

Discriminating between absorption and scattering coefficients in optical characterisation measurements on gold nanoparticle based photoacoustic contrast agents

C. Ungureanu*, A. Amelink⁺, Henricus J. C. M. Sterenborg⁺, S. Manohar, T. G Van Leeuwen

Biomedical Technological Institute (BMTI) & Faculty of Science and Technology, Biophysical Engineering Group, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands;

⁺Center for Optical Diagnostics and Therapy, Department of Radiation Oncology, Erasmus Medical Center, Westzeedijk 118, P.O. Box 2040, 3000 CA Rotterdam, The Netherlands

ABSTRACT

Plasmon resonant nanoparticles such as gold nanoshells and gold nanorods can be tuned to possess sharp interaction peaks in the near-infrared wavelength regions. These have great importance as contrast agents in photoacoustic imaging and as photothermal agents for therapeutic applications due to their high absorptions. While the optical properties of the particles can be described using Mie theory and/or numerical methods such as the Discrete Dipole Approximation, discriminating between their optical absorption and scattering in experiments is not easy. In this paper we discuss for the first time a novel method based on a two-fiber spectrometer that allows measurement of the scattering and absorption coefficients of gold nanoparticles in solution.

This technique, called Differential Path length Spectroscopy, has been developed earlier for measurement in highly diffusive media such as tissue. We demonstrate this concept on gold nanospheres and nanoshells of various sizes. We believe that this will develop into a fast and reliable method able to work on small samples (<1 ml) of nanoparticles to obtain scattering and absorbing spectra.

Keywords: gold nanoparticles, optical properties, photoacoustic imaging, contrast agents, differential path length spectroscopy.

1. INTRODUCTION

Gold nanoparticles have great potential to be used as contrast agents and therapeutic vectors in medicine. This is due to their special optical properties as a consequence of the surface plasmon effect. By tuning the size and shape of the particles they can be made to strongly absorb or scatter different wavelengths of the incident light [1]. Further, they can be bioconjugated with appropriate antibodies. In this way, once injected into blood they will accumulate at tumor sites. The accumulation of particles can be detected using various imaging techniques. Particles with a high absorption cross section can be detected using photoacoustic imaging [2, 3]. The high scattering cross section can be used efficiently by techniques like OCT [4] and Raman [5]. Further, a high absorption cross section can be used efficiently to convert the incident light into heat transforming the gold nanoparticles into therapeutic vectors [6]. The localized heat generated can be sufficiently high to induce apoptosis and/ or necrosis at the tumor site.

However, gold nanoparticles can be found in various sizes and shapes, each one with their advantages and disadvantages. Firstly, it must be determined 1) at what wavelength the extinction, absorption and scattering plasmon peaks are located and 2) how large the extinction, absorption and scattering cross sections are. These two main parameters will decide the application domain. For example: gold spheres (20 – 80 nm) absorb and scatter in the green wavelength region (520-550 nm). This limits the application of these particles to investigation of superficial tissue, because light at 520 nm is heavily absorbed by hemoglobin and is also highly scattered by tissue components. The increase in size of these particles will lead to an increase of the scattering cross section and not to a substantial red shift of the absorption

* c.ungureanu@tnw.utwente.nl; phone: +31-(0)53-489 3112; fax:+31-(0)53-489 1105

plasmon peak. This drawback is eliminated by the use of gold nanorods (spherical capped cylinder). Here, because of the asymmetry, two plasmon bands exist: one is situated in the green wavelength region due to the oscillation of plasmon on the transversal axis, while the other plasmon peak (the longitudinal plasmon peak) is red shifted in proportion to the aspect ratio of the particle.

Once the aspect ratio of the particles is chosen to tune the longitudinal plasmon peak to the desired wavelength, we have to answer the question of how large the extinction (Q_{ext}), absorption (Q_{abs}) and scattering efficiencies (Q_{sca}) are. The answer to this question is of high importance because if we intend to use photoacoustic imaging techniques to detect particles, we need particles with high Q_{abs} to obtain sufficient contrast. The easiest way to estimate Q_{abs} is to measure the extinction spectrum. When particles are small enough (< 20 nm) we can use the following approximation $\mu_{ext} \approx \mu_{abs}$.

However, particles at this size have a low Q_{abs} which do not recommend them as contrast agents for photoacoustic imaging. On the other hand, if the particle size increases, the simple assumption of $\mu_{ext} \approx \mu_{abs}$ is not valid anymore due to an increase of the scattering component (Q_{sca}). This is a question which needs to be addressed: how can we accurately determine the values of μ_{abs} and μ_{sca} for prepared gold nanoparticles.

One of the solutions is to simulate optical properties of the particles using various computer models based on e.g. Mie theory for spheres and shells and on Discrete Dipole Approximation for generalized particles. From these simulations Q_{ext} , Q_{abs} , Q_{sca} can be obtained for the sizes and shapes measured using electron microscopy and μ_{ext} can be determined using a spectrophotometer. Using that information, the concentration of particles can be calculated as $N_{part} (cm^{-3}) = \frac{\mu_{ext} (cm^{-1})}{Q_{ext} * \pi * r^2 (cm^2)}$, where r is the radius of the particle. Further, the absorption and scattering coefficient can be calculated using

$$\mu_{abs,sca} = N_{part} * Q_{abs,sca} * \pi * r^2. \quad (1)$$

However, this can not be always done correctly because different theoretical models provide different Q_{ext} , Q_{abs} , and Q_{sca} for the same particle [7] which confounds estimation of the efficiencies. In some cases, the absorption coefficient can be measured using collimated transmission of light with some experimental limitations [8]. Methods like the double integrating sphere technique were used with some success for different gold nanoparticles [9].

Nevertheless, currently there is no easy and reliable experimental method to discriminate between absorption and scattering properties for a solution containing gold nanoparticles. In this article we report a new experimental method based on the combined use of differential path length spectroscopy (DPS) and extinction measurements, which can be used to differentiate the absorption and scattering properties of gold nanoparticles. This approach allows us to obtain the backscattering coefficient (μ_{bk}) spectrum and the extinction spectrum (μ_{ext}) for the gold nanoparticles. Further, the scattering phase function can be estimated for the investigated particles from computer simulation.

We have found that scattering phase function is almost independent of choosing the dielectric function used for simulation. Using the phase function at 180° , ($p(180)$) estimated from computer simulation and μ_{bk} , the scattering coefficient can be calculated. Finally, the absorption coefficient can be calculated as extinction coefficient minus scattering coefficient.

The primary results in the case of gold spheres with 25 nm diameter compare well with the theory. Further this method can be used to determine the absorption and scattering coefficient for gold nanorods, gold nanoshells and in principle to any particles which scatter the light and have a calculable phase function.

2. EXPERIMENTAL SECTION: MATERIAL AND METHODS

2.1 Differential path length spectroscopy setup

We have used an optical fiber based system to measure the backscattering spectrum of investigated particles. The system is well described in [12] but a short description of the setup is provided here. The schematic diagram of the setup is shown in figure 1. The device contains a spectrophotometer (SD 2000; Ocean Optics; Duiven, The Netherlands) and halogen light source (HL-2000-FHSA; Ocean Optics; Duiven, The Netherlands). During measurement, photons travel from the light source through one arm of a bifurcated fiber and through the *dc* fiber, after which it exits into the sample. Reflected photons that are collected by the *dc* fiber travel through the second arm of the bifurcated fiber and into the first

channel of the spectrophotometer. Reflected photons that are collected by the c fiber travel directly into the second channel of the spectrophotometer. Spectral reflections at the probe tip due to refractive index mismatch between the fiber and sample are minimized by polishing the DPS probe tip at an angle of 15 deg.

A calibration procedure, described in detail elsewhere [10] was utilized to account for other internal reflections, variability in lamp-specific output, and in fiber specific transmission properties.

The difference between the collected signal from the dc and c-fiber is called the differential reflectance signal. When single scattering events are detected, the differential reflectance signal was shown to be equal to $C_{app} * p(180) * \mu_{sca}$, where C_{app} is the apparatus constant which depends on the distance between the probe tip and the reference material. The apparatus constant was determined using measurements on polystyrene beads of 0.2 micron diameter (2.65% solid latex, 90003-53-8) purchased from Polysciences Inc.

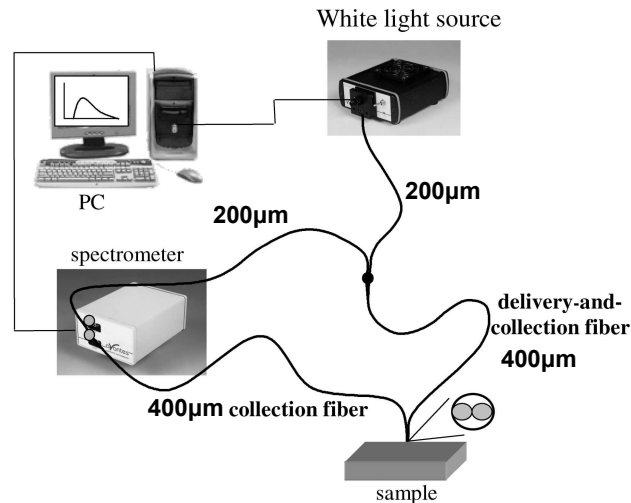


Figure1. Schematic drawing of the DPS setup.

The main advantage of using DPS is that it interrogates small volumes, which is helpful when big volumes of nanoparticles are not available.

2.2 Determination of the extinction spectrum

The extinction spectrum of gold nanoparticles was measured using the Shimadzu PC3101 UV-Vis-NIR spectrophotometer using plastic cuvette with 1 cm path length.

2.3. Gold nanoparticles

Gold nanoparticles of 25 nm diameter were purchased from Aurion with a bulk concentration of $3.3 * 10^{10}$ p/ml.

2.4 Optical property simulations

Optical properties of the gold nanospheres were simulated using the DDSCAT code [11].

The important input parameters in the simulations are:

- Size
- Shape
- Dielectric function of the nanoparticle
- Local refractive index.

Gold spheres with 25 nm diameter and a size corrected dielectric function of gold from Palik [12] were used. The size correction of dielectric function was done using the approach mentioned in [13]. A number of 40000 dipoles was used for simulations. Water with refractive index of 1.33 was chosen as medium in which nanoparticles are dispersed.

The output parameters are Q_{ext} , Q_{abs} , Q_{sca} , Q_{bk} (backscattering efficiency) and $p(180)$ which is the phase function at 180° .

2.5. EXPERIMENTAL PROTOCOL TO DETERMINE OPTICAL PROPERTIES

The experiment consists of the following steps:

1. Calibration of the DPS setup (determine C_{app} using polybeads).
2. Measure the extinction spectrum of the solution of gold nanoparticles (determine μ_{ext}).
3. Measure the backscattering spectrum of the investigated solution using DPS (determine μ_{bk}).
4. Simulate optical properties of gold spheres using DDSCAT (determine $p(180)$).
5. Using :

$$\mu_{bk} = 4 \cdot \pi \cdot p(180) \cdot \mu_{sca} \tag{2}$$

determine μ_{sca}

6. Using $\mu_{ext} = \mu_{abs} + \mu_{sca}$, (3)

determine μ_{abs}

3.RESULTS AND DISCUSSION

3.1 Polybeads (0.2µm)

Figure 2 shows the DPS signal from the solution of 0.2 micron polybeads diluted to ensure that we have only single scattering events, plotted along with the Q_{bk} calculated using DDSCAT code.

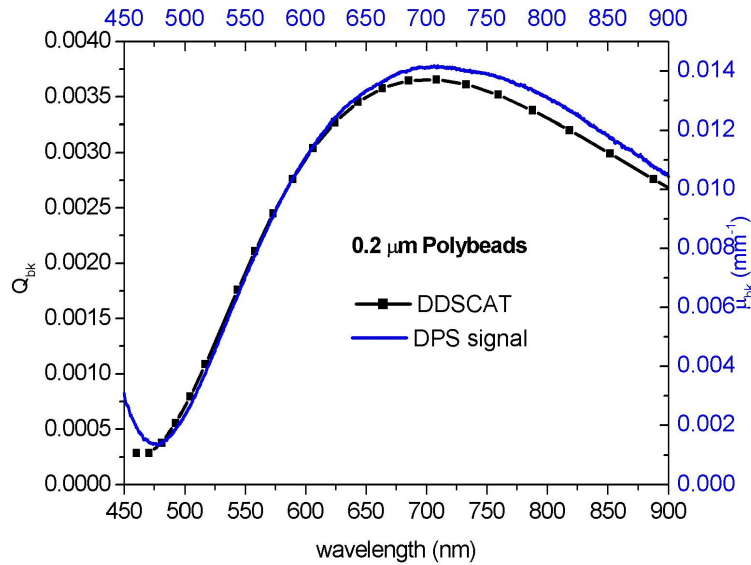


Figure 2. Differential signal from 0.2 micron beads together with backscattering efficiency calculated using DDSCAT.

The two graphs from figure 2 have the same shape indicating the agreement between experiments and simulations. Differences in spectrum shape are probably due to polydispersity of beads used in calibration.

We have determined the scattering coefficient of polystyrene beads using formula (1) and the concentration of beads provided by the producer. Figure 3 shows the simulated phase function at 180° of polystyrene beads using DDSCAT. The μ_{sca} calculated using formula (1) and μ_{sca} determined using DPS, $p(180)$ and formula (2) are both plotted in figure 4.

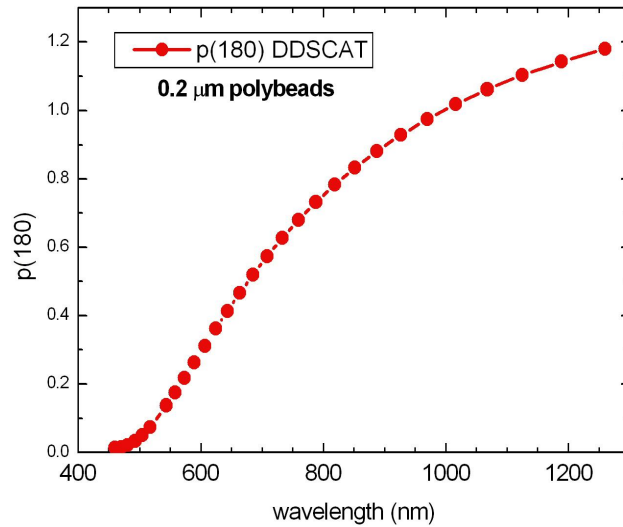


Figure 3. Simulated $p(180)$ of polybeads using DDSCAT.

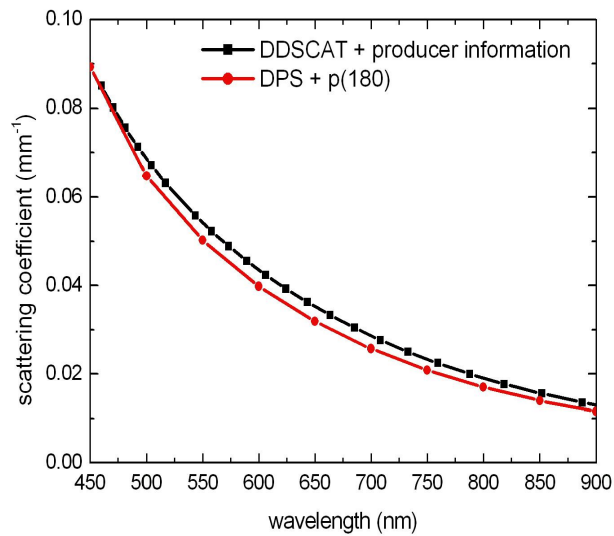


Figure 4. μ_{sca} determined from DPS and $p(180)$ and μ_{sca} calculated using Q_{sca} from DDSCAT and concentration of particles from the producer.

Results from figure 4 compares well one to another, however for a perfect match the apparatus constant must be perfectly determined.

3.2 Gold spheres 25 nm diameter

The measured extinction spectrum and simulated extinction efficiency of the gold nanospheres are shown in figure 5. The position of the plasmon peak predicted by simulations matches the experimental determined one. Figure 6 shows the comparison between the μ_{bk} from DPS and Q_{bk} from computer simulation.

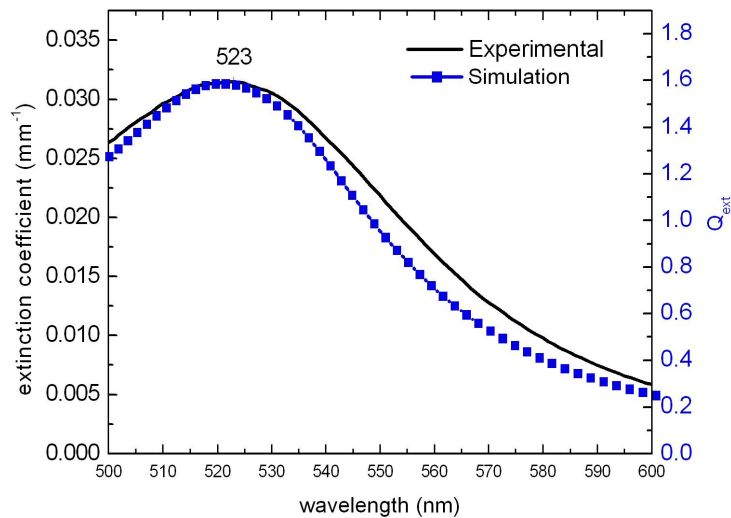


Figure 5. Experimental extinction spectrum and simulated extinction efficiency using size corrected Palik dielectric function for 25 nm gold spheres.

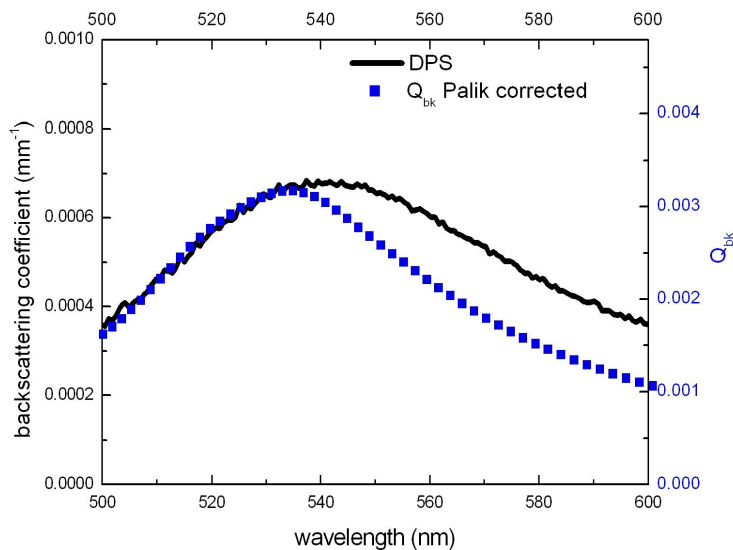


Figure 6. Experimental backscattering coefficient and simulated backscattering efficiency.

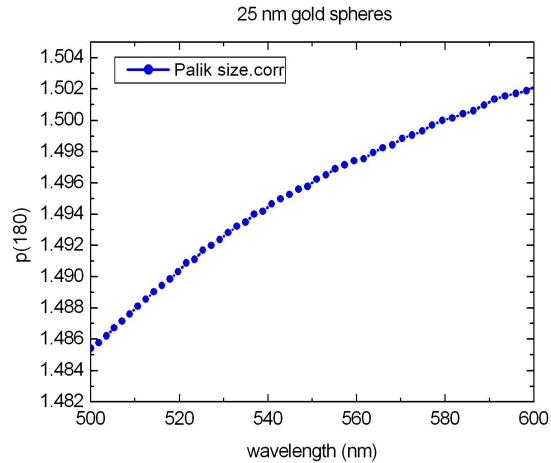


Figure 7. Simulated $p(180)$ for 25 nm gold spheres.

Again, the results from simulation agree in general with the experimental measured spectrum (see figure 6). The shape differences could be due to polydispersity of the gold nanoparticles and the fact that simulations are done for a single particle. The simulated phase function at 180° for the 25 nm gold sphere as a function of wavelength is plotted in figure 7.

Using the above information, μ_{sca} of the gold spheres can be determined using formula (2) and in a different approach using formula (1). Results using both methods are plotted in figure 8.a. Both methods predict the same values of scattering coefficient at the plasmon peak.

However, the method based on formula (1) has several drawbacks. One major drawback is that the results strongly depend on the dielectric function. If a different dielectric is chosen then results can strongly vary in amplitude. Secondly, the shape of the scattering spectrum does not follow the real curve because of the assumptions of monodispersity in the simulation.

Further, knowingly that we are in the single scattering regime, the absorption coefficient can be calculated using formula (3). The extinction, absorption and scattering coefficient spectrum determined with DPS and $p(180)$ is plotted in figure 8.b.

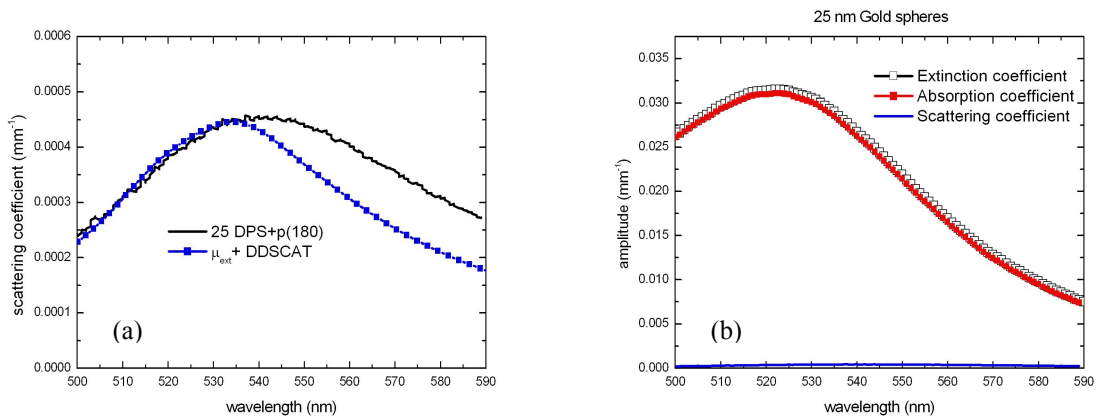


Figure 8.a. Scattering coefficient spectrum determined using both methods and figure 8.b. Absorption and scattering coefficient determined using DPS and $p(180)$.

Figure 9 shows the simulated Q_{ext} , Q_{abs} and Q_{sca} for 25 nm gold spheres.

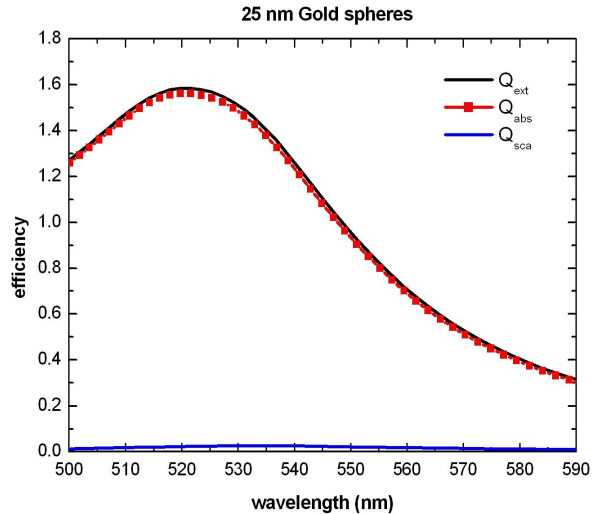


Figure 9. Simulated Q_{ext} , Q_{abs} and Q_{sca} for 25 nm gold spheres using size corrected dielectric function from Palik [12].

The experimental results (figure 8.5 and figure 9) compare well with simulations, showing that at this size of the particles extinction is dominated by the absorption.

Some sources of error which can affect our approach of determining optical properties of gold nanoparticles were identified. These are the following:

- For the initial calibration, to determine C_{app} , the size and concentration of particles used for calibration must be accurate.
- The probes must be diluted enough to avoid multiple scattering events.
- For particles with strange shapes (stars, diamonds), the phase function at 180° function of wavelength may not be easy to determine.
- The size of the particles and material must be available for a right determination of the phase function.

4. CONCLUSION

For the first time, we demonstrate the application of differential path length spectroscopy to measure scattering properties of gold nanoparticles. This setup can provide qualitative (shape of the backscattering spectrum) and quantitative information (magnitude of the backscattering coefficient).

Further, using information about the phase function, the scattering coefficient can be determined from DPS measurements on low-scattering samples. If the extinction spectrum is available, then the absorption coefficient can be determined by subtracting scattering from extinction. The results on 25 nm gold nanospheres compare well with the theory proving the feasibility of the method.

This method can be easily extrapolated to determine the absorption and scattering coefficient for gold nanoparticles with various sizes and shapes. This is the subject of future work.

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