Protocols

Graphical programming for Icy

a.k.a. programming, for the rest of us
Foreword: Reproducible Research

- Quote: "Results aren't much if they can’t be reproduced!"  
  (your boss, your reviewers, your colleagues, you!)
Foreword: Reproducible Research

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- Fact: Most journals now reject papers without proper quantification
  
  [...] Image quantification was carefully conducted using Photoshop. [...]
Foreword: Reproducible Research

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- Conclusion: Image quantification is a protocol in its own right...
Foreword: Reproducible Research

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- Fact: Most journals now reject papers without proper quantification
- Conclusion: Image quantification is a protocol in its own right...
- Icy makes these protocols easy to read / write / use / adapt
Foreword: Reproducible Research

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- Conclusion: Image quantification is a protocol in its own right...

- 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.
Protocols in Icy

- A protocol is a workflow linking processing blocks together

http://icy.bioimageanalysis.org/protocol/Extract_channels
Protocols in Icy

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

**Extra actions**
- Collapse
- Remember results
- Remove block

**Run protocol until this block (inclusive) and stop**

**Block execution priority** (starts at 0, click to prioritize)

**Name (can be modified)**

**Execution state**

"Drop zones" (link from other blocks)

"Drag zones" (link to other blocks)

**Input parameters (0 or more)**
- Input
- Gaussian pre-filter
- Frame
- Intensity classes
- Min object size (px)
- Max object size (px)
- Min object intensity

**Output (0 or more)**
- Active Sequence
- ALL
- list of ROI

**Arbitrary color code indicates parameter types**
Protocols in Icy

- The protocols editor: [http://icy.bioimageanalysis.org/plugin/Protocols](http://icy.bioimageanalysis.org/plugin/Protocols)
Protocols in Icy

- Blocks are organised by groups

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

- Question: how would you find the nuclei in this image?
Protocols in Icy

- 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…)*
Protocols in Icy

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

Outline:

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Menu: Sequence > Extract Channel

NOTE: channel index starts at 0...
Protocols in Icy

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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!
Protocols in Icy

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Menu: Blocks > KMeans Threshold

Finds the optimal separation(s) between the histogram modes (i.e. intensity classes)
2 modes => 1 threshold (3 => 2, etc.)
Protocols in Icy

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

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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.
Protocols in Icy

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Menu: Blocks > Label Extractor

Extracts objects from a labeled image using connected component analysis
Protocols in Icy

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Menu: ROI > ROI Statistics

Calculates size, dimensions, intensity statistics, etc.
Protocols in Icy

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

*don't forget me!*
Protocols in Icy

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if you want to see something...
Protocols in Icy

Improving the results - what we can do?
Protocols in Icy

Improving the results - filtering ROI
Protocols in Icy

Improving the results - filling holes
Protocols in Icy

Improving the results - filling holes
Protocols in Icy

Then save your statistics on disk!
Protocols in Icy

- How about batch processing?

*Embed your protocol inside a Sequence File Batch box*
Protocols in Icy

- How about batch processing?

Connect the first block

Change XLS file save strategy to "merge"
Protocols in Icy

- How about batch processing?

Select folder you want to process here (ex: “batch\hcs”)

Select the destination XLS file and click on “run”!
Protocols in Icy

- Make your protocol nicer: build your own block with “workflow”!

Copy / paste “K Means”, “Thresholder” and “Label Extractor” blocks inside the workflow block

Add a new workflow block
Protocols in Icy

- Make your protocol nicer: build your own block with "workflow"!

Remove previous block (select them and press “Delete”)

Expose “Input” variable of “K Means” block and “Extracted ROI” of “Label Extractor” block
Protocols in Icy

- Make your protocol nicer: build your own block with “workflow”!

Collapse the workflow block and rename it “Threshold”
Protocols in Icy

- Make your protocol nicer: build your own block with "workflow"!

Reconnect links and reorganize your blocks.
Protocols in Icy

- Make your protocol nicer: build your own block with "workflow"!

Much nicer isn't it? :)

Image of a protocol workflow in Icy software.
Protocols in Icy

- Make your protocol nicer: you can also better tool ;)
Protocols in Icy

- 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
Protocols in Icy

- 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

Your parameters, your code, you control everything!
Protocols in Icy

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

Support:

icy.bioimageanalysis.org/support
Go further...

Every other Thursday

Image Analysis
Open Desk

9h30-12h30
Pasteur - François Jacob Building