KymoFlow is a macro written in ImageJ that takes a kymograph and returns a kymograph where the local pixel intensity is equal to the slope of a high contrast line in units of pixel/frame.

To install:

1. Place the following macros directly in the macro folder in ImageJ.

KymoFlow.ijm

LKMTA core.ijm

LKMTA Fourwayrotion.ijm

2. Place the following plugins in the ImageJ plugin folder and use the Install function in the plugin drop down menu to create the class files. Afterwards these will showup in the plugins menu.

Z Project Ignore NaNs.java

Z Project StDev Ignore NaNs

NaN to Zero.java

In addition, the TransformJ plugin may need to be installed by ImageJ users by manually downloading the following two jar-files to the plugins folder.

> TransformJ\_.jar (version 4.0.0)

> imagescience.jar (version 3.0.0)

These can currently be found at <https://imagescience.org/meijering/software/transformj/>

After the files have been installed, take an image of a kymograph where traces have been drawn over the motion of interest. Black lines with a width of 3 pixels drawn on a white background work well. Make the image the active window and then run the KymoFlow macro. For an input image of roughly 300 x 300 pixels, output will be produced in a couple of minutes. The memory used by ImageJ may need to be increased by using the Edit>Options>Memory and Threads command.

A test file with an angle of -33º (i.e. ~1.53 pixels/frame) is included as well as the output. Note the output is in a 32-bit format and is most easily viewed using ImageJ.