Periphyton Analysis

Periphyton species diversity and numbers are usually assessed by one of two approaches: 1) scraping, peeling, or using a sticky material to remove algae from a determined area of natural substrate (rocks or plant surfaces) and examining the scrapings microscopically and for chlorophyll or other pigment content, or 2) by placing artifical substrates in the water for later retrieval, followed by taxonomic and chlorophyll analyses. Commonly used artificial substrates include unglazed clay tiles and glass slides. Slides are not thought to represent natural substrates as well as tiles, but they don't need to be scraped (which can damage the algae and disrupt community structure) in order to examine them by light microscopy. Non-transparent surfaces can be studied by SEM (which, however, is expensive).

I like to use glass slides because they can easily be mounted in slide holders and anchored at various depths in the water. One can also observe ecologically-significant internal features of the algae, such as parasites or endosymbionts, and see algae at the bottom of a stack or aggregation of cells. Upon retrieval, slides bearing algae can be transported back to the lab in 1% glutaraldehyde; this prevents decomposition or other changes. Back at the lab, slides are rinsed to remove sand and sediment, and algae removed from one side by wiping with a paper towel. Then the algae are dehydrated by submerging slides in increasing concentrations of ethanol for 15 minutes each, and stained with fast green dissolved in 95% ethanol. Rinsing with 100% ethanol removes excess stain and completes the dehydration process. Slides should then be submersed briefly in xylenes in a fume hood, whereupon they are removed and a drop of permount added to the algae-coated surface. A large cover slip is added at an angle to avoid bubble formation, then the slide is left to dry on a slide drier. Addition of a lead weight helps to flatten the preparation. When the permount is hard, excess permount is removed and slides are ready to label, store, and examine by LM.

Percent cover of species can be estimated in small quadrats marked on the cover slip, or you can use an ocular grid (Whipple disk) to define areas for analysis. Biovolume can be estimated for various species using data provided in Hillebrand, et al. (1999)-- Biovolume calculation for pelagic and benthic microalgae, Journal of Phycology 35:403-424. Algae that were dead when collected can be recognized by lack of evidence of cytoplasmic contents, which normally stain well with fast green. This stain also aids in recognition of taxonomically important features such as chloroplast shape and presence of pyrenoids.

If SEM examination is required, the slides or other substrates are treated as above except that they are not stained with fast green. Ethanol-submerged substrates are then critical-point dried and gold-coated prior to examination with the SEM.

Chlorophyll may be extracted from slides or samples scraped from substrates; Dimethyl sulfoxide (DMSO) is the best solvent for use with green algae, and is as good a solvent as 90% acetone for diatoms and cyanobacteria (Shoaf and Lium, 1976, Oceanogr. & Limnol. pp 927). Algae are filtered onto a 0.45 micrometer membrane filter that is soluble in DMSO and the filter ground in 3-4 ml of DMSO for 3 minutes at room temperature. After grinding, an equal volume of 90% acetone is added, mixed, then centrifuged for 10 minutes at about 5,000g. Absorbance of the supernatant is determined at appropriate wavelengths, 663 nm for chla and 645 nm for chl b. Equations used for acetone extractions are also appropriate for DMSO. Although toxicity of DMSO is low, it penetrates skin and thus contact with this solvent should be limited.