

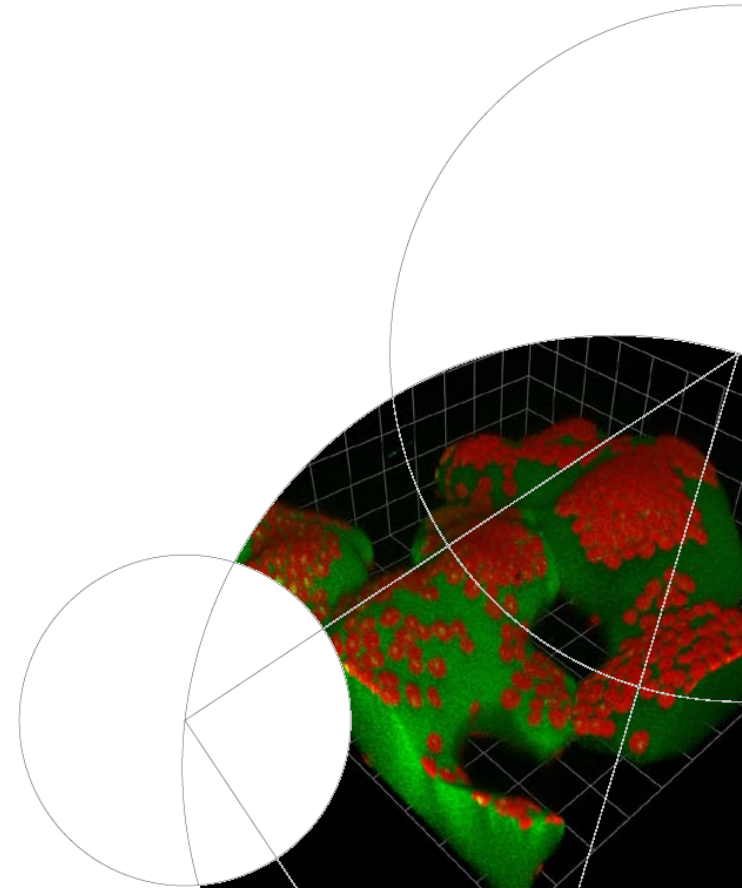
Department of Plant Biology and Biotechnology



High Speed 4D Bioimaging

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Why we use Confocal Laser-Scanning Microscopes:

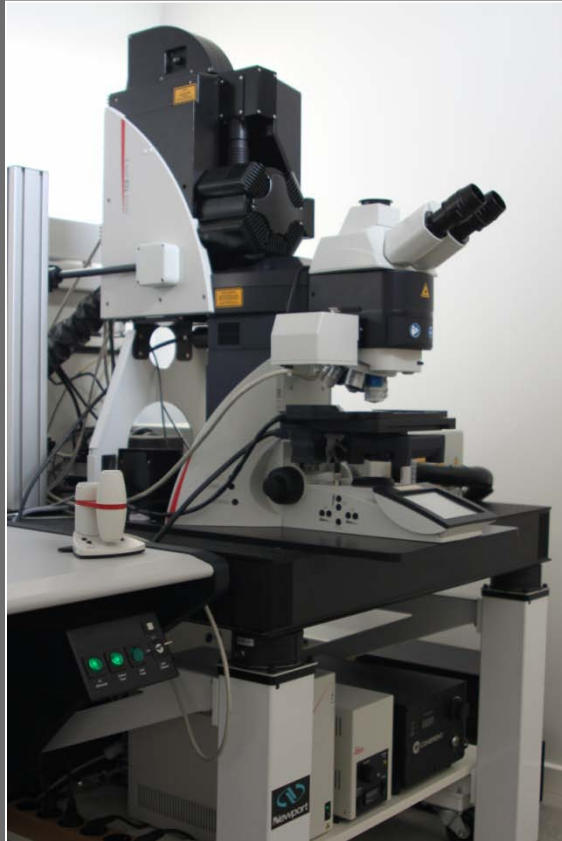
High contrast and high resolution even deep inside specimen

through ...single-point excitation

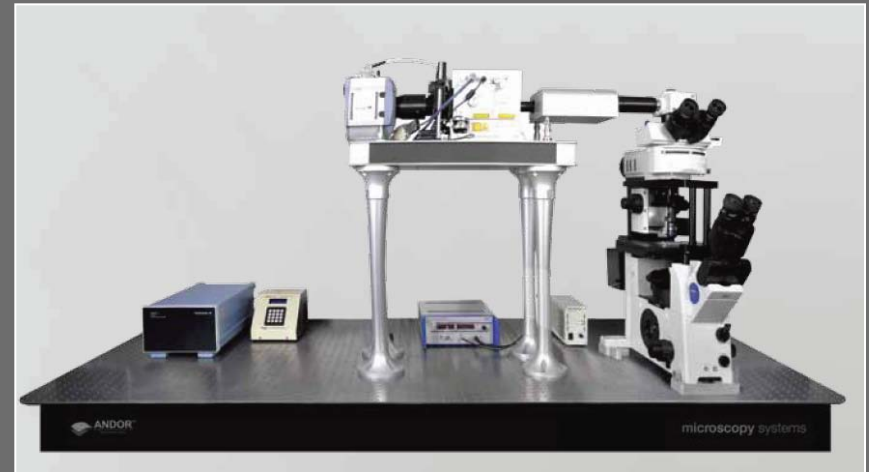
...elimination of out-of-focus light



The Microscopes



Leica SP5-X with resonant scanner

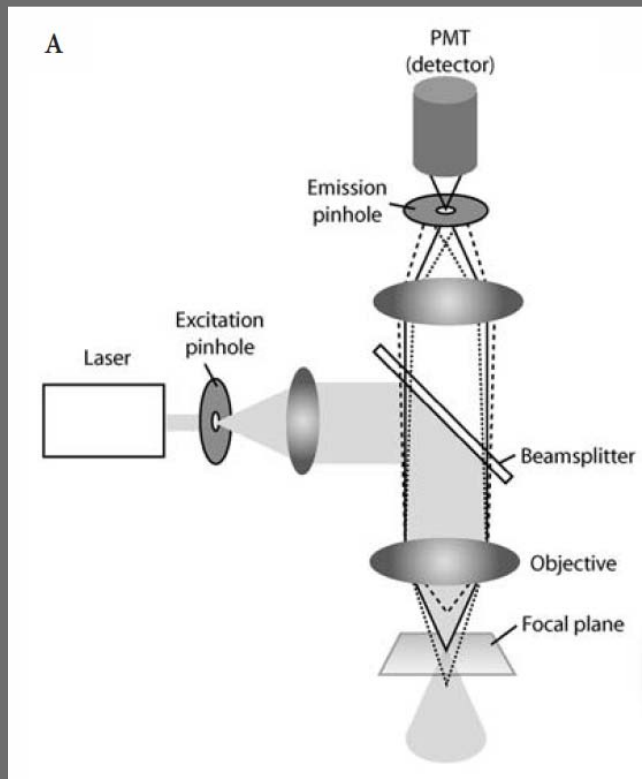


Andor Revolution XD Spinning Disk
Confocal

High Speed: the principle

Resonant scanner

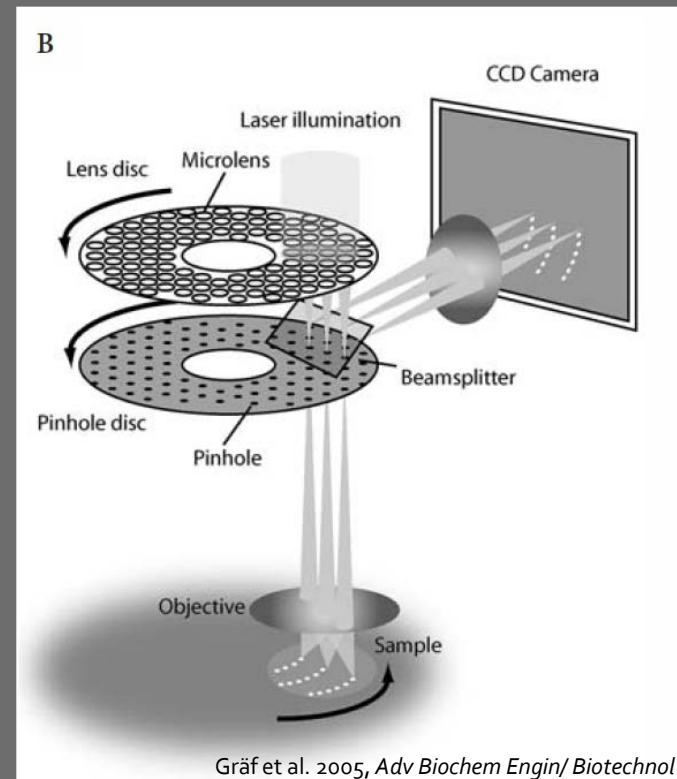
Beam path in conventional CLSM



Scan mirror moves 20x faster

25 fps

Spinning disc



Gräf et al. 2005, *Adv Biochem Engin/ Biotechnol*

Parallelized scanning with multiple beams

50 fps



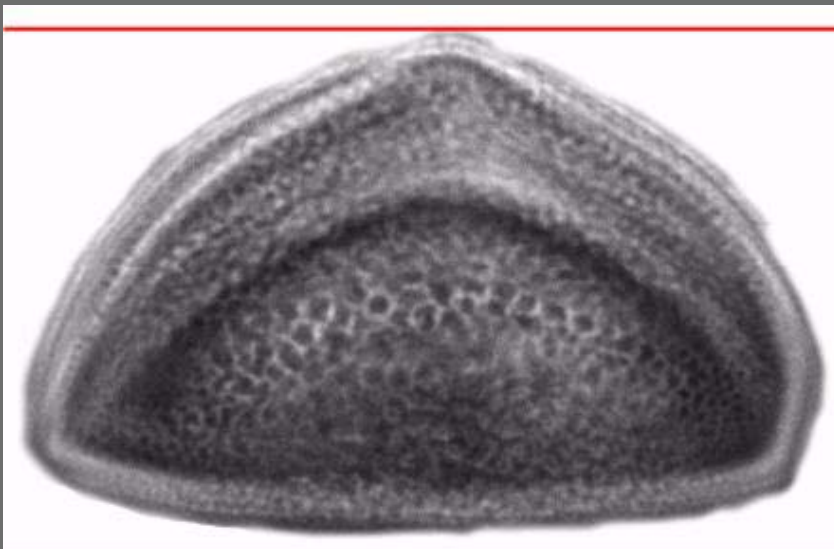
Head-to-head comparison

	Resonant scanner	Spinning disc
Flexibility	+	-
Photobleaching	-	+
Sensitivity	-	+



Confocal imaging in 3D

Object: Pollen grain



— Focus plane



Maximum intensity projection

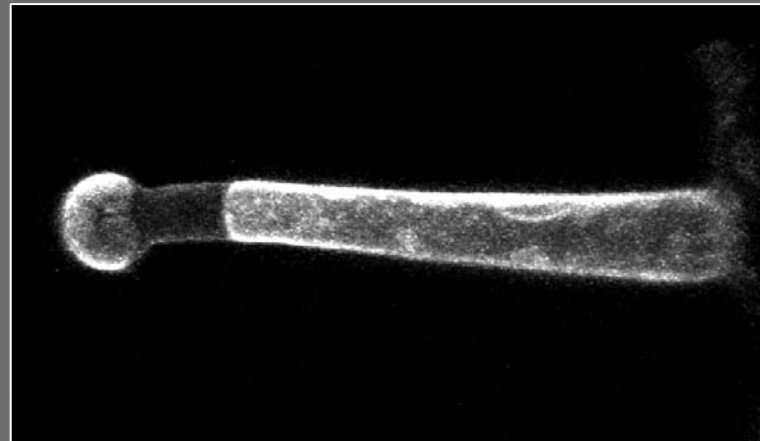
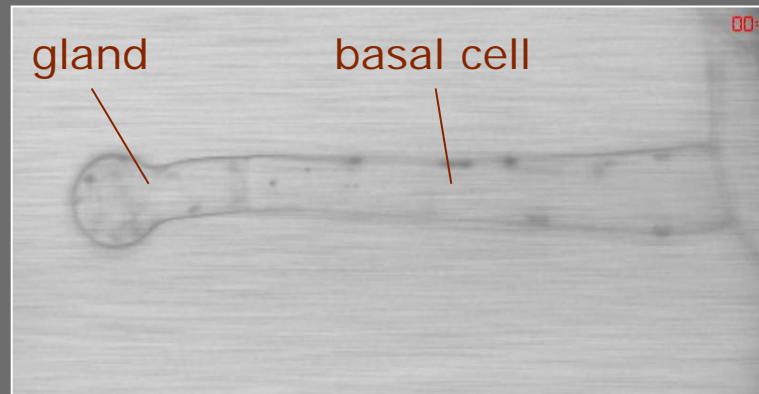


4D fluorescence redistribution

FRAP = Fluorescence Redistribution After Photobleaching

Aim: Quantify intra- and intercellular connectivity

Object: Tobacco leaf trichome



Endoplasmic reticulum stained with DiOC₆

4D fluorescence redistribution

ER stained with DiOC₆



Maximum intensity projection

pre-bleach



bleaching



post-bleach



Image analysis

Velocity - [2011-02-22 DiOC reso 1.mv42]

File Edit Actions Tools Library Measurements Window Help

XYZ

Library

- 2011-02-22 DiOC reso 1.JF
 - Series002 (spliced)
 - 512 x 256 x 46 x 1517 x 20 (1.7 GB)
 - Series002 (spliced) (cropped)
 - 481 x 206 x 46 x 1517 x 20 (1.2 GB)
 - target
 - no bleach target half
 - neighbor

Image Sequence Measurements Colocalization Notes

Find Objects Using % Intensity

Channel: 1

Lower: 10

Upper: 100

Exclude Objects by Size

Exclude objects: < 2 μm^2

00:02:35.705

10,00 μm 20,00 μm

Findings

- Find Objects
- Find Objects in ROIs
- Find Spots
- Processing
 - Close Objects
 - Dilate Objects
 - Erode Objects
 - Fill Holes in Objects
 - Open Objects
 - Remove Noise From Objects
 - Separate Touching Objects
- Filtering
 - Clip Objects to ROIs
 - Exclude Objects by Shape
 - Exclude Objects by Size
 - Exclude Objects Not Touching ROIs
 - Exclude Objects Touching Edge of Image
- Combining
 - Combine Objects
 - Exclude Non-Touching Objects
 - Link via Touching Objects

Measurements Histogram

Filter: All

Name	ID	Color	Min (1)	Max (1)	Mean (1)	Sum (1)	Standard ... (1)	Min (2)	Max (2)	Mean (2)	Sum (2)
Object 1	1	Blue	26	80	39,741935	1232	13,161448	195	212	202,258...	6270
Object 2	2	Green	26	75	41,4	1035	12,155657	198	222	210,58	5267
Object 3	3	Red	26	76	39,71875	2542	11,418829	138	190	162,921...	10427
Object 4	4	Cyan	26	54	38,206333	917	8,509696	181	212	197,583...	4742
Object 5	5	Magenta	26	77	40,884615	1063	14,170193	185	211	199,576...	5189
Object 6	6	Yellow	26	62	34,32	858	8,365262	172	194	186,6	4665
Object 7	7	Dark Blue	26	126	45,714286	8262	16,916003	163	202	182,595...	34503
Object 8	8	Light Blue	26	77	40,8125	1306	12,102266	202	214	209,15625	6693
Object 9	9	Red	26	66	39	1560	10,915585	204	227	217,55	8702
Object 10	10	Cyan	27	80	39,26087	903	13,191622	191	219	203,913...	4690
Object 11	11	Magenta	26	73	38,682353	3288	11,597375	215	238	227,388...	19328
Object 12	12	Yellow	30	98	44,173913	1016	17,856498	201	226	212,36087	4882
Object 13	13	Dark Blue	26	57	36,625	879	8,380595	201	223	212,583...	5102
Object 14	14	Light Blue	26	80	37,253333	2794	9,589012	209	234	221,026...	16577
Object 15	15	Red	26	53	33,521739	771	7,008501	216	242	228,608...	5258
Object 16	16	Green	26	66	36,52	913	10,27276	210	223	215,4	5385
Object 17	17	Dark Blue	26	95	39,108434	3246	12,65674	178	207	193,277...	16042

00:02:35.705 T: 57

Ready

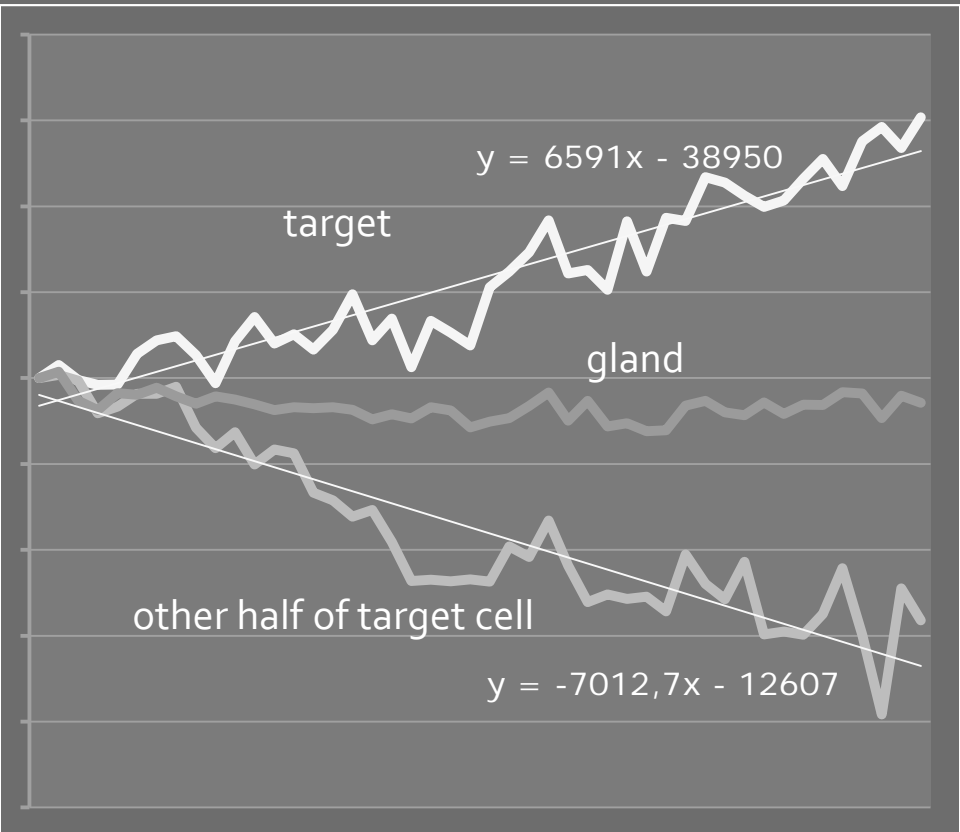
start Velocity - [2011-02-22... 2011-02-22 DiOC reso 1

Selected 0 of 46 objects

Snapshot from Velocity (PerkinElmer)



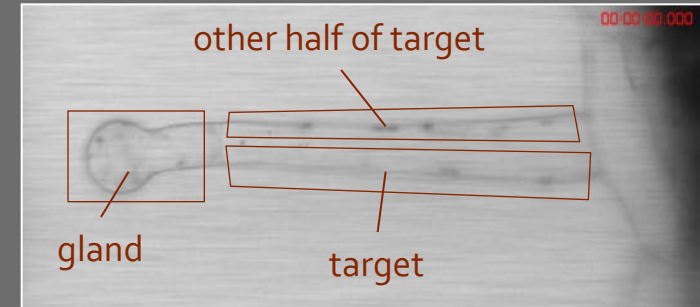
Post-bleach fluorescence redistribution



303,686

- 28,583

- 281,946



Result:
Signal recovery in target
area due to intracellular
redistribution

Summary

Resonant scanner and Spinning disc confocal increase the temporal resolution of live-cell imaging

Benefit of 4D:

Fluorescence from the whole cell can be accounted for in FRAP experiments



Acknowledgements



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@ Department of Plant Biology and
Biotechnology, KU LIFE

