OMEGA: Shared resources for single particle tracking and for analyzing the dynamics of intracellular trafficking

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The Image Problem: can the quantitative content of images be fully exploited for systems biology and integrated with other types of data?

- Are they only pretty pictures?
- Are they measurements?
- Are results biased?
- Are results reproducible?
- Can image data and analysis results be re-used and re-interpreted?
- Can results be combined with other types of data?
Outline

• **WHO AM I AND WHAT I AM DOING DEALING WITH THESE STUFF?**

• **BIOLOGICAL MOTIVATION** → real-time trafficking of HIV-1 in target cells

• **APPROACH** → develop SHARABLE TOOLS, METADATA DESCRIPTORS and ERROR ACCOUNTING METHODS to:
  • Streamline the workflow
  • Capture the full flow of information and data across the workflow
  • Account for the propagation of error along the analysis routine

• **PROGRESS REPORT** → so far we have:
  • Produced a tool called OMEGA
  • Produced a data model / metadata standard called MIAPTE
  • Developed a sharable method to assess the error associated with motion type classification
Tracking fluorescently-labeled viral particles movement to gain insight into the underlying biology

VIRAL ENTRY

Infectious HIV-1 virus: < 1/10

Adapted from, McDonald Nature Methods (2006) 3: 782
Tracking fluorescently-labeled viral particles movement to gain insight into the underlying biology → VIRAL ASSEMBLY
Moving fluorescently-labeled virions are imaged at high temporal and spatial resolution.
Particle tracking: estimation of the trajectory followed by individual sub-diffraction particles
Particle Tracking output

particle \( p_k \)

\[
\begin{align*}
  t_0 & \quad x_0 \quad y_0 \\
  t_1 & \quad x_1 \quad y_1 \\
  \ldots & \\
  \ldots & \\
  \ldots & \\
  \ldots & \\
  t_n & \quad x_n \quad y_n
\end{align*}
\]

\((x_0, y_0)\)

\((x_1, y_1)\)

\((x_n, y_n)\)
Motion Analysis: computation of “biologically meaningful” metrics from individual trajectories

Capture time-series

Viral Particle Path

End

Estimated trajectory

Start

Uniform Segments

Tracking Measures

Motion Analysis

Segmentation

Linking

End

Start

Tracking Measures

Motion Analysis
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  • Streamline the workflow
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• PROGRESS REPORT → so far we have:
  • Produced the MIAPTE particle tracking data model / metadata standard
  • Produced the OMEGA particle tracking tool
  • Developed a sharable method to assess the error associated with motion type classification
Particle Tracking and Motion Analysis minimum reporting guidelines:

Minimum Information About Particle Tracking Experiments

- Available on github.org
  https://github.com/OmegaProject/MIAPTE

- Deposited on fairsharing.org
  https://fairsharing.org/bsg-s000671

- Posted to biorXiv.org:
  https://www.biorxiv.org/content/early/2017/07/13/155036
MIAPTE: based on the tracking workflow and on the resulting trajectory data structure.
MIAPTE v03 – Trajectory Elements
MIAPTE v03 (4DN) – Analysis Elements

Diagram showing relationships between MIAPTE v03 (4DN) analysis elements:

- **Algorithm**
  - ID
  - Name
  - AuthorName
  - ReleaseDate
  - Publication
  - Version
  - Description

- **RunDefinition**
  - ID
  - AlgorithmInformation
  - ParameterList

- **AnalysisRun**
  - ID
  - Name
  - AnalysisDate
  - Experimenter

- **Parameter**
  - ID
  - Name
  - Type
  - Value
  - Unit

- **PhotonCount**
  - CaliberationMethod [manual/camera]
  - Gain
  - GeoCenterDefinition [centroid/symmetry/local max]

- **LocalizationError**
  - LocalizationErrorDefinition [std/FWHM/median]
  - XLocalizationError
  - YLocalizationError
  - ZLocalizationError

- **SNREstimation**
  - Particle Area
  - Mean Intensity
  - LocalBackground
  - LocalNoise
  - LocalSNR
  - PlaneSignalIntensity
  - PlaneBackground
  - PlaneNoise
  - PlaneSNR

- **MobilityMeasures**
  - TotalTimeTraveled
  - TotalCurvDistTraveled
  - TotalNetStraightDistTraveled
  - MaxCumStraightDistTraveled
  - ConfinementRatio
  - DirectionalPersistence

- **VelocityMeasures**
  - AverageCurvSpeed
  - AverageStraightSpeed
  - ArrestCoefficient
  - ForwardProgressionLinearly

- **Tracking Measures**
  - TrajectorySegmentMap

- **Intensity Measures**
  - MaxPeakIntensity
  - MinPeakIntensity
  - AveragePeakIntensity
  - MaxCentroidIntensity
  - MinCentroidIntensity
  - AverageCentroidIntensity
  - MaxMeanIntensity
  - MinMeanIntensity
  - AverageMeanIntensity
  - MaxLocalBackground
  - MinLocalBackground
  - AverageLocalBackground
  - MaxLocalNoise
  - MinLocalNoise
  - AverageLocalNoise
  - MaxLocalSNR
  - MinLocalSNR
  - AverageLocalSNR

- **Diffusivity Measures**
  - MSDofn Array
  - ObservedDiffusionConstant2Lin
  - ObservedDiffusionConstant2Log
  - MomentScalingSpectrum [Array]
  - SlopeLog-LogMSD
  - SlopeMSS

- **Diff. Meas. Error Estimation**
  - MinDetectODC2
  - UncertaintyODC2
  - UncertaintySMSS

- **Anisotropy Measures**
  - RadiusGyrX
  - RadiusGyrY
  - RadiusGyrZ
  - Asymmetry
A tool for the management, analysis, and dissemination of intracellular trafficking data that incorporates motion type classification and quality control

Open Microscopy Environment inteGrated Analysis

- Posted to biorXiv.org
  http://biorxiv.org/cgi/content/short/251850v1

- Available on github.org
  https://github.com/OmegaProject/Omega
The goal is to automate the Single Particle Tracking and Motion Analysis workflow and capture the entire data life-cycle...

... with the purpose of fostering the reproducibility of the process, the (re-)use and (re-)interpretation of results and the standardization of error reporting
... the solution is to integrate both work-flow and data-flow in a unified framework
OMEGA: integrates workflow, dataflow and data provenance.
OMEGA: easy to use graphical user interface
OMEGA: motion analysis workflow
OMEGA: example use case – Nocodazole abrogates mobility during MPMV assembly
OMEGA data browser: analysis results management

Image ID: NA
Owner: Caterina Strambio De Castiglia
Name: VIRUS snr 7 density low.tif
Times Analyzed: 1
Acquired: 2015-03-03 12-03-34
Imported: 2015-03-03 12-06-57
Dimensions (XY): 512 x 512
Pixel Type: uint8
Dimensions (ZTC): 1 x 100 x 1

Parameters:
- Radius: 2
- Cutoff: 0.001
- Percentile: 0.1
- Absolute Percentile: false
- Analyzed Z-plane: 0
- Analyzed Channel: 0
Executed: 2017-11-08 10-31-28
Times Analyzed: 2
Average number of spots found per time point: 77.51
Max number of spots found per time point: 97
Min number of spots found per time point: 60

Parameters:
- Displacement: 20.0
- Link Range: 1
- Movement Type: Brownian
- Object Feature: 1.0
- Dynamics: 1.0
- Optimizer: Greedy
- Min Track Length: 25
Executed: 2017-11-08 10-50-36
Times Analyzed: 1
Total number of trajectories: 59
Average trajectory length: 32.728813559322035
Max trajectory length: 49
Min trajectory length: 26

Parameters:
- Displacement: 20.0
- Link Range: 1
- Movement Type: Brownian
- Object Feature: 1.0
- Dynamics: 1.0
- Optimizer: Greedy
- Min Track Length: 25
Executed: 2017-11-08 10-50-36
Times Analyzed: 1
Total number of trajectories: 59
Average trajectory length: 32.728813559322035
Max trajectory length: 49
Min trajectory length: 26
OMEGA data browser: analysis results management

Image 1

Detection run 1

Particles 1

Linking run 1

Trajectories 1

Editing run 1

Edited Trajectories 1

Segmentation run 1

Trajectory segments 1

PD1 analysis definition

PL1 analysis definition

TE1 analysis definition

TS1 analysis definition

Detection run n

Linking run n

Editing run n

Segmentation run n
FAIR requirements of Particle Tracking Data

Data acquisition description
Raw data

OMERO
OME-xml

Data analysis definition and execution
Analysis results + Error
Results Visualiz.

Biological Source
Experimental Process
Sample description

ISA-tab
Shared ontologies
MIBBI guidelines
MIACME

OMEGA
MIAPTE

MIACME guidelines

University of Massachusetts Medical School
umassmed.edu
Data management: **OMEGA vs. OMERO**

- Image data/metadata management
- Image processing (with ImageJ/Fiji)
- User interaction
- Single Particle Tracking
- Trajectory management
- Movement analysis
- Statistical analysis
- Results visualization and exploration
- Work- and data-flow integration
- Data provenance
- Error propagation
- Data semantics
Accounting the effect of particle detection uncertainty on motion type estimation

A Monte Carlo simulation method to assess motion type estimation error in multiple particle tracking

• In preparation
Motion type classification in OMEGA is based on a method developed by the MOSAIC group.

The motion type is evaluated by the evaluation of four plots:

1. x - y Trajectory plot
2. Log-log MSD vs. Δt plot
3. Moment Scaling Spectrum (MSS) plot
4. Slope of MSS vs. Observed Diffusion Constant phase space plot
The behavior of trajectories on the four plots allows to classify their dynamics and understand their biological functioning.
How can we be confident about motion type classification? What are the sources of error in motion analysis?

1) Localization error: inversely proportional to PRECISION and ACCURACY

2) Sampling error: the shorter the trajectory (i.e. the fewer detected points) the more likely one will make an error in calculating metrics that describe the type of motion
Monte Carlo simulation method to empirically estimate the accuracy and precision of each particle localization algorithm

1. Generate artificial images containing individual simulated point sources

2. Simulate particle observation in 12 representative image quality scenarios (i.e. Peak Intensities)

3. Estimate particle position using the desired Particle Detector

4. Compute $DGT_{PosV}$ by subtracting expected from observed particles coordinates

5. Store distributions of $\Delta x$ and $\Delta y$ values for each Detector and input Peak Intensity value

6. For each Detector, plot $\Delta x$ and $\Delta y$ values as a function of input Peak Values and compare with published results

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Expected

(x\text{Exp}, y\text{Exp})

Observed

(x\text{Obs}, y\text{Obs})

$DGT_{PosV}$

($\Delta x$, $\Delta y$)

Particle Detector$_i$, Input Peak Intensity$_k$

Image$_0$ > ($\Delta x_0$, $\Delta y_0$)

Image$_i$ > ($\Delta x_i$, $\Delta y_i$)

... Image$_n$ > ($\Delta x_n$, $\Delta y_n$)
Simulating the effect of position error on artificial trajectories

When simulating the effect of position error on artificial trajectories, we sample uniformly at random directly from distributions of empirically observed x and y offsets, to “modify” the position of each point along the particle path.

\[
(x_{1\_noise}, y_{1\_noise}) = (x_1 + \Delta x, y_1 + \Delta y)
\]

Infinitely precise trajectory

“Noisy” trajectory
Assessing and reporting motion type estimation error

1. Load image into OMEGA
2. Run Particle Detector
3. Estimate local SNR values and compute aggregate SNR values for each trajectory
4. Compute ODC and SMSS for each trajectory
5. Compile trajectory lists including Length, SNR, ODC and SMSS values
6. Extrapolate Std.Dev. for ODC and SMSS from stored 4D matrices
7. Report confidence intervals on Phase Space

Particle Pk
\[ t_0 x_0 y_0 I_0 \quad \text{Bkg}_0 \quad \text{Ns}_0 \quad \text{SNR}_0 \]
\[ t_1 x_1 y_1 I_1 \quad \text{Bkg}_1 \quad \text{Ns}_1 \quad \text{SNR}_1 \]
\[ \cdots \]
\[ t_n x_n y_n I_n \quad \text{Bkg}_n \quad \text{Ns}_n \quad \text{SNR}_n \]

MSD/\Delta t (log-log)

MSS

Phase Space

Image ij
\[ T_0 L_0 \quad \text{SNR}_0 \quad \text{ODC}_0 \quad \text{SMSS}_0 \]
\[ T_k L_k \quad \text{SNR}_k \quad \text{ODC}_k \quad \text{SMSS}_k \]
\[ \cdots \]
\[ T_n L_n \quad \text{SNR}_n \quad \text{ODC}_n \quad \text{SMSS}_n \]
OMEGA: example use case – Reporting error using particle tracking benchmarking datasets
Thank you from the OMEGA team

Project co-founders

Image acquisition

Software engineering and infrastructure

Motion analysis and algorithms integration