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应用不同光谱技术, 动物RBC, Hb及人皮肤表面血流氧化-还原状态检测

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摘 要 目的: 检测与分析实验动物血液RBC(RBC. O_2, RBC. CO_2), Hb(HbO_2, HbCO_2)和人体皮肤表面流动血液氧化·还原状态的成像与未成像可见光谱领域OD值特征, 并为该技术对白癜风病表皮黑色素颗粒检测中的应用尊定基础。方法: 利用不同光谱技术和*in vitro*和*in vivo*检测手段, 统计分析血液不同状态下波长与位置的OD值信息。结果: *in vitro*检测: 动物血液Hb O_2和RBC. O_2两者在可见领域均有367, 414(Soret带) nm与541, 576(Q带) nm的吸收峰位; 血液Hb CO_2和RBC. CO_2均有432(Soret带)与和553(Q带) nm的波长吸收峰位; 血液RBC状态和Hb溶血状态波长吸收峰位无改变, 只是在氧化与还原状态下有完全独立的吸收峰位, 血液RBC状态和Hb溶血状态波长吸光度OD值之间, 有显著性差异($p < 0.01$)。浓度为1.5×10^7 cell·mL^{-1}的RBC. O_2和Hb. O_2在576 nm的吸收峰位吸光度(y)与红细胞浓度(x)做成两条回归曲线: 既, Hb. O_2(b_1) $\hat{y} = 0.05 + 0.983x$; RBC. O_2(b_2) $\hat{y} = 0.127 + 1.934x$, 两者之间差异有显著性($p < 0.01$)。*in vivo*检测: 在人手背皮肤表面ImSpector图像中RBC. O_2状态在540, 576 nm, RBC. CO_2状态在555和755 nm处有吸收峰。选择(a: 指甲, b: 指, c: 手背)三个点位分别进行波长检测, 每点($n = 10$) 545 nm吸收峰的平均OD值, 依次为0.83 ± 0.001, 0.73 ± 0.001和0.62 ± 0.001, 其三处测定点的OD值之间有显著性差异($p < 0.01$)。结论: *in vitro*检测的RBC与Hb两者波长吸收峰位不变, 但吸光度OD值不同, 认为RBC状态测定结果更接近于活体组织血管内原始状态。*in vivo*检测对人体无任何侵袭与损伤, 灵敏度高, 测试时间短, 并且同时获得被测样品的波长与位置信息画面等优势, 有望表皮中黑素等有色颗粒的直接检测。

关键词 光谱; 吸光度值; RGB像; 活细胞; 皮肤表面
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引 言

随着光谱技术在医学领域应用研究的深入开展, "光学力学的診斷" (photodynamic diagnosis: PDD)以及"光学力学的治疗" (photodynamic therapy: PDT)[1-3]等一门有发展潜力和应用前景的"医学光谱学"逐渐形成, 使在国内外基础医学和临床医学领域中发挥着主要作用, 人类使用光谱学这一技术对机体微观世界进行着进一步探讨并得到了大量有价值的诊疗情报[4]。另外认为在现代医学诊疗检测方法发展的过程中, 尽量获取被检材料原始状态的动态信息, 保证检测结果的代表性与准确性作为今后的发展趋势[5-7]。

红血球(red blood cell, RBC)里的血红蛋白(hemoglobin, Hb)是在血液循环中, 氧化·还原(Oxy.-Deoxy.)[8-11]两种血球形式存在。要得到氧化·还原状态RBC可信情报, 只用血液O_2与CO_2分压测定方法为现阶段主要检测手段[12-14]。Hb的光谱波长, 只使血球溶血后检测为主要手段[15-17], 而且由依靠Hb测定结果来推断RBC的功能。另外也有使用荧光及近红外光谱波长领域OxyHb, DeoxyHb[18, 19], HbNO[20, 21]等体外测定的报道。这样就, 一是RBC整体离开机体血液循环, 而是血球溶血, 使RBC和Hb原有存在环境人为的发生改变, 影响了测定结果的准确性。本文利用体外(*in vitro*)和体内(*in vivo*)活细胞研究方法, 以RBC, Hb各种型态与活体皮肤表面血流为列, 其氧化和

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还原状态 RGB(red, green, blue)的构造 OD(optical density)值分别进行检测与分析。

1 材料与方法

1.1 家兔血液 *in vitro* absorption spectrum

U-3200 型自记分光光度计(Hitachi, Ltd Tokyo Japan)。被检血液来自雌性白色家兔, 体重 3 kg。测定波长固定为 300~ 800 nm 范围, 血样调制[2]分为氧化·还原血红蛋白(HbO_2, $Hb.CO_2$)型和氧化·还原红血球($RBC.O_2$, $RBC.CO_2$)型等四种型态。

1.2 人体皮肤表面 *in vivo* imaging spectrum

ImSpector (Spectral Imaging Oulu Finland Led Company)。波长范围为 400~ 800 nm, 被测像面范围一点的大小指定为 4 mm^2, 一次被测像面的总面积大小为 140 mm^2。测定人左右手背表面皮肤血流氧化型($RBC.O_2$)为人体安定自然与光线充足的条件下进行, 只是测定还原型($RBC.CO_2$)时, 手腕部用绷血带压迫 1.5 min 后再进行测定。该检测系统与方法详见参考文献[3]。

2 结 果

2.1 *in vitro* 检测结果

实验兔的血液 HbO_2 和 $RBC.O_2$ 两者在可见光范围内

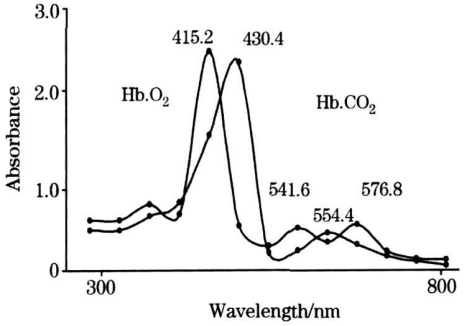

Fig. 1 U-3200 spectrometer *in vitro* determination of rabbit blood dissolved the condition expressed of oxy and deoxy hemoglobin wavelength absorption peak position

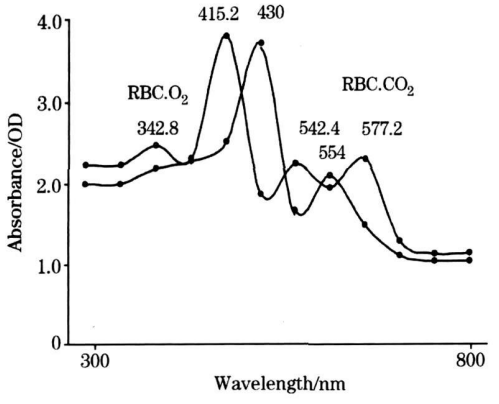

Fig. 2 U-3200 spectrometer *in vitro* determination of rabbit blood condition expressed of oxy and deoxy erythrocyte wavelength absorption peak position

均持有 367, 414 nm(Soret 带)与 541, 576 nm(Q 带)的吸收峰; 血液 $Hb.CO_2$ 和 $RBC.CO_2$ 均有 432 nm(Soret 带)与和 554 nm(Q 带)的吸收峰, Hb 与 RBC 由氧化峰位向右还原峰位移动的距离均为约 15 nm 左右(见图 1 和图 2)。

检测 RBC 和 Hb 与 CO_2 结合的时间经过是有所不同, 即从氧化变成还原状态时所化的时间, 前者约为 120 s, 后者为 180 s, RBC 状态比 Hb 快 60 s(见图 3 和图 4)。

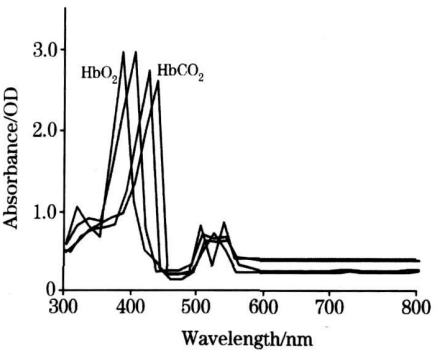

Fig. 3 Rabbit HbO_2 absorption wavelength changes to the $Hb-CO_2$ wavelength time process, altogether 180 s (each curve scanning need 60 s)

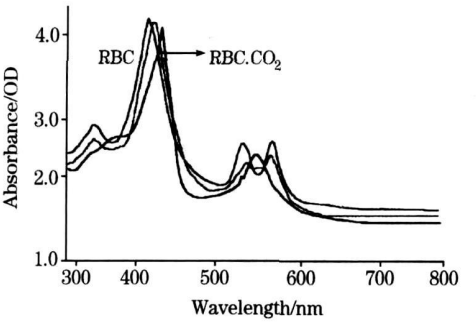

Fig. 4 Rabbit $RBC.O_2$ absorption wavelength changes to $RBC.CO_2$ wavelength time process, altogether 120 s (each curve scanning need 60 s)

利用直线回归(Linear regression)统计方法, 浓度为 1.5×10^7 cell·mL 的 $Hb.O_2$ 和 $RBC.O_2$ 在 576 nm 的吸收位置吸光度($\hat{y}$)与红细胞浓度(x)计算相关系数(r)与列出回归方程($\hat{y} = a + bX$); 对 $Hb.O_2$, $r = 1.705$, 差异有高度显著性($p < 0.0005$), $\hat{y} = 0.05 + 0.983X$; $RBC.O_2$, $r = 1.074$, 差异有高度显著性($p < 0.0005$), $\hat{y} = 0.127 + 1.934x$, 结果说明 $RBC.O_2$ 与 $Hb.O_2$ 均存在直线回归关系, 两条回归系数(b_1 与 b_2)的差别也有显著性($p < 0.01$)(见图 5)。

为了进一步证实直线回归结果, 又算出波长范围 520~ 600 nm 之间的波长面积(Integral), 其结果如下, $Hb.O_2$ 为 69.69, $Hb.CO_2$ 为 70.81 Absc; $RBC.O_2$ 为 222.30, $RBC.CO_2$ 为 200.55 Absc。$RBC.O_2$ 和 $RBC.CO_2$ 波长面积比 $Hb.O_2$ 和 $Hb.CO_2$ 的波长面积比分别大于 152.61 和 129.74 Abs ($p < 0.005$)(见图 6)。

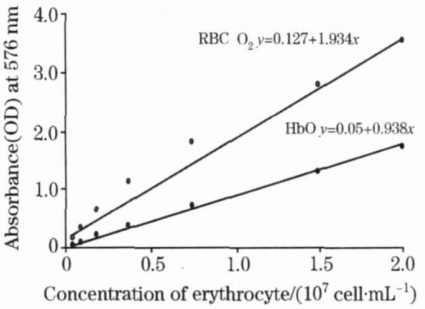

Fig. 5　linear regression of rabbit oxy erythrocyte and hemoglobin condition, in wavelength at 576 nm

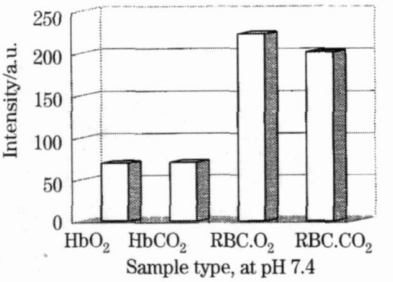

Fig. 6　Rabbit oxy and deoxy hemoglobin and erythrocytes domain integral area, in wavelength area at 520～600 nm

2.2　*in vivo* 检测

采用 ImSpector, 人手皮肤表面指定位置氧化和还原流动血液直接进行观测, 其中图像 7(a) 为未进行血流阻断的人手背表皮 RBC O_2 状态, 与图 8(a) 所表示的 540 和 576 nm 的吸收峰对应; 图 7(b) 为进行血流阻断的人手背表皮 RBC CO_2 状态, RBC CO_2 状态与在 555 和 755 nm 处特有图[图 8(b)] 所表示的吸收峰对应, 活体皮肤表面 RBC CO_2 状态具有在 775 nm 处矮小的特殊吸收峰位, 这是 *in vitro* 方法未能测定到的(见图 7 和图 8)。

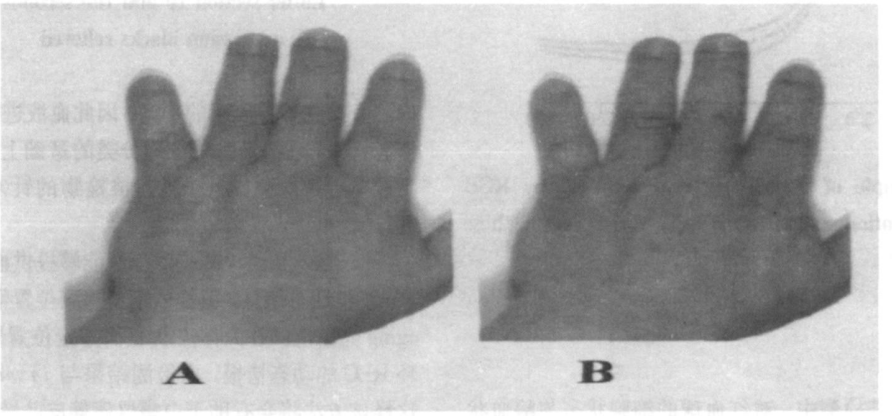

Fig. 7　An example of RGB reconstitution image at back of hand in normal subject. Arrow (a) shows an image on normal blood flow and arrow (b) on obstructed blood flow

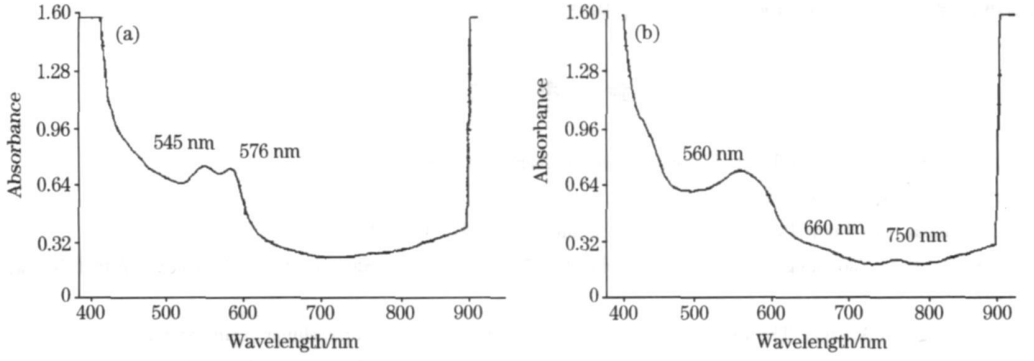

Fig. 8　Absorbance imaging spectrum on normal blood flow (a) (oxygenized state) and on obstructed blood flow (b) (deoxygenized state), (a) and (b) corresponds the recording position at (a) and (b) in Fig 7

在自然状态下, 人左手像指甲 *a*、手指皮肤 *b* 与手背表面 *c* 三处(图 9 中位置 *a*, *b*, *c*), 均具有 RBC O_2 特有的 545 和 576 nm 的吸收峰(见图 10 中光谱曲线 *a*, *b*, *c*)。

可由波长 576 nm 吸收位峰, 计算出皮肤表面组织血管内 RBC O_2 变化为 RBC CO_2 的氧化·还原的速度。皮肤与指甲由 RBC O_2 变化为 RBC CO_2 所需时间是 10 s, 由 RBC CO_2 转变为 RBC O_2 所需时间是, 皮肤处为 20 s, 指甲处为 10 s(见图 11)。

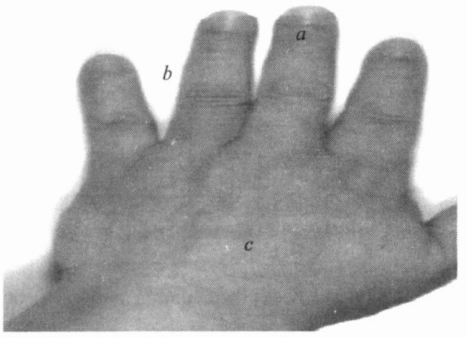

Fig. 9 Imaging spectrum of RGB reconstitution image at three positionsin normal subject

a: Nail, *b*: Finger, *c*: Back of hand

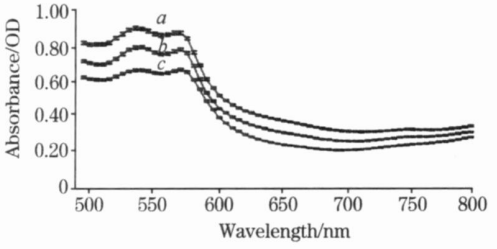

Fig. 10 An example of actual spectrum recorded by RGB reconstitution system. *a*, *b* and *c* correspond to those in Fig. 9

3 结 论

在 *in vitro* 光谱检测中, 对红血球的细胞状态与溶血状态时的吸光度, 反应速度及波长面积等进行了广泛性的对比, 结果为 RBC 的各检测指标均高于 Hb 状态, 由于血液 RBC 近似于血管内状态, 虽然受到大气压和稀释液影响, 但有完整的细胞膜起调解作用, 细胞内血红蛋白在检测周期

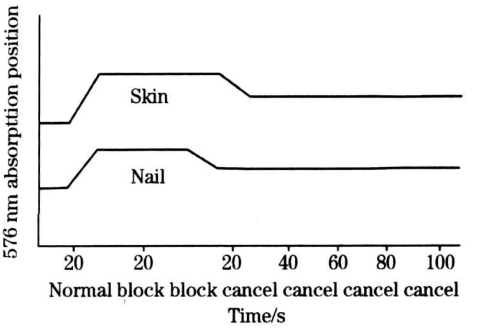

Fig 11 Uses by the imaging spectrum examine on the hand back skin and nail surface blood flow 576 nm absorption wavelength peek position, the blood flow blocks before and after absorption and time variation Front end X axis 10 and 20 seconds between are expression blood flows normal. Middle 10 and 30 seconds between are expressed the blood flow blocks Latter section 10 and 100 seconds between partially is this expression blocks relieved

内不直接受外界因素的干扰。因此血液进行光谱检测分析时作者认为, 在血液进行细胞分类的基础上, 保证血细胞完整为前提进行检测, 能够提高被检物的针对性和结果的准确性。

in vitro 光谱检测技术虽然能够提供血样被检物 RBC 及 Hb 波长动态信息, 但提供不了检测位置情报, 因此利用 Imaging 光谱能够在人体皮肤表面指定位置同时得到皮肤微循环 RBC 的动态情报, 而检测结果与 *in vitro* 结果是一致的。这样该方法将会有助于白癜风病等皮肤疑难症的诊疗, 特别是对微循环细胞分子学研究领域, 利用光敏剂等对癌症的早期确诊以及观测白癜风患者皮肤移植后皮肤的再生, 表皮中检测黑色素颗粒, 酪氨酸酶活性等可望有一定的应用和研究价值。

参 考 文 献

[1] Aizawa K, Kuroiwa Y, Tsuchida T, et al. Jpn. J. Cancer Chemother, 1996, 23(1): 22.
[2] Aizawa K. Physics Application, 1996, 65,(1): 24.
[3] Aizawa K, Okunaka T, Ohtani T, et al. Photochem. Photobiol., 1987, 46: 789.
[4] RUAN Ping, HUANG Yaoxiong, LI Dan(阮 萍, 黄耀熊, 李 丹). Spectroscopy and Spectral Analysis(光谱学与光谱分析), 2005, 25(7): 1121.
[5] Mansur Arkin, Katsuo Aizawa: The Development of Image Spectrum Device of Intracellular Granular Pigment. Oversea Scholars Academic Conference of Xinjiang Uighur Autonomous Region Progrram Hope. Chiba University, Japan. October 7, 2001. 14.
[6] LI Liming, Aizawa Katsuo, Arkin Mansur, et al. Real-Time Imeging Spectrometery for Photodynamic Diagnosis. Monograph on Photonics Science and Technology. Edited by Hiroyuki. Sasabe et al. Japan: Adachi PWC Publishing Chitose, 2003, 51.
[7] Mansur Arkin, Rie Kubota, Masao Kanazawa, et al. Journal of Tokyo Medical University, 2004, 62(5): 523.
[8] Bakhtiar R, Leung K H: Rapid Communications in Mass Spectrometry. John Wiley Sons, 11. Ltd, 1997. 1935.
[9] Sugawara Y, Kadono E, Suzuki A, et al. Acta Physiologica Scandinica, 2003, 107: 49.
[10] John W Smalley, Andrew J Birss, Robert Withall, et al. Biochem. J., 2002, 362: 239.
[11] Kim D Vandegriff, Ronald J Rohlfs, Michael D Magde, et al. Analytical Biochemistry, 1998, 256: 107.
[12] Yukio Hamada, Hiroya Utahashi, Kazuhiro Aoki, et al. Int. J. Pediatric Otorhinolaryngology, 2002, 64(1): 41.
[13] Detr B, Cambier C, Frans A, et al. The Veterinary Journal, 2003, 1165: 258.

[14] Yasuhiro Morimoto, Mali Mathru, Julian F, et al. Journal of Neurosurgical Anesthesiology, 2001, 13: 33.
[15] Adar F, Gouterman M, Aronowitz S. J. Phys. Chem., 1976, 80: 2184.
[16] Hiromi Sakai, Yoji Suzuki, Megumi Kinoshita, et al. American Journal of Physiology-Heart and Circulatory Physiology, 2003, 285: 2543.
[17] LI Renqiang, Nagai Yukifumi, Nagai Masako, et al. Chirality, 2000, 12: 216.
[18] Vlad Toronov, Scott Walker, Rajarsi Gupta, et al. NeuroImage, 2003, 19: 1521.
[19] Rendell M, Anderson E, Schlueter W, et al. Clin. Lab. Haem., 2003, 25: 93.
[20] Kazuyoshi Kirima, Koichiro Tsuchiya, Hiroyoshi Sei, et al. American Journal of Physiology-Heart and Circulatory Physiology, 2003, 285: 589.
[21] Sonia Cacciaa U, Ilya Denisova, Michele Perrellab, et al. Biophysical Chemistry, 1999, 76: 63.
[22] Suga S, Xiahedin I, Hayashi N, et al. Journal of Tokyo Medical University, 1996, 54(1): 3.

Examination of Animal RBC, Hb and Human Skin Surface Blood Stream in Oxygenation-Deoxidization Conditions Using Different Spectrum Techniques

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Abstract The authors examined and analyzed animal blood RBC (RBC O_2, RBC CO_2), Hb (HbO_2, $HbCO_2$) and the human body's skin surface flowing blood in oxygenation-deoxidization conditions and revealed the characteristic of OD value in the imaginable and unimaginable visible spectrum domain and this gave a foundation for the technology to be applied in the vitiligo sickness epidermis melanin pellet examination. Methods: Using different spectrum and *in vitro* and *in vivo* methods, the authors did statistics for the information of OD value under different state and wavelength. Results: Examination of *in vitro*: Experimented rabbit's blood Hb O_2 and RBC O_2 both have 367, 414 nm (the Soret cingulum) and 541, 576 (the Q cingulum) nm absorption peaks in the visible domain and both have 432 nm (Soret cingulum) and 553 nm (Q cingulum) absorption peaks, but blood had no change in the absorption peak position. No matter under what RBC and Hb condition, there was only completely independent absorption peak under the acidification and deoxidization condition. There is a significant difference ($p < 0.01$) between OD values under conditions of blood RBC cell and Hb hemolysis. Examination of *in vivo*: By using back skin surface specimen of the *in vivo* hand, absorption peaks were found at 540 and 576 nm for RBC O_2 condition and at 555 and 755 nm for RBC CO_2;. Having selected the specimen of hand back skin (a: Nail, b: refers to skin, c: hand back skin), wavelengths were examined for the three dots. Among them, 545 nm absorption peak has average OD values of absorbency, which are 0.83 ± 0.001, 0.73 ± 0.001 and 0.62 ± 0.001, and differences are notable ($p < 0.01$). Conclusion: Each absorption peak position of *in vitro* examination for RBC and Hb is invariable, but OD value of absorbency is different. Examination results under RBC condition are close to the alloriginality of RBC in vivo blood cell organization. The *in vivo* examination does not show any attack and damage to the human body, its sensitivity is high, testing time is short, and it has the superiority of taking in-phase test for the wavelength and the position information and so on. It is hopeful for the direct examination of epidermis black element and colored pellet.

Keywords Spectrum; Absorbance value; RGB image; Living cell; Skin surface

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