







ImageJ Macro Programming

Robert Haase

With material from

Anne Esslinger, Alberti Lab, Biotec, MPI CBG

Martin Weigert, EPFL, Lausanne

Benoit Lombardot, Scientific Computing Facility, MPI CBG

Gayathri Nadar, Scientific Computing Facility, MPI CBG

Jens Ehrig, CMCB, TU Dresden

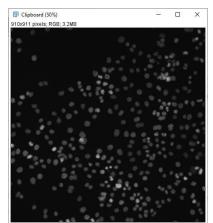
Noreen Walker, Scientific Computing Facility, MPI CBG

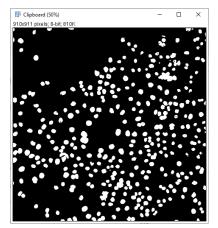
Virtually at CCI Gothenburg, October 2021

ImageJ macro



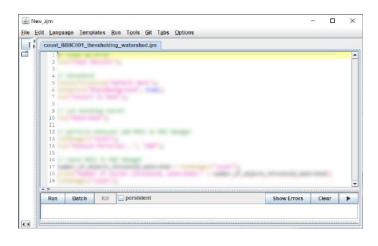
Day 1: Scripting in ImageJ / Fiji allows reproducible workflows.



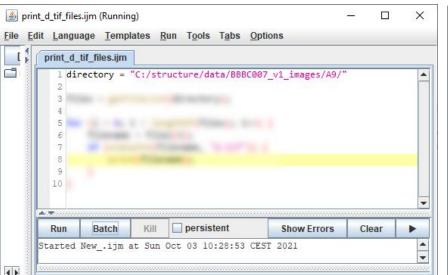


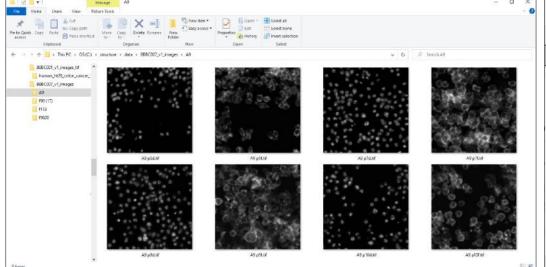


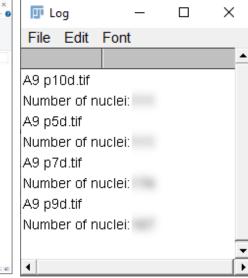




Day 2: Run workflows on hundreds over folders of images!





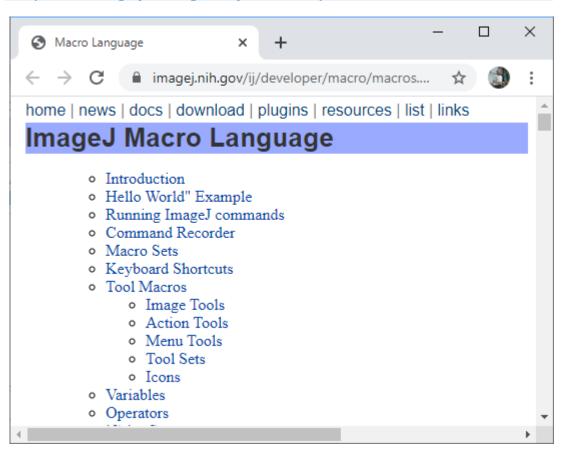


Online documentation

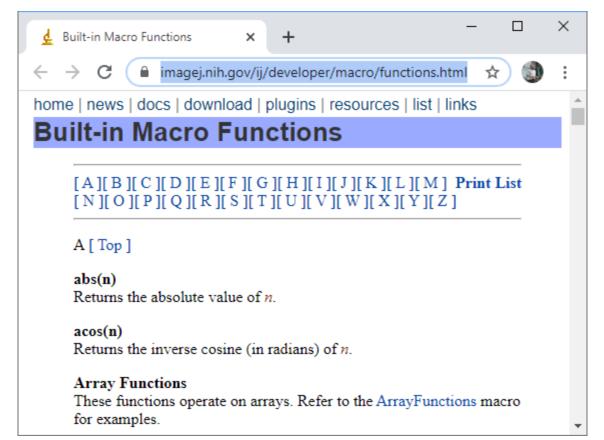


Important ImageJ macro websites

https://imagej.nih.gov/ij/developer/macro/macros.html



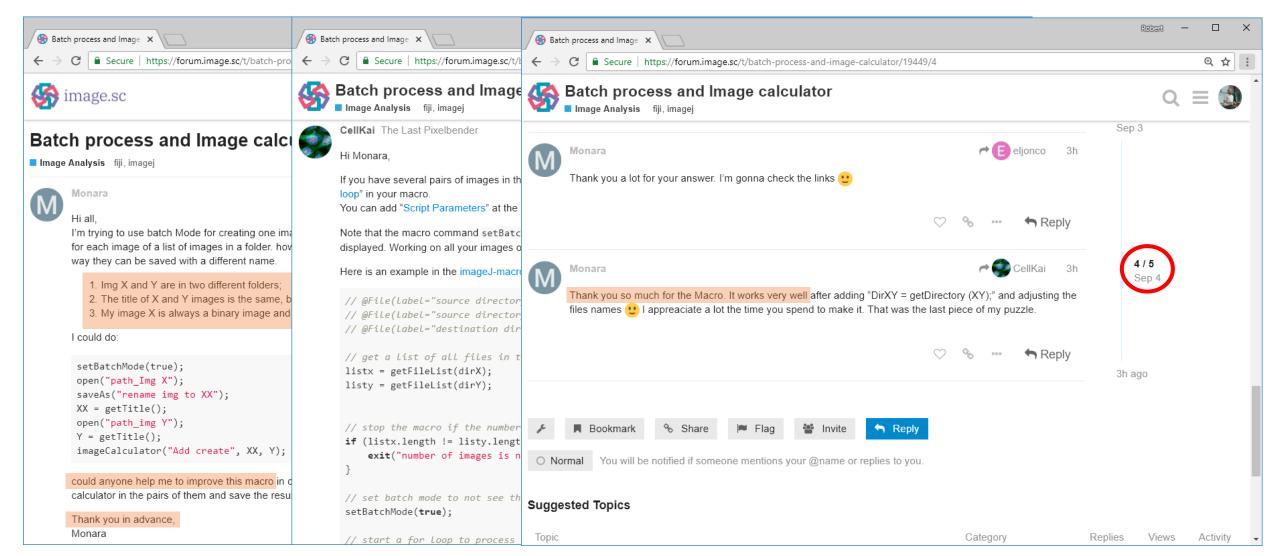
https://imagej.nih.gov/ij/developer/macro/functions.html



ImageJ, Fiji & friends: community



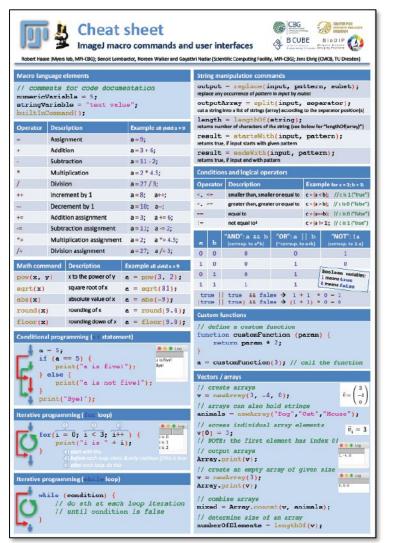
Visit http://forum.image.sc!

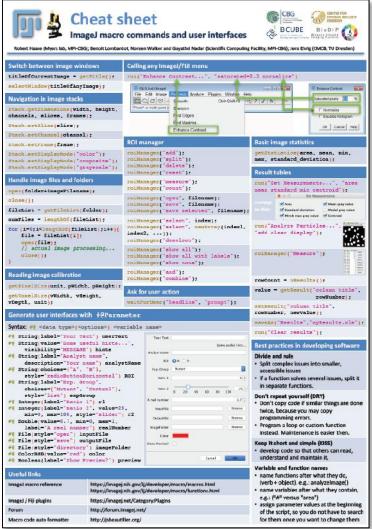


Cheat sheets!



https://github.com/BiAPoL/imagej-macro-cheat-sheet/blob/master/ImageJ macro cheatsheet.pdf



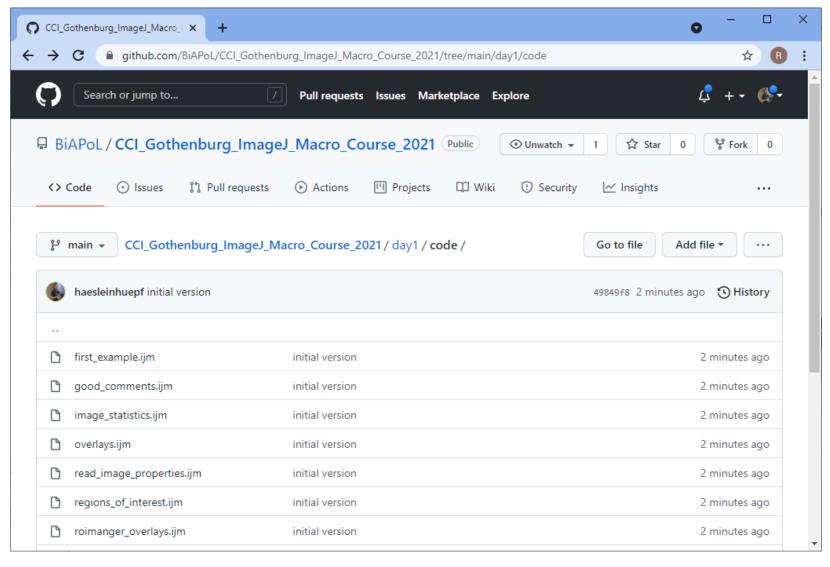


Thanks to
Benoit Lombardot,
Gayathri Nadar,
Jens Ehrig and
Noreen Walker for
contributing to and
maintaining this document!

Example code for this course



https://github.com/BiAPoL/CCI Gothenburg ImageJ Macro Course 2021



How to eat a banana the computational way

using ImageJ mactor





- banana0008.tif banana0009.tif banana0010.tif banana0011.tif banana0012.tif

- Remove shell
- Repeat until nothing left:
 - Take a bite
 - Chew
 - Swallow
- Digest

- Access folder
- Repeat for all images:
 - Open an image file
 - Segment the banana slice
 - Analyse it
- Save measurements

```
folder = "C:/structure/teaching/lecture applied bioimage an
filelist = getFileList(folder);
for (i = 0; i < lengthOf(filelist); i++) {</pre>
    // get the nth entry from the filelist array
    file = filelist[i];
    if (endsWith(file, ".tif")) {
        // open the image
        open(folder + file);
        // segment the object in the image
        setAutoThreshold("Default dark");
        setOption("BlackBackground", true);
        run("Convert to Mask");
        run("Fill Holes");
        // measure the position of this one single object
        run("Set Measurements...", "centroid redirect=None
        run("Create Selection");
        run("Measure");
        setResult("Slice", nResults() - 1, i);
        // close the image
        close();
// save the positions as CSV to disc
result filename = "Position.csv"
saveAs("Results", folder + result filename);
```









ImageJ macro programming: Variables and operations

Robert Haase

With material from

Benoit Lombardot, Scientific Computing Facility, MPI CBG

Virtually at CCI Gothenburg, October 2021



Variables



Variables hold values and can be connected using operators

```
// initialise program
a = 1;
b = 2.5;

// run complicated algorithm
final_result = a + b;

// print the result
print( final_result );
```

```
8 ← Log
3.5
```

variables.ijm

Mathematical operations



• Math commands supplement operators to be able to implement any form of calculations

Command	Description	Example
pow(x, y)	x to the power of y	a = pow(3, 2);
sqrt(x)	square root of x	a = sqrt(81);
abs(x)	absolute value of x	a = abs(-9);
round(x)	rounding of x	a = round(9.4);
sin(x)	sinus of x given in radians	b = sin(PI);
cos(x)	cosinus of x given in radians	b = cos(PI);
tan(x)	tangens of x given in radians	b = tan(PI);



Comments should contain <u>additional information</u> such as

- User documentation
 - What does the program do?
 - How can this program be used?
- Your name / institute in case a reader has a question
- Comment why things are done.
- Do <u>not</u> comment what is written in the code already!

```
// This program sums up two numbers.
// Usage:
// * Run it in FIJI (www.fiji.sc)
// Author: Robert Haase, MPI CBG,
           rhaase@mpi-cbg.de
// July 2016
// initialise program
a = 1;
b = 2.5;
// run complicated algorithm
final result = a + b;
// print the final result
print( final result );
```

good comments.ijm







- Write your own ImageJ macro notebooks!
- Use:

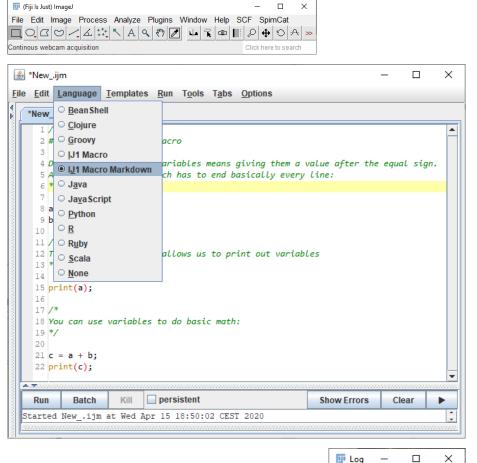
```
/*
```

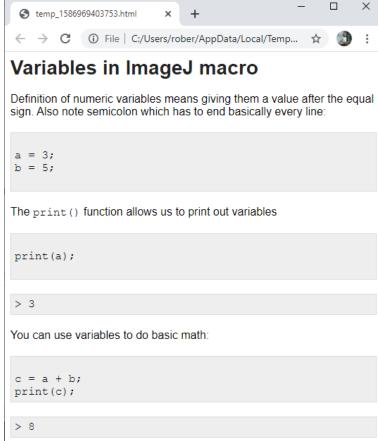
Headlines

* Bullet points

[Links] (http://fiji.sc)

* /





Installation

• https://github.com/haesleinhuepf/imagejmacromarkdown

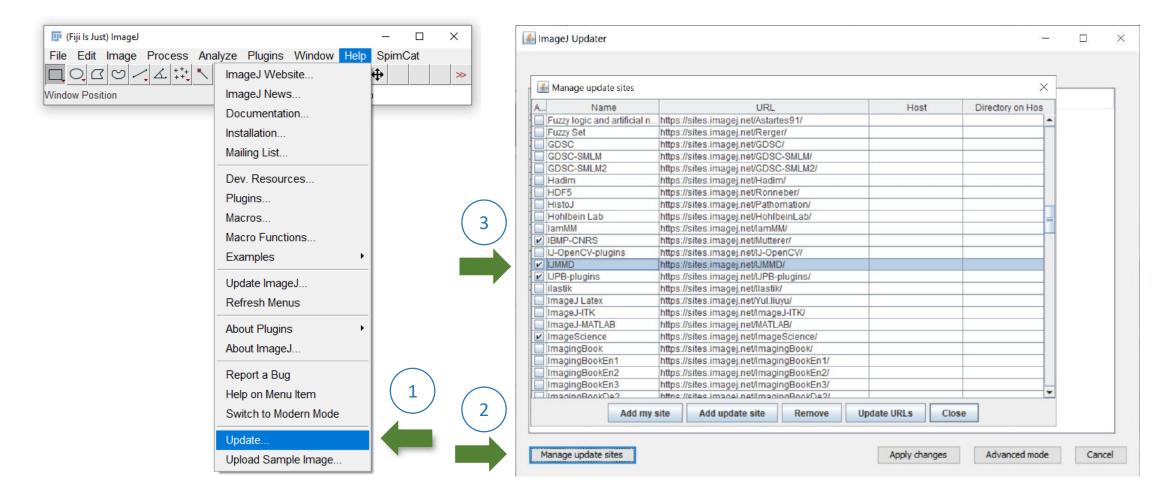


File Edit Font

ImageJ Macro Markdown installation



Available via the IJMMD update site







Strings



- Texts are called "strings" in the world of programming
- They represent a chain of characters

```
// initialise program
firstname = "Robert";
lastname = "Haase";

// run complicated algorithm
name = firstname + " " + lastname;
print("Hello " + name + "!");
```

string_variables.ijm

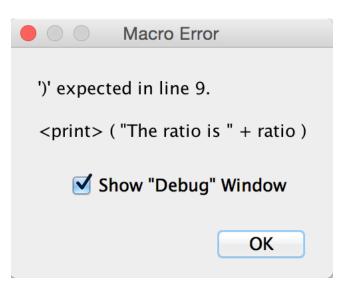


Code highlights



- Colours help us to read and understand what we see:
 - comments / documentation
 - commands / action
 - values (numbers, vectors, texts)
- If something is wrong with the colour, likely something is wrong with the program.

```
1 a = 5;
2 b = 7;
3
4 sum = a + b;
5 ratio = a / b;
6
7 print("The sum is + sum);
8 print("The ratio is " + ratio);
9
10
```

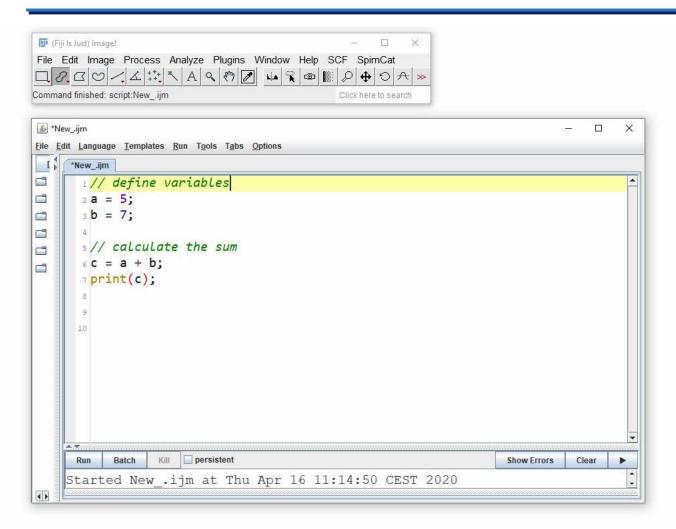


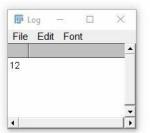




Learning by doing!

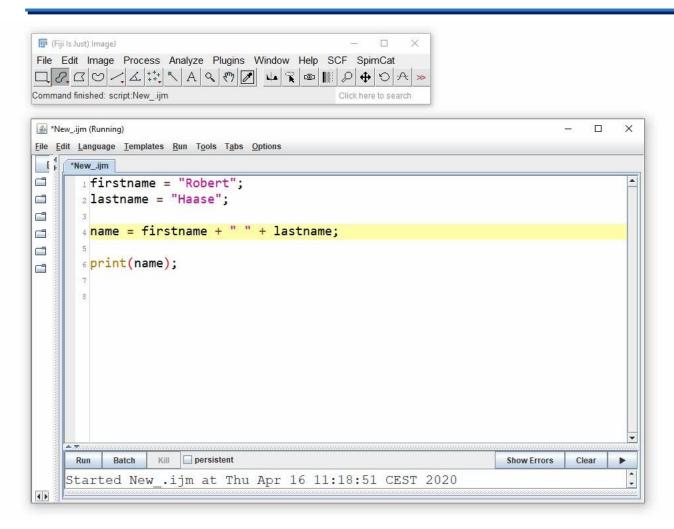






Learning by doing!





III Log − □ ×

File Edit Font

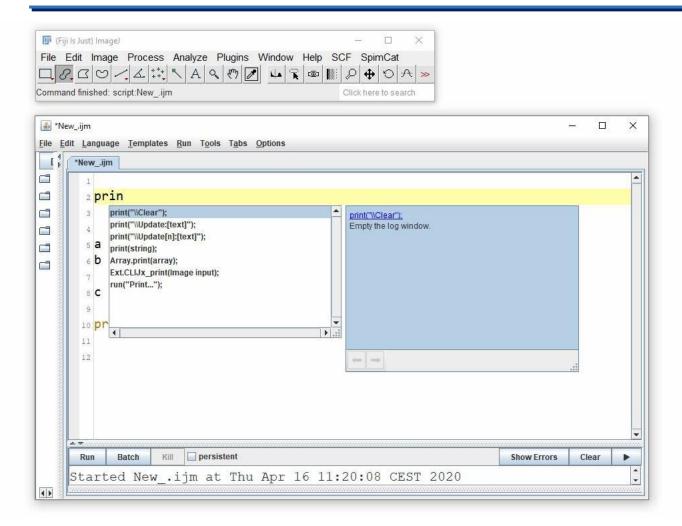
Robert; Haase

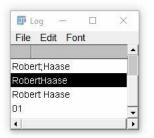
RobertHaase

Robert Haase

Learning by doing!















ImageJ macro programming: Recording macros & working with images

Robert Haase

With material from

Benoit Lombardot, Scientific Computing Facility, MPI CBG

Virtually at CCI Gothenburg, October 2021

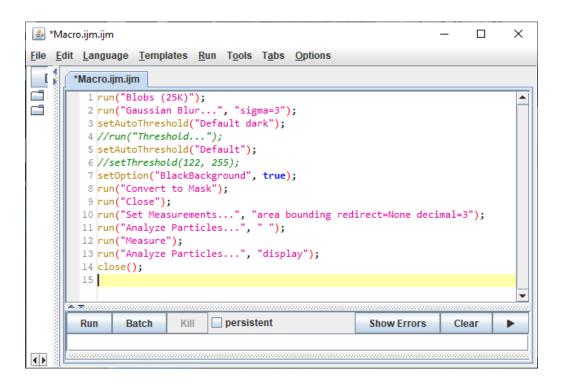




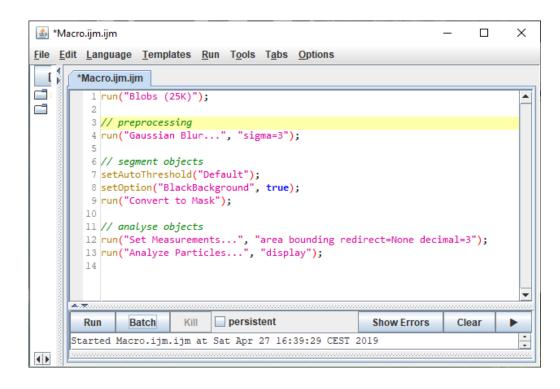
Macro recording



- Editing recorded macros needs to be trained. It's 80% reading and 20% writing
- Hints:
 - Put comments first. Try to understand what was recorded and why.
 - Do it in tiny steps. As soon as you have a working workflow consisting of 4-5 steps, create a macro.
 - Collect macros. When you do something new, do cherry picking from the old macros.



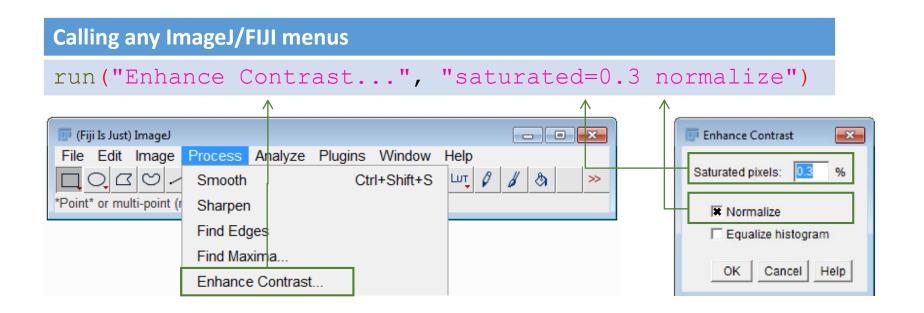




The run() command



- The first parameter of the run command is the menu you want to call
- The following parameter contains the entries of the dialog in case the menu has one.
 - Numeric values: "label=value"
 - Checkboxes: "label" in case it is activated.



Working with image files



• Open and close commands allow handling image files.

```
// initialise program
imageFilename = "/Users/rhaase/images/blobs.gif";

open( imageFilename );

// process the image
// ...
close();
```

Built-in command	Description	Parameters
open(filename);	open an image	filename of the image
close();	close current image	



Working with image files



• Firstly, lets read image properties

Built-in command	Description	Parameters
width = getWidth(); height = getHeight();	return the width/height of the current image	
getPixelSize(unit, pWidth, pHeight)	get size of a pixel	variables where physical unit, width and height will be stored
<pre>getVoxelSize(vWidth, vHeight, vDepth, unit)</pre>	get size of a voxel	variables where width, height, depth and physical unit will be stored

Working with image files



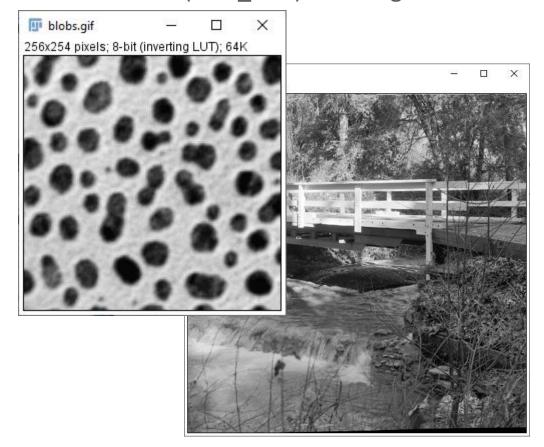
• Let's do some pixel value statistics

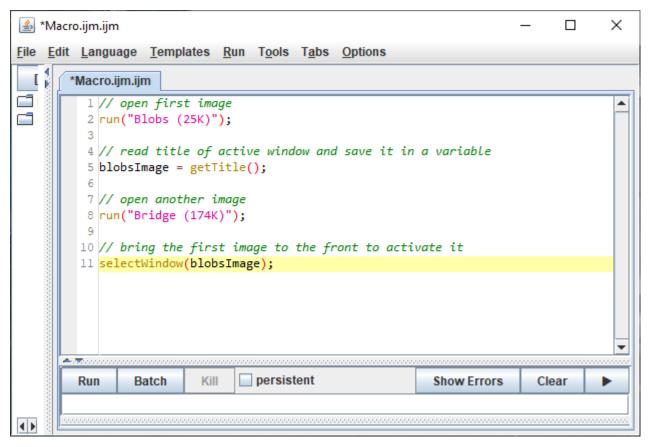
Built-in command	Description	Parameters
getStatistics(area, mean, min, max, std)	get statistics of an image or a region in the image	area in physical units, mean average grey value, minimum grey value, maximum grey value, standard deviation of grey value

Switching between windows



- If you work with several images, you may want to switch between them. Use
 - getTitle() to get the headline of the current window
 - selectWindow(title) to select a window to bring it to the front
 - rename(new_title) to change the name of a window

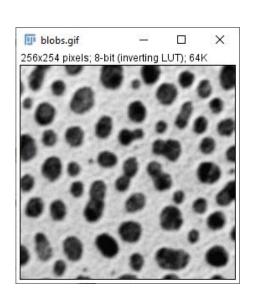




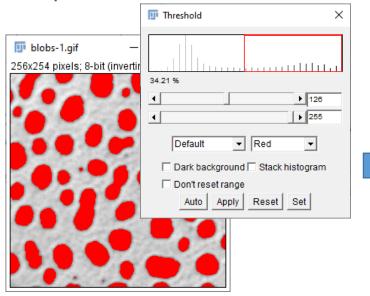
Switching between windows



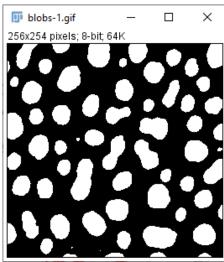
Before "destroying" an image, make a duplicate!



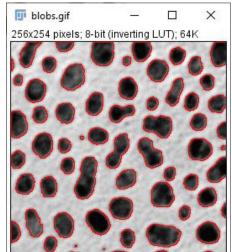


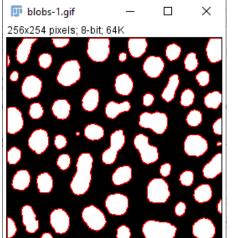


















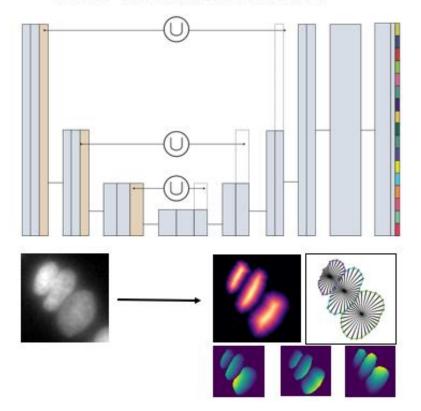
Deep-learning based segmentation: StarDist



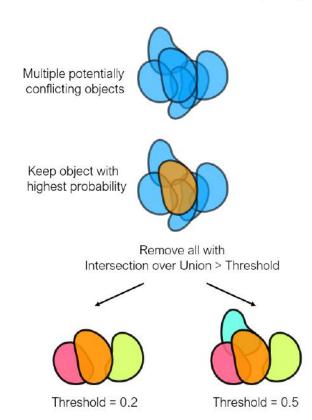
Better segmentation using modern computational methods (deep learning)

and additional constraints

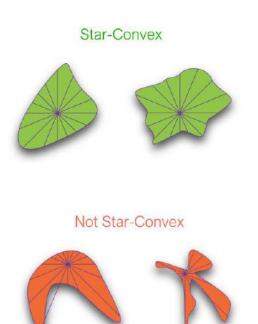
Dense Candidate Prediction



Non-Maximum-Suppression (NMS)



Important: Assumes objects are star-convex







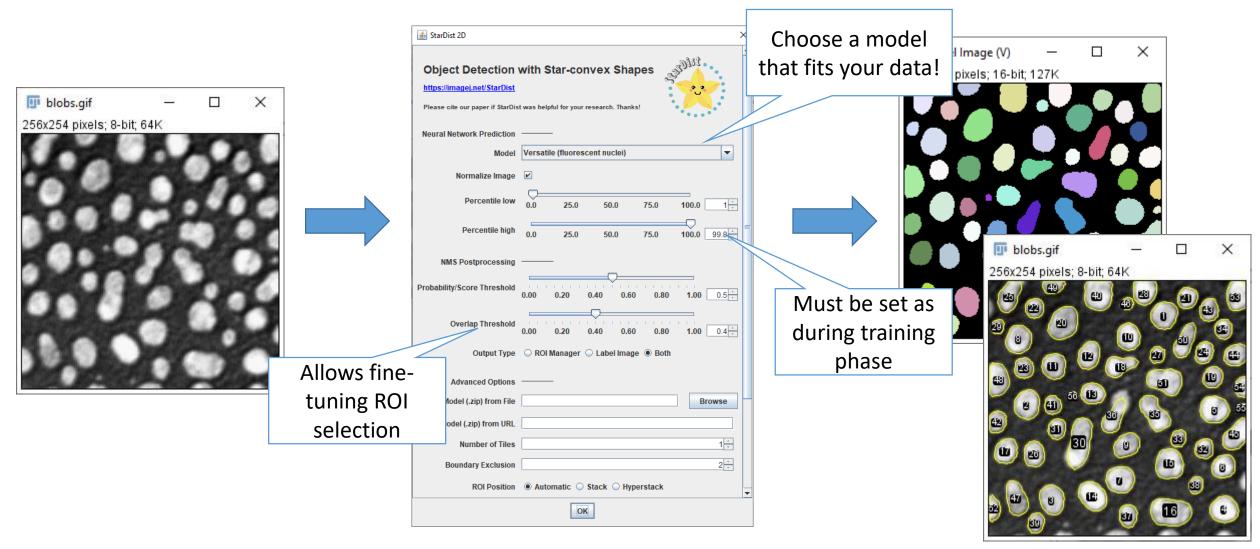




Deep-learning based segmentation: StarDist



Deep-learning based image segmentation



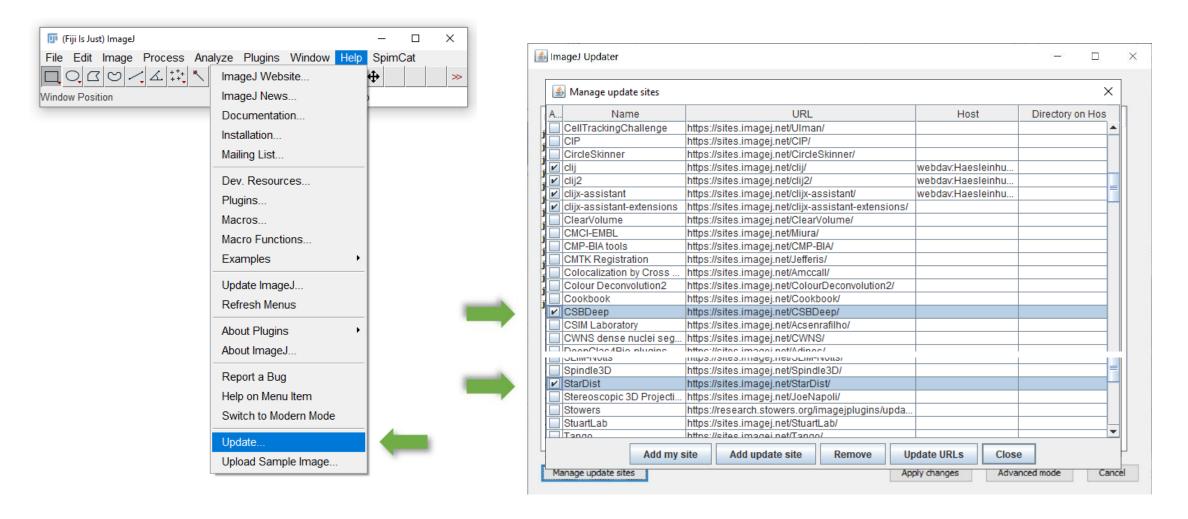




Exercise: StarDist Installation



Activate the "CSBDeep" and "StarDist" update sites.











Break: 15 min









Image data flow graphs

Robert Haase

With materials from
Elisabeth Kugler, The University of Sheffield,
Daniela Vorkel, MPI CBG / CSBD

Image processing in life-sciences



State-of-the-art software for more than 20 years: ImageJ / Fiji



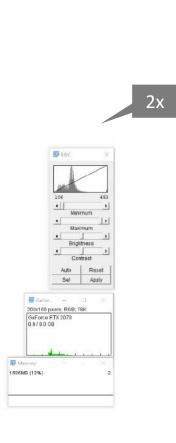


How image processing is supposed to be



• (... in my honest opinion)

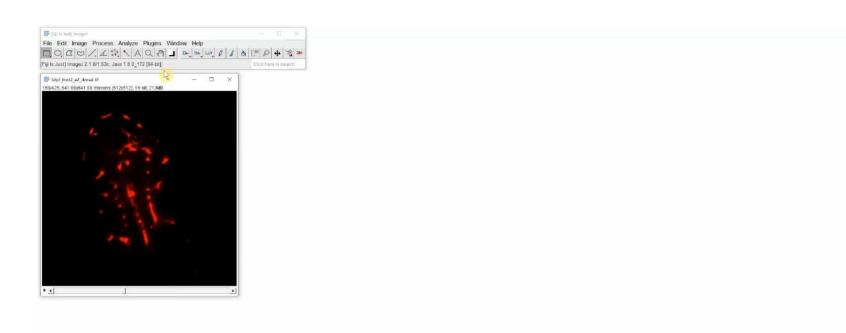




GPU acceleration + code generation



• After setting up the workflow, generate code!



Special thanks to Elisabeth Kugler!



Elisabeth Kugle @KuglerElisabeth



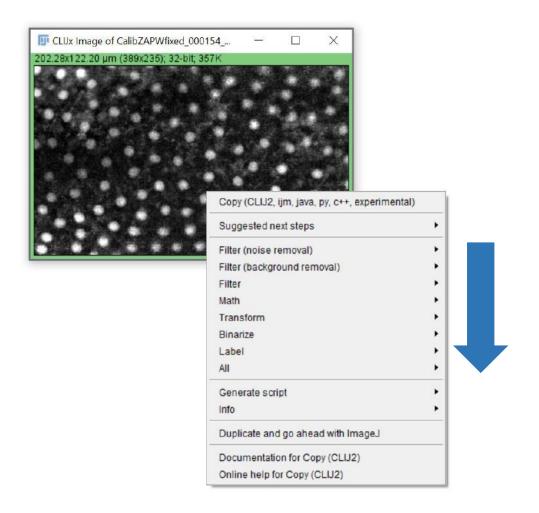
Image data source: Elisabeth Kugler; labs of Tim Chico and Paul Armitage, The University of Sheffield (UK)" https://zenodo.org/record/4204839#.X8DCRGj7Q2w



Image Data Flow Graph design



• The menu order is intentional: From preprocessing to analysis

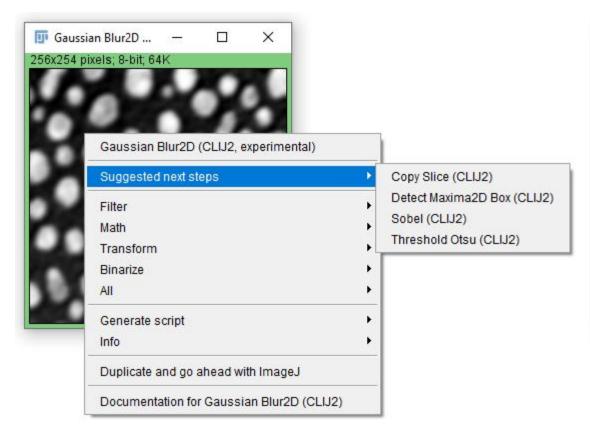


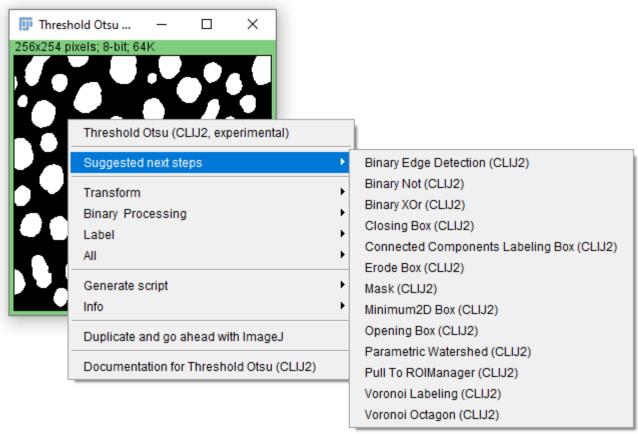


Expert system: Suggestions



Explore suggestions!

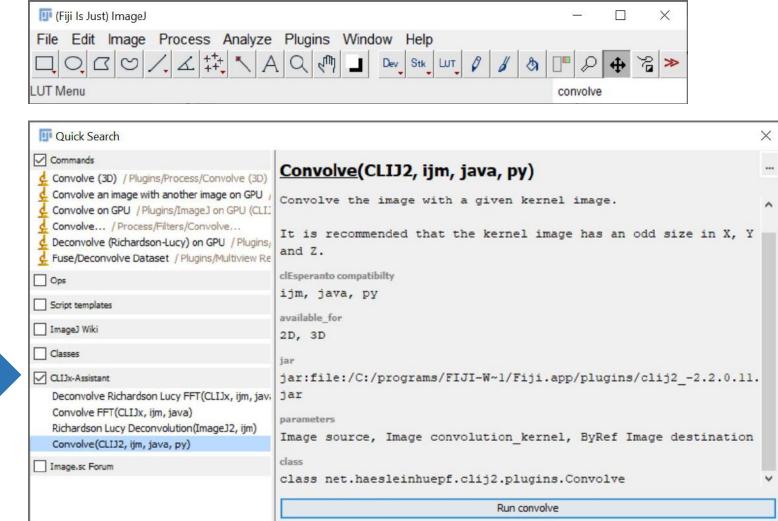




Fiji search bar



In Fijis search bar result, there is a new category: CLIJx-assistant which offers IDFG operations

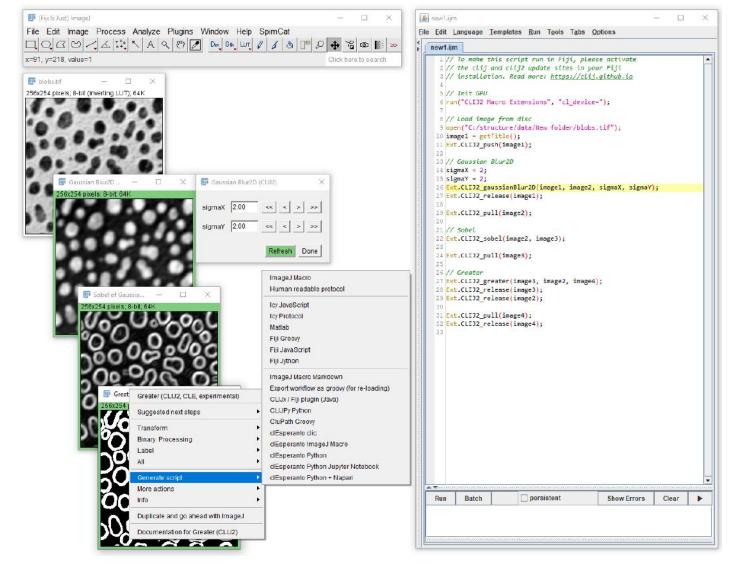




Generate macros

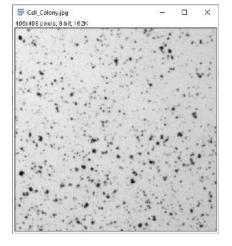


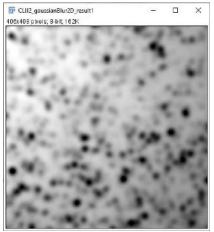
Generate scripts in multiple languages from a given Image Data Flow Graph



CLIJ2: What every ImageJ Macro script must have



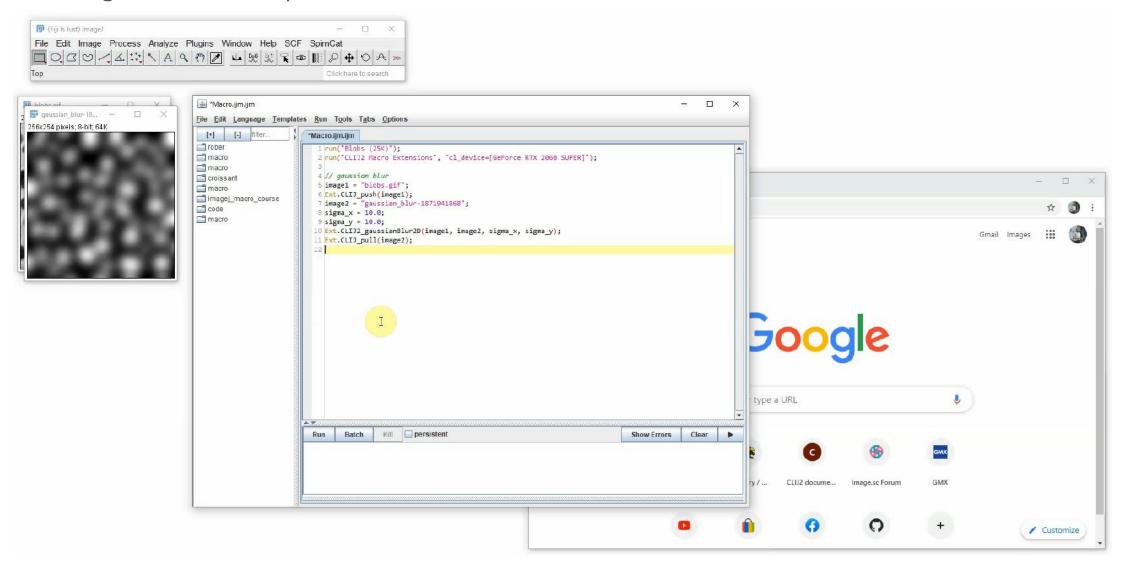




CLIJ2 macro editing



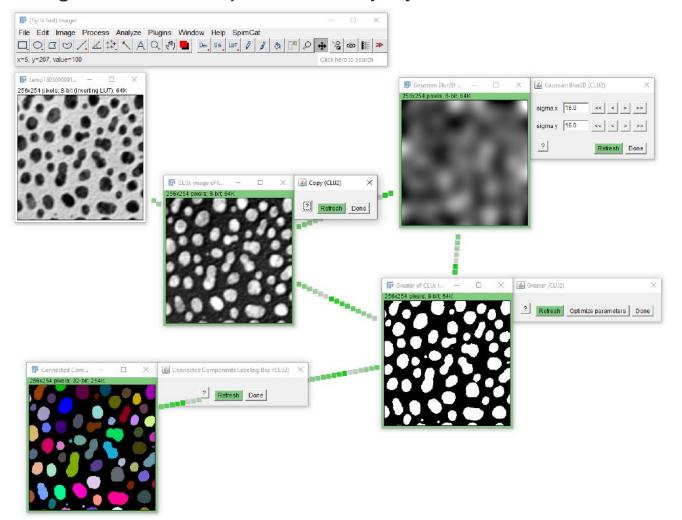
• Use the integrated online help!



Demo: Segment blobs.gif



- Design a workflow for segmenting blobs.gif (File > Open Samples...)
- Export the workflow as ImageJ Macro script for and Fiji Jython.

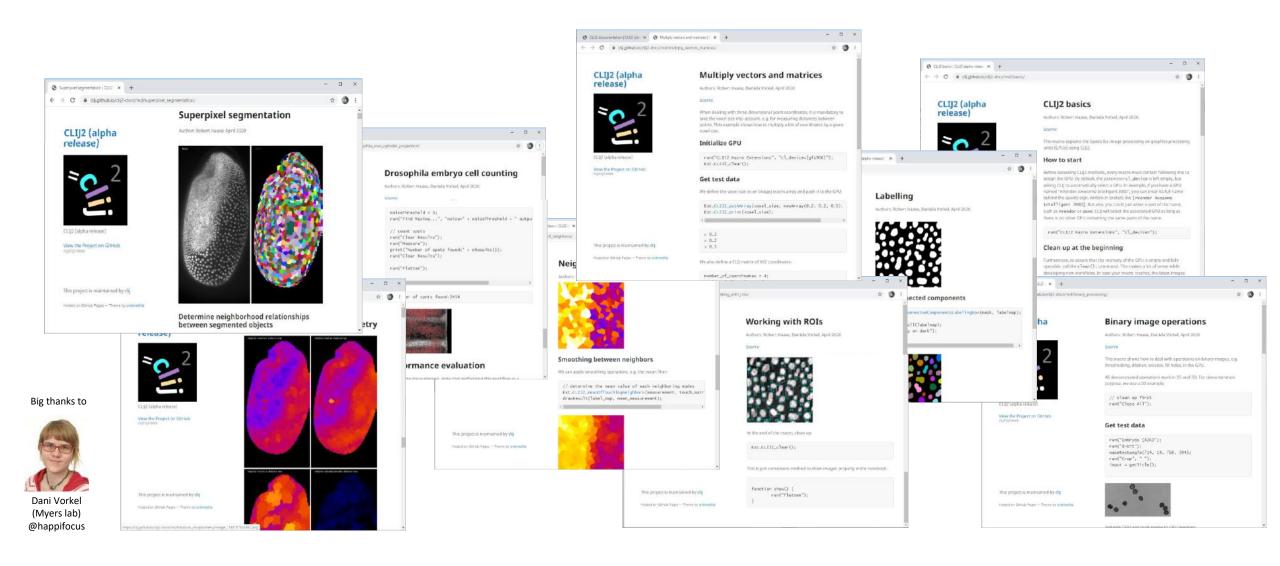






Online documentation: tutorials

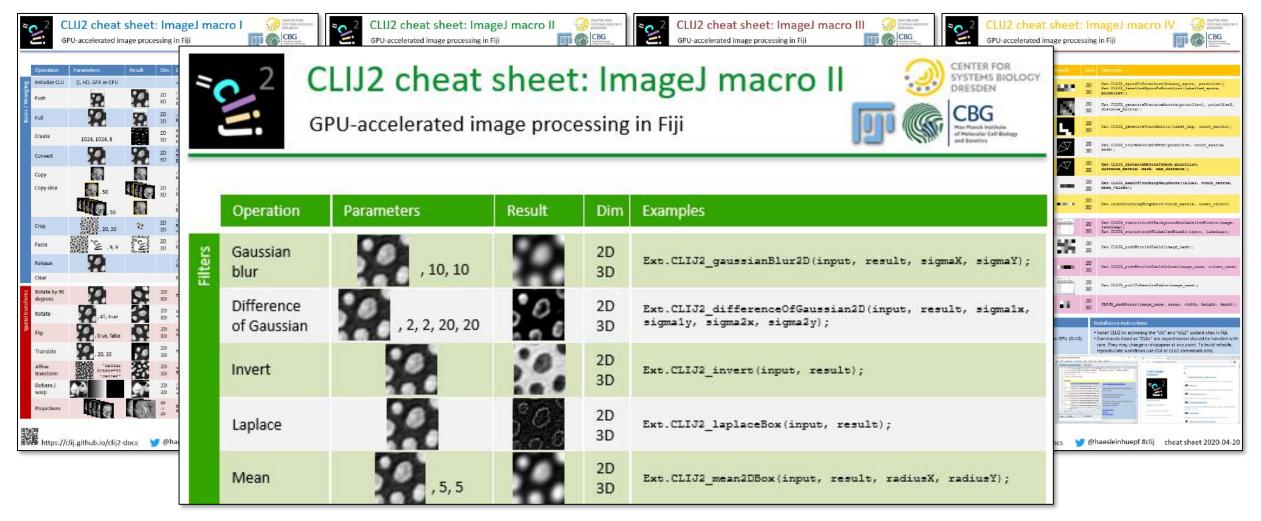




Online documentation: Cheat sheets



Cheat sheets show the most important methods with input and output parameters visually.



Last but not least: citability



• If you work with CLIJ and friends, please cite the paper(s). It'll be hard to apply for grants otherwise.



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Exercises

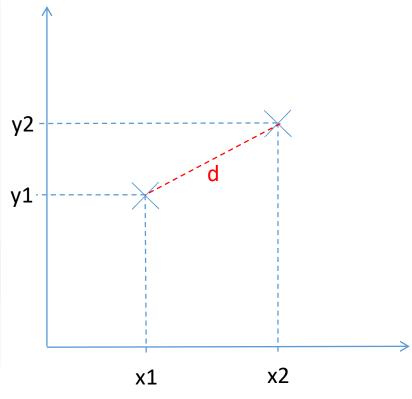
With material from
Anne Esslinger, Albert Lab Biotec, MPI CBG
Benoit Lombardot, Scientific Computing Facility, MPI CBG

Exercise: basic math with variables



Calculate the distance of two points!

```
// initialise program
x1 = 3;
y1 = 5;
x2 = 7;
y2 = 8;
// run complicated algorithm
d = sqrt(pow(x1 - x2, 2) +
         pow(y1 - y2, 2));
print("The distance is " + d);
                         exercise math.ijm
```



Optional Exercise: Write a function doing this! Hint:

```
function distance(x1, y1, x2, y2) {

}

function distance(x1, y1, x2, y2) {
}
```

Optional exercise: Type conversion



• Play with the macro language. Try things like the following and observe what's happening.

```
// initialise program
a = "1";
b = 2;

// do some calculations
print(a + b);
print(b + a);
```

```
// initialise program
a = "1";
b = 2;

// do some calculations
print(a / b);
print(b / a);
```

```
// initialise program
a = "1";
b = 2;

// do some calculations
print(0 + a + b);
print("" + b + a);
```

```
// initialise program
a = "1";
b = 2;

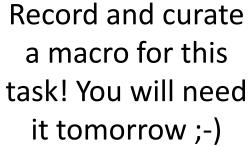
// do some calculations
print(a / b);
print(b / a);
```

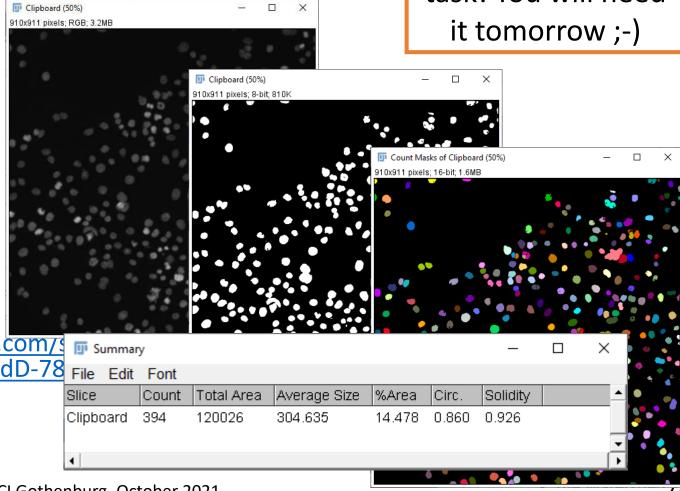
Exercise: Segmentation

PoL
Physics of Life
TU Dresden

- Download and install Fiji
 - http://fiji.sc/Downloads
- Download the BBBC001 dataset (TIF), unzip it and count nuclei in the first image file
 - https://bbbc.broadinstitute.org/BBBC001
 - AS_09125_050118150001_A03f00d0.tif
- Hints:
 - Use automatic thresholding and the Watershed algorithm
 - Summarize your measurement in the Particle Analyzer
 - Select "Show: Count Masks"
 - Use the "Glasbey on dark" lookup table / color map
 - Enter your count here: https://docs.google.com/

 23Soro5XZ3y1kJHpvaTaa1f4n2C7G3WX0qddD-78
 - Be a good scientist: Don't cheat! ;-)

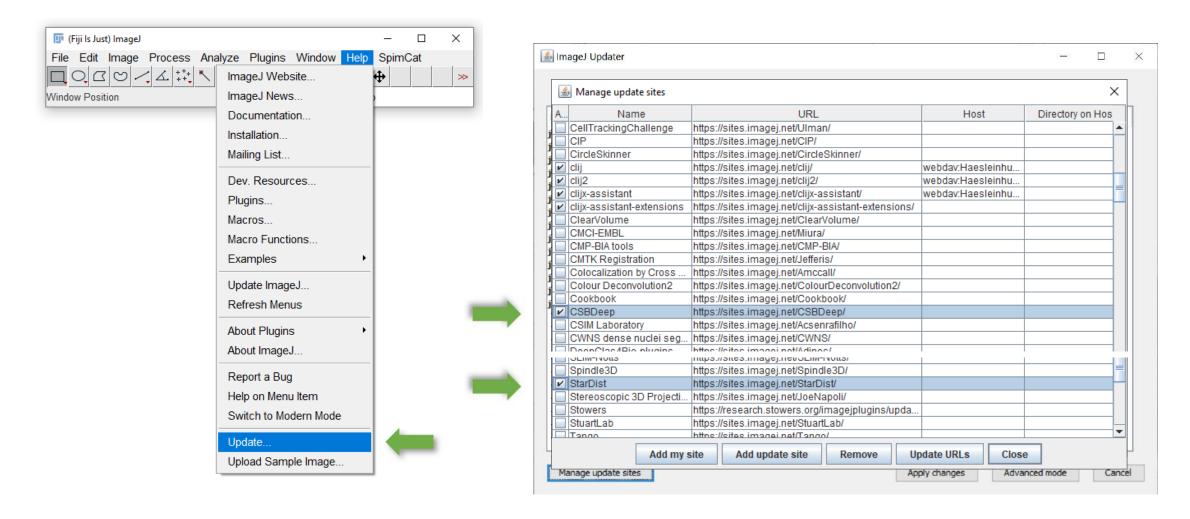




Exercise: StarDist Installation



Activate the "CSBDeep" and "StarDist" update sites.



Exercise

Pol Physics of Life TU Dresden

- Download and install Fiji
 - http://fiji.sc/Downloads
- Download the BBBC001 dataset (TIF), unzip it and count nuclei in the first image file
 - https://bbbc.broadinstitute.org/BBBC001
 - AS 09125 050118150001 A03f00d0.tif
- Hints:
 - Use Plugins > StarDist > StarDist2D to segment the nuclei
 - Use Analyze > Set Measurements and Analyze > Measure to determine the maximum intensity in the label image (= number of labels)

- Enter your count here: https://docs.google.com/spreadsheets/d/1Ek-23Soro5XZ3y1kJHpvaTaa1f4n2C7G3WX0qddD-78/edit?usp=sharing
- Be a good scientist: Don't cheat! ;-)

Record and curate a macro for this task! You will need it tomorrow ;-)

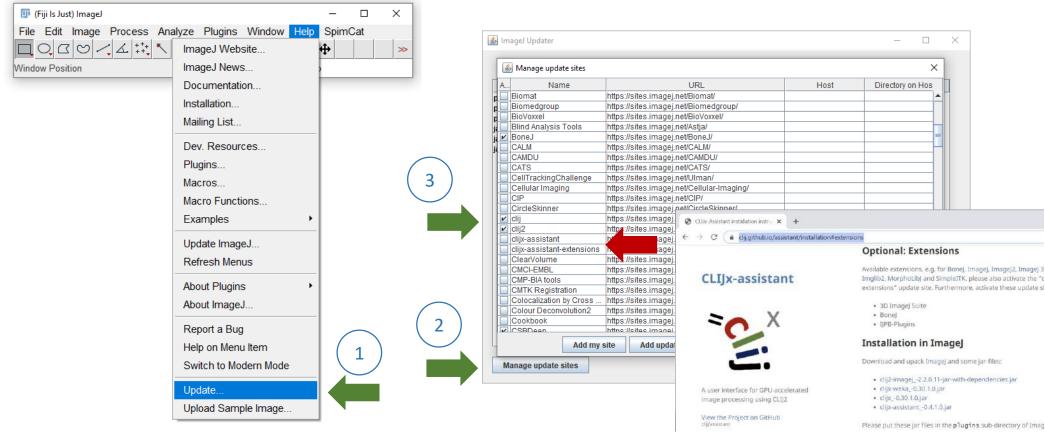


Exercise CLIJ Installation



- Just activate the CLIJ and CLIJ2 update sites, in menu Help > Update...
 - Do not install clijx! That's experimental stuff you're free to try out later.

Linux users: vou mav need to install OpenCL, e.g. apt-get install ocl-icd-devel



Exercise

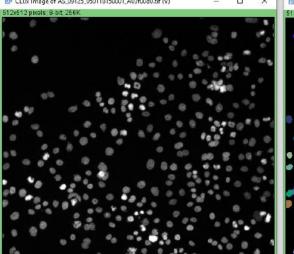
Pol Physics of Life TU Dresden

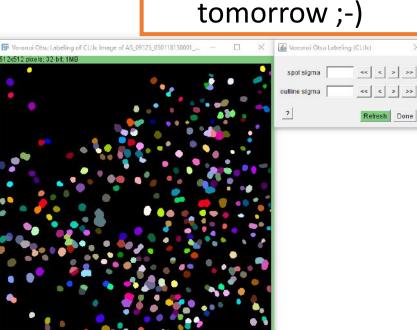
Generate a macro

for this task! You

will need it

- Download and install Fiji
 - http://fiji.sc/Downloads
- Download the BBBC001 dataset (TIF), unzip it and count nuclei in the first image file
 - https://bbbc.broadinstitute.org/BBBC001
 - AS_09125_050118150001_A03f00d0.tif
- Hints:
 - Use CLIJ Assistant [Right-click menu] Label > Voronoi-Otsu-Labeling to segment the nuclei
 - Use Analyze > Set Measurements and Analyze > Measure to determine the maximum intensity in the label image (= number of labels)





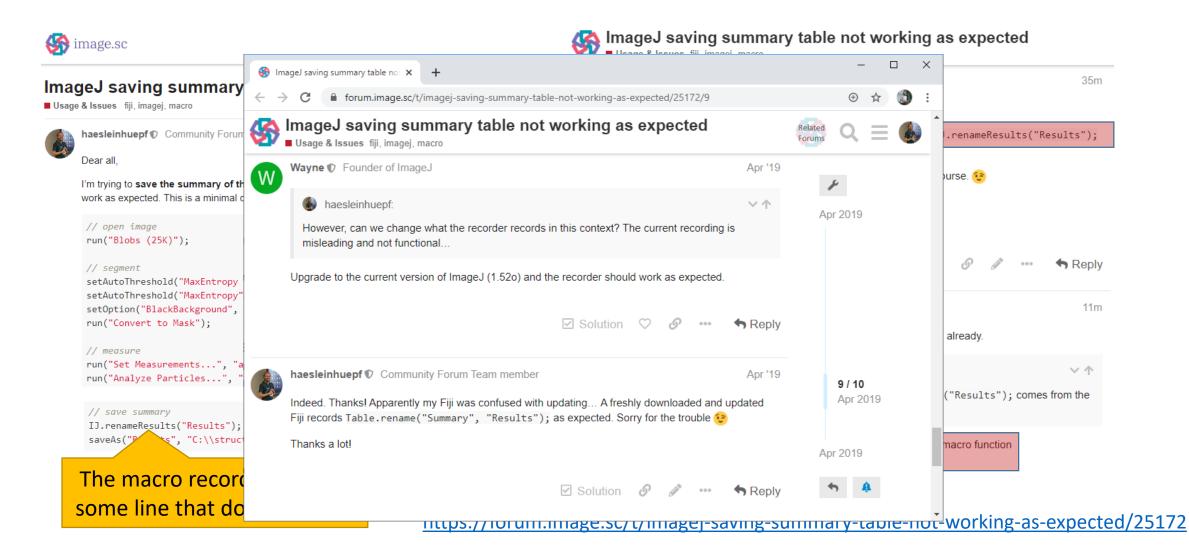
- Enter your count here: https://docs.google.com/spreadsheets/d/1Ek-23Soro5XZ3y1kJHpvaTaa1f4n2C7G3WX0qddD-78/edit?usp=sharing
- Be a good scientist: Don't cheat! ;-)



Hint: image.sc



In case you run into trouble: Visit image.sc and ask. They may ask within 5 hours, even Saturdays.

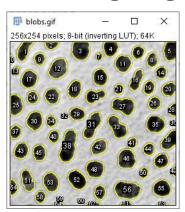


Summary



Today, you learned

- ImageJ macro
 - Variables
 - Working with files and images
 - Macro recording
 - Using the ROI manager
 - Custom functions
- Exercises:
 - Macro recording and
 - image segmentation





Coming up next

- ImageJ macro
 - Loops and conditions
 - Interacting with tables
 - Custom dialogs
- How to write readable code.









Break: 21h

@haesleinhuepf









CENTER FOR SYSTEMS BIOLOGY DRESDEN

ImageJ macro programming

Robert Haase

With material from

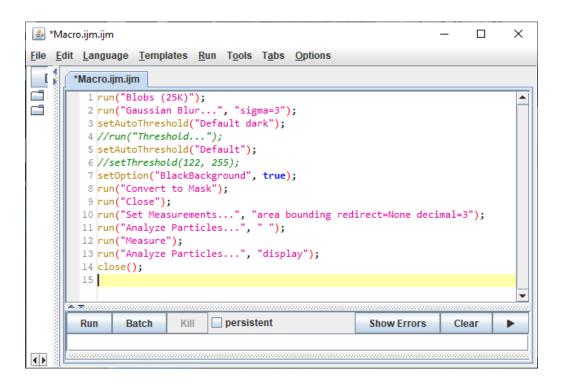
Benoit Lombardot, Scientific Computing Facility, MPI CBG

Virtually at CCI Gothenburg, October 2021

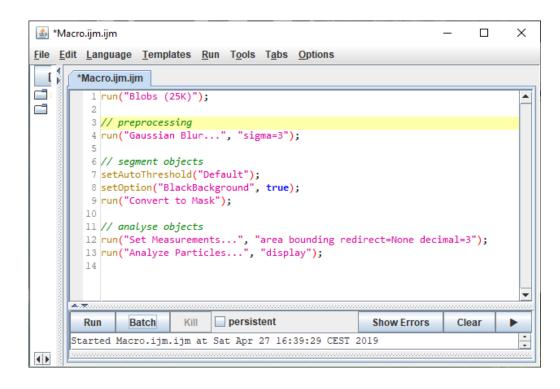
Follow up: Macro recording



- Editing recorded macros needs to be trained. It's 80% reading and 20% writing
- Hints:
 - Put comments first. Try to understand what was recorded and why.
 - Do it in tiny steps. As soon as you have a working workflow consisting of 4-5 steps, create a macro.
 - Collect macros. When you do something new, do cherry picking from the old macros.















ImageJ macro programming: Conditions and loops

Robert Haase

With material from

Benoit Lombardot, Scientific Computing Facility, MPI CBG

Virtually at CCI Gothenburg, October 2021





• Image processing workflows rarely look like this

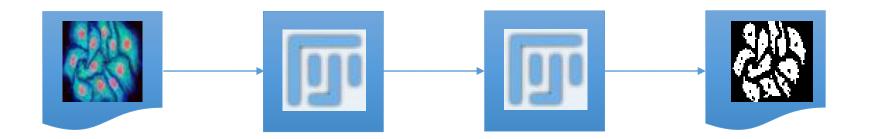
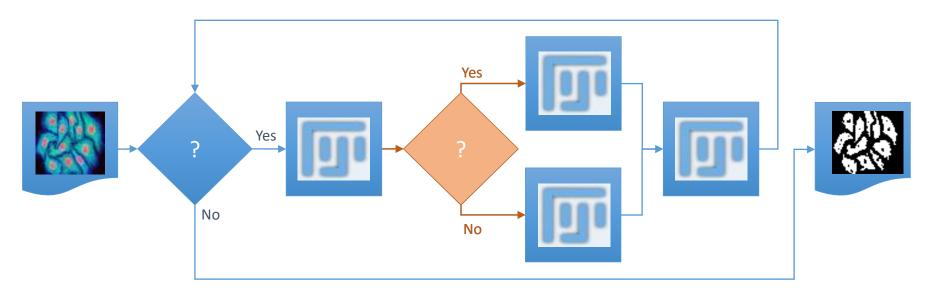




Image processing workflows rather look like this

Conditional statement





Depending on a condition, some lines of code are executed or not.

```
if (condition {
    // do something if
    // condition is true
}

do a final thing
```



• Depending on a condition, some lines of code are executed or not.

```
if (condition) {
// do something if
// condition is true
}

do a final thing
```



• The else statement allows to program alternatives.

```
if ( condition ) {
    // do something if
    // condition is true
}
else{
    // do something else if
    // condition is false
}
// do a final thing
```



- The if / else statement allows to program alternatives.
- Depending on a condition, either the one or the other block is executed.
- Curly brackets {} are used to mark where a block starts and ends.
- Indentation helps reading blocks.

```
Note: this line does not end with a; semicolon!

if (condition) {
    // do something if
    // condition is true
}
else {
    // do something else if
    // condition is false
}
// do in any case
```



• Comparison operators always have true or false as results.

```
// initialise program
quality = 99.5;

// evaluate result
if (quality > 99.9) {
    print("Everything is fine.");
} else {
    print("We need to improve!");
}
```

Operator	Description	Example
<, <=	smaller than, smaller or equal to ¹	a < b
>, >=	greater than, greater or equal to ¹	a > b
==	equal to ¹	a == b
!=	not equal to ¹	a != 1

¹ these operators work also with string values

Combined conditions



- Logic operators always take conditions as operands and result in a condition.
- Conditions can be either true (1) or false (0).

```
// initialise program
quality = 99.9;
age = 3;

if ((quality >= 99.9) && !(age > 5)) {
    print("The item is ok.");
}
```

Operator	Description	Example
&&	and	a < b && b < c
П	or	a < b b > c
!	negation ("not")	!(a == b)



Why is indentation important?



Does this program say "Yin" or "Yang"?

```
// initialise program
a=5;b=3;c=8;
// execute algorithm
d=(a+b)/c;
// evaluate result
if (b>0) {
if (a==5)
print("Yin");
else
if (c<5)
if (d!=0)
print("Yang");
```

```
// initialise program
a = 5;
b = 3;
c = 8;
// execute algorithm
d = (a + b) / c;
// evaluate result
if (b > 0) {
    if (a == 5) {
       print("Yin");
    } else {
        if (c < 5) {}
    if (d != 0) {
        print("Yang");
```

Yes, it's the same program as on the left ©

Yin

Yang

Readable code



Rules for readable code

- Every command belongs on its own line
- Insert <u>empty lines to separate</u> important processing steps
- Put <u>spaces</u> between operators and operands, because:

This is easier to read thanthat, or isnt'it?

- Indent every conditional block (if/else) using the TAB key
- Hint: put the "{" behind the if; it makes your program shorter.
- Make use of tools: http://jsbeautifier.org

```
// initialise program
a = 5;
b = 3;
c = 8;

// execute algorithm
d = (a + b) / c;

// evaluate result

if (a == 5) {
    print("Yin");
} else {
    print("Yang");
}
```

Troubleshooting

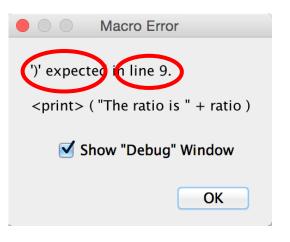


If your program throws error messages:

- Don't panic.
- "There are two ways to write error-free programs; only the third one works."

Alan J. Perlis, Yale University

- Read <u>where</u> the error happened. You may see your fault immediately, when looking at the right point.
- Read what appears to be wrong. If you know, what's missing, you may see it, even if it's missing in a slightly different place.
- Don't take error messages to strict. It's just a program reporting about an issue with another program. It may also have issues.



Troubleshooting

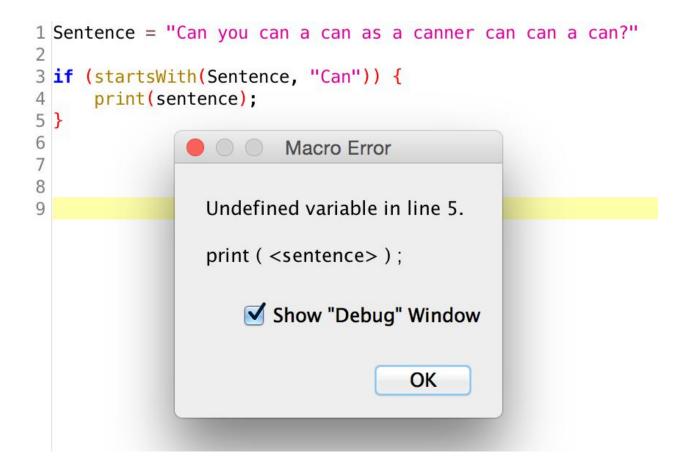


```
Sentence = "Can you can a can as a canner can can a can?"

if (startsWith(Sentence, "Can") {
   print(sentence);
}
```

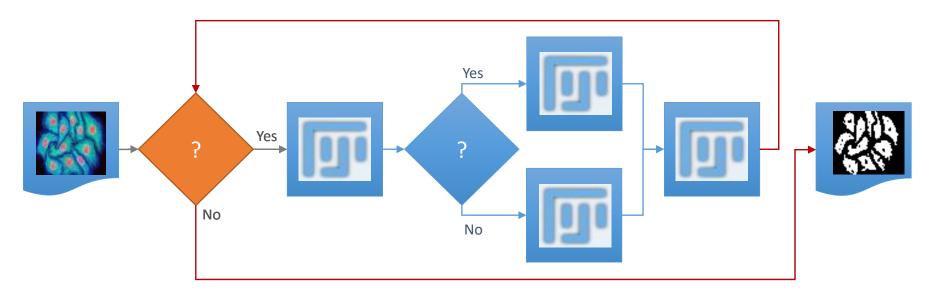
Troubleshooting







• To repeat actions, you run code in loops



Loop statement



• The for statement allows us to execute some lines of code for several times

```
Note: this line does not
end with a; semicolon!

for( <initializer>; <condition>; <iterator>) {
    // do something repeatedly
}

// do something
```



- The for statement allows to execute some lines of code for several times
 - The initializer is only executed once; at the beginning.

1. start: initialize counter i

```
for((i = 0) i < 10; i++ ) {
   // do something for 10 times
}</pre>
```



- The for statement allows to execute some lines of code for several times
 - The initializer is only executed once; at the beginning.
 - The condition is checked every time before the whole block is executed.



- The for statement allows to execute some lines of code *for* several times
 - The initializer is only executed once; at the beginning.
 - The condition is checked every time before the whole block is executed.
 - After block execution, the counter is increased.

```
3. increase counter

// do something

for( i = 0; i < 10; i++) {
    // do something for 10 times
}</pre>
```



- The for statement allows to execute some lines of code for several times
 - The initializer is only executed once; at the beginning.
 - The condition is checked every time before the whole block is executed.
 - After block execution, the counter is increased.



- The for statement allows to execute some lines of code *for* several times
 - The initializer is only executed once; at the beginning.
 - The condition is checked every time before the whole block is executed.
 - After block execution, the counter is increased.

```
// do something once
for( i = 0; i < 10; i++) {
    // do something for 10 times
}</pre>
```

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- The for statement allows to execute some lines of code *for* several times
 - The initializer is only executed once; at the beginning.
 - The condition is checked every time before the whole block is jumped over.
 - After block execution, the counter is increased.

```
2. check condition
If false: exit the loop

// do something once
for (i 0, i < 10 i++ ) {
    // do something for 10 times
}</pre>
```



- The for statement allows to execute some lines of code *for* several times
 - The initializer is only executed once; at the beginning.
 - The condition is checked every time
 - After block execution, the counter is increased.

```
for( i = 0; i < 10; i += 1 ) {
    // do something at each
    // iteration of the loop
}

// do another thing once</pre>
```

- The counter is initialized.
- 2. check condition:
 - if true: execution code in the loop
 - if false: exit the loop
- 3. increase counter

Step 2 and 3 are repeated until the condition is false and the loop exits

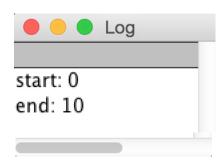
Troubleshooting: Tracing



• *Tracing* means printing something out after every line of code to ensure the path of execution is gone right.

```
print("start: " + start);
print("end: " + end);

// print numbers
for ( i = start; i > end; i += 1 ) {
    print("is this ever printed?");
    if (i > 5) {
        print(i);
    }
}
```



troubleshooting_tracing.ijm

Troubleshooting: Tracing



 Tracing means printing something out after every line of code to ensure the path of execution is gone right.

```
print("start: " + start);
print("end: " + end);

// print numbers
for ( i = start; i < end; i += 1 ) {
    print("is this ever printed?");
    if (i > 5) {
        print(i);
    }
}
```

troubleshooting tracing.ijm



Don't forget to remove the "traces" after you found the bug!

Working with image files in a folder

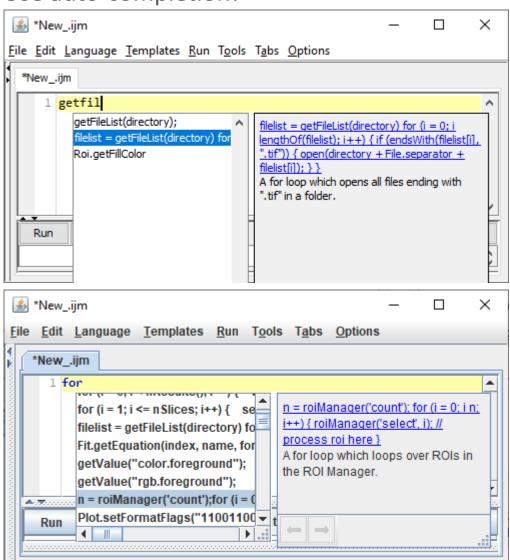


• The files in a folder are represented as an array []

```
foldername = "/Users/rhaase/temp/";
// get all files in the folder as array
list = getFileList( foldername );
// print out the array; item by item
for (i = 0; i < lengthOf(list); i += 1 ) {</pre>
    filename = list[i];
                                                         Log
    print( filename );
                                       sphericity.png
                                                                                    process folder.ijm
                                       sphericity2.png
                                       spim_histo.png
                                       spim_histo2.png
                                       spimdata1.png
                                       spimdata2.png
                                       spimdata3.png
```



Use auto-completion!



```
×
File Edit Language Templates Run Tools Tabs Options
  *New_.ijm
     1 filelist = getFileList(directory)
     2 for (i = 0; i < lengthOf(filelist); i++) {</pre>
           if (endsWith(filelist[i], ".tif")) {
               open(directory + File.separator + filelist[i]);
           Batch
                        persistent
                                              Show Errors
                                                           Clear
*New_.ijm
                                                              ×
File Edit Language Templates Run Tools Tabs Options
   *New_.ijm
      1 n = roiManager('count');
     2 for (i = 0; i < n; i++) \{
           roiManager('select', i);
           // process roi here
     5 }
                             persistent
    Run
             Batch
                                           Show Errors
                                                          Clear
```











ImageJ macro programming: Arrays, Plots and Tables

Robert Haase

Recap: Variables



• Variables are memory blocks where you can store stuff

Computer memory

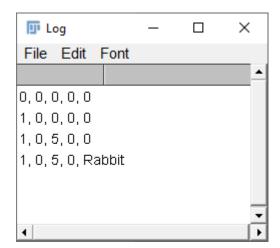
measurement name "Drosophila" combination "Drosophila5"

Arrays

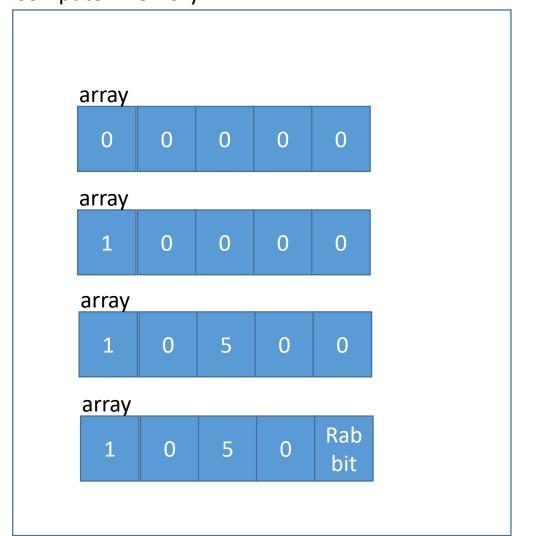


• Arrays are variables, where you can store multiple stuff

```
1
2 array = newArray(5);
3 Array.print(array);
```



Computer memory



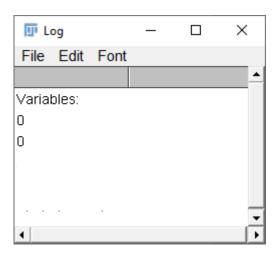
arrays_access.ijm

Arrays: pitfalls



- Variable assignment: by value
- Array assignment: by reference

```
1 // working with variables
2 print("Variables:");
3 a = 0;
4 print(a);
5
6 b = a;
7 b = 5;
8 print(a);
```



 $arrays_variable_comparion.ijm$

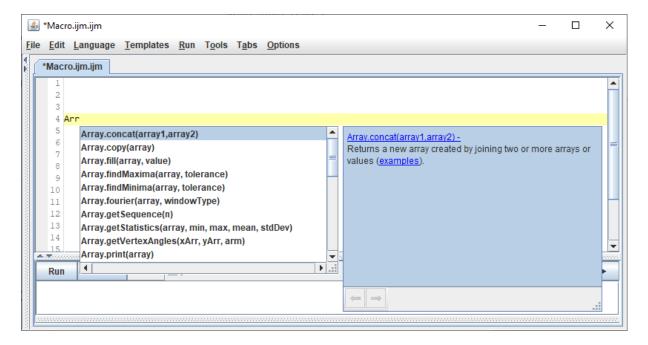
Arrays



Arrays can be initialized with values (more than 1)

```
v = newArray(3, -4, 0);
animals = newArray("dog", "cat", "mouse");
```

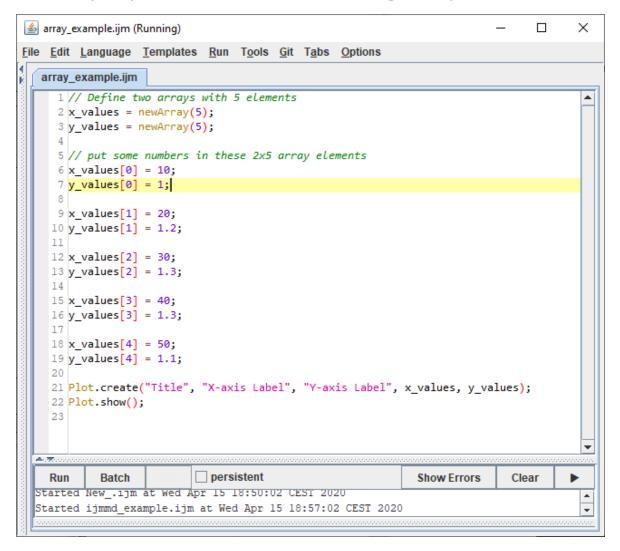
- Arrays can be concatenated: mixed = Array.concat(v, animals);
- Find out what else you can do with Arrays by starting typing:

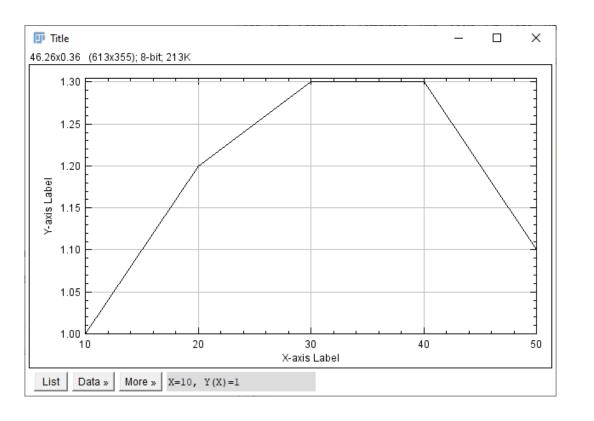


Arrays and plots



Simple plots can be drawn using arrays















Break: 15 min









Working with tables

Robert Haase

With materials from
Benoit Lombardot, Scientific Computing Facility, MPI CBG

@haesleinhuepf

Results tables



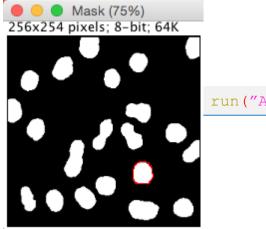
- ImageJ/FIJIs tool for collecting data/measurements
- programmable as well
 - Create tables with measurements
 - Read cells
 - Write cells
 - Add columns
 - Save as CSV or XLS file

		Results	
	Area	X	Υ
1	658	108.8298	13.0684
2	477	247.3092	14.0451
3	501	174.4381	33.6916
4	660	73.8045	41.7788
5	448	138.9688	55.2455
6	520	28.8481	57.9981
7	506	8.3617	98.0217
8	676	175.2855	99.5828
9	545	37.5569	122.7312
10	593	224.4410	127.8558

Creating results tables

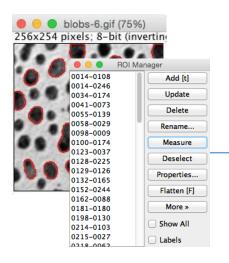


Two ways for creating result tables



run("Analyse Particles...", "display");

roiManager("Measure");

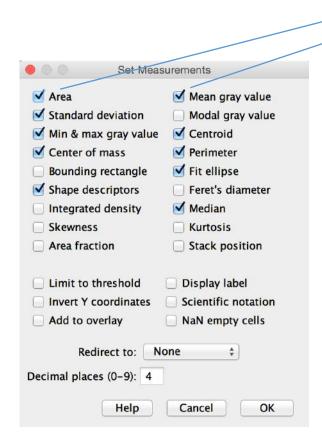


Results Area 658 108.8298 13.0684 477 247.3092 14.0451 501 174.4381 33.6916 660 73.8045 41.7788 448 138.9688 55.2455 520 28.8481 57.9981 506 8.3617 98.0217 676 175.2855 99.5828 9 545 37.5569 122.7312 10 593 224.4410 127.8558

Creating results tables



- Configure what to measure...
- Menu Analyze > Set measurements...



run("Set Measurements...",
"area mean standard min centroid center
perimeter fit shape median redirect=None
decimal=4");

... by entering the first word behind the checkbox.

Reading from tables



Reading elements in tables



Built-in command

value = getResult ("column title", rowNumber);

rowCount = nResults();

Writing in tables



Manipulating (and adding) elements in a table



Handle with care!

Built-in command

setResult ("column title", rowNumber, newValue);



Logging, working with text files



There is an alternative to write simple tables and text files to disc

```
1 path = "C:/structure/teaching/lecture applied bioimage analysis/06 example code/test.csv";
3 headline = "Number, number squared";
4
5 File.append(headline, path);
7 for (i = 0; i < 10; i++)
      contentline = "" + i + ", " + pow(i, 2);
      File.append(contentline, path);
10 |}
save_csv_file.ijm
```

```
test.csv ×
        Number, number squared
        0, 0
        1, 1
        2, 4
        3, 9
        4, 16
        5, 25
        6, 36
        7, 49
        8, 64
1.0
        9, 81
11
```

That might be useful in the exercise tomorrow.











ImageJ macro programming: ROI and Overlays

Robert Haase

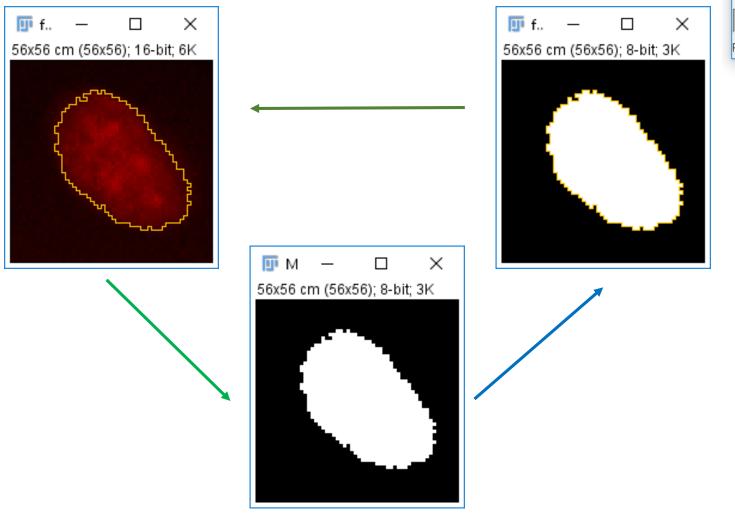
With material from

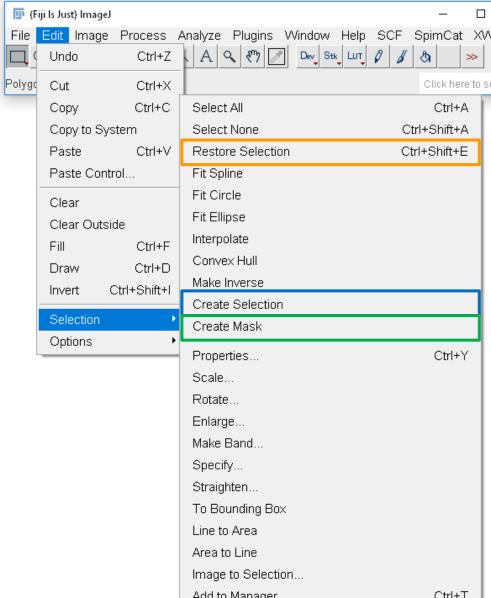
Benoit Lombardot, Scientific Computing Facility, MPI CBG

Selection versus masks



• Selections and masks are exchangeable.

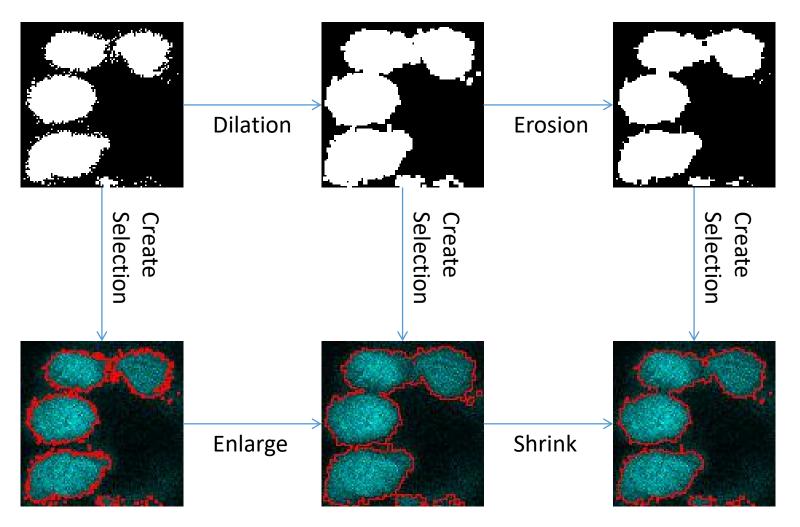


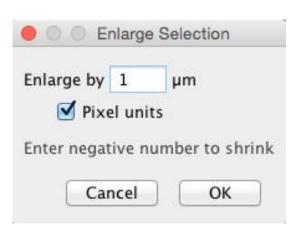


Selections versus masks



Some operations for selections you know already from masks.

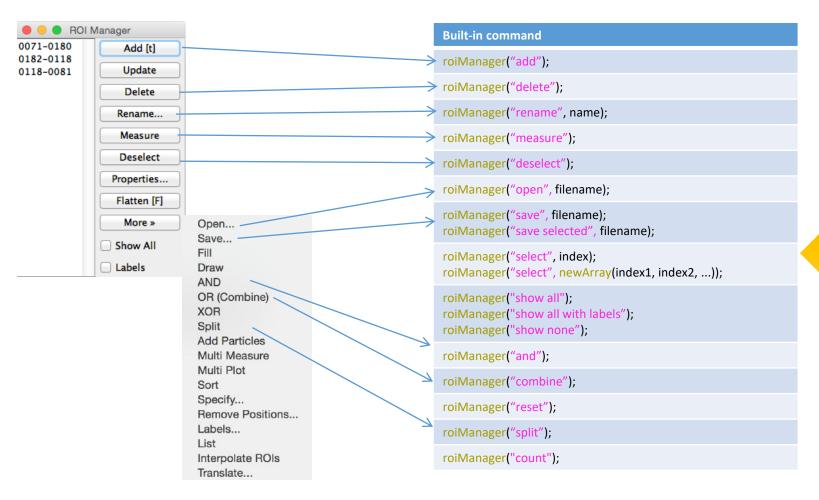


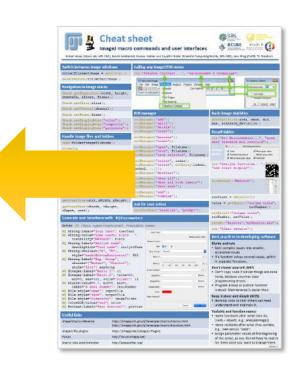


ROI Manager



• Every button in the ROI Manager can be called from a macro.





Hint: Use the <u>macro recorder!</u>

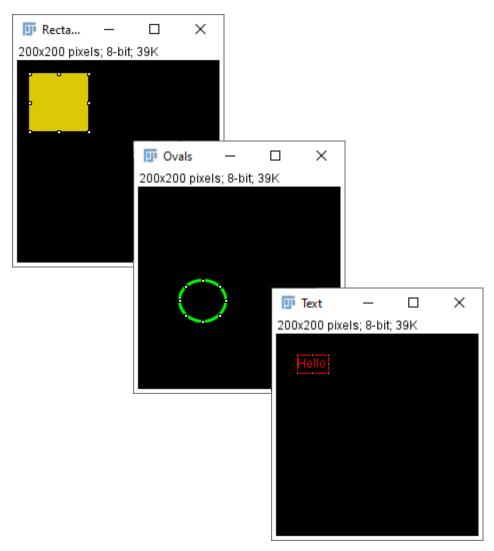


Regions of interest



• ROIs can be used for visualization of (intermediate) results

```
3 // Draw rectangles
4 newImage("Rectangles", "8-bit black", 200, 200, 1);
5 makeRectangle(11, 12, 59, 58);
6 Roi.setFillColor(220, 200, 4);
```

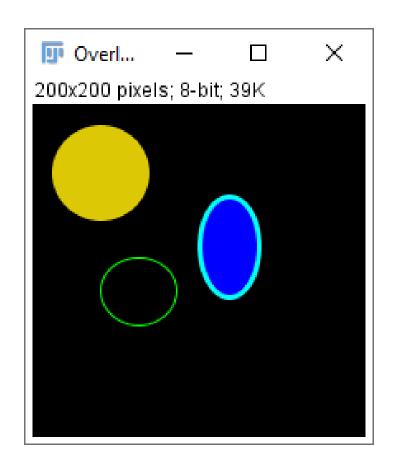


Overlay



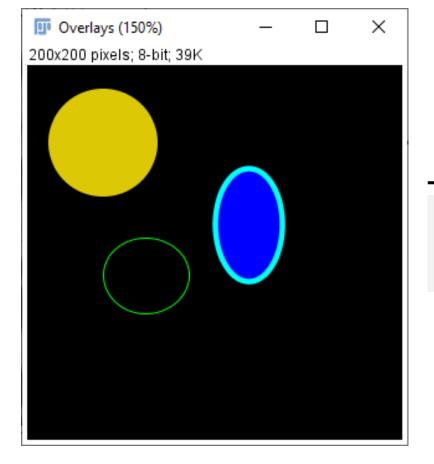
You can collect ROIs in the "Overlay"

```
3 // Draw filled elipses
 4 newImage("Overlays", "8-bit black", 20
 5 makeOval(11, 12, 59, 58);
 6 Roi.setFillColor(220, 200, 4);
 7 Overlay.addSelection();
 9 // Draw outline elipses
10 makeOval(40, 92, 46, 41);
11 Roi.setStrokeColor("Green");
12 Overlay.addSelection();
13
14 // Draw filled elipses, with outline
15 makeOval(100, 55, 36, 61);
16 Overlay.addSelection("", 0, "blue");
17 makeOval(100, 55, 36, 61);
18 Overlay.addSelection("Cyan", 3);
                                overlays.ijm
```

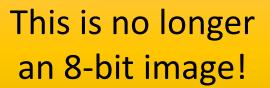


Overlay

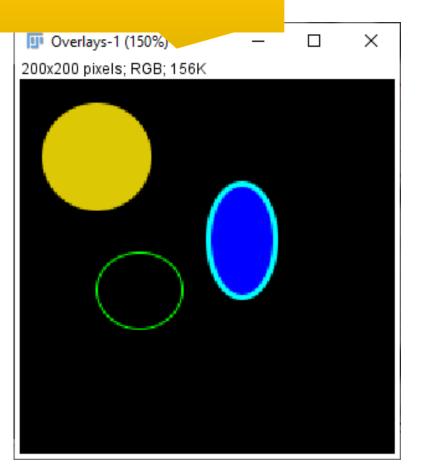
• Overlays can be flattened – for visualization purposes



```
// burn in the overlay
// in the image
run("Flatten");
```





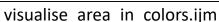


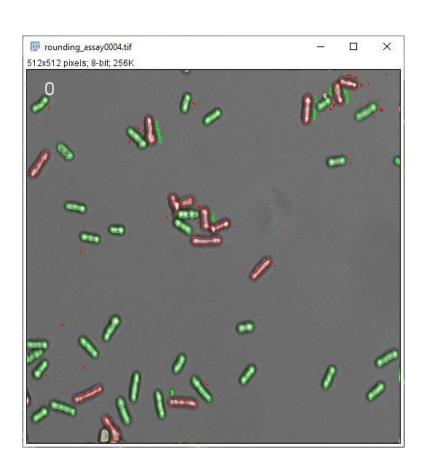
ROI Manager, Results tables and Overlays



Custom visualization with a for loop

```
17 // connected components analysis -> send results to ROI Manager
18 run("Analyze Particles...", " show=Nothing add");
20 // switch back to original image
21 selectWindow(original_image_title);
22 roiManager("Show None");
24 number_of_regions = roiManager("count");
25 for (i = 0; i < number_of_regions; i++ ) {
26
      // measure area ( = pixel count)
      run("Set Measurements...", "area redirect=None decimal=3");
      roiManager("Select", i);
30
      roiManager("Measure");
      pixel count = getResult("Area", nResults - 1);
32
33
      // visualise if ROIs are too small or not
34
      if (pixel count > 10) {
          Overlay.addSelection("green");
36
      } else {
          Overlay.addSelection("red");
38
39 }
```

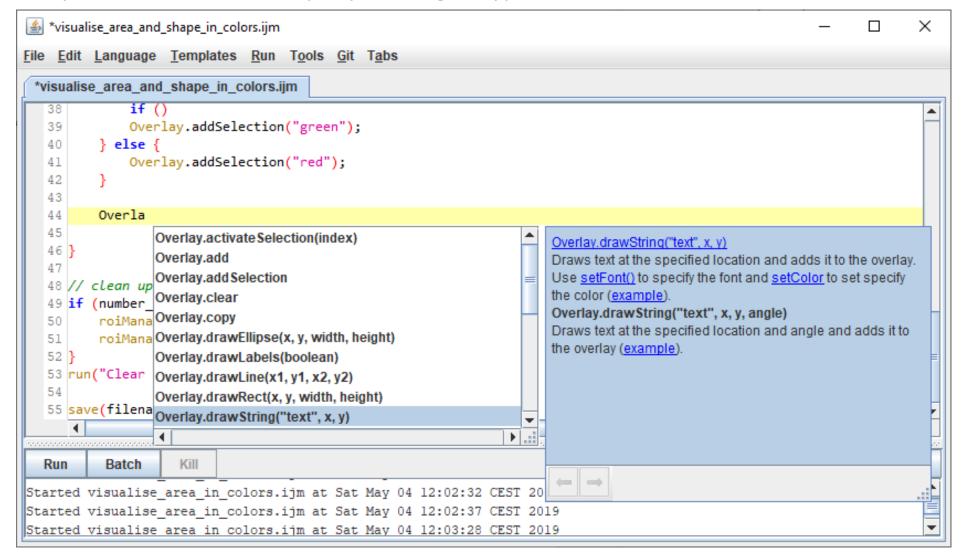




Overlay



Explore what you can do with overlays by starting to type...













Custom Macro Functions

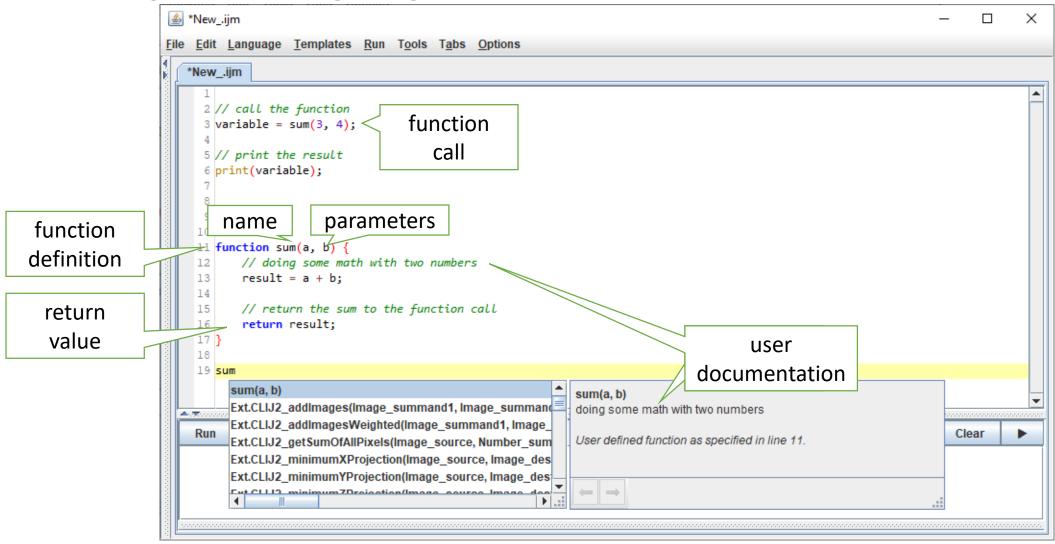
Robert Haase

October 2021

Functions



For reusing code and for organizing code: Use functions!













Exercises

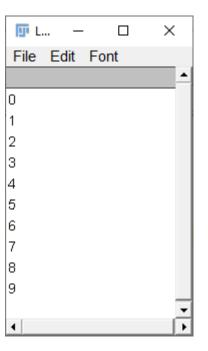


```
// initialisation
loop_count = 10;

// print 10 numbers
for( i = 0; i < loop_count; i++ ) {
         print(i);
}</pre>
```

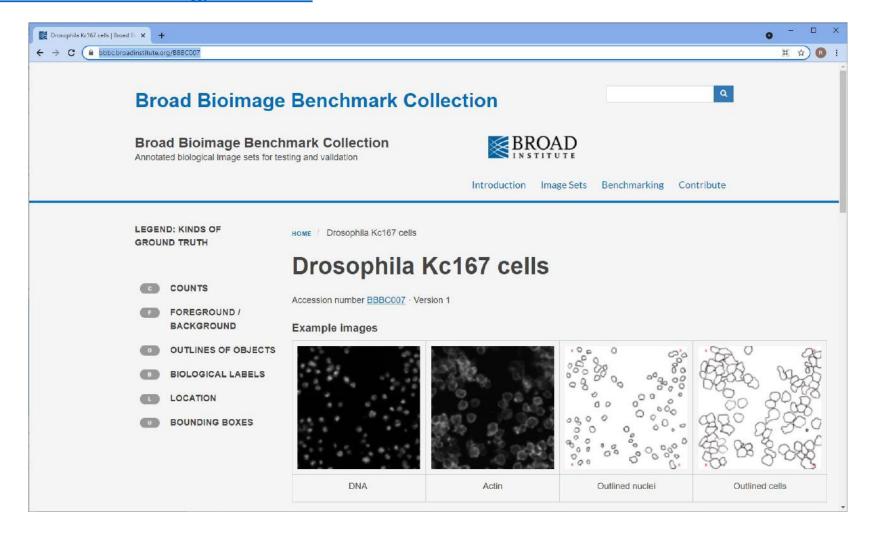
exercise_for_loop.ijm

• Use this example code to program a for loop which counts backwards from a given number to 0.



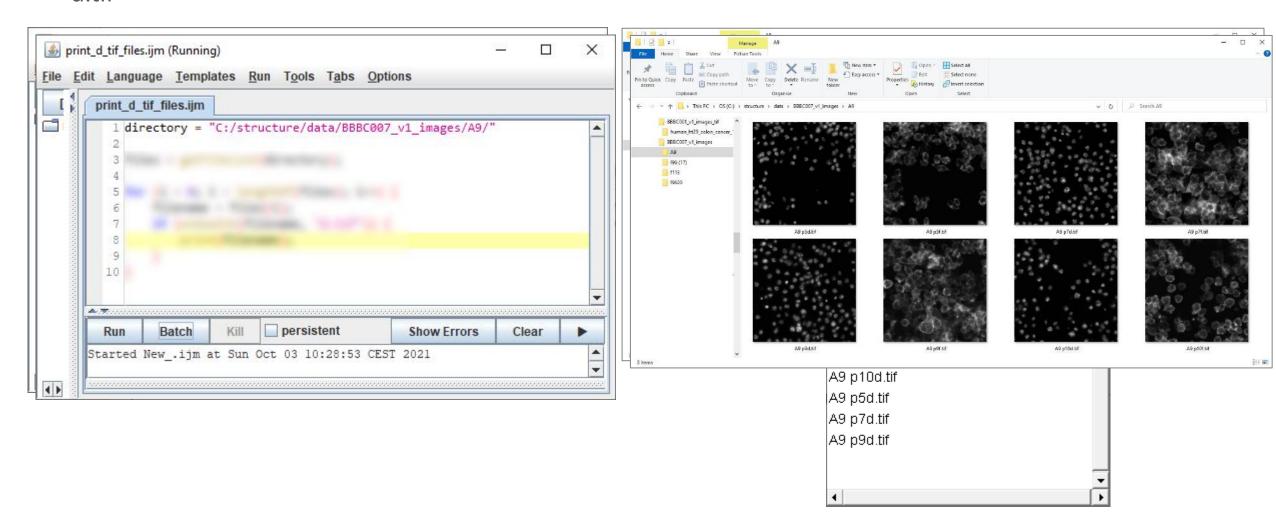


 Visit the Broad Bioimage Benchmark Collection Website and download BBBC007 https://bbbc.broadinstitute.org/BBBC007



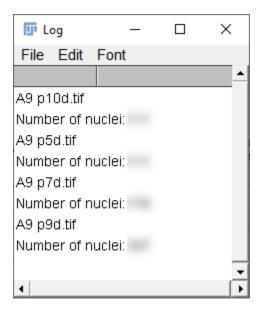


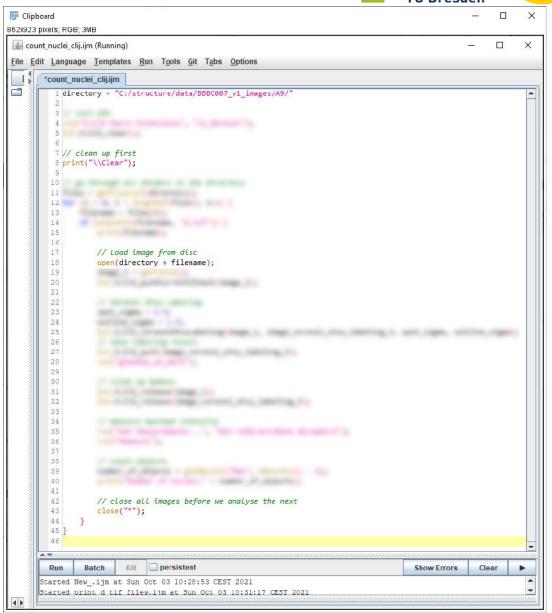
• Write a for-loop that goes through the A9 folder of BBBC007 and prints out all filenames ending with "d.tif"



Pol Physics of Life TU Dresden

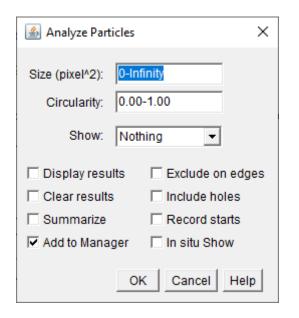
- Extend your script so that it counts nuclei in these images using one method of your choice.
- Hint: The number of objects in a label-image equals the maximum intensity of that image.

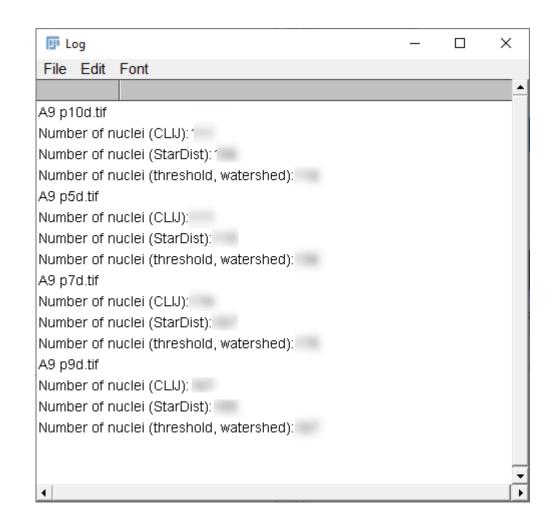






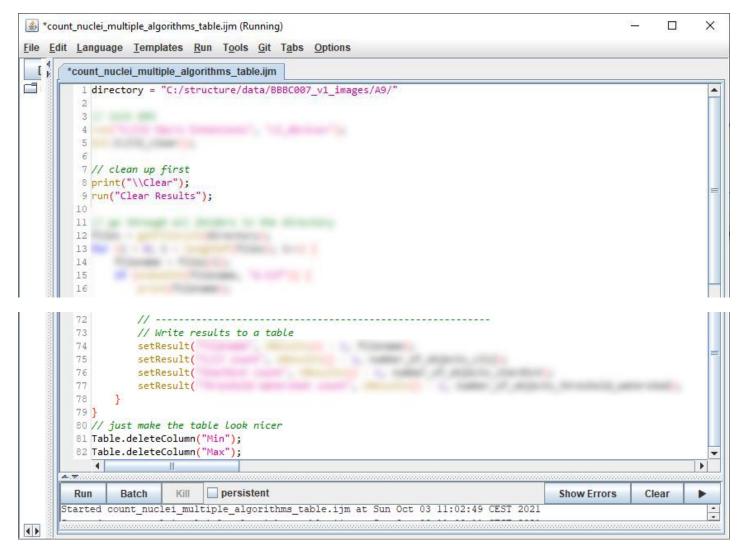
- Extend your script so that it applies the algorithms from yesterday to your image
 - Thresholding + Watershed
 - CLIJ
 - StarDist
- Hint: If you make the algorithms save ROIs to the ROI manager, you can count the elements in the ROI manager to know how many objects there are in the image.

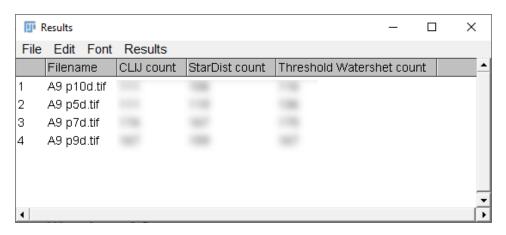






Extend your script so that it outputs the results to a table.

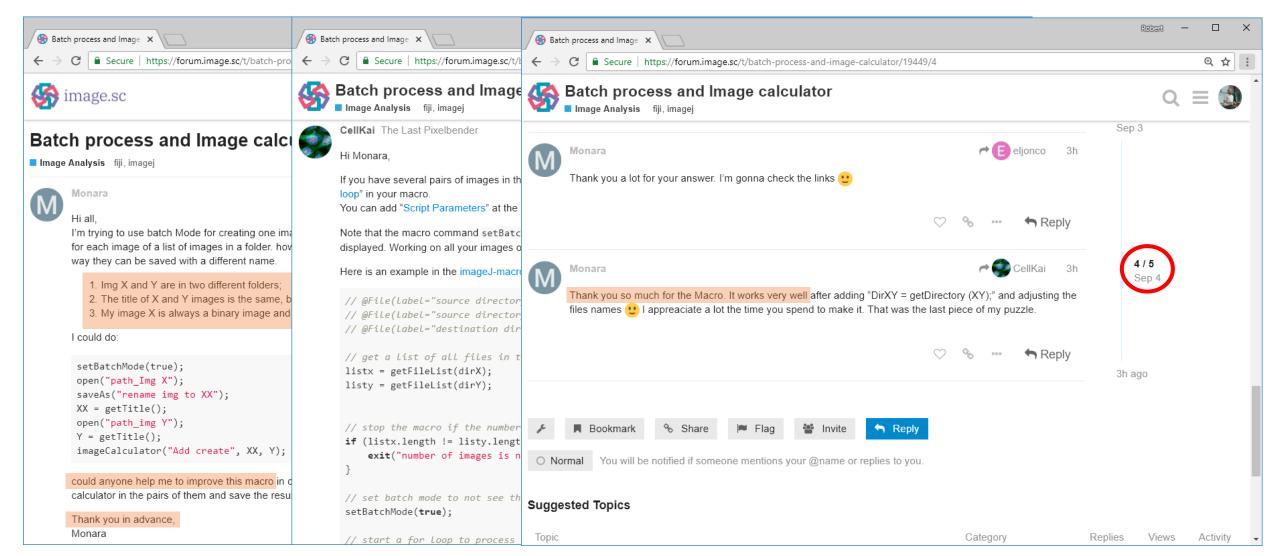




ImageJ, Fiji & friends: community



Visit http://forum.image.sc!



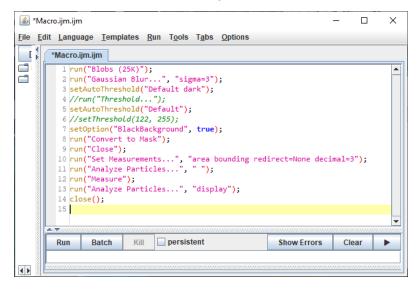
Summary

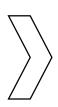


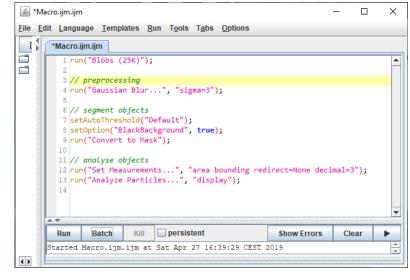
Today, you learned

- Conditions (if)
- Loops (for)
- Results tables
- Arrays
- Plots
- Tables
- Processing folders

Reminder: Clean up recorded code! Write comments!







```
for( i = 0; i < 10; i += 1 ) {
    // do something at each
    // iteration of the loop
}
// do another thing</pre>
```



