

FILE FORMAT

ODT-SAS enables 3D segmentation of ODT images saved in a .mat file format. The MAT-file should consist of 4 variables with specific names:

- “REC” or “RECON” – tomographic reconstruction;
- “n_imm” or “n_immersion” – refractive index of object immersion medium;
- “dx” or “dxo” – object space sample size in XY [um];
- “rayXY” – xy-coordinates of illumination vectors (when the variable does not exist, it is synthetically generated – the effectiveness of 3D segmentation may then be less).

KEY STEPS FOR RUNNING ODT_SAS

The main script is **ODT_SAS.mat**

1. Select type of segmentation:
 - a) 'Cell'; → 3D segmentation of whole cell
 - b) 'Organelle'; → 3D segmentation of nucleolus and lipid droplets (*requires the biological cell mask to be loaded*)
 - c) 'Cell_Organelle'; → 3D segmentation – cell + organelle
2. Set parameters for 3D cell segmentation and 3D organelle segmentation (the satisfactory level of 3D segmentation can be achieved if the parameters will be properly selected).
3. Run **ODT_SAS.mat**

GUI FOR MANUAL NUCLEOLI MARKERS DETECTION

When choosing ‘Organelle’ or ‘Cell_Organelle’ segmentation, the user is asked to mark nucleoli and diameter through the largest nucleolus.

GUI commands:

- select nucleoli markers – press **S** key;
- stop selecting nucleoli markers – press right mouse button;
- mark diameter through the largest nucleolus – press **R** key;
- save nucleoli markers and diameter – press “**Save Seed Points**” button.