

# Fluorescent Carbon Nanoparticles: Synthesis, Characterization, and Bioimaging Application

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Fluorescent carbon nanoparticles (CNPs) 2–6 nm in size with a quantum yield of about ~3% were synthesized via nitric acid oxidation of carbon soot, and this approach can be used for milligram-scale synthesis of these water-soluble particles. These CNPs are nanocrystalline with a predominantly graphitic structure and show green fluorescence under UV exposure. Nitric acid oxidation induces nitrogen and oxygen incorporation into soot particles, which afforded water solubility and a light-emitting property; the isolation of small particles from a mixture of different sized particles improved the fluorescence quantum yield. These CNPs show encouraging cell-imaging applications. They enter into cells without any further functionalization, and the fluorescence property of these particles can be used for fluorescence-based cell imaging applications.

## Introduction

The emergence of fluorescence carbon nanoparticles (CNPs) shows high potential in biological labeling, bioimaging, and other different optoelectronic device applications.<sup>1–13</sup> These carbon nanoparticles are biocompatible and chemically inert,<sup>2,6,14–18</sup> which has advantages over conventional cadmium-based quantum dots.<sup>19</sup> However, these fluorescent carbon nanoparticles are poorly studied compared with other carbon-based materials, such as carbon nanotubes and fullerenes. In addition, the understanding of the origin of fluorescence in carbon nanoparticle is far from sufficient.<sup>4,5,7,9</sup> For example, information on the microstructure and surface ligands remains unclear and details of the organic passivation is not sufficient to aid understanding of the surface states beneficial for light emission.

Common routes in making fluorescent carbon nanoparticles include high-energy ion beam radiation based creation of point defects in diamond particles, followed by annealing;<sup>1,3</sup> laser ablation of graphite, followed by oxidation and functionalization;<sup>4,7</sup> thermal decomposition of organic compounds,<sup>10,11,13</sup> electrooxidation of graphite,<sup>9</sup> and oxidation of candle soot with nitric acid.<sup>8</sup> A wide range of fluorescent carbon particles of different colors can be prepared by those approaches; for example, octadecylamine-functionalized diamond nanoparticle showed blue fluorescence,<sup>12</sup> nitrogen-doped diamonds showed red fluorescence,<sup>1a</sup> and candle soot derived particles,<sup>8</sup> thermal decomposition method,<sup>11,13</sup> or laser ablation method<sup>4</sup> produced particles with multiple colors. However, the quantum yield of most of these particles is too low (<1%),<sup>8,9</sup> with few exceptions.<sup>4,13</sup> In addition, the synthetic methods are cumbersome and inefficient. For example, in the high-energy ion beam radiation based method, it is difficult to introduce a large number of point defects into ultrafine nanocarbon particles (<10 nm) for bright luminescence.<sup>1,3</sup> Thermal decomposition based methods produce a low yield of soluble and fluorescent particles with a significant fraction of insoluble product.<sup>10,11,13</sup> Soot-based synthesis produces a particle mixture of different colors, and isolation of different colored particles by gel electrophoresis is a difficult task.<sup>8</sup> A recent report showed that surface passivation can lead to a significant increase

in fluorescence quantum yield (4–15%); however, the exact mechanism is not yet clear.<sup>4,13</sup> Thus, simple, efficient, and large-scale synthesis of fluorescent carbon nanoparticles and their isolation, purification, and functionalization are very challenging.

Among all these synthetic methods, the soot-based approach is simple and straightforward.<sup>8</sup> However, the quantum yield of fluorescent carbon nanoparticles is too low (<0.1%) for any useful application.<sup>8</sup> Herein, we report an improved soot-based method of synthesizing fluorescent carbon nanoparticles (CNPs) 2–6 nm in size with a quantum yield of ~3%. There are three distinct improvements in our modified method. First, we developed a simple separation method of small size and fluorescent carbon particles from the heterogeneous particle mixture. The method is applicable for milligram-scale synthesis of these particles. Second, small particles are more fluorescent than larger ones and thus isolation of small particles improves the quantum yield from <0.1% to ~3%. Third, we observed that these small carbon particles enter into cells without any further functionalization and the fluorescence property of the particles can be used for fluorescence-based cell-imaging applications.

## Experimental Procedures

**Synthesis of Carbon Particles.** Carbon soot (25 mg, collected from a burning candle) was mixed with 15 mL of 5 M nitric acid in a 25 mL three-necked flask. It was then refluxed at 100 °C for 12 h with magnetic stirring. After that, the black solution was cooled and centrifuged at 3000 rpm for 10 min to separate out unreacted carbon soot. The light brownish-yellow supernatant was collected, which shows green fluorescence under UV exposure. The aqueous supernatant was mixed with acetone (water/acetone volume ratio was 1:3) and centrifuged at 14 000 rpm for 10 min. The black precipitate was collected and dissolved in 5–10 mL of water. The colorless and nonfluorescent supernatant was discarded. This step of purification separates excess nitric acid from the carbon nanoparticles. This concentrated aqueous solution, having almost neutral pH, was taken for further use. The same synthesis technique was also performed for 6 h of reflux and 18 h of reflux. The supernatant obtained from the 6 h reflux was pale yellow and, for the 18 h reflux, was dark yellow. We weighed the unreacted carbon soot, which was removed as precipitate, in order to find

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