UNIVERSITÉ DE RENNES 1
Faculté de Médecine

Array-CGH and FISH applications for diagnosis and study of gliomas

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Cancer

- **Chromosome numerical aberrations / segmental alterations**
- **Restricted to tumor cells → selective advantage**

→ Spécific aberrations (*bcr-abl*) or not (-3, +8, +7, …)

→ Gains: oncogenes (amplification)

→ Losses: Tumor Supressor Genes

→ Translocations → gene overexpression / fusion genes

… mutations …

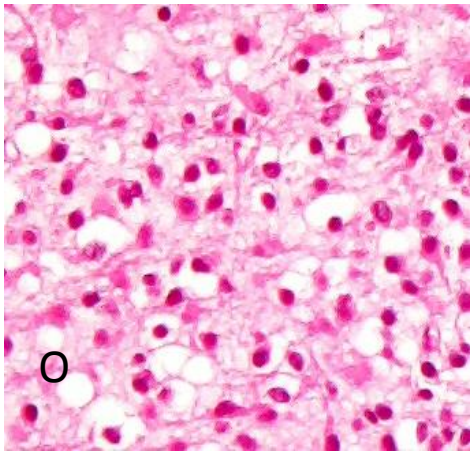
Clinical impact: **- Diagnosis**

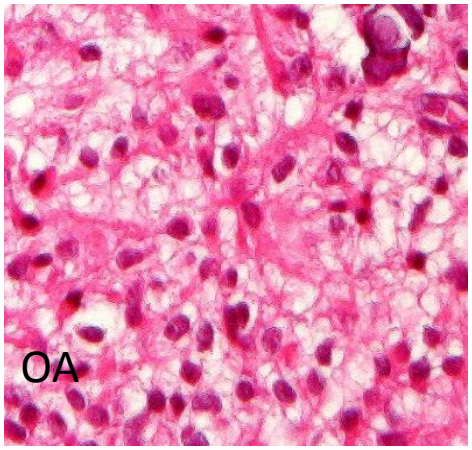
- Prognosis

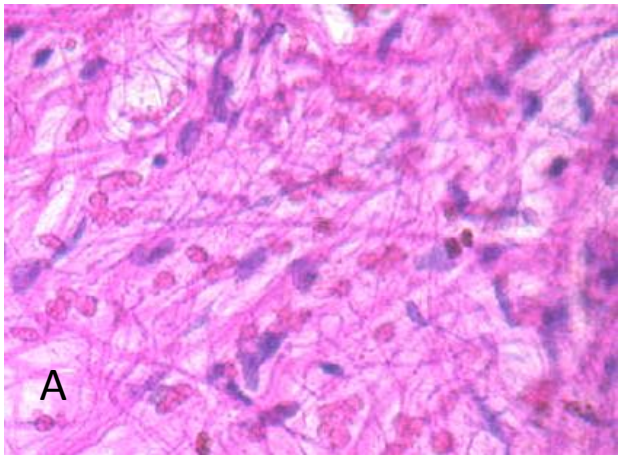
- Treatment response

Gliomes

- Diagnostic histologique difficile





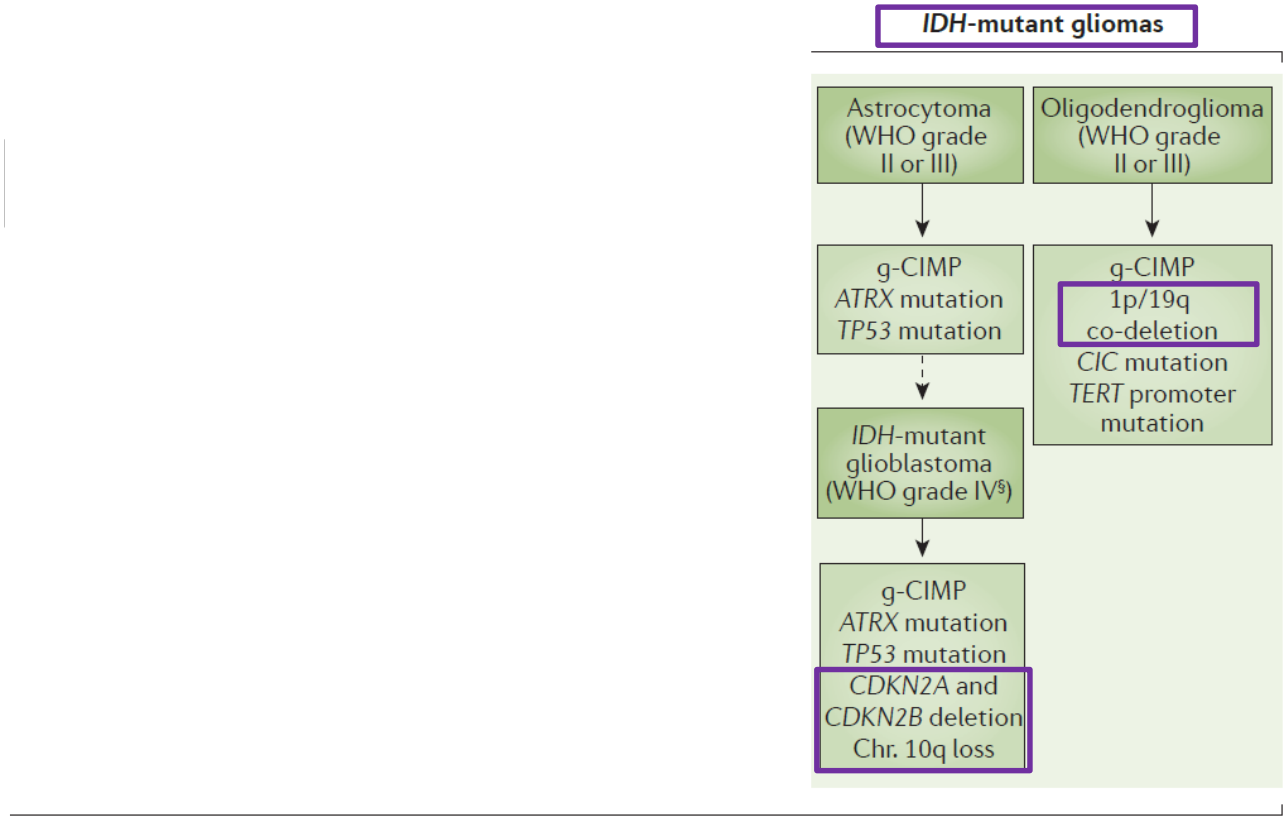


- Impact pronostic des co-délétions 1p36 et 19q13
  - Chimiosensibilité (réponse PCV)
  - Survie globale plus longue

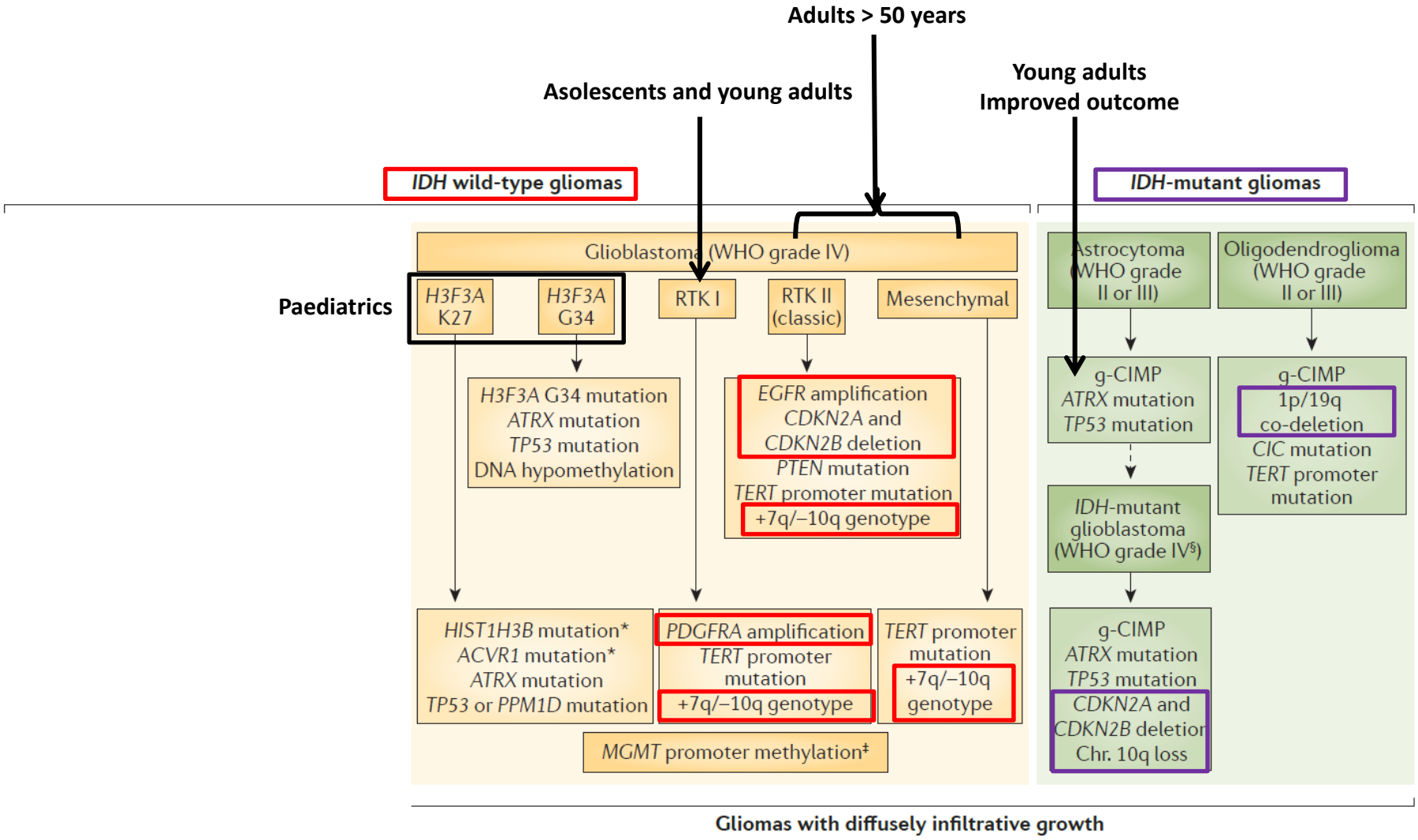
Glioma

Michael Weller[1], Wolfgang Wick[2], Ken Aldape[3], Michael Brada[4], Mitchell Berger[5], Stefan M. Pfister[6,7], Ryo Nishikawa[8], Mark Rosenthal[9], Patrick Y. Wen[10], Roger Stupp[11] and Guido Reifenberger[12]

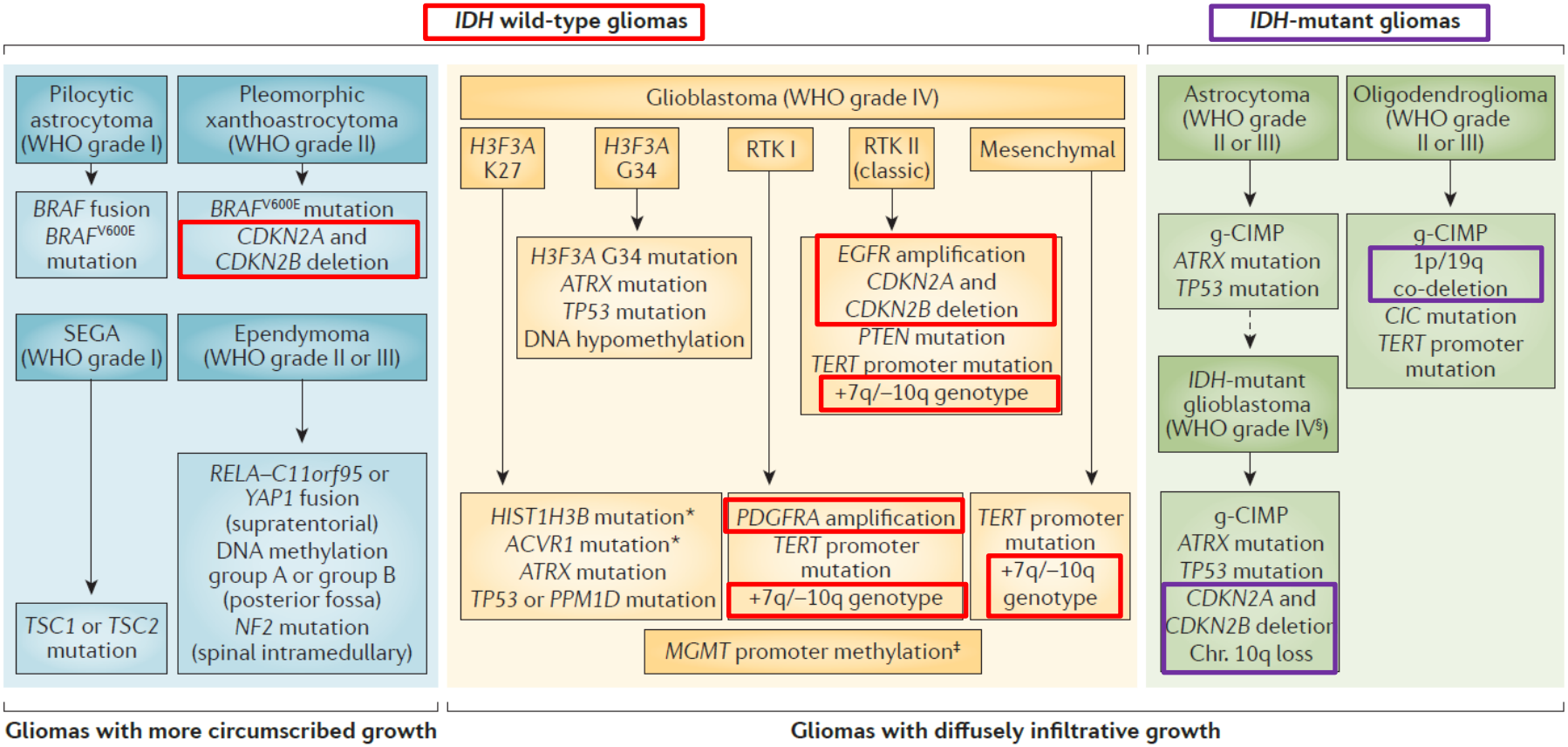
Abstract | Gliomas are primary brain tumours that are thought to derive from neuroglial stem or progenitor cells. On the basis of their histological appearance, they have been traditionally classified as astrocytic, oligodendroglial or ependymal tumours and assigned WHO grades I–IV, which indicate different degrees of malignancy. Tremendous progress in genomic, transcriptomic and epigenetic profiling has resulted in new concepts of classifying and treating gliomas. Diffusely infiltrating gliomas in adults are now separated into three overarching tumour groups with distinct natural histories, responses to treatment and outcomes: isocitrate dehydrogenase (*IDH*)-mutant, 1p/19q co-deleted tumours with mostly oligodendroglial morphology that are associated with the best prognosis; *IDH*-mutant, 1p/19q non-co-deleted tumours with mostly astrocytic histology that are associated with intermediate outcome; and *IDH* wild-type, mostly higher WHO grade (III or IV) tumours that are associated with poor prognosis. Gliomas in children are molecularly distinct from those in adults, the majority being WHO grade I pilocytic astrocytomas characterized by circumscribed growth, favourable prognosis and frequent *BRAF* gene fusions or mutations. Ependymal tumours can be molecularly subdivided into distinct epigenetic subgroups according to location and prognosis. Although surgery, radiotherapy and alkylating agent chemotherapy are still the mainstay of treatment, individually tailored strategies based on tumour-intrinsic dominant signalling pathways and antigenic tumour profiles may ultimately improve outcome. For an illustrated summary of this Primer, visit: http://go.nature.com/TXY7Ri



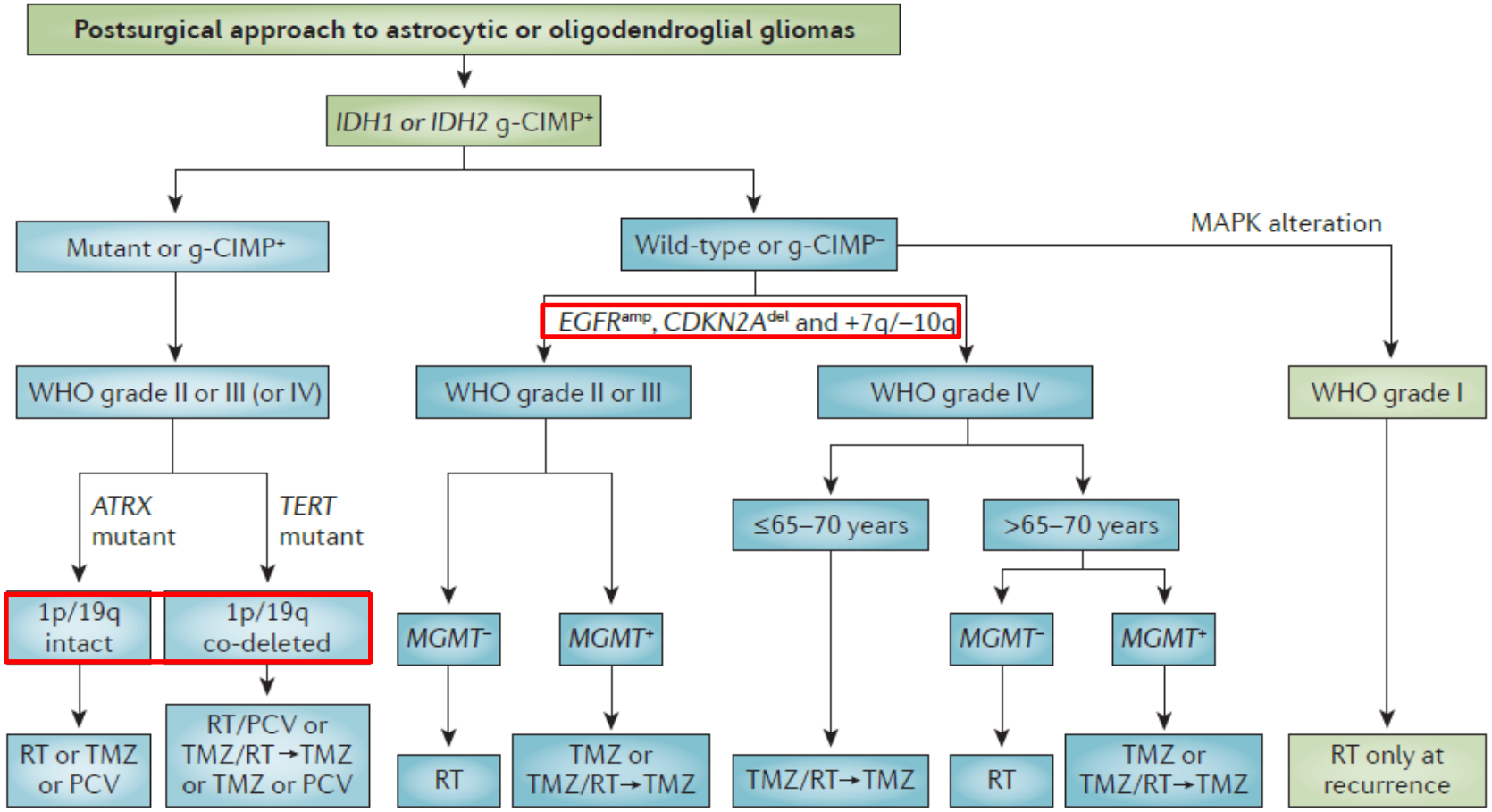
Gliomas with diffusely infiltrative growth


Adults > 50 years
Asolescents and young adults
Young adults
Improved outcome
IDH wild-type gliomas
IDH-mutant gliomas
Paediatrics
Glioblastoma (WHO grade IV)
H3F3A K27
H3F3A G34
RTK I
RTK II (classic)
Mesenchymal
Astrocytoma (WHO grade II or III)
Oligodendroglioma (WHO grade II or III)
H3F3A G34 mutation
ATRX mutation
TP53 mutation
DNA hypomethylation
EGFR amplification
CDKN2A and CDKN2B deletion
PTEN mutation
TERT promoter mutation
+7q/–10q genotype
g-CIMP
ATRX mutation
TP53 mutation
g-CIMP
1p/19q co-deletion
CIC mutation
TERT promoter mutation
IDH-mutant glioblastoma (WHO grade IV§)
HIST1H3B mutation*
ACVR1 mutation*
ATRX mutation
TP53 or PPM1D mutation
PDGFRA amplification
TERT promoter mutation
+7q/–10q genotype
TERT promoter mutation
+7q/–10q genotype
g-CIMP
ATRX mutation
TP53 mutation
CDKN2A and CDKN2B deletion
Chr. 10q loss
MGMT promoter methylation‡
Gliomas with diffusely infiltrative growth

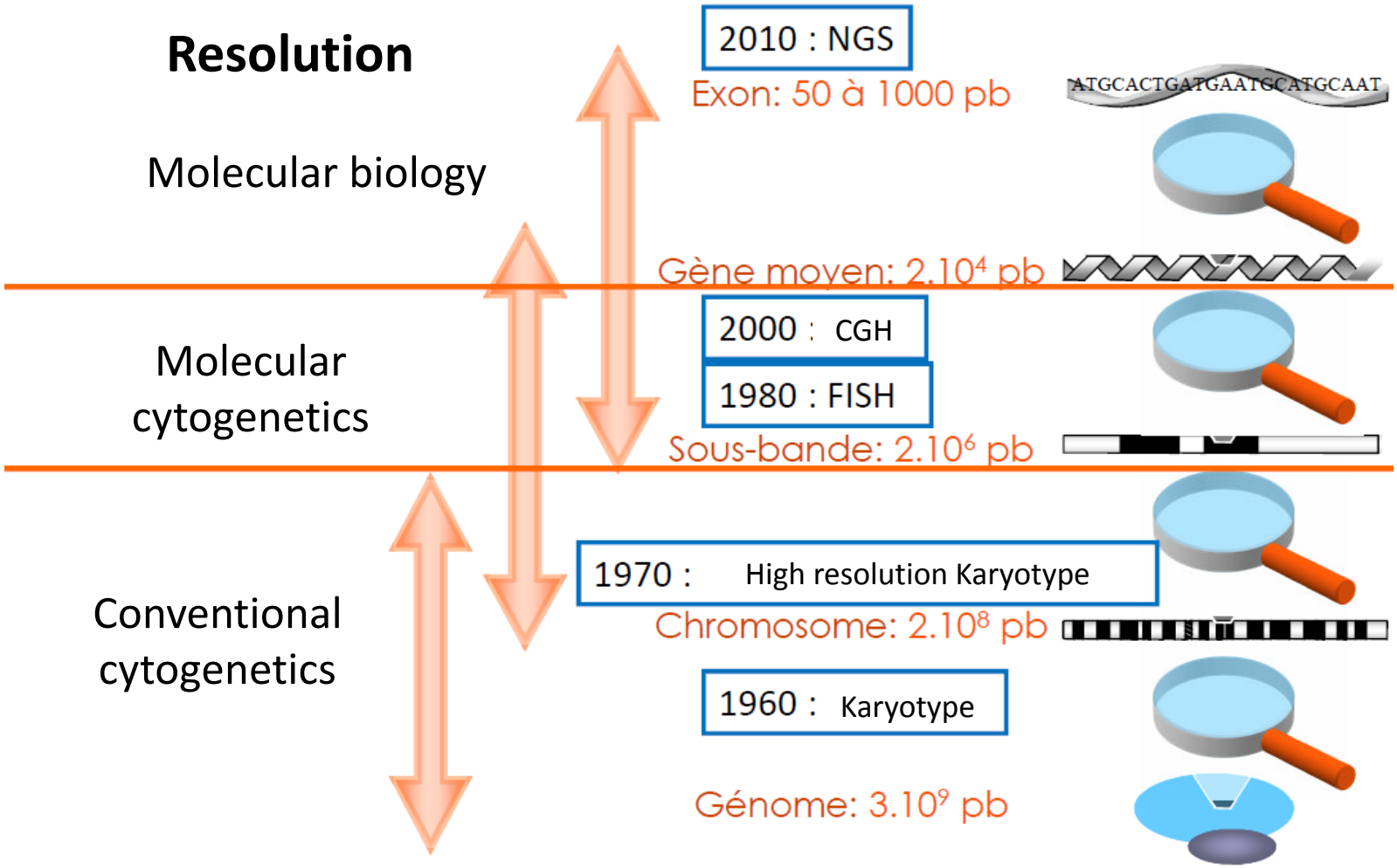
Gliomas


IDH wild-type gliomas
IDH-mutant gliomas
Pilocytic astrocytoma (WHO grade I)
Pleomorphic xanthoastrocytoma (WHO grade II)
BRAF fusion
BRAFV600E mutation
BRAFV600E mutation
CDKN2A and CDKN2B deletion
SEGA (WHO grade I)
Ependymoma (WHO grade II or III)
TSC1 or TSC2 mutation
RELA–C11orf95 or YAP1 fusion (supratentorial)
DNA methylation group A or group B (posterior fossa)
NF2 mutation (spinal intramedullary)
Glioblastoma (WHO grade IV)
H3F3A K27
H3F3A G34
RTK I
RTK II (classic)
Mesenchymal
H3F3A G34 mutation
ATRX mutation
TP53 mutation
DNA hypomethylation
EGFR amplification
CDKN2A and CDKN2B deletion
PTEN mutation
TERT promoter mutation
+7q/–10q genotype
HIST1H3B mutation*
ACVR1 mutation*
ATRX mutation
TP53 or PPM1D mutation
PDGFRA amplification
TERT promoter mutation
+7q/–10q genotype
TERT promoter mutation
+7q/–10q genotype
MGMT promoter methylation‡
Astrocytoma (WHO grade II or III)
Oligodendroglioma (WHO grade II or III)
g-CIMP
ATRX mutation
TP53 mutation
g-CIMP
1p/19q co-deletion
CIC mutation
TERT promoter mutation
IDH-mutant glioblastoma (WHO grade IV§)
g-CIMP
ATRX mutation
TP53 mutation
CDKN2A and CDKN2B deletion
Chr. 10q loss
Gliomas with more circumscribed growth
Gliomas with diffusely infiltrative growth

Clinical impact of a-CGH analysis



Genome analysis



Sanlaville 2014 from http://www.calender-science.com/Cytogenetique-1.pdf

→ **Which technique to be chosen?**

1 – Hybridation in situ en fluorescence : FISH

Principe de la FISH

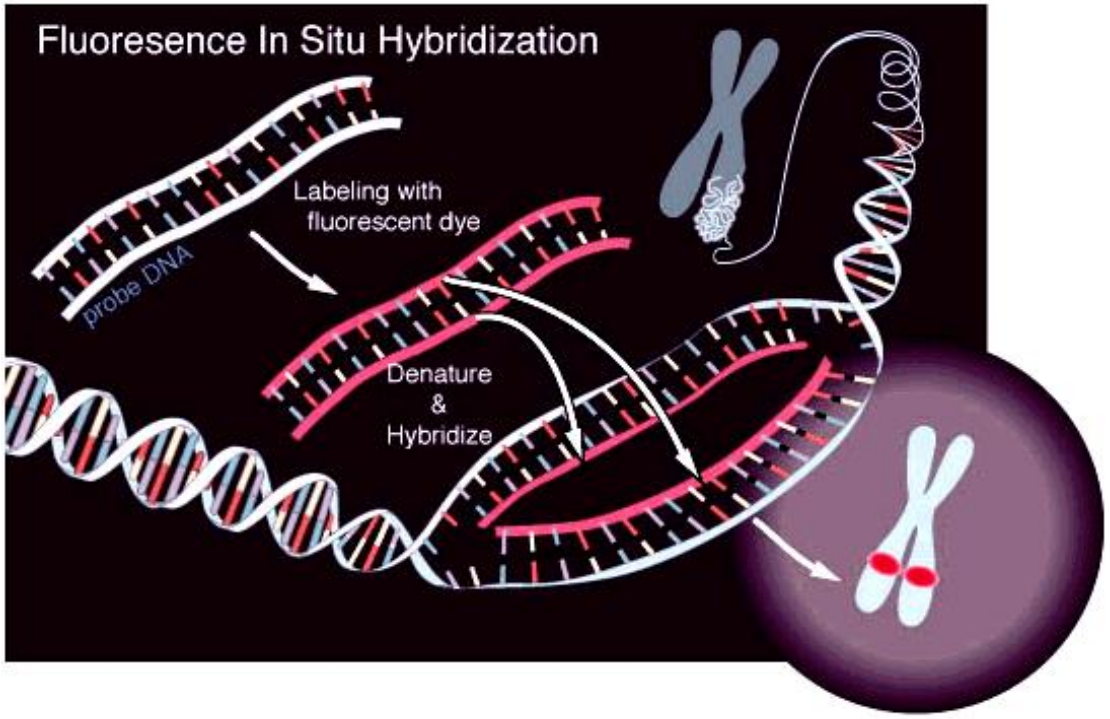
Hybridation d'une séquence d'ADN cible par des sondes ADN marquées par un fluorochrome

1 - **Marquage** de la sonde

2 - **Dénaturation** de l'ADN et de la sonde

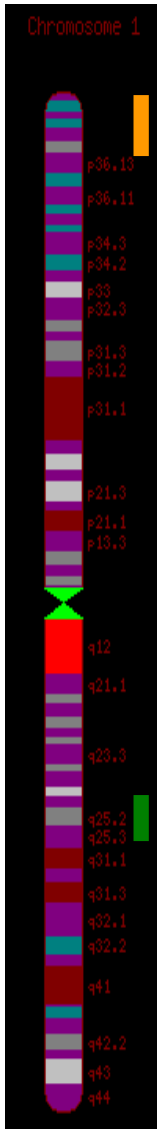
3 - **Hybridation**

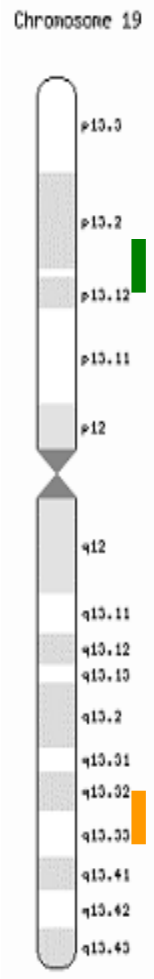
4 - **Lecture**

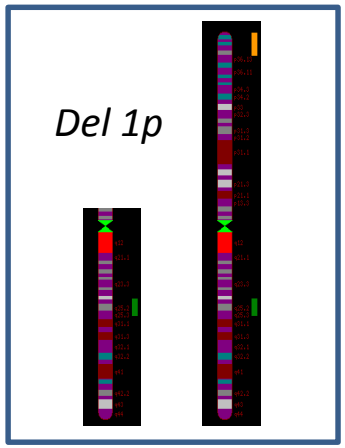


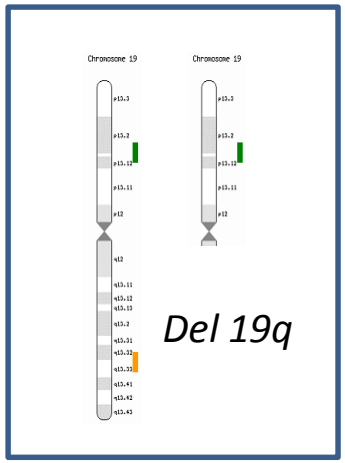
FISH analysis

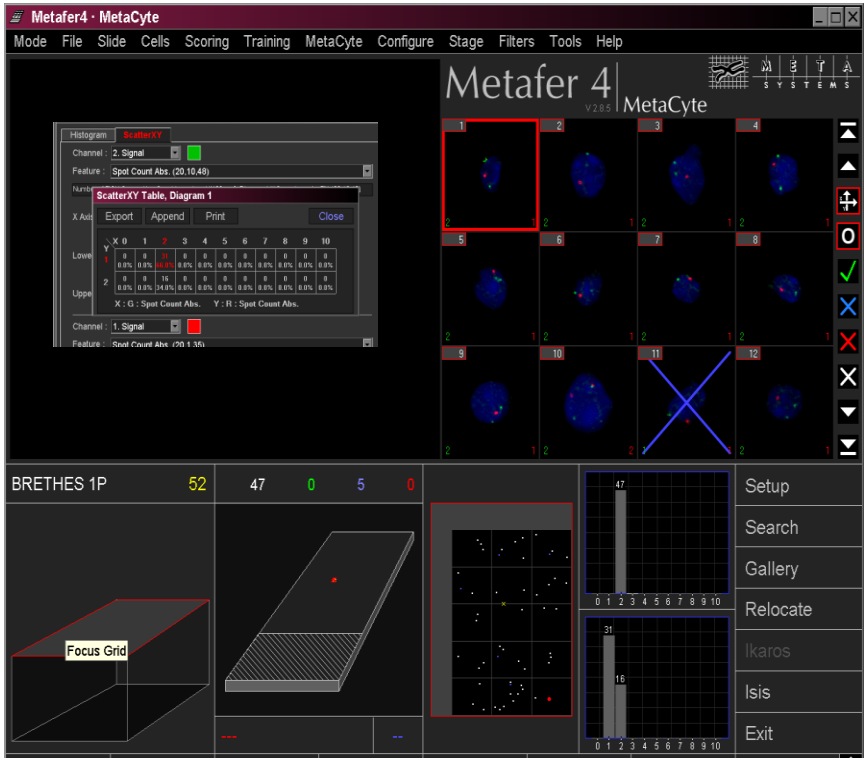
Acta Neuropathologica 2006







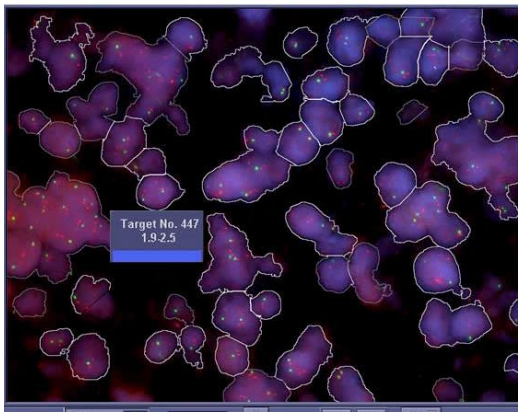




FISH analysis

- **Routine screening**

Acquisition station
Slide charger



Automatic nuclei segmentation

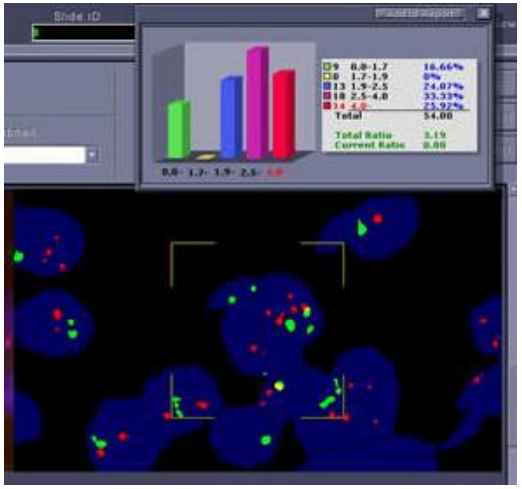
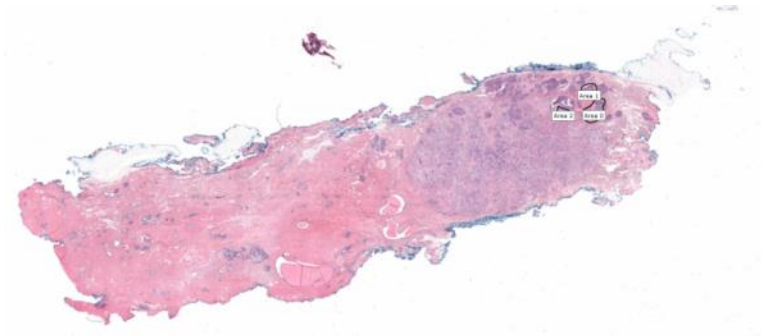
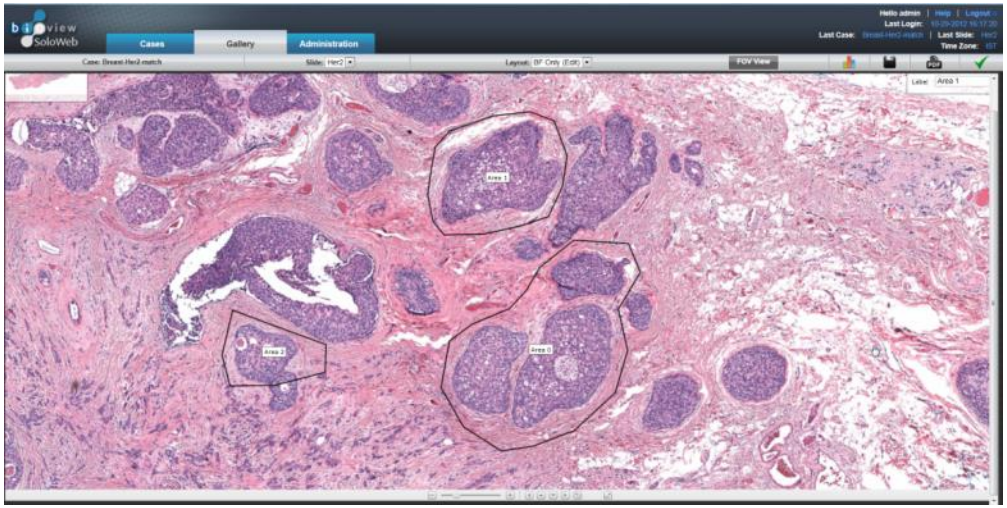


Image analysis

BioView's Solution

- H&E / IHC region of interest selection can be performed from any location via standard web browsers on a digital slide

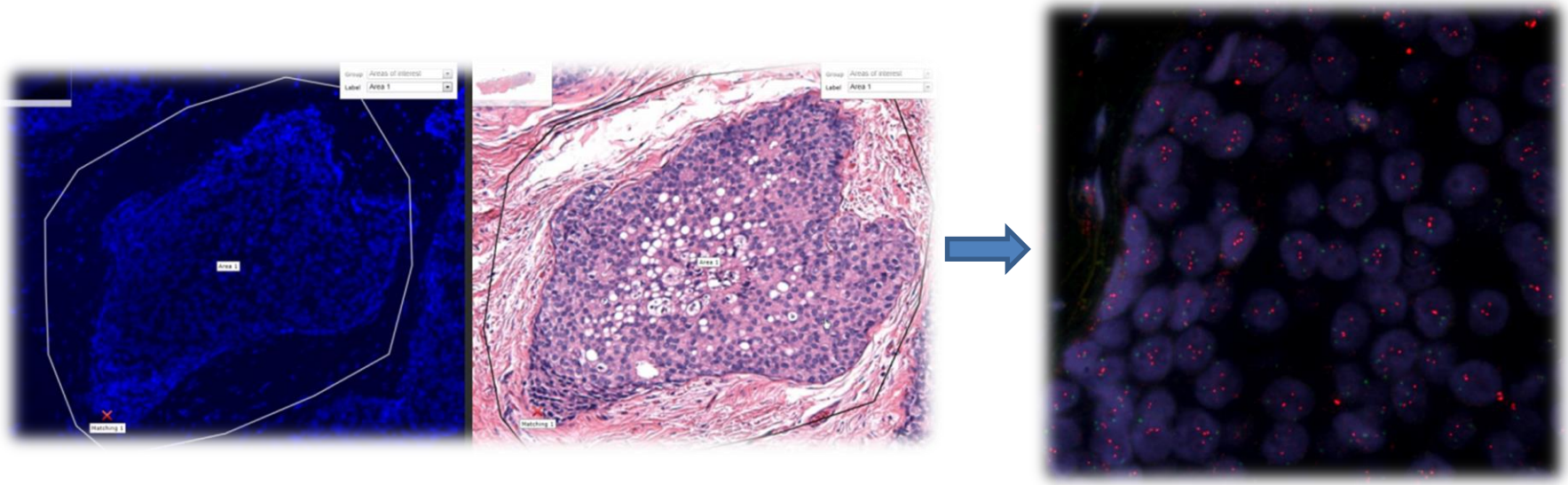




H&E digitally review and marked using BioView's SoloWeb application

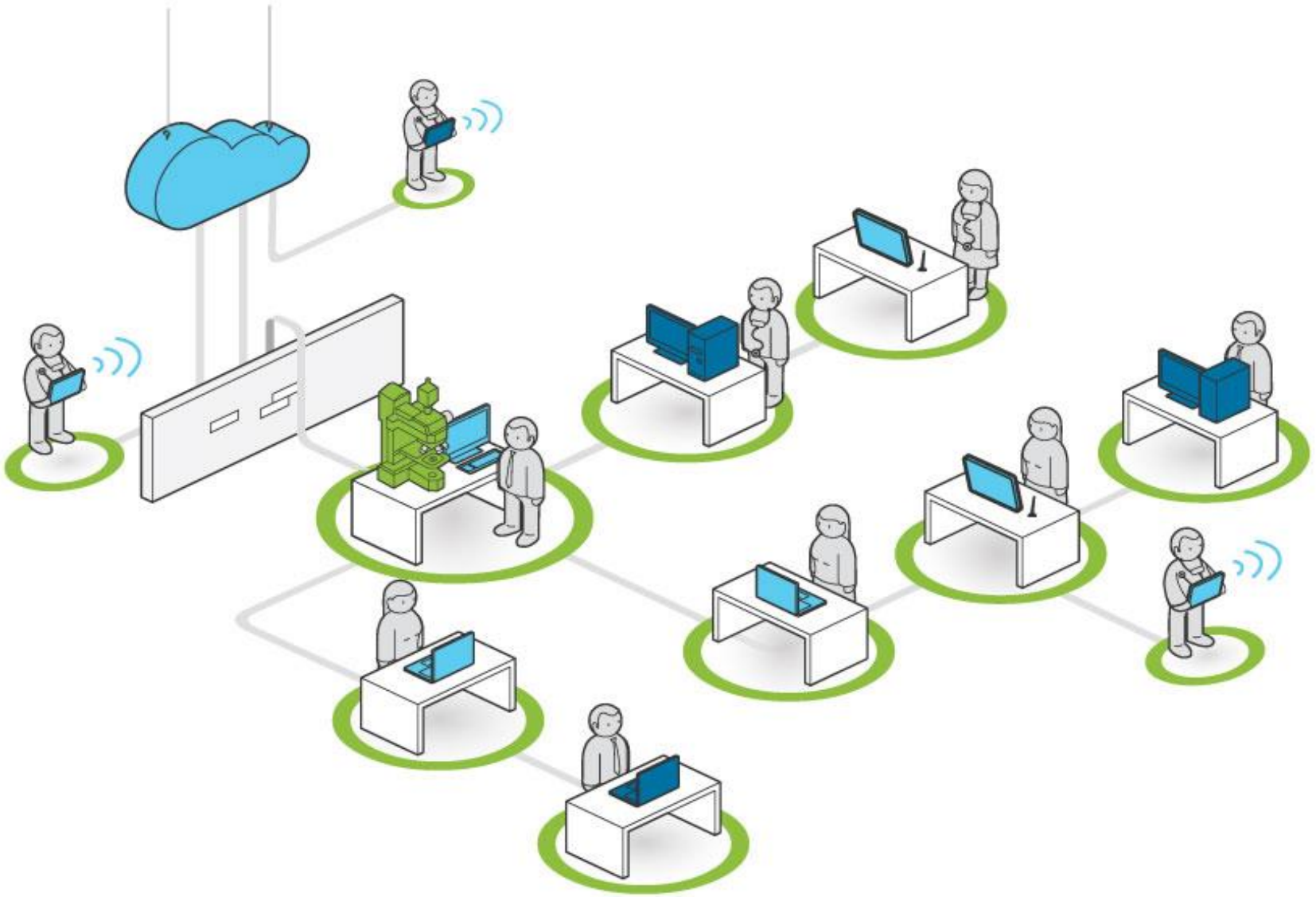
BioView's Solution

- Integrated solution to allow matching between H&E /IHC and FISH slides prepared on parallel sections



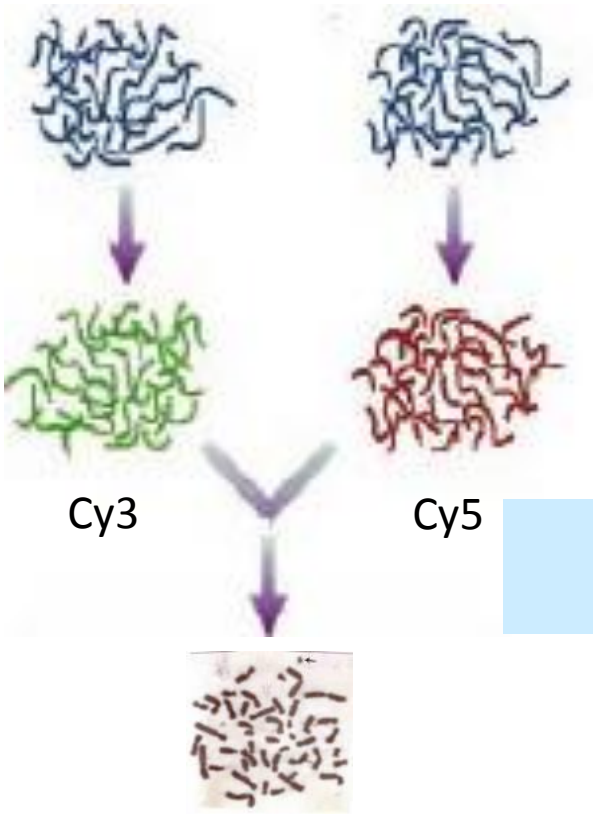
FISH analysis is performed according to pathologist guidance

System Typical Layout



Comparative Genomic Hybridization (CGH)

Metaphasis (Kallioniemi et al., 1992)



Technical steps

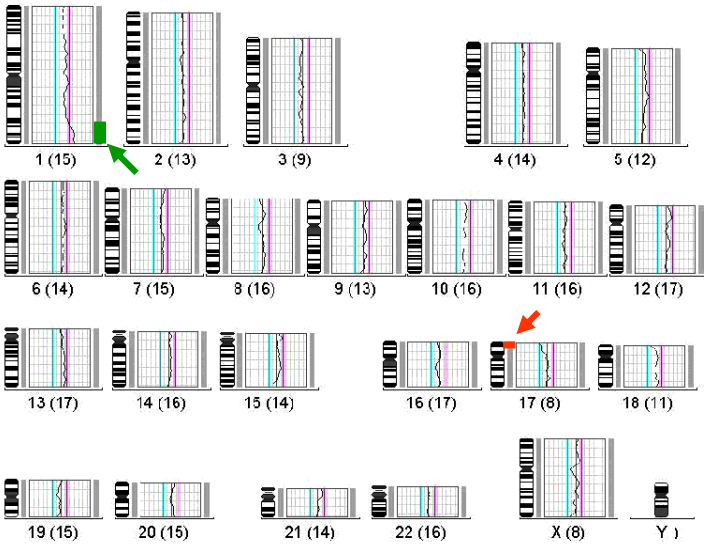
DNA extraction

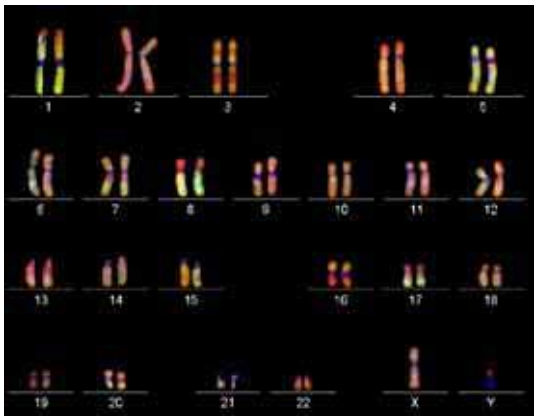
Labelling

Hybridization
(metaphasis)

Scanning

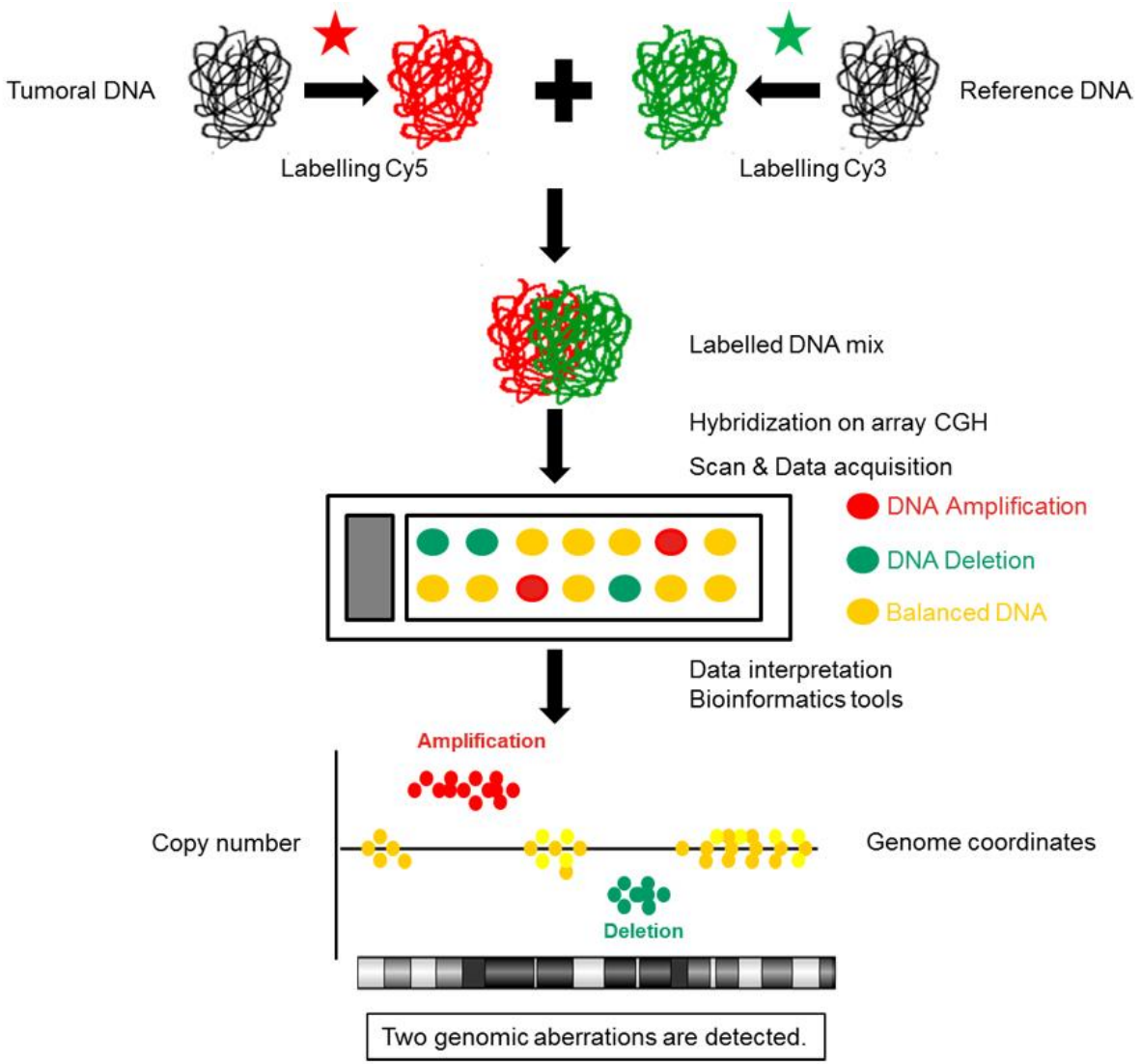
analysis

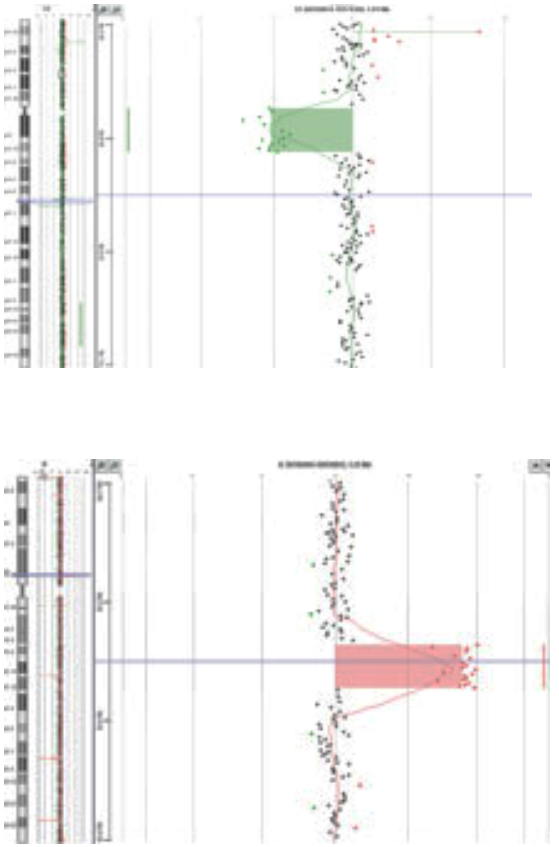




Resolution: 10 – 20 Mb

Comparative Genomic Hybridization (CGH)





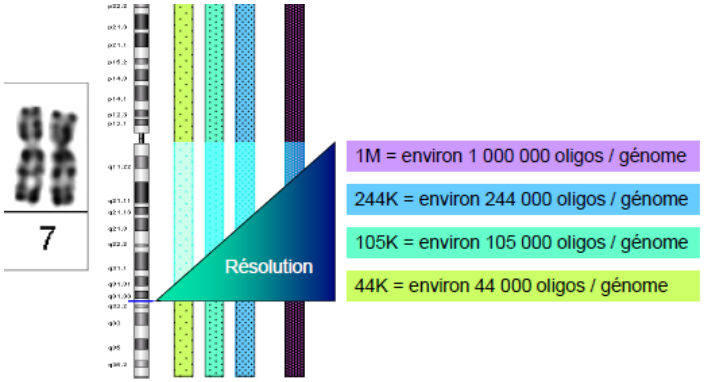
High resolution

http://www.helixio.fr/contenu/support-fr/technologies-fr

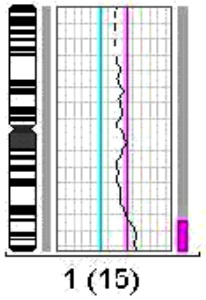
Comparative Genomic Hybridization (CGH)

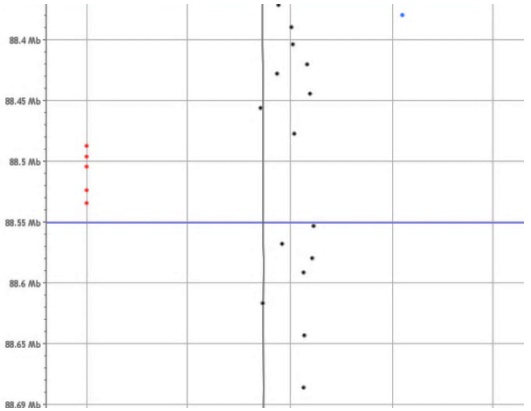
Resolution : number and distribution of clones

- **Metaphasis : Karyotype bands (5-10 Mb)**
- **BACs : ~ 170 kb**
- **Oligonucleotides**



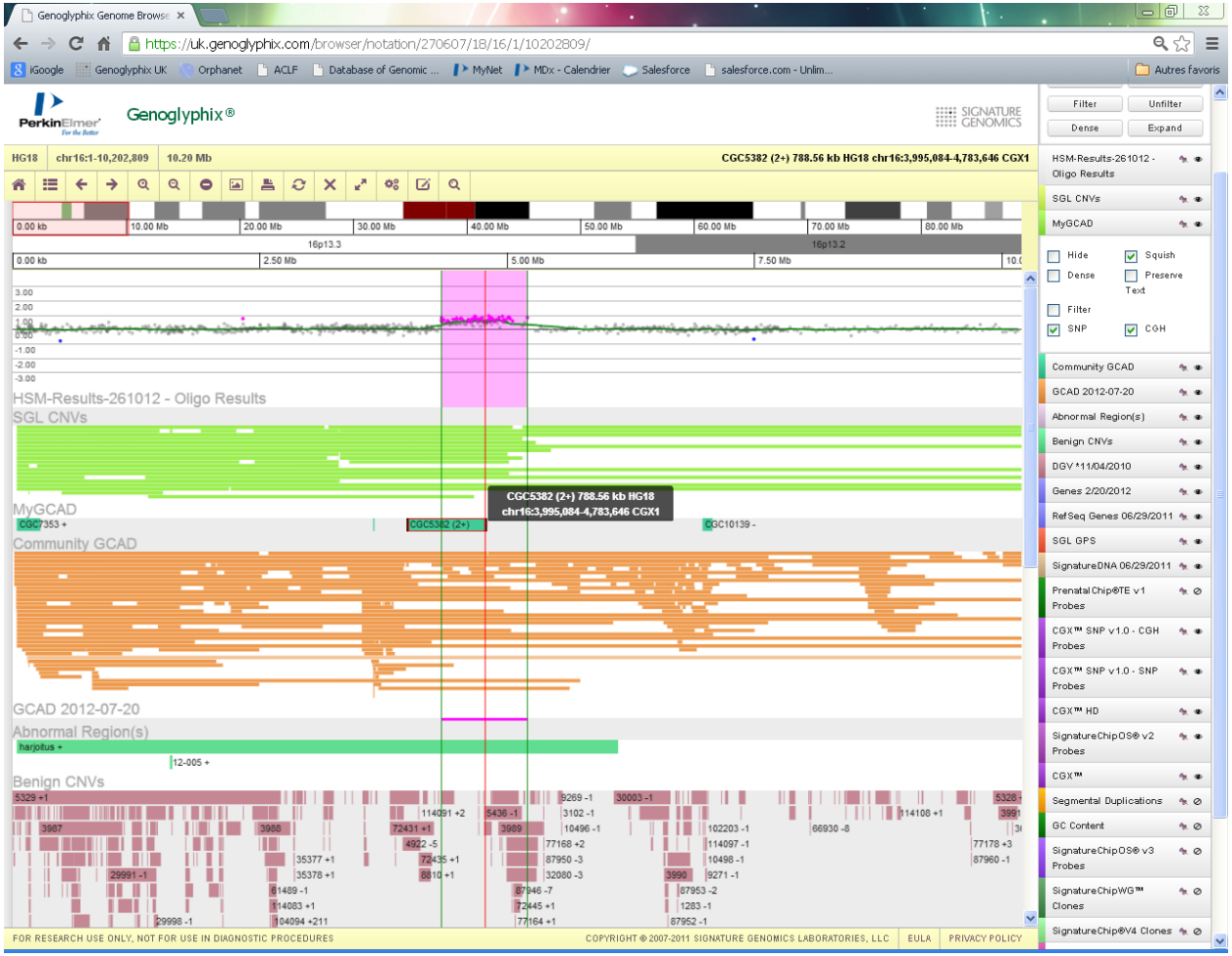
180K : 30-50kb resolution
200x higher than karyotype





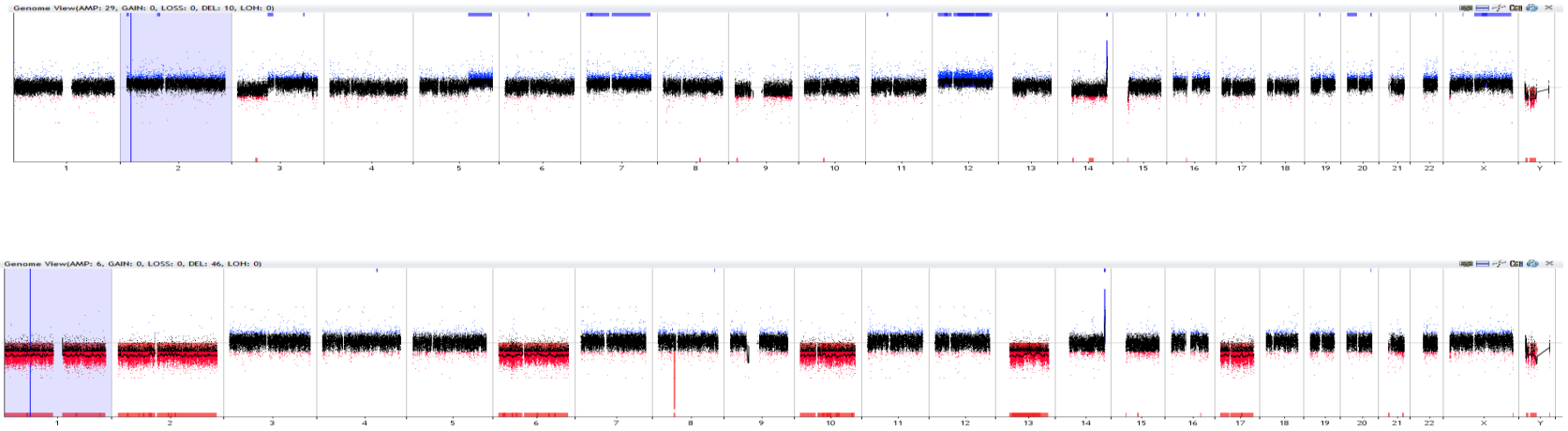
Sanlaville 2014 from http://www.calender-science.com/Cytogenetique-1.pdf

Data analysis



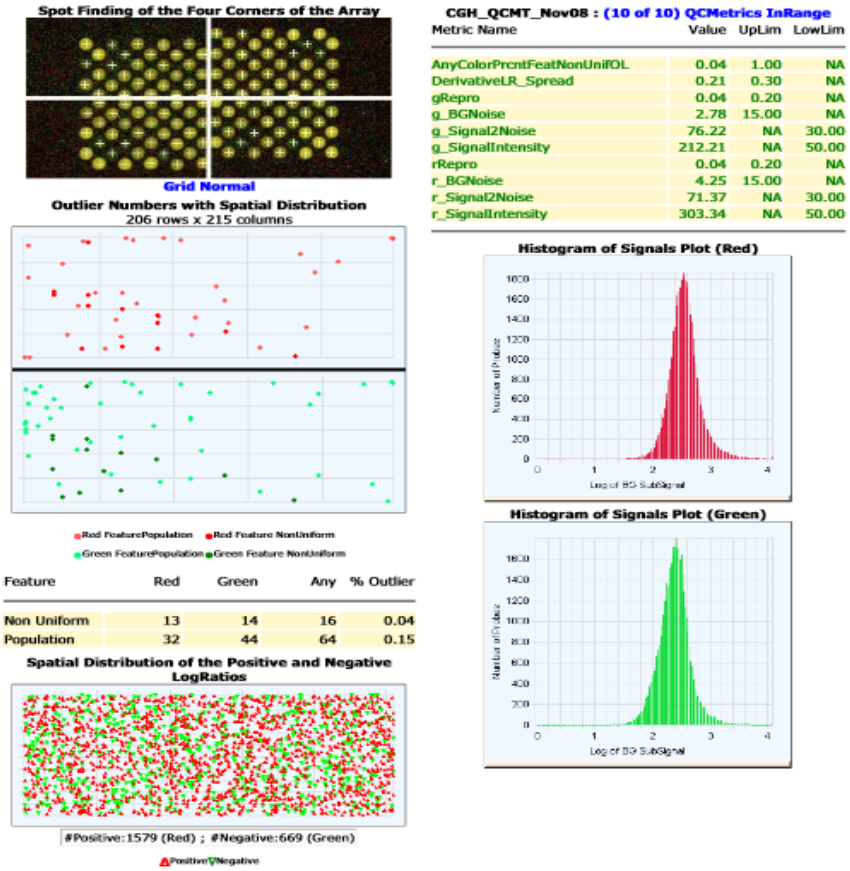
Array-CGH for tumor classification in routine

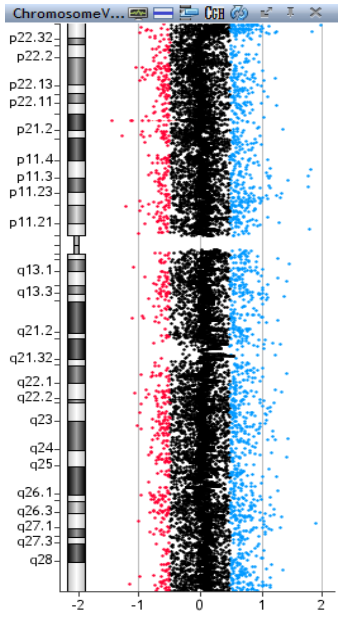
- Genomic copy number profiles can distinguish distinct subgroups within histologically defined disease entities
- Tumor type specific copy number patterns can be used for efficient tumor classification and patient stratification



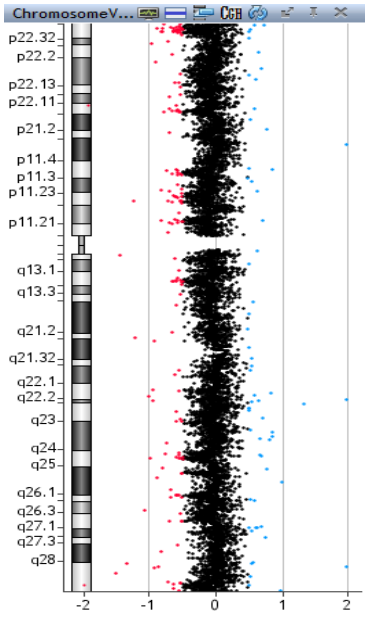
Array-CGH: Limitations

➢ Technical





Background



Waves (GC)

Array-CGH: Limitations

➢ Technical

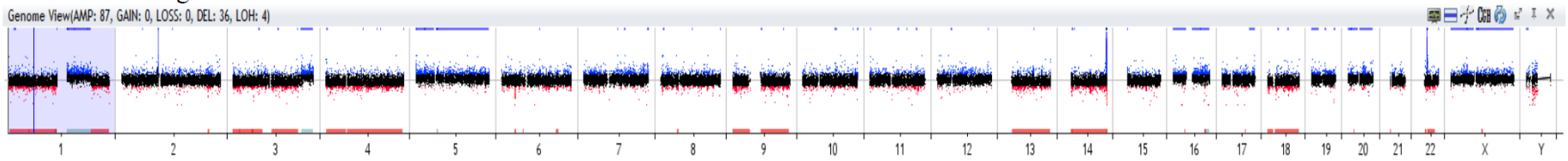
Samples:

- Fresh: Blood, BM, other liquids

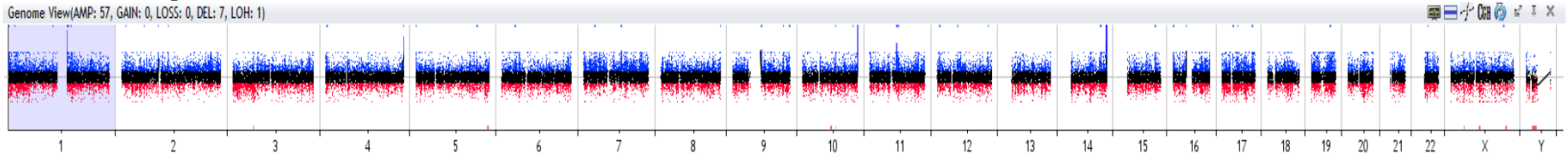
- Frozen tissues (cancer)

FFPE tissues

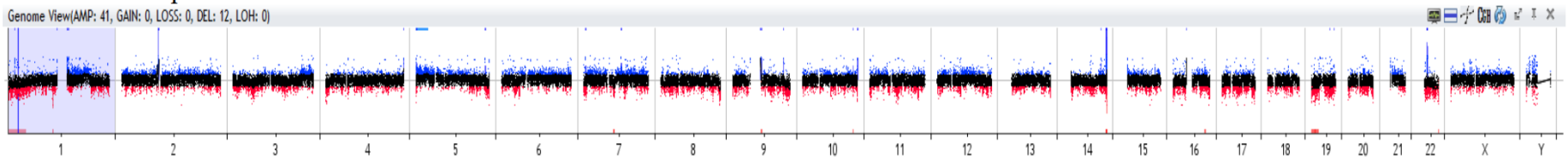
Echantillon congelé



Echantillon du protocole A1



Echantillon du protocole B1



Array-CGH: Limitations

➢ Analysis

- DNA origin (% of tumoral cells, reactive cells)
- Balanced chromosomal aberrations: translocations, inversions
- Subclones, ploïdy
- Driver chromosomal aberrations → Genomic databases
- Constitutional abnormalities (predisposition genes), mutations
- Chromosomal mechanism: FISH, karyotype

FISH et ACPA : analyse comparative

	FISH	ACPA
Formation, GBEA et C.Q.	Indispensable	
Mise en œuvre	+++	
Tissus fixés	+++	+/-
Analyse ciblée des C tumorales	+++	-
Infiltrat tumoral	10%	> 30 %
Automatisation	+++	+/-
Coût / test	+	++
Etude pangénomique	-	+++
Résolution	+	+++

Clinical impact of Array-CGH analysis

1p / 19q co-deletion

***CDKN2A* / *CDKN2B* deletion**

10q loss / 7q gain

***PDGFRA* or *EGFR* amplification**

Tumor classification

Prediction of patient outcome

Post surgical approach

Conclusion

- **Diagnostic et pronostic individuel, choix du traitement**
- **ACPA :**
  - Tissu frais, congelé (→ FFPE samples)
  - Analyse pangénomique de haute résolution
- **ACPA : limites**
  - Taille du prélèvement, % de C tumorales, clones, ploïdie, hétérogénéïté tumorale …
  - FISH en 2ème intention ou pour confirmation
  - Réseau (CHU Angers)
- **Facturation : RiHN / B**

Acknowledgments

Medical and Technical Teams:

- Cytogenetic and Cellular Biology Department
- Pathology Department
- Clinical cancer center
- PFGMC