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Methods Article

Rapid cell transfer by means of nylon mesh to improve cellular diagnosis: the role of immunocytochemistry

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Short Title: Rapid cell transfer method

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Abstract

Immunocytochemistry (ICC) is an important ancillary technique in clinical cytology for not only identifying and characterizing tumor cells but also gaining prognostic or therapeutic information. Although cell blocks are often prepared for immunocytochemical evaluation of body cavity fluid and fine-needle aspiration specimens, they are not suitable for hypocellular samples. Liquid-based cytology can help prepare additional smears from residual cytological specimens. However, since conventional methods are used for nongynecological specimens in most laboratories, ICC is often limited by the number of cytological smears. Cell transfer methods permit to evaluate several immunocytochemical markers in a single cytological smear. Yet, these methods have some limitations; for example, they are time-consuming (about 3–40 h), and medium membranes with their attached cells are occasionally stretched or torn when peeled off the slides. Therefore, in an attempt to solve these problems, we developed a rapid and reliable cell transfer method using a nylon mesh. Our method requires no special equipment or reagent and can significantly reduce the turnaround time, as compared to previous methods.

Introduction

Immunocytochemistry (ICC) is an important ancillary technique in clinical cytology for not only identifying and characterizing tumor cells but also gaining prognostic or therapeutic information. Even though cell block cytology is frequently used to examine body cavity fluid and fine-needle aspiration specimens, it is not a suitable technique for analyzing samples with a small number of cells [1, 2]. Furthermore, while liquid-based cytology can help prepare additional smears from residual cytological specimens, most laboratories use traditional methods for the evaluation of nongynecological specimens [3]. Therefore, ICC is often limited by the number of cytological smears obtained by conventional methods.

Previous studies have reported the efficacy of transferring cells from a single cytological smear to multiple slides using a mounting medium [4], followed by adding multiple primary antibodies to perform ICC [1, 2, 5, 6]. Nonetheless, it takes a long time (about 5 to 40 h) to implement this technique. Moreover, Itoh et al. [7] have proposed a modified cell transfer method that entails using a diluted mounting medium and is practicable in about 3 h. In this method, however, medium membranes with their attached cells are occasionally stretched or torn when peeled away from slides.

Hence, we strived to overcome the drawbacks of previous methods by developing a rapid and reliable cell transfer method that involves using a nylon mesh.

Methods

Previous Methods

Briefly, after removing the cover glass, a thin layer of the mounting medium is spread uniformly over the top of the smear. The mounting medium is then allowed to dry by incubating the slide at 37°C–80°C for 30 min or up to overnight. Afterward, the slide is soaked in warm water at 45°C–60°C for 30 min to 2 h to soften. The medium membrane is peeled away from the slide by using a scalpel blade and is subsequently placed in a 37°C–80°C oven for 30 min up to overnight. Finally, the medium membrane is cut into pieces, each of which is later transferred to another slide [1, 2, 5–9]. As described above, previous methods of cell transfer require a period of 3 to 40 h.

Rapid Cell Transfer Method

Our method includes the following steps (Fig. 1a): (1) Papanicolaou-stained slides are immersed in xylene (in a 60°C oven) to remove the coverslips and residual mounting medium; (2) a nylon mesh sample pack for pathological materials (Sample pack KA1000; 45 × 74 mm, Eiken Chemical, Tokyo, Japan) is cut to an appropriate size (the same width as the slide and about 1 cm longer than the smear), and patient information is written in its margin (Fig. 1b); (3) smear is overlaid with a piece of nylon mesh and covered with 0.5 mL of a mounting medium (Malinol 550cps, Muto Chemicals, Tokyo, Japan or Entellan new for cover slipper, Merck, Darmstadt, Germany) diluted threefold with xylene (Fig. 1c); (4) the slides are placed on a hot plate (80°C) for 5 min to help the mounting medium dry; (5) a permanent marker is used on the surface of the dried medium to divide each slide into multiple cellular areas for subsequent immunocytochemical analysis; (6) the slides are soaked in 60°C warm water for 5 min so that the mounting medium becomes soft; (7) the medium membrane with its attached smear is peeled away from slide while the margin of the nylon mesh is held (Fig. 1d); (8) using scissors, the membrane is cut along the marked areas into several pieces (Fig. 1e); (9) each piece is transferred to a moistened, positively charged slide (Muto Chemicals) (Fig. 1f); (10) each piece is gently compressed to remove air bubbles; (11) the slides are placed on an 80°C hot plate for 5 min to ensure good attachment; and (12) the slides are dipped in xylene for 5 min to remove the nylon mesh and mounting medium (Fig. 1-4). The major steps of our method and those suggested by previous methods are summarized in Table 1. Our cell transfer method can be completed in about 30 min after coverslip removal, and ICC results are correlated with formalin-fixed cell block (Fig. 2-4).

Discussion

It has been shown in previous studies that cell transfer methods are a useful tool for the evaluation of multiple immunocytochemical markers when only a single conventional cytological specimen is available [1, 2, 5–9]. On the other hand, previously proposed methods of cell transfer have the disadvantage of being time-consuming and usually result in an extension of the turnaround time by 1 day [2, 9]. In comparison with these methods, Ito's method approach is capable of reducing the procedure time significantly, mainly because the time required for the medium membrane to dry and soften is shortened as a result of diluting the mounting medium to make the resultant membrane thinner [7]. However, thinning of the membrane reduces its strength and consequently

makes it prone to stretching or tearing. Therefore, our method involves using a nylon mesh as a supporting structure for the membrane, thereby preventing the membrane from stretching or tearing when peeled off the slide. Furthermore, previous methods require that a scalpel blade be used to peel the membrane off the slide, whereas our method involves grabbing the margin of the nylon mesh instead, hence offering improved reliability and safety. The following are the points to be aware of while performing cell transfer: the orientation (top and bottom) of the mounting medium must be maintained to ensure that the smear will adhere to new slides [5]; in addition, when processing multiple patient samples simultaneously, one medium membrane may be confused with another. In our method, these problems can be avoided by inscribing patient information (e.g., patient number) in the margin of the nylon mesh. If the same products (a mounting medium and nylon mesh) in our method are not available, another maker's similar products can probably be used.

Recently, cancer subtype identification in patients with non-small cell carcinoma of the lung and breast cancer has gained importance for modern personalized cancer treatment. Particularly in lung cancer cases, almost 70% of which are at an advanced stage and therefore cannot be operated on, small biopsy and/or cytological specimens serve as fundamental diagnostic materials [3, 10]. Even in such a situation, cell transfer methods allow for immunohistochemical analysis of several markers—including thyroid transcription factor-1 (TTF-1), p40, p63, and cytokeratin 5/6—with conventional direct smears [2]. In breast cancer, ICC of formalin-fixed cell blocks from cytological specimens is considered a reliable method for assessing markers, such as estrogen receptor (ER), progesterone receptor (PR), and human epithelial growth factor receptor 2 (HER2) [11, 12]. However, cell blocks often lack adequate cellularity, even when direct smears are cellular, due to the tumor type and operator's skills in obtaining cytological specimens. A previous study comparing formalin-fixed cell block/biopsy with cell transfer methods for alcohol-fixed smears across the same cases reported a strong correlation of ICC results regarding ER, PR, and HER2 between both methods [9]. Therefore, ICC of smears prepared from conventional smears via cell transfer methods is especially useful in case of a lack of cell blocks.

The cell transfer method described in this study requires no special equipment or reagent and can significantly decrease the turnaround time, as compared to previous methods. Consequently, our method has the ability to contribute to the rapid analysis of multiple immunocytochemical markers in a single, conventional, direct smear.

Conflict of Interest Statement

The authors declare no conflicts of interest.

Funding Sources

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Author Contributions

S.M. and T. N. developed this new cell transfer method. R. T., S. K., H. Y., and T. I. offered expert supervision and evaluated the quality of immunostaining. H. O. provided close coordination and wrote the manuscript.

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Figure Legends

Fig. 1. The new rapid cell transfer method. **a** Materials needed to perform this method: a nylon mesh sample pack for pathological materials, scissors, diluted mounting medium, and dropper. **b** Overlay a piece of nylon mesh on the smear. **c** Cover the nylon mesh with the diluted mounting medium. **d** Hold the margin of the nylon mesh and peel the membrane away from the slide. **e** Cut the membrane along the marked areas into several pieces. **f** Transfer each membrane piece to another slide.

Fig. 2. Invasive ductal carcinoma. **a** Ethanol-fixed direct smear prepared from fine-needle aspiration specimen. **b** Immunocytochemistry of HER2 on our cell transfer method.

Fig. 3. Adenocarcinoma of the lung (**a** to **e** is the same sample). **a** Ethanol-fixed direct smear prepared from pleural effusion. **b** Immunocytochemistry of TTF-1 on our cell transfer method. **c** Formalin-fixed cell block (TTF-1). **d** Air-dried methanol-fixed direct smear (May-Giemsa staining). **e** Immunocytochemistry of TTF-1 on our cell transfer method. The positive rate of TTF-1 decreased in air-dried smears.

Fig. 4. Urothelial carcinoma. **a** Alcohol-fixed direct smear prepared from urine. **b** Immunocytochemistry of p53 on our cell transfer method.

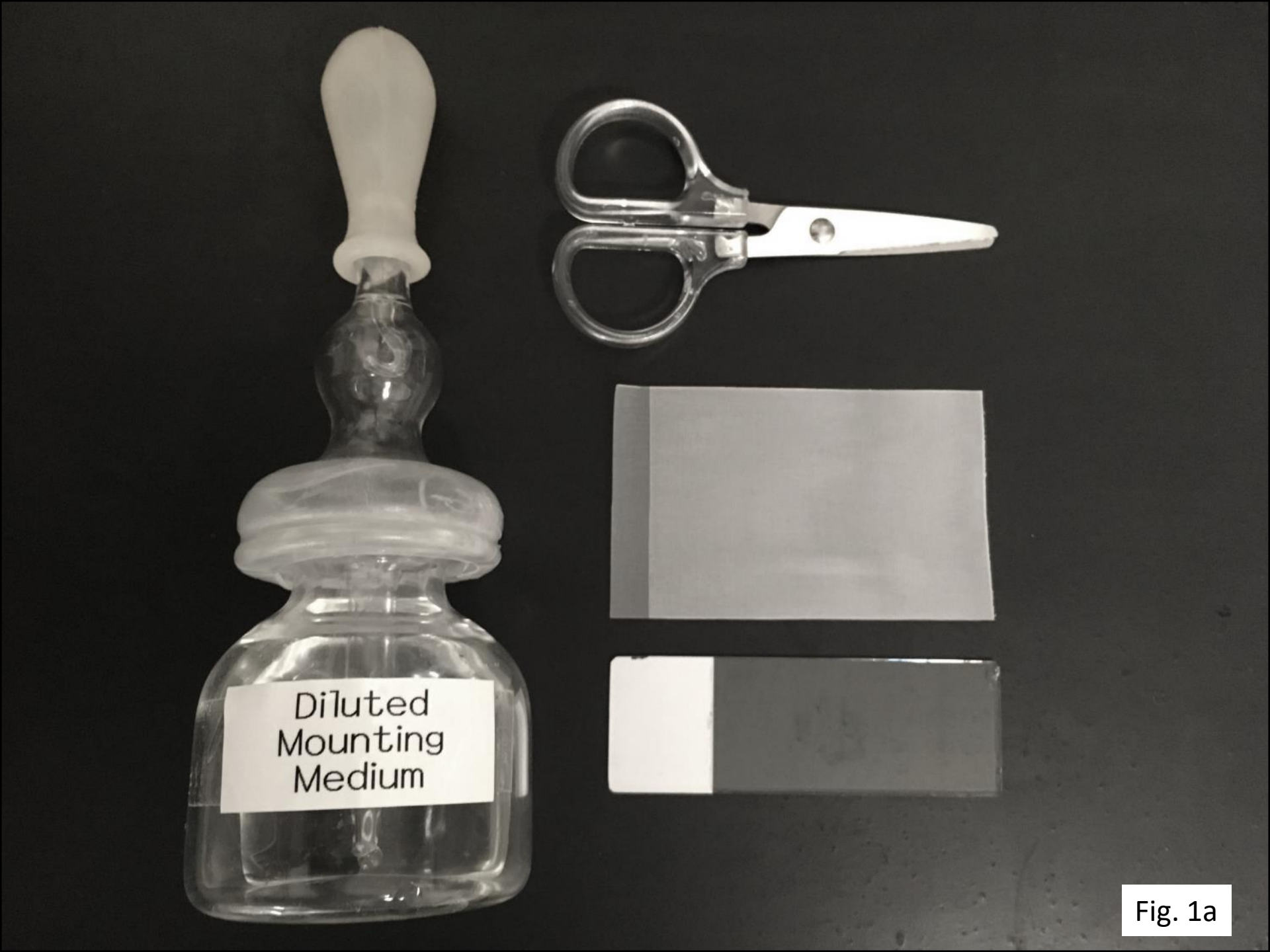


Fig. 1a

Fig. 1b

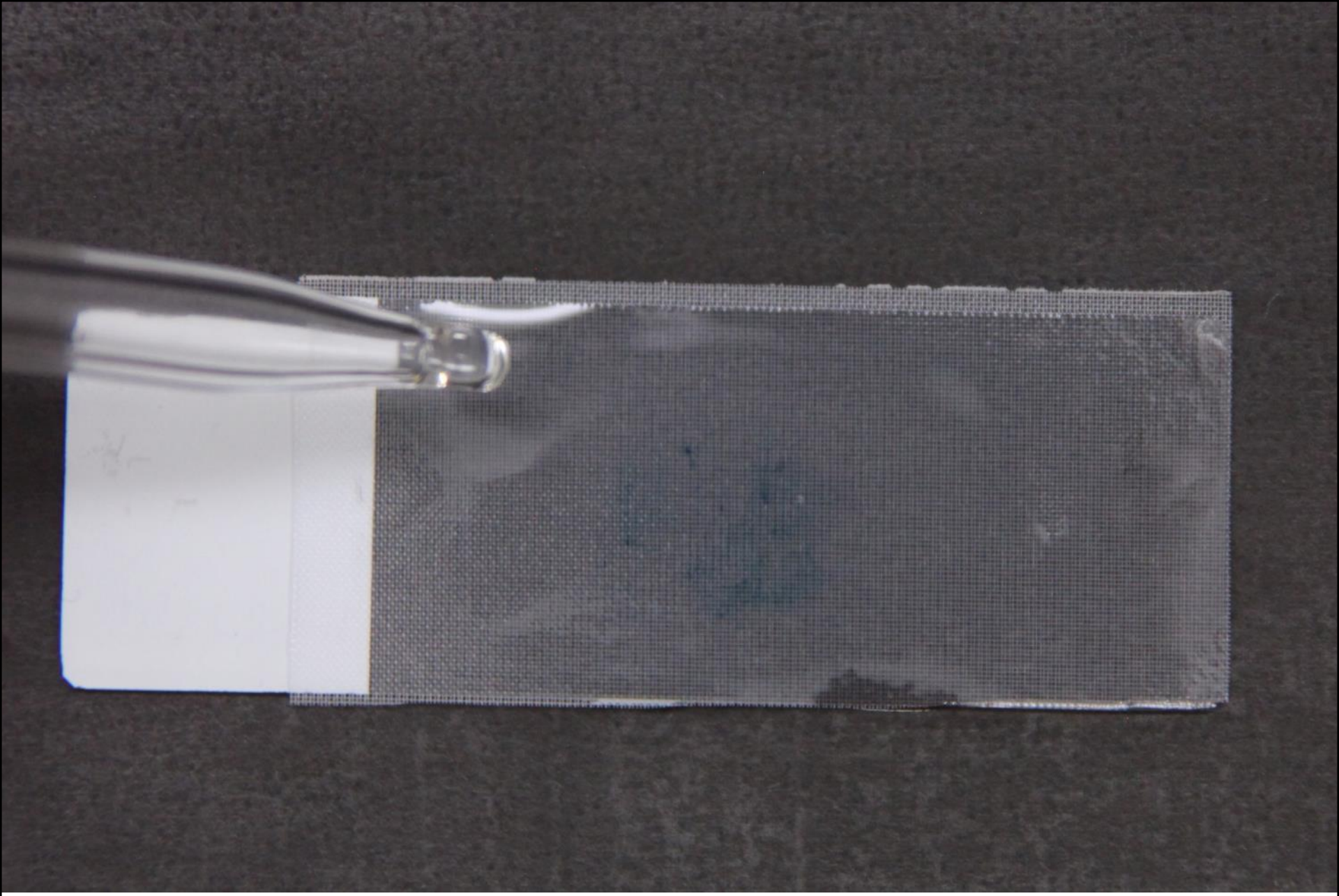


Fig. 1c

Fig. 1d

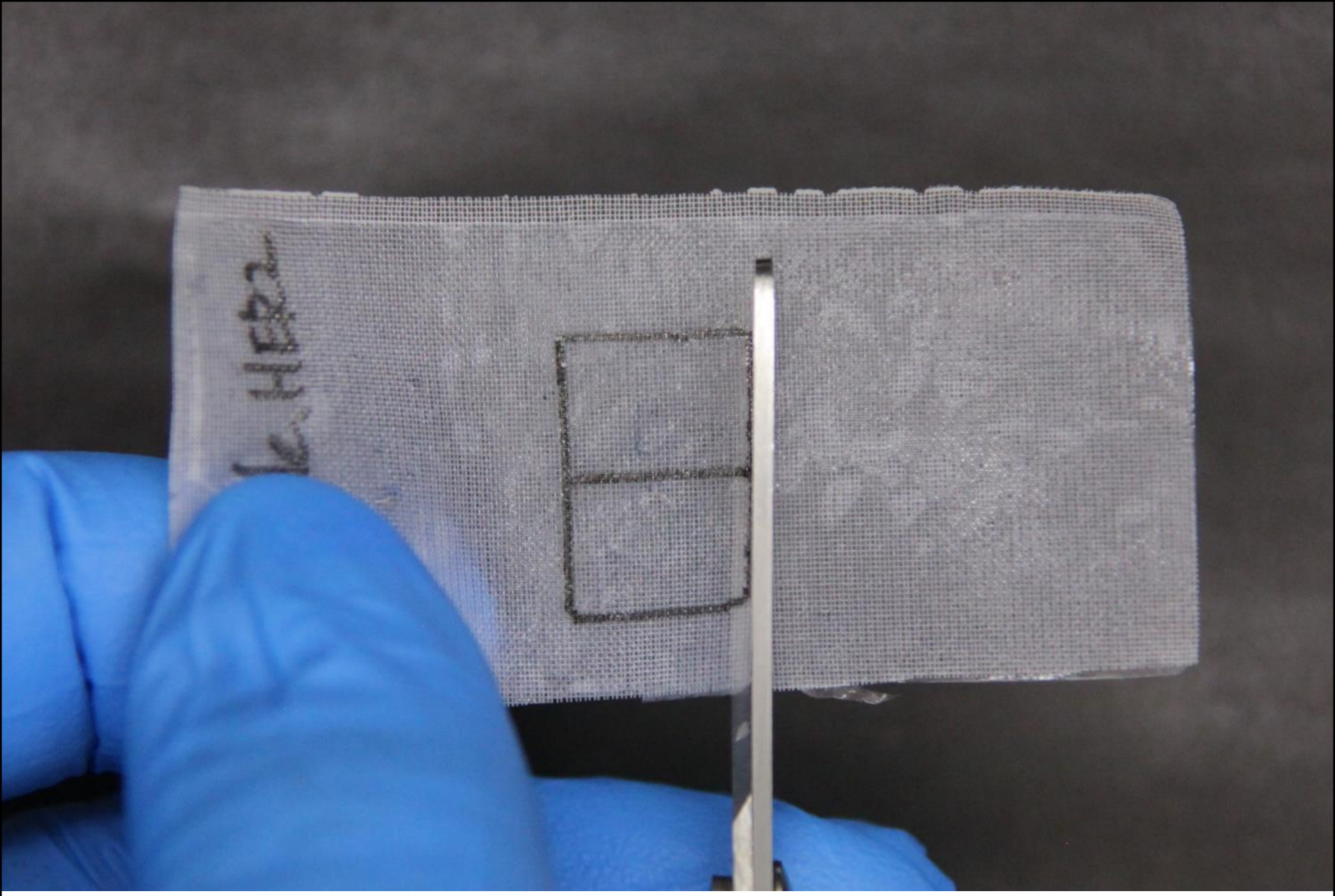


Fig. 1e

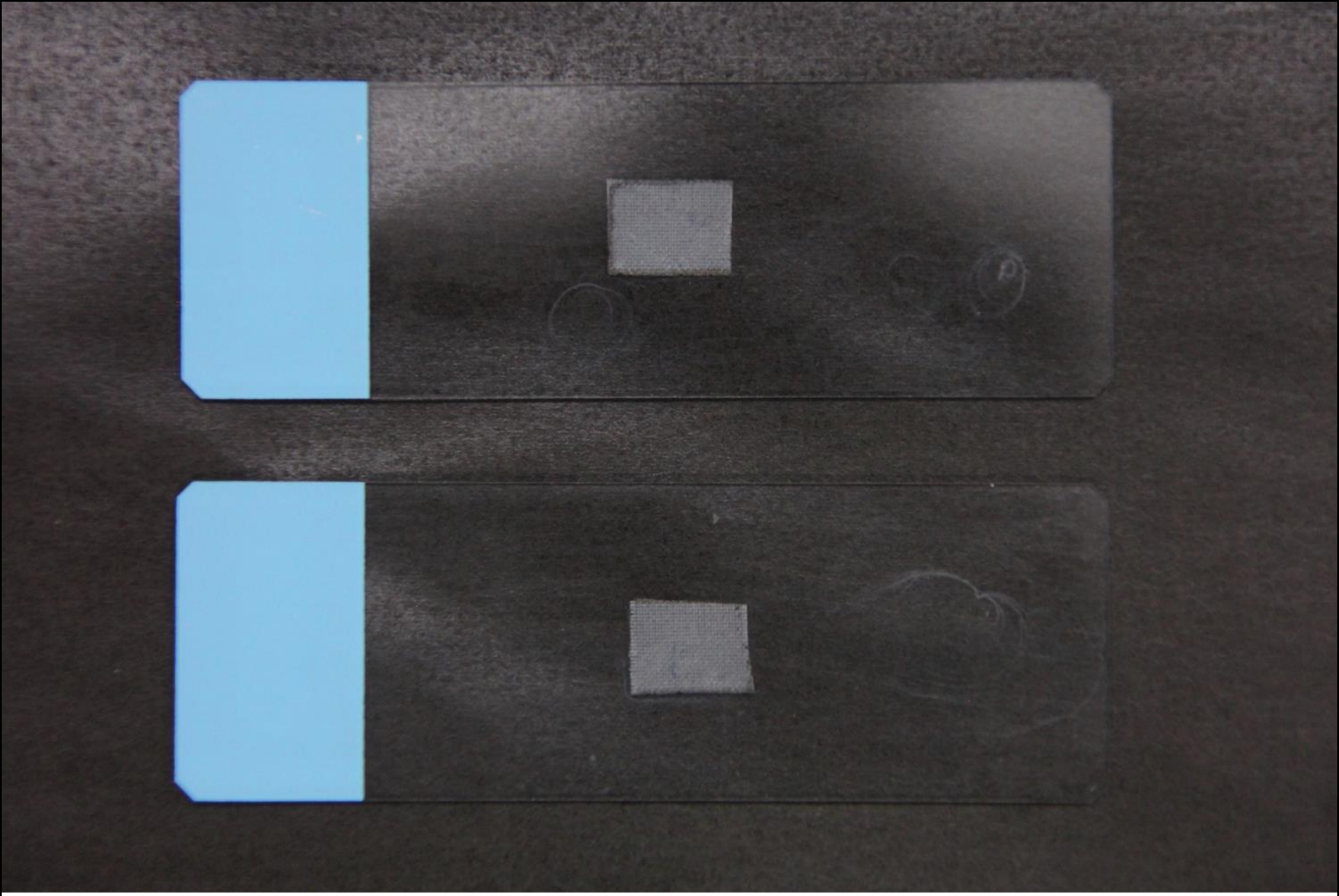


Fig. 1f

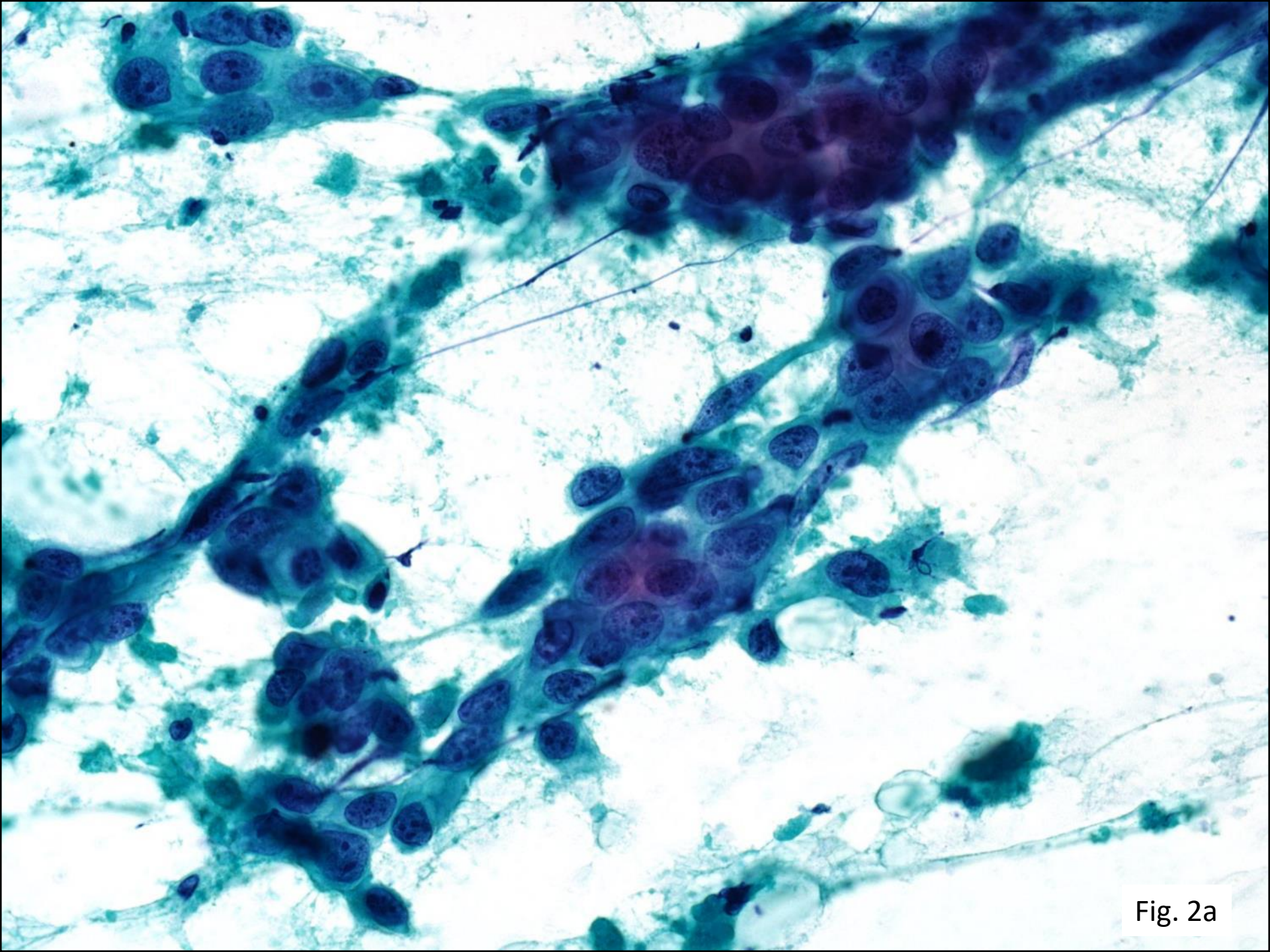


Fig. 2a

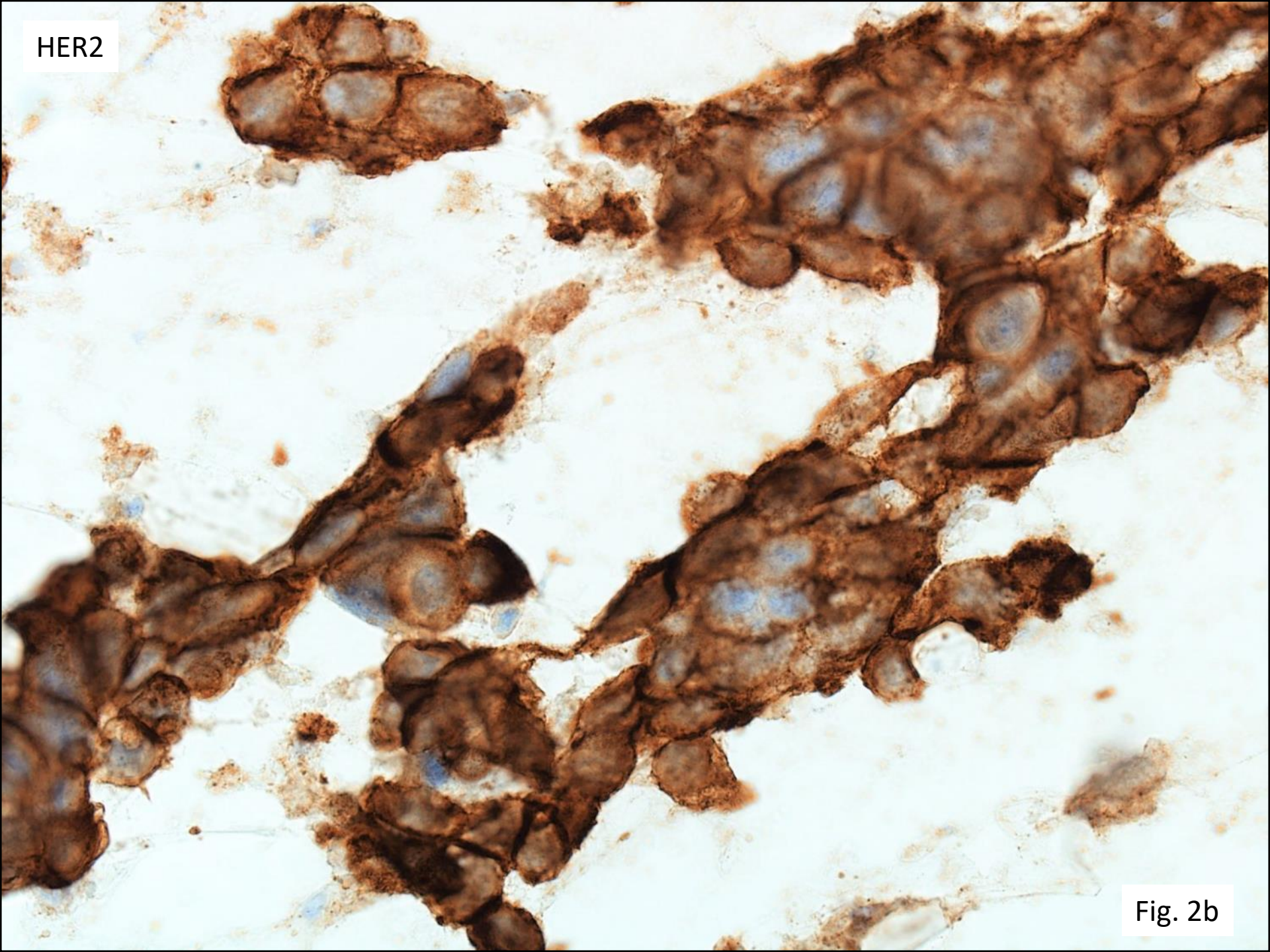


Fig. 2b

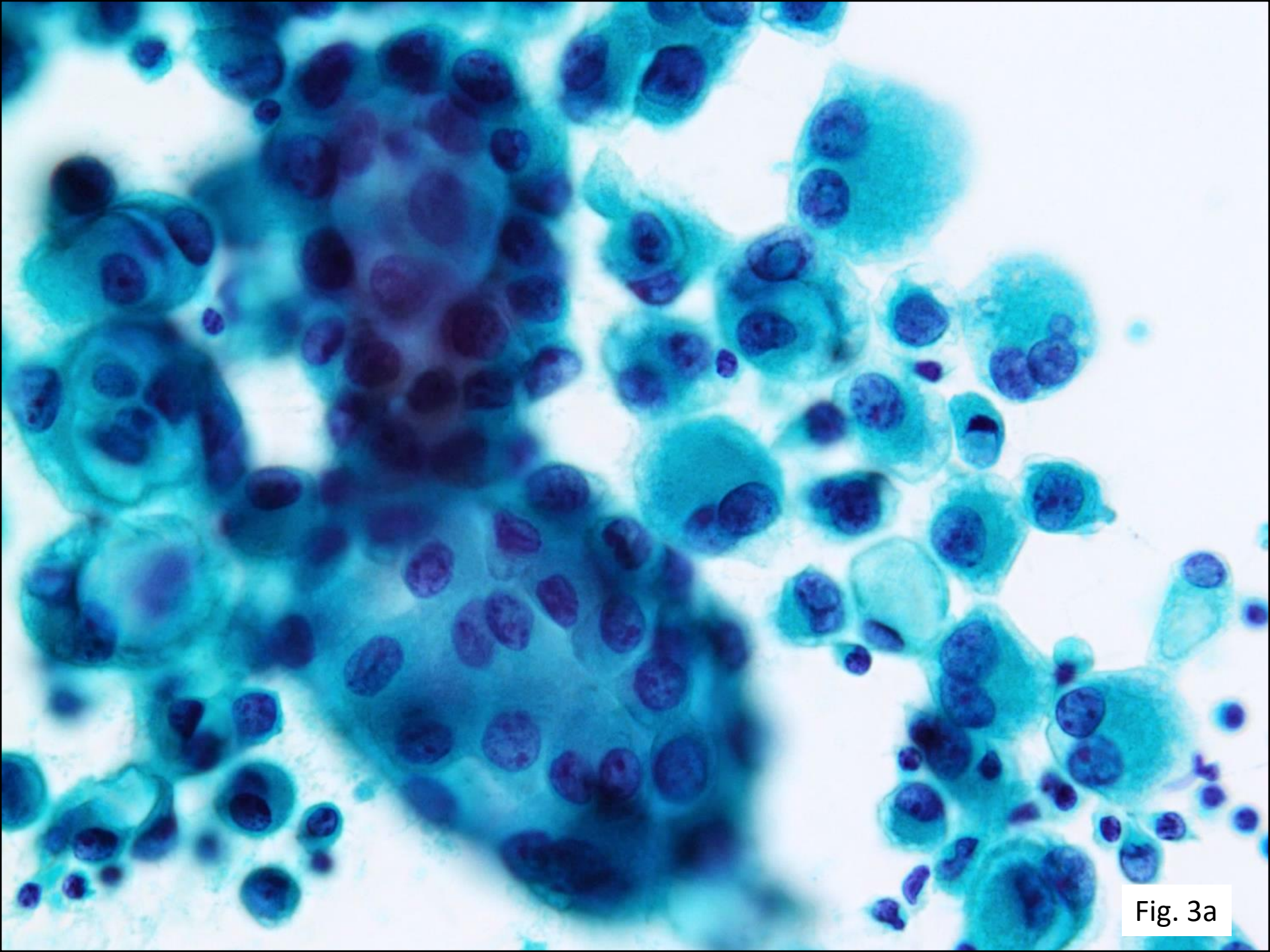


Fig. 3a

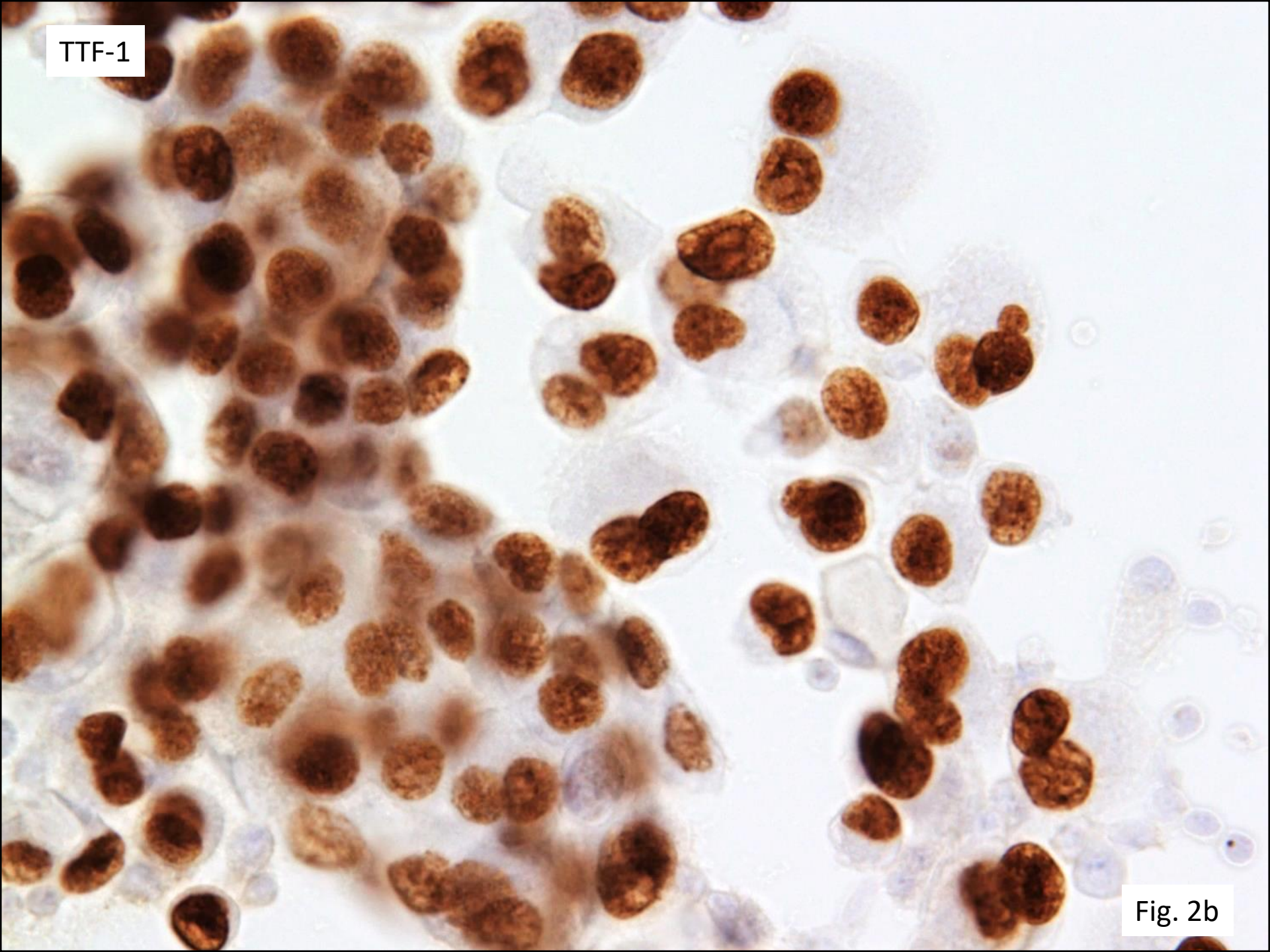


Fig. 2b

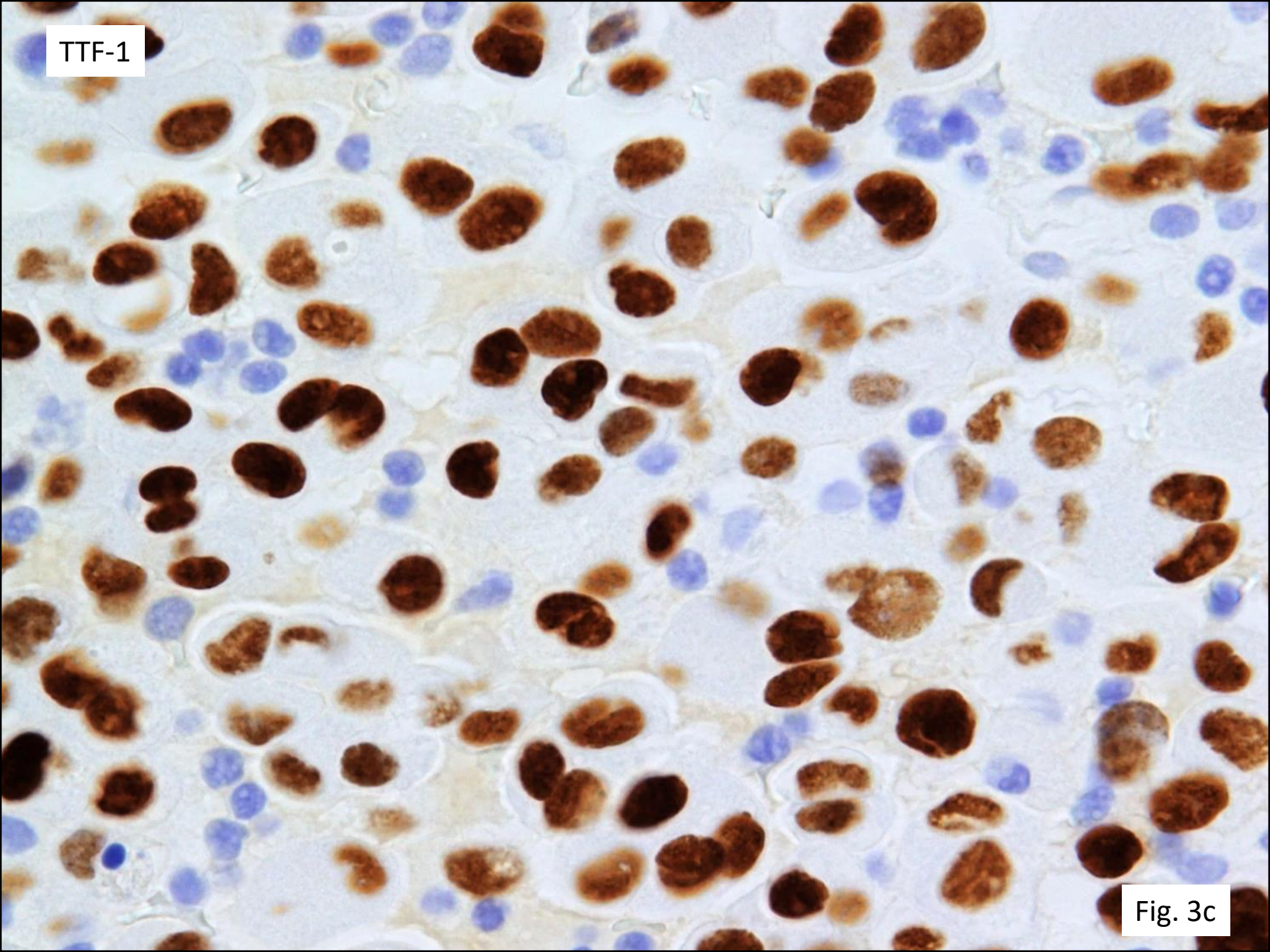
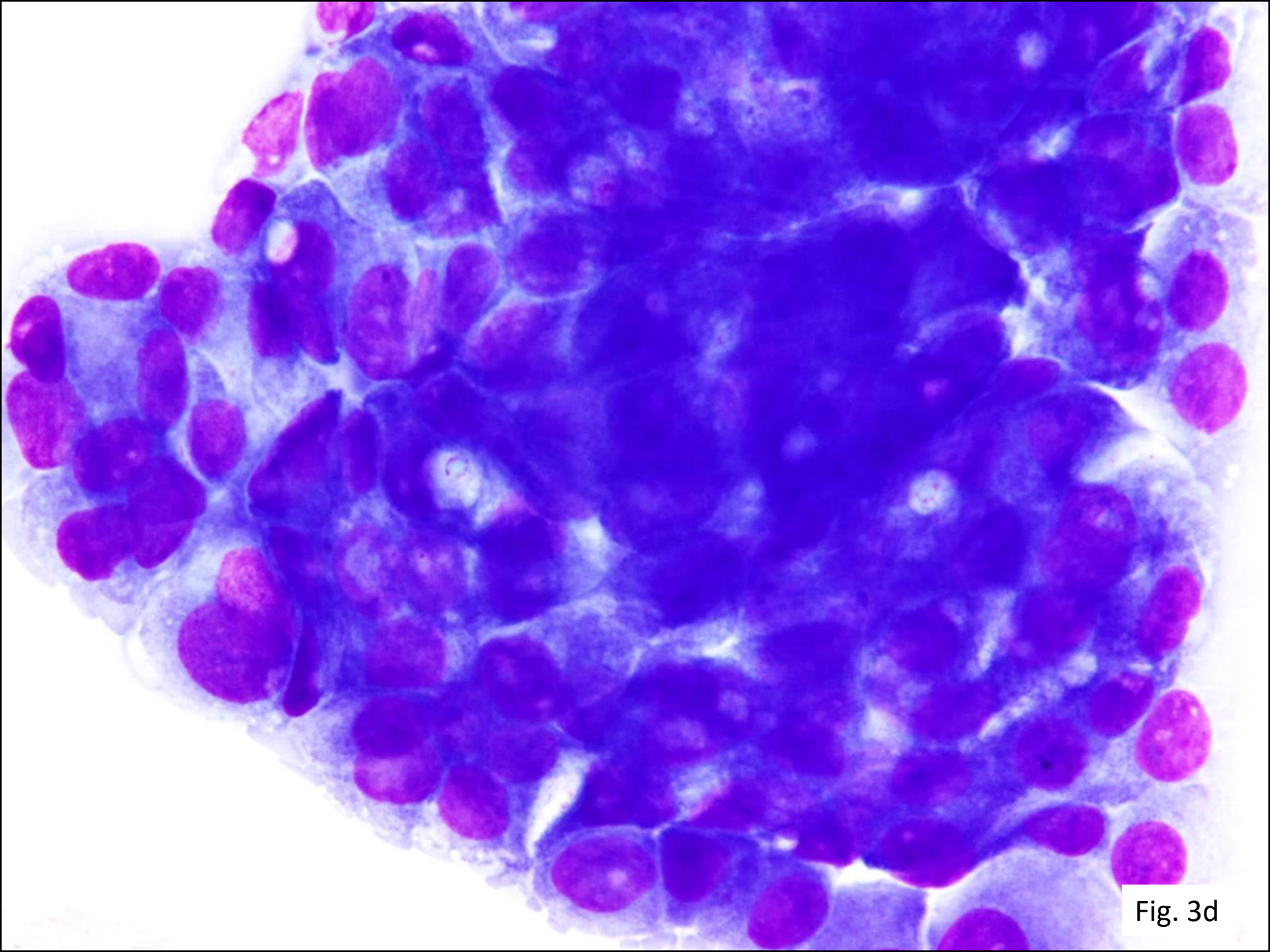


Fig. 3c



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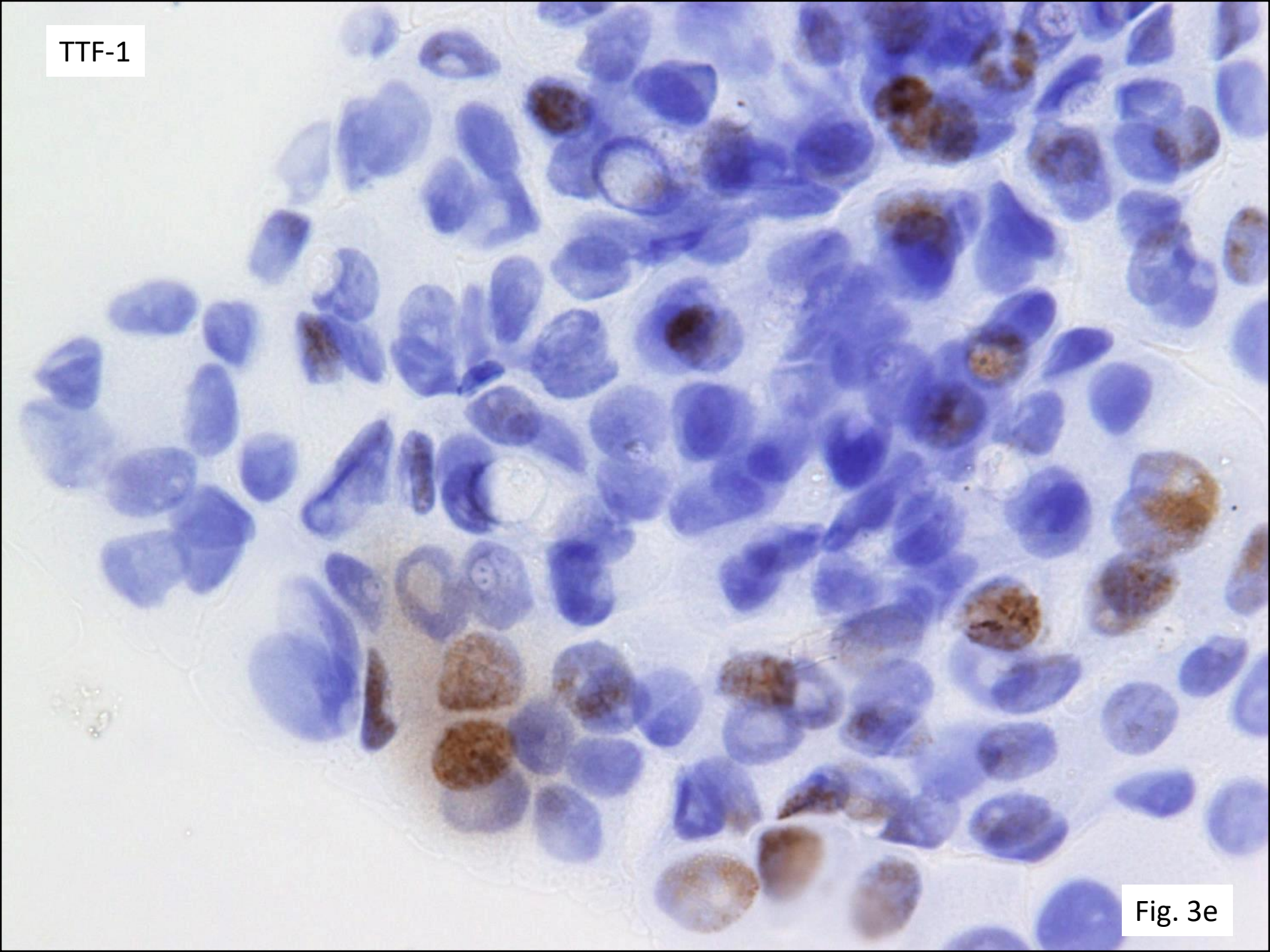

Fig. 3e

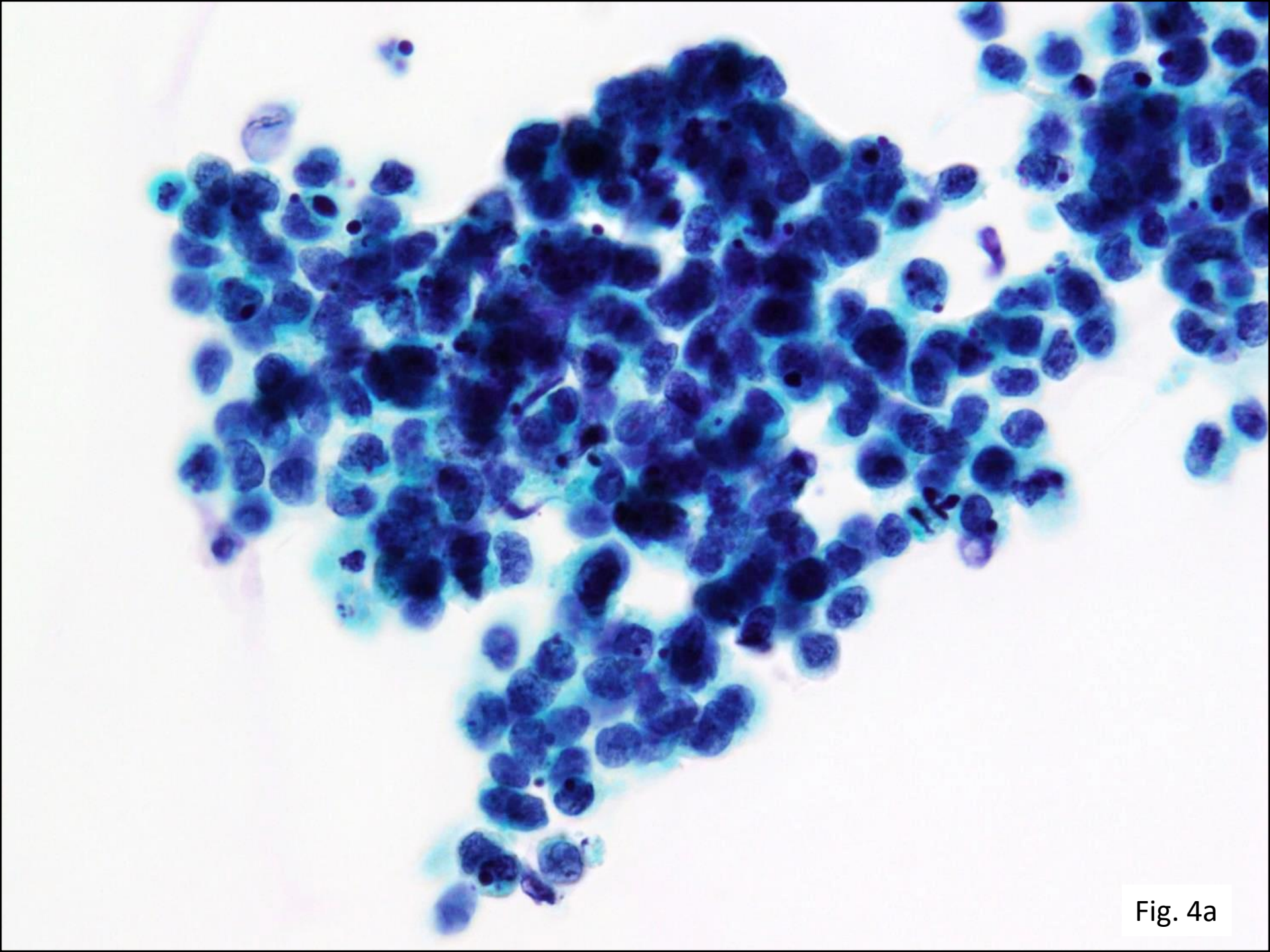

Fig. 4a

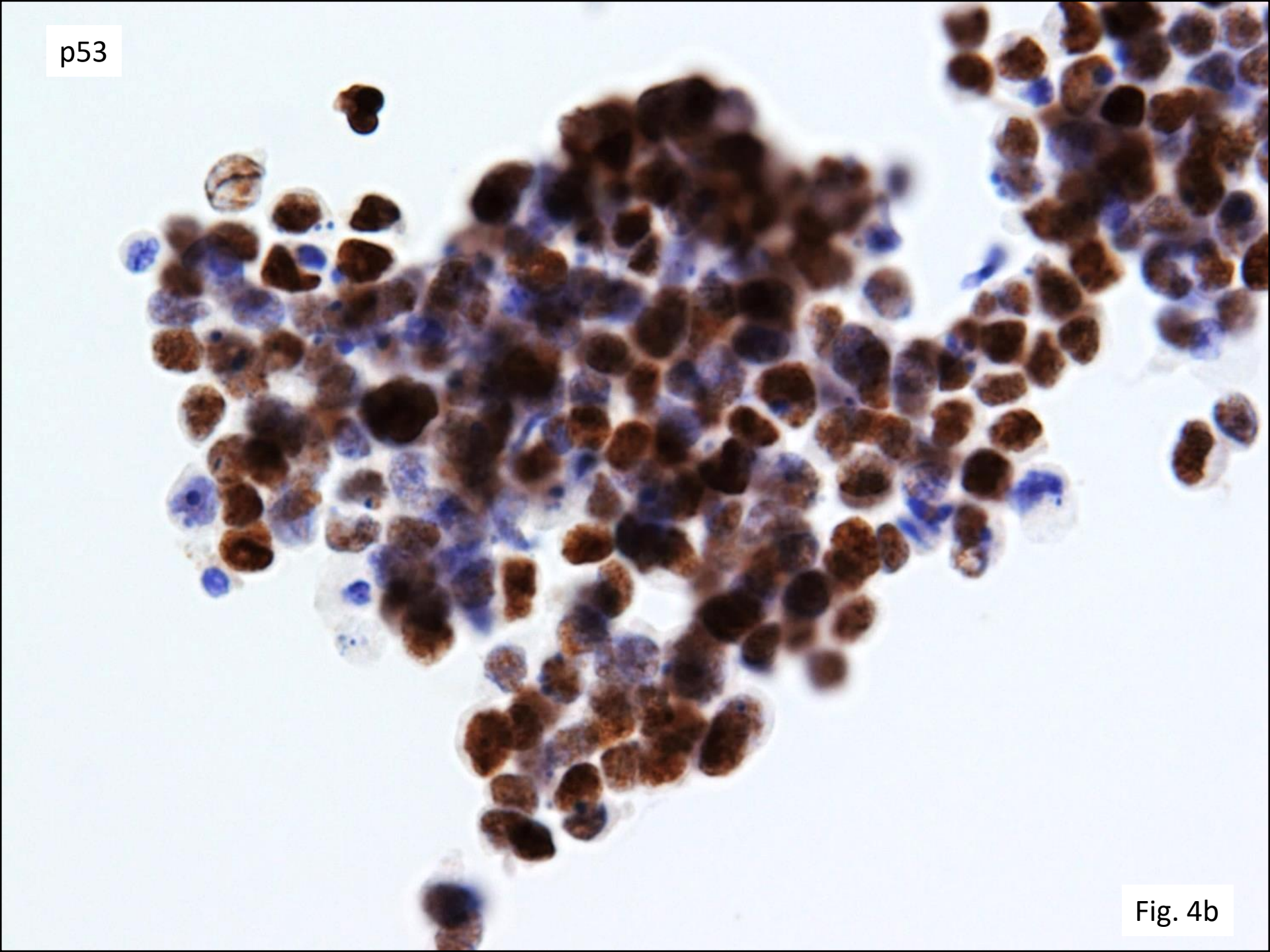

Fig. 4b

Table 1. Comparison between our method and previous methods

	Our method	Time	Previous methods [1, 2, 5-9]	Time
1	Remove the coverslips and residual mounting medium with 60°C xylene		Remove the coverslips and residual mounting medium with xylene	
2	Cover the smear with a nylon mesh and diluted mounting medium		Cover the smear with a mounting medium	
3	Place the slide on an 80°C hot plate	5 min	Place the slide in a 37°C–80°C oven	30 min to overnight
4	Soak the slide in 60°C warm water	5 min	Soak the slide in 45°C–60°C warm water	30 min to 2 h
5	Peel the membrane off the slide while holding the margin of the nylon mesh		Peel the membrane off the slide with a scalpel blade	
6	Transfer each membrane piece to another slide		Transfer each membrane piece to another slide	
7	Place the slides on an 80°C hot plate	5 min	Place the slides in a 37°C–80°C oven	30 min to overnight
8	Remove the residual mounting medium with xylene	5 min	Remove the residual mounting medium with xylene	12 min to 1 h
9	Rehydrate the slide with alcohol and water		Rehydrate the slide with alcohol and water	