Holographic Tomographic Microscopy for Nano-Scale Dose Calculation and Assessing Gold Nanoparticle Uptake in Live Cells

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Purpose

- To assess whether the three-dimensional density distribution and localization of gold nanoparticles (GNP) within live cancer cells can be obtained with Holographic Tomographic Microscopy (HTM)
- The exact location of GNP is crucial for their radiosensitizing properties due to the short range of emitted secondary electrons.

Methods

Holographic Tomographic Microscopy (HTM) *[1]*

- Non-invasive
- Quantitative
- 3D (tomography)
- Resolution 100nm (x, y), 300nm (z)
- 520nm low-power(0.2mW/mm^2) laser rotating at 45 degree angle
- Field-of-view: ~80µm lateral, ~30µm depth
- GPU-accelerated complex deconvolution

Cell Lines

- Human fibroblasts, NCI-H1703, Calu6, MCF7, A459

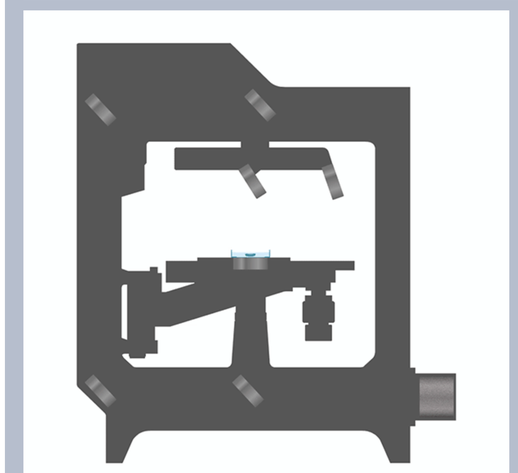


Fig. 1: Principle of (HTM):

- beam split into sample/reference beam
- sample positioned between rotational arm and high-NA air objective
- illuminated at 45^o angle, rotating 360^o

Fig. 2: example tomography of a live neuron, colors indicate different range of refractive index within the cell (with permission of Nanolive SA)

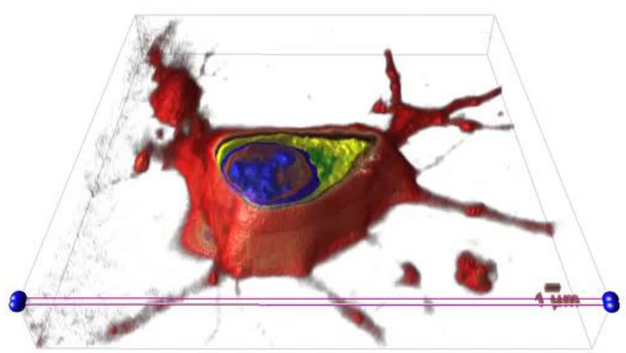
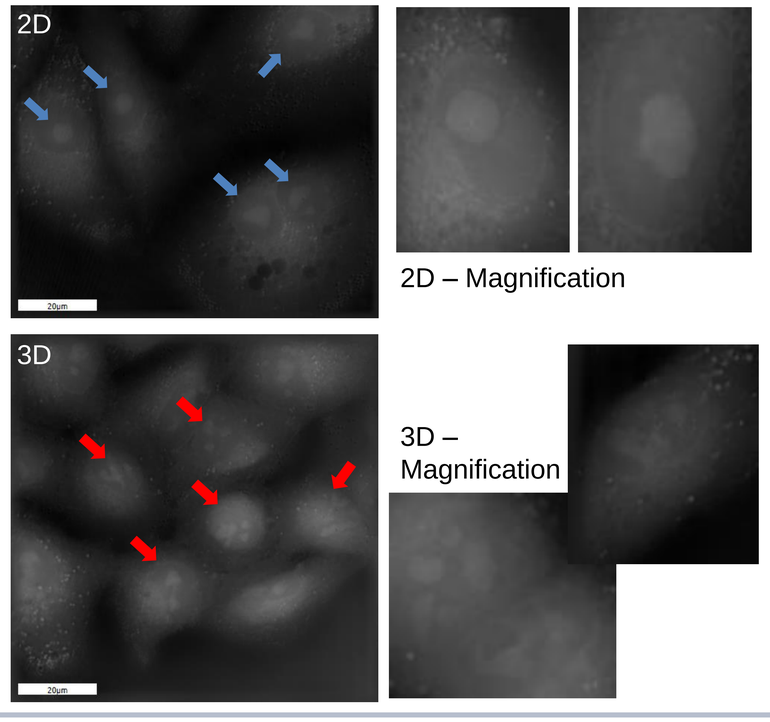


Fig. 3: A549 cells grown in 2D (top) and 3D (bottom) cell culture exhibiting different types of nuclear substructure



Results

•All cell lines showed excellent sample contrast and we performed long-term imaging of the same cells >48h (using laser-etched grids on fluoroscopy plates)

•Clear differentiation possible of:

- Cytoplasm
- Endoplasmic reticulum
- Organelles (mitochondria, lysosomes, ribosomes)
- Nuclear membrane
- Nucleoli

•*Optical Density differences*

- The optical density differences between mitochondria and cytoplasm were largest, 1.19±0.04 vs. 1.72±0.04 (mean±SD) and similar among cell lines
- Within the nucleus, large differences were observed between chromatin phases, with variations up to 0.32±0.04 with an average nuclear density of 1.4. (see Figure 4)
- The densest organelles observed have optical densities ~2.0 and are suspected to be ribosomes.
- When H1703 and A549 cells are grown as tumor spheroids we detected larger & more heterogeneous areas of condensed chromatin in the nucleus compared to traditional 2D-grown cells (see Figure 3).

•*Gold Nano Particles (GNP)*

- 1.9 nm GNP added to H1703 and MCF7 cell lines in concentrations of 10-250µg/ml could be localized within cells due to their high absorption of light around 510nm (see Figure 5).
- The images indicate that GNP preferentially accumulate around organelles close to the nuclear envelope.

Conclusion

•(HTM) is a valuable tool to provide

- structural input for nanometer-scale dose calculations (see Figure 6)
- Measurements of density differences within cells in vitro.

•GNP strong absorbing properties at the laser's wavelength could enable localization of very small amounts of nanometer-size GNP in live samples

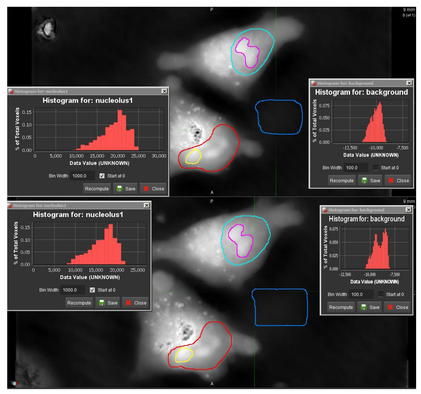


Fig. 4: measurement of optical density differences between different parts of the cell and nucleus.

Fig. 5: MCF7 cells treated with GNP: after 4h (left, tomographic acquisition) and after 12h (right, bright field)

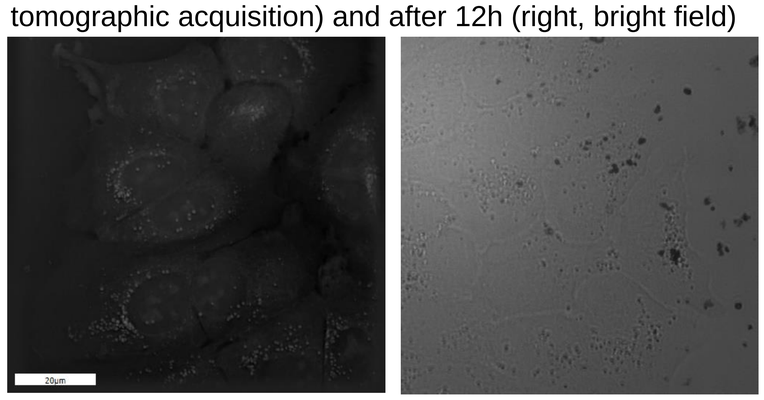
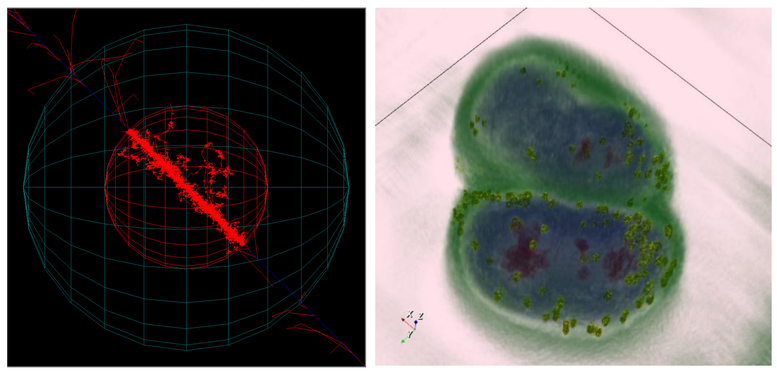


Fig. 6: nano-dosimetric Monte Carlo simulation on idealized geometry (left) and 3D cell tomography (right)



References

[1] Cotte, Yann, et al. "Marker-free phase nanoscopy." Nature Photonics 7.2 (2013): 113-117.

Acknowledgements

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