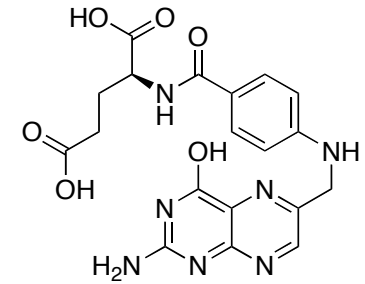


Intraoperative tumor-specific fluorescence imaging in ovarian cancer by folate receptor- α targeting: first in-human results

Gooitzen M van Dam, George Themelis, Lucia M A Crane, Niels J Harlaar, Rick G Pleijhuis, Wendy Kelder, Athanasios Sarantopoulos, Johannes S de Jong, Henriette J G Arts, Ate G J van der Zee, Joost Bart, Philip S Low & Vasilis Ntziachristos

Presented by Zhuzhu Wang
2/18/2012

What is folate?



- A water-soluble B vitamin that occurs naturally in food. Folic acid is the synthetic form that is found in supplements.
- A basic component of cell metabolism and DNA synthesis and repair.
- Is especially important during periods of rapid cell division (ex: tumor) and growth (ex: infancy, pregnancy).

Folate binding proteins

- Low affinity binders
 - Consisting of the reduced flate carrier (RFC).
 - Responsible for the majority of folate transport across cell membrane.
- Cytoplasmic-binding proteins
 - Consisting of specific enzyme involved in one-carbon metabolism
- Half-affinity binders
 - Consisting of folate receptor (FR).
 - Mediate folate uptake by endocytosis.

Ann Rev Nutr, **1990**, 10, 319-335

- Folate receptor α (FR α) distribution:
 - Restricted expression in normal adult epithelial tissues.
 - highly expressed in various nonmucinous tumors of epithelial origin.
 - The reasons for its expression and function in tumor relative to normal tissue are unknown

TABLE II – EXPRESSION OF FOLATE RECEPTOR ISOFORMS (FR α , FR β , FR γ) IN NORMAL AND MALIGNANT HUMAN TISSUES¹

System	FR α	FR β	FR γ
Normal tissues			
Genitourinary	Placenta ^{49,112} Kidney ⁵ Bladder ⁵ Testes ¹¹³	Placenta ¹¹²	
Central nervous	Choroid plexus ¹¹⁴ Cerebrospinal fluid ²⁷		
Hematopoetic		Spleen ¹¹² Thymus ¹¹² Neutrophils ⁵¹	Hematopoetic ⁵⁰
Gastrointestinal	Submandibular salivary glands ⁴ Colorectum ⁵		
Respiratory	Lung ⁵		
Endocrine	Human milk ^{51,115}		
Malignant tissues			
High expression	Nasopharyngeal epidermoid carcinoma ^{20,49} Non-mucinous ovarian carcinoma ⁵ Cervical carcinoma ⁶³ Uterine carcinoma ⁵⁶ Metastatic endometrial carcinoma ⁵ Primary renal cell carcinoma ⁵ Metastatic pancreatic carcinoma ⁵	Leukemia ⁵¹ Lymphoma ⁵	Leukemia ⁵¹
Low or negligible expression	Mucinous ovarian carcinoma ⁵ Primary endometrial carcinoma ⁵ Metastatic renal cell carcinoma ⁵ Lung carcinoma and adenocarcinoma ⁵ Primary breast carcinoma ⁵ Primary bladder carcinoma ⁵ Primary pancreatic carcinoma ⁵ Colorectal carcinoma ^{5,64} Primary prostate carcinoma ⁵ Primary brain carcinoma ^{4,58} Primary liver carcinoma ⁵ Primary head and neck carcinoma ⁵		

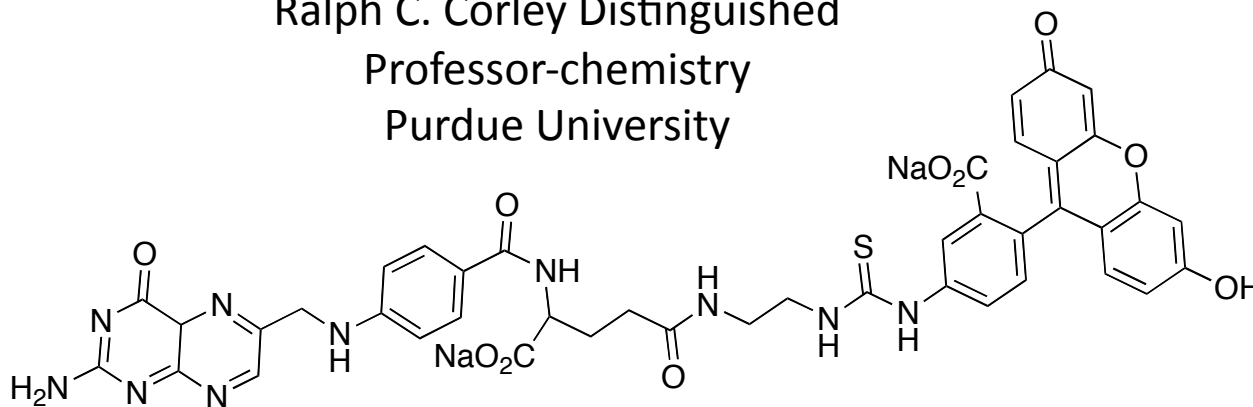
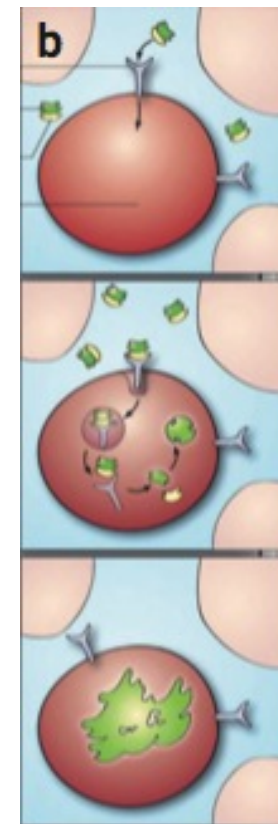
¹Adapted from *Folate Receptors*.⁴

Folate-FITC (Fluorescein isothiocyanate)



Philip Low

Ralph C. Corley Distinguished
Professor-chemistry
Purdue University



Optical imaging of metastatic tumors using Folate-FITC

M 109 cells, a murine lung carcinoma cell line of BALB/c mouse origin.

L 1210 cells, a lymphocyte derived cell line of DBA mouse origin.

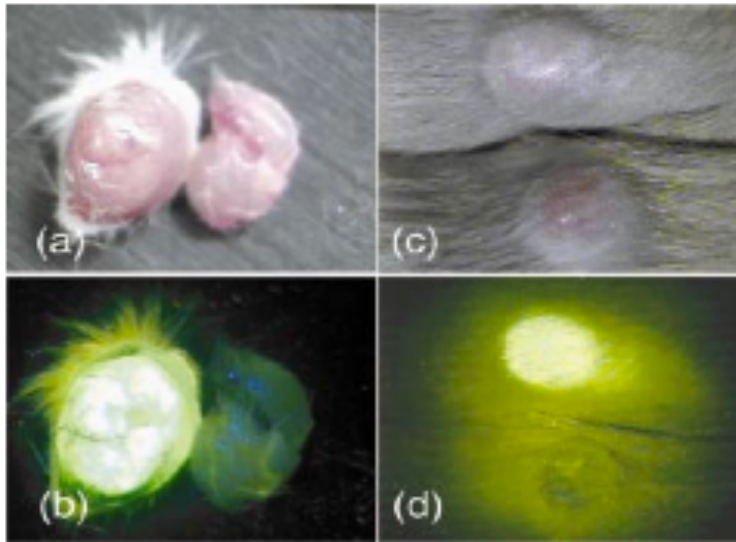


Fig. 1 (a) and (b) display M109 subcutaneous tumors (left tissue sample) next to muscle (right) tissue isolated from the same mouse. (c) shows the white light image and (d) shows the fluorescent image of L1210 subcutaneous tumors from a mouse injected with folate-fluorescein (top tumor) or folate-fluorescein plus 100-fold molar excess of free folate (bottom tumor)

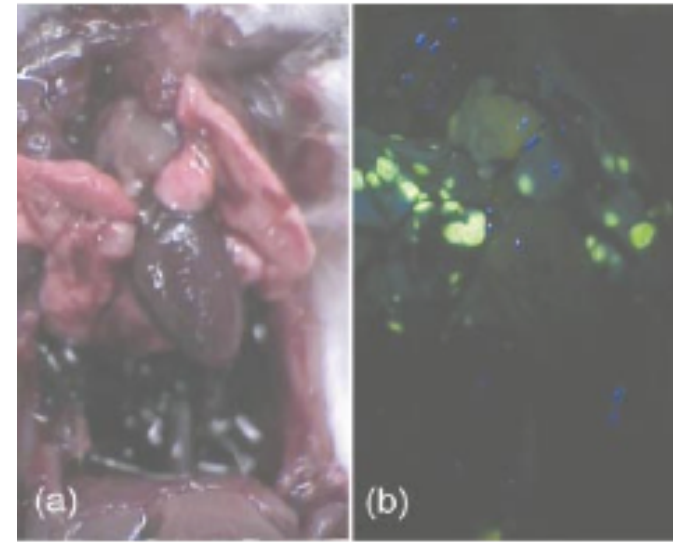


Figure. 3 Optical images of multiple metastatic M109 tumor nodules in a mouse lung (a) Normal light image of the opened chest cavity. (b) Same chest cavity visualized under 488-nm argon laser illumination

Journal of Biomedical Optics 8 (4), 636-641 (October 2003)

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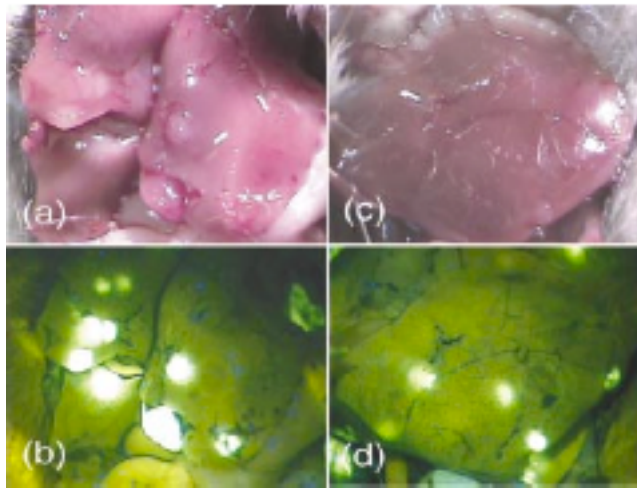
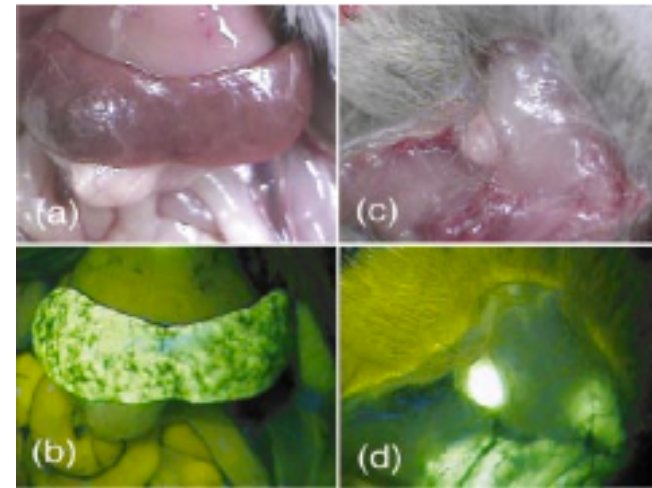


Fig. 4 Images of L1210 metastatic tumor nodules in the liver of a DBA mouse. (a) and (b) show the white light and fluorescent light images of the lower lobe of the liver, (c) and (d) display the same images of the upper lobe of liver



Fig, 5 L1210 tumor nodules in the spleen and muscle tissue of a DBA mouse. (a) and (b) show the white light and fluorescent light images of a tumor-infiltrated spleen, (c) and (d) display the same images of a tumor nodule in the skeletal muscle of the neck

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- Application suggestion:

“The ability of highly fluorescent conjugates to reveal a tumor’s location under direct illumination might be exploited to guide a surgeon’s knife during tumor resection”

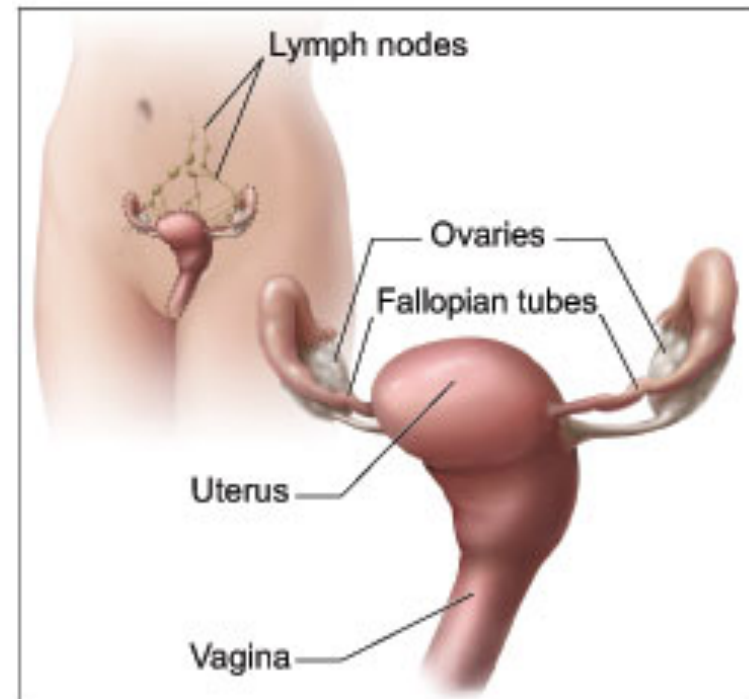
Such as.....

Ovarian cancer

- Epithelial ovarian cancer (EOC) is one of the most common gynecologic malignancies and the fifth most frequent cause of cancer death in women.
- The overall 5 - year survival rate is 45%, and for stages III and IV it is only 20 – 25%.
- The American Cancer Society estimate that there would be 22,280 new cases of ovarian cancer in 2012 and 15,500 deaths from this disease.
- The cause of ovarian cancer is still unknown, but risk factor including: Family history of cancer; Personal history of cancer; Age over 55; Never pregnant; Menopausal hormone therapy
- Early ovarian cancer may not cause obvious symptoms, often result in the disease being diagnosed only at more advanced stages.

Ovarian Cancer: Treatment

- Combination of cytoreductive surgery and chemotherapy.
- Different procedures depending on severity and malignancy of the disease.



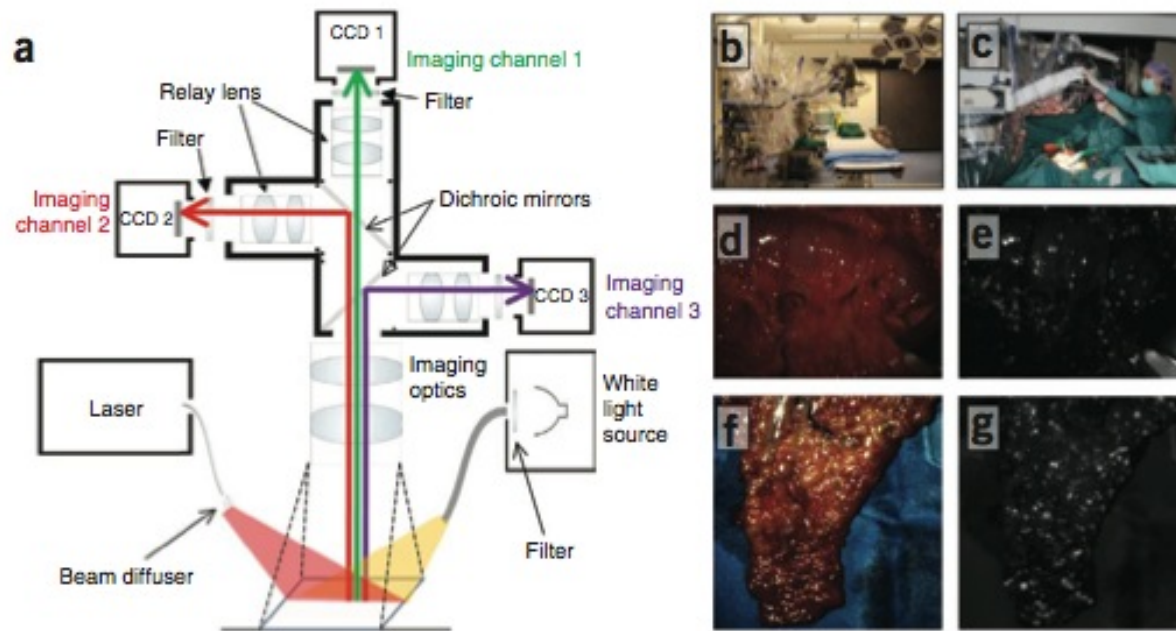
“With ovarian cancer it is clear that the more cancer you can remove, the better the prognosis for the patient, that is why we chose to begin with ovarian cancer. It seemed like the best place to start to make a difference in people’s lives”

“Ovarian cancer is notoriously difficult to see, and this technique allowed surgeon to spot a tumor 30 times smaller (1 / 10 of a millimeter) than the smallest they could detect using standard techniques”

“By dramatically improving the detection of the cancer – by literally lighting it up – cancer removal is dramatically improved”

--- Philip Low

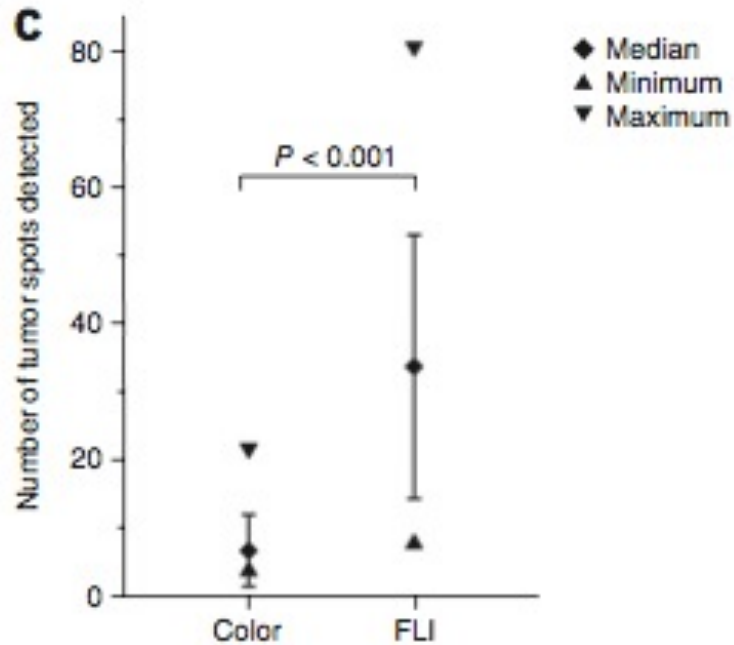
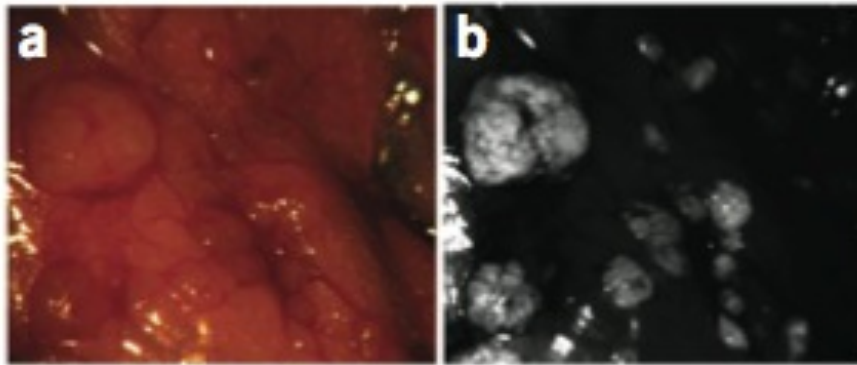
Multispectral fluorescence camera



Under leadership of Gooitzen M. van Dam in Department of Surgery, Division of Surgical Oncology at the University Medical Center Groningen

[See video](#): Application of multispectral intraoperative fluorescence imaging technology for intraoperative detection of the sentinel lymph node (SLN) in patients with cervical and vulvar cancer. *Journal of Visualized Experiments*, **2010**, 44, 1-4

Quantification of tumor deposits ex vivo



Quantification of tumor deposits ex vivo

[See video](#) : Application of the intra-operative multispectral imaging system

Summary

- The first in-human results of intraoperative utilization of tumor specific fluorescence imaging for diagnostic staging and cytoreductive surgery using the systemically administered targeted fluorescent agent folate-FITC.
- 34 tumors deposits using this technique vs. 7 tumor deposits using visual and tactile observation.
- However, FR- α is overexpressed in “only” 90-95% of malignant tumors, only FR- α -positive patients are suitable for FR- α targeted image-guided surgery.
- Will this technology be approved for general use in a short time?

Perspective

- Overexpression of FR- α varies between different solid tumors originating from different organs. Thus identification of tumor-specific targets for other solid tumors for the tumor-specific fluorescence imaging in cancer surgery.
- Development of new fluorescent agent in the near-infrared spectrum (red fluorescent dye), which allows for identification of more deeply seated tumors.