JEasyTFM - Input and output files and folders names and formats

Data formats and folders of the images to be analyzed

For a TFM time-lapse experiment, z-stack and time-series images need to be gathered for each acquired channel (within them need at least to be a bead and cell image) and xy position (i.e. cell). Once the time-series images are acquired, z-stack images of the beads at equilibrium (named reference images later in this document) need to be taken as well for each xy position. The input files must be in OME TIF format with the z-stack (slices) images followed by the t-series (frames) images within a single file. Each xy stage position (corresponding to a cell) and channel have to be saved within a single file. Additionally, the filenames need to finish with the cell number as a string followed by an underscore (i.e. "_") and an iterated channel number padded to two digits (i.e. the images for the cell 1 with 3 acquired channels will finish with "1_01.tif", "1_02.tif" and "1_03.tif"). For example, for the cell number 12 with 3 acquired channels, the filenames should finish with

"12_34.tif"

"12_35.tif"

"12_36.tif"

The numbers 34, 35 and 36 being respectively calculated with the equation

[3 × (cell_number – 1) + channel_number]

which gives for our taken example of the cell number 12 respectively the values

[3 × (12 – 1) + 1] = 34

[3 × (12 – 1) + 2] = 35

[3 × (12 – 1) + 3] = 36.

The **Beads_reference_images** of a given experiment need to be saved within a folder having the same name than the one of the kinetic acquisition followed by "_-_Trypsine". The conditions on the filenames and formats are similar to the one described for the kinetic acquisition images.

Data folder and file names definitions

If we have for example image filenames for the cell number 12 being:

"ProtocolCombination_14.11.2018_15_51_21_Cell_12_34.tif"

"ProtocolCombination_14.11.2018_15_51_21_Cell_12_35.tif"

"ProtocolCombination_14.11.2018_15_51_21_Cell_12_36.tif"

We define as "prefixes" the beginning of the filename that has to be defined in the text field **Sequence_images** or **Reference_images** as described below. In the example, this will be:

"ProtocolCombination_14.11.2018_15_51_21_Cell_"

given that the ends of the filenames (i.e. "12_34.tif", "12_35.tif" and "12_36.tif") are automatically deduced by the algorithm.

Job files backups

Each time a job is launched, a copy of the job file

"JEasyTFM.txt" file (saved in the "ImageJ/plugins" folder)

used for the given launch is saved in a folder named "jobs" with the name

"JEasyTFM_y_begin.txt"; y corresponding to the number of launches made

This means that on the first job launch within the given experiment folder, the copied job name will be

"JEasyTFM_1_begin.txt"

on the second launch it will be

"JEasyTFM_2_begin.txt"

and so on, giving thus traceability of the different launches made.

On top of this, each time an analysis section or iteration for a cell number or folder within a section is completed, an updated version of the job file (i.e. corresponding to the analysis that needs still to be performed) is saved in the "jobs" folder with the name

"JEasyTFM_y_end.txt"; y corresponding to the given number of launch made.

Thus in the case a job has been stopped or canceled before completion, the user has just to overwrite the

"JEasyTFM.txt" file within the "ImageJ/plugins" folder by the "JEasyTFM_y_end.txt" file saved within the "jobs" folder to restart the last job where it had been stopped or canceled.

Images of the cells extraction

The **Cells_images** analysis creates a folder for each cell number defined within **Cells_starting_folder** and **Cells_ending_folder** within which all following analysis data will be saved.

In this part of the analysis, the best focused images for all the frames of the acquired channels for all the acquired cells will be extracted. The outputted images will be named

"Cells_Ch1_xx.tif"

"Cells_Ch2_xx.tif"

"Cells_Ch3_xx.tif"

depending on the channel they represent and

"Cells_xx.tif"

in the case there is only one acquired channel; xx corresponding to the given frame of the time series.

The images will be saved in a folder named

"Cell z"; z corresponding to the given cell number.

Beads reference images best focus extraction

In this part of the analysis the best focused images of the beads at equilibrium (i.e. after the addition of trypsin also called reference images in this document) for all the acquired cells will be extracted. The images will be named

"BeadsReference.tif"

In the case of the activation of the checkbox

Beads1

the images will be named

"BeadsReference_1.tif"

and in the case of the activation of the checkbox

Beads2

the images will be named

"BeadsReference_2.tif"

The images will be saved in a folder named

"Cell z"; z corresponding to the given cell number.

Beads sequence images best focus extraction

In this part of the analysis the best focused images for all the frames of the beads for all the acquired cells will be extracted. The images will be named

"BeadsAfter_xx.tif"; xx corresponding to the given frame of the time series.

In the case of the activation of the checkbox

Beads1

the images will be named

"BeadsAfter_1_xx.tif"

and in the case of the activation of the checkbox

Beads2

the images will be named

"BeadsAfter_2_xx.tif"

The images will be saved in a folder named

"Cell z"; z corresponding to the given cell number.

Traction force analysis

In this section of the package, the main features of the traction force analysis will be performed divided into different sections.

Traction force alignment output files: The **Traction_force_alignment** part of the analysis is composed of an **Alignment_make** section which aligns the best focused images for all the frames of the beads taking as reference the best focused image of the beads at equilibrium and this for all the acquired cells. The translation of images with respect to the reference image done by the **Alignment_make** section will introduce borders that have to be eliminated. To do so, the **Alignment_crop** section opens the images of the beads, measure the position of the borders and crop them out. Finally the images of the beads created by the **Alignment_make** algorithm will be overwritten. Similarly, all the images of the cells created by the previous algorithm are opened, cropped and overwritten.

Alignment make output files: The **Alignment_make** section aligns the best focused images for all the frames of the beads taking as reference the best focused image of the beads at equilibrium and this for all the acquired cells.

The output images will be named

"CorrBeadsAfter_xx.tif"; xx corresponding to the given frame of the time series

and saved in a folder named

"Cell z/CorrBeadsAfter"; z corresponding to the given cell number.

In the case of the activation of the checkbox

Beads1

the images will be named

"CorrBeadsAfter_1_xx.tif"

and saved in a folder named

"Cell z/CorrBeadsAfter_1"

In the case of the activation of the checkbox

Beads2

the images will be named

"CorrBeadsAfter_2_xx.tif"

and saved in a folder named

"Cell z/CorrBeadsAfter_2"

The images of the beads at equilibrium for all the acquired cells will be named

"CorrBeadsReference.tif"

and saved in a folder named

"Cell z/Analysis"

together with a file named

"Transformation.txt"

which records the x and y translation values that have been applied between the

"BeadsAfter_xx.tif"

And

"CorrBeadsAfter_xx.tif"

images.

In the case of the activation of the checkbox

Beads1

the reference images will be named

"CorrBeadsReference_1.tif"

and saved in a folder named

"Cell z/Analysis_1"

together with the

"Transformation.txt"

file.

In the case of the activation of the checkbox

Beads2

the reference images will be named

"CorrBeadsReference_2.tif"

and saved in a folder named

"Cell z/Analysis_2"

together with the

"Transformation.txt"

file.

The translation transformations that have been applied on the

"CorrBeadsAfter_xx.tif"

images (and saved in the "Transformation.txt" file) are similarly applied on the best focused images for all the frames of the acquired channels for all the acquired cells.

The outputted images will be named

"CorrCells_Ch1_xx.tif"

"CorrCells_Ch2_xx.tif"

"CorrCells_Ch3_xx.tif"

depending on the channel they represent and

"CorrCells_xx.tif"

in the case there is only one acquired channel and saved in a folder named

"Cell z/CorrCells"

In the case of the activation of the checkbox

Beads1

the images will be named

"CorrCells_1_Ch1_xx.tif"

"CorrCells_1_Ch2_xx.tif"

"CorrCells_1_Ch3_xx.tif"

depending on the channel they represent and

"CorrCells_1_xx.tif"

in the case there is only one acquired channel and saved in a folder named

"Cell z/CorrCells_1"

In the case of the activation of the checkbox

Beads2

the images will be named

"CorrCells_2_Ch1_xx.tif"

"CorrCells_2_Ch2_xx.tif"

"CorrCells_2_Ch3_xx.tif"

depending on the channel they represent and

"CorrCells_2_xx.tif"

in the case there is only one acquired channel and saved in a folder named

"Cell z/CorrCells_2"

Alignment crop output files: The translation of images with respect to the reference image done by the **Alignment_make** section will introduce borders that have to be eliminated.

To do so, the **Alignment_crop** section opens the images of the beads, measures the position of the borders, crops them out and adds the border positions data to the previously generated "Transformation.txt" file.

Finally the images of the beads created by the **Alignment_make** algorithm will be overwritten.

Similarly, all the images of the cells created by the previous algorithm are opened, cropped and overwritten.

Traction force calculation output files: The **Traction_force_calculation** part of the analysis is composed of a **PIV_calculation** section which applies a PIV (Particle Image Velocimetry) algorithm between the reference images of the beads and the images for all the frames of the beads generated at the **Traction_force_alignment** section.

Next, the **Force_calculation** section will generate the corresponding force maps using a FTTC (Fourier Transform Traction Cytometry) algorithm.

And the **Force_superposition** section will combine all the cells with all the force maps images.

PIV calculation output files: The **PIV_calculation** section which will apply a PIV (Particle Image Velocimetry) algorithm between the reference images of the beads and the images for all the frames of the beads generated at the **Traction_force_alignment** section.

The results are outputted in the form of text in a file named

"Stack_xx.txt"; xx corresponding to the given frame of the time series

together with a image representing the beads displacement magnitude and vectors named

"PIV3_Stack_xx.tif"

and saved in a folder named

"Cell z/Analysis"; z corresponding to the given cell number.

In the case of the activation of the checkbox

Beads1

the output files will be named

"Stack_1_xx.txt"

And

"PIV3_Stack_1_xx.tif"

and saved in a folder named

"Cell z/Analysis_1".

In the case of the activation of the checkbox

Beads2

the output files will be named

"Stack_2_xx.txt"

And

"PIV3_Stack_2_xx.tif"

and saved in a folder named

"Cell z/Analysis_2".

Lambda calculation output files: The **Lambda_calculation** section will read all the previously generated

Stack_xx.txt; xx corresponding to the given frame of the time series

files and determine the regularization or Lagrange parameter λ value so that the calculated force maps using an FTTC (Fourier Transform Traction Cytometry) algorithm will stand within the **Precision_for_the_Regularization_factor_calculation** value of the optimal force and this for all Frames.

The results are outputted in the form of text in a file named

lambda.txt

saved in a folder named

Cell z/Analysis; z corresponding to the given cell number.

In the case of the activation of the checkbox

Beads1

the file will be saved in a folder named

Cell z/Analysis_1.

In the case of the activation of the checkbox

Beads2

the file will be saved in a folder named

Cell z/Analysis_2.

Force calculation output files: The **Force_calculation** section first reads all the previously generated

"Stack_xx.txt"; xx corresponding to the given frame of the time series

files and generates all the corresponding force maps using an FTTC (Fourier Transform Traction Cytometry) algorithm to evaluate the minimal and maximal values of the obtained force vectors.

These values are then stored in a text file named

"scale.txt"

and saved in the folder

"Cell z/Analysis"; z corresponding to the given cell number.

Following the obtained maximal value is ceil rounded by a "nice number" and used for defining the maximum force vector value so that the force scaling is normalized through the whole acquired images.

The obtained force outputs are then saved in a text file named

"Traction_Stack_xx.txt"

in the folder

"Cell z/Analysis".

The images of the vector and magnitude maps are named

"Traction_Vector_Plot_Traction_Stack_xx.tif"

And

"Traction_Magnitude_Plot_Traction_Stack_xx.tif"

and saved in a folder named

"Cell z/Traction_Vector_Plot".

In the case of the activation of the checkbox

Beads1

the output files will be named

"scale_1.txt"

And

"Traction_Stack_1_xx.txt"

and saved in the folder

"Cell z/Analysis_1"

as well as

"Traction_Vector_Plot_Traction_Stack_1_xx.tif"

And

"Traction_Magnitude_Plot_Traction_Stack_1_xx.tif"

which are saved in a folder named

"Cell z/Traction_Vector_Plot_1".

In the case of the activation of the checkbox

Beads2

the output files will be named

"scale_2.txt"

And

"Traction_Stack_2_xx.txt"

and saved in the folder

"Cell z/Analysis_2"

as well as

"Traction_Vector_Plot_Traction_Stack_2_xx.tif"

And

"Traction_Magnitude_Plot_Traction_Stack_2_xx.tif"

which are saved in a folder named

"Cell z/Traction_Vector_Plot_2".

Force superposition output files: The **Force_superposition** section will combine all the cells with all the force maps images.

Thus the outputted images will be named:

"Superposition_with_Traction_Vector_Plot_Ch1_xx.jpg"

"Superposition_with_Traction_Magnitude_Plot_Ch1_xx.jpg"; xx corresponding to the given frame of the time series

or

"Superposition_with_Traction_Vector_Plot_Ch2_xx.jpg"

"Superposition_with_Traction_Magnitude_Plot_Ch2_xx.jpg"

or

"Superposition_with_Traction_Vector_Plot_Ch3_xx.jpg"

"Superposition_with_Traction_Magnitude_Plot_Ch3_xx.jpg"

depending on the channel they represent and:

"Superposition_with_Traction_Vector_Plot_xx.jpg"

"Superposition_with_Traction_Magnitude_Plot_xx.jpg"

in the case there is only one acquired channel and saved in a folder named

"Cell z/PicCells"; z corresponding to the given cell number.

In the case of the activation of the checkbox

Beads1

the images will be named:

"Superposition_with_Traction_Vector_Plot_1_Ch1_xx.jpg"

"Superposition_with_Traction_Magnitude_Plot_1_Ch1_xx.jpg"

or

"Superposition_with_Traction_Vector_Plot_1_Ch2_xx.jpg"

"Superposition_with_Traction_Magnitude_Plot_1_Ch2_xx.jpg"

or

"Superposition_with_Traction_Vector_Plot_1_Ch3_xx.jpg"

"Superposition_with_Traction_Magnitude_Plot_1_Ch3_xx.jpg"

depending on the channel they represent and:

"Superposition_with_Traction_Vector_Plot_1_xx.jpg"

"Superposition_with_Traction_Magnitude_Plot_1_xx.jpg"

in the case there is only one acquired channel and saved in a folder named

"Cell z/PicCells_1".

In the case of the activation of the checkbox

Beads2

the images will be named:

"Superposition_with_Traction_Vector_Plot_2_Ch1_xx.jpg"

"Superposition_with_Traction_Magnitude_Plot_2_Ch1_xx.jpg"

or

"Superposition_with_Traction_Vector_Plot_2_Ch2_xx.jpg"

"Superposition_with_Traction_Magnitude_Plot_2_Ch2_xx.jpg"

or

"Superposition_with_Traction_Vector_Plot_2_Ch3_xx.jpg"

"Superposition_with_Traction_Magnitude_Plot_2_Ch3_xx.jpg"

depending on the channel they represent and:

"Superposition_with_Traction_Vector_Plot_2_xx.jpg"

"Superposition_with_Traction_Magnitude_Plot_2_xx.jpg"

in the case there is only one acquired channel and saved in a folder named

"Cell z/PicCells_2".

Cells analysis

In the **Cells_analysis** part of the analysis, the segmentation of the images of the cells as well as the force integration over the cells over all the frames will be performed.

The **Cells_segmentation** part of the analysis applies several filters to the acquired images of the cells and the **Cells_selection** part of the analysis requires the user to draw a ROI (Region Of Interest) delimiting the displacements of the cell of interest over all the frames as well as to define a threshold window for the given cell over all the frames in order to generate cells segmentation ROIs.

The **Force_integration** part of the analysis makes a superposition of the different cell images together with the vector and magnitude force representations images and the cell ROI together with the measured integrated values.

Cells segmentation output files: The **Cells_segmentation** part of the analysis applies several filters to the acquired images of the cells and output images named

"focused.tif"

And

"binaries.tif"

that are saved in the folder

"Cell z"; z corresponding to the given cell number.

This intermediate step aims to reduce the waiting time for the user between the analyses of each cell through the final segmentation algorithm that will have manual steps.

Cells selection output files: The **Cells_selection** part of the analysis requires the user to draw a ROI (Region Of Interest) delimiting the displacements of the cell of interest over all the frames as well as to define a threshold window for the given cell over all the frames.

The output will be a ROI file for all the frames named

"RoiSet.zip"

and saved in the folder

"Cell z/Analysis".

As well as a stack image named

"segmentation.tif"

and saved in the folder

"Cell z"

that is a superposition of the images of the cells will all the calculated ROIs.

In the case of the activation of the checkbox

Beads1

the output ROI file

"RoiSet.zip"

will be saved in the folder

"Cell z/Analysis_1"

and in the case of the activation of the checkbox

Beads2

it will be saved in the folder

"Cell z/Analysis_2".

Force integration output files: The **Force_integration** part of the analysis makes a copy of the previous PIV output text file

"Stack_xx.txt"; xx corresponding to the given frame of the time series

in a folder named

"Cell z/Integration_calculation"; z corresponding to the given cell number

and generates similarly to the **Force_calculation** section all the files generated by the FTTC algorithm, i.e.:

"Traction_Stack_xx.txt"

"Traction_Vector_Plot_Traction_Stack_xx.tif"

"Traction_Magnitude_Plot_Traction_Stack_xx.tif"

"Traction_Vector_Scale.tif"

in the same folder.

The generation of all these files in a new folder may be redundant, but it is a very fast process and above all, it is able to compartmentalize the creation of

"Plot_Traction"

images on which the parameters can be played around in order for example to increase or decrease the vector lengths or relative intensity for the superimposed images generated at the next section.

In the case of the activation of the checkbox

Beads1

the files will be named

"Stack_1_xx.txt"

"Traction_Stack_1_xx.txt"

"Traction_Vector_Plot_Traction_Stack_1_xx.tif"

"Traction_Magnitude_Plot_Traction_Stack_1_xx.tif"

"Traction_Vector_Scale.tif"

and saved in a folder named

"Cell z/Integration_calculation_1".

In the case of the activation of the checkbox

Beads2

the files will be named

"Stack_2_xx.txt"

"Traction_Stack_2_xx.txt"

"Traction_Vector_Plot_Traction_Stack_2_xx.tif"

"Traction_Magnitude_Plot_Traction_Stack_2_xx.tif"

"Traction_Vector_Scale.tif"

and saved in a folder named

"Cell z/Integration_calculation_2".

Next the superposition of the different cell images together with the vector and magnitude force representations images and the cell ROI as well as measured integrated values are generated and named

"Integration_with_Traction_Vector_Plot_Ch1_xx.jpg"

"Integration_with_Traction_Magnitude_Plot_Ch1_xx.jpg"; xx corresponding to the given frame of the time series

or

"Integration_with_Traction_Vector_Plot_Ch2_xx.jpg"

"Integration_with_Traction_Magnitude_Plot_Ch2_xx.jpg"

or

"Integration_with_Traction_Vector_Plot_Ch3_xx.jpg"

"Integration_with_Traction_Magnitude_Plot_Ch3_xx.jpg"

depending on the channel they represent and:

"Integration_with_Traction_Vector_Plot_xx.jpg"

"Integration_with_Traction_Magnitude_Plot_xx.jpg"

in the case there is only one acquired channel and saved in a folder named

"Cell z/Integration_results"; z corresponding to the given cell number.

In the case of the activation of the checkbox

Beads1

the images will be named:

"Integration_with_Traction_Vector_Plot_1_Ch1_xx.jpg"

"Integration_with_Traction_Magnitude_Plot_1_Ch1_xx.jpg"

or

"Integration_with_Traction_Vector_Plot_1_Ch2_xx.jpg"

"Integration_with_Traction_Magnitude_Plot_1_Ch2_xx.jpg"

or

"Integration_with_Traction_Vector_Plot_1_Ch3_xx.jpg"

"Integration_with_Traction_Magnitude_Plot_1_Ch3_xx.jpg"

depending on the channel they represent and:

"Integration_with_Traction_Vector_Plot_1_xx.jpg"

"Integration_with_Traction_Magnitude_Plot_1_xx.jpg"

in the case there is only one acquired channel and saved in a folder named

"Cell z/Integration_results_1".

In the case of the activation of the checkbox

Beads2

the images will be named:

"Integration_with_Traction_Vector_Plot_2_Ch1_xx.jpg"

"Integration_with_Traction_Magnitude_Plot_2_Ch1_xx.jpg"

or

"Integration_with_Traction_Vector_Plot_2_Ch2_xx.jpg"

"Integration_with_Traction_Magnitude_Plot_2_Ch2_xx.jpg"

or

"Integration_with_Traction_Vector_Plot_2_Ch3_xx.jpg"

"Integration_with_Traction_Magnitude_Plot_2_Ch3_xx.jpg"

depending on the channel they represent and:

"Integration_with_Traction_Vector_Plot_2_xx.jpg"

"Integration_with_Traction_Magnitude_Plot_2_xx.jpg"

in the case there is only one acquired channel and saved in a folder named

"Cell z/Integration_results_2".

All the integration data are resumed in a table saved in a text file named

"Integration_results.txt"

and saved in the folder

"Cell z".

Beads positions tracking

In the **Traction_high_force** part of the analysis, the tracking of the beads between the reference images of the beads and the images for all the frames of the beads generated at the **Traction_force_alignment** section will be performed.

The **Traction_high_force** part of the analysis is composed of a **high_PIV_calculation** section which takes the data previously obtained from the **PIV_calculation** section of the **Traction_force_calculation** part of the analysis to push the calculation further to have a more refined definition of the beads displacement map.

The **Particle_tracker** section will use the more refined definition of the beads displacement map to compute the displacement of the beads.

High PIV calculation output files: The **High_PIV_calculation** section which will take the data previously obtained from the **PIV_calculation** section of the **Traction_force_calculation** part of the analysis, saved in the

"Stack_xx.txt"; xx corresponding to the given frame of the time series

file to push the calculation further to have a more refined definition of the beads displacement map.

The results are outputted in the form of text in a file named

"Stack_xx_2.txt"

and saved in the folder

"Cell z/Analysis"; z corresponding to the given cell number.

In the case of the activation of the checkbox

Beads1

the output files will be named

"Stack_1_xx_2.txt"

and saved in the folder

"Cell z/Analysis_1".

And in the case of the activation of the checkbox

Beads2

the output files will be named

"Stack_2_xx_2.txt"

and saved in the folder named

"Cell z/Analysis_2".

Particle tracker output files: The **Particle_tracker** section uses the previously generated

"Stack_xx_2.txt"; xx corresponding to the given frame of the time series

file in order to compute the displacement of the beads.

The obtained outputs are saved in a text file named

"Stack_xx_2_Trajectories.txt"

in the folder

"Cell z/Analysis"; z corresponding to the given cell number.

As the images of the beads displacements they are named

"Stack_xx_Trajectories.tif"

and saved in a folder named

"Cell z/Trajectories".

In the case of the activation of the checkbox

Beads1

the output files will be named

"Stack_1_xx_2_Trajectories.txt"

and saved in the folder

"Cell z/Analysis_1"

as well as

"Stack_1_xx_Trajectories.tif"

and saved in a folder named

"Cell z/Trajectories_1".

And in the case of the activation of the checkbox

Beads2

the output files will be named

"Stack_2_xx_2_Trajectories.txt"

and saved in the folder

"Cell z/Analysis_2"

as well as

"Stack_2_xx_Trajectories.tif"

and saved in a folder named

"Cell z/Trajectories_2".

FA segmentation

Within the FA_segmentation section, a segmentation of focal adhesion algorithm outputs a ROI file named

"RoiSet_xx.zip"; xx corresponding to the given frame number.

as well as a stack image named

"CorrCells_xx.tif"

and saved in the folder

"Cell z/FA_Analysis"; z corresponding to the given cell number.

In the case of the activation of the checkbox

Beads1

the files will be named

"RoiSet_1_xx.zip"

"CorrCells_1_xx.tif"

and saved in a folder named

"Cell z/FA_Analysis_1".

In the case of the activation of the checkbox

Beads2

the files will be named

"RoiSet_2_xx.zip"

"CorrCells_2_xx.tif"

and saved in a folder named

"Cell z/FA_Analysis_2".

Traction force applied on FA

The Traction_force_applied_on_FA7-10 algorithm outputs

text files named

"FA_positions_xx.txt"

"Beads_displacements_xx.txt"

"Fitting_parameters_data_xx.tif"

and images named

"Beads_displacement_plot_xx.tif"

"Beads_displacement_plot_with_image_xx.tif"

"Regularization_parameter_plot_xx.tif"

"Force_plot_xx.tif"

"Force_plot_with_image_xx.tif"; xx corresponding to the given frame of the time series

and saved in the folder

"Cell z/Traction_force_on_FA"; z corresponding to the given cell number.

If one of the checkboxes Ch1 and/or Ch2 and/or Ch3 is/are activated, this algorithm will then be applied to the beads displacements and FA positions data merge of all the selected beads colors.