

# Protocols

### Graphical programming for Icy

a.k.a. programming, for the rest of us

• Quote: "Results aren't much if they can't be reproduced!" (your boss, your reviewers, your colleagues, you!)

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- Fact: Most journals now **reject** papers without proper quantification [...] Image quantification was <u>carefully</u> conducted using Photoshop. [...]

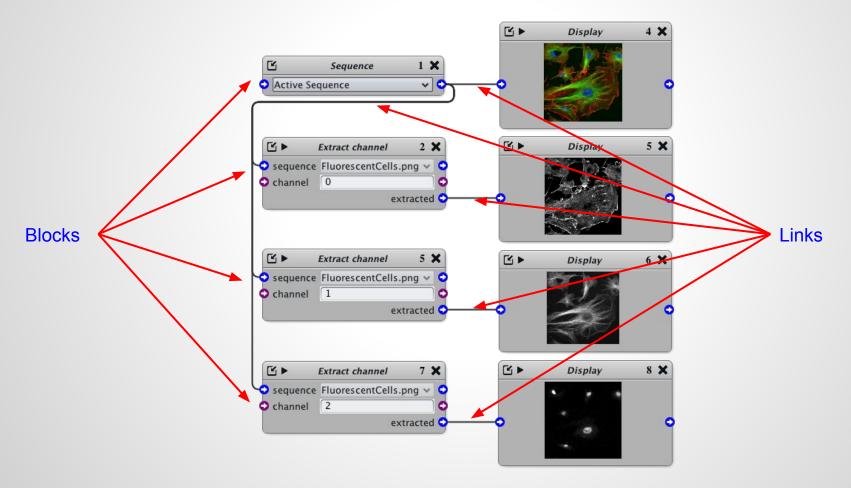
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- Quote: "Results aren't much if they can't be reproduced!"
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- Icy makes these protocols easy to read / write / use / adapt
  - **Design** a protocol once, run on thousands of images
  - **Upload** your protocol and share with the world (within publications)
  - **Download** other protocols, run them out-of-the-box
  - Extend any protocol to meet your needs and share/publish again

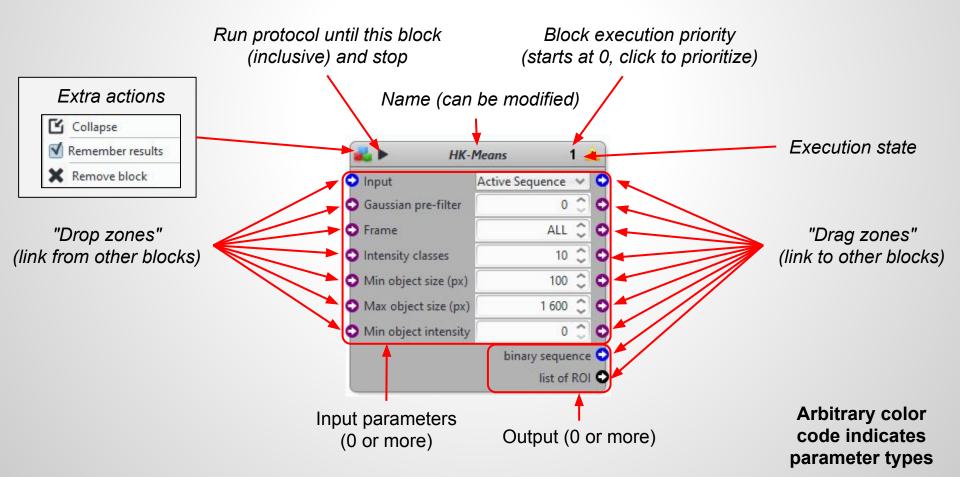
all in just a few clicks, no programming knowledge required.

A protocol is a <u>workflow</u> linking processing <u>blocks</u> together

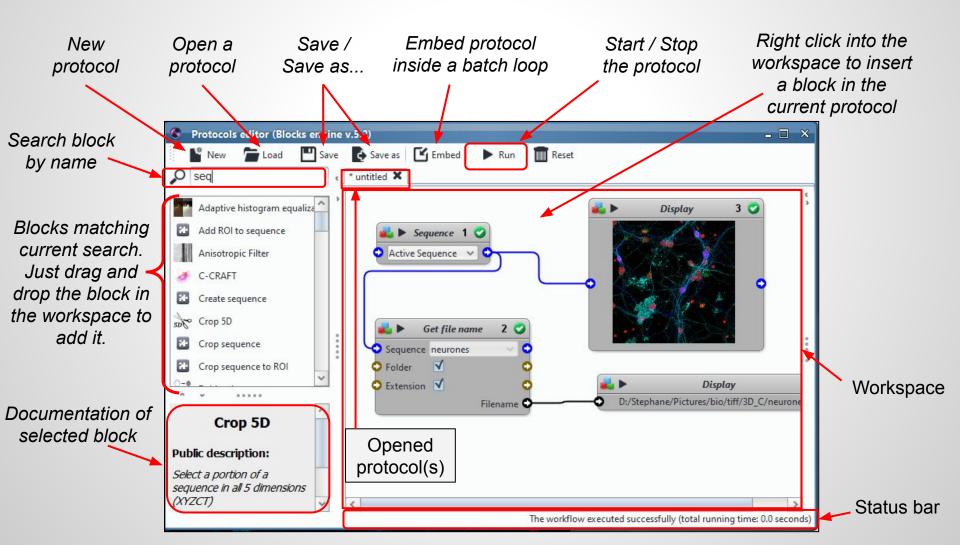


#### http://icy.bioimageanalysis.org/protocol/Extract-channels

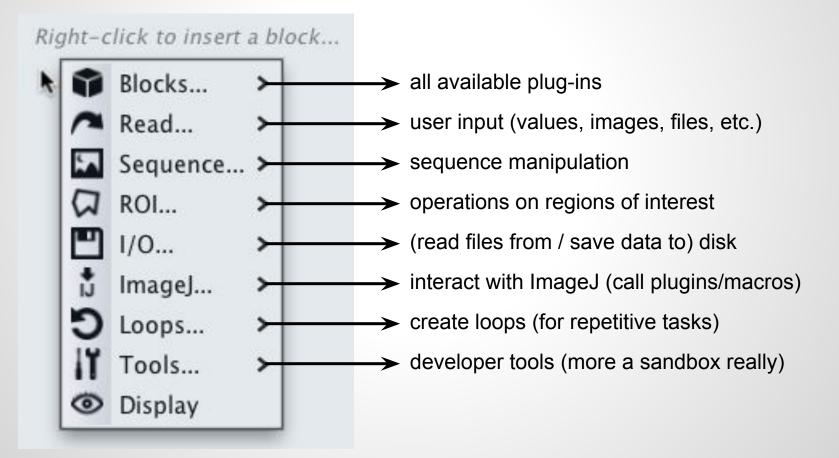
- Standardised design: all blocks look the same
- Strong modularity: one block = one task



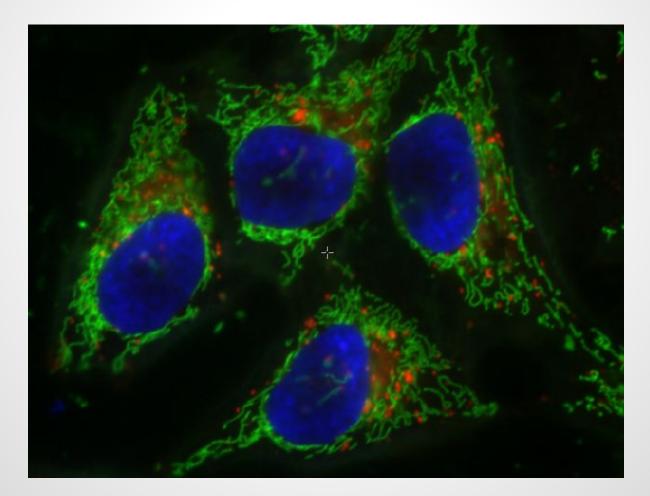
• The protocols editor: <u>http://icy.bioimageanalysis.org/plugin/Protocols</u>



• Blocks are organised by groups



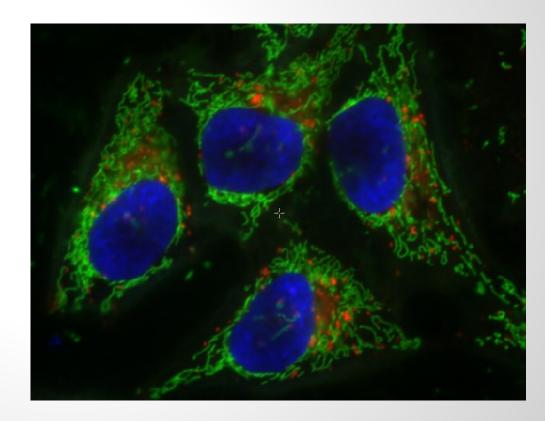
More ideas on how to tidy things up? Let us know!



• Question: how would you find the nuclei in this image?

#### Outline:

- 1. Extract the channel of interest
- 2. Clean the data
- 3. Find an intensity threshold
- 4. Threshold the image
- 5. Extract the regions of interest
- 6. Quantify



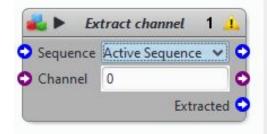
(notice how generic this outline is...)

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Menu: Sequence > Extract Channel



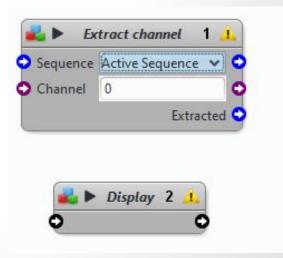
NOTE: channel index starts at 0...

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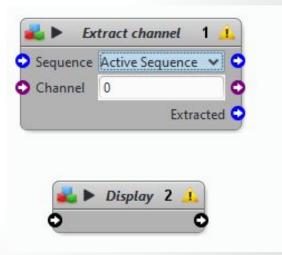
NOTE: adding a Display box is helpful to see what we are doing !

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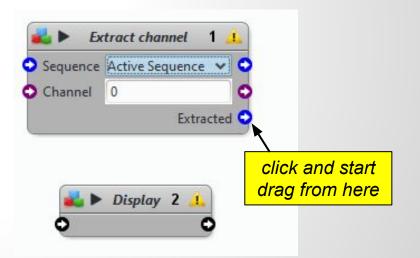
We connect blocks by dragging Output on Input. Let's see that !

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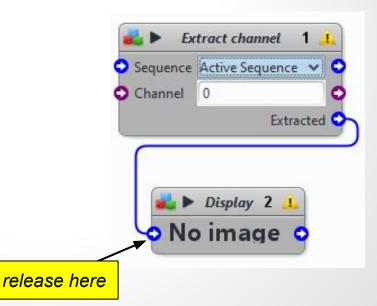
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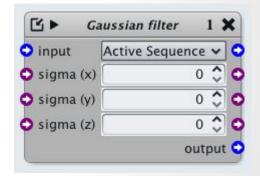
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#### Menu: Blocks > Gaussian Filter



Diffuses the intensity contained in each pixel (i.e. makes the image look blurry)

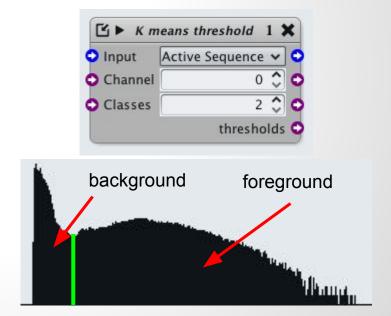
Adapt the diffusion to the image noise Too much diffusion: edges fade away!

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#### Menu: Blocks > KMeans Threshold



Finds the optimal separation(s) between the histogram modes (i.e. intensity classes)

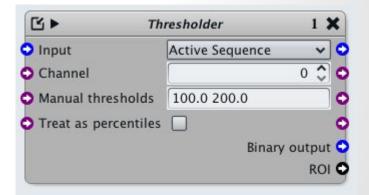
 $2 \mod > 1$  threshold ( $3 \Rightarrow 2$ , etc.)

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#### Menu: Blocks > Thresholder



Creates a labeled image by classifying pixel intensities according to the threshold(s)

1 threshold => [0;1] (binary) image 2 thresholds => [0;1;2] (labeled) image etc.

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Menu: Blocks > Label Extractor



Extracts objects from a labeled image using connected component analysis

• Question: how would you find the nuclei in this image?

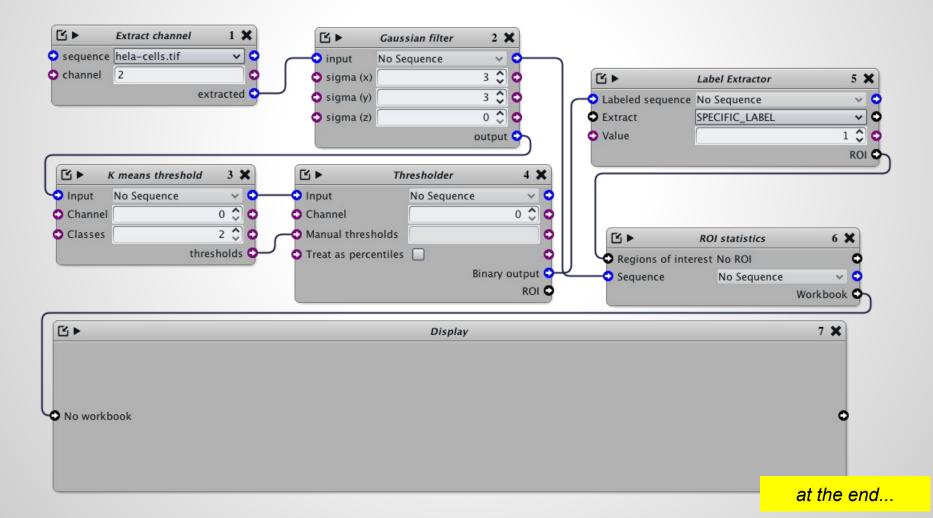
#### Outline:

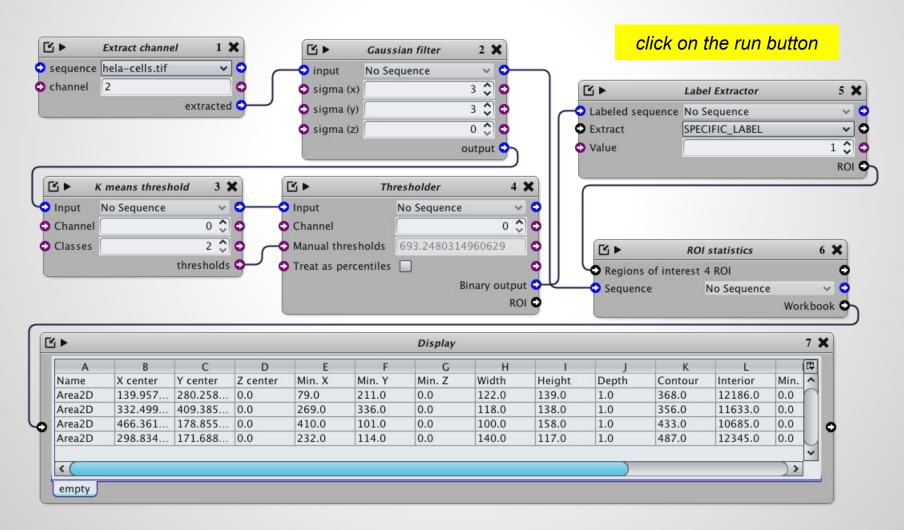
- 1. Extract the channel of interest
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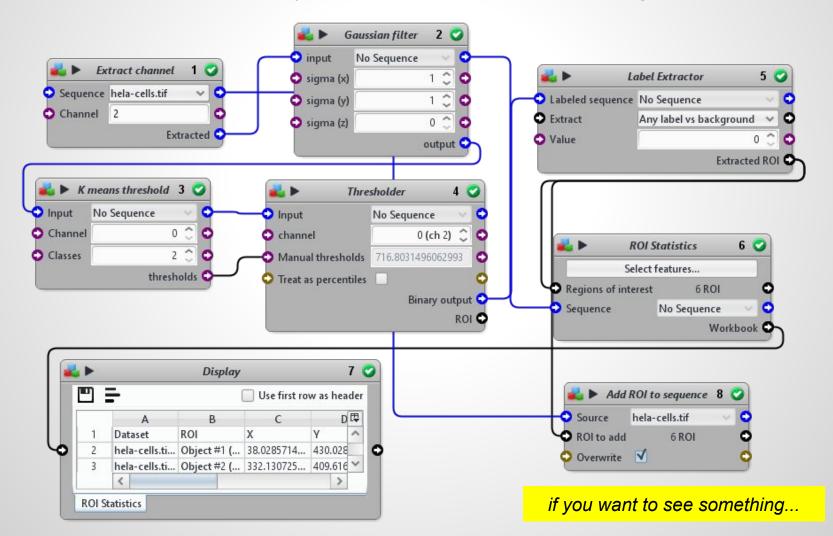
Menu: ROI > ROI Statistics



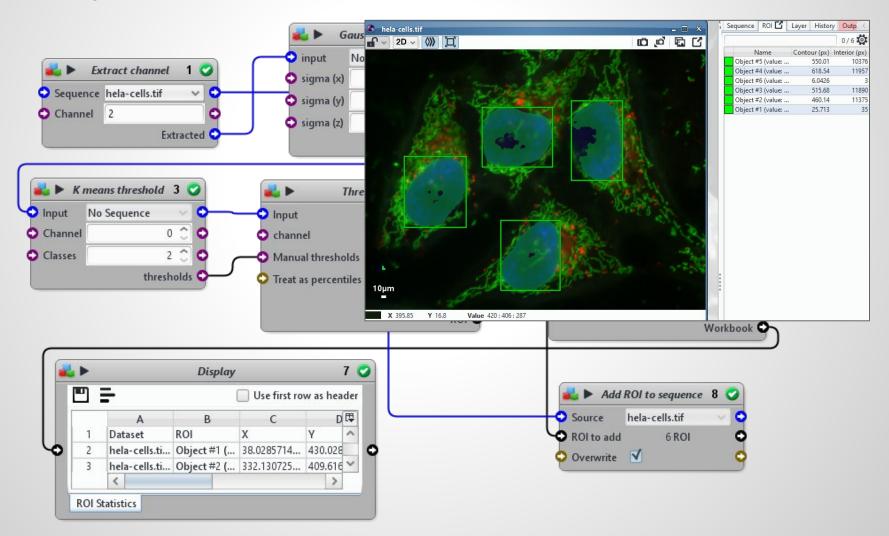
Calculates size, dimensions, intensity statistics, etc.

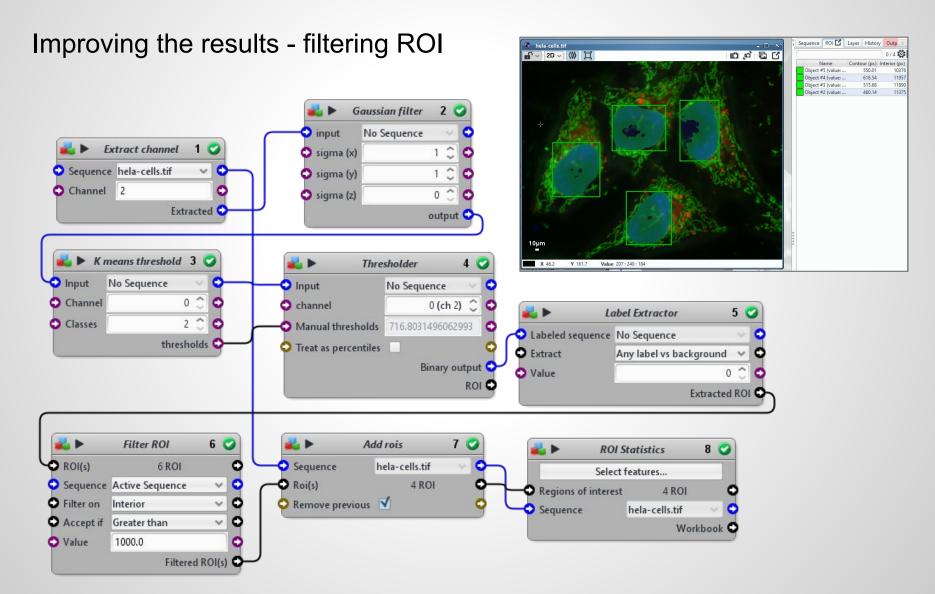


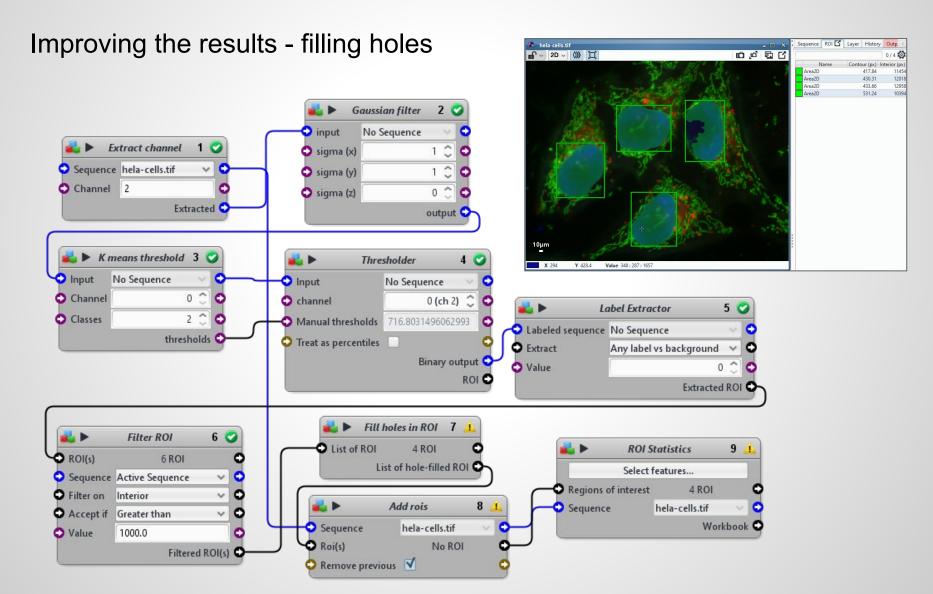




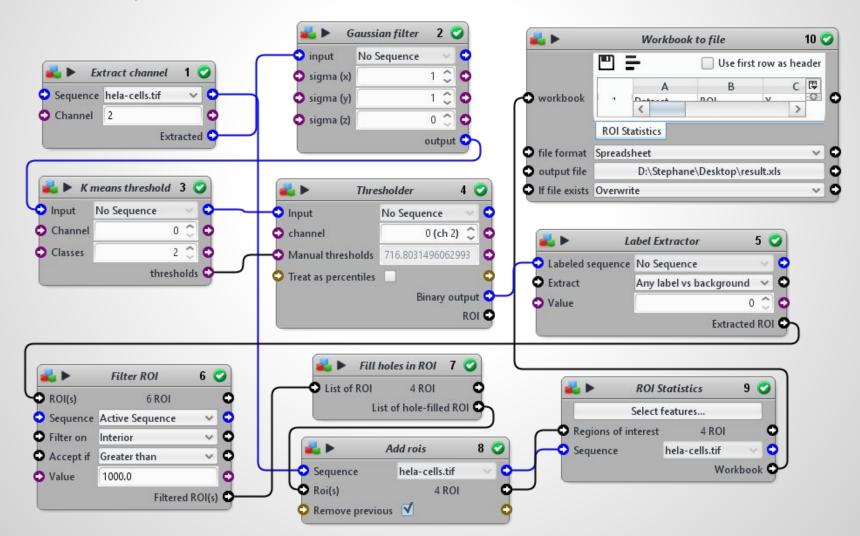
Improving the results - what we can do?



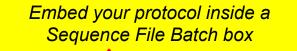


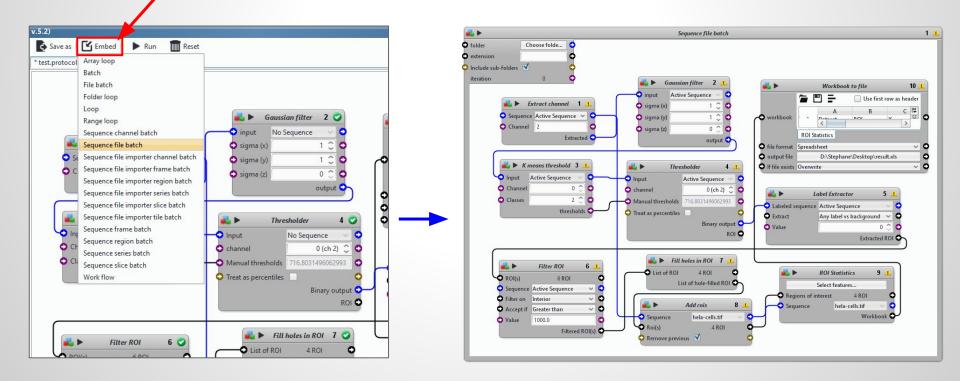


Then save your statistics on disk !

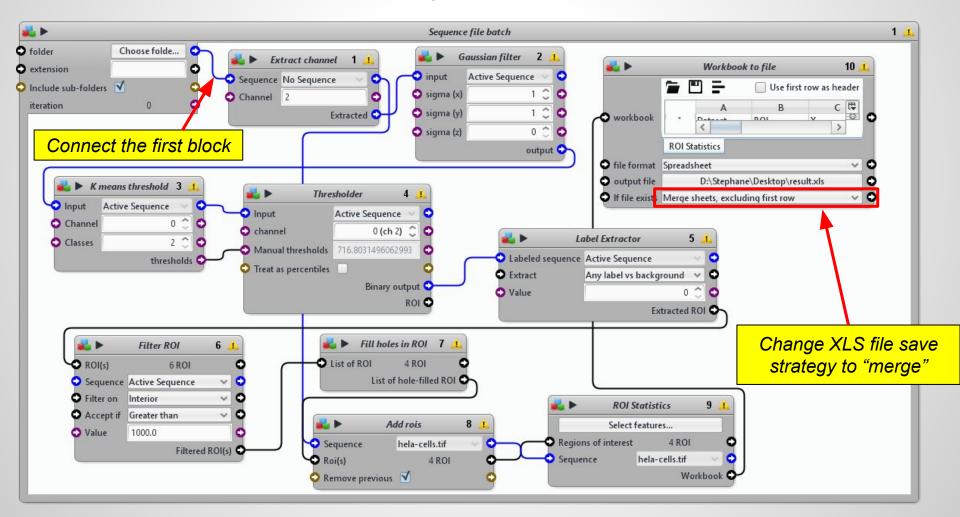


• How about batch processing ?

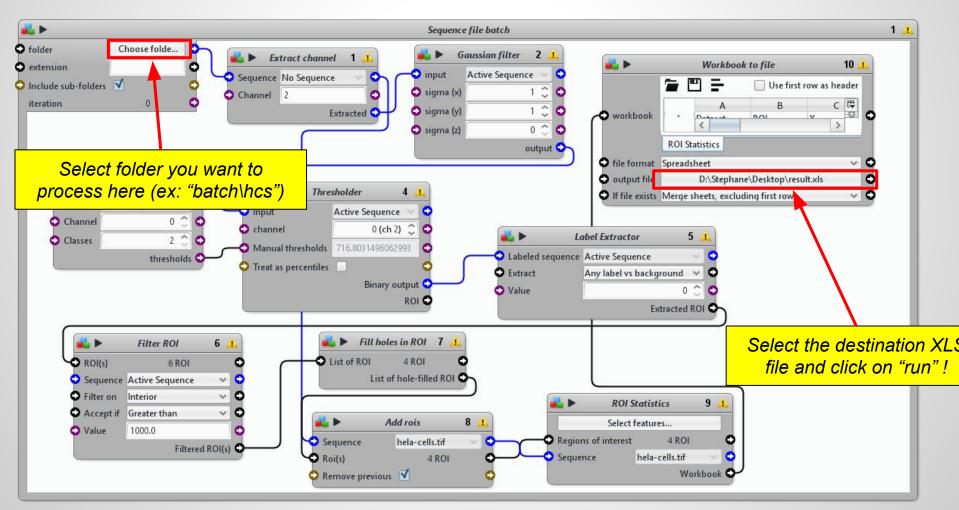


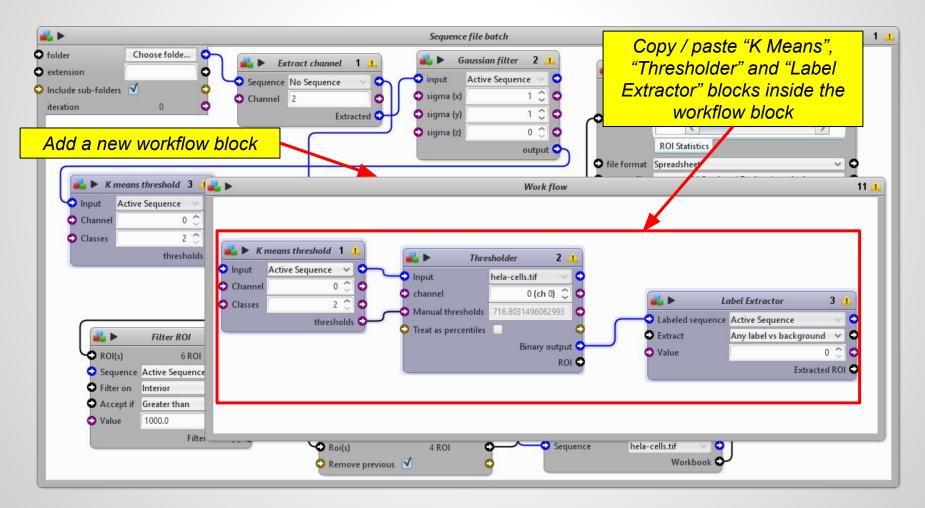


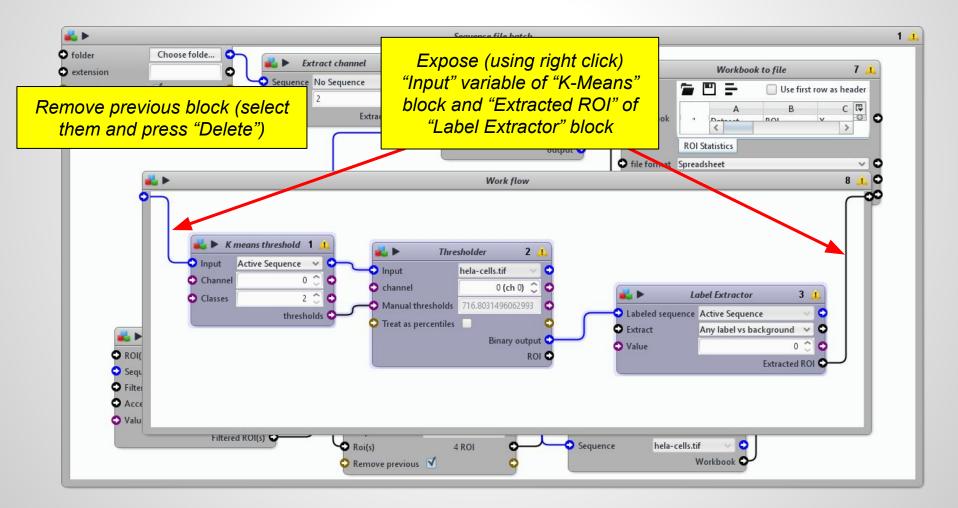
#### How about batch processing ?

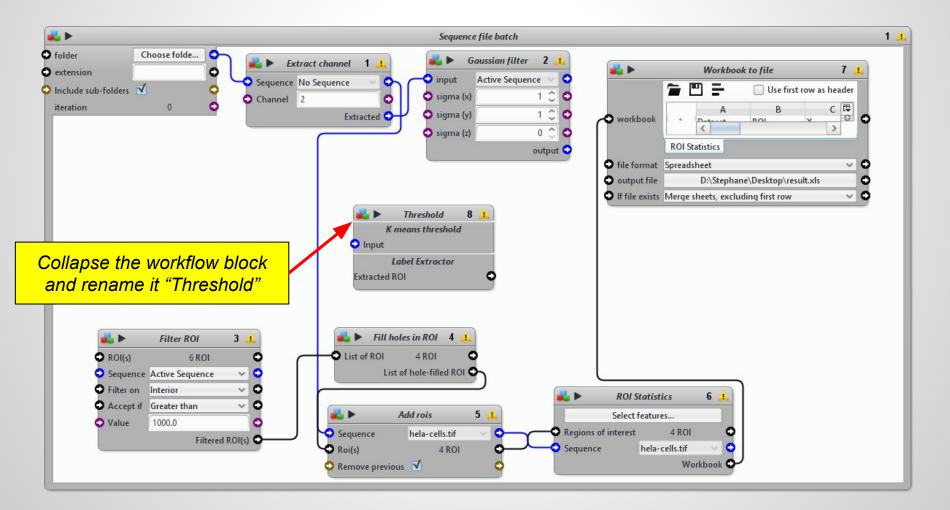


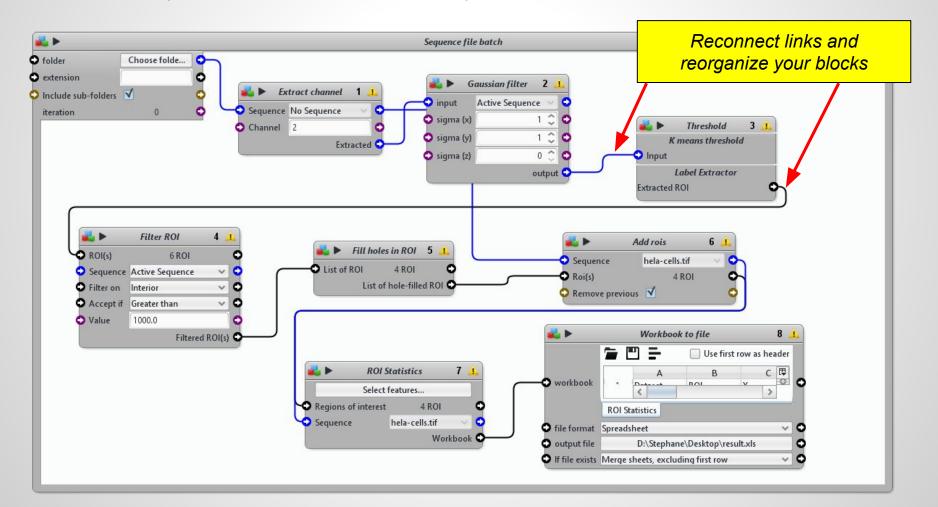


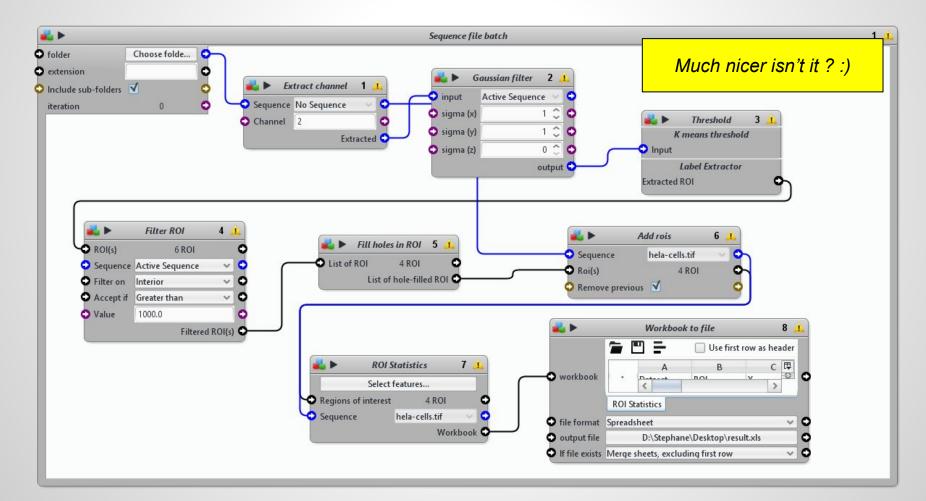




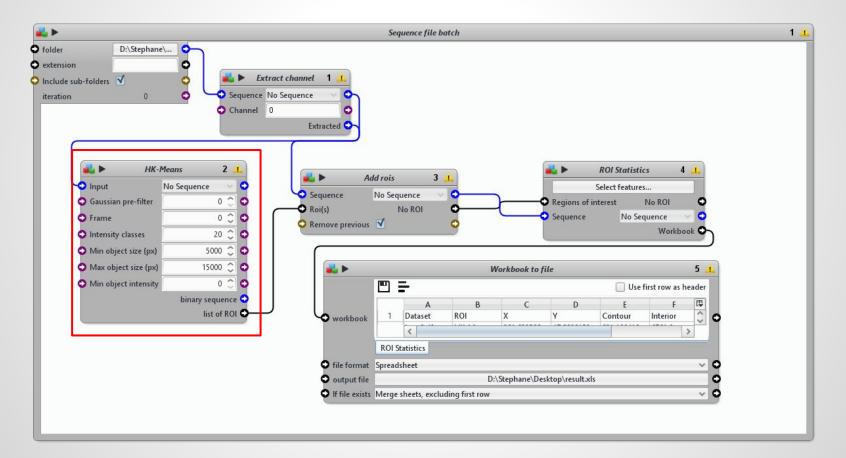








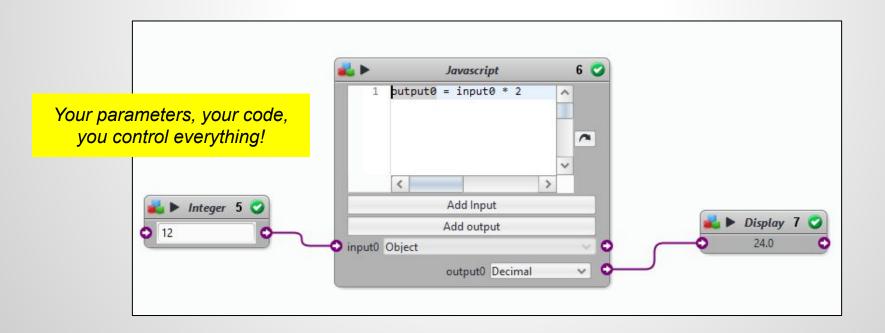
• Make your protocol nicer: you can also better tool ;)



- Most plugins have their corresponding Block
- What if the one you need isn't there (yet)?
  - #1: Leave a comment on the plug-in's page online

	This resource needs	
PLUGIN KMeans Color Quantization	MaskEditor	PLUGIN
Publication ID: ICY-1411F1	NHerve Toolbox	PLUGIN
SHORT DESCRIPTION		
DOCUMENTATION	One review on "KMeans Color Quantization"	
1 REVIEW		
DEPENDENCIES	Hi! very nice plugin, i was wondering if it was possible to call it in block editor?	RATED
(f) (d) (d) (d) (d)	Leave a comment & rating	EDIT
	LEAVE A REVIEW VIEW FULL CHANGELOG	

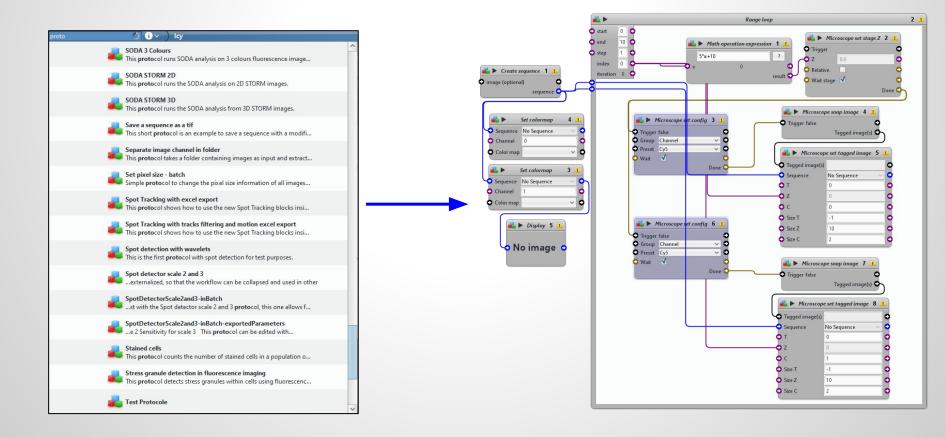
- Most plugins have their corresponding Block
- What if the one you need isn't there (yet)?
  - #1: Leave a comment on the plug-in's page online
  - #2: The "DIY" (Do It Yourself) approach



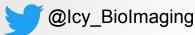
 Protocols can be used in *headless* mode (command line) ! See the plugin documentation to get more information about it: <u>http://icy.bioimageanalysis.org/plugin/protocols/</u>

And our protocol is ready. Here comes the magic spell:		
icy.sh -hl -x plugins.adufour.protocols.Protocols protocol=/my/great.protocol input1=/my /inputFile output1=/my/outputFile		
Let's briefly analyse this line:		
<ul> <li>icy.sh is the lcy startup command (NB: replace by icy.exe on windows; you can also</li> </ul>		
generically use "java -jar icy.jar" instead)		
<ul> <li>"-hl" is short for "headless", which means icy will run on the command line and no</li> </ul>		
graphical interface will appear		
• "-x" tells icy to run a specific plugin at startup (this plugin is "Protocols", as you might have		
guessed)		
<ul> <li>"protocol=" indicates the path to the protocol file to run</li> </ul>		
<ul> <li>"input1=" and "output1=" are used to pass all the necessary parameters to the protocol,</li> </ul>		
using the IDs specified above.		

A good way to learn: use online protocols and adapt them to your needs !



# Keep in touch !



### Support forum

http://icy.bioimageanalysis.org/support

# Image Analysis Hub Open Desk

Every other Thursday 9h30-12h30

Pasteur - François Jacob Building

https://research.pasteur.fr/en/news/ima ge-analysis-opendesk/

Don't forget to cite and acknowledge us :)



