Live imaging

- Systems evolution
  - Confocal microscopy / spinning disk
- Higher sensor sensitivity
- Fluorophores research

- Fast acquisition of 3D, multichannel
- stacks in time
- Tracking: Keeping trace of the displacement of detected objects.
- The ability of many types of cells to migrate is central to processes of major importance in biological research. Insight into many dynamic processes has been derived from tracking the movement of fluorescently labelled structures.

- Some examples: Progenitor cells in embryonic development, neuronal steam cells displacement fields, chromosomes motion, immune system cells migration, etc.
Acquisition tips

- Choose a time interval coherent with the dynamics you want to observe
- Consider that Signal to Noise Ratio decreases for higher frame rates
Acquisition tips

• **BLEACHING** OF YOUR SAMPLE DUE TO HIGH NUMBER OF ACQUISITIONS.
Basic Movement analysis

- PROJECTING ALL TIME POINTS ONTO A SINGLE FRAME YIELDS TRAJECTORIES (MAX INTENSITY, SDEV)

Standard Deviation

MIP

BG Corrected MIP
Manual tracking

- The position of the object in each frame is marked manually
  - Very slow and work intensive procedure (particularly in 3D)
  - Accuracy of obtained values is not very high/biased.
  - Repeated measurements might be required for higher accuracy.
  - Marking of particles can be inexact if the objects are ‘big’ or with high density.
  - Difficult to reproduce results.

Common image processing software offer tools to facilitate manual tracking and to map particles displacement and speed.
Automatic and semi-automatic tracking

1. De-noising: Gaussian, Median, Wavelets
2. Bleaching correction: Fit Exponential
3. Perform Tracking

### MAIN APPROACHES

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Automatic and semi-automatic tracking

ADVANTAGES & DRAWBACKS

- Local maxima / objects segmentation and analysis in time:
  - Easiest to implement
  - Follows variegated trajectories in a specific way
  - Need single objects segmentation (contrast, noise)
  - Need small displacement between frames and continuity
  - Cell-cell contact difficult to resolve

- Intensity-based image correlation:
  - Useful if cells are recognizable by morphology
  - Better results with cell-cell contact and discontinuity problems
  - Less influenced by noise and poor contrast
  - Follows only the global displacement
  - Computationally slow
  - Cell morphology cannot change dramatically in following time points
  - Works poorly if cells density is really high and the cells look the same
Objects analysis in time

• The neighborhood of a detected object is analysed in the following time point to find object correlations.

• Some examples of correlation strategies:

  ➢ **Brownian motion**
    - Define the region in which the object will move from one time point to the next
    - Random motion

  ➢ **Autoregressive algorithm**
    - The most probable motion between $T_1$ and $T_2$ is the same as between $T_0$ and $T_1$
    - It is possible to define a smaller radius $r$

Typical input parameters

- **MaxDistance**: 20
- **MaxGapSize**: 3

*PTBIOP Course: Tracking, February 2014*
Objects analysis in time

• A CASE STUDY
  – Characterization of gel visco-elastic properties by Brownian motion analysis
  – High-frequency acquisition through resonant scanner confocal microscopy.

The features finding code finds high intensity peaks in the image as candidate features, none within a given distance from another candidate.

The tracking code links the positions found in successive frames into trajectories. The algorithm tries to minimize the sum of the distances between features in two successive frames. A maximum displacement limits the matching to only those features closer than what the user knows to be extremely unlikely distances the feature may travel between two frames. Any feature with no match in the successive frame will be assigned a distance of this maximum allowable displacement. The algorithm can be made to remember features over more than immediately successive frames, so that if a feature disappears momentarily (out of the focal plane, typically), it can be matched to its previous trajectory segment when it reappears.
Common problems

Excessive displacement or shape change

- Possible failure with both objects segmentation and images correlation methods
- Increase image speed (higher memory need, longer time for analysis, eventually lower SNR)
- Decrease your magnification (loss of resolution)

Cells-cells interaction

- Decrease cells seeding density (limited by the biological system)
- Restrict rate of cells growth
- Use software which allows user intervention
Common problems

- **Cells entering and leaving the field of view**
  - Drop cells which are leaving the field of view / the focal plane.
  - Use software which allows user intervention.

- **Mitosis**
  - Pattern recognition.
  - Eventually work backwards in time.

- **Image drift**
  - Need a stationary reference in the field of view (dead cell, bead...).
  - Track the reference and then subtract out your reference movement from the movement of the cells.
  - Drift correction algorithms.
Common problems

- **REFERENCE DRIFT, CASE STUDY**

- Calculation of Global Displacement (ImageJ)
- Measure of Real Displacement (Metamorph)
- Evaluation of Specific Displacement (Real-Global)
Common problems

• REFERENCE DRIFT, CASE STUDY

Original Film

After StackReg Registration
Common problems

- REFERENCE DRIFT
- MetaMorph manual tracking
- Evaluation of global displacement and cells specific motion
• POST-PROCESSING: FILTERING

Different types of filters:
Track duration, Length, Average or Instantaneous speed, Number of Time Points, Objects Intensity.
Representing results

- **PER TRACK EVOLUTION**

- **MEASUREMENT DISTRIBUTIONS**

  The diffusion coefficient describes how the square of the distance from the track origin grows over time.
Representing results

- BIRTH / DEATH
- MAPS

- FLOW MAPS

An individual displacement vector can be considered a sample of a displacement field.

To estimate the displacement field, each actual displacement vector is weighted by a Gaussian kernel (width=25) in forming a local, averaged displacement vector.