

Materials List for:

Mapping Molecular Diffusion in the Plasma Membrane by Multiple-Target Tracing (MTT)

Vincent Rouger^{1,2,3}, Nicolas Bertaux^{4,5,6}, Tomasz Trombik^{1,2,3}, Sébastien Mailfert^{1,2,3}, Cyrille Billaudeau^{1,2,3}, Didier Marguet^{1,2,3}, Arnaud Sergé^{1,2,3}

¹Institut National de la Santé et de la Recherche Médicale, UMR 631, Parc scientifique de Luminy

²Centre National de la Recherche Scientifique, UMR 6102, Parc scientifique de Luminy

³Centre d'Immunologie de Marseille-Luminy, Aix-Marseille University

⁴École Centrale Marseille, Technopôle de Château-Gombert

⁵Institut Fresnel, Aix-Marseille University

⁶Centre National de la Recherche Scientifique, UMR 6133, Aix-Marseille University

Correspondence to: Arnaud Sergé at serge@ciml.univ-mrs.fr

URL: <https://www.jove.com/video/3599>

DOI: [doi:10.3791/3599](https://doi.org/10.3791/3599)

Materials

Name	Company	Catalog Number	Comments
Cos-7 cell line	ATCC	CRL-1651	5,000 cells/well
HBSS without Ca ²⁺	GIBCO, by Life Technologies	14175	1 ml
0.05% Trypsin EDTA	GIBCO, by Life Technologies	25300	1 ml
8-well Lab-tek	Nalge Nunc international	155441	1
QDot-605 streptavidin	Invitrogen	Q10101MP	20 mM
Biotinylated Fab (for Fab synthesis, see reference ²¹)			
Fab from mAb 108	ATCC	HB-9764	200 µg
NHS-Biotin	Thermo Fisher Scientific, Inc.	21435	18.5 µg
Complete medium			
DMEM	GIBCO, by Life Technologies	41965	500 ml
Fetal Bovine Serum	Sigma-Aldrich	F7524	50 ml
L-Glutamine	GIBCO, by Life Technologies	25030	5 ml
HEPES	GIBCO, by Life Technologies	15630	5 ml
Sodium Pyruvate	GIBCO, by Life Technologies	11360	5 ml
Imaging medium			
HBSS with Ca ²⁺	GIBCO, by Life Technologies	14025	25 ml
HEPES	GIBCO, by Life Technologies	15630	250 µl
Inverted microscope	Nikon Instruments	Eclipse TE2000U	
Fluorescent lamp	Nikon Instruments	Intensilight C-HGFIE	
1.3 NA 100x objective	Nikon Instruments	Plan Fluor 1.30	
1.49 NA 100x objective	Nikon Instruments	APO TIRF 1.49	
Camera	Roper Scientific	Cascade 512 B	
Thermostated box	Life Imaging Services	The Box	

Appendix: example Script of MTT supplementary analysis

```
function MTT_example(file_name)
%%% Basic examples showing how to recover MTT output results
%%% to plot each trace and to build the histogram
%%% of fluorescence intensities
```

```
if nargin<1 % no file_name provided?
files = dir('*.stk');
```

<pre> if isempty(files), disp('no data in current dir'), return, end file_name = files(1).name; % default: first stk file disp(['using' file_name 'by default']) end </pre>
<pre> file_param = [file_name '_tab_param.dat']; % output file </pre>
<pre> %% Load data cd('output23') % or ('output22'), according to version used % Disclaimer: version 2.2 only generates 7 parameters, % an extra parameter, noise, was added in version 2.3 </pre>
<pre> % To read all parameters at once, in a single table % tab_param = fread_all_param(file_param); % tab_i = tab_param(2:8:end, :); tab_j = ... </pre>
<pre> % To read all parameters (except frame_number) in separate tables % [tab_i,tab_j,tab_alpha,tab_radius,tab_offset,tab_blk,tab_noise] = fread_all_data_spt(file_param); </pre>
<pre> tab_i = fread_data_spt(file_param, 3); % index is 3 because trace number & frame number, non informative, are discarded! tab_j = fread_data_spt(file_param, 4); tab_alpha = fread_data_spt(file_param, 5); tab_blk = fread_data_spt(file_param, 8); </pre>
<pre> %% Loop over traces N_traces = size(tab_i,1); % Tables are N_traces lines by N_frames columns </pre>
<pre> for itrc = 1:N_traces No_blk_index = tab_blk(itrc, :)>0; % non blinking steps only plot(tab_i(itrc, No_blk_index), tab_j(itrc, No_blk_index)) xlabel('i (pixel)'), ylabel('j (pixel)') title(['trace # ' num2str(itrc)]) disp('Please strike any key for next trace'), pause end </pre>
<pre> %% Fluo histogram N_datapoints = sum(tab_blk(:)>0); % non blinking steps only hist(tab_alpha(tab_blk>0),2*sqrt(N_datapoints)) % using 2sqrt(N) bins xlabel('intensity (a.u.)'), ylabel('occurrence') title('histogram of particles fluorescence intensity') </pre>