2D INTEGRATED SUPER RESOLUTION CORRELATIVE MICROSCOPY

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Recent years have seen many developments in Correlative Light and Electron Microscopy (CLEM). The number of applications and technical developments have rapidly increased. Light (fluorescence) microscopy and electron microscopy are to a great extend complementary imaging modalities. Light (LM) microscopy is capable of rapidly identifying locations of fluorescent labels with high sensitivity in large fields of view and electron microscopy is capable of imaging the ultrastructure and context of these locations with nm resolution.
Our group focused on the development and application of integrated CLEM approaches; here the optical microscope is integrated inside the electron microscope. For several types of applications this is advantageous and in general it affords simple and reliable tracing back regions of interest identified by LM in the EM. This is accomplished by making use of the same sample stage that can be positioned in LM or EM mode. However, in general the correlation accuracy is no better than 0.5 - 1 μm [1].
To improve the accuracy we developed a high accuracy correlation method based on the use of fiducial markers visible in both fluorescence microscopy (FM) and TEM. The 120 nm diameter fiducial markers consist of fluorescently labeled silica coated gold nanoparticles. The correlation method relies on first correlating the positions of the fiducial markers in FM and low magnification TEM images. Next, the low magnification TEM image is correlated with a high magnification TEM image using auto correlation. Both steps result in linear transformation matrices and multiplication of the matrices result in the overall transformation matrix. The method is not sensitive to shrinkage of the specimen and requires only a low density of fiducial markers. The mean correlation accuracy was found to be better than 30 nm.
In addition we incorporated localization based super-resolution (SR) microscopy inside a TEM. A compact SR module was developed that is mounted on one of the side ports of a TEM (Technai 12). An important challenge is to minimize background fluorescence and optimize imaging conditions.
Here, the high accuracy correlation method and several applications will be discussed including applications of the integrated SR CLEM in material science [2] and biology.

REFERENCES

[1] A.V. Agronskaia, J.A. Valentijn, L.F. van Driel, C.T.W.M. Schneijdenberg, B.M. Humbel, P.M.P. van Bergen en Henegouwen, A.J. Verkleij, A.J. Koster and H.C. Gerritsen, "Integrated fluorescence and transmission electron microscopy: a novel approach to correlative microscopy". *J. Struct. Biol.* **164,** 183-189 (2008).
[2] F.C. Hendriks, S. Mohammadian, Z. Ristanović, S. Kalirai, F. Meirer, E.T.C.Vogt, P.C.A. Bruijnincx, H.C. Gerritsen, B.M. Weckhuysen, "Integrated Transmission Electron and Single-Molecule Fluorescence Microscopy Correlates Reactivity with Ultrastructure in a Single Catalyst Particle" *Angew. Chem. Int. Ed.*, **57**(1), 1521-3773 (2018)