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Brief note

# Utilization of interferometric light microscopy for the rapid analysis of virus abundance in a river

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**Abstract**

There is a constant need for direct counting of biotic nanoparticles such as viruses to unravel river functioning. We used, for the first time in freshwater, a new method based on interferometry differentiating viruses from other particles such as membrane vesicles. In the French Marne River, viruses represented between 42 and 72% of the particles. A spring monitoring in 2014 revealed their increase ($2.1 \times 10^7$ to $2.1 \times 10^8$ $mL^{-1}$) linked to an increase in algal biomass and diversity of bacterial plankton. Predicted virus size distributions were in agreement with transmission electron microscopy analysis suggesting a dominance of large viruses (≥60 nm).



*Keywords:* Virus; Membrane vesicle; Interferometric light microscopy; River

## 1. Introduction

Water from the Marne River, one of the major tributaries of the Seine River, is used for the production of drinking water for most of the inhabitants of Paris and surroundings. The Marne River used to be subjected to high levels of nutrients (nitrogen and phosphorus), originating from intensive agriculture and urban impact which entailed uneven and recurrent development of algae. These algal blooms were mostly due to diatoms, and secondarily to Chlorophyceae, cyanobacteria forming only low populations in lotic systems [1−3]. Since the 2000s, with the EU Water Framework Directive (2000/60/CE), phosphorus has been considerably reduced [4,5]. Algal blooms have become scarce, except in dry years. These blooms are mainly observed in spring, when the dilution rate by the discharge becomes lower than algal growth rate. These algal blooms constitute a significant inconvenience for the production of drinking water as, in addition to clogging filters, they increase the water pH [2]. Algal dynamics have been extensively studied for 25 years in order to improve their growing prediction by modeling [6], without explicitly considering the ecological effect of viruses.

In any aquatic environment, viruses, about ten times more abundant than bacteria, control the algal and microbial diversity by lysis and may influence biogeochemical cycles. The abundance of aquatic viruses fluctuates over time, especially

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with the seasons. A peak of concentration is usually observed during spring–summer periods in surface waters; conversely, a significant decline occurs in autumn–winter [7]. The most common aquatic viruses of phytoplankton are double-stranded DNA-tailed viruses (Caudovirales) such as the following families: Myovirus (head size: 60–145 nm), Podovirus (60–70 nm) and Siphovirus (40–80 nm) which infect prokaryotes while PhycoDNAviruses (large DNA viruses, 100–220 nm) or picorna-like viruses (RNA viruses, 35 nm) infect eukaryotic algae [7].

Viral direct counts in any environments remain a critical step requiring a reliable and accurate method [8]. Transmission electron microscopy (TEM) is one of the best techniques since it allows virus morphotypes to be counted and characterized [9], but is highly time-consuming. Optical methods based on epifluorescence microscopy to detect double-stranded DNA binding fluorophores such as DAPI or SYBR are used extensively. However, these methods are adapted neither for detection of single-stranded DNA, nor RNA viruses [10]. Flow cytometry is also frequently used to enumerate viruses in natural viral assemblages [11]. Most previous methods need expensive equipment and do not distinguish viruses from extracellular membrane vesicles. Extracellular membrane vesicles are another type of aquatic environmental nanoparticle. Produced by organisms from the three domains of life, they are made of lipids and proteins and sometimes contain genetic material, and could thus be mistaken for viruses [12].

We used a new method suitable for all genetic material of viruses and able to differentiate viruses from membrane vesicles. The interferometric light microscope (ILM) combines two measurements for a single nanoparticle (30–100 nm): its scattered signal and its Brownian motion. The scattered signal is a function of the size and refractive index (related to density) of the particle, while the Brownian motion is a function only of the size of the particle [13]. ILM was utilized to distinguish virus from other particles and then tested to explore the variations of viral communities in the Marne River in spring 2014, expected to be the algal bloom period. The discharge averaging 180 $m^3\ s^{-1}$ in January–March decreased to 60 $m^3\ s^{-1}$ during the studied period (April–May). The diversity of eukaryotic and bacterial communities was determined simultaneously.

## 2. Material and methods

### 2.1. Sample preparation

Water was collected at Saint Maurice (5 km upstream from Paris, 48°48′58.16″ North, 002°25′27.35″ East), the outlet of the Marne River, at three dates in 2014: April 1st and 22nd and May 14th. Samples were kept in an ice cooler during transport and filtered as soon as they arrived at the laboratory. Two liters of water were first filtered through 0.22 µm filters (PolyVinyliDene Fluoride, Millipore) to collect eukaryotic and prokaryotic plankton. The filters were replaced every 0.5 L to avoid clogging. Filtrates (nanometric fraction) were then

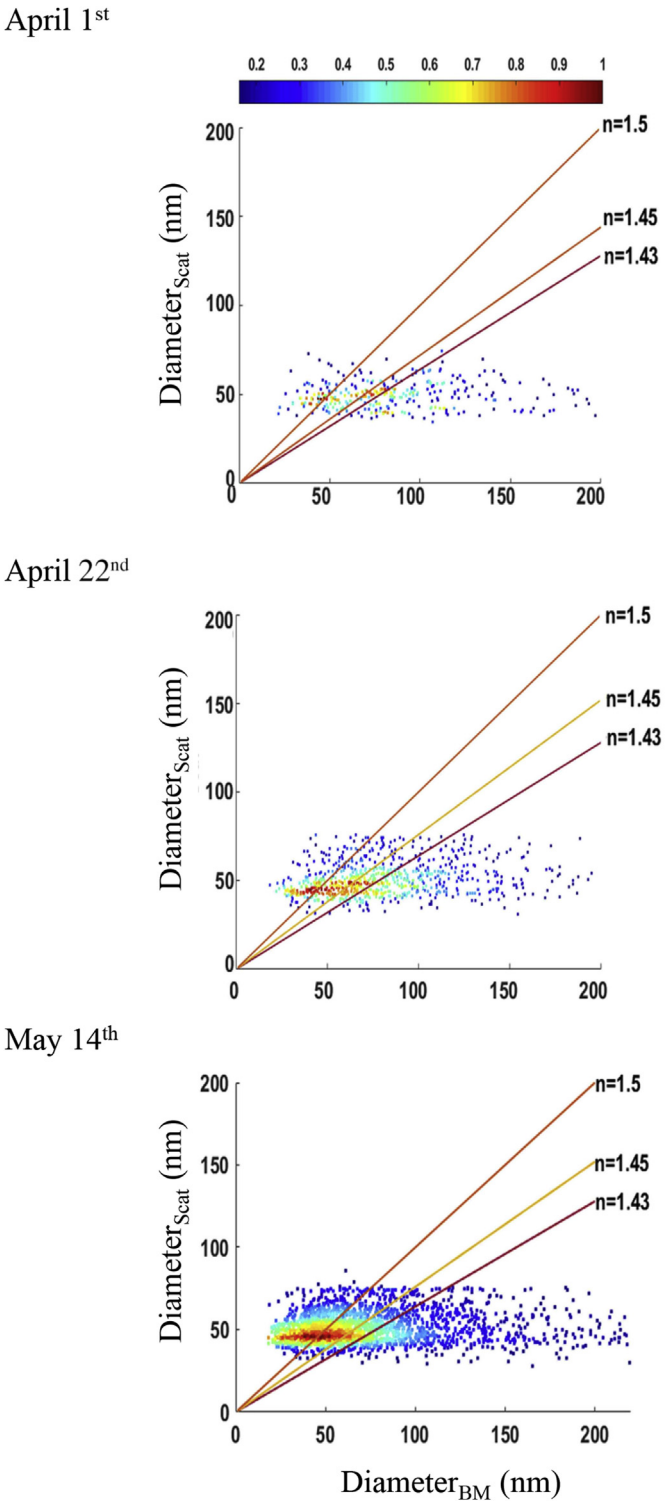


Fig. 1. Analysis of particles present at the Saint Maurice station, an outlet of the Marne River. Plot of the diameters of particles computed with their scattered signals (Diameter$_{_scat}$) and Brownian motion (Diameter$_{_BM}$). Lines indicate refractive indexes of the different particles (viruses and vesicles). The colored bar corresponds to the relative number of characterized particles: the different colors correspond to the Matlab histogram plotted in normalized linear scale. The viruses that possess a refractive index of 1.5 are likely close to this line, while the rest of the particles could be vesicles.

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