

2.1 Organization of work, type of data

Why is it important to process experimental data continuously?

Avoid this approach:

„I am experimenting this year. During the next one I am going to evaluate what I got“

Types of data:

- **Numerical data** - raw data or data converted to the numerical form, may be obtained with simple tools (ruler etc.) or more complex instrumentation (spectrophotometer, etc.)
- **Graphical data** - very frequent type of data, their quantification is extremely important
- **Structural data** - analytical data, outputs from sequencing machines, mass spectrometers, etc.

2.1 Organization of work, type of data

Software used in modern laboratory:

- **Software controlling machines** - very often quite complex. For the user, the most important is the form of the output (table, text)
- **General, „office“-type software** - text and spreadsheet editors, graphical software, presentation software, typically products of Microsoft at the Charles University
- **Specialized software** - very important in the field of image analysis and processing of sequence data
- **Open source software** - very good tools, often problems with the compatibility
- **Online software** - mainly in bioinformatics, structure analysis, but there are also a plenty of software available for various office applications

2.1 Organization of work, type of data

Windows Explorer or similar software (Windows Commander)

Individual folders should be named according to their date, ideally as **YYMMDD (211104)**. The date must correspond well to the one in the laboratory book.

Never modify folder with raw data - copying to the folder with other files accompanying the experiment.

Back up data - external HDD, shared laboratory HDD drives, or online **cloud repositories**.

Do not postpone backuping!!!

Experiment must be always finished with **complete documentation**, do not start the new one **without finishing the previous**.

2.2 Data explorers, correct data handling and saving

Windows Explorer

The screenshot shows a Windows Explorer window titled '050420'. The left pane displays a folder tree with the following structure:

- Protokoly
 - 2002
 - 2003
 - 2004
 - 2005
 - kvasinky
 - MDRs
 - PBA
 - PINs**
 - 050302
 - 050308
 - 050316
 - 050317
 - 050323
 - 050406
 - 050420
 - 050614
 - 050624
 - AdrianakvanifikaceGE
 - materiály pro alignme

The right pane shows a table of files in the '050420' folder:

Název	Velikost	Typ	Změněno
2005 04 20 PIN7 _ 3H IAA vs NPA a BFA .JNB	312 kB	SigmaPlot 9.0 Note...	22.4.2005 13:54
2005 04 20 PIN7 _ 3H IAA vs NPA a BFA .xls	135 kB	List aplikace Microso...	21.4.2005 18:14
050420.doc	30 kB	Dokument aplikace ...	20.4.2005 16:02

2.2 Data explorers, correct data handling and saving

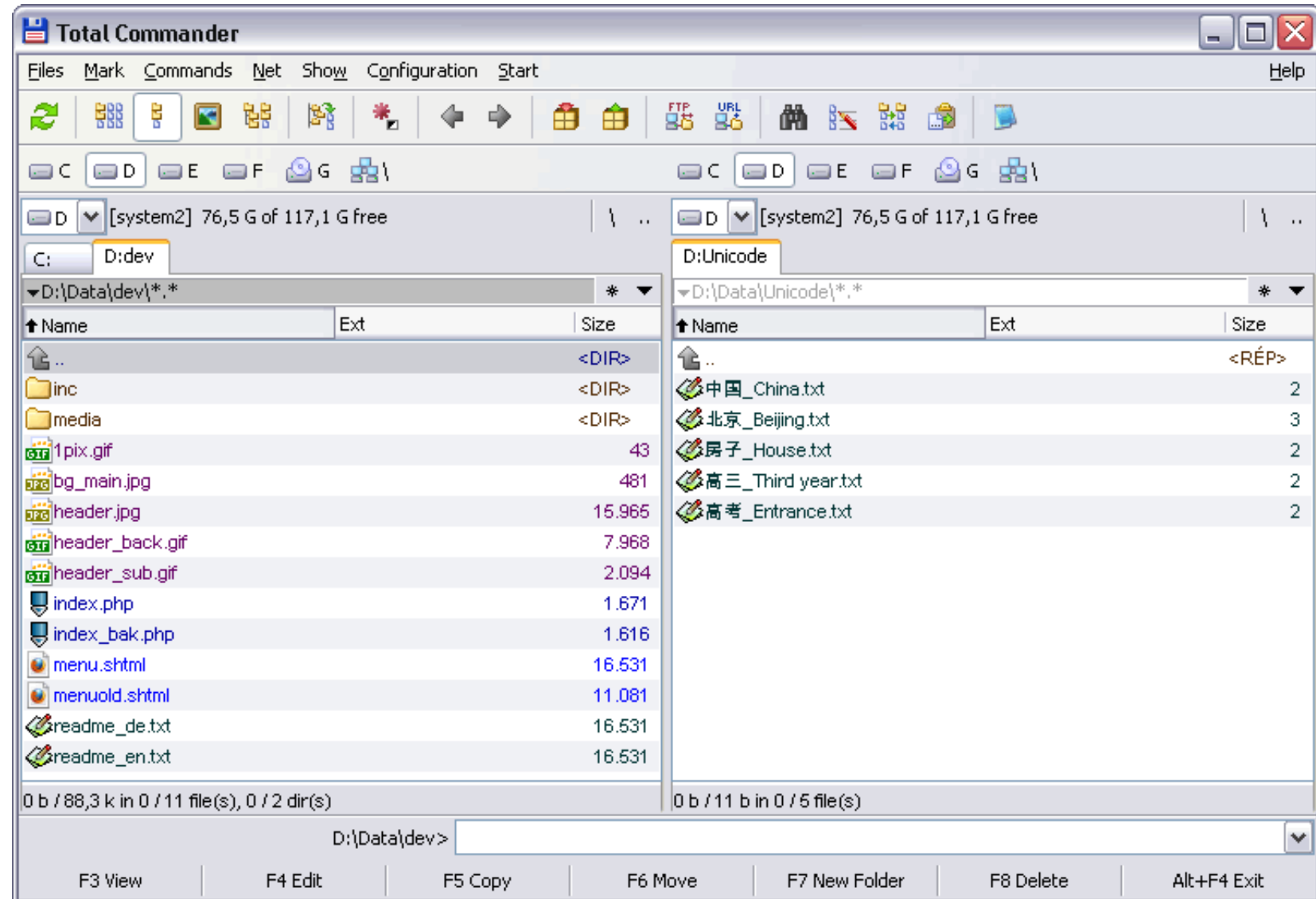
070420_PIN1GFP_ACC_AVG	070420-0h	
070423_PIN1GFP_pINTAMPINGFP_	070422-48h	
070424_PIN1GFP_ACCxAVG	070423-72h	
070427_PIN1-GFP_DR5GFP_AVG_2	070420.doc	26 kB
070503_PIN1-GFP_AVG_ACC_STS	report070420.cdr	17 158 kB
070504_PIN1-GFP_SN_ACC_AVG_s	report070420.pdf	1 066 kB
070509_PIN1-GFP_ACC_AVG_exp	Záloha_report070420.cdr	17 158 kB
070510_DR5-GFP_ACC_AVG_stac		
070510_PIN1-GFP_DR5GFP_MCP		
070514_PIN1-GFP_Et_gasing_1ppn		
070605_PIN1-GFP_ACCxAVG		
070607_BY-2wt_BRs1_2_3_Kohout		
070608_DR5rev-GFP_Et_gasing_1p		
070614_Et-gas_AVG_PIN1-GFP		
070614-PIN1GFP-BRs1_2_3_Kohou		
070618_DR5_BRs1_2_3_Kohout		
070619_Et-gas-AVG_PIN1-GFP		
070621_Et-gas-AVG_PIN1-GFP		
070626_Et-gas-AVG_PIN1-GFP_inte		
070627_PIN3-GFP_test		
070702_PIN1_trafficking		
071105_BRs_Kohout_GVGPIN7		
071110_BRs_Kohout_GVGPIN7_d2		

2.2 Data explorers, correct data handling and saving



Total Commander

- shareware
- the biggest advantage is the existence of two identical windows



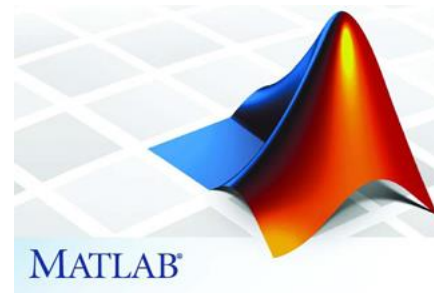
2.3 Spreadsheets, statistical software, graph editors

Bulk data processing, their editing and graphical output



Microsoft Excel - suitable for all basic operations including statistics and various graphical outputs

Specialised mathematical or statistical software - [Statgraphics](#), [NCSS](#), [Matlab](#), or the open source alternative [R](#)



2.3 Spreadsheets, statistical software, graph editors

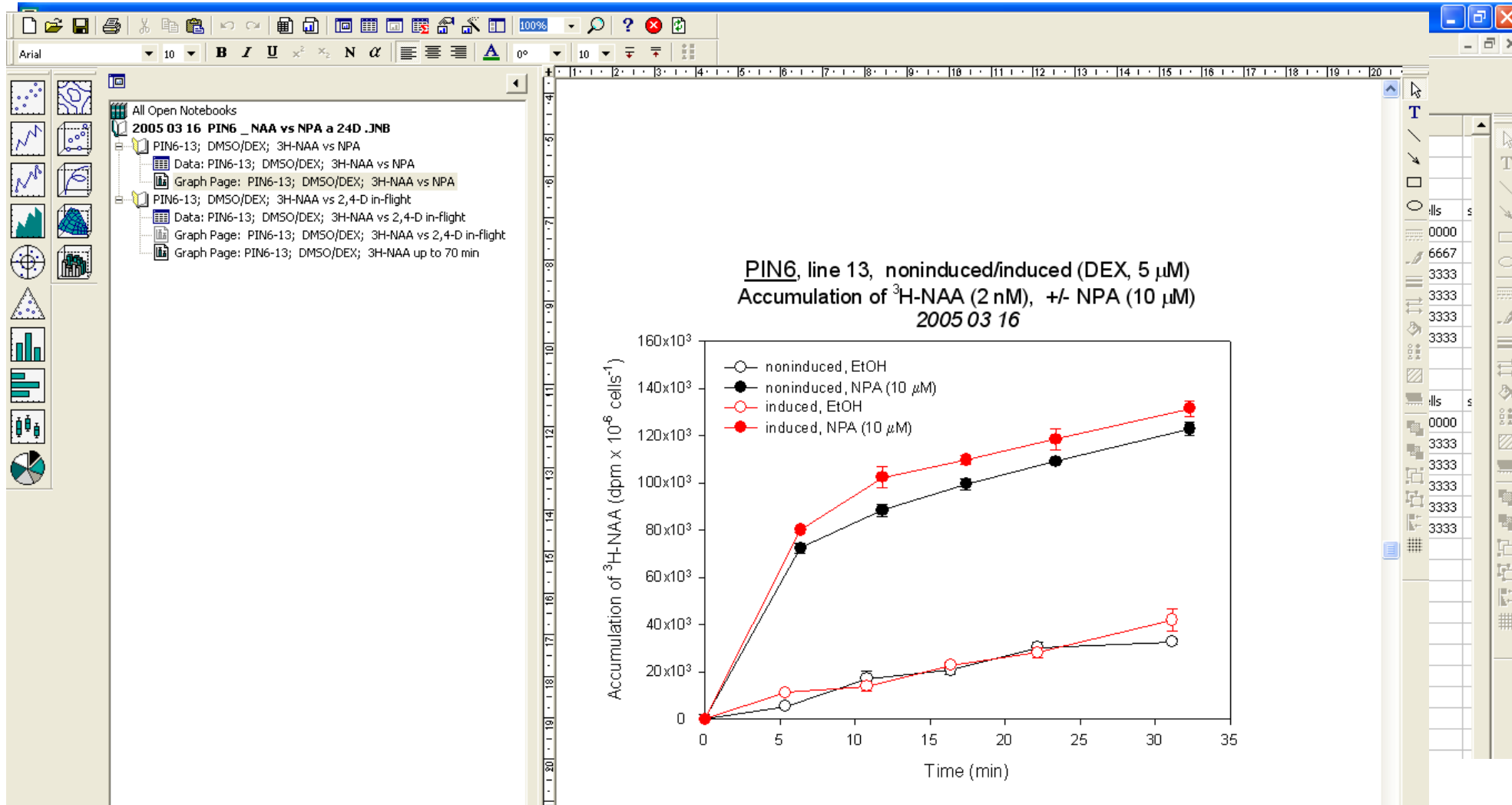
Keeping a complete log of the experiment, automation of numerical operations



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1														
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3														
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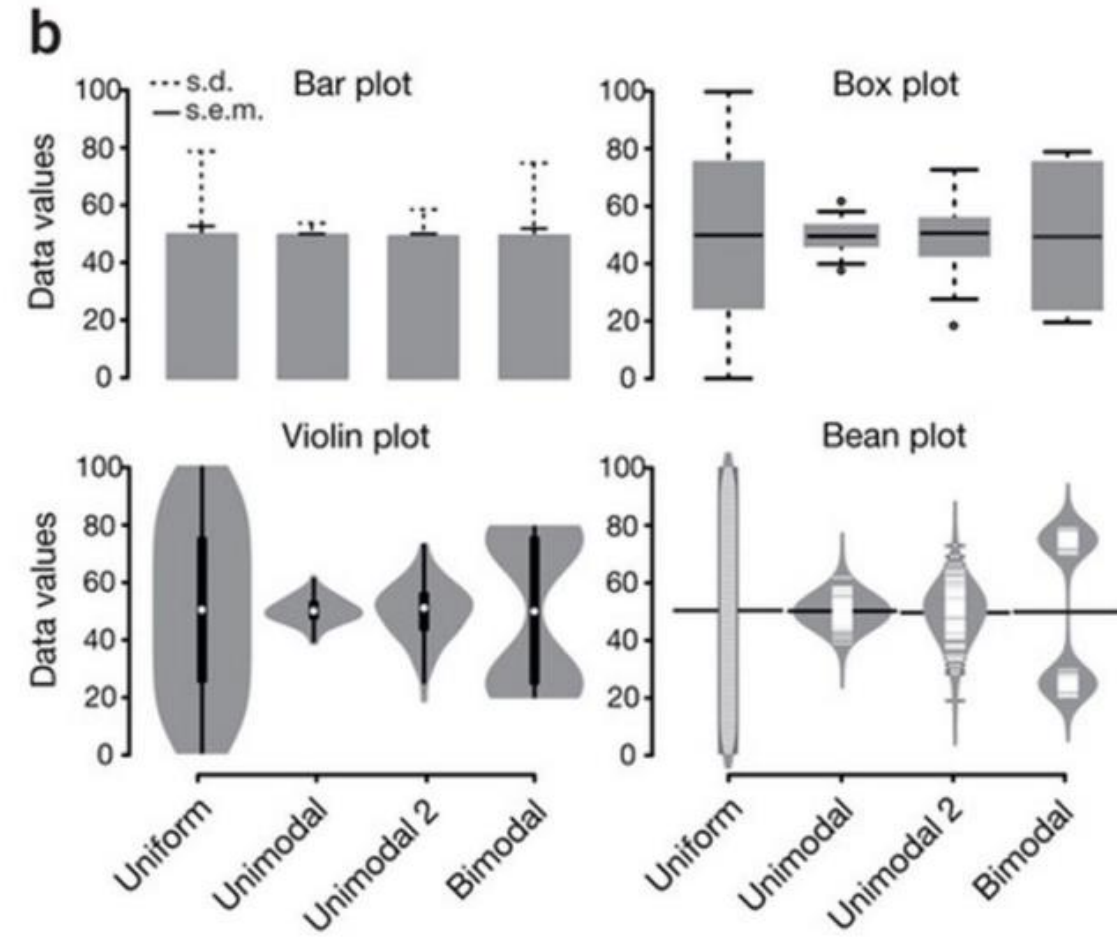
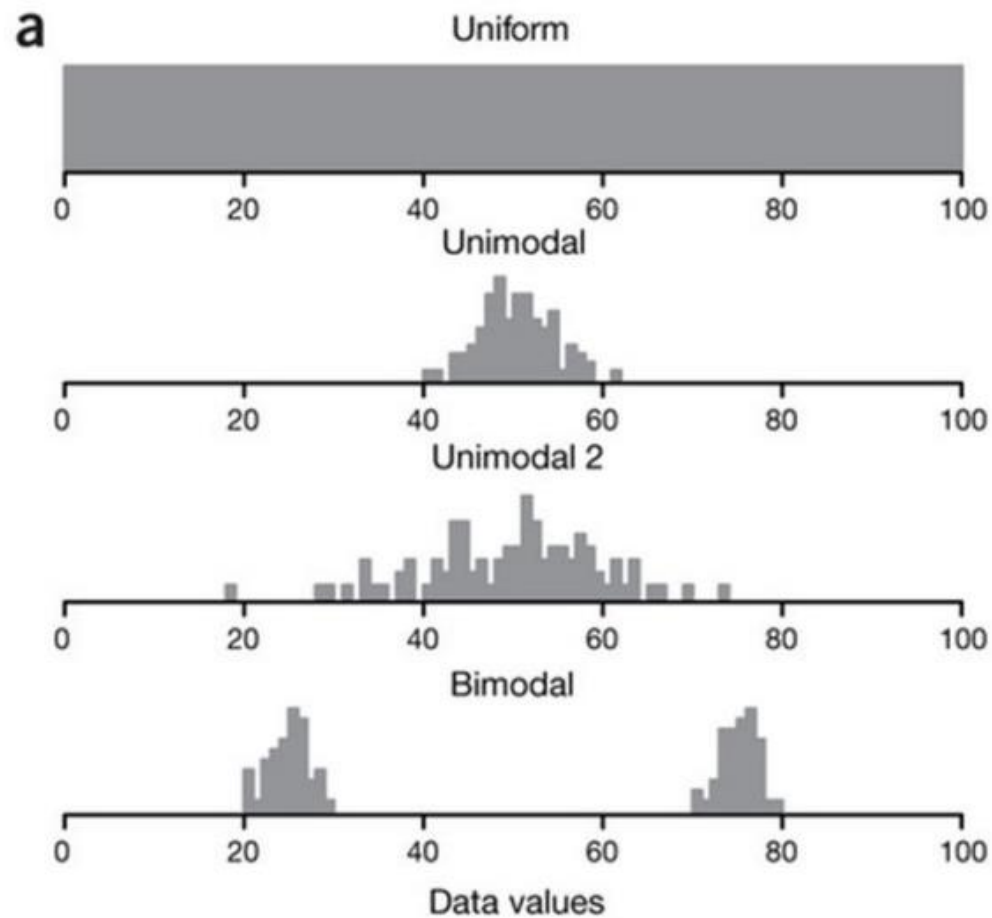
2.3 Spreadsheets, statistical software, graph editors

Keeping a complete log of the experiment, automation of numerical operations



2.3 Spreadsheets, statistical software, graph editors

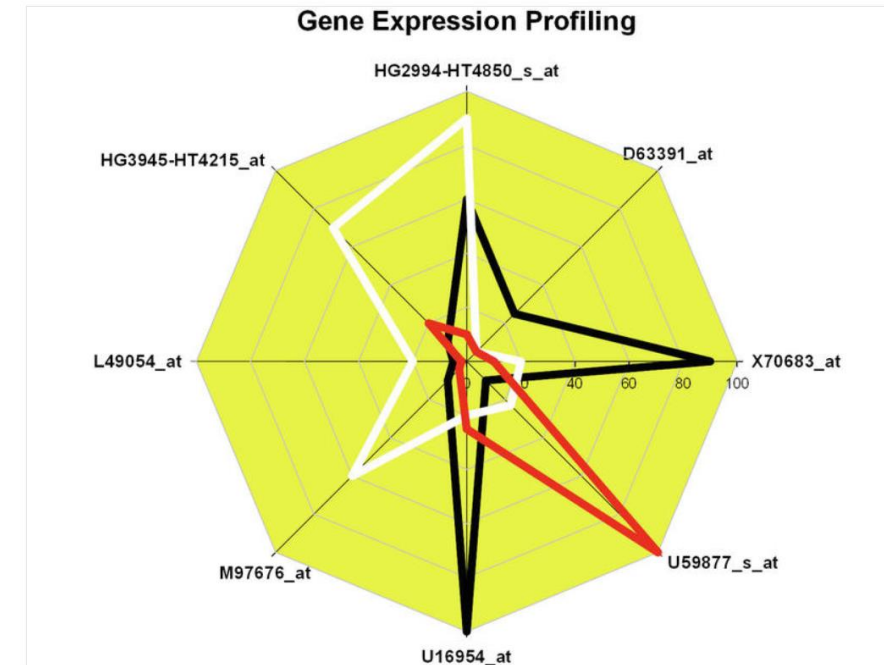
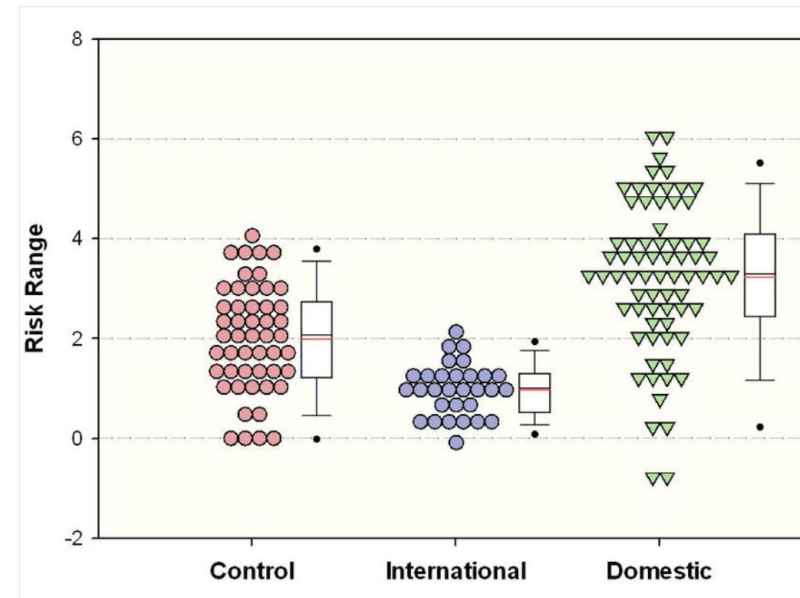
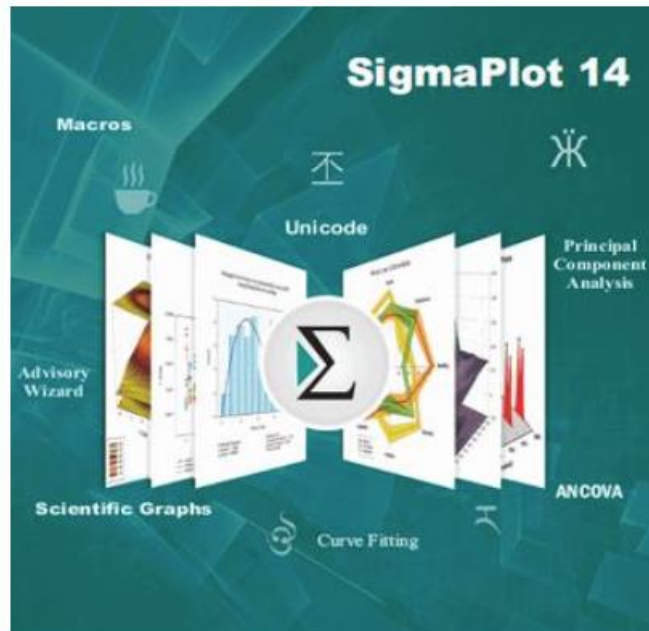
Bar plot versus box plot - what is better?



Spitzer M, Wildenhain J, Rappsilber J, Tyers M (2014) BoxPlotR: a web tool for generation of box plots. Nat Methods 11:121–122.

2.3 Spreadsheets, statistical software, graph editors

Creating graphs - line/scatter plots, bar charts, box plots, histograms

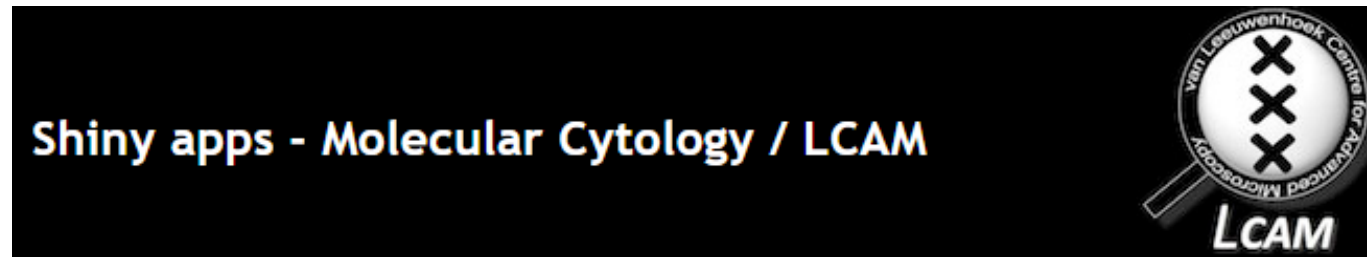


2.3 Spreadsheets, statistical software, graph editors

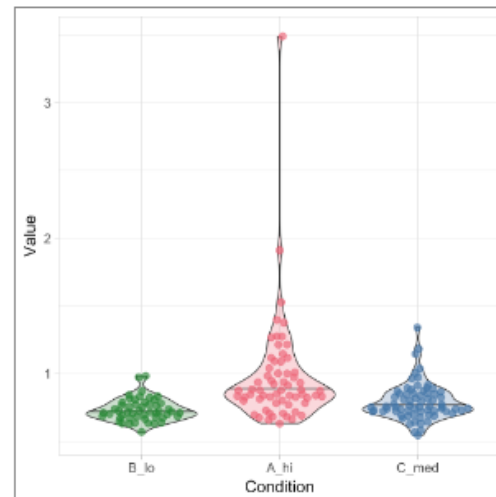
Creating graphs - line/scatter plots, bar charts, box plots, histograms

In biology, it is good to plot **all data** together with **their summaries and statistics**

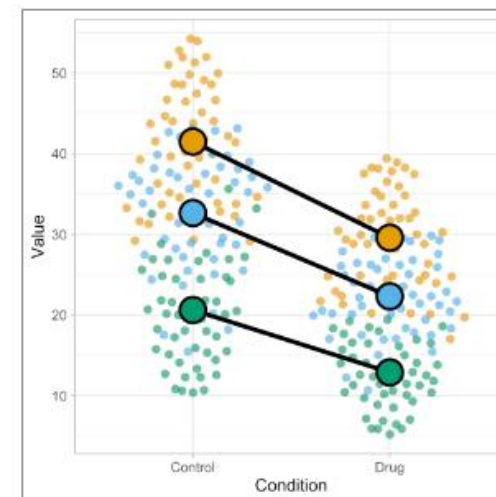
Shiny apps - R-based web tools for creating plots



PlotsOfData



SuperPlotsOfData

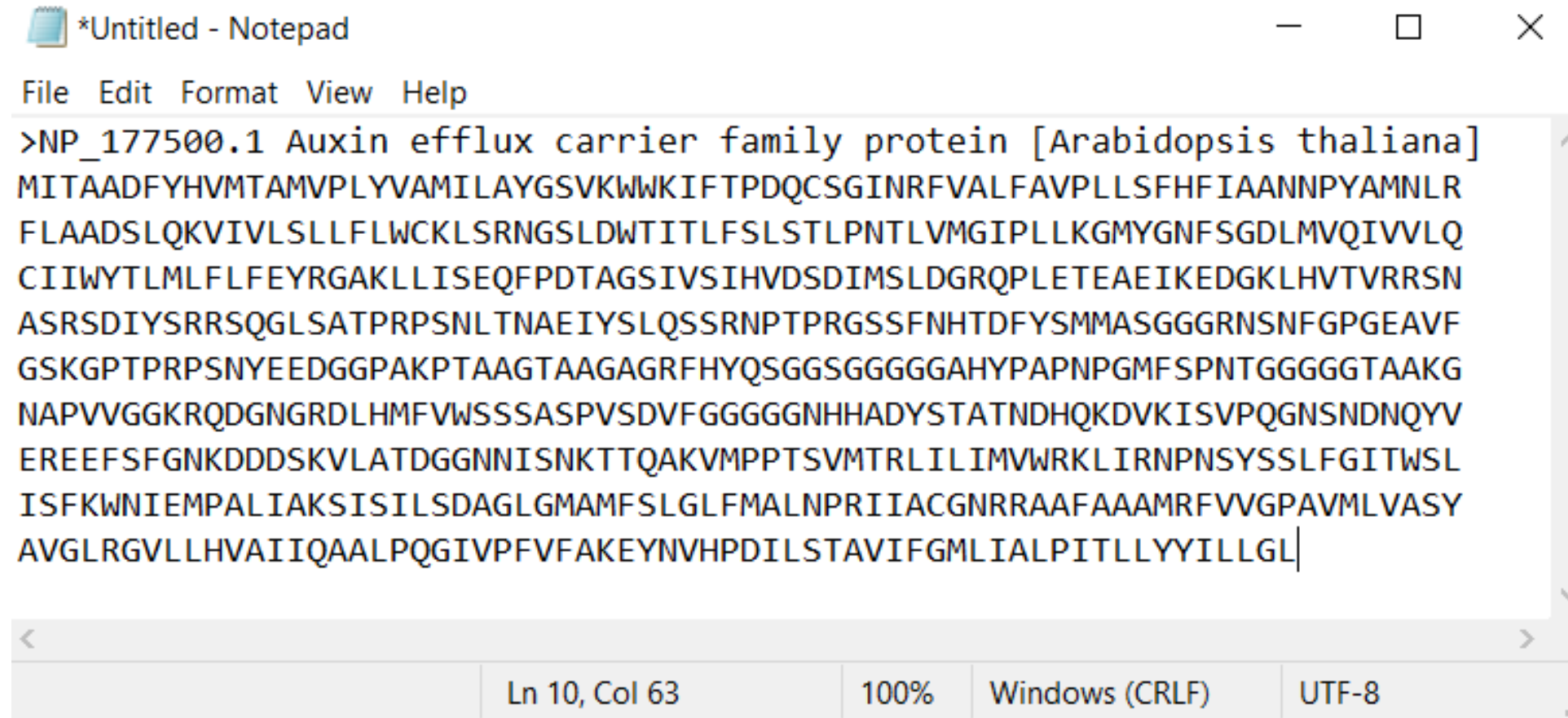


2.4 Processing of structural and sequence data

Notepad - a digital notebook, which is still an invaluable helper

It is important not to use formatting to write nucleotide or amino acid sequences

The most commonly used is the so-called **FASTA** format



The screenshot shows a Notepad window titled '*Untitled - Notepad'. The text inside is a FASTA format sequence for NP_177500.1. The sequence is as follows:

```
>NP_177500.1 Auxin efflux carrier family protein [Arabidopsis thaliana]
MITAADFYHVMTAMVPLYVAMILAYGSVKWWKI FTPDQCSGINRFVALFAVPLLSFHFIAANNPYAMNLR
FLAADSLQKVIVLSLLFLWCKLSRNGSLDWTITL FSLSTLPNTLVMGIPLLKGMYGNFSGDLMVQIVVLQ
CIIWYTLMLFLFEYRGAKLLISEQFPDTAGSIVSIHVSDIMSLDGRQPLETEAEIKEDGKLHVTVRRSN
ASRSDIYSRRSQGLSATPRPSNL TNAEIYSLQSSRNPTPRGSSFNHTDFYSMMASGGGRNSNFGPGEAVF
GSKGPTPRPSNYEEDGGPAKPTAAGTAAGAGRFHYQSGGSGGGGGGAHYPAPNPGMFSPNTGGGGGTAAKG
NAPVVGGKRQDGNRDLHMFVWSSSASPVSDVFGGGGGNHHADYSTATNDHQKDVKISVPQGN SNDNQYV
EREEFSFGNKDDDSKVLATDGGNNISNKTTQAKVMPPT SVMTRLILIMVWRKLIRNPNSYSSLFGITWSL
ISFKWNIEMPALIAKSISILSDAGLGMAMFSLGLFMALNPRIIACGNRRAAF AAAMRFVVGPAVMLVASY
AVGLRGVLLHVAIIQAALPQGIVPFVFAKEYNVHPDILSTAVIFGMLIALPITLLYYILLGL|
```

The status bar at the bottom of the Notepad window shows: Ln 10, Col 63; 100%; Windows (CRLF); UTF-8.

2.4 Processing of structural and sequence data

SnapGene

Geneious



Restriction Analysis

Get a restriction profile of your DNA based on cutting sites, frequency. Vector NTI Advance offers the most comprehensive REN analysis scheme than any other softwares.



Digestion/Ligation Cloning

Create recombinants from individual fragments that have compatible ends, and modify the ends if they are not compatible. NEW in version 11, this is all done in a graphical manner.



Gateway® Cloning

Using the intuitive Gateway Cloning Wizard, you can rapidly clone DNA fragments into multiple expression vectors simultaneously.

[Read more](#)



TOPO® Cloning

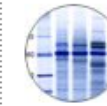
The integrated TOPO® Cloning Wizard greatly facilitates vector construction using the most comprehensive collection of vectors made by Invitrogen.

[Read more](#)



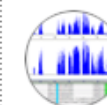
Design Primers for:

- Amplification
- Sequencing
- Multiplex PC
- Hybridization



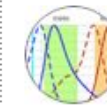
Gel Simulation

- Run digested DNA on Agarose or Acrylamide gels as if you would in the lab
- Get prepared before loading the real stuff



Multi-Sequence Alignment

- Fast, accurate alignment by ClustalW algorithm
- Alignment statistics, phylogenetic guide tree, repeat identification



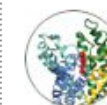
Contig Assembly

- Fast, accurate assembly by the CAP3 algorithm
- Flexible ends trimming and vector contamination trimming
- Chromatogram data analysis and editing; consensus creation using Quality Values



Use BioAnnotator to analyze proteins for:

- PFAM, ProSite, BLOCKS, Proteolytic Cleavage, Spidey, Sim4



View Protein Structure in 3-D

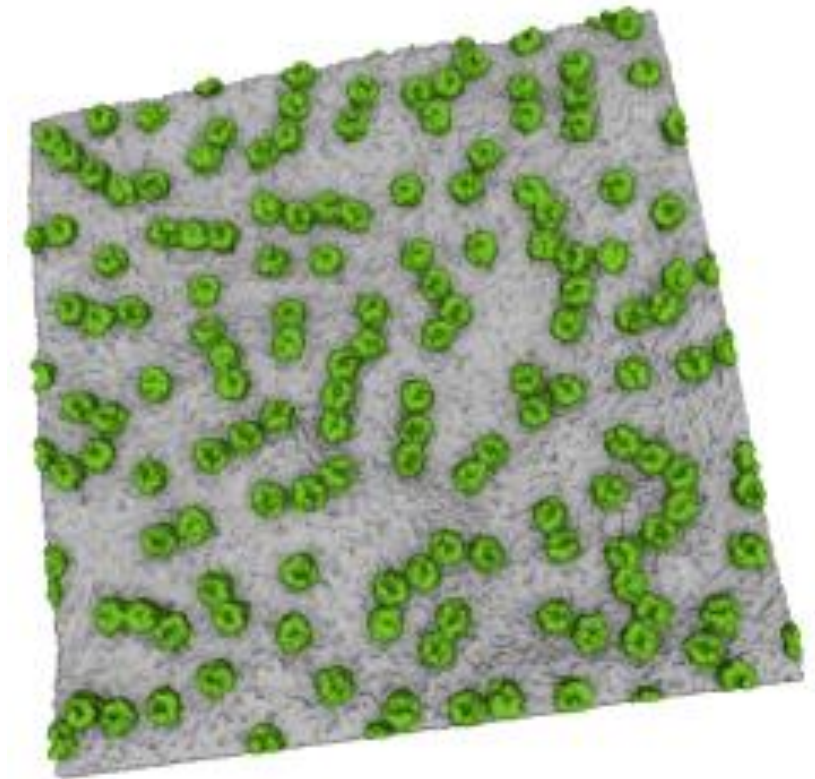
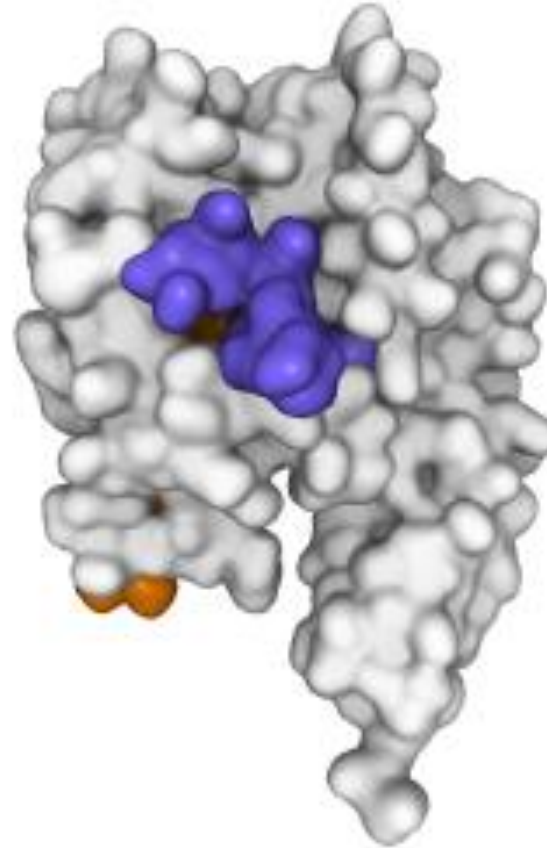
- View protein structures in stereo mode
- Freedom to configure how you

2.4 Processing of structural and sequence data

3D structures of macromolecules – Molstar viewer

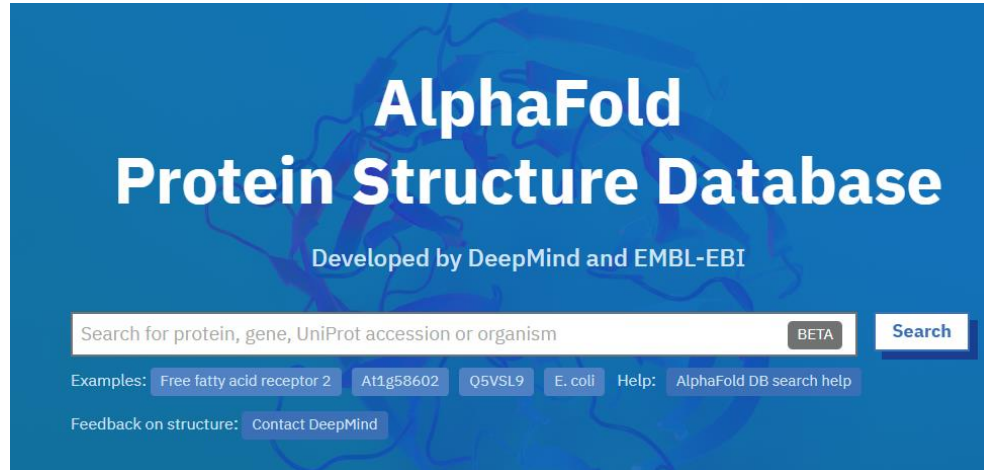


Mol* (*'molstar'*) is a modern web-based open-source toolkit for visualisation and analysis of large-scale molecular data



2.4 Processing of structural and sequence data

3D structures of macromolecules - AI-based solution for „*in silico*“ translation



AlphaFold2.ipynb
File Edit View Insert Runtime Tools Help

+ Code + Text Copy to Drive

ColabFold: AlphaFold2 using MMseqs2

Easy to use protein structure and complex prediction using [AlphaFold2](#) and [Alphafold2-multimer](#). Sequence alignments/templates are generated through [MMseqs2](#) and [HHsearch](#). For more details, see [bottom](#) of the notebook, checkout the [ColabFold GitHub](#) and read our manuscript. Old versions: [v1.0](#), [v1.1](#), [v1.2](#), [v1.3](#)

[Mirdita M, Schütze K, Moriwaki Y, Heo I, Ovchinnikov S, Steinegger M. ColabFold: Making protein folding accessible to all. Nature Methods, 2022](#)

Input protein sequence(s), then hit Runtime -> Run all

```
query_sequence: "PIAQIHILEGRSDEQKETLIREVSEAIRSLDAPLTSVRVIITEMAKGHFGIGGELASK"
```

- Use : to specify inter-protein chainbreaks for **modeling complexes** (supports homo- and hetro-oligomers). For example `PI...SK:PI...SK` for a homodimer

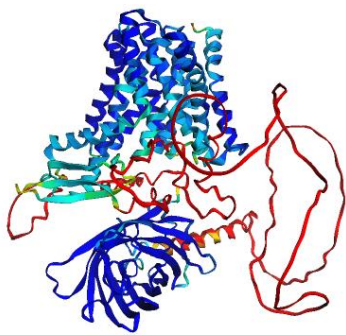
```
jobname: "test"
```

```
use_amber: 
```

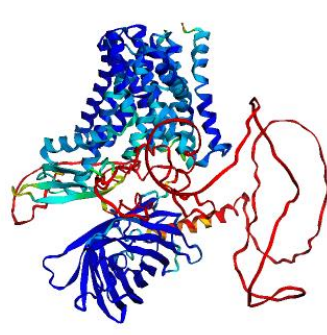
```
template_mode: none
```

- "none" = no template information is used, "pdb70" = detect templates in pdb70, "custom" - upload and search own templates (PDB or mmCIF format, see [notes below](#))

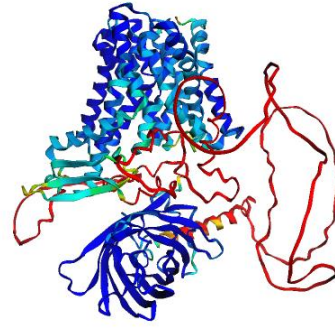
Show code



NtPIN2T



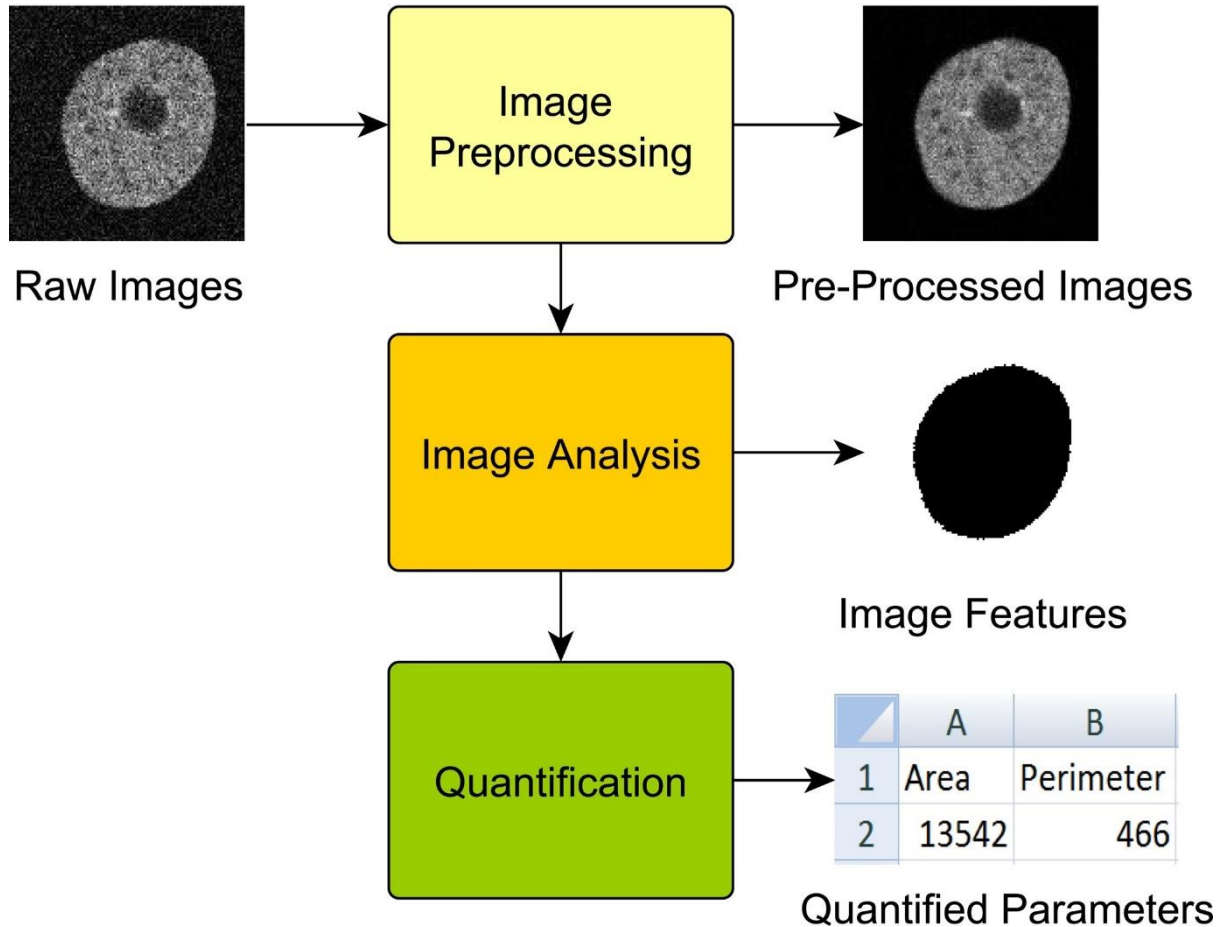
NtPIN3bT



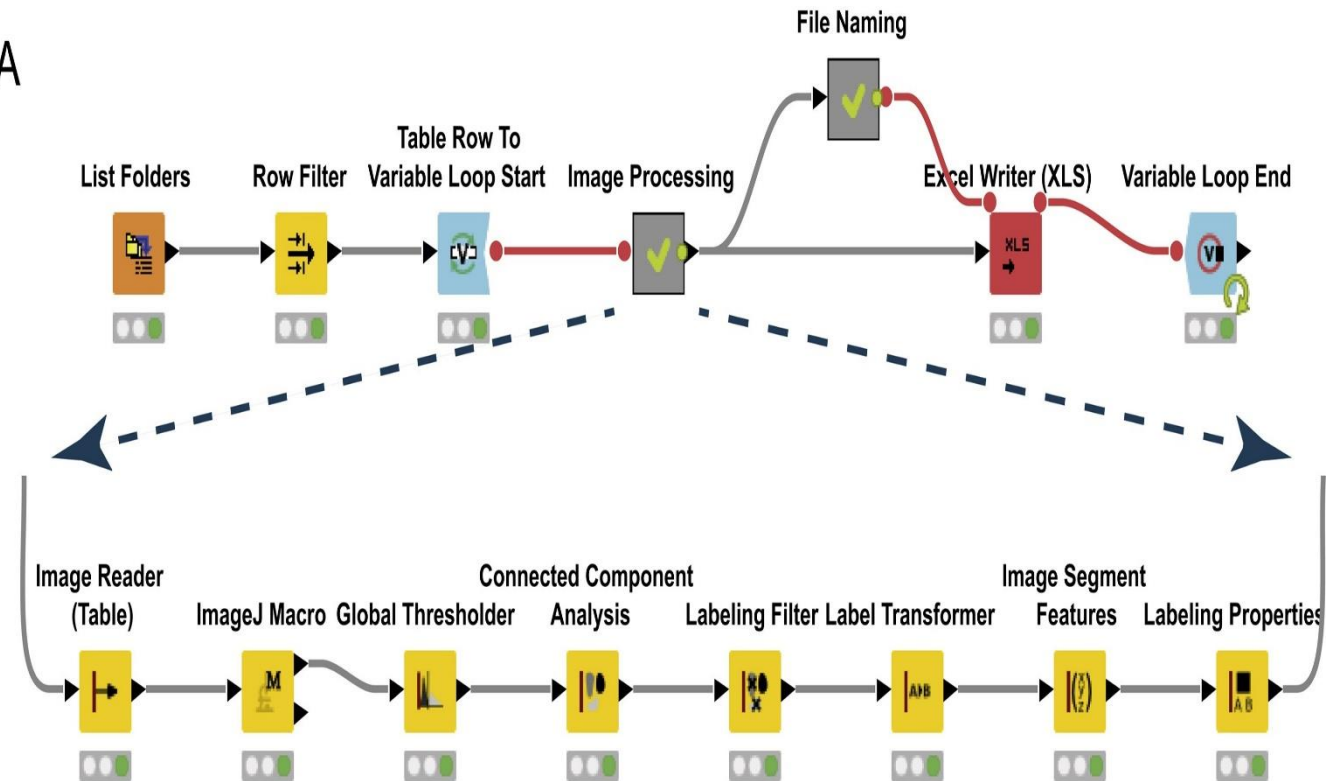
NtPIN11T

2.5 Image analysis, graphical software, presentation software

The task of **image analysis** is to **evaluate features that are often not apparent at first glance**



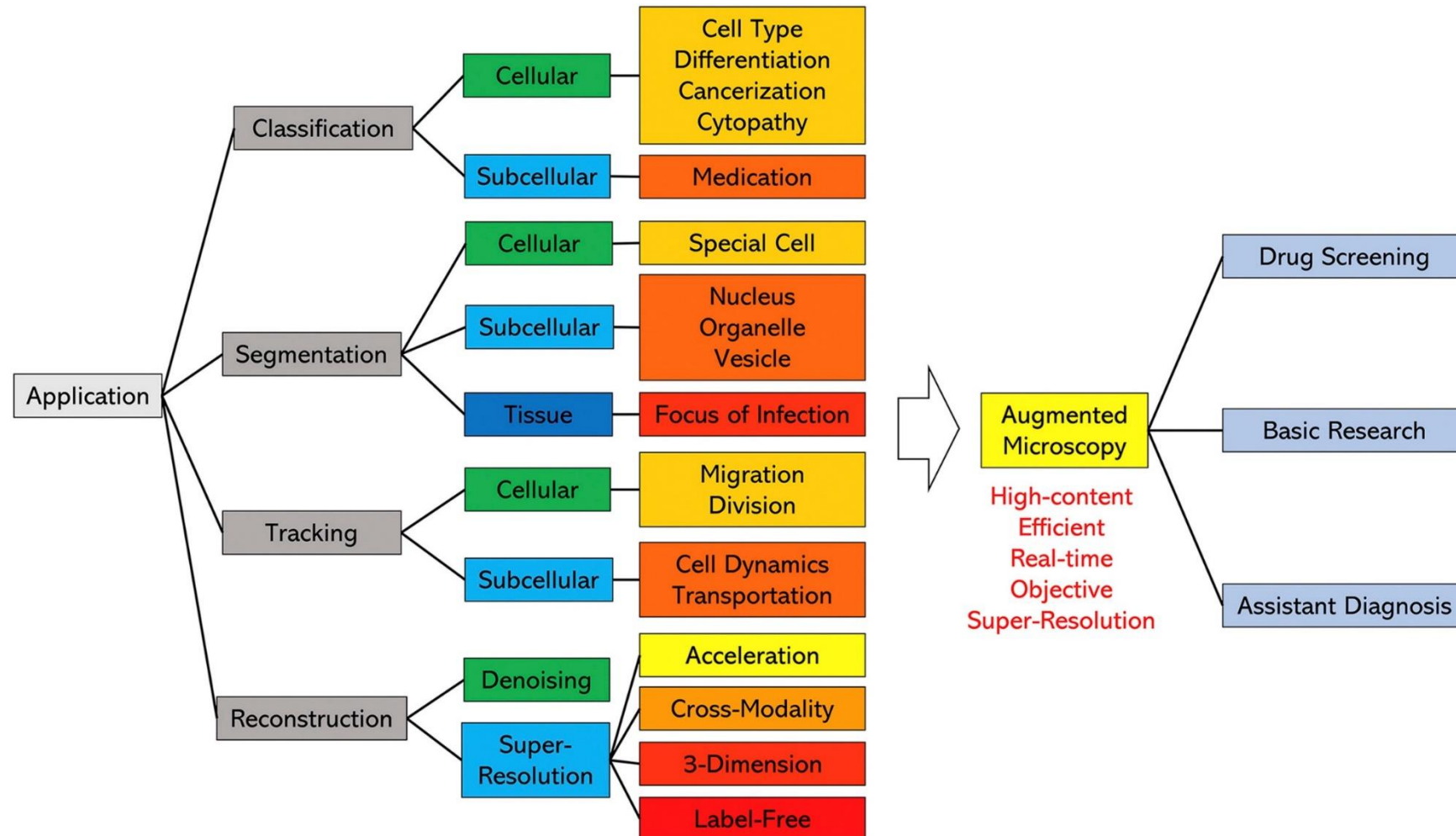
A



Wollmann T et al., J Biotechnol 261:70-75, 2017

2.5 Image analysis, graphical software, presentation software

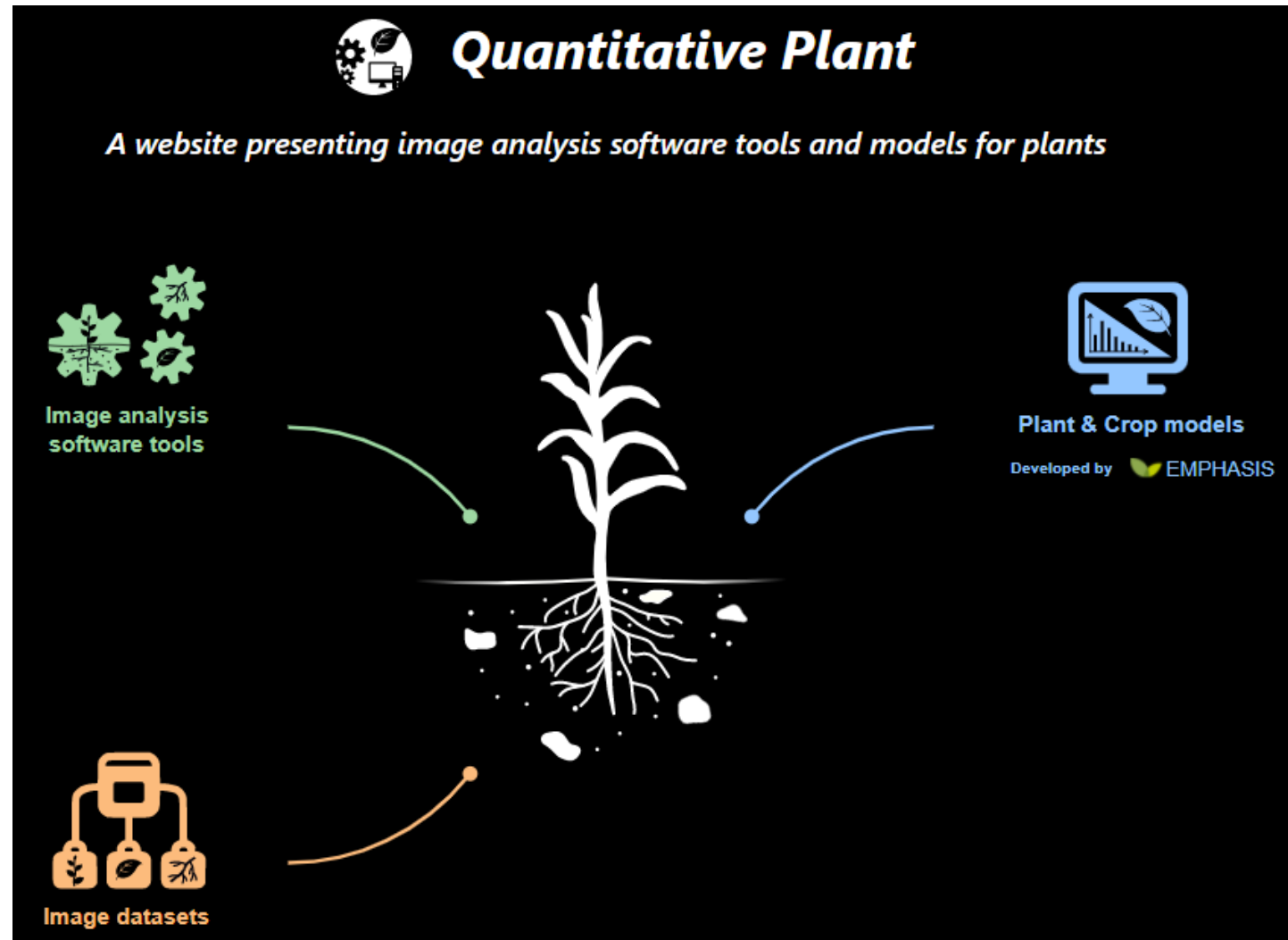
Artificial intelligence in image analysis



Liu et al., Comput Biol Med 134:104523, 2021

2.5 Image analysis, graphical software, presentation software

Summary of image analysis tools for plant research



2.5 Image analysis, graphical software, presentation software

Summary of image analysis tools for plant research

The screenshot displays the Quantitative Plant website interface. At the top, there is a navigation bar with links for 'Image software tools', 'Image datasets', 'Plant and Crop models', 'API', 'Submit', 'About', 'Cite us', and a search bar. Below the navigation bar, there are several filter dropdowns: 'root-system', 'Measurements', 'Operating system', 'License', and 'Automation level'. The main content area shows a grid of tool cards. Each card includes a representative image of the tool's output, the tool's name, its category, supported operating systems (Windows, macOS, Linux), release date, and a list of measurable parameters.

Tool Name	Category	Release Date	Measurable Parameters
ARIA	root-system	2014-10	length, convex-hull, shape, count
DART	root-system	2011-06	length, topology, insertion
DIRT	root-system	2014-09	length, shape, diameter
DynamicRoots	root-system	2015-06	length, topology, growth, count, gravitropism
ER			
GLO-Roots			

2.5 Image analysis, graphical software, presentation software

ImageJ - open source image analysis



Acquisition

Algorithm, Filters, ...

Processing

Automation

Analysis

Image J

Image J ecosystem



<https://cif.unil.ch/cif-wiki/several-image-processing-workflow-examples/>



2.5 Image analysis, graphical software, presentation software

[ImageJ \(NIH\)](#)

ImageJ
Image Processing and Analysis in Java

[Fiji](#)

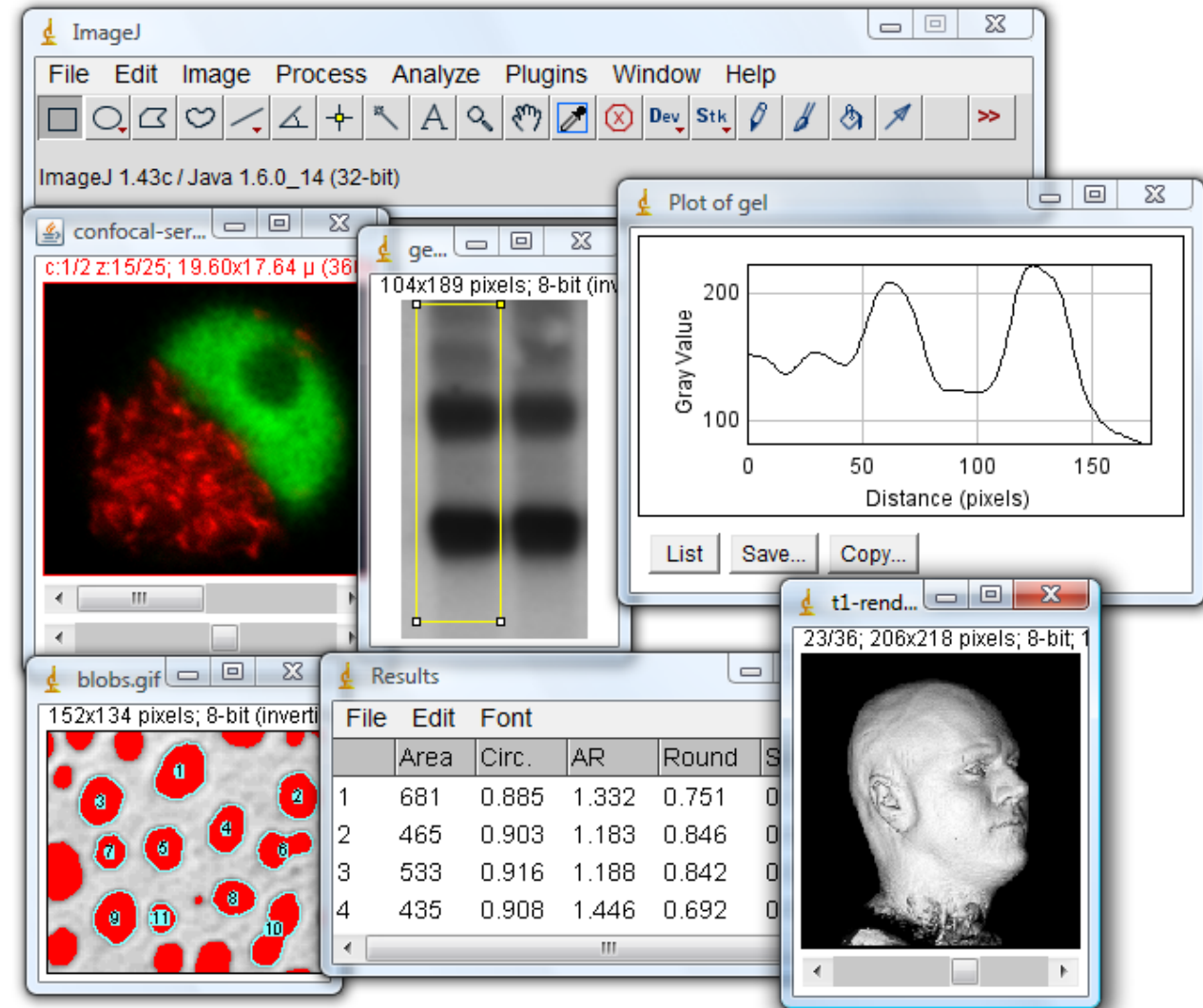
Imaging tutorials

[Scientific](#)

[Imaging](#)

[Tutorials](#)

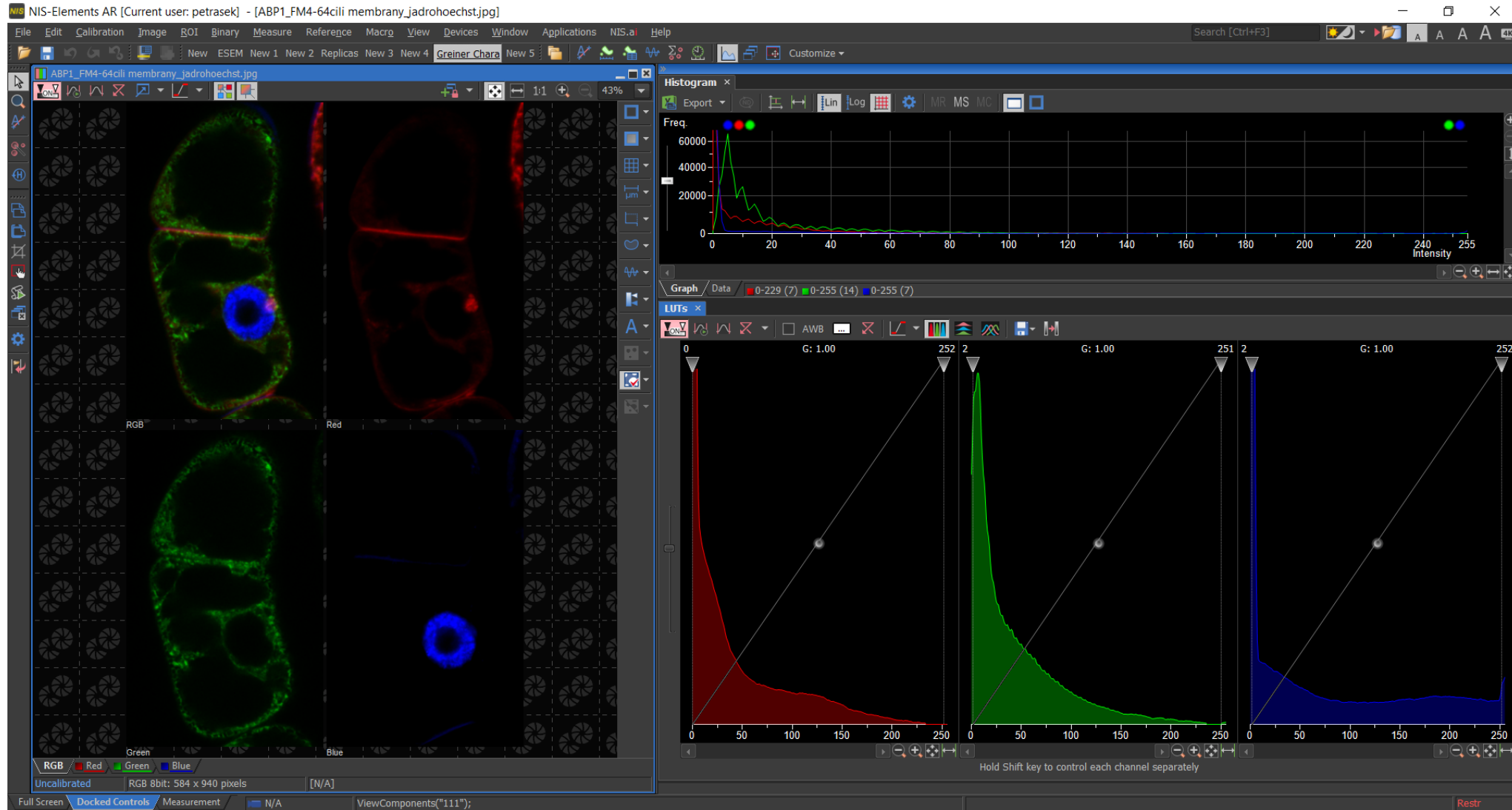
[\(imagej.net\)](http://imagej.net)



<http://rsb.info.nih.gov/ij/docs/concepts.html>

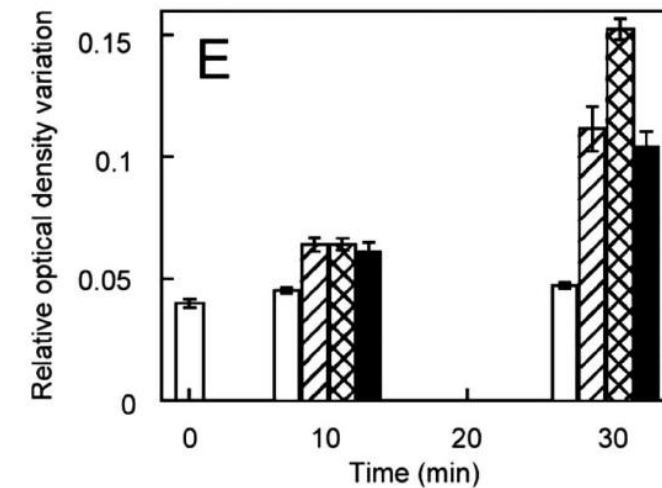
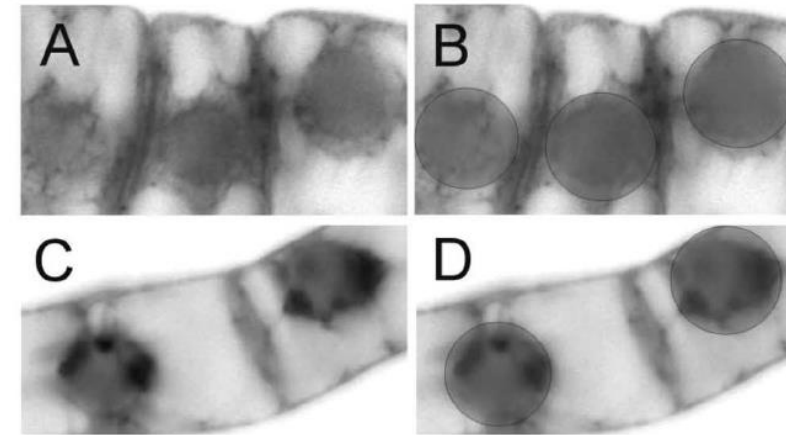
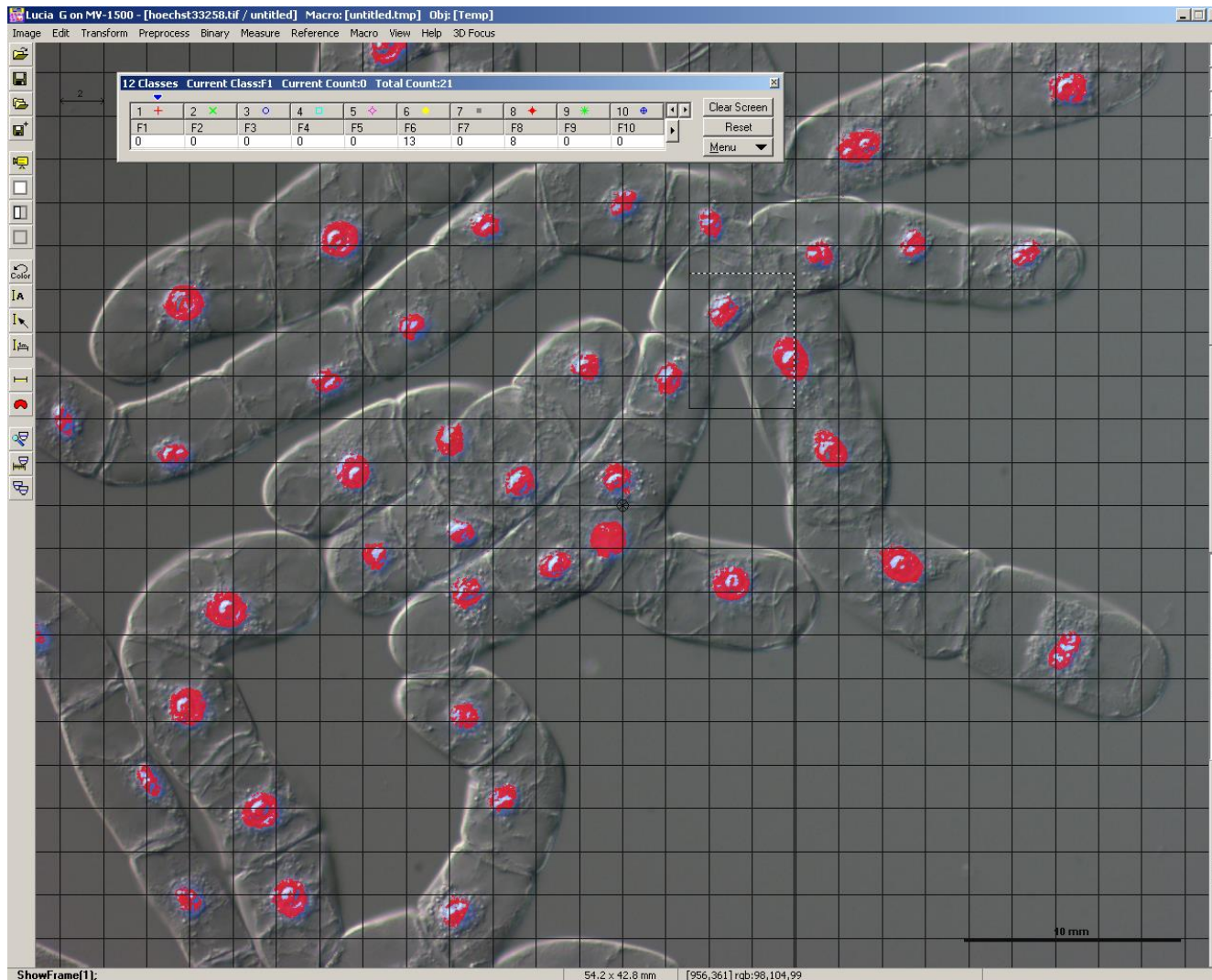
2.5 Image analysis, graphical software, presentation software

NIS Elements – good example of commercially available, comprehensive image analysis tool



2.5 Image analysis, graphical software, presentation software

NIS Elements – good example of commercially available, comprehensive image analysis tool



2.5 Image analysis, graphical software, presentation software

Graphical software for grabbing and processing of micro- and macroscopical images

Leica



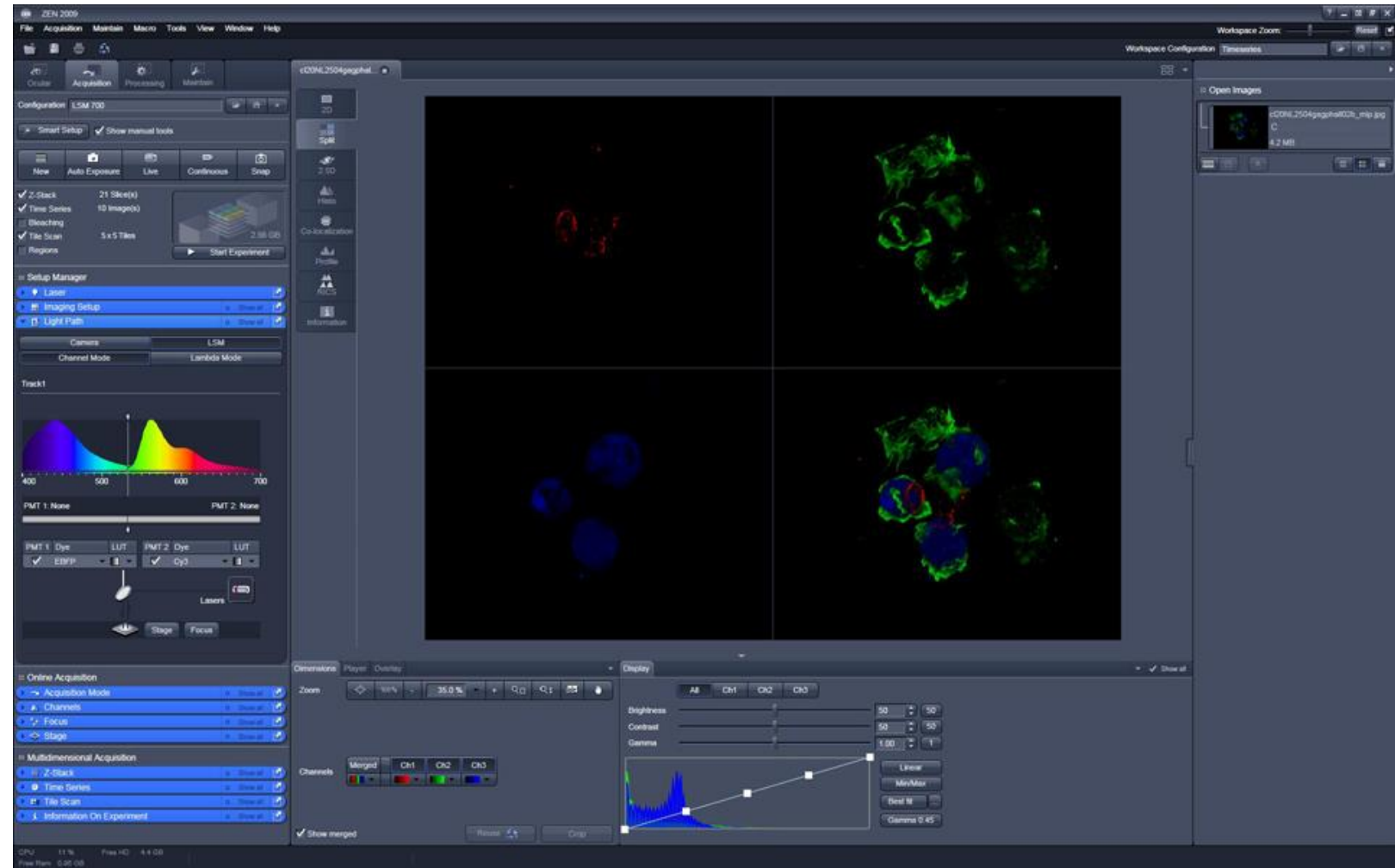
Zeiss



Nikon



Olympus



2.5 Image analysis, graphical software, presentation software

Colour depth in biological imaging

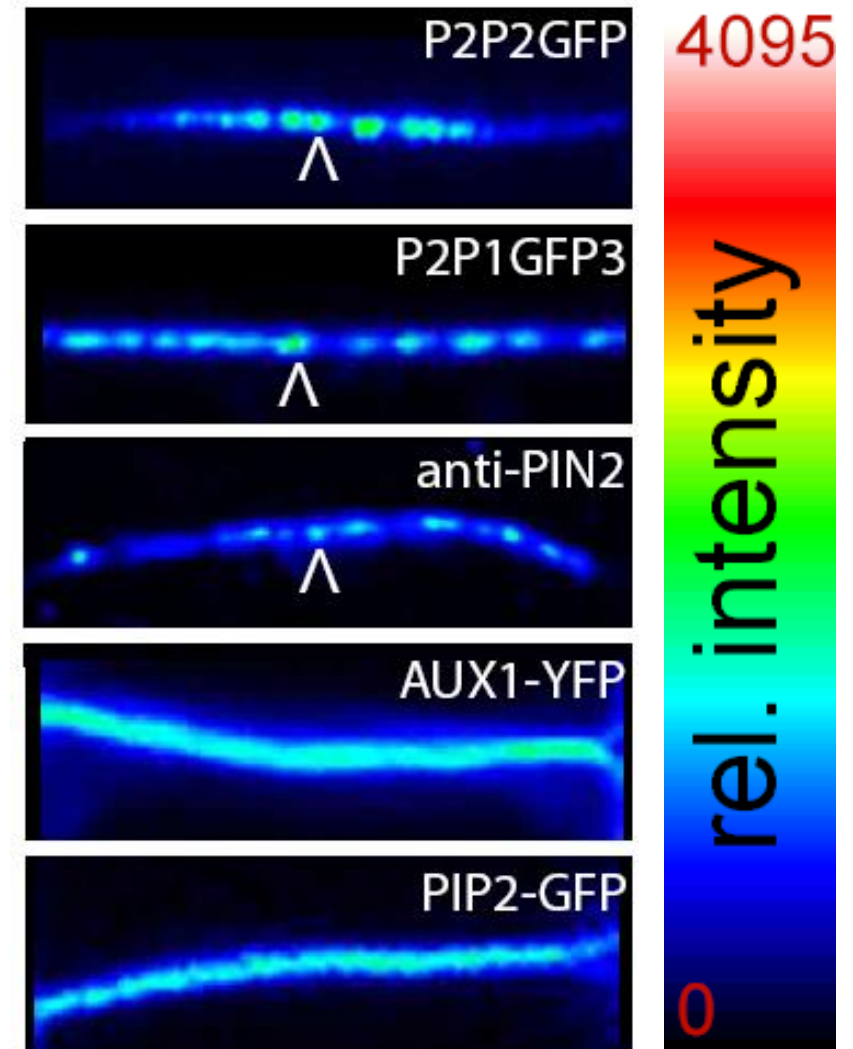
8 bit - 256 levels of grey (2^8)

12 bit - 4096 levels of grey (2^{12})

16 bit - 65536 levels of grey (2^{16})

24 bit - 16 777 216 levels of grey (2^{24})

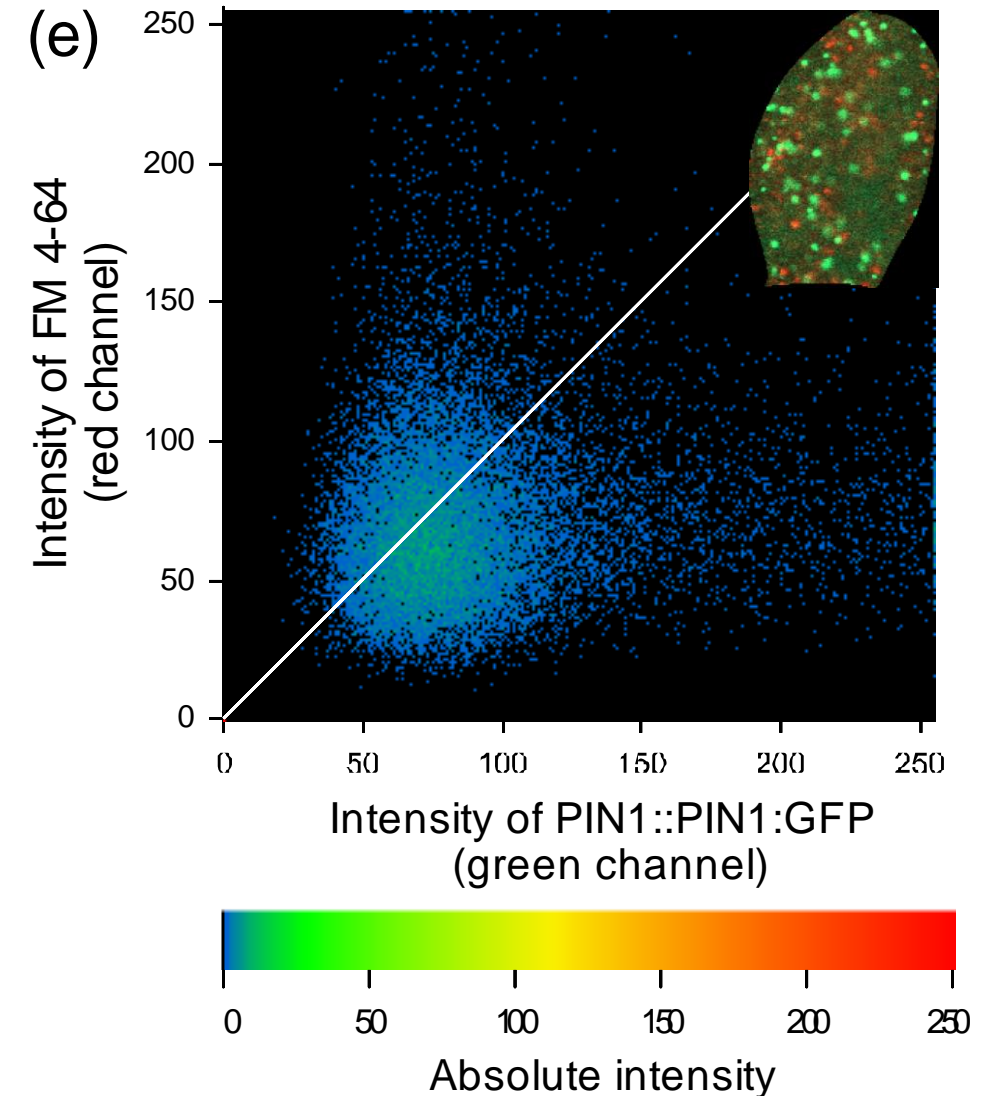
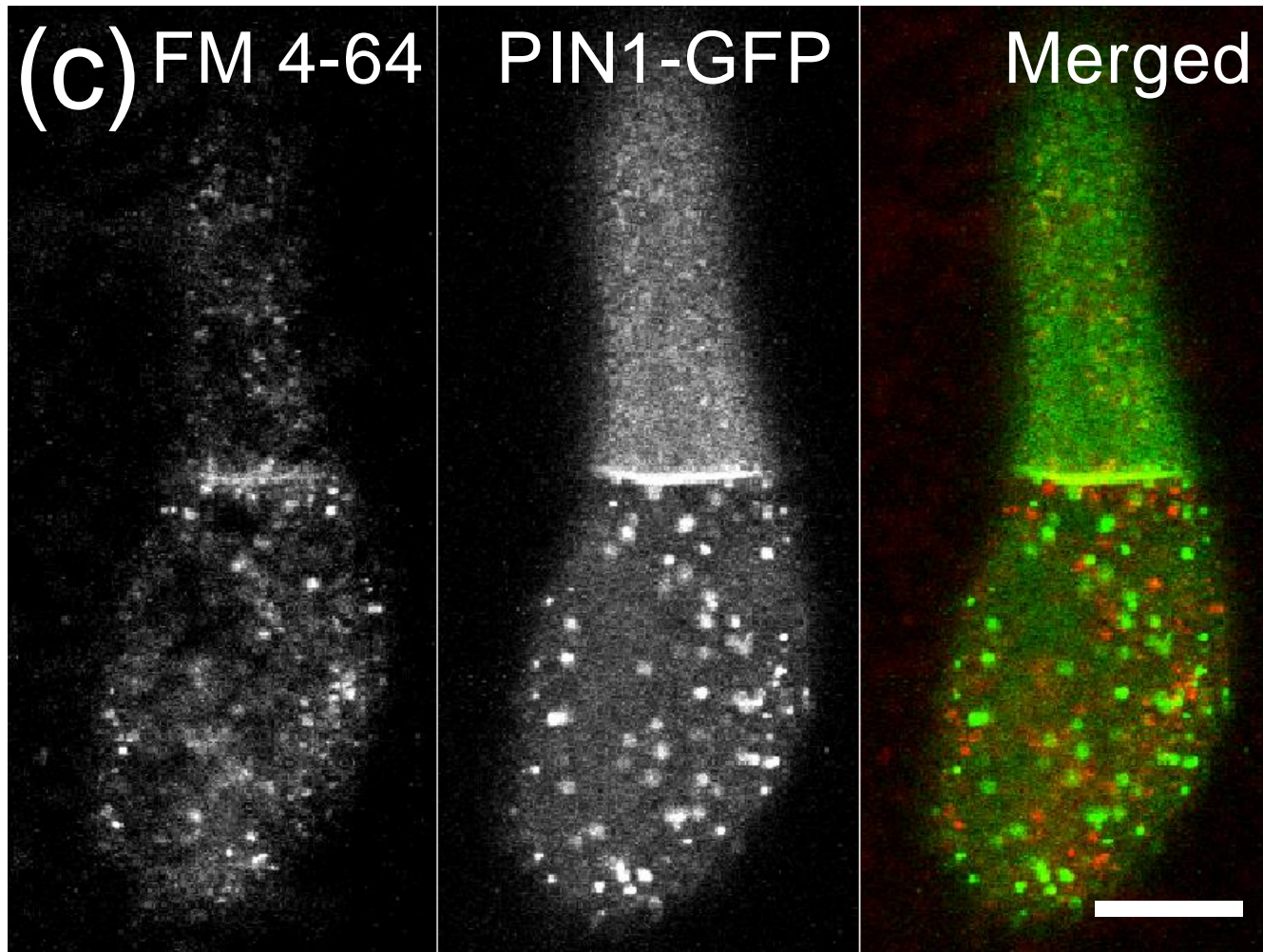
A suitable **LUT**, i.e. **Look Up Table**, is the key to correct presentation of the image



Kleine-Vehn, Mol Syst Biol 7:540, 2011

2.5 Image analysis, graphical software, presentation software

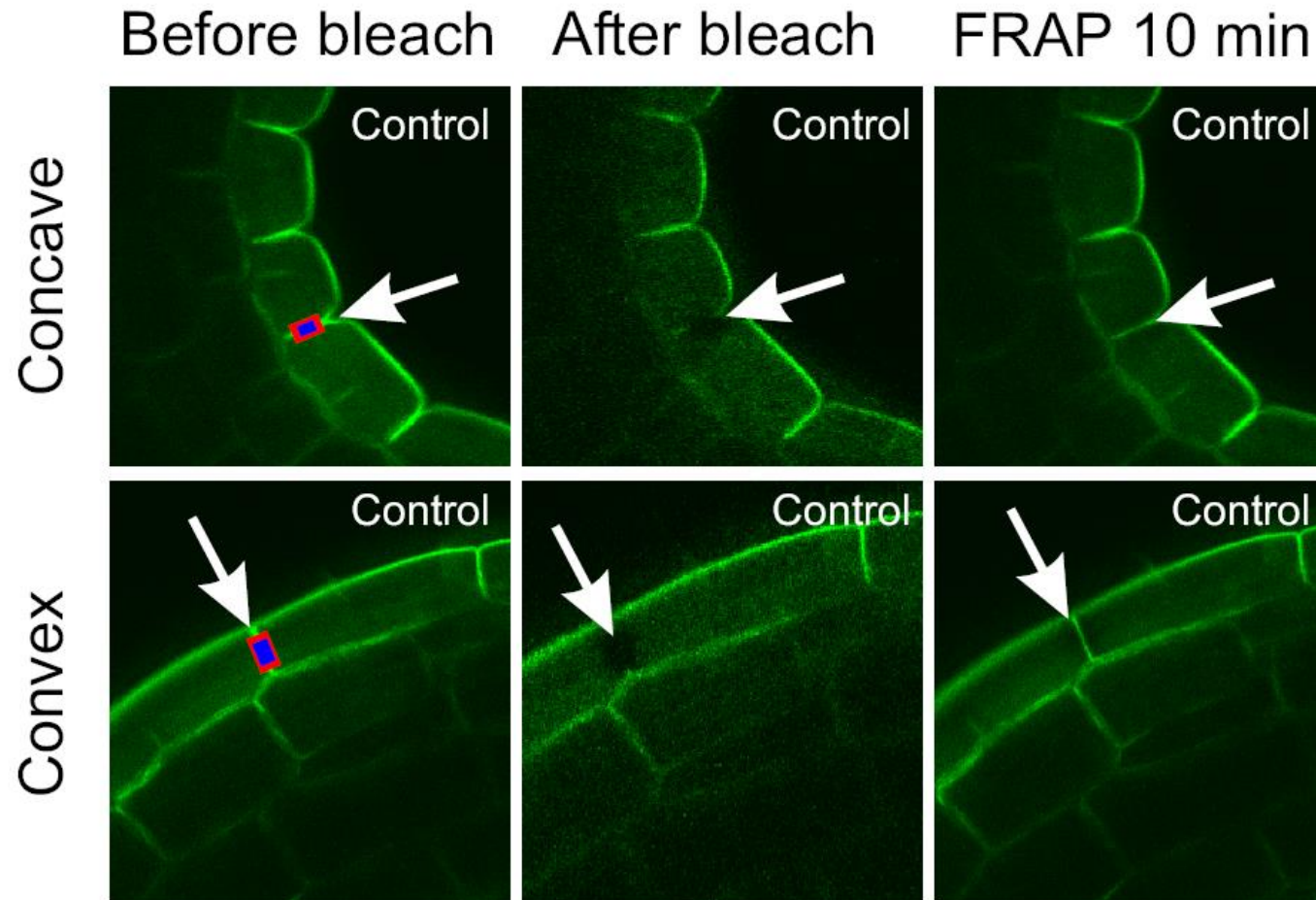
Analysis of colocalization



Jelínková et al., *Plant Journal* 61, 883-891, 2009

2.5 Image analysis, graphical software, presentation software

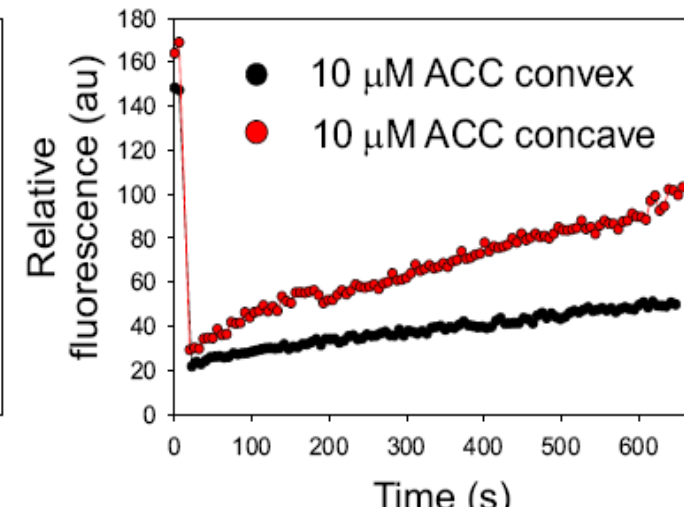
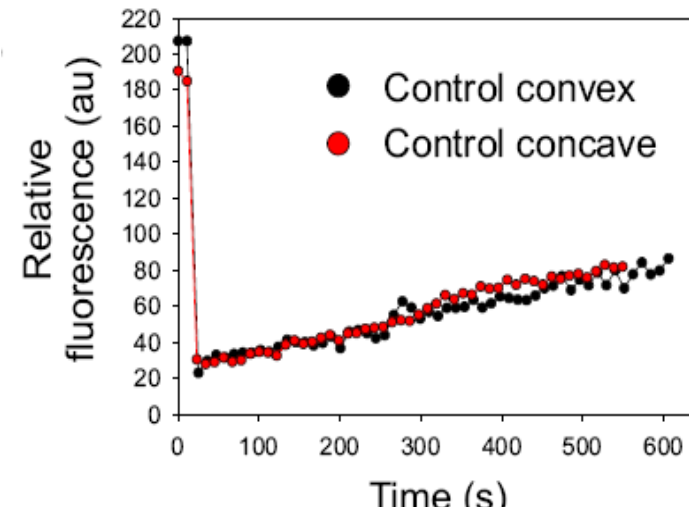
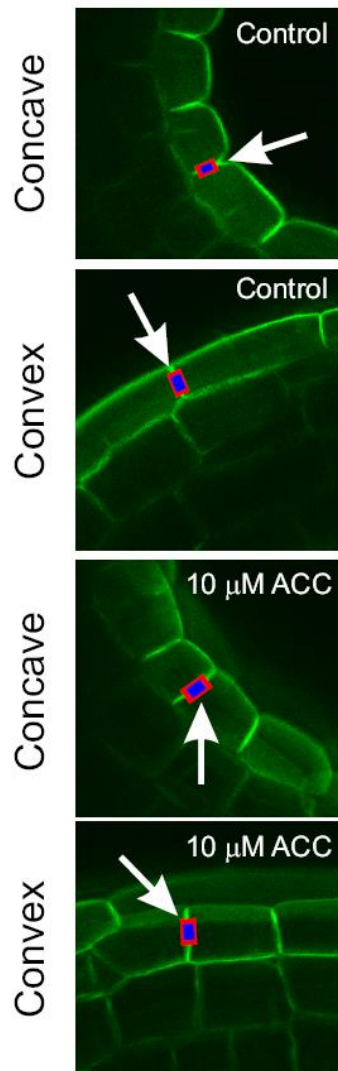
Analysis of intracellular protein dynamics



Vandenbussche et al., 2009, Development 137, 597-606

2.5 Image analysis, graphical software, presentation software

Analysis of intracellular protein dynamics

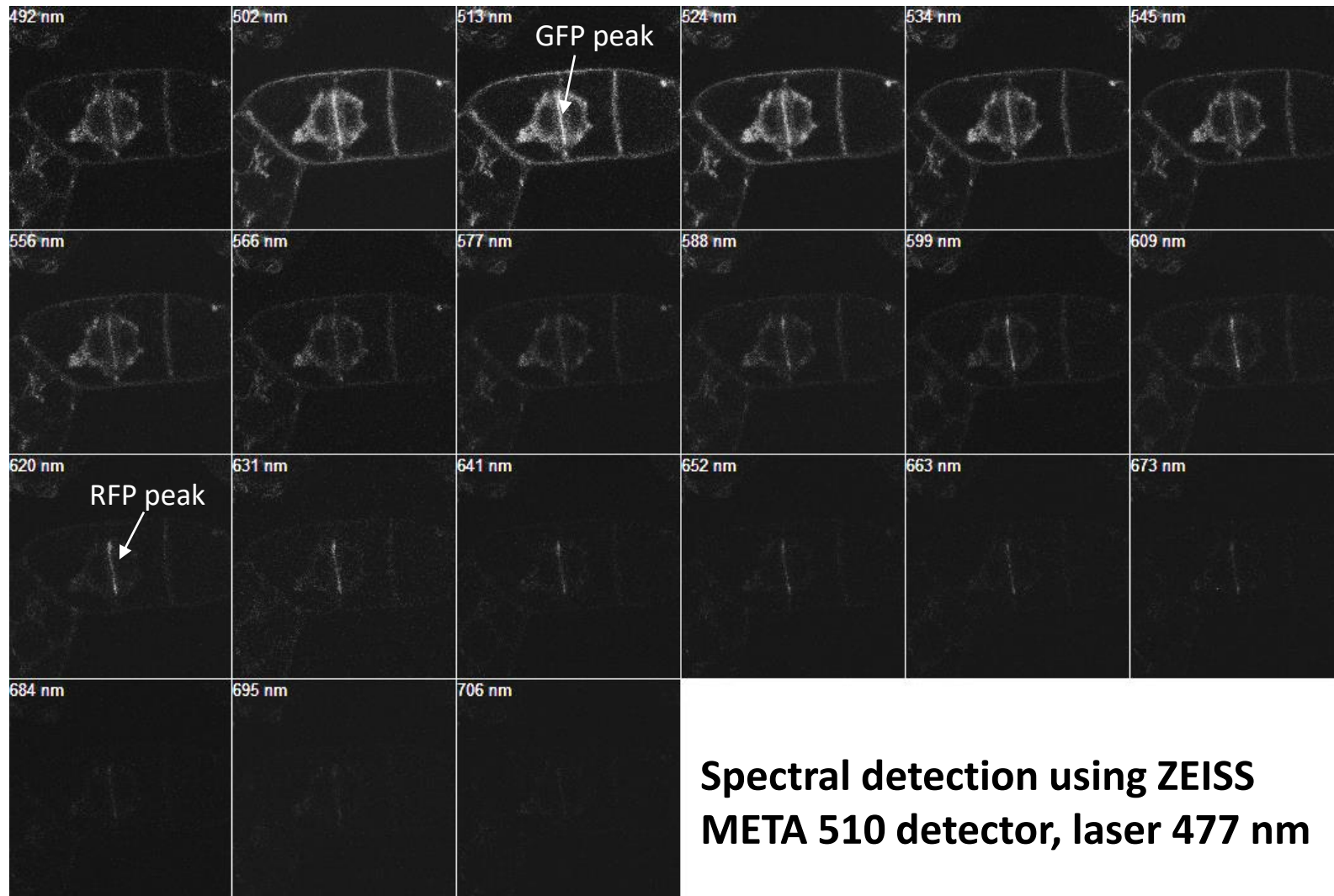


Net increase of relative fluorescence after 10 minutes of FRAP (% of initial fluorescence)					
Control			10 μ M ACC		
Concave	Convex	Concave/Convex ratio	Concave	Convex	Concave/Convex ratio
25.42	14.96	1.70	22.33	3.63	6.16
11.21	14.37	0.78	14.42	11.34	1.27
17.25	24.75	0.70	32.08	23.99	1.34
26.12	27.66	0.94	44.09	20.53	2.15
17.41	33.49	0.52	30.25	12.61	2.40
38.86	36.01	1.08	20.91	19.05	1.10
9.78	22.48	0.43	29.18	8.20	3.56
			31.18	24.07	1.30
			30.84	18.44	1.67
			21.48	28.29	0.76
			28.87	18.77	1.54
			24.06	17.32	1.39

Vandenbussche et al., 2009, Development 137, 597-606

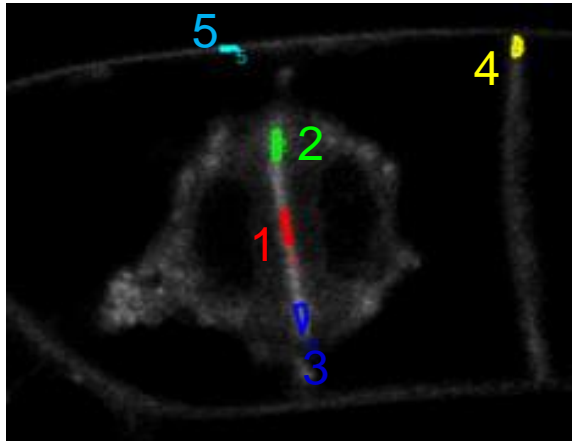
2.5 Image analysis, graphical software, presentation software

Interaction between two proteins - FRET analysis *in vivo* (PIN1-ADL1)



2.5 Image analysis, graphical software, presentation software

Interaction between two proteins - FRET analysis *in vivo* (PIN1-ADL1)

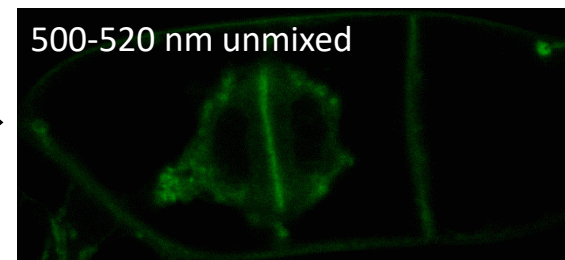
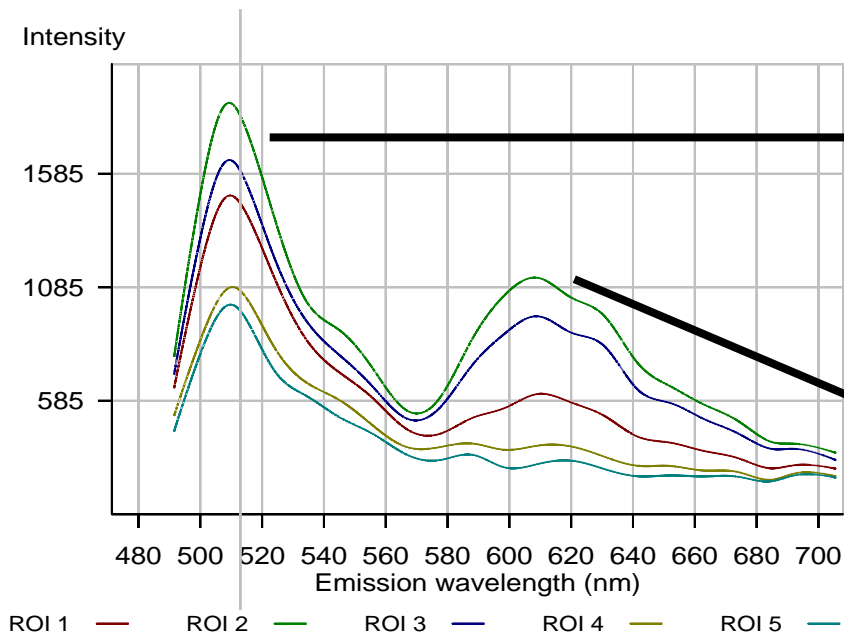


Average fluorescence intensity measured over the emission spectrum in the following region of interests:

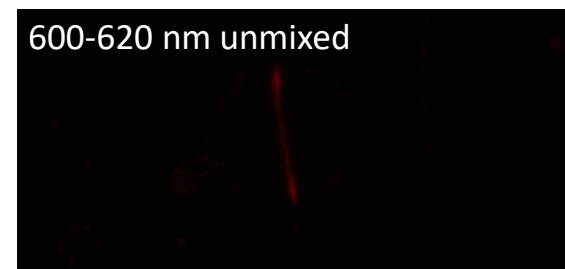
ROI1 - middle cell plate

ROI2, ROI3 - growing ends of the cell plate

ROI4, ROI5 - transversal and longitudinal plasma membranes



ROI1 - low FRET



ROI2, ROI3 - high FRET

ROI4, ROI5 - no FRET

2.5 Image analysis, graphical software, presentation software

Irfan View - the ideal tool for daily viewing of experimental documentation



IRFANVIEW GRAPHIC VIEWER

- ▶ Fast and compact (just 5 MB)
- ▶ Freeware for non-commercial use
- ▶ Supports Windows XP, Vista, 7, 8, and 10
- ▶ 32 and 64 bit version
- ▶ Multi language support
- ▶ Unicode support
- ▶ Designed to be simple but powerful

[More information about IrfanView](#)

GET IRFANVIEW (VERSION 4.58)

The program is available in 32 and 64 bit.
Which version should I download?
[See 64-bit info.](#)

32-BIT	64-BIT
 DOWNLOAD Current version 4.58	 DOWNLOAD Current version 4.58

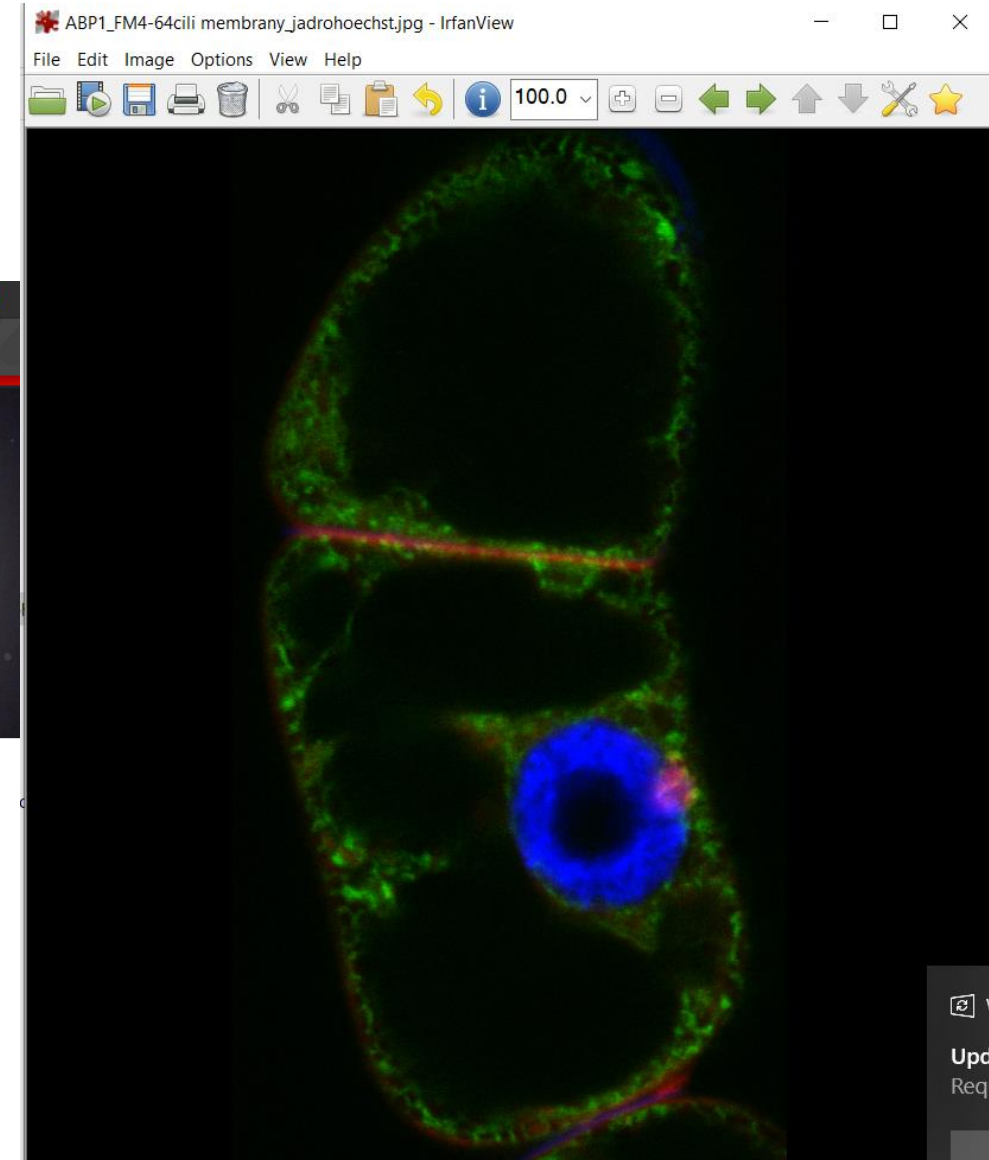
USEFUL PARTNER SITES

Stempel bestellen:
www.stempelSERVICE.de

scams.info:
www.scams.info

Lunacy, Sketch for Windows:
icons8.com

Top 5 UI/UX design agencies:
[unlabeled.com](#)



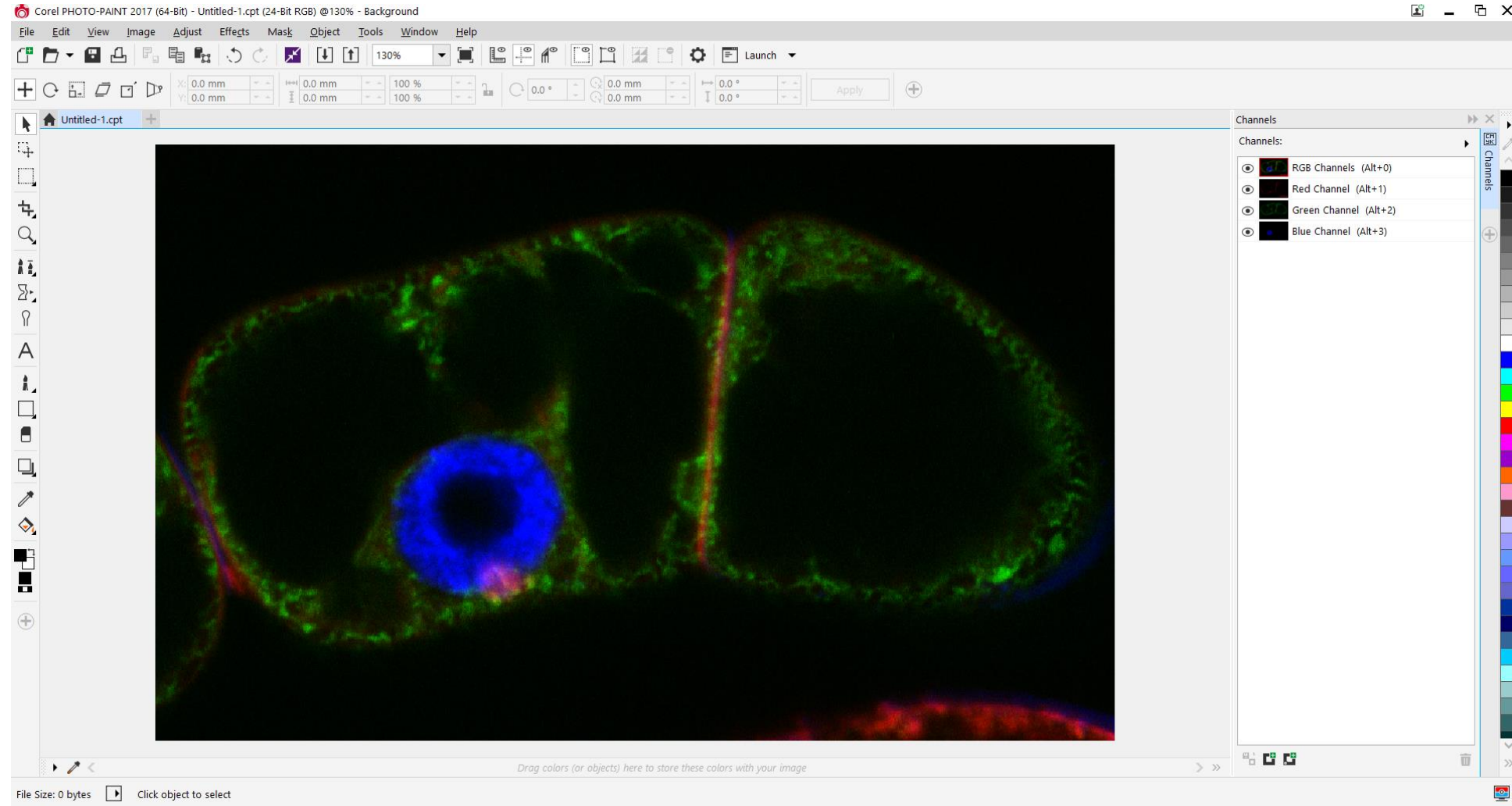
2.5 Image analysis, graphical software, presentation software

Raster graphics and its editing using software editors

Corel Photo Paint

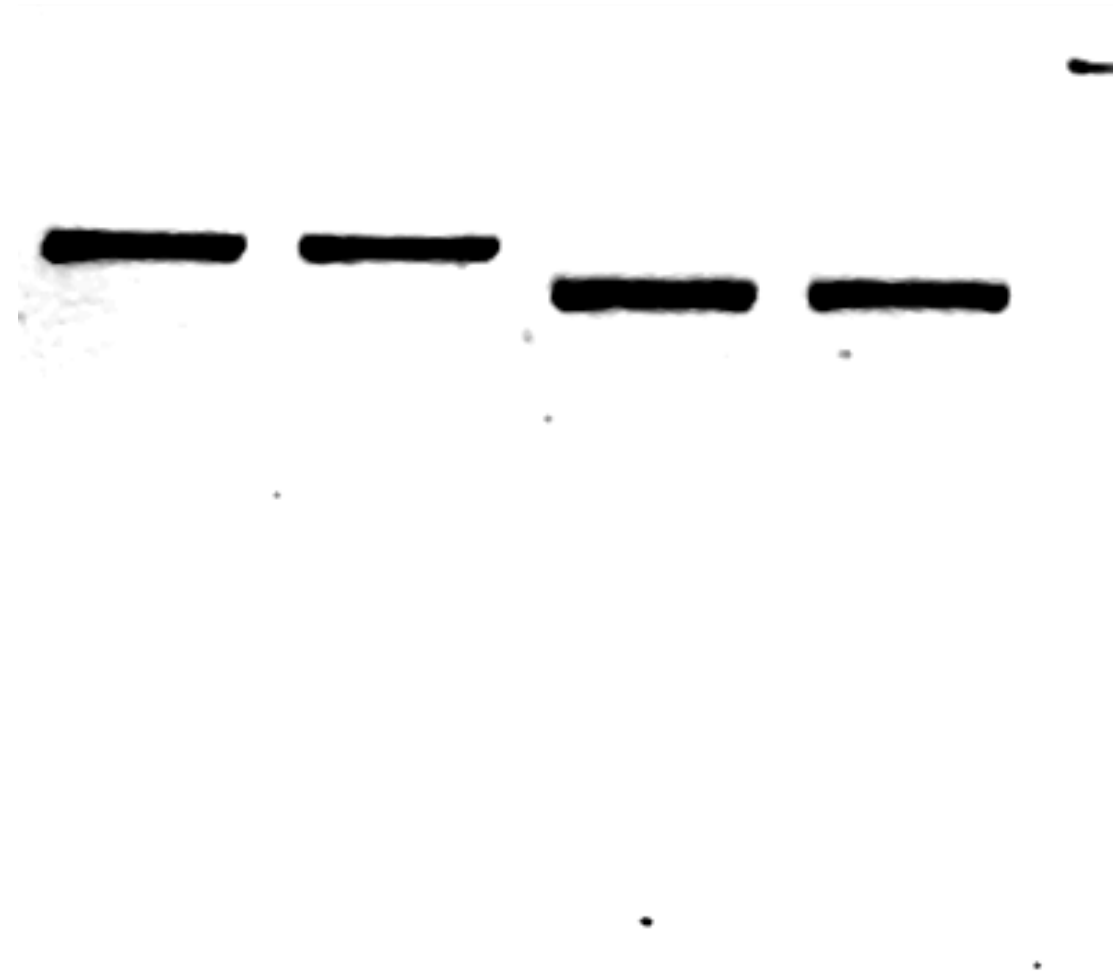
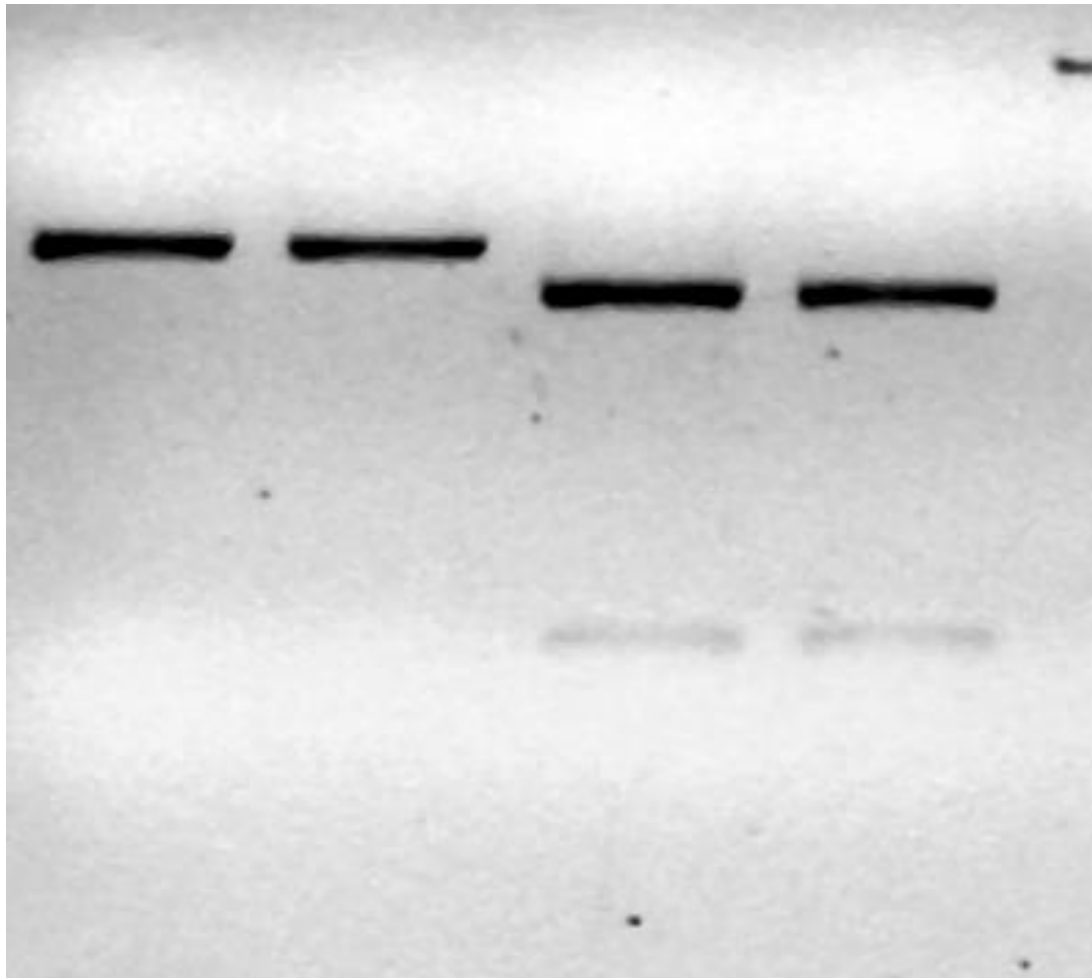


Adobe Photoshop



2.5 Image analysis, graphical software, presentation software

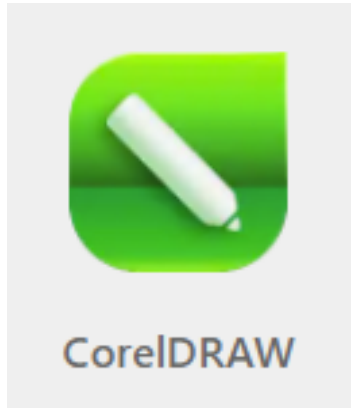
Beware of too extensive modifications!!!!



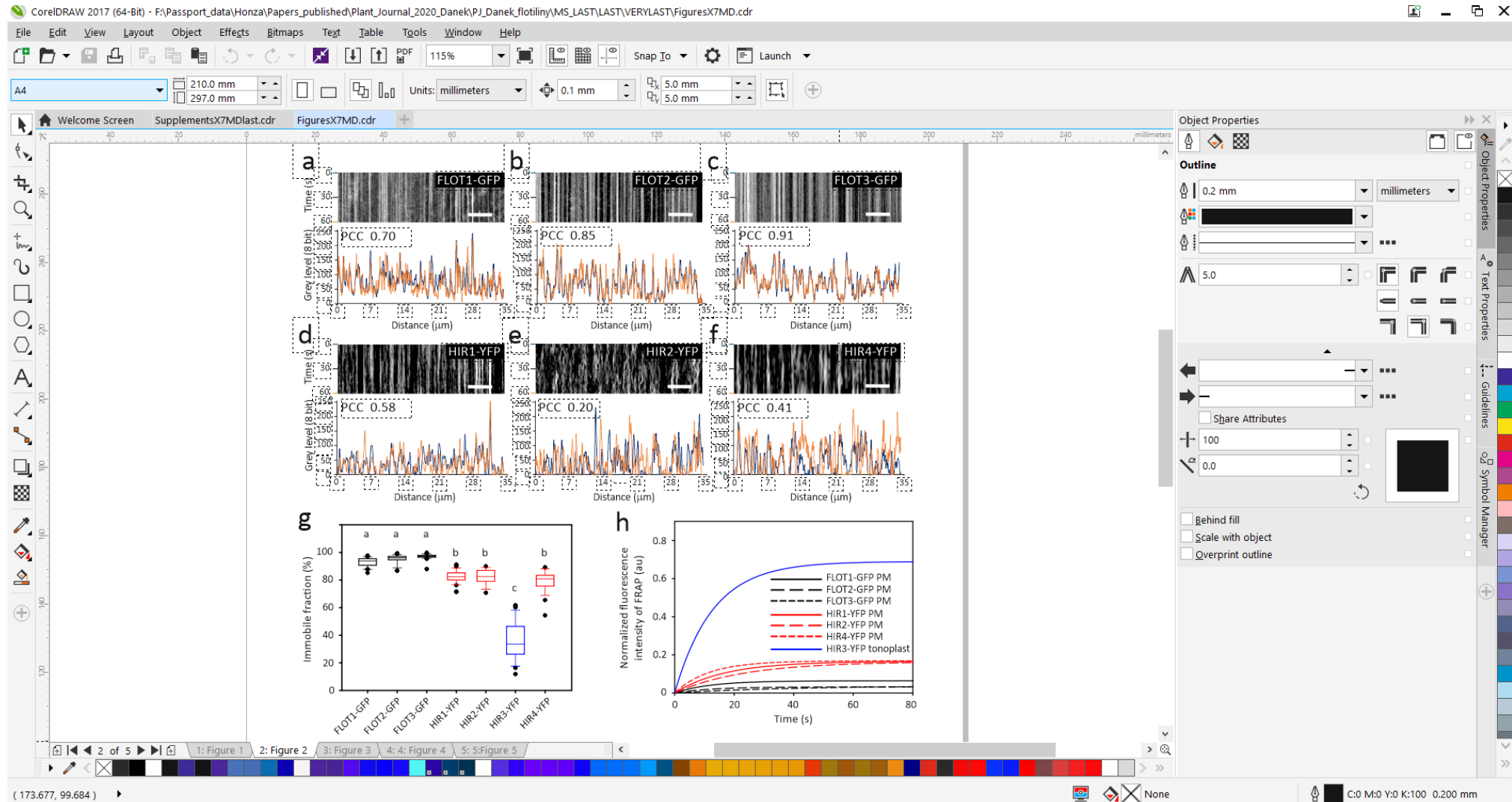
2.5 Image analysis, graphical software, presentation software

Vector graphics combined with raster graphics - ideal for creating images for scientific publications

Corel Draw



Adobe Illustrator



2.6 Open source graphical software

Both vector and raster graphics can be edited using freely available programs, but for the purpose of presenting scientific outputs these tools are rather not suitable

GIMP



Inkscape

