/glass] coating [PLL, gelatine, laminin, TBD]
4	Pump program	Specimen adherence, viability	pump speed [$<70\mu\text{L}/\text{min}$ - TBD] frequency [continuous, pulsed]
5	Air bubbles	Amount/size of air bubbles in slide	pump speed [$<70\mu\text{L}/\text{min}$ - TBD] frequency [continuous, pulsed], degassing
6	Specimen growth	Growth speed	medium supply
7	Staining solution stability	Staining efficiency, fluorescence intensity	2°C warmer than targeted slide temperature
8	Upload temperature	Specimen viability	temperature range and fluctuations [20°C - 37°C]
9	Upload g forces	Specimen viability, adherence	vibrations, hypergravity profile of upload [vibration TBD, hypergravity $<4g$]
10	pH stability	pH value of growth medium	pH buffering, CO_2 filling
11	EB humidity	relative air humidity	different gas filling humidity values [80% - 95% TBC]
12	Laser intensity	fluorescence signal quality	laser power settings [1% - X%]
13	SIM image averaging number	fluorescence signal quality	number of line scan images [multiples of 1 line scan image in ms (minimum 7 images)]
14	Fluorophore/staining bleaching	fluorescence signal intensity over time	laser power, SIM image averaging number
15	Stage move speed x/y	required tile image time	tile image size [$\mu\text{m}/\text{FoVs}$] stage move speed [TBD $\mu\text{m}/\text{s}$]
16	Focus depth	acceptable focus depth for z-stack	z-stack focus range [$10\mu\text{m}$ - TBD μm]
17	Focus stability / autofocus	focus stability for tile images, autofocus	x/y moving range
18	Z stack step size	Over/undersampling of Z axis, time	z-stack step size [nm]
19	Image frame rate	optimal frame rate to image features of interest	SIM mode or line scan mode, number of fluorescence channels
20	Fluorescence bleed-through	custom multi bandpass emission filter	choose fluorophores according to filter
	etc...		

Ibidi FLUMIAS 4-Channel Slide



- Glass slides coated with PLL allowed good cell attachment and growth
- Handling can be improved (leaking, bubbles)

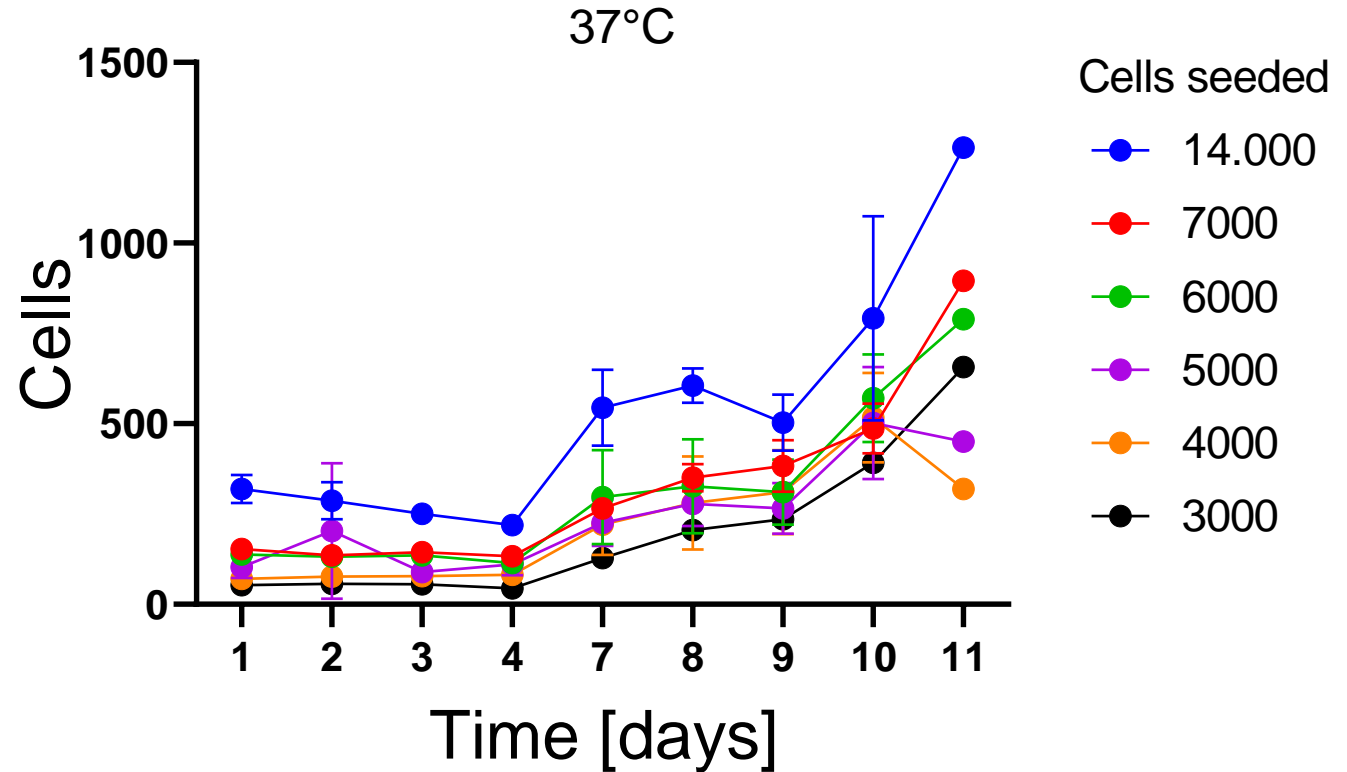
Optimize Seeding Density for Worst / Best Upload Scenario



Time until EB integration into FLUMIAS

Best: 1-2 days

Worst: 7+ days



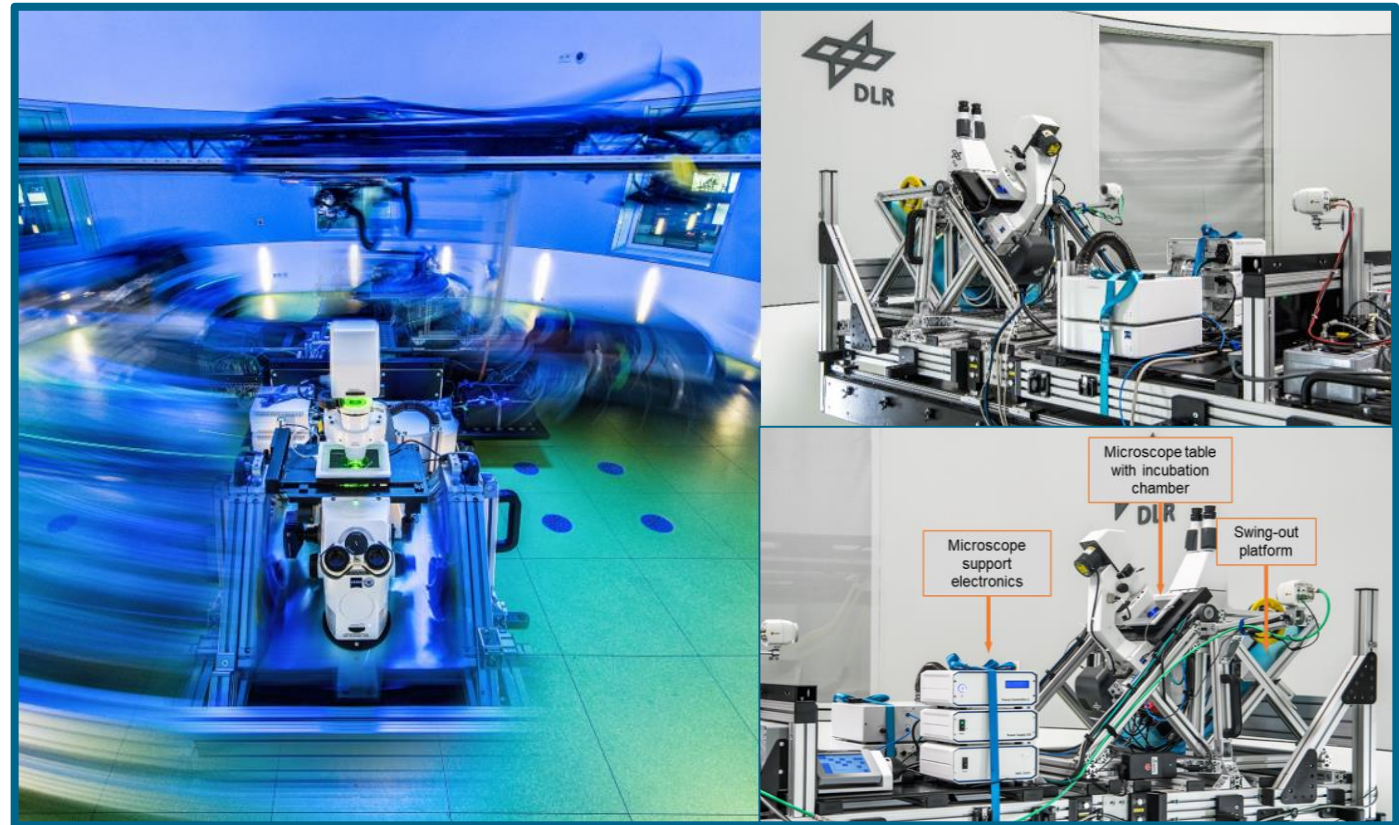
- Repeat assay at 20°C and 30°C (no electrical heating during upload)
- Account for limited medium refresh (only sporadic pumping)

Upload Hypergravity Control

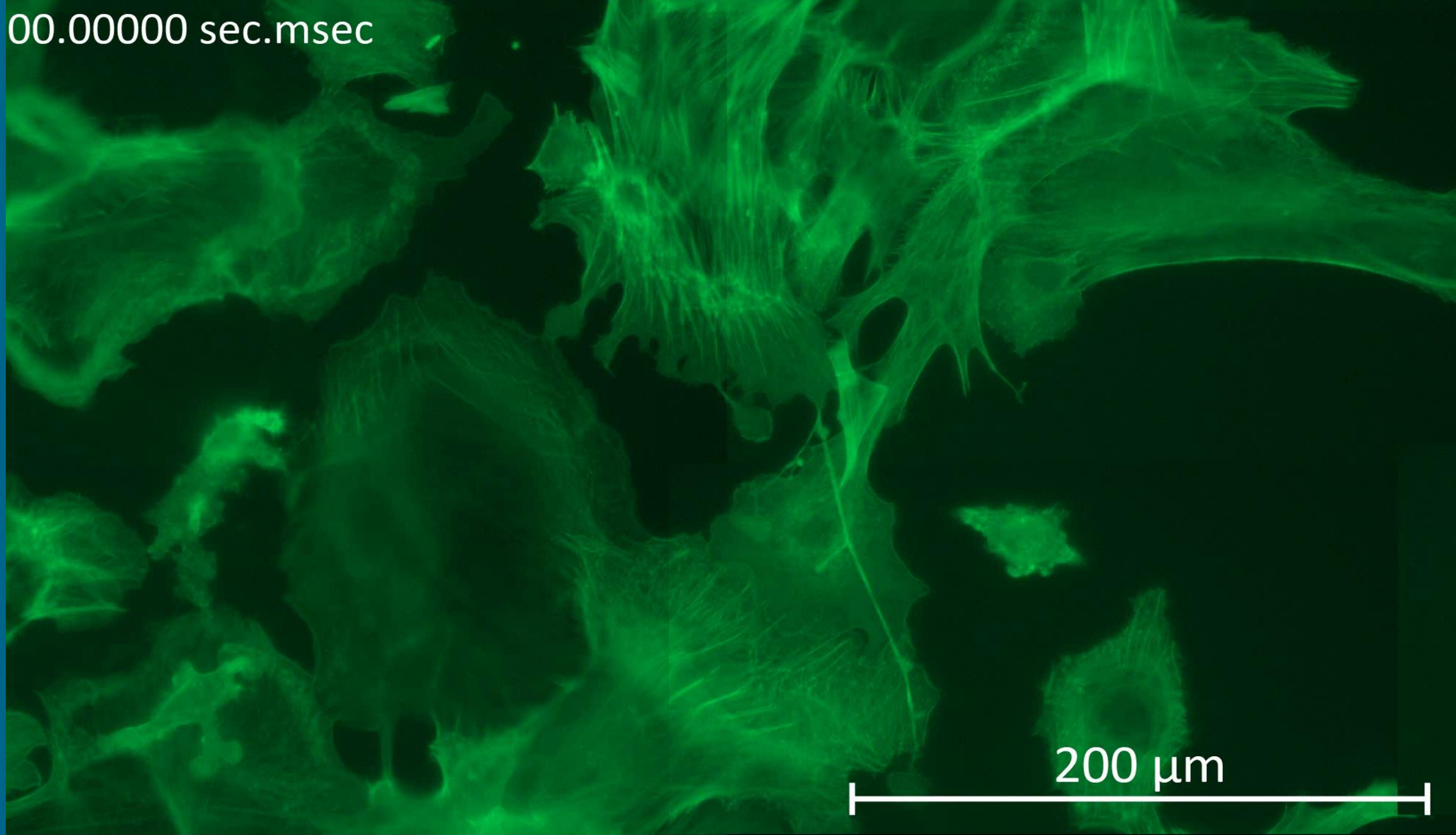
Multi-Sample Incubator Centrifuge



Centrifuge Microscope (Hyperscope)



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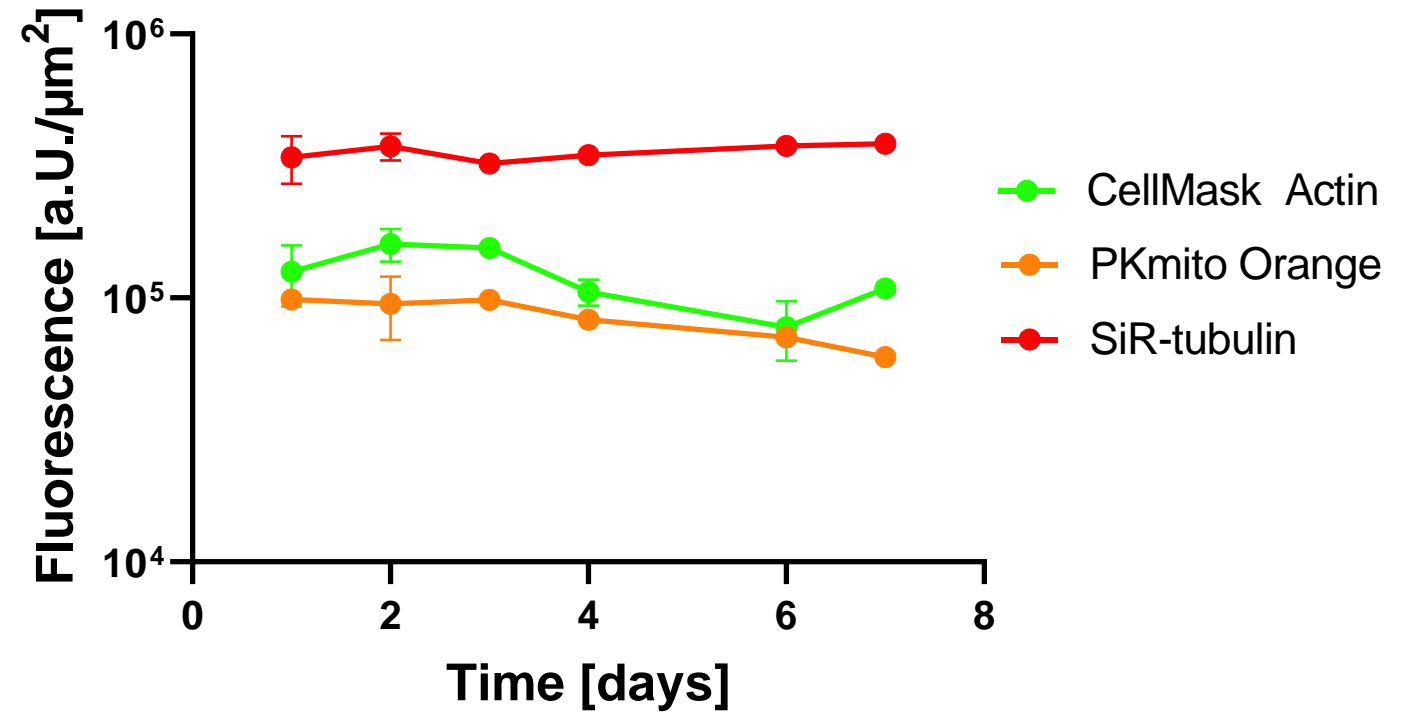
200 μm



Staining Solution Stability at 37°C

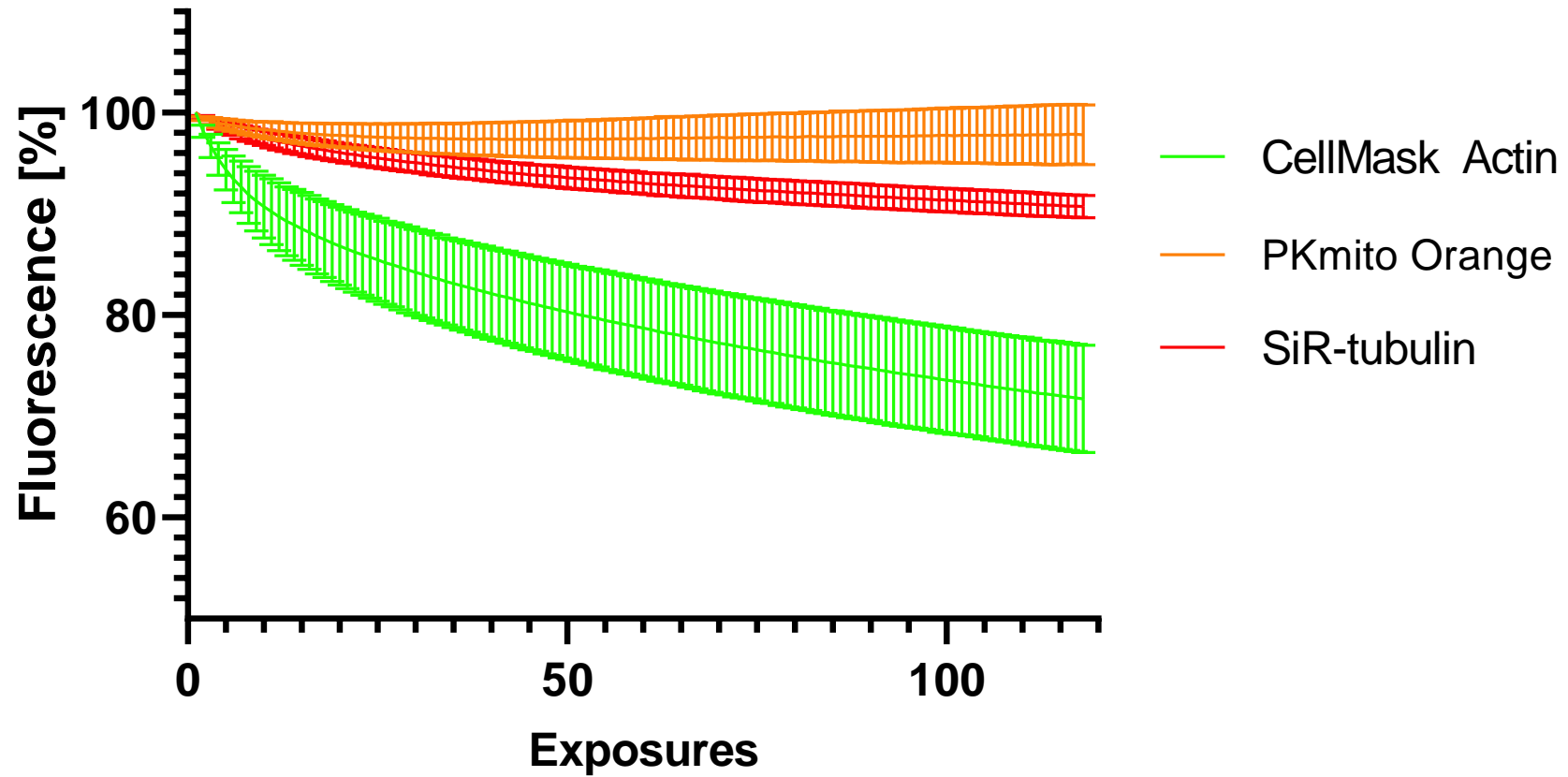


Staining / medium reservoirs in 37°C
Fluorescence intensity after incubation

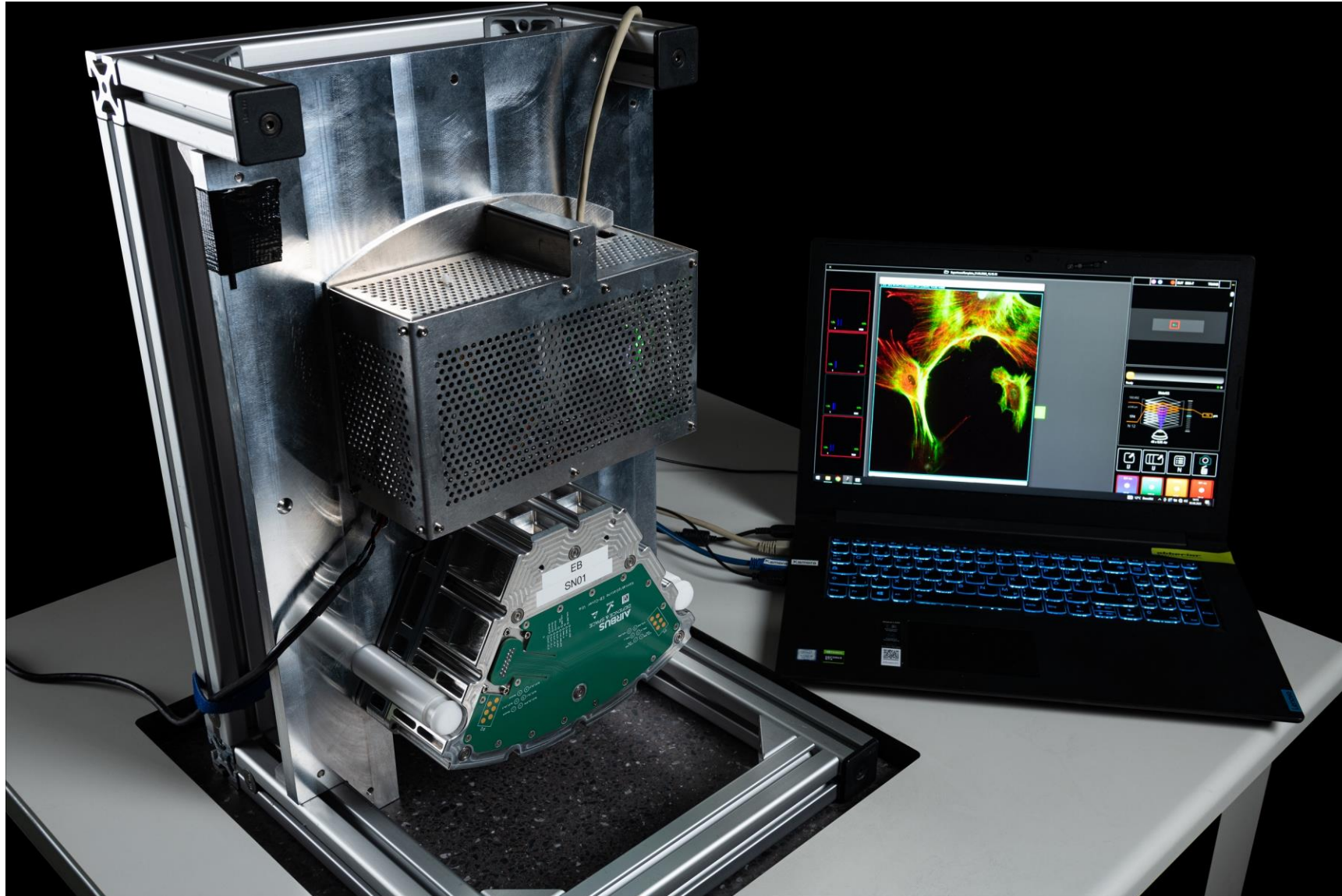


- Staining solution stable for several days at 37°C
- >50% fluorescence remaining after 3 months at 37°C

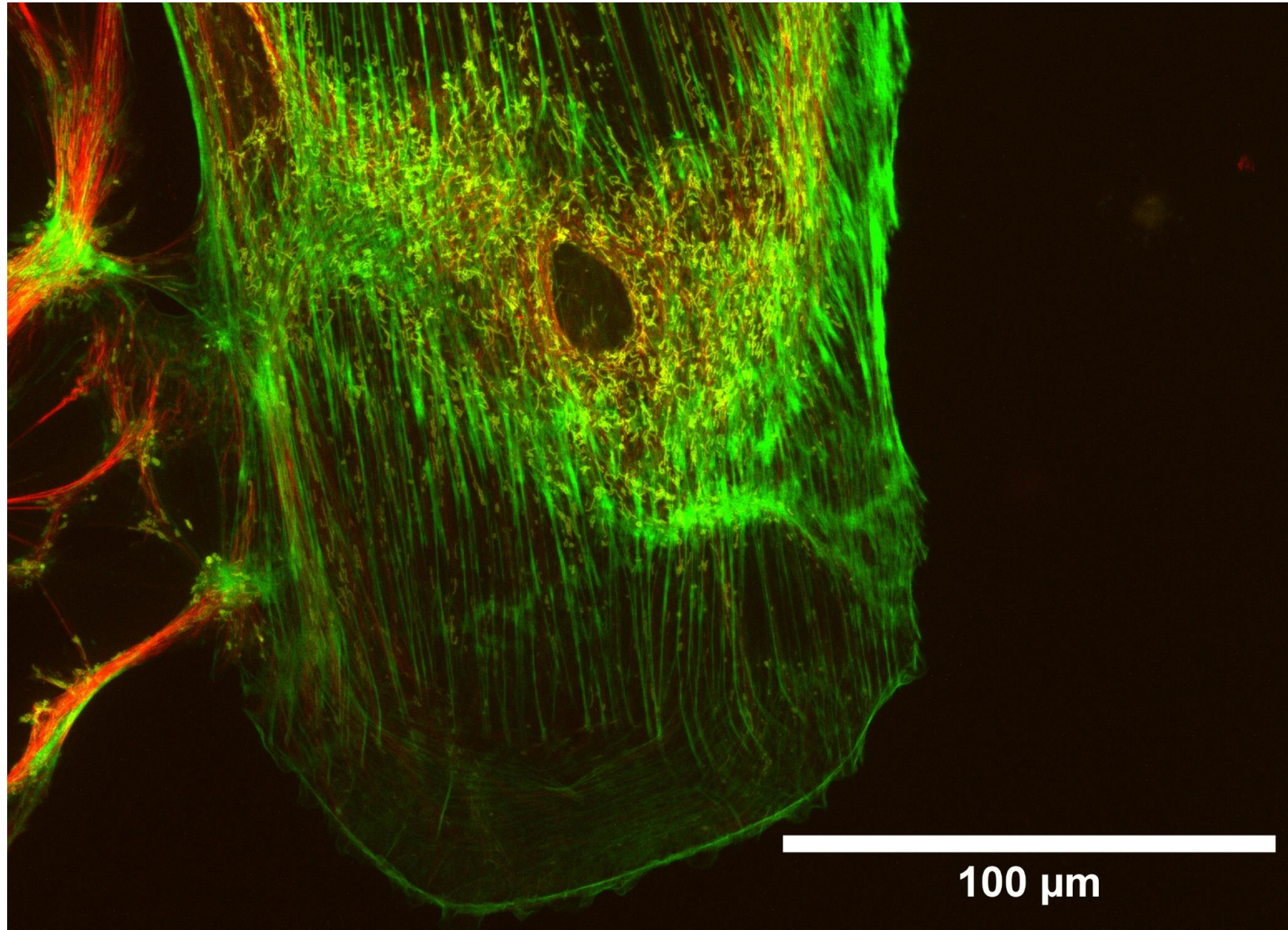
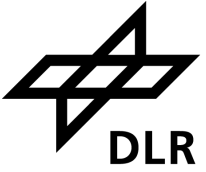
Bleaching



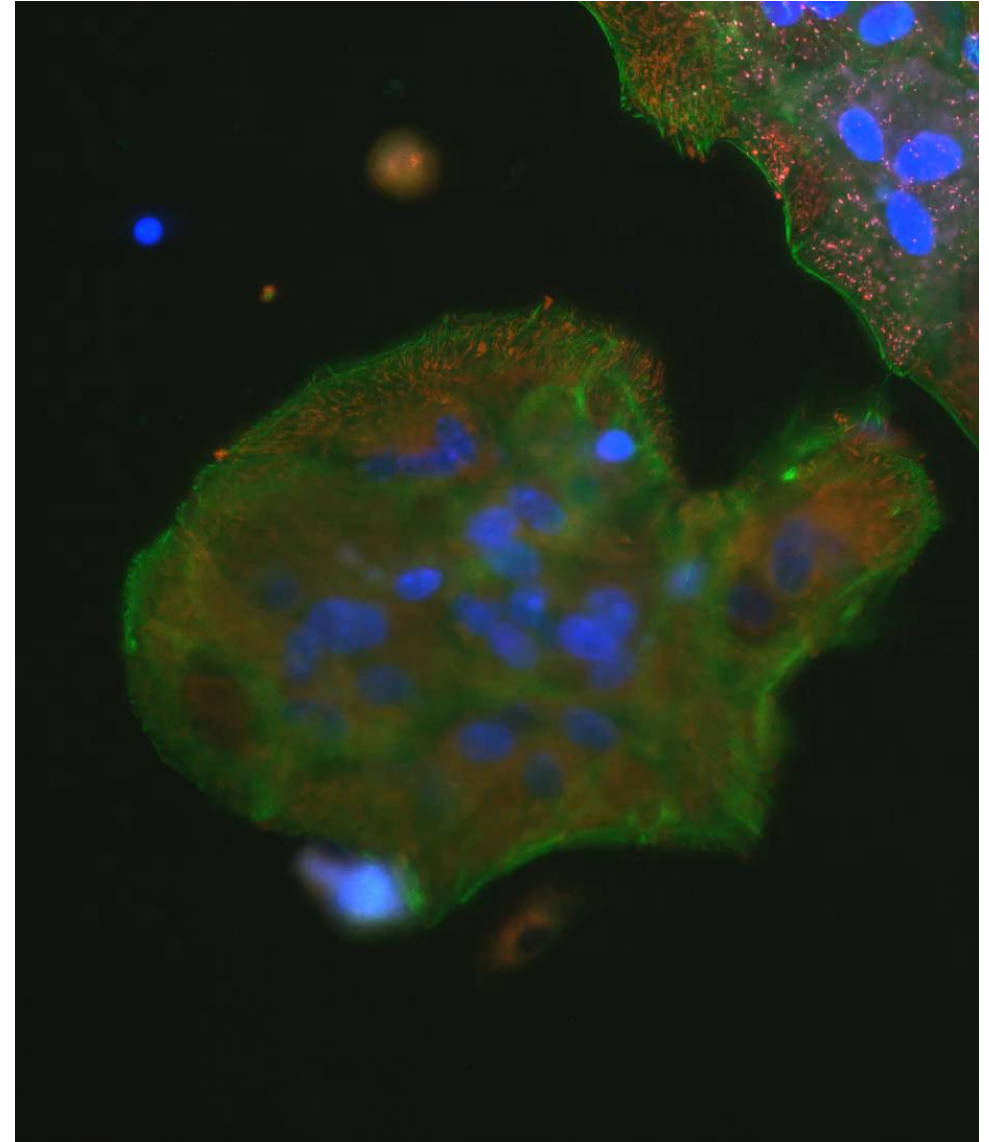
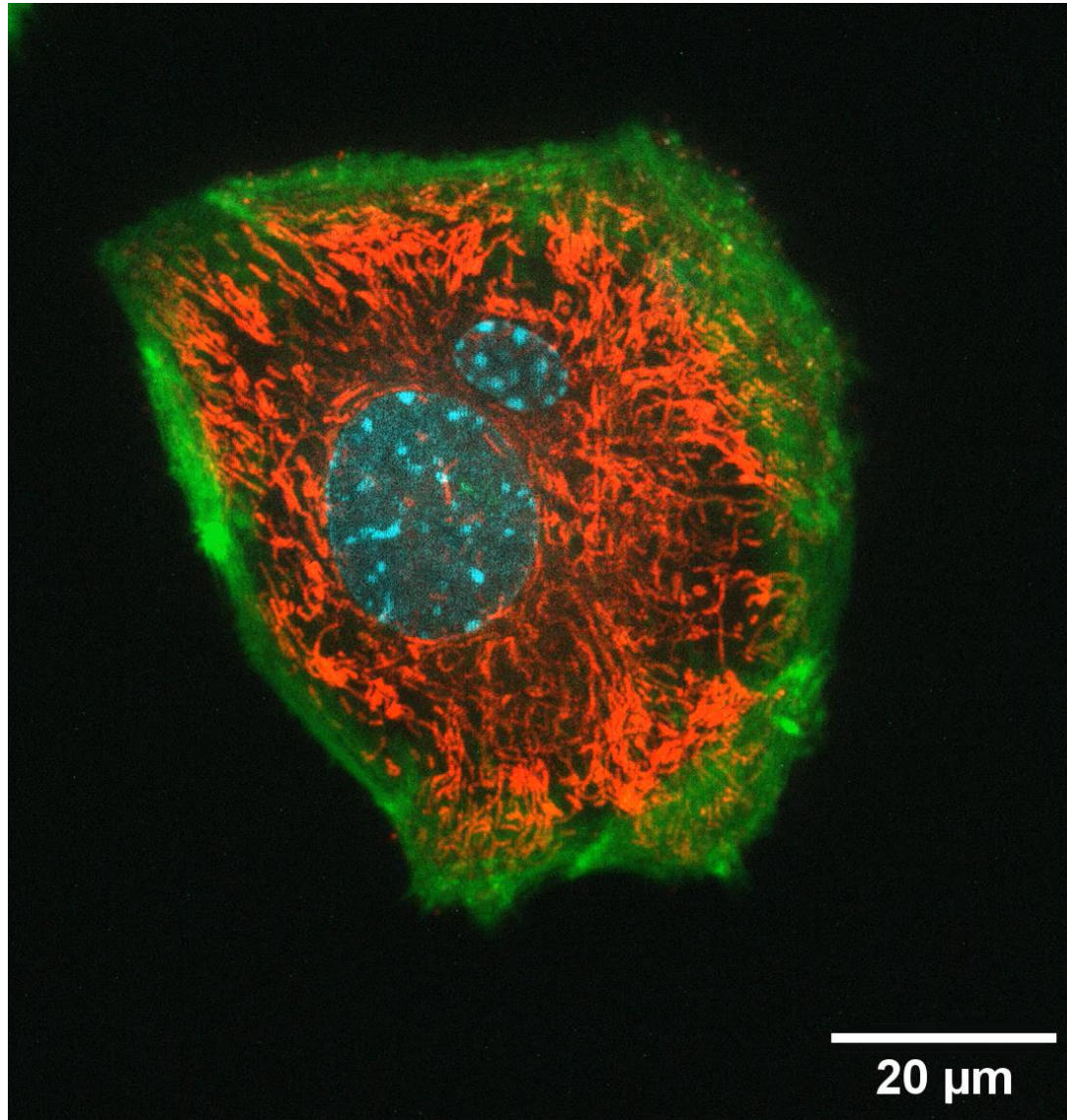
Imaging Procedure Optimization at FLUMIAS SRM



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Acknowledgements



Lisa Mühlbeyer

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Sources



Ibidi 4 Channel Slide: Ibidi

FLUMIAS CAD Model: Airbus / DLR Space Agency

SpaceX Falcon Launch / Dragon Coasting: Wikimedia

Graphics done with Biorender