Functional AStrocyte Phenotyping (FASP)

Introduction

Functional AStrocyte Phenotyping (FASP) is a Fiji plugin that automatically analyzes and characterizes the spatiotemporal functional status of astrocytes from time-lapse Ca^{2+}-fluorescence microscopy imaging data. Being totally unsupervised, it automatically detects astrocyte functionally independent units (FIUs), extracts functional features of them, and further characterizes the functional status.

FASP explicitly models and well handles the intracellular propagation phenomena of astrocytic Ca^{2+} fluctuations, the major reason why it's difficult to repurpose the existing methods of neuron spatiotemporal analysis for astrocytes. Besides, considering the complex nature of Ca^{2+} signaling and low signal to noise ratio, FASP is designed to be data-driven and probabilistically principled, to flexibly account for complex patterns and perform robustly with noisy data. Parameter tuning is purposely designed to be very easy: the only two parameters for users to tune have either probabilistic meaning or physical (scale) meanings.

How to cite FASP

The FASP Fiji plugin is based on algorithm introduced in our following paper which is submitted and under review:

Yinxue Wang, Guilai Shi, David J. Miller, Yizhi Wang, Congchao Wang, Gerard Broussard, Yue Wang, Lin Tian, and Guoqiang Yu. "Automated functional analysis of astrocytes from chronic time-lapse calcium imaging data." Submitted.

Tutorial

Installation

Download file "FASP_.jar" from https://github.com/VTcbil/FASP. To install the FASP plugin, simply save or copy it into the folder "\Fiji.app\plugins\", then call *"Help ▶ Refresh Menus"* or restart Fiji/ImageJ. The FASP plugin will become available in Fiji/ImageJ's *"Plugins"* menu.
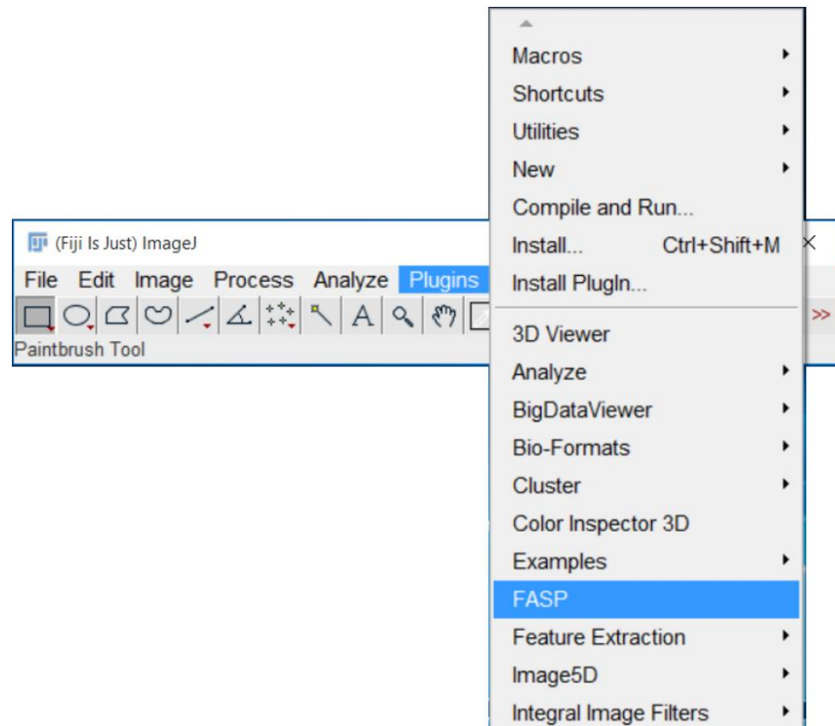
The plugin has been tested and successfully run on ImageJ version ≥ 1.49q. If users encounter problems using older versions of ImageJ, please update your ImageJ to a newer version ≥ 1.49q.
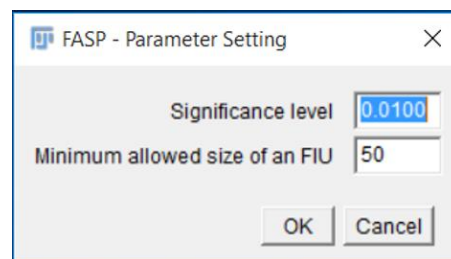
Input

FASP is designed for single-channel gray-scale time lapse image stack. So far we only support 8-bit or 16-bit images of any file format supported by Fiji/ImageJ. Open the image stack of interest in Fiji/ImageJ.
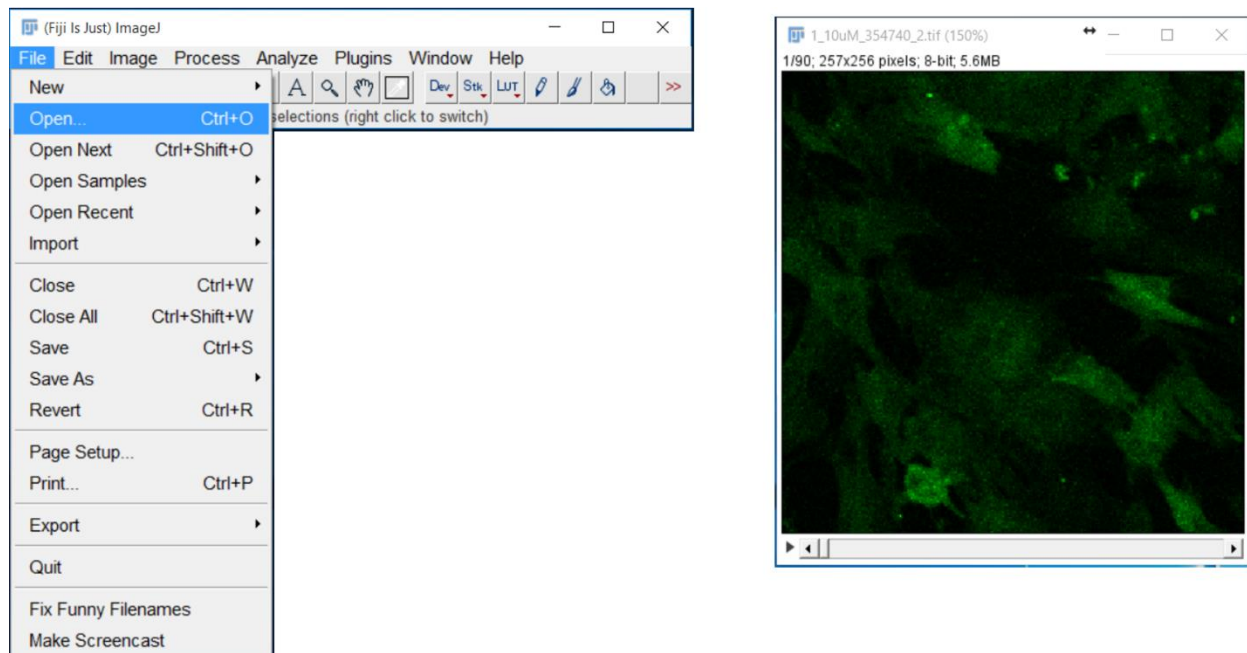
(Note: Fiji/ImageJ typically provides users with "Lookup Tables" under "Image" menu that can be applied to single-channel grayscale images or image stacks to produce false-color images. Some colors may make manual proofreading easier, such as green color. FASP can be applied to single-channel grayscale image stacks of any false color, and the results will be just shown as overlay on the input false-color image stack.)

Parameter setting

With the input image stack opened, run FASP from the plugins menu. A dialogue window will occur, allowing users to adjust the input parameters. Suggested values of parameters are given as default in the textboxes. After the parameter setting is done, click "OK".


Processing procedure

Since FASP is a totally unsupervised algorithm, NO interactive user input is needed while FASP is running. The progress of the program will be shown using a progress bar in the bottom right corner of Fiji/ImageJ toolbar. Besides computing platform, the running time of FASP also depends on the activity level of the imaged cells, the spatiotemporal scales of input image stack, and the minimum allowed size of FIUs.
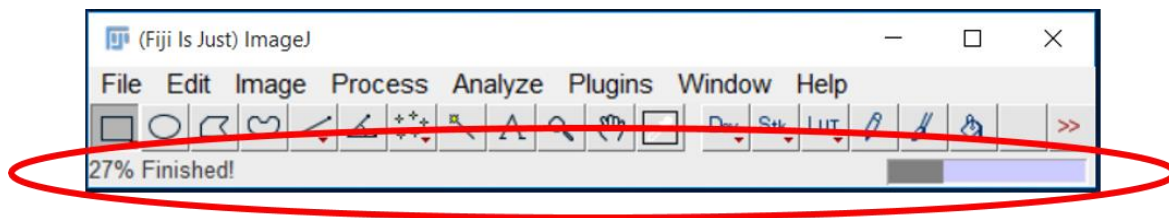
Outputs
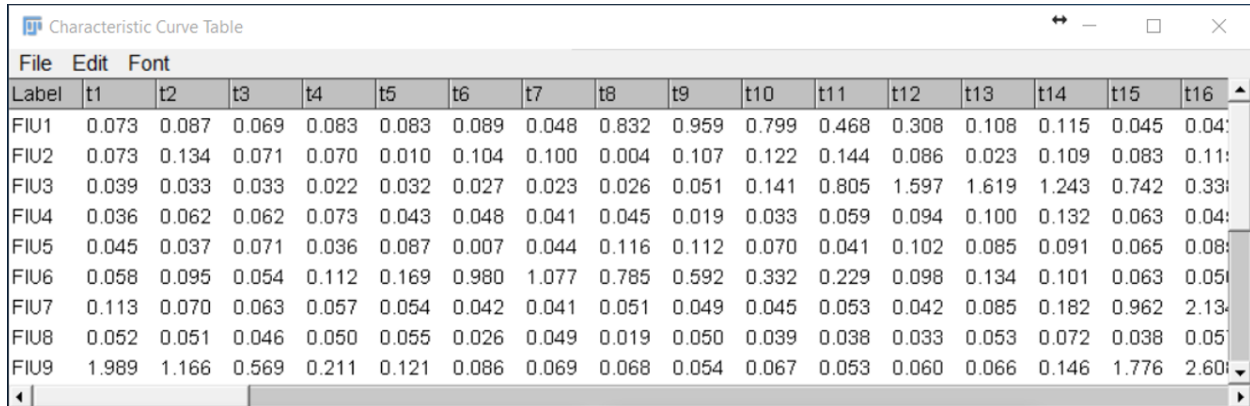
The results are given using tables and the ROI manager of Fiji/ImageJ. All FIUs are circled and labeled on the original image stack. The edges of FIUs are indicated as yellow, and a label is put on each FIU to show the ID of this FIU. If the yellow edges disappear due to some reason/operation, users may reset the edge overlay by checking the checkbox "show all" on the bottom right.
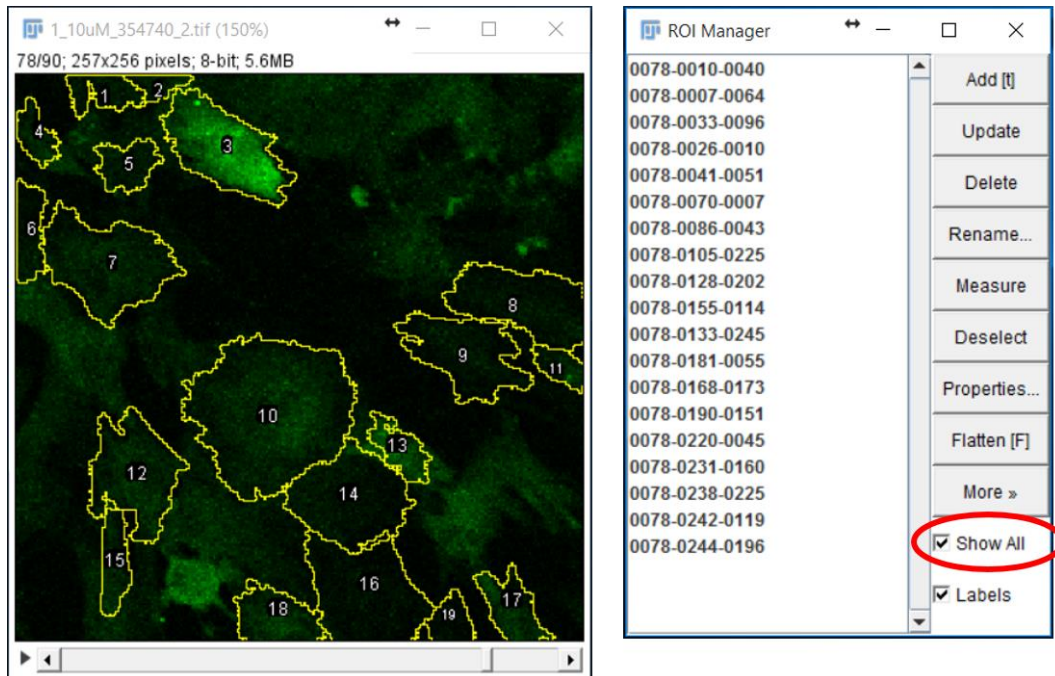
If the user is particularly interested in one FIU, she/he can click on the label of the FIU of interest to inspect its characteristic curve (in $\Delta F/F_0$). The selected FIU will be highlighted with blue edges on the image stack.
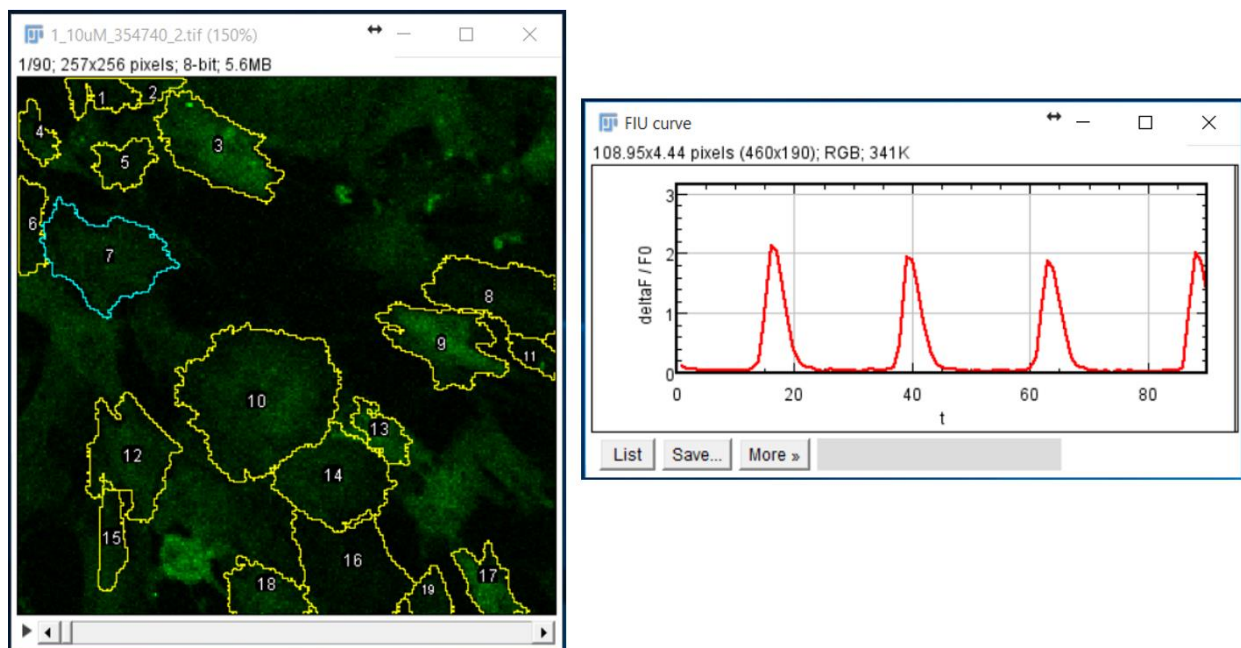
Besides, to make it easier for users to save the results, a table is also given containing the characteristic curves (in $\Delta F/F_0$) of all FIUs.

Characteristic Curve Table

File Edit Font

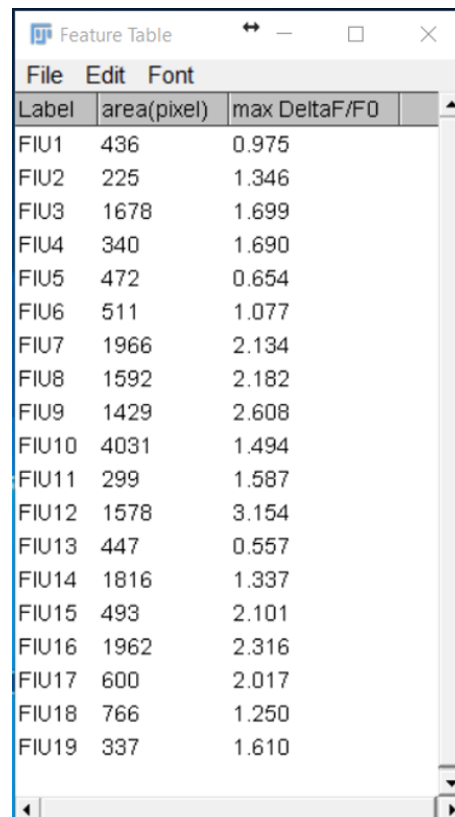| Label	t1	t2	t3	t4	t5	t6	t7	t8	t9	t10	t11	t12	t13	t14	t15	t16
FIU1	0.073	0.087	0.069	0.083	0.083	0.089	0.048	0.832	0.959	0.799	0.468	0.308	0.108	0.115	0.045	0.04?
FIU2	0.073	0.134	0.071	0.070	0.010	0.104	0.100	0.004	0.107	0.122	0.144	0.086	0.023	0.109	0.083	0.11?
FIU3	0.039	0.033	0.033	0.022	0.032	0.027	0.023	0.026	0.051	0.141	0.805	1.597	1.619	1.243	0.742	0.33?
FIU4	0.036	0.062	0.062	0.073	0.043	0.048	0.041	0.045	0.019	0.033	0.059	0.094	0.100	0.132	0.063	0.04?
FIU5	0.045	0.037	0.071	0.036	0.087	0.007	0.044	0.116	0.112	0.070	0.041	0.102	0.085	0.091	0.065	0.08?
FIU6	0.058	0.095	0.054	0.112	0.169	0.980	1.077	0.785	0.592	0.332	0.229	0.098	0.134	0.101	0.063	0.05?
FIU7	0.113	0.070	0.063	0.057	0.054	0.042	0.041	0.051	0.049	0.045	0.053	0.042	0.085	0.182	0.962	2.13?
FIU8	0.052	0.051	0.046	0.050	0.055	0.026	0.049	0.019	0.050	0.039	0.038	0.033	0.053	0.072	0.038	0.05?
FIU9	1.989	1.166	0.569	0.211	0.121	0.086	0.069	0.068	0.054	0.067	0.053	0.060	0.066	0.146	1.776	2.60?

The basic information of each FIU is listed in a table, including its area (in pixel) and the magnitude (max $\Delta F/F_0$) of this characteristic curve.

Feature Table

File Edit Font

Label	area(pixel)	max DeltaF/F0
FIU1	436	0.975
FIU2	225	1.346
FIU3	1678	1.699
FIU4	340	1.690
FIU5	472	0.654
FIU6	511	1.077
FIU7	1966	2.134
FIU8	1592	2.182
FIU9	1429	2.608
FIU10	4031	1.494
FIU11	299	1.587
FIU12	1578	3.154
FIU13	447	0.557
FIU14	1816	1.337
FIU15	493	2.101
FIU16	1962	2.316
FIU17	600	2.017
FIU18	766	1.250
FIU19	337	1.610

And some overall assessment of the sample is also provided in a summary table.

Number of FIUs	Total area(pixel) of FIUs	Average area of FIUs	Std of areas	Average of max DeltaF/F0	Std of max DeltaF/F0
19	20978	1104.105	3720.430	1.673	2.611

To save the results, the user can use the export/save function of Fiji/ImageJ for ROI manager, images and tables.
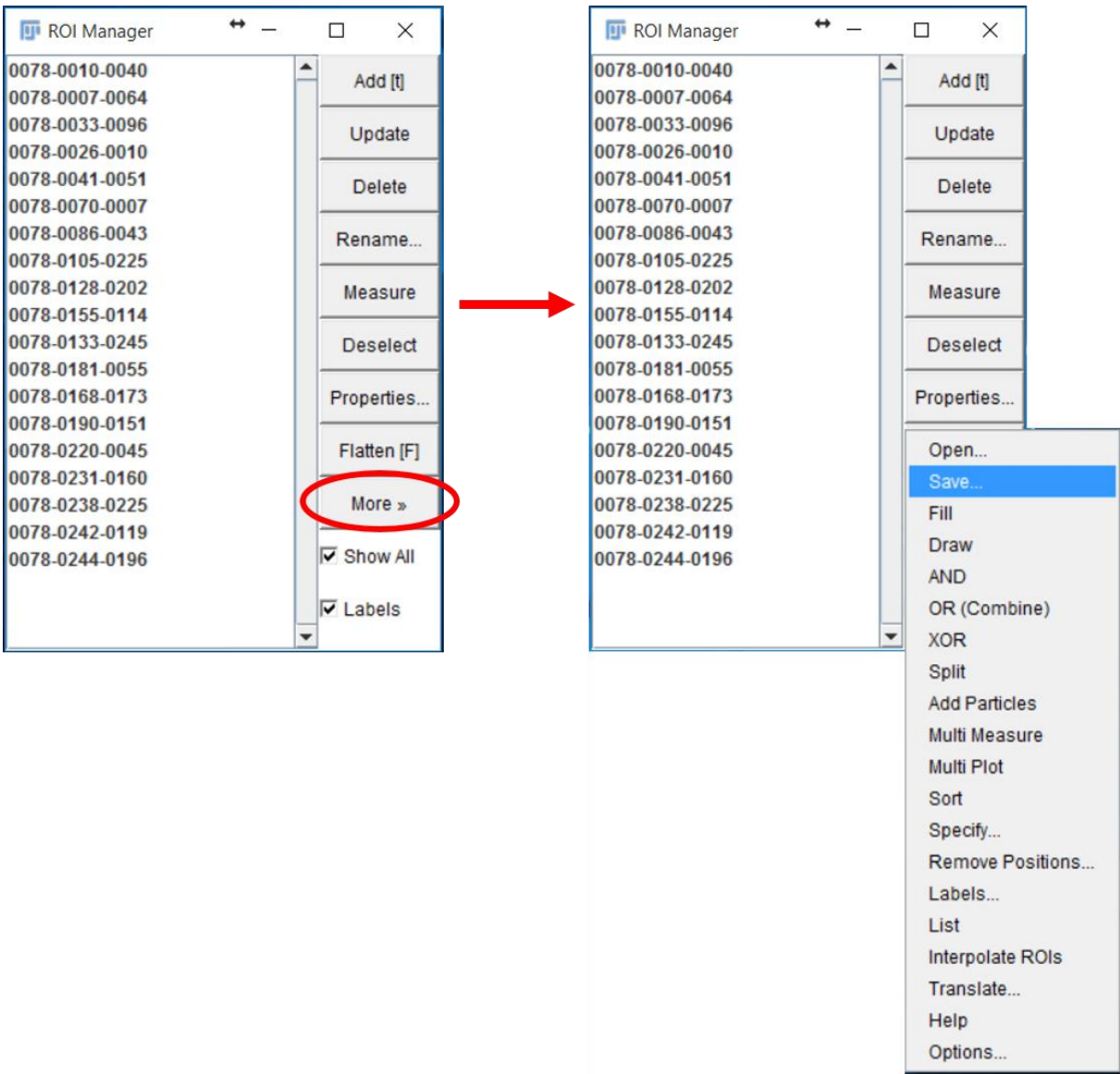
Characteristic Curve Table

File	Edit	Font

			t3	t4	t5	t6	t7	t8	t9	t10	t11	t12	t13	t14	t15	t16
Save As...	**Ctrl+S**		0.069	0.083	0.083	0.089	0.048	0.832	0.959	0.799	0.468	0.308	0.108	0.115	0.045	0.04:
Rename...			0.071	0.070	0.010	0.104	0.100	0.004	0.107	0.122	0.144	0.086	0.023	0.109	0.083	0.11:
Duplicate...			0.033	0.022	0.032	0.027	0.023	0.026	0.051	0.141	0.805	1.597	1.619	1.243	0.742	0.33i
FIU4	0.036	0.062	0.062	0.073	0.043	0.048	0.041	0.045	0.019	0.033	0.059	0.094	0.100	0.132	0.063	0.04:
FIU5	0.045	0.037	0.071	0.036	0.087	0.007	0.044	0.116	0.112	0.070	0.041	0.102	0.085	0.091	0.065	0.08:
FIU6	0.058	0.095	0.054	0.112	0.169	0.980	1.077	0.785	0.592	0.332	0.229	0.098	0.134	0.101	0.063	0.05(
FIU7	0.113	0.070	0.063	0.057	0.054	0.042	0.041	0.051	0.049	0.045	0.053	0.042	0.085	0.182	0.962	2.13₄
FIU8	0.052	0.051	0.046	0.050	0.055	0.026	0.049	0.019	0.050	0.039	0.038	0.033	0.053	0.072	0.038	0.05'
FIU9	1.989	1.166	0.569	0.211	0.121	0.086	0.069	0.068	0.054	0.067	0.053	0.060	0.066	0.146	1.776	2.60i