

## Color-coded Live Imaging of Heterokaryon Formation and Nuclear Fusion of Hybridizing Cancer Cells

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**Abstract.** *Background/Aim:* Fusion of cancer cells has been studied for over half a century. However, the steps involved after initial fusion between cells, such as heterokaryon formation and nuclear fusion, have been difficult to observe in real time. In order to be able to visualize these steps, we have established cancer-cell sublines from the human HT-1080 fibrosarcoma, one expressing green fluorescent protein (GFP) linked to histone H2B in the nucleus and a red fluorescent protein (RFP) in the cytoplasm and the other subline expressing RFP in the nucleus (mCherry) linked to histone H2B and GFP in the cytoplasm. *Materials and Methods:* The two reciprocal color-coded sublines of HT-1080 cells were fused using the Sendai virus. The fused cells were cultured on plastic and observed using an Olympus FV1000 confocal microscope. *Results:* Multi-nucleate (heterokaryotic) cancer cells, in addition to hybrid cancer cells with single-or multiple-fused nuclei, including fused mitotic nuclei, were observed among the fused cells. Heterokaryons with red, green, orange and yellow nuclei were observed by confocal imaging, even in single hybrid cells. The orange and yellow nuclei indicate nuclear fusion. Red and green nuclei remained unfused. Cell fusion with heterokaryon formation and subsequent nuclear fusion resulting in hybridization may be an important natural phenomenon between cancer cells that may make them more malignant. *Conclusion:* The ability to image the complex processes following cell fusion using reciprocal color-coded cancer cells will allow greater understanding of the genetic basis of malignancy.

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**Key Words:** Green fluorescent protein, red fluorescent protein, dual-color HT-1080, cell fusion, Sendai virus, heterokaryon, nuclear fusion, cell hybrid, imaging.

Cell fusion has been subjected to extensive research over many decades (1-4). However, the steps of cell hybridization, including heterokaryon formation and nuclear fusion, have not been thoroughly observed in real time. Color-coded imaging of hybrid cancer cells expressing spectrally-distinct fluorescent proteins suggested that this method could be used to visualize the steps of cell hybridization (5).

In 1998, the laboratory of Geoff Wahl created a fusion gene of histone H2B and green fluorescent protein (GFP) to study nuclear dynamics in live cells (6). Subsequently, our laboratory created dual-color cells with H2B-GFP expressed in the nucleus and red fluorescent protein (RFP) expressed in the cytoplasm (7, 8), which enabled real-time simultaneous imaging of nuclear and cytoplasmic behavior.

The dual-color cancer cells have been used to demonstrate novel cellular behavior, including nuclear and cytoplasmic destruction during infection by tumor-targeting *Salmonella typhimurium* A1-R (9); nuclear and cytoplasmic deformation by cancer cells trafficking in narrow capillaries (10, 11); clasmocytosis (destruction of the cytoplasm) of cancer cells *in vivo* in real time in the liver, circulation and other organs (12, 13); nuclear-cytoplasmic behavior in real time of cells trafficking in blood vessels (14, 15) and lymphatic vessels (16); nuclear-cytoplasmic separation during cancer cell death (17); real-time imaging of apoptosis *in vitro* (7, 18) and *in vivo* (19-21); real-time imaging of mitosis *in vitro* (7) and *in vivo* (22); macrophage ingestion and digestion of cancer-cell nuclei and cytoplasm (23-25) and the role of nuclear rigidity in cancer-cell migration (10, 11, 26).

In the present study, we imaged cell and nuclear fusion using reciprocally color-coded sublines of a human fibrosarcoma (HT-1080), one of which expresses GFP in the nucleus and RFP in the cytoplasm (HT-1080-H2B-GFP-RFP) and a sister subline expressing RFP in the nucleus and GFP in the cytoplasm (HT-1080-H2B-mCherry-GFP).

## Materials and Methods

**Cell lines and culture.** HT-1080-H2B-GFP-RFP cells and HT-1080-H2B-mCherry-GFP (7-27) cells were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin and streptomycin (Gibco-BRL). The cell lines were cultured at 37°C with 5% CO<sub>2</sub>.

**HVJ (Sendai virus)-mediated cell fusion.** HT-1080-H2B-GFP-RFP cells were fused with HT-1080-H2B-mCherry-GFP cells using Sendai virus (Hemagglutinating Virus of Japan: HVJ) envelope, GenomONE-CF (Ishihara Sangyo Kaisha, Ltd, Osaka, Japan) according to the manufacturers' instructions. The cells were fused in suspension.

**Imaging.** An FV1000 confocal microscope, Olympus Corp. (Tokyo, Japan), was used in this study, with two-laser diodes (473 nm and 559 nm [28]). Scanning and image acquisition were controlled by Fluoview software (Olympus).

## Results and Discussion

**Imaging the steps of cell hybridization.** Reciprocal color-coded sister sublines of dual-color HT-1080 human fibrosarcoma cells, HT-1080-H2B-mCherry-GFP and HT-1080-H2B-GFP-RFP, were used. Figure 1 shows images of co-cultured dual-color HT-1080-H2B-mCherry-GFP and HT-1080-H2B-GFP-RFP cells captured by FV1000 confocal microscopy. Spontaneous cell fusion was not observed in co-cultures of the reciprocal sister sublines.

Cell fusion was effected by HVJ. Highly unusual heterokaryons were observed. For example, in Figure 2A, a heterokaryon with 7 GFP-expressing nuclei is shown. This may represent a fusion of 7 HT-1080-H2B-GFP-RFP cells with each other, which maintained only the GFP-expressing nuclei.

Figure 2B shows a heterokaryon with 3 orange nuclei, 1 yellow nucleus and 5 green nuclei, resulting from multiple cell- and nuclear-fusion events. Various nuclei with 3 different fluorescence colors were observed. It appears the green nuclei are unfused; the yellow nucleus represents a fusion of a red and green nucleus and the orange nuclei may be the result of two red nuclei and one green nucleus fusing. Thus, we have demonstrated that it is possible to image heterokaryon formation and subsequent nuclear fusion after fusion of the reciprocal color-coded HT-1080 sublines.

Figure 2C shows two heterokaryons, one with two yellow nuclei and a green cytoplasm and another heterokaryon with two yellow nuclei that appears to have just divided along with multiple small green nuclei and a yellow cytoplasm. The results in Figure 2C suggest that it is possible for nuclei to fuse without cytoplasmic fusion as seen in the heterokaryon with fused and unfused nuclei with the green cytoplasm that appears not fused. It seems also possible for cytoplasm to fuse, in case of the latter heterokaryon, with a yellow cytoplasm, which also contains fused yellow nuclei as well as unfused nuclei.

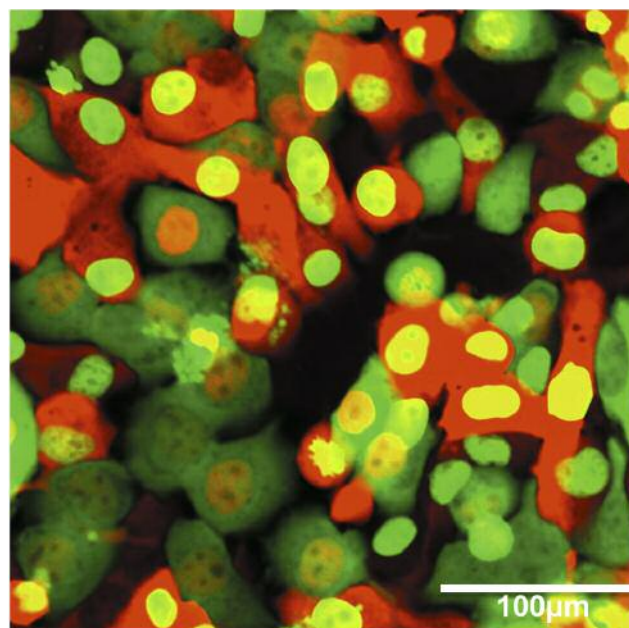


Figure 1. Co-culture of HT-1080 human fibrosarcoma H2B-mCherry-GFP and HT-1080-H2B-GFP-RFP cells in a 1:1 ratio in vitro (bar=100 μm). In the co-culture, no cell fusion was observed. Images were captured with an Olympus FV1000 confocal microscope.

**Imaging of a dividing fully hybridized cell.** Figure 3A shows a fused HT-1080-H2B-mCherry-GFP-HT-1080-H2B-GFP-RFP hybrid cell undergoing mitosis of its fused yellow nucleus. This cell was observed in the process of mitosis. Figure 3B shows a schematic of this cell.

Future studies will use hybrid cells, as described in this report, to visualize color-coded chromosomes within them in order to observe chromosome loss, gain, and recombination in real time during cancer cell progression in order to thereby determine each chromosome's role in cancer-cell behavior in real time.

## Conflicts of Interest

None of the authors have any conflict of interest in regard to this study.

## Dedication

This paper is dedicated to the memory of A. R. Moossa, MD and Sun Lee, MD.

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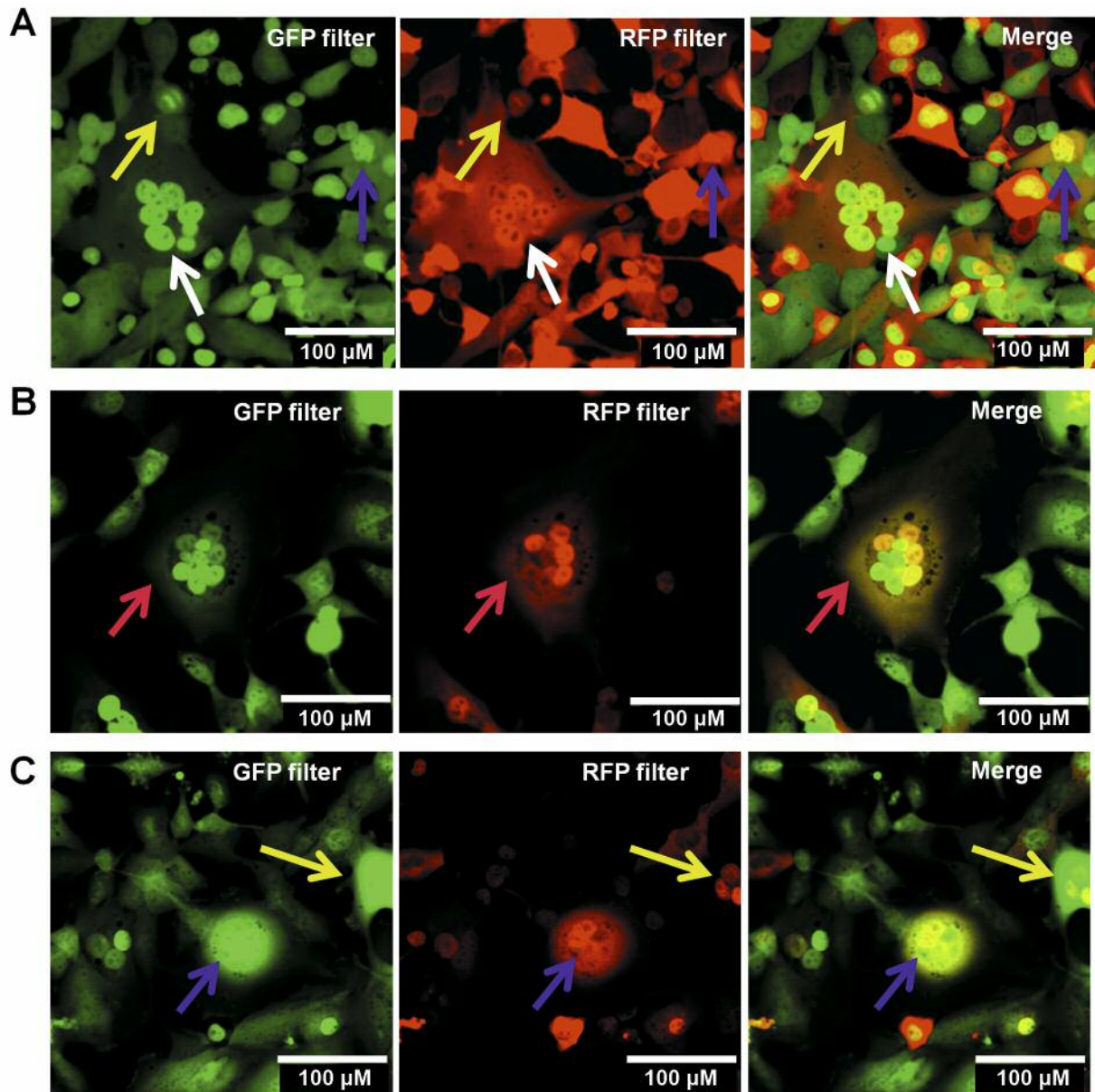


Figure 2. Imaging the steps of cell hybridization. Reciprocal color-coded sister sublines of human fibrosarcoma cells: HT-1080-H<sub>2</sub>B-mCherry-GFP cells and HT-1080-H<sub>2</sub>B-GFP-RFP cells were fused. A) A heterokaryon with 7 GFP-expressing nuclei is shown. B) A heterokaryon with 3 orange nuclei, 1 yellow nucleus and 5 green nuclei is shown. C) Two heterokaryons are shown, one with a green cytoplasm and 3 yellow nuclei and one with a yellow cytoplasm and yellow nuclei and multiple small green nuclei

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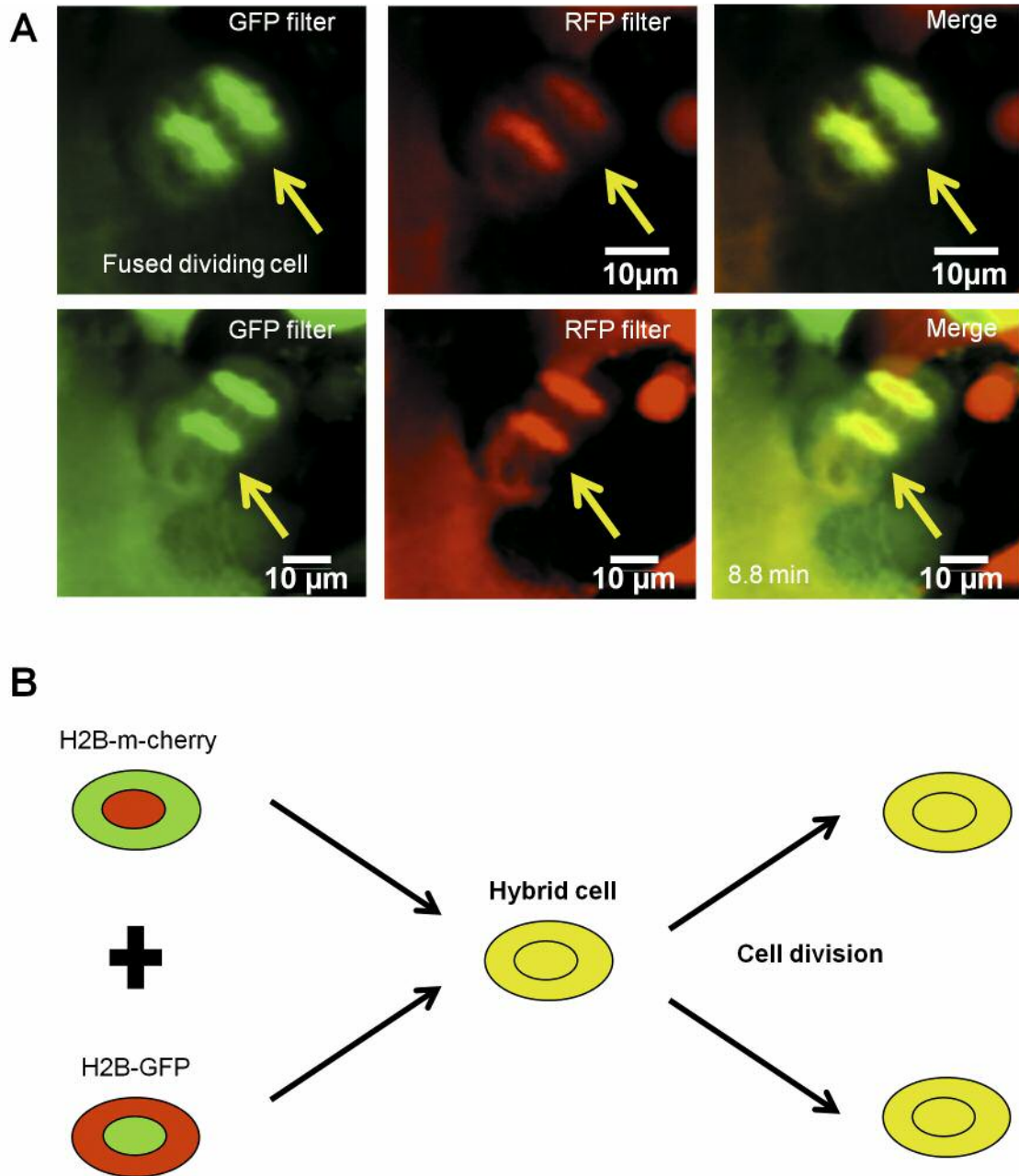


Figure 3. Imaging of a dividing hybrid cell. A) A hybrid fused HT-1080-H2B-mCherry-GFP – HT-1080-H2B-GFP-RFP cell, with a yellow nucleus, was observed to divide. This cell was observed in the process of mitosis over 8.8 minutes. B) A schematic of Figure 3A.

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