

# Correlative transmission electron microscopy and photoluminescence microscopy revealing enhanced fluorescence in nitrogen vacancy containing nanodiamond

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Fluorescent nanodiamonds (FNDs) are diamond nanoparticles containing color centers that emit visible light at room temperature. Among the color centers in FNDs, the nitrogen-vacancy centers (NV) have drawn the most attention due to its exceptionally stable optical properties and great prospect in sensing and biomedical diagnostic applications [1]. The current understanding of the optical properties of the FNDs relies largely on the optical measurement methods from either ensemble of materials or from few single particles. As most of the FND fabrication is a top-down process where larger diamond crystals are milled to desired nanometer size, FNDs generally have broad size distribution and irregular shapes [2]. Therefore understanding of FNDs structure-property relationship using ensemble measurements only is insufficient.

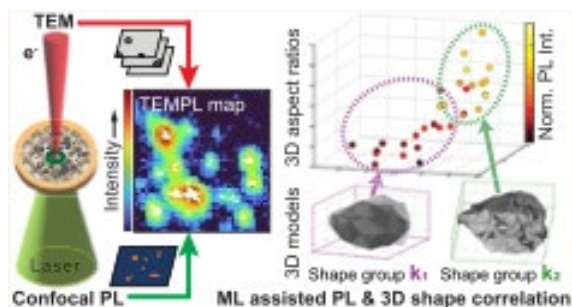
We have developed a new method based on correlative transmission electron microscopy and photoluminescence (TEMPL) [3]. TEMPL allows a direct correlation of the fluorescence brightness and three-dimensional size and shape of individual nanoparticles. PL provides optical information with exquisite energy resolution, and TEM provides structural information with exquisite spatial resolution. Unsupervised machine learning (ML) with the generalized 3D shape descriptors, is used to analyse correlations between the PL brightness and 3D shape of FND particles. The automation provided by machine learning allows TEMPL to be applied to large sample areas (2-3 orders of magnitude larger than a typical TEM field of view) containing a statistically significant number of particles.

Using this new method, we directly reveal that the volume-averaged PL brightness of thin, flake-like nanodiamond particles is up to several times greater than that of three-dimensional-shaped, thicker particles provided the particle diameter is less than the sub-wavelength limit. With the assumption that the number of NVs within a particle is proportional to its volume, this implies that individual NVs within thinner particles are brighter. This experimental observation is supported by the theoretical simulations on simplified particle geometries on a range of supporting substrate and

surrounding medium. The simulations indicate that the comparative brightness of thinner particles, either on a thin supporting substrate or in a low-index medium, is attributable, at least in part, to the constructive interference of partial light waves in these particles. With increasing particle thickness, such an effect becomes damped. The sub-wavelength dimension of the substrate plays an important role, as it results in higher effective reflectivity comparable to diamond particles in a low-index medium (e.g., a low-index solution).

We have introduced a machine-learning-assisted correlative TEMPL method, which enables direct elucidation of the 3D morphological–fluorescence relationship of fluorescent nanoparticles. We emphasize that the method provides three-dimensional morphological information, which is potentially crucial for a variety of fluorescent nanoparticle systems but rarely achieved. Another significant advantage is that, in contrast to ensemble-based methods, TEMPL is performed ultimately at the level of individual particles. Notwithstanding this, machine learning assistance permits the analysis of a statistically meaningful number of particles. By using the TEMPL method to analyse the 3D morphology–fluorescence relationship of NV-containing nanodiamonds, we directly reveal that the volume-averaged brightness of thin, flake-like nanodiamond particles is up to several times greater than that of three-dimensional-shaped, thicker particles.

#### Graphic:



#### Keywords:

Correlative microscopy, Machine Learning, fluorescence

#### Reference:

- [1] Xiao et al, *Nanoscale Research Letters* (2021) 16, pp.167
- [2] J Storteboom et al, *Optics Express* (2015) 23, 9, pp.11327-11333
- [3] H Wen et al, *ACS Nano* (2022) 17, pp.16491