



University
of Glasgow



Customized image analysis software

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My two roles

- Lecturer in Institute of Cardiovascular and Medical Sciences, University of Glasgow
- **SULSA Technologist**

The **SULSA Imaging Technologist** at the University of Glasgow will support the biological imaging community in Scotland by providing technical advice and support on a range of imaging systems including two-photon confocal microscopy, whole organ/body fluorescence/luminescence imaging and structured light applications, writing customised open source software for image analysis for university labs across Scotland, and coordinating and teaching computational techniques associated with imaging.

~3 days/week

My background

- Ion channels in skeletal & cardiac muscle membrane
- Membrane biophysics
- Ca handling in skeletal muscle
- Whole heart electrophysiology
 - arrhythmias
 - electrode arrays
 - optical mapping
 - 2P confocal linescan recording of V_m from single cells (isolated and *in situ*)



software

Case studies

- Chromosome segregation in *E. Coli*
Prof. David Leach, Edinburgh
- Tracking lymphocytes in lymphoid tissue
Prof. Pasquale Maffia, Glasgow
- Collection & analysis of images of membrane voltage in cardiac tissue
Cairn Research Ltd., scientific instruments manufacturer

Case study 1

(Customer: Prof. Leach + team, Edinburgh)

nature

Vol 455 | 30 October 2008 | doi:10.1038/nature07282

LETTERS

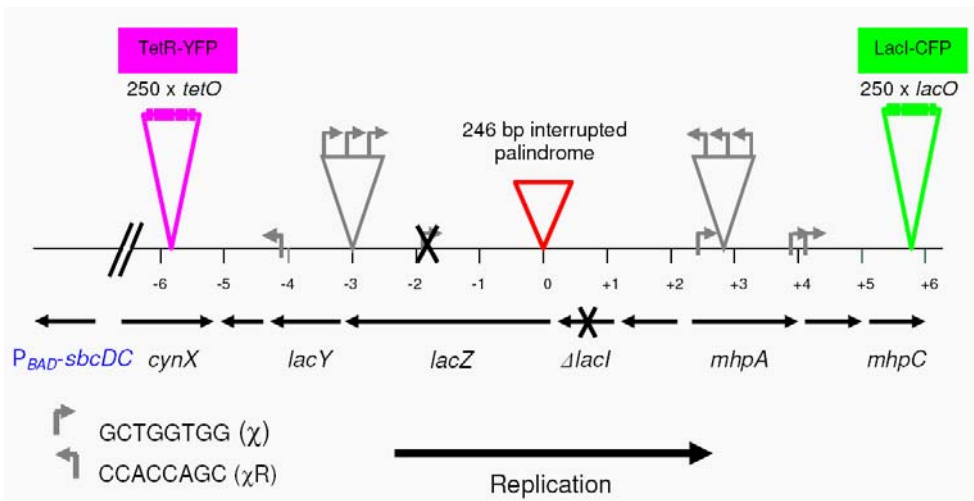
Non-random segregation of sister chromosomes in *Escherichia coli*

Martin A. White¹, John K. Eykelenboom¹, Manuel A. Lopez-Vernaza¹, Emily Wilson¹ & David R. F. Leach¹



Background

- In *E.Coli*, domains of L & R chromosome arms (replichores) occupy distinct cellular locations
- During replication, domain segregation results in translational symmetry of chromosome arms
- Track position of palindrome region as it is replicated and segregated



TetR – tetracycline repressor protein
LacI – lac repressor protein

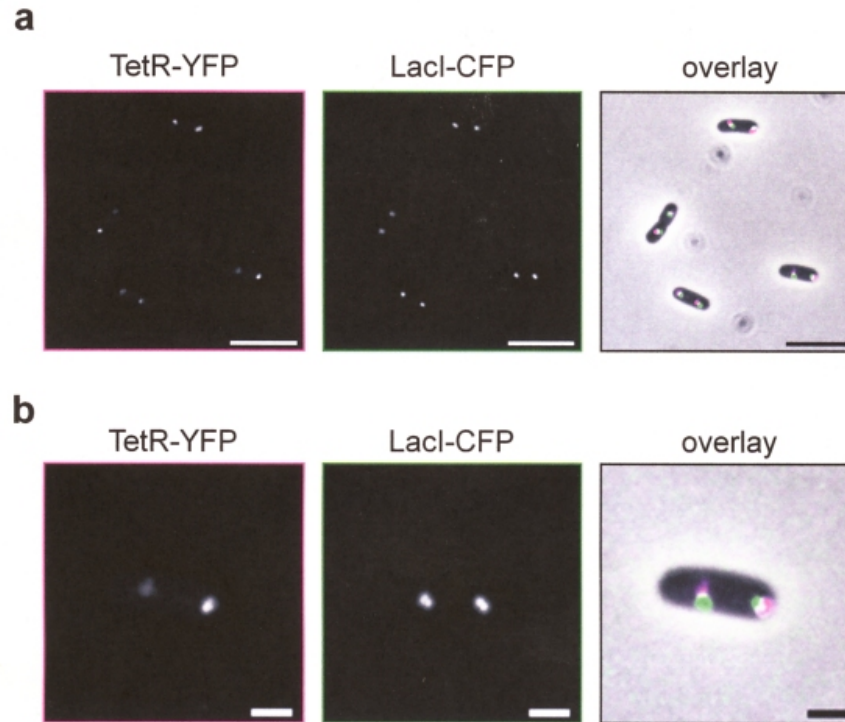
- Arrays of tetO & lacO sites visualized on binding with TetR-YFP & LacI-CFP
- Twin YFP-CFP spot

Existing setup

- Zeiss Axiovert 200 + Photometrics cooled CCD
- 0.129 μm per pixel resolution
- Acquisition: MetaMorph 6.3r2 software
- Analysis: CFP, YFP images \rightarrow foci
 - Autodeblur: blind deconvolution
 - MetaMorph: standard threshold + centroid \rightarrow (x,y)
- Analysis: Brightfield images \rightarrow cell outlines
 - MetaMorph: 'fibre length' function
- Foci and cells counted & correlated **manually!**

Notes for Francis Burton

Typical Data Set



Images typically acquired at a resolution of 129 nm per pixel, but have the ability to acquire at a resolution of 64.5 nm per pixel (possibly higher with optivar). Calibration bar in **a** shows 5 μm , **b** shows 1 μm . *fluorescence images deconvolved.*

Main quantifications required:

- Number of foci per cell
- Location of foci within cells

• centroid to nearest cell pole.

* Time-lapse

- migration of YFP/CFP with respect to each other and location within cell.

Typical cell size

- Nutrient rich growth medium: 3 – 6 μm , 2 – 16 YFP and CFP foci
- Nutrient poor growth medium: 1 – 3 μm , 1 or 2 YFP and CFP foci

Issues with thresholding

- Thresholding cells is fine
- Thresholding foci: issues of heterogeneity of fluorescence intensity and dividing overlapping foci.

Solutions considered

- Software to correlate MetaMorph data
 - read files exported from MM
 - perform calcs inside MM using VB extension
 - ~~X~~ MM 'journals' (macros) not suitable
- Custom program to analyze cell & foci images and to correlate data

Two recent papers

OPEN ACCESS Freely available online

PLoS COMPUTATIONAL BIOLOGY

PSICIC: Noise and Asymmetry in Bacterial Division Revealed by Computational Image Analysis at Sub-Pixel Resolution

2008

Jonathan M. Guberman¹, Allison Fay², Jonathan Dworkin², Ned S. Wingreen¹, Zemer Gitai^{1*}

¹ Department of Molecular Biology, Princeton University, Princeton, New Jersey, United States of America, ² Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York, New York, United States of America

IOP PUBLISHING

PHYSICAL BIOLOGY

Phys. Biol. 4 (2007) 220–227

[doi:10.1088/1478-3975/4/3/008](https://doi.org/10.1088/1478-3975/4/3/008)

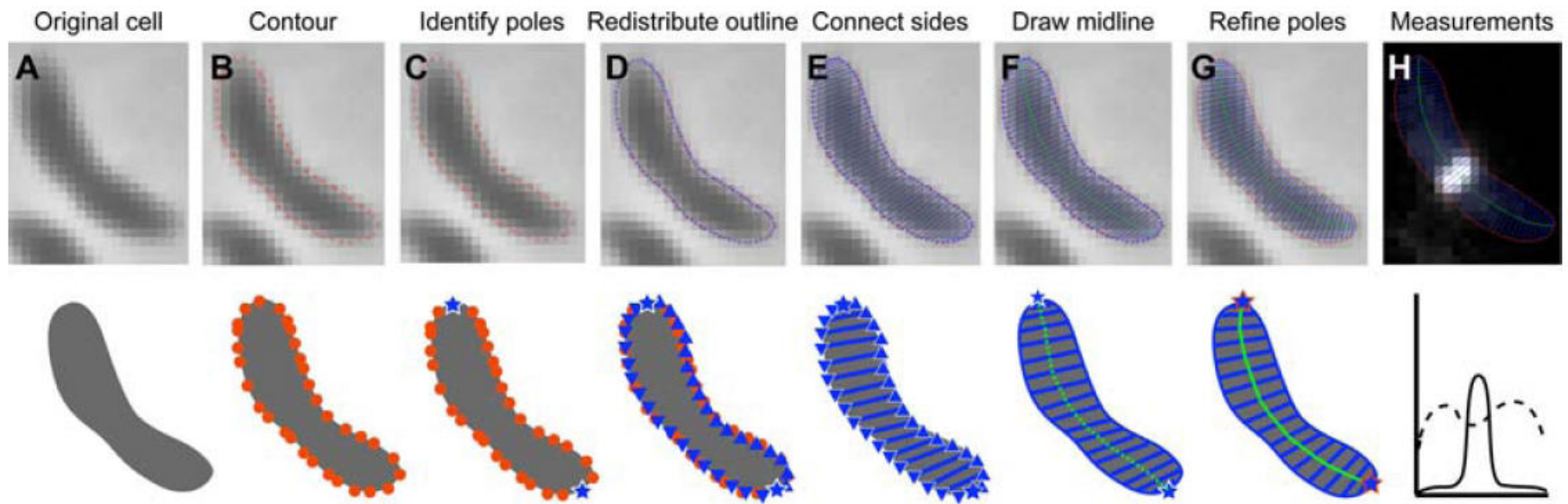
Precise particle tracking against a complicated background: polynomial fitting with Gaussian weight

Salman S Rogers, Thomas A Waigh, Xiubo Zhao and Jian R Lu

2007

School of Physics and Astronomy, University of Manchester, Manchester M60 1QD, UK

Cell outline analysis (Guberman et al., 2008)

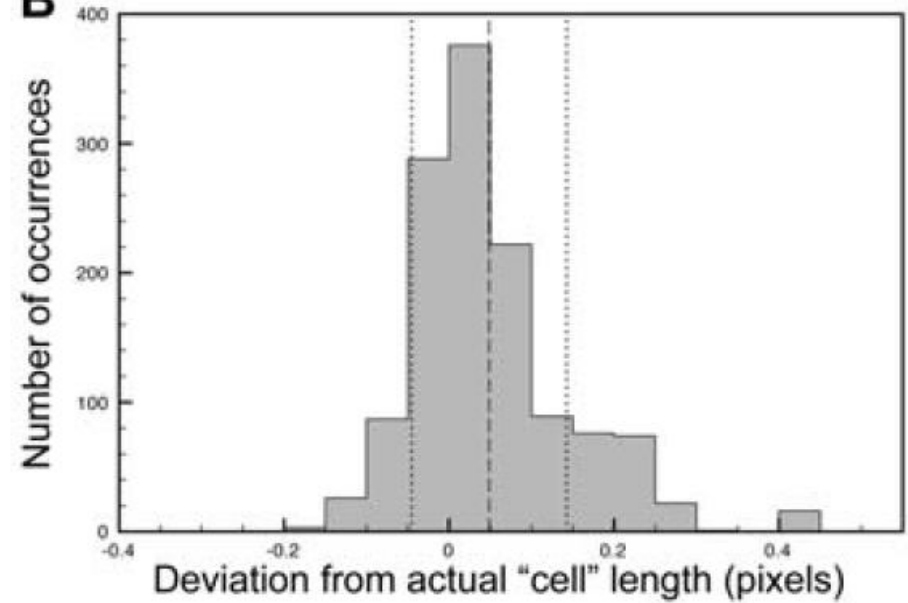


Cell outline analysis (Guberman et al., 2008)

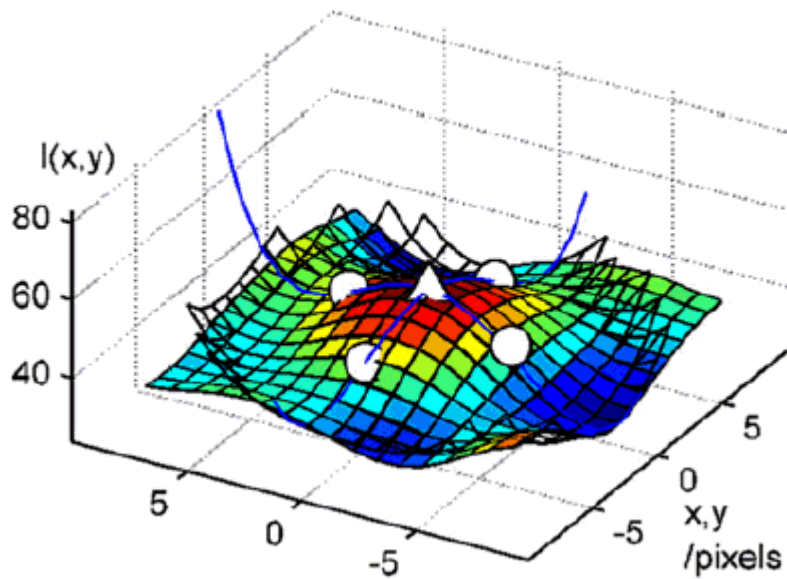
A



B



Foci analysis (Rogers et al., 2007)



$$\theta = \frac{1}{2} \cot^{-1} \frac{P_{20} - P_{02}}{P_{11}},$$

$$e = \frac{1 + \frac{\sqrt{(P_{20} - P_{02})^2 + P_{11}^2} + (P_{20} + P_{02})}{\sqrt{(P_{20} - P_{02})^2 + P_{11}^2} - (P_{20} + P_{02})}}$$

$$I_{\text{fit}}(x, y) = \sum_{i+j=4}^{i=0, j=0} P_{ij} (x - x_n)^i (y - y_n)^j$$

$$W(x, y) = \exp \left(-\alpha \frac{(x - x_n)^2 + (y - y_n)^2}{R^2} \right)$$

$$R = \left(\frac{AB}{36CD} \right)^{1/4},$$

where

$$A = P_{20} \cos^2 \theta - P_{11} \cos \theta \sin \theta + P_{02} \sin^2 \theta,$$

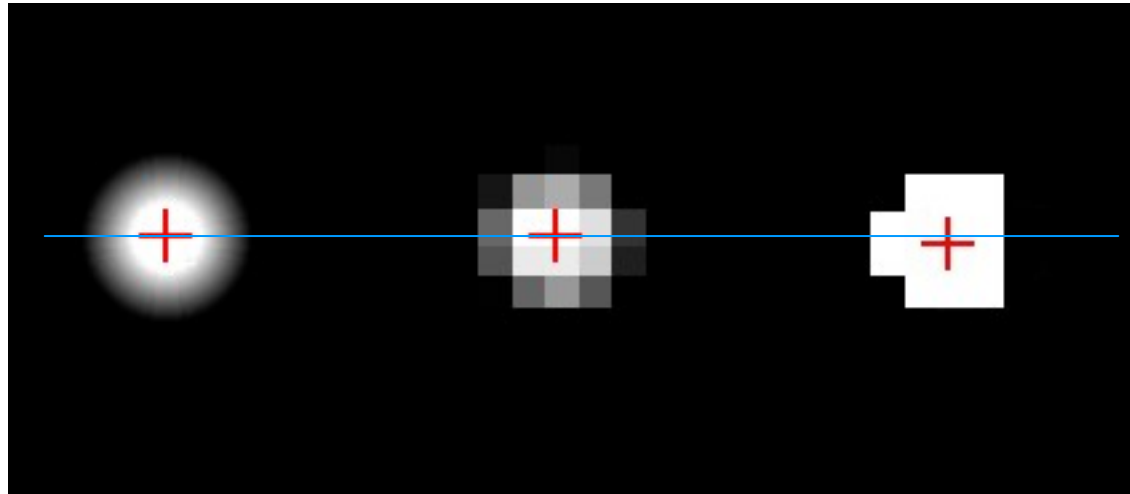
$$B = P_{20} \sin^2 \theta - P_{11} \cos \theta \sin \theta + P_{02} \cos^2 \theta,$$

$$C = P_{40} \cos^4 \theta - P_{31} \cos^3 \theta \sin \theta + P_{22} \cos^2 \theta \sin^2 \theta - P_{13} \cos \theta \sin^3 \theta + P_{04} \sin^4 \theta,$$

$$D = P_{40} \sin^4 \theta + P_{31} \sin^3 \theta \cos \theta + P_{22} \sin^2 \theta \cos^2 \theta + P_{13} \sin \theta \cos^3 \theta + P_{04} \cos^4 \theta.$$

$$\text{skewness} = \frac{|P_{30}| + |P_{21}| + |P_{12}| + |P_{03}|}{P_{20}P_{02} - P_{11}^2/4} R$$

Precision: fitting vs centroid

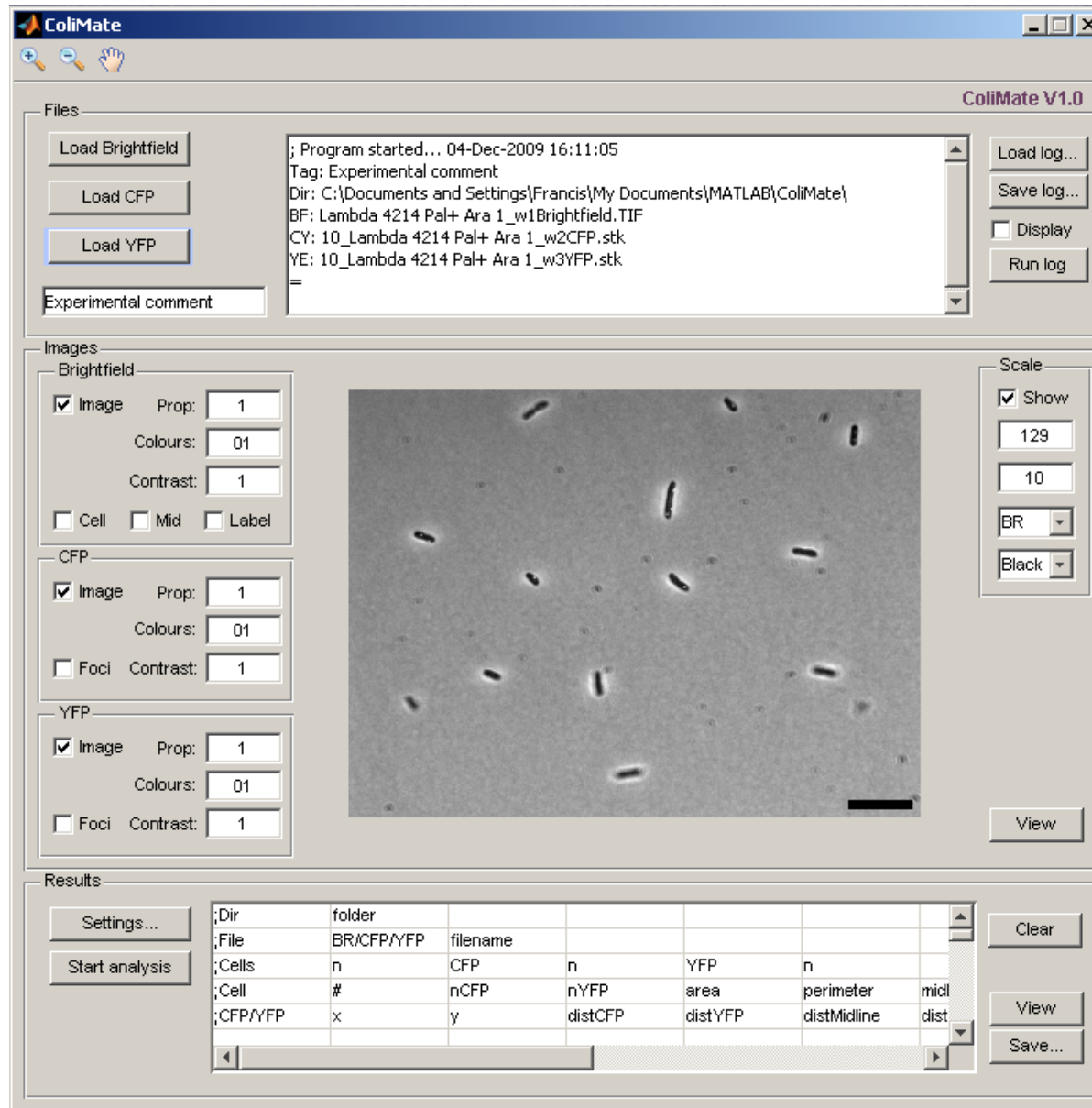


Mean absolute deviation: ~0.01 pixel

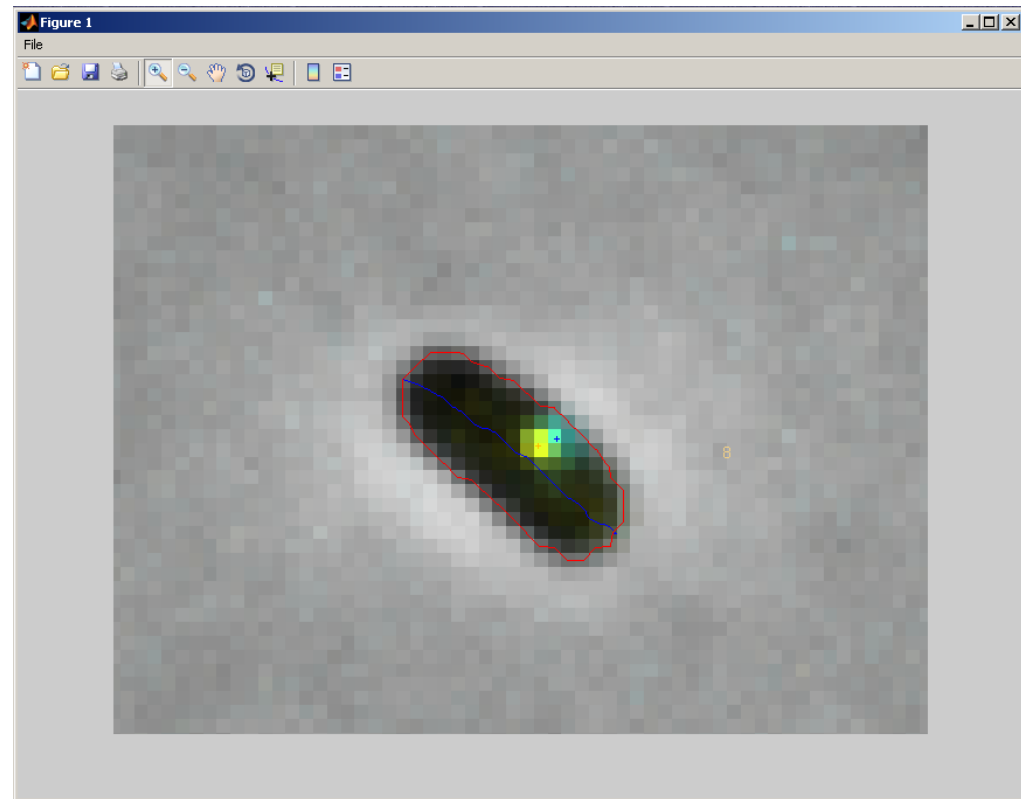
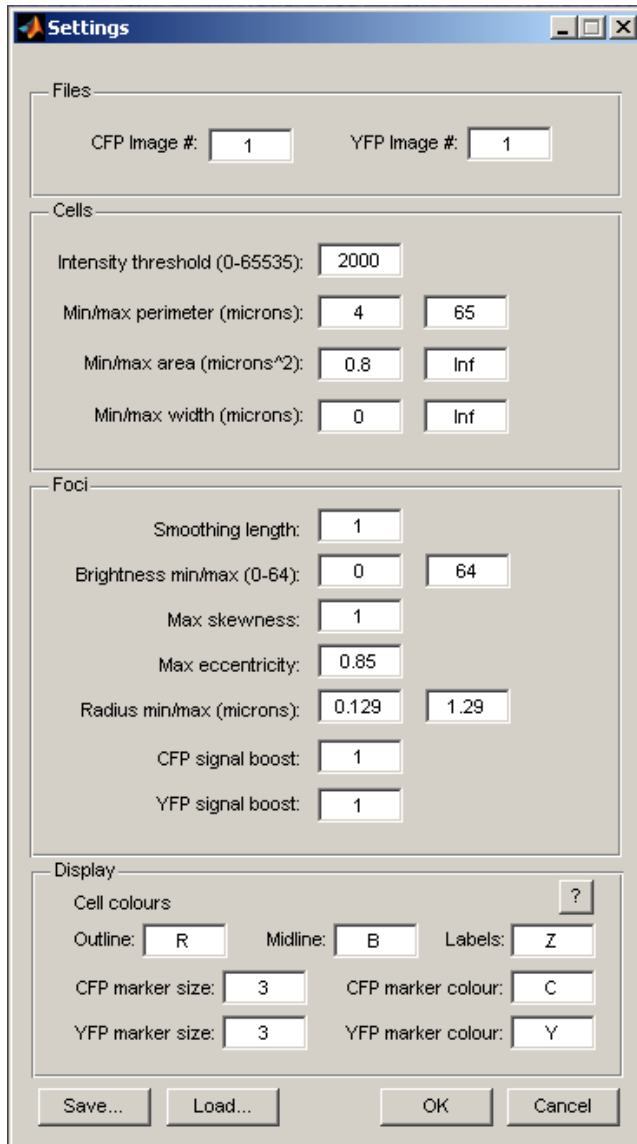
~0.1 pixel

handles close/overlapping foci

Software: ColiMate (MATLAB)



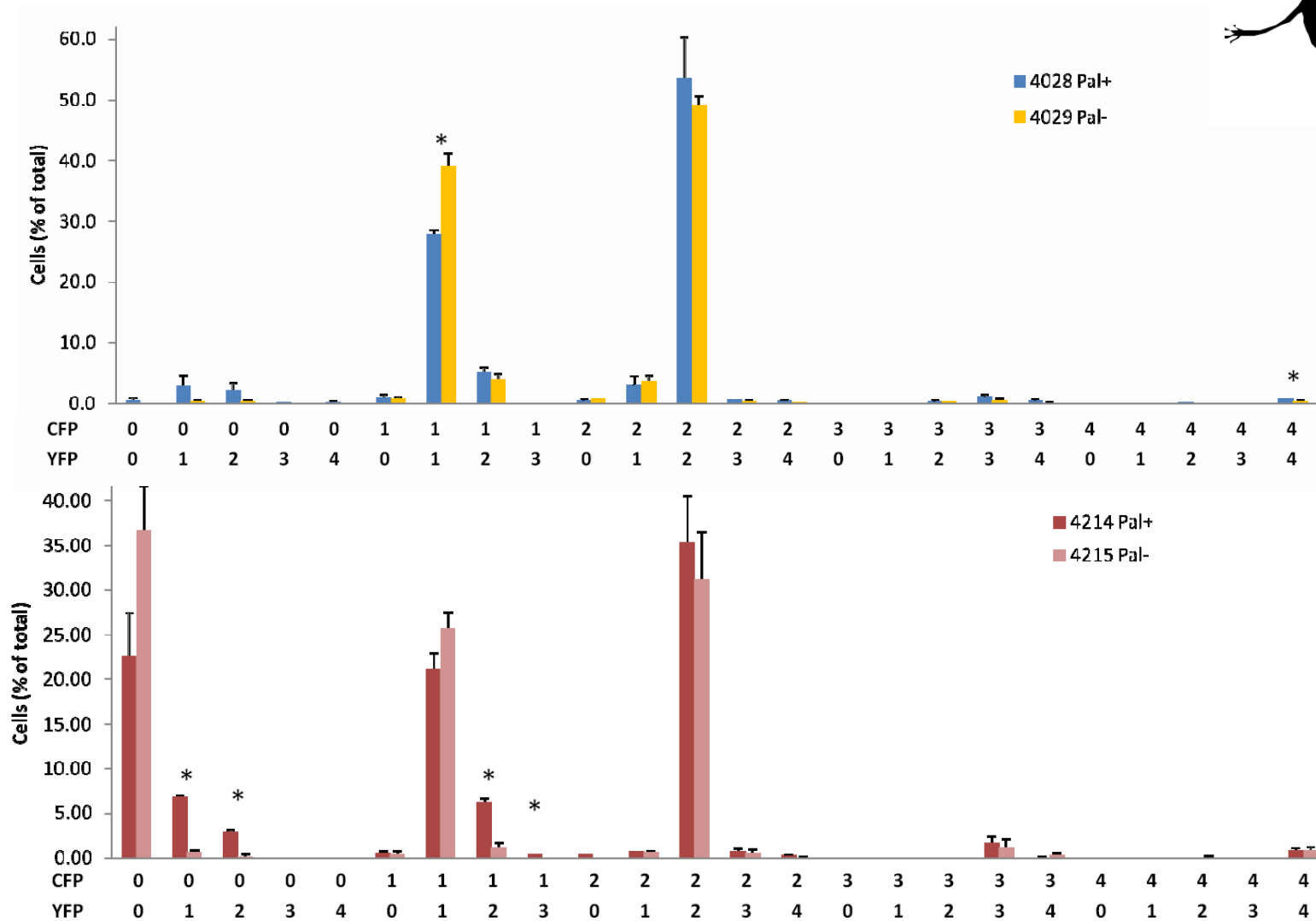
Software: ColiMate



Software: ColiMate

Results												
	1	2	3	4	5	6	7	8	9	10	11	12
1	;Dir	folder										
2	;File	BR/CFP/YFP	filename									
3	;Cells	n	CFP	n	YFP	n						
4	;Cell	#	nCFP	nYFP	area	perimeter	midlength	maxwidth				
5	;CFP/YFP	x	y	distCFP	distYFP	distMidline	distPole	brightness	radius	eccentricity	skewness	rotation
6												
7	Dir	C:\Document...										
8	File	BR	Lambda 421 ...									
9	File	CFP	10_Lambda ...									
10	File	YFP	10_Lambda ...									
11	Cells		13 CFP		9 YFP		6					
12												
13	Cell	1	0	0	2.0743	6.1973	2.5744	1.0751				
14												
15	Cell	2	1	0	2.9411	7.9542	3.4538	1.0529				
16	CFP	79.3889	81.7731	0	0	0.1209	0.8925	3.8700	2.2859	0.4396	0.3358	2.0926
17												
18	Cell	3	2	1	3.1703	8.2911	3.5880	1.1030				
19	CFP	11.5266	66.8754	1.4816	0.2315	0.0245	1.1771	5.3397	1.7936	0.1544	0.0112	0.1354
20	CFP	12.6903	65.9584	1.4816	1.6926	0.4150	0.9866	5.3611	1.7943	0.2329	0.0373	-0.2584
21	YFP	11.3003	66.9241	0.2315	0	0.0130	0.9514	4.4496	2.1427	0.2490	0.2978	0.5454
22												
23	Cell	4	0	0	3.0499	8.1489	3.4858	1.0667				
24												
25	Cell	5	0	0	3.5302	9.2227	4.0770	1.1204				
26												
27	Cell	6	0	0	2.4349	6.7947	2.8247	1.0314				
28												
29	Cell	7	0	0	4.4486	10.7948	4.7298	1.2238				
30												
31	Cell	8	1	1	2.1873	6.2088	2.5620	1.0603				
32	CFP	29.3088	60.2136	0	0.1936	0.3188	1.0162	8.5422	2.0664	0.3763	0.0531	2.2192
33	YFP	29.1279	60.1445	0.1936	0	0.1432	1.0664	6.1911	1.9457	0.4471	0.1307	-0.7611
34												
35	Cell	9	1	0	2.5464	6.6081	2.7828	1.1239				
36	CFP	60.3481	87.1805	0	0	0.1893	0.9334	7.2989	1.8429	0.3746	0.0197	-0.6357

Final figures: Derived histograms



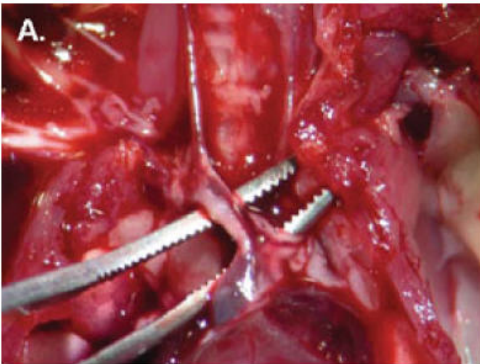
"... most definitely exceeded all of our expectations" Martin White

Case study 2

(Customer: Prof. Pasquale Maffia, Glasgow)

Imaging leucocyte dynamics in aorta
adventitia of apolipoproteinE^{-/-} mice

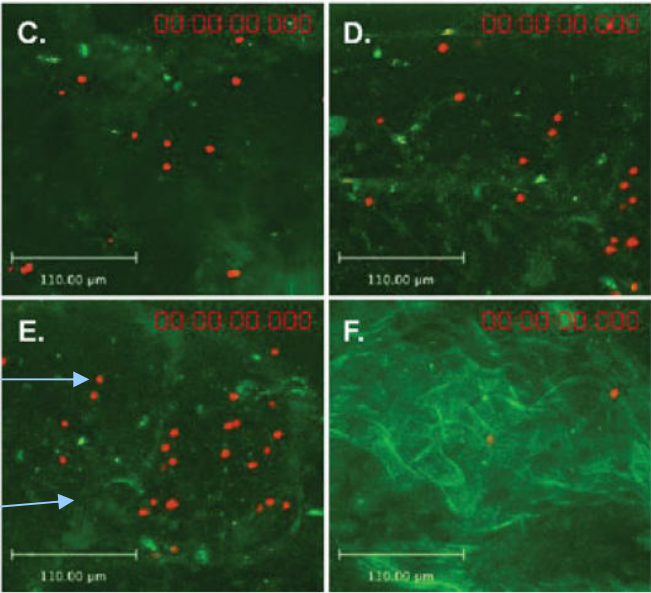
sub-diaphragmatic
region of
abdominal artery
of mouse



para-aortic
lymph node

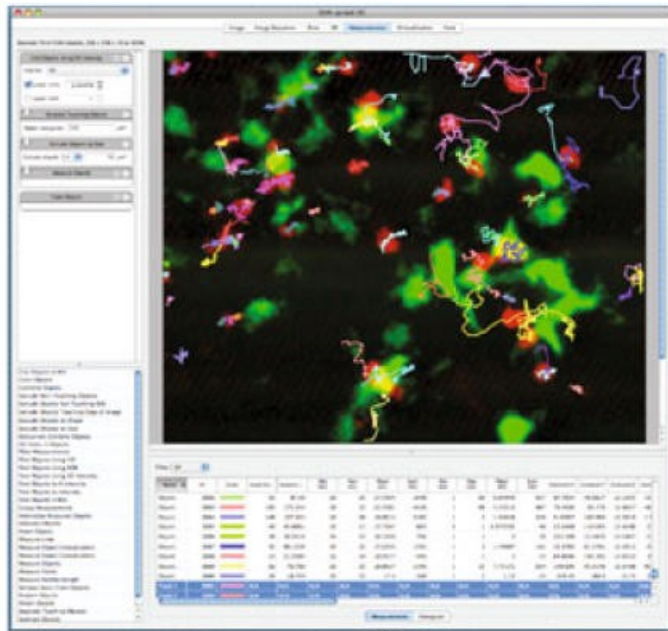
T lymphocytes
Cell Tracker™ Red

Adventitia elastin
autofluorescence

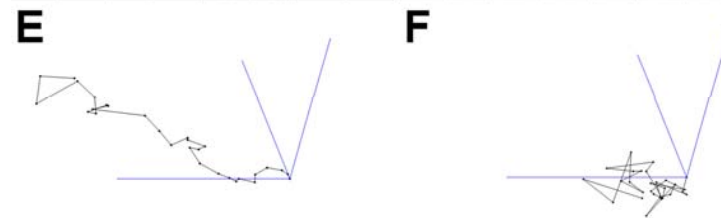
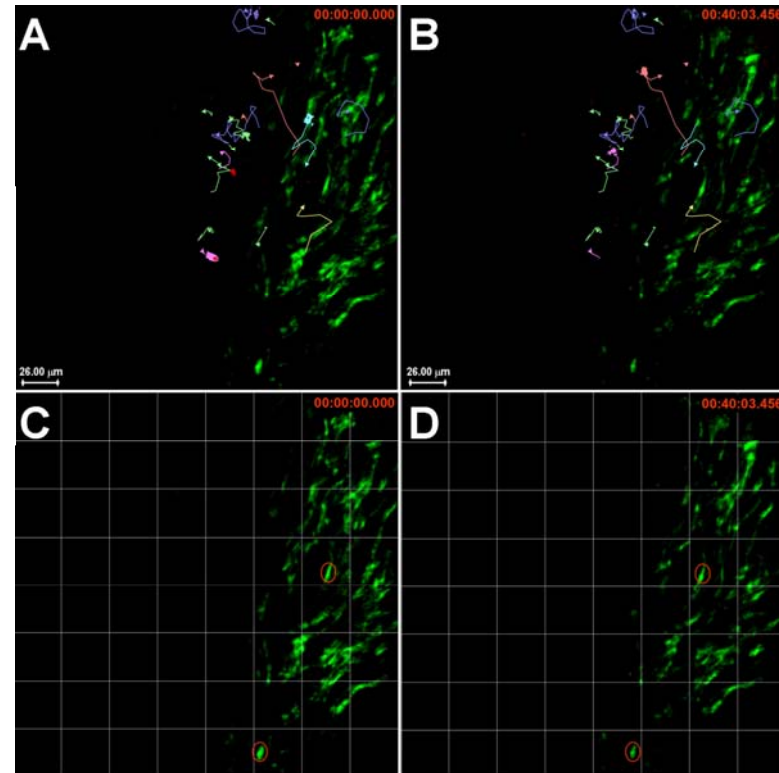


MPLSM of ATLO
(artery tertiary lymphoid organs)

Problem: Tissue drift



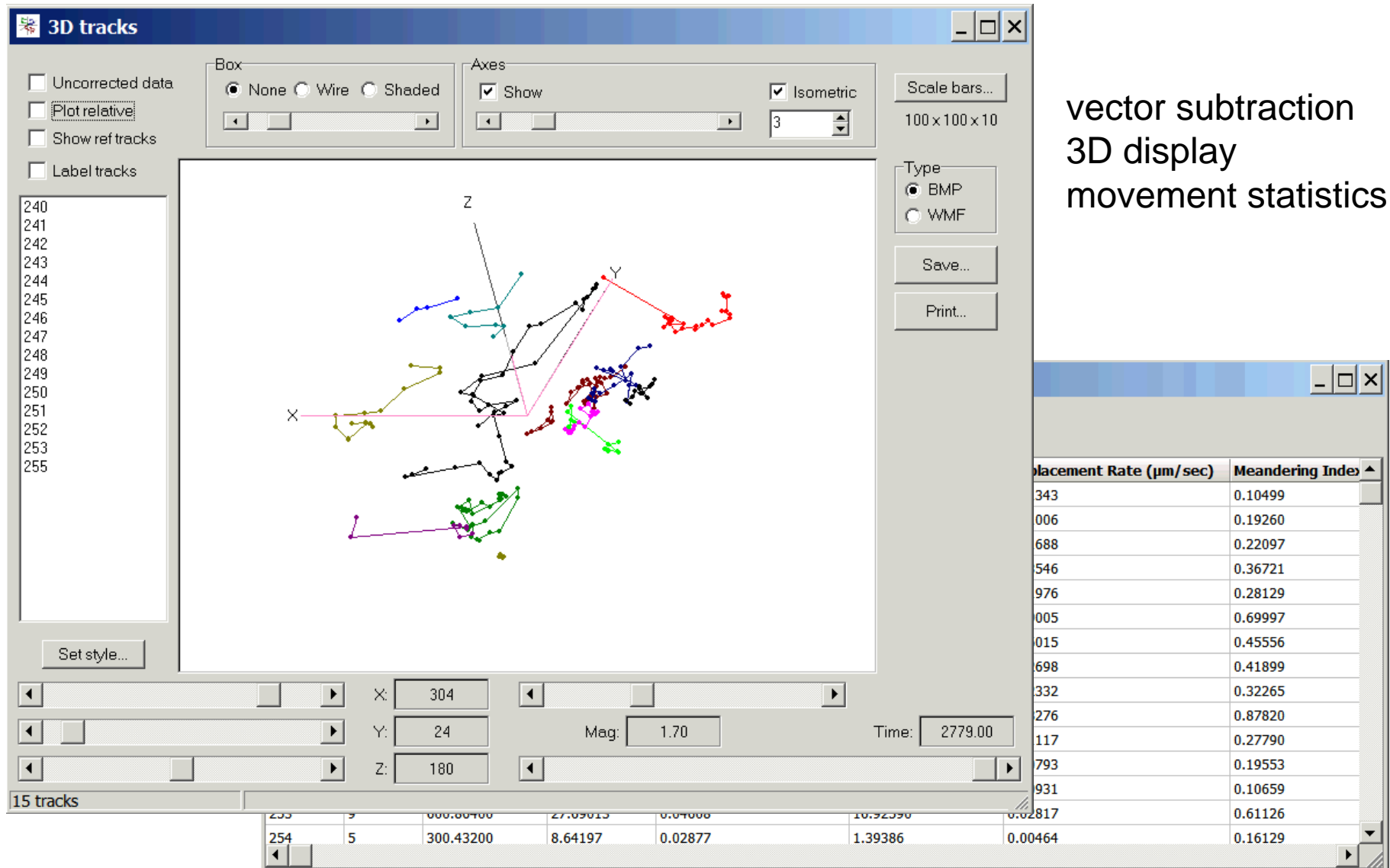
Velocity 5 cell tracking module
no drift correction



uncorrected

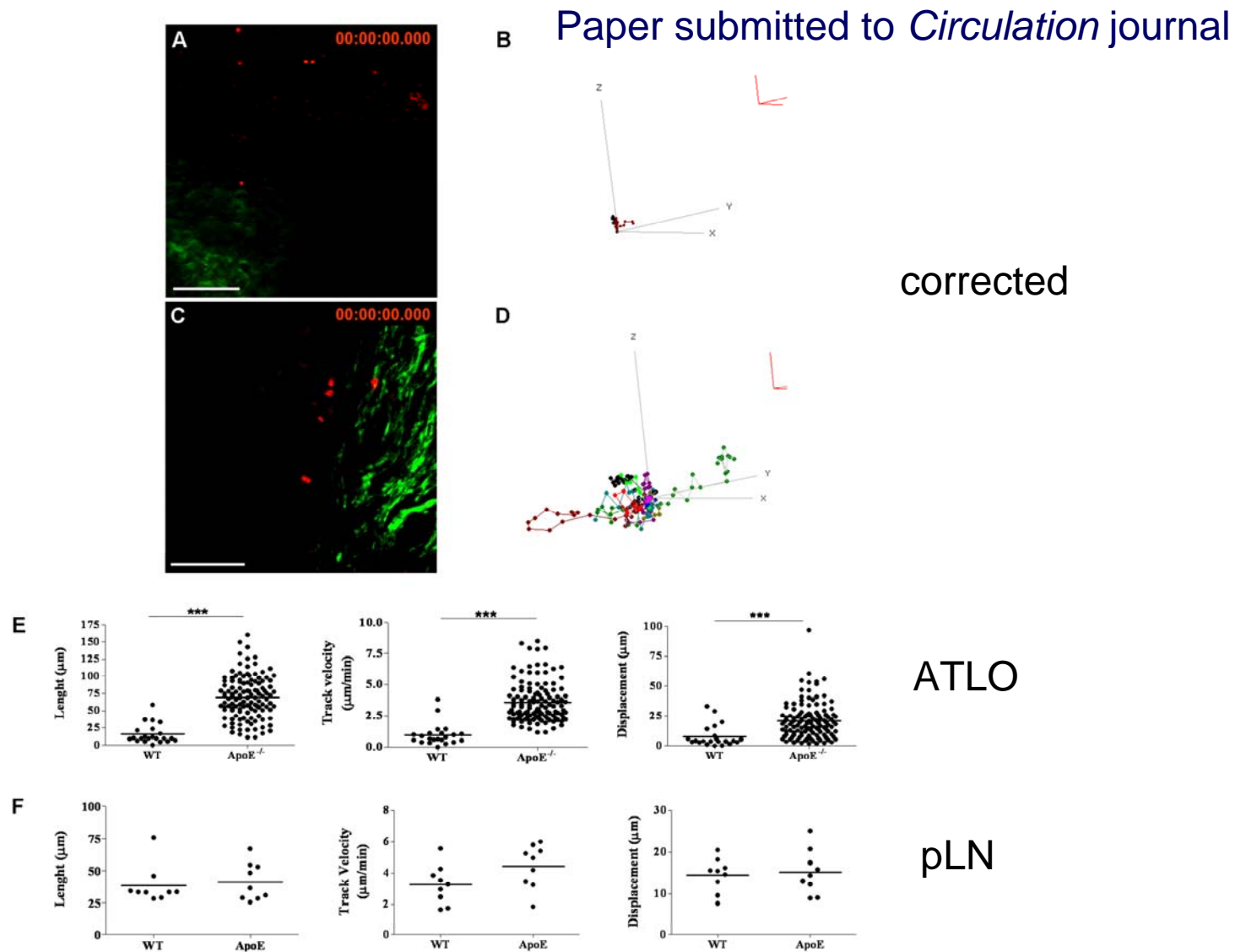
corrected

Solution: Custom software



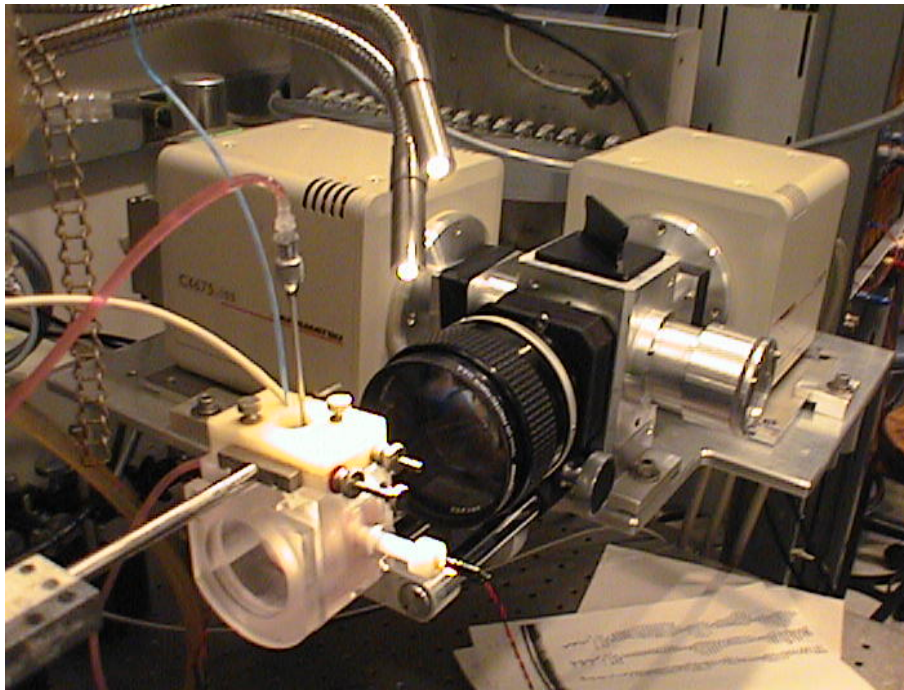
vector subtraction
3D display
movement statistics

Leukocyte invasion associated with local T- and B cell-mediated immune responses in atherosclerosis

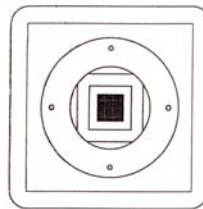
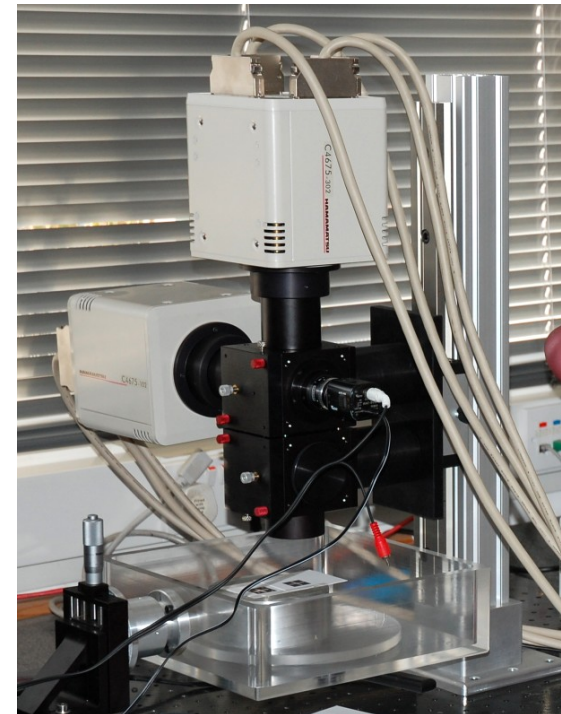


Case study 3: Real-time imaging of membrane voltage in cardiac tissue

(Customer: Cairn Research Ltd, scientific instruments manufacturer)



Hamamatsu photodiode array
16x16 (256) pixels
1000 frames/sec
16 bit digitization

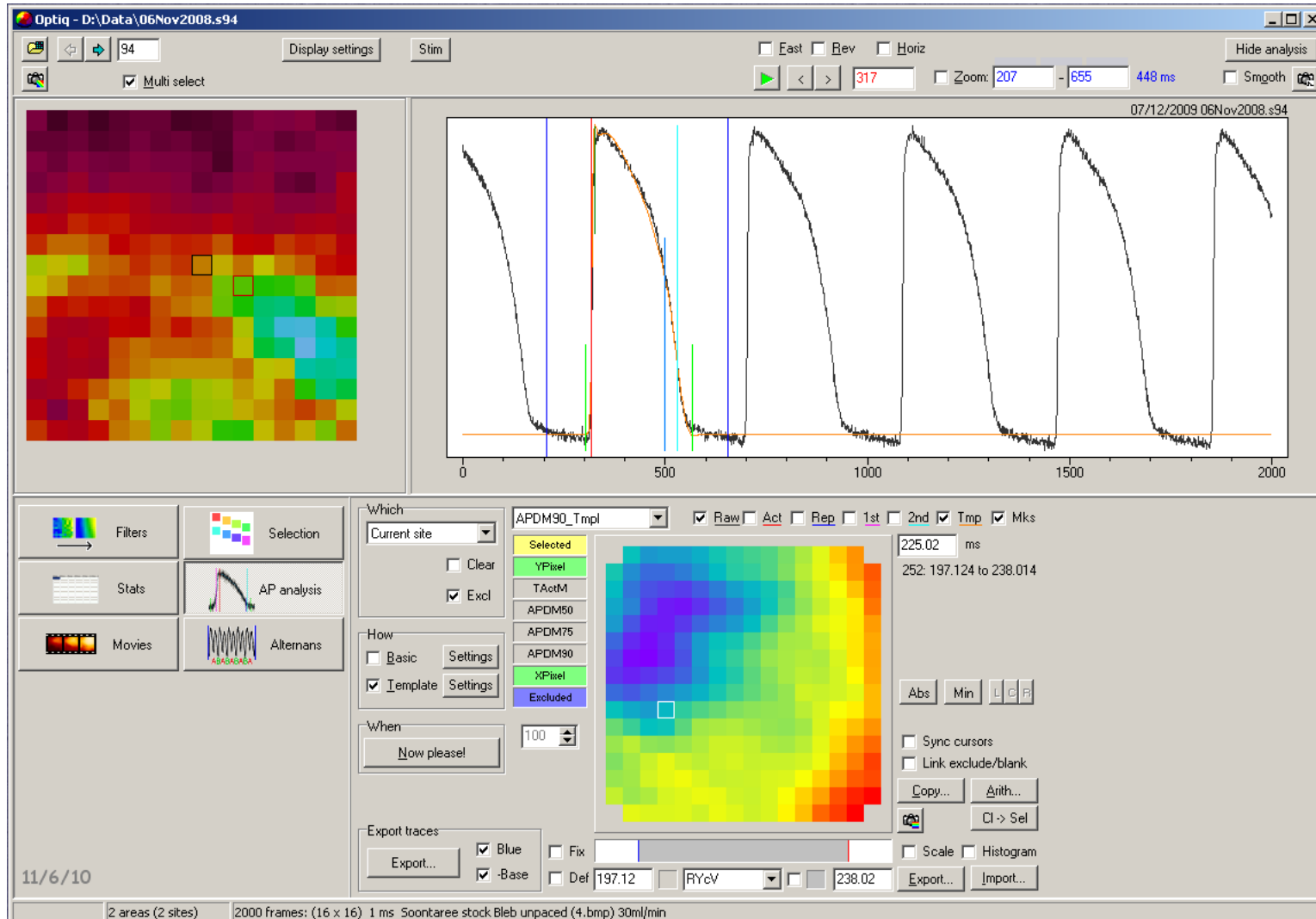


Recording software: QRecord (Delphi)

The screenshot displays the QRecord software interface, which is divided into several functional windows:

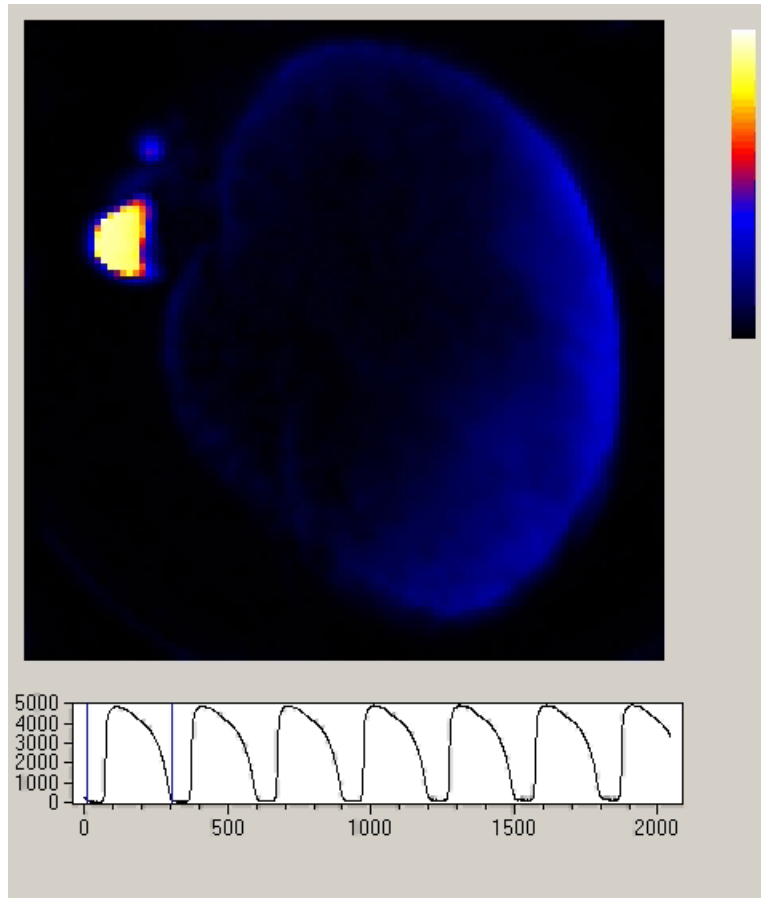
- QRecord: Files:** Shows the data file directory (D:\Data\2009\Dec\071209), file name template (07Dec2009.s*), and a table of saved files. The table includes columns for Cam, Filename, Start, Dur, and Comment.
- QRecord: Monitor:** Features a camera selection dropdown (Camera 1, 2), map scaling options (Fixed, Inst, Grow), and a real-time waveform plot. The plot shows a signal oscillating between approximately -100 and 100.
- QRecord: Setup:** Configures recording parameters such as Sampling rate (1000Hz), Input mode (Ref SE), and Gain. It also includes checkboxes for Pixel map, Acquiring, and Use analogue inputs.
- QRecord: Notes:** Displays a log of saved files with timestamps and details, such as "15:22:56: Saved 07Dec2009.s3 - 15:22:56 (2s) Soontaree stock Bleb vpace 60ms (4 bmp) 30ml/min".
- QRecord: Rec:** Shows recording presets (A, B, C) and acquisition settings. It includes a "Queued" counter (0) and a "Captured" counter (12).
- QRecord: View:** Displays a grid of recorded traces (red and green) and a zoomed-in view of a single trace (red) with a scale from -12400 to -11800.
- QRecord: Movie:** Provides a movie playback interface with a color-coded heatmap of the recorded data and playback controls (Play, Stop, Reverse).

Analysis software: Optiq (Delphi)

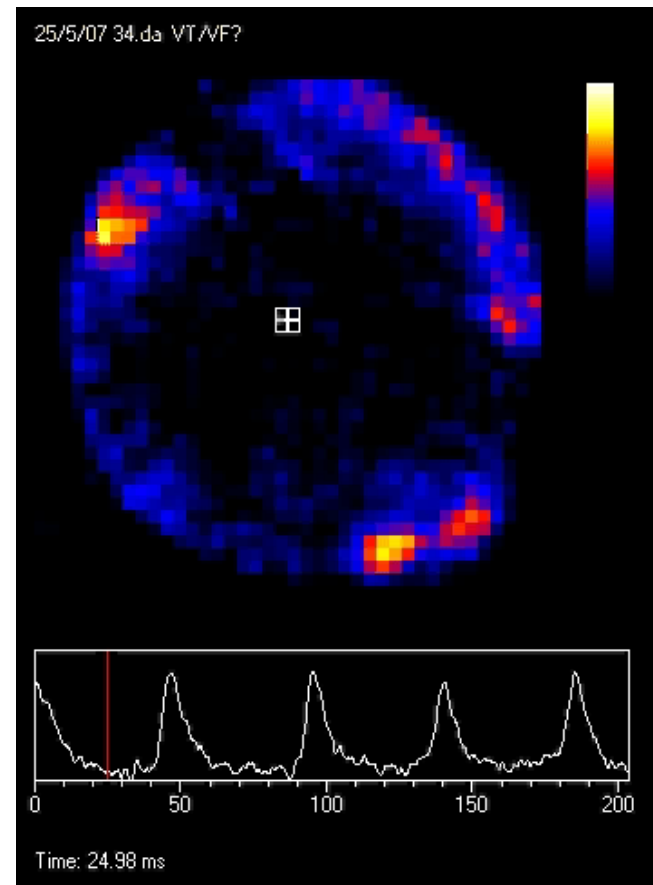


So far, software in use in 5 UK laboratories and UC Davis

Example of use: Visualising electrical waves in hearts



epicardial pacing (rabbit)



ventricular fibrillation (rat)

Services available

- free email/phone consultation on imaging hardware and image analysis software
- free visits to lab to discuss research problem and potential solutions
- training in use of existing commercial or freeware software (if suitable for task)
- development of custom software (if existing software unsuitable)
- ImageJ macros and plugins
- MATLAB scripts, functions and graphical user interfaces
- C/C++, Delphi, Java or Python standalone programs
- real-time or performance-critical drivers and standalone programs
- hardware interfacing
- training in user-level programming

Main areas of software development (& teaching)

- **ImageJ**

- macros & plugins

Java

Javascript

- **MATLAB**

- scripts & GUIs
- good built-in support (toolkit) for image processing

IDL

ITK

Python

Perl

- **Delphi** (compiled language)

- ideal GUI development language
- a few image processing libraries: PixeLook, GraphicEx, Intel IPL, ImgSource

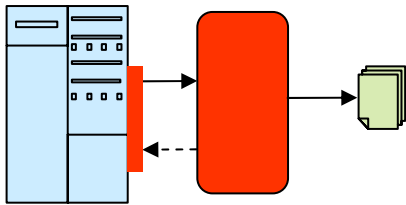
R statistics

Origin

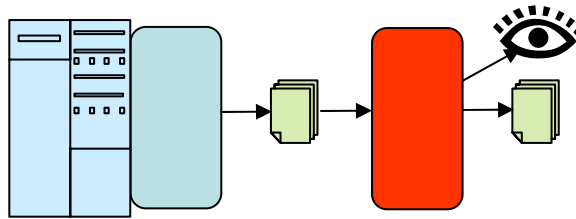
Excel

C / C++

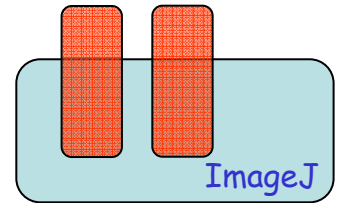
Solution patterns



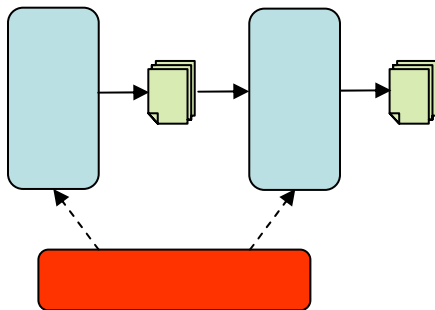
data acquisition



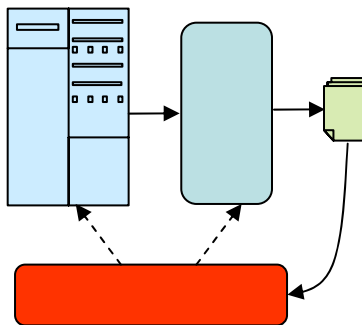
custom analysis



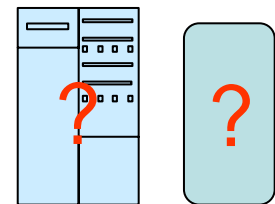
macros / plugins



scripting



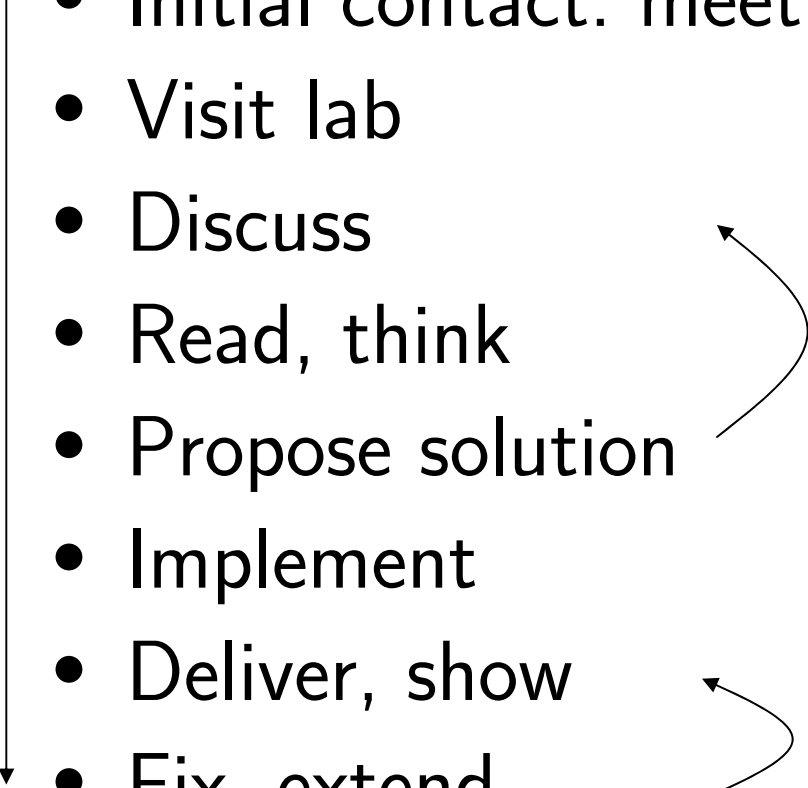
coordinating



advice

Blue = existing Red = custom

Typical process

- Initial contact: meeting, email
 - Visit lab
 - Discuss
 - Read, think
 - Propose solution
 - Implement
 - Deliver, show
 - Fix, extend
- 

Funding model

- service not tied to specific labs or equipment
- no consumables or equipment maintenance
- no charge to users of the 'facility'

Guiding principles

- Simple as possible
- Not re-invent wheel
 - but be prepared to develop new methods
- Cheap as possible
 - all software freely available
 - (charge for hardware development)
- Happy, productive customers
 - publications!