Scientific Software and Databases

Johannes A. Schmid





Bookmark slide





Software for	Commercial Software	Freeware
Writing Calculating Presenting	MS-Word MS-Excel MS-Powerpoint	OpenOffice LibreOffice Office-Online (Word, Excel,) Google Docs
Literature Citation managing	Endnote (Reference Manager)	Myendnoteweb Sciwheel (<u>https://sciwheel.com</u>) Zotero Bibus <u>Mendeley</u>
Pdf-editing	Adobe Acrobat Prof.	Pdf-XChange Editor
Image Processing	Adobe-Photoshop Adobe Illustrator both: (Vers. CS2:free)	Gimp, Irfanview, Paint.net InkScape
Image Analysis		ImageJ, Fiji, CellProfiler
Curve fitting Data analysis	GraphPad-Prism Sigmaplot, MS-Excel	CurveExpert
Cytometry	CellQuest, DIVA,	CytExpert Cytoflow
X-ray structure analysis		Chimera, Rasmol, RasTop CN3D (iCN3D)
Sequence analysis	Snapgene, VectorNTI CloneManager	CLC Sequence Viewer UGENE, <mark>SnapGene Viewer</mark> , SerialCloner, ApE
Network visualization		Cytoscape



Download-links for software

Online Office packages:

- OnlineWord, Excel, Powerpoint..: <u>https://www.office.com</u> (You need a free MS-account: hotmail or outlook) - GoogleDocs, Google sheets etc: <u>https://docs.google.com</u> ...

- OnlyOffice: similar to Word.. <u>https://www.onlyoffice.com</u>
- LibreOffice: <u>https://de.libreoffice.org</u>

- Offline alternatives to MS-Office

- OpenOffice: <u>https://www.openoffice.org/de/</u>
- *Mendeley*: <u>https://www.mendeley.com/</u> .. If you don't have Endnote
- MyEndnoteWeb: <u>https://www.myendnoteweb.com/</u> free alternative to Endnote
- Sciwheel: <u>https://sciwheel.com</u>
- *pdf-xchange* editor: <u>https://www.tracker-software.com/products</u>
- free (older) versions of Adobe Photoshop and Adobe Illustrator: <u>https://www.computerbild.de/download/Adobe-Photoshop-CS2-Vollversion-8040793.html</u> <u>https://www.computerbild.de/download/Adobe-Illustrator-CS2-Vollversion-8043129.html</u>
- *Gimp*: <u>https://www.gimp.org</u> , <u>Inkscape</u>: <u>https://inkscape.org/</u>
- Fiji-version of ImageJ: <u>https://fiji.sc</u>
- CellProfiler: <u>http://cellprofiler.org</u>
- Graphpad Prism (trial version): <u>http://www.graphpad.com/scientific-software/prism/</u>



Download-links for software II

- Cytometry: *Flowing Software*: <u>http://www.uskonaskel.fi/flowingsoftware/</u> Cytexpert: <u>https://www.beckman.com/coulter-flow-cytometers/cytoflex/cytexpert</u> Cytoflow: <u>https://cytoflow.github.io</u>
- 3D-molecular structure viewer *ChimeraX*: <u>https://www.cgl.ucsf.edu/chimerax/</u> *CN3D* (from NCBI): <u>https://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml</u>
- Sequence analysis (DNA, RNA, protein, plasmids): SnapGene Viewer: <u>http://www.snapgene.com/products/snapgene_viewer/</u> UGENE: <u>http://ugene.net</u> Serial Cloner: <u>http://serialbasics.free.fr/Serial_Cloner.html</u>
- Network visualization: *Cytoscape*: <u>http://www.cytoscape.org</u>
- LinRegPCR for realtime PCR (qPCR) analysis: <u>http://www.hartfaalcentrum.nl/index.php?main=files&sub=LinRegPCR</u>
- Plagiarism check: VIPER: <u>http://scanmyessay.com/</u>



Content of the 1st session

- MS-Word
- Endnote (citation manager software)
- Mendeley (free citation manager software)
- SciWheel Workspace
- PubMed database: professional search and link to citation manager



Microsoft-Word From 2003 to 2007-2010-2013-2016... Microsoft 365

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lt's a great way to keep track of where you are or quickly move your content around.	2 1	opens a detailed submenu			
To get started, go to the Home tab and apply Heading styles to the headings in your document.	- - - M				



Some Hints for MS-Word

- There are three different ENTER commands:
 - ENTER alone: new paragraph
 - SHIFT / ENTER: new line (e.g. in bulleted lists)
 - CTRL (Strg) / ENTER: new page
- <u>Use rather tables instead of tabulators (e.g.</u> when you write a CV; you can make the lines invisible)
- You can copy all different kinds of files into a Word-file and maintain the original file in the background (e.g. Excel graphs...) by OLE (<u>Object</u> <u>Linked Embedding</u>)
- You can generate <u>hyperlinks</u> to other files or websites
- Use "Insert symbol" for Greek letters instead switching to Symbol font (this maintains the symbols if you are switching the font later)
- You can apply the <u>auto-correction feature for frequently used text</u> (including formats such as symbols: e.g. "NF" is replaced by "NF- κ B" or TNF is replaced by TNF α)



Finding a command

 using the search window in the toolbar, you can find most commands quickly:

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	autocorrect" 0 results	
	See more search results for autocorrect	



Using the auto-correction feature

General abc Change how Word corrects and formats your text. Display AutoCorrect: German (Germany) Proofing Save Language Base of Access Advanced OutoCorrect optio Advanced OutoCorrect optione buttons German Use p Ignore words i Outoc Access Toolbar Add-ins Trust Center Suggest from Custom Dictions French modes: Spanish modes: Spanish modes: Spanish modes: Show readabil Check sgalling Math correctings Men correctings Men correctings Show readabil Check sgalling Show readabil Choose th check Writing Style: G	Word Options					?	×
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File Menu > Options > Proofing > Autocorrection - you can delete unwanted entries (e.g. DNA> DANN) - you can specify that it shouldn't capitalize letters after a period (e.g. abbreviation p53) - you can define your own abbreviation-autotext entries (even in a formated manner including Greek symbols e.g. NF > NF- κ B) - you can define auto-text entries, e.g. that "phos" is always extended to "phosphorylation"

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Customizing Word

There is a fast access toolbar that can be customized

	Word Options				?	\times
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Customizing Word



Language and spell checking settings



Language is usually automatically set. You might consider to set it specifically to US-English (e.g. if you are submitting a manuscript to a US-journal)



Spell-Checking in MS-Word



There is a typographic error in this sentence.

There is a grammatical inconsistences in this sentence.

abc Grammar >	Double-check whether the noun is singular or plural			
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[<u>∎</u> <u>С</u> ору	Ignore Once			
Paste Options:	D <u>o</u> n't check for this issue			
	Options for "Grammar"			
Search "inconsistences"	See More			

mistakes are underlined in red; right-clicking on the wrong word opens a context menu:

suggestions for the right word – or: option to add the unknown word to the dictionary

(the latter makes sense to add scientific terms to the dictionary). It's recommendable to use this feature for correct scientific writing.

You find your custom dictionary at:

C:\Users*username*\AppData\Ro aming\Microsoft\UProof: CUSTOM.DIC (can be transferred to other PC)



The Thesaurus: Shift-F7 (or right-click in a word)



gives you suggestions for synonyms
 (quite convenient, when you write a longer text, where you
 don't want to use the same words again and again)



Other useful features: Count words, characters

Word Count	?	×
Statistics:		
Pages	1	
Words	256	
Characters (no spaces)	1.450	
Characters (with spaces)	1.699	
Paragraphs	7	
Lines	24	

Include textboxes, footnotes and endnotes

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Background and Aims

It has been reported that IKB kinase 2 (IKK2), the central enzyme of the inflammatory NF-KB pathway, is involved in platelet activation as megakaryocyte/platelet-specific deletion of exons 6/7 of IKK2 resulted in platelet degranulation defects and prolonged bleeding. We aimed to investigate the role of IKK2 in platelet physiology in more detail, using a plateletspecific IKK2 knockout via excision of exon 3, which comprises the active site of the enzyme.

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Mice expressing Cre recombinase downstream of the megakaryocyte-specific Pf4-promoter were crossed with mice, where exon-3 of IKK2 was flanked by loxP sites. Excision was verified on the genomic level and deletion on the level of RNA and protein. Platelet function was investigated in vitro by aggregometry, flow cytometry and degranulation assays; as well as in vivo by measuring bleeding times and thrombus formation induced in mesenteric arteries by FeCl₃. A potential role of IKK2 in human platelets was studied by applying the specific IKK2 inhibitors TPCA-1 and BMS-345541 followed by flow cytometry.

Results

Platelets with a complete deletion of IKK2 did not show any functional impairment in vivo or in vitro. Bleeding time and thrombus formation were not increased. Moreover, platelet aggregation, GPIIb/IIIa activation and degranulation were unaltered. Pharmacological inhibition of IKK2 with TPCA-1 or BMS-345541 did not affect activation of murine or human platelets over a wide concentration range.

Conclusions

In contrast to previous claims, our results imply that IKK2 is not required for platelet function. However, this does not exclude an effect of inflammation or active IKK2 on platelet

D Focus

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Page 1 of 1 256 of 271 words

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reactivity.

Compare and merge documents (if several people work on the same document)





The Reviewing Mode of MS-Word: As Reviewer



Background and Aims

It has been <u>reported-shown</u> that IκB kinase 2 (IKK2), the central enzyme of the inflammatory NF-κB pathway, is involved in platelet activation as megakaryocyte/platelet-specific deletion of exons 6/7 of IKK2 resulted in platelet degranulation defects and prolonged bleeding. We <u>aimed-intended</u> to investigate the role of IKK2 in platelet physiology in more detail, using a platelet-specific IKK2 knockout via excision of exon 3, which comprises the active site of the enzyme.

- 1. Click on the reviewing tab
- 2. Activate the "track changes mode" (Änderungen nachverfolgen)
- 3. Type your changes into the document (deleted text will appear struckthrough), changes are marked in a different colour (according to the reviewer-computer settings)
- 4. If necessary: add comments
- 5. You should deactivate the trackchanges mode again at the end



The Reviewing Mode of MS-Word II: accept or reject changes done by the reviewer



of exons 6/7 of IKK2 resulted in platelet degranulation defects and prolonged b



Insert a Table of Contents

When you **define headings in your document**, these can be used for inserting a table of contents



Headings: usually: Ctrl +1 (Heading 1) Ctrl +2 (Heading 2)..

IKB kinase 2 (IKK2) is not essential for platelet activation

Background and Aims

It has been shown that IκB kinase 2 (IKK2), the central enzyme of the inflammatory NF-κB pathway, is involved in platelet activation as megakaryocyte/platelet-specific deletion of exons 6/7 of IKK2 resulted in platelet degranulation defects and prolonged bleeding. We intended to investigate the role of IKK2 in platelet physiology in more detail, using a <u>platelet-specific</u> IKK2 knockout via excision of exon 3, which comprises the active site of the enzyme.

Methods

Mice expressing Cre recombinase downstream of the megakaryocyte-specific Pf4-promoter were crossed with mice, where exon-3 of IKK2 was flanked by loxP sites. Excision was verified on the genomic level and deletion on the level of RNA and protein. Platelet function was investigated *in vitro* by aggregometry, flow cytometry and degranulation assays; as well as *in vivo* by measuring bleeding times and thrombus formation induced in mesenteric arteries by FeCl₃. A potential role of IKK2 in human platelets was studied by applying the specific IKK2 inhibitors TPCA-1 and BMS-345541 followed by flow cytometry.



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"Ctrl + click" on an entry lets you jump to the respective page of the document

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	Results	1
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Copying Excel Graphs into Word (as Excel file)

In Excel: click on the graph and copy it (Ctrl-C) > in Word (Home tab): click: Paste special: and paste as Excel object





Opening Excel Graphs in Word (as Excel file)



Double clicking on the graph in the Word file opens a small Excel Window (the frame changes): > you can change the appearance of the graph

(as in Excel)you can also go into the data and change the data that is shown etc.

This procedure can be done with every file that allows OLE (Object Linked embedding)



The different tabs and toolbars





The different tabs and toolbars





Developments since MS-Office 2013



- Usability on tablets and convertibles
- Strong connection to Onedrive the cloud-solution of Microsoft (with hotmail or outlook account)
- New licensing strategy: Microsoft365: always newest Office : (Word, Excel, Powerpoint, Outlook, OneNote, Access, Publisher): rental for students and academics possible – but not supported by our University for employees (due to data safety issues).
- Microsoft365 is free for most students with some restrictions at the Med.Univ Vienna (does not include the 1TB Onedrive space)
- pdf's can be converted in Word documents (format maintained, editable)
- Integration of online videos or pictures, recording of the screen...

Add-ins within Word 2013 and later





Apps in Word 2013







Mendeley Add-In for citations

Adding of figure legends

Eintrag

festlegen





You can open the same document twice (e.g. to work on 2 different positions simultaneously)

(Dragging the window to the left margin of the monitor will scale it exactly to the left half of the monitor >> and vice versa on the right side)



The Navigation Window

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Some features since Word 2016

- Better sharing options
- Authors can work simultaneously on the same document on the cloud (Onedrive)
 → You can work with others on a manuscript or grant proposal simultaneously and see changes in real time: BUT: frequent error messages with large or complex documents



can be checked and previous versions can be restored





Chat function for co-authors

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Smart Look up function within a document (by right-clicking a word)

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Inserting of 3D models

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Johannes A. Schmid, Inst. of Vascular Biology and Thrombosis Research

Some features of Office 365 users: Opening of Word-files, which had been received as email attachment:

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Translations within a document

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wir auch verstärkt mit der <u>Complex</u> Systems Grup	₽	Ne <u>u</u> er Kommentar		This requires the appropriate computer performance and the possibility to analyze

Translator

algorithms

Einfügen

larger amounts of data with more complex



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Working with images in Office files

Double click on an image and perform brightness/contrast adjustments, changes of colors or removal of background...





Working with images in Office files



Easier background removal





The "Researcher" function

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Weitere Themen

Speech recognition (Dictating text)

Included into the Start-tab of the latest version without the need of an Add-in





Newest functions in Office 365

Transparency of images



Integration of animated 3D





Google Docs for text documents



Good MS-Word-alternative for shared documents (joint publications, grant applications etc.)

Hämostaseologie Tagung 2022

Florian Prüller, Graz: Gerinnungsdiagnostik

1. Fall: aPTT sehr hoch 2. Fall: ähnlich: PZ: 14%, aPTT 150

Faktor VIII nimmt <u>durch Lagerung</u> rasch ab! (30% in <u>8h bei</u> 4°C) <u>Unterfüllung</u> von Röhrchen beeinflusst <u>aPTT</u> sehr stark. Hämolyse -> reduziert die Gerinnungszeiten -> bei <u>verringerter</u> Blutgerinnung kann es dann zu <u>rel.</u> normalen Werten kommen. Es gibt sehr unterschiedliche <u>aPTT</u> Reagenzien -> die Werte unterschiedlicher Labors können oft nicht verglichen werden.

Ingrid Pabinger: Hämophilie...

Fallbeispiel: Tonsillektomie -> Nachblutung Bei Blutungen: 72% : unbekannte Ursache. Häufig auftretendes Nasenbluten: wichtiges Indiz Hämophilie A: Faktor VIII reduziert; B: Faktor IX <u>Faktorspiegel</u> < 1% : schwere Hämophilie 5-40% : Schwere Blutung nur bei zusätzlichen Risikosituationen Unterschied moderat: mild: Gelenks- und Muskelblutung



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Google Docs features



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Google Docs features

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- Filters can be applied (left side)



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Search

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User Guide



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3 results

- Betulinic Acid-Mediated Apoptosis in Human Prostate Cancer Cells Involves p53
- 1 and Nuclear Factor-Kappa B (NF-kappaB) Pathways.
- Cite Shankar E, Zhang A, Franco D, Gupta S.

Molecules (IF: 3.27; Q1). 2017 Feb 10;22(2):264. doi: 10.3390/molecules22020264.



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Items 1-18 of 18 (Display the 18 citations in PubMed)

 <u>Reactive oxygen species trigger NF-κB-mediated NLRP3 inflammasome activation</u> involvement in low-dose CdTe QDs exposure-induced hepatotoxicity. Pang Y, Wu D, Ma Y, Cao Y, Liu Q, Tang M, Pu Y, Zhang T.



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Writing a research paper with co-authors

- Make sure you are using the same text software ideally one which allows working simultaneously on a shared document
- Make sure you are using the same citation management software: Sciwheel allows to share projects with your coauthors
- Agree on a common software for generating figures (recommendation: use freeware such as Gimp for bitmap images – and Inkscape to create scalable vector graphics – so that all co-authors, students etc. can use that software)



AI tools for scientific literature

- <u>https://sciwheel.com</u> > create projects for specific topics > AI will find suggestions for further literature fitting to the topics in the project
- https://www.semanticscholar.org/
- https://www.chatpdf.com/
- <u>https://typeset.io/resources/introducing-copilot-ai-assistant-explains-research-papers/</u>

https://typeset.io

https://elicit.org



Writing scientific manuscripts

- Professional translation of German phrases to English:
- <u>https://www.deepl.com/translator</u>

Apps for Windows, Mac, Android and iOS available (e.g., mark text in any application > press Ctrl + CC > marked text is copied to a DeepL window and translated – and you can then insert the translated text in your document)

• Improved writing: https://www.deepl.com/write#en/



Endnote TM

Software for scientific reference handling (citation manager)

- There are Plug-Ins for MS-Word and Open Office so that you do not have to type the citations – and it also takes over the formating and the numbering of the citations
- You can search PubMed directly from Endnote or Ref.Manager
- From most of the Journals there are export links available for these programs
- You can use these programs to generate your own specific literature database
- URLs (weblinks) and pdf-Links can be added to the references



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Connections to Databases (Pubmed)

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Formatting as requested by the editors of the Journals

"Output styles" - in the Edit Tab





Endnote- Journal Styles

Styles can be edited (e.g. to add doi-hyperlinks)

Formatting as requested by the FWF in grant application

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Link to MS-WordTM

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- The reference is inserted with the Journal style and a reference list is added at the end of the document (in the Journal style as well); this can be done immediately ("instant formatting" on) or after clicking: "Update Citations and Bibliography"
- When you add citations later (e.g. in between other citations), the program automatically updates the numbering of the references in the text ("Cite while you write" feature)

Link to MS-WordTM



 From a Word-file containing Endnote references an Endnote library can be extracted, which can then be extended (e.g. if 2 authors work on a manuscript) → you don't need to send Endnote libraries via email to co-authors, as they are already "embedded" into the Word file.



Groups and Smart Groups of references







Other Endnote features

- direct import of pdf-files (even of whole folders): the Journal information is detected in the pdf-file and imported into the respective fields (>useful to import old literature folders and generating Endnote files directly from them)
- MyEndnoteWeb a web version of the Endnote (to access endnote library files from other computers and to share citations)
- Auto-hyperlink between in-text citations and the bibliography in EndNote X4 and Microsoft Word
- Creating new groups by comparing, combining and suppressing existing groups.
- Wildcards (word stem plus "*")can be added within search terms for better search results (e.g. NF* for all versions of NF-kappa B)
- pdf-preview window (editable)



Importing of pdf-files into Endnote

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Reference Preview

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style of the citation, when the reference is inserted into a Word document



The pdf-preview and editing tab



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Endnote Full-Text Search feature

Open URL Path: currently not active for MedUni Wien

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Further features

- library sharing with up to 14 colleagues
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- Mac[®] and Windows[®] compatibility install on up to 3 computers of either type
- Background synchronization
- More than 6,000 reference styles
- Reference types such as interview, podcast, conference paper and press release
- Plug-in for adding citations to Microsoft[®] PowerPoint[®] slides (Windows only)
- Online storage of pdf-files: unlimited (X5 and X6: 5 GB, X4: 2GB)

Free online Endnote: MyEndnoteWeb

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Free online Endnote: MyEndnoteWeb

http://www.myendnoteweb.com

Plug-in available for inserting references into Word

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Cite While You Write™

Mit dem EndNotePlug-In können Sie Referenzen einfügen und Zitate und Bibliografien automatisch formatieren, während Sie Ihre Dokumente in Word erstellen. Darüber hinaus können Sie mit dem Plug-In Onlinereferenzen in Ihrer Bibliothek in Internet Explorer für Windows speichern.

US-Patent 8,082,241

Siehe Installationsanweisungen und Systemanforderungen.

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Übernehmen: Referenz erfassen

Um das Capture-Tool zu installieren, ziehen Sie einfach die Schaltfläche **Referenz übernehmen** auf die Lesezeichenleiste (auch als "Favoritenleiste" oder "Lesezeichen-Symbolleiste" bezeichnet). In manchen Browsern müssen Sie die rechte Maustaste klicken und "Zu Favoriten hinzufügen" oder "Zu den Lesezeichen hinzufügen" auswählen. Um das Tool zu verwenden, rufen Sie die gewünschte Seite auf

und klicken Sie in der Lesezeichenleiste auf die Schaltfläche **Referenz übernehmen**. Das Fenster "Referenz übernehmen" wird geöffnet. Folgen Sie den Anweisungen im Fenster.



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Mendeley-Freeware alternative to Endnote

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Mendeley Web Importer and Plugins for Word and OpenOffice





pdf's can be dragged to the program window and are recognized; they can also be edited in the software (highlighting, adding notes...)





Other Citation Managers

Zotero: <u>https://www.zotero.org/</u>



Zotero Standalone

Zotero Standalone runs as a separate application and plugs into your choice of browser.



 Papers (Mac and Windows): <u>http://www.papersapp.com/</u> Recommendation: When co-authoring a manuscript find an agreement on the citation software before starting to write.



Task1: Citing literature in text documents

(Pubmed / GoogleDoc / Sciwheel)

- Register for a gmail account (to use Google Docs)
- Register for an account on the Sciwheel literature database platform: <u>https://sciwheel.com</u> – and get the Browser extension of Sciwheel and the Google Addon (under Tools)
- Register for a Pubmed account and search for papers that contain the word roots "inflammat*" and "thrombo*" as well as the word "cancer" in the title and save it to your Sciwheel account.
- Start a new Google document and write any text.
- Insert the citation of Franco et al. 2015
- Format citations and bibliography with the style of "The Lancet"
- How many words does the bibliography have including the preceding number of the citation? (Highlight the text and use the tool: Word count)
 Put this number into the results field and upload a screenshot of your Google Doc.



Microsoft Excel ^{тм}

Some hints:

You can use auto-fill in functions:

- e.g. type in "0" and "0.2"
- click on lower right corner
- drag down up to the desired value
- the increment will always be as the first one
- this also works for dates, weekdays etc.

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Microsoft Excel ^{тм}

Some hints:

By default text is left-aligned and numbers are right-aligned

If you have troubles that numbers (with commas) are recognized as text: You can change that in the Excel options:

- specify whether "." or "," is used as decimal point

Excel Options

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Working with Excel: Calculations

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Working with Excel: Calculations

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Executing a calculation on a whole column



- Grabbing a calculation cell at the bottom right corner and dragging it down, executes the calculation based on the cells next to that column
- double clicking onto the bottom right corner of the cell with the equation, performs the calculation for the whole column (even if it's very long!) (the cursor changes its shape to a thin cross, when it is at that corner)



If you want to calculate a long column double click onto the lower right corner of the cell with the equation

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NF.

Tricks: If you want to keep a field constant in an equation: type a "\$" in between column-letter and row-number

× ✓	<i>f</i> _x =C22*1	100/C\$18	
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- clicking into an equation shows the cells that are used for it
- Formulas > Trace precedents: shows equation traces



Tricks: If you want to keep a field constant in an equation: You can also mark the cell (or the range) and press F4 It will automatically add the \$-signs correctly

×	\checkmark	$f_x = 18^*$	100/MAX(\$I\$8:	\$1\$15)
		Area	% of maximum	
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	3	14454.569	77.49908907	
	4	18651.276	100	
	5	6828.619	36.61207416	
	6	10535.861	56.48868742	
	7	10792.983	57.86726334	
	8	9740.569	52.22467889	



Checking whether a cell is contained in another column (somewhere)

=ZÄHLENWENN(\$F\$11:\$F\$1000;E11)

ENGLISH: "COUNTIE (*E\$11:*E\$1000;E11)

range to compare; specific cell

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Uniprot A	NK
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Q7Z6K4	93
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Removing of duplicate rows

- Select the range (or the whole sheet)
- In the data tools tab: click on "Remove duplicates"





Filtering of sheets





Filtering functions

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You can perform frequently used calculations by using predefined Excel sheets (as templates)

Excel-templates on my website (under "For Scientists"): http://www.meduniwien.ac.at/user/johannes.schmid/tools.html

MEDICAL UNIVERSITY OF VIENNA

Research Group of Johannes A. Schmid

	<u>Home</u>	<u>Team</u>	Projects	Publications	Lectures	For Scientists
Protoce	ols: mobile	e phone ve	ersion			MS-Excel Templates
• DNA-c	onstructs	(Google s	preadsheet)			DNA-calculations
Addger	ne plasmid	s	. ,			<u>Biorad Protein Assay</u>

- ___.
- <u>Links to plasmids</u> with maps (powered by GenomeCompiler)
- Research Material (plasmids, primers, cells)
- Molarity Calculator
- Macros for ImageJ

MS-Excel Templates

- DNA-calculations
- Biorad Protein Assay
- BCA-Protein Assay (Harald Freudenthaler)
- <u>FLIP</u>(fluorescence loss in photobleaching)
- FRAP (fluorescence recovery after photobleaching)

- BCA-Protein Assay (Harald Freudenthaler)
- FLIP (fluorescence loss in photobleaching)
- FRAP (fluorescence recovery after photobleaching)
 template for single values
 - template for single values
 template for 5 replicates + curve fit
 - template for 5 replicates + curve fittingNEW (Solver Add-In has to be activated)
- template for 5 replicates
- template for single values with control region
- FRET: correction factors, Youvan and Xia values
- Molarity calculations
- <u>realtime PCR</u> (quantitative PCR, qPCR)
 with dilution curve to determine PCR effciency
- <u>realtime PCR</u> with quantification of the PCR efficiency based on the amplification curve
- <u>SDS-PAGE</u>: MW-calculations





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Highlight a certain cell area

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- press "Strg" or "Ctrl" if you want to activate separate areas
- include the title row (if you want an appropriate legend)
- click the Insert tab and the desired graph type
 - note: don't use the "Line" type if you want to generate a normal x/y-graph, but the "scatter" type with connecting line ("Line" type generates equal x-axis distances regardless of the data)

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UU) OF VIENNA

Johannes A. Schmid, Inst. of Vascular Biology and Thrombosis Research

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 - press "Strg" or "Ctrl" if you want to activate separate areas
 - include the title row (if you want an appropriate legend)
- click the Insert tab and the desired graph type
 note: don't use the "Line" type if you want to generate a normal x/y-g type with connecting line ("Line" type generates equal x-axis distances

Changes can be made by right-clicking or double clicking graph elements



Format Axis

Axis Options 🗸

Text Options

×

- Clicking on the graphs activates additional tabs
 - (Chart Design, Format)
- Predesigned styles





 Quick Layout options. legends, titles... etc.









• Adding chart elements such as trendlines



• Right-clicking on data points or clicking on the "+" sign next to graphs allows adding a trendline, as well





Adding error bars

- · Chart elements: Error bars
- Choose the last option (more options)



Adding error bars

- Chart elements: Error bars
- Choose the last option (more options)
- Specify the column containing the error bars





Graph elements can be filtered





Generating graphs with two y-axes

• Right-click on a dataset > format data series





Generating graphs with two y-axes

- Right-click on a dataset > format data series
- Secondary Axis





Tabs

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Add-ins



Importing of data from text or csv sources...

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Transform Data

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Development tools: Macros, Add-Ins...

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Since Excel 2016



6 new graph types



Forecasting functions

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Forecasting functions

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3D maps

Tell me... function



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... suggest available functions, which can be executed quickly

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3-dimensional scattergramm (bubble charts)





 Colors of graph elements cannot be controlled by a numerical value (> R ggplot2 graphs)





Johannes A. Schmid, Inst. of Vascular Biology and Thrombosis Research

• Excel does not have sophisticated graphs like violin plots or parallel coordinate plots





• Excel has limitations for multi-dimensional graphs





• Excel has limitations for automated multiple graphs (like scatterplot matrices)





Data analysis and Solver functions

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Data Analysis Functions

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Tab: Data >



Use the Analysis ToolPak to perform complex data analysis

Excel for Microsoft 365, Excel for Microsoft 365 for Mac, Excel 2021, Excel 2021 for Mac, More...

If you need to develop complex statistical or engineering analyses, you can save steps and time by using the Analysis ToolPak. You provide the data and parameters for each analysis, and the tool uses the appropriate statistical or engineering macro functions to calculate and display the results in an output table. Some tools generate charts in addition to output tables.

The data analysis functions can be used on only one worksheet at a time. When you perform data analysis on grouped worksheets, results will appear on the first worksheet and empty formatted tables will appear on the remaining worksheets. To perform data analysis on the remainder of the worksheets, recalculate the analysis tool for each worksheet.

The Analysis ToolPak includes the tools described in the following sections. To access these tools, click **Data Analysis** in the **Analysis** group on the **Data** tab. If the **Data Analysis** command is not available, you need to load the Analysis ToolPak add-in program.

Link to website

z-Test: Two Sample for Means



Defining variables for equations

- 1. Parameters for equations can be defined in the Formula tab ("Define name") e.g. *k* and *d* of a linear equation
- 2. These parameters can then be used in the function mode in combination with cells
- 3. Changing the parameters (k and d) also changes the results in y-column

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Defining variables for equations

- 1. Parameters for equations can be defined in the Formula tab ("Define name") e.g. *k* and *d* of a linear equation
- 2. These parameters can then be used in the function mode in combination with cells
- 3. Changing the parameters (k and d) also changes the results in y-column
- 4. An equation can be used to calculate expected y-values (dependent values) for given x-values using any starting values defined for the variables. (in this case k and d as defined in the green cells; the x-value is taken from column B)

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Defining more complex equations



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Curve fitting with the Solver function



MEDICAL UNIVERSITY

OF VIENNA

- 1. An equation is defined, which should be used for curve fitting
- 2. The parameters for this equation are defined as names and some initial values are given
- This equation is used to calculate expected y-values (dependent values) for given x-values using any starting values defined for the parameters.
- 4. The difference between calculated values and measured vales is calculated and squared
- 5. The best curve fit for a given equation is obtained, when the sum of the squared differences is minimal – this can be optimized using the solver function.

Curve fitting with the Solver function II

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Example of a single and double curve fit with Excel

(from my website at: <u>http://www.meduniwien.ac.at/user/johannes.schmid/protocols.htm</u> - Excel templates: FRAP template for single values + curve fitting)

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Curve fitting incl. R² calculation

Example: dose-response curve fit (Link on my website)

R² = 1.0 - (SSres/SStot) SSres: Sum of squared residuals (sum of squared differences (measured – fitted)) SStot: Sum of squares total: sum of squared differences (measured – mean of measured)

	А	В	С	D	E	F	G	Н	I.	J	K	L	М	Ν
1	Put your dat	ta into the green cells, t	he rest is calcu	lated, if necessary	you can extend the	columns								
2	log(agonist)) vs. response Varia	ble slope (fou	r parameters)										
3	Y=Bottom +	(X^Hillslope)*(Top-Bot	tom)/(X^HillSl	ope + EC50^HillSlop	pe)									
4	bottom	0												
5	Hillslope	0.815975958		mean y	SSres: sum of (fitte	ed-measured) ² :	12.88988	SStot: sum of (fi	tted - mean) ² :	15649.7505				
6	Тор	89.19929346		78.8132	(Sum of squared re	siduals)		(Sum of squares	total)					
7	EC50	444.5469734												
8	dose ng/ml	response measured	resp_fitted	fitted-measured	(fitted-measured) ²	2		(fitted - mean) ²						
9	100000	90	88.13779	-1.86221	3.46781			86.947612						
10	10000	80	82.68093	2.68093	7.18738			14.95913732						
11	1000	60	58.83584	-1.16416	1.35528			399.0960043						
12	100	20	20.37351	0.37351	0.13951			3415.199886						
13	10	3	3.85915	0.85915	0.73815			5618.112589						
14	1	0.57	0.61198	0.04198	0.00176			6115.43523						
15										Data > Solver				
16								Solver Parar	neters					X
17	100					$R^2 = 1.0 - (SS)$	res/SStot)							
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Johannes A. Schmid, Inst. of Vascular Biology and Thrombosis Research

Curve Fitting Software: CurveExpert: Fitting many equations in a batch mode

- <u>https://www.curveexpert.net/</u> free trial version with quite low restrictions available, commercial: not that expensive (79\$)
- Data can be copied/pasted from Excel
- Data points are fitted against about 60 built-in equations, which are then scored according to the goodness of fit

		Name	Kind	Score
CurveFinder	×	🐼 Ratkowsky Model	Regression	995
	CurveFinder calculates all results for your data and searches for the best fit.	Logistic	Regression	995
		Sector Logistic Power	Regression	994
	Linear Regression	🐼 Vapor Pressure Model	Regression	991
	Polynomial Regressions from degree 2 🛓 to 20	🔗 Gompertz Relation	Regression	990
		🔊 DR-LogProbit	Regression	990
	Built-in nonlinear regressions (or available)	🔗 Gaussian Model	Regression	989
-	Custom nonlinear regressions (2 available)	🚰 Hoerl	Regression	984
	Savitzky-Golay Smoothing	💑 Rational Model	Regression	975
Weighting: Default ~	All Off	💑 Modified Exponential	Regression	965
		🔊 Lowess Smoothing	Smoother	964
		🔗 Sinusoidal	Regression	958
	OK Cancel	🚱 Weibull Model	Regression	949
		Modified Hoerl	Regression	938
		A Bleasdale-VD	Regression	932



Curve Fitting Software: CurveExpert

 Double clicking on a model opens a detailed graph-result Data: COVID infections, Austria, 1st wave





QTiPlot – a powerful Excel alternative

- Small (20MB) and powerful spreadsheet program available in many languages: <u>https://www.qtiplot.com</u>
- Rich graph features beyond Excel graphs
- Sophistical curve fitting is possible
- Statistical analyses beyond Excel
- Available as professional software for 10€ from our IT-dept.



QTiPlot – graphs

stacked curves

70

60

50

40

30

20

10

700

Intensity (a. u.)



violin plots



contour plots and heatmaps







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QTiPlot – data analysis

- Correlation, interpolation smoothing
- Curve fitting: Logistic, Boltzmann, Gauss, Lorentz, Multi-peak fit; custom equations







QTiPlot – statistics and data handling

- Non-parametric tests like Mann-Whitney,
- Different types of ANOVA + posthoc tests (Bonferroni, Tukey, Sidak..)
- data exchange with Excel (including graphs with OLE), Origin, LabView
- Import from SQL-databases, MS-Access and Matlab
- direct data import via serial port
- Python scripts and automated data analysis
- Plots can have 2 or more layers





Curve Fitting Software: GraphPad Prism

- <u>www.graphpad.com</u>
- Rental license available at the MedUni (84€/year)
- Data can be copied from Excel
- Structured according to scientists' needs
- 1st step: Definition of the data structure

here: version 5

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	Welcome to GraphPad Prism	×
SraphPad PRIM®		Version 5.01
Learn to use Prism Open a file	Available analyses • Linear regression • Nonlinear regression • Correlation (Pearson or Spearman) • Organization of data table	
New table & graph:	Sample data	
vv	Start with an empty data table	
AT	O Use sample data Dose-response - X is log(dose)	\sim
Column	Choose a graph	
Grouped	Table only No graph	
	Subcolumns for replicates or error values	
Survival	X error bar: Enter X error values to plot horizontal error bars	
Clone from:	Y: C Enter and plot a single Y value for each point	
Opened project	💿 Enter 🔋 📑 replicate values in side-by-side subcolumns,	
Describert	and plot Mean and Error 🗸 SEM 🗸	
Recent project	Enter and plot error values already calculated elsewhere	
Saved example	Enter: Mean, SD, N	
Shared example		
	Cancel	Create

GraphPad user interface

5.4

Data tables → (data can be excluded with "Ctrl-E")

<u>Results</u> tables \rightarrow

<u>Graphs</u>: automatically generated (including error bars, if replicates or SD are specified)

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GraphPad Graphs

- error bars are drawn automatically
- graphs and results
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 the same
 data sheet
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Analyzing data (curve fitting)

			Grap	phPad Prism
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MEDICAL UNIVERSITY	Johannes	A. Schmid. Inst. of		Help Cancel OK

Analysis options

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	Pa	arameters: N	onlinear Re	gressio	n	
Fit	Compare Constrain	Weights Initial v	alues Range	Output	Diagnostics	
Choo	se an equation					
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- One can choose a fitting algorithm from a large set of equations
- If you are not sure about the correct option you can click on "Learn about this equation"



Equation: Two phase association

M-

Optionen

GraphPad Prism 5 Help

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Introduction

9

Drucken

An exponential decay equation models many chemical and biological processes. It is used whenever the rate at which something happens is proportional to the amount which is left.

A two-phase model is used when the outcome you measure is the result of the sum of a fast and slow exponential decay.

Entering data

Create an XY data table. Enter time into X, and response (binding, concentration ..) into Y. If you have several experimental conditions, place the first into column A, the second into column B, etc.

After entering data, click Analyze, choose nonlinear regression, choose the panel of exponential equations, and choose Two phase association.

Consider constraining Plateau to a constant value of zero

If you have subtracted off any background signal, then you know the curve has to plateau at Y=0. In this case, you should constrain the parameter Plateau to be a constant value equal to zero. To do this, go to the Constrain tab of the nonlinear regression dialog, set the drop down next to Plateau to "Constant equal to" and enter the value 0.0.

Model

SpanFast=(Plateau-Y0)*PercentFast*.01 SpanSlow=(Plateau-Y0)*(100-PercentFast)*.01 Y=Y0+ SpanFast*(1-exp(-KFast*X)) + SpanSlow*(1-exp(-KSlow*X))





Fitting parameters I

Fit Compare Constrain Weights Initial values Range Output D What question are you asking? Image: No comparison Image: No comparison Image: No comparison	liagnostics
What question are you asking?	
No comparison	
<u> </u>	
O For each data set, which of two equations (models) fits best?	
O Do the best-fit values of selected parameters differ between data sets?	
\bigcirc For each data set, does the best-fit value of a parameter differ from a hyp	pothetical value?

Parameters: Nonlinear Regression

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Fit Comp	pare Const	rain Weights	Initial values	Range	Output	Diagnostics	
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Fitting parameters II



Fitting parameters III

Parameters: Nonlinear Regression	×
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Define the curve	
Minimum X value: O Choose automatically	Fit Compare Constrain Weights Initial values Range Output Diagnostics
◯ Start the curve at X = 0.0	Summary table of the best-fit values of selected parameters
Maximum X value: Ochoose automatically	Create summary table and graph
C End the curve at X = 9.60055	Parameter(s) to include Create a:
Table of XY coordinates Create a table of XY coordinates of 150 points that define the curve Check this option only if you want to copy or export the curve to another program	 ✓ Plateau ✓ ParcentFast ✓ KFast ✓ KSlow ✓ Fast HalfLife ✓ Slow HalfLife ✓ Ratio of rate const ● Bar graph labeled with "A", "B" ● State and the sta
	Report: Parameters with SE V
	Additional output
	Dose-ratios for Schild plot
	Ki from IC50. KD= [ligand]=
	Location of interpolated X values
	Column, with replicate values stacked Y column, maintaining the side-bu-side arrangement of replicates
	Number of digits in output
MEDICAL UNIVERSITY OF VIENNA Johannes A. Schmid	Show 4 significant digits

Diagnostics of curve fitting

Darameters: Nor	linear Regression
Parameters. Nor	
Fit Compare Constrain Weights Initial valu	ues Range Output Diagnostics
Do the initial parameter values define a cur	ve near the data?
O Don't rit the curve. Instead plot the curve derin	ted by the initial values of the parameters
Fit the curve. Maximum number of iterations	1000
How precise are the best-fit values of the p	oarameters?
SE of parameters	
✓ Cl of parameters: 95%	Dutput Format: Range ("1.23 to 4.56")
✓ Plot 95% confidence band ∨	
How to quantify goodness-of-fit?	
🗹 R squared 🛛 🗹 Sum-of-Squares 🖓	✔ Sy.x
Normality tests. Are the residuals Gaussian	?
 D'Agostino-Pearson (recommended) 	
🗌 Shapiro-Wilk	
Kolmogorov-Smirnov (not recommended)	
Does the curve systematically deviate from	the points?
Runs test Replicates test	🗹 Residual plot (create a separate graph)
Are the parameters intertwined or redundan	ıt?
Covariance of parameters	Dependency
Could outliers impact the results?	
 Count the outliers 	
Would it help to use stricter convergence of	criteria?
Medium 🗸 🗹 Automatically switch to	o strict convergence when needed
✓ Make these diagnostics choices the default for fit	uture fits





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Results Window

Ì	Nonlin fit Table of results	A Data Set-A				
1		Y				
1	Two phase association					
2	Best-fit values					
3	Plateau	80.74				
4	YO	30.54				
5	PercentFast	59.48				
6	KFast	0.2918				
7	KSlow	2.130				
8	Fast HalfLife	2.376				
9	Slow HalfLife	0.3254				
10	Ratio of rate const	0.1370				
11	Std. Error					
12	Plateau	1.012				
13	YO	1.234				
14	PercentFast	4.611				
15	KFast	0.04414				
16	KSlow	0.4424				
17	Ratio of rate const	0.01844				
18	95% Confidence Intervals					
19	Plateau	78.75 to 82.72				
20	YO	28.12 to 32.96				
21	PercentFast	50.44 to 68.52				
22	KFast	0.2053 to 0.3783				
23	KSlow	1.263 to 2.997				
24	Fast HalfLife	1.832 to 3.377				
25	Slow HalfLife	0.2313 to 0.5488				
26	Ratio of rate const	0.1008 to 0.1731				

- gives the fitted values for equation variables, their standard error, 95% confidence intervals, the goodness of fit (R²) and diagnostic parameters

Goodness of Fit	
Degrees of Freedom	787
R²	0.8495
Absolute Sum of Squares	15043
Sy.x	4.372
Normality of Residuals	
D'Agostino & Pearson omnibus K2	1.149
P value	0.5629
Constraints	
Plateau	Plateau < 100.0
YO	Y0 > 0.0
PercentFast	0 < PercentFast < 100.0
KFast	KFast > 0.0
KSlow	KSlow > 0.0
Number of points	
Analyzed	792
Outliers (not excluded, Q=1.0%)	16

Analysis Change Arrange Draw Write Text Image <

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∃Sho₩ a	rea fill						
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Position:	Below	\sim					

< the appearance of individual datasets can be changed (or all at once) < plotting of SEM or SD error bars can be set < symbols, borders, lines, error bars and legends can be defined



Exporting of high resolution graphs

You can either copy/paste the graph (e.g. into Word as GraphPad object) or export it with defined specifications (e.g. at high resolution for publication: monochrome, TIF, 1200 dpi)

2								
12	<u>F</u> ile	<u>E</u> dit	<u>V</u> iew	<u>I</u> nsert	<u>C</u> hange	<u>A</u> rrange	<u>W</u> indow	<u>H</u> elp
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	Exp	ort						
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	Sen	d to W	ord				Ct	l+K





Creating layouts of several graphs

Create New Layout
 Add one more graph to the page Array of graphs: 1 v across by 1 v down Standard arrangement Arrangement of graphs Image of graphs will be nicely aligned
Orientation:
Background color:
Include master title on top of page
Help Cancel OK





Calculating values from a standard curve (after non-linear regression). Example: dose-response

Prism	File	Sheet	Undo	Clipboard	Analysis	Cha	nge	Import	Draw	Write	Text	Export P	rint S
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		🗙 🔆 New 🗸	1 7-	6 •	💳 Analyze 🛅	🍯 ## 🗷	1.23	XML		ΤΤα	$\overrightarrow{\mathbf{A}} \stackrel{\bullet}{\mathbf{A}} \stackrel{\bullet}{\mathbf{B}} I \stackrel{U}{\underline{U}} \mathbf{X}^2 \mathbf{X}_2 \stackrel{\bullet}{\underline{\Box}} \stackrel{\bullet}{\mathbb{P}} \equiv \mathbf{\overline{\cdot}}$		3
	amily		Та	ble format:	X			Α					_
ė. 👝 🛛	Data Tables	;		XY	og(Conce	entration	Da	ta Set-	A		How the data are organized		
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Ī[👌 RIA dat	a		Titlo		6.0	7	51	77	1	rows represent unknowns.		
🔁 L	ayouts			Title		-0.0		26	200	2	The goal		
⊡ ⊡ F	loating No	tes	0	The		-5.0	3.	30	300		To fit a dose-response curve, a	nd then	
<u>+</u> …[🗎 Data wit	th notes	6	litle		-4.0	- 32	28	212	2	Interpolate concentrations for the	ne three	
			7	Title		-3.0	20	07	307	7	unknown values.		
			8	Unknown	1		112	23			How to fit a dose response cu	rve	
			9	Unknown	2		134	45			1. Click Analyze, choose Nonline	ear regres	ssion
			10	Unknown	3		14	56			Dose-response (inhibition) panel	of equat	ions
			11	Title			98	87			and choose the equation: [Ant	agonist] v	/s.
			12	Title							2. Check the option on the bott	om of the	e first
			13	Title							tab of the nonlinear regression of	lialog:	
			14	Title	_						Interpolate unknowns from star	dard cur	ve.
			15	Title	_						 LOOK at the graph to make su fit nicely. 	re the cu	irve
			16	Titlo							4. To find the concentrations co	rrespond	ing
			17	Title							to the unknown values, go to the	e results	76

Calculating values from a standard curve (after non-linear regression). Example: dose-response

Fit	Compare	Constrain	Weights	Initial values	Range	Output	Diagnostics	
Chas		ation						
Lnoos	se an equ	ation						
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	i wo p	nase associ	ation					Details
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	Dose-res	ponse - Jr	hibition					
E E	Dose-res	nonse - Si	necial					
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E E	Bindina -	Competiti	ve					
	Binding -	Kinetics						
+	Enzymel	kinetics						
÷	Exponen	tial						
+	Lines							
÷	Polynomi	ial						
+	Gaussiar	n						
÷	Sine way	/es						
+	Classic e	equations (from prior	versions of	Prism			
-If) -If) 0.0 log	X is not alre you have su). (inhibitor) va	ady the log ubtracted off s. response	of dose, go fany basa Variable :	back and tran response, con slope	sform you Isider con	r data. straining E	Bottom to a co	nstant value of ut this equation
Fitting	1 method							
	east square	es (ordinaru)	fit OF	Robust fit	Automa	tic outlier	elimination	
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	iterpolate u	riknowns ffo	nn standart	i curve, conna	ence intel			
					Lea	am	Cancel	OK







Statistics with GraphPad-Prism

http://cdn.graphpad.com/docs/prism/6/Prism-6-Statistics-Guide.pdf Excellent Statistics Guide! (402 pages).

GraphPad PRIM®	
Learn to use Prism Open a file	Available analyses • t test (one-sample, paired and unpaired) • Mann-Whitney • Wilcoxon • Column statistics (including normality tests) • Correlation matrix
New table & graph.	Sample data
XY	Start with an empty data table
Column	Use sample data How is a Column data table organized?
Grouped	Choose a graph How is a Column data table organized? Making a column bar graph Frequency distribution and histogram
Contingency	t test - Unpaired t test - Unpaired
Survival	Crest - One sample One-way ANOVA - Ordinary One-way ANOVA - Repeated measures Dec-way ANOVA - Repeated measures
Clone from:	Bland-Altman method comparison
Opened project	Selected graph: Scatt Metanalysis (Forest) plot
Recent project	Plot: Mean with SEM ~
Saved example	



ANOVA (Analysis of Variances)

	Α	В	С	D							
	Control	Treated	Treated+Antagonist	Title	٦						
	Y	Y	Y	Y							
1	54.	87	45		_						
2	23	98	39								
3	45	64	51								
4	54	77	49								
5	45	89	50								
6	47		55								
7											
8	How the data are organized										
9	The columns	define three	treatments. Note th	at, unlike many							
10	statistics programs, Prism does not define groups by using a grouping variable. Instead, the groups are defined by the columns. Note that one value is missing. This is fine for ordinary										
11											
12	one-way ANOVA (but not for repeated measures).										
13	The goals										
14	- To determine if the differences between the group means										
15	- To determin	ie the 95% c	onfidence interval fo	r the difference							
16	between the	e pairs of gro	oup means (post test	s).							
17	How to perfe	orm one-wa	Y ANOVA								
18	Click Analyze	e, choose on	e-way ANOVA from t	he list of column							
19	analyses, and Click the link	then accep below for de	t all the default choi tailed instructions, a	ces on the dialog. nd to learn about							
20	one way ANOVA.										



ANOVA

Choose test									
You may either choose a test by checking the two option boxes, or you may choose a test by name below.									
Repeated measures test. Values in each row represent matched observations.									
Nonparametric test. Don't assume Gaussian distributions.									
Test name: One-way analys	Test name: One-way analysis of variance								
Post test									
Test name: Bonferroni: Com	est name: Bonferroni: Compare all pairs of columns.								
Significance level. Alpha	= 0.05 (95% confidence intervals)	~							
Control column:	A:Control \sim	Select							
Significant digits									
Show 4 🗸 significant digits									
Output Create a table of descriptive	ve statistics for each column								

ANOVA alone just tells you, whether the columns (groups) are the same or not; it does not tell you, which groups differ significantly from each other. The latter question can be answered with a "Post test" such as Bonferroni



ANOVA Results

	One-way ANOVA		One-way ANOVA uala
Table Analyzed	data	150	
One-way analysis of variance			
P value	< 0.0001		
P value summary	***		
Are means signif. different? (P <		100-	
0.05)	Yes		
Number of groups	3		
F	22.57		_
R squared	0.7633		
		50-	
Bartlett's test for equal variances			-
Bartlett's statistic (corrected)	2.986		
P value	0.2247	•	
P value summary	ns		
Do the variances differ signif. (P <		0	
0.05)	No	Cont	rol Treated Treated+Antagonist
ANOVA Table	SS	df	MS
Treatment (between columns)	4760	2	2380
Residual (within columns)	1476	14	105.4
Total	6236	16	

One way ANOVA data



Results of the Post-test (Bonferroni) to detect, which groups differ from each other

Bonferroni's Multiple			Significant?		
Comparison Test	Mean Diff.	t	P < 0.05?	Summary	95% CI of diff
Control vs Treated Control vs	-38.33	6.165	Yes	***	-55.23 to -21.43
Treated+Antagonist Treated vs	-3.500	0.5904	No	ns	-19.61 to 12.61
Treated+Antagonist	34.83	5.602	Yes	***	17.93 to 51.73



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ROC curves in GraphPad Prism

(Receiver Operator Characteristics – to choose a cut-off value that separates for instance normal from disease

Prism	File	Sheet	Undo	Clipboard	Analysis		Change	Import	Draw	Write		Text	
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Family Data Tables ROC curve data		đ	Α	B		C D		D		E	F		
			Controls	Patients		Title	Т	itle	Ti	tle	Title	T	
			Y	Y		Y		Y		Y	Y		
	nro DProjecti	info 1	1	97.9	112.	7							
Results			2	94.9	9 104.	0	How the data are organized						
Graphs			3	98.6	5 12 6.	7	A lab test was performed in controls and patients. These two groups are defined by two columns. Note that, unlike many statistics programs, Prism does not use a grouping variable. Instead, the groups are defined by columns. The values in each row are not paired in any way. Also note that the sample sizes are not equal, which is fine. The goal You want to choose a cutoff value that separates 'normal' from 'abnormal' test results. To help make the decision, plot the tradeoff of sensitivity vs. specificity as a Receiver Operator Characteristic (ROC) curve						· -
ROC curve data		4	77.3	3 123.	3								
Gaybuls Gaybuls Floating Notes Gaybuls Gaybuls			5	97.9	9 120.	5							
			6	99.	7 130.	3							that
		7	83.0) 129.	6								
		8	102.	5 140.	2								
		9	104.	5 119.	7	m							
			10	108.9	9 139.	9							
			11	93.2	2 134.	2							
			12	101.3	3 137.	5							
			13	99.8	3 131.	2	Oetailed help for creating and understanding a ROC curve						
1				1	-			1		1		I	1


Analyze > column analysis > ROC





ROC curve concept



In a Receiver Operating Characteristic (ROC) curve the true positive rate (Sensitivity) is plotted in function of the false positive rate (100-Specificity) for different cut-off points. Each point on the ROC plot represents a sensitivity/specificity pair corresponding to a particular decision threshold. A test with perfect discrimination (no overlap in the two distributions) has a ROC plot that passes through the upper left corner (100% sensitivity, 100% specificity). Therefore the closer the ROC plot is to the upper left corner, the higher the overall accuracy of the test (Zweig & Campbell, 1993).











Missing Controls

Missing Patients

0

0

Kaplan Meier analysis (survival curves)

SraphPad PRIM®				
Learn to use Prism Open a file	Available analyses • Kaplan-Meier • Log-rank • Wilcoxon-Gehan Organization of data tat	ole		
New table & graph:	Sample data Start with an empty Use sample data	data table Comparing two groups		~
Column	Choose a graph			
Grouped	<u> </u>			
Contingency	Selected graph: Sta	ircase, points, no error bars (s	starting at 100%)	
Survival	Show result as:	Plot symbols at:	Error bars:	
	 Fractions 	All points	None	
Clone from:	Percents	Censored points only	⊖ se	
Opened project			🔘 95% CI	
Recent project				



The data table structure of Kaplan Meier analysis

🗉 🛅 Family	Table	format:	X	Α	В	С	D	E	F	G
📄 🛅 Data Tables	Sur	vival	Days	Standard	Experimenta	Title	Title	Title	Title	Title
Two groups		X	Y	V	V	<u>v</u>	N N	N/	N/	v
- 🕞 Info			^	<u> </u>						
Project info 1	1		90	1		How th	e data are org	anized		
🖓 🦳 Results	2	Title	142	1		Each ro	w represents or	ne subject. The	X values are tir	ne. The Y
Survival of Two groups	3	Title	150	1		values a	are entered into The Y value is	"1" when the s	nat define the ti ubject died at t	reatment he
Graphs	4	Title	269	1		specifie	d time, and "0"	when the subje	ct's data was o	ensored at
Layouts	5	Title	291	1		that tim	ne (either becau	ise we don't kno	w what happen	ed after
- 🫅 Floating Notes	6	Title	468	0		protoco	l was not being	followed). Not	te that unlike so	ome
🗄 🛅 Data with notes	7	Title	680	1		program	is, you don't en	ter group ID int	o a column in Pr	ism, but
	8	Title	837	1		from: Ta	able 3.3 of D Ma	chim, YB Cheung	g, and MK Parm	ar, Surivival
	9	Title	890	0		Analysis	: A Practical App	proach. Second e	edition, John Wile	ey, 2006.)
	10	Title	1037	1		The go	als			
	11	Title	1090	0		- To cre	eate a Kaplan-M	leier survival cu	rve.	
	12	Title	1113	0		- lo de is mo	termine whethe pre than expect	r the difference ed by chance.	between surviv	al curves
	13	Title	1153	1				,		
	14	Title	1297	1		Surviva	g the results Lanalysis is unic	ue. You don't n	eed to click the	Analyze
	15	Title	1429	1		button	because Prism a	utomatically an	alyzes survival	data.
	16	Title	1577	0		Simply	view the linked i	results sheet an	d graph. Click l	below to
	17	Title	272		1	lean m		ai anaiysis.		
	18	Title	362		1	🕐 Step	by step instruction	s for analyzing surv	ival data	

Data is automatically analyzed; you don't need to press the "Analyze" button !



Kaplan-Meier curve

Survival of Two groups: Survival proportions





Results tables: Survival Proportions

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	🖞 Two gro	oups		Х			Y	•	Y	
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	Surviva	l of Two groups	3	14	2 000		87 500			
	Surv	ival proportions	4	15	0.000		81 250			
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🖨 🦳 🔤	iraphs		7	29	1.000		68.750			-
	Iwo gro avouts	oups	8	36	2.000				85.7	14
- 👝 F	loating No	tes	9	37	3.000				78.5	71
÷	🗋 Data wit	h notes	10	38	3.000				78.5	71
			11	46	8.000		68.750			
			12	51	9.000				78.5	71
			13	56	3.000				78.5	71
			14	65	0.000				78.5	71
			15	68	0.000		61.875			
			16	82	7.000				67.3	47



Results tables: Nr. of subjects at risk

Prism	File	Sheet	Undo	Clipboard	Ana	alysis	Interpret	Change	Drav
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	Two gro	oups		Х			Y	Y	
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	Surviva	of Two groups	3	1/	2 000		15		
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	🚹 # of	subjects at risk	4	10	0.000		14		
	- K Curv	e comparison	5	26	9.000		13		
1~	🔤 🛐 Data	summary	6	27	2.000				14
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	avouts	Jups	8	36	2.000				13
🛓 🦲 F	loating No	tes	9	37	3.000				12
÷(🛅 Data wit	h notes	10	38	3.000				11
		11	11	46	8.000		11		
			12	51	9.000				10
			13	56	3.000				9
			14	65	0.000				8
			15	68	0.000		10		
			16	82	7 000				7



Results tables: Curve comparison

Comparison of Survival Curves

Log-rank (Mantel-Cox) Test	
Chi square	1.682
df	1
P value	0.1947
P value summary	ns
Are the survival curves sig different?	No
Gehan-Breslow-Wilcoxon Test	
Chi square	1.392
df	1
P value	0.2380
P value summary	ns
Are the survival curves sig different?	No
Median survival	
Standard	1037
Experimental	1307
Ratio	0.7934
95% CI of ratio	0.4460 to 1.141
Hazard Ratio	
Ratio	1.927
95% CI of ratio	0.7151 to 5.191



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Results tables: Data summary

🕀 🛅 Family	NH.	Survival	Α	B
🗖 🦳 Data Tables		Data summary	Standard	Experimental
Two groups			Y	Y
Info	1	Number of rows	30	30
Results	2	# of blank lines	14	16
Survival of Two groups	3	# rows with impossible data	0	0
Survival proportions # of subjects at risk	4	# censored subjects	5	9
Curve comparison	5	# deaths/events	11	5
🔤 Data summary	6			
Graphs	7	Median survival	1037	1307
		1	1	I I



GraphPad Prism 6: New features

- Sharing concept: LabArchives
- Improved sample data sets
- More statistical analyses (e.g. adjusted P-values for multiple comparisons; new post tests...)
- More graph types (Pie charts, superimposing of individual points and columns or box/whiskers...)
- New curve fitting modes (implicit and differential)



WAND:

If you like MAGIC, you'll love the WAND. While MAGIC reformats graphs, the WAND analyzes and graphs a new data table to match what you have done with another...



Examples for customized graphs







pO₂

- Draw zig-zag and elbow lines
- Show ticks above and below axis
- Show right axis only

- Plot line to divide groups of bars
- Superimpose individual points with bars (or box-whiskers)
- · Show two line column titles

- · Plot depth charts
- Draw zig-zag and elbow lines



Newest version: GraphPad 9.0 (annual rental license at our Univ. 84€/year)

Highlights

- New analyses: Principal Component Analysis (PCA) and Principal Component Regression (PCR)
- · More automation: Automatically add multiple comparison results to graphs ("Stars on Graph")
- · New Graphs: Bubble plots and other multiple variables graphs from multiple variables data tables
- New Graphs: Estimation plots automatically generated from t tests
- · New Graphs: Actual vs Predicted plot from nonlinear regression
- Expanded analyses: New options for Multiple t test analyses (paired, nonparametric, and more)
- Expanded analyses: Interpolation from Multiple Linear Regression
- · More data: Increased data table limits
- More data: Text variables and variable types (continuous, categorical, label) for multiple variables data tables



Newest version: GraphPad 9.0



One-Click Regression Analysis

No other program simplifies curve fitting like Prism. Select an equation and Prism does the rest—fits the curve, displays a table of results and function parameters, draws the curve on the graph, and interpolates unknown values.

Start a Free Trial

Automate Your Work Without Programming

Automatically add multiple pairwise comparisons to your analysis with a single click. For customization options of these lines and asterisks, simply click the toolbar button again. Make adjustments to the data or the analysis, and the results displayed on the graph will update automatically.

Start a Free Trial



Newest version: GraphPad 9.0

Countless Ways to Customize Your Graphs

Focus on the story in your data, not manipulating your software. Prism makes it easy to create the graphs you want. Choose the type of graph, and customize any part—how the data is arranged, the style of your data points, labels, fonts, colors, and much more. The customization options are endless.

Start a Free Trial









Violin plots

Violin plots show data distributions much more clearly than do box-whisker or bar graphs.











Curve Fitting Software: SigmaPlot



Rental license: 21€/year at our university

Features:

Creating Exact Graphs Data Visualization More than 100 2-D and 3-D Graph Types Customizing Details of Charts and Graphs Plotting Data from Existing Graph Templates Publishing Charts and Graphs Sharing Graphs on the Web Data analysis tools Integration into Microsoft Excel Regression Wizard to fit data easily Plotting Mathematical Functions Macros: Automation



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Generating graphs II

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Johannes A. Schmid, Inst. of Vascular Biology and Thrombosis Research

Statistics and non-linear Regression

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Non-linear Regression



Johannes & Schmid Inst of V

OF VIENNA

Curve fitting by right-clicking on the curve





Graph Export Functions





Statistics Features

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Statistics Features II





Other Features

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Further Menus



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Index •	Insert Graphs into Word	Exploratory Enzyme Kinetics.
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- Register SigmaPlot...
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Additional functions

- Inserting graphs into Word or Powerpoint
- Batch processing of Excel file
- ROC curves (Receiver Operating Characteristics);
 e.g. diseased versus healthy)

In a Receiver Operating Characteristic (ROC) curve the true positive rate (Sensitivity) is plotted in function of the false positive rate (100-Specificity) for different cut-off points. Each point on the ROC plot represents a sensitivity/specificity pair corresponding to a particular decision threshold. A test with perfect discrimination (no overlap in the two distributions) has a ROC plot that passes through the upper left corner (100% sensitivity, 100% specificity). Therefore the closer the ROC plot is to the upper left corner, the higher the overall accuracy of the test (Zweig & Campbell, 1993).





Pharmacology functions

Pharmacology Window Help

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Exploratory Enzyme Kinetics...

Shel<u>f</u> Life...

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Toolbars

The Standard Toolbar



The Formatting Toolbar





2D Graph Toolbar



3D Graph Toolbar



Page Toolbar



Integration of Sigmaplot into Excel



Sigmaplot 12.0 – Features

Office 2007-like ribbon user interface including a quick access toolbar



Create pdf-button added

PDF

Office

Graph Output

- New curve fitting features (including: implicit function curve fitting, when the equation is not known)
- New statistics functions (e.g. normal distribution comparison)
- New analysis features (e.g. enzyme kinetics)
- New graphing features (including transparent graph objects such as symbols)



Current version: Sigmaplot 14

<u>https://systatsoftware.com/sigmaplot/</u>

Designed Specifically to Meet the Needs of Scientists, Professional Researchers and Engineers

With an award-winning interface and intuitive wizard technology that guides users step-by-step through the graph creation and data analysis process, SigmaPlot provides the flexibility to create compelling graphs and statistical analysis you simply can't achieve with basic spreadsheet software.

Learn More

Overview

Product Features What's New in SP14.5 Graphing Features Statistics Transforms System Requirements Run SigmaPlot On a MAC Brochure

Add On Modules

Graph Showcase 21 CFR 11 Section 508 SigmaPlot Instrumentation Framework Smoothing Routines WebViewer for SigmaPlot Licensing




GNU R - Bioconductor

• Introduction by Alexander Tolios



Bioconductor **R** additional slides

- R is a language and environment for statistical computing and graphics: Programming environment for many different applications in life sciences including biostatistics, data analysis, sophisticated plotting, etc. (https://cran.r-project.org/manuals.html)
- <u>https://bioconductor.org</u> searching something: <u>https://rseek.org</u>
- Many packages available for tailored tasks: All Packages

Bioconductor version 3.16 (Release)

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 Coverage (153)
 DifferentialExpression (364)
 DifferentialMethylation (58)
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 DifferentialSplicing (36)
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 DriverMutation (1)
 FunctionalPrediction (26)
 GeneFusionDetection (2)
 GeneRegulation (111)
 GeneSetEnrichment (146)

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Packages found under Software:

Rank based on number of downloads: lower numbers are more frequently downloaded.

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<u>S4Vectors</u>	Hervé Pagès	Foundation of vector-like and list- like containers in Bioconductor	2
BiocVersion	Bioconductor Package Maintainer	Set the appropriate version of Bioconductor packages	3
GenomeInfoDb	Hervé Pagès	Utilities for manipulating chromosome names, including modifying them to follow a particular naming style	4
IRanges	Hervé Pagès	Foundation of integer range manipulation in Bioconductor	5



RStudio – now: Posit (<u>https://posit.co</u>)

- Graphical user interface for Windows:
- <u>https://posit.co/download/rstudio-desktop/</u> requires R install.
- <u>https://rstudio-conf-2020.github.io/r-for-excel/</u>

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Basics

ggplot2 is based on the **grammar of graphics**, the idea that you can build every graph from the same few components: a **data** set, a set of **geoms**—visual marks that represent data points, and a **coordinate system**.



To display data values, map variables in the data set to aesthetic properties of the geom like **size**, **color**, and **x** and **y** locations.



Complete the template below to build a graph.



ggplot(data = mpg, **aes(**x = cty, y = hwy**))** Begins a plot that you finish by adding layers to. Add one geom function per layer.

aesthetic mappings data geom

qplot(x = cty, y = hwy, data = mpg, geom = "point") Creates a complete plot with given data, geom, and mappings. Supplies many useful defaults.

last_plot() Returns the last plot

ggsave("plot.png", width = 5, height = 5) Saves last plot as 5' x 5' file named "plot.png" in working directory. Matches file type to file extension.



<u>https://exts.ggplot2.tidyverse.org/gallery/</u>

120 registered extensions available to explore



Showing 102 of 120



patchwork 💿 Star 2173

Easy composition of ggplot plots using arithmetic operators

- author: thomasp85
- tags: visualization, composition

js libraries:





'ggstatsplot' provides a collection of functions to enhance 'ggplot2' plots with results from statistical tests. • author: IndrajeetPatil • tags: visualization, statistics • js libraries:



esquisse 🖲 Star 1582

Explore and Visualize Your Data Interactively with ggplot2

- author: dreamrs
- tags: visualization, interface
- js libraries:



<u>https://exts.ggplot2.tidyverse.org/gallery/</u>



hrbrthemes 💿 Star (1093)

A compilation of extra {ggplot2} themes, scales and utilities, including a spell check function for plot label fields and an overall emphasis on typography.

- author: hrbrmstr
- tags: theme, typography
- js libraries:



See Star 701

Visualisation Toolbox for 'easystats' and Extra Geoms, Themes and Color Palettes for 'ggplot2'



ggrepel 🖲 Star 1059

Repel overlapping text labels away from each other.

- author: slowkow
- tags: visualization, general
- js libraries:



ggiraph (estar) 638 htmlwidget to make 'ggplot' graphics interactive.







ggraph is tailored at plotting graph-like data structures (graphs, networks, trees, hierarchies...).

author: thomasp85

tags: visualization, general

js libraries:



COWPLOT (Star 636

Streamlined plot theme and plot annotations for 'ggplot2'





ggpubr () star 956 'ggplot2' Based Publication Ready Plots • author: kassambara • tags: visualization, statistics • js libraries:



ggalt 🖲 Star 623

A compendium of 'geoms', 'coords' and 'stats' for 'ggplot2'.

- <u>http://r-statistics.co/Top50-Ggplot2-Visualizations-</u> <u>MasterList-R-Code.html</u>
- <u>https://r-graph-gallery.com</u>





GGPLOT2 – some own examples

Scattergram in ggplot2:

Data: COVIDexample (Tag, Infizierte)

Code for scatter with smoothing:

ggplot(COVIDexample,aes(x=Tag, y=Infizierte))+geom_point(size=3,colour="red")+geom_smooth(method="gam")





Johannes A. Schmid, Inst. of Vascular Biology and Thrombosis Research

Bar graphs with colors depending on values

Pathway	activ	p_value	Ratio
Integrin Signaling	-1.225	5.01E-20	1.09E-01
Actin Cytoskeleton Signaling	-1.213	3.98E-13	8.12E-02
Phospholipase C Signaling	-1.414	2.51E-12	7.39E-02
IL-8 Signaling	-1.807	1.20E-10	7.44E-02
Calcium Signaling	-1.89	6.31E-10	7.28E-02

forR: Excel table

ggplot (forR,aes(x=Pathway,y=activ,fill=p_value)) +

geom_bar(stat="identity") +

coord_flip() +

scale_fill_distiller(palette = "Blues")



230 of 370

Column + line-graph + gradient





Define global parameter first

>p=ggplot(forR, aes(x=Function,y=zscore,fill=Ratio))

#Defines that "p" is the variable for a ggplot with "forR" as data and x, y, and fill-values defined as global aesthetics # x=Function,y=zscore,fill=Ratio > afterwards, x and y and fill don't have to be defined again.





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Violin plots with box plot inset

ggplot(**pivot**,aes(name,value)) + geom_violin(aes(fill=name)) + scale_x_discrete(limits=c("C3", "C14", "C14T3")) + theme(text = element_text(size = 14,face="bold")) + geom_boxplot(width=0.1)

... if you want to plot several datasets on the same x-axis you have to use the pivot function





Parallel coordinate plots

• Different possibilities: a) ggplot2 and ggally or b) plotly (interactive)

a) ggplot2/ggally:

- ggparcoord function RDocumentation
- # loading of the necessary packages
- library(GGally)
- library(ggplot2)
- library(svglite)

ggparcoord(data, columns=1:21,groupColumn = "sex")

In contrast to parcoord from plotly; ggparcoord can plot more than 21 axes (but it is not interactive)





Interactive parallel coordinate plots

```
packages = c('GGally', 'plotly', 'parcoords', 'tidyverse')
for(p in packages){
    if(!require(p, character.only = T)){
        install.packages(p)
    }
    # data[,1:20] means: all row
parcoords(
    data[,1:20],
    reorderable = T,
    # reorderable = T (Theorem and the parcoords package call
```







Interactive parallel coordinate plots: gating

Gates for several parameters can be defined to select the corresponding lines

(by hovering with the mouse over a range on an axis).

Gates are by default combined with AND operand

using an alphaOnBrushed command, the non-chosen lines have and alpha-density reduction

while the chosen lines are shown with full density (alpha=1)

parcoords(data[,1:20], reorderable = T, brushMode = '1D-axes', alphaOnBrushed = 0.3)









Bar graph colored

library(ggplot2)

ggplot(IPA,aes(y=reorder(Functions,neglogp),x=neglogp,fill=zscore)) + geom_col(colour="black")+ scale_fill_gradient2(low="blue",high ="red")





SVG-graphs with ggplot

Svg-Export > to Inkscape: to consider:

Font in Inkscape do not work, if svg-file was exported from ggplot2.

Workaround:install.packages("svglite")

- Save Plot extra :
- > plot=ggplot(Rcourse,aes(x=Tag, y=Infizierte))+geom_point(size=3,colour="red")
- > ggsave(file="Scatter.svg", plot=plot, width=10, height=10)

The svg-file will be in the standard folder (Documents); in Inkscape it is possible to change the font and type.

Work around 2: export as pdf file and open the pdf file with Inkscape



Customizing ggplot2 Visualizations With ggThemeAssist

```
install.packages("ggThemeAssist")
```

library(ggThemeAssist)

library(ggplot2)

#Example:

plot=ggplot(IPA,aes(y=reorder(Functions,neglogp),x=neglogp,fill=zscore)) +
geom_col(colour="black")+ scale_fill_gradient2(low="blue",high ="red")

ggThemeAssistGadget(plot)





Scatterplot matrix

- Command:
- pairs(forR[1:10])





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Scatterplot matrix with ggally

install.packages("plotly")
library(plotly)
library(devtools)
install_github("ggobi/ggally")
install.packages("GGally")
library(GGally)
p <- ggpairs(forR[1:10])
ggplotly(p)</pre>





Johannes A. Schmid, Inst. of Vascular Biology and Thrombosis Research

MS-Powerpoint

Master view:

to change general settings

Font type and size can be adjusted
Footnotes or icons can be customized
Background and Layout
at the end the Master view has to be closed again





MS-Powerpoint





Some Powerpoint Hints

- Exact positioning of objects and exact drawing: when you press the "Alt" Key, while you are dragging or drawing
- Pixel-wise shifting of objects: "Strg" (Ctrl) + arrow keys
- Drawing: "Start" tab > Drawing
- Arrange button (Anordnen): contains buttons for grouping of objects, flipping and mirroring of, positioning forward or backward....
- usefull short cuts: Ctrl-D: Duplicate Ctrl-C, Ctrl-V for copy/paste









Some Powerpoint Hints

- insertion of more sophisticated objects: movies (*.avi-files, later versions: mp4-files); pictures, equations...
- Screen recording if you want to record part of a screen (e.g. a movie running on the 2nd screen – or recording your own presentation)
- objects that can be activated: select an object > add a user-defined animation (object action > activate content): You can use this to activate "foreign" programs (e.g. crystal structure program, when you want to show a molecule at a presentation)









Hints for using Animations

- You can use animated bulleted lists to guide the auditory through list text parts
- Use animations just when it is suited • to improve the presentation (not just for fun, and not too much)
- vou can use animations for instance to build up a complicated slide step by step
- you can use animations to ٠ emphasize important data
- You can use animations in drawings • to illustrate "reactions" or movements.



SCF complex

IEDICAL UNIVERSITY

Example for an animated drawing

- add a user-defined animation path







- select the objects

Hints from the San Francisco Edit Company

http://www.sfedit.net

Many communication experts agree that there is a right and a wrong way to use presentation software like PowerPoint or Keynote. If used correctly, the software can greatly enhance your presentation. Here are some technical tips to assist you in developing an appropriate presentation.

- 1. Learn to use the software. Use the software's defaults for font size, margins, and placement.
- 2. Keep the layout and style as consistent as possible.
- 3. Choose colors with care. The text and background colors should contrast, dark letters on a light background for small rooms, light letters on a dark background for large rooms. The background should be a solid color, no fading, photos, etc. Avoid red-green combinations because a significant fraction of the human population is red-green colorblind.
- 4. Use animated features in moderation. Overuse of these effects, such as slide transitions and custom animations, can be distracting. Focus should be on the content.
- 5. Strive for simplicity and visibility. Eliminate any words, lines, and diagrams that do not add essential information to the slide.



- 6. Display data using diagrams and figures instead of tables; they are easier for the audience to comprehend.
- 7. Keep tables simple. There should only be one table per slide. The font size for the data should not be smaller than 22 point. If you need to decrease the font size to have the table fit on the slide, eliminate some of the data or do not use the table.
- 8. Use fonts at least 36 point in size for titles, 28 point for main bullets, and 24 point for sub-bullets. If it can't be, read it's a waste.
- 9. Limit text blocks to no more than two lines each. Do not have large text blocks containing paragraphs; the audience will spend time reading the text and ignore what you are saying.
- 10. Use a heading on every slide.
- 11. Limit the number of items on each slide. Each slide should make just one or two points using 7-9 lines maximum.
- 12. Avoid using too many words in bold, italics, or capital letters.
- 13. Use the same font throughout to avoid distraction. Sans serif fonts (e.g., Arial) are easier to read and more attractive than fonts with serifs (e.g., Times New Roman).
- 14. Using "builds" in diagrams and text slides can be very useful. Text builds can be made even more effective if you darken previous text as new material is added.
- 15. Control the number of slides. Budget 2-3 minutes per slide (e.g. 30 minute talk = 10-15 slides).
- 16. Practice with feedback and then practice some more.

Some other features

... various design options

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... SmartArts:









MS-Powerpoint 2013 and later...

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OF VIENNA

Nach Onlinevorlagen und -designs suchen Empfohlene Suchbegriffe: Fotoalben Geschäftliches Kalender Diagramme Willkommen bei PowerPoint HOLZART NET7

- Better speaker mode
- also suited for 16:9 screens
- Improved video- and audio features (also plays mp4- or flash-videos)
- new designs
- etc. etc.



Speaker Mode

The speaker sees the speaker mode screen, the audience only the presentation. Laser point can be activated, a zoom function can be used, etc.





Keine Notizen.

Notes that you added will be shown here (while they are not visible to the audience)

OF VIENNA

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Powerpoint 2016

- Tell me... function (to access functions quickly)
- Smart Look-up (Wikipedia...)
- Ink Equations (Hand-written equations are converted to proper math)
- Sharing with others, including "conflict resolution")

Johannes Schmid

Bildschirmaufzeichnung einfügen

Audio Bildschirmaufzeichnung

Zeichnen Sie Ihren Computerbildschir zugehörige Audiosi Sie die Aufzeichnur

Medien

einfügen.

Q Freigeben

Insert Screen recording function

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New recording functions

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If you want to record your presentation (e.g. for online talks) Settings: +/- microphone and camera (to record the video of the speaker)



Microsoft Access 2016

- Relational database management system
- You can generate tables, queries, forms, reports ... to access data in a professional manner – so that the relations between data features are maintained

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- Notebook platform
- works best with a Microsoft account (Hotmail or Outlook)
- There is a desktop version, a free Windows10-version, an online version – and also versions for Android and iPad
- You can have several notebooks
- you can create sections and pages





sections	pages
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search function (searches in all notebooks)

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specific categories and task options

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Kategorien



you can send a section as task to Outlook



You can add a calendar event to the page



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You can insert many elements:

- attachments
- Excel spreadsheets
- images or screenshots
- videos
- Hyperlinks to websites
- you can record audio or video
- you can insert time stamps
- equations
- symbols



Many drawing features (particularly useful with graphics tablets)



Microsoft Onenote for Windows 10

Has the same basic features (but less), slightly different appearance





Microsoft Onenote Browser-Version





Cell phone version (Android)





OpenOffice and LibreOffice: Alternatives to MS-Office

http://www.openoffice.org/ driven by Apache

	Willkommen bei OpenOffice.org
	Ein neues Dokument erstellen
	Präsentation
	Datenbank
	Yorlagen
	Ein Dokument öffnen
Sun.	t, 😰 💪 🥏

https://www.libreoffice.org/ LibreOffice Datei Extras Hilfe Datei öffnen Datei offnen Datei verwendete Dateien Vorlagen Erstellen: Writer Textdokument



Calc Tabellendokument



Impress Präsentation



D<u>r</u>aw Zeichnung



Math Formeldokument

<u>B</u>ase Datenbank



OpenOffice Features

- Look a bit like MS-Office 2003 (Button appearance)
- OpenOffice applications can read MS-Office files (and also save in MS-Office format- although the later with some restrictions: some formatings might be lost)
- OpenOffice is available for many languages and platforms (including Macintosh and Linux)
- Bibliography support (similar to Endnote Plug-In) possible via <u>www.zotero.org</u> (free extension for Firefox) – and more recently also with Mendeley and Endnote



OpenOffice-Writer

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Pdf-Viewers / Editors and generating of pdf's

Viewing and editing

- PDF-Xchange Viewer: small (about 40 MB), very fast, files can be edited (highlighting, comments, stamps...)
- Follow-up: <u>PDF-xchange editor</u> (340 MB)
- pdf24: extract pages or combine files to a joint pdf...
- (Acrobat Professional Commercial): huge file, slow
 but can extract and delete pages

Generating pdf-files

- Office 2007 and later
- OpenOffice
- DoPDF: installs a pdf-printer driver (printer command in any software > choose DoPDF as printer > pdffile is generated instead of a printout)
- Foxit pdf-viewer also installs a pdfprinter driver

Translation of Text

Online Services

- LEO: <u>http://dict.leo.org/</u>
- Google: <u>http://translate.google.at</u>

Translation of phrases: DeepL https://www.deepl.com/

(when desktop program is installed: Ctrl+CC translates selected text in all programs

😑 💮 Text 🖉 Write 🖂 Bilder 😑 Dateien 💭 Gespeiche	ert DeepL Pro testen (2) Anmelden $ \Box$ \times
Deutsch (erkannt) 🗸	
X Die mit diesen Inhalten verbundene Onlineaufgabe, erfordert eine professionelle und koordinierte Nutzung der vorgestellten Instrumente und Datenbanken, um die gestellte Aufgabe erfüllen zu können.	The online task associated with this content requires a professional and coordinated use of the tools and databases presented in order to be able to fulfill the task set.



Improving English text with DeepL Write

$\equiv \bigoplus$ Text	🖉 Write	🖂 Bilder	Dateien	Gespeic	hert		DeepL Pro teste	n	② Anmelden	_		×
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Translation of Text offline

Offline program: LingoPad: <u>http://www.ego4u.de/de/lingopad</u> Installs a dictionary locally

LingoPad 2.6, Deutsch-I	nglisch Wört	terbuch		- • ×					
🛄 👻 🔳 DE 📧 EN	G	🕶 🏹 💼 🛛 🤯 Programm	🕶 😡 Info 💌	📧 Englisch Lernen					
kreativ -	≓ Engl	isch	Deutsch						
kreativ	 creati 	vely	kreativ adv						
kreative	 creati 	ve	kreativ; schöpfe	erisch adj					
kreatives	crea	tive work	kreative Arbeit	; schöpferische					
Kreativität			Tätigkeit						
Kreatur			_						
Kreaturen									
Krebs									
krebsartig	:								
Krebse									
krebserregend									
Krebses									
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Deutsch (de-DE Wörterbuch	eutsch (de-DE Wörterbuch), 173124 Stichwörter, 377294 Verweise, Ladezeit 2.725 Sekunden								

ImageJ





•

ImageJ Plugins and Macros – and new ImageJ: Fiji

Many plugins and macros available:

Acquisition Analysis Collections Color Filters Graphics Input/Output Programming Examples Stacks Utilities

- Improved version with additional built-in plugins and automated updates:
 - <u>Fiji</u>: <u>https://fiji.sc</u>

With an integrated search field

List of extensions:

https://imagej.net/list-of-extensions









ImageJ	
File Edit Image Process Analyz	ze Plugins Window Help
New	Image Strg+N
Open Strg+O	Hyperstack
Open Next Strg+Umschalt+O	Text Window Strg+Umschalt+N
Open Samples	Internal Clipboard
Open Recent	System Clipboard Strg+Umschalt+V
Import	Image Sequence
Close Stra+W	Raw
Close All	LUT
Save Strg+S	Text Image
Save As	Text File
Revert Strg+R	Results
	- URL
Page Setup	Stack From List
Print Strg+P	TIFF Virtual Stack
Quit	AVI
	Exif Data
	Selected as a Stack
	Open List
	*.PICT; *.PSD; *.ICO
	Virtual Stack
	*.GIF Stack
	*.AVI
	*.TIF Improvision TIFF file
	*.PIC
MEDICAL UNIVERSITY	*.TXT Leica SP2 series of Vascular Biology and Thr
	Animated Gif

The File Menu

- New images (e.g. pasting from the Clipboard)
- Opening of images
- Importing of images: ٠ Many different file formats
- Saving •
- Reverting: opening the last saved version of an image
- Page setup and printing •

The Edit Menu



- Copy/Paste, Undo comand (also: Copy to System)
- Paste control: allows pasting with additional operations: blending, transparent (white pixels copied as transparent) logical operations difference.
- Draw (a line along selection)
- Invert the image

 (also inverts the grey scale)^{Divide}



Blend

AND.

OR.

XOR Add

Difference

Transparent

"Blending"of fluorescence and phase contrast

HeLa N2 C Phako.tif



	🛓 Paste Control	
-4	Transfer Mode:	Blend 🔽



Selection tools

- July - Law						
ile	Edit	Image	Process	Analy		
ļ	Unc	o	S	Strg+Z		
lygc	Cut		S	Strg+X		
	Cop	ру	S	Strg+C		
	Copy to System					
	Pas	ste	S	Strg+V		
	Paste Control					
	Cle	ar				
	Clear Outside					
	Fill		S	Strg+F		
	Dra	W	S	trg+D		
	Invert Strg+Umschalt+I					
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ImageJ

F

-		
2	Select All	Strg+A
x	Select None	Strg+Umschalt+A
C	Restore Selection	Strg+Umschalt+E
	Fit Spline	
v	Fit Circle	
	Fit Ellipse	
	Convex Hull	
	Make Inverse	
=	Create Selection	
D	Create Mask	
H	Properties	Strg+Y
•	Rotate	
•	Enlarge	
	Make Band	
	Specify	
	Straighten	
	To Bounding Box	
	Line to Area	Original Selection
	Area to Line	
	Add to Manager	Strg+T

- Restore selection: places a selection at exactly the same position in a different image (Strg+Shift E)
- Fitting of selections: modifies a • freehand or polygonal selection with different criteria



Editing of selections

example: make band around nucleus to measure perinuclear area



Johannes A.



Create Mask

Creates a new 8-bit image called "Mask" whose pixels have a value of 255 inside the selection and 0 outside. By default, this image has an inverting LUT, so black is 255 and white is 0. Check "Black Background" in *Process/Binary/Options* before using *Create Mask* and an inverting LUT will not be used (black will be 0 and white 255).

Create Selection

Creates a selection from a thresholded image or a binary mask. This command is based on the Threshold_To_Selection plugin written by Johannes Schindelin.

Rotate ...

Rotates the selection by the specified number of degrees (negative number indicate counter-clockwise rotation).

Enlarge...

Grows an area selection by a specified number of pixels. Enter a negative value to shrink the selection. Enter zero to convert a composite selection into a polygon selection.

Make Band...

Takes an area selection and creates a band with a thickness of the specified number of pixels. If you imagine the band as a doughnut shape, then the original selection corresponds to the hole (i.e. the band is made by growing out the original selection).

Specify ...

Opens a dialog that allows your to define a rectangular or elliptical selection. *Width* and *Height* are the dimensions of the selection. *X Coordinate* and *Y Coordinate* define the position of the selection. Check *Oval* to create an elliptical selection. If *Centered* is checked, the selection is positioned so *X Coordinate* and *Y Coordinate* define the center of the selection, otherwise they define the upper left corner.

Add to Manager

Adds the current selection to the <u>ROI Manager</u>. If there is no selection then it opens the ROI Manager. As a shortcut, press "t". (Except when using the text editor, you do not have to hold down the control key to use menu shortcuts).

General Options of ImageJ

geJ					
Edit	Image	Process	Analyz		
Undo		S	Strg+Z		
Cut		S	Strg+X		
Сору		S	Strg+C		
Copy to System					
Paste		S	strg+V		
Paste Control					
Cle	Clear				
Cle	ar Outs	side			
Fill		S	Strg+F		
Dra	Draw Strg+D		trg+D		
Inve	Invert Strg+Umschalt+I		halt+l		
Sel	ection		•		
Opt	tions		×		

Line Width	
Input/Output	
Fonts	
Profile Plot Options	
Arrow Tool	
Point Tool	
Wand Tool	Colore
Colors	S Colors
Appearance	Foreground:
Conversions	Background:
Memory & Threads	Selection:
Proxy Settings	Geleciion.
Compiler	0
DICOM	
Misc	



The Image Menu > Brightness/Contrast





The Image Menu > Threshold





Splitting of colour of an RGB image





Working with image stacks

🛓 ImageJ						
File Edit	Image Process	Analyze Plugins \	Vindow Help	[
	Туре	•) Dev Stk 0 1 8 8 1 🗡 😕			
Polygon sele	Adjust	► Stra+l		(els;		
	Properties	Strg+Umschalt+P				
	Stacks	•	Add Slice		d Make Montag	e X
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	Crop	Strg+Umschalt+X	Previous Slice [<]		Rows:	4
	Rename	Strg+Umschalt+D	Set Slice		Scale Factor:	0.25
	Scale	Strg+E	Images to Stack		First Slice:	1
	Transform	•	Stack to Images		Last Slice:	5/
	Zoom	+	Make Montage		Increment:	3
	Overlay	•	Reslice [/]		Font Size:	12
	Lookup Tables	•	Orthogonal Views Strg+Umschalt+H		T ON COLC.	
			Z Project		ces	
			3D Project		Use Fore	ground Color
			Label		ОК	Cancel
			Tools >			



Image stack montage





Johannes A. Schmid, Inst. of Vascular Biology and Thrombosis Research

Z-stacks: maximum projections

Max Intensity





MAX_flybrain-z-stack blu...

Standard Deviation Average Intensity


3D projections of z-stacks

🛓 ImageJ			
File Edit	Image Process	Analyze Plugins	Window Help
	Туре	+	Dev Stk 0 1 3 1 >>
Oval, elliptic	Adjust Show Info Properties Color Stacks Hyperstacks Crop	Strg+Umschalt+P	Add Slice Delete Slice Next Slice [>] Previous Slice [<]
	Rename	ong. on och an o	Set Slice
	Scale Transform Zoom Overlay	Strg+E	Images to Stack Stack to Images Make Montage Reslice [/]
	Lookup Tables	•	Orthogonal Views Strg+Umschalt+H
			3D Project
			Plot Z-axis Profile
			Label
			Tools





Reslicing of image stacks

Reslice...

Reconstructs one or more orthogonal slices through the image volume represented by the current stack. Before using this command, create a straight line or rectangular selection to specify were the reconstructions will be done. A dialog box allows you the specify the *Z-Spacing* (displacement between slices) of the source volume. Multiple slices are reconstructed and saved as a stack if you create a rectangular selection or set *Slice Width* greater than one.





Additional Image Menu Options

Image	Process	Analyze	Plugins	V
Туре				١
Adjus	t			•
Show	Info		Strg+I	
Prope	erties	Strg+Un	nschalt+P	
Color				۲
Stack	s			۲
Crop				
Dupli	cate	Strg+Urr	nschalt+D	
Rena	me			
Scale			Strg+E	
Rotat	e			۲
Zoom	I			۲
Looku	up Tables			F

- Cropping: Cut out relevant part of the image
- Duplicate image
- Rename image
- Scaling: enlarge or reduce (changes the resolution)
- Rotate: flipping, 90° or arbitrary
- Zoom: enlarge or reduce without changing the resolution
- Lookup-Tables: Gray-Values, Pseudo-Colours



The Process Menu

Process	Analyze	Plugins	Window	Help
Smooth	i i	Str	g+Umscha	alt+S
Sharper	n			
Find Ed	ges	Sti	rg+Umsch	alt+F
Enhanc	e Contras	t		
Noise				
Shadov	VS			
Binary				▶ →
Math				
FFT				
Filters				
Image (Calculator			
Subtrac	t Backgro	und		
Repeat	Comman	d Str	g+Umscha	alt+R

Make Binary
Convert to Mask
Find Maxima
Erode
Dilate
Open
Close-
Options
Outline
Fill Holes
Skeletonize
Distance Map
Ultimate Points
Watershed

- Smooting, Sharpening
- Find edges: areas of significant contrast change
- Enhance contrast
- Add noise or shadows
- Binary: Thresholded images can be adjusted and modified



Options with Binary images (after thresholding)



Segmentation of thresholded binarized image

- Make Binary
- Convert to Mask
- Find Maxima...
- Erode

Dilate

Open Close-

Options...

Outline

Fill Holes

Skeletonize

Distance Map Ultimate Points

Watershed



Thresholded Cells

EDM and UEPs

After Watershed Segmentation

- <u>EDM: Euklidian Distance Map</u>: Each foreground pixel in the binary image is replaced with a gray value equal to that pixel's distance from the nearest background pixel.
- <u>Ultimate Points</u>: Generates the ultimate eroded points (UEPs) of the EDM. Requires a binary image as input. The UEPs represent the centers of particles that would be separated by segmentation.
- <u>Watershed</u>: automatically separating or cutting apart particles that touch. It first calculates the Euclidian distance map (EDM) and finds the ultimate eroded points (UEPs). It then dilates each of the UEPs (the peaks or local maxima of the EDM) as far as possible - either until the edge of the particle is reached, or the edge of the region of another (growing) UEP.



Image Mathematics (with Constants)

Process	Analyze	Plugins	Window	Help	
Smooth	i	Str	g+Umscha	alt+S	
Sharpe	n				
Find Ed	lges	Str	g+Umscha	alt+F	
Enhand	e Contras	t			А
Noise				+	S
Shadov	VS			+	N
Binary				+	
Math				Þ	Δ
FFT				÷	
Filters				•	X
Image (Calculator				N
Subtrac	t Backgro	und			N
Repeat	Comman	d Str	g+Umscha	alt+R	G

Fourier Transformation - can be applied to reduce noise and other more specific applications

.dd.... ubtract... 1ultiply... ivide... ND... R... 0R... 1in... 1ax... amma... Log Exp Square Square Root Reciprocal NaN Background

Abs

Add... Adds a constant to the image. With 8-bit images, results greater than 255 are set to 255. With 16-bit signed images, results greater than 65,535 are set to 65,535.

- Subtract...Subtracts a constant from the image. With 8-bit and 16-bit images, results less than 0 are set to 0.
- Multiply.. Multiplies the image by the specified real constant. With 8-bit images, results greater than 255 are set to 255. With 16-bit signed images, results greater than 65,535 are set to 65,535.
- Divide...Divides the image by the specified real constant. Attempts to divide by zero will be ignored.
 - AND...Does a bitwise AND of the image and the specified binary constant.

Min..Pixels in the image with a value less than the specified constant are replaced by the constant.

- Max...Pixels in the image with a value greater than the specified constant are replaced by the constant.
- Gamma...Applies the function f(p) = (p/255)^gamma*255 to each pixel (p) in the image or selection, where 0.1 <= gamma <= 5.0. For RGB images, this function is applied to all three color channels. For 16-bit images, the image min and max are used for scaling instead of 255.
- Log..For 8-bit images, applies the function f(p) = log(p) * 255/log(255) to each pixel (p) in the image or selection. For RGB images, this function is applied to all three color channels. For 16-bit images, the image min and max are used for scaling instead of 255. For float images, no scaling is done. To calculate log10 of the image, multiply the result of this operation by 0.4343 (1/log(10).
- Reciprocal: Generates the reciprocal of the active image or selection. Only works with 32-bit float images.
- NaN Background: Sets non-thresholded pixels in 32-bit float images to the NaN (Not a Number) value. For float images, the "Apply" option in Image/Adjust Threshold runs this command. Pixels with a value of Float.NaN (0f/0f), Float.POSITIVE_INFINITY (1f/0f) or Float.NEGATIVE_INFINITY (-1f/0f) are ignored when making measurements on 32-bit float images
- Abs: Generates the absolute value of the active image or selection. Only works with 32-bit float images.



Process > Filters

Process	Analyze	Plugins	Window	Help		
Smooth		Str	g+Umscha	alt+S		
Sharpe	n					
Find Ed	lges	Str	g+Umscha	alt+F		
Enhanc	e Contras	t				
Noise						
Shadow	/s					Convo
Binary				•		Gauss
Math						Media
FFT						Mean.
Filters			-		►	Minimu
Image (`alculator					Maxim
Subtrac	t Packaro					Unsha
Repeat	Comman	d Str	q+Umscha	alt+R		Varian
•			-			Show

- lve... ian Blur... n... ım... um... rp Mask... се... Circular Masks... Anisotropic Diffusion Kalman Stack Sigma Filter Median 3D Pseudo Flatfield
- Gaussian Blur...Smooths the current image by doing a convolution using a square, Gaussian (bell-shaped) kernel. The width of the kernel, in pixels, is 2*radius+1, where radius is entered into a dialog box.
- **Median...**Reduces noise in the active image by replacing each pixel with the median of the neighboring pixel values.
- **Mean...**Smooths the current image by replacing each pixel with the neighborhood mean. The size of the neighborhood is specified by entering its radius in a dialog box.
- Minimum...This filter does grayscale erosion by replacing each pixel in the image with the smallest pixel value in that pixel's neighborhood.

Maximum...This filter does grayscale dilation by replacing each pixel in the image with the largest pixel value in that pixel's neighborhood.

Unsharp Mask...Sharpens and enhances edges by subtracting a blurred version of the image (the unsharp mask) from the original. The unsharp mask is created by Gaussian blurring the original image and then multiplying by the "Mask Weight" parameter. Increase the Guassian blur radius to increase contrast and increase the "Mask Weight" value for additional edge enhancement.

Variance...Highlights edges in the image by replacing each pixel with the neighborhood variance.

Show Circular Masks: Generates a stack containing examples of the circular masks used by the *Median*, *Mean*, *Minimum*, *Maximum* and *Variance* filters for various neighborhood sizes.



Image Mathematics (with 2 Images)

Process	Analyze	Plugin	s Window	Help
Smooth		S	Strg+Umscha	alt+S
Sharper	n			
Find Ed	ges	S	Strg+Umscha	alt+F
Enhanc	e Contras	t		
Noise				
Shadow	/s			
Binary				
Math				
FFT				
Filters				•
Image C	Calculator.			
Subtrac	t Backgro	und		
Repeat	Comman	d S	itrg+Umscha	alt+R

MEDICAL UNIVERSITY

OF VIENNA



Add	img1 = img1+img2
Subtract	img1 = img1-img2
Multiply	img1 = img1*img2
Divide	img1 = img1/img2
AND	img1=img1 AND img2
OR	img1 = img1 OR img2
XOR	img1 = img1 XOR img2
Min	img1 = min(img1,img2)
Max	img1 = max(img1,img2)
Average	img1 = (img1+img2)/2
Difference	img1 = img1-img2

Subtract Background...(dark objects on bright background) Removes smooth continuous backgrounds from gels and other images. Uses a rolling ball algorithm inspired by Stanley Sternberg's article, "Biomedical Image Processing", IEEE Computer, January 1983. The Rolling Ball Radius should be at least as large as the radius of the largest object in the image that is not part of the background



Johannes A. Schmid, Inst. of Vascular Biolog

Before

The Analyze Menu: Measuring

Analyze Plugins	Window			
Measure	Strg+M			
Analyze Particle	S			
Summarize			🛓 Set Measurements	
Distribution			🔽 Area	🔽 Mean Gray Val
Label			🗖 Standard Deviation	🔲 Modal Gray Val
Clear Results			🥅 Min & Max Gray Value	🗖 Centroid
Set Measureme	nts ——	•	🗖 Center of Mass	🗖 Perimeter
			🗖 Bounding Rectangle	🗖 Fit Ellipse
Set Scale			🔲 Circularity	🔲 Feret's Diame
Calibrate			Integrated Density	🗖 Median
Histogram	Strg+H		🗖 Skewness	🗖 Kurtosis
Plot Profile	Strg+K		🗖 Area Fraction	🗖 Slice Number
Surface Plot				
Gels	+		🗌 Limit to Threshold	🗖 Display Label
Tools	•		Invert Y Coordinates	
			Redirect To:	None
			Decimal Places (0-9):	2

- Based on the options checked • in the "Set Measurement" menu, different values of the selected regions can be measured (by clicking "Measure" or Strg-M)
- > a Results window is opened, ٠ showing the data – these can be copied into MS-Excel or other programs (or saved as .xls file)





OK.

-

Cancel

X

Measurement Options I

- Area Area of selection in square pixels. Area is in calibrated units, such as square millimeters, if Analyze/Set Scale was used to spatially calibrate the image.
- Mean Gray Value Average gray value within the selection. This is the sum of the gray values of all the pixels in the selection divided by the number of pixels. Reported in calibrated units (e.g., optical density) if Analyze/Calibrate was used to calibrate the image. For RGB images, the mean is calulated by converting each pixel to grayscale using the formula gray=0.299red+0.587green+0.114blue or the formula gray=(red+green+blue)/3 if "Unweighted RGB to Grayscale Conversion" is checked in Edit/Options/Conversions.
- Standard Deviation- Standard deviation of the gray values used to generate the mean gray value.
- Modal Gray Value Most frequently occurring gray value within the selection. Corresponds to the highest peak in the histogram.
- Min & Max Gray Level Minimum and maximum gray values within the selection.
- Centroid The center point of the selection. This is the average of the x and y coordinates of all of the pixels in the image or selection. Uses the X and Y Results table headings.
- Center of Mass This is the brightness-weighted average of the x and y coordinates all pixels in the image or selection. Uses the XM and YM headings. These coordinates are the first order spatial moments.
- Perimeter The length of the outside boundary of the selection.
- Bounding Rectangle The smallest rectangle enclosing the selection. Uses the headings BX, BY, Width and Height, where BX and BY are the coordinates of the upper left corner of the rectangle



Measurement Options II

- **Fit Ellipse** Fit an ellipse to the selection. Uses the headings Major, Minor and Angle. Major and Minor are the primary and seconday axis of the best fitting ellipse. Angle is the angle between the primary axis and a line parallel to the x-axis of the image. Note that ImageJ cannot calculate the major and minor axis lengths if Pixel Aspect Ratio in the Set Scale dialog is not 1.0.
- **Circularity** 4pi(area/perimeter^2). A value of 1.0 indicates a perfect circle. As the value approaches 0.0, it indicates an increasingly elongated polygon. Values may not be valid for very small particles.
- **Feret's Diameter** The longest distance between any two points along the selection boundary. Also known as the caliper length. The Feret's Diameter macro will draw the Feret's Diameter of the current selection on the image.
- Integrated Density The sum of the values of the pixels in the image or selection. This is equavalent to the product of Area and Mean Gray Value.
- Median- The median value of the pixels in the image or selection.
- Skewness- The third order moment about the mean.
- Kurtosis- The fourth order moment about the mean.
- Area Fraction- The percentage of pixels in the image or selection that have been highlighted in red using Image/Adjust/Threshold. For non-thresholded images, the percentage of non-zero pixels.
- Limit to Threshold If checked, only thresholded pixels are included in measurement calculations. Use Image/Adjust/Threshold to set the threshold limits.
- <u>Display Label -</u> If checked, the image name and slice number (for stacks) are recoded in the first column of the results table.
- Invert Y Coordinates If checked, the XY origin is assumed to be the lower left corner of the image window instead of the upper left corner.
- Redirect To The image selected from this popup menu will be used as the target for statistical calculations done by the Measure and Analyze Particles commands. The Redirect To feature allows you to outline a structure on one image and measure the intensity of the corresponding region in another image. With ImageJ 1.35d or later this feature also works with stacks.
- **Decimal Places** This is the number of digits to the right of the decimal point in real numbers displayed in the results table and in histogram windows



Analyze Particles (or objects of interest)





Additional Analyze Options

•

Analyze	Plugins	Window			
Measur	re	Strg+M			
Analyze	e Particle	s			
Summa	irize				
Distribu	ition				
Label					
Clear R	esults				
Set Measurements					
Set Scale					
Calibra	te				
Histogr	am	Strg+H			
Plot Profile		Strg+K			
Surface					
Gels		+			
Tools		+			

- Summarize: For each column in the results table, calculates and displays the mean, standard deviation, minimum and maximum of the values in that column
- Distribution: Calculates a binary distribution
 (histogram) for a list of results
 - Label: labels the analyzed objects in the image ("Centroid" option has to be checked in "Set Measurements" options)

- Set Scale: Can be used to convert a distance in pixel (specified with the line tool) into a known distance in *mm* or similar
- Calibrate: Can be used to calibrate an image to a set of density standards, for example radioactive isotope standards
- Histogram: Calculates and displays a histogram of the distribution of gray values in the active image or selection





Plot Profiles

Analyze	Plugins	Window			
Measur	е	Strg+M			
Analyze	e Particle	s			
Summa	rize				
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Label					
Clear R	esults				
Set Measurements					
Set Sca	ale				
Calibra	te				
Histogra	am	Strg+H			
Plot Pro	ofile	Strg+K			
Surface	e Plot				
Gels		+			
Tools		+			

Plot Profile: For each column in the results table, calculates and displays the mean, standard deviation, minimum and maximum of the values in that

column



1 g HMWPStat3-2.tif

384x421 pixels; 8-bit; 157K



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Surface Plots

		 Surface Ple
Analyze Plugins	Window	a three-di
Measure	Strg+M	graph of t
Analyze Particle	S	intensities
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Histogram	Strg+H	
Plot Profile	Strg+K	10.0
Surface Plot		ALC: 1
Gels	•	
Tools	+	SF 50 _ 3
		11000

Surface Plot: Displays a three-dimensional graph of the intensities of pixels in a grayscale or pseudo color image HeLa N2 C Fluo.tif 4x322 pixels; 8-bit; 105K





Analyzing Gels









Method 1 (line background connecting "valleys")

Method 2 (rectangle + draw Ctrl+D better quantification)



(correct averaging of the intensities over the lane width > peaks)



Method 3

(rectangle over the half of the peak, which goes down to the correct background + draw Ctrl+D > measure area with the magic stick > multiply by 2: best quantification of the band)



Method 4

(horizontal measurement lane, fast method, but not completely correct, quantification still quite good)

(averaging perpendicular to the lane > not showing proper peaks, but the lateral heterogeneity of the bands)





Johannes A. Schmid, Inst. of Vascular Biology and Thrombosis Research

Plugins Window Help					
Macros	•				
Shortcuts	•				
Utilities	+				
New					
Edit					
Compile and Run					
Fret Analyzer					
Intensity vs. Time plot	Strg+1				
Online Help [F1]					
Enhance Image	Strg+E				
Colocalisation Analysis	+				
Colour functions	+				
Deconvolution	+				
FRETcalc v1					
LUT	+				
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Particle Analysis	•				
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Spatial calibration					
Stacks - Building					
Stacks - Reducing					
Stacks - Shuffling					
Stacks - T-functions					
Stacks - Z-functions	•				
Toolbars	×				
TransformJ	+				

The Plugins Menu

 Many freely available plugins can be loaded into ImageJ, which appear in this menu for a list see:

http://rsb.info.nih.gov/ij/plugins/index.html

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Eraser Tool Options		
Smooth Tool Options		
XYZ Points Options		

 Macros can be recorded to automatize frequently used commands

Plugin Example: FRAP Analysis by Intensity vs. Time Plot or Plugin: Stacks > measure stack



- Images recorded with LSM510: time stack of bleached area
- circle selected for quantification
- click Strg.1 (Intensity vs. Time Plot) in the Plugin menu > graph appears
- Click "List" in the graph > List of results appears
- The results can be copied into MS-Excel > into curve fitting software (e.g. Graphpad Prism)



Macros

Shortcuts

Utilities

New...

Edit...

Compile and Run...

Fret Analyzer	
Intensity vs. Time plot	Strg+1
Online Help [F1]	
Enhance Image	Strg+E
Colocalisation Analysis	•
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ROI Segmentation Spatial calibration Stacks - Building Stacks - Reducing Stacks - Reducing Stacks - Shuffling Stacks - T-functions Stacks - Z-functions Toolbars	

Plugin: Example 2: Nucleus Counter for measuring multiple cells

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SpotEnhancingFilter:





Example 3: FRET analysis with PixFRET

PixFRET	PixFRET	PixFRET
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Donor SBT Model Step 1: Background Determination Define a ROI and click "Get" or choose values and click "Set"	Acceptor SBT Model Step 1: Background Determination Define a ROI and click "Get" or choose values and click "Set"	Background FRET 0.0
then click "Accept" to go to the step 2.	then click "Accept" to go to the step 2.	Donor 0.0
Step 2: Model Parameters Determination Define a ROI and click "Get" or choose values and click "Set", then click "Accept" to go to the FRET computation. Background Donor FRET 0	Step 2: Model Parameters Determination Define a ROI and click "Get" or choose values and click "Set", then click "Accept" to go to the FRET com Background Acceptor FRET 0 Accept	Acceptor 0.0 Reset Get Parameters Gaussian blur 1.0 (0.0 = No blur) Threshold 0.2 Correction Factor Output FRET/sqrt(Donor*Acceptor) Computation BTdon = 0.13341
Reset Get Accept	Reset	BTacc = 0.36910
Model Donor	Model Acceptor	Show blurred images Compute FRET
Gaussian Blur 2.0 (2.0 recommended) Constant Constant	Gaussian Blur 2.0 (2.0 recommendation © Constant a 0.36910 © Linear a 0.42037 b -0.00003 © Expo. a 0.38521 b 0.14985 Reset Reset General constant co	Save Parameters Close



Fiji: "Fiji Is Just ImageJ"

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Label		Compile and Run	
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Set Measurements			-
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Plot Profile Strg+K		Feature Extraction	
Surface Plot		Image5D	
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		Tracking	•
		Transform	> _
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		Volume Viewer	
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Task 3a (curve fitting)

- 1. Download CurveExpert Pro from https://www.curveexpert.net/
- 2. Download data from here:

https://www.meduniwien.ac.at/user/johannes.schmid/2023Task3a.xlsx and copy days (as x) and accumulated cases (as y) into CurveExpert

- 3. Search for the best equations using the Curve finder tool (checking only the built-in non-linear regressions)
- 4. Choose the result with the highest score and check the equation by double clicking on this result.
- 5. In the original Excel file: Define the variables of this equation (with name manager); type in the correct equation and perform a curve fit in Excel using the Solver function and minimizing the sum of residuals squared
- 6. Type the expected number of accumulated cases at day 100 into the online text field of the task and upload your Excel file



Task 3b Professional graphs with R-ggplot2

- Download and install R
- Download and install R-Studio
- Download the Excel file pathwaysR.xlsx
- Create a ggplot2 bubble graph with pathway names on yaxis, neglogp on x-axis, color gradient of bubbles defined by zscore (with positive in red, zero=white and negative in blue) and size of bubble=Ratio)



Example Macro for ImageJ

• Record macro to define nuclei via the DAPI channel and measure fluorescence of the nuclei in another channel for a composite image

rename("test.tif"); run("Split Channels"); selectWindow("C1-test.tif"); setAutoThreshold("Default dark no-reset"); //run("Threshold..."); setOption("BlackBackground", false); run("Convert to Mask"); run("Watershed"); setAutoThreshold("Default no-reset"); run("Analyze Particles...", "size=10 pixel show=Outlines display exclude clear include summarize add"); selectWindow("C2-test.tif"); selectWindow("ROI Manager"); roiManager("Measure"); selectWindow("Results");







www.cellprofiler.org



Image Analysis & Quantification



Image-centric Data Analysis

- **Process** large sets of images
- Identifies and measures objects
- Export data for further analysis





CellProfiler - Basics

Establish a "Pipeline" of a predefined workflow:

- Load images: based on parts of the file name...
- Improve images if necessary (brightness, contrast, background subtraction...)
- Identify objects (primary, secondary, tertiary objects)
- **Measure** object features (mean intensity, shape factors: diameter, perimeter...)
- Visualize data (e.g. density plots...)
- Export data (database format, Excel-csv-format)
 - Pipeline: Steps of image processing in CellProfiler
 - *Module:* One step of a pipeline
 - Primary Objects: Key objects used to identify cells
 - Secondary Objects: Other parts of cells, attached to primary objects

CellProfiler-Interface

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Module: Loading of Images

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Other modules

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Other modules

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Module Categor File Processing Image Processing Object Processing Measurement Data Tools Worm Toolbox Other All	ies CalculateMath CalculateStatistics DisplayDataOnImage DisplayDensityPlot DisplayHistogram DisplayPlatemap DisplayScatterPlot ExportToDatabase ExportToSpreadsheet FlagImage MergeOutputFiles
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Help for module, "DisplayScatterPlot"

<u>F</u>ile <u>E</u>dit

DisplayScatterPlot

Display Scatter Plot plots the values for two measurements

A scatter plot displays the relationship between two measurements (that is, features) as a collection of points. If there are too many data points on the plot, you should consider using **DisplayDensityPlot** instead.

The module will show a plot shows the values generated for the current cycle. However, this module can also be run as a Data Tool, in which you will first be asked for the output file produced by the analysis run. The resultant plot is created from all the measurements collected during the run.

See also DisplayDensityPlot, DisplayHistogram.

Settings:

Type of measurement to plot

You can plot two types of measurements:

- Image: For a per-image measurement, one numerical value is recorded for each image analyzed. Per-image measurements are produced by many modules. Many have MeasureImage in the name but others do not (e.g., the number of objects in each image is a per-image measurement made by IdentifyObject modules).
- Object: For a per-object measurement, each identified object is measured, so there may



Object separation

- Clump identification: Two options
 - Intensity: Works best if objects are brighter at center, dimmer at edges



Shape: Works best if objects have indentations where clumps touch (esp. if objects are round)






Primary, secondary and tertiary objects

- **Goal:** Identify *tertiary objects* by removing the primary objects from secondary objects
 - "Subtract" the nuclei objects from cell objects to obtain cytoplasm





CellProfiler- Data visualization

Data from thousands of cells can be stored in Spreadsheet (Excel) of Database formats and visualized in different ways:





GIMP (GNU Image Manipulation Program) (free alternative to Adobe Photoshop)

- https://www.gimp.org/: for generating publication quality figures
- to create professional images of research results (microscopy images, Western Blots...)
- Can be set to different languages (default: system language)
- Theme and icons can be set to different modes: system, light, gray, dark (under: Edit > preferences)

If you prefer Photoshop: an older version (CS2) is available at: <u>https://www.computerbild.de/download/Adobe-Photoshop-CS2-Vollversion-8040793.html</u>



GIMP (GNU Image Manipulation Program) (free alternative to Adobe Photoshop)





Some Features

- GIMP works with layers, that means that you can change, move... just one layer (e.g. text) while leaving the others unaltered. This also includes changing of contrast and brightness.
- Colour pictures usually work in RGB mode (Red-Green-Blue) called channels (Kanäle). You can select one channel (by highlighting it) and modify it. You can watch all channels (clicking on the eye-symbol left of RGB), while changing just one.
- You can copy/paste image data in one channel alone (> you can generate a merged colour image from monochrome microscopy images generated with different filters)



Example of an image in GIMP

Tools window Untitled]-6.0 (RGB color 8-bit gamma integer, GIMP built-in sRGB, 1 layer) 1844x1646 – GIMP File Edit Select View Image Layer Colors Tools Filters Windows Help Brushes Patterns Aa Fonts 🔬 Document History . 🗆 🔍 🔨 🖉 💹 🖒 . X 2. Hardness 050 (51 × 51) 🗶 🥏 🕹 🕺 🚱 🚱 🖉 🤤 0 o , P , N , & 🖣 😤 😥 👔 А Color Picker 0 Measure Shift+M **教信事業会いの** Tool Options 🔍 Devices 🥎 Undo 🔳 Images tools options Color Picker Sample average Basic, Sample merged 10,0 🕽 Spacing Pick Target (Ctrl) Pick only ╋ ⊵ C 0 Set foreground color Set background color 🛿 Layers 🛃 Channels 🎊 Paths layers, channels and paths \rightarrow Add to palette Normal V Mode Use info window (Shift) 0-100,0 Opacity Lock: 🖉 💠 📖 MEDICAL UNIVERSITY ۵ Pasted Layer Johannes A. Schmid, Inst. of Vascular Biology and T OF VIENNA

Image Modes: Gray, RGB, 8 bit, 16 bit...









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Image Adjustments: intensity levels II

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Image Adjustments: intensity levels of colour channels

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Image Adjustments: intensity levels of colour channels

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OF VIENNA	Johannes A. Schmid, Inst. of Vascular I	Biology and Thrombosis Re	search		343

The image resolution (pixels per inch, dpi)



Ctrl (STRG) and scroll > enlargens or shrinks the canvas



The Scale Image window

width and height in pixels this defines the file size

> resolution > 300 dpi is minimum for publications (for decent text quality you need at least 150 dpi)

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Scaling = re-calculation of images (intra- or extrapolation) - you can specify whether the proportions are maintained



Resizing the canvas



= Set Image Canvas Size [Untitled]-12 Canvas Size - Ø Width: 400 Height: 600 \sim px 400 × 600 pixels 300 ppi Offset • <u>X</u>: 62 ▲ ▼ <u>Y</u>: 66 Center рх \sim Layers Resize layers: All layers \sim White Fill with: \sim Resize text layers <u>H</u>elp Reset Resize Cancel

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🏰 Set Image Canvas Size



Adding text to images

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Image Layers (e.g. with text)

- Layers can be modified separately
- Saving the image in GIMP or Photoshop format (*.psd) also saves the layer information (> you can later on change them again, e.g. the text of figures !)
- You can merge layers (for saving in JPG- or TIF-format)





Image processing: Rules for correct usage of features

- Changes (levels, brightness, contrast) should always be made on the whole image (and not parts of it; changing parts of the image, e.g. single bands is of course not allowed)
- You should not discard any image information (e.g. the background by making it completely white)
- Computer experts can prove that something was changed so be carefull when changing anything



Gimp Menu: File



< open from clipboard





Gimp Menu: Edit

Undo history



Edit Select View Image Layer Colors Tools

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Undo Move Text Layer

Undo History

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Redo

Gimp Menus: Select and View

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Gimp Menus: Image and Layer

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Gimp Menus: Colors, Tools and Filters



Hot...

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Opening of Photoshop-files and saving in Photoshop-format is possible

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IrfanView



Freeware, which can handle many file types (with plugins: even flash-videos)

Some nice features:

- very fast
- batch conversion of images
- (e.g. to change the resolution)
- available in many languages
- slideshow option
- also contains Paint-tools and a text tool
- rotating of images
- changing canvas size or resolution ...



Inkscape (free alternative for Adobe Illustrator)

- <u>https://inkscape.org</u>
- for generating publication quality figures
- Creates scalable vector graphics files (svg-files) and also other file formats, where images can be expanded without losing the resolution (e.g. perfectly suited for posters and figures to prevent pixelated images)
- Good tutorials and documentation at: <u>https://inkscape.org/learn/</u>
- If you prefer Adobe Illustrator: an older version (CS2) is available at: <u>https://www.computerbild.de/download/Adobe-Illustrator-CS2-Vollversion-8043129.html</u>



Difference between bitmap and vector graphics

Gimp, jpg-file (enlarged):

This is a test

Inkscape:

This is a test

Bitmap Graphics	Vector Graphics
Made up pixels with different colours	 Made of points, lines and shapes based on mathematical equations
Loss of image quality due to the creation of new pixels when enlarge	 No change in the image quality when enlarge
 Loss of image quality due to the loss of pixels when shrunk 	 No change in the image quality when shrunk
 File size depends on the number of pixels the image is made up of 	 File size depends on the number of objects and their mathematical information
	AD



What is Inkscape?

- Vector Graphics Editor
- Free Software
- Cross Platform
- Easy to use
- Good for:
 - Compositing
 - Drawing
- Not for:
 - Bitmap editing





Scaling figures

- Vector images can be scaled freely without loss of quality
- Bitmap images can be scaled down, but not up



Images are made by points and their connections. Connections can be straight or smooth



The start screen

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Exercise 1: set up a canvas

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Document Properties (Shift+Ctrl+D)					
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	Show border shadow				
	Use antialiasing				
	Background colour:				
	Border <u>c</u> olour:				

- File > Document Properties
 - Shows page in view
 - Doesn't restrict drawing
 - Useful as a guide
- Change background colour to white
- Change to landscape



Moving around

- Panning
 - Scroll bars on bottom / right
 - Scroll up/down, Shift+scroll for left/right
- Zooming in / out
 - Click to zoom in, shift+click to zoom out \mathbb{Q}
 - Control + Scroll Up/Down to zoom in/out to cursor
- Shortcuts

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6

– Fit 🔍 page, 🚳 drawing, 🔍 selection in window

Create basic shapes

- Select tool from toolbar
- Click and drag on canvas
 - Box selects the bounds of the new shape
 - Colours are remembered from the last shape
- Basic options appear in top toolbar
 - Number of spokes on stars
 - Rounded corners on rectangles
 - Circle vs segment vs arc

The main toolbar

Selection tool, F1 Edit nodes tool, F2 Sculpt tool Zoom tool, F3 Measurement tool Make rectangles, F4 Make 3D boxes Make ellipses / arcs, F5 Make polygons / stars Make spirals, F9 ൭

The main toolbar

- Selection tool, F1
 Edit nodes tool, F2
 Sculpt tool
 Zoom tool, F3
 Measurement tool
 Make rectangles, F4
 Make 3D boxes
 Make ellipses / arcs, F5
 Make polygons / stars
 Make spirals, F9
- Draw freehand lines, F6
 Draw straight lines / curves
 Calligraphy tool
 Add text, F8
 Sculpt with spray
 Erase
 Fill
 Edit gradients
 Select colour
 Create diagram connectors

press Strg (Ctrl) to force an exact horizontal/vertical line or 15° angles

Control nodes

• Use the Edit Nodes tool



- Two types of control points, squares and circles
 - Squares generally change the size of the shape
 - Circles change the appearance





Resize / Rotate



- Can use shift/control keyboard modifiers as before
- For rotation you can move the crosshair to change the centre of rotation

Grouping

- Grouping (right click > Group or Ctrl + G)
 - -Combine multiple objects into a single object
 - Reversible: Ungroup (Control+Shift+G)

Transform Shortcuts



- Rotate 90 degrees anticlockwise
- Rotate 90 degrees clockwise
- Mirror object around the vertical axis
- Mirror object around the horizontal axis


Aligning and distributing

- Object > Align and Distribute
 - Align = Give objects the same centre/edge position
 - Distribute = Space objects evenly
- Align relative to
 - First/Last selected object in group
 - Largest/Smallest object in group
 - Page
 - Drawing
- Never align anything by eye!

🗣 Align and Distribute 🗖 🔳 💌
🖺 Align and Distribute (Shift+ Ctrl+ A) 🖪 🔳
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Relative to: First selected 💌
Treat selection as group:
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Remove overlaps
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Fill and Stroke

- Fill = Colour/ Gradient/ Pattern within a shape
- Stroke = The line around a shape
- Object > Fill and Stroke (Shift+Ctrl+F)
- Edit
 - Colours
 - Opacity
 - Blur





Stroke Options

- Width of line
- Shape of corners
- Shape of line ends
- Dashes
- Arrowheads

余 Fill and Stroke (Shift+Ctrl+F) 📃 🔳 💌
Fill and Stroke (Shift+Ctrl+F)
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Creating and Editing Paths

- Created using freehand or line tool
- Can convert other objects to become a path
- Paths are composed of nodes and segments
- There are different types of node and segment

Nodes and Segments

- Segment types
 Lines (1)
 - Curves (2)
- Node types
 - End (3)
 - Corner (4)
 - Smooth (5)
 - Normal
 - Symmetric
 - Auto





- Use nodes toolbar to add, remove or convert nodes or segments
- Select nodes or segments to make their handles visible
- Drag handles to change the arc of curves



Combining Paths



Opacity / Blur

- Applies to whole object
- Separate from alpha in colours
- Works on all Objects







Gradients

- Standard colour option
- Set multiple colours / opacities to go through
- Set the direction and extent of the gradient







Z axis - Ordering

- New objects sit over the top of old objects
- Objects obscure those underneath them (except for transparency)





- Send object to bottom of z-stack
- Lower object one level
- Raise object one level
- Bring object to top





Working with bitmaps (photos)

- Inkscape can include bitmaps in images
- Appear as objects alongside vector objects
- Can't edit the images
- Can't increase the resolution of the image
- Transparency (from PNG etc) is preserved
- File > Import
- Formats: PNG, JPEG, SVG, PDF etc.



Text toolbar sans-serif Image: Image:

Text and font settings: font, alignment, spacing

Text and Font (Shift+Ctrl+T)		۹
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Example for a Figure template in Inkscape











Cytometry Software: FlowExplorer - freeware

Scattergrams (Dot plots): each event is a dot









Contour plots and density plots

Dot plots are 2D-graphs they show values of two parameters Density plots show a pseudocolor visualization of dot density



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next file

markers and quadrants can be generated by clicking on the border and dragging into the graph

Pressing the "Stats On" Button shows statistics



Histograms (frequency of events for a parameter)



choosing Frequency (Histogram) for the Y-axis generates a histogram



Overview of cytometry plots



colour coding of the

contours of the frequency (similar to the height lines in a



Time course of fluorescence in flow analysis

Some processes can be measured by analyzing the time course of fluorescence (e.g. calcium influx with Fluo-4 calcium sensitive fluorophores). The time parameter can be recorded at the data acquisition – and also visualized with WinMDI



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Mathematical operations (e.g. ratios)

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Mathematical Calculations with Parameters
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Y2 = (FSC-H) FSC-Height
Convert LOG to LIN for calculations
Math Formula
(× = Y1/Y2*500
C X= Y1/Y2
Output Parameter (Always converted to LIN)
× = (FSC-H) FSC-Height ▼
Vew Label: Calculated Parameter
File Renaming (if empty file overwrite)
File Prefix: R_new
Apply Calculation to File
Cancel OK



Cytometry software: Flowing Software 2

http://www.flowingsoftware.com

File Settings Help	
Viain File Edit Create Visualization tools > MetaData tools > Collection tools > Automation tools > Parameter modification tools > Data modification tools > Data reduction tools > Region control tools > Layout tools >	File Settings Help Main File Edit Create Open FCS Strg+O Open TXT (tab delimited) Next file in directory Strg+N Previous file in directory Strg+P File Sort Method Image: Sort Method



Gating can be done in different graphs than the one used for defining the region

Regions can be used as locigal "Gates" to accept or reject data (e.g. to exclude cell debris)





FL2-Height



Flowing Software 2 – Regions and statistics









Cell cycle analysis with Flowing Software





Multi-parallel coordinate plots for visualization of several parameters in parallel





Can be done with Freeware (Mondrian: http://www.theusrus.de/Mondrian/index.html)

One event (e.g. one cell) is represented by a line linking several y-axis (for the different parameters e.g. fluorescence signals); a "population" can be selected and is highlighted also for the other parameters. The data density can be reduced (using a so called alphafactor) to obtain better visibility of numerous data points.

MatLab-Visualization of a parallel coordinate plot



Parallel coordinates with gating



https://bl.ocks.org/jasondavies/1341281



Molecular Structure Analysis Software (Visualization and analysis of crystallographic data)

- Chimera (UCSF): <u>http://www.cgl.ucsf.edu/chimera/</u>
- Rasmol: <u>http://www.openrasmol.org/</u>
- RasTop: <u>http://www.geneinfinity.org/rastop/</u>
- Protein Explorer: <u>http://www.umass.edu/microbio/rasmol/</u>
- Cn3D: <u>http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml</u>
- Jmol: <u>http://jmol.sourceforge.net/</u>



Rasmol

- simple small tool (400 kB) to visualize molecular structures from *.pdb files (protein data bank)
- Molecules can be moved back and forth
- Molecules can be rendered in different ways (wire, cartoon, space fill..)
- Molecules can be colored in different ways
- Distances can be measured in Angstroem
- You can also incorporate a Rasmol application into Powerpoint







FP structure.pdb

Display modes in RasMol: Rendering and colour







Johannes A. Schmid, Inst. of Vascular Biology and Thrombosis Research

Measure Distances with RasMol







RasTop (modern Rasmol)

- small (1 MB) and fast
 3D molecule viewer
 based on Rasmol
- allows loading of more than one molecule
- allows surface views and many other features





http://www.geneinfinity.org/rastop/

Cn3D Molecule Viewer (NCBI)

- structure window for visualization of the 3D structure of the molecule: you can turn the molecule, render it in different ways and colour it in different ways
- sequence window: shows the amino acid sequences of proteins: residues can be selected (Mouse Mode: rectangle, column or row): sequence homologies can be aligned
- Import window: activated from the sequence window: can be used to import additional molecules from NCBI





Cn₃D structure and sequence window

ILDK_B maqssskspellarycdsllkkssknpeeaeledtlnqvmvvfkyiedkdvfqkfyakmlakrlvh ILDK_C kkrfevkkwnavalwawdivvdncaicrnhimdlciecqanqasatseectvawgvcnhafhfhci ILDK_D psiklqssdgeifevdveiakqsvtiktmledlgmdpvplpnvnaailkkviqwcthhkddppppe **▲**[



Cn3D Rendering and Colouring



- Molecules can be rendered in different ways (worms, tubes, wire, balls+sticks, space fill). The appearance can be customized ("Edit Global Style)
- Colours can be adjusted to distinguish domains, molecules, secondary structures, charges and even alignements.





ILDK_E wds1pde111gifsc1c1pe11kvsgvckrwyr1asdes1w

Selection of molecules or domains

sequences selected in the sequence window are highlighted in the structure window





Identification of interaction domains with the help of Cn3D

Select first the molecule for which you want to define the potentially interacting amino acids (Mouse mode: rows). From the Show/Hide menu of the structure window chose "Select by Distance" and "Other Molecule" > define the cutoff in Angstroem.

➤ the amino acids in the vicinity of the selected molecule will be highlighted.

press the Ctrl (Strg) button and click at the previously selected molecule to de-select it > only the nearby residues of the other
 molecules are highlighted >
 interaction domains can be
 identified.

Customized appearance of selected residues



Selected residues can be rendered and coloured differently: Menu "Style" > Annotate > New > Edit Style > the appearance can be adjusted for the selected residues (e.g. space fill, colouring by charge)


Style options: adding amino acid numbers

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Importing sequences and aligning them

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From the sequence window: click "Imports" and "Show Imports" -then the Import window comes up > in the menu "Edit" select "Import Sequences" > define with which molecule the imported sequence will be aligned > define the molecule to be imported (either by accession number or the sequence in FASTA format) > The two sequences will be shown aligned in the Import window >Alignments > "Merge all" aligns the two chains in the sequence window > aligned residues can be visualized... ➤When you use a structure file for import you will also see the two structures superimposed after saving the



Example of two aligned structures





VAST (Vector Alignement Search Tool) for defining structural neighbours

S 1NDD neighbors - Cn3D 4.1	
<u>File</u> ⊻iew Show/ <u>H</u> ide <u>S</u> tyle <u>W</u> indow <u>C</u> DD <u>H</u> elp	
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1NDD neighbors - Sequence/Alignment Viewer	×
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Chimera

- <u>http://www.cgl.ucsf.edu/chimera/</u>
- capable of showing surfaces (also in transparency mode)
- very good 3D rendering and visualization tools
- fancy preset visualization options
- several molecules can be loaded into the same window
- many tools (e.g. side view, cutting through molecules, rotation, movie recorder...)







Preset: Publication1 + 60%

transparent surface



Preset hydrophobicity surface view





New Chimera X

Chime	eraX																
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Johannes A. Schmid, Inst. of Vascular Biology and Thrombosis Research

New Chimera X

... allows virtual reality view of molecules with a VR headset:

ChimeraX Virtual Reality

Warning: ChimeraX virtual reality (VR) capabilities are a work in progress. We recommend using Windows 10 where VR equipment works best. It is possible to use on Mac and Linux but less stable, see details for Mac VR, Linux VR.

ChimeraX allows display and analysis of structures and density maps using virtual reality headsets such as HTC Vive, Vive Pro, Oculus Rift, Samsung Odyssey and Windows Mixed Reality. For multi-person VR sessions, see meeting. To add buttons for easy command execution from within VR, see buttonpanel. ChimeraX can also record 360° movies.

Starting VR Mode Hand-Controller Modes Other Icons: Changing the Display, Etc. Limitations of Interactive Viewing







Free Molecular Biology Software

- website showing an overview of available freeware (a bit outdated): <u>http://molbiol-</u> <u>tools.ca/molecular_biology_freeware.htm</u>
- UGENE <u>http://ugene.unipro.ru/</u>
- GenomeProfiler: obsolet: Flash-based <u>https://designer.genomecompiler.com/app</u> online or standalone
- Gene Designer: <u>https://www.atum.bio/resources/tools/gene-designer</u> obsolet (Adobe Air-based)
- SnapGene Viewer: https://www.snapgene.com/snapgene-viewer
- SerialCloner: <u>http://serialbasics.free.fr/Serial_Cloner.html</u>
- ApE: <u>https://jorgensen.biology.utah.edu/wayned/ape/</u>

UGENE

http://ugene.unipro.ru/

Powerful freeware, quite fast, also contains smart web-based analyses





UGENE

- includes a 3D molecule viewer





UGENE

- alignments

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Ugene-Workflows



Workflows (scripts) can be generated to facilitate repeated actions

NGS (Next generation sequencing) data is supported



Some key UGENE features

- Alignments: <u>ClustalW</u>, <u>ClustalO</u>, <u>MUSCLE</u>, <u>Kalign</u>, <u>MAFFT</u>, <u>T-Coffee</u>, optimized <u>Smith-Waterman</u> algorithm
- PCR *in silico*; Integrated <u>Primer3</u> package for PCR primer design
- <u>Search through online databases</u>: NCBI, PDB, UniProtKB/Swiss-Prot, UniProtKB/TrEMBL, DAS servers
- Local and NCBI Genbank BLAST search
- <u>Restriction enzyme finder with integrated REBASE restriction enzymes list; Open reading frames finder</u>
- Plasmid construction and annotation; Cloning in silico by designing of cloning vectors
- Genome mapping short reads with <u>Bowtie</u>, <u>BWA</u> and <u>UGENE Genome Aligner</u>
- Raw NGS data processing
- Visualization of next generation sequencing data (BAM files) using UGENE Assembly Browser
- RNA-seq data analysis with <u>Tuxedo</u> pipeline (TopHat, Cufflinks, etc.)
- ChIP-seq data analysis with <u>Cistrome</u> pipeline (MACS, CEAS, etc.)
- <u>HMMER2</u> and <u>HMMER3</u> packages integration
- <u>Chromatogram viewer</u>
- Search for transcription factor binding sites (TFBS) with <u>weight matrix</u> and <u>SITECON</u> algorithms
- Search for direct, inverted and <u>tandem repeats</u> in DNA sequences
- Building (using integrated <u>PHYLIP Neighbor Joining</u>, <u>MrBayes</u> or <u>PhyML Maximum Likelyhood</u>) and editing phylogenetic trees
- · Combining various algorithms into custom workflows with UGENE Workflow Designer
- Contigs assembly with <u>CAP3</u>
- <u>3D Structure viewer</u> for files in PDB and MMDB formats, anaglyph view support
- Protein secondary structure prediction with GOR IV and PSIPRED algorithms
- Constructing <u>dotplots</u> for nucleic acid sequences
- Creating and using a <u>shared storage</u> (e.g. for a lab)
- Search for complex signals with <u>ExpertDiscovery</u>
- Search for a pattern of various algorithms' results in a nucleic acid sequence with <u>UGENE Query</u> <u>Designer</u>



CloneManager

- commercial software from Scientfic Education: <u>http://www.scied.com</u>
- very well suited for "in silico cloning" and handling of DNA constructs, primer design, alignments....
- Supports Genbank format
- Import from Entrez, NCBI
- basic license: 575\$
- professional license: 1.100 \$

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SNAPGene-Viewer (freeware: with restrictions)









LinRegPCR Software for realtime PCR

http://www.hartfaalcentrum.nl/index.php?main=files&sub=LinRegPCR

Nucleic Acids Research Advance Access published February 22, 2009

Nucleic Acids Research, 2009, 1–12 doi:10.1093/nar/gkp045

Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data

J. M. Ruijter^{1,*}, C. Ramakers², W. M. H. Hoogaars¹, Y. Karlen³, O. Bakker⁴, M. J. B. van den Hoff¹ and A. F. M. Moorman¹



classical methods (like the $\Delta\Delta$ Ct-method) require correct baseline settings for correct quantification



the amplification cu starting point N₀

LinRegPCR uses the logarithmic part of the amplification curve to calculate a starting point N_0





Read values from Excel for LinRegPCR	
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O MJ Research	Rotor-Gene (Corbett Research)
C Bio_rad iCycler	C Eppendorf Realplex
C LightCycler 480 (converted raw data)	C Applied Biosystems (5 leading columns)
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New Web-based LinRegPCR tools

<u>https://www.gear-genomics.com/rdml-tools/</u>

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New Web-based LinRegPCR tools

<u>https://www.gear-genomics.com/rdml-tools/</u>

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2	A2	gDNA	unkn	cDNA	Exon 1	non- saturating DNA binding dye		baseline error; instable baseline; no plateau	130.788	5	0		1.841	0.134			Yes	Yes	Yes	No	No	No	No
3	A3	gDNA	unkn	cDNA	Exon 2	non- saturating DNA binding dye		baseline error; instable baseline; no plateau	130.788	6	0		1.911	0.184			Yes	Yes	Yes	No	No	No	No



Radioactivity software: RadPro Calculator

Online: http://www.radprocalculator.com/

Download link: <u>http://www.radprocalculator.com/RadProDownloads.aspx</u>

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VIPER – anti-plagiarism software

http://scanmyessay.com/

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Plagiarism check by SimTexter

https://people.f4.htw-berlin.de/~weberwu/simtexter/app.html





Plagiarism check by using Google Scholar for table values or similar text.

Example: Detection of an "octapaper" (the same figures or data sets published in 8(!) different articles, with different authors and institutions in China:

https://scienceintegritydigest.com/2020/06/03/the-octopaper/

(apparently sold as service for students, who need a paper for promotion)

It took a Google Scholar search for table values to detect that all eight papers are connected.





https://cytoflow.github.io

Cytoflow: Better quantitative flow cytometry.



Cytoflow is a **point-and click program** and a **Python library** for analyzing flow cytometry data. It was written by Brian Teague to address shortcomings in currently-available flow software.



Cytoflow

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⊠ B1+H> B1_H	
⊠ B1-W> B1_W	
FSC-A> FSC_A	
K FSC-H> FSC_H	
K FSC-W> FSC_W	
HDR-T> HDR_T	
🗵 SSC-A> SSC_A	
SSC-H> SSC_H	
🗵 SSC-W> SSC_W	
🕅 V2-A> V2_A	
⊠ V2-H> V2_H	
🗵 V2-W> V2_W	
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X Y2-H> Y2_H	
X Y2-W> Y2_W	
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Events: 20000	
Set up experiment	
Import!	

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 Cytometry analysis 		
Export figure		A.
Experiment Browser		
Help		7
✓ Plot Parameters		
✓ View Properties		
✓ Workflow		1

User manual link:



https://cytoflow.readthedocs.io/en/stable/user_manual/user_manual.html







Violin Plots





CytExpert (for Cytoflex Beckman Coulter)

https://www.beckman.com/flow-cytometry/research-flow-cytometers/cytoflex/software

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MEDICAL UNIVERSITY

OF VIENNA

Recent Compensation

Close Experiment

Exit

Floreada – web-based flow cytometry analysis <u>https://floreada.io/</u>





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Floreada features



- Histogram overlays
- Compensation matrices
- Exporting of images
- Gating
- Saving analyses on workspaces
- Cell cycle analysis
- t-SNE plots




Databases

Image databases

- <u>https://imagescience.org/images/</u>
- <u>https://histologyguide.com</u>
- mouse histology:

https://www.emouseatlas.org/emap/home.html



Histology Guide virtual microscopy laboratory

HOME

SLIDE BOX

ELECTRON MICROSCOPY

QUIZ

SEARCH

INDEX

HELP

Slide Box

This virtual slide box contains 275 microscope slides for the learning histology.



Fig 023 Types of Tissue

Cells and Tissues

Tissues are classified into four basic types: epithelium, connective tissue (includes cartilage, bone and blood), muscle, and nervous tissue.







€mouseatlas

Anatomy and Histology EMAP

Gene Expression About

Help

Velcome to the eMouseAtlas community resource.

Search all data using the resource access buttons or the Zegami image browser below





Mouse embryo anatomy, ontology and staging ... more info



Histology atlas including Kaufman's Atlas of Mouse Development ... more info



A database of spatially mapped gene expression, enhancer and lineage data ... more info



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Mouse Databases

- Jackson Mouse database: <u>http://www.informatics.jax.org</u>
- <u>https://www.mousephenotype.org</u>
- Mouse repository MMHC

https://frederick.cancer.gov/resources/repositories/nci-mouse-repository



Search

Mouse Genome Informatics

ownload 🔻 More Resources 🔻	Submit Data Find Mice (IMSR)	💥 Analysis Tools	Contact Us Browsers				
	Keywords, Symbols, or IDs		**NEW: MOUSE RESOURCES FOR COVID-19 RESEARCH**				
	Or use topic specific search and analysis to	ols:	integrated genetic, genomic, and biological data to facilitate the study of l health and disease.	human			
	Genes		About Us MGI Publications Cite Us	f 🔁			
	Phenotypes & Mutant Alleles			-			
	Human-Mouse: Disease Con	nection	More Filters for Expression Summaries				
	Gene Expression Database (G	GXD)	Results Associations				
	Recombinase (cre)		Filter expression by:				
	100 Function		Theller Stage T Molecular Function T Assay Type T Biological Process T				
	Strains, SNPs & Polymorphism	ms	Detected? 7 Cellular Component 7 TPM Level 7 Phenotype 7				
	Vertebrate Homology		Wild type? 7 Disease 7				
	Mouse Models of Human Can	cer	000	•000			
	Batch Data and Analysis Tool	s	What's new at MGI updated September 2 MGI is updated to the reference genome Build 39. Read more	20, 2021			
	Nomenclature		 Multiple Genome Viewer updated with human and rat genomes transcripts. Read more 	and			



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Nucleotide Databases

• Addgene Plasmid Repository plasmid collection: very comprehensive non-profit collection (appr.	www.addgene.org							
65\$/plasmid)	www.ensembl.org							
Ensembl Genome Browser	www.ncbi.nlm.nih.gov/geo							
Gene Expression Omnibus GEO								
microarray data	www.genecards.org							
 GeneCards: gene overview with many 								
U UGENE – Integrated Bioinf × GeneCards - Human Gene ×	http://bandlifeesiancedb.jp/							
← → C f www.genecards.org								
🏢 Apps 🥩 MolCalc 📋 Geräte 📋 MUW 📋 Science 🦓 AAI 💋 Bank A. 🔕 BioGPS 👩 I	Doodle 💠 Dropbox 🛐 G-Scholar							
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Search by keyword(s) for Search Advance	ed Search About V3 Search							



GeneCards Screenshot

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🔅 GeneCardsSuite GeneCa	rds MalaCards LifeMa	ap Discovery Path	Cards Gene Analy	tics GeneALaCa	rt VarElect (Genes LikeMe G	ene Loc
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lome User Guide Analysi	s Tools - News And Vi	iews About -			My Genes	Log In / S	Sign Up
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UniProtKB/Swiss-Prot Summary for IKBKB Gene

> Serine kinase that plays an essential role in the NF-kappa-B signaling pathway which is activated by multiple stimuli such as inflammatory cytokines, bacterial or viral products, DNA damages or other cellular stresses (PubMed:30337470). Acts as part of the canonical IKK complex in the conventional pathway of NF-kappa-B activation. Phosphorylates inhibitors of NF-kappa-B on 2 critical serine residues. These modifications allow polyubiquitination of the inhibitors and subsequent degradation by the proteasome. In turn, free NF-kappa-B is translocated into the nucleus and activates the transcription of hundreds of genes involved in immune response, growth control, or protection against apoptosis. In addition to the NF-kappa-B inhibitors, phosphorylates several other components of the signaling pathway including NEMO/IKBKG, NF-kappa-B subunits RELA and NFKB1, as well as IKK-related kinases TBK1 and IKBKE (PubMed:11297557, PubMed:20410276). IKK-related kinase phosphorylations may prevent the overproduction of inflammatory mediators since they exert a negative regulation on canonical IKKs. Phosphorylates FOXO3, mediating the TNF-dependent inactivation of this pro-apoptotic transcription factor (PubMed:15084260). Also phosphorylates other substrates including NCOA3, BCL10 and IRS1 (PubMed:17213322). Within the nucleus, acts as an adapter protein for NFKBIA degradation in UV-induced NF-kappa-B activation (PubMed:11297557). Phosphorylates RIPK1 at 'Ser-25' which represses its kinase activity and consequently prevents TNF-mediated RIPK1-dependent cell death (By similarity). Phosphorylates the C-terminus of IRF5, stimulating IRF5 homodimerization and translocation into the nucleus (PubMed:25326418). IKKs_HUMAN,O14920



UniProt	UniProtKB -							
BLAST Align Retrieve/ID mapping	g Peptide search SPARQL							
UniProt. The new UniProt website is here! Take me to Uni								
UniProtKB - O1	4920 (IKKB_HUMAN)							
Display Help video	Selast ≥ Align Server Add to basket O History							
Entry	Protein Inhibitor of nuclear factor kappa-B kinase subunit beta							
Publications	Gene IKBKB							
Feature viewer	Organism Homo sapiens (Human)							
Feature table	Status Reviewed - Annotation score: ••••• - Experimental evidence at protein level ¹							
None	Function							
Function								
Names & Taxonomy	Serine kinase that plays an essential role in the NF-kappa-B signaling pathway which is activated by multiple stimuli such a callular strasses (RubMed: 20227470)							
Subcellular location	Acts as part of the canonical IKK complex in the conventional pathway of NF-kappa-B activation. Phosphorylates inhibitors							
Pathology & Biotech	polyubiquitination of the inhibitors and subsequent degradation by the proteasome. In turn, free NF-kappa-B is translocate							
PTM / Processing	Involved in immune response, growth control, or protection against apoptosis. In addition to the NF-kappa-B inhibitors, photomological NEMO/IKBKG, NF-kappa-B subunits RELA and NFKB1, as well as IKK-related kinases TBK1 and IKBKE (PubMed:11297557,							
Expression	IKK-related kinase phosphorylations may prevent the overproduction of inflammatory mediators since they exert a negativ							
✓ Interaction	dependent inactivation of this pro-apoptotic transcription factor (PubMed:15084260).							
Structure	Within the nucleus, acts as an adapter protein for NFKBIA degradation in UV-induced NF-kappa-B activation (PubMed:1129							
Family & Domains	Phosphorylates RIPK1 at 'Ser-25' which represses its kinase activity and consequently prevents TNF-mediated RIPK1-depe							
	Phosphorylates the C-terminus of IRF5, stimulating IRF5 homodimerization and translocation into the nucleus (PubMed:25:							
Sequences (4+)	By similarity - 10 Publications -							
Similar proteins	Catalytic activity							
Cross-references	 ATP + L-seryl-[I-kappa-B protein] = ADP + H⁺ + O-phospho-L-seryl-[I-kappa-B protein] 							



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Display

Help video PTM / Processingⁱ

Entry

Publications

Feature viewer

Feature table

Names & Taxonomy
 Subcellular location

Pathology & Biotech

PTM / Processing

Family & Domains

Expression
 Interaction
 Structure

Function

Amino	acid	modifications

Molecule processing

Chainⁱ (PRO_000086013)

None	Feature key	Position(s)	Description
None	Cross-link ⁱ	163	Glycyl lysine isopeptide (Lys-Gly) (interchain with G-Cter in ubiquitin) 🕜 1 Publication 👻
	Modified residue ¹	177	Phosphoserine; by TBK1 and PKC/PRKCZ 🕜 3 Publications 👻
	Modified residue ¹	179	S-nitrosocysteine 🕜 1 Publication 👻
	Modified residue ⁱ	180	(Microbial infection)O-acetylthreonine; by Yersinia YopJ 🕜 1 Publication 🚽
	Modified residue ⁱ	181	Phosphoserine; by TBK1, PKC/PRKCZ and PDPK1 🕜 4 Publications 👻
	Modified residue ¹	191	Hydroxyproline 🗣 1 Publication 👻
	Modified residue ¹	670	Phosphoserine; by autocatalysis 🕜 1 Publication 👻
	Modified residue ¹	672	Phosphoserine 🕜 Combined sources 👻 🗣 1 Publication 👻
	Modified residue ¹	675	Phosphoserine 🕜 Combined sources 👻
	Modified residue ¹	675	Phosphoserine; by autocatalysis 🕜 Combined sources 👻 🔗 1 Publication 👻
	Modified residue ¹	682	Phosphoserine; by autocatalysis 🕜 1 Publication 🚽

1 - 756 Inhibitor of nuclear factor kappa-B kinase subunit beta



C)isplay	Help video	Sequence	s (4+) ⁱ						
E	intry		Sequence status	ⁱ : Complete.						
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2	Names & Taxonomy		* Hide							
2	Subcellular location		10	20	96	40	50			
2	Pathology & Biotech		MSWSPSLTTQ	TCGAWEMKER	LGTGGFGNVI	RWHNQETGEQ	IAIKQCRQEL			
	PTM / Processing		60	70	80	90	100			
	Expression		SPRNRERWCL 110	EIQIMRRLTH 120	PNVVAARDVP 130	EGMQNLAPND 140	LPLLAMEYCQ 150			
	Interaction		GGDLRKYLNQ	FENCCGLREG	AILTLLSDIA	SALRYLHENR	IIHRDLKPEN			
			160	170	180	190	200			
\leq	Structure		IVLQQGEQRL	IHKIIDLGYA	KELDQGSLCT	SFVGTLQYLA	PELLEQQKYT			
2	Family & Domains		210	220	230	240	250			
	Sequences (4+)		260	270	280	290 WHSKVKQKSE	300			



GEO microarray analysis

Gene Expression Omnibus	
NCBI > GEO > Accession Display 2	IONS FAQ MIAME EMAI Not logged in L
VCaP Search GEO help: Mouse over screen elements for information.	
Scope: Self 👽 Format: НТМL 💽 Amount: Quick 👽 GEO accessio	n: GSE49287 G0
Browse Content	
Repository Browser Query Da	aSets for GSE49287
DataSets: 3413 Status Public on Jul 28, 2013	
Series: AR and c-Myb depletion effects in prostate cancer ce	ls
Organism Homo sapiens	
Platorms. 12217 Experiment type Expression profiling by array Summary This SuperSeries is composed of the SubSeries liste	bolow
Samples: 1025616 Summary This SuperSeries is composed of the Subseries liste	Delow.
Analyze with CEO2R	
Download family Format	
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 Samples 		✓ Define groups			
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Group	Accession	♦ Title	si-control	GSM1196774	LNCaP/RM/NC siRNA #1
-	GSM1196774	LNCaP/RM/NC siRNA#1	si-AR	GSM1196775	LNCaP/RM/AR siRNA#1
		LNCaP/RM/AR siRNA #1	si-control	GSM1196776	LNCaP/CSS/NC siRNA #1
Top 250	Save all results	LNCaP/CSS/NC siRNA #1	si-AR	GSM1196777	LNCaP/CSS/AR siRNA #1



GEO2R	Value distribution	Options	Profile graph	R script	
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Quick start

Recalculate if you changed any options. Save all results Select columns

ID	adj.P.Val	P.Value	t	В	logFC	Gene.symbol	Gene.title
ILMN_2305407	0.353	0.00000748	8.86	-0.209	0.839	ZBTB16	zinc finger and B
▶ ILMN_2306540	1	0.00004872	7.06	-0.67	0.473	PDE9A	phosphodiester
ILMN_1730986	1	0.00012875	6.24	-0.968	0.356	MALT1	mucosa associa
ILMN_2402817	1	0.00012916	6.24	-0.969	0.484	ZBTB16	zinc finger and B
ILMN_2188862	1	0.0004045	-5.35	-1.374	-0.544	GDF15	growth differenti
▶ ILMN_1668619	1	0.00046526	-5.25	-1.428	-0.293	KIAA1467	KIAA1467

ID	adj.P.Val	P.Value	t	В	logFC	Gene.symbol	Gene.title
▼ ILMN_2305407	0.353	0.00000748	8.86	-0.209	0.839	ZBTB16	zinc finger and B



GSE49287/ILMN_2305407/ZBTB16



BioGPS: Customizable collection of websites:

http://biogps.org

One search entry looks for results in many different databases







The subsites can be extended to full window





The subsites, which are opened can be customized to different layouts or an own collection layout can be saved (after free registration)

Pathway databases

Biocarta Nature Signaling Gateway Pathway Interaction database Pathways - Biolegend Reactome	http://www.biocarta.com/ http://www.signaling-gateway.org/ http://pid.nci.nih.gov/ http://www.biolegend.com/index.php?page=pa thways http://www.reactome.org/

STRING database for functional interactions and signaling networks:

http://string-db.org





Protein Databases and other tools

Human Protein references	http://www.hprd.org/
Lipidomics	http://www.lipidmaps.org/
Macromol. Movements	http://www.molmovdb.org/
Phosphorylation sites	http://www.phosida.com/
Protein Database	https://www.rcsb.org
ScanProsite	https://www.expasy.org/resources/scanprosite
Transcription factor database	https://jaspar.genereg.net
Uniport Protein database	https://www.uniprot.org/

Centrifugation	http://www.sciencegateway.org/tools/rotor.htm
DNA /protein calculator	
Fermentas Double digest	https://at.promega.com/resources/tools/biomath/
Molarity Calculator	http://www.fermentas.com/en/tools/doubledigest
Molarity Calculator	http://www.graphpad.com/quickcalcs/Molarityform.cfm
Primer3 primer design	http://www.meduniwien.ac.at/user/johannes.schmid/MolarityJava.htm
	http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi



Other webtools

- Medical images: <u>https://en.wikiversity.org/wiki/WikiJournal_of_Medicine/Medical_gallery_of_Blausen_Medical_2014</u>
- Biodigital images: <u>https://human.biodigital.com/login?returnUrl=/explore</u>
- Interactive presentations: <u>https://www.mentimeter.com</u>
- Protocols, methods: <u>https://experiments.springernature.com</u>
- biological drawing (svg-files): https://bioicons.com
- Signaling pathways: <u>https://apps.pathwaycommons.org</u>
- Interactive fluorescent protein database: <u>https://www.fpbase.org/chart/</u>
- Gene set enrichment analysis: <u>https://www.gsea-msigdb.org/gsea/msigdb/</u>
- Medical biochemistry: <u>https://themedicalbiochemistrypage.org</u>
- NF-κB target genes: <u>https://www.bu.edu/nf-kb/gene-resources/target-genes/</u>
- Microvesicle database: <u>http://microvesicles.org/index.html</u>
- Austrian data portal: <u>https://www.data.gv.at</u>



Other webtools

- Open Cell protein localization/images: <u>https://opencell.czbiohub.org</u>
- Cancer guidelines: https://www.onkopedia-guidelines.info/en/onkopedia/guidelines
- Online curve fitting: <u>https://www.mycurvefit.com</u>
- Cell line collection: <u>https://www.dsmz.de/collection/</u>
- Cell lines: <u>https://www.cellosaurus.org</u>
- Primer database: <u>https://pga.mgh.harvard.edu/primerbank/</u>
- Transcriptional start sites: <u>https://dbtss.hgc.jp</u>
- European Bioinformatics Institute: <u>https://www.ebi.ac.uk</u>
- Addgene plasmid repository: <u>https://www.addgene.org</u>



Updated Links

- Nucleotide databases
- Ensembl Genome Browser
- Gene Expression Omnibus (GEO)
- <u>FuncAssociate 2.0: The Gene Set Functionator</u>
- ENCODE Project at UCSC
- EPD The Eukaryotic Promoter Database
- Pathways
- BioGRID | Database of Protein and Genetic Interactions
- <u>KEGG PATHWAY Database</u>
- <u>Reactome pathways</u>



Updated Links

- **STRING**: functional protein association networks
- <u>PhosphoSitePlus: a resource for protein phosphorylation and</u> <u>other post-translational modifications</u>
- <u>neXtProt exploring the universe of human proteins</u>



- <u>Primer3Plus</u>
- <u>Addgene: CRISPR/Cas Plasmids for Genome Editing</u>
- <u>Animal Experimentation Design Assistant: https://eda.nc3rs.org.uk</u>
- <u>ArrayExpress < EMBL-EBI</u>
- BCCM/LMBP plasmids catalogue: <u>https://bccm.belspo.be</u>
- Broad-Novartis Cancer Cell Line Encyclopedia
- <u>cBioPortal for Cancer Genomics</u>
- <u>Clinical Trials Register</u>



- <u>Complex Portal < EMBL-EBI</u>
- <u>COXPRES co-regulated gene database</u>
- <u>CRISPR design</u>
- <u>CRISPR Design 2</u>
- <u>CRISPR DESKGEN</u>
- Datasearch Elsevier
- <u>EBI</u>
- <u>eClinPath | A Resource for Veterinary</u> <u>Clinical Pathology</u>
- EMBL-EBI Train online |
- ENCODE at UCSC

- <u>Enrichr</u>
- European Data Portal
- <u>Expression Atlas < EMBL-EBI</u>
- Fluorescence SpectraViewer | Life
 Technologies
- <u>fluorophores.org</u>
- <u>Garland Science Instructor Resource</u> <u>Center</u>
- GeneCopoiea cDNA and shRNA Clones
- <u>GeneInfinity</u>
- GENEVESTIGATOR
- <u>Genevisible</u>
- <u>ImageJ</u>
- InterPro protein sequence analysis & classification < InterPro < EMBL-EBI



- IPA Ingenuity Pathway Analysis
- IPA Login
- <u>iPathwayGuide</u>
- <u>iPathwayGuide</u>
- MIRUMIR miRNA survival data
- Molecular Movies Home
- <u>Mouse Phenotyping Consortium</u>
- Mouse Strain Resource
- <u>nebiocalculator.neb.com/#!/</u>
- <u>NEBuilder</u>
- <u>Open Microscopy Environment</u> <u>OME</u>

- Platelet Web Systems Biology
- PolyGene Customized Transgenic
 Mouse and Rat Models
- PRIMEGENS isoform specific PCR
- <u>Primer Plasmids Enzymes | Tools</u>
 <u>for Genomics Scientists</u>
- Prostate Cancer Cell Lines Database
- <u>Proteins and Proteomics Opening</u>
 <u>Page</u>
- <u>Proteomics PRIDE Archive</u>
- <u>Rad Pro Calculator: Online Nuclear</u> <u>Calculations and Free Health Physics</u> <u>Software</u>
- real-time PCR primer and probe database



- <u>RNAi library Screeninc</u>.
- <u>RNAseq Exiqon XploreRNA</u>
- <u>ScanProsite</u>
- <u>Science Events: Scientific</u> <u>conferences, courses,</u> <u>meetings and more at</u> <u>Natureevents Directory</u>
- <u>SignalPeptide 4.1 Predictor</u>
- TargetScanHuman 5.1
- The Medical Biochemistry Page
- transcriptomics ExAtlas
- Tronolab Lentiviral Systems
- Tsien lab Website

- WikiPathways
- <u>super-enhancers in mouse and</u> <u>human genome dbSUPER</u>
- <u>GUILDify: Web server for</u> phenotypic characterization of genes
- <u>Phosphatome phosphatase</u>
 <u>database</u>
- Fluorescent protein properties
- <u>BBMRI.at Biobanks</u>
- Human Cell Atlas
- miRNA expression atlas
- <u>CIBERSORT Immune cell profiling</u>
- <u>Phospho.ELM</u>

Cytoscape

www.cytoscape.org: an open source platform for network visualization and analysis



Cytoscape for any network data

Example:

• Correlation between clinical data from a routine health check and lifestyle factors, as well as psychic traumas in childhood

		.			InflammScore		InflammY/N
	(Jorrelati	on mati	IX monocytes	-0.2686503	monocytes	-0.1644713
				MCHC	-0.246242	reading_h_per_week	0.1584925
	InflammY/N	InflammScore	pltsperleukos	plts _l embolism_in_family	0.1584836	weightpersize	0.1589208
InflammY/N		0.4887223			0.1736061	waist	0.1717746
InflammScore	0.4887223			bloodpressure_sys	0.1782497	leukocytes	0.1761921
pltsperleukos				weight_kg	0.2053307	thrombosis_in_family	0.1768258
pltsperneutros			0.5779366	bilirubin	0.2095422	disease_at_diagnosis	0.1775
pltspermonos	0.189353	0.2351637	0.2614663	weightpersize	0.2335143	BMI	0.184894
pltsperlympho				pltspermonos	0.2351637	triglycerides	0.1890771
Neutrotolymph			-0.3045387	BMI	0.2455855	pltspermonos	0.189353
plttolymph				young_neutro	0.2795951	liquor_L_per_week	0.1992718
traumascore				waist	0.2815638	hipsize cm	0.3040328
traumaY/N				ery sedim2	0.3663316	CRP	0.4833983
bedtime				hipsize cm	0.4368706	InflammScore	0.4887223
sleepduration				InflammY/N	0.4887223	prior infections	0.5794086
rice time	1			ery sedim1	0.4996148	· _	
				CRP	0.9719415		

correlation matrix can be imported into Cytoscape with the App: aMatReader (take care to use "." and not "," for the decimal point!)







Mind-Mapping software:

- XMind
- •

Edraw MindMap

- Freemind
- Mindmanager

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CMapTools



Edraw Mind Map



<mark>Network analyst</mark>: <u>www.networkanalyst.ca</u>

- <u>Tutorials: http://www.networkanalyst.ca/faces/docs/Tutorial.xhtml</u>
- Nature Protocols article: <u>https://www.nature.com/articles/nprot.2015.052</u>



Features of Network Analyst

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- Web application for meta-analysis and visualization of genes and proteins
- <u>Statistics</u>: summary-level data from multiple datasets; differential analysis: nested comparisons, time series, pair-wise comparisons... 2-factor analysis (e.g. donor/gender effects when comparing different treatments)



Data input I: List of predefined differentially expressed genes



(choosing human might sometimes provide a richer network, even in case the experiment was done in mice!)

Enter your gene/protein lis	t below: 😮
Specify organism	H. sapiens (human)
Set ID type	Official Gene Symbol
Data label:	Auto
IGFBP5 4.67 IogFC CCND2 4.87 IogFC TGFBI 4.46 HERC6 3.65 DDX58 3.42 ULBP1 3.83 CCND2 5 CCDC81 4.26 HES2 4.8 AIF1L -3.83 IFIT3 5.02 TAGLN 3.82 RSAD2 4.7 ISG15 3.85 OAS3 7.04 IFITM1 3.94 FBLN5 3.64 SCARA3 -3.44 -3.44	Example: Genes of endothelial-mesenchymal transition – extracted from a GEO dataset and analyzed with Geo2R
O Upload	Proceed



Visual analytics options

Please choose a suitable visual analytics method to proceed:





Chose a protein interactome database

	Name	Information	Parameters
•	IMEx Interactome	Literature-curated comprehensive data from InnateDB (<u>Breuer K. et al</u>)	None
	STRING Interactome	STRING interactome with medium (400) - high (1000) confidence score (<u>Szklarczyk D et al</u>)	Confidence score cutoff: Require experimental evidence:
	Rolland Interactome	Experimentally validated binary human PPI data (<u>Rolland T et al</u> .	None


Chose a network

By default the 1st oder network is calculated, if this is too dense (>2000 nodes), it is advisable to switch to zero-order network or to minimum network (the lowest number of nodes required to link all the seeds of the differentially expressed genes).

If it is too sparse, you may switch to 2nd order network)





Adjustable network view









Calculation of significantly altered functions (functional enrichment analysis > Function Explorer)

Choose color here





Zero order network of the same data set

235 nodes (instead of 5325), 409 edges, 238 seeds (from 988 diff. regulated genes)

Mostly the same pathways are enriched





Different databases can be searched for the network

KEGG, Reactome, Gene Ontologies:

GO:BP (biological process), GO:MF (molecular function), GO:CC (cell. compartments), **motif** (transcription factor binding motifs)





Different databases can be searched for the network





Module explorer

Modules are tightly clustered subnetworks with more internal connections than expected randomly in the network (module members are likely to cooperate in biological functions)

You can make all module node names visible by using "More Options" > highlighted nodes > increase





Data input II: A single gene expression data (raw data)





Input II: A single gene expression data (raw data)

The data has to be in the following format (adding: #NAME and #CLASS):

#NAME	Column2	Column3	Column4	Column5	Column6
#CLASS:EXP	C4-4BO	D4-4IL	E4-4PD	F4-4CON	C6-3BO
#CLASS:TREATMENT	IL-1a_PDGF	IL-1a	PDGF	control	IL-1a_PDGF
DDX11L1	0.424898092812381	0.700476587867491	0.314804949796422	0.964096737476719	0.395740854386969
CICP27	0.48738310646126	0.794771513157345	0.839479866123791	0.471569056374482	0.949778050528727
RP4-669L17.10	5.06128610555924	4.66086345004138	4.00064623699619	6.26662879359867	5.87015600674005
MTND1P23	78.6061471702905	74.1427526850513	52.9396990574316	74.4974315948043	271.623331089403
MTND2P28	643.233227504296	423.451568069511	365.016339288951	494.110057269182	229.358207840875
hsa-mir-6723	986.863311564944	869.466564690523	852.268817209271	1014.82708863025	1062.90716943615

Example data: Transition of smooth muscle cells from a contractile to a synthetic phenotype by IL-1 and PDGF





Input of all the relevant parameter

Clicking on question marks provides very good support for chosing the correct options

Annotation	Specify organism	H. sapiens (human)	•	↓
	ID type	Official Gene Symbol	2	Submit
	Gene-level summarization	Sum	2	
Algorithms	Data Filtering:	Variance (IQR%):	⊇ 4	
	Statistical method	Limma		Submit
	Normalization	Log2-counts per millon	le View Data	
	Primary factor			



Upload and process your data below or try our examples

Data Upload	Datei auswählen Keine ausgewählt	Submit
Annotation	Specify organismH. sapiens (human)Image: Comparison of the sapiens (human)Data typeRNA-seq data (counts)Image: Comparison of the sapiens (human)ID typeOfficial Gene SymbolImage: Comparison of the same	Submit
Algorithms	Data Filtering: Variance (IQR%): Statistical method Normalization Log2-counts per millon Log2-counts per millon View Data	Submit
Comparisons	 Specific comparison Use a common control Nested comparisons Control vs. IL_1_PDGF versus Control vs. IL_1_PDGF Interaction only ?? Pairwise comparisons ? Time series ? 	Submit
Feature Selection	Adjusted p-value (FDR): 0.05 Log2 fold change: 1.0 Result name: Auto	Submit
	Try Examples Proceed	



Results of differential gene expression analysis

Sort table by: ID 🔻	Sorting order: Ascending V	Update				± Downloa	ad Table
		(1 of 47)	1 2 3 4 5 6 7	8 9 10 🕨 🕨 20	•		
ID	logFC	AveExpr	t	P.Value	adj.P.Val	В	View
CDCP1	-5.3061	3.4349	-15.019	3.2035E-10	2.4121E-6	13.483	
CXCL3	-6.0038	2.2239	-14.551	4.9327E-10	2.4121E-6	13.107	
CXCL6	-8.3072	4.6153	-13.253	1.7423E-9	4.8358E-6	11.986	
<u>MIR146A</u>	-4.9065	1.521	-13.128	1.9778E-9	4.8358E-6	11.871	
CYBRD1	1.7647	8.7588	12.468	3.9414E-9	7.4932E-6	11.245	
DEPTOR	3.9595	5.2192	12.24	5.0321E-9	7.4932E-6	11.021	
CXCL1	-8.5609	5.8266	-12.182	5.3632E-9	7.4932E-6	10.962	
ST3GAL1	-2.0796	5.729	-11.698	9.1587E-9	1.1196E-5	10.467	
CXCL5	-7.9698	3.71	-11.504	1.1414E-8	1.2403E-5	10.263	
LPXN	-2.902	3.1178	-11.38	1.3152E-8	1.2863E-5	10.131	
TNFAIP3	-4.712	5.3096	-11.196	1.6278E-8	1.4472E-5	9.9311	
CXCL2	-5.1335	2.7298	-10.872	2.3847E-8	1.7124E-5	9.5724	
ATP6V1C2	-1.2638	6.5612	-10.855	2.4335E-8	1.7124E-5	9.5533	

O Previous

→ Visual Exploration



Diagnostics of the analysis





Principal component analysis (PCA)













Protein interaction network





Path Explorer

...defines the shortest path between two nodes





New visual mode:





Gene-miRNA network

Function explorer often leads to very similar results as the protein interaction network analysis





Transcription factor – gene interaction network





Transcription factor – gene interaction network





Protein – drug interaction network





Protein – drug interaction network





Protein – chemical interaction network





Reduction of complexity by using filters





Protein – chemical interaction network





Heatmap clustering





Heatmaps





