

## Wet SEM: A Novel Method for Rapid Diagnosis of Brain Tumors

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The authors present the application of wet SEM for histopathological assessment, a technology for imaging fully hydrated samples at atmospheric pressure in a scanning electron microscope (SEM). Both transmission and scanning electron microscopy techniques usually require long and complex sample preparation of the tissues. In marked contrast, a rapid preparation of tissues is described for evaluation by SEM imaging. The wet SEM technology successfully demonstrated both histological and ultrastructural features of several CNS tumors: Rosette formation and intracytoplasmic lumens were observed in ependymoma; numerous fibrillary processes in fibrillary astrocytoma; and focal rosette formation with no intracytoplasmic lumens in medulloblastoma. Application of this method simultaneously with frozen section may improve rapid intraoperative diagnosis of these intracranial tumors.

*Keywords brain tumors, ependymoma, fibrillary astrocytoma, medulloblastoma, microscopy, wet SEM*

Progress in the processing of wet tissues, without the need of complex preparation procedures, may facilitate the microscopic examination of tissues for clinical diagnosis as well as diverse areas of research in the life sciences. We present here the application of wet SEM for histopathology assessment, a technology for imaging fully hydrated samples at atmospheric pressure in a scanning electron microscope (SEM), that can be applied intraoperatively and complement the frozen section diagnosis [1–3].

Ependymomas constitute no more than 5% of all primary central nervous system (CNS) neoplasms, but

comprise approximately 10% of intracranial neoplasms in the pediatric population and up to 30% of those encountered in children under 3 years of age [4]. In the ultrastructural level, cellular ependymomas exhibit a number of specialized cytoplasmic features [5], such as zipper-like junctional complexes (zonula adherents) that bind their constituent cells and are likely responsible for the typical cohesive growth pattern and “pushing” margins and prominent arrays of slender, curving microvilli and cilia, which sprout into the lumina [5]. Tumors that should be considered in the differential diagnosis in the region of the fourth ventricle are fibrillary astrocytoma and medulloblastoma [6].

Transmission electron microscopy (TEM) requires specially prepared ultrathin sections (typically 50 nm), and reveals a wealth of subcellular information not available on light microscopy, which is limited by diffraction to 0.25  $\mu\text{m}$ . Its use is encumbered by extensive processing of the tissue sample, which may alter its structure significantly. Preparation of samples also requires specific skills and takes at least a few days

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FIG. 1 Wet SEM specimen capsule.

to achieve. In addition, the very thin slices present a very limited (and often arbitrary) portion of the sample, necessitating the imaging of multiple serial sections [7, 8]. SEM occupies a more restricted niche, usually limited to surface imaging. It is used to investigate surface topography, and imaging of intracellular structure by SEM requires specialized preparatory techniques, such as fracturing and etching. In marked contrast, we present a rapid preparation of tissues for evaluation by SEM imaging.

**MATERIALS AND METHODS**

The principles of the wet SEM technology were recently described in great detail [1–3]. For the evaluation of the technology for diagnostic neuropathology, cases of ependymoma, fibrillary astrocytoma, and medulloblastoma were selected from the pathology repository of the Department of Pathology Sheba Medical Center after taking samples for diagnostic purposes. All samples were kept in glutaraldehyde prior

to SEM examination. A few small fragments [1–3], several millimeters each, were taken from each tumor. Tissue samples were washed several times in water, incubated for 5 min with 1% tannic acid, and stained with uranyl acetate, 0.1%, pH 3.5, for 10 min. Samples were placed in the wet SEM specimen capsule (Figure 1) and visualized in the SEM with a backscattered electron detector. Imaging was performed on JEOL JSM 6060 LV. Evaluation was done in magnifications of  $\times 6,400$ , although most of the information was achieved at  $\times 800$  and  $\times 1,600$ .

The cases were also examined using TEM, and revisions of the hematoxylin and eosin and immunostains were performed as well.

**RESULTS**

**Ependymoma**

The tumor is composed of elongated cells arranged in rosettes. In many of the cells intracytoplasmic lumens are observed. Some structures suggestive of cilia are present within the lumens, but are not clearly identified. Multiple junctional complexes are present between adjacent cells. Since the microscopic examination is based on surface elements, other features such as blepharoplasts are not recognized. Smaller elements such as microvilli were not demonstrated at the magnifications used for this study (Table, Figures 2 and 3).

**Fibrillary Astrocytoma**

The tumor is composed of elongated cells rich in fibrillary processes. No rosette formation was noted. The cells lack the intracytoplasmic lumens that were observed in ependymoma. In addition, there were no developed junctional complexes (Table, Figure 2).

**Medulloblastoma**

Rosette formation is focally observed. No intracytoplasmic lumens can be identified (Table, Figure 2).

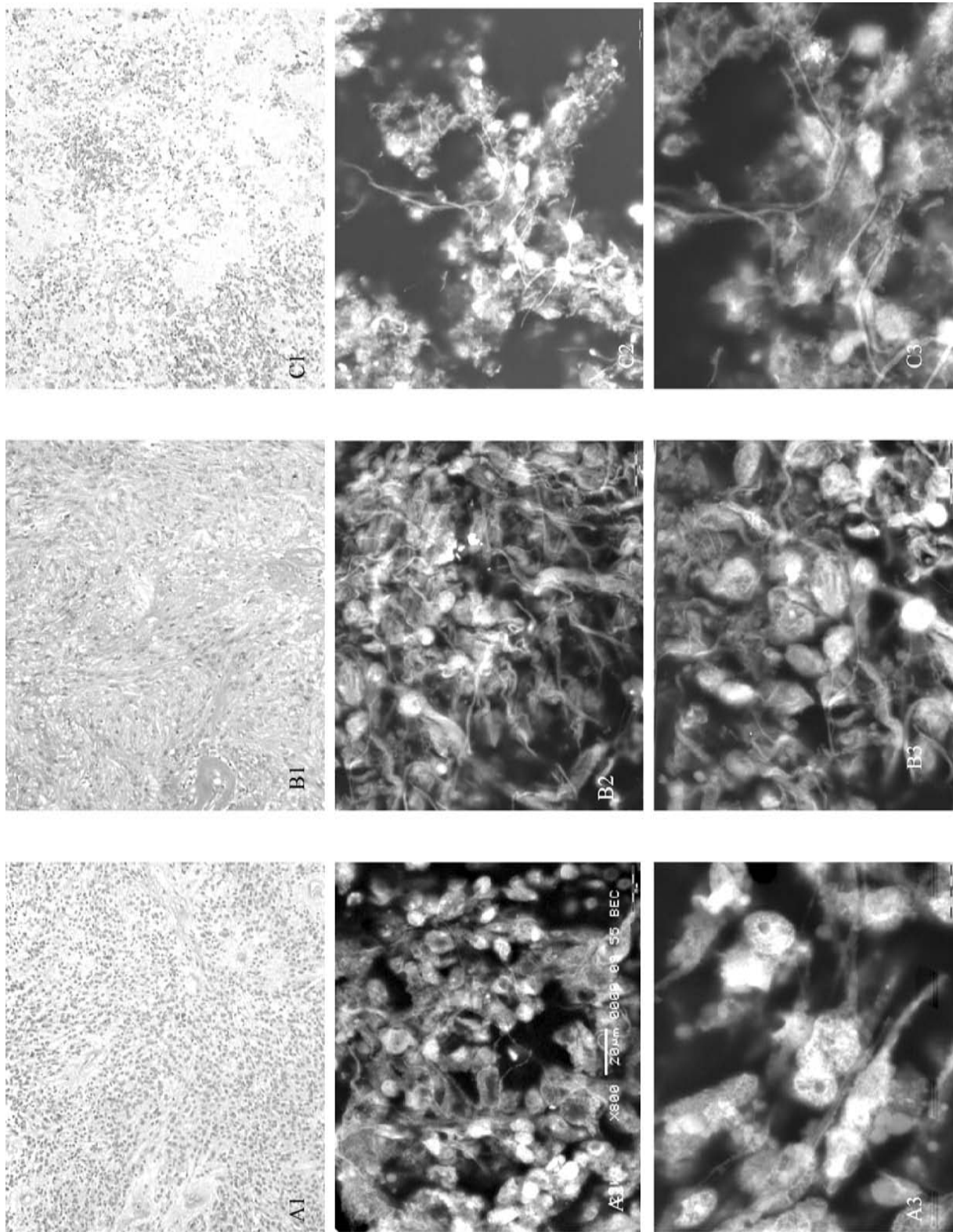
**DISCUSSION**

We present a rapid and simple technology based on a scanning electron microscopy imaging for histopathological evaluation that can be performed on wet

TABLE Wet SEM Characteristics of Ependymoma, Fibrillary Astrocytoma, and Medulloblastoma

	Ependymoma	Fibrillary astrocytoma	Medulloblastoma
Rosette formation	Present	Not identified	Present
Intracytoplasmic lumen	Easily identified in many cells	Not identified	Not identified
Cell processes	Few	Abundant	Poorly developed
Junctional complexes	Present	Not identified	Not identified

Note. The evaluation was made in magnifications up to  $\times 6400$ .



**FIG. 2** (A1) Ependymoma in H&E stain. Rosettes and pseudorosettes are present. (A2, A3) Ependymoma in wet SEM technique ( $\times 800$ ,  $\times 1,800$ ). Rosettes are identified and intracytoplasmic lumens are easily recognized. (B1) Fibrillary astrocytoma (H&E). (B2, B3) Prominent cell processes by wet SEM ( $\times 900$ ,  $\times 1,300$ ). (C1) Medulloblastoma with rosettes formation. (C2, C3) Rosettes structures without intracytoplasmic lumens ( $\times 800$ ,  $\times 1,600$ ).

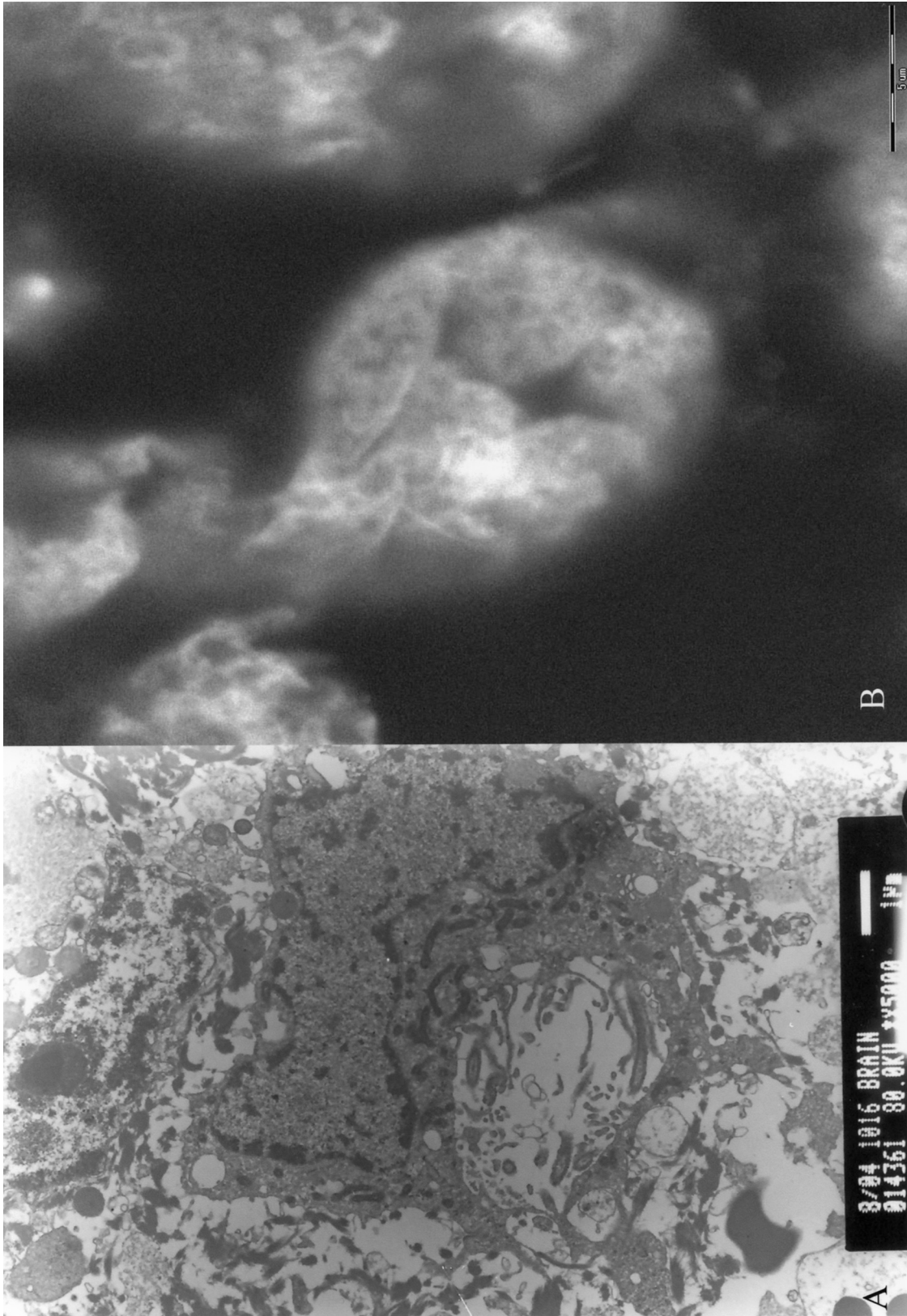


FIG. 3 A cell of ependymoma with intracytoplasmic lumen: (A) by TEM, (B) by wet SEM.

tissues. Our technology is based on two principles. First, samples are imaged in sealed specimen capsules (Figure 1), which completely isolate the specimen from the vacuum in the electron microscope chamber. The capsules contain a thin, partition membrane that is strong enough to sustain a 1-atm pressure difference but is electron-transparent (QuantomiX, Israel). Thus, biological samples placed in the capsule, such as cells or tissue fragments, sustain in their wet state, whereas the electron beam passes in the vacuum and probes the sample through the partitioning membrane. Second, imaging is done in a SEM, based on detection of backscattered electrons (BSE). This setup results in 3 unique characteristics: The first is that the samples can be imaged in an electron microscope, at resolutions up to 20 nm, in a fully wet state, without dehydration or coating. The second is that backscattered electrons result from interactions of the scanning electron beam with an internal layer of a few microns into the sample. As a result, the SEM can now be used to probe the inside of the wet specimen (cells or tissue fragments) and yields a wealth of structural information. In addition, any material present beyond the layer of beam penetration is irrelevant to the imaging process and thus, thick specimens, for example, tissue fragments of a few millimeters thick, can be imaged without thin sectioning, embedding or freezing. A 10-min fixation in formaldehyde may be required, however. The third characteristic feature is based on the fact that backscattering efficiency is sensitive to material differences within the sample, allowing for contrast mechanisms that are distinct from those operative in other microscopy methods [1–3]. The material contrast in untreated samples can rely on their natural structures or can be enhanced by general staining with heavy metals, which highlight some internal structural features in the specimen.

In this study we present some features of CNS tumors that are demonstrated by this technology. Electron microscopy (EM) is still an essential tool in modern diagnostic neuropathology [9]. Tumors of the CNS for which EM is useful include unusual variants of meningioma, ependymoma, schwannoma, and small “blue cell” tumors of childhood [10], among which EM findings are most contributory for the diagnosis of ependymoma. The use of SEM for the differentiation between CNS tumors is less contributory, and the most recent advances in the field were published in the late 1980s [11]. Both techniques require long and tedious processing of the tissue, such as dehydration, embedding, critical point drying, coating, and thin slicing. Intraoperative differentiation between astrocytic and ependymal tumors is crucial because the diagnosis of fibrillary astrocytic tumor prompts termination of the procedure, while in the case of ependymal tumor, total excision is the only chance, at least temporarily, of cure. Intraoperative differentiation between astrocytoma and ependymoma in frozen section can be difficult, especially if epithelial features are absent and perivascular pseudorosettes are inconspicuous. The freezing process may exacerbate

the problem [6]. The usefulness of cytology applied simultaneously with frozen section in the intraoperative diagnosis of CNS tumors has been shown [12], but there are still some difficulties in the frozen section diagnosis of pediatric CNS tumors [13].

Typical ependymomas are well-demarcated solid or cystic tumors of childhood and young adults that frequently protrude into the lumen of the ventricle or into the subarachnoid space [14]. Microscopically, these neoplasms consist of clusters or strands of fusiform or polygonal cells that form variable numbers of Flexner-type rosettes with a small central lumen, or perivascular pseudorosettes in which capillaries are surrounded by long, tapering ependymal cell processes [14, 15]. Blepharoplasts (ciliary basal bodies) usually are readily demonstrated in the apical cytoplasm of the cuboidal to columnar ependymal cells forming the lumens of the ependymal rosettes. Papillary and tubular formations of ependymal cells are rare.

On the ultrastructural level, the ependymal cells in the solid areas of ependymoma and in the areas of true rosettes and perivascular pseudorosettes are similar [15, 16]. The round or elongated nuclei contain finely dispersed chromatin. The cytoplasm contains no distinctive organelles or inclusions, but intracytoplasmic lumens with variable numbers of microvilli and cilia are the characteristic cytoplasmic feature and periluminal ciliary basal bodies (blepharoplasts) may be seen. Scattered foci of randomly oriented glial filaments are found in the cytoplasm of some cells. In myxopapillary ependymomas the cells are separated by extracellular spaces of varying size containing mucoid substances [17]. Astrocytomas are composed of cells invested with delicate processes. On ultrastructural study, these processes contain bundles of cytoplasmic filaments [18]. Medulloblastoma is an extremely cellular tumor, with sheets of anaplastic cells. The tumor may express neural phenotypes as Homer Wright rosettes. Medulloblastomas exhibit a broad repertoire of differentiating potential [19].

In this study, the novel wet SEM technology successfully demonstrated both histological and ultrastructural features of several CNS tumors: Rosette formation and intracytoplasmic lumens were observed in ependymoma; numerous fibrillary processes in fibrillary astrocytoma; and focal rosette formation with no intracytoplasmic lumens in medulloblastoma. Application of this method simultaneously to frozen section may improve rapid intraoperative diagnosis of these intracranial tumors. Evaluation of large series for each tumor is necessary to establish the capacity of the technology and to optimize and minimize the tissue preparation protocols. The technology may also be appropriate for other types of intraoperative surgeries requiring rapid diagnosis. In summary, we present a novel wet SEM method for the rapid preparation and examination of CNS tumor samples and the possible application of this method alongside intraoperative frozen section evaluation for differential diagnosis between astrocytic and ependymal tumors and medulloblastoma.

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