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

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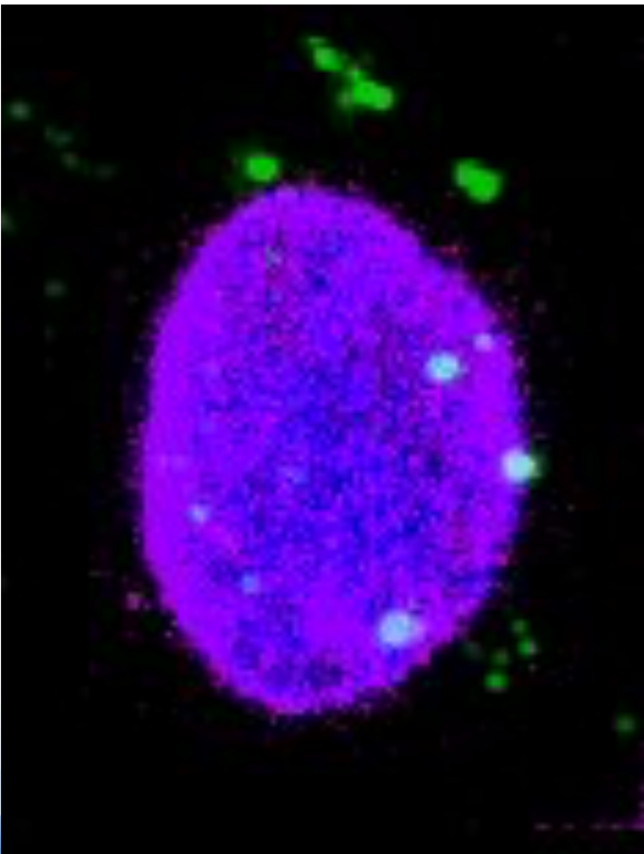
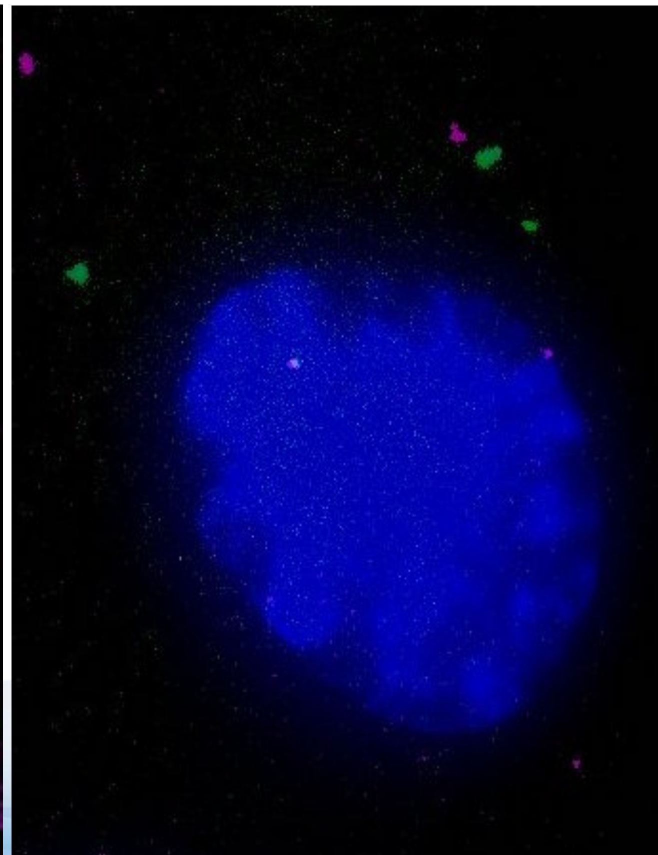
2018-5-30, 2018 OME Meeting, Dundee

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





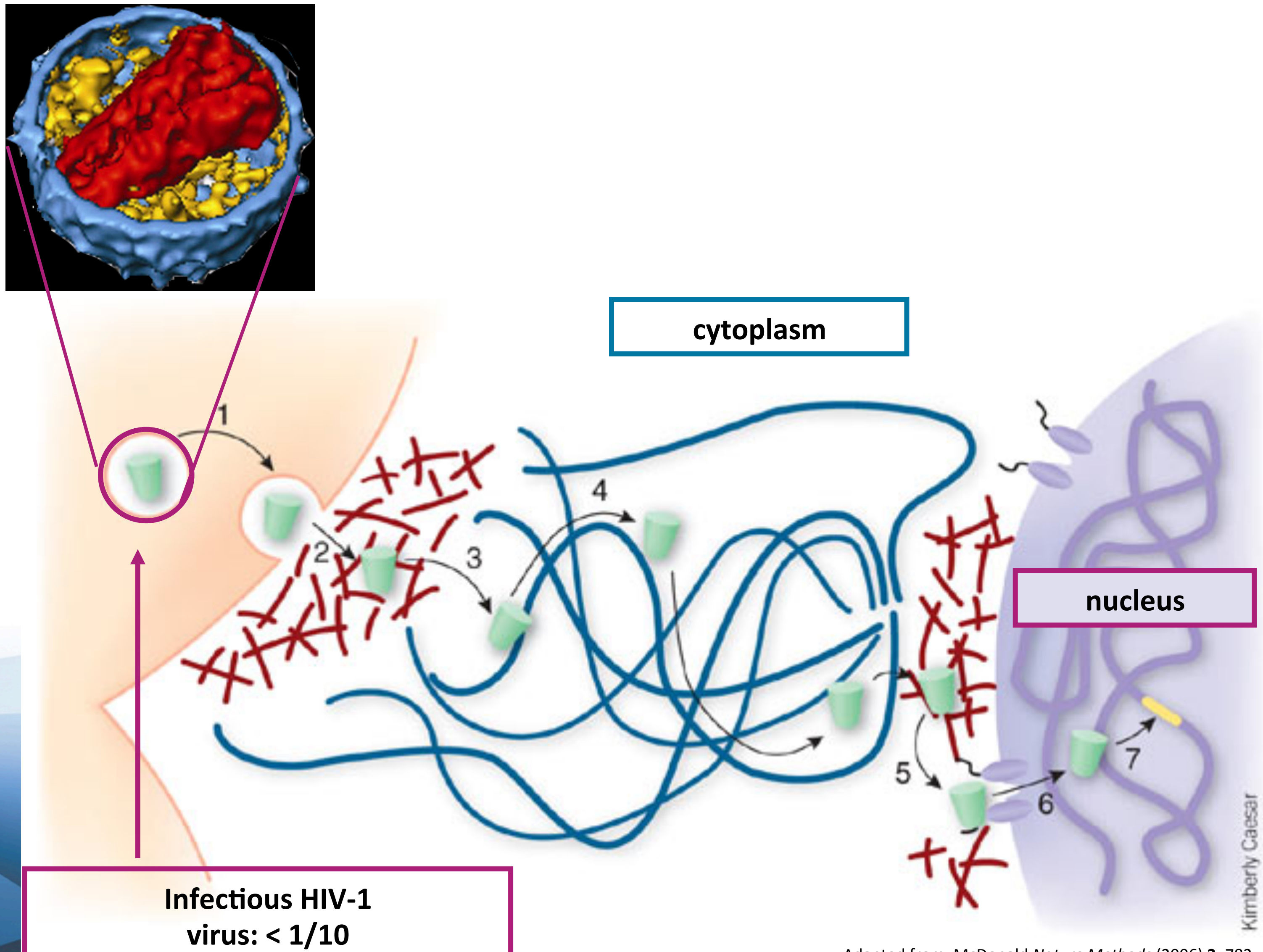
- Are they only pretty pictures?
- Are they measurements?
- Are results biased?
- Are results reproducible?
- Can image data and analysis results be re-used and re-interpreted?
- Can results be combined with other types of data?

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

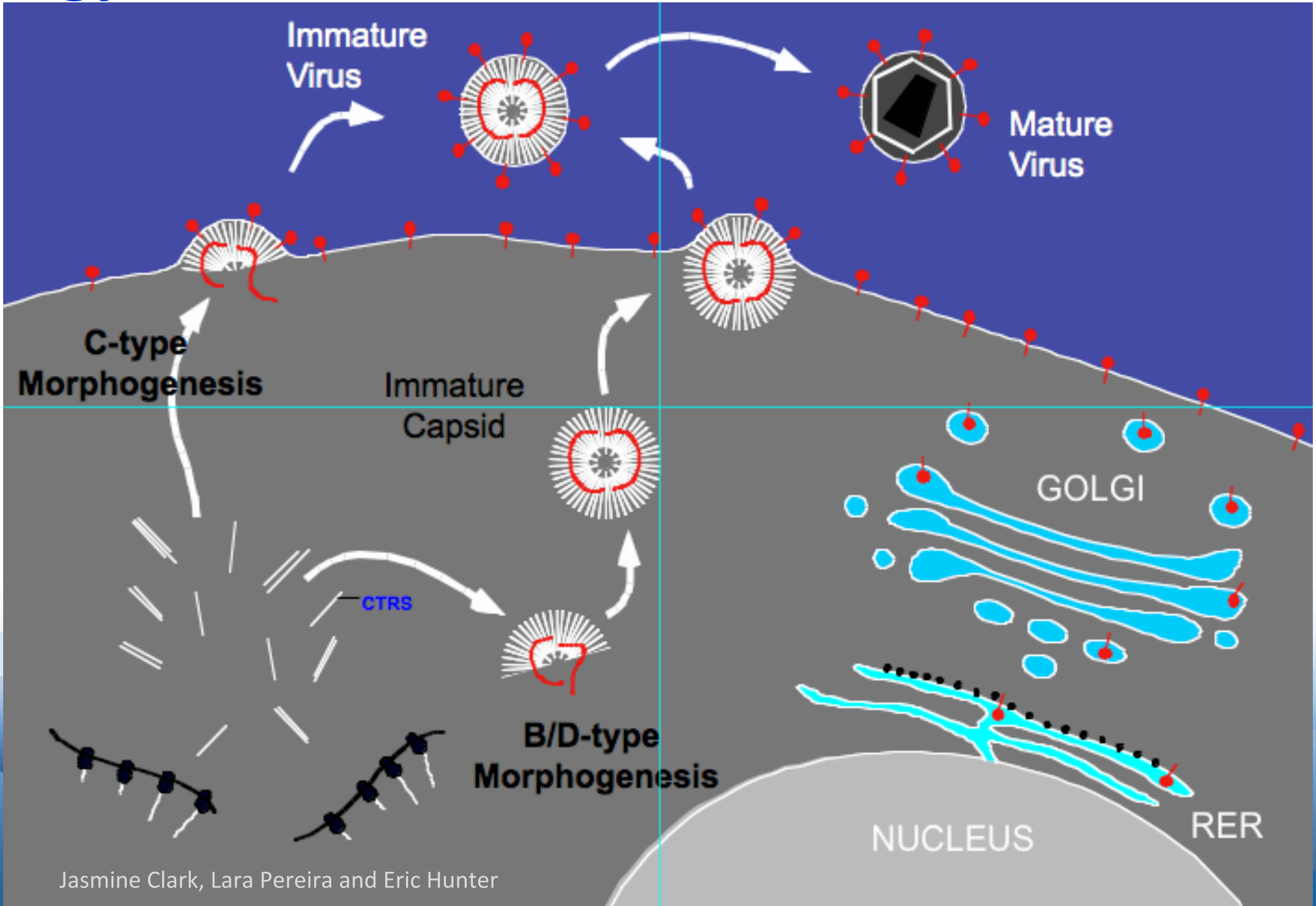
Tracking fluorescently-labeled viral particles movement to gain insight into the underlying biology

→ VIRAL ENTRY

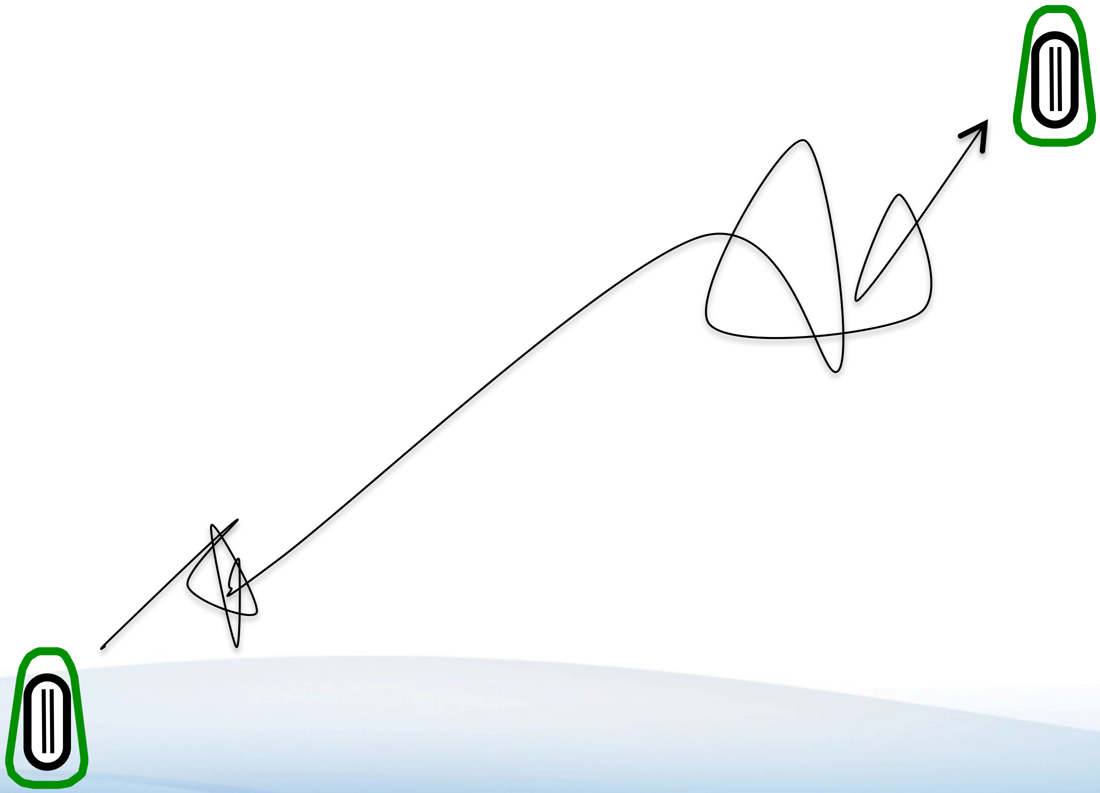


Adapted from, McDonald *Nature Methods* (2006) **3**: 782

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



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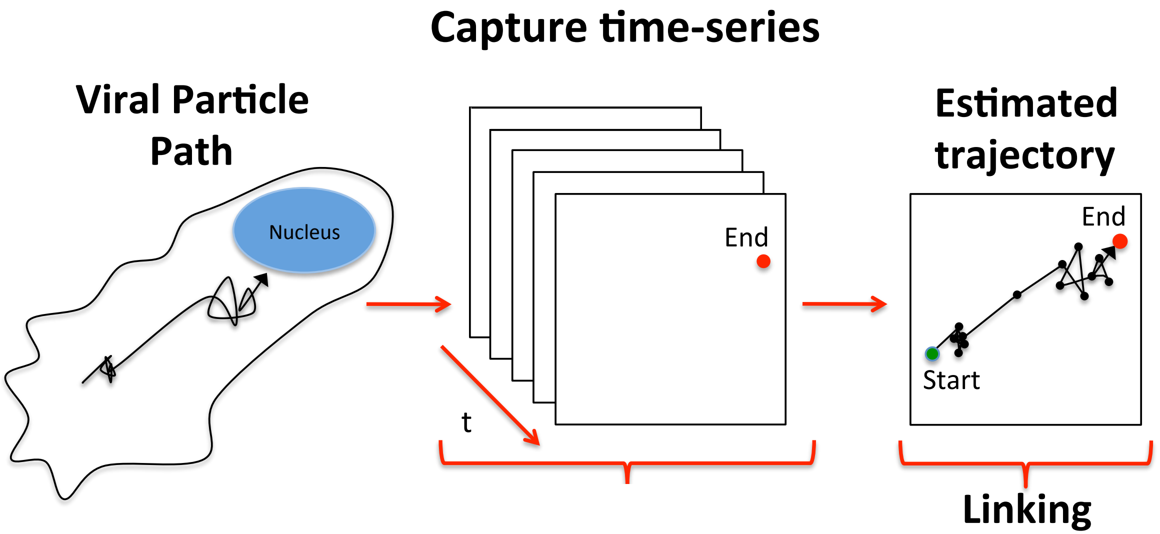
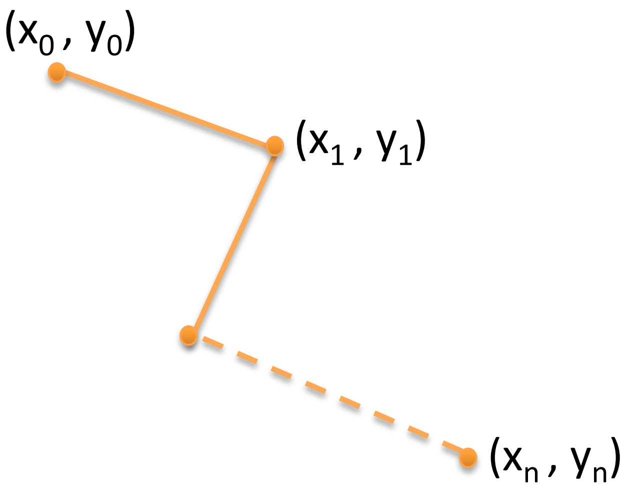
Particle tracking : estimation of the trajectory followed by individual sub-diffraction particles



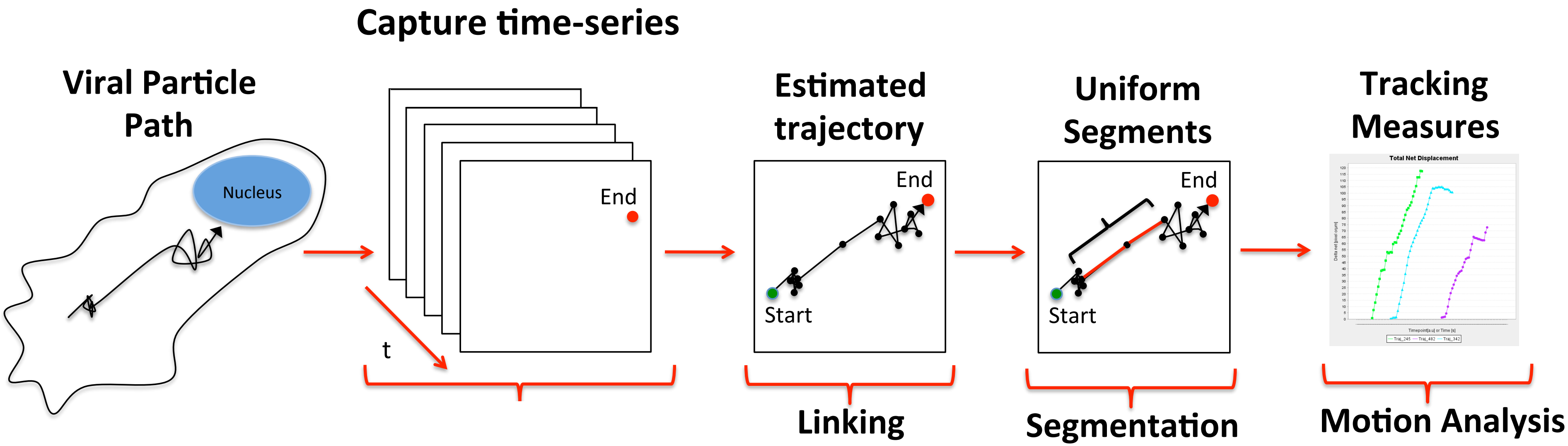
Particle Tracking output

particle p_k

$t_o \quad x_0 \quad y_0$
$t_1 \quad x_1 \quad y_1$
…
…
…
…
$t_n \quad x_n \quad y_n$



Motion Analysis: computation of "biologically meaningful" metrics from individual trajectories



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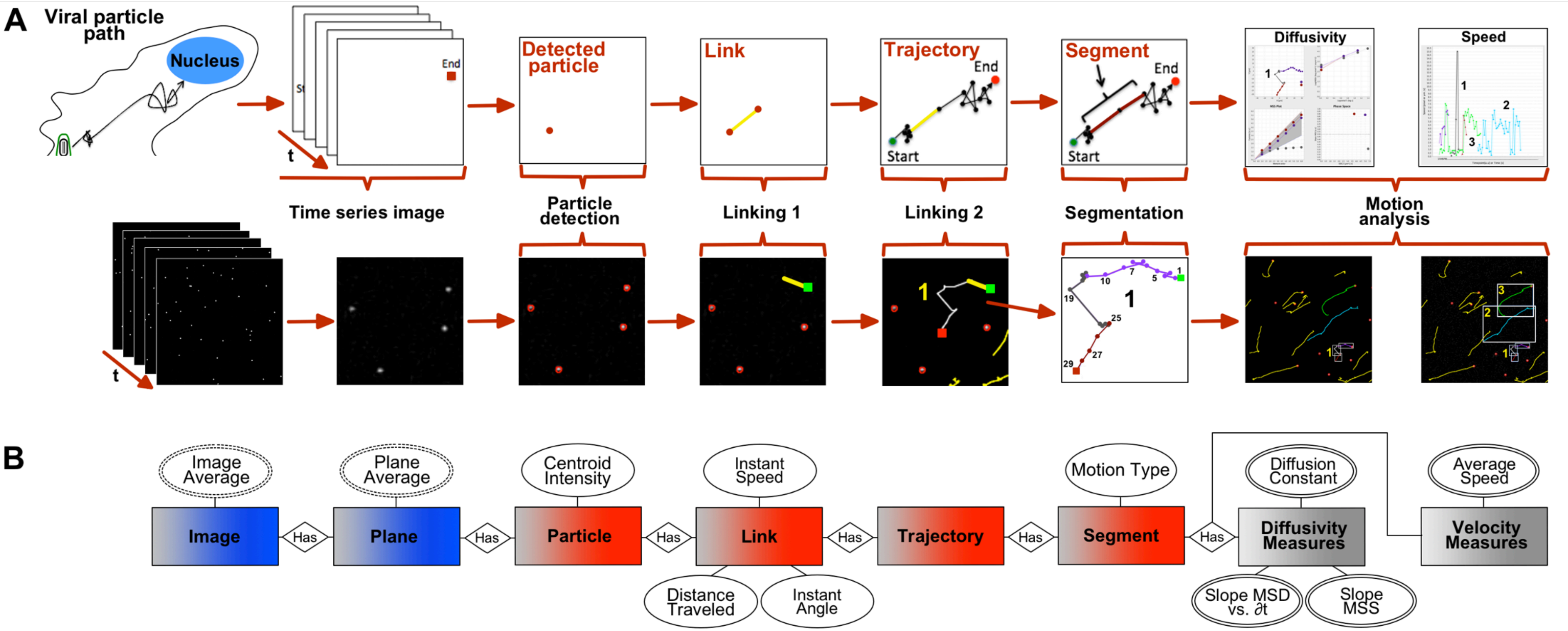
- **PROGRESS REPORT** → so far we have:
    - Produced the MIAPTE particle tracking data model / metadata standard
    - Produced the OMEGA particle tracking tool
    - Developed a sharable method to assess the error associated with motion type classification

Particle Tracking and Motion Analysis minimum reporting guidelines:

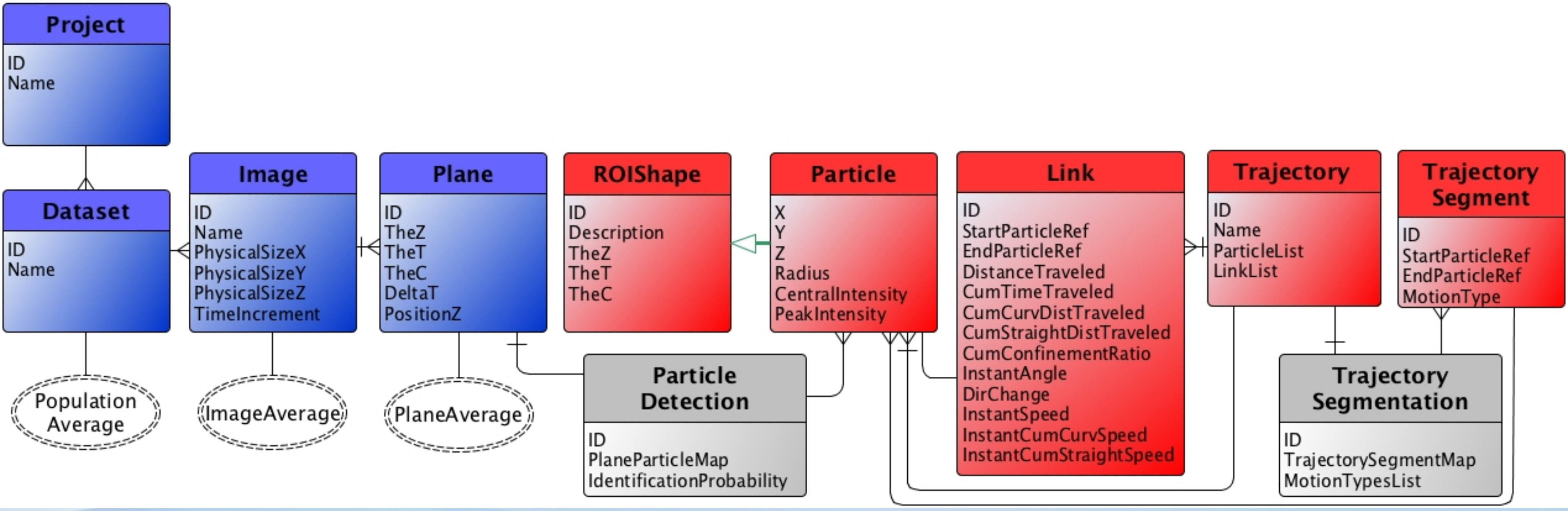
Minimum Information About Particle Tracking Experiments

- 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

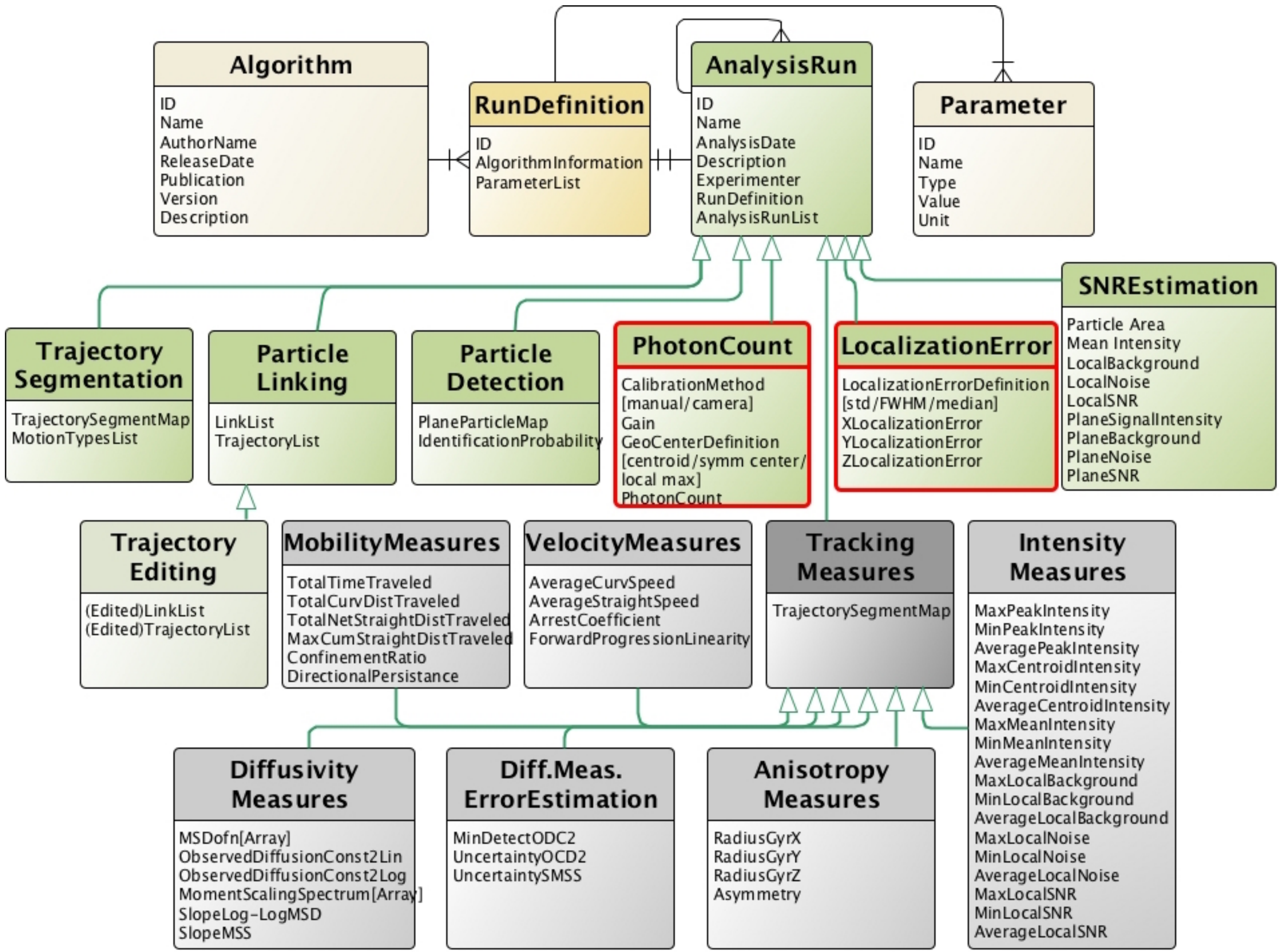
MIAPTE: based on the tracking workflow and on the resulting trajectory data structure



MIAPTE v03 – Trajectory Elements



MIAPTE v03 (4DN) – Analysis Elements



A tool for the management, analysis, and dissemination of intracellular trafficking data that incorporates motion type classification and quality control

Open **M**icroscopy **E**nvironment inte**G**rated **A**nalysis

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

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

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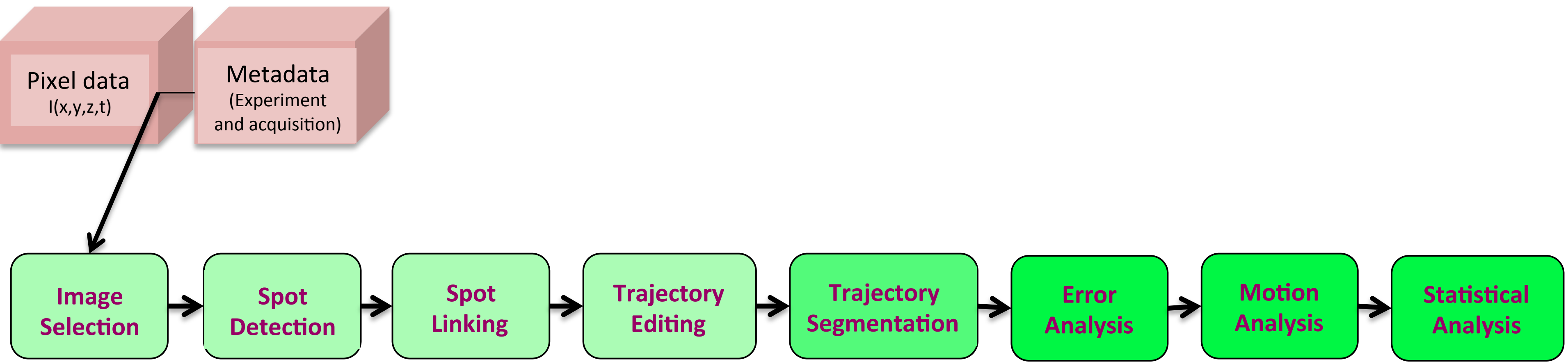
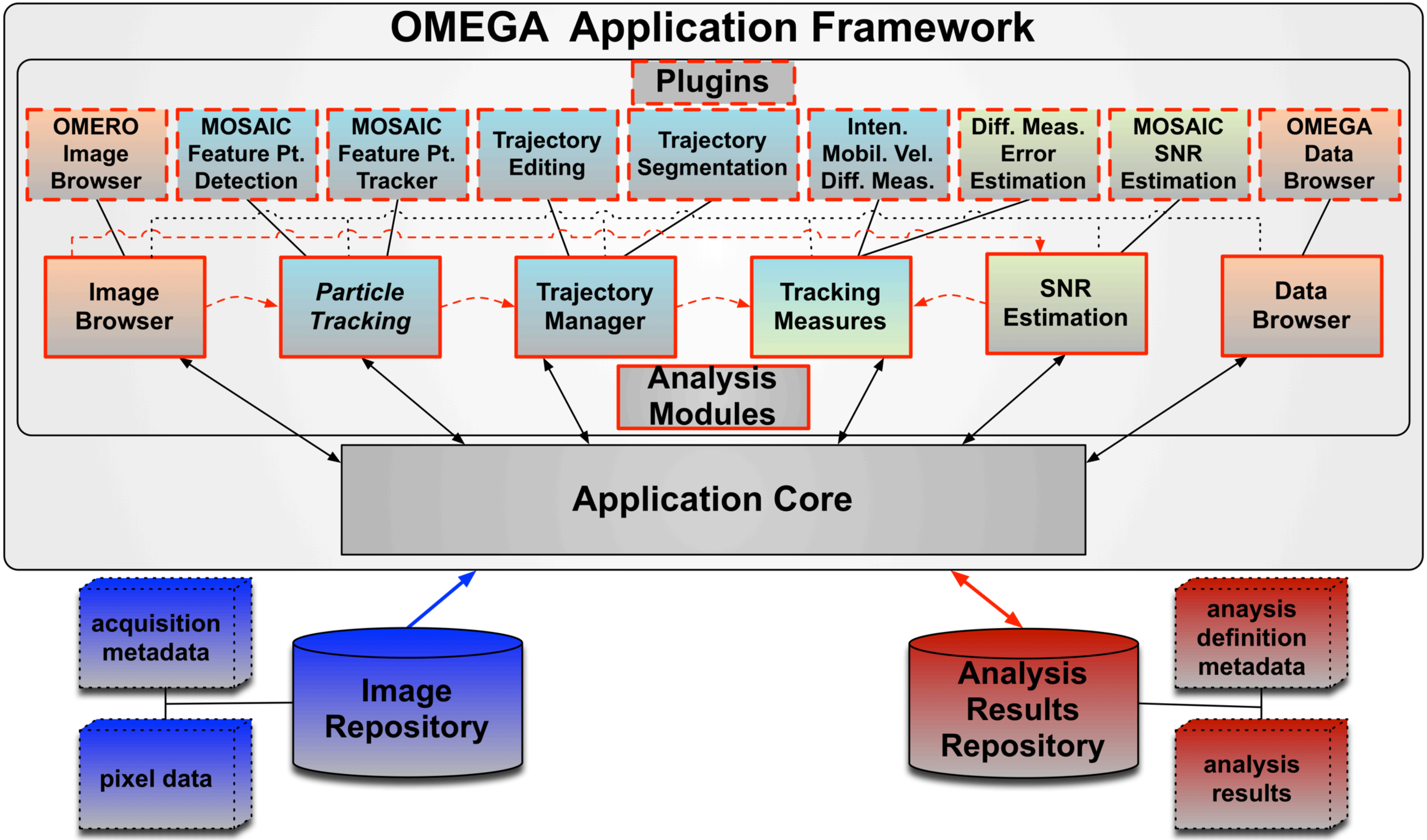
The goal is to automate the Single Particle Tracking and Motion Analysis workflow and capture the entire data life-cycle...

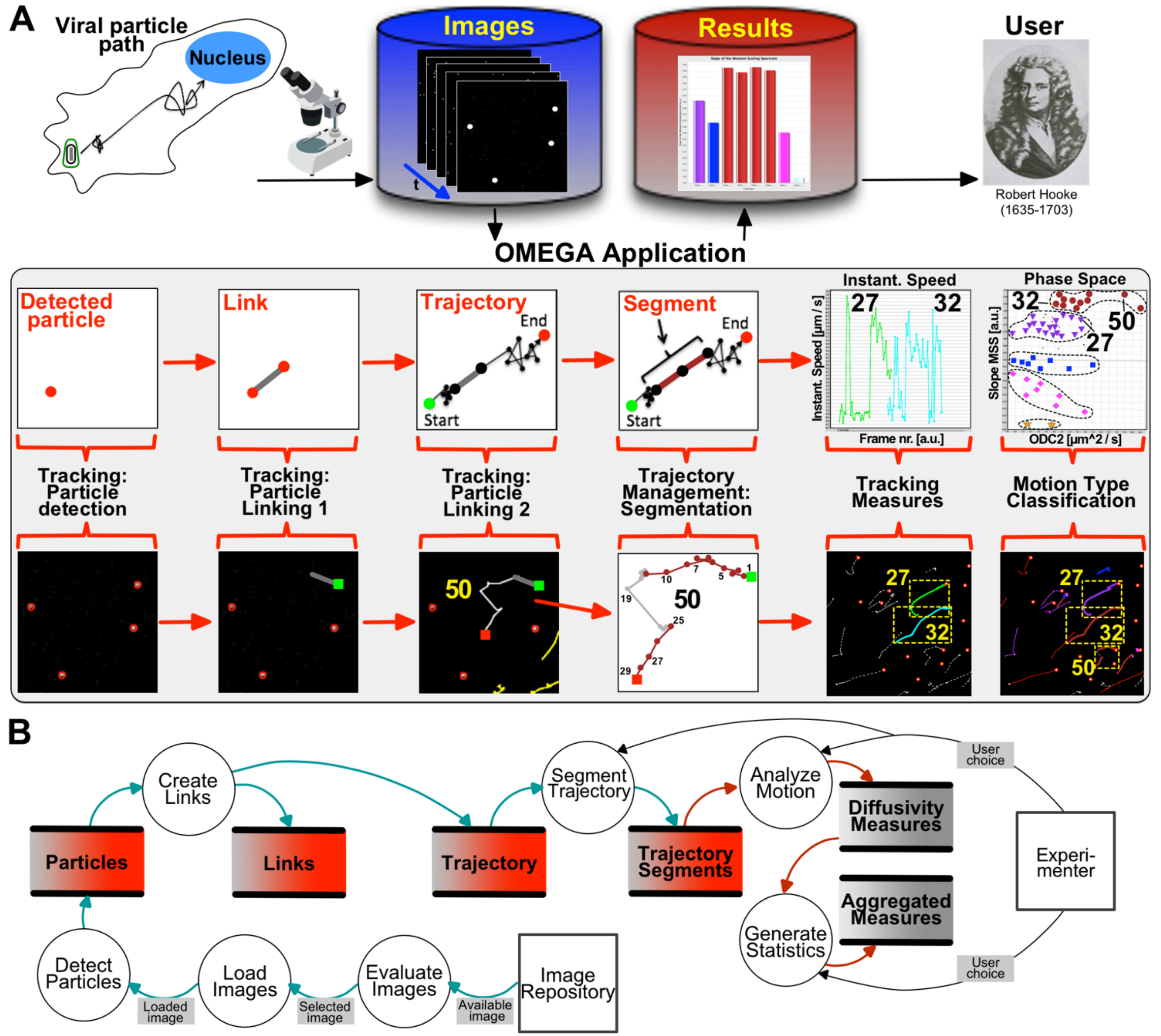


... with the purpose of fostering the reproducibility of the process, the (re-)use and (re-)interpretation of results and the standardization of error reporting

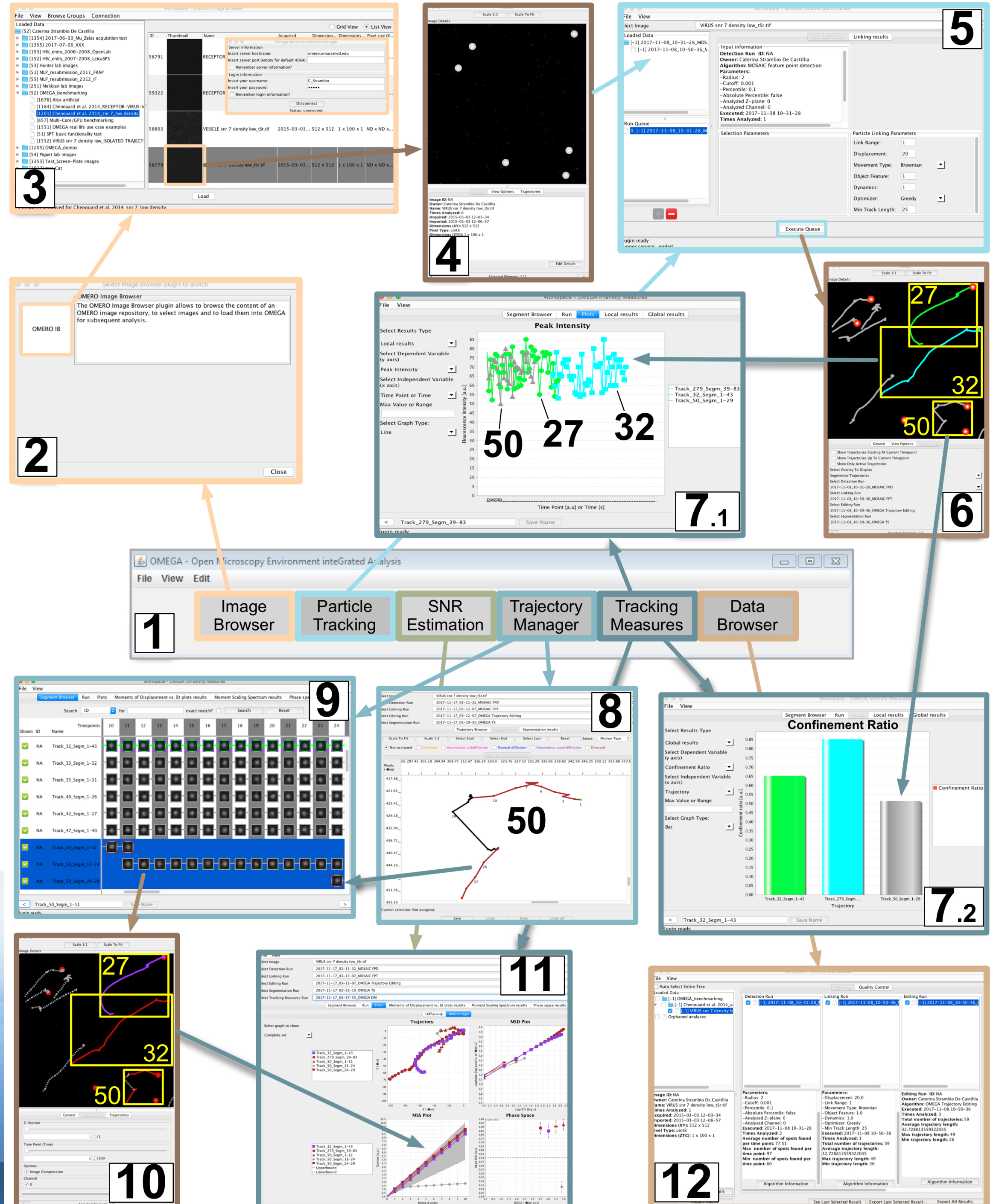
… the solution is to integrate both work-flow and data-flow in a unified framework



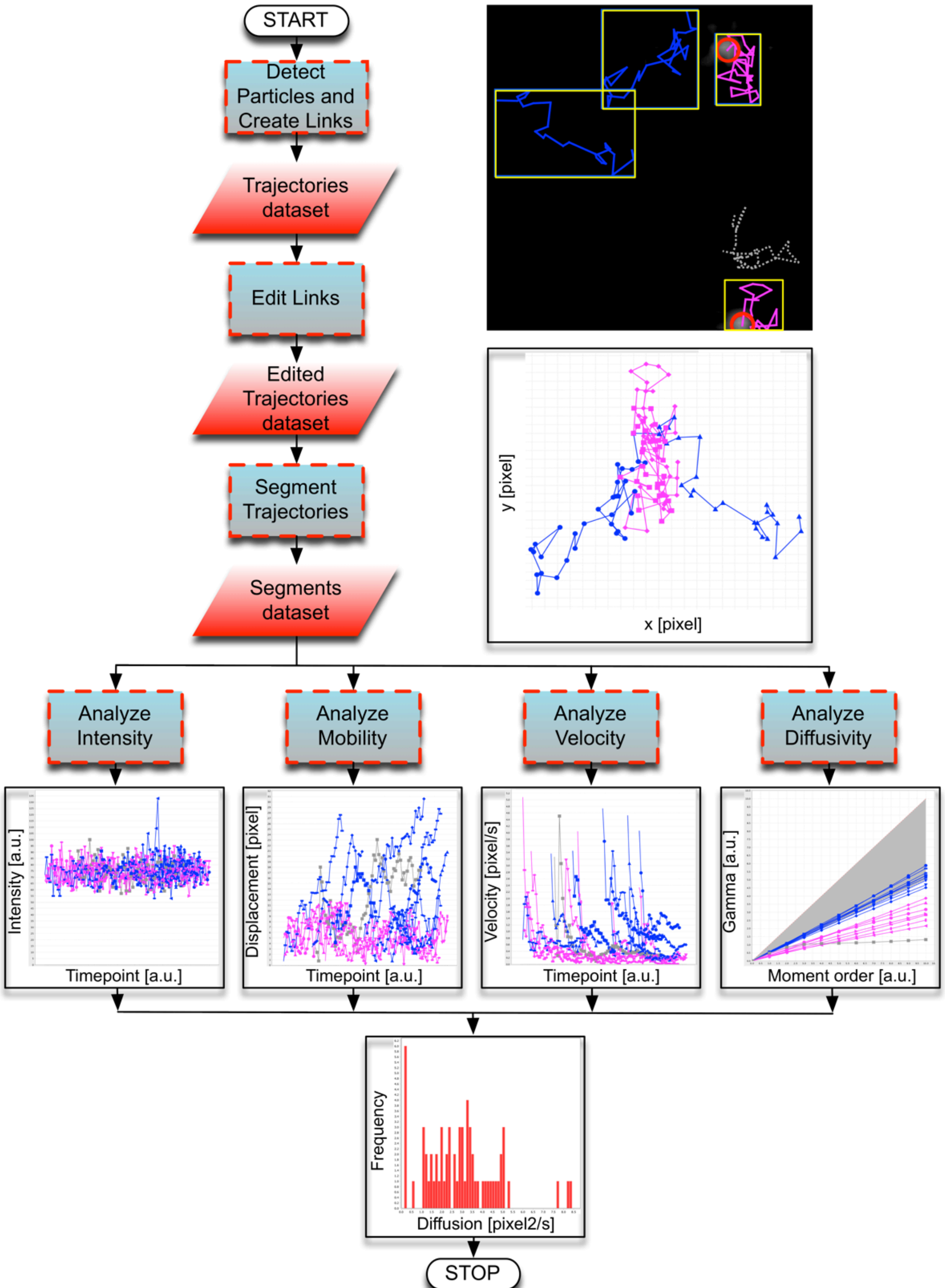
OMEGA: integrates workflow, dataflow and data provenance



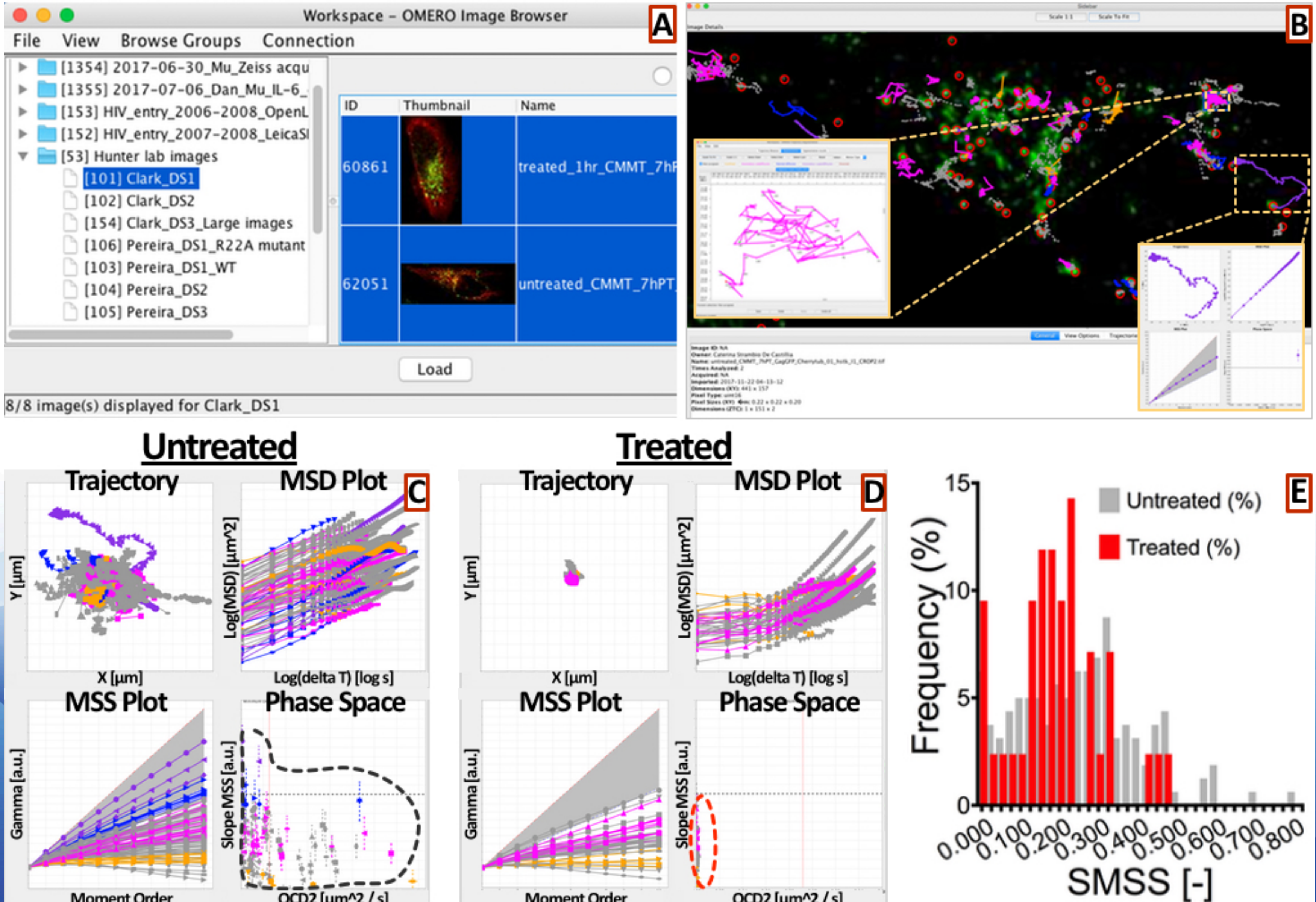
OMEGA: easy to use graphical user interface



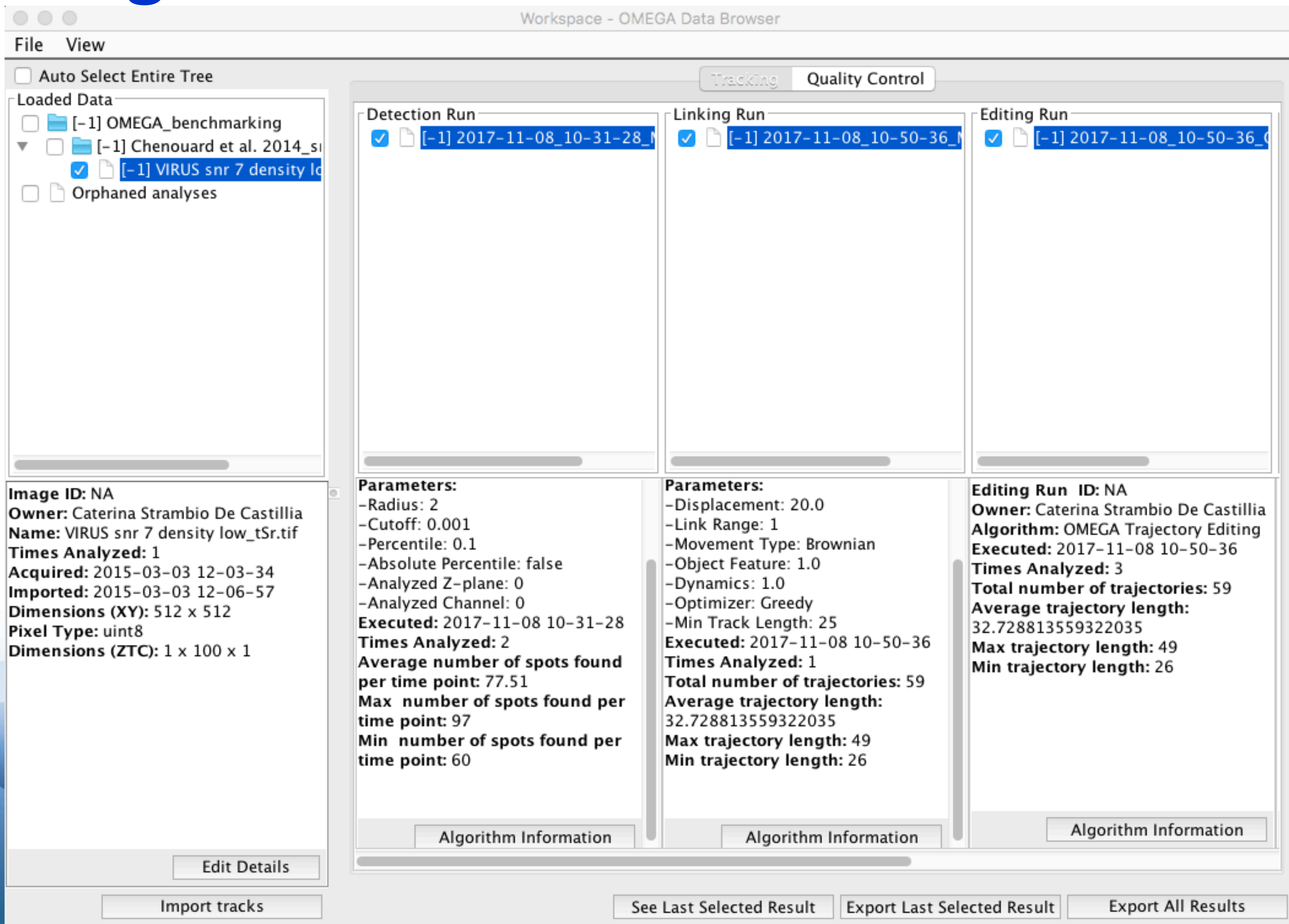
OMEGA: motion analysis workflow



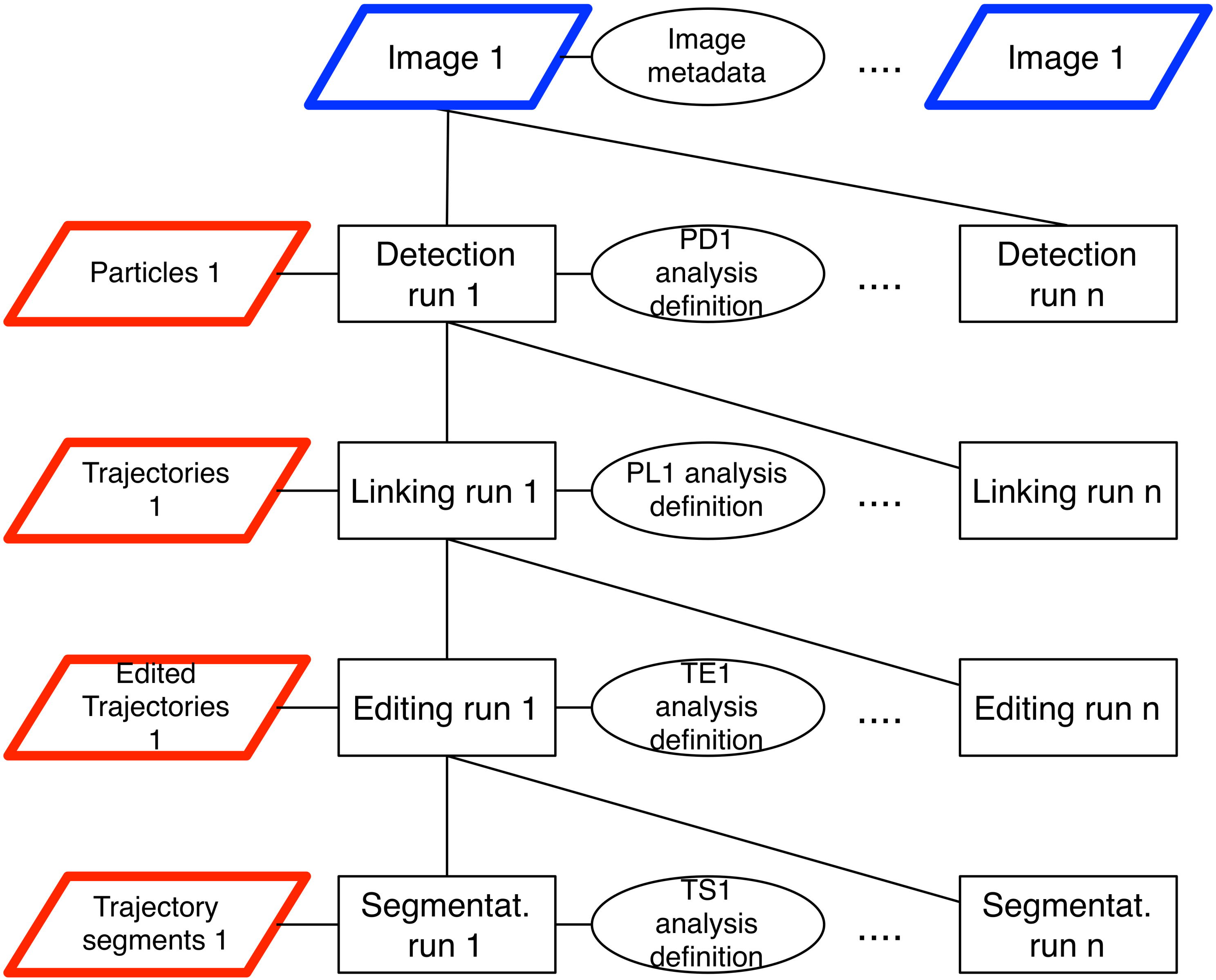
OMEGA: example use case – Nocodazole abrogates mobility during MPMV assembly



OMEGA data browser: analysis results management



OMEGA data browser: analysis results management



FAIR requirements of Particle Tracking Data

OMERO

OME-xml

Data acquisition description

Raw data

Biological Source

Experimental Process

Sample description

Data analysis definition and execution

Analysis results + Error

Results Visualiz.

ISA-tab

Shared ontologies

MIBBI guidelines

MIACME

OMEGA

MIAPTE

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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

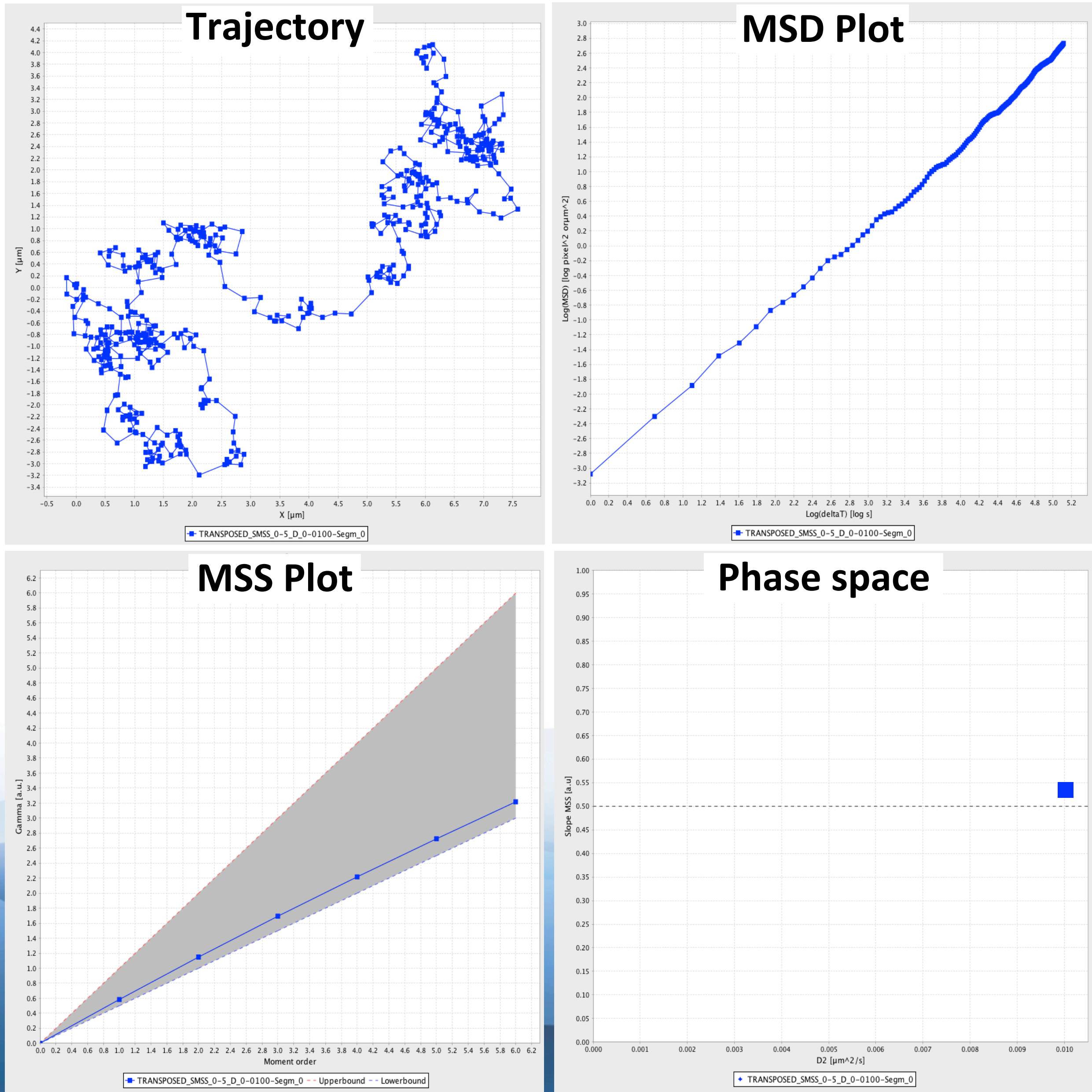
- Work- and data-flow integration
- Data provenance
- Error propagation
- Data semantics

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

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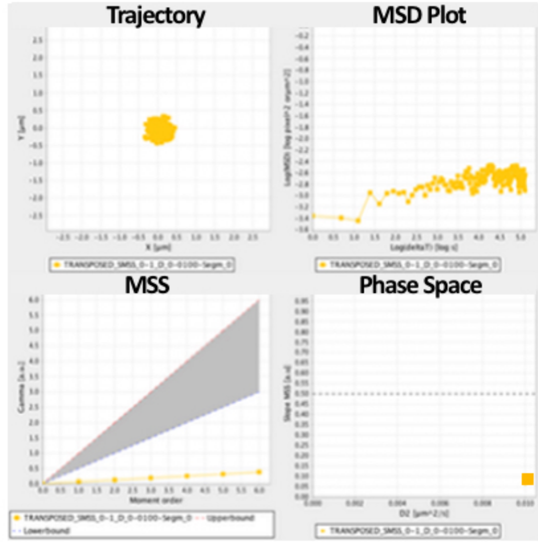
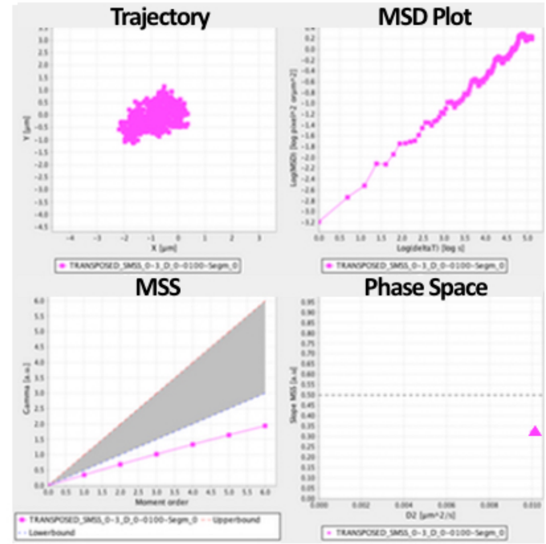
The behavior of trajectories on the four plots allows to classify their dynamics and understand their biological functioning

Motion types

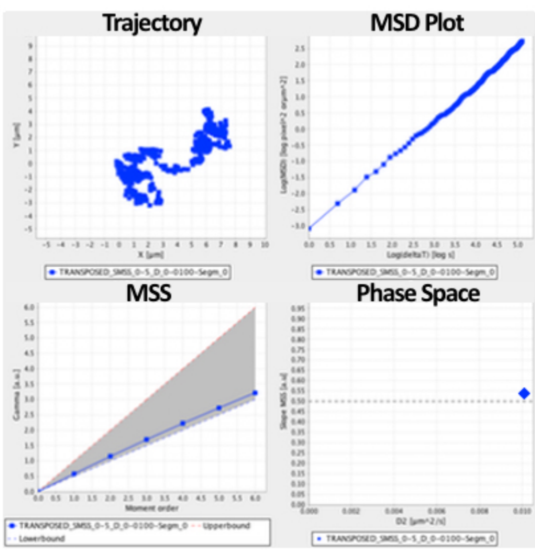
- Interaction with immobile structure (e.g. NPC) → Confined
- Corraled motion → Sub diffusion
- Default state → Normal diffusion
- Interaction with drifting structure (e.g. actin cytoskeleton) → Super diffusion
- Directed motion (e.g. along microtubules) → Directed



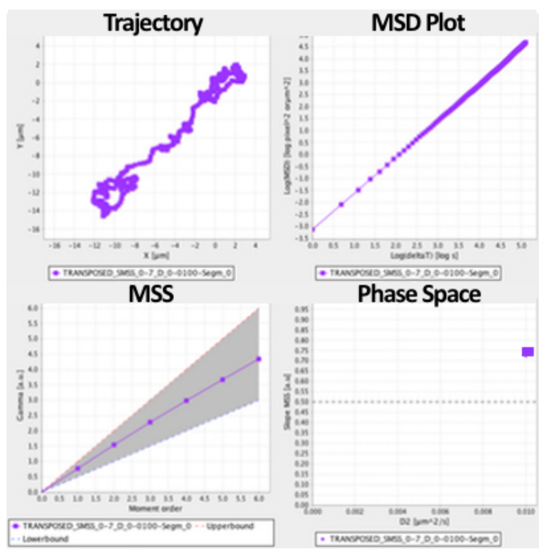
Confined



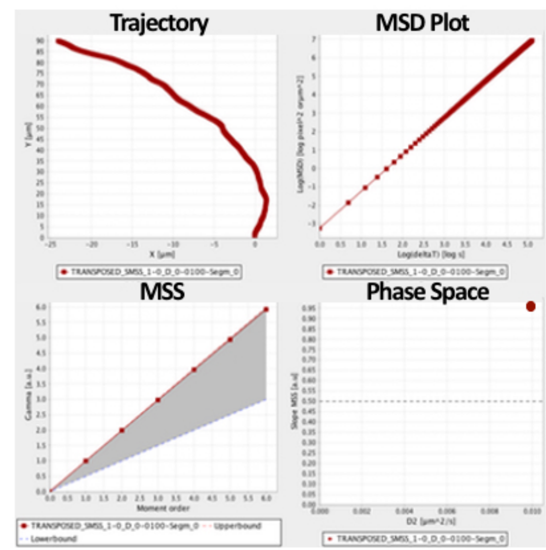
Subdiffusion



Normal diffusion



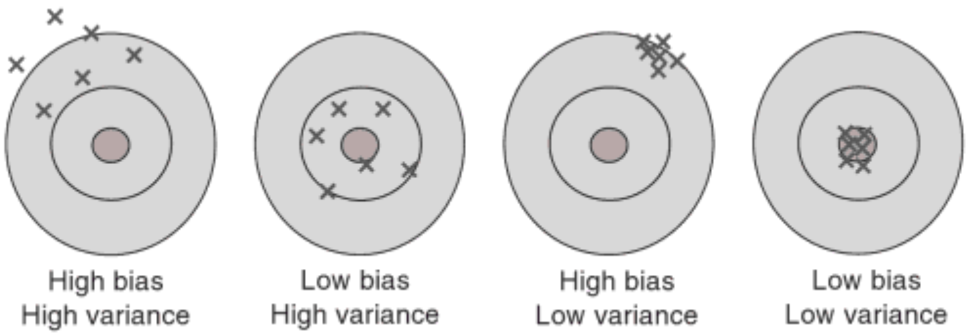
Superdiffusion



Directed

How can we be confident about motion type classification? What are the sources of error in motion analysis?

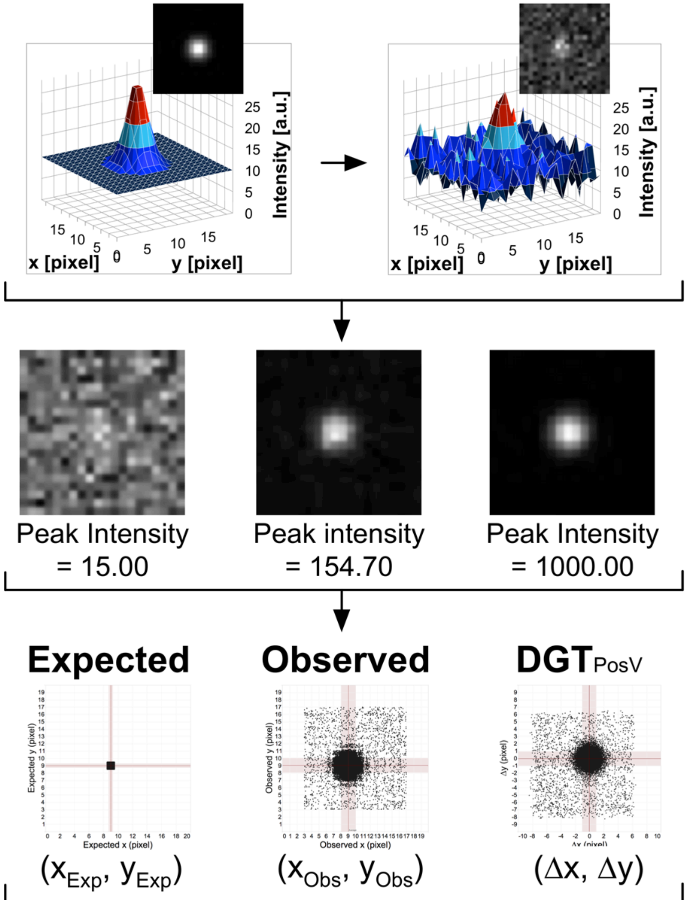
1) Localization error: inversely proportional to PRECISION and ACCURACY



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 Δx and Δy values for each Detector and input Peak Intensity value
6. For each Detector, plot Δx and Δy values as a function of input Peak Values and compare with published results

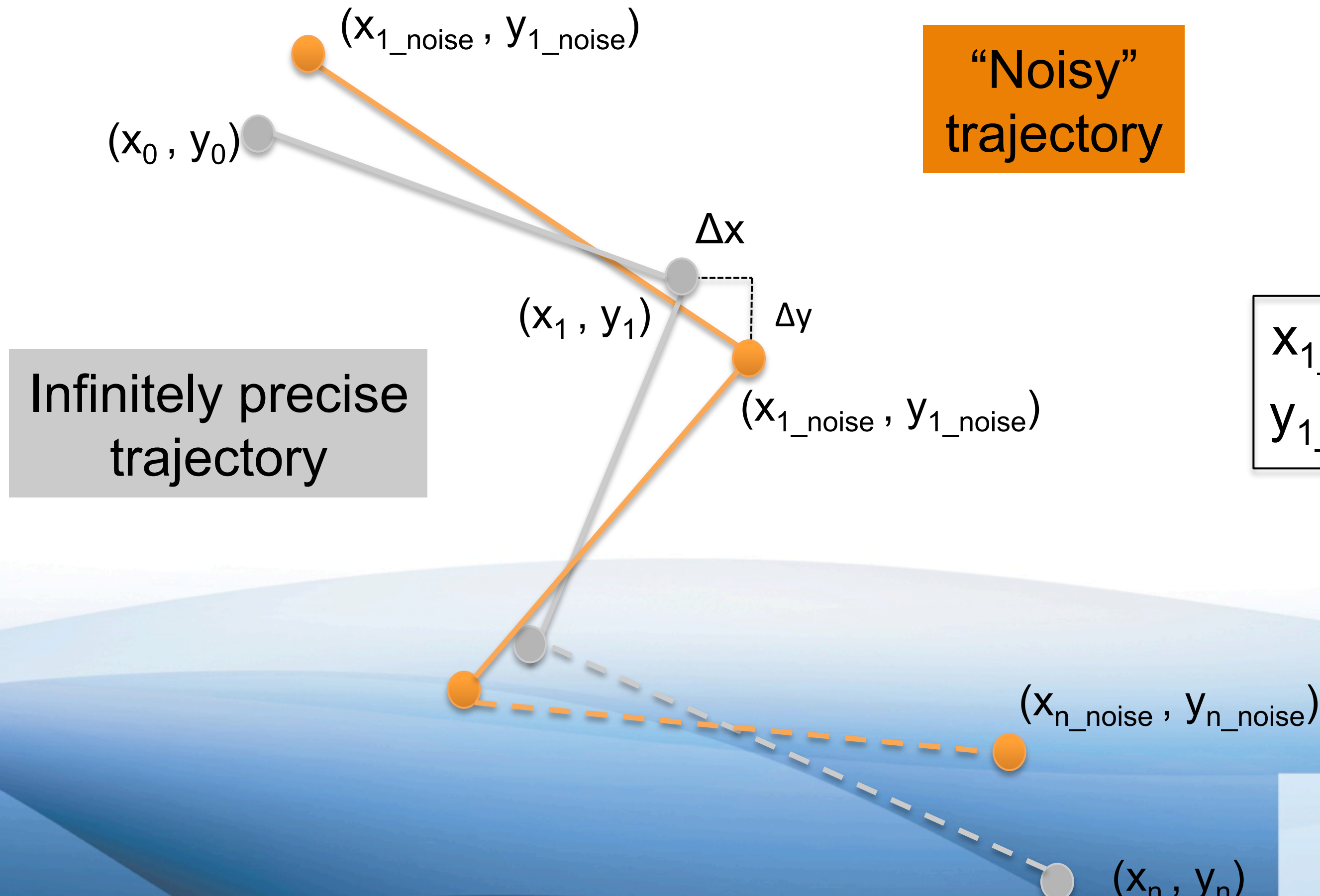


Particle Detector$_i$
Input Peak Intensity$_k$

Image$_0$ > (Δx_0, Δy_0)
Image$_i$ > (Δx_i, Δy_i)
…
Image$_n$ > (Δx_n, Δy_n)

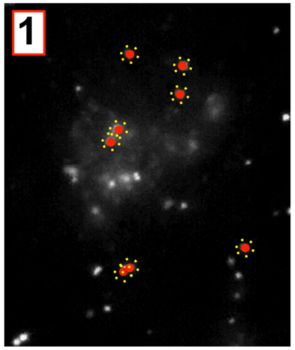
Simulating the effect of position error on artificial trajectories

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

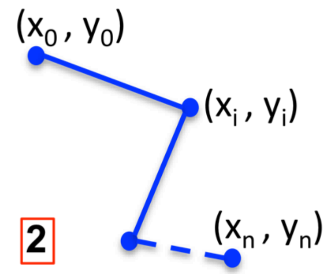


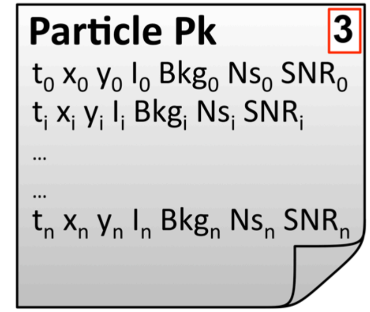
$$x_{1\_noise} = x_1 + \Delta x$$
$$y_{1\_noise} = y_1 + \Delta y$$

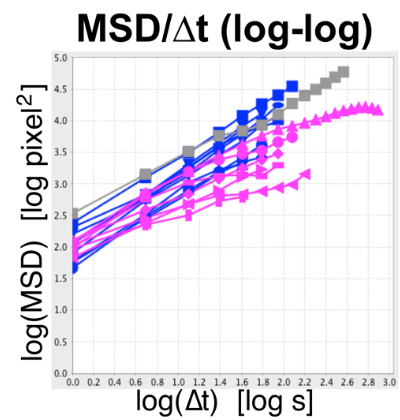
Assessing and reporting motion type estimation error

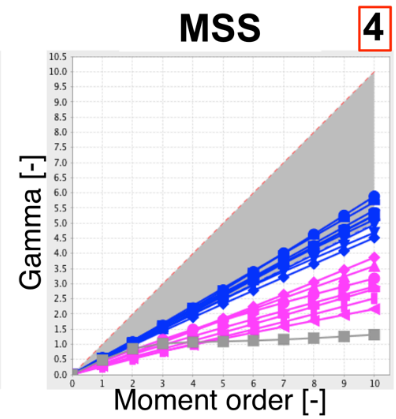


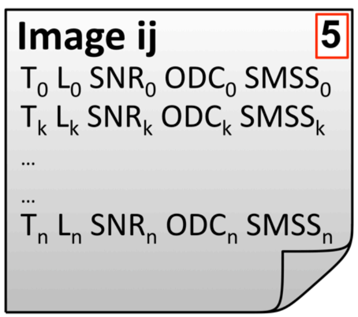
1. Load image into OMEGA
2. Run Particle Detector
2. Run Particle Linker
3. Estimate local SNR values and compute aggregate SNR values for each Trajectory
4. Compute ODC and SMSS for each trajectory
5. Compile trajectory lists including Length, SNR, ODC and SMSS values
6. Extrapolate Std.Dev. for ODC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

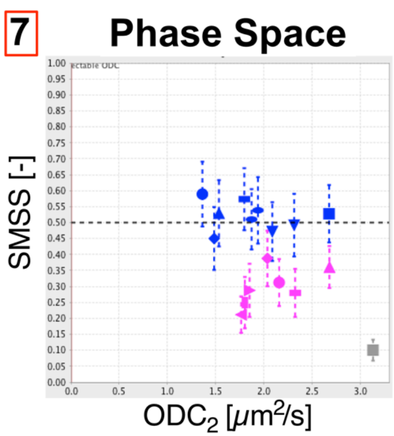


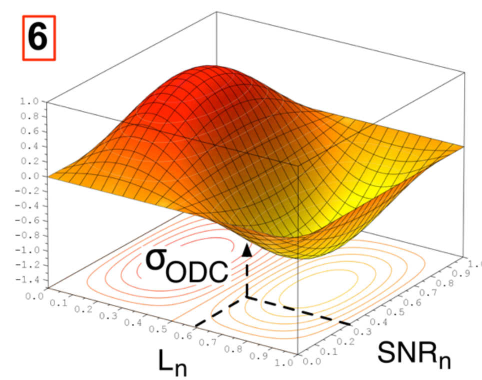




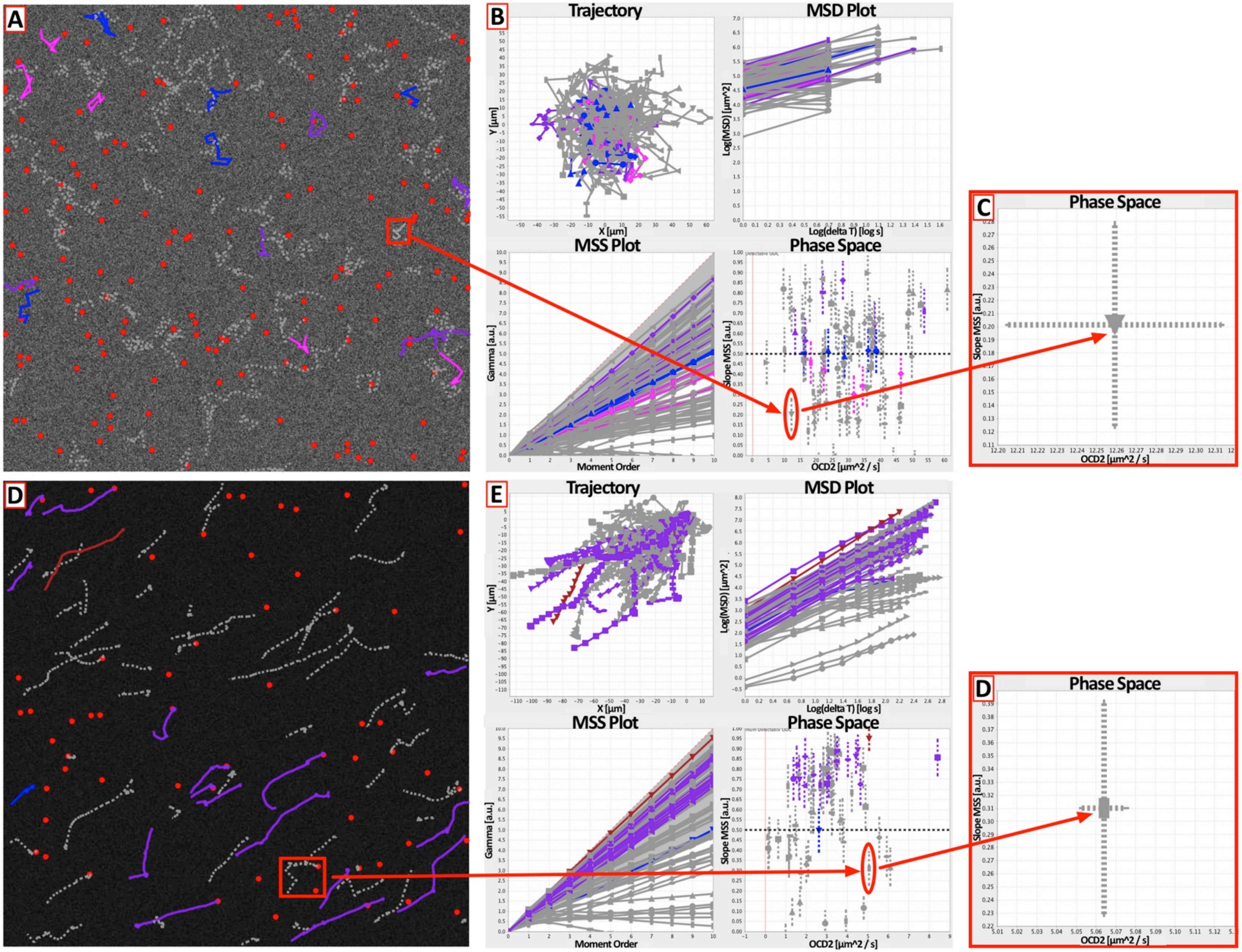








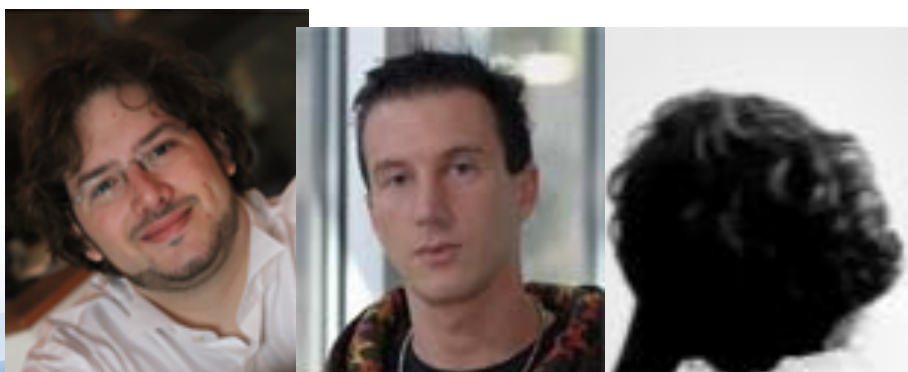
OMEGA: example use case – Reporting error using particle tracking benchmarking datasets



Thank you from the OMEGA team

Project co-founders

Image acquisition



Software engineering and infrastructure

Motion analysis and algorithms integration

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