整理：
已有LSFM图像处理方案

畅星兆

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参考文献

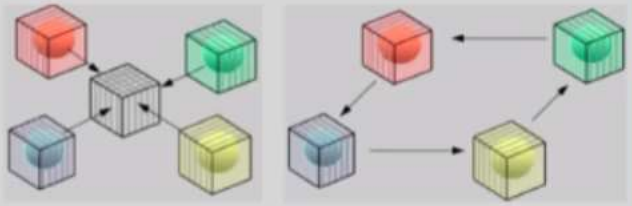
1. Whole-animal functional and developmental imaging with isotropic spatial resolution, 2015.
2. Inverted selective plane illumination microscopy (iSPIM) enables coupled cell identity lineaging and neurodevelopmental imaging in Caenorhabditis elegans, 2011.
3. 3D high- and super-resolution imaging using single-objective SPIM, 2014.
4. Reconstruction of Zebrafish Early Embryonic Development by Scanned Light Sheet Microscopy,2008.
5. OpenSPIM: an open-access light-sheet microscopy platform, 2013.
6. πSPIM: high NA high resolution isotropic light-sheet imaging in cell culture dishes, 2016.

方案1：IsoView 1. 图像配准（image registration）

Intensity based

- No embedding necessary
- Sample independent

- Typically slow
- Hard to cope with developing samples
- Result hard to verify automatically

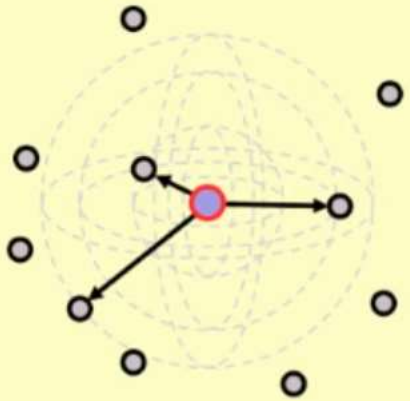


P. Shaw et al., in *Biophysical Journal* ***55***, 1989.
C. J. Cogswell et al., in *Proceedings of SPIE*, 1996.
R. Heintzmann et al., in *Analytical Cellular Pathology* ***20***, 2000.
R. Heintzmann et al., in *Journal of Microscopy* ***206***, 2002.
J. Huisken et al., in *Science* ***305***, 2004.
J. Swoger et al., in *Optics Express* ***15***, 2007.
P. Verveer et al., in *Nature Methods* ***4***, 2007.
W. Wein et al., in *proceedings of MICCAI*, 2007.
S. Preibisch et al., in *IEEE ISBI*, 2008.

Bead based

- Very fast
- Sample independent
- Easy use with developing samples
- Automatic verification

- Embedding in rigid medium

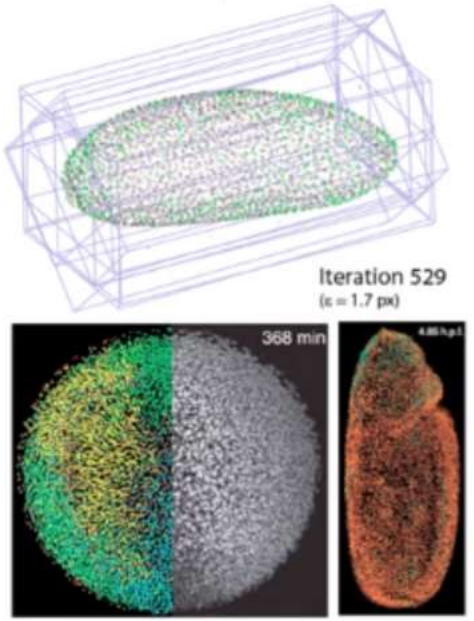


S. Preibisch, S. Saalfeld T. Rohlfing, P. Tomancak, in *SPIE Medical Imaging 2009.*
S. Preibisch, S. Saalfeld, J. Schindelin, P. Tomancak,

Segmentation based

- Potentially fast
- Automatic verification possible
- No embedding necessary

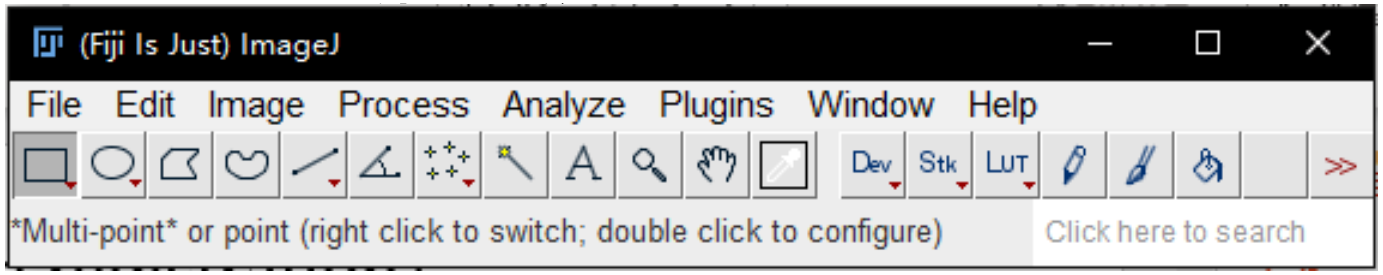
- Staining dependent
- Hard to cope with developing samples

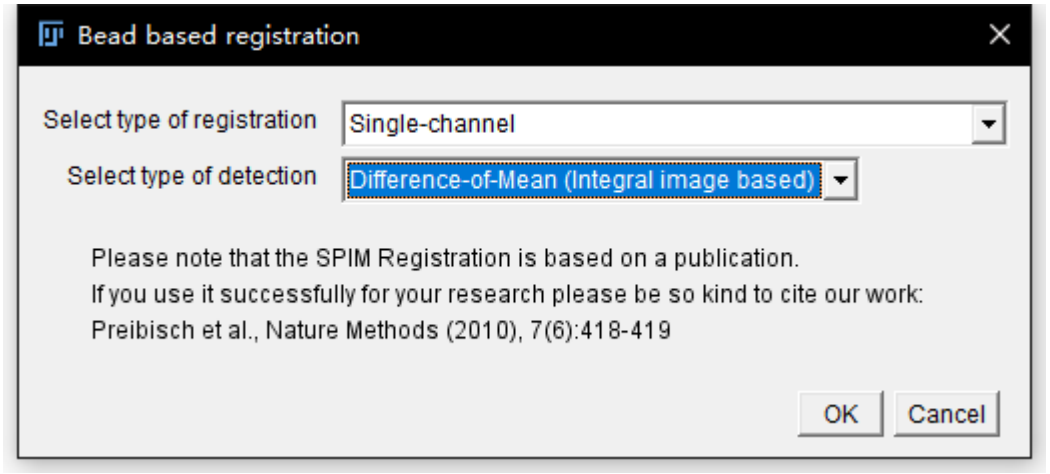


S. Preibisch, in *PhD Thesis*, 2010.
P. Keller et al., in *Science*, 2008.

系统采用Bead-based方法。

IsoView

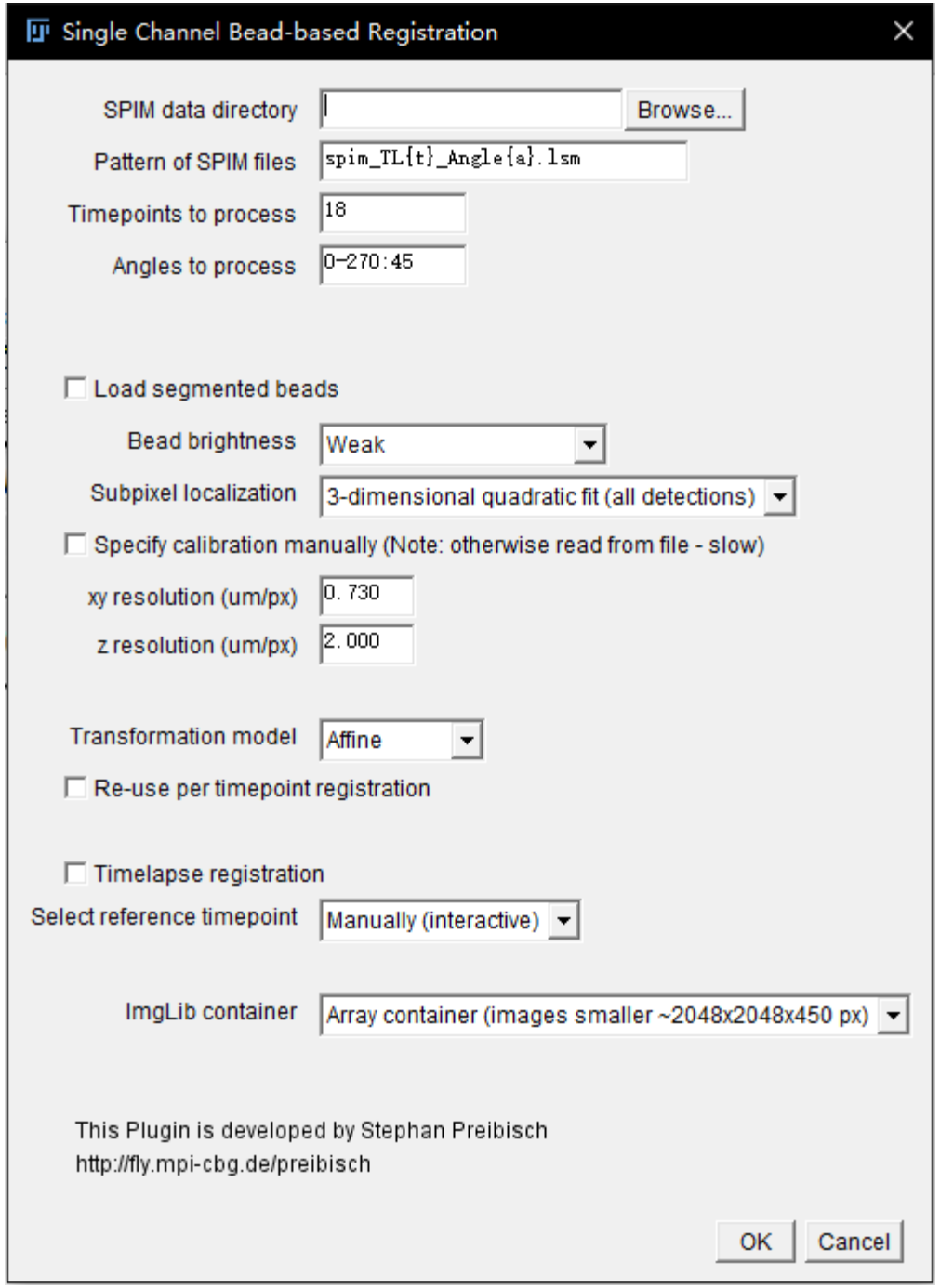




Fiji 用户界面

软件实现：Fiji bead-based registration plug-in

Fiji(ImageJ): 基于java公共图像处理软件，开放结构，支持用户自定义插件和宏。

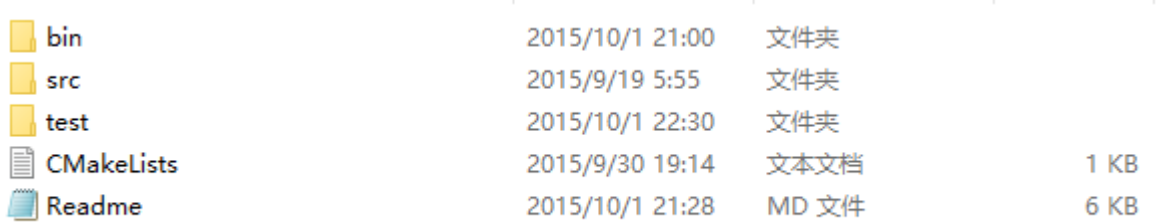

Single Channel Bead-based Registration
SPIM data directory
Browse...
Pattern of SPIM files
spim_TL{t}_Angle{a}.lsm
Timepoints to process
18
Angles to process
0-270:45
Load segmented beads
Bead brightness
Weak
Subpixel localization
3-dimensional quadratic fit (all detections)
Specify calibration manually (Note: otherwise read from file - slow)
xy resolution (um/px)
0.730
z resolution (um/px)
2.000
Transformation model
Affine
Re-use per timepoint registration
Timelapse registration
Select reference timepoint
Manually (interactive)
ImgLib container
Array container (images smaller ~2048x2048x450 px)
This Plugin is developed by Stephan Preibisch
http://fly.mpi-cbg.de/preibisch
OK
Cancel

参数界面

IsoView 1. 多角度图去卷积（muliview deconvolution）

算法：Lucy-Richardson three-dimensional miltiview deconcolution algorithm
代码基于CUDA架构。CUDA是显卡厂商NVIDIA推出的运算平台，使用C、C++语言架构编写程序，并使GPU能够解决复杂的计算问题。所编写出的程序可以在支持CUDA的处理器上以超高性能速度运行。

软件组成



-"bin": Windows 7 64-bit executables for running the code.
-"src": All source code files.
-"test": A cropped IsoView test data set (anterior region of a Drosophila first instar larva expressing GCaMP6s panneuronally), consisting of four registered views with corresponding PSFs.

验证该的所需条件

1. 确认电脑的配置可以搭载CUDA软件
2. 下载CUDA软件架构
3. 准备相应XML文件

XML语言，通用标记语言。在此方法中，使用XML文件将相应参数和原始图像路径传递给算法文件。所包含的参数有：背景噪声值、

3. 运行.exe程序

注：源代码中提供了相应的原始图像，以及XML文件。

```
<?xml version="1.0" encoding="UTF-8"?>
- <document>
    <view A="1.000000000000 0.000000000000 0.000000000000 0.000000000000 0.000000000000 1.000000000000 0.000000000000
      0.000000000000 0.000000000000 0.000000000000 1.000000000000 0.000000000000 0.000000000000 0.000000000000 0.000000000000
      1.000000000000" psfFilename="D:\Supplementary_Software\test\psfReg_1.klb"
      imgFilename="D:\Supplementary_Software\test\imReg_1.klb"/>
    <view A="1.000000000000 0.000000000000 0.000000000000 0.000000000000 0.000000000000 1.000000000000 0.000000000000
      0.000000000000 0.000000000000 0.000000000000 1.000000000000 0.000000000000 0.000000000000 0.000000000000 0.000000000000
      1.000000000000" psfFilename="D:\Supplementary_Software\test\psfReg_2.klb"
      imgFilename="D:\Supplementary_Software\test\imReg_2.klb"/>
    <view A="1.000000000000 0.000000000000 0.000000000000 0.000000000000 0.000000000000 1.000000000000 0.000000000000
      0.000000000000 0.000000000000 0.000000000000 1.000000000000 0.000000000000 0.000000000000 0.000000000000 0.000000000000
      1.000000000000" psfFilename="D:\Supplementary_Software\test\psfReg_3.klb"
      imgFilename="D:\Supplementary_Software\test\imReg_3.klb"/>
    <view A="1.000000000000 0.000000000000 0.000000000000 0.000000000000 0.000000000000 1.000000000000 0.000000000000
      0.000000000000 0.000000000000 0.000000000000 1.000000000000 0.000000000000 0.000000000000 0.000000000000 0.000000000000
      1.000000000000" psfFilename="D:\Supplementary_Software\test\psfReg_4.klb"
      imgFilename="D:\Supplementary_Software\test\imReg_4.klb"/>
    <deconvolution verbose="1" numIter="20" lambdaTV="0.000000" imBackground="100.000000" blockZsize="-1"/>
  </document>
```

XML代码

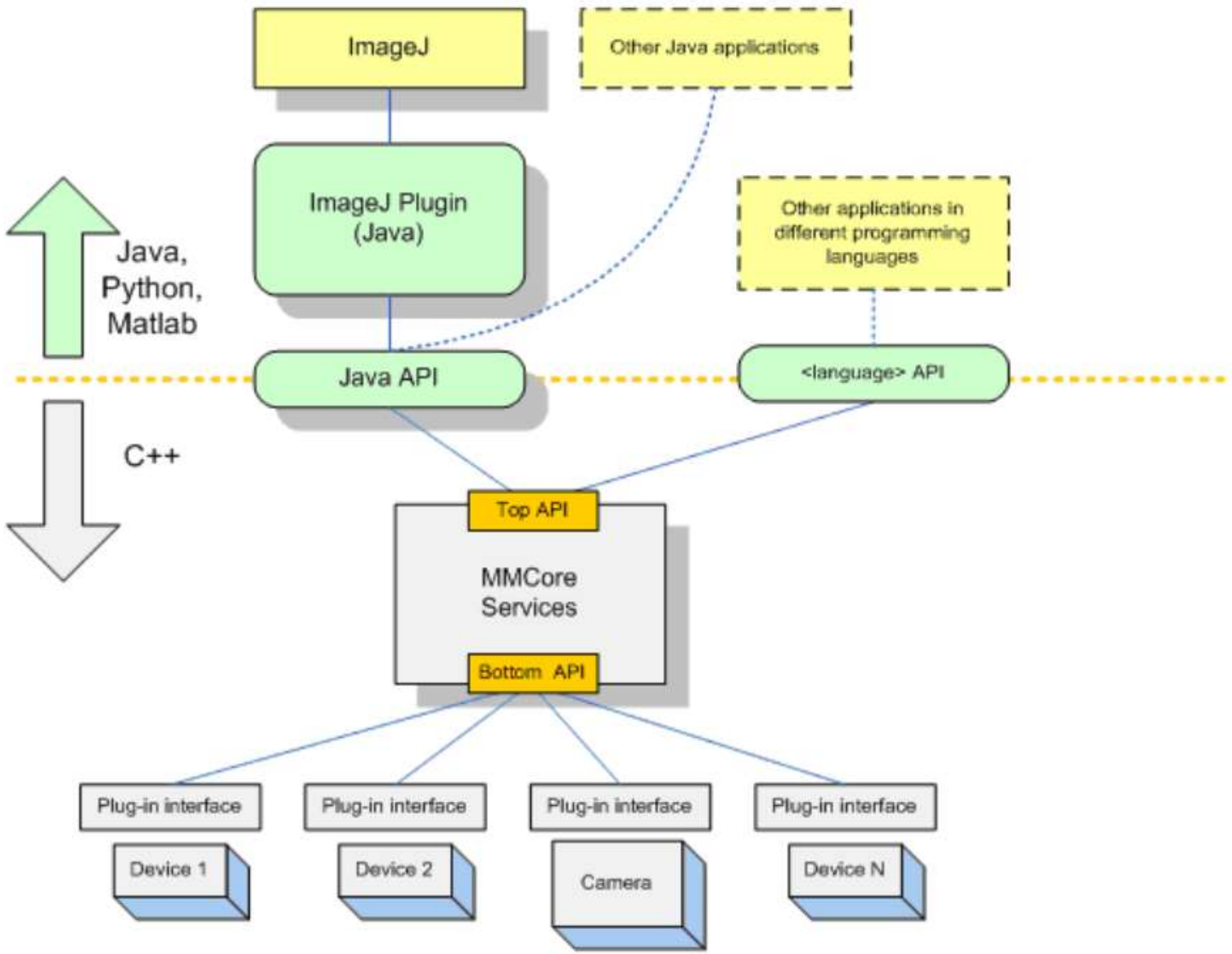
Fiji插件局限性：无法实现实时图像采集以及图像处理（real-time），需要其他插件的辅助。方法：

1. IJ_webcam_plugin
2. Micro-Manager

ImageJ插件：Micro-Manager

μManager is software for control of microscopes. It works with almost all microscopes, cameras and peripherals on the market, and provides an easy to use interface that lets you run your microscopy-based experiments.
the current hardware list: https://micro-manager.org/wiki/Device_Support

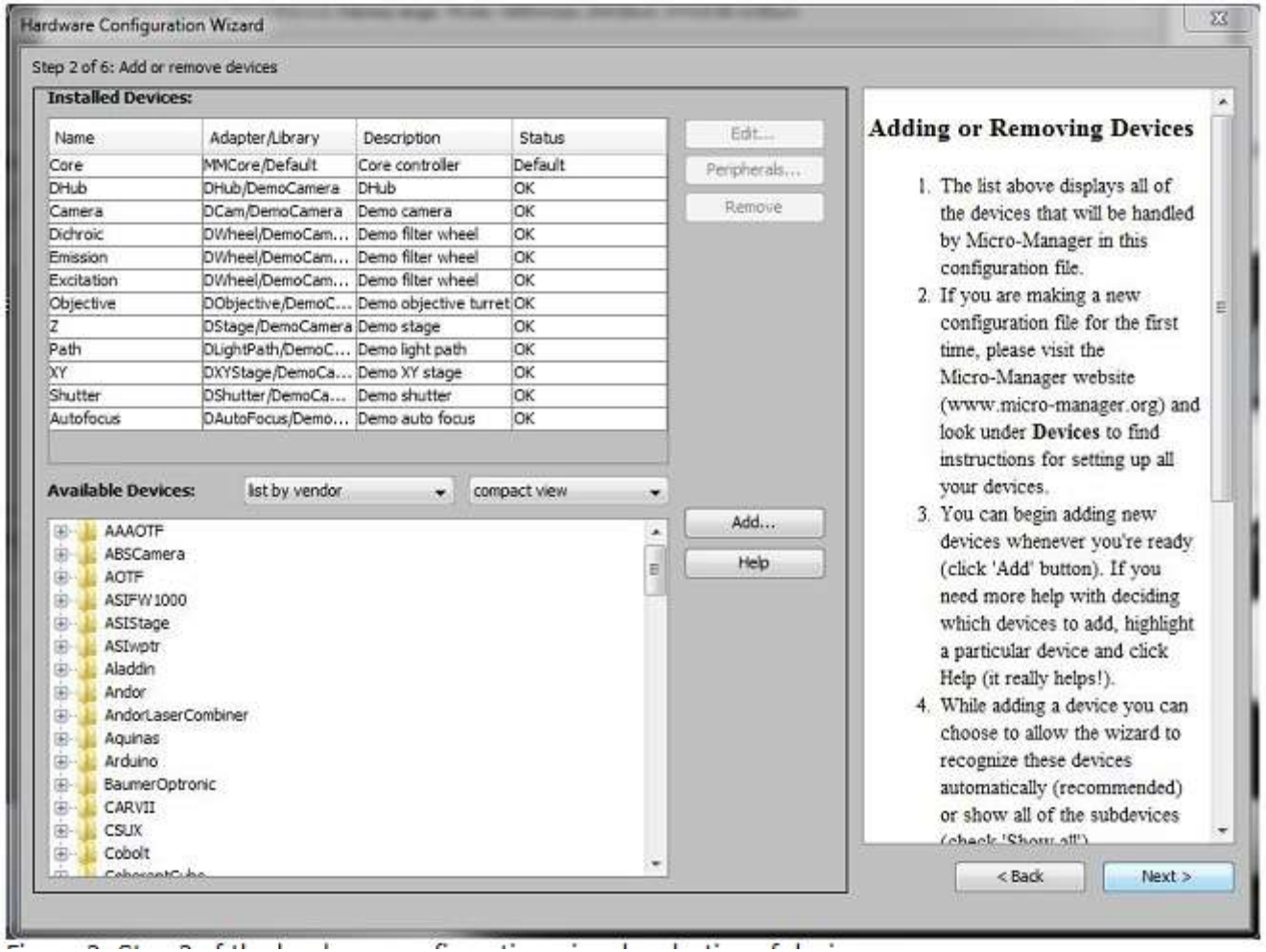
ImageJ插件：Micro-Manager



Programmatic interfaces to 3rd-party analysis environments (such as Matlab) to enable "intelligent data collection" -- i.e. analysis driven acquisition.

右图为软件结构框图

https://micro-manager.org/



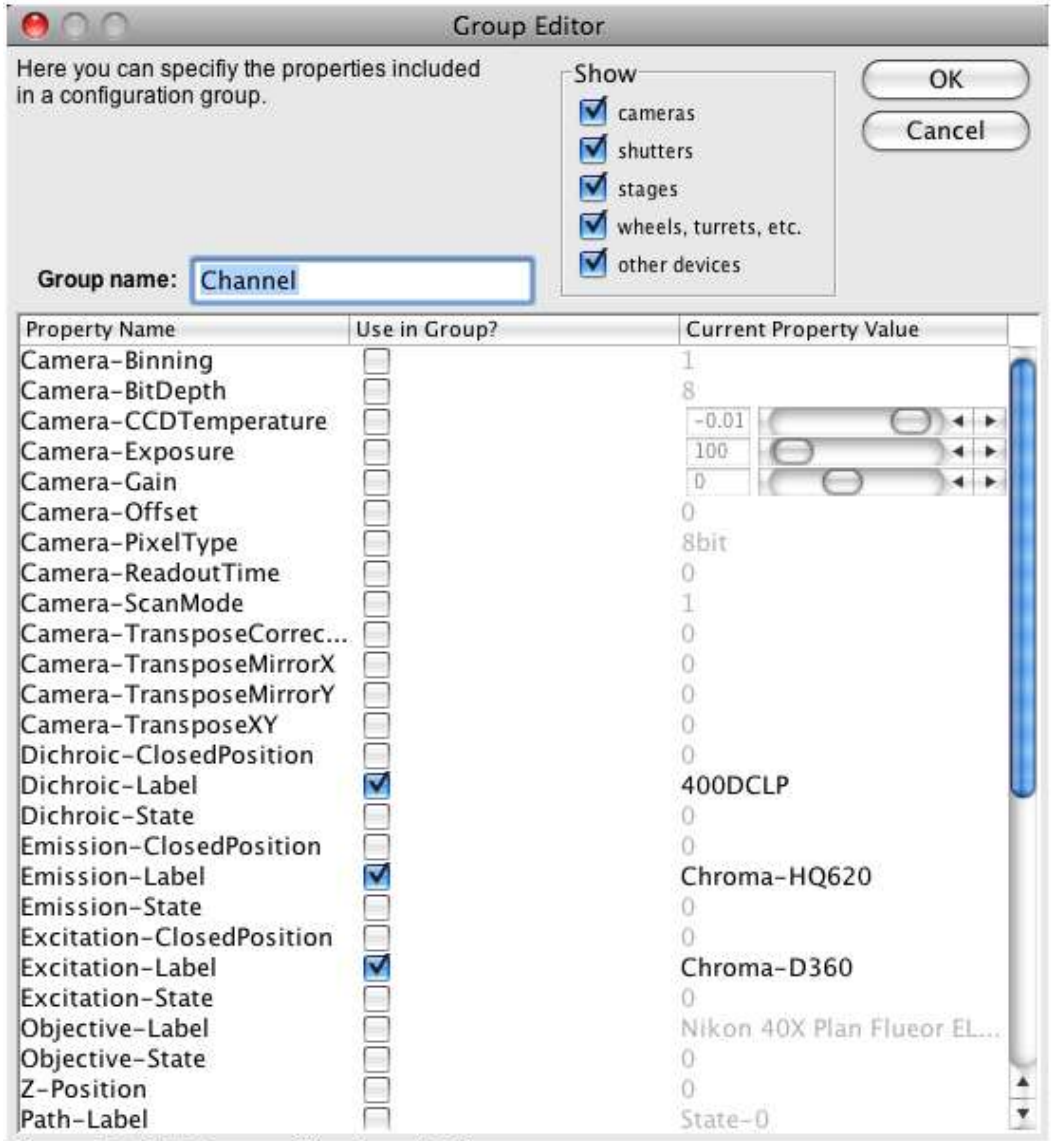


Figure 4. Initial Screen of the Preset Editor

用户界面

方案2：inverted SPIM (iSPIM)

图像处理在Matlab以及ImageJ中实现，包括：

matlab: 人工图像剪切（cropping）、最大强度投影（max-intensity projection）

Deconvolution Lab插件：Richardson–Lucy deconvolution algorithm

DeconvolutionLab——standalone application

完全开源，可以被常见的图像处理平台（ImageJ, Fiji, ICY, Matlab）所调用, 但却独立于调用软件。

参考文献：DeconvolutionLab2: An open-source software for deconvolution microscopy 叙述了算法实现细节、调参等

源代码：https://github.com/Biomedical-Imaging-Group/DeconvolutionLab2

ImageJ、Fiji调用

Put the file DeconvolutionLab_2.jar in the **plugins** folder and restart ImageJ or Fiji. Check the menu Plugins » DeconvolutionLab2.

1. » DeconvolutionLab2 Lab: Start the complete user interface of DeconvolutionLab2
2. » DeconvolutionLab2 Run: Run headless a deconvolution command given as a macro
3. » DeconvolutionLab2 Launch: launch the GUI for a deconvolution command provided as a macro

Example of ImageJ macro

```
image = " -image synthetic Cube 10.0 1.0 size 200 100 100"
psf = " -psf synthetic Double-Helix 3.0 30.0 10.0 size 200 100 100"
algorithm = " -algorithm RIF 0.1000 -out mip MI1 -path home"
run("DeconvolutionLab2 Launch", image + psf + algorithm + parameters)
```

Matlab调用

Put the DeconvolutionLab_2 into the java folder of Matlab

Example of Matlab commands

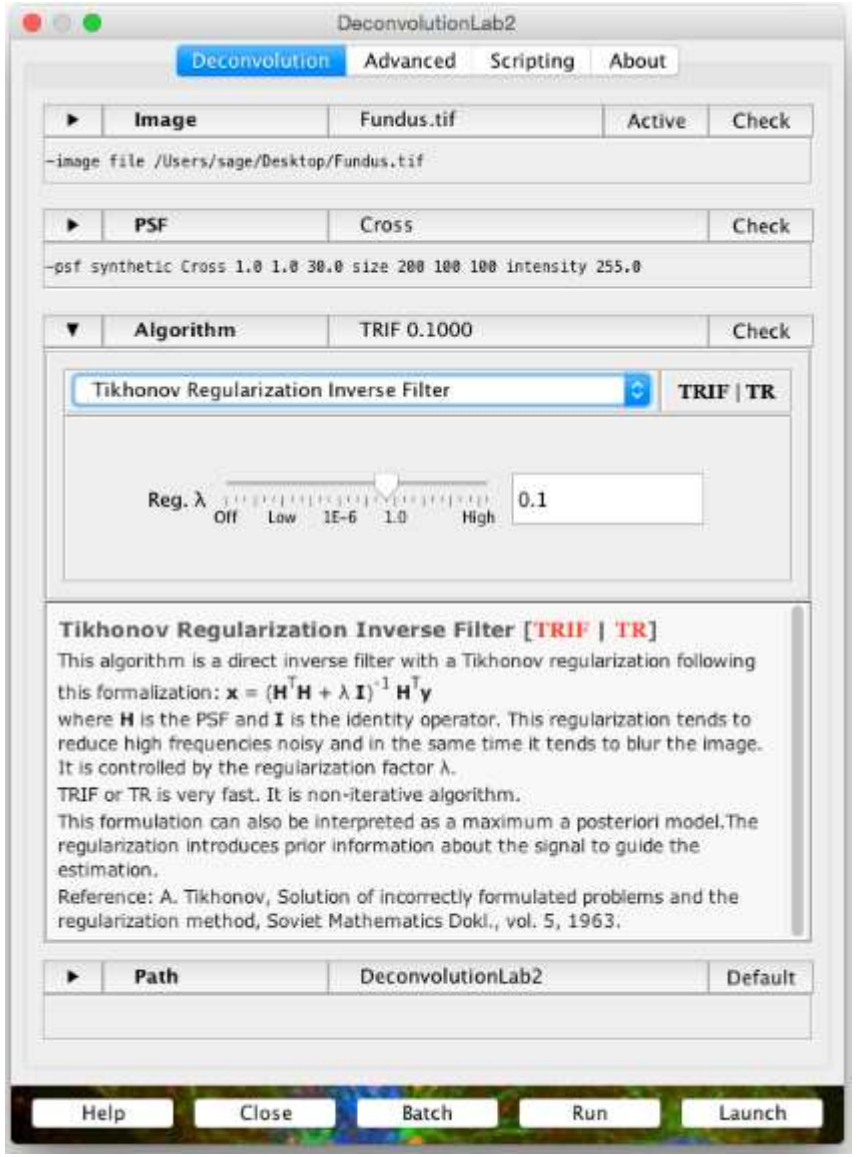
```
javaaddpath([matlabroot filesep 'java' filesep 'DeconvolutionLab_2.jar'])
result = DL2.RIF(rand(40, 40, 30), rand(30, 30, 30), 0.125, '-out mip rand');
```

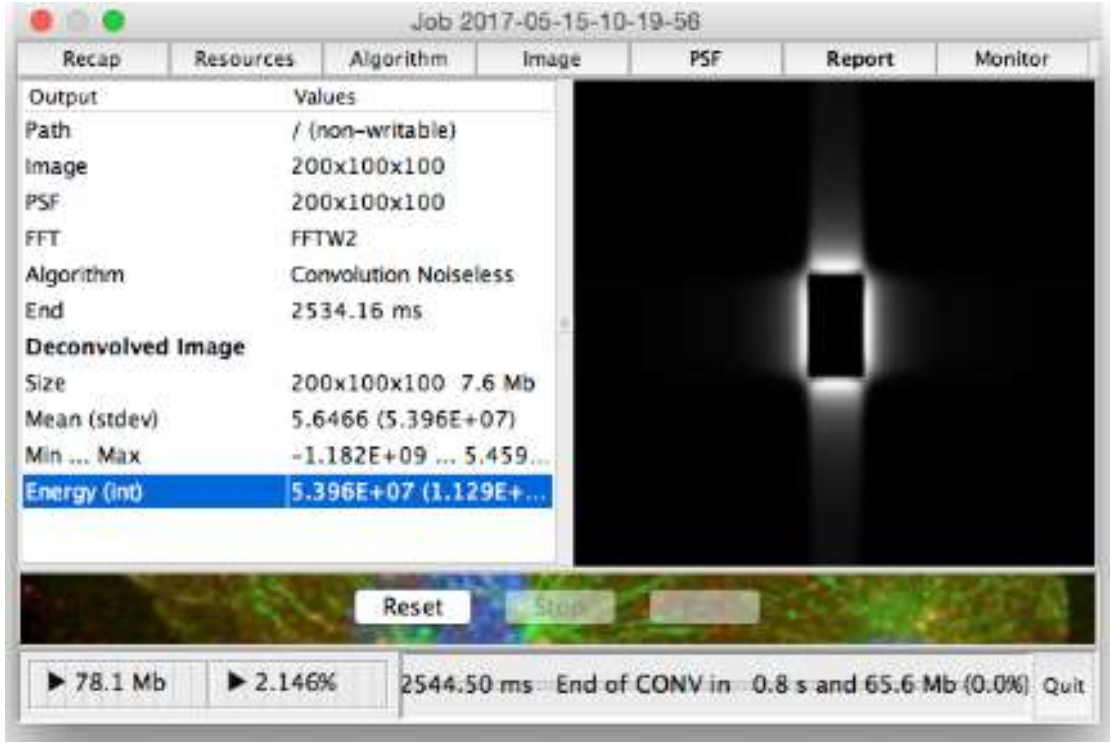
Java程序（或被调用的子程序）

Use the DeconvolutionLab2 as a Java library

Snippet of Java code

```
RealSignal r = Lab.getImage("fundus.tif");
RealSignal h = new DirectionalMotionBlur(2, 20, 20).generate(r.nx, r.ny, r.nz);
Simulation sim = new Simulation(0, 1, 0);
RealSignal y = sim.run(r, h);
TikhonovRegularizedInverseFilter trif = new TikhonovRegularizedInverseFilter(0.001);
RealSignal x = trif.run(y, h);
Lab.show(x, "TRIF " + i);
```


DeconvolutionLab2
Deconvolution
Advanced
Scripting
About
Image
Fundus.tif
Active
Check
-image file /Users/sage/Desktop/Fundus.tif
PSF
Cross
Check
-psf synthetic Cross 1.0 1.0 30.0 size 200 100 100 intensity 255.0
Algorithm
TRIF 0.1000
Check
Tikhonov Regularization Inverse Filter
TRIF | TR
Reg. λ
Off
Low
1E-6
1.0
High
0.1
Tikhonov Regularization Inverse Filter [TRIF | TR]
This algorithm is a direct inverse filter with a Tikhonov regularization following this formalization: x = (H^T H + λ I)^-1 H^T y
where H is the PSF and I is the identity operator. This regularization tends to reduce high frequencies noisy and in the same time it tends to blur the image. It is controlled by the regularization factor λ.
TRIF or TR is very fast. It is non-iterative algorithm.
This formulation can also be interpreted as a maximum a posteriori model.The regularization introduces prior information about the signal to guide the estimation.
Reference: A. Tikhonov, Solution of incorrectly formulated problems and the regularization method, Soviet Mathematics Dokl., vol. 5, 1963.
Path
DeconvolutionLab2
Default
Help
Close
Batch
Run
Launch


Job 2017-05-15-10-19-56
Recap
Resources
Algorithm
Image
PSF
Report
Monitor
Output
Values
Path
/ (non-writable)
Image
200x100x100
PSF
200x100x100
FFT
FFTW2
Algorithm
Convolution Noiseless
End
2534.16 ms
Deconvolved Image
Size
200x100x100 7.6 Mb
Mean (stdev)
5.6466 (5.396E+07)
Min ... Max
-1.182E+09 ... 5.459...
Energy (int)
5.396E+07 (1.129E+...
Reset
Stop
78.1 Mb
2.146%
2544.50 ms End of CONV in 0.8 s and 65.6 Mb (0.0%)
Quit

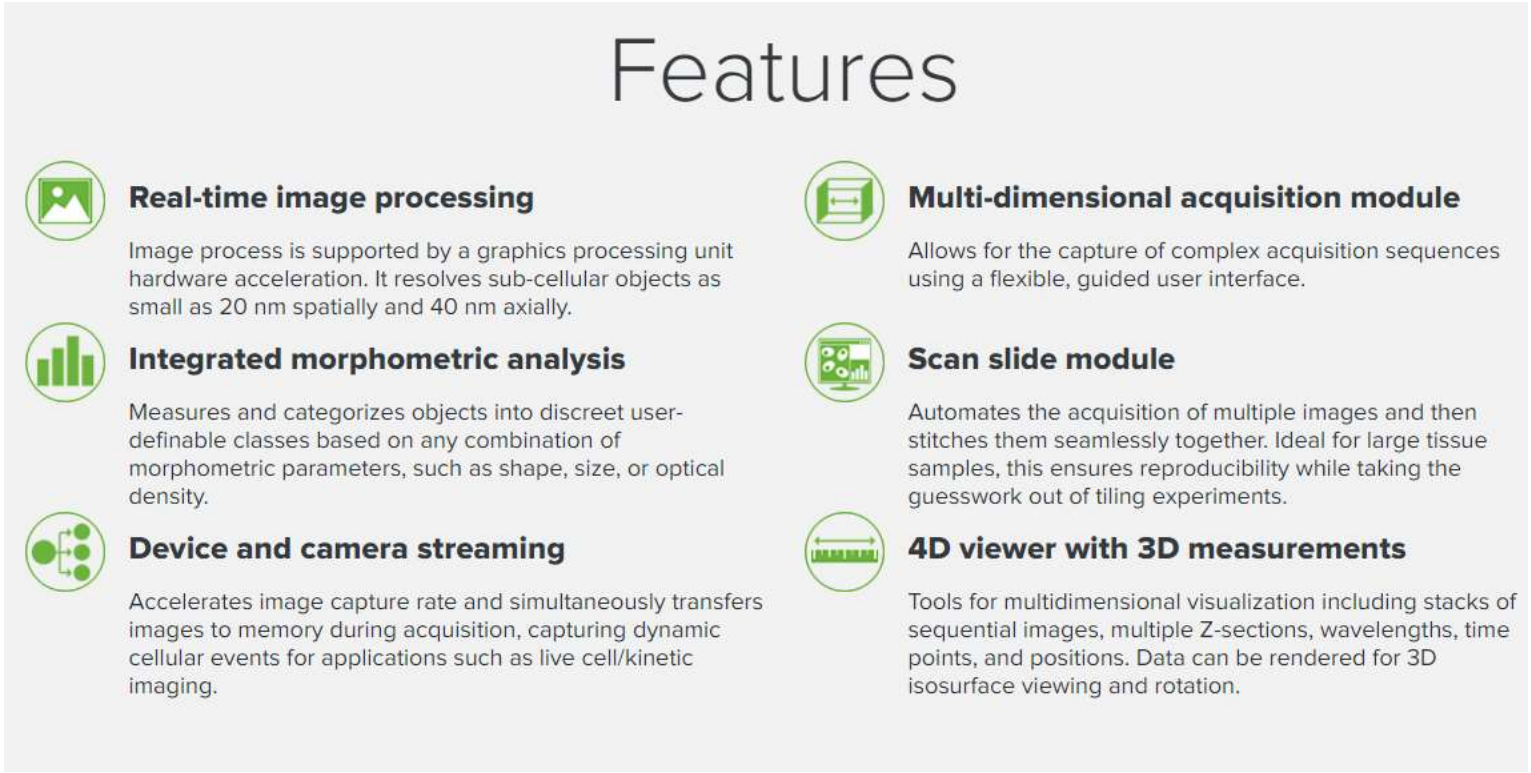
用户界面

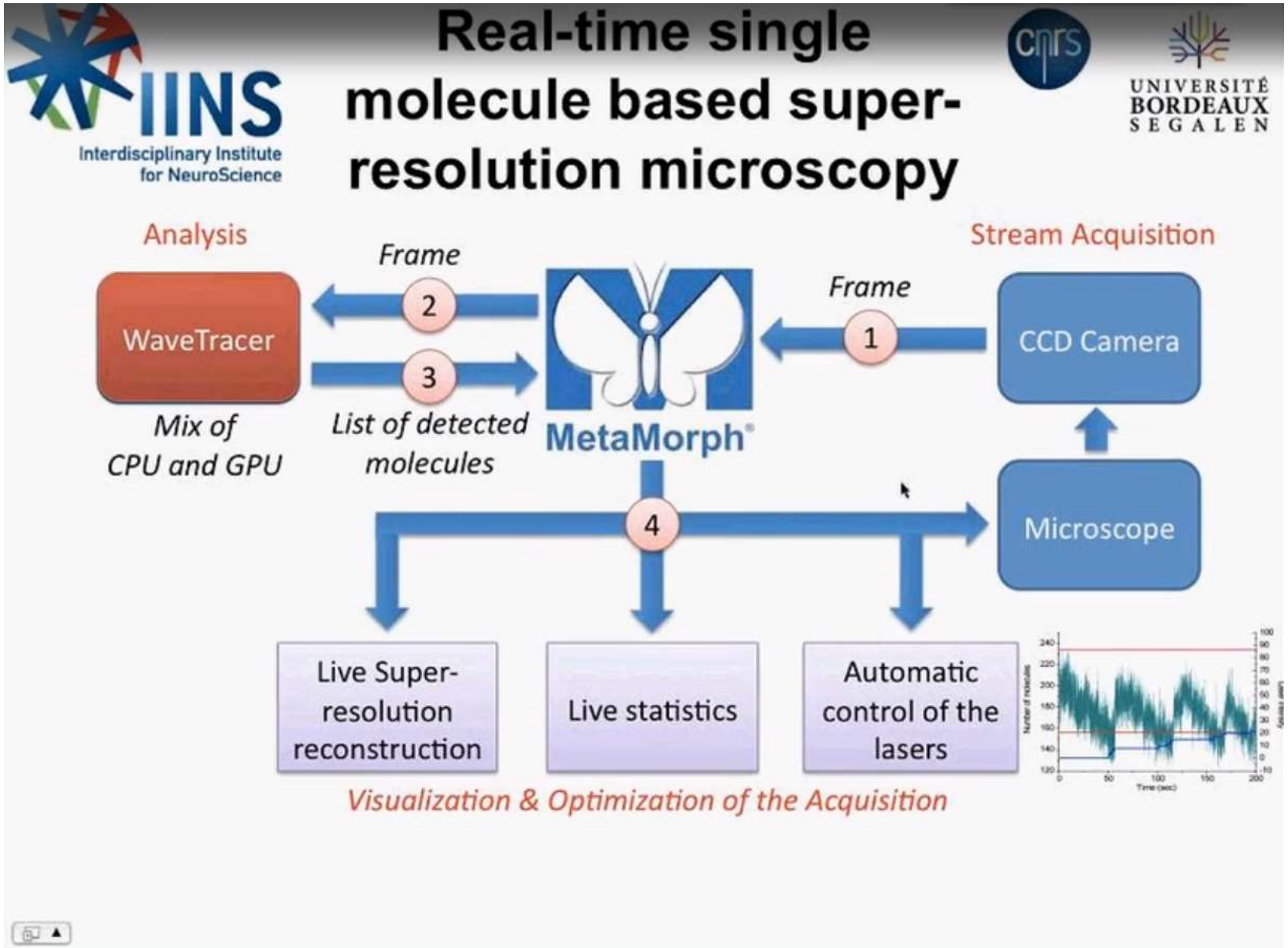
方案3：single-objective (soSPIM)

图像处理在MetaMorph中实现，包括：

MetaMorph super-resolution system

https://www.moleculardevices.com/products/cellular-imaging-systems/acquisition-and-analysis-software/metamorph-microscopy




IINS
Interdisciplinary Institute for NeuroScience
Real-time single molecule based super-resolution microscopy
cnrs
UNIVERSITÉ BORDEAUX SEGALEN
Analysis
Stream Acquisition
Frame
2
WaveTracer
3
Mix of CPU and GPU
List of detected molecules
MetaMorph®
Frame
1
CCD Camera
Microscope
4
Live Super-resolution reconstruction
Live statistics
Automatic control of the lasers
Visualization & Optimization of the Acquisition

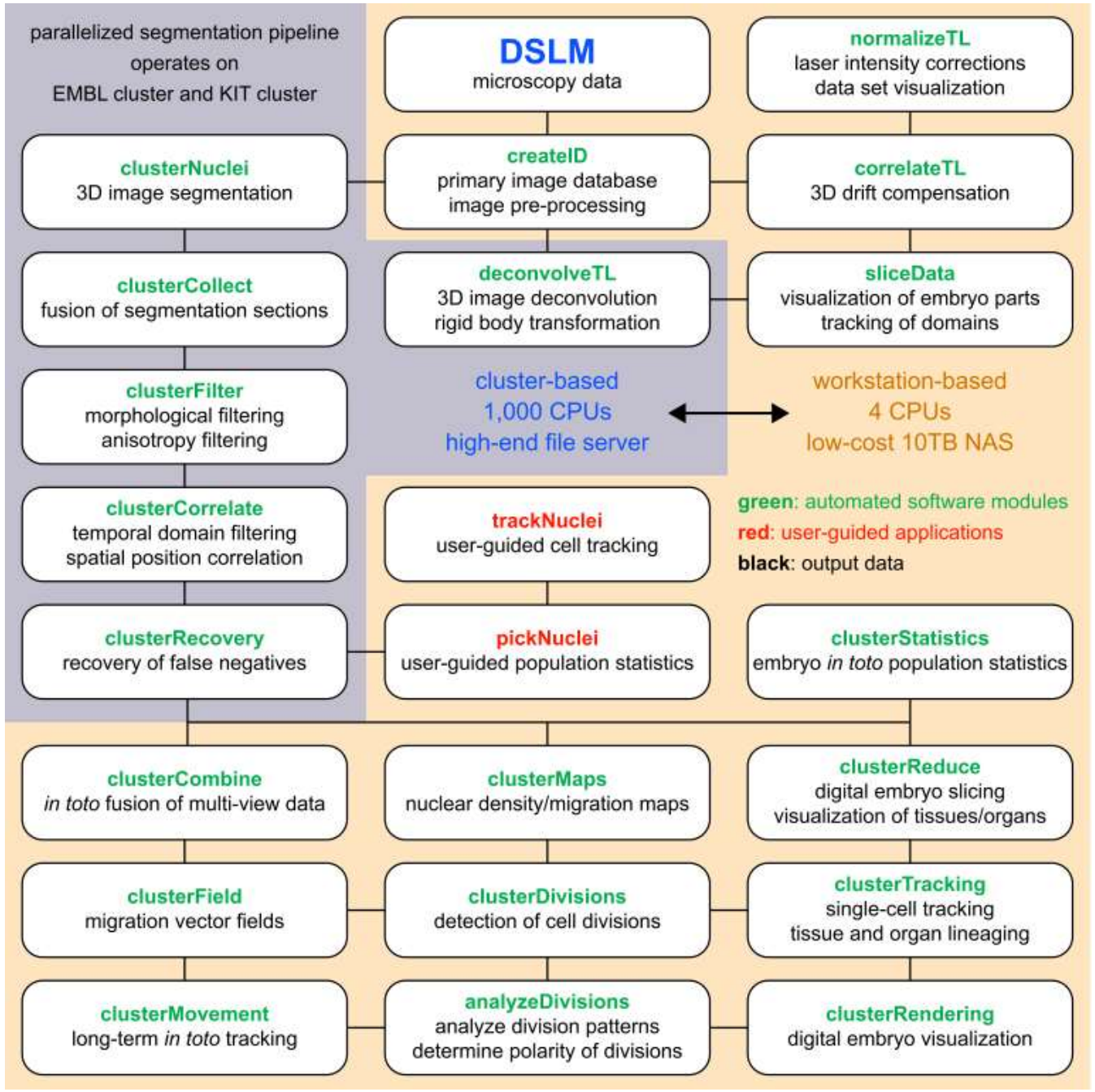
运行框架

方案4：DSLM

图像处理在.NET framework和Matlab混合编程：

.NET framework：用户界面、低层硬件控制（图像获取）

matlab：图像处理



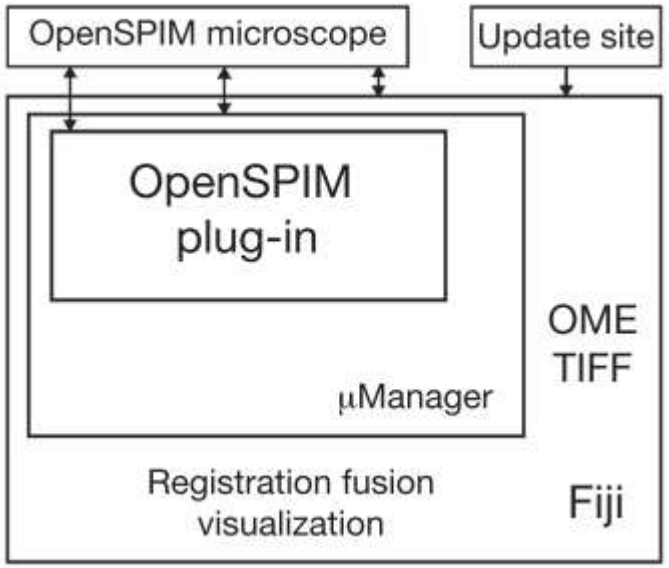
图像处理：compensation, reconstruction(restoration), tracking等

源代码：https://www.embl.de/digitalembryo/

方案5：openSPIM

图像处理：将micro manager和Fiji两个插件集成到一起（ extending micro manager running inside Fiji ）

百科： https://openspim.org，提供详细的安装指导、软件使用等支持。



总结

1. 图像处理可以分为两步：图像获取（acquisition）和图像处理（processing）
2. 已搜集的方案总结如下

图像获取	图像处理
LabView	Fiji
Micro Manager	Fiji
Micro Manager + Fiji	
.NET framework + Matlab	