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Research paper

The use of circularly polarized light for biometry, identification and estimation of mass of coccoliths

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ABSTRACT

The most commonly used tool for observing and identifying coccoliths and other calcareous nannofossils is a transmitted light microscope equipped with crossed polarizers. Such microscopes can readily be modified to produce circularly polarized light (CPL) instead of linearly polarized light, and this can overcome some disadvantages of linearly polarized light. With circularly polarized light there is no extinction of small coccoliths at any orientation, so the whole specimen can be seen at once and radial coccoliths no longer show the pseudo-extinction crosses typical of crossed polarized light, so their appearance does not vary with orientation and some aspects of their morphology are much easier to observe. Finally the combination of circularly polarized light and the oblique illumination obtained with the microscope condenser means that coccolith brightness is less dependent on inclination of the crystal c-axis than is generally assumed and so that mass estimates based on integrated brightness are more reliable than might be expected. This illumination method thus has a wide range of applications in the study of calcareous nannofossils, including biometry, taxonomic identification, and calculations of coccolith size and mass for a wide range of taxa. Here we describe this method combined with the development of a new computer program for analysis of images captured under circularly polarized light. We then focus on applying this technique to calculations of coccolith size and mass of various taxa, comparing results with previous studies.

1. Introduction

Coccolithophore algae possess small calcareous plates called coccoliths that they secrete to form a cell covering. Calcium carbonate ($CaCO_3$) has three anhydrous crystalline polymorphs: vaterite, aragonite, and calcite. Traces of aragonite and vaterite precipitated with calcite have been reported in modern coccoliths (i.e., Wilbur and Watabe, 1963), however these findings have not been corroborated (Young et al., 1991) and neither aragonite nor vaterite have been found in nannofossil sediments because they are unstable minerals that revert to calcite. Therefore, fossil coccoliths are composed of calcite. The most common method for observing and identifying calcareous nannofossils is using a transmitted light microscope equipped with crossed polarizers (also called crossed nicols). The sample is illuminated with linearly polarized light and observed through a second linear polarizer, usually called the analyzer, orientated at 90° to the first polarizer. Isotropic materials appear dark, as do anisotropic materials viewed along an optical axis. Other anisotropic materials show interference colors depending on their shape, thickness and orientation.

Since Kamptner (1954) demonstrated that coccoliths of different taxa show different extinction patterns, taxonomic identification has routinely made use of diagnostic extinction patterns. However, this traditional technique, using linearly polarized light, has some disadvantages. For example, when observed with linearly polarized light, coccoliths with a radial fabric and circular or elliptical shape show distinct extinction crosses, with some areas where the extinction of light is total, so they appear black, and others where brightness is at a maximum. Although these extinction crosses can be useful for identifying taxa, they can also be a disadvantage for some applications because the brightness depends not only on the thickness and shape, but also on the orientation of the coccolith with respect to the axes of the polarizers. Furthermore, for specimens formed of single crystal units, such as *Florisphaera profunda* nannoliths, the whole specimen is invisible at some angles, which means that the observer has to rotate the microscope stage in order to see them. These disadvantages can be overcome if circularly polarized light is used instead of linearly polarized light. Furthermore, a method of estimating the mass of a single coccolith originally proposed by Beaufort (2005) using images taken with linearly polarized light can be improved if circularly polarized light is used.

Although circular polarization is not a new technique and indeed is commonly used in several fields of science, it has not yet been widely applied to the study of coccoliths and has the potential to become an important technique in the field, as demonstrated recently by Fuertes et al. (2013) and Bollmann (2014). Here, we describe this technique in detail and present a new computer program for coccolith size

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and mass determination using images captured under circularly polarized light, and then apply this method to some common Pleistocene coccolithophore taxa.

2. Materials and methods

In this study we used a Nikon Eclipse LV100 POL polarized light microscope with a 100× H/N2 objective, two λ/4 plates and a Nikon DS-Fi1 Digital Camera. The images were captured using Nis-Elements software, and processed with a customized program developed in MatLab, named C-Calcita. The sample slides were prepared using the decantation method of Flores and Sierro (1997), which creates a homogenous distribution and therefore allows calculations of the number of coccoliths per gram of sediment and coccolith fluxes to be made.

2.1. Description of the microscope technique

When a linear polarizer microscope is used, the light ray that passes through a birefringent material is split into two perpendicular rays, the ordinary and the extraordinary, that are propagated through the sample with different velocities. The retardation of the slow ray with respect to the fast one means that when both rays emerge from the sample they have a phase difference (φ) that depends on the difference in the refractive index of the two rays (n_o and n_e), the thickness of the sample (d), and the wavelength of the light (λ), such that:

$$\varphi = 2\ \pi\ d(n_e\text{-}n_o)/\lambda.$$

The ordinary and extraordinary rays are recombined when they pass through the second polarizer, where they interfere. The interference image obtained depends on the phase difference between the two rays. As calcite is a highly birefringent material, coccoliths can easily be seen in linearly polarized light and they show an extinction pattern when viewed between the crossed nicols of a polarizing microscope.

A linear polarizer microscope can be transformed into a circular polarizer microscope by using two λ/4 retardation plates (Fig. 1a), placing one of them above the lower linear polarizer at an angle of 45° relative to it and the other one before the upper linear polarizer, also at 45°.

Linearly polarized light can be described as two orthogonal components, Ex and Ey, which are equal and in phase. When light falls on the λ/4 plate with an angle of 45° relative to its optical axis, the components travel along it with different velocities, so that when they exit the plate they maintain the same angle but have a 90° phase shift: when one of them is maximal, the other is minimal. This means that light emerging from the plate is circularly polarized (e.g., Saleh and Teich, 1991; Hecht, 2002, Bass, 2009).

The circularly polarized light then falls on the sample. When a ray of light falls on a birefringent material such as calcite, it is split into two rays, as described above. When rays of light fall perpendicular to the optical axis, as is the case in the R units of heterococcoliths (Young et al., 1992; Young and Henriksen, 2003) or in nannoliths like *F. profunda* (Kameo and Furukawa, 2007), the trajectories of the ordinary and extraordinary rays are the same, but they travel along the crystal with different velocities, and when they emerge they have a phase shift. In the specific case when the incident rays are parallel to the optic axis, they are not split into two rays and the calcite behaves as if it were optically isotropic (e.g., Murphy, 2001).

When circularly polarized light passes through the calcite element and is split into two components, both components travel with the same angular velocity: the phase shift remains constant and the emerging light remains circularly polarized. Subsequently, the light passes through a second λ/4 plate which turns the circularly polarized light linear again, so it can be analyzed by the upper linear polarizer, producing an image where only the birefringent materials are seen and with no

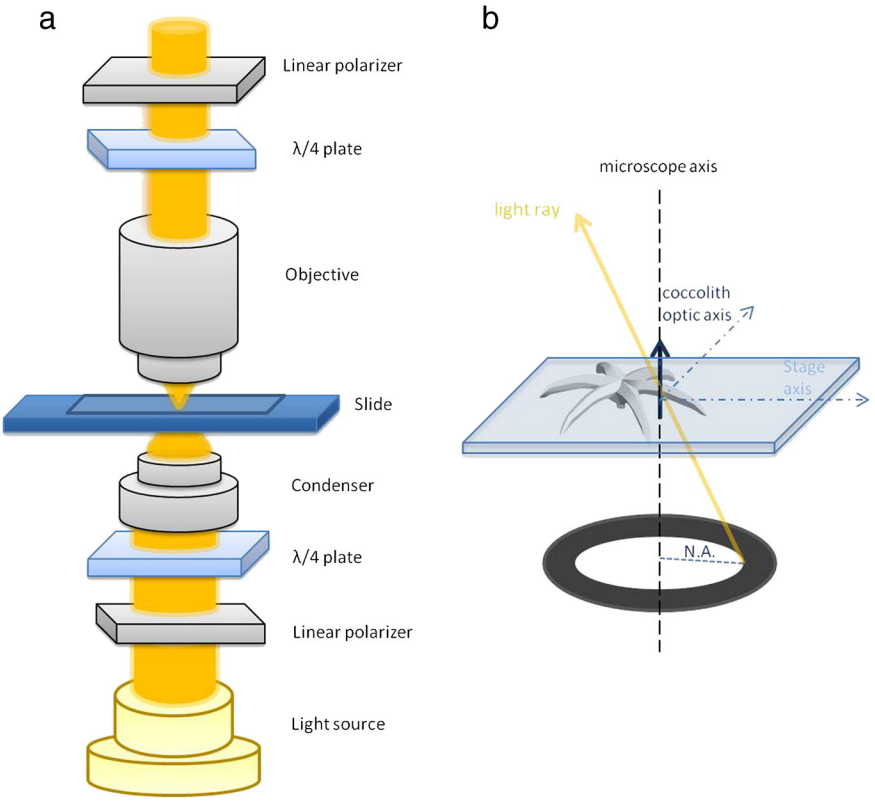

Fig. 1. a: Diagram illustrating how a linearly polarized light microscope can be transformed into a circular one, using two λ/4 plates. b: Diagram illustrating the relative positions of one light ray, the microscope axis, the optical axis of a coccolith (based on Kameo and Furukawa, 2007) and the stage axis.