

■ LiDRoS Analysis Tool - Help Manual

■■ Important Instructions ■■

- The images **must always be stored in the Root Folder chosen as input**.
- Inside this Root Folder, you should have the following structure:
 - CellLine1 → (with its respective LDs/ROS structure)
 - CellLine2 → (with its respective LDs/ROS structure)
 - CellLine3 → (and so on...)
- The folder you select as **Input Folder** in the program corresponds to this **Root Folder**.
- The **Output Folder** can be any folder you want, but remember: this will be the folder later selected when running **Statlysis**.

■ Folder Structure Requirements

Your root folder must follow this structure for proper metadata extraction:

CellLine1/	→ LDs/ → Co60/ → NP-treated/ → 0/ 2/ 10/ → Untreated/ → 0/ 10/ → ROS/ (same structure as LDs)
------------	---

■ Expected Folder Levels

Level 1 – Cell Line: e.g., HCT116, A549, MCF7

Level 2 – Theme: 'LDs' or 'ROS'

Level 3 – Radiation Source: Co60, LINAC_6MV, Xray, etc.

Level 4 – Nanoparticles: 'NP-treated' or 'Untreated'

Level 5 – Dose: '0', '0 Gy', '2 Gy', '10 Gy', etc.

Level 6+ – (Optional) Objective or replicate name (parsed automatically)

■ Example

```
HCT116/  
→ LDs/  
→ Co60/  
→ NP-treated/  
→ 2 Gy/  
→ image01.tif
```

■■ Common Issues

- Using wrong names like 'C60' instead of 'Co60'
- Skipping LDs/ROS folders
- Missing dosage folders under treatment
- Placing images directly under ROS/LDs without nested folders

■ Outputs

- **Excel:** Global Metrics, Nucleus Metrics, LD/ROS Metrics
- **PNGs:** Overlays for masks and segmentation (LDRed, LDGreen, LDColoc, etc.)

■ Contact

Developed by: Marco Ferreira, FCUL-IST (2025)
Email: fc60327@alunos.fc.ul.pt