glass 2 inside the imaging box 1. The object glass provides a defined volume at a distance from a digital camera 6. The defined volume is provided by a space between the two glass plates constituting the object glass 2. The space between the two glass plates can be chosen depending on the desired sample volume, and the focus depth of the camera. For high densities a shorter space should be chosen to reduce the risk of rotifers overlapping in the picture. The rotifers in the volume are photographed by the digital camera and the digital image processed by image processing to obtain a rotifer density inside the fixed volume. Figure 2 shows an arrangement of light sources 7, object glass 2 and camera lens 6 of the prior art rotifer counter shown in Figure 1. Lighting is provided by 16 light emitting diodes 7 mounted in a square with four diodes along each side. The square is arranged so the cone visible to the camera (camera line of sight) falls in between the light emitting diodes 7. This set-up provides darkfield conditions, where light is reflected by particles in the water, causing rotifiers and other particles to appear in the images as bright spots against a dark background. Darkfield conditions provide images with better contrast than bright field conditions.

The principles of the rotifer counter can be used for organisms that move sufficiently slowly or do not respond strongly to stimuli such as pumping or light. However, organisms such as copepodites of *Acartia*, and many other species, move rapidly, react to pressure gradients caused by pumping, and show strong taxis toward light. When pumping samples from a tank, this behavior invalidates the assumption that the plankton density in the measurement volume equals the density in the tank.

SUMMARY OF THE INVENTION

The invention provides a solution to the problems identified above.

In a first aspect the invention provides a method for density measurement of zooplankton in situ in an aqueous solution, the method comprising:

- acquiring at least one image of a volume V of the aqueous solution;
- processing the at least one image and identifying particles in the image;
- analyzing the identified particles based on a sharpness of each particle, and
- identifying zooplankton to be counted;

wherein analyzing a sharpness of each particle further comprises tracing a boundary of each identified particle, calculating an intensity gradient for each pixel along the boundary of each particle from the at least one image based on a rate of change of intensity between neighbouring pixels, and calculating a mean intensity gradient of all the pixels along the boundary of each particle. Analyzing a sharpness of each particle may further comprise correcting the mean intensity gradient for a general contrast level in the at least one image.

The method may further comprise filtering of the identified particles in the at least one image based on shape parameters identifying which particles are zooplankton to be counted. An illumination device for illumination of the volume of the aqueous solution may be controlled in interaction with image acquiring and processing.

In a second aspect the invention provides a system for density measurement of zooplankton in situ in an aqueous solution, comprising an imaging device for acquiring at least one image of a volume V of the aqueous solution; a plate arranged at a distance from the imaging device, the distance and a view angle of the imaging device defining the volume V of the aqueous solution imaged by the imaging device; and an image processing device performing image processing of the at least one image from the imaging device identifying particles in the image, analyzing the identified particles based on a sharpness of each particle, and identifying zooplankton to be counted; wherein analyzing a sharpness of each particle further comprising: tracing a boundary of each identified particle, calculating an intensity gradient for each pixel along the boundary of each particle from the at least one image based on a rate of change of intensity between neighbouring pixels, and calculating a mean intensity gradient of all the pixels along the boundary of each particle.

In a further embodiment, the imaging device may comprise a camera housing including at least one digital camera. At least one illuminating device is provided illuminating the volume of the aqueous solution. A controller may be provided for

controlling the imaging device and the illuminating device based on feedback from the image processing device.

In further aspects, the invention provides use of the system and method above for establishing a density of zooplankton copepods in an aqueous solution. The