Installation

- 1. Install KNIME Analytics Platform (from thumb drive)
- 2. Help > Install New Software > Add (> Archive):
 - 00_InstallationFiles/CommunityContributions_trunk.zip
 - <u>https://update.knime.org/community-contributions/trunk</u>
- 3. Available Software Sites:
 - Enable KNIME AP 3.5 Update Site
 - Enable KNIME Community Contributions (trunk)
- 4. Select KNIME Community Contributions (trunk)
- 5. Install KNIME Image Processing and KNIME Image Processing - OMERO Integration





OMERO and KNIME Workshop

Stefan Helfrich, Christian Dietz KNIME



Learning goals

- How to get around KNIME Analytics Platform (AP)
- How to get your images from OMERO into KNIME AP
- How to extract quantitative data from your images
- How to use analytics and visualization functionality in KNIME AP



What is KNIME Analytics Platform?

- A tool for data analysis, manipulation, visualization, and reporting
- Based on the graphical programming paradigm
- Provides a diverse array of extensions:
 - Text Mining
 - Network Mining
 - Cheminformatics
 - Image Informatics
 - Many integrations, such as Java, R, Python, Weka, etc.



The KNIME® Analytics Platform





Over 2000 native and embedded nodes included:





The KNIME Workbench





Creating New Workflows, Importing and Exporting

- Right-click anywhere in KNIME Explorer to create a new workflow or workflow group or to import a workflow
- Right-click on workflow or workflow group to export the selected workflow



EXAMPLES (knime-guest@http://public				
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exercises	Export KNIME Workflow			
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Exercise 00 – Importing the Exercises

- File > Import KNIME Workflow > Browse
 - 01_KNIME-OMERO/OME-UGM-2018.knar



Visual KNIME Workflows



Nodes are combined to create **WORKFLOWS**



Random Forest



A node can have 3 states:



Not Configured:

The node is not yet configured and cannot be executed with its current settings.



File Reader

Configured:

The node has been set up correctly, and may be executed at any time



Executed:

┣+ ►

The node has been successfully executed. Results may be viewed and used in downstream nodes.



Inserting and Connecting Nodes

- Insert nodes into workspace by dragging them from the Node Repository or by double-clicking in the Node Repository
- Connect nodes by left-clicking output port of Node A and dragging the cursor to (matching) input port of Node B
- Common port types:





Node Configuration

- Most nodes require configuration
- To access a node configuration window:
 - Double-click the node

OR

• Right-click > Configure

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Node Execution

- Right-click node
- Select Execute in context menu
- If execution is successful, status shows green light
- If execution encounters errors, status shows red light





Node Outputs and Views

- Right-click executed node
- Select View option in context menu

OR

• Select output port (last item) to inspect execution results







New node: OMERO Connection

- Uses server settings (IP address, port) and credentials to establish a connection
- Doesn't download any images

	Job Man	luger serection	Memory Foncy
Server			
Hostname			
		— — — — — — —	ad Connection
port 4,064		Encrypt	ed Connection
Workflow credentials			
	Use Workflow	Credentials	
	credentials < N	IONE > 🗘	
Manual Credentials			
usern	ame		
passv	word		
	Test conne	ection	





Exercise 01 – Connecting to OMERO

- Create a connection to an OMERO instance:
 - IP: ...
 - User: ...
 - **PW**: ...
- Which port(s) do you see on the *OMERO Connection* node?



Exercise 01 - Solution

OMERO Connection

Node 1



New node: List Remote Files

- Creates file locations for a set of files (usually all files in a folder \rightarrow List Files)
- Doesn't load any images yet



List from: ome://c Directory /dataset/67 Include sub fc Filter: Extension(s) / E none regular exp	 Files on ome:// KNIME Demo Examples (Project; id:67) ome-ugm-data1 (Dataset; id:67) xyz-1 H3K9-Cy3 DAPI 1.Ism.ome (Image; id:67) xyz-1 H3K9-Cy3DAPI 2.Ism.ome (Image; id:68) xyz-1 H3K9-Cy3DAPI 3.Ism.ome (Image; id:69) xyz-2 H3K9-Cy DAPI 3.Ism.ome (Image; id:70) xyz-2 H3K9-Cy3DAPI 1.Ism.ome (Image; id:71) xyz-2 H3K9-Cy3DAPI 2.Ism.ome (Image; id:72) ome-ugm-data2 (Dataset; id:68) 	e
_	Select a directory OK Cancel	0



New node: Image Reader (Table) Remote

- Input table has to contain file locations
- Uses Bio-Formats to open images and store them into an Img column





Exercise 02 – Downloading Images

- Use the existing connection to select a dataset from our OMERO instance:
 - *ome-ugm-data1* or *ome-ugm-data2*
- What does the output table look like?
- Download images from the selected dataset and inspect the images



Exercise 02 - Solution





New Node: Joiner

- Combines columns from 2 different tables
- Top port contains "Left" data table
- Bottom port contains the "Right" data table



Joiner Settings	S Column Selection	Flow Variables	Job Manager Selection	Memory Policy
oin Mode	loin mo	da (_	
	Join mo	Inner Join		
vining Columns				
 Match all of the 	following 📀 Match ar	y of the following	3	
Top Inp	ut ('left' table)	Bottom	nput ('right' table)	
I CustomerKey		Custome	rKey ᅌ	+ -
erformance Tuning	Maximum number of	open files:		
erformance Tuning	Maximum number of	open files: 200		
erformance Tuning	Maximum number of	open files: 200		
Performance Tuning	Maximum number of	open files: 200		
Performance Tuning Row IDs	Maximum number of Enable hiliting	open files: 200		
Performance Tuning Row IDs	Maximum number of Enable hiliting Row ID se	open files: 200 parator in joined	table:	
erformance Tuning	Maximum number of Enable hiliting Row ID se	open files: 200 parator in joined	table:	
Performance Tuning	Maximum number of Enable hiliting Row ID se	open files: 200 parator in joined	table:	
Performance Tuning Row IDs	Maximum number of Enable hiliting Row ID se	open files: 200 parator in joined	table:	
Performance Tuning Row IDs	Maximum number of Enable hiliting Row ID se	open files: 200	table:	



Joining Columns of Data





Joining Columns of Data





Exercise 03 – Processing Images

- Segment the first channel of each image:
 - Smoothing (Gaussian Convolution), Global Thresholding (Yen), Fill Holes, and Connected Component Analysis
 - Replace the result from the previous step!
- Join the resulting Labeling with the input data to recover a raw version of the first channel
- Remove cells that are touching the border image
 Use Labeling Filter



Exercise 03 - Solution





New Node: Row Filter

- Row filtering with in- and exclude option according to certain criteria
 - Certain value or pattern in a selectable column

- Row number
- Row ID

	Filter Criteria Flow	Variables Job Manager Selection	Memory Policy	
		Row number range First row number 10 to the end of the table Last row number 10		
			•••	Dialog - 0:279 - Row Filter
	 Include rows by attribute value Exclude rows by attribute value 		Filter Criteria Flow	Variables Job Manager Selection Memory Policy
Row Filter → → → →	Include rows by number Exclude rows by number Include rows by row ID Exclude rows by row ID		 Include rows by attribute value Exclude rows by attribute value Include rows by number 	Column value matching Column to test: S CITY C Filter based on collection elements Matching criteria • use pattern matching constance case sensitive match contains wild cards regular expression
Delete superfluous rows		ОК Арріу	Exclude rows by number Include rows by row ID Exclude rows by row ID	use range checking lower bound: upper bound: only missing values match
			L	OK Apply Cancel ?

Dialog - 2:46 - Row Filter (Delete superfluous)



New Node: String Manipulation

- Create and edit values in string columns
 - Clean up capitalization (e.g. Lowercase)
 - Search and Replace, join
- Modify existing strings or create new columns





Exercise 04 – Visualizing Results

- Extract mean intensity from 2nd channel for each cell in an image (*Image Segment Features*)
- Extract the cell area (NumPix) for each cell in an image (Segment Features)
- *Join* the information for each cell
- Filter cells according to their size (*Row Filter*)
- Extract original file name for each cell (*Labeling Properties*)



Exercise 04 – Visualizing Results (continued)

• Extract class (xyz-1 or xyz-2) from Name column

- *String Manipulation* to extract first 5 characters

- Compute a p-value for the (independent) mean intensities distributions (*Independent groups t-test*)
- Generate a box plot of the NumPix properties (per group):
 - Use the Conditional Box Plot (JavaScript)



Exercise 04 - Solution





- Learning/LEARNING HUB: <u>https://www.knime.com/learning-hub</u>
- Learning/NODE GUIDE: <u>https://www.knime.com/nodeguide</u>
- Community/Forum: <u>https://forum.knime.com/</u>

KNIME TV on YouTube https://www.youtube.com/user/KNIMETV



KNIME Image Processing and the ImageJ Ecosystem





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