

Materials List for:

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

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

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## Materials

Name	Company	Catalog Number	Comments
Cos-7 cell line	ATCC	CRL-1651	5,000 cells/well
HBSS without Ca <sup>2+</sup>	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
<b>Biotinylated Fab</b> (for Fab synthesis, see reference <sup>21</sup> )			
Fab from mAb 108	ATCC	HB-9764	200 µg
NHS-Biotin	Thermo Fisher Scientific, Inc.	21435	18.5 µg
<b>Complete medium</b>			
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
<b>Imaging medium</b>			
HBSS with Ca <sup>2+</sup>	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');
```

```

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

file_param = [file_name '_tab_param.dat']; % output file

%% 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

% 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 = ...

% 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);

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);

%% Loop over traces
N_traces = size(tab_i,1);
% Tables are N_traces lines by N_frames columns

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

%% 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')

```